Improved Formulation of *Lecanicillium lecanii* (Zimmermann) Zare and Gams and its Evaluation Against Sucking Pests

by

NITHYA P. R.

(2013-11-125)

Thesis submitted in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM – 695 522

KERALA, INDIA

2015

DECLARATION

I, hereby declare that the thesis entitled **"**Improved Formulation of *Lecanicillium lecanii* (Zimmermann) Zare and Gams and its Evaluation against Sucking Pests**"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani, **Nithya P. R.**

Date: **(2013-11-125)**

CERTIFICATE

Certified that this thesis entitled **"**Improved Formulation of *Lecanicillium lecanii* (Zimmermann) Zare and Gams and its Evaluation against Sucking Pests**"** is a record of bonafide research work done independently by Ms. Nithya P. R. (2013-11-125) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellayani,

Date:

Dr. Reji Rani O. P.

(Major Advisor, Advisory Committee)

Assistant Professor

Department of Agricultural Entomology

College of Agriculture, Vellayani

CERTIFICATE

We, the undersigned members of the advisory committee of Miss. Nithya P. R. (2013-11-125), a candidate for the degree of **Master of Science in Agriculture** with major in Agricultural Entomology, agree that this thesis entitled **"**Improved Formulation of *Lecanicillium lecanii* (Zimmermann) Zare and Gams and its Evaluation against Sucking Pests**"** may be submitted by Ms. Nithya P. R., in partial fulfilment of the requirement for the degree.

Dr. Reji Rani O. P.
(Chairman, Advisory Committee)
Assistant Professor
Department of Agricultural Entomology
College of Agriculture, Vellayani

Dr. K. Sudharma
(Member, Advisory Committee)
Professor
Department of Agricultural Entomology
College of Agriculture, Vellayani

Dr. M.S. Sheela
(Member, Advisory Committee)
Associate Director and Head
Department of Agricultural Entomology
College of Agriculture, Vellayani,

Dr. A. Naseema
(Member, Advisory Committee)
Professor
Department of Plant Pathology
College of Agriculture, Vellayani

EXTERNAL EXAMINER
(Name and Address)

ACKNOWLEDGEMENT

My humble obeisance to the **Almighty** who held my hands from the day of conceiving this venture till its completion. I bow before you for the blessings, showered upon me at all stages of my journey. Whenever I was in trouble, you helped me..... Whenever I felt I can't do anything, you did it for me..... Whenever I crossed the limits you put me back in place. Thank you for reminding me that you haven't given upon me. I am indebted to you more than any one, during the past two years.

I cannot fully express in words my sincere gratitude for the friendship and guidance that my major advisor, **Dr. Reji Rani O. P.** has extended throughout my post graduate life. Her concern for my growth as a professional and as an individual has always transcended the boundaries of the workplace. Her approach to mentoring provided for me the freedom to explore. She had tremendous faith in my ability to overcome what seemed to be a log in my journey. Her logical thinking, thoughtful advice, theoretical and practical knowledge of entomopathogenic fungi, and constructive criticism helped me turn into a professional.

I would like to express my sincere gratefulness to **Dr. M. S. Sheela,** Associate Director & Head, Department of Agricultural Entomology, for her insightful, timely and valuable advice during the course of my PG programme. I express my heartfelt gratitude to my co-advisor, **Dr. K. Sudharma** for her ever ending support and guidance. Her doors were always open for me and she always provided kind and insightful advice. She was instrumental in the foundation and development of this research. I take this opportunity to thank **Dr. A. Naseema** Professor, Dept. of Plant Pathology for her support and suggestions throughout the course of this dissertation.

A word of gratitude will not be sufficient to Dr. C. Nandakumar and Dr. Hebsy Bai for their enthralling personality which always inspired me. Special thanks to Dr. Nisha M. S. for the love, support and encouragement she rendered, from the start of my PG life. I am

grateful to all my dear teachers in Dept. of Agricultural Entomology. Dr. Thomas Biju Mathew, Dr. T. Jiji, Dr. Prathapan, Dr. Anitha, Dr. Amritha, Dr. Ambily Paul, Dr Nazeema, Dr. Premila, Dr. Narayanan, Dr. Faizal and Dr. Devaneshan for the courage and enthusiasm they instilled in me. I would like to thank Dr. Thomas George, Dr. Sumam George, Dr. Meenakumari, Dr. Jayalekshmi and Dr. Soni for their kindness in providing me with necessary facilities. A word of thankfulness to Dr. Anit for his open mindness in allowing me to utilize his lab facilities at any time. I am indebted to Mrs. Brigit Joseph for her constant patience and teachings, including statistical analyses and interpretation of the results (thanks a lot m'am).

I wish to express my sincere gratitude to Shifa chechi. Without her "this" would not have happened. My first lessons of lab was from her and you are not less than a teacher to me. A word of gratitude towards my junior friend, Nithin and his family for their support in the course of this thesis work.

No words can express how grateful am I to my dear dearest friends Murali and Lishma (Puchi). Thank you for helping me so much in completing my lab and field works. I am grateful for the unconditional love, care and support you two have extended, which helped me to overcome all hardships. I am grateful to yet another dear friend of mine, Anisharan Nair for his love, care and support. Thank you for being a patient listener of my worries and for giving enough strength and courage to aim high.

I cannot forget Divya (K. K.), for her kind and sensible advice which many times helped me to get hold of myself. Many thanks to Aswathy Vishwanathan (achu) for keeping the charm in me with her silly talks and 'Korean' thoughts! Thanks Soumya ji for your help with my stat! Special thanks to Milsha, Akshay, Sujitha, Shilpa and Aiswarya for keeping the spirit of Kallisto in Vellayani too. I express my sincere thanks to Sreeja, Geogy, Vaishakhi, Vidya chechi and Lekshmi chechi for their support and spirit. Special thanks for their night

company which kept me awake many nights. My deepest sense of gratitude to Thanuja, Revathy, Keerthisree, Ardra, Aswathy, Sachu chechi and Anila for their inspiring words. Thanks to the friendliness and support extended to me by Srinivas, Nagaraju and Srinivas chettan. My deepest sense of gratitude and indebtedness to my beloved seniors and guiding lights, Malini chechi and Lokesh chettan. I thank all the seniors and juniors for their love, support and guidance. I hereby acknowledge photo courtesy to my dear junior brother, Jithin George. Heartfelt love and thanks to my dear juniors Thamizharashi and Shivamoorthy for being so supportive and encouraging especially during my falls. No words can express my thankfulness to Naveeda chechi and her family for their homely love and support. All my tensions and worries faded away when I was with Zaira (my motta).

I render my profound thanks to Jiji chechi, Sujith, Sujitha, Renjitha, Rasiya, Anjitha, Akisha and Santhosh chettan for their kindness and helpfulness for the successful completion of this work. My sincere thanks to Babukuttan for his brotherly care and affection. His words inspired me and gave me enough courage during the last days of my research. I am obliged to all the labourers who helped in successful completion of my field work.

Words cannot express my indebtedness to my family – achan, amma, mathu (nimya) and manu. My gratitude towards you never ends.... Thank you for being so sportive and supportive. I can't express my gratitude towards achan and amma for letting me complete my post-graduation and believing in me. Mathu....., I started seeing this world with u, thank you for being with me when I was seeing things in my own ways. You are such a wonderful person. All thanks to my dear little brother for keeping the spirit in me.

This work is an outcome of the help and support from many people. Special thanks to all my critics....thanking you all,

Nithya P. R.

CONTENTS

LIST OF TABLES

15	Effect of carrier materials on the viability of *L. lecanii* formulations stored under refrigeration	
16	Effect of carrier materials on the spore count of *L. lecanii* formulations stored at room temperature	
17	Effect of carrier materials on the spore count of *L. lecanii* formulations stored under refrigeration	
18	Effect of adjuvants on the viability of chitin enriched groundnut oil formulation *L. lecanii* stored at room temperature	
19	Effect of adjuvants on the viability of chitin enriched groundnut oil formulation *L. lecanii* stored under refrigeration	
20	Effect of adjuvants on the spore count of chitin enriched groundnut oil formulation *L. lecanii* stored at room temperature	
21	Effect of adjuvants on the spore count of chitin enriched groundnut oil formulation *L. lecanii* stored under refrigeration	
22	Effect of adjuvants on the viability of chitin enriched sunflower oil formulation *L. lecanii* stored at room temperature	
23	Effect of adjuvants on the viability of chitin enriched sunflower oil formulation *L. lecanii* stored under refrigeration	
24	Effect of adjuvants on the spore count of chitin enriched	

25	sunflower oil formulation *L. lecanii* stored at room temperature effect of adjuvants on the spore count of chitin enriched sunflower oil formulation *L. lecanii* stored under refrigeration	
26	Effect of adjuvant combination on the viability of *L. lecanii* in chitin enriched groundnut oil formulation stored at room temperature and refrigerated conditions	
27	Effect of adjuvant combination on the spore count of *L. lecanii* in chitin enriched groundnut oil formulation stored at room temperature and refrigerated conditions	
28	Effect of UV protectants on the viability of *L. lecanii*	
29	Effect of boric acid on the viability of *L. lecanii* on groundnut oil formulations after UV irradiation with different wavelength and irradiation periods	
30	Efficacy of chitin enriched groundnut oil formulations of *L. lecanii* on *A. craccivora* in cowpea	
31	Efficacy of chitin enriched groundnut oil formulations of *L. lecanii* on *B. tabaci* in cowpea	
32	Efficacy of chitin enriched groundnut oil formulations of *L. lecanii* on *A. biguttula biguttula* in cowpea	
33	Efficacy of chitin enriched groundnut oil formulations of *L. lecanii* on *Tetranychus* sp. in cowpea	
34	Effect of *L. lecanii* formulations on yield of cowpea	

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

@	At the rate of
^{o}C	Degree Celsius
CC	Colloidal chitin
C	Chitin
CD	Critical difference
Cfu	Colony forming unit
Cm	Centimetre
CS	Chitosan
DAI	Days after inoculation
DAS	Days after storage
DAS	Days after spraying
DAT	Days after treatment
EPF	Entomopathogenic fungi
et al.	And others
Fig.	Figure
g	Gram
g^{-1}	Per gram
GNO	Groundnut oil
HAT	Hours after treatment
H	Hours
KAU	Kerala Agricultural University
l^{-1}	Per litre
mg	Milligram
mg^{-1}	Per milligram
ml	Millilitre
ml^{-1}	Per millilitre
Min	Minutes
nm	nanometre
NS	Non Significant

PEG	Polyethylene Glycol
POX	Polyoxyethylene
ppm	Parts per million
SFO	Sunflower oil
sp. or spp.	Species (singular and plural)
rpm	Revolutions per minute
viz.	Namely

Introduction

1. INTRODUCTION

Over exploitation of chemical pesticides has been condemned in different parts of the world with the arising problems of pesticide resistance, pest resurgence, and pesticide residue and health concerns. Consequently, need for viable, sustainable and environmentally benign alternatives had been thought of to trim down the harmful effects of chemical insecticides on environment and humanity.

An attractive alternative method to chemical pesticides is biological control. Crop protection based on microbial bio control agents like bacteria, virus, fungi and nematodes had been recognized as a valuable tool in ecofriendly pest management. Among the various bio control agents, entomopathogenic fungi have ample scope in managing pests of important crops *viz*. cereals, pulses, vegetables and other horticultural crops. They are the most important among all the biological control agents owing to their wide host range, route of pathogenicity and ability to control sucking pests as well.

To date, diverse species such as *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Beauveria bassiana* (Balsamo) Vuillemin, *Lecanicillium* (*Verticillium*) *lecanii* (Zimmermann) Zare and Gams, *Paecilomyces lilacinus* (Thom) Samson and *Nomuraea rileyi* (Farlow) Samson have been used to control crop pests. Genus *Lecanicillium* is known for its pathogenicity to insects, mites as well as nematodes infesting various crop plants. *L. lecanii* is a potential species that has the ability to control a wide range of insect pests belonging to Orders Homoptera, Orthoptera, Coleoptera and Lepidoptera (Goettel *et al*., 2008).

Fungus based biopesticides have overwhelming advantages of high selectivity to target pests, safety to humans and other non-target organisms, suitability for their use in organic agriculture, as well as production of export-oriented agricultural commodities. Despite several advantages, the rate of consumption of biopesticides is

not comparable with chemical pesticides. More than 750 species of fungi have been reported as entomopathogenic (Rabindra and Ramanujan, 2007). Yet, their commercial products cover only a small percentage of the total insecticide market. There are many factors that limit their use, such as expensive production methods, poor stability in storage, susceptibility to environmental conditions and field efficacy. Large scale utilization of mycoinsecticides calls for standardization of commercially viable mass production and formulation techniques. The main bottle neck in commercialization of microbial products is their short shelf life. This necessitates research to improve the properties that fulfill long term storage. Formulation development is thus becoming an important area of research.

Development of formulation is mandatory in order to enhance shelf life and enable easiness in application. Microbial formulations that are target-specific, safe and acceptable in terms of storage properties are difficult to develop. The reason is that, apart from their requirement for good physical properties and convenience in use, formulated product must also keep its biological agent functional throughout storage and during application. Woods (2003), emphasized the need of development of a microbial formulation that aims at preserving the organism with good physical properties and delivering them to the target area without hampering its pathogenicity.

Formulation technology must take into account the biological, biochemical as well as physical properties of the organism. Entomopathogens have been formulated in different types such as dry formulations that include dustable powders, wettable powders, granules, water dispersible granules, micro granules, pellets, briquettes and even nanocapsules and liquid formulations, such as suspensions and emulsions, suspension concentrates, suspo-emulsions, oil dispersions and ultra low volume formulations. While dry formulations are easier to prepare, liquid formulations are better in terms of viability and virulence during storage as well as efficacy during field application.

Liquid formulation technology is a promising substitute to overcome the inhalation hazards of conventional dry formulations of bio pesticides. Oil formulations are dispersions of organisms in oil, intended for dilution before use. Formulating entomopathogenic fungi in oils increases their effectiveness by preventing conidial desiccation, increasing adhesion, spreading the inoculum over the host's body even into crevices, and overcoming the defensive nature of the cuticle. They have better infective properties as well as spreading and penetration power. They also have the distinguishing character of delivering hydrophobic conidia to the targeted area. Moreover they are more compatible with adjuvants which can increase and broaden the pest control potential (Burges, 1998). Vernner and Bauer (2007) emphasized the need for careful assortment of the inert components in a formulation, to prevent instability problems.

The prime factors that determine the quality of a fungus based bio formulation are the viability and virulence of the infective propagule that is usually the conidia. Loss of these properties during long term storage prevents their large scale adoption. This situation can be overcome through formulation improvements (Vimaladevi and Prasad, 1996).

Above all, the acceptance of the bio formulation is eventually determined by its field performance.

In light of afore said facts, the present investigation was carried out with an aim to improve the conidial viability and virulence of *L. lecanii* in bio formulations and to evaluate their effectiveness in controlling sucking pests. It emphasizes the significance of optimization of growth media, carrier material, adjuvants and UV protectants in developing a bio formulation.

L. lecanii being a promising bio agent for sucking pests, cowpea *Vigna unguiculata* (L.) Walp. *sesquipedalis* that succumbs to an array of sucking pests was selected for evaluating the formulations.

Review of Literature

2. REVIEW OF LITERATURE

Pest management is currently taking a new turn, which is more concerned with ecosystem safety as well as safety to users and beneficiaries than merely eradicating pests. Microbial control has gained much attention in the past two decades in the pest control arena of many important crops.

Though 90 genera and nearly 700 species are expected to be entomopathogenic, only few members belonging to the Entomophthorales and Hyphomycetes are exploited. The fungi like *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Lecanicillium lecanii* (Zimmermann) Zare and Gams are the most widely exploited biocontrol agents. *L. lecanii* is a promising fungal bio agent primarily infecting sucking pests belonging to the Order Homoptera *viz.* aphids, scales mealy bugs and whiteflies, offering great scope for insect pest management (Ekbom, 1979; Kanagaratnam *et al.* 1982; Hall and Papierok, 1982; Cuthbertson *et al.* 2005; Diaz *et al.* 2009; Park and Kim, 2010; Ujjan and Shahzad, 2012*).* *L. lecanii* is also highly pathogenic to a wide range of other insect hosts belonging to Orthoptera, Coleoptera and Lepidoptera (Banu, 2013).

L. lecanii was first reported by Viegas in 1939 on scale insect *Coccus* (*Lecanium*) *viridis* Green. In India, its pathogenicity was first studied and demonstrated by Easwaramoorthi and Jayaraj (1978) on *C. viridis*. The genus *Verticillium* has been recently redefined using its rDNA sequencing, after which, all the insect pathogenic species were placed into a new genus *Lecanicillium* (Zare *et a*l., 2000; Zare and Gams, 2001). *L. lecanii* is one such species which was formerly designated as *V. lecanii*. The present classification given by Zare and Gams (2001) is as follows

Kingdom	Fungi
Phylum	Ascomycota
Class	Sordariomycetes
Order	Hypocreales
Family	Hypocreaceae
Genus	*Lecanicillium*

2.1 PATHOGENICITY

Lecanicillium species exhibits variability in host infectivity owing to their capacity to exploit a wide range of hosts, extending from arthropod pests and nematodes.

2.1.1 Aphids

First report on pathogenicity of *L. lecanii* to aphids was that of Hall (1976). He found that the chrysanthemum aphid, *Macrosiphoniella sanborni* Gillette when treated with the spore suspension at 2.33 x 10^5 spores ml^{-1} caused 50 per cent mortality. Rapid mortality of aphids, *Myzus persicae* (Sulzer) and *Aphis gossypii* Glover was observed by Yokomi and Gottwald (1988) when treated with *L. lecanii* at 10^6 to 10^7 conidia ml^{-1}. Of the three isolates evaluated, isolate VL4 at 10^7 spores ml^{-1} was found to cause 100 per cent mortality within eight days. When the concentration was increased from 10^6 to 10^{10}, the mortality of *A. craccivora* Koch raised from 10 to 63.7 per cent on the second day after treatment (Sahayaraj and Namashivayam, 2000). Under laboratory conditions, *L. lecanii* isolates were more virulent to *A. gossypii* and *M. persicae* compared to *B. bassiana, Paecilomyces* sp. and *M. anisopliae* (Loureiro and Moino, 2006). When the spore concentration of *L. lecanii* was increased from 10^3 to 10^8, mortality rate of *A. craccivora* also increased (Saranya *et al.,* 2010). The mortality observed was 28 and 100 per cent respectively, on the seventh day of treatment. Salam and Hawary (2011) reported

high virulence of *L. lecanii* to both adult and nymphal stages of *A. craccivora*. They observed 100 per cent mortality in adults and nymphs after three days, when treated with 5 x 10^6 spores ml^{-1} and 1 x 10^6 spores ml^{-1}. Suresh *et al.* (2012) reported 73.99 and 57.73 per cent mortalities of adults and nymphs of *A. craccivora*, respectively at a higher dose of 1 x 10^8 conidia ml^{-1}.

2.1.2 Whiteflies

Kanagaratnam *et al.* (1982) noted heavy infestations of cucumber whitefly *Trialeurodes vaporariorum* Westwood in glass houses which could effectively be controlled by fortnightly or monthly sprays of *L. lecanii* at 10^7 spores ml^{-1} causing 85 to 95 per cent mortality. Mor *et al.* (1995) evaluated 36 isolates of *L. lecanii* from different hosts (insects and rusts) against *Bemisia tabaci* Gennadius and found that the virulence to larvae of *B. tabaci* within these isolates ranged from 0 to 83 per cent. Pathogenicity studies conducted by Cuthbertson and Walters (2005) revealed the efficacy of *Lecanicillium* sp. to *B. tabaci* on host plants like tomato, chrysanthemum, verbena, cucumber and poinsettia when treated with 10^7 spores ml^{-1} .They recorded more than 90 per cent mortality of the pest on all the host plants, seven days after treatment. Dose dependent mortality of *B. tabaci* against different concentrations of *L. lecanii* was described by Raheem *et al.* (2009). The mortality of *B. tabaci* was found to increase with increase in concentration from 10^5 to 10^7 spores ml^{-1}. The mortality rates were 60.60, 87.64 and 100 respectively, seven days after treatment. Park and Kim (2010) identified *Lecanicillium* isolates, Btab01 and 4078 as the most effective isolate against *B. tabaci.* Bioassay studies using these isolates proved their effectiveness against egg, larval and adult stages causing 71 to 77 per cent, 75 to 84 per cent and 93 to 96 per cent mortality, respectively. Zhu and Kim (2011) while investigating the susceptibility of sweet potato white fly, *B. tabaci* biotype Q to EPF found *L. lecanii* as highly virulent compared to *B. bassiana, Isaria fumosorosea* Wize and *Aschersonia aleurodes* Webber.

2.1.3 Scales

L.lecanii is an effective pathogen against scale insects. Easwaramoorthy and Jayaraj (1978) found that the white halo fungus *L. lecanii* was highly effective to coffee green scale, *C. viridis* Green, under field conditions @ 16 x 10^6 spores ml^{-1} causing 73.10 per cent mortality after two applications. Evans and Prior (1990) summarized the entomopathogens on the diaspidid scale insects and reported the incidence of *L. lecanii* on different species *viz. Aspidiotus nerii* Buche, *Aspidiotus* sp., *Pseudaulacaspis pentagona* Targioni-Tozzetti, *Chionaspis salicis, Lepidosaphes ulmi* Linnaeus, *Lepidosaphes* sp., *Mytilaxpis* sp. and *Unaspis citri* Comstock, infesting various crops. Of the various EPF tested for biological control of scales, *viz. Fusarium*, *Penicillium*, *Lecanicillium*, *Cladosporium*, *Trichoderma*, *Glomerella*, *Phomopsis*, *Alternaria*, and *Beauveria bassiana, L. lecanii* was found to be most potent species as it was infective to nearly 40 species of scale insects (Zare and Gams, 2001; Goettel *et al*., 2008). Ying-ping *et al.* (2012) reported that *L. lecanii and L. dimorphism* as pathogenic to purple scale of citrus, *Lecanium beckii* Newman and *L. psalliotae* pathogenic to Red date scale, *Phoenicococcus marlatti* Cockerell. Liu *et al.* (2014) studied pathogenicity of *L. lecanii, L. fungicola* and *Fusarium incarnatum-equiseti* against Japanese pine bast scale, *Matsucoccus matsumurae* Kuwana at 5 x 10^7 spores ml^{-1} and reported *L. lecanii* as most virulent, causing 100 per cent mortality eight days after treatment.

2.1.4 Mites

Fiedler *et al*. (2002) observed *L. lecanii* as highly effective against mite *Tetranychus ludeni* Zacher under greenhouse conditions. Simova and Draganova (2003) evaluated the virulence of four isolates of *B. bassiana*, one isolate each of *M. anisopliae*, *I. farinosa* and *L. lecanii*, at 2 x 10^9 conidia ml^{-1} against two-spotted spider mite, *T. urticae* Koch and found that except one isolate of *B. bassiana,* all other isolates were effective in managing the pest. Chandler *et al*.

(2005) assessed 40 isolates of EPF from six genera, *viz. Hirsutella, Metarhizium, Beauveria, Lecanicillium, Tolypocladium and Paecilomyces* against *T. urticae* with a spore suspension of 1 x 10^7 spores ml^{-1} and reported that only three isolates, *Metarhizium* 442.99, *Hirsutella* 457.99 and *Lecanicillium* 450.99, were pathogenic. *L. lecanii* isolated by Koike *et al.* (2005), named A-2 and B-2 were pathogenic to *T. urticae*, but the levels of virulence varied between the isolates. Wenzel and Filho (2011) reported 47 to 76 per cent mortality of *T. urticae* when treated with 1 x 10^7 conidia ml^{-1} of *L. lecanii*, six days after treatment. Amjad *et al.* (2012) studied the effect of *L. lecanii* on *T. urticae* and reported that spore loads at 10^6, 10^7 and 10^8 were pathogenic to both the eggs and adults.

2.2 OPTIMISATION OF MEDIA SUPPLEMENTS

EPF are to be mass produced in an appropriate media which favours its growth, sporulation, viability and virulence. Composition of the media is crucial in obtaining infective propagules of superior quality and quantity. Many attempts have been made in the past to improve the growth and sporulation by amending the media with various supplements.

St. Leger *et al.* (1986a) studied the effects of different carbon sources such as chitin, chitosan, N-acetylglucosamine, cellulose, pectin, sucrose, starch etc. on the growth of *M. anisopliae* and observed appreciable growth in all the tested sources. But chitinase activity was found to be high only in cultures supplied with chitin. Bidochka and Khachatourians (1987) stated that presence of chitin in the culture media induced chitinolytic activity which in turn enhanced fungal virulence. Matsumoto *et al*. (2004) detected extracellular N-acetyl-hexaminidase when *L. lecanii* was cultured on shrimp waste which is a natural source of chitin. Whereas,cultures to which sucrose was added as a sole carbon source produced no enzyme. The growth, development and sporulation of *B. bassiana* was studied by Hegedus *et al*. (1990) in a media containing chitin monomers and suggested that

submerged conidia production was better in media containing N-acetylglucosamine than that contained yeast-extract-peptone glucose. They observed 86.3 per cent improvement in conidia production of *B. bassiana* in chitin amended media. Similar effect of enhanced sporulation with chitin addition was noticed for the same fungus when cultured in rice bran (Liu *et al*., 1990). Wu *et al.* (2010) cultured three isolates of *M. anisopliae* on six media having different compositions on the basis of chitin, and other carbon and nitrogen sources and noted that chitin peptone nutrient media produced the highest colony growth, but maximum conidial yield was from the medium that have high C/N ratio. Balakrishnan *et al*. (2011) while studying the effect of chitin supplementation in culture media at 0.1 to 0.6 per cent, reported an increase in biomass of *M. anisopliae* with no change in spore yield and a decrease in biomass of *B. bassaina* with increase in spore yield. The response of *B. brongniartii* to chitin supplementation was found to be positive in terms of biomass and spore yield (Srikanth and Santhalakshmi, 2012).

Studies conducted by Agus *et al*. (2015) to determine the viability and spore production of entomopathogenic *Pencillium* sp., showed higher conidial production (1.43×10^8 conidia ml^{-1}) and viability (83.26 per cent) using chitin containing liquid media.

Chitosan, the partially deacetylated form of chitin is composed of β-1, 4-glucosamine subunits. This compound generally exhibits antifungal activity against plant pathogenic fungi. Lafontaine and Benhamou (1996) reported that chitosan supplementation to tomato plants developed resistance to infection by *Fusarium oxysporum* f. sp. *radicis-lycopercisi*. Palma-Guerrero *et al.* (2008) while investigating the effects of chitosan on growth and spore germination of various biocontrol fungi reported that these fungi were not affected by chitosan. The growth of entomopathogens such as *L. psalliotae* and *Pochonia chlamydosporia* Goddard was not affected by chitosan @ one to two mg ml^{-1}, while *B. bassiana* showed an

enhancement in growth. They observed that spore germination was less affected in 0.01 to one mg ml^{-1} chitosan amended media. Another study by Palma-Guerreo *et al.* (2010a) reported an increase in conidiation of *L. psalliotae B. bassiana, M. anisopliae, P. chlamydosporia*, *P. rubescens* and *Paecilomyces lilacinus* (Thom) Samson when cultured in chitin amended medium. They also discovered that addition of chitin is inhibitory to plant pathogenic fungi, whereas the higher membrane rigidity of entomopathogenic fungi (Palma-Guerreo *et al.,* 2010b) makes their membranes more resistant to chitosan and hence non inhibitory to their growth.

Media supplementation with yeast extract increased growth and sporulation of entomopathogenic fungi, as it is an excellent source of vitamin B, nitrogen and amino acids. Rombach *et al*. (1988) observed largest number of conidia mg^{-1} of *B. bassiana* with a concentration of 0.75 per cent yeast extract. Hsiao *et al.* (1992) also reported enhanced growth and sporulation of *B. bassiana* and *M. anisopliae* in media supplemented with yeast extract. Kamp and Bidochka (2002) assessed conidia production of *M. anisopliae*, *B. bassiana* and *L. lecanii* on different media, like corn meal agar (CMA), nutrient agar (NA), malt extract agar (MEA), sabouraud dextrose agar (SDA), and yeast peptone dextrose agar (YPDA) and potato dextrose agar (PDA) and reported maximum conidiation of *L. lecanii* in YPDA. Purwar and Sachan (2006) stated the suitability of yeast extract in increasing the biomass and conidial counts of *B. bassiana* by supplementing yeast extract. Wenzel *et al*. (2007) evaluated the effect of 0.5, one, two, three and five per cent yeast extract on growth and sporulation of *L. lecanii* and reported that yeast supplementation at any concentration stimulated its growth and sporulation. Highest sporulation was observed with one per cent concentration. Derakhshan *et al*. (2008a) evaluated different liquid media, *viz.* Molasses Yeast Broth (MYB), Potato Dextrose Broth (PDB), Potato Carrot Broth (PCB), Jaggery Yeast Broth (JYB), Sucrose Yeast Broth (SYB) and Potato Sucrose Broth (PSB) and established that MYB supported maximum sporulation (8.33×10^7 spores ml^{-1}) and biomass production (746 mg /

100 g). In a study conducted by Pandey and Kanaujia (2010), maximum biomass, conidial count and viability was observed in *B. bassiana* and *M. anisopliae* when culture media was *s*upplemented with 10 per cent yeast.

Addition of chemical compounds like Tween-80 and polyethylene glycol (PEG) to mass production systems can have different responses in terms of biomass, blastospore production, viability and virulence of entomopathogenic fungi. In a study with *M. anisopliae*, Kleespies and Zimmermann (1992) noticed an increased blastospore yield of 7.7 fold with addition of five per cent PEG. Geetha and Balaraman (2001) observed a positive increase in blastospore yield of *B. bassiana* with PEG as additive. Higher spore yield of *M. anisopliae* with addition of PEG was further stated by Leland *et al.* (2005). Balakrishnan *et al.* (2011) reported positive correlation for biomass and spore yield of *M. anisopliae* and *B. bassiana* in PEG amended molasses media. In their study, PEG one to six per cent significantly enhanced biomass, radial growth and spore yield of *B. bassiana*. In *M. anisopliae,* PEG two per cent enhanced biomass while the higher concentrations (five and six per cent) were not favourable, whereas spore output was maximum at highest concentration of PEG (six per cent). Srikanth and Santhalakshmi, (2012) observed that PEG at one to six per cent concentration improved the biomass as well as spore yield of *B. brongniartii* in molasses media.

Studies were conducted by various workers to test the suitability of commonly available nitrogenous fertilizers as nitrogen source for the mass production of biocontrol agents. Jayaraj and Ramabadran (1998) recorded least growth of *Trichoderma harzianum* Rifai in urea supplementation compared to ammonium sulphate and ammonium chloride supplementation. In 2002, Desai and Kulkarni reported the lethal effects of urea on *T. viride* Pers. and *T. harzianum.* Complete inhibition of growth and sporulation was noted at a concentration of 1000 ppm in

potato dextrose agar medium. Inhibitory action was found to be less at lower concentration, 100 ppm.

Effect of several vegetable oils on germination of *L. lecanii* was studied by Verhaar *et al.* (1999). There was an increase in germination rate after 24 h when the growth media was supplemented with 0.5 per cent arachid oil. It was also found that arachid oil with tween-80, stimulated germination of *L. lecanii* spores.

2.3 DEVELOPMENT OF FORMULATIONS

Currently, bio pesticides are formulated mainly in solid carriers like talc, peat, lignite, clay, etc. However, these solid formulations suffer from major setbacks like shorter shelf life, high contamination and low field performance (Hedge, 2002). Liquid formulations offer longer shelf life of two years, with high purity, carrier-free activity, easiness in handling and application, convenience in storage and transport, better quality parameters and enhanced export potential (Pindi and Satyanarayana, 2012). In addition, they are also compatible with machinery on large farms and are preferred by farmers and industries.

The essential constituents of a bio formulation are the technical ingredient or the living entity, an inert carrier material, adjuvants such as stabilizing agent, humectants, wetting or spreading agent, an emulsifier, a binder or a combination of these. The literature pertaining to bio formulation is reviewed below.

2.3.1 Carrier material

According to Jones and Burges (1998), a dry formulation consists of 50 to 80 per cent technical ingredient, 20 to 50 per cent inert carrier and one to 10 percent dispersant/surfactant whereas a liquid formulation carries 20 to 80 per cent technical ingredient 35 to 65 per cent liquid carrier one to five percent dispersant and three to eight per cent surfactant.

Formulating entomopathogens in oil was found to increase their efficiency (Prior *et al.,* 1988). Oils help the conidia to stick on to the insect cuticle and enable the fungus to kill insects even at low humidity (Vimaladevi and Prasad, 1996; Inyang *et al.,* 2000). Verhaar *et al.* (1999) reported groundnut oil as the better carrier for *L. lecanii* compared to sunflower oil, as germination in the former was found to be excellent. Use of oil based formulations of mycoinsecticides has been extensively studied by Lomer and Lomer (2001). They observed comparatively higher germination of *M. anisopliae* and *B. bassiana* in diesel: sunflower oil mixture (7:3) than diesel: groundnut oil mixture (7:3). Vimaladevi *et al*. (2002) prepared oil formulation of *Nomurea rileyi* Farlow Samson with sunflower oil in the ratio 50:50. Batta *et al*. (2003) regarded 50:50 oil combination of coconut oil and soya bean oil mixed with spore suspension of *M. anisopliae* in distilled water as the best in maintaining viability for 30 months. They also reported that the invert emulsion was effective against the whitefly, *B. tabaci* and the red spider mite, *T. cinnabarinus* in eggplant. Derakhshan *et al*. (2008b) prepared talc based formulations by mixing *L. lecanii* culture and sterilized talc in 50:50 ratio and studied the effect of storage temperature on the viability and virulence of *L. lecanii.* Banu (2013) prepared dry formulations by mixing *L. lecanii* multiplied in PDB and sabouraud dextrose broth (SDB) with yeast extract with talc in the ratio 1:2.

Stathers *et al.* (1993) obtained better viability for conidia of *M. anisopliae* when stored in peanut oil, rapeseed oil and paraffin oil based formulations for 14 weeks. Alves *et al.* (2002) studied the effect of refined paraffin oil and vegetable oils such as peanut oil, sunflower oil and soybean oil on the conidial viability of *M. anisopliae* and reported that formulations based on groundnut oil maintained viability more than 90 per cent after 40 weeks of storage. Sunflower oil was found to be superior in retaining viability of *L. lecanii* spores compared to talc based formulations as reported by Banu and Gopalakrishnan (2012).

Additives can be incorporated in bio formulations for improving their shelf life. Presence of chitin in formulation enhanced *M. anisopliae* (St. Leger *et al.,* 1986b). Pavlyushin *et al.* (2005) attained better retention of conidial viability of *T. viride* in chitin and chitosan based talc formulations, even after three months of storage. Addition of chitin in wheat bran was reported to induce conidia production in *B. bassaina* formulations (Gerding-gonzalez *et al.,* 2007). Sriram *et al.* (2010) used chitin as additive in talc formulations of *T. harzianum* at concentrations varying from one to five per cent and reported that two or five per cent chitin helped in maintaining the number of colony forming units (10^6 cfu g^{-1}) in the formulation upto six months of storage. They observed that chitin addition resulted in an extension of shelf-life of talc formulation by two months. They also reported that addition of colloidal chitin at 0.2 per cent in production medium enhanced the shelf-life by additional two months. Abdel-Kader *et al.* (2012) reported that carriers, sawdust + talc + chitosan and sawdust + chitosan maintained viability of *T. harzianum* in a dry formulation over a period of three and five months, respectively.

2.3.2 Adjuvants

Adjuvants can be humectants, surfactants or emulsifiers used in a formulation to improve the keeping quality as well as field efficacy. Various adjuvants have been tried previously in bioformulations. Santharam *et al*. (1977), reported the effectiveness of glycerol as adjuvant in *L. lecanii* formulations. Burges (1998) regarded glycerol as a humectant and stabilizer for mycoformulations. Glycerine was reported to enhance viability of spores suspended in oil formulations by reducing the loss of water through evaporation. According to Curtis *et al.* (2003) spray mixture of glycerol and egg powder resulted in high germination of *L. lecanii*.

Surfactants are substances added in a formulation to improve its ability to spread evenly on the target. The effectiveness of tween-80 as an adjuvant in *L. lecanii* formulations was documented by Easwaramoorthi and Jayaraj (1978).

Prior *et al.* (1988) proved the effectiveness of tween-80 as an adjuvant in oil and water formulations of *B. bassiana*. They described tween-80 as a wetting agent which facilitated suspension of hydrophobic conidia. Luz and Batagin (2005) also reported relatively less toxic effect of the surfactants tween-20 and tween-80 in oil formulations of *B. bassiana* while studying the effects of surfactants on germination. Mishra *et al.* (2013) found that the surfactant, sodium-dodecyl sulphate had a negative impact on the conidial germination of *B. bassiana,* whereas tween-20 and tween-80 did not inhibit germination of the conidia.

The effectiveness of PEG in enhancing viability of *M. anisopliae* was reported by Kleespies and Zimmermann (1998). Derakhshan *et al.* (2008a) reported that talc based product of *L. lecanii* prepared from molasses yeast broth supplemented with two per cent PEG maintained conidial viability.

Chavan and Kadam (2008) studied the effect of adjuvants, glycerol (two, five, eight and 10 per cent), tween-80 (one, two, three, four and five per cent) and arachid oil (one, two, three, four and five per cent) in liquid formulations of *L. lecanii*. The prepared formulations were inoculated to PDB and biomass as well as surface area covered by the fungus was observed. They observed an increase in surface area covered and biomass produced, with increase in concentration of adjuvants. Arachid oil two to five per cent and glycerol five to 10 per cent were the most promising adjuvants followed by tween-80 one to five per cent. Batta *et al*. (2011) prepared water-in-oil formulation in the ratio 50:50, along with adjuvants. The aqueous phase consisted of sterile de-ionized water (42.25 per cent w/w), glycerine (4 per cent w/w) and water-soluble emulsifier (0.75 per cent w/w). The oil phase consisted of a mixture of rape seed oil (24 per cent w/w) and tea oil (24 per cent w/w) with oil-emulsifier tween-20 (two per cent w/w). They observed better maintenance of viability of *Zoophthora radicans* (Brefeld) Batko for four weeks compared to unformulated conidia. Boruah *et al*. (2015) while evaluating adjuvants suitable for

liquid formulations of *M. anisopliae* reported that glycerol five, and 10 per cent and tween-80 one per cent were promising, as these formulations when inoculated in PDB enhanced growth and development of the fungus.

Effect of adjuvant combinations on the growth of *L. lecanii* was examined by Chavan and Kadam (2009a; 2010a) and found that glycerol two per cent + tween-80 one per cent + arachid oil 0.5 per cent and glycerol five per cent + tween-80 one per cent + arachid oil two per cent were the best adjuvant combinations for *L. lecanii* formulations.

2.3.3 UV protectants

Fungal spores are highly susceptible to solar radiation. Exposure to sunlight inactivates *L. lecanii* within hours (Moore *et al*., 1993). UV-A (315-400 nm) and UV-B (280-315 nm) can lower the efficacy of biocontrol agents by inactivating their structures due to genetic and morphological changes (Braga *et al*., 2001). UV-C with a lower wavelength of 100-280 nm was also reported to be highly deleterious to entomopathogens. (Lee *et al.,* 2006).

The relative culturability of *L. lecanii* was reduced to nearly zero by four hours of exposure to UV-B (Braga *et al*., 2002). UV protectants like montmorillonite (one per cent) and polyoxyethelene (one per cent) gave nearly 80 per cent survival when treated with UV-C for 30 min and about 93 per cent survival when treated with UV-B for 6 h and was not affected by UV-A (Lee *et al.,* 2006). Chavan and Kadam (2010b) studied the effect of UV radiation on viability of *L. lecanii* and reported that liquid formulations of *L. lecanii* with boric acid gave good protection against UV (320 nm) at 10, 20, 30, 40 and 50 minutes of exposure while those with indigo lacked this quality. They observed more detrimental effect with increase in exposure time. Grand and Cliquet (2013) studied the impact of culture age on conidial yields, germination and tolerance of conidia of *L. lecanii, B. bassiana* and *M. anisopliae* to

UV exposure (UV-B: 280-315 nm and UV-A: 315-400 nm) and reported 20 to 80 per cent reduction in conidial germination. Galvao and Bettilol (2014) reported low conidial germination of *Lecanicillium* spp after 60 min exposure to UV-B (290 nm) irradiation. They observed better conidial germination at lower exposure periods of 15 min.

2.3.4 Storage temperature

Storage temperature is the most important factor which determines the keeping quality of a formulation. Zhang *et al*. (1992) observed that wettable powder formulation of *B bassiana* had more than 85 per cent germination, when stored for eight months at 10-20 ºC. Alves *et al*. (2002) tested the effect of different water-based and oil-based formulations on conidial viability of *M. anisopliae* stored at 10 ºC and 27 ºC and found that formulation stored at 10 ºC was more viable (91 per cent germination) than that stored at 27 ºC, 40 weeks after storage.

High storage temperature lowers the viability of *L. lecanii* conidia. Conidial viability declined rapidly when stored at 35 ºC and was declined to nearly zero after three months while, spores retained viability when stored at 15 ºC and 25 ºC (Chen *et al*., 2008).

Derakhshan *et al.* (2008b) reported that the viability of *L. lecanii* stored in refrigerated condition was significantly higher than that stored in room temperature. Viability of talc and oil formulations of *L. lecanii* under refrigerated condition was found to be more than that stored at room temperature (Banu and Gopalakrishnan, 2012; Banu, 2013).

2.4 FIELD EVALUATION

Dry formulations have their own disadvantages such as spore settlement in spray fluid, settlement in sprayers, blocking of sprayers, dehydration on exposure and

occasionally, inhalation hazards. Formulation of entomopathogenic fungi in oils increases its effectiveness (Prior *et al*., 1988) probably by preventing conidial desiccation and increasing adhesion (Vimaladevi and Prasad, 1996).

Field trials carried out by Jayaraj (1989) to determine the effectiveness of *L. lecanii* in controlling the coffee scale *C. viridis* concluded that the fungus caused 73.10 per cent mortality of the pest when applied at 1.60 x 10^6 spores ml^{-1} twice at fortnightly intervals. 97.60 per cent mortality was observed when tween-20 was added as a surfactant.

Bioassay with oil formulation of *N. rileyi* showed significant reduction in population of *Spodoptera litura* Fabricius larvae in groundnut under field conditions (Vimaladevi *et al*., 2002). Manjula *et al.* (2003) reported that groundnut oil formulation of *B. bassiana* recorded 100 per cent mortality of adults of *B. tabaci* in groundnut while formulations based on coconut oil, sunflower oil and castor oil resulted in 97.80, 85.60 and 64.40 per cent respectively. Chavan and Kadam (2009a) evaluated oil based formulations of *L. lecanii* and found that two formulations, one with glycerol two per cent + tween-80 one per cent + arachid oil 0.5 per cent and the other with glycerol five per cent + tween-80 one per cent + arachnid oil two per cent were the best in terms of viability and virulence against *Macronellicoccus hirsutus* Green in guava. Mortality observed after 14 days was 81.28 and 81.93 per cent, respectively. Liquid formulation of *L. lecanii* caused 92.34 per cent mortality of whitefly, *Aleurodicus disperses* Russell in guava (Chavan and Kadam, 2009b). Application of oil based formulations of *L. lecanii* was found to reduce the sucking pests in Okra (Naik and Shekharappa, 2009). The mean number of leafhoppers, aphids, whiteflies, thrips and mites after second spray was 7.75, 7.75, 2.70, 3.00 and 11.00 per three leaves, after two sprays. Banu and Gopalakrishnan (2012) observed that oil based formulations of *L. lecanii* recorded maximum mortality of papaya mealy bug, *Paracoccus marginatus* Williams & Granara de Willinkthan compared to

talc based products. They reported a maximum mortality of 75 per cent with oil based formulation of *L. lecanii* sprayed at fortnightly interval. Boruah and Dutta (2014) evaluated *M. anisopliae* formulation made of 10 per cent glycerol and 0.5 per cent sunflower oil for managing *A. craccivora* in cowpea and attained 50 per cent mortality after 15 days of treatment.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled 'Improved formulation of *Lecanicillium lecanii* (Zimmermann) Zare and Gams and its evaluation against sucking pests' was carried out at the Biocontrol Laboratory for Crop Pest Management, Department of Entomology, College of Agriculture, Vellayani during 2013-2015 and the field trial was conducted at the Instructional Farm, Vellayani.

3.1. PATHOGENICITY STUDIES

Laboratory experiments were carried out to assess the pathogenicity of *L. lecanii* to different groups of sucking pests infesting cowpea *viz*.

1. Jassid (*Amrasca biguttula biguttula* Ishida)
2. Cow bug (*Anchon pilosum* Walker)
3. Pea aphid (*Aphis craccivora* Koch)
4. White fly (*Bemisia tabaci* Gennadius)
5. Mealy bug (*Ferrisia virgata* Cockerell)
6. Pod bug (*Riptortus pedestris* Fabricius)
7. Scale (*Lecanium* sp.)
8. Red spider mite (*Tetranychus* sp.)

Since scale infestation was not observed in cowpea during the course of study it was undertaken using scales *Lecanium* sp. infesting brinjal.

3.1.1 Maintenance of Stock Culture of Test Organisms

The test organisms were reared on cowpea plants. The seeds of cowpea V*igna ungulata* variety Vellayani Jyothika obtained from the Department of Olericulture, College of Agriculture, Vellayani were sown in polybags of 45 cm diameter. Sequential planting was done to obtain enough number of cowpea plants for rearing the test organisms continuously.

3.1.1.1 A. biguttula biguttula

Adults and nymphs collected from field were released into potted plants kept in rearing cage made of brass and wood measuring 164 x 100 x 118 cm^3. The plants were watered daily and insects were allowed to multiply. The adult hoppers were collected using inverted test tubes and used for the study.

3.1.1.2 A. pilosum

Adults and nymphs collected from cowpea field were confined in rearing jars (17 x 10 cm^2) provided with tender cowpea twigs. The cut end of the twig was wound with moist cotton to maintain its turgidity. The culture was kept under observation for ruling out any infection. Healthy adults were separated for pathogenicity studies.

3.1.1.3 A. craccivora

Gravid females of *A. craccivora* were collected from the field and released into potted plants as described in para 3.3.3.1. The emerging young ones were collected and transferred to new plants and two days later the second instar nymphs that emerged were used for pathogenicity studies.

3.1.1.4 B. tabaci

Adults and nymphs collected from cowpea fields were brought to the lab and allowed to multiply as described in para 3.1.3.2. The adults obtained from the subsequent generations were collected using inverted test tubes and used for pathogenicity studies.

3.1.1.5 F. virgata

Colonies were collected from field and released into potted plants (3.1.3.1). The adults from subsequent generations were utilized for pathogenicity studies.

3.1.1.6 R. pedestris

Early instar nymphs collected from cowpea field were confined in rearing jars (17 x 10 cm^2) provided with tender cowpea pods. The pods were replaced with fresh ones on alternate days. The culture was kept under observation for ruling out any infection. Healthy adults were separated for pathogenicity studies.

3.1.1.7 Scale

Twigs of scale infested brinjal plants were detached with a slanting cut and the cut end of the twig was wound with moist cotton to maintain its turgidity and then placed in petridishes. It was kept under observation for two to three days to rule out any infection. Healthy scales were utilized for pathogenicity studies.

3.1.1.8 Tetranychus sp.

Rearing was initiated from the field collected mites. Stock culture was maintained in potted plants confined in the rearing cage (3.1.3.1). On establishment of colonies the adults were separated using fine camel hair brush and were used for testing pathogenicity.

3.1.2 Maintenance of Fungal Culture

L. lecanii isolate (Vl8) obtained from National Bureau of Agricultural Insect Resources (NBAIR), was used for the study. The fungus was subcultured and maintained in Sabouraud Dextrose Agar (SDA). Fourteen day old cultures were stored under refrigeration for carrying out various experiments. The virulence of the culture was maintained by spraying of spore suspension of the fungus on *A. craccivora* and reisolation of the fungus from the insect cadaver.

3.1.2.1 Mass Multiplication

The fungus was mass multiplied in Sabouraud Dextrose Broth (SDB) Himedia, India. One litre SDB was sterilized at 121°C for 15 minutes at 1.06 kg cm^{-2} pressure.

The sterilized media was inoculated with one ml of the fungal culture and incubated for 14 days for further studies.

3.1.2.2 Preparation of Spore Suspension

Spore suspension was prepared from 14 day old cultures. The culture was blended in an ordinary mixer and filtered through double layered muslin cloth to remove mycelial mat. The resultant spore suspension was used of for assessing the pathogenicity to test organisms.

