NOVEL BIOFORMULATIONS OF ENTOMOPATHOGENIC FUNGI AND THEIR EFFICACY AGAINST BANANA WEEVILS

by

REMYA S.
(2016-11-001)

THESIS

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

2018

DECLARATION

I, hereby declare that this thesis entitled **"Novel bioformulations of entomopathogenic fungi and their efficacy against banana weevils"** is a bonafide record of research work done by me during the course of research and that 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,

23/06/2018

Remya.S

(2016-11-001)

CERTIFICATE

Certified that this thesis entitled **"Novel bioformulations of entomopathogenic fungi and their efficacy against banana weevils"** is a record of research work done independently by Ms. Remya. S under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Vellayani,
23/06/2018

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

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Remya. S, a candidate for the degree of **Master of Science in Agriculture** with major in Agricultural Entomology, agree that this thesis entitled "Novel bioformulations of entomopathogenic fungi and their efficacy against banana weevils" may be submitted by Ms. Remya. S, in partial fulfilment of the requirement for the degree.

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

Dr. N. Anitha
(Member, Advisory Committee)
Professor and Head
Department of Agricultural Entomology
College of Agriculture, Vellayani.

Dr. Amritha V. S.
(Member, Advisory Committee)
Assistant professor
Department of Agricultural Entomology
College of Agriculture, Vellayani.

Dr. Susha S. Thara
(Member, Advisory Committee)
Assistant Professor
Department of Plant Pathology
College of Agriculture, Vellayani.

23.6.18
EXTERNAL EXAMINER
Dr. K. Sudharma
Professor (Entomology) Retd.
Kerala Agricultural University

ACKNOWLEDGEMENT

I would like to express my gratitude and sincere thanks to Dr. Reji Rani O. P., Assistant Professor, Department of Agricultural Entomology for her valuable guidance and constructive suggestions throughout the period of study without which this endeavor would not have been successful.

I express my sincere gratitude to Dr. N. Anitha, Professor and Head, Department of Agricultural Entomology, for the timely advice, valuable suggestions and support provided at all times of this work.

I convey my heartfelt thanks to Dr. Amritha V. S., Assistant Professor, Department of Agricultural Entomology, for the valuable advice and suggestions during the course of my research work.

I would like to thank Dr. Susha S Thara, Asst. Professor, Department of Plant Pathology, for her guidance and support during the period of study.

I wish to express my earnest thanks to my teachers, Dr. Sudharma, Dr. Jiji. T, Dr. M. H. Faizal, Dr. R. Krishnakumar, Dr. K. D. Prathapan, Dr. Ambily Paul and Dr. Santhosh for their timely help rendered during the work.

I owe my heartfelt gratitude to Smt. Brigit Joseph, Assoc. Professor and Dr. Vijayaraghava Kumar, Prof. and Head, Department of Agricultural Statistics for their valuable advices which helped me to complete the statistical analysis.

I am obliged to Jiji Chechi, Sam Chettan and Smitha Chechi for their timely support, guidance, care and friendly approach given during all the stages of my research work. A word of special thanks to Asha Chechi and Chinchu Chechi who have really helped me a lot to reduce my work load.

I express my heartfelt thanks and love to my dearest batchmates, especially Binseena, Jyothis, Priyatha, Raesa, Muhsina, Geethu, Nivya and Pooja for their care, support, help and inspiration during my hardest times. I