3.1.3 Assessment of Pathogenicity

Pathogenicity test of *L. lecanii* was carried out using detached leaf method (Yokomi and Gottwald, 1988). Tender leaf of cowpea was detached and the leaf petiole was covered with moistened cotton to maintain the turgidity of the leaf and was kept upside down position in a petri dish (9 cm diameter) lined with filter paper moistened to saturation level. The test organisms were released separately into these petri dishes. *A. craccivora* were transferred by tapping the leaves gently while *A. biguttula biguttula*, *F. virgata* and *Tetranychus* sp. were transferred using a soft and fine bristled brush moistened with water.

In the case of *B. tabaci,* to facilitate easy transfer, the adults collected in a test tube were kept in a refrigerator for two to five minutes and later transferred to glass jars (10.5 x 6 cm ^{2}) to enable free movement of the insect. Treated insects of *R. pedestris* and *A. pilosum* were transferred into fresh cowpea pods and twigs respectively and placed in rearing jars (17 x 10 cm^2) secured with muslin cloth at the top.

The spore suspension was applied topically on *A. craccivora, F. virgata*, *R. pedestris, A. pilosum* and *Tetranychus* sp. using an atomizer, while tender leaves pretreated with spore suspension were used for *B. tabaci* and *A. biguttula biguttula* so as to reduce the risk of damage to wings.

To conduct pathogenicity test in scales, the method suggested by Nirmala (2003) was followed. The adults were released on shoots of brinjal which were kept turgid by moist cotton. A pretreatment time of one hour was given for acclimatization. Later, each shoot containing scales was dipped for one or two minutes in spore suspension containing 2.14 x 10^7 spores ml^{-1} of the fungus.

Thirty insects were used in a treatment and each treatment was replicated five times. Insects treated with sterile water served as control. The treated insects were observed for development of symptoms and mortality was recoreded at 24 h interval. The dead insects were removed daily and placed over a wet filter paper inside a petridish for the development of mycelia. Observations were continued till 100 per cent mortality was observed in any one of the treatment. Percentage mortality was corrected using Abbot's formula (Abbot, 1925). For confirmation of pathogenicity, the fungus was reisolated from the cadavers and examined under microscope for mycelial and spore characteristics.

3.1.4 Determination of effective dose

The insects found susceptible to *L. lecanii* in tests described under 3.1.3, were selected to determine effective dose. Spore count of the suspension prepared as in para 3.1.2.2 was enumerated using a Neubauer haemocytometer. Six spore concentrations ranging from 10^3 to 10^8 spores ml^{-1} were prepared by serial dilution method. Each concentration was tested separately for its effectiveness using the procedure adopted for pathogenicity test (para 3.1.3). Organisms sprayed with sterile water served as control. Each treatment was replicated thrice and each replication contained 30 insects. Mortality of the insects were recorded at 24 h interval. Dead insects were removed after each observation.

3.2 OPTIMIZATION OF CULTURE MEDIA FOR IMPROVED SPORULATION AND CONIDIAL VIABILITY

In order to find out the best media supplement and its optimum dose an experiment was carried out in CRD with 28 treatments and 3 replications. Different media supplements, their source and concentrations are listed in Table 1.

Table 1. Media supplements, source and concentrations

Treatments	Supplements	Source	Tested concentrations (%)
T_1, T2, T_3	Chitin crude	MATSYAFED	0.5, 2.0, 5.0
T_4, T_5, T_6	Chitin extrapure	HIMEDIA	0.5, 2.0, 5.0
T_7, T_8, T_9	Chitosan crude	MATSYAFED	0.5, 2.0, 5.0
T_{10}, T_{11}, T_{12}	Chitosan extrapure	HIMEDIA	0.5, 2.0, 5.0
T_{13}, T_{14}, T_{15}	Yeast	HIMEDIA	1.0, 2.0, 3.0
T_{16}, T_{17}, T_{18}	Urea	HIMEDIA	0.5, 1.0, 2.0
T_{19}, T_{20}, T_{21}	Polyethylene glycol (PEG)	HIMEDIA	1.0, 3.0, 5.0
T_{22}, T_{23}, T_{24}	Arachid oil	HIMEDIA	1.0, 3.0, 5.0
T_{25}, T_{26}, T_{27}	Tween-80	HIMEDIA	1.0, 3.0, 5.0
T_{28}	SDB (Check)	-	-

To 250 ml conical flasks each containing 100 ml SDB, 0.5, two and five g of chitin and chitosan based supplements were added and mixed thoroughly. Similarly one, two and three g of yeast, 0.5, one and two g of urea, two, four and five ml of polyethylene glycol (PEG) and one, three and five ml of arachid oil and tween-80 were dissolved thoroughly with 100 ml SDB. These were then sterilized at 121°C for 15 minutes at 1.06 kg cm^{-2} pressure.

The media were then inoculated with one millilitre of 14 day old culture broth of *L. lecanii* and incubated at room temperature for 14 days. SDB without any supplements served as check. The biomass yield, spore count, colony forming units (cfu) and mortality of test insects were recorded on 14 days after inoculation. All the treatments were replicated thrice and the analysis was carried out in a completely randomized design.

3.2.1 Determination of fungal biomass

Fungal biomass was determined using the method suggested by Hall and Bell (1961). The 14 day old culture (100 ml broth) was filtered through a pre-dried and weighed Whatman No. 1 filter paper and the mat collected was dried at 100 º C for 24 h and weighed till two subsequent weights were constant.

3.2.2 Enumeration of spore count

The spore count was enumerated from a 14 day old culture broth using a haemocytometer and calculated using the formula,

Spores ml^{-1} = (n) x 10^4, where 'n' is the average number of spores in the four one millimetre corner squares of haemocytometer.

3.2.3 Estimation of colony forming units (cfu)

Colony forming units (cfu) were estimated by dilution plate method (Aneja, 1996 and 2003). One ml of 100 times diluted culture was poured on each Petri dish. To this 15 ml molten SDA media was added and gently rotated for uniform spreading of the spore suspension. The plates were then incubated at room temperature. Three replications were maintained for each treatment. Cfu was estimated after seven days of incubation, using the formula,

$$\text{cfu} = \frac{\text{number of colonies} \times \text{dilution factor } (10^2)}{\text{volume of sample (ml)}}$$

3.2.4 Determination of Mortality

For this study the spores collected from 14 day old cultures from different growth media were used. Tender cowpea leaf was kept upside down in a 9 cm plastic petri dish lined with a moist filter paper. Leaf petiole was covered with moistened cotton to maintain turgidity. Second instar nymphs of *A. craccivora* collected from laboratory culture (para 3.1.3.2) were released into the petridish using a fine moistened camel hairbrush @ 30 aphids per petri dish. The spore suspension (20 ml) was applied using an atomizer. Three replications were maintained for each treatment. Test insects sprayed with water alone served as control. Care was taken not to drown the insects in spore suspension. Observations were recorded at 12 h interval on the number of insects died and was continued till 100 per cent mortality was observed in any one of the treatments. Pathogenicity of *L. lecanii* was confirmed by reisolating the fungus from cadavers and examining under microscope.

The spores harvested from the best medium were used for development of formulations.

3.3 DEVELOPMENT OF FORMULATIONS WITH IMPROVED SHELF LIFE AND UV TOLERANCE

The basic components used for developing the formulations were

1. Spore concentrate in distilled water
2. Carriers *viz.* sunflower oil, groundnut oil and talc
3. Additives – colloidal chitin, chitin and chitosan

3.3.1 Preparation of Spore Concentrate

Spores were harvested from selected media following the procedure of Kim*, et al.* (2007) with some modifications. The conidia were separated from mycelium by filtering through sterilized Whatman No.1 filter paper. The spores were centrifuged at 12000 rpm for 25 min in Hermile labortechnic Z323K centrifuge. The

pellet was repeatedly washed with sterile distilled water and resuspended in 10 ml sterile distilled water and this concentrated spore suspension was utilized for preparation of formulations.

3.3.2 Preparation of Carrier materials

Liquid carriers, *viz.* groundnut oil (GNO), sunflower oil (SFO) and distilled water as well as a dry carrier, talc were sterilized at 121 º C for 15 minutes at 1.06 kg cm^{-2} pressure. It was cooled and utilized for preparation of formulations.

3.3.2 Preparation of Additives

Colloidal chitin was added as additive to liquid formulations while chitin and chitosan were added to dry formulations. These additives were sterilized at 121 º C for 15 minutes at 1.06 kg cm^{-2} pressure and added with the carriers at desired concentrations.

3.3.2.1 Preparation of Colloidal Chitin

Crude chitin (40 g) was slowly added into 250 ml of cold 0.25 N HCl with vigorous stirring and kept overnight at 4 º C in a refrigerator. The mixture was filtered through glass wool into 2 L ice cold water with rapid stirring using a magnetic stirrer. The gelatinous white material formed beneath was separated by filtration through a Whatmann No. 1 filter paper. The chitin pellet was washed repeatedly with tap water until the pH became neutral (Roberts and Selitrennikoff, 1988) (Plate 1).

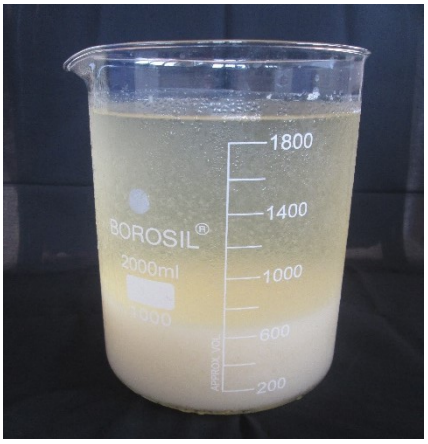
3.3.2.2 Determination of Optimum Concentration of Additives

To determine the optimum dose of additive (colloidal chitin) for preparing the liquid formulation, the spore suspension was mixed with colloidal chitin at different concentrations (0.1, 0.5 and 1.0 per cent) and they were assessed based on cfu and spore count. Similarly, for preparing dry formulations the spore suspension was

A) Crude Chitin

B) Crude Chitin + HCl

C) Filtration through glass wool



D) Colloidal Chitin

E) Filtration through Whatmann No. 1

F) Dried Colloidal Chitin

Plate 1. Preparation of Colloidal Chitin

mixed with talc and the additives *viz.* chitin and chitosan were added different concentrations (0.5, 2 and 5 per cent) and checked for cfu and spore count.

3.3.3 Standardization of Carriers

To standardize the carrier material for preparing formulations, the spore suspension of known concentration was used. The concentration was determined as 8.12×10^{8} spores ml^{-1} using a haemocytometer.

Liquid formulations were prepared by suspending *L. lecanii* spores @ 8.12×10^{8} spores ml^{-1} in oil carriers (GNO, SFO) and dry formulations were prepared using talc as the carrier. In addition to these basic formulations, five enriched formulations were prepared by adding additives to each of the basic carriers. The concentration of additives was determined following the procedure under 3.3.2.2. The carriers, additives and proportions of spore suspension used for preparing the formulations are given in Table 2.

Each of the formulations were prepared to a volume of 30 ml / 30 g. The liquid formulations were stored in glass vials with bakelite lid and dry formulations were stored in polypropylene covers at two different temperatures, room temperature and under refrigeration. Spores suspended in sterile distilled water alone served as control and all the treatments were replicated thrice. Spore count and cfu were estimated at fortnightly interval for a period of three month and the data were subjected to Analysis of Variance.

Table 2. List of carriers, additives and proportions of spore suspension used for preparing the formulations

Type of formulation	Carrier + additive	Proportion (Carrier : Spore suspension)
Basic	Sunflower oil (100 %)	50 :50
		60 :40
		65:35
	Groundnut oil (100 %)	50 :50
		60 :40
		65:35
	Talc	50 :50
		60 :40
		65:35
Enriched	Sunflower oil (99.90 %) + Colloidal chitin (0.1 %)	50 :50
		60 :40
		65:35
	Groundnut oil (99.90 %) + Colloidal chitin (0.1 %)	50 :50
		60 :40
		65:35
	Talc (95 %) + chitin (5 %)	50 :50
		60 :40
		65:35
	Talc (99.5 %) + chitosan (0.5 %)	50 :50
		60 :40
		65:35
Sterile distilled water (99.90 %) + colloidal chitin (0.1 %)		
Sterile distilled water (100 %) (Check)		

Two effective carrier + additive combinations were selected for further studies.

3.3.6. Standardization of adjuvants

To the selected formulations (para 3.3.5) the following adjuvants were incorporated at concentrations as detailed in Table 3.

Table 3. List of adjuvants and their concentrations

Sl. No.	Adjuvants	Concentrations (%)
1	Polyethylene glycol (PEG)	0.5, 1.0, 2.0
2	Polyoxyethylene (POX)	0.5, 2.0, 5.0
3	Tween-80	1.0, 3.0, 5.0
4	Glycerol	1.0, 3.0, 5.0

Oil + sterile distilled water + adjuvant combination was rated visually in terms of miscibility before incorporating spores. The proportion of formulation was maintained in such a way that addition of adjuvants was adjusted in the volume of carriers as detailed in the Table 4. All the treatments were replicated thrice. The formulations were assessed for spore count and cfu at fortnightly intervals and the data was statistically analyzed. Suitable concentration of each of the adjuvants was selected for studying their combination effect.

Table 4. Components of the formulation

Formulation			Adjuvant (ml)
Carrier + additive (ml)		Spore suspension (ml)	
Groundnut oil + colloidal chitin	19.35	10.5	PEG 0.15
	19.2	10.5	PEG 0.3
	18.9	10.5	PEG 0.6
	19.35	10.5	POX 0.15
	18.9	10.5	POX 0.6
	18	10.5	POX 1.5
	19.2	10.5	Tween-80 0.3
	18.6	10.5	Tween-80 0.9
	18	10.5	Tween-80 1.5
	19.2	10.5	Glycerol 0.3
	18.6	10.5	Glycerol 0.9
	18	10.5	Glycerol 1.5
Sunflower oil + colloidal chitin	19.35	10.5	PEG 0.15
	19.2	10.5	PEG 0.3
	18.9	10.5	PEG 0.6
	19.35	10.5	POX 0.15
	18.9	10.5	POX 0.6
	18	10.5	POX 1.5
	19.2	10.5	Tween-80 0.3
	18.6	10.5	Tween-80 0.9
	18	10.5	Tween-80 1.5
	19.2	10.5	Glycerol 0.3
	18.6	10.5	Glycerol 0.9
	18	10.5	Glycerol 1.5

3.3.6.1. Standardization of effective adjuvant combination

The adjuvants' concentrations determined under para 3.3.6 were used for preparing another set of formulations in order to study the effect of adjuvants when added in combinations.

Table 5. Composition of formulation with adjuvant combinations

Sl. No.	**Treatments**	**Ingredients of formulations (volume in ml)**		
		Carrier + additive	Spore suspension	Adjuvants
1	Tween-80 (1 %)	19.2	10.5	0.3
2	PEG (2 %)	18.9	10.5	0.6
3	Glycerol (5 %)	18	10.5	1.5
4	POX (0.5 %)	19.35	10.5	0.15
5	Tween-80 (1 %) + PEG (2 %) (*AC1)	18.6	10.5	0.3 + 0.6
6	Tween-80 (1 %) + Glycerol (5 %) (*AC2)	17.7	10.5	0.3 + 1.5
7	Tween-80 (1 %) + POX (0.5 %) (*AC3)	19.05	10.5	0.3 + 0.15
8	PEG (2 %) + Glycerol (5 %) (*AC4)	17.4	10.5	0.6 + 1.5
9	PEG (2 %) + POX (0.5 %) (*AC5)	18.75	10.5	0.6 + 0.5
10	Glycerol (5 %) + POX (0.5 %) (*AC6)	17.85	10.5	1.5 + 0.15
11	Tween-80 (1 %) + PEG (2 %) + Glycerol (5 %) (*AC7)	17.1	10.5	0.3 + 0.6 + 1.5
12	Tween-80 (1 %) + PEG (2 %) + POX (0.5 %) (*AC8)	18.45	10.5	0.3 + 0.6 + 0.15
13	Tween-80 (1 %) + Glycerol (5 %) + POX (0.5 %) (*AC9)	17.55	10.5	0.3 + 1.5 + 0.15
14	PEG (2 %) + Glycerol (5 %) + POX (0.5 %) (*AC10)	17.25	10.5	0.6 + 1.5 + 0.15
15	Tween-80 (1 %) + PEG (2 %) + Glycerol (5 %) + POX (0.5 %) (*AC11)	16.95	10.5	0.3 + 0.6 + 1.5 + 0.15

* AC = Adjuvant Combination

Each formulation was replicated thrice and their effectiveness was assessed based on cfu and spore count at monthly intervals. The effective formulations were selected for standardizing UV protectant.

3.3.8. Standardization of UV protectants

The materials tested for their UV tolerance are listed in Table 6.

Table 6. UV protectants tested

Sl. No.	UV protectants	Concentrations (%)
1	Arachid oil	0.5, 1.0, 2.0
2	Sunflower oil	0.5, 1.0, 2.0
3	Boric acid	0.5, 1.0, 2.0
4	Indigo	0.5, 1.0, 2.0

The spore suspension of *L. lecanii* (10^8 spores^{-1} ml as mentioned in para 3.1.4) mixed with each of the UV protectants at the concentrations mentioned above were kept in glass bottles and exposed to two different UV length, 365 nm and 620 nm. The source of UV light was Deep Vision UV chamber. The distance between exposed suspension and UV light source was 0.15 m. Exposure duration was 10, 20 and 30 min. UV tolerance was assessed based on spore count and cfu before and after exposure. The material that exhibited maximum tolerance was selected as the UV protectant for preparing tank mix formulations for field evaluation.

3.4. EVALUATION OF FORMULATIONS AGAINST SUCKING PESTS OF COWPEA UNDER FIELD CONDITIONS

A field experiment was conducted to evaluate the efficacy of *L. lecanii* formulations against sucking pests of cowpea. The formulations tested under field conditions were

Formulation I – Chitin enriched groundnut oil + AC 11 + boric acid 1 %
Formulation II – Chitin enriched groundnut oil + AC 2 + boric acid 1 %
Formulation III – Chitin enriched groundnut oil + AC 9 + boric acid 1 %
Standard Check - Talc based formulation
Chemical check - Chlorpyriphos 0.05 %

The cowpea variety Vellayani Jyothika was raised in the Instructional Farm, College of Agriculture, Vellayani adopting the Package of Practices Recommendations of Kerala Agricultural University (KAU, 2014). The experiment was laid out in randomized block design with six treatments including an untreated control and was replicated four times. The spacing adopted was 1.5 m × 45 cm and unit plot size was 3.2 m × 2.0 m (Plate 2).

First spraying was done at five per cent infestation level of the major sucking pest, *A. craccivora* (vegetative phase of the crop). Second spraying was done at 50 per cent infestation level (reproductive phase of the crop). Pretreatment counts of *A. craccivora* and other sucking pests *viz. B. tabaci, A. biguttula biguttula* and *Tetranychus* sp. were taken. Post treatment counts were taken at third, seventh and 14 th DAT.

Care was taken to avoid spray drift by placing sun pack screens in between the treatments. Spraying was done during evening hours.

Plate 2. Experimental layout

3.4.1 Assessment of Population of *A. craccivora*

The pretreatment count of aphids was taken following the sampling technique of Banks (1952). The aphid population was grouped into five classes.

Class	Description
1. Zero (N)	No aphids
2. Very light (VL)	One aphid to small colony of some scattered individuals confined to young leaves.
3. Light (L)	Scattered aphid colonies present on the stem and leaves and not confined to crown and upper leaves.
4. Medium (M)	Aphid present in large numbers not in recognizable colonies but diffused and infesting a large portion of the stem and leaves.
5. Heavy (H)	Very large number, very dense, infesting all leaves and stem. Stem usually black with aphids

The collection of samples and estimation of aphid population were done following the method of Srikanth (1985). Ten shoots in each class were collected from the experimental field. The sample shoots were cut with a sharp blade ensuring that the number of aphids falling from the shoots was reduced to a minimum. The shoots were then transferred to plastic containers with provision for ample aeration and were brought to the laboratory.

Each sample shoot was then transferred to a white paper and were gently tapped to dislodge the aphids. The mean number of aphids (all stages) per twig in each class was recorded as detailed in Table 7.

Table 7. Mean number of aphids in each class

Class	Number of aphids / sample										Mean number of aphids
	1	2	3	4	5	6	7	8	9	10	
VL	5	0	1	3	4	15	8	1	12	10	6.5
L	48	52	74	32	42	80	28	38	35	66	49.5
M	174	168	190	152	270	148	253	165	220	158	189.8
H	456	760	622	800	560	590	610	425	368	512	570.3

3.4.2 Assessment of population of other sucking pests

Population of *B. tabaci, A. biguttula biguttula and Tetranychus* sp. were recorded following sampling techniques of Rawat and Shau (1973). Sixteen plants were selected from each treatment (four plants per replication). Ten leaves from each plant were observed randomly from top, middle and bottom for recording the insect populations.

3.4.3 Estimation of yield

Yield was recorded separately from each treatment during harvests and the data was subjected to analysis of variance.

Results

4. RESULTS

4.1 PATHOGENECITY STUDIES

Pathogenicity studies of *L. lecanii* to different sucking pests of cowpea revealed that the fungus was pathogenic to aphids *A. craccivora* Koch, jassids *A. biguttula biguttula* Ishida, white fly *B. tabaci* Gennadius, mealy bug *F. virgata* Cockerell, mite *Tetranychus* sp. and brinjal scale *Lecanium* sp. It was found to be non infective to cow bug, *A. pilosum* Walker and pod bug, *R. pedestris* Fabricius. The symptoms exhibited by the insects when treated with spore suspension @ 2.14 x 10^7 spores ml^{-1} are described below.

4.1.1 Symptoms of Mycosis

4.1.1.1 A. biguttula biguttula

Initially the treated adults were highly mobile. Movement was arrested 48 h after treatment (HAT). Mortality was recorded from 72 HAT. The body was shrunken.

4.1.1.2 A. craccivora

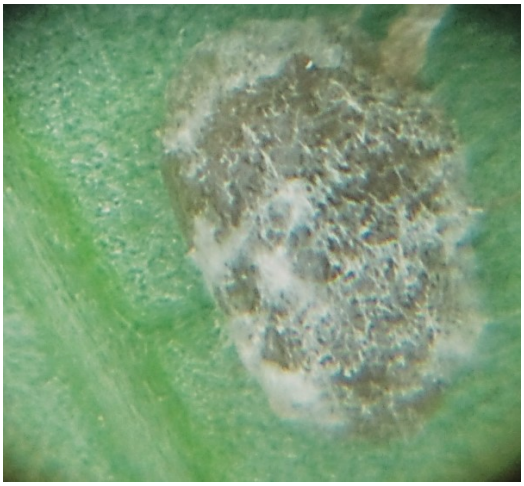
The treated nymphs were feeding normally without any impairment in movement initially. Lethargic movement was observed along with cessation of feeding at 24 HAT. Mortality initiated at 24 HAT. All the insects were dead by 120 HAT. The cadaver was found to be covered with white mycelial network at 72 HAT (Plate 3. A).

4.1.1.3 B. tabaci

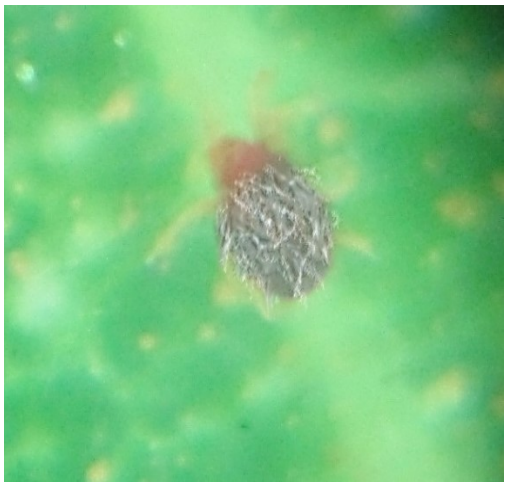
The infected whiteflies were found to be less active. At 48 h the dead insects were fixed to the leaf surface and abdomen was shrunken. Later mycelia emerged from the abdominal region. By 96 h the whole cadaver was found to be covered with mycelia except in the wing region (Plate 3. B).

(A) *Aphis craccivora*

(B) *Bemisia tabaci*



(C) *Lecanium* sp.



(D) *Tetranychus* sp.

Plate 3. *L. lecanii* infected insects and mite

4.1.1.4 F. virgata

The treated insects did not show any symptoms of infection in the initial days after treatment. Mortality initiated four days after treatment. White mycelial mat covered the dead cadaver by 48 h after death.

4.1.1.5 Lecanium sp.

Movement was arrested by 24 HAT. Death was observed from 48 HAT. Mycelial growth originated from the body margins which covered the entire body later (Plate 3. C).

4.1.1.6 Tetranychus sp.

The movement was completely arrested after 24 h of treatment. The body shrunk and mortality was observed 24 h onwards. The cadavers were dry and stiff with mycelial strands all over (Plate 3. D).

4.1.2 Effective Dose of *L. lecanii*

Dose mortality response of organisms to *L. lecanii* are presented in Tables 8 - 11.

4.1.2.1 A. craccivora

The mortality of *A. craccivora* treated with different doses of *L. lecanii* is furnished in Table 8. None of the tested concentrations could result in mortality 24 hours after treatment (HAT) except with 10^8 spores ml^{-1} which resulted in negligible mortality of 3.33 per cent. At 42 HAT, concentrations ranging from 10^6 to 10^8 resulted in 16.67 to 26.67 per cent mortality. Spore concentrations of 10^7 and 10^8 spores ml^{-1} resulted in 50 and 53.33 per cent mortality 72 HAT respectively. Complete mortality was observed with 10^8 spores ml^{-1}, while mortality was 90 per cent with 10^7 spores ml^{-1} at 120 HAT. At the end of the observational period (144

Table 8. Dose-mortality response of *A. craccivora* to *L. lecanii*

Dose (spores ml^{-1})	**Cumulative per cent mortality* at different intervals after treatment (N=90)**					
	24 h	**48 h**	**72 h**	**96 h**	**120 h**	**144 h**
10^8	3.33	16.67	53.33	86.67	100	100
10^7	0	26.67	50	76.67	90	100
10^6	0	16.67	40	50	76.67	83.33
10^5	0	20	33.33	46.67	56.67	60
10^4	0	6.67	16.67	23.33	33.33	43.33
10^3	0	0	10	23.33	26.67	33.33
Control	0	0	0	0	0	0

*Mean of three replications

Table 9. Dose-mortality response of *B. tabaci* to *L. lecanii*

Dose (spores ml^{-1})	**Cumulative per cent mortality* at different intervals after treatment (N=90)**						
	24 h	**48 h**	**72 h**	**96 h**	**120 h**	**144 h**	**168 h**
10^8	3.33	13.33	23.33	50	63.33	70	73.33
10^7	0	6.67	20	46.67	53.33	66.67	70
10^6	0	3.33	13.33	26.67	30	40	53.33
10^5	0	0	3.33	10	16.67	23.33	30
10^4	0	0	0	3.33	13.33	16.67	23.33
10^3	0	0	0	0	13.33	13.33	16.67
Control	0	0	0	0	0	0	0

*Mean of three replications

DAT), 10^6, 10^7 and 10^8 were found to be effective resulting in 83.33, 100 and 100 per cent mortality respectively.

4.1.2.2 B. tabaci

Dose- mortality response of *B. tabaci* to *L. lecanii* is presented in Table 9. At 24 HAT, only 3.33 per cent mortality was observed with 10^8 spores ml^{-1}. After 48 h, the mortality ranged from 3.33 to 13.33 per cent when treated with 10^6 to 10^8 spores ml^{-1}. Fifty per cent mortality was recorded with 10^8 spores ml^{-1} at 72 HAT. At 120 HAT 53.33 and 63.33 per cent mortality was recorded with 10^7 and 10^8 spores ml^{-1}. At the end of 144 HAT the mortality rate observed was 66.67 and 70 per cent with the spore concentrations 10^7 and 10^8 spores ml^{-1}. Lower dose of 10^6 resulted in 53.33 per cent while it was much lesser with doses 10^5, 10^4 and 10^3 (30, 23.33 and 16.67 per cent respectively).

***4.1.2.3 Lecanium* sp.**

The mortality observed with *Lecanium* sp. at different spore concentrations of *L. lecanii* is presented in Table 10. None of the insects were dead in the first 24 HAT. Mortality initiated only after 48 HAT. The mortality ranged from 23.33 per cent to 6.67 per cent with 10^8 to 10^6 spores ml^{-1} at this point of time. At 96 HAT, 56.67 and 63.33 per cent mortality was observed with spore concentrations 10^7 and 10^8 spores ml^{-1}. By 144 HAT, 10^5, 10^6, 10^7 and 10^8 spores ml^{-1} were found to be effective with 56.67, 63.33, 83.33 and 90 per cent mortality. The lower spore concentrations, 10^4 and 10^3 spores ml^{-1} resulted in 43.33 and 10 per cent mortality.

***4.1.2.4 Tetranychus* sp.**

The dose mortality responses of *Tetranychu*s sp. to different doses of *L. lecanii* is furnished in Table 11. The mortality observed at 24 HAT with spore concentrations 10^8 to 10^3 spores ml^{-1} varied from 73.33 to 0 per cent. By 72 HAT, the mortality rate observed with 10^8 and 10^7 spores ml^{-1} was 96.67 and 90 per cent.

Table 10. Dose-mortality response of *Lecanium* sp. to *L. lecanii*

Dose (spores ml^{-1})	**Cumulative per cent mortality* at different intervals after treatment (N=90)**					
	24 h	**48 h**	**72 h**	**96 h**	**120 h**	**144 h**
10^8	-	23.33	46.67	63.33	70	90
10^7	-	13.33	40	56.67	66.67	83.33
10^6	-	6.67	16.67	33.33	56.67	63.33
10^5	-	-	10	30	46.67	56.67
10^4	-	-	3.33	13.33	26.67	43.33
10^3	-	-	-	6.66	10	10
Control	-	-	-	-	-	-

*Mean of three replications

Table 11. Dose-mortality response of *Tetranychus* sp. to *L. lecanii*

Dose (spores ml^{-1})	**Cumulative per cent mortality* at different intervals after treatment (N=90)**				
	24 h	**48 h**	**72 h**	**96 h**	**120 h**
10^8	73.33	86.67	96.67	100	100
10^7	36.67	56.67	90	90	100
10^6	23.33	36.67	60	83.33	90
10^5	6.67	36.67	56.67	73.33	86.67
10^4	3.33	16.67	30	46.67	56.67
10^3	0	0	13.33	36.67	36.67
Control	0	0	0	0	0

*Mean of three replications

At 120 HAT, cent per cent mortality was recorded with higher doses, 10^8 and 10^7 spores ml^{-1}. Lower doses, 10^4, 10^5 and 10^6 spores ml^{-1} resulted in 56.67, 86.67 and 90 per cent mortality, respectively. The mortality observed with 10^3 spores ml^{-1} was much lower compared to rest of the doses tested (36.67 per cent).

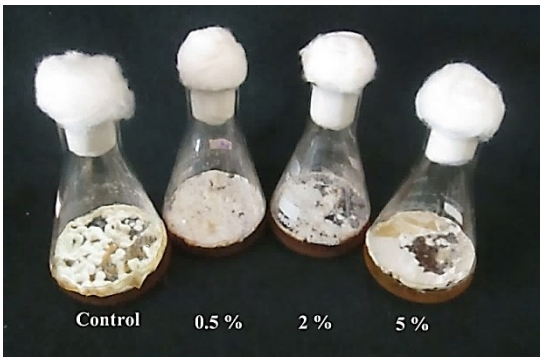
In case of all the susceptible insects, the spore doses of 10^7 spores ml^{-1} and 10^8 spores ml^{-1} were effective.

4.2 OPTIMIZATION OF CULTURE MEDIA FOR IMPROVED SPORULATION AND CONIDIAL VIABILITY

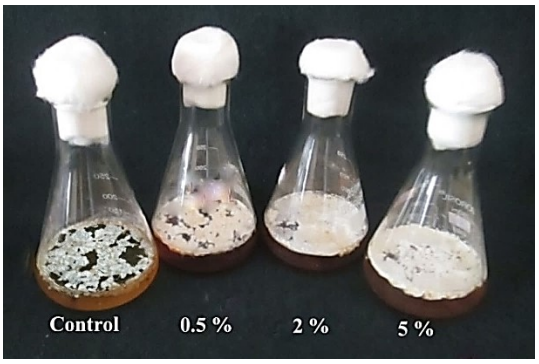
With a view to optimize the culture media for improved sporulation and conidial viability, the fungus was cultured in Sabouraud Dextrose Broth (SDB) amended with media supplements like chitin and chitosan, yeast, polyethylene glycol, tween 80 and arachid oil as detailed in para 3.3. The biomass yield, spore count, colony forming units and mortality *on A. craccivora* were assessed. The results are presented in Table 12 and 13.

4.2.1 Biomass

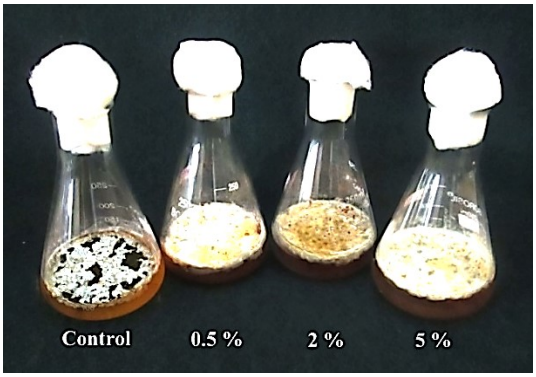
Biomass determined from 100 ml broth of 14 day old culture (Plates 4. A to I) revealed that PEG two per cent with a biomass of 2.67 g was found to be significantly superior over other treatments (Table 12). This was followed by the biomass observed with PEG four and five per cent, chitosan five per cent (extra pure and crude forms) and arachid oil five per cent each (2.21 g, 1.96 g 2.00 g, 1.97 g and 1.94 g, respectively). Biomass recorded from chitin crude and extra pure five per cent, chitosan extra pure 0.5 and two per cent, chitosan crude 0.5 and two per cent and arachid oil three per cent were statistically on par, the biomass being 1.89 g, 1.78 g, 1.88 g, 1.83 g, 1.75 g, 1.85 g and 1.81 g, respectively. Biomass of media amended with arachid oil one per cent was 1.37 g, which was significantly superior to unamended media (0.62 g). This was followed by yeast one per cent (0.97 g). All other supplements namely, yeast two and three per cent (0.80 g and 0.64 g), extra



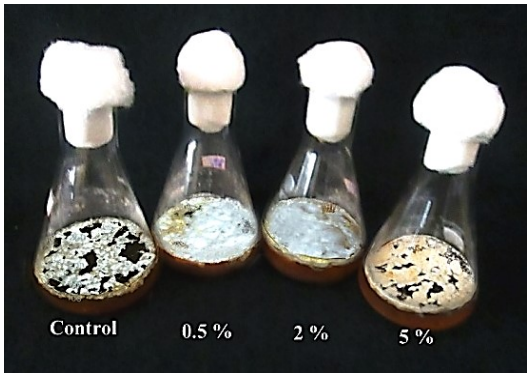
(A) Chitin (crude)



(B) Chitin (extra pure)

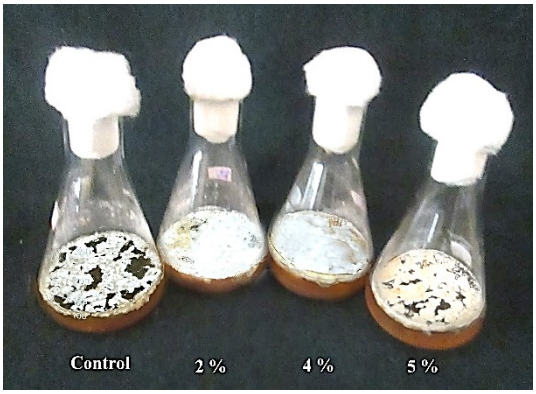


(C) Chitosan (crude)

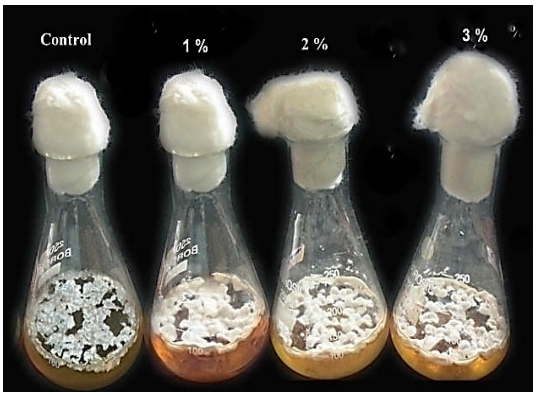


(D) Chitosan (extra pure)

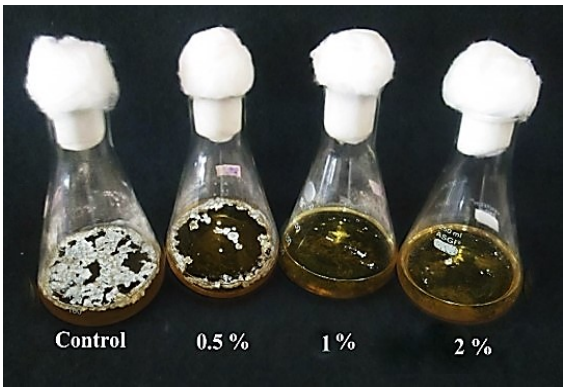
Plate 4. Growth of *L. lecanii* in different media supplements (14 days after inoculation).



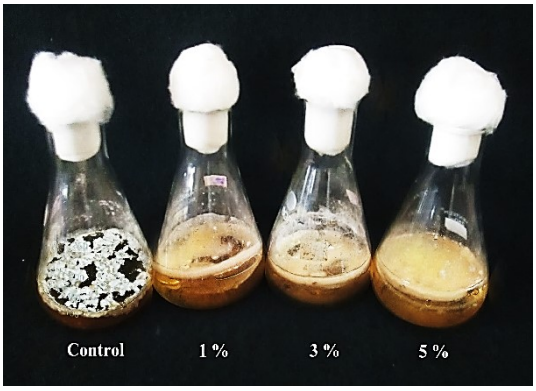
(E) Polyethylene glycol



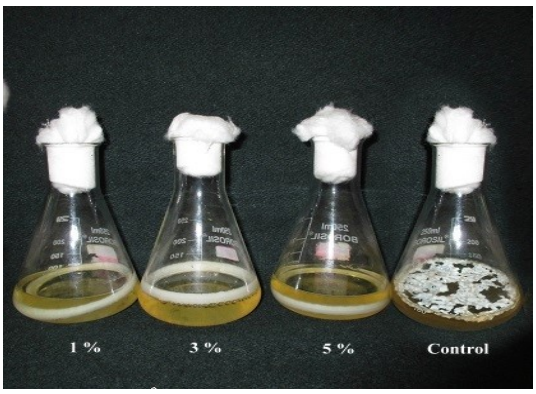
(F) Yeast



(G) Urea



(H) Arachid oil



(I) Tween-80

Plate 4. Growth of *L lecanii* in different media supplements
(14 days after inoculation)

pure chitin 0.5 and two per cent (0.64 g and 0.77 g), crude chitin 0.5 and two per cent (0.52 g and 0.56 g) and tween-80 one, three and five per cent (0.48 g, 0.47 g and 0.43 g) were on par with unamended media. Mycelial yield obtained in urea 0.5, one and two per cent amended media was significantly lower (0.19 g, 0.014 g and 0.01g) than that in unamended media.

4.2.2 Spore count

The spore yield from chitin, chitosan and yeast supplemented media was notably higher (1.12 x 10^8 spores ml^{-1} to 9.34 x 10^8 spores ml^{-1}) than that obtained from other supplements *viz.* PEG, urea, tween-80 and arachid oil (0.04 x 10^8 spores ml^{-1} to 0.55 x 10^8 spores ml^{-1}) (Table 12).

Chitosan extra pure five per cent was found to be the best media supplement for enhancing sporulation, the spore yield being 9.34 x 10^8 spores ml^{-1}. This was followed by chitosan extra pure two per cent which yielded 7.83 x 10^8 spores ml^{-1}. Chitosan crude five per cent yielded 4.52 x 10^8 spores ml^{-1}, followed by chitosan crude 0.5 and two per cent and chitosan extra pure 0.5 per cent (3.77 x 10^8 spores ml^{-1}, 4.21 x 10^8 spores ml^{-1} and 4.09 x 10^8 spores ml^{-1}) which were on par with yeast one per cent (3.22 x 10^8 spores ml^{-1}).

The spore count obtained in media supplemented with crude chitin five and 0.5 per cent were statistically on par (3.01 x 10^8 spores ml^{-1} and 2.53 x 10^8 spores ml^{-1}) but significantly lower than the earlier mentioned supplements. The spore yield of yeast two per cent, chitin extra pure five per cent and chitin crude and extra pure two per cent were statistically on par (2.39 x 10^8 spores ml^{-1}, 2.38 x 10^8 spores ml^{-1}, 1.92 x 10^8 spores ml^{-1} and 1.86 x 10^8 spores ml^{-1}, respectively). The lowest spore yield was recorded from media amended with chitin extra pure 0.5 per cent (1.32 x 10^8 spores ml^{-1}) and yeast three per cent (1.12 x 10^8 spores ml^{-1}) which were however superior to control.

Table 12. Effect of media supplements on growth and sporulation of *L. lecanii*

Sl no.	Media supplements		Biomass (g/100 ml)	Spore count (x 10^8 spores ml^{-1})	cfu (x 10^5 ml^{-1})
1	Chitin crude	0.5 %	0.52 (1.23)	2.53 (1.88)	1.46 (1.57)
2		2 %	0.56 (1.25)	1.92 (1.71)	1.37 (1.54)
3		5 %	1.89 (1.7)	3.01 (2.00)	1.57 (1.60)
4	Chitin extrapure	0.5 %	0.64 (1.28)	1.32 (1.52)	1.37 (1.54)
5		2 %	0.77 (1.33)	1.86 (1.69)	1.37 (1.54)
6		5 %	1.78 (1.67)	2.38 (1.84)	1.53 (1.59)
7	Chitosan crude	0.5 %	1.75 (1.66)	3.77 (2.18)	1.43 (1.56)
8		2 %	1.85 (1.69)	4.21 (2.28)	1.53 (1.59)
9		5 %	1.97 (1.72)	4.52 (2.35)	1.50 (1.58)
10	Chitosan extrapure	0.5 %	1.88 (1.7)	4.09 (2.26)	1.45 (1.57)
11		2 %	1.83 (1.68)	7.83 (2.97)	1.49 (1.58)
12		5 %	2.00 (1.73)	9.34 (3.22)	1.53 (1.59)
13	Yeast	1 %	0.97 (1.4)	3.22 (2.06)	1.55 (1.60)
14		2 %	0.8 (1.34)	2.39 (1.84)	1.46 (1.57)
15		3 %	0.64 (1.28)	1.12 (1.46)	1.43 (1.56)
	CD (0.05)		-	**0.155**	-
16	Polyethylene glycol (PEG)	2 %	2.67 (1.92)	0.55 (1.24)	1.23 (1.49)
17		3 %	2.21 (1.79)	0.49 (1.22)	0.87 (1.37)
18		5 %	1.94 (1.72)	0.53 (1.24)	1.31 (1.52)
19	Arachid oil	1 %	1.37 (1.54)	0.34 (1.16)	1.17 (1.47)
20		3 %	1.81 (1.68)	0.16 (1.07)	0.75 (1.32)
21		5 %	1.96 (1.72)	0.10 (1.05)	0.59 (1.26)
22	Tween 80	1 %	0.48 (1.22)	0.20 (1.10)	2.00 (1.73)
23		3 %	0.47 (1.21)	0.20 (1.10)	1.16 (1.47)
24		5 %	0.43 (1.2)	0.16 (1.08)	0.86 (1.37)
25	Urea	0.5 %	0.19 (1.09)	0.06 (1.03)	1.16 (1.47)
26		1 %	0.01 (1.01)	0.04 (1.02)	0
27		2 %	0.01 (1.00)	0.04 (1.02)	0
28	Unamended check		0.62 (1.27)	0.36 (1.17)	1.43 (1.56)
	CD (0.05)		**0.081**	**0.032**	**0.174**

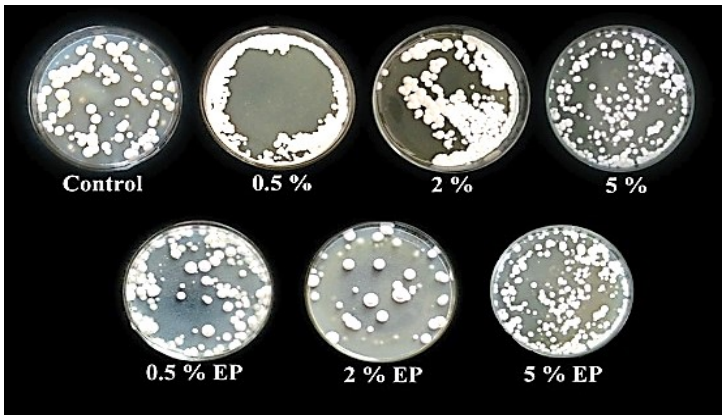
Figures in parentheses are values after $\sqrt{x + 1}$ transformation

The media with PEG, urea, tween-80 and arachid oil were found to be inferior compared to other supplements, with the spore count varying from 0.04 x 10^8 spores ml^{-1} to 0.55 x 10^8 spores ml^{-1}). PEG two, four and five per cent with spore yield of 0.55 x 10^8 spores ml^{-1}, 0.55 x 10^8 spores ml^{-1} and 0.49 x 10^8 spores ml^{-1}, respectively were significantly higher than the unamended media. Tween-80 one per cent was found to yield a spore count (0.34 x 10^8 spores ml^{-1}), which was on par with the control. All other treatments, namely arachid oil one, three, five; tween-80 three, five and urea 0.5, one and two per cent recorded lower spore count with respect to unamended media.

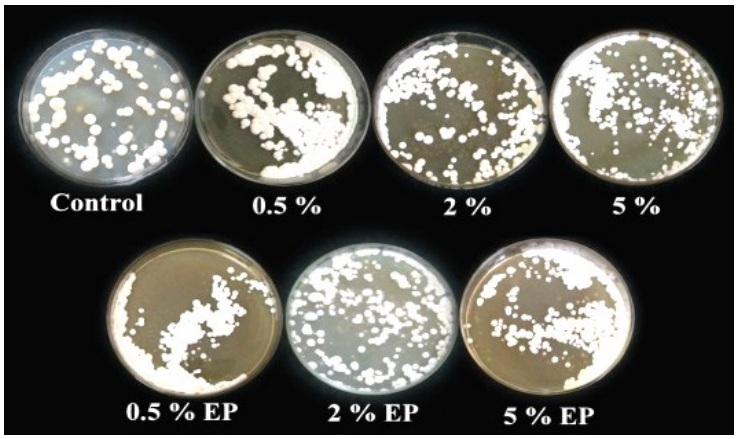
In general, all the tested concentrations, both of crude and extra pure chitin and chitosan (0.5, two and five per cent) as well as yeast (one, two and three per cent) could significantly enhance sporulation. Maximum sporulation was observed with extra pure chitosan five per cent (9.34 x 10^8 spores ml^{-1}) while tween-80, arachid oil and urea were found to inhibit sporulation.

4.2.3 cfu (colony forming units)

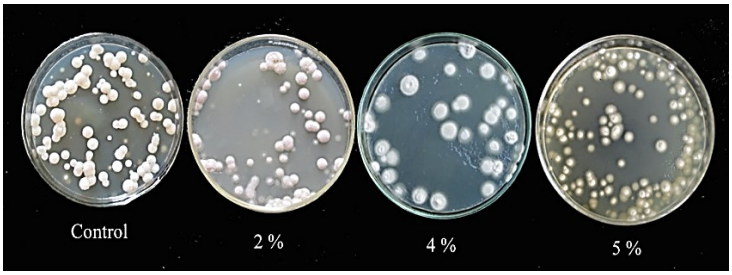
Media supplements were found to influence the number of viable colonies significantly (Plates 5. A to G). The maximum number of colony forming units were observed in media amended with one per cent arachid oil (2.00 x 10^5 ml^{-1}) which was on par with chitin crude five per cent (1.57 x 10^5 ml^{-1}), yeast one per cent (1.55 x 10^5 ml^{-1}), chitin extra pure five per cent (1.53 x 10^5 ml^{-1}), chitosan crude and extra pure five per cent (1.50 x 10^5 ml^{-1} and 1.53 x 10^5 ml^{-1}) and chitosan crude and extra pure two per cent (1.53 x 10^5 ml^{-1} and 1.49 x 10^5 ml^{-1}). The other media supplements *viz.* chitin crude and extra pure 0.5 and two per cent (1.46 x 10^5 ml^{-1}, 1.37 x 10^5 ml^{-1}, 1.37 x 10^5 ml^{-1} and 1.37 x 10^5 ml^{-1}, respectively), chitosan crude and extra pure 0.5 per cent (1.43 x 10^5 ml^{-1} and 1.46 x 10^5 ml^{-1}), yeast two and three per cent (1.45 x 10^5 ml^{-1} and 1.43 x10^5 ml^{-1}), PEG five and two per cent (1.23 x 10^5 ml^{-1} and 1.31 x 10^5 ml^{-1}), tween-80 one per cent (1.17 x 10^5 ml^{-1}), urea 0.5



(A) Chitin crude and extra pure (EP)

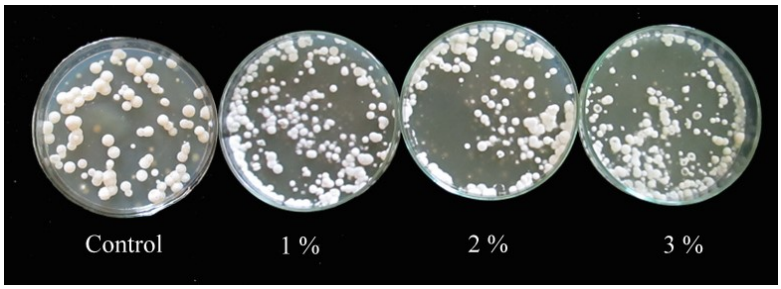


(B) Chitosan crude and extra pure (EP)

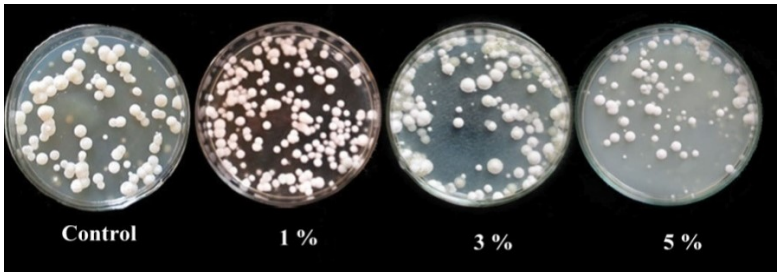


(C) Polyethylene glycol

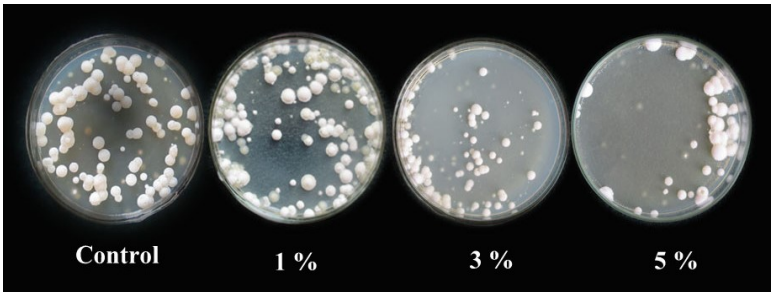
Plate 5. Comparative viability of *L. lecanii* in different media supplements (7 days after inoculation).



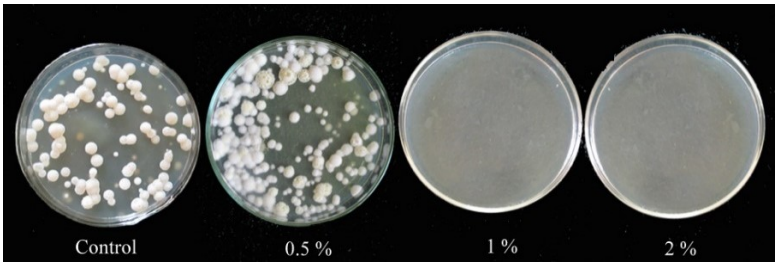
(A) Yeast



(B) Arachid oil



(C) Tween-80



(D) Urea

Plate 5. Comparative viability of *L. lecanii* in different media supplements (7 days after inoculation)

per cent (1.16×10^5 ml^{-1}) and arachid oil three per cent (1.16×10^5 ml^{-1}) were on par with unamended media.

The cfu in media amended with PEG four per cent, arachid oil five per cent, tween-80 three and five per cent were lower than the unamended media, cfu being 0.87×10^5 ml^{-1}, 0.86×10^5 ml^{-1}, 0.75×10^5 ml^{-1} and 0.59×10^5 ml^{-1} respectively. None of the spores were viable in media amended with one and two per cent urea.

4.2.4 Mortality

Efficacy of the *L. lecanii* cultured on various media supplements was evaluated on *A. craccivora.* The data on mortality recorded at 12 h interval are given in Table 13. After 12 h, irrespective of the treatment, mortality rate was very low, varying from 0 to 26.45 per cent. PEG, tween-80 and urea supplements showed no mortality in the first 12 h of observation while arachid oil five per cent gave 26.45 per cent mortality followed by chitosan crude and extra pure five per cent with 23.12 per cent mortality.

After 24 h, maximum mortality was noticed with chitosan extra pure two per cent and arachid oil five per cent (36.50 per cent each). This was followed by chitosan extra pure five per cent (36.12 per cent), chitosan crude two per cent (33.18 per cent), chitin extra pure five per cent (29.45 per cent), chitosan crude 0.5 and extra pure five per cent (26.45 per cent), arachid oil three per cent (26.45 per cent) and chitin extra pure 0.5 per cent (25.93 per cent). These treatments were statistically on par and significantly superior to rest of the media supplements. None of the aphids died at this point of time when treated with one and two per cent urea supplemented culture broth.

At the end of 36 h, mortality observed with chitosan crude five per cent, arachid oil five per cent and chitosan extra pure 0.5 per cent were 53.23 per cent, 53.23 per cent and 50 per cent respectively. These were followed by chitosan extra pure five per cent, chitin crude five per cent, chitosan extra pure two per cent, arachid

Table 13. Efficacy of *L. lecanii* cultured on different media supplements

Sl no.	Amended culture broth		Mean mortality of *A. craccivora* (%) at 12 h intervals					
			12 h	**24 h**	**36 h**	**48 h**	**60 h**	**72 h**
1	SDB + Chitin crude	0.5 %	05.47 (2.54)	16.31 (4.16)	33.18 (5.85)	49.67 (7.12)	66.35 (8.21)	79.79 (8.99)
2		2 %	07.80 (2.97)	23.11 (4.91)	36.50 (6.12)	56.57 (7.59)	66.35 (8.21)	86.60 (9.36)
3		5 %	10.48 (3.39)	29.47 (5.52)	46.56 (6.90)	69.76 (8.41)	86.42 (9.35)	100 (10.05)
4	SDB + Chitin extrapure	0.5 %	02.14 (1.77)	25.93 (5.19)	39.59 (6.37)	56.57 (7.59)	69.76 (8.41)	83.27 (9.18)
5		2 %	05.47 (2.54)	23.11 (4.91)	36.50 (6.12)	59.72 (7.79)	73.26 (8.62)	86.60 (9.36)
6		5 %	16.31 (4.16)	29.45 (5.52)	43.21 (6.65)	69.76 (8.41)	83.27 (9.18)	100 (10.05)
7	SDB + Chitosan crude	0.5 %	12.98 (3.74)	26.46 (5.24)	39.59 (6.37)	56.57 (7.59)	76.59 (8.81)	76.59 (8.81)
8		2 %	20.00 (4.58)	33.18 (5.85)	43.21 (6.65)	62.97 (8.00)	69.76 (8.41)	86.60 (9.36)
9		5 %	23.12 (4.91)	36.09 (6.09)	53.23 (7.36)	66.06 (8.19)	79.79 (8.99)	89.82 (9.53)
10	SDB + Chitosan extrapure	0.5 %	12.98 (3.74)	23.11 (4.91)	50.00 (7.14)	59.72 (7.79)	69.76 (8.41)	86.60 (9.36)
11		2 %	16.31 (4.16)	36.50 (6.12)	46.56 (6.90)	66.35 (8.21)	72.82 (8.59)	89.82 (9.53)
12		5 %	23.12 (4.91)	36.12 (6.09))	49.67 (7.12)	73.26 (8.62)	86.60 (9.36)	100 (10.05)
13	SDB + Yeast	1 %	03.81 (2.19)	23.11 (4.91)	26.45 (5.24)	42.78 (6.62)	53.23 (7.36)	66.06 (8.19)
14		2 %	02.14 (1.77)	12.99 (3.74)	16.31 (4.16)	25.93 (5.19)	36.12 (6.09)	63.25 (8.02)
15		3 %	0	12.99 (3.74)	16.31 (4.16)	23.12 (4.91)	33.18 (5.85)	59.82 (7.79)
16	SDB + Polyethylene glycol	2 %	0	23.11 (4.91)	29.45 (5.52)	33.18 (5.85)	53.23 (7.36)	69.76 (8.41)
17		4 %	0	12.99 (3.74)	23.12 (4.91)	23.12 (4.91)	36.50 (6.12)	43.21 (6.65)
18		5 %	0	07.82 (2.97)	07.80 (2.97)	12.98 (3.74)	26.45 (5.24)	32.60 (5.80)
19	SDB + Tween 80	1 %	0	16.31 (4.16)	23.12 (4.91)	26.45 (5.24)	36.12 (6.09)	59.72 (7.79)
20		3 %	0	05.45 (2.54)	12.98 (3.74)	23.12 (4.91)	23.12 (4.91)	23.12 (4.91)
21		5 %	0	02.13 (1.77)	16.31 (4.16)	26.45 (5.24)	26.45 (5.24)	26.45 (5.24)
22	SDB + Arachid oil	1 %	03.81 (2.19)	23.11 (4.91)	36.50 (6.12)	53.23 (7.36)	66.58 (8.22)	79.79 (8.99)
23		3 %	16.31 (4.16)	26.45 (5.24)	46.56 (6.90)	69.72 (7.79)	69.76 (8.41)	82.88 (9.16)
24		5 %	26.45 (5.24)	36.50 (6.12)	53.23 (7.36)	66.58 (8.22)	76.59 (8.81)	100 (10.05)
25	SDB + Urea	0.5 %	0	02.13 (1.77)	02.14 (1.77)	12.98 (3.74)	16.31 (4.16)	20.00 (4.58)
26		1 %	0	0	0	05.47 (2.54)	12.98 (3.74)	16.31 (4.16)
27		2 %	0	0	0	10.00 (3.32)	12.98 (3.74)	12.98 (3.74)
28	Unamended Check		02.14 (1.77)	12.99 (3.74)	25.93 (5.19)	39.59 (6.37)	53.23 (7.36)	59.74 (7.79)
	CD (0.05)		**2.092**	**1.350**	**1.157**	**1.091**	**0.957**	**0.878**

Figures in parentheses are values after $\sqrt{x + 1}$ transformation

oil three per cent, chitin extra pure five per cent and chitosan crude two per cent with 50, 49.67, 46.56, 46.56, 46.56, 43.21 and 43.21 per cent mortality respectively. The mortality observed with all other treatments was less than 50 per cent. Mortality observed with urea amended media was negligible.

At 48 HAT, the mortality observed with higher concentration of both crude and extra pure chitosan and chitin (two and five per cent) as well as arachid oil (three and five per cent) ranged from 56.57 to 73.26 per cent whereas the unamended check (SDB) recorded only 39.59 per cent mortality of *A. craccivora.*

A similar trend was observed after 60 HAT. Maximum mortality was recorded with chitosan five per cent both extra pure and crude (86.60 and 79.79 per cent), chitin extra pure and crude five per cent (83.27 and 86.42 per cent), chitosan crude 0.5 per cent (76.59 per cent), chitosan extra pure two per cent (73.26 per cent) and arachid oil five per cent (76.59 per cent). Of which chitosan extra pure five per cent, chitin crude five per cent were found to be significantly superior. Mortality rate observed with lower concentrations of the above set of treatments was found to range from 66.35 to 69.76 per cent which were significantly higher than control (53.23 per cent). The corresponding mortality observed with arachid oil and yeast one per cent, chitin crude 0.5 and two per cent and PEG two per cent, were on par with control, mortality being 66.58, 53.23, 66.35 and 53.23 per cent respectively.

At the end of the experiment (72 HAT), cent per cent mortality was observed in chitin crude and extra pure five per cent, chitosan extra pure five per cent and arachid oil five per cent amended media, which was on par with chitosan crude five per cent and extra pure two per cent (89.82 per cent) and chitin crude and extra pure two per cent, chitosan crude two per cent and extra pure 0.5 per cent (86.60 per cent each).

The mortality achieved in media amended with chitin, chitosan and arachid oil ranging from 76.59 to 100 per cent was superior to that obtained in control (59.74 per cent).

In general, PEG (two and four per cent) improved the biomass while chitin, chitosan and yeast at all tested concentrations improved sporulation. Arachid oil (one per cent) could improve viability. Mortality was high with higher concentrations of chitin, chitosan and arachid oil.

4. 3 FORMULATION OF *L. lecanii*

The formulations prepared vide para 3.3, stored at room temperature and refrigeration, were checked for their retention of conidial viability and spore count.

Of the various concentrations of additives tested as per para 3.3.2.2, 0.1 per cent colloidal chitin was the best additive for liquid formulations in terms cfu and spore count. In talc based formulations, chitin five per cent and chitosan 0.5 per cent were the superior additives. Various formulations prepared for the study are presented in Plates 6 to 8.

4.3.1 Effect of Carrier Materials on Viability and Spore Count

4.3.1.1 Viability

The viability of the formulations stored at room temperature (Table 14) and refrigeration (Table 15) was assessed in terms of cfu at fortnightly intervals.

4.3.1.1.1 Room temperature

At 15 days after storage (DAS), maximum number of cfu was observed with the enriched carriers, groundnut oil (GNO) + chitin 65:35 (3.4×10^6 ml^{-1}) and GNO + chitin 60:40 (3.30×10^6 ml^{-1}). This was followed by sunflower oil (SFO) + chitin 60:40 and GNO + chitin 50:50 (3.13×10^6 ml^{-1}) which were statistically on par with SFO + chitin 65:35 and SFO + chitin 50:50 (3.07×10^6 ml^{-1} and 3.13×10^6 ml^{-1}). The

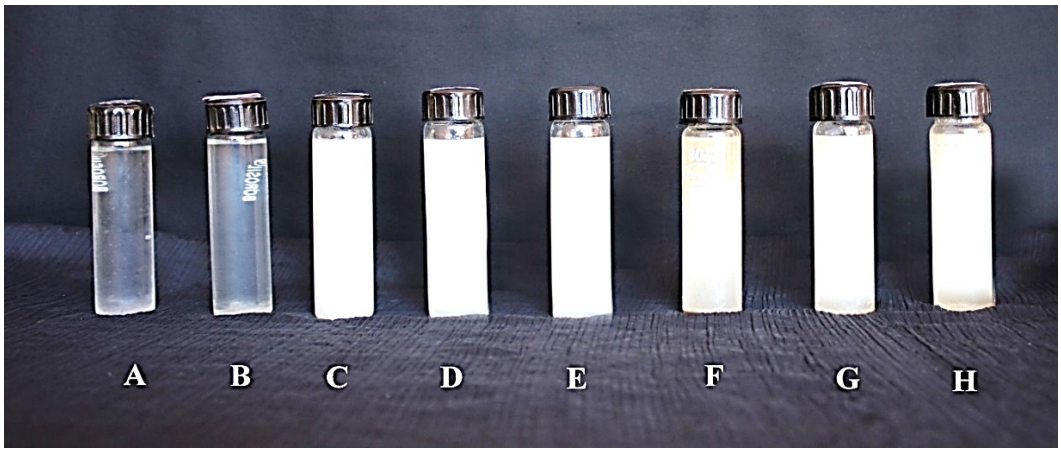


Plate 6. *L. lecanii* formulations in groundnut oil (GNO)

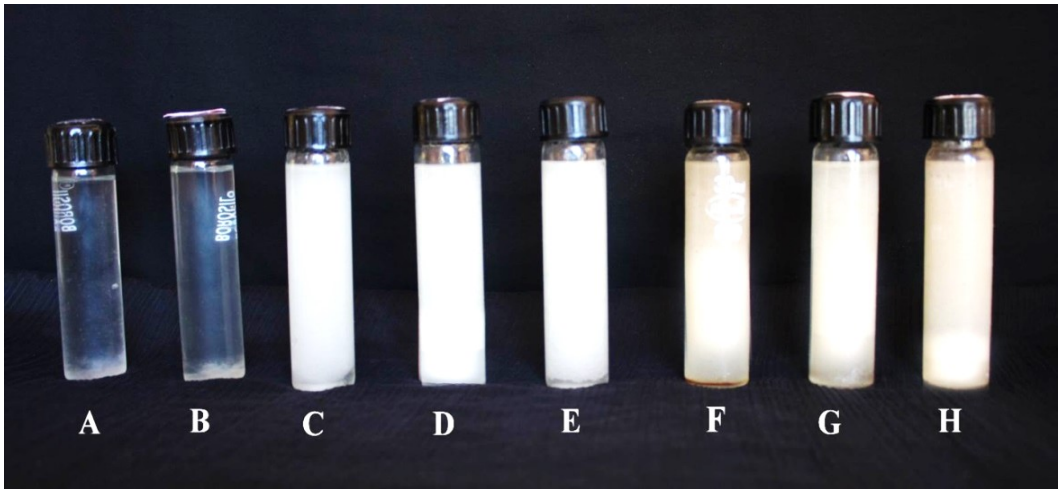


Plate 7. *L. lecanii* formulations in sunflower oil (SFO)

A. Distilled water, B. Distilled water + chitin, C. GNO / SFO + Spore concentrate (SS) 50:50, D. GNO / SFO + SS 60:40, E. GNO / SFO + SS 65:35, F. (GNO / SFO + chitin) + SS 50:50, G. (GNO / SFO + chitin) + SS 60:40, H. (GNO / SFO + chitin) + SS 65:35

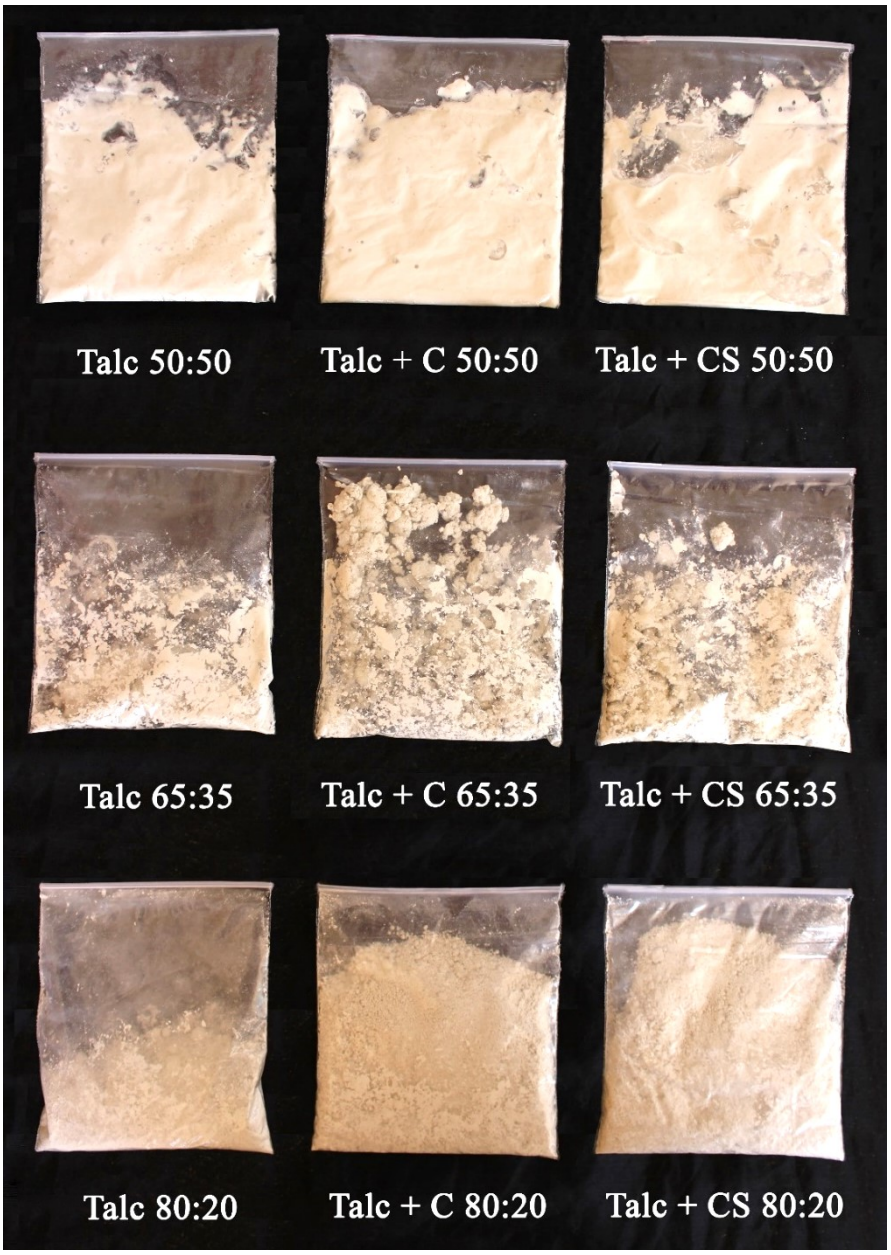


Plate 8. *L. lecanii* formulations in talc (C- chitin; CS- Chtisan)

cfu obtained with the basic formulations, GNO (65:35, 60:40 and 50:50) and SFO (65:35 and 60:40) were statistically similar to that of SFO + chitin 50:50 (2.93 x 106 ml-1, 2.90 x 106 ml-1, 2.83 x 106 ml-1, 2.90 x 106 ml-1, 2.77 x 106 ml-1 and 2.97 x 106 ml-1). The cfu obtained with talc formulations, viz. talc + chitosan 80:20, talc + chitin 65:35, talc + chitin 80:20 and talc + chitosan 65:35 (2.63 x 106 ml-1, 2.57 x 106 ml-1, 2.53 x 106 ml-1 and 2.50 x 106 ml-1, respectively) were in line with that of SFO 50:50 (2.67 x 10^6 ml^{-1}). The cfu of talc + chitosan 50:50, talc + chitin 50:50, distilled water (DW) + chitin and spore suspension alone (2.43 x 10^6 ml^{-1}, 2.13 x 10^6 ml^{-1}, 1.93 x 10^6 ml^{-1} and 1.73 x 10^6 ml^{-1}, respectively) were significantly different from each other. Minimum cfu was recorded with talc formulations 80:20, 65:35 and 50:50 (1.50 x10^6 ml^{-1}, 1.40 x 10^6 ml^{-1} and 1.30 x 10^6 ml^{-1}, respectively).

At 30 DAS, chitin enriched GNO formulations at proportions, 65:35 and 60:40 were found to be the best carriers in terms of their cfu values (3.23 x 10^6 ml^{-1} and 3.20 x 10^6 ml^{-1}). This was followed by GNO + chitin 50:50, SFO + chitin 60:40 and SFO + chitin 65:35, their cfu values being 3.10 x 10^6 ml^{-1}, 3.00 x 10^6 ml^{-1} and 2.90 x 10^6 ml^{-1}, respectively. SFO + chitin 50:50 with a cfu of 2.63 x 10^6 ml^{-1} was statistically similar to GNO and SFO at all tested proportions (65:35, 60:40 and 50:50). The cfu recorded were 2.57x10^6 ml^{-1}, 2.50, 2.53 x 10^6 ml^{-1}, 2.46 x 10^6 ml^{-1}, 2.43 x 10^6 ml^{-1} and 2.43 x 10^6 ml^{-1}, respectively. The viability of spores stored in chitin and chitosan enriched talc formulations *viz.* talc + chitosan 80:20 (2.40 x 10^6 ml^{-1}), talc + chitin 65:35 (2.37 x 10^6 ml^{-1}). Talc + chitin 80:20 (2.30 x 10^6 ml^{-1}) and talc + chitosan 65:35 (2.26 x 10^6 ml^{-1}), did not differ significantly from each other and were on par with SFO + chitin 60:40 and 50:50. Talc formulations of chitin and chitosan 50:50 were found to retain viability of spores (1.90 x 10^6 ml^{-1} and 1.73 x 10^6 ml^{-1}) as that of spore suspension in distilled water with chitin (1.87 x 10^6 ml^{-1}) and were significantly different from the viability observed in control, *i.e.,* spore suspension in distilled water (1.50 x 10^6 ml^{-1}). Talc 65:35

(1.37 x 10^6 ml^{-1}) retained viability as that of control, but talc 80:20 and 50:50 was found to be inferior to the control.

A similar trend was observed at 45 DAS. The viability of spores in GNO + chitin 65:35 and GNO + chitin 60:40 were significantly higher (3.16 x 10^6 ml^{-1} and 2.90 x 10^6 ml^{-1}). This was followed by SFO + chitin 65:35 (2.70 x 10^6 ml^{-1}), GNO + chitin 50:50, SFO + chitin 60:40 and SFO + chitin 50:50 with 2.67 x 10^6 ml^{-1}, 2.63 x 10^6 ml^{-1} and 2.60 x 10^6 ml^{-1}, respectively. The chitin enriched SFO 50:50 and 60:40 did not differ significantly from GNO 50:50 (2.30 x 10^6 ml^{-1}), 65:35 (2.37 x 10^6 ml^{-1}), 60:40 (2.40 x 10^6 ml^{-1}) and SFO 65:35 (2.30 x 10^6 ml^{-1}). Enriched formulations of talc, *viz.* talc + chitin 65:35 and 80:20, talc + chitosan 65:35 and 80:20 (2.23 x 10^6 ml^{-1}, 2.20 x 10^6 ml^{-1}, 2.13 x 10^6 ml^{-1} and 2.20 x 10^6 ml^{-1}, respectively) were found to be on par with basic oil formulations of GNO and SFO. Talc + chitin and talc + chitosan at 50:50 were found to retain viability of spores (1.46 x 10^6 ml^{-1} and 1.72 x 10^6 ml^{-1}) as that of DW + chitin (1.57 x 10^6 ml^{-1}). Less viability was observed with talc 80:20, 65:35, 50:50 (1.29 x 10^6 ml^{-1}, 1.33 x 10^6 ml^{-1} and 1.10 x 10^6 ml^{-1}) which was on par with the control (1.23 x 10^6 ml^{-1}).

The cfu observed at 60 DAS also revealed that the viability of spores in the enriched carrier GNO + chitin 65:35 (2.59 x 10^6 ml^{-1}) was significantly higher than that in rest of the tested carriers. But at this point of time, GNO + chitin 65:35 was found to be on par with GNO + chitin 50:50, GNO 60 :40, SFO + chitin 65:35, SFO + chitin 60:40, GNO 60:40 and 65:35 (2.53 x 10^6 ml^{-1}, 2.53 x 10^6 ml^{-1}, 2.50 x 10^6 ml^{-1}, 2.27 x 10^6 ml^{-1}, 2.37 x 10^6 ml^{-1} and 2.46 x 10^6 ml^{-1}, respectively). The number of cfu observed with rest of the treatments, namely SFO 50:50, 60:40 and 65:35 (2.03 x 10^6 ml^{-1}, 1.97 x 10^6 ml^{-1} and 2.09 x 10^6 ml^{-1}) GNO 50:50 (2.13 x 10^6 ml^{-1}), talc + chitin 65:35 and 80:20 (1.83 x 10^6 ml^{-1} and 1.93 x 10^6 ml^{-1}) and talc + chitosan 65:35 and 80:20 (1.69 x 10^6 ml^{-1} and 1.83 x 10^6 ml^{-1}) as well as DW + chitin (1.40 x 10^6 ml^{-1}) were significantly higher than the talc + chitin and talc + chitosan

50:50 (1.06 x 10^6 ml^{-1} and 1.11 x 10^6 ml^{-1}), talc 65:35 (0.93 x 10^6 ml^{-1}), 80:20 (1.09 x 10^6 ml^{-1}) 50:50 (0.79 x 10^6 ml^{-1}) and control (0.99 x 10^6 ml^{-1}).

At 75 DAS also the number of cfu observed with GNO + chitin 65:35 and SFO + chitin 65:35 was superior (2.46 x 10^6 ml^{-1} and 2.36 x 10^6 cfus ml^{-1}). This was followed by GNO + chitin 60:40 (2.23 x 10^6 ml^{-1}), GNO + chitin 50:50 (2.20 x 10^6 ml^{-1}) and SFO + chitin 60:40 (2.13 x 10^6 ml^{-1}). Enriched formulation, SFO + chitin 50:50 was on par with basic formulations, GNO 50:50, 60:40, 65:35 and SFO 50:50, 60:40, 65 :35 with cfu 1.86 x 10^6 ml^{-1}, 1.66 x 10^6 ml^{-1}, 1.73 x 10^6 ml^{-1}, 1.73 x 10^6 ml^{-1}, 1.56 x 10^6 ml^{-1}, 1.63 x 10^6 ml^{-1} and 1.70 x 10^6 ml^{-1}, respectively. The viability of spores in DW + chitin (1.26 x 10^6 ml^{-1}) was similar to that observed in talc + chitin 65:35, 80:20, talc + chitosan 65:35, 80:20 (1.19 x 10^6 ml^{-1}, 1.30 x 10^6 ml^{-1}, 1.13 x 10^6 ml^{-1}, 1.34 x 10^6 ml^{-1} and 1.26 x 10^6 ml^{-1}, respectively). Viability of spores in talc + chitin 50:50, talc 65:35 and talc 50:50 (0.63 x 10^6 ml^{-1}, 0.50 x 10^6 ml^{-1} and 0.33 x 10^6 ml^{-1}, respectively) was significantly lower than that in control (0.82 x 10^6 ml^{-1}).

After 90 DAS, the enriched formulations, GNO + chitin 65:35, SFO + chitin 65:35, GNO + chitin 60:40 and GNO + chitin 50:50 were found to be superior, with a cfu of 2.27 x 10^6 ml^{-1}, 2.20 x 10^6 ml^{-1}, 2.16 x 10^6 ml^{-1} and 2.10 x 10^6 ml^{-1}, respectively. Enriched formulation, SFO + chitin 60:40 was on par with the basic formulation, GNO 65:35 (1.66 x 10^6 ml^{-1} each). This was followed by the enriched formulation, SFO + chitin 50:50 which was on par with the basic formulations *viz.* GNO 60:40, SFO 65:35, GNO 50:50 and SFO 50:50 with their cfu 1.60 x 10^6 ml^{-1}, 1.53 x 10^6 ml^{-1}, 1.49 x 10^6 ml^{-1}, 1.36 x 10^6 ml^{-1} and 1.33 x 10^6 ml^{-1}, respectively. Viability of spores in SFO 50:50 and 60:40 was found to be on par with DW + chitin (1.16 x 10^6 ml^{-1}). The cfu observed in talc + chitin and talc + chitosan 80:20 (0.90 x 10^6 ml^{-1} and 0.85 x 10^6 ml^{-1}) were significantly higher than that of control (0.57 x 10^6 cfu ml^{-1}). The viability of spores in talc + chitin and talc + chitosan 65:35

Table 14. Effect of carrier materials on the viability of *L. lecanii* formulations stored at room temperature

Sl no.	Carrier : Spore concentrate	*Mean number of cfu (x 10^6 spores ml^{-1})					
		15 DAS	**30 DAS**	**45 DAS**	**60 DAS**	**75 DAS**	**90 DAS**
1	SFO + SC (50 : 50)	2.67 (1.63)	2.43 (1.56)	2.23 (1.49)	2.03 (1.43)	1.56 (1.25)	1.33 (1.15)
2	SFO + SC (60 : 40)	2.77 (1.66)	2.43 (1.56)	2.23 (1.49)	1.97 (1.40)	1.63 (1.28)	1.29 (1.14)
3	SFO + SC (65 : 35)	2.90 (1.70)	2.46 (1.57)	2.30 (1.52)	2.09 (1.45)	1.70 (1.30)	1.49 (1.22)
4	(SFO + CC 0.1 %) + SC (50 : 50)	2.97 (1.72)	2.63 (1.62)	2.60 (1.61)	2.13 (1.46)	1.86 (1.37)	1.60 (1.26)
5	(SFO + CC 0.1 %) + SC (60 : 40)	3.13 (1.77)	2.90 (1.70)	2.63 (1.62)	2.27 (1.51)	2.13 (1.46)	1.66 (1.29)
6	(SFO + CC 0.1 %) + SC (65 : 35)	3.07 (1.75)	3.10 (1.76)	2.7 (1.64)	2.50 (1.58)	2.36 (1.54)	2.20 (1.48)
7	GNO + SC (50 : 50)	2.83 (1.68)	2.53 (1.59)	2.3 (1.52)	2.13 (1.46)	1.66 (1.29)	1.36 (1.17)
8	GNO + SC (60 : 40)	2.90 (1.70)	2.50 (1.58)	2.37 (1.54)	2.37 (1.54)	1.73 (1.32)	1.53 (1.24)
9	GNO + SC (65 : 35)	2.93 (1.71)	2.57 (1.60)	2.4 (1.55)	2.46 (1.57)	1.73 (1.32)	1.66 (1.29)
10	(GNO + CC 0.1 %) + SC (50 : 50)	3.13 (1.77)	3.00 (1.73)	2.67 (1.63)	2.53 (1.59)	2.20 (1.48)	2.16 (1.47)
11	(GNO + CC 0.1 %) + SC (60 : 40)	3.30 (1.82)	3.20 (1.79)	2.90 (1.70)	2.53 (1.59)	2.23 (1.49)	2.10 (1.45)
12	(GNO + CC 0.1 %) + SC (65 : 35)	3.40 (1.84)	3.23 (1.80)	3.16 (1.78)	2.59 (1.61)	2.46 (1.57)	2.27 (1.51)
13	Talc + SC (50 : 50)	1.30 (1.14)	1.30 (1.14)	1.13 (1.06)	0.79 (0.89)	0.33 (0.58)	0.06 (0.24)
14	Talc + SC (65 : 35)	1.40 (1.18)	1.37 (1.17)	1.10 (1.05)	0.93 (0.96)	0.50 (0.71)	0.06 (0.24)
15	Talc + SC (80 : 20)	1.50 (1.22)	1.30 (1.14)	1.30 (1.14)	1.09 (1.05)	0.90 (0.95)	0.33 (0.58)
16	(Talc + C 5 %) + SC (50 : 50)	2.13 (1.46)	1.73 (1.32)	1.46 (1.21)	1.06 (1.03)	0.63 (0.79)	0.06 (0.24)
17	(Talc + C 5 %) + SC (65 : 35)	2.57 (1.60)	2.37 (1.54)	2.23 (1.49)	1.83 (1.35)	1.19 (1.09)	0.66 (0.81)
18	(Talc + C 5 %) + SC (80 : 20)	2.53 (1.59)	2.30 (1.52)	2.20 (1.48)	1.93 (1.39)	1.30 (1.14)	0.85 (0.92)
19	(Talc + CS 0.5 %) + SC (50 : 50)	2.43 (1.56)	1.90 (1.38)	1.72 (1.31)	1.11 (1.05)	0.73 (0.85)	0.07 (0.26)
20	(Talc + CS 0.5 %) + SC (65 : 35)	2.50 (1.58)	2.26 (1.50)	2.13 (1.46)	1.69 (1.30)	1.13 (1.06)	0.63 (0.79)
21	(Talc + CS 0.5 %) + SC (80 : 20)	2.63 (1.62)	2.40 (1.55)	2.20 (1.48)	1.83 (1.35)	1.33 (1.15)	0.90 (0.95)
22	SC+ CC	1.93 (1.39)	1.87 (1.37)	1.57 (1.25)	1.40 (1.18)	1.26 (1.12)	1.16 (1.08)
23	SC	1.73 (1.32)	1.50 (1.22)	1.23 (1.11)	0.99 (1.00)	0.82 (0.91)	0.63 (0.79)
	CD (0.05)	**0.064**	**0.072**	**0.107**	**0.134**	**0.128**	**0.378**

*Mean of three replications, DAS: Days after storage; SFO: Sunflower oil; GNO: Groundnut oil; SC: Spore Concentrate; CC: Colloidal Chitin; C: Chitin; CS: Chitosan, Figures in parentheses are √x transformed values

were on par with that of control while talc 50:50 with chitin and chitosan as well as talc alone formulations were inferior to the control (0.33 to 0.06 x 10^6 ml^{-1}).

4.3.1.1.2 Refrigeration

The viability of spores suspended in various carriers was found to improve when stored under refrigeration (Table 15).

At 15 DAS, enriched formulation, GNO + chitin 65:35 recorded maximum number of cfu, 4.19 x 10^6 ml^{-1}. This was followed by GNO + chitin 60:40, SFO + chitin 65:35, GNO + chitin 50:50, SFO + chitin 60:40 and SFO + chitin 50:50, their cfu being 3.94 x 10^6 ml^{-1}, 3.83 x 10^6 ml^{-1}, 3.73 x 10^6 ml^{-1}, 3.73 x 10^6 ml^{-1} and 3.60 x 10^6 ml^{-1}, respectively. The viability observed in basic oil formulations, GNO (50:50, 60:40, 65:35) and SFO (50:50, 60:40, 65:35) was on par with all the enriched formulations of talc (50:50, 65:35, 80:20) with cfu ranging from 3.00 x 10^6 ml^{-1} to 3.53 x 10^6 ml^{-1}. All the basic formulations with talc (50:50, 65:35, 80:20) was inferior in terms of cfu (2.33 to 2.57 x 10^6 ml^{-1}) when compared to spore suspension in DW + chitin (3.23 x 10^6 ml^{-1}) as well as spore suspension in distilled water alone (2.97 x 10^6 ml^{-1}).

Viability recorded at the end of one month after storage (30 DAS) also showed a similar trend with GNO + chitin 65:35 (3.73 x 10^6 ml^{-1}) being the superior treatment. This was followed by the enriched formulations, GNO + chitin 60:40, SFO + chitin 65:35, GNO + chitin 50:50, SFO + chitin 60:40, SFO + chitin 50:50 and basic formulation, GNO 65:35. The cfu recorded were 3.56 x 10^6 ml^{-1}, 3.53 x 10^6 ml^{-1}, 3.53 x 10^6 ml^{-1}, 3.43 x 10^6 ml^{-1}, 3.27 x 10^6 ml^{-1} and 3.27 x 10^6 ml^{-1}, respectively. All the basic oil formulations except GNO 65:35 were on par enriched talc formulations *viz.* talc + chitin (65:35 and 80:20), talc + chitosan 65:35 and DW + chitin, the cfu values ranging from 3.23 to 2.66 x 10^6 ml^{-1}. Least number of viable colonies was recorded in talc 50:50 (2.06 x 10^6 ml^{-1}).

At 45 DAS, maximum viability was observed with the enriched formulations, GNO + chitin (60:40, 65:35, 50:50) and SFO + chitin 65:35. The cfu recorded was 3.57 x 10^6 ml^{-1}, 3.50 x 10^6 ml^{-1}, 3.47 x 10^6 ml^{-1} and 3.30 x 10^6 ml^{-1}, respectively. This was followed by the enriched formulations, SFO + chitin 60:40 (3.10 x 10^6 ml^{-1}) and SFO + chitin 50:50 (2.97 x 10^6 ml^{-1}) which was on par with the basic formulation, GNO 65:35 (3.00 x 10^6 ml^{-1}). Viability in basic oil formulations *viz.* GNO (60:40, 50:50), SFO (65:35, 60:40, SFO 50:50) were found to be on par with enriched formulations of talc *viz.* talc + chitin 80:20 and talc + chitosan (65:35, 80:20) and spore suspension in DW + chitin. The cfu recorded were 2.90 x 10^6 ml^{-1}, 2.87 x 10^6 ml^{-1}, 2.90 x 10^6 ml^{-1}, 2.80 x 10^6 ml^{-1}, 2.83 x 10^6 ml^{-1}, 2.80 x 10^6 ml^{-1}, 2.80 x 10^6 ml^{-1} and 2.73 x 10^6 ml^{-1}, respectively. The viability of talc formulations (talc + chitosan 50:50, talc 80:20 and talc 65:35) did not differ significantly from control (2.00 x 10^6 ml^{-1}, 2.16 x 10^6 ml^{-1}, 2.23 x 10^6 ml^{-1} and 2.43 x 10^6 ml^{-1}, respectively) and but superior to talc (50:50) (2.06 x 10^6 ml^{-1}).

A similar trend was observed by the end of second month of storage. Enriched formulations, *viz.* GNO + chitin (65:35, 60:40 and 50:50) and SFO + chitin 65:35 were found to be superior and the cfu recorded were 3.46 x 10^6 ml^{-1}, 3.43 x 10^6 ml^{-1}, 3.33 x 10^6 ml^{-1} and 3.23 x 10^6 ml^{-1}, respectively. The enriched formulations, SFO + chitin (50:50, 60:40) ranked were on par with the basic formulations, GNO (50:50, 60:40, 65:35) and SFO (65: 35, 60:40), the cfu being 2.87 x 10^6 ml^{-1}, 2.97 x 10^6 ml^{-1}, 2.97 x 10^6 ml^{-1}, 2.97 x 10^6 ml^{-1}, 2.70 x 10^6 ml^{-1}, 2.67 x 10^6 ml^{-1}, and 2.73 x 10^6 ml^{-1}, respectively. The basic oil formulation, SFO 50:50 did not differ significantly from the enriched talc formulations, *viz.* talc + chitin (65:35, 80:20) and talc + chitosan (65:35, 80:20) and spore suspension in DW + chitin (2.60 x 10^6 ml^{-1}, 2.40 x 10^6 ml^{-1}, 2.50 x 10^6 ml^{-1}, 2.46 x 10^6 ml^{-1}, 2.37 x 10^6 ml^{-1} and 2.57 x 10^6 ml^{-1}, respectively). The viability observed with talc (65: 65) (1.83 x 10^6 ml^{-1}) were on par with the control (2.03 x 10^6 ml^{-1}). Lowest cfu was observed with talc + chitosan (50:50), talc + chitin

(50:50) and talc 50:50 (1.50 x 10^6 ml^{-1}, 1.33 x 10^6 ml^{-1} and 1.16 x 10^6 ml^{-1}, respectively).

At 75 DAS, the enriched oil formulation, GNO + chitin 65:45 was found to be superior with cfu 3.33 x 10^6 ml^{-1}. This was followed by GNO + chitin (60:40; 50:50), SFO + chitin (65:35, ;60:40) and GNO 65:35, their cfu being 3.27 x 10^6 ml^{-1}, 3.23 x 10^6 ml^{-1}, 3.00 x 10^6 ml^{-1}, 2.82 x 10^6 ml^{-1} and 2.80 x 10^6 ml^{-1}, respectively. The viability recorded with enriched formulation, SFO + chitin 50:50 (2.67 x 10^6 ml^{-1}) was on par with the basic oil formulations, GNO (50:50, 60:40), SFO (50:50, 60:40, 65:35), enriched talc formulations, talc + chitosan (65:35, 80 :20), talc + chitin 80:20 (3.00 - 2.33 x 10^6 ml^{-1}) and spore suspension in DW + chitin (2.80 x 10^6 ml^{-1}, 2.73 x 10^6 ml^{-1}, 2.37 x 10^6 ml^{-1}, 2.43 x 10^6 ml^{-1}, 2.53 x 10^6 ml^{-1}, 2.25 x 10^6 ml^{-1}, 2.25 x 10^6 ml^{-1}, 2.23 x 10^6 ml^{-1} and 2.33 x 10^6 ml^{-1}, respectively). The cfu observed with talc 65:35 and 80:20 (1.66 x 10^6 ml^{-1} and 1.53 x 10^6 ml^{-1}) was statistically similar to that of control (1.72 x 10^6 ml^{-1}). Lowest cfu was recorded in talc + chitin 50:50, talc + chitosan 50:50 and talc 50:50 (0.86 x 10^6 ml^{-1}, 0.83 x 10^6 ml^{-1} and 0.67 x 10^6 ml^{-1}, respectively).

By the end of the experimental period (90 DAS), enriched formulation, GNO + chitin 65:35 was found to be superior with 3.13 x 10^6 cfu ml^{-1}. This was followed by GNO + chitin (60:40 and 50:50) and SFO + chitin 65:35 (2.92 x 10^6 ml^{-1}, 2.83 x 10^6 ml^{-1} and 2.80 x 10^6 ml^{-1}, respectively). The viability observed with enriched formulations, GNO + chitin 50:50 and SFO + chitin 65:35 did not differ significantly from those obtained from SFO + chitin 50:50 (2.50 x 10^6 ml^{-1}), SFO + chitin 60:40 (2.63 x 10^6 ml^{-1}) and the basic formulations, GNO 50:50, 60:40 and 65:35 (2.49 x 10^6 ml^{-1}, 2.53 x 10^6 ml^{-1} and 2.53 x 10^6 ml^{-1}, respectively). The enriched talc formulations, namely talc + chitin and talc + chitosan (65:35 and 80:20) retained viability as that of all the tested proportions of basic oil formulation of SFO (50:50, 60:40 and 65:35). Their cfu values being 2.03 x 10^6 ml^{-1}, 2.06 x 10^6 ml^{-1}, 1.92 x 10^6 ml^{-1}, 1.99 x 10^6 ml^{-1}, 2.13 x 10^6 ml^{-1}, 2.10 x 10^6 ml^{-1} and 2.20 x 10^6 ml^{-1},

Table 15. Effect of carrier materials on the viability of *L. lecanii* formulations stored under refrigeration

Sl no.	Carrier : Spore concentrate	*Mean number of cfu (x 10^6 spores ml^{-1})					
		15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
1	SFO + SC (50 : 50)	3.23 (1.80)	3.13 (1.77)	2.83 (1.68)	2.60 (1.61)	2.37 (1.54)	2.13 (1.46)
2	SFO + SC (60 : 40)	3.33 (1.83)	3.16 (1.78)	2.80 (1.67)	2.67 (1.63)	2.43 (1.56)	2.10 (1.45)
3	SFO + SC (65 : 35)	3.43 (1.85)	3.16 (1.78)	2.90 (1.70)	2.73 (1.65)	2.53 (1.59)	2.20 (1.48)
4	(SFO + CC 0.1 %) + SC (50 : 50)	3.60 (1.90)	3.27 (1.81)	2.97 (1.72)	2.87 (1.69)	2.67 (1.63)	2.50 (1.58)
5	(SFO + CC 0.1 %) + SC (60 : 40)	3.73 (1.93)	3.43 (1.85)	3.10 (1.76)	2.97 (1.72)	2.82 (1.68)	2.63 (1.62)
6	(SFO + CC 0.1 %) + SC (65 : 35)	3.83 (1.96)	3.53 (1.88)	3.30 (1.82)	3.23 (1.80)	3.00 (1.73)	2.80 (1.67)
7	GNO + SC (50 : 50)	3.27 (1.81)	3.07 (1.75)	2.87 (1.69)	2.97 (1.72)	2.70 (1.64)	2.49 (1.58)
8	GNO + SC (60 : 40)	3.53 (1.88)	3.23 (1.80)	2.90 (1.70)	2.97 (1.72)	2.80 (1.67)	2.53 (1.59)
9	GNO + SC (65 : 35)	3.50 (1.87)	3.27 (1.81)	3.00 (1.73)	2.70 (1.64)	2.73 (1.65)	2.53 (1.59)
10	(GNO + CC 0.1 %) + SC (50 : 50)	3.73 (1.93)	3.53 (1.88)	3.47 (1.86)	3.33 (1.83)	3.23 (1.80)	2.83 (1.68)
11	(GNO + CC 0.1 %) + SC (60 : 40)	3.94 (1.99)	3.56 (1.89)	3.57 (1.89)	3.43 (1.85)	3.27 (1.81)	2.93 (1.71)
12	(GNO + CC 0.1 %) + SC (65 : 35)	4.19 (2.05)	3.73 (1.93)	3.50 (1.87)	3.46 (1.86)	3.33 (1.83)	3.13 (1.77)
13	Talc + SC (50 : 50)	2.33 (1.53)	2.06 (1.44)	1.69 (1.30)	1.16 (1.08)	0.67 (0.82)	0.16 (0.39)
14	Talc + SC (65 : 35)	2.57 (1.60)	2.50 (1.58)	2.23 (1.49)	1.83 (1.35)	1.66 (1.29)	0.18 (0.43)
15	Talc + SC (80 : 20)	2.46 (1.57)	2.40 (1.55)	2.16 (1.47)	1.63 (1.28)	1.53 (1.24)	1.30 (1.14)
16	(Talc + C 5 %) + SC (50 : 50)	3.13 (1.77)	2.49 (1.58)	1.97 (1.40)	1.33 (1.15)	0.86 (0.93)	0.23 (0.48)
17	(Talc + C 5 %) + SC (65 : 35)	3.10 (1.76)	3.07 (1.75)	2.63 (1.62)	2.40 (1.55)	2.37 (1.54)	2.03 (1.43)
18	(Talc + C 5 %) + SC (80 : 20)	3.23 (1.80)	3.07 (1.75)	2.80 (1.67)	2.50 (1.58)	2.23 (1.49)	2.06 (1.44)
19	(Talc + CS 0.5 %) + SC (50 : 50)	3.00 (1.73)	2.56 (1.60)	2.00 (1.41)	1.50 (1.22)	0.82 (0.91)	0.24 (0.49)
20	(Talc + CS 0.5 %) + SC (65 : 35)	3.20 (1.79)	3.03 (1.74)	2.80 (1.67)	2.46 (1.57)	2.25 (1.50)	1.92 (1.39)
21	(Talc + CS 0.5 %) + SC (80 : 20)	3.16 (1.78)	2.83 (1.68)	2.70 (1.64)	2.37 (1.54)	2.25 (1.50)	1.99 (1.41)
22	SC+ CC	3.23 (1.80)	3.03 (1.74)	2.73 (1.65)	2.57 (1.60)	2.33 (1.53)	2.02 (1.42)
23	SC	2.97 (1.72)	2.66 (1.63)	2.43 (1.56)	2.03 (1.43)	1.72 (1.31)	1.08 (1.04)
	CD (0.05)	**0.091**	**0.082**	**0.089**	**0.093**	**0.160**	**0.128**

*Mean of three replications, DAS: Days after storage; SFO: Sunflower oil; GNO: Groundnut oil; SC: Spore Concentrate; CC: Colloidal Chitin; C: Chitin; CS: Chitosan, Figures in parentheses are √x transformed values

respectively. Talc 80:20 (1.30 x 10^6 ml^{-1}) was on par with control (1.08 x 10^6 ml^{-1}). Least cfu was observed with talc + chitin 50:50, talc + chitosan 50:50, talc 65:35 and talc 50:50 (0.24 x 10^6 ml^{-1} to 0.16 x 10^6 ml^{-1}).

Enriched formulations using GNO + chitin as carrier in proportions 65:35, 60:40 and 50:50 and SFO + chitin 65:35 were the best carrier materials that could improve the viability. Enriched as well as basic oil formulations were found to be better than enriched talc formulations (65:35 and 80:20). Basic talc formulations (80:20) was equally good as spore suspension in distilled water + chitin.

Refrigeration was found to improve viability of all the formulations when compared to storage under room temperature.

4.3.2. Spore Count

The effect of carrier materials on the spore count of *L. lecanii* formulations are furnished in Table 16 and 17.

4.3.2.1. Room temperature

The spore count of *L. lecanii* formulations stored under room temperature using different carriers showed considerable variations under storage (Table 16).

At 15 DAS, chitin enriched formulations of GNO (60:40, 50:50 and 65 : 65) were found to be superior with spore count being 5.33 x 10^8 spores ml^{-1}, 5.23 x 10^8 spores ml^{-1} and 5.21 x 10^8 spores ml^{-1}, respectively. The enriched formulations of SFO (60:40 and 65:35) were ranked second (4.56 x 10^8 spores ml^{-1} and 4.53 x 10^8 spores ml^{-1}). The basic formulation, GNO 65:35 with 4.19 x 10^8 spores ml^{-1} did not differ significantly from the enriched formulation, SFO + chitin 50:50 (4.14 x 10^8 spores ml^{-1}). This was followed by rest of the basic formulations, *viz.* GNO (60:40 and 50:50) and SFO (65:35, 60:40 and 50:50). The spore count recorded were 3.94 x 10^8 spores ml^{-1}, 3.79 x 10^8 spores ml^{-1}, 3.77 x 10^8 spores ml^{-1}, 3.63 x 10^8 spores ml^{-1} and 3.36 x 10^8 spores ml^{-1}. SFO 50:50

with 3.40 x 10^8 spores ml^{-1} was inferior to all other basic and enriched oil formulations. The enriched talc formulations, talc + chitin (65:35, 80:20) and talc + chitosan (65:35, 80:20), with 3.12 x 10^8 spores ml^{-1}, 3.08 x 10^8 spores ml^{-1}, 2.89 x 10^8 spores ml^{-1} and 2.86 x 10^8 spores ml^{-1} were significantly superior to DW + chitin (2.44 x 10^8 spores ml^{-1}). Talc + chitosan 50:50 (2.27 x 10^8 spores ml^{-1}) and talc alone 80:20, 65:35 (2.28 x 10^8 spores ml^{-1} and 2.25 x 10^8 spores ml^{-1}) recorded spore count similar to the control (2.31 x 10^8 spores ml^{-1}). Least spore count were noted with talc + chitin 50:50 and talc 50:50 formulations (2.07 x 10^8 spores ml^{-1} and 1.71 x 10^8 spores ml^{-1}).

At 30 DAS, also chitin enriched GNO formulations at all the tested proportions were found to be superior. The spore count observed were 4.67 x 10^8 spores ml^{-1} (50:50), 4.65 x 10^8 spores ml^{-1} (65:35) and 4.56 x 10^8 spores ml^{-1} (60:40). This was followed by chitin enriched formulations of SFO, their spore count being 4.19 x 10^8 spores ml^{-1}(65:35), 4.13 x 10^8 spores ml^{-1} (60:40) and 3.85 x 10^8 spores ml^{-1} (50:50). The basic formulations of GNO 65:35 (3.56 x 10^8 spores ml^{-1}) was ranked next, followed GNO 60:40 (3.36 x 10^8 spores ml^{-1}) and 50:50 (3.01 x 10^8 spores ml^{-1}). Enriched talc formulations, talc + chitosan and talc + chitin (80:20) recorded spore count on par with the basic formulations of SFO (50:50, 60:40 and 65:35) and spore suspension in DW + chitin. The values of spore count were 2.55 x 10^8 spores ml^{-1}, 2.55 x10^8 spores ml^{-1}, 2.61 x 10^8 spores ml^{-1}, 2.57 x 10^8 spores ml^{-1}, 2.66 x 10^8 spores ml^{-1} and 2.35 x 10^8 spores ml^{-1}, respectively. The spore count in talc 80:20, 65:35 (1.91 x 10^8 spores ml^{-1} and 1.76 x 10^8 spores ml^{-1}) and talc + chitin 50:50 (1.76 x 10^8 spores ml^{-1}) did not differ significantly from control (1.82 x 10^8 spores ml^{-1}). Spore count in talc + chitosan 50:50 and talc 50:50 were the lowest, 1.50 x 10^8 spores ml^{-1} and 1.11 x 10^8 spores ml^{-1}. At 45 DAS, enriched formulation, GNO + chitin 65:35 recorded the maximum spore count of 3.94 x 10^8 spores ml^{-1}, followed by rest of the chitin

enriched formulations of GNO (50:50 and 60:40) and SFO (65:35). The spore count recorded were 3.70 x 10^8 spores ml^{-1}, 3.52 x 10^8 spores ml^{-1} and 3.78 x 10^8 spores ml^{-1}, respectively. The enriched formulation, SFO + chitin at 60:40 with 3.26 x 10^8 spores ml^{-1} as ranked third. This was followed by the chitin enriched SFO (50:50), and basic formulations of GNO (60:40 and 65:35). The spore count observed were 2.87 x 10^8 spores ml^{-1}, 2.84 x 10^8 spores ml^{-1} and 2.76 x 10^8 spores ml^{-1}, respectively. The basic formulations, SFO 65:35 (2.29 x 10^8 spores ml^{-1}) was found to be on par with SFO 60:40 (2.24 x 10^8 spores ml^{-1}) and GNO 50:50 (2.12 x 10^8 spores ml^{-1}). The basic oil formulation, SFO 50:50 did not differ significantly from the enriched talc formulations *viz*. talc + chitosan (65:35 and 80:20) and talc + chitin 80:20 and spore suspension in DW + chitin, their spore count being 2.05 x 10^8 spores ml^{-1}, 1.98 x 10^8 spores ml^{-1}, 1.82 x 10^8 spores ml^{-1}, 1.81 x 10^8 spores ml^{-1} and 1.95 x 10^8 spores ml^{-1}, respectively. The enriched talc formulation, talc + chitin 65:35 (1.53 x 10^8 spores ml^{-1}) was superior to talc + chitosan 50:50, talc + chitin 50:50, talc 80:20 and talc 65:35. The spore count recorded in these formulations was on par with that of control (1.27 x 10^8 spores ml^{-1}, 1.19 x 10^8 spores ml^{-1}, 1.18 x 10^8 spores ml^{-1}, 1.14 x 10^8 spores ml^{-1} and 1.29 x 10^8 spores ml^{-1}, respectively). Lowest spore count was observed with talc 50:50, 1.04 x 10^8 spores ml^{-1}.

After two months of storage (60 DAS), the enriched formulations, GNO + chitin (65:35, 60:40, and 50:50) and SFO + chitin (65:35, 60:40) were superior, their spore count being 2.82 x 10^8 spores ml^{-1}, 2.68 x 10^8 spores ml^{-1}, 2.74 x 10^8 spores ml^{-1}, 2.82 x 10^8 spores ml^{-1} and 2.58 x 10^8 spores ml^{-1}, respectively. The spore count (2.82 x 10^8 spores ml^{-1}) recorded with the chitin enriched formulation of SFO (50:50) was statistically similar to the basic formulation, GNO 65:35 (2.23 x 10^8 spores ml^{-1}). The basic formulations, GNO 60:40 and 50:50 with spore count, 1.94 x 10^8 spores ml^{-1} and 1.79 x 10^8 spores ml^{-1}, did not differ significantly from each other. The spore count observed with the rest of the basic oil

formulations, SFO 65:35, 60:40 and 50 : 40 (1.60 x 10^8 spores ml^{-1}, 1.59 x 10^8 spores ml^{-1} and1.51 x 10^8 spores ml^{-1}, respectively) did not differ significantly from enriched talc formulations, talc + chitosan 80:20 (1.41 x 10^8 spores ml^{-1}) and talc + chitin 80:20 (1.36 x 10^8 spores ml^{-1}) and spore suspension in DW + chitin (1.57 x 10^8 spores ml^{-1}). The enriched talc formulations, talc + chitosan 65:35 (1.21 x 10^8 spores ml^{-1}) and talc + chitin 65:35 (1.15 x 10^8 spores ml^{-1}) were on par with control (1.08 x 10^8 spores ml^{-1}). A significantly lower spore count was obtained with other enriched talc formulations, talc + chitosan and talc + chitin (50:50) and basic talc formulations talc (65:35 and 80:20). Spore count recorded was 0.84 x 10^8 spores ml^{-1}, 0.73 x 10^8 spores ml^{-1}, 0.72 x 10^8 spores ml^{-1} and 0.82 x 10^8 spores ml^{-1}, respectively. The least spore count was observed with talc 50:50 (0.54 x 10^8 spores ml^{-1}).

A more or less similar trend was noted after 75 DAS also. Chitin enriched GNO (50:50, 60:40 and 65:35) and SFO (50:50, 60:40 and 65:35) were the superior formulations with spore count, 2.19 x 10^8 spores ml^{-1}, 2.29 x 10^8 spores ml^{-1}, 2.32 x 10^8 spores ml^{-1}, 2.03 x 10^8 spores ml^{-1}, 2.11 x 10^8 spores ml^{-1} and 2.24 x 10^8 spores ml^{-1}, respectively. The basic formulations of GNO and SFO with spore count 1.45 x 10^8 spores ml^{-1} (65:35), 1.41 x 10^8 spores ml^{-1} (60:40), 1.36 x 10^8 spores ml^{-1} (50:50) and 1.22 x 10^8 spores ml^{-1} (65:35) were ranked second. Rest of the basic SFO formulations *viz*., SFO 60:40 (1.06 x 10^8 spores ml^{-1}) and SFO 50:50 (1.05 x 10^8 spores ml^{-1}) was on par with enriched talc formulation, talc + chitin 80:20 (0.90 x 10^8 spores ml^{-1}) and spore suspension in DW + chitin (1.00 x 10^8 spores ml^{-1}). The spore count observed with enriched talc formulations, talc + chitosan (80:20, 65:35), talc + chitin 65:35 did not differ significantly from control, their spore count being 0.83 x 10^8 spores ml^{-1}, 0.79 x 10^8 spores ml^{-1}, 0.74 x 10^8 spores ml^{-1} and 0.66 x 10^8 spores ml^{-1}, respectively. Talc + chitosan 50:50 (0.52 x 10^8 spores ml^{-1}) and talc + chitin 50:50 (0.44 x 10^8 spores ml^{-1}) were statistically similar. The basic talc formulations, talc (80:20, 65:35 and 50:50) recorded the lowest spore count

Table 16. Effect of carrier materials on the spore count of *L. lecanii* formulations stored at room temperature

Sl no.	Carrier : Spore Concentrate	*Mean spore count (x 10^8 spores ml^{-1})					
		15 DAS	**30 DAS**	**45 DAS**	**60 DAS**	**75 DAS**	**90 DAS**
1	SFO + SC (50 : 50)	3.36(1.83)	2.61(1.62)	2.05(1.43)	1.51(1.23)	1.05(1.03)	0.76(0.87)
2	SFO + SC (60 : 40)	3.63(1.91)	2.57(1.60)	2.24(1.50)	1.59(1.26)	1.06(1.03)	0.76(0.87)
3	SFO + SC (65 : 35)	3.79(1.95)	2.66(1.63)	2.29(1.51)	1.60(1.27)	1.22(1.10)	0.83(0.91)
4	(SFO + CC 0.1 %) + SC (50 : 50)	4.15(2.04)	3.84(1.96)	2.87(1.69)	2.35(1.53)	2.03(1.42)	1.38(1.18)
5	(SFO + CC 0.1 %) + SC (60 : 40)	4.56(2.14)	4.13(2.03)	3.26(1.81)	2.58(1.61)	2.11(1.45)	1.51(1.23)
6	(SFO + CC 0.1 %) + SC (65 : 35)	4.53(2.13)	4.19(2.05)	3.78(1.95)	2.82(1.68)	2.24(1.50)	1.65(1.29)
7	GNO + SC (50 : 50)	3.77(1.94)	3.01(1.74)	2.12(1.46)	1.79(1.34)	1.36(1.17)	0.72(0.85)
8	GNO + SC (60 : 40)	3.94(1.99)	3.36(1.83)	2.84(1.68)	1.94(1.39)	1.41(1.19)	0.72(0.85)
9	GNO + SC (65 : 35)	4.19(2.05)	3.56(1.89)	2.76(1.66)	2.23(1.49)	1.45(1.20)	0.86(0.93)
10	(GNO + CC 0.1 %) + SC (50 : 50)	5.23(2.29)	4.67(2.16)	3.52(1.88)	2.74(1.66)	2.19(1.48)	1.54(1.24)
11	(GNO + CC 0.1 %) + SC (60 : 40)	5.33(2.31)	4.56(2.14)	3.70(1.92)	2.68(1.64)	2.29(1.51)	1.60(1.27)
12	(GNO + CC 0.1 %) + SC (65 : 35)	5.21(2.28)	4.65(2.16)	3.94(1.98)	2.82(1.68)	2.32(1.52)	1.67(1.29)
13	Talc + SC (50 : 50)	1.71(1.31)	1.11(1.05)	1.04(1.02)	0.54(0.74)	0.23(0.48)	0.02(0.13)
14	Talc + SC (65 : 35)	2.25(1.50)	1.67(1.29)	1.14(1.07)	0.72(0.85)	0.28(0.53)	0.05(0.22)
15	Talc + SC (80 : 20)	2.28(1.51)	1.91(1.38)	1.18(1.09)	0.82(0.91)	0.31(0.55)	0.06(0.24)
16	(Talc + C 5 %) + SC (50 : 50)	2.07(1.44)	1.76(1.33)	1.19(1.09)	0.73(0.85)	0.43(0.65)	0.07(0.27)
17	(Talc + C 5 %) + SC (65 : 35)	3.12(1.77)	2.02(1.42)	1.53(1.24)	1.15(1.07)	0.74(0.86)	0.39(0.63)
18	(Talc + C 5 %) + SC (80 : 20)	3.08(1.75)	2.54(1.59)	1.81(1.34)	1.36(1.17)	0.90(0.95)	0.60(0.77)
19	(Talc + CS 0.5 %) + SC (50 : 50)	2.27(1.51)	1.51(1.23)	1.27(1.13)	0.84(0.92)	0.52(0.72)	0.08(0.29)
20	(Talc + CS 0.5 %) + SC (65 : 35)	2.89(1.70)	2.31(1.52)	1.98(1.41)	1.21(1.10)	0.79(0.89)	0.56(0.75)
21	(Talc + CS 0.5 %) + SC (80 : 20)	2.86(1.69)	2.55(1.60)	1.82(1.35)	1.41(1.19)	0.83(0.91)	0.53(0.73)
22	SC+ CC	2.44(1.56)	2.35(1.53)	1.95(1.40)	1.57(1.25)	1.00(1.00)	0.69(0.83)
23	SC	2.32(1.52)	1.82(1.35)	1.29(1.14)	1.08(1.04)	0.66(0.81)	0.33(0.57)
	CD (0.05)	**0.057**	**0.119**	**0.090**	**0.100**	**0.115**	**0.124**

*Mean of three replications, DAS: Days after storage; SFO: Sunflower oil; GNO: Groundnut oil; SC: Spore Concentrate; CC: Colloidal Chitin; C: Chitin; CS: Chitosan, Figures in parentheses are √x transformed values

(0.31 x 10^8 spores ml^{-1}, 0.28 x 10^8 spores ml^{-1} and 0.23 x 10^8 spores ml^{-1}, respectively).

At the end of experimental period (90 DAS) too enriched oil formulations, GNO + chitin (65:35, 60:40 and 50:50) and SFO + chitin (65:35, 60:40 and 50:50) were found superior. The spore count recorded were 1.67 x 10^8 spores ml^{-1}, 1.60 x 10^8 spores ml^{-1}, 1.54 x 10^8 spores ml^{-1}, 1.65 x 10^8 spores ml^{-1}, 1.51 x 10^8 spores ml^{-1} and 1.38 x 10^8 spores ml^{-1}, respectively. The basic formulations, GNO 65:35 and SFO 65:35 were ranked second (0.86 x 10^8 spores ml^{-1} and 0.83 x 10^8 spores ml^{-1}). Chitin enriched talc formulation (80:20) recorded a spore count which was on par with the basic oil formulations, GNO (60:40, 50:50), SFO (50:50, 60:40 and 65:35) and spore suspension in DW + chitin (0.60 x 10^8 spores ml^{-1}, 0.72 x 10^8 spores ml^{-1}, 0.72 x 10^8 spores ml^{-1}, 0.76 x 10^8 spores ml^{-1}, 0.76 x 10^8 spores ml^{-1}, 0.83 x 10^8 spores ml^{-1} and 0.69 x 10^8 spores ml^{-1}, respectively). Enriched talc formulations, talc + chitosan 65:35 (0.56 x 10^8 spores ml^{-1}), talc + chitosan 80:20 (0.53 x 10^8 spores ml^{-1}) and talc + chitin 65:35 (0.39 x 10^8 spores ml^{-1}) did not differ significantly from each other. The spore counts noticed with enriched talc formulations, talc + chitosan and talc + chitin (50:50) and basic talc formulations, talc (80:20, 65:35 and 50:50) were lower to the control, the spore count being 0.08 x 10^8 spores ml^{-1}, 0.07 x 10^8 spores ml^{-1}, 0.06 x 10^8 spores ml^{-1}, 0.05 x 10^8 spores ml^{-1}, 0.02 x 10^8 spores ml^{-1} and 0.33 x 10^8 spores ml^{-1}, respectively.

4.3.2.2 Refrigeration

The spore count of various formulations stored under refrigeration was found to be high when compared to that under room temperature (Table 17).

At 15 DAS, chitin enriched formulations of GNO (65:35, 60:40, 50:50) and SFO (65:35, 50:50, 60:40) were found to be superior, spore count being 7.13 x 10^8 spores ml^{-1}, 6.95 x 10^8 spores ml^{-1}, 6.81 x 10^8 spores ml^{-1}, 7.02 x 10^8 spores ml^{-1}, 6.98 x 10^8 spores ml^{-1} and 6.92 x 10^8 spores ml^{-1}, respectively.

The spore count observed with basic oil formulations, GNO 65:35 (5.61 x 10^8 spores ml^{-1}), GNO 60:40 (5.21 x 10^8 spores ml^{-1}), GNO 50:50 (5.19 x 10^8 spores ml^{-1}), SFO 65:35 (5.27 x 10^8 spores ml^{-1}), SFO 60:40 (5.18 x 10^8 spores ml^{-1}), SFO 50:50 (5.14 x 10^8 spores ml^{-1}) were on par with chitin and chitosan enriched talc formulations, talc + chitosan 65:35 (5.09 x 10^8 spores ml^{-1}), talc + chitin 80:20 (5.05 x 10^8 spores ml^{-1}), talc + chitin 65:35 (5.03 x 10^8 spores ml^{-1}) and talc + chitosan 80:20 (5.00 x 10^8 spores ml^{-1}). This was followed by DW + chitin, talc 80:20 and talc 65:35, spore count being 4.36 x 10^8 spores ml^{-1}, 4.17 x 10^8 spores ml^{-1} and 3.86 x 10^8 spores ml^{-1}, respectively. The spore count obtained in talc 80:20 did not differ significantly from that of control (3.50 x 10^8 spores ml^{-1}). The lowest spore count was observed in talc 50:50 (2.86 x 10^8 spores ml^{-1}), talc + chitosan 50:50 (2.63 x 10^8 spores ml^{-1}) and talc + chitin 50:50 (2.47 x 10^8 spores ml^{-1}).

At 30 DAS also, maximum spore count was recorded with chitin enriched formulations of GNO and SFO. The spore count recorded were 7.00 x 10^8 spores ml^{-1} (GNO + chitin 65:35), 6.94 x 10^8 spores ml^{-1} (GNO + chitin 50:50), 6.74 x 10^8 spores ml^{-1} (GNO + chitin 60:40), 6.89 x 10^8 spores ml^{-1} (SFO + chitin 65:35), 6.86 x 10^8 spores ml^{-1} (SFO + chitin 60:40) and 6.79 x 10^8 spores ml^{-1} (SFO + chitin 50:50). This was followed by basic formulations, SFO 65:35 (5.55 x 10^8 spores ml^{-1}), 60:40 (5.21 x 10^8 spores ml^{-1}) and 50:50 (5.04 x 10^8 spores ml^{-1}). Spore count recorded with basic formulation of GNO 65:35 (4.88 x 10^8 spores ml^{-1}), 60:40 (4.84 x 10^8 spores ml^{-1}) and 50:50 (4.83 x 10^8 spores ml^{-1}) showed no significant difference from chitin and chitosan enriched talc formulations *viz*. talc + chitin 65:35 (4.76 x 10^8 spores ml^{-1}), talc + chitin 80:20 (4.67 x 10^8 spores ml^{-1}), talc + chitosan 80:20 (4.66 x 10^8 spores ml^{-1}) and talc + chitosan 65:35 (4.52 x 10^8 spores ml^{-1}). Talc 65:35 with 3.88 x 10^8 spores ml^{-1} was on par with spore suspension in DW + chitin (3.93 x 10^8 spores ml^{-1}). Talc 80:20 (3.32 x 10^8 spores ml^{-1}) recorded spore count

similar to that of control (3.50 x 10^8 spores ml^{-1}). Lowest spore count was noted with 50:50 formulations of talc alone, talc + chitin and talc + chitosan i.e., 2.47 x 10^8 spores ml^{-1}, 2.42 x 10^8 spores ml^{-1} and 2.16 x 10^8 spores ml^{-1}, respectively.

As that of earlier, at 45 DAS, chitin enriched formulations of GNO (65:35, 50:50 and 60:40) and SFO (65:35, 60:40 and 50:50) recorded maximum spore count of 6.82 x 10^8 spores ml^{-1}, 6.77 x 10^8 spores ml^{-1}, 6.54 x 10^8 spores ml^{-1}, 6.75 x 10^8 spores ml^{-1}, 6.69 x 10^8 spores ml^{-1} and 6.54 x 10^8 spores ml^{-1}, respectively. The basic formulation, GNO 65:35 with 5.31 x 10^8 spores ml^{-1} was the next best, followed GNO 60:40 (4.94 x 10^8 spores ml^{-1}). The basic oil formulations, *viz.* GNO (50:50) and SFO (50:50, 60:40 and 65:35) did not differ significantly from chitin and chitosan enriched talc formulations, talc + chitin (65:35 and 80:20) and talc + chitosan (65:35 and 80:20). The spore count being, 4.72 x 10^8 spores ml^{-1}, 4.65 x 10^8 spores ml^{-1}, 4.58 x 10^8 spores ml^{-1}, 4.65 x 10^8 spores ml^{-1}, 4.45 x 10^8 spores ml^{-1}, 4.47 x 10^8 spores ml^{-1}, 4.13 x 10^8 spores ml^{-1} and 4.41 x 10^8 spores ml^{-1}, respectively). Basic talc formulations, talc (65:35 and 80:20) with spore count of 3.08 x 10^8 spores ml^{-1} and 3.16 x 10^8 spores ml^{-1} were found to be on par with spore suspension in DW + chitin (3.43 x 10^8 spores ml^{-1}). Talc + chitin 50:50, talc + chitosan 50:50 and talc 50:50, were inferior to control (2.08 x 10^8 spores ml^{-1}, 1.88 x 10^8 spores ml^{-1} and 1.73 x 10^8 spores ml^{-1} and 2.31 x 10^8 spores ml^{-1}, respectively).

A similar trend was observed 60 DAS also. Maximum spore count was noticed with chitin enriched GNO (50:50, 60:40 and 65:35) and SFO (50:50, 60:40 and 65:35) formulations, whose spore count were 6.77 x 10^8 spores ml^{-1}, 6.54 x 10^8 spores ml^{-1}, 6.82 x 10^8 spores ml^{-1}, 6.50 x 10^8 spores ml^{-1}, 6.69 x 10^8 spores ml^{-1} and 6.75 x 10^8 spores ml^{-1}, respectively. Basic formulation, GNO 65:35 was found to be the next best (5.04 x 10^8 spores ml^{-1}). The spore count observed in basic oil formulations, GNO (50:50, 60:40, 65:35) and SFO (50:50, 60:40, 65:35) was on par with the chitin as well as chitosan enriched formulations of

talc (65:35 and 80:20). The values being 4.56 x 10^8 spores ml^{-1}, 4.65 x 10^8 spores ml^{-1}, 5.04 x 10^8 spores ml^{-1}, 4.38 x 10^8 spores ml^{-1}, 4.49 x 10^8 spores ml^{-1}, 4.52 x 10^8 spores ml^{-1}, 4.32 x 10^8 spores ml^{-1}, 4.25 x 10^8 spores ml^{-1}, 4.08 x 10^8 spores ml^{-1} and 4.23 x 10^8 spores ml^{-1}, respectively. This was followed by spore suspension in DW + chitin, talc 65:35, talc 80:20 which were similar to control, with spore count of 2.71 x 10^8 spores ml^{-1}, 2.65 x 10^8 spores ml^{-1}, 2.58 x 10^8 spores ml^{-1} and 2.19 x 10^8 spores ml^{-1}, respectively. At this period of storage also, 50:50 formulations of talc + chitin, talc + chitosan, and talc alone at 50:50 proportion recorded lowest spore count ranging from 1.85 x 10^8 spores ml^{-1} to 1.35 x 10^8 spores ml^{-1}.

At 75 DAS, enriched formulation, GNO + chitin 65:35 with 6.55 x 10^8 spores ml^{-1} was found to be superior, followed by rest of the enriched formulations, *viz.* SFO + chitin (50:50, 60:40 and 65:35) and GNO + chitin (50:50 and 60:40), with spore count 5.85 x 10^8 spores ml^{-1}, 6.07 x 10^8 spores ml^{-1}, 6.37 x 10^8 spores ml^{-1}, 6.11 x 10^8 spores ml^{-1} and 6.36 x 10^8 spores ml^{-1}. As that of earlier, all the basic oil formulations with GNO (50:50, 60:40 and 65:35) and SFO (50:50, 60:40 and 65:35) did not differ significantly from enriched talc formulations, talc + chitin (65:35 and 80:20) and talc + chitosan (65:35 and 80:20). The spore count recorded were 3.70 x 10^8 spores ml^{-1}, 3.81 x 10^8 spores ml^{-1}, 3.66 x 10^8 spores ml^{-1}, 3.71 x 10^8 spores ml^{-1}, 3.79 x 10^8 spores ml^{-1}, 3.81 x 10^8 spores ml^{-1}, 3.44 x 10^8 spores ml^{-1}, 3.58 x 10^8 spores ml^{-1}, 3.39 x 10^8 spores ml^{-1} and 3.51 x 10^8 spores ml^{-1}, respectively. The spore count obtained with talc 65:35 (2.34 x 10^8 spores ml^{-1}), talc 80:20 (2.10 x 10^8 spores ml^{-1}) and DW + chitin (2.55 x 10^8 spores ml^{-1}) was on par with each other and superior to control (1.63 x 10^8 spores ml^{-1}). Lowest spore count of 0.74 x 10^8 spores ml^{-1}, 0.66 x 10^8 spores ml^{-1} and 0.65 x 10^8 spores ml^{-1} was observed with talc + chitosan 50:50, talc + chitin 50:50 and talc 50:50, respectively.

Table 17. Effect of carrier materials on the spore count of *L. lecanii* formulations under refrigeration

Sl no.	Carrier : Spore concentrate	*Mean spore count (x 10^8 spores ml^{-1})					
		15 DAS	**30 DAS**	**45 DAS**	**60 DAS**	**75 DAS**	**90 DAS**
1	SFO + SC (50 : 50)	5.14(2.27)	5.04(2.25)	4.65(2.16)	4.38(2.09)	3.71(1.93)	3.44(1.85)
2	SFO + SC (60 : 40)	5.18(2.28)	5.20(2.28)	4.58(2.14)	4.49(2.12)	3.79(1.95)	3.53(1.88)
3	SFO + SC (65 : 35)	5.27(2.30)	5.55(2.36)	4.65(2.16)	4.52(2.13)	3.81(1.95)	3.55(1.89)
4	(SFO + CC 0.1 %) + SC (50 : 50)	6.81(2.61)	6.79(2.61)	6.50(2.55)	6.23(2.50)	5.85(2.42)	5.62(2.37)
5	(SFO + CC 0.1 %) + SC (60 : 40)	6.95(2.64)	6.86(2.62)	6.69(2.59)	6.41(2.53)	6.07(2.46)	5.75(2.40)
6	(SFO + CC 0.1 %) + SC (65 : 35)	7.13(2.67)	6.89(2.63)	6.75(2.60)	6.48(2.55)	6.37(2.52)	6.19(2.49)
7	GNO + SC (50 : 50)	5.19(2.28)	4.83(2.20)	4.72(2.17)	4.56(2.14)	3.70(1.92)	3.49(1.87)
8	GNO + SC (60 : 40)	5.21(2.28)	4.84(2.20)	4.94(2.22)	4.65(2.16)	3.81(1.95)	3.56(1.89)
9	GNO + SC (65 : 35)	5.61(2.37)	4.88(2.21)	5.31(2.31)	5.04(2.25)	3.66(1.91)	3.58(1.89)
10	(GNO + CC 0.1 %) + SC (50 : 50)	6.92(2.63)	6.94(2.63)	6.77(2.60)	6.46(2.54)	6.11(2.47)	5.87(2.42)
11	(GNO + CC 0.1 %) + SC (60 : 40)	6.98(2.64)	6.74(2.60)	6.54(2.56)	6.43(2.54)	6.36(2.52)	6.08(2.47)
12	(GNO + CC 0.1 %) + SC (65 : 35)	7.02(2.65)	7.00(2.65)	6.82(2.61)	6.72(2.59)	6.55(2.56)	6.20(2.49)
13	Talc + SC (50 : 50)	2.86(1.69)	2.47(1.57)	1.73(1.32)	1.35(1.16)	0.65(0.81)	0.16(0.40)
14	Talc + SC (65 : 35)	4.16(2.04)	3.88(1.97)	3.08(1.75)	2.65(1.63)	2.34(1.53)	2.24(1.50)
15	Talc + SC (80 : 20)	3.86(1.96)	3.32(1.82)	3.16(1.78)	2.58(1.61)	2.10(1.45)	2.08(1.44)
16	(Talc + C 5 %) + SC (50 : 50)	2.47(1.57)	2.16(1.47)	2.08(1.44)	1.85(1.36)	0.66(0.81)	0.16(0.40)
17	(Talc + C 5 %) + SC (65 : 35)	5.03(2.24)	4.76(2.18)	4.45(2.11)	4.32(2.08)	3.44(1.86)	3.18(1.78)
18	(Talc + C 5 %) + SC (80 : 20)	5.05(2.25)	4.67(2.16)	4.47(2.11)	4.25(2.06)	3.58(1.89)	3.16(1.78)
19	(Talc + CS 0.5 %) + SC (50 : 50)	2.63(1.62)	2.42(1.56)	1.88(1.37)	1.67(1.29)	0.74(0.86)	0.22(0.47)
20	(Talc + CS 0.5 %) + SC (65 : 35)	5.08(2.25)	4.52(2.13)	4.13(2.03)	4.08(2.02)	3.39(1.84)	2.83(1.68)
21	(Talc + CS 0.5 %) + SC (80 : 20)	5.00(2.24)	4.66(2.16)	4.41(2.10)	4.23(2.06)	3.51(1.87)	3.20(1.79)
22	SC+ CC	4.36(2.09)	3.93(1.98)	3.43(1.85)	2.71(1.65)	2.55(1.60)	2.48(1.58)
23	SC	3.51(1.87)	3.50(1.87)	2.31(1.52)	2.19(1.48)	1.63(1.28)	0.80(0.90)
	CD (0.05)	**0.165**	**0.143**	**0.149**	**0.179**	**0.122**	**0.227**

*Mean of three replications, DAS: Days after storage; SFO: Sunflower oil; GNO: Groundnut oil; SC: Spore Concentrate; CC: Colloidal Chitin; C: Chitin; CS: Chitosan, Figures in parentheses are √x transformed values

At the end of 90 days of storage under refrigeration, chitin enriched formulations of GNO and SFO at all tested proportions were found to be superior. The spore count recorded was 6.20 x 10^8 spores ml^{-1} (GNO + chitin 65 :35), 6.19 x 10^8 spores ml^{-1} (SFO + chitin 65:35), 6.08 x 10^8 spores ml^{-1} (GNO + chitin 60:40), 5.87 x 10^8 spores ml^{-1} (GNO + chitin 50:50), 5.75 x 10^8 spores ml^{-1} (SFO + chitin 60:40) and 5.62 x 10^8 spores ml^{-1} (SFO + chitin 50:50). This was followed by basic oil formulations, GNO (50:50, 60:40 and 65:35) and SFO (50:50, 60:40 and 65:35) which were statistically similar to chitin and chitosan enriched talc formulations (65:35 and 80:20) .The spore count values were 3.49 x 10^8 spores ml^{-1}, 3.56 x 10^8 spores ml^{-1}, 3.58 x 10^8 spores ml^{-1}, 3.44 x 10^8 spores ml^{-1}, 3.53 x 10^8 spores ml^{-1}, 3.55 x 10^8 spores ml^{-1}, 3.18 x 10^8 spores ml^{-1}, 3.16 x 10^8 spores ml^{-1}, 2.83 x 10^8 spores ml^{-1} and 3.20 x 10^8 spores ml^{-1}, respectively. Talc 65:35 (2.24 x 10^8 spores ml^{-1}) and talc 80:20 (2.08 x 10^8 spores ml^{-1}) were on par with spore suspension in DW + chitin (2.48 x 10^8 spores ml^{-1}). Talc + chitosan 50:50, talc + chitin 50:50 and talc 50:50 (0.22 x 10^8 spores ml^{-1}, 0.16 x 10^8 spores ml^{-1} and 0.16 x 10^8 spores ml^{-1}) were inferior to the control (0.80 x 10^8 spores ml^{-1}).

The overall observations based on spore count revealed that the chitin enriched formulations with GNO and SFO as carriers in proportions 65:35, 60:40 and 50:50 were the best. Chitin and chitosan enriched talc formulations (65:35 and 80:20) were equally good as basic formulations of GNO and SFO (65:35, 60:40 and 50:50).

Better retention of spore count was noted when the formulations were stored under refrigeration.

4.3.2 Effect of Adjuvants on Viability and Spore Count of oil formulations

The chitin enriched formulations based on GNO and SFO which were found to be superior in experiment 4.3.1 were amended with adjuvants namely polyethylene glycol (PEG), polyoxyethylene (POX), tween-80 and glycerol at varying

concentrations. Among the adjuvants used tween-80 and POX were found to improve the miscibility of formulations (Plate 9. A to D and Plates 10. A to D).

The results on conidial viability and spore count of these formulations stored at two different temperatures (room temperature and refrigeration) are presented below.

4.3.2.1 Chitin enriched ground nut oil formulation

4.3.2.1.1 Viability

Viability was assessed based on the number of cfu recorded at fortnightly intervals.

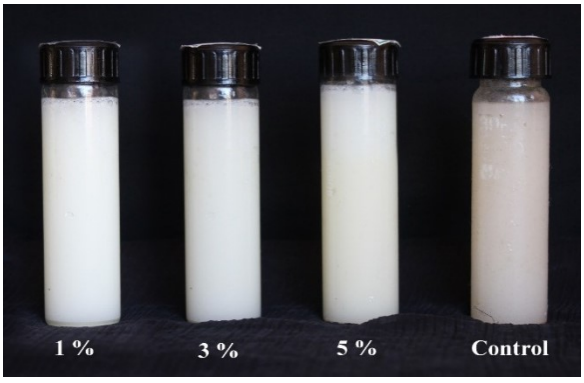
4.3.2.1.1.1 Room temperature

The data on number of cfu are furnished in Table 18. At 15 DAS, maximum number of cfu (4.66 x 106 cfu ml-1) was recorded in formulations to which glycerol five per cent was added as adjuvant. This was closely followed by tween-80 one per cent, PEG two per cent, POX 0.5 per cent and glycerol three per cent with cfu 4.51 x 106 ml-1, 4.46 x 106 ml-1, 4.43 x 106 ml-1 and 4.38 x 106 ml-1, respectively. The cfu recorded from these formulations as well as those from glycerol one per cent, PEG 0.5 per cent, tween-80 three per cent and PEG one per cent were statistically on par with GNO formulation without any adjuvant (check). The number of cfu recorded was 4.23 x 106 ml-1, 4.12 x 106 ml-1, 4.12 x 106 ml-1 and 4.11 x 106 ml-1 and 4.26 x 106 ml-1, respectively. The lowest number of cfu was recorded in formulations with tween-80 five per cent (3.81 x 106 spores ml-1), POX two per cent (3.75 x 106 spores ml-1) and POX five per cent (3.72 x 106 spores ml-1) as adjuvants.

At 30 DAS the viability of formulations with various adjuvants at different concentrations exhibited no significant difference among each other.

(A) PEG + GNO

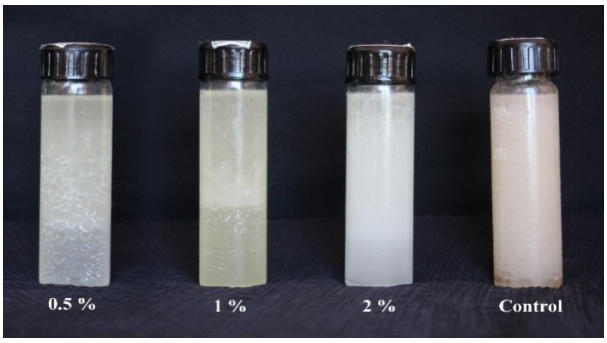
(B) POX + GNO



(C) Tween-80 + GNO

(D) Glycerol + GNO

Plate 9. Chitin enriched groundnut oil (GNO) formulations with adjuvants



(A) PEG + SFO

(B) POX + SFO

(C) Tween-80 + SFO

(D) Glycerol + SFO

Plate 10. Chitin enriched sunflower oil (SFO) formulations with adjuvants

At 45 DAS, maximum cfu was recorded with glycerol five per cent, followed by tween-80 one per cent, POX 0.5 per cent, glycerol three per cent, PEG two per cent and glycerol one per cent, their cfu being 3.65 x 10^6 cfu ml^{-1}, 3.55 x 10^6 cfu ml^{-1}, 3.52 x 10^6 cfu ml^{-1}, 3.48 x 10^6 cfu ml^{-1}, 3.47 x 10^6 cfu ml^{-1} and 3.29 x 10^6 cfu ml^{-1}, respectively. This was on par with the cfu obtained in formulation without adjuvants (3.54 x 10^6 cfu ml^{-1}). PEG one per cent, tween-80 three per cent, POX two per cent, PEG 0.5 per cent, POX five per cent and tween-80 five per cent yielded a cfu of 3.25 x 10^6 ml^{-1}, 3.17 x 10^6 ml^{-1}, 3.16 x 10^6 ml^{-1}, 3.13 x 10^6 ml^{-1}, 2.93 x 10^6 ml^{-1} and 2.88 x 10^6 cfu ml^{-1}, which were statistically which were not significant.

By the end of 60 DAS, glycerol five per cent with 3.44 x 10^6 cfu ml^{-1} was the best adjuvant, closely followed by tween-80 one per cent, POX 0.5 per cent and PEG two per cent with cfu being 3.30 x 10^6 ml^{-1}, 3.24 x 10^6 ml^{-1} and 3.17 x 10^6 ml^{-1}. PEG one per cent recorded 3.05 x 10^6 cfu ml^{-1} which was similar to the check formulation (2.83 x 10^6 ml^{-1}). The cfu obtained with glycerol one and three per cent (2.61 x 10^6 ml^{-1} and 2.65 x 10^6 ml^{-1}), tween-80 three per cent (2.66 x 10^6 ml^{-1}) and POX two per cent (2.61 x 10^6 ml^{-1}) was similar to that of check formulation. PEG 0.5 per cent (2.56 x 10^6 ml^{-1}), tween-80 five per cent (2.52 x 10^6 ml^{-1}) and POX five per cent (2.44 x 10^6 cfu ml^{-1}) had lesser number of cfu. The lowest cfu was noted in POX five per cent.

At 75 DAS also, glycerol five per cent recorded maximum cfu of 2.75 x 10^6 ml^{-1} which was on par with POX 0.5 per cent (2.72 x 10^6 ml^{-1}), tween-80 one per cent (2.70 x 10^6 ml^{-1}) and PEG two per cent (2.63 x 10^6 ml^{-1}), the latter two being on par with PEG one per cent and tween-80 three per cent (2.46 x 10^6 ml^{-1} and 2.43 x 10^6 ml^{-1}). The rest of the adjuvants namely, glycerol (three and one per cent), PEG 0.5 per cent, POX (two and five per cent) and tween-80 five per cent retained viability as that of GNO (2.36 x 10^6 cfu ml^{-1}). Their cfu values being 2.34 x 10^6 ml^{-1}, 2.24 x 10^6 ml^{-1}, 2.33 x 10^6 ml^{-1}, 2.24 x 10^6 ml^{-1}, 2.21 x 10^6 ml^{-1} and 2.22 x 10^6 ml^{-1}, respectively.

Table 18. Effect of adjuvants on the viability of *L. lecanii* in chitin enriched groundnut oil formulations stored at room temperature

Sl no.	Adjuvants	Concentration (%)	*Mean number of cfu (x 10^6 ml^{-1})					
			15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
1	Polyethylene Glycol (PEG)	0.5	4.12 (2.03)	3.46 (1.86)	3.13 (1.77)	2.56 (1.60)	2.33 (1.53)	1.65 (1.29)
2		1	4.11 (2.03)	3.52 (1.88)	3.25 (1.80)	3.05 (1.75)	2.46 (1.57)	1.72 (1.31)
3		2	4.46 (2.11)	3.66 (1.91)	3.47 (1.86)	3.17 (1.78)	2.63 (1.62)	2.32 (1.52)
4	Polyoxyethylene (POX)	0.5	4.43 (2.10)	3.72 (1.93)	3.52 (1.88)	3.24 (1.80)	2.72 (1.65)	2.33 (1.53)
5		2	3.75 (1.94)	3.42 (1.85)	3.16 (1.78)	2.61 (1.62)	2.24 (1.50)	1.85 (1.36)
6		5	3.72 (1.93)	3.37 (1.84)	2.93 (1.71)	2.44 (1.56)	2.21 (1.49)	1.46 (1.21)
7	Tween-80	1	4.51 (2.12)	3.73 (1.93)	3.55 (1.88)	3.30 (1.82)	2.70 (1.64)	2.43 (1.56)
8		3	4.12 (2.03)	3.60 (1.90)	3.17 (1.78)	2.66 (1.63)	2.43 (1.56)	1.53 (1.32)
9		5	3.81 (1.95)	3.46 (1.86)	2.89 (1.70)	2.52 (1.59)	2.22 (1.49)	1.47 (1.21)
10	Glycerol	1	4.23 (2.06)	3.52 (1.88)	3.29 (1.81)	2.61 (1.62)	2.24 (1.50)	1.74 (1.24)
11		3	4.38 (2.09)	3.54 (1.88)	3.48 (1.86)	2.65 (1.63)	2.34 (1.53)	1.75 (1.32)
12		5	4.66 (2.16)	3.83 (1.96)	3.65 (1.91)	3.44 (1.85)	2.75 (1.66)	2.52 (1.59)
13	Formulation without adjuvant		4.26 (2.06)	3.6 (1.90)	3.54 (1.88)	2.83 (1.68)	2.36 (1.54)	1.75 (1.32)
	CD (0.05)		**0.100**	**NS**	**0.069**	**0.071**	**0.088**	**0.075**

* Mean of three replications, DAS: Days after storage, Figures in parentheses are √x transformed values

By the end of the experimental period (90 DAS), the best adjuvants were glycerol five per cent, tween-80 one per cent, POX 0.5 per cent and PEG two per cent with cfu being 2.52 x 10^6 ml^{-1}, 2.43 x 10^6 ml^{-1}, 2.33 x 10^6 ml^{-1} and 2.32 x 10^6 ml^{-1}, respectively. The viability recorded with POX two per cent, glycerol three and one per cent, PEG one and 0.5 per cent were statistically on par with that of the formulation without any adjuvant (1.85 x 10^6 cfu ml^{-1}, 1.75 x 10^6 cfu ml^{-1}, 1.74 x 10^6 cfu ml^{-1}, 1.72 x 10^6 cfu ml^{-1}, 1.65 x 10^6 cfu ml^{-1} and 1.76 x 10^6 ml^{-1}, respectively). The lowest cfu values were obtained in formulations with tween-80 three and five per cent (1.53 x 10^6 ml^{-1} and 1.47 x 10^6 ml^{-1}) and POX five per cent (1.46 x 10^6 ml^{-1}).

4.3.2.1.1.2 Refrigeration

GNO formulation with various adjuvants at different concentrations, when stored under refrigeration did not show significant variation in their viability (number of cfu) till 45 DAS (Table 19). The number of cfu ranged from 4.2 x 10^6 ml^{-1} to 4.84 x 10^6 ml^{-1}.

But by 60 DAS, maximum number of viable colonies (4.72 x 10^6 ml^{-1} and 4.67 x 10^6 ml^{-1}) were observed in formulations to which glycerol five per cent and tween-80 one per cent were added as the adjuvants. This was closely followed by PEG two per cent, glycerol three per cent, POX 0.5 per cent and tween-80 three per cent with cfus 4.56 x 10^6 ml^{-1}, 4.50 x 10^6 ml^{-1}, 4.43 x 10^6 ml^{-1} and 4.35 x 10^6 ml^{-1}, respectively. The latter two were on par with formulation without adjuvant (4.16 x 10^6 ml^{-1}). The cfu recorded with PEG 0.5 and one per cent (4.11 x 10^6 ml^{-1} and 4.24 x 10^6 ml^{-1}), glycerol one per cent (4.22 x 10^6 ml^{-1}), POX two and five per cent (4.18 x 10^6 ml^{-1} and 4.13 x 10^6 ml^{-1}) and tween-80 three and five per cent (4.35 x 10^6 ml^{-1} and 4.17 x 10^6 ml^{-1}) were also statistically on par to that of check formulation.

Table 19. Effect of adjuvants on the viability of *L. lecanii* in chitin enriched groundnut oil formulation stored under refrigeration

Sl no.	**Adjuvants**	**Concentration (%)**	***Mean number of cfu (x 10^6 ml^{-1})**					
			15 DAS	**30 DAS**	**45 DAS**	**60 DAS**	**75 DAS**	**90 DAS**
1	Polyethylene Glycol	0.5	4.66 (2.16)	4.53 (2.13)	4.24 (2.06)	4.11 (2.03)	3.69 (1.92)	3.15 (1.78)
2		1	4.68 (2.16)	4.55 (2.13)	4.33 (2.08)	4.24 (2.06)	3.76 (1.94)	3.60 (1.90)
3		2	4.86 (2.20)	4.59 (2.14)	4.38 (2.09)	4.56 (2.14)	4.10 (1.96)	3.65 (1.91)
4	Polyoxyethylene	0.5	4.73 (2.17)	4.66 (2.16)	4.54 (2.13)	4.43 (2.11)	3.98 (1.99)	3.62 (1.90)
5		2	4.56 (2.13)	4.42 (2.10)	4.21 (2.05)	4.18 (2.04)	3.80 (1.95)	3.43 (1.85)
6		5	4.52 (2.13)	4.44 (2.11)	4.24 (2.06)	4.13 (2.03)	3.72 (1.93)	3.27 (1.81)
7	Tween-80	1	5.14 (2.27)	4.99 (2.23)	4.71 (2.17)	4.67 (2.16)	4.25 (2.06)	4.09 (2.02)
8		3	4.75 (2.18)	4.65 (2.16)	4.51 (2.12)	4.35 (2.09)	3.77 (1.94)	3.55 (1.88)
9		5	4.65 (2.15)	4.52 (2.13)	4.49 (2.12)	4.18 (2.04)	3.75 (1.94)	3.42 (1.85)
10	Glycerol	1	5.11 (2.26)	4.85 (2.20)	4.64 (2.15)	4.22 (2.06)	3.84 (2.03)	3.65 (1.91)
11		3	5.19 (2.28)	4.74 (2.18)	4.68 (2.16)	4.50 (2.12)	4.20 (2.05)	3.82 (1.95)
12		5	5.33 (2.31)	5.19 (2.28)	4.84 (2.20)	4.72 (2.17)	4.32 (2.08)	4.12 (2.03)
13	Formulation without adjuvant		4.62 (2.15)	4.47 (2.11)	4.22 (2.05)	4.16 (2.04)	3.66 (1.91)	3.33 (1.82)
14	**CD (0.05)**		**NS**	**NS**	**NS**	**0.069**	**0.087**	**0.100**

* Mean of three replications, DAS: Days after storage, Figures in parentheses are √x transformed values

After 75 DAS, glycerol five and three per cent and tween-80 one per cent with cfu, 4.32 x 10^6 ml^{-1}, 4.25 x 10^6 ml^{-1} and 4.20 x 10^6 ml^{-1} were found to be the best adjuvants. The second best adjuvant was PEG two per cent which recorded a cfu of 4.10 x 10^6 ml^{-1}. The rest of the formulations with POX 0.5, two and five per cent, glycerol one per cent, tween-80 three and five per cent and PEG 0.5 and one per cent were statistically on par with the formulation without any adjuvants. The number of colonies observed were 3.98 x 10^6 ml^{-1}, 3.80 x 10^6 ml^{-1}, 3.72 x 10^6 ml^{-1}, 3.84 x 10^6 ml^{-1}, 3.77 x 10^6 ml^{-1}, 3.75 x 10^6 ml^{-1}, 3.69 x 10^6 ml^{-1}, 3.76 x 10^6 ml^{-1} and 3.66 x 10^6 ml^{-1}, respectively.

By the end of 90 DAS, glycerol five per cent and tween-80 one per cent were found to be the superior adjuvants followed by glycerol five per cent, PEG two per cent, POX 0.5 per cent, PEG one per cent, tween-80 three per cent and glycerol one per cent. Their cfu being 3.83 x 10^6 ml^{-1}, 3.65 x 10^6 ml^{-1}, 3.62 x 10^6 ml^{-1}, 3.60 x 10^6 ml^{-1}, 3.55 x 10^6 ml^{-1} and 3.48 x 10^6 ml^{-1}, respectively. The above said formulations except glycerol three per cent, POX two and five per cent (3.43 x 10^6 $ml^{-1}$and 3.27 x 10^6 ml^{-1}), tween-80 five per cent (3.42 x 10^6 ml^{-1}) and PEG 0.5 per cent (3.15 x 10^6 ml^{-1}) were found to be in parity with the cfu obtained in check formulation.

Of the various adjuvants tested, formulation with glycerol five per cent, tween one per cent, PEG 2 per cent and POX 0.5 per cent could maintain conidial viability over three months of storage both in room temperature and refrigeration. Under room temperature, tween-80 and POX five per cent significantly reduced the number of viable colonies than the check formulation. The number of viable colonies in all the formulations were maintained to the level of 10^6 though there was a narrow reduction at 45 DAS. But the number of cfu noted was always more in storage under refrigeration.

4.3.2.1.2 Spore Count

4.3.2.1.2.1 Room temperature

The spore count observed at 15 DAS indicated that glycerol 5 per cent, tween-80 one per cent, PEG two per cent and POX 0.5 per cent were the superior adjuvants, the spore count being 5.81 x 10^8 spores ml^{-1}, 5.74 x 10^8 spores ml^{-1}, 5.73 x 10^8 spores ml^{-1} and 5.70 x 10^8 spores ml^{-1}, respectively (Table 20). The spore count in formulations with adjuvants PEG 0.5 and one per cent, glycerol one and three per cent, tween-80 three per cent and POX two per cent (5.53 x 10^8 spores ml^{-1}, 5.57 x 10^8 spores ml^{-1}, 5.56 x 10^8 spores ml^{-1}, 5.51 x 10^8 spores ml^{-1}, 5.44 x 10^8 spores ml^{-1} and 5.40 x 10^8 spores ml^{-1}) was found to be statistically similar to that of the formulation with no adjuvants (5.54 x 10^8 spores ml^{-1}). Formulations with POX and tween-80 five per cent recorded a spore count significantly lesser than that observed in check (5.23 x 10^8 spores ml^{-1} and 5.25 x 10^8 spores ml^{-1}).

At 30 DAS, glycerol five per cent was found to have maximum spore count (5.35 x 10^8 spores ml^{-1}). This was closely followed by tween-80 one per cent, PEG two per cent, POX 0.5 per cent and glycerol three per cent (5.31 x 10^8 spores ml^{-1}, 5.28 x 10^8 spores ml^{-1}, 5.23 x 10^8 spores ml^{-1} and 5.06 x 10^8 spores ml^{-1}, respectively) which was on parity with the spore count in formulation without adjuvant (5.09 x 10^8 spores ml^{-1}). The spore count in one per cent glycerol and one per cent PEG were found to be in parity with the spore count in formulation without adjuvant, the spore count being 4.94 x 10^8 spores $ml^{-1}$and 4.89 x 10^8 spores ml^{-1}. PEG 0.5 one per cent, tween-80 three and five per cent, POX two and five per cent recorded a spore count lower than that observed in check formulation. The spore counts recorded were 4.67 x 10^8 spores ml^{-1}, 4.87 x 10^8 spores ml^{-1}, 4.61 x 10^8 spores ml^{-1}, 4.64 x 10^8 spores ml^{-1} and 4.63 x 10^8 spores ml^{-1}, respectively.

After 45 DAS, glycerol at five per cent, tween-80 one per cent and POX 0.5 per cent recorded maximum spore count of 4.78 x 10^8 spores ml^{-1},

4.72 x 10^8 spores ml^{-1} and 4.70 x 10^8 spores ml^{-1} which was on par with PEG two per cent (4.58 x 10^8 spores ml^{-1}). Glycerol one and three per cent (4.29 x 10^8 spores ml^{-1} and 4.40 x 10^8 spores ml^{-1}), PEG 0.5 and one per cent (4.28 x 10^8 spores ml^{-1} and 4.26 x 10^8 spores ml^{-1}), tween-80 three per cent (4.50 x 10^8 spores ml^{-1}), POX two and five per cent (4.21 x 10^8 spores ml^{-1} and 4.08 x 10^8 spores ml^{-1}) recorded a spore count that was on par with that of check formulation (4.31 x 10^8 spores ml^{-1}). The lowest spore count was observed with five per cent tween-80 with spore count being 3.89 x 10^8 spores ml^{-1}.

At 60 DAS, the highest spore count was observed with glycerol five per cent (4.34 x 10^8 spores ml^{-1}) followed by tween-80 one per cent, POX 0.5 per cent and PEG two per cent with their spore count being 4.21 x 10^8 spores ml^{-1}, 4.21 x 10^8 spores ml^{-1} and 4.02 x 10^8 spores ml^{-1}, respectively. Tween-80 three per cent, POX two per cent, glycerol one per cent and three per cent and PEG one per cent recorded similar spore counts of 3.76 x 10^8 spores ml^{-1}, 3.68 x 10^8 spores ml^{-1}, 3.65 x 10^8 spores ml^{-1}, 3.63 x 10^8 spores ml^{-1}, 3.61 x 10^8 spores ml^{-1}, respectively and were not significantly different from check (3.58 x 10^8 spores ml^{-1}). The spore count noted with PEG 0.5 per cent , POX five per cent and tween-80 five per cent was the lowest, their values being 3.25 x 10^8 spores ml^{-1}, 3.19 x 10^8 spores ml^{-1} and 3.15 x 10^8 spores ml^{-1}, respectively.

Spore count recorded at 75 DAS indicated that formulations with glycerol five per cent, tween-80 one per cent, PEG two per cent and POX 0.5 per cent as adjuvants were superior to rest of the formulations. The spore counts of these formulations were 3.66 x 10^8 spores ml^{-1}, 3.63 x 10^8 spores ml^{-1}, 3.60 x 10^8 spores ml^{-1} and 3.59 x 10^8 spores ml^{-1}. Glycerol one (3.26 x 10^8 spores ml^{-1}) and glycerol three per cent (3.26 x 10^8 spores ml^{-1}) were on statistical parity with formulation without adjuvants (3.31 x 10^8 spores ml^{-1}). PEG one per cent and tween-80 three per cent recorded a spore count (2.83 x 10^8 spores ml^{-1}) lesser than the check formulation. The lowest spore counts were observed with PEG 0.5 per cent (2.60 x 10^8 spores ml^{-1}),

Table 20. Effect of adjuvants on the spore count of *L. lecanii* in chitin enriched groundnut oil formulation stored at room temperature

Adjuvants	**Concentration (%)**	***Mean spore count (x 10^8 spores ml^{-1})**					
		15 DAS	**30 DAS**	**45 DAS**	**60 DAS**	**75 DAS**	**90 DAS**
Polyethylene Glycol	0.5	5.54 (2.35)	4.67 (2.16)	4.28 (2.06)	3.25 (1.80)	2.60 (1.61)	1.63 (1.27)
	1	5.57 (2.36)	4.89 (2.21)	4.26 (2.06)	3.61 (1.89)	2.83 (1.68)	1.75 (1.32)
	2	5.73 (2.39)	5.28 (2.29)	4.58 (2.14)	4.02 (2.01)	3.60 (1.90)	2.61 (1.62)
Polyoxyethylene	0.5	5.70 (2.38)	5.23 (2.28)	4.70 (2.17)	4.21 (2.05)	3.59 (1.89)	2.62 (1.62)
	2	5.40 (2.32)	4.64 (2.15)	4.21 (2.05)	3.68 (1.91)	2.60 (1.61)	1.71 (1.31)
	5	5.23 (2.28)	4.63 (2.15)	4.08 (2.02)	3.19 (1.78)	2.54 (1.59)	1.59 (1.26)
Tween-80	1	5.74 (2.39)	5.31 (2.30)	4.72 (2.17)	4.21 (2.05)	3.63 (1.91)	2.63 (1.62)
	3	5.44 (2.33)	4.87 (2.20)	4.50 (2.12)	3.76 (1.93)	2.83 (1.68)	2.39 (1.54)
	5	5.25 (2.29)	4.61 (2.14)	3.89 (1.97)	3.15 (1.77)	2.54 (1.59)	1.57 (1.25)
Glycerol	1	5.51 (2.34)	4.94 (2.22)	4.29 (2.07)	3.65 (1.91)	3.26 (1.80)	2.12 (1.46)
	3	5.56 (2.35)	5.06 (2.25)	4.40 (2.10)	3.63 (1.91)	3.26 (1.80)	2.55 (1.60)
	5	5.81 (2.41)	5.35 (2.31)	4.78 (2.19)	4.34 (2.08)	3.66 (1.91)	2.71 (1.65)
Formulation without adjuvant		5.54 (2.34)	5.09 (2.26)	4.31 (2.07)	3.58 (1.89)	3.31 (1.82)	2.41 (1.55)
CD (0.05)		**0.032**	**0.052**	**0.063**	**0.064**	**0.052**	**0.060**

* Mean of three replications, DAS: Days after storage, Figures in parentheses are √x transformed values

POX two and five per cent (2.60 x 10^8 spores ml^{-1} and 2.54 x 10^8 spores ml^{-1}) and tween-80 five per cent (2.54 x 10^8 spores ml^{-1}).

By the end 90 DAS, a similar trend was observed. Formulations with glycerol five per cent, tween-80 one per cent, POX 0.5 per cent and PEG two per cent as adjuvants recorded the maximum spore count of 2.71 x 10^8 spores ml^{-1}, 2.63 x 10^8 spores ml^{-1}, 2.62 x 10^8 spores ml^{-1} and 2.61 x 10^8 spores ml^{-1}, respectively. Glycerol three per cent with a spore count of 2.55 x 10^8 spores ml^{-1}) and tween-80 three per cent (2.39 x 10^8 spores ml^{-1}) was also found to be on parity with the check. Glycerol one per cent, PEG 0.5 and one per cent, POX two and five per cent and tween-80 five per cent were the inferior adjuvants as they recorded a spore yield lesser than that of the check formulation without any adjuvant, their spore count being 2.12 x 10^8 spores ml^{-1}, 1.63 x 10^8 spores ml^{-1}, 1.75 x 10^8 spores ml^{-1}, 1.71 x 10^8 spores ml^{-1}, 1.59 x 10^8 spores ml^{-1} and 1.57 x 10^8 spores ml^{-1}, respectively.

4.3.2.1.1.2 Refrigeration

The spore counts observed with various adjuvants at different concentrations when stored under refrigeration did not show significant variation till two month after storage (Table 21). The spore count ranged from 5.81 x 10^8 spores ml^{-1} to 6.50 x 10^8 spores ml^{-1}.

But by 75 DAS, formulations with glycerol five per cent, tween-80 one per cent, POX 0.5 per cent and PEG two per cent recorded maximum spore counts, 6.23 x 10^8 spores ml^{-1}, 6.19 x 10^8 spores ml^{-1}, 6.11 x 10^8 spores ml^{-1} and 6.05 x 10^8 spores ml^{-1}, respectively. PEG one per cent (5.86 x 10^8 spores ml^{-1}) was the next best adjuvant. This was followed by glycerol one and three per cent (5.79 x 10^8 spores ml^{-1} and 5.82 x 10^8 spores ml^{-1}), tween-80 three and five per cent (5.74 x 10^8 spores ml^{-1} and 5.57 x 10^8 spores ml^{-1}), PEG 0.5 per cent (5.68 x 10^8 spores ml^{-1}), POX two and five per cent (5.64 x 10^8 spores ml^{-1} and

Table 21. Effect of adjuvants on the spore count of *L. lecanii* in chitin enriched groundnut oil formulation stored under refrigeration

Adjuvants	**Concentration (%)**	***Mean spore count (x 10^8 spores ml^{-1})**					
		15 DAS	**30 DAS**	**45 DAS**	**60 DAS**	**75 DAS**	**90 DAS**
Polyethylene Glycol	0.5	6.55 (2.56)	6.49 (2.54)	6.40 (2.53)	6.37 (2.52)	5.68 (2.38)	5.35 (2.31)
	1	6.61 (2.57)	6.54 (2.55)	6.49 (2.54)	6.46 (2.54)	5.86 (2.42)	5.54 (2.35)
	2	6.73 (2.59)	6.62 (2.57)	6.56 (2.56)	6.47 (2.54)	6.05 (2.46)	5.85 (2.41)
Polyoxyethylene	0.5	6.70 (2.58)	6.61 (2.57)	6.56 (2.56)	6.48 (2.54)	6.11 (2.47)	5.84 (2.41)
	2	6.67 (2.58)	6.58 (2.56)	6.56 (2.56)	6.35 (2.52)	5.64 (2.37)	5.48 (2.34)
	5	6.67 (2.58)	6.57 (2.58)	6.51 (2.55)	6.37 (2.52)	5.58 (2.36)	5.43 (2.33)
Tween-80	1	6.73 (2.59)	6.68 (2.58)	6.58 (2.56)	6.49 (2.54)	6.18 (2.48)	5.88 (2.42)
	3	6.66 (2.58)	6.61 (2.57)	6.53 (2.55)	6.40 (2.53)	5.74 (2.39)	5.50 (2.34)
	5	6.69 (2.58)	6.56 (2.56)	6.50 (2.55)	6.43 (2.53)	5.57 (2.36)	5.29 (2.30)
Glycerol	1	6.68 (2.58)	6.55 (2.56)	6.59 (2.56)	6.49 (2.58)	5.79 (2.40)	5.47 (2.33)
	3	6.66 (2.58)	6.62 (2.57)	6.55 (2.56)	6.49 (2.54)	5.82 (2.41)	5.61 (2.36)
	5	6.72 (2.59)	6.63 (2.57)	6.55 (2.56)	6.46 (2.54)	6.23 (2.49)	5.89 (2.42)
Formulation without adjuvant		6.66 (2.58)	6.67 (2.58)	6.59 (2.56)	6.49 (2.54)	5.72 (2.39)	5.48 (2.34)
CD (0.05)		**NS**	**NS**	**NS**	**NS**	**0.050**	**0.070**

* Mean of three replications, DAS: Days after storage, Figures in parentheses are √x transformed values

5.58 x 10^8 spores ml^{-1}), which did not differ from the check formulation (5.72 x 10^8 spores ml^{-1}).

At the end of three months, glycerol five per cent recorded the highest spore count of 5.89 x 10^8 spores ml^{-1}. This was closely followed by tween-80 one per cent, PEG two per cent and POX 0.5 per cent, their spore counts being 5.88 x 10^8 spores ml^{-1}, 5.85 x 10^8 spores ml^{-1} and 5.84 x 10^8 spores ml^{-1}, respectively. As that of earlier, rest of the formulations *viz.* glycerol one and three per cent, PEG 0.5 per cent, tween-80 three and five per cent, POX two and five per cent were similar to that of the check in terms of spore count. The spore counts observed were 5.47 x 10^8 spores ml^{-1}, 5.61 x 10^8 spores ml^{-1}, 5.35 x 10^8 spores ml^{-1}, 5.54 x 10^8 spores ml^{-1}, 5.50 x 10^8 spores ml^{-1}, 5.29 x 10^8 spores ml^{-1}, 5.48 x 10^8 spores ml^{-1} and 5.43 x 10^8 spores ml^{-1}, respectively.

The formulations to which glycerol five per cent, tween-80 one per cent, PEG two per cent and POX 0.5 per cent were added as adjuvants recorded the highest spore count over three months of storage. PEG 0.5 per cent, POX and tween-80 five per cent significantly reduced the spore counts under room temperature while under refrigeration, the remaining concentrations of adjuvants retained spore count more or less similar to that of check.

4.3.2.2 Chitin Enriched Sunflower Oil Formulation

4.3.2.2.1 Viability

4.3.2.2.1.1 Room temperature

The data on cfu (Table 22) revealed that after 15 days of storage, the formulation with glycerol five per cent with 3.89 x 10^6 ml^{-1} was found to be the best adjuvant. The viability observed with glycerol three per cent (3.72 x 10^6 ml^{-1}), PEG one and two per cent (3.52 x 10^6 ml^{-1} and 3.70 x 10^6 ml^{-1}), tween-80 one and three per cent (3.65 x 10^6 $ml^{-1}$and 3.51 x 10^6 ml^{-1}) POX 0.5 per cent (3.63 x 10^6 ml^{-1}), glycerol

one per cent (3.58 x 10^6 ml^{-1}), POX two per cent (3.48 x 10^6 ml^{-1}) and PEG 0.5 per cent (3.46 x 10^6 ml^{-1}) were on par with that of the formulation without any adjuvants (3.67 x 10^6 ml^{-1}). Tween-80 five per cent and POX five per cent recorded the lowest cfu of 3.45 x 10^6 ml^{-1} and 3.42 x 10^6 ml^{-1}.

After 30 DAS, glycerol one per cent recorded maximum cfu of 3.61 x 10^6 ml^{-1} which was found to be on par with PEG 2 (3.50 x 10^6 ml^{-1}), tween-80 one per cent (3.46 x 10^6 ml^{-1}), POX 0.5 and two per cent (3.36 x 10^6 ml^{-1} and 3.29 x 10^6 ml^{-1}), glycerol one and three per cent (3.31 x 10^6 ml^{-1} and 3.34 x 10^6 ml^{-1}) and PEG one per cent (3.23 x 10^6 ml^{-1}). Lowest cfu was recorded with tween-80 five per cent, PEG 0.5 per cent and POX five per cent (3.14 x 10^6 ml^{-1}, 3.08 x 10^6 ml^{-1} and 3.08 x 10^6 ml^{-1}, respectively) which did not differ significantly from that of the formulation without any adjuvant (2.94 x 10^6 ml^{-1}).

The cfu at 45 DAS indicated that tween-80 one per cent, glycerol five per cent, PEG two per cent and POX 0.5 were the suitable concentrations of adjuvants with cfu 3.44 x 10^6 ml^{-1}, 3.34 x 10^6 ml^{-1}, 3.32 x 10^6 ml^{-1} and 3.24 x 10^6 ml^{-1}, respectively. This was followed by PEG one per cent, tween-80 three per cent, glycerol one per cent and POX two per cent (3.18 x 10^6 ml^{-1}, 3.13 x 10^6 ml^{-1}, 3.10 x 10^6 ml^{-1} and 3.08 x 10^6 ml^{-1}, respectively). The check formulation (2.86 x 10^6 m^{-1}) did not show any significant difference in cfu from POX two per cent (3.08 x 10^6 ml^{-1}), glycerol three per cent (2.96 x 10^6 ml^{-1}), PEG 0.5 per cent (2.86 x 10^6 ml^{-1}), POX five per cent (2.84 x 10^6 ml^{-1}) and tween-80 five per cent (2.70 x 10^6 ml^{-1}).

By the end of 60 DAS, glycerol five per cent and tween-80 one per cent recorded maximum cfu of 3.25 x 10^6 ml^{-1} and 3.16 x 10^6 ml^{-1}. The next high values of cfu were obtained with PEG two per cent, POX 0.5 and two per cent with 2.72 x 10^6 ml^{-1}, 2.70 x 10^6 ml^{-1} and 2.65 x 10^6 ml^{-1}, respectively). These were followed by tween-80 three and five per cent, glycerol one and three per cent, PEG

Table 22. Effect of adjuvants on the viability of *L. lecanii* in chitin enriched sunflower oil formulation stored under room temperature

Sl no.	Adjuvants	Concentration (%)	*Mean number of cfu (x 10^6 ml^{-1})					
			15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
1	Polyethylene Glycol	0.5	3.46(1.86)	3.14(1.77)	2.86(1.69)	2.44(1.56)	1.85(1.36)	1.43(1.20)
2		1	3.52(1.88)	3.23(1.80)	3.18(1.78)	2.52(1.59)	1.93(1.39)	1.46(1.21)
3		2	3.70(1.92)	3.50(1.87)	3.32(1.92)	2.72(1.65)	2.29(1.51)	1.78(1.33)
4	Polyoxyethylene	0.5	3.63(1.91)	3.36(1.83)	3.24(1.83)	2.70(1.64)	2.42(1.55)	1.83(1.35)
5		2	3.48(1.87)	3.29(1.81)	3.08(1.81)	2.65(1.63)	2.24(1.50)	1.35(1.16)
6		5	3.42(1.85)	3.08(1.75)	2.84(1.75)	2.43(1.56)	1.62(1.27)	1.25(1.12)
7	Tween-80	1	3.65(1.91)	3.46(1.86)	3.44(1.86)	3.16(1.78)	2.66(1.63)	2.06(1.43)
8		3	3.51(1.87)	3.20(1.79)	3.13(1.79)	2.55(1.60)	2.23(1.49)	1.32(1.15)
9		5	3.45(1.86)	3.18(1.78)	2.70(1.78)	2.33(1.53)	1.43(1.19)	1.17(1.08)
10	Glycerol	1	3.58(1.89)	3.31(1.82)	3.10(1.82)	2.46(1.57)	2.15(1.47)	1.53(1.24)
11		3	3.72(1.93)	3.34(1.83)	2.96(1.83)	2.53(1.59)	2.07(1.44)	1.54(1.24)
12		5	3.89(1.97)	3.61(1.90)	3.34(1.90)	3.25(1.80)	2.53(1.59)	2.17(1.47)
13	Formulations without adjuvant		3.67(1.92)	2.94(1.71)	2.86(1.71)	2.27(1.51)	1.44(1.20)	1.34(1.16)
	CD (0.05)		**0.058**	**0.088**	**0.080**	**0.091**	**0.091**	**0.119**

*Mean of three replications, DAS: Days after storage, Figures in parentheses are √x transformed values

0.5 and one per cent and POX five having per cent with cfu values, 2.55 x 10^6 ml^{-1}, 2.33 x 10^6 ml^{-1}, 2.46 x 10^6 ml^{-1}, 2.53 x 10^6 ml^{-1}, 2.44 x 10^6 ml^{-1}, 2.52 x 10^6 ml^{-1} and 2.43 x 10^6 ml^{-1}, respectively which were statistically on par with the formulation without any adjuvant (2.27 x 10^6 ml^{-1}).

The cfu recorded after 75 days of storage revealed that tween-80 one per cent was the best adjuvant, followed by glycerol five per cent, POX 0.5 per cent and PEG two per cent, their cfu values being 2.66 x 10^6 ml^{-1}, 2.53 x 10^6 ml^{-1}, 2.42 x 10^6 ml^{-1} and 2.29 x 10^6 ml^{-1}, respectively. The cfu obtained with POX two per cent (2.24 x 10^6 ml^{-1}), tween-80 three per cent (2.23 x 10^6 ml^{-1}), glycerol one and two per cent (2.15 x 10^6 ml^{-1} and 2.07 x 10^6 ml^{-1}) and PEG 0.5 and one per cent (1.85 x 10^6 ml^{-1} and 1.93 x 10^6 ml^{-1}) were found to be significantly higher than the check formulation. Lowest cfu obtained with POX and tween-80 five per cent were statistically similar to that of the formulation without any adjuvant, their values being 1.62 x 10^6 ml^{-1}, 1.44 x 10^6 ml^{-1} and 1.43 x 10^6 ml^{-1}, respectively.

By end of 90 DAS, the best adjuvant was found to be glycerol five per cent with cfu being 2.27 x 10^6 ml^{-1}. Tween-80 one per cent (cfu 2.06 x 10^6 ml^{-1}), POX 0.5 per cent (1.81 x 10^6 spores ml^{-1}) and PEG two per cent (1.78 x 10^6 ml^{-1}) were ranked next. The cfu observed with rest of the formulations with adjuvants, *viz.* glycerol one and three per cent, PEG 0.5 and one per cent, POX two and five per cent and tween-80 three and five per cent did not differ significantly from that of the formulation without adjuvant. The cfu recorded were 1.53 x 10^6 ml^{-1}, 1.54 x 10^6 ml^{-1}, 1.43 x 10^6 ml^{-1}, 1.46 x 10^6 ml^{-1}, 1.35 x 10^6 ml^{-1}, 1.25 x 10^6 ml^{-1}, 1.32 x 10^6 ml^{-1}, 1.17 x 10^6 spores ml^{-1} and 1.34 x 10^6 spores ml^{-1}, respectively.

The adjuvants viz. glycerol, tween-80, PEG and POX at five, one , two and 0.5 per cent respectively were found to be desirable for retaining viability of the formulations based on chitin enriched sunflower oil.

4.3.2.2.1.2 Refrigeration

Upto one month of storage, the viability observed with chitin enriched sunflower oil formulations with various adjuvants at different concentrations did not show any significant variation. The cfu values ranged from 4.42 x 10^6 ml^{-1} to 5.18 x 10^6 ml^{-1} (Table 23).

By 45 DAS, glycerol one per cent recorded maximum number of viable colonies, 3.65 x 10^6 ml^{-1}. This was followed by tween-80 one per cent, PEG two per cent, POX 0.5 per cent, glycerol three and one per cent, which were on par with the formulation without any adjuvant. Their cfu values were 3.55 x 10^6 ml^{-1}, 3.54 x 10^6 ml^{-1}, 3.53 x 10^6 ml^{-1}, 3.48 x 10^6 ml^{-1}, 3.47 x 10^6 ml^{-1} and 3.29 x 10^6 ml^{-1}, respectively. PEG one and 0.5 per cent, tween-80 three per cent and POX two per cent with 3.25 x 10^6 ml^{-1}, 3.13 x 10^6 ml^{-1}, 3.17 x 10^6 ml^{-1} and 3.16 x 10^6 ml^{-1} cfu respectively were similar to that of the check. POX and tween-80 five per cent recorded lowest cfu of 2.93 x 10^6 ml^{-1} and 2.88 x 10^6 ml^{-1} and were inferior to the check formulation.

A similar trend was observed at 60 DAS also. Glycerol five per cent with 3.54 x 10^6 ml^{-1} cfu was the best adjuvant, followed by glycerol three and one per cent, tween-80 one per cent, POX 0.5 per cent and PEG two per cent, which were on par with the formulation without adjuvants. The cfu recorded with these formulations were 3.32 x 10^6 ml^{-1}, 3.08 x 10^6 ml^{-1}, 3.30 x 10^6 ml^{-1}, 3.24 x 10^6 ml^{-1}, 3.17 x 10^6 ml^{-1} and 3.06 x 10^6 ml^{-1}, respectively. The rest of the formulations with PEG 0.5 and one per cent, tween-80 three and five per cent, POX two and five per cent were statistically on par with the formulation without any adjuvants. The number of colonies observed were 2.74 x 10^6 ml^{-1}, 3.05 x 10^6 ml^{-1}, 2.83 x 10^6 ml^{-1}, 2.69 x 10^6 ml^{-1}, 2.74 x 10^6 ml^{-1} and 2.71 x 10^6 ml^{-1}, respectively.

As that of earlier, at 75 DAS also maximum cfu was observed with glycerol five per cent (3.24 x 10^6 ml^{-1}). This was followed by tween-80 one per cent

Table 23. Effect of adjuvants on the viability of *L. lecanii* chitin enriched sunflower oil formulation stored under refrigeration

Sl no.	Adjuvants	Concentration (%)	*Mean number of cfu (x 10^6 ml^{-1})					
			15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
1	Polyethylene Glycol	0.5	4.12 (2.03)	3.39 (1.84)	3.13 (1.77)	2.74 (1.65)	2.57 (1.06)	2.21 (1.49)
2		1	4.25 (2.06)	3.52 (1.88)	3.25 (1.8)	3.05 (1.75)	2.74 (1.65)	2.34 (1.53)
3		2	4.46 (2.11)	3.66 (1.91)	3.54 (1.88)	3.17 (1.78)	2.89 (1.70)	2.69 (1.64)
4	Polyoxyethylene	0.5	4.53 (2.13)	3.72 (1.93)	3.52 (1.88)	3.24 (1.80)	2.94 (1.71)	2.53 (1.59)
5		2	4.08 (2.02)	3.42 (1.85)	3.16 (1.78)	2.74 (1.66)	2.67 (1.63)	2.45 (1.56)
6		5	3.98 (1.99)	3.37 (1.84)	2.93 (1.71)	2.71 (1.65)	2.56 (1.60)	2.23 (1.49)
7	Tween-80	1	4.47 (2.11)	3.73 (1.93)	3.55 (1.88)	3.30 (1.82)	3.10 (1.76)	2.73 (1.65)
8		3	4.12 (2.03)	3.60 (1.90)	3.17 (1.78)	2.83 (1.68)	2.77 (1.66)	2.46 (1.57)
9		5	4.01 (2.00)	3.45 (1.86)	2.88 (1.70)	2.69 (1.64)	2.61 (1.61)	2.25 (1.50)
10	Glycerol	1	4.29 (2.07)	3.52 (1.88)	3.47 (1.86)	3.08 (1.75)	2.72 (1.65)	2.30 (1.52)
11		3	4.38 (2.09)	3.54 (1.88)	3.48 (1.86)	3.32 (1.82)	2.81 (1.68)	2.47 (1.57)
12		5	4.52 (2.13)	3.83 (1.96)	3.65 (1.91)	3.54 (1.88)	3.24 (1.80)	2.91 (1.71)
13	Formulation without adjuvant		4.26 (2.06)	3.6 (1.90)	3.29 (1.81)	3.06 (1.78)	2.73 (1.65)	2.37 (1.54)
	CD (0.05)		**NS**	**NS**	**0.069**	**0.116**	**0.068**	**0.061**

*Mean of three replications, DAS: Days after storage, Figures in parentheses are √x transformed values

(3.10 x 10^6 ml^{-1}), POX 0.5 per cent (2.94 x 10^6 ml^{-1}) and PEG two per cent (2.89 x 10^6 ml^{-1}). The cfu obtained with remaining formulations with adjuvants, *viz.* glycerol one and three per cent, tween-80 three and five per cent, PEG 0.5 and one per cent, POX two and five per cent (2.56 x 10^6 ml^{-1} to 2.81 x10^6 ml^{-1}) were more or less similar to the check formulation (2.73 x 10^6 ml^{-1}).

By the end of the experimental period (90 DAS), glycerol five per cent was found to be the superior adjuvant followed by tween-80 one per cent, PEG two per cent and POX 0.5 per cent. PEG one per cent, tween-80 three per cent and glycerol one per cent, their cfu being 2.91 x 10^6 ml^{-1}, 2.73 x 10^6 ml^{-1}, 2.69 x 10^6 ml^{-1} and 2.53 x 10^6 ml^{-1}, respectively. The viability observed with rest of the formulations, *viz.* glycerol one and three per cent (2.30 x 10^6 ml^{-1} and 2.47 x 10^6 ml^{-1}), tween-80 three and five per cent (2.46 x 10^6 ml^{-1} and 2.25 x 10^6 ml^{-1}), POX two and five per cent (2.45 x 10^6 $ml^{-1}$and 2.23 x 10^6 ml^{-1}), PEG 0.5 and one per cent (2.21 x 10^6 ml^{-1} and 2.34 x 10^6 ml^{-1}) did not differ significantly from the check formulation (2.37 x 10^6 ml^{-1}).

The formulations with glycerol five per cent, tween-80 one per cent, PEG two per cent and POX 0.5 per cent were found to improve the viability both in room temperature as well as under refrigeration.

4.3.2.2.2 Spore count

4.3.2.2.2.1 Room temperature

The spore count of *L. lecanii* formulations with various adjuvants like polyethylene glycol (PEG), polyoxyethylene (POX), tween-80 and glycerol at different concentrations did not exhibit any significant difference from each other as well as from that of the formulation without any adjuvant, till one month after storage. The spore count values ranged from 3.15 x 10^8 spores ml^{-1} to 3.87 x 10^8 spores ml^{-1} (Table 24).

But by 45 DAS, formulation with glycerol five per cent was found to be superior with a spore count of 3.87 x 10^8 spores ml^{-1} which was on par with tween-80 one per cent (3.80 x 10^8 spores ml^{-1}). This was followed by POX 0.5 per cent (3.68 x 10^8 spores ml^{-1}), PEG two per cent (3.64 x 10^8 spores ml^{-1}) and glycerol three per cent (3.46 x 10^8 spores ml^{-1}), which was on par with that of the check formulation (3.52 x 10^8 spores ml^{-1}). POX two and five per cent, PEG 0.5 and one per cent, glycerol one per cent, tween-80 three and five per cent recorded a spore count lesser than the check formulation, their spore count being 3.35 x 10^8 spores ml^{-1}, 3.20 x 10^8 spores ml^{-1}, 3.31 x 10^8 spores ml^{-1}, 3.34 x 10^8 spores ml^{-1}, 3.26 x 10^8 spores ml^{-1}, 3.21 x 10^8 spores ml^{-1} and 3.15 x 10^8 spores ml^{-1}, respectively.

Glycerol five per cent was found to be the superior adjuvant for formulating *L. lecanii* with spore count being 3.54 x 10^8 spores ml^{-1} after 60 DAS. The next best adjuvant was tween-80 one per cent with a spore count of 3.44 x 10^8 spores ml^{-1} which was on par with POX 0.5 per cent (3.31 x 10^8 spores ml^{-1}). This was followed by glycerol three per cent, PEG two per cent and glycerol one per cent with 3.28 x 10^8 spores ml^{-1}, 3.15 x 10^8 spores ml^{-1} and 3.14 x 10^8 spores ml^{-1} and were significantly superior to rest of the treatments. Tween-80 three per cent (2.95 x 10^8 spores ml^{-1}), PEG one per cent (2.91x 10^8 spores ml^{-1}) and PEG 0.5 per cent (2.86 x 10^8 spores ml^{-1}) showed statistical similarity with formulation without adjuvants (2.94 x 10^8 spores ml^{-1}). The lowest spore count was observed with higher concentrations of POX (2 and 5 per cent) and tween-80 (5 per cent), with spore count 2.69 x 10^8 spores ml^{-1}, 2.41 x 10^8 spores ml^{-1} and 2.37 x 10^8 spores ml^{-1}, respectively.

By the end of 75 DAS also glycerol at five per cent was the best adjuvant (2.68 x 10^8 spores ml^{-1}). This was followed by tween-80 one per cent with 2.51 x 10^8 spores ml^{-1}, on par with POX 0.5 per cent (2.43 x 10^8 spores ml^{-1}). The PEG and POX two per cent, glycerol one and three per cent were statistically on par with the spore count obtained with formulation without adjuvants, the spore count being 2.45 x 10^8 spores ml^{-1}, 2.30 x 10^8 spores ml^{-1}, 2.23 x 10^8 spores ml^{-1},

Table 24. Effect of adjuvants on the spore count of *L. lecanii* in chitin enriched sunflower oil formulation stored at room temperature

Sl no.	Adjuvants	Concentration (%)	*Mean spore count (x 10^8 spores ml^{-1})					
			15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
1	Polyethylene Glycol	0.5	4.53 (2.13)	4.21 (2.05)	3.31 (1.82)	2.86 (1.69)	2.13 (1.46)	1.18 (1.08)
2		1	4.65 (2.16)	4.26 (2.06)	3.34 (1.83)	2.91 (1.71)	2.16 (1.47)	1.24 (1.12)
3		2	4.66 (2.16)	4.36 (2.09)	3.64 (1.91)	3.15 (1.78)	2.45 (1.57)	1.60 (1.26)
4	Polyoxyethylene	0.5	4.56 (2.14)	4.34 (2.08)	3.68 (1.92)	3.31 (1.82)	2.53 (1.59)	1.65 (1.28)
5		2	4.58 (2.14)	4.24 (2.06)	3.35 (1.83)	2.69 (1.64)	2.30 (1.52)	1.19 (1.09)
6		5	4.57 (2.14)	4.13 (2.03)	3.20 (1.79)	2.37 (1.54)	2.07 (1.44)	0.88 (0.94)
7	Tween-80	1	4.74 (2.18)	4.49 (2.12)	3.80 (1.95)	3.44 (1.86)	2.61 (1.62)	1.68 (1.30)
8		3	4.55 (2.13)	4.32 (2.08)	3.21 (1.79)	2.95 (1.72)	2.11 (1.45)	1.26 (1.12)
9		5	4.43 (2.11)	4.08 (2.02)	3.15 (1.77)	2.41 (1.55)	2.04 (1.43)	1.04 (1.02)
10	Glycerol	1	4.47 (2.12)	4.28 (2.07)	3.26 (1.81)	3.14 (1.77)	2.23 (1.49)	1.55 (1.25)
11		3	4.50 (2.12)	4.30 (2.07)	3.46 (1.86)	3.28 (1.81)	2.31 (1.52)	1.58 (1.26)
12		5	4.64 (2.15)	4.55 (2.13)	3.87 (1.97)	3.55 (1.88)	2.68 (1.64)	1.71 (1.31)
13	Formulation without adjuvant		4.69 (2.17)	4.45 (2.11)	3.52 (1.88)	2.94 (1.72)	2.38 (1.54)	1.20 (1.10)
	CD (0.05)		**NS**	**NS**	**0.045**	**0.049**	**0.061**	**0.700**

* Mean of three replications, DAS: Days after storage, Figures in parentheses are √x transformed values

2.31 x 10^8 spores ml^{-1} and 2.38 x 10^8 spores ml^{-1}, respectively. The spore count observed with PEG 0.5 and one per cent (2.13 x 10^8 spores ml^{-1} and 2.16 x 10^8 spores ml^{-1}), tween-80 three and five per cent (2.11 x 10^8 spores ml^{-1} and 2.04 x 10^8 spores ml^{-1}) and POX five per cent (2.07 x 10^8 spores ml^{-1}) were the lowest and inferior to that of the check formulation.

After 90 DAS, the spore count noticed with glycerol five, three per cent and one per cent, tween-80 one per cent, POX 0.5 per cent and PEG two per cent were the adjuvants. The values recorded were 1.71 x 10^8 spores ml^{-1}, 1.58 x 10^8 spores ml^{-1}, 1.55 x 10^8 spores ml^{-1}, 1.68 x 10^8 spores ml^{-1}, 1.65 x 10^8 spores ml^{-1} and 1.60 x 10^8 spores ml^{-1}, respectively. Rest of the formulations with adjuvants namely, tween-80 three per cent, PEG 0.5 and one per cent and POX two per cent were on par with the check formulation. The spore count recorded were 1.26 x 10^8 spores ml^{-1}, 1.18 x 10^8 spores ml^{-1}, 1.24 x 10^8 spores ml^{-1}, 1.19 x 10^8 spores ml^{-1} and 1.20 x 10^8 spores ml^{-1}, respectively. Lowest spore count was recorded with tween-80 and POX five per cent (1.04 1.20 x 10^8 spores $ml^{-1}$and 0.88 1.20 x 10^8 spores ml^{-1}).

4.3.2.2.2.2 Refrigeration

Under refrigeration, the formulations with different adjuvants at varying concentrations did not show any significant difference in spore counts till two months of storage. The values ranged from 5.56 x 10^8 spores ml^{-1} to 5.86 x 10^8 spores ml^{-1} (Table 25).

But by 75 DAS, maximum spore count was recorded with glycerol five per cent (5.86 x 10^8 spores ml^{-1}). The spore counts observed with rest of the formulations *viz.* tween-80 one, three and five per cent (5.71 x 10^8 spores ml^{-1}, 5.46 x 10^8 spores ml^{-1} and 5.38 x 10^8 spores ml^{-1}), PEG 0.5, one and two per cent (5.50 x 10^8 spores ml^{-1}, 5.57 x 10^8 spores ml^{-1} and 5.68 x 10^8 spores ml^{-1}), POX 0.5 and two per cent (5.65 x 10^8 spores ml^{-1} and 5.41 x 10^8 spores ml^{-1}), glycerol one and three per cent (5.63 x 10^8 spores ml^{-1}) were more or less similar to the formulation

Table 25. Effect of adjuvants on the spore count of *L. lecanii* in chitin enriched sunflower oil formulation stored under refrigeration

Sl no.	Adjuvants	Concentration (%)	Mean spore count (x 10^8 spores ml^{-1})					
			15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
1	Polyethylene Glycol	0.5	6.11 (2.47)	5.84 (2.41)	5.79 (2.40)	5.65 (2.37)	5.50 (2.34)	5.22 (2.29)
2		1	6.09 (2.46)	5.93 (2.43)	5.83 (2.41)	5.72 (2.39)	5.57 (2.36)	5.34 (2.31)
3		2	6.15 (2.48)	6.01 (2.45)	5.95 (2.44)	5.82 (2.41)	5.68 (2.38)	5.54 (2.35)
4	Polyoxyethylene	0.5	6.06 (2.46)	5.95 (2.44)	5.86 (2.42)	5.76 (2.40)	5.65 (2.37)	5.53 (2.35)
5		2	6.08 (2.46)	5.94 (2.43)	5.77 (2.40)	5.65 (2.37)	5.41 (2.32)	5.34 (2.31)
6		5	6.01 (2.45)	5.91 (2.43)	5.80 (2.41)	5.56 (2.35)	5.21 (2.28)	4.84 (2.20)
7	Tween-80	1	6.14 (2.47)	6.03 (2.46)	5.91 (2.43)	5.86 (2.42)	5.71 (2.34)	5.56 (2.36)
8		3	6.09 (2.46)	5.93 (2.44)	5.83 (2.41)	5.75 (2.39)	5.46 (2.33)	5.46 (2.34)
9		5	5.99 (2.44)	5.87 (2.42)	5.91 (2.43)	5.62 (2.37)	5.38 (2.31)	5.16 (2.27)
10	Glycerol	1	6.11 (2.47)	6.02 (2.45)	5.77 (2.40)	5.68 (2.38)	5.62 (2.37)	5.36 (2.32)
11		3	6.21 (2.49)	5.98 (2.45)	5.80 (2.41)	5.76 (2.40)	5.63 (2.37)	5.36 (2.32)
12		5	6.12 (2.47)	6.08 (2.47)	5.87 (2.42)	5.81 (2.41)	5.72 (2.39)	5.60 (2.37)
13	Formulation without adjuvant		6.21 (2.49)	6.02 (2.45)	5.79 (2.41)	5.70 (2.38)	5.52 (2.34)	5.35 (2.31)
	CD (0.05)		**NS**	**NS**	**NS**	**NS**	**0.054**	**0.065**

* Mean of three replications, DAS: Days after storage, Figures in parentheses are √x transformed values

without any adjuvants (5.52 x 10^8 spores ml^{-1}). Lowest spore count was recorded with POX five per cent, the spore count being 5.21 x 10^8 spores ml^{-1}.

By end of 90 DAS, glycerol five per cent, tween-80 one per cent, PEG two per cent and POX 0.5 per cent were the superior adjuvants, the spore count being 5.60 x 10^8 spores ml^{-1}, 5.56 x 10^8 spores ml^{-1}, 5.54 x 10^8 spores ml^{-1} and 5.53 x 10^8 spores ml^{-1}, respectively. These were followed by tween-80 three and five per cent (5.46 x 10^8 spores ml^{-1} and 5.16 x 10^8 spores ml^{-1}), PEG 0.5 and one per cent (5.22 x 10^8 spores ml^{-1} and 5.34 x 10^8 spores ml^{-1}), POX two per cent (5.34 x 10^8 spores ml^{-1}), glycerol one and three per cent (5.36 x 10^8 spores ml^{-1}) and were statistically similar to the check formulation (5.35 x 10^8 spores ml^{-1}). Lowest spore count was recorded with POX five per cent, the spore count being 4.84 x 10^8 spores ml^{-1}.

Addition of adjuvants did not cause any significant difference in spore count when the formulations were stored under refrigeration till two months. Glycerol five per cent, tween-80 one per cent, PEG two per cent and POX 0.5 per cent were the suitable concentrations that can be utilized in formulations.

The viability and spore count in formulations added with adjuvants, viz. glycerol five per cent, tween-80 one per cent, PEG two per cent and POX 0.5 per cent were significantly higher than the formulation without any adjuvants. The effect of combinations of these attempted for further improvement of formulation is presented in Table 19 to 20.

4.3.3. Effect of Adjuvant Combinations in *L. lecanii* Formulations

The viability and spore count of formulations with various adjuvant combinations were assessed at room temperature and refrigeration.

4.3.3.1. Viability

4.3.3.1.1. Room temperature

The viability of GNO + chitin formulation with adjuvant combinations, stored at room temperature is presented in Table 26.

The viability observed at 30 DAS revealed that the combination, tween-80 one per cent + glycerol five per cent (AC2) gave maximum number of cfu, 5.08 x 10^6 ml^{-1}. This was followed by tween-80 one per cent + glycerol five per cent + POX 0.5 per cent (AC9), tween-80 one per cent + glycerol five per cent + POX 0.5 per cent + PEG two per cent (AC11) and tween-80 one per cent + glycerol five per cent + PEG two per cent (AC7) with 4.92 x 10^6 cfu ml^{-1}, 4.77 x 10^6 cfu ml^{-1} and 4.81 x 10^6 cfu ml^{-1}, respectively. AC11 and AC7 were found to be on par with AC10 (glycerol five per cent + POX 0.5 per cent + PEG two per cent) with cfu being 4.66 x 10^6 cfu ml^{-1}. The next best combination was found to glycerol five per cent + POX 0.5 per cent (AC6) with a cfu of 4.47 x 10^6 ml^{-1} which was on statistical parity with the combinations, PEG two per cent + glycerol five per cent, AC4 (4.37 x 10^6 ml^{-1}) and tween-80 one per cent + PEG two per cent + POX 0.5 per cent, AC8 (4.27 x 10^6 ml^{-1}). The formulations, tween-80 one per cent + PEG two per cent (AC1), PEG two per cent + POX 0.5 per cent (AC5) and tween-80 one per cent + POX 0.5 (AC3) per cent were not statistically different from their individual check formulations viz. tween-80 one per cent, PEG two per cent and glycerol five per cent (3.70 x 10^6 ml^{-1}, 3.68 x 10^6 ml^{-1} and 3.70 x 10^6 ml^{-1}, respectively). Minimum cfu of 3.57 x 10^6 ml^{-1} was observed with POX 0.5 per cent.

A more or less similar trend was observed by the end of 60 DAS. Three combinations namely, AC2 (4.23 x 10^6 ml^{-1}), AC9 (4.18 x 10^6 ml^{-1}) and AC11 (4.02 x 10^6 ml^{-1}) recorded maximum number of viable colonies. This was followed by four combinations *viz.* AC10, AC7, AC4 and AC6, with cfu values, 3.72 x 10^6 ml^{-1}, 3.63 x 10^6 ml^{-1}, 3.54 x 10^6 ml^{-1} and 3.52 x 10^6 ml^{-1}, respectively. As observed in

earlier observations, cfu of the combinations AC1 (3.15 x 10^6 ml^{-1}), AC5 (3.13 x 10^6 ml^{-1}) and AC8 (3.24 x 10^6 ml^{-1}) were not significantly superior to their individual check formulations (2.89 x 10^6 ml^{-1} to 3.24 x 10^6 ml^{-1}). The lowest cfu of 2.85 x 10^6 ml^{-1} and 2.89 x 10^6 ml^{-1} was observed in POX 0.5 per cent (individual check) and AC3 adjuvants.

By the end of 90 DAS also, the best combination was AC1 (3.71 x 10^6 ml^{-1}), followed by AC9 (3.62 x 10^6 ml^{-1}) AC11 (3.35 x 10^6 ml^{-1}). The combinations AC10 and AC7 were statistically on par, their cfu being 3.06 x 10^6 ml^{-1} and of 2.94 x 10^6 ml^{-1}. The viability recorded with AC6, AC8 and AC4 were statistically on par with each other. The cfu values recorded were 2.60 x 10^6 ml^{-1}, 2.59 x 10^6 ml^{-1} and 2.55 x 10^6 ml^{-1}, respectively. This was followed by AC3 and AC1 with cfu being 2.31 x 10^6 ml^{-1} each. These were not significantly different from their individual checks *viz.* tween-80 one per cent and glycerol five per cent (2.14 x 10^6 ml^{-1}, 2.20 x 10^6 ml^{-1}). The lowest cfu values were obtained with POX 0.5 per cent (2.01 x 10^6 ml^{-1}AC5 (2.01 x 10^6 ml^{-1}) and PEG 2 per cent (1.69 x 10^6 ml^{-1}).

4.3.2.1.1.2 Refrigeration

The results on viability of GNO + chitin formulation with adjuvant combinations, stored at refrigeration is presented below (Table 26).

Under refrigeration, the viability observed at 30 DAS was maximum with the combination, AC2 (5.62 x 10^6 ml^{-1}). This was followed by AC10 (5.50 x 10^6 ml^{-1}), AC11 (5.48 x 10^6 ml^{-1}) and AC4 (5.34 x 10^6 ml^{-1}). The latter was found to be on par with AC9 (5.16 x 10^6 ml^{-1}) and AC7 (5.14 x 10^6 ml^{-1}). The combination AC8 with a cfu of 4.86 x 10^6 ml^{-1} was on statistical parity with the combinations, AC3 (4.80 x 10^6 ml^{-1}), AC4 (4.76 x 10^6 ml^{-1}), AC1 (4.65 x 10^6 ml^{-1}) and AC4 (4.55 x 10^6 ml^{-1}). The lowest spore counts were observed with individual checks, tween-80one per cent, PEG two per cent, glycerol five per cent and POX 0.5 per cent (4.15 x 10^6 ml^{-1} to 4.34 x 10^6 ml^{-1}).

Table 26. Effect of adjuvant combination on the viability of *L. lecanii* in chitin enriched groundnut oil formulation stored at room temperature and under refrigerated conditions

Sl no.	Formulations	*Mean number of cfu (x 10^6 ml^{-1})					
		1 MAS		2 MAS		3 MAS	
		Room temperature	Refrigeration	Room temperature	Refrigeration	Room temperature	Refrigeration
1	Tween 80 1 %	3.70 (1.92)	4.32 (2.08)	3.19 (1.79)	4.06 (2.01)	2.14 (1.46)	3.45 (1.86)
2	PEG 2 %	3.68 (1.92)	4.28 (2.07)	3.00 (1.73)	3.85 (1.96)	1.69 (1.30)	3.23 (1.80)
3	Glycerol 5 %	3.74 (1.93)	4.34 (2.08)	3.24 (1.80)	4.18 (2.04)	2.20 (1.48)	3.56 (1.89)
4	POX 0.5 %	3.57 (1.89)	4.15 (2.04)	2.89 (1.70)	3.61 (1.90)	2.01 (1.42)	2.94 (1.71)
5	AC1	3.66 (1.91)	4.65 (2.16)	3.15 (1.77)	4.35 (2.09)	2.31 (1.52)	3.87 (1.97)
6	AC2	5.08 (2.25)	5.62 (2.37)	4.23 (2.06)	5.29 (2.30)	3.71 (1.93)	4.87 (2.21)
7	AC3	3.62 (1.90)	4.80 (2.19)	2.85 (1.69)	4.12 (2.03)	2.31 (1.52)	3.55 (1.89)
8	AC4	4.38 (2.09)	4.55 (2.13)	3.54 (1.88)	4.24 (2.06)	2.55 (1.60)	3.62 (1.90)
9	AC5	3.74 (1.94)	4.76 (2.18)	3.13 (1.77)	4.28 (2.07)	2.01 (1.42)	3.28 (1.81)
10	AC6	4.92 (2.22)	5.34 (2.31)	3.52 (1.88)	4.63 (2.15)	2.60 (1.61)	4.15 (2.04)
11	AC7	4.77 (2.18)	5.14 (2.27)	3.63 (1.91)	4.76 (2.18)	2.94 (1.72)	3.65 (1.91)
12	AC8	4.26 (2.06)	4.86 (2.21)	3.24 (1.80)	4.29 (2.07)	2.59 (1.61)	3.57 (1.89)
13	AC9	4.47 (2.12)	5.16 (2.27)	4.18 (2.04)	5.24 (2.29)	3.62 (1.90)	4.87 (2.21)
14	AC10	4.66 (2.16)	5.50 (2.35)	3.72 (1.93)	4.90 (2.21)	3.06 (1.75)	4.55 (2.13)
15	AC11	4.81 (2.19)	5.48 (2.34)	4.02 (2.01)	5.16 (2.27)	3.35 (1.83)	4.73 (2.18)
	CD (0.05)	**0.052**	**0.055**	**0.097**	**0.058**	**0.077**	**0.054**

*Mean of three replications, MAS: Months after storage, Figures in parentheses are √x transformed values, AC1- Tween-80 1 % + PEG 2 %, AC2- Tween-80 1 % + glycerol 5 %, AC3- Tween-80 1 % + POX 0.5 %, AC4- PEG 2 % + glycerol 5 %, AC5- PEG 2 % + POX 0.5 %, AC6- Glycerol 5 % + POX 0.5 %, AC7- Tween-80 1 % + PEG 2 % + glycerol 5 %, AC8- Tween-80 1 % + PEG 2 % + POX 0.5 %, AC9- Tween-80 1 % + glycerol 5 % +POX 0.5 %, AC10- PEG 2 % + glycerol 5 % +POX 0.5 %, AC11- Tween-80 1 % + PEG 2 % + POX 0.5 % + glycerol 5 %

At 60 DAS, AC 2(5.29 x 10^6 ml^{-1}) and AC9 (5.24 x 10^6 spores ml^{-1}) obtained maximum spore count. The next superior combinations were AC11 (5.16 x 10^6 ml^{-1}) and AC10 (4.90 x 10^6 ml^{-1}). This was followed by the adjuvant combination AC7 (4.76 x 10^6 ml^{-1}) which was on par with the combinations, AC6 (4.63 x 10^6 ml^{-1}). The cfu of combinations AC1 (4.35 x 10^6 ml^{-1}), AC8 (4.29 x 10^6 ml^{-1}, AC5 (4.28 x 10^6 ml^{-1}), AC4 (4.24 x 10^6 ml^{-1}) and AC3 (4.12 x 10^6 ml^{-1}) were not significantly superior to the individual check formulation, glycerol five per cent (4.18 x 10^6 ml^{-1}). The lowest cfu of 4.06 x 10^6 ml^{-1}, 3.85 x 10^6 ml^{-1} and 3.61 x 10^6 ml^{-1} was observed in tween-80 one per cent, PEG two per cent and POX 0.5 per cent, respectively.

A similar trend was noticed by the end of 90 DAS. Maximum cfu was observed with adjuvant combinations, AC2 (4.87 x 10^6 ml^{-1}) and AC9 (4.87 x 10^6 ml^{-1}), followed by AC11 (4.73 x 10^6 ml^{-1}) and AC10 (4.55 x 10^6 ml^{-1}). The adjuvant combination AC6 (4.15 x 10^6 ml^{-1}) was ranked third. The cfu obtained with combinations AC1 (3.87 x 10^6 ml^{-1}), AC7 (3.65 x 10^6 ml^{-1}, AC4 (3.62 x 10^6 ml^{-1}), AC8 (3.57 x 10^6 ml^{-1}) and AC3 (3.55 x 10^6 ml^{-1}) were not significantly superior to the individual check formulation, glycerol five per cent (3.56 x 10^6 ml^{-1}). The viability recorded with AC5, did not differ significantly from that of tween-80one per cent and PEG two per cent, their cfu being 3.28 x 10^6 ml^{-1}, 3.45 x 10^6 ml^{-1} and 3.23 x 10^6 ml^{-1}, respectively. Lowest cfu was obtained with POX 0.5 per cent (2.94 x 10^6 ml^{-1}).

4.3.2.1.2 Spore count

4.3.2.1.2.1 Room temperature

The data on spore count of GNO + chitin formulation with adjuvant combinations, stored at room temperature is furnished in Table 27.

At 30 DAS, AC2, AC9 and AC11 recorded the maximum spore count, 5.93 x 108 spores ml-1, 5.91 x 108 spores ml-1 and 5.72 x 108 spores ml-1, respectively.

These were followed by AC7 and AC10 per cent with a spore count of 5.44 x 10^8 spores ml^{-1} each. The combinations, AC8 (5.36 x 10^8 spores ml^{-1}), AC6 (5.34 x 10^8 spores ml^{-1}), AC4 (5.32 x 10^8 spores ml^{-1}), AC3 (5.25 x 10^8 spores ml^{-1}), AC5 (5.23 x 10^8 spores ml^{-1}) and AC1 (5.13 x 10^8 spores ml^{-1}) were superior to the individual checks *viz.* tween-80 one per cent (4.32 x 10^8 spores ml^{-1}), PEG two per cent (4.28 x 10^8 spores ml^{-1}), glycerol five per cent (4.54 x 10^8 spores ml^{-1}) and POX 0.5 per cent (4.36 x 10^8 spores ml^{-1}).

The spore count observed at 60 DAS, revealed that maximum spore counts were obtained with adjuvant combinations, AC2, AC9, AC11, AC10 and AC7. The spore counts recorded were 5.19 x 10^8 spores ml^{-1}, 5.12 x 10^8 spores ml^{-1}, 5.04 x 10^8 spores ml^{-1}, 4.81 x 10^8 spores ml^{-1} and 4.72 x 10^8 spores ml^{-1}, respectively. AC6 (4.27 x 10^8 spores ml^{-1}), AC4 (4.15 x 10^8 spores ml^{-1}), AC3 (4.14 x 10^8 spores ml^{-1}), AC1 (4.10 x 10^8 spores ml^{-1}) and AC5 (4.08 x 10^8 spores ml^{-1}) did not differ significantly from each other. The spore count recorded with the adjuvant combination, AC8 (4.04 x 10^8 spores ml^{-1}) was found to be on par with individual check, *viz.* glycerol five per cent (3.66x 10^8 spores ml^{-1}). Rest of the individual check formulations with the adjuvants, tween-80 one per cent, PEG two per cent and POX 0.5 per cent recorded significantly lower spore count (3.47 x 10^8 spores ml^{-1}, 3.19 x 10^8 spores ml^{-1} and 3.23 x 10^8 spores ml^{-1}, respectively) than the combinations.

By the end of experimental period (90 DAS), AC 2 and AC9t were the superior adjuvant combinations, with spore count of 3.44 x 10^8 spores ml^{-1} and 3.40 x 10^8 spores ml^{-1}. The next best adjuvant combinations were AC11 with a spore count 3.00 x 10^8 spores ml^{-1}. This was followed by AC7, AC10, AC8 and AC6 (2.85 x 10^8 spores ml^{-1}, 2.80 x 10^8 spores ml^{-1}, 2.59 x 10^8 spores ml^{-1} and 2.55 x 10^8 spores ml^{-1}, respectively). The spore count obtained with AC1 (2.39 x 10^8 spores ml^{-1}), AC5 (2.39 x 10^8 spores ml^{-1}), AC4 (2.37 x 10^8 spores ml^{-1}, AC3 (2.33 x 10^8 spores ml^{-1}) were on par with the individual check formulations *viz.*

glycerol five per cent (2.27 x 10^8 spores ml^{-1}), tween-80one per cent (2.20 x 10^8 spores ml^{-1}) and POX 0.5 per cent (2.05 x 10^8 spores ml^{-1}). Lowest spore count was recorded with PEG 0.5 per cent, spore count being 1.84 x 10^8 spores ml^{-1}.

4.3.2.1.1.2 Refrigeration

None of the formulations showed significant variation in spore count during first two months of storage under refrigeration (Table 27). The spore count values ranged from 4.81 x 10^8 spores ml^{-1} to 5.41 x 10^8 spores ml^{-1}.

But by 90 DAS, a similar trend as in room temperature was observed with the spore count. The adjuvant combinations, *viz.* AC2, AC9, AC10 and AC11 recorded the maximum spore count of 4.59 x 10^8 spores ml^{-1}, 4.50 x 10^8 spores ml^{-1}, 4.49 x 10^8 spores ml^{-1} and 4.48 x 10^8 spores ml^{-1}, respectively. This was followed by AC6 (4.39 x 10^8 spores ml^{-1}), AC7 (4.38 x 10^8 spores ml^{-1}) and AC8 (4.27 x 10^8 spores ml^{-1}). The adjuvant combinations, AC4, AC5, AC1 and AC3 recorded spore count which was on par with that of the individual checks, glycerol five per cent, tween-80 one per cent and PEG two per cent (3.95 x 10^8 spores ml^{-1}, 3.79 x 10^8 spores ml^{-1}, 3.76 x 10^8 spores ml^{-1} and 3.71 x 10^8 spores ml^{-1}, respectively). The lowest spore count was obtained in formulation with POX 0.5 per cent as adjuvant, value being 3.39 x 10^8 spores ml^{-1}.

Out of the 11 adjuvant combinations tested, AC 2 (glycerol five per cent + tween-80 one per cent), AC 9 (tween-80one per cent + glycerol five per cent + POX 0.5 per cent) and AC 11 (tween-80 one per cent + PEG two per cent + glycerol five per cent + POX 0.5 per cent were found to be effective in maintaining the viability and spore count of chitin enriched ground nut oil formulations.

4.3.4. Effect of UV protectants on *L. lecanii*

The observations on viability and spore count of *L. lecanii* spore suspension with various UV protectants, after exposure to 365 (long wave) and 260 (short wave) nm for 10, 20 and 30 minutes are presented below.

Table 27. Effect of adjuvant combinations on the spore count of *L. lecanii* in chitin enriched groundnut oil formulation stored at room temperature and under refrigerated conditions

Sl no.	Formulations	*Mean number of cfu (x 10^6 ml^{-1})					
		1 MAS		2 MAS		3 MAS	
		Room temperature	Refrigeration	Room temperature	Refrigeration	Room temperature	Refrigeration
1	Tween 80 1 %	4.32 (2.08)	5.88 (2.43)	3.47 (1.86)	4.81 (2.19)	2.20 (1.48)	3.57 (1.89)
2	PEG 2 %	4.28 (2.07)	5.82 (2.41)	3.19 (1.79)	4.81 (2.19)	1.84 (1.36)	3.48 (1.87)
3	Glycerol 5 %	4.54 (2.13)	5.78 (2.40)	3.66 (1.91)	5.22 (2.28)	2.27 (1.51)	3.66 (1.91)
4	POX 0.5 %	4.36 (2.09)	5.85 (2.42)	3.23 (1.80)	4.99 (2.23)	2.05 (1.43)	3.39 (1.84)
5	AC1	5.13 (2.27)	5.91 (2.43)	4.10 (2.02)	4.92 (2.22)	2.39 (1.55)	3.76 (1.94)
6	AC2	5.93 (2.44)	6.03 (2.46)	5.19 (2.28)	5.39 (2.32)	3.40 (1.84)	4.59 (2.14)
7	AC3	5.25 (2.29)	6.03 (2.46)	4.14 (2.03)	5.23 (2.29)	2.33 (1.53)	3.71 (1.93)
8	AC4	5.32 (2.31)	5.79 (2.41)	4.15 (2.04)	4.85 (2.20)	2.37 (1.54)	3.95 (1.99)
9	AC5	5.23 (2.29)	5.76 (2.40)	4.08 (2.02)	4.87 (2.21)	2.38 (1.54)	3.79 (1.95)
10	AC6	5.36 (2.32)	6.05 (2.46)	4.27 (2.07)	5.40 (2.32)	2.55 (1.60)	4.39 (2.10)
11	AC7	5.44 (2.33)	5.92 (2.43)	4.72 (2.17)	5.29 (2.30)	2.85 (1.69)	4.38 (2.09)
12	AC8	5.34 (2.31)	5.82 (2.41)	4.04 (2.01)	5.25 (2.29)	2.59 (1.61)	4.27 (2.07)
13	AC9	5.91 (2.43)	5.89 (2.43)	5.12 (2.26)	5.29 (2.30)	3.44 (1.86)	4.50 (2.12)
14	AC10	5.44 (2.33)	6.07 (2.46)	4.81 (2.19)	5.41 (2.33)	2.80 (1.67)	4.49 (2.12)
15	AC11	5.72 (2.39)	6.07 (2.46)	5.04 (2.25)	5.30 (2.30)	3.00 (1.73)	4.48 (2.12)
	CD (0.05)	**0.059**	**NS**	**0.102**	**NS**	**0.127**	**0.126**

*Mean of three replications, MAS: Months after storage, Figures in parentheses are √x transformed values, AC1- Tween-80 1 % + PEG 2 %, AC2- Tween-80 1 % + glycerol 5 %, AC3- Tween-80 1 % + POX 0.5 %, AC4- PEG 2 % + glycerol 5 %, AC5- PEG 2 % + POX 0.5 %, AC6- Glycerol 5 % + POX 0.5 %, AC7- Tween-80 1 % + PEG 2 % + glycerol 5 %, AC8- Tween-80 1 % + PEG 2 % + POX 0.5 %, AC9- Tween-80 1 % + glycerol 5 % +POX 0.5 %, AC10- PEG 2 % + glycerol 5 % +POX 0.5 %, AC11- Tween-80 1 % + PEG 2 % + POX 0.5 % + glycerol 5 %

4.3.4.1. Viability

The data on viability of spore suspension after adding UV protectants are given in Table 28. On exposure to long wave UV radiation for 10 minutes, maximum viable colonies (251.79 x 10^4 ml^{-1}) were observed with boric acid one per cent, which was on par with that of the unexposed control (293.85 x 10^4 ml^{-1}). This was followed by boric acid 0.5 percent, cfu being 227.42 x 10^4 ml^{-1}. Boric acid two per cent was superior to rest of the UV protectants, arachid oil and sunflower oil (0.5 to two per cent). Sunflower oil at 0.5, one and two per cent recorded a cfu of 19.73 x 10^4 ml^{-1}, 18.77 x 10^4 ml^{-1} and 31.99 x 10^4 ml^{-1}, respectively. The cfu observed with arachid oil added spore suspension was 17.36 x 10^4 ml^{-1} (0.5 per cent), 16.92 x 10^4 ml^{-1} (one per cent) and 24.95 x 10^4 ml^{-1} (two per cent). Indigo, at two per cent recorded the lowest cfu (3.32 x 10^4 ml^{-1}) whereas, 0.5 and one per cent indigo failed as UV protectant.

Similar trend was noted at 20 and 30 minutes after exposure. At these exposure levels, all the UV protectants recorded significantly lower cfu than unexposed control.

At 20 minutes of exposure, boric acid one per cent was the best UV protectant which recorded a cfu of 182.79 x 10^4 ml^{-1}. The next best UV protectant was boric acid 0.5 per cent with 142.91 x 10^4 ml^{-1}.This was followed by one per cent boric acid (30.53 x 10^4 ml^{-1}). Sunflower oil and arachid oil two per cent were statistically similar (17.05 x 10^4 ml^{-1} and 16.28 x 10^4 ml^{-1}). The cfu observed with sunflower oil (0.5 and one per cent), arachid oil (0.5 and one per cent) and indigo (two per cent) ranged from 1.28 x 10^4 ml^{-1} to 10.35x 10^4 ml^{-1}.

Maximum viable colonies (118.48 x 10^4 ml^{-1}) was recorded with boric acid one per cent after 30 minutes of long wave UV exposure. Boric acid 0.5 per cent was the protectant that was ranked second (86.53 x 10^4 ml^{-1} cfu). This was followed by two per cent arachid oil and one per cent boric acid with cfu, 6.67 x 10^4 ml^{-1} and 6.51x 10^4 ml^{-1}. Sunflower oil and arachid oil recorded significantly lower cfu ranging

from 4.26x 10^4 ml^{-1} to 1.62 x 10^4 ml^{-1}. No viable colonies were observed in indigo added spore suspensions.

On exposure to short wave UV radiation also, maximum number of cfu was obtained with boric acid.

After an exposure period of 10 minutes, boric acid one and 0.5 per cent recorded highest number of cfu, 192.74x 10^4 ml^{-1} and 190.52 x 10^4 ml^{-1}. Boric acid two per cent with 53.45 x 10^4 ml^{-1} number of viable colonies was the next best UV protectant. Rest of the treatments, *viz.* sunflower oil and arachid oil (0.5, one and two per cent) recorded significantly lower cfu (15.33 x 10^4 ml^{-1} to 23.85 x 10^4 ml^{-1}).

A similar trend was observed with 20 and 30 minutes of short wave UV exposure. After 20 minutes exposure, boric acid one per cent was found to be best (159.77 x $10^4 ml^{-1}$). This was followed by boric acid 0.5 per cent (117.77x 10^4 ml^{-1}). Boric acid one per cent (33.50 x 10^4 ml^{-1}) was ranked third. Sunflower oil and arachid oil recorded lowest cfu ranging from 9.27 x 10^4 ml^{-1} to 16.45 x 10^4 ml^{-1}.

After 30 minutes exposure also, boric acid one per cent was the best with cfu 95.67 x 10^4 ml^{-1}, followed by boric acid 0.5 per cent (53.49 x 10^4 ml^{-1}). Boric acid two per cent (4.26 x 10^4 ml^{-1}), sunflower oil 0.5 (1.62 x 10^4 ml^{-1}), one (2.94 x 10^4 ml^{-1}), and two per cent (2.31 x 10^4 ml^{-1}), arachid oil 0.5 (4.26 x 10^4 ml^{-1}), one (2.83 x 10^4 ml^{-1}) and two per cent (3.22 x 10^4 ml^{-1}) recorded significantly lower cfu and were statistically similar.

The assessment of cfu after UV exposure, indicated that boric acid one per cent is an effective UV protectant to *L. lecanii*.

The assessment of spore count did not showed any variation before and after UV irradiation.

Table 28. Effect of UV protectants on the viability of *L. lecanii*

Sl no.	UV protectants (%)		cfu after UV exposure (x 10^4 ml^{-1})					
			10 minute		20 minute		30 minute	
			365 nm	260 nm	365 nm	260 nm	365 nm	260 nm
1	Boric acid	0.5	227.47 (15.08)	190.53 (13.80)	142.87 (11.95)	117.85 (10.85)	86.53 (9.30)	53.49 (7.31)
2		1	251.76 (15.87)	192.74 (13.88)	182.79 (13.52)	159.77 (12.64)	118.48 (10.89)	95.67 (9.78)
3		2	60.59 (7.78)	53.45 (7.31)	30.53 (5.53)	33.50 (5.79)	6.51 (2.55)	4.26 (2.07)
4	Indigo	0.5	0	0	0	0	0	0
5		1	0	0	0	0	0	0
6		2	3.32 (1.82)	0	1.28 (1.13)	0	0	0
7	Sunflower oil	0.5	19.73 (4.44)	17.63 (4.20)	10.35 (3.22)	12.62 (3.55)	2.31 (1.52)	1.62 (1.27)
8		1	18.77 (4.33)	17.18 (4.15)	8.99 (3.00)	10.28 (3.21)	1.90 (1.38)	2.94 (1.71)
9		2	31.99 (5.66)	23.85 (4.88)	17.05 (4.13)	16.45 (4.06)	2.94 (1.71)	2.31 (1.52)
10	Arachid oil	0.5	17.36 (4.17)	15.63 (3.95)	9.96 (3.16)	11.32 (3.36)	4.26 (2.07)	3.32 (1.82)
11		1	16.92 (4.11)	15.33 (3.92)	9.96 (3.16)	9.27 (3.05)	1.62 (1.27)	2.83 (1.68)
12		2	24.95 (5.00)	17.53 (4.19)	16.28 (4.04)	11.27 (3.36)	6.67 (2.58)	4.26 (2.07)
12	Untreated control		293.85 (17.14)	272.65 (16.51)	293.85 (17.14)	272.65 (16.51)	293.85 (17.14)	272.65 (16.51)
14	Treated control		0	0	0	0	0	0
	CD (0.05)		**1.467**	**0.872**	**0.696**	**0.810**	**0.765**	**0.832**

Figures in parentheses are √x transformed values, Long wave UV: 365 nm, Short wave UV: 260 nm

4.3.4.1. Effect of UV protectants in Chitin Enriched Groundnut Oil Formulations

Chitin enriched ground nut oil formulation with selected adjuvant combinations *viz.* AC2 (tween 80 one per cent + glycerol five per cent), AC11 (tween 80 one per cent + PEG two per cent + POX 0.5 per cent + glycerol five per cent), and AC9 (tween 80 one per cent + glycerol five per cent + POX 0.5 per cent) were tested for UV protectancy with boric acid one per cent.

4.3.4.1.1. Viability

The number of cfu noted with the above mentioned formulations is furnished in Table 29. All the formulations with adjuvant combinations *viz.* AC2, AC9 and AC11 with boric acid one per cent were found to be viable even after exposure to UV both at short and long wavelengths. The cfu recorded with these formulations did not differ significantly among themselves as well as with the check (unexposed GNO formulation without boric acid).The number of viable colonies ranged from 1.43 x 10^6 ml^{-1} to 1.46 x 10^6 ml^{-1} in long wave exposure and 1.42 x 10^6 ml^{-1} to 1.54 x 10^6 ml^{-1} short wave exposure.

The assessment of spore count did not showed any variation before and after UV irradiation.

The chitin enriched groundnut oil formulations with adjuvant combinations, AC11 and one per cent boric acid (Formulation I), AC2 and one per cent boric acid (Formulation II) and AC9 and one per cent boric acid (Formulation III) and the UV protectant, boric acid one per cent were superior in terms of viability and UV tolerance.

Table 29. Effect of boric acid on the viability of *L. lecanii* in groundnut oil formulations after UV irradiation with different wavelength and irradiation periods

Sl no.	Formulations	Cfu (x 10^6 ml^{-1})						
		0 min	10 min		20 min		30 min	
			365 nm	260 nm	365 nm	260 nm	365 nm	260 nm
1	AC2	1.70 (1.30)	1.68 (1.30)	1.60 (1.27)	1.56 (1.25)	1.51 (1.23)	1.49 (1.22)	1.45 (1.21)
2	AC9	1.63 (1.28)	1.56 (1.25)	1.61 (1.27)	1.53 (1.24)	1.55 (1.25)	1.45 (1.21)	1.42 (1.19)
3	AC11	1.64 (1.28)	1.57 (1.25)	1.56 (1.25)	1.55 (1.25)	1.57 (1.25)	1.43 (1.20)	1.54 (1.24)
4	Control	1.46 (1.21)	1.46 (1.21)	1.46 (1.21)	1.46 (1.21)	1.46 (1.21)	1.46 (1.21)	1.46 (1.21)
	CD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

AC2 - Tween 80 one per cent + glycerol five per cent, AC9 - Tween 80 one per cent + POX 0.5 per cent + glycerol five per cent, AC11 - Tween 80 one per cent + PEG two per cent + POX 0.5 per cent + glycerol five per cent, Figures in parentheses are √x transformed values

4.4 FIELD EFFICACY OF FORMULATIONS AGAINST SUCKING PESTS OF COWPEA

Efficacy of selected formulations in the management of *Aphis craccivora, Bemisia tabaci, Amrasca biguttula biguttula* and *Tetranychus* sp. are presented in Tables 30 to 33.

4.4.1 *A. craccivora*

4.4.1.1 First spraying (Five per cent level of infestation)

The aphid population assessed from 15 cm terminal shoot is expressed in Table 30. The population did not differ significantly among the plots, prior to imposition of treatments. The average number of aphids ranged from 130.14 to 253.16.

Three days after spraying (DAS), the population was significantly reduced in all the treated plots. The least population was observed with Formulation I (9.23) which was on par with chlorpyriphos 0.05 % treated plots where there was complete control of the pest. In plots treated with formulation III and II, the population recorded was 33.00 and 45.77 which were on par with the standard check *ie.,* KAU talc formulation (77.8). The corresponding population in untreated plots was 224.25.

By seven DAS, further decrease in population was observed in treated plots. As that of earlier, least population was found in plots treated with Formulation I (1.61) which was on par with the cent per cent control obtained with chlorpyriphos treatment. The aphid population in Formulation II and III treated plots were on par with the standard check (21.17, 13.40 and 24.48, respectively). The average number of aphids in untreated plots was 198.83.

At 14 DAS, Formulation I was statistically superior with no aphids in the treated plots and was on par with chlorpyriphos 0.05 %. The population levels were very low in plots treated with Formulation II, III and standard check, the count being

(8.91, 4.51 and 9.20, respectively). The corresponding count in untreated check was 268.38.

4.4.1.2 Second spraying (Fifty per cent level of infestation)

At the time of second spraying, aphid population was very high in all the plots ranging from 553.47 to 619.84 aphids per 15 cm of terminal twig, but the levels did not differ significantly.

At three DAS, the population was very much reduced in plots treated with Formulation I and chlorpyriphos 0.05 % (73.63 and 148.24). This was followed by Formulation II and III (171.21 and 198.76). The standard check (KAU talc formulation) recorded maximum population (265.49) compared to other treatments. The population in untreated plots was significantly high (621.22).

Considerable reduction in number of aphids was noticed seven DAS. Least number was observed with Formulation I treated plots and chlorpyriphos 0.05 % and (17.38 and 12.08). Formulation II and III were also effective in bringing down the population (37.92 and 56.94) but they were inferior to Formulation I and chemical check. All the oil formulations exhibited superiority over the KAU talc formulation. The average number of population in untreated plots was 615.53.

Similar trend was observed at 14 DAS also. Formulation I was on par with the chemical check in reducing the population (3.46 and 2.23), followed by Formulation II and Formulation III (11.02 and 11.37), which were superior to standard check (21.72). The average population in untreated plots was very high (616.62).

4.4.2 *B. tabaci*

The average population before and after treatment recorded from ten leaves per plant is presented in Table 31. The population levels did not differ significantly among the plots and it ranged from 13.14 to 17.55 whiteflies per 10 leaves. At three DAS, all the oil formulations of *L. lecanii* were equally effective in controlling

Table 30. Efficacy of chitin enriched groundnut oil formulations of *L. lecanii* on *A. craccivora* in cowpea

Treatments		Number of aphids/ 15 cm terminal shoot							
		At five per cent level of infestation				At fifty per cent infestation level			
		Precount	**3 DAS**	**7 DAS**	**14 DAS**	**Precount**	**3 DAS**	**7 DAS**	**14 DAS**
Formulation I	AC11 (Tween-80 1 % + PEG 2 % + POX 0.5 % + glycerol 5 %) + Boric acid 1 %	133.18 (11.58)	9.23 (3.2)	1.61 (1.61)	0 (1)	578.05 (24.06)	148.24 (12.22)	17.38 (4.29)	3.46 (2.11)
Formulation II	AC2 (Tween-80 1 % + glycerol 5 %) + Boric acid 1 %	130.14 (11.45)	45.77 (6.84)	21.17 (4.71)	8.91 (3.15)	619.84 (24.92)	171.21 (13.13)	37.92 (6.24)	11.02 (3.47)
Formulation III	AC9 (Tween 80 1 % + POX 0.5 % + glycerol 5 %) + Boric acid 1 %	146.27 (12.14)	33.00 (5.83)	13.40 (3.79)	4.51 (2.35)	605.79 (24.65)	198.76 (14.13)	56.94 (7.61)	11.37 (3.52)
Standard Check	Talc based product (KAU)	156.04 (12.53)	77.8 (8.88)	24.48 (5.05)	9.20 (3.19)	606.27 (24.64)	265.49 (16.32)	77.3 (8.85)	21.72 (4.77)
Chemical check	Chlorpyriphos 0.05 %	171.21 (13.12)	0 (1)	0 (1)	0 (1)	553.47 (23.55)	73.63 (8.64)	12.08 (3.62)	0 (1)
Untreated		253.16 (15.94)	224.25 (15.01)	198.83 (14.14)	268.38 (16.41)	568.17 (23.86)	621.22 (24.94)	615.53 (24.83)	616.62 (24.85)
CD (0.05)		**NS**	**2.838**	**2.687**	**1.926**	**NS**	**4.115**	**1.359**	**0.717**

DAS – Days after spraying; Figures in parentheses are $\sqrt{x + 1}$ transformation.

whiteflies as that of the chlorpyriphos 0.05 % (6.43, 6.62, 5.71 and 1.91) and superior over the KAU talc formulation (10.79). The corresponding population in untreated plots was 17.25. At seven DAS, Formulation I recorded least whitefly population (2.28) which was on par with the population observed in plots treated with chlorpyriphos 0.05 % (0.87). Formulation III and I ranked second with average population of 3.24 and 3.63 respectively. All the oil formulations were found to be superior to the KAU talc formulation, the average population recorded being 6.96. The untreated plots recorded an average population of 18.20. At 14 DAS, least population was recorded in *L. lecanii* formulation, Formulation I (0.66) which was to be on par with other two oil formulations, Formulation II and III as well as with chlorpyriphos 0.05 % (1.30, 1.22 and 1.48). The untreated plots recorded high population of 20.79.

4.4.3 *A. biguttula biguttula*

The jassid population did not differ significantly in the treatment plots before spraying. The average population was 6.52 to 10.09 jassids per 10 leaves (Table 32).

All the formulations of *L. lecanii*, *viz.* Formulation I, II and III and KAU talc formulation, were on par with each other till seven DAS the population being 3.42, 3.85, 3.68 and 4.41, respectively while complete control was recorded in chlorpyriphos 0.05 % treated plots. The corresponding population in untreated plots was 8.07.

By 14 DAS, Formulation I, II and III were equally effective as chlorpyriphos 0.05 %. The average population recorded was 1.46, 2.01, 1.75 and 1.53 respectively. KAU talc formulation was also effective in reducing jassid population (3.30) but was inferior to the test formulations. Population recorded in untreated plots was significantly high (6.00).

Table 31. Efficacy of chitin enriched groundnut oil formulations of *L. lecanii* on *B. tabaci* in cowpea

Treatments		**Number of whiteflies / 10 leaves**				
		Precount	**3 DAS**	**7 DAS**	**14 DAS**	***21 DAS**
Formulation I	AC11 (Tween-80 1 % + PEG 2 % + POX 0.5 % + glycerol 5 %) + Boric acid 1 %	14.75 (3.97)	6.43 (2.73)	2.28 (1.81)	0.66 (1.29)	0
Formulation II	AC2 (Tween-80 1 % + glycerol 5 %) + Boric acid 1 %	13.14 (3.76)	6.62 (2.76)	3.63 (2.15)	1.30 (1.52)	0
Formulation III	AC9 (Tween 80 1 % + POX 0.5 % + glycerol 5 %) + Boric acid 1 %	13.80 (3.85)	5.71 (2.59)	3.24 (2.06)	1.22 (1.49)	0
Standard Check	Talc based product (KAU)	14.63 (3.95)	10.79 (3.43)	6.96 (2.82)	2.77 (1.94)	0
Chemical check	Chlorpyriphos 0.05 %	15.72 (4.09)	1.91 (1.71)	0.87 (1.37)	1.48 (1.57)	0
Untreated		17.55 (4.31)	17.25 (4.27)	18.20 (4.38)	20.79 (4.67)	0
CD (0.05)		**NS**	**1.291**	**0.665**	**0.604**	

DAS: Days after spraying; Figures in parentheses are $\sqrt{x + 1}$ transformation, * No infestation was observed thereafter

Table 32. Efficacy of chitin enriched groundnut oil formulations of *L. lecanii* on *A. biguttula biguttula* in cowpea

Treatments		**Number of jassids / 10 leaves**				
		Precount	**3 DAS**	**7 DAS**	**14 DAS**	***21 DAS**
Formulation I	AC11 (Tween-80 1 % + PEG 2 % + POX 0.5 % + glycerol 5 %) + Boric acid 1 %	10.09 (3.33)	5.80 (2.61)	3.42 (2.10)	1.46 (1.57)	0
Formulation II	AC2 (Tween-80 1 % + glycerol 5 %) + Boric acid 1 %	6.52 (2.74)	5.07 (2.46)	3.85 (2.20)	2.01 (1.74)	0
Formulation III	AC9 (Tween 80 1 % + POX 0.5 % + glycerol 5 %) + Boric acid 1 %	7.75 (2.96)	4.37 (2.32)	3.68 (2.16)	1.75 (1.66)	0
Standard Check	Talc based product (KAU)	6.31 (2.70)	5.48 (2.55)	4.41 (2.33)	3.3 (2.07)	0
Chemical check	Chlorpyriphos 0.05 %	8.50 (3.08)	1.29 (1.51)	0 (1)	1.53 (1.59)	0
Untreated		6.64 (2.76)	8.11 (3.02)	8.07 (3.01)	6 (2.65)	0
CD (0.05)		**NS**	**0.563**	**0.566**	**0.493**	

DAS: Days after spraying; Figures in parentheses are $\sqrt{x + 1}$ transformation. * No infestation was observed thereafter

4.4.4 *Tetranychus* sp.

Pre treatment count of mites did not differ significantly (Table 33). The average population ranged from 54.91 to 68.35 per 10 leaves. Significant reduction in population observed at 3 DAS in plots treated with Formulation I, II and III were on par with each other (26.95, 23.33 and 28.60, respectively). The lowest population was observed with chlorpyriphos 0.05 % treated plots (5.18) while the corresponding population in KAU talc formulation treated plots was 47. The population in untreated plots was 67.53.

At seven DAS Formulation I, II and III effectively controlled mite population (4.98, 7.67 and 5.32) and were superior to KAU talc formulation (14.83). Cent per cent control was observed in chlorpyriphos 0.05 %. The population in untreated plots remained high (55.96). By 14 DAS, Formulation I, II and III treated plots recorded complete control of mites as that of chlorpyriphos 0.05 %. The KAU talc formulation reduced the mite population to significantly lower levels (5.88) but was inferior to oil formulations as well as chlorpyriphos 0.05 %. The population in untreated plots was significantly high (45.42).

4.4.5 Average yield

The average yield obtained from plots treated with bioformulations and chemical insecticide is presented in Table 34. Among the bioformulations, Formulation I and II recorded highest yield of 7.03 and 6.74 kg per plot respectively which was significantly lower than the yield obtained from Chlorpyriphos 0.05 % treated plots (8.57 kg). The yield obtained from plots treated with KAU talc formulation (5.56 kg) did not differ significantly from that of Formulation III treated plots (6.26). Lowest yield was obtained from untreated control (4.44 kg).

Formulation I was equally effective as that of chlorpyriphos 0.05 per cent (chemical control) in controlling aphids both at five and fifity per cent level of infestation. The other two oil formulations, Formulation II and III significantly

Table 33. Efficacy of chitin enriched groundnut oil formulations of *L. lecanii* on *Tetranychus* sp. in cowpea

Treatments		**Number of mites / 10 leaves**				
		Precount	**3 DAS**	**7 DAS**	**14 DAS**	***21 DAS**
Formulation I	AC11 (Tween-80 1 % + PEG 2 % + POX 0.5 % + glycerol 5 %) + Boric acid 1 %	58.23 (7.70)	26.95 (5.29)	4.98 (2.44)	0 (1)	0
Formulation II	AC2 (Tween-80 1 % + glycerol 5 %) + Boric acid 1 %	56.29 (7.57)	23.33 (4.93)	7.67 (2.94)	0 (1)	0
Formulation III	AC9 (Tween 80 1 % + POX 0.5 % + glycerol 5 %) + Boric acid 1 %	68.35 (8.33)	28.60 (5.44)	5.32 (2.51)	0 (1)	0
Standard Check	Talc based product (KAU)	54.91 (7.48)	47.00 (6.93)	14.83 (3.98)	5.88 (2.62)	0
Chemical check	Chlorpyriphos 0.05 %	56.74 (7.60)	5.18 (2.48)	0 (1)	0 (1)	0
Untreated		64.45 (8.09)	67.53 (8.28)	55.96 (7.55)	45.42 (6.81)	0
CD (0.05)		**NS**	**0.896**	**0.540**	**0.623**	

DAS: Days after spraying; Figures in parentheses are $\sqrt{x + 1}$ transformation,* No infestation was observed thereafter

Table 34. Effect of different formulations of *L. lecanii* on yield of cowpea

Sl. No.	Treatments	Yield per plot* (kg)
1	Formulation I	7.03
2	Formulation II	6.74
3	Formulation III	6.26
4	Talc based formulation of *L. lecanii* (Standard Check)	5.56
5	Chlorpyriphos 0.05 % (Chemical check)	8.57
6	Untreated	4.44
	CD (0.05)	**2.20**

*12 plants per plot; Formulation I- Chitin enriched groundnut oil + (PEG 2 % + POX 0.5 % + tween-80 1 % + glycerol 5 %) + Boric acid 1 %, Formulation II- Chitin enriched groundnut oil + (tween-80 1 % + glycerol 5 %) + Boric acid 1 %, Formulation III- Chitin enriched groundnut oil + (POX 0.5 % + tween-80 1 % + glycerol 5 %) + Boric acid 1 %

reduced the number of aphids at fifty per cent level of infestation levels than the standard check. At lower infestation levels oil formulations showed on par effect with that of the KAU talc formulation (standard check). In case of whiteflies and jassids, these oil formulations were effective in managing them as that of chlorpyriphos 0.05 per cent by 14 DAS. Complete control of mites was obtained with these formulations by 14 DAS. Thus, the sucking pests in cowpea can be effectively managed by (GNO + chitin) 65:35 + (tween-80 one per cent + PEG two per cent +POX 0.5 per cent + Glycerol five per cent) + boric acid one per cent.

Among the bioformulations, Formulation I and II recorded highest yield per plot which was significantly lower than the yield obtained from Chlorpyriphos 0.05 % treated plots. The yield obtained from plots treated with KAU talc formulation was on par with Formulation III treated plots.

Major findings

- *L. lecanii* was infective to sucking pests of cowpea, *viz. A. craccivora*, *B. tabaci, A. biguttula biguttula*, *F. virgata*, *Tetranychus* sp. and brinjal scale, *Lecanium* sp.
- The effective dose was 10^7 spores ml^{-1}
- The best media supplement to improve viability and sporulation of *L. lecanii* was chitosan extra pure 5 %
- The formulations based on chitin enriched groundnut oil and sunflower oil were the best in maintaining viability and virulence during storage
- The ideal proportion of carrier : technical ingredient was 65:35 in the case of oil based formulations
- The ideal proportion of carrier : technical ingredient was 80:20 in the case of talc based formulations
- Best adjuvant combination was tween-80 1 % + PEG 2 % + POX 0.5 % + glycerol 5 %

- Boric acid 1 % was the best UV protectant
- Chitin enriched groundnut oil formulation with adjuvant combination (tween-80 1 % + PEG 2 % + POX 0.5 % + glycerol 5 %) and UV protectant (boric acid 1 %) was equally effective as chlorpyriphos 0.05 % in controlling aphids, whiteflies, jassids and mites infesting cowpea.

Discussion

5. DISCUSSION

In pest management programmes microbial agents are alternative tools to conventional pesticides. Entomopathogenic fungi (EPF) has an important position among all the biocontrol agents because of their route of pathogenicity, broad host range and ability to manage both sucking pests and chewing ones with no hazardous effects on human health and environment. Yet, they cover only a small percentage of the total insecticide market owing to their low shelf life. Improvements are needed to fulfill the requirements for high market share.

Development of an entomopathogen as a biopesticide requires basic research on its pathogenicity.

PATHOGENICITY OF *L. lecanii*

Pathogenicity is a qualitative trait referring to the inherent, genetic capacity of a microorganism to cause disease, which is mediated by virulence factors that results in specific host-pathogen interactions. Study on this aspect revealed that the *L. lecanii* isolate Vl8 exhibited difference in their infectivity to various sucking pests. Though there was no remarkable difference in symptoms exhibited by infected insects, considerable variation was noted in mortality rate. When 100 per cent mortality of the aphid, *A. craccivora* and the mite, *Tetranychus* sp. were observed on the fifth day, the mortality was only 63 to 70 per cent in the case of the whitefly, *B. tabaci* and the scale, *Lecanium* sp. Interestingly, *A. pilosum* and *R. pedestris* belonging to the same order did not take infection. Variation in host infectivity is a highly variable biological trait of entomopathogens. Such heterogeneic nature of EPF, already explained by Chandler (2009) may be due to the short generation time of the pathogen and the host, as well as the wide range of habitats they dwell in. Brodeur also (2012) opined this type of variability in *Lecanicillium* is due to its capacity to exploit a wide range of hosts, from arthropod pests to plant pathogenic fungi owing to the difference in mode of action. The interactions between host and fungus are thus

diverse and dynamic. However, evolutionary analyses by researchers such as Ebert (1994), Ewald (1995) and Myers and Rothman (1995) indicated that virulence (death of the host) can be a highly adaptive trait where a pathogen is benefitted by its multiplication within the host.

Dose-mortality response of the aphid *A. craccivora* to *L. lecanii* unveiled that as the spore concentration increased from 10^3 to 10^8 spores ml^{-1}, the per cent mortality increased from 26.67 to 100 on the fifth day after treatment (DAT), whereas on sixth day, the concentrations 10^7 as well as 10^8 resulted in 100 per cent mortality. A similar correlation between mortality and spore concentration was reported in *L. lecanii* infesting *A. craccivora*, by Sahayaraj and Namashivayam (2000) and Saranya *et al.* (2010).

The present study also indicated a similar response of *B. tabacii* to varying spore concentrations. The mortality observed was 13.33 to 63.33 per cent on the fifth day when the dose was increased from 10^3 to 10^8. There are similar reports in this line. Raheem *et al.* (2009) tested three concentrations of *L. lecanii* 10^5, 10^6 and 10^7 and found an increase in mortality rate of *B. tabaci* from 67.6 to 100 per cent with increase in concentration. Similar observations on *L. lecanii* were also reported by Park and Kim (2010) and Lokesh (2014).

The most susceptible pest species to *L. lecanii* was *Tetranychus* sp., which resulted in 100 per cent mortality with 10^8 spores ml^{-1} at four DAT. This observation is in accordance with Feidler *et al*. (2002) who reported *L. lecanii* as highly virulent against the mite *T. ludeni* Simova and Draganova (2003) and Koike *et al.* (2005) also proved the effectiveness of *L. lecanii* on *T. urticae*.

The infectivity of *L. lecanii* to brinjal scale *Lecanium* sp. is supported by Zare and Gams (2001) who regarded it as the most potential EPF against scale insects as it is pathogenic to 40 different species of scales affecting various crops.

The effective dose of *L. lecanii* determined in this work *i.e.,* 10^7 spores ml^{-1} against the sucking pests *s*uch as aphids, scales, whiteflies and mites with a mortality of 100, 83.33, 70 and 100 respectively is similar to the reports of Nirmala *et al.* (2006) who reported 80.80 per cent mortality of *A. craccivora* with 10^7 spores ml^{-1}. Wenzel and Filho (2011) also reported 76 per cent mortality of *T. urticae* with *L. lecanii* @ 10^7 spores ml^{-1}.

OPTIMISATION OF CULTURE MEDIA

Large scale production of EPF needs production of biomass of high quantity and quality (Kleepies and Zimmermann, 1998). Manipulation of cultural and nutritional parameters can be exploited to favour the production of infective propagules *i.e.*, the conidia. Culture media and production conditions not only affect the growth and sporulation of the fungi but also the spore viability and virulence (Kmitowa and Popowska-Nowak, 1995; Kleespiess and Zimmermann, 1998). Hence, optimization of culture media is necessary for supporting mycelial growth as well as conidiation.

Addition of media supplements to improve growth and sporulation can overcome limitations in the mass production of EPF.

In this study, polyethylene glycol (PEG) two per cent was the best supplement that improved the mycelial growth followed by five per cent each of chitosan and arachid oil. A significantly higher biomass production was noticed in chitin and yeast amended media too. Tween-80 and urea at all the tested concentrations were found to inhibit growth of the fungus.

Several other studies pertaining to supplementation of PEG in culture media also indicated its suitability in enhancing the biomass of a wide variety of EPF. Kleespies and Zimmermann (1992) attributed this quality of PEG to its water regulation capacity which in turn prolongs the fungal growth. Leland *et al.* (2005) and Balakrishnan *et al*. (2011) noted a similar response of *M. anisopliae* to two per

cent PEG supplemented molasses media while Srikanth and Santhalakshmi (2012) observed a similar behavior of B. bassiana and B. brongniartii cultures.

Comparison studies on the effect of media supplements on conidial yield of the fungus (Fig. 1), showed that chitin, chitosan (0.5, two and five per cent) as well as

yeast (one, two and three per cent) significantly improved the sporulation of *L. lecanii* by tenfold (10^8 spores ml^{-1}). The increase in spore yield noted in PEG amended media was negligible. As observed in the case of biomass, tween-80 and urea were found to inhibit sporulation. Though arachid oil was found to enhance mycelial growth it inhibited sporulation.

Chitosan increased sporulation without reducing growth, spore viability, and pathogenicity is in harmony with the investigations of Palma-Guerrero *et al*. (2008, 2010a, 2010b) who explained that the resistance of EPF to chitosan is due to their plasma membrane permiabilization, membrane rigidity and chitosanolytic activity (Palma-Guerrero *et al*., 2010b).

The finding that chitosan is an inducer of conidiation without inhibiting the biomass significantly is in contradiction to the commonly accepted fact by fungal physiologists that conditions favouring rapid mycelial growth reduces sporulation. A slight reduction in biomass observed with chitosan when compared to PEG might have favoured conidiation, while maximum mycelial growth observed in PEG might have reduced conidiation. Palma-Guerrero *et al*. (2010a) observed that two per cent chitosan profoundly increased conidiation of these fungi five to sixty times and stated that though chitosan has antifungal activity on plant pathogens they have a positive effect on entomopathogens which are resistant to chitosan. This resistance is attributed to membrane rigidity which, makes their membranes more resistant after chitosan binding. Further research is required to elucidate the mechanism involved in conidiation induction by chitosan in EPF.

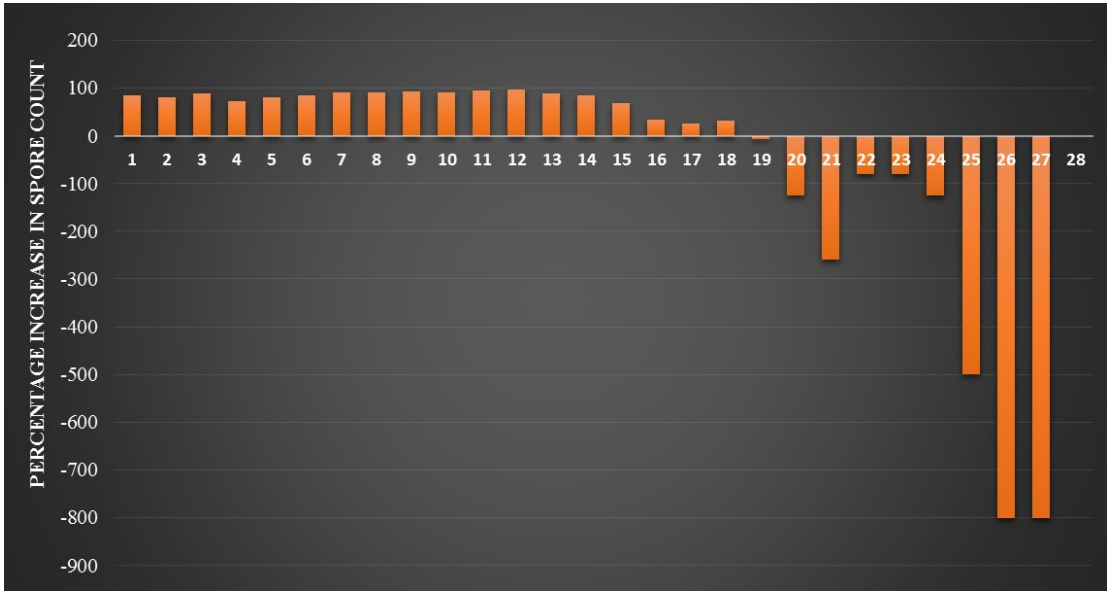


Fig 1. Effect of media supplements on sporulation of *L. lecanii*

1- Chitin crude 0.5 %, 2- Chitin crude 2 %, 3- Chitin crude 5 %, 4- Chitin extra pure 0.5 %, 5- Chitin extra pure 2 %, 6- Chitin extra pure 5 %, 7- Chitosan crude 0.5 %, 8- Chitosan crude 2 %, 9- Chitosan crude 5 %, 10- Chitosan extra pure 0.5 %, 11- Chitosan extra pure 2 %,12- Chitosan extra pure 5 %, 13- Yeast 1 %, 14- Yeast 2 %, 15- Yeast 3 %,16- PEG 2 %, 17- PEG 4 %, 18- PEG 5%, 19- Tween 80 1 % , 20- Tween 80 3 %, 21- Tween 80 5 %, 22- Arachid oil 1 %, 23- Arachid oil 3 %, 24- Arachid oil 5 %, 25- Urea 0.5 %, 26- Urea 1 %, 27- Urea 2%, 28- Unamended

The present study also revealed proportionate increase in biomass with increase in concentration of chitin. Similar report on the differential response to different concentrations of chitin was in line with the report of Pandey and Kanaujia (2010) and Balakrishnan *et al*. (2011). The latter observed that chitin @ 0.1 to 0.6 per cent could enhance the biomass of *M. anisopliae* whereas it reduced the growth of *B. bassiana.* In the case of *B. brongniartii* also a positive response was observed in chitin amended growth media (Srikanth and Santhalakshmi, 2012). Nutritional value of carbon source is likely to play a significant role in mycelial growth. Such differential patterns of fungal growth to the same media supplement and its concentration suggests the prevalence of an optimum nutrient availability for utilization.

The role of chitin in enhancing sporulation of EPF in this study is substantiated by the findings of Hegedus *et al*., 1990; Liu *et al*., 1990; Sun and Liu, 2006; Gerding-Gonzalez *et al*., 2007; Wu *et al*., 2010 and Sreekanth and Santhalekhmi, 2012. Agus *et al.* (2015) explained that it was the carbon content of chitin that induced sporulation.

Yeast at different concentrations evaluated in this study, one, two and three per cent were found to promote biomass of *L. lecanii*. This finding was in line with that of Kamp and Bidochka (2002) who observed increased biomass of *L. lecanii* in yeast peptone-agar-extract. Wenzel *et al*. (2007) reported the superiority of yeast one per cent in promoting biomass of *L. lecanii*. Similarly in case of other EPF *viz. M. anisopliae* and *B. bassiana* also, yeast was found to be a growth promoter (Pandey and Kanaujia, 2010).

Tween-80 and urea were found to inhibit growth as well as sporulation. Though arachid oil was found to enhance mycelial growth it inhibited sporulation. Inhibitory action of urea on the growth of *L. lecanii* observed in this study may be due to the increased nitrogen content which might have altered the ideal C: N ratio

required for mycelial growth. Desai and Kulkarni (2000) noted similar effect of urea on growth and sporulation of the biocontrol agents, *Trichoderma viride* and *T. harzianum*. Complete inhibition in growth and sporulation was noted at 1000 ppm concentration. The total inhibition observed is attributed to the presence of nitrate form of nitrogen in urea, while the preference is for ammoniacal form.

Thus it can be concluded that conidiation enhances with inhibition of mycelia, but not at the total expense of mycelia.

Conidial germination of EPF are generally influenced by the abiotic factors like humidity, temperature, light as well as the nutrient components in the culture media (Ignoffo, 1992). Among the nutrient factors, carbohydrate and protein content are the essential constituents that determine the viability. The data recorded on the number of colony forming units (cfu) of *L. lecanii* in culture broth amended with different media supplements revealed that arachid oil one per cent, was the best media supplement followed by chitin and chitosan (five per cent) and yeast one per cent. It may be noted that, though the spore yield was highest in chitosan amended media followed by chitin and yeast, the number of cfu was highest in arachid oil amended media followed by chitin, chitosan and yeast. The difference in viability observed on the seventh day may be due to the fact that arachid oil might have stimulated the conidia to germinate earlier. Verhaar *et al.* (1999) while comparing the germination of spores of *L. lecanii* suspended in different vegetable and mineral oils observed that arachid oil and paraffin oil could stimulate germination after 24 hours.

The enhanced viability of *L. lecanii* in chitin and chitosan amended media observed in this experiment is attributed to their carbohydrate and protein content as the former is a natural polymer composed of simple sugar molecule and the latter is a derivative of N-acetyl-D-Glucosamine. Nevertheless, Palma-Guerrero *et al*. (2010a) reported that the conidia of EPF grown in chitosan amended media, showed no significant difference in germination when compared to that observed in the media

without chitosan. The present finding that chitin and chitosan enhanced the viability of the fungus is in concurrence with Agus *et al.* (2015) who observed 83.32 per cent increase in the viability of entomopathogenic *Penicillium* sp.

The increase in viability of *L. lecanii* observed in yeast amended media is in line with the observations of Purwar and Sachan (2006) and Pandey and Kanaujia (2010) who noted maximum viability of *B. bassiana* and *M. anisopliae* in yeast supplemented SDB.

In the present experiment, PEG and tween-80 amended media did not show any improvement in viability over unamended media. Kleespies and Zimmermann (1998) reported a similar observation with PEG five per cent, where they observed less viability of *M. anisopliae*, which they have accounted towards the reduction in half-life of blastospores. The finding of Verhaar *et al*. (1999) that germination of *L. lecani* spores was not affected by tween-80 was once again proved in this study.

The suppression of spore germination in urea amended media observed in the present study may be attributed to the inhibition of over growth and sporulation of the fungus in the presence of nitrates present in urea. This result was in agreement with the observations of Jayaraj and Ramabadran (1998) and Desai and Kulkarni (2000). They noted a similar effect of urea on germination of the anatagonistic fungi, *T. harzianum* and *T. viride*. Increase in viability of these fungi was noted when nitrogen source was in the form of ammonium sulphate and ammonium chloride. Effect of urea on growth and sporulation of EPF was not studied earlier.

Virulence is the primary factor used for selection of EPF for its development as a biopesticide. Generally, virulence of entompathogens is assessed in terms of mortality of susceptible host insect. In the present work, 100 per cent mortality of *A. craccivora* was observed with spores cultured in media amended with higher concentrations of chitin and chitosan (two and five per cent) and arachid oil (five per cent). Mortality observed with high concentrations of PEG (four and five per cent),

tween-80 (three and five per cent) and all tested concentrations of urea was comparatively low when compared to that observed with unamended media.

The high mortality observed with *L. lecanii* cultured in chitin and chitosan supplemented media may be due the enhanced chitinolytic activity which might have possibly enhanced the virulence, as chitin induces chitinase production. Several studies suggested that virulence was correlated, at least in part, with chitinase activity (El-Sayed *et al.,* 1989, 1993 and St. Leger *et al*., 1996). Extracellular chitin induces chitinases which synergically act with proteases to hydrolyze insect cuticle (Mohanty and Prakash, 2004; Fang *et al*., 2005).

The virulence of *M. anisopliae* cultured in PEG amended media did not show any difference from that of the unamended media (Kleespies and Zimmermann, 1998).

The effect of tween-80 and arachid oil as supplements in culture media is studied for the first time. However, the present trial included these supplements to get a preliminary assessment of carriers and adjuvants that can be selected for developing formulations.

DEVELOPMENT OF FORMULATIONS

The essential constituents of a formulation are the technical ingredient, an inert carrier and stabilizing agents or additives which can either be a humectant, a wetting or spreading agent, an emulsifier, a binder or a combination of these. Formulation technology involves standardization of each of these constituents in such a way as to maintain the viability and virulence of the infective propagules. A dry formulation consists of 50 to 80 per cent technical ingredient, 20 to 50 per cent inert carrier and one to 10 percent dispersant/surfactant whereas a liquid formulation carries 20 to 80 per cent technical ingredient, 35 to 65 per cent liquid carrier, one - five percent dispersant and three to eight per cent surfactant (Burges and Jones, 1998).

The experiment demonstrated that for oil based liquid formulations 65:35 was the ideal ratio of oil carrier and spore concentrate in sterile distilled water and for talc based dry formulation it is 80:20. The present finding points out to the possibility of formulating an entomopathogen with lesser proportion of technical ingredient (80:20) as against the proportion (50:50) suggested by Burges and Jones (1998). But, there is a dearth of knowledge regarding standardization of the proportion in which carrier is to be mixed with technical ingredient while formulating a mycoinsecticide. However, perusal of literature revealed that various workers have tested the efficacy of mycoinsecticide formulations prepared in oil *i.e.,* formulation of *Nomuraea rileyi* F. in the ratio 50:50 based on sunflower oil (Vimaladevi *et al*., 2002); 50:50 oil combination of coconut oil and soya bean oil mixed with spore suspension of *M. anisopliae* in distilled water (Batta, 2003). Dry formulations of *V. lecanii* based on talc prepared by earlier workers were in the ratio 50:50 (Derakhshan *et al.,* 2008b) and 65:35 (Banu, 2013). Conversely, in the present work 50:50 formulations based on talc were inferior to others.

The carrier material and other constituents used in a bioformulation should be non-inhibitory to the infective propagule *i.e.,* conidia, inert on the target crop plant, at the same time should maintain viability during storage.

The results proved the superiority of two carriers, one using enriched ground nut oil (GNO + chitin) and the other using enriched sunflower oil (SFO + chitin) in terms of viability of *L. lecanii*. The viability observed before and after three months of storage was 10^6. However, there was a slight decline in the number of viable colonies over a period of storage of three months (3.07×10^6 ml^{-1} @ 15 DAS to 2.20×10^6 ml^{-1} @ 90 DAS). Among the basic oil formulations, both GNO and SFO (without chitin) were better in maintaining viability of spores when compared to talc based basic formulation. The decline in number of viable colonies ranged from 2.67×10^6 ml^{-1} at 15 DAS to 1.29×10^6 ml^{-1} at 90 DAS in basic oil formulations and 1.30×10^6 ml^{-1} at 15 DAS to 6.00×10^4 ml^{-1} at 90 DAS in basic talc formulations.

However, chitin enriched talc formulation was superior to basic talc formulation (2.13×10^6 ml^{-1} @ 15 DAS to $6.00\ 10^4$ ml^{-1} @ 90 DAS). Earlier workers too reported GNO and SFO as ideal carriers for formulating EPF. Verhaar *et al*. (1999) reported an excellent germination of *L. lecanii* spores in arachid (ground nut) oil compared to sunflower oil. While studying the viability of *M. anisopliae* in various formulations Alves *et al.* (2002) found that ground nut oil was superior for retention of viability than mineral oils, where the conidial viability was more than 90 per cent even after 40 weeks of storage. They also observed that the number of viable spores declined over storage time. High viability of *L. lecanii* spores in SFO formulation compared to talc based formulations was earlier reported by Banu and Gopalakrishnan (2012). The increased viability observed with arachid oil and sunflower oil may be due to the protective action of these on the mucilaginous outer envelope of *L. lecanii* spores which is usually lost while drying them for preparation of formulations.

Chitin addition improved the bioformulations by suppressing the common contaminant like *Penicillium* (Knudsen *et al.,* 1990) attributing to the fact that saprophytic organisms are unable to utilize chitin as carbon source. Pavlyushin *et al.* (2005) observed better retention of viability of *T. viride* in chitin and chitosan based formulations, even after a longer period of storage. Addition of chitin two or three per cent to wheat bran induced higher conidia production in alginate pelleted formulations of *B. bassiana* (Gerding-gonzalez *et al.,* 2007). Chitin addition (two or five per cent) in talc formulations helped in maintaining high cfus in talc based formulations of *T. harzianum* and also enhanced shelf life by additional two months (Sriram *et al.,* 2010). However, enrichment of vegetable oil with chitin to improve storage properties of EPF was not seen attempted earlier.

Conidia are infective propagules in EPF ultimately leading to mortality of susceptible insects. The virulence of entomopathogens is generally assessed in terms of mortality of susceptible host insect. In this context apart from spore count,

estimation of cfu is more relevant for assessing the effectiveness of formulations over the period of storage.

The data on spore count revealed that the chitin enriched formulations with GNO and SFO were superior with respect to spore count. Though the spore count observed was 10^8 spores ml^{-1} even after three months of storage, there was a slight decline in the number at the end of three months (4.15×10^8 spores ml^{-1} @ 15 DAS to 1.38×10^8 spores ml^{-1} @ 90 DAS). The spore count in chitin and chitosan enriched talc formulations were equally good as basic formulations of GNO and SFO. The decline in number of spores ranged from 2.86×10^8 spores ml^{-1} @ 15 DAS to 3.9×10^7 spores ml^{-1} @ 90 DAS in enriched talc formulations and 3.36×10^8 spores ml^{-1} @ 15 DAS to 7.20×10^7 spores ml^{-1} @ 90 DAS in basic oil formulations. However, chitin enriched talc formulation was superior to basic talc formulation (1.17×10^8 spores ml^{-1} @ 15 DAS to 2.00×10^6 spores ml^{-1} @ 90 DAS). Presence of chitin in the formulation may enhance virulence, as chitin induces production of chitinases, which are important cuticle degrading enzymes (St. Leger *et al.,* 1986b; Mohanty and Prakash, 2004; Fang *et al*., 2005).

To wrap up, enrichment of oil formulations (GNO and SFO) with chitin could sustain viability as well as spore count of *L. lecanii* till the end of experimental period (three months), but as indicated in Fig. 2. It was the groundnut oil (GNO) formulation that outnumbered in spore count and cfu.

The stability and activity of technical ingredient in a bioformulation can be enhanced with addition of adjuvants. Emulsifier is an inevitable constituent of oil formulations. Wetter is also an essential component of liquid formulations to wet and spread the hydrophobic surfaces of insect and leaf cuticles. So also surfactants have been activators of fungi (Woertz and Kinney, 2004). However, inherent properties of some of the surfactants do have some inhibiting effect on spores, which affects germination.

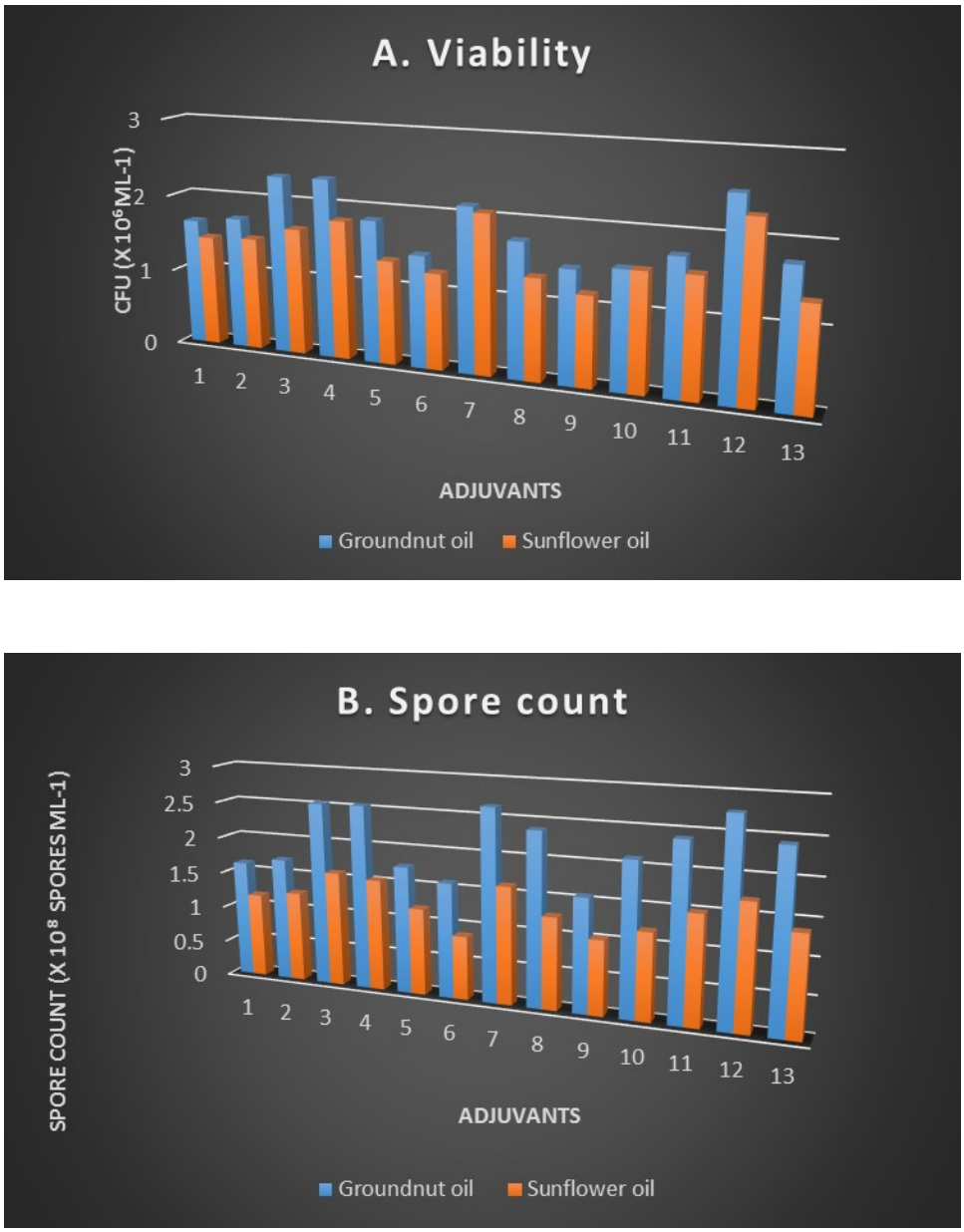


Fig. 2 Comparison of viability and spore count of *L. lecanii* in groundnut oil (GNO) and sunflower oil (SFO) formulations

1- PEG 0.5 %, 2 – PEG 1 %, 3 – PEG 2 %, 4- POX 0.5 %, 5- POX 2 %, 6- POX 5 %, 7- Tween 80 1 %, 8 – Tween 80 3 %, 9 – Tween 80 5 %, 10 – Glycerol 1 %, 11- Glycerol 3 %, 12 – Glycerol 5 %, 13 – control

The trial to evaluate different adjuvants for their suitability to chitin enriched GNO formulations of L. lecanii revealed that glycerol five per cent, tween-80 one per cent, PEG two per cent and POX 0.5 per cent were the adjuvants that could maintain conidial viability over three months of storage. Higher concentrations of tween-80 and POX (five per cent) significantly reduced the number of viable colonies than the check formulation. The number of viable colonies in all the formulations were maintained to the level of 106, though there was a narrow reduction after 45 DAS.

The suitability of glycerol observed in this study is in concurrence with the reports of Santharam et al. (1977), who noted that it improved the efficacy of L. lecanii spores. It acts as a powerful humectant with nutrient qualities (Burges, 1998), as a stabilizer (Jones and Burges, 1998) and as a depressor of water molecule activity (Batta *et al*., 2011).

The finding that tween-80 can be used as an adjuvant was in line with the observations of Easwaramoorthi and Jayaraj (1977). Burges (1998) opined this property of tween-80 to its ability to rehydrate fungal spores. So also, Luz and Batagin (2005) reported its relatively less toxic effect on spores of *B. bassiana*. Tanuja *et al.* (2010) also opined that tween-80 has the ability to increase cell permeability that hastens germination (Tanuja *et al*., 2010). This was further confirmed by the findings of Mishra *et al.* (2013) who reported that tween-20 and tween-80 did not inhibit germination of the *B. bassiana* conidia.

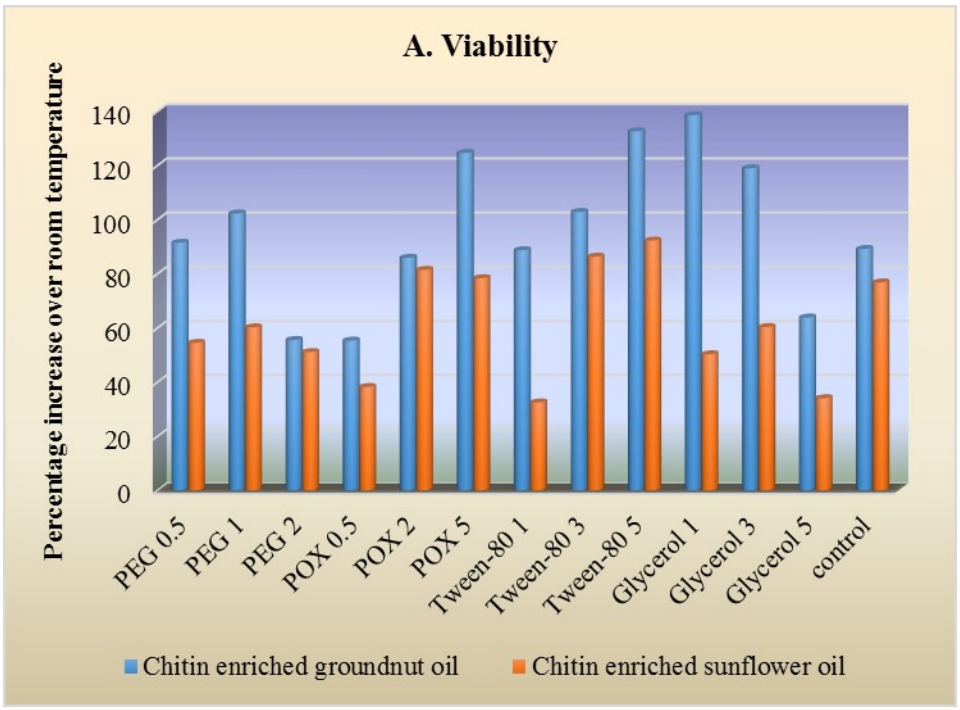
The utility of PEG observed in this study was supported by Hallsworth and Magan (1994) who stated that addition of PEG can lead to accumulation of trehalose in spores and that trehalose increases spore resistance against heat and desiccation. Its effectiveness in enhancing viability of *M. anisopliae* was also reported by Kleespies and Zimmermann (1998) and Derakhshan *et al.* (2008b). Polyoxyethylene tried as adjuvant in this study was a non-ionic surfactant that could effectively be utilized in fungal oil formulations to maintain their shelf life.

Further evaluation to study the combination effect of adjuvants disclosed that glycerol five per cent + tween-80 one per cent (AC2), tween-80 one per cent + glycerol five per cent + POX 0.5 per cent (AC9) and tween-80 one per cent + PEG two per cent + glycerol five per cent + POX 0.5 per cent (AC11) were equally effective in maintaining the viability and spore count of Chitin enriched GNO formulations. Chavan and Kadam (2010a) reported some analogous combinations *viz*. glycerol two per cent + tween-80 one per cent + arachid oil 0.5 per cent and glycerol five per cent + tween-80 one per cent + arachid oil two per cent as the best adjuvant combinations for *L. lecanii* formulations.

There were no previous report on chitin enriched bioformulations of *L. lecanii*. Therefore, the study represents the first attempt to investigate the suitability of such a bioformulation.

Storage temperature is the most important abiotic factor that affects the shelf life of biological formulations by maintaining them in a state of low metabolic activity. Comparison of viability and virulence of all the formulations, stored at room temperature and refrigeration revealed that at both the temperatures, obviously there was a decline in number of cfu as well as spores over the period of storage, but the level was maintained at 10^6 in the case of cfu and 10^8 in the case of spores. Neverthless, their performance during long time storage needs to be elucidated.

Unsurprisingly, the values were always high in refrigerated formulations. There was 58.37 to 138.57 per cent increase in viability, under refrigeration in all the groundnut oil (GNO) formulations tried with various adjuvants, while it was 32.52 to 92.31 per cent in sunflower oil (SFO) formulations with added adjuvants (Fig. 3 A). Similarly, with respect to spore count also, refrigeration was better. The per cent increase in spore count over room temperature ranged from 112.68 to 229.63 in chitin enriched GNO formulations, while it was 227.49 to 450 per cent with chitin enriched SFO formulations (Fig 3. B), indicating the suitability of lower temperature for



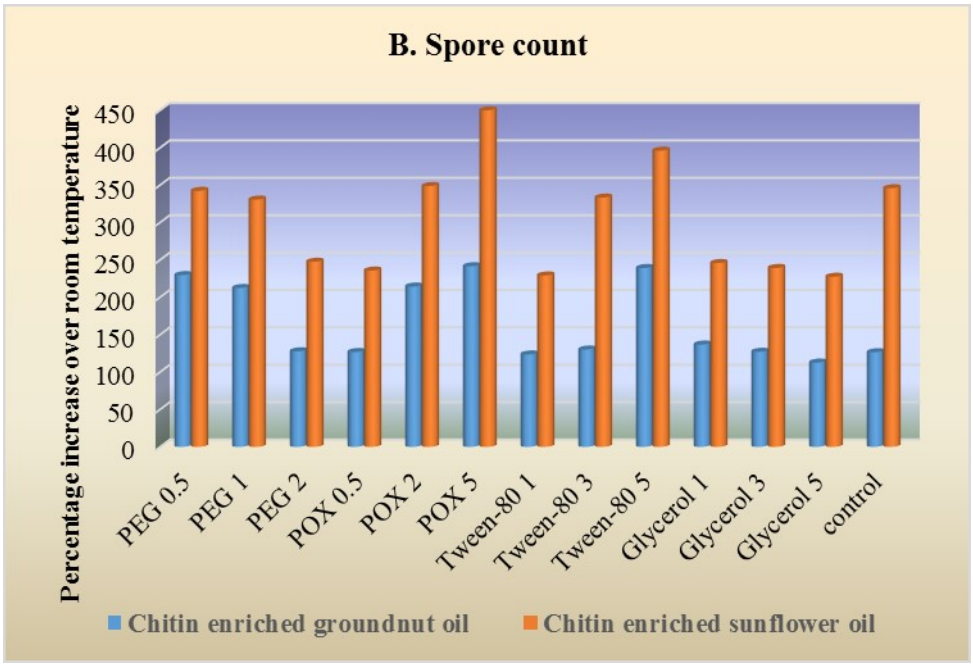


Fig. 3 Effect of refrigeration on viability and spore count of oil formulations

prolonged storage. Chen et al. (2008) and Derakhshan et al. (2008) too reported high viability of L. lecanii formulations stored under refrigeration. This was further substantiated by Banu and Gopalakrishnan (2012) and Banu (2013). They reported that viability of sunflower oil based formulations and talc based formulations of L. lecanii were significantly higher under refrigeration, the values ranging from 78 to 70 per cent at the end of six months of storage and that the talc formulations stored under refrigeration (9 ± 2˚C) supported maximum viability than those stored at room temperature (27 ± 2˚C).

Fungal spores are highly susceptible to solar radiation. Incorporation of photoprotective or UV protective agents can overcome this deleterious effect. Among the various UV protectants tested at different concentrations, boric acid one per cent was the one which showed maximum tolerance to UV irradiation in terms of germination and spore count, compared to indigo (0.5, one and two per cent). Chitin enriched GNO formulations developed as mentioned earlier with each of the adjuvant combinations (AC2, AC9 and AC11) were equally compatible with one per cent boric acid. The germination and spore count of these formulations with this UV protectant were unaffected on UV irradiation at 365 nm (UV-A) and 260 nm (UV-C) for 10, 20 and 30 minute at 15 cm distance. Importance of UV protectants in bio formulations was earlier studied by Braga *et al*. (2001) who observed that sunlight inactivates the fungus due to genetic and morphological changes and that UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (100-280 nm) are highly deleterious to entomopathogens. The detrimental effect of sunlight on EPF was pointed out earlier by Grand and Cliquet (2013) and Galvao and Bettilol (2014).

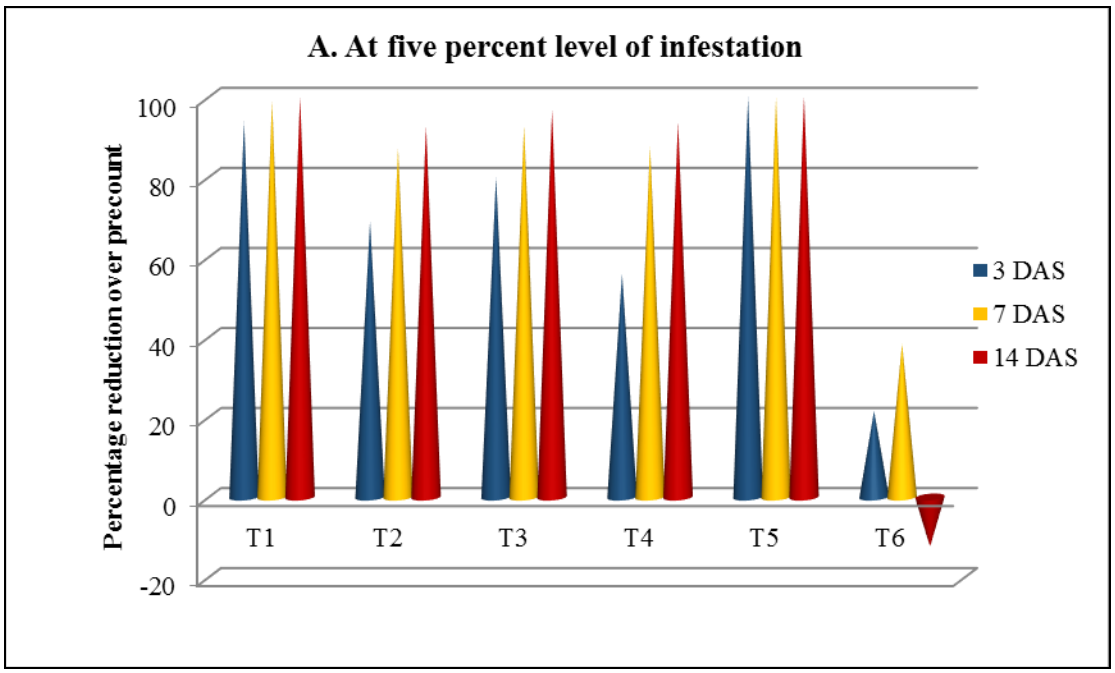
The present finding that boric acid one per cent is an ideal UV protectant compared to indigo is exactly in agreement with the report of Chavan and Kadam (2010b).

FIELD EVALUATION

Field evaluation conducted using the effective formulations *viz*. Formulation I (Chitin enriched GNO + tween-80 one per cent + PEG two per cent + POX 0.5 per cent + glycerol five per cent and one per cent boric acid), Formulation II (Chitin enriched GNO + tween-80 one per cent + glycerol five per cent and one per cent boric acid) and Formulation III (Chitin enriched GNO + tween-80 one per cent + POX 0.5 per cent + glycerol five per cent and one per cent boric acid) revealed that at lower infestation level of five per cent, Formulation I at one per cent strength, was found to be equally effective as chlorpyriphos 0.05 per cent in controlling aphid, *A. craccivora* in cowpea. Per cent reduction over pre count was 93 per cent at three days after spraying (DAS) and 99 per cent at seven DAS. Almost cent per cent reduction was observed at 14 DAS (Fig 4. A). Formulation II and III ranked second resulting in 68 and 80 per cent reduction, respectively at three DAS, 87 and 92 per cent, respectively at seven DAS and 97 and 96 per cent, respectively at 14 DAS. The corresponding per cent reduction was 55 per cent, 87 per cent and 93 per cent with talc based product of KAU.

Even at higher infestation level too, Formulation I was equally good as chlorpyriphos 0.05 per cent in controlling aphids. Per cent reduction over pre count was 71 per cent at 3 DAS, 96 per cent at seven DAS and 99, per cent at 14 DAS (Fig 4. B). Formulation II ranked second with per cent reduction of 70, 93 and 98 per cent at three, seven and 14 DAS. The corresponding reduction with Formulation III was 64, 89 and 97 per cent respectively. Per cent reduction in population noted with talc based product was 52, 86 and 96 on three, seven and 14 DAS.

The efficacy of oil formulations of EPF observed in this study is supported by the previous reports of Prior et al., 1988; Bateman et al., 1993 and Inglis et al., 1996. Batta (2003) attributed this efficacy to the prolonged period of wetness in oil formulations which allow conidial germination and penetration into host tissues



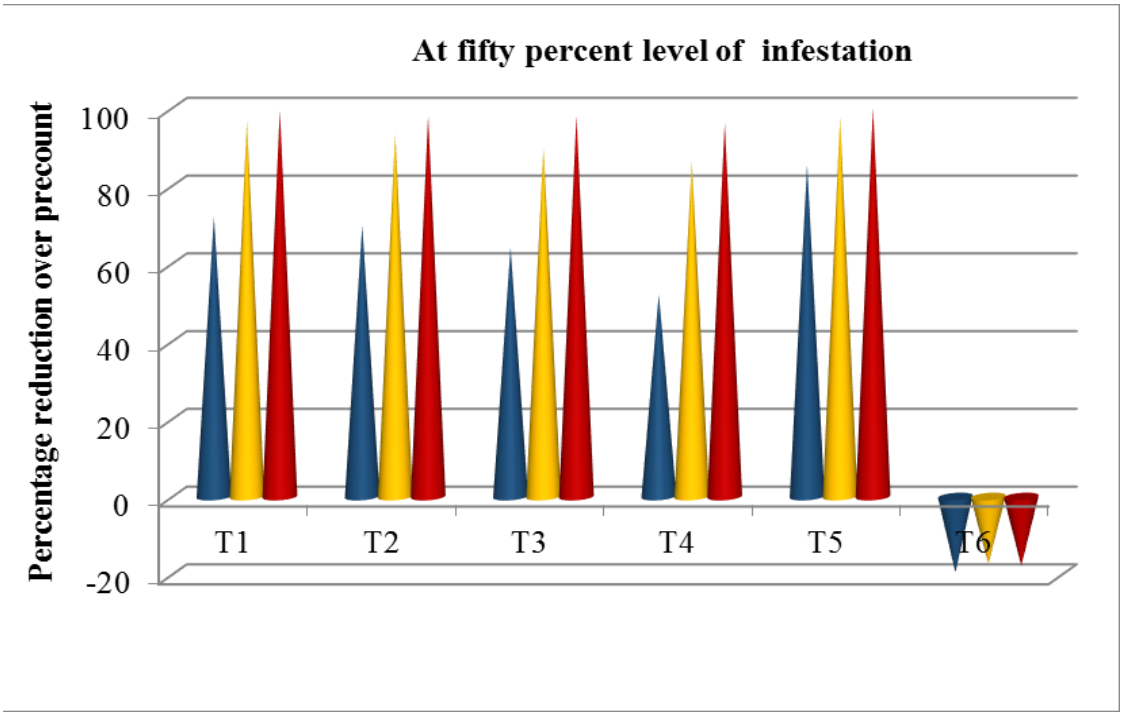


Fig 4. Efficacy of chitin enriched groundnut oil (GNO) formulations on

T1 – Chitin enriched GNO formulation + Tween-80 1 % + PEG 2 % + POX 0.5 % + glycerol 5 % + boric acid, T2 – Chitin enriched GNO formulation + Tween-80 1 % + Glycerol 5 % + boric acid, T3 – Chitin enriched GNO formulation + Tween-80 1 % + Glycerol 5 % + POX 0.5 % + boric acid, T4 – Talc based formulation of *L. lecanii*, T5 - Chlorpyriphos 0.05 %, T6 - Untreated control

Ramle *et al*. (2004) suggested that this is due to the enhanced adhesion of the conidia to insect cuticle through hydrophobic interaction between the spore and cuticle surface. The enhanced virulence was further explained by Bandani and Esmailpour (2006) due to the action on lipid layer of epicuticle which is the primary site of establishment of mycosis. Efficacy of oil based formulations of EPF against sucking pests of okra was earlier demonstrated by Naik and Shekharappa (2009) too. Banu and Gopalakrishnan (2012) reported that oil based formulations of *L. lecanii* recorded maximum mortality of mealy bugs than that of talc based products.

The bioformulations were effective in controlling whiteflies, jassids and mites too. When 50 per cent control was observed in whitefly population on third DAS, the per cent reduction observed in jassids was comparatively less. It took more than 14 days to get an effective control. In case of mites, nearly 50 per cent reduction was observed at three DAS. By 14 DAS, 100 per cent control was obtained (Fig 5, 6 and 7). Bioefficacy of oil formulations of *M. anisopliae* on whiteflies and mites was earlier reported by Batta in 2003. Likewise, Andrew et al. (2004) reported that oil formulation of L. muscarium reduced the whitefly population in tomato.

The above findings clearly indictated the efficacy of chitin enriched GNO formulation with adjuvant combinations in controlling sucking pests. The carrier ground nut oil itself can act as a lubricating agent to the protective mucilaginous layer of L. lecanii conidia. Enrichment with chitin might have favored easier cuticle penetration in soft bodied insects.

Moreover the adjuvants tween- 80 + PEG + POX + glycerol acted as an ideal combination of surfactant, humectant, emulsifier and wetting agent as well which might have facilitated better adherence, earlier germination and easier penetration on the treated insects. The efficacy of tween-80 in improving the field performance of B. bassiana oil formulations was attributed to its non-ionic nature which prevents reaction with ions present if any, in water (Field and Dastgheib, 1996). The property

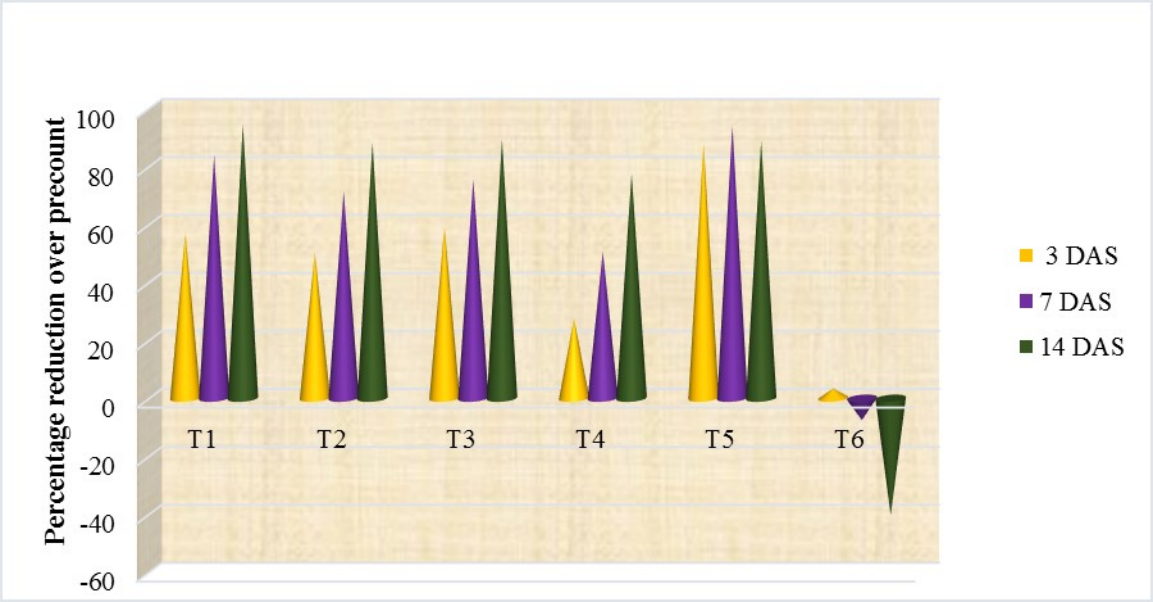


Fig 5. Efficacy of chitin enriched groundnut oil (GNO) formulations on *B. tabaci*

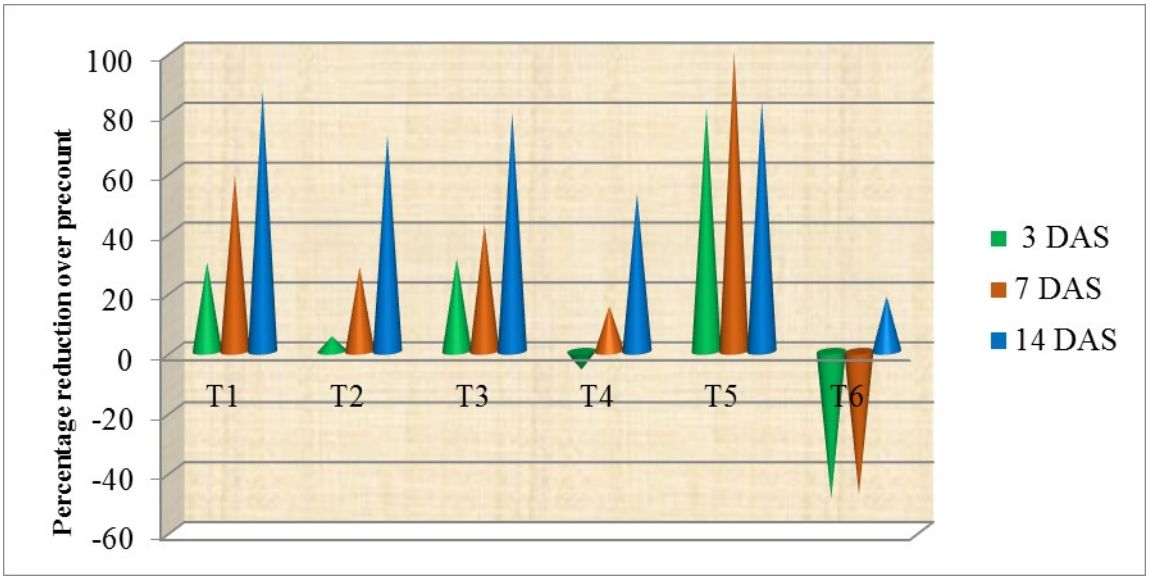


Fig 6. Efficacy of chitin enriched groundnut oil (GNO) formulations on *A. biguttula biguttula*

T1 – Chitin enriched GNO formulation + Tween-80 1 % + PEG 2 % + POX 0.5 % + glycerol 5 % + boric acid, T2 – Chitin enriched GNO formulation + Tween-80 1 % + Glycerol 5 % + boric acid, T3 – Chitin enriched GNO formulation + Tween-80 1 % + Glycerol 5 % + POX 0.5 % + boric acid, T4 – Talc based formulation of *L. lecanii*, T5 - Chlorpyriphos 0.05 %, T6 - Untreated control

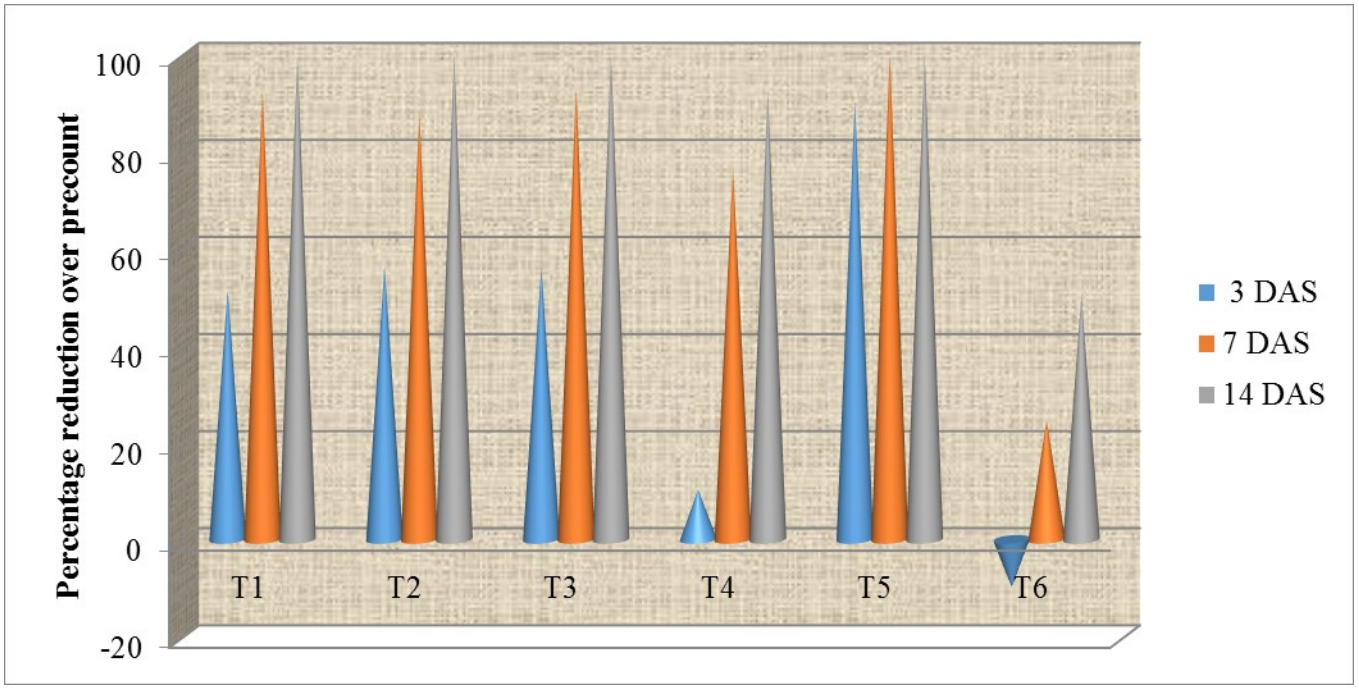


Fig 7. Efficacy of chitin enriched groundnut oil (GNO) formulations on *Tetranychus*sp.

T1 – Chitin enriched GNO formulation + Tween-80 1 % + PEG 2 % + POX 0.5 % + glycerol 5 % + boric acid, T2 – Chitin enriched GNO formulation + Tween-80 1 % + Glycerol 5 % + boric acid, T3 – Chitin enriched GNO formulation + Tween-80 1 % + Glycerol 5 % + POX 0.5 % + boric acid, T4 – Talc based formulation of *L. lecanii*, T5 - Chlorpyriphos 0.05 %, T6 - Untreated

of PEG in stimulating conidial germination (Kleespies and Zimmermann 1998) and preventing conidial dehydration (Burges, 1998) under field conditions is because of its ability to function as an osmotic regulator.

Literature pertaining to betterment of formulation using adjuvant combination is meager. Boruah and Dutta (2014) reported a combination of glycerol (10 per cent) + sunflower oil (0.5 per cent) in *M. anisopliae* liquid formulation could result in highest mortality of *A. craccivora* in cowpea at 15 DAS.

When the formulations were ranked in terms of the mean yield, though Formulation I and II recorded highest yield per plot among the bioformulations, the yield obtained from chlorpyriphos 0.05 per cent treated plots was significantly higher than these. The yield recorded from plots treated with talc based product was on par with that of Formulation III. Increase in yield obtained in chemical treatment may be due to the broad spectrum effect of the chemical which might have checked the pod borers and pod bugs also.

From the foregoing results it is obvious that *L. lecanii*, an effective entomopathogen to sucking pests can successfully be formulated using groundnut oil as carrier. Conidial viability and virulence of bioformulations based on *L. lecanii* can significantly be improved by enriching with chitin and incorporating a combination of adjuvants, tween-80 + glycerol + PEG + POX and the field application of this formulation with one per cent boric acid could effectively control the sucking pest complex in cowpea. The efficacy of these formulations in managing a wide variety of sucking pests possibly leads to its application in other crops as well.

Summary

6. SUMMARY

The investigation entitled "Improved formulation of the entomopathogenic fungus *Lecanicillium lecanii* Zimmermann (Zare and Gams) and its evaluation against sucking pests" was carried out at the Biocontrol Laboratory for Crop Pest Management and Instructional farm, College of Agriculture, Vellayani (2013-2015). The study aimed at developing an improved bio formulation of the fungus with better conidial viability and virulence. Attempts were also made to enhance the efficacy of the formulation with better spread and adhesion under field conditions. Pathogenicity of the fungus to different sucking pests was initially assessed to ensure its practical utility. Possibility of producing more infective propagules by optimising the media supplements, formulating in a compatible and stable carrier material, assorted usage of adjuvants and incorporation of a suitable UV protectant in the formulation, were the prime areas explored during this research. Formulations developed in the course of study were eventually validated for its efficacy in managing sucking pests of vegetable cowpea, a major pulse crop of Kerala.

Pathogenicity studies carried out using spore suspension of *L. lecanii* in sucking pests infesting cowpea revealed that the fungus was infective to cowpea aphid *Aphis craccivora* Koch, white fly *Bemisia tabaci* Gennadius, the jassid *Amrasca biguttula biguttula* Ishida, mealy bug *Ferrisia virgata* Cockerell and red spider mite *Tetranychus* sp. The scale insect that infests brinjal, *Lecanium* sp. was also found susceptible to the fungus. It was not infective to the pod bug *Riptortus pededstris* Fabricius and cow bug *Anchon pilosum* Walker.

Symptoms of mycosis observed after treatment with spore suspension did not show much variation among the test organisms. Lethargic movement and cessation of feeding was observed in all the susceptible species. The cadavers were dry and stiff with mycelial strands all over.

The time taken for mortality ranged from 24 to 96 hours after treatment (HAT). *L. lecanii* took lesser time to cause mortality of *A. craccivora, B. tabaci*,

L*ecanium* sp. and *Tetranychus* sp. when compared to *A. bigutulla bigutulla* and *F. virgata*.

Dose mortality studies in highly susceptible sucking pests *viz. A. craccivora*, *B. tabaci, Lecanium* sp. and *Tetranychus* sp. using spore concentrations ranging from 10^3 to 10^8 spores ml^{-1} revealed that mortality increased with increase in spore concentrations. The mortality observed in *A. craccivora* treated with spore concentrations *i.e.,* 10^6, 10^7 and 10^8 spores ml^{-1} were 83.33, 100 and 100 per cent, respectively at the end of observational period (144 HAT). The corresponding mortality observed in whiteflies was 66.67 and 70 per cent with the spore concentrations 10^7 and 10^8 spores ml^{-1} respectively. The mortality observed with *Lecanium* sp. @ 10^5, 10^6, 10^7 and 10^8 spores ml^{-1} was found to be 56.67, 63.33, 83.33 and 90 per cent respectively. The mite species recorded 100 per cent mortality with higher doses, 10^8 and 10^7 spores ml^{-1}, 20 HAT. Though high mortality was observed at 10^8 spores ml^{-1}, the lesser dose (10^7) which was normally the spore yield of *L. lecanii* cultures, was also found to bring about effective control of the pests under laboratory conditions.

In the experiment to optimize media supplement for enhanced growth and sporulation, chitin, chitosan, yeast, polyethylene glycol (PEG), arachid oil, tween-80 and urea were added to the standard medium, Sabouraud dextrose broth. Data on biomass revealed that maximum yield was obtained with PEG two per cent (2.67g / 100 ml), followed by that of PEG four per cent, crude and extra pure chitosan five per cent and arachid oil five per cent. Chitin and yeast amended media were significantly superior to unamended media.

Maximum number of viable colonies (2×10^5 ml^{-1}) were recorded in media supplemented with arachid oil one per cent. Chitosan at 0.5, two and five per cent, chitin (five per cent), and yeast (one, two and three per cent), yielded 1.53 to 1.57×10^5 colonies ml^{-1}. None of the spores were viable in media amended with one and two per cent urea.

Maximum sporulation was found in media amended with five per cent chitosan extra pure, which yielded 9.34 x 10^8 spores ml^{-1}, followed by chitosan extra pure two per cent (7.83 x 10^8 spores ml^{-1}). The efficacy of the amended culture broth of *L. lecanii* where treated on test insect, *A. craccivora* recorded 100 per cent mortality within 72 h of treatment in chitosan five per cent, crude chitin five per cent and arachid oil five per cent.

Of the basic and enriched carrier materials evaluated for their ability to improve conidial viability and spore count, chitin enriched ground nut oil (GNO + Chitin) and chitin enriched sunflower (SFO + chitin) were observed as the best carriers. Ideal proportion of carrier: technical ingredient in oil formulations was 65:35. The number of cfu observed with chitin enriched GNO and SFO by the end of three months of storage was 2.27 x 10^6 ml^{-1} and 2.20 x 10^6 ml^{-1}. The corresponding spore count was 1.67 x 10^8 spores ml^{-1} and 1.65 x 10^8 spores m^{-1}, respectively. The basic as well as chitin enriched oil formulations were found to be superior in terms of viability than the chitin and chitosan enriched talc formulations. The enriched talc formulations were better than basic talc formulations in maintaining viability.

Refrigeration was found to improve viability and spore count of all the formulations. The number of cfus ranged from 0.06 to 2.27 x 10^6 ml^{-1} at room temperature while it ranged from 0.16 to 2.93 10^6 spores ml^{-1} under refrigeration. The spore count observed at room temperature ranged from 0.02 to 1.67 10^8 spores ml^{-1} at room temperature while under refrigeration it varied from 0.16 to 6.20 10^8 spores ml^{-1}. Out of the four adjuvants tested at varying concentrations, PEG two per cent, POX 0.5 per cent, tween-80 one per cent and glycerol five per cent were found to be effective of better viability and spore count during the three months of storage. Under room temperature, higher concentration (five per cent) of tween-80 and POX significantly reduced the number of viable colonies than the check formulation (without adjuvants). The number of viable colonies in all the

formulations were observed to the level of 10^6 though there was a narrow reduction after 45 days of storage (DAS). But the number of cfu noted was always higher during storage under refrigeration. PEG 0.5 per cent, POX and tween-80 five per cent significantly reduced the spore count under room temperature while under refrigeration, the remaining concentrations of adjuvants retained spore count more or less similar to that of check.

Among the 11 adjuvant combinations evaluated, the effective adjuvant combinations in maintaining viability and spore count were found to be tween-80 one per cent + PEG two per cent + glycerol five per cent + POX 0.5 per cent (AC1), tween-80 one per cent + Glycerol five per cent (AC2) and tween-80 one per cent + glycerol five per cent + POX 0.5 per cent (AC3).

From the experiment to standardize the UV protectant, it was inferred that the UV rays, both short (260 nm) and long wave rays (365 nm) were detrimental to *L. lecanii*. The detrimental effects of UV increased with increase in exposure period and decrease in wavelength. From the four UV protectants, boric acid one per cent was found to exhibit better UV protectancy for *L. lecanii*. Indigo, at all the tested concentrations (0.5, one and two per cent) lacked UV protectability.

The three effective formulations, tested under field conditions for management of sucking pests in cowpea were Formulation I - Chitin enriched groundnut oil + AC1 + boric acid one per cent; Formulation II - Chitin enriched groundnut oil + AC2 + boric acid one per cent and Formulation III - Chitin enriched groundnut oil + AC3 + boric acid one per cent. Formulation I was equally effective as chlorpyriphos 0.05 per cent resulting in 98.93 per cent control at five per cent level of infestation and 96.74 per cent at fifty per cent level of infestation levels of aphids, seven days after spraying (DAS). When treated at five per cent infestation levels, there was 100 per cent reduction in population 14 DAS. Similarly, at fifty per cent level of infestation also the aphid population was meagre in these plots.

The percentage reduction noted in population of mites, jassids and whiteflies was 92.58, 58.81 and 96.74 per cent, respectively at seven DAS. By 14 DAS, the three biofomulations were equally effective. Complete control of mites was achieved14 DAS, in Formulation I, II and III treated plots.

Among the bioformulations, Formulation I and II recorded highest yield of 7.03 and 6.74 kg per plot respectively, which was significantly lower than the yield obtained from chlorpyriphos 0.05 per cent treated plots (8.57 kg).

It is concluded that viability and virulence of *L. lecanii* formulations can be improved using chitin enriched groundnut oil as carrier and PEG two per cent + POX 0.5 per cent + tween 80 one per cent + glycerol five per cent as adjuvant. The formulation when sprayed at one per cent concentration using boric acid one per cent as UV protectant, effectively controlled aphids, whiteflies, jassids and mites infesting cowpea, under field conditions.

References

REFERENCES

Abbot, W. S. 1925. A method for computing the effectiveness of insecticides. *J. Econ. Entomol*. 18: 265-267.

Abdel-Kader, M. M., El-Mougy, N. S., Aly, M. D. E., and Lashin, S. M. 2012. Long activity of stored formulated bio-agents against some soil-borne plant pathogenic fungi causing root rot of some vegetables. *J. Appl. Sci. Res*. 8(4): 1882-1892.

Agus, N., Saranga, A. P., Rosmana, A., and Sugiarti, A. 2015. Viability and Conidial Production of Entomopathogenic Fungi *Pencillium* sp. *Int. J. Sci. Technol*. 4(1): 193-195.

Alves, R. T., Bateman, R. P., Gunn, J., Prior, C., and Leather, S. R. 2002. Effects of Different Formulations on Viability and Medium-Term Storage of *Metarhizium anisopliae* Conidia. *Neotrop. Entomol.* 31(1): 91-99.

Amjad, M., Bashir, M. H., Afazal, M., Sabri, M. A., and Javed, N. 2012. Synergistic effect of some Entomopathogenic Fungi and Synthetic Pesticides against two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). *Pakistan J. Zool.* 44(4): 977-984.

Aneja, K. R. 1996. *Experiments in Microbiology, Plant Pathology, Tissue culture and Mushroom cultivation*. Vishwa Prakashan, New Delhi, 451p.

Aneja, K. R. 2003. Experiments in Microbiology, Plant Pathology and Biotechnology. New Age International Limited (P) Ltd, New Delhi, 460p.

Balakrishnanan, S., Srikanth, J., Santhalakshmi, G., Hari, K., and Sankaranarayanan, C. 2011. Response of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* to molasses media fortified with supplements. *J. Sugarcane Res.* 1(2): 57-65.

Bandani, A. R. and Esmailpour, N. 2006. Oil formulation of entomopathogenic fungus, *Beauveria bassiana*, against Sunn pest, *Eurygaster integriceps* Puton (Heteroptera: Scutelleridae). *Commun. Agric. Appl. Biol. Sci*. 71 (2): 8-443.

Banks, C. J. 1952. A method of estimating population and counting large number of *Aphis fabae* Scopoli. *Bull. Entomol. Res*. 45: 751-756.

Banu, J. G. 2013. Effect of different storage conditions on spore viability of *Lecanicillium lecanii* formulations and infectivity to mealybug, *Paracoccus marginatus*. *Int. J. Plant Prot*. 6(2): 334-337.

Banu, J. G. and Gopalakrishnan, N. 2012. Development of formulations of a native entomopathogenic fungus, *Lecanicillium lecanii* and testing virulence against mealybug, *Paracoccus marginatus* infesting cotton. *Indian J. Plant Prot.* 40(3): 182-186.

Bateman, R. P., Carey, M., Moore, D. and Prior, C. 1993. The enhanced infectivity of *Metarhizium anisopliae* in oil formulations to desert locusts at low humidities. *Ann. App. Biol*. 122: 145-152.

Batta, Y. A. 2003. Production and testing of novel formulations of the entomopathogenic fungus *Metarhizium anisopliae* (Metschinkoff) Sorokin (Deuteromycotina: Hyphomycetes). *Crop Prot*. 22: 415–422.

Batta, Y. A., Rahman, M., Powis, K., Baker, G., and Schmidt, O. 2011. Formulation and application of the entomopathogenic fungus: *Zoophthora radicans* (Brefeld) Batko (Zygomycetes: Entomophthorales). *J. Appl. Microbiol*. 110: 831-839.

Bidochka, M. J., Pfeifer, T. A., and Khachatourians, G. G. 1987. Development of the entomopathogenic fungus *Beauveria bassiana* in liquid cultures. *Mycopathologia* 99: 77-83.

Boruah, S. and Dutta, P. 2014. Preliminary evaluation of bioformulations of *Metazhizium anisopliae* against cowpea aphid, *Aphis craccivora*. *Insect Environ*. 20(2): 54-56.

Boruah, S., Dutta, P., Puzari, K. C., and Hazarika, G. N. 2015. Liquid bioformulation of M*etarhizium anisopliae* is effective for the management of cow pea mosaic disease. *Int. J. Appl. Biol. Pharma. Technol.* 6(1): 178-185.

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

Braga, G. U. L., Rangel, D. E. N., Miller, C. D., Anderson, A. J., and Roberts, D. W. 2002. Damage and recovery from UV-B exposure in conidia of the entomopathogens *Verticillium lecanii* and *Aphanocladium album*. *Mycologia* 94(6): 912-920.

Brodeur. 2012. Host specificity in biological control: insights from opportunistic pathogens. *Evol. Appl.* 5(5): 470-480.

Burges, H. D. 1998. Formulation of mycoinsecticides. In: Burges, H. D. (ed.), *Formulation of Microbial Biopesticides: Beneficial Microorganisms, Nematodes and Seed treatments* (1st Ed.). Kluwer Academic Publishers, New York, pp.131-185.

Burges, H. D. and Jones, K. A. 1998. Trends in Formulation of Microorganisms and Future Research Requirements. In: Burges, H. D. (ed.), *Formulation of Microbial Biopesticides: Beneficial Microorganisms, Nematodes and Seed treatments* (1st Ed.). Kluwer Academic Publishers, New York, pp. 311-332.

Chandler, D., Davidson, G., and Jacobson, R. J. 2005. Laboratory and glasshouse evaluation of entomopathogenic fungi against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), on tomato, *Lycopersicon esculentum*. *Biocontrol Sci. Technol*. 15: 37-54.

Chandler, D. 2009. Understanding the evolution and function of entomopathogenic fungi [on-line]. Available: http:// http://www2.warwick.ac.uk/fac/sci/lifesci/research/entomopathogenicfungi/understanding_the_evolution_and_function_of_entomopathogenic_fungi.pdf [12 June 2015].

Chavan, B. P. and Kadam, J. R. 2008. Effect of various adjuvants on growth and development of the entomopathogenic fungi, *Verticillium lecanii* (Zimmermann) Viegas. *J. Biol. Control* 22(2): 75-81.

Chavan, B. P. and Kadam, J. R. 2009a. Effect of combination of adjuvants on liquid formulations of *Verticillium lecanii* (Zimmermann) Viegas and their efficacy. *J. Biol. Control* 23(1): 73-77.

Chavan, B. P. and Kadam, J. R. 2009b. Potential of liquid formulations of *Verticillium lecanii* (Zimmermann) against spiralling whitefly, *Aleurodicus disperses* Russel. *J. Biol. Control* 23(2): 151-154.

Chavan, B. P. and Kadam, J. R. 2010a. Effect of liquid formulations of *Pochonia (Verticillium) lecanii* (Zimm.) Viegas on viability and virulence against Mealy bug. *Ann. Plant Protec. Sci.* 18(1): 63-66.

Chavan, B. P. and Kadam, J. R. 2010b. Effect of Ultraviolet Light on viability of liquid formulation of *Lecanicillium lecanii* (Zimmermann) Zare and Gams with various adjuvants. *J. Biol. Control* 24(2): 147-152.

Chen, A., Shi, Z., and Zhang, L. 2008. The effects of some storage conditions on viability of *Lecanicillium lecanii* conidia to whitefly (Homoptera: *Trialeurodes vaporariorum*). *Biocontrol Sci. Technol.* 18: 267-278.

Curtis, J. E., Price, T. V., and Ridland, P. M. 2003. Initial development of spray formulation which promotes germination and growth of the fungus entomopathogen, *Verticillium lecanii* (Zimm.) Viegas (Deuteromycotina: Hyphomycetes) on capsicum leaves (*Capsicum annum* grossum Sendt. Var. California Wonder) and infection of *Myzus persicae* Sulzer (Homoptera: Aphididae). *Biocontrol Sci. Technol*. 13: 35-46.

Cuthbertson, A. G. S., Walters, K. F. A., and Northing, P. 2005. The susceptibility of immature stages of *Bemisia tabaci* (G.) to the entomopathogenic fungus *Lecanicillum muscarium* (*V. lecanii* (Zimm.) on tomato and verbena foliage. *Mycopathologia* 159: 23-29.

Derakhshan, A., Rabindra, R. J., Ramanujam, B., and Rahimi, M. 2008a. Evaluation of Different Media and Methods of Cultivation on the Production and Viability of Entomopathogenic Fungi, *Verticillium lecanii* (Zimm.) Viegas. *Pakistan J. Biol. Sci.* 11: 1506-1509.

Derakhshan, A., Rabindra, R. J., and Ramanujam, B. 2008b. Effect of storage of formulations on viability of *Verticillium lecanii* (Zimmermann) Viegas and its virulence to *Brevicorne brassicae* (L). *J. Biol. Sci.* 8: 498-501.

Desai, S. A. and Kulkarni, S. 2002. Effect of Urea on the Growth and Sporulation of *Macrophomina phaseolina* (Tassi) Gold and Native Isolates of *Trichoderma viride* Pers. and *Trichoderma harzianum* Rifai. *Karnataka J. Agric. Sci*. 15(1): 77-79.

Diaz, B. M., Oggerin, M., Lastra, C. C. L., Rubio, V., and Fereres, A. 2009. Characterization and virulence of *Lecanicillium lecanii* against different aphid species. *Biocontrol* 54 (6): 825–835.

Easwaramoorthi, S. and Jayaraj, S. 1978. The effect of temperature, pH and media on the growth of the fungus, *Cephalosporium lecanii*. *J. Invertebr. Pathol*. 29: 399-400.

Ebert, D. 1994. Virulence and local adaptation of a horizontally transmitted parasite. *Science* 265: 1084-1086.

Ekbom, B. S. 1979. Investigations on the potential of a parasitic fungus (*Verticillium lecanii*) for biological control of greenhouse whitefly (*Trialeurodes vaporariorium*). *Swedish J. Agric. Res.* 9: 129-138.

El-Sayed, G. N., Coudron, T. A., Ignoffo, C. M. and Riba, G. 1989. Chitinolytic activity and virulence associated with native and mutant isolates of an entomopathogenic fungus, *Nomuraea rileyi*. *J. Invertebr. Pathol*. 54: 394-403.

El-Sayed, G. N., Ignoffo, C. M., Leathers, T. D. and Gupta, S. C. 1993. Effects of cuticle source and concentration on expression of hydrolytic enzymes by an entomopathogenic fungus, *Nomuraea rileyi*. *Mycopathologia* 122: 149-152.

Evans, H.C. and Prior, C. 1997. Entomopathogenic fungi. In: Rosen, D. (ed.), *World crop pests, armored scale insects, their biology, natural enemies and control* (4 th Ed.). Elsevier Science Publisher, New York. pp. 3-17.

Ewald, P. W. 1995. The evolution of virulence: a unifying link between parasitology and ecology. *J. Parasitol.* 81: 659-669.

Fang, W., Leng, B., Xiao, Y., Jin, K., Ma. J., Fan, Y., Feng, J., Yang, X., Zhang, Y., and Pei, Y. 2005. Cloning of *Beauveria bassiana* chitinase gene *Bbchit*1 and

its application to improve fungal strain virulence. *Appl. Environ. Microbiol.* 71: 363-370.

Field, R. J. and Dastgheib, F. 1996. Enhancing uptake and translocation of systemic active ingredients. In: Foy, C. L. and Pritchard, D. W. (eds), *Pesticide Formulation and Adjuvant Technology*. Boca Raton, Florida, CRC press, 363p.

Fiedler, Z., Sosnowska, D., and Baranowski, T. 2002. Fungal pathogens active substance if Fitoverm - abamectin in biological control against greenhouse pests. *Prog. Plant Prot.* 42(2): 420-423.

Galvao, J. A. H. and Bettiol, W. 2014. Effects of UV-B radiation on *Lecanicillium* spp., biological control agents of the coffee leaf rust pathogen. *Trop. Plant Pathol.* 39(5): 392-400.

Geetha, I. and Balaraman, K. 2001. Biomass and blastospore production in *Beauveria bassiana* (Bals.) Vuill. as influenced by media components. *J. Biol. Control* 15(1): 93-96.

Gerding-Gonzalez, M., France, A., Sepulveda, M. E., and Campos, J. 2007. Use of chitin to improve a *Beauveria bassiana* alginate-pellet formulation. *Biocontrol Sci. Technol*. 17(1): 105-110.

Goettel, M. S., Koike, M., Kim, J. J., Aiuchi, D., Shinya, R., and Brodeur, J. 2008. Potential of *Lecanicillium* spp. for management of insects, nematodes and plant diseases. *J. Invertebr. Pathol*. 98: 256-261.

Grand, M. L. and Cliquet, S. 2013. Impact of culture age on conidial germination, desiccation and UV tolerance of entomopathogenic fungi. *Biocontrol Sci. Technol*. 23(7): 847-859.

Hall, I. M. and Bell, J. V. 1961. Further studies on the effect of temperature on the growth of some entomophthoraceous fungi. *J. Insect. Pathol.* 8: 589-596.

Hall, R. A. 1976. A bioassay of the pathogenicity of *Verticillium lecanii* conidiospores on the aphid, *Macrosiphoniella sanborni. J. Invertebr. Pathol.* 27: 41-48.

Hall, R. A. and Papierok, B. 1982. Fungi as biocontrol agents of arthropods of agricultural and medical importance. *Parasitology* 84: 205-240.

Hallsworth, J. E. and Magan, N. 1994. Effects of KCl concentration on accumulation of acyclic sugar alcohols and trehalose in conidia of three entomopathogenic fungi. *Lett. Appl. Microbiol.* 18: 8-11.

Hegde, S.V. 2002. Liquid biofertilizers in Indian agriculture. *Biofertilizer News Lett.* 12: 17-22.

Hegedus, D. D., Bidochka, M. J., and Khachatourians, G. G. 1990. *Beauveria bassiana* submerged conidia production in a defined medium containing chitin, two hexosamines or glucose. *Appl Microbiol Biotech.* 33(6): 641-647.

Hsiao, W. F., Bidochka, M. J., and Khachatourians, G. G. 1992. Effect of temperature and relative humidity on the virulence of the entomopathogenic fungus *Verticillium lecanii*, toward the oat-bird aphid, *Rhopalosiphum padi* (Hom., Aphididae). *J. Appl. Ent.* 114: 484-490.

Ignoffo, C. M. 1992. Environmental factors affecting persistence of entomopathogens. *Florida Entomologist* 75(4): 516-525.

Inglis, G. D., Johnson, D. L., and Goettel, M. S. 1996. Effect of bait substrate and formulation on infection of grasshopper nymphs by *Beauveria bassiana*. *Biocontrol Sci. Technol.* 6:35-50.

Inyang, E., McCartney, H., Oyejola, B., Ibrahim, L., Pye, B., Archer, S., and Butt, T. 2000. Effect of formulation, application and rain on the persistence of the entomogenous fungus *Metarhizium anisopliae* on oilseed rape. *Mycol. Res*. 104: 653-661.

Jayaraj, J. and Ramabadran, R. 1998. Effect of certain nitrogenous sources on the *in vitro* growth, sporulation and production of antifungal substances by *Trichoderma harzianum. J. Mycol. Plant Pathol*. 28: 23-25.

Kamp, A. M. and Bidochka, M. J. 2002. Conidium production by insect pathogenic fungi on commercially available agars. *Lett. Appl. Microbiol*. 35: 74-77.

Kanagaratnam, P., Hall, R. A., and Burges, H. D. 1982. Control of glasshouse whitefly by an aphid strain of the fungus, *Verticillium lecanii. Ann. Appl. Biol.* 100: 213-219.

KAU (Kerala Agricultural University). 2014. *Package of Practices Recommendations: Crops* (15th Ed.). Kerala Agricultural University, Thrissur, 360p.

Kim, J. J., Goettel, M. S., and Gillespie, D. R. 2007. Potential of *Lecanicillium* species for dual control of aphids and the cucumber powdery mildew fungus, *Sphaerotheca fuliginea. Biol. Control* 40: 327-332.

Kleespies, R.G. and Zimmermann, G. 1992. Production of blastospores by three strains of *Metarhizium anisopliae* (Metch.) Sorokin in submerged culture. *Biocontrol Sci. Technol.* 2: 127-135.

Kleespies, R. G. and Zimmermann, G. 1998. Effect of additives on the production, viability and virulence of blastospores of *Metarhizium anisopliae*. *Biocontrol Sci. Technol.* 8(2): 207-214.

Kmitowa, K. and Popowska-Nowak, E. 1995. The effect of culture methods on the pathogenicity of different strains and species of entomopathogenic fungi. *Pol. Ecol. Stud.* 21(1): 51-56.

Knudsen, G. R., Johnson, J. B., and Eschen, D. J. 1990. Alginate pellet formulation of a *Beauveria bassiana* (Fungi: Hyphomycetes) isolate pathogenic to cereal aphids. *J. Econ. Entomol*. 83: 2225-2228.

Koike, M., Kodama, T., Kikuchi, A., Okabe, M., Kuramoti, K., and Saito, Y. 2005. Effects of *Verticillium lecanii* (*Lecanicillium* spp.) against two-spotted spider mite, *Tetranychus urticae* and its natural enemy, *Phytoseiulus persimilis* [abstract]. In. Abstracts, *Annual Meeting of the Society for Invertebrate Pathology*; 7-11, August, 2005, *Anchorage. Society for Invertebrate Pathology*, Anchorage, Alaska, 45p.

Lafontaine, P. J and Benhamou, N. 1996. Chitosan Treatment: An emerging strategy for Enhancing Resistance of Greenhouse Tomato plants to infection by *Fusarium oxysporum* f.sp. *radices-lycopersici*. *Biocontrol Sci. Technol*. 6(1): 111-124.

Lee, J. Y., Kang, S. W., Yoon, C. S., Kim, J. J., Choi, D. R., and Kim, S. W. 2006. *Verticillium lecanii* spore formulation using UV protectant and wetting agent and the biocontrol of cotton aphids. *Biotechnol. Lett*. 28: 1041-1045.

Leland, J. E., Mullins, D. E., Vaughan, L. J., and Warren, H. L. 2005. Effects of media composition on submerged culture spores of the entomopathogenic fungus, *Metarhizium anisopliae* var. *acridum* Part 2: Effects of media

osmolality on cell wall characteristics, carbohydrate concentrations, drying stability, and pathogenicity. *Biocontrol Sci. Technol.* 15(4): 393-409.

Liu, S. D., Chang, Y. C., and Huang, Y. S. 1990. Application of entomopathogenic fungi as biological control of Leucaena psyllid, *Heteropsylla cubana* Crawford (Homoptera: Psyllidae) in Taiwan. *Pl. Prot. Bull*. 32(1):49-58.

Liu, W., Xie, Y., Dong, J., Xue, J., and Zhang, Y. 2014. Pathogenicity of Three Entomopathogenic Fungi to *Matsucoccus matsumurae*. *PLoS ONE.* 9(7): 72-83.

Lokesh, S. 2014. Evaluation of entomopathogenic fungi against pest complex of chilli (*Capsicum annuum* L.). M. Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 161p.

Lomer, C. H. and Lomer, C. J. 2001. Collection of Insect Pathogens. *Lubilosa Tech. Bull.* 2: 24p.

Loureiro, E. S. and Moino, A. Jr. 2006. Pathogenicity of Hyphomycete fungi to aphids *Aphis gosyypii* Glover and *Myzus persicae* (Sultzer) (Hemiptera: Aphididae). *Neotrop. Entomol*. 35: 660-665.

Luz, C. and Batagin, I. 2005. Potential of oil-based formulations of *Beauveria bassiana* to control *Triatoma infestans*. *Mycopathologia* 160: 51-62.

Manjula, K., Rao, A., and Nagalingam, B. 2004. Record of *Nomuraea rileyi* (Farlow) Sampson on *Helicoverpa armigera* Hubner in kharif Groundnut. *Indian J. Plant Prot*. 32: 125-128.

Matsumoto, Y., Saucedo, G., Revah, S., and Shirai, K. 2004. Production of β-*N*-acetylhexosaminidase of *Verticillium lecanii* by solid state and submerged

fermentations utilizing shrimp waste silage as substrate and inducer. *Process Biochem.* 39: 665-671.

Mishra, S., Kumar, P., and Malik, A. 2013. Evaluation of *Beauveria bassiana* Spore Compatibility with Surfactants. *Int. J. Biol. Biomol. Agric. Food Biotechnol. Eng*. 7(1): 7-11.

Mohanty, S. S. and Prakash, S. 2004. Extracellular metabolites of *Trichophyton ajelloi* against *Anopheles stephensi* and *Culex quinquefasciatus* larvae. *Curr. Sci.* 86: 323-325.

Moore, D. and Prior, C. 1993. The potential of mycoinsecticides. *Biocontrol News Info.* 14: 31–40.

Mor, H., Gindin, G., Ben-Zeev, I. S., Geschtovt, N. U., Arrkhozhina, N., and Barash, I. 1995. Diversity among isolates of *Verticillium lecanii* as expressed by DNA polymorphism and virulence towards *Bemisia tabaci*. *Phytoparasitica.* 24: 111-118.

Myers, J. H. and Rothaman, L. E. 1995. Virulence and transmission of infectious diseases in humans and insects: evolutionary and demographic patterns. *Trends Ecol. Evol.* 10: 194-98.

Naik, H. P. R. and Shekharappa. 2009. Field evaluation of different entomopathogenic fungal formulations against sucking pests of okra. *Karnataka J. Agric. Sci*. 22(3): 575-578.

Nirmala, R. 2003. Evaluation of Entomopathogenic Fungi against sugarcane wooly aphid, *Ceratovacuna lanigera* Zehhntner (Homoptera: Aphididae) and other aphids. *Hands on Training Report submitted to Project Directorate of Biological Control.* pp. 19-20.

Nirmala, R., Ramanujam, B., Rabindra, R. J. and Rao, N. S. 2006. Effect of entomofungal pathogens on mortality of three aphid species. *J. Biol. Control*. 20: 89-94.

Palma-Guerrero, J., Jansson, H-B., Salinas, J., and Lopez-Llorca, L.V. 2008. Effect of chitosan on hyphal growth and spore germination of plant pathogenic and biocontrol fungi. *J. Appl. Microbiol.* 104: 541-553.

Palma-Guerrero, J., Larriba, E., Guerri-Agullo, B., Jansson, H-B., Salinas, J., and Lopez-Llorca, L. V. 2010a. Chitosan increases conidiation in fungal pathogens of invertebrtaes. *Appl. Microbiol. Biotechnol.* 87: 2237-2245.

Palma-Guerrero, J., Lopez-Jimenez, J. A., Perez-Berna, A. J., Huang, I. C., Jansson, H, B., Salinas, J., Villalain, J., Read, N. D., and Lopez-Llorca, L. V. 2010b. Membrane fluidity determines sensitivity of filamentous fungi to chitosan. *Mol. Microbiol.* 75:1021-1032.

Pandey, A. K. and Kanaujia, K. R. 2010. Suitability of different synthetic and grain based media for the mass culture of entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin. *J. Ent. Res.* 34: 305-309.

Park, H. and Kim, K. 2010. Selection of *Lecanicillium* strains with high virulence against developmental stages of *Bemisia tabaci*. *Mycobiol.* 38: 210-214.

Pavlyushin, V. A., Tyuterev, S. L., Novikova, I. I., Popova, E. V. Bykova, G. A., Boikova, I. V., and Khatskevich, L. K. 2005. New preparations for combined protection of plants against diseases of various etiology. *Russ. Agric. Sci*. 12: 7-12.

Pindi, P. K. and Satyanarayana, S. D. V. 2012. Liquid Microbial Consortium: A Potential Tool for Sustainable Soil Health. *J. Biofertil. Biopestici*. [e-journal]

3(4). Available: http://www.omicsonline.org/liquid-microbial-consortium-a-potential-tool-for-sustainable-soil-health.pdf. ISSN 2155-6202 [20 February 2015].

Prior, C., Jollands, P., and Patourel, G. L. 1988. Pathogenicity test of *Beauveria bassiana* (Balsamo) against oil palm bagworm (*Metisa plana* Wlk). *Elaeis*. 5(2): 92-101.

Purwar, J. P. and Sachan, G.C. 2006. Evaluation of media for growth and sporulation of entomogenous fungi. *Ann. Plant Protec. Sci*. 14: 484-485.

Rabindra, R. J. and Ramanujam, B. 2007. Microbial control of sucking pests using entomopathogenic fungi. *J. Biol. Control.* 21: 21-28.

Raheem, A. M., Sabry, K. H., and Rajab, Z. A. 2009. Effect of Different Fertilization rates on control of *Bemisisa tabaci* (Genn.) by *Verticillium lecanii* and *Beauveria bassiana* in Potato crop. *Egypt. J. Biol. Pest Control* 19(2): 129-133.

Ramle, M., Basri, W. M., Ramlah, A. A., and Norman, K. 2004. The effects of oils on germination of *Beauveria bassiana* (Balsamo) Vuillemin and its infection against the oil palm bagworm, *Metisa plana* (Walker). *J. Oil Palm Res*. 16 (2): 78-87.

Rawat, R. R. and Shau, H. R. 1973. Estimation of losses in growth and yield of okra due to *Empoasca devantans* and *Earias* spp. *Indian J. Entomol.* 35:252-254.

Roberts, W. K. and Selitrennikoff, C. P. 1988. Plant and bacterial chitinases differ in antifungal activity. *J. Gen. Microbiol.* 134: 169-176.

Rombach, M. C. and Gillepsie, A. T. 1998. Entomogenous hypomycetes for insect and mite control of greenhouse crops. *Biocontrol News and Inf.* 9(1): 7-8.

Sahayaraj, K. and Namasivayam, S. K. R. 2007. Bioefficacy of entomopathogenic fungi against *Aphis craccivora* in groundnut. *Indian J. Plant Prot*. 35(2): 352-353.

Salam, A. M. F. and Hawary, F. M. A. 2011. Lethal and pathogenic effects of *Beauveria bassiana* and *Lecanicillium lecanii* on the adult and nymph of *Aphis craccivora* Koch. *Arch. Phytopathol. Plant Prot*. 44(1): 56-57.

Santharam, G., Easwaramoorthi, S., Regupathy, A., and Jayaraj, S. 1977. Possibility of increasing the pathogenicity of the white halo fungus, *Cephalosporium lecanii* on the coffee green bug, *Coccus viridis* during summer. *J. Plant. Crops* 5: 121-122.

Saranya, S., Ushakumari, R., Jacob, S., and Philip, B. M. 2010. Efficacy of different entomopathogenic fungi against cowpea aphid, *Aphis craccivora* (Koch). *J. Biopestic.* 3(1): 138-142.

Simova, S. and Draganova, S. 2003. Virulence of entomopathogenic fungi to *Tetranychus urticae* Koch (Tetranychidae, Acarina). *Rasteniev dni Nauki* 40: 87-90.

Srikanth, J. 1985. Studies on cowpea aphid *Aphis craccivora* Koch (Hemiptera: Aphididae) and its predatory coccinellid *Menochilus sexmaculatus* F. (Coleoptera: Coccinellidae) M.Sc. Agricultural Thesis, University of Agricultural Sciences, Bangalore, India, 134 p.

Srikanth, J. and Santhalakshmi, G. 2012. Effect of Media additives on the Production of *Beauveria brongniartii,* an Entomopathogenic fungus of *Holotrichia serrata*. *Sugar Tech.* 14(3): 282-290.

Sriram, S., Palanna, K. B., and Ramanujam, B. 2010. Effect of chitin on the shelf- life of *Trichoderma harzianum* in talc formulation. *Indian J. Agric. Sci.* 80(10): 80-82.

Stathers, T. E., Moore, D., and Prior, C. 1993. The effect of different temperatures on the viability of *Metarhizium flavoviride* conidia stored in vegetable and mineral oils. *J. Invertebr. Pathol.* 62(2): 111-115.

St. Leger, R. J., Cooper, R. M., and Charnley, A. K. 1986a. Cuticle degrading enzymes of entomopathogenic fungi: regulation of production of chitinolytic enzymes. *J. Gen. Microbiol.* 132: 1509-1517.

St. Leger, R. J., Charnley, A. K., and Cooper, R. M. 1986b. Cuticle degrading enzymes of entomopathogenic fungi. Synthesis in culture on cuticle. *J. Invertebr. Pathol*. 47:167-177.

St. Leger, R. J., Joshi, L., Bidochka, M. J., and Roberts, D. W. 1996. Construction of an improved mycoinsecticide overexpressing a toxic protease. *Proc. Natl. Acad. Sci. USA* 93: 6349-6354.

Sun, M. and Liu, X. 2006. Carbon requirements of some nematophagous, entomopathogenic and mycoparasitic hyphomycetes as fungal biocontrol agents. *Mycopathologia* 161(5): 295-305.

Suresh, B. C., Khan, H. K., and Prasanna, P. M. 2012. Efficacy of different entomopathogenic fungi against cowpea aphid, *Aphis craccivora* Koch under laboratory conditions. *Int. J. Plant Prot*. 5(1): 68-71.

Tanuja, K., Hemalatha, K., Karuna, R., and Sashidhar, R. B. 2010. Effect of various surfactants (cationic, anionic and non-ionic) on the growth of *Aspergillus parasiticus* (NRRL 2999) in relation to aflatoxin production. *Mycotox. Res.* 26: 155–170.

Ujjan, A. A. and Shahzad, S. 2012. Use of entomopathogenic fungi for the control of mustard aphid (*Lipaphis erysimi*) on canola (*Brassica napus* L.). *Pak. J. Bot.* 44(6): 2081-2086.

Verhaar, M. A., Hijwegen, T., and Zadoks, J. C. 1999. Improvement of the efficacy of *Verticillium lecanii* used in biocontrol of *Sphaerotheca fuliginea* by addition of oil formulations. *BioControl* 44: 73-87.

Vernner, R. and Bauer, P. 2007. Q-TEO, a formulation concept that overcomes the incompability between water and oil. *Pfalzenschutz-Nachrichten Bayer* 60(1): 7-26.

Viegas, A. P. 1939. Um amigo do fazendeiro *Verticillium lecanii* (Zimm.) n. comb., o causador do halo branco do *Coccus viridis* (Green). *Revista do Instituto de Cafe do Estado de Sao Paulo* 14: 754-772.

Vimaladevi, P. S. and Prasad, Y. G. 1996. Compatibility of oils and antifeedants of plant origin with the entomopathogenic fungus, *Nomuraea rileyi*. *J. Invertebr. Pathol*. 68: 91-93.

Vimaladevi, P. S., Prasad, Y. G., and Chowdary, A. 2002. Effect of drying and formulation of conidia on virulence of the entomofungal pathogen, *Nomuraea rileyi* (Farlow) Samson. *J. Biol. Control* 16(1): 43-48.

Wenzel, I. M., Monteiro, A. C., and Pereira, G. T. 2007. Performance of *Lecanicillium lecanii* on culture medium containing vitamins and yeast extract concentrations. *Bragantia* 66(3): 413-421.

Wenzel, I. M. and Filho, B. A. 2011. Effect of pesticides on the pathogenicity of conidia of *Lecanicillium lecanii* on *Tetranychus urticae*. *Arq. Inst. Biol*. 78(2): 261-266.

Woertz, J. R. and Kinney, K. A. 2004. Influence of sodium dodecyl sulphate and Tween 20 on fungal growth and toluene degradation in a vapour-phase bioreactor. *J. Environ. Eng.* 130: 292-299.

Woods, T. S. 2003. Pesticide Formulations, in AGR 185. In: *Encyclopedia of Agrochemicals.* Wiley and Sons, New York, pp 1-11.

Wu, J., Ali, S., Huang, Z., Ren. S. X., and Cai, S. J. 2010. Media composition influences growth, enzyme activity and virulence of the entomopathogen *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae). *Pakistan J. Zool.* 42(4): 451-459.

Yokomi, R. K. and Gottwald, T. R. 1988. Virulence of *Verticillium lecanii* isolated in aphids determined by detached leaf bioassay. *J. Invertebr. Pathol*. 51: 250–258.

Ying-Ping, X., Jiao-Liang, X., Zhi-Juan, Z., Wei-Min, L., Qian, Y., and Jin-Hua, F. 2012. Entomopathogenic fungal parasites of scale insects and their potential in biological control. *Mycosystema* 31(3): 307-321.

Zare, R., Gams, W., and Culham, A. 2000. A revision of *Verticillium* section *Prostrata* I. Phylogenetic studies using ITS sequences. *Nova Hedwig* 71: 465-480.

Zare, R. and Gams, W. 2001. A revision of *Verticillium* section *Prostrata*. IV. The genera *Lecanicillium* and *Simplicillium*. *Nova Hedwig.* 73: 1-50.

Zhang, A. W., Liu, W. Z., Nong, X. Q., Deng, C. S., Guo, W. L., and Jang, B. 1992. A trial production of wettable powder of *Beauveria bassiana*. *Chin. J. Biol. Control* 8(3): 118-120.

Zhu, H. and Kim, J. J. 2011. Susceptibility of the tobacco whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotype Q to entomopathogenic fungi. *Biocontrol Sci. Technol*. 21(12): 1471-1483.

Abstract

Improved Formulation of *Lecanicillium lecanii* (Zimmermann) Zare and Gams and its Evaluation Against Sucking Pests

by
NITHYA P. R.
(2013-11-125)

Abstract of the thesis

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE
Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM – 695 522
KERALA, INDIA

2015

ABSTRACT

The work entitled "Improved formulation of *Lecanicillium lecanii* (Zimmermann) Zare and Gams and its evaluation against sucking pests" was conducted at College of Agriculture, Vellayani during the period 2013-15 with the objective to improve the conidial viability and virulence of *L. lecanii* in bioformulations and evaluation of their effectiveness in controlling sucking pests.

Pathogenicity studies revealed that the fungus is infective to sucking pests of cowpea, *viz.* the aphid, *Aphis craccivora* Koch, the white fly, *Bemisia tabaci* Gennadius, the jassid *Amrasca biguttula biguttula* Ishida, the mealy bug, *Ferrisia virgata* Cockerell, red spider mie *Tetranychus* sp. and the brinjal scale *Lecanium* sp., causing mortality of 90 %, 53.33 %, 26.67 %, 6.67 %, 100 % and 70 %, respectively, five days after treatment. It was not infective to *Riptortus pedestris* Fabricius and *Anchon pilosum* Walker. Observations on symptoms of mycosis did not exhibit much variation among the test organisms. Lethargic movement followed by cessation of feeding was noticed in all species. The cadaver was covered with white mycelia by 72 hours after treatment (HAT) in aphids and mealy bugs, while mycelia covered the cadaver of whitefly except the wings. In scales, radiating white mycelial strands were observed from the margins of body. Mites took infection within 24 HAT and surface of the cadaver was blanketed with white mycelia. The effective dose of *L. lecanii* was determined as 10^7 spores ml^{-1}.

The media supplement to improve viability and sporulation was assessed based on biomass, number of spore count, colony forming units (cfu) and mortality caused to *A. craccivora.* Maximum biomass yield was obtained with PEG 2 % (2.67g / 100 ml), followed by that of PEG 4 %, crude and extra pure chitosan 5 % and arachid oil 5 %. Based on number of cfu, arachid oil (1 %), crude and extra pure chitosan (0.5, 2 and 5 %), crude and extra pure chitin (5 %), and yeast (1, 2 and 3 %) were found to be superior media supplements with cfu ranging from 1.53 – 1.57 x 10^5 ml^{-1}. Sporulation was found to be maximum with

5 % chitosan extra pure yielding 9.34 x 10^8 spores ml^{-1}, followed by chitosan extra pure 2 % (7.83 x 10^8 spores ml^{-1}). The spore suspensions from extra pure chitosan 5 %, crude chitin 5 % and arachid oil 5 %, sprayed on *A. craccivora* resulted in cent per cent mortality within 72 h of treatment.

Chitin enriched ground nut oil (GNO + Chitin) and chitin enriched sunflower (SFO + chitin) were the best carriers suitable for formulation. The viability by the end of three months of storage was 2.27 x 10^6 ml^{-1} and 2.20 x 10^6 ml^{-1}. The corresponding spore count was 1.67 x 10^8 spores ml^{-1} and 1.65 x 10^8 spores m^{-1}.

PEG 2 %, POX 0.5 %, tween-80 1 % and glycerol 5 % were found to be the effective adjuvant concentrations. The effective adjuvant combinations were found to be tween-80 1 % + PEG 2 % + glycerol 5 % + POX 0.5 % (AC1), tween-80 1 % + Glycerol 5 % (AC2) and tween-80 1 % + glycerol 5 % + POX 0.5 % (AC3). Among the four materials tried for their UV protectance, boric acid 1 % was the best.

The three effective formulations, tested under field conditions for management of sucking pests in cowpea were Formulation I - Chitin enriched groundnut oil + AC1 + boric acid 1 % ; Formulation II - Chitin enriched groundnut oil + AC2 + boric acid 1 % and Formulation III - Chitin enriched groundnut oil + AC3 + boric acid 1 %. Field evaluation revealed that Formulation I was equally effective as chlorpyriphos 0.05 % resulting in 98.93 % control at 5 % level of infestation and 96.74 % at fifty per cent infestation levels, seven days after treatment. The percentage reduction noted in population of mites, jassids and whiteflies was 92.58 %, 58.81 % and 96.74 %, respectively after seven days of spraying. Among the bioformulations, Formulation I and II recorded highest yield of 7.03 and 6.74 kg $plot^{-1}$ respectively which was significantly lower than the yield obtained from chlorpyriphos 0.05 % treated plots (8.57 kg).

It is concluded that viability and virulence of *L. lecanii* formulations can be improved using chitin enriched groundnut oil as carrier and PEG 2 % + POX 0.5 % + tween-80 1 % + glycerol 5 % as adjuvant. The formulation at 1 % concentration with boric acid 1 % as UV protectant effectively controlled aphids, whiteflies, jassids and mites the common sucking pests in cowpea, under field conditions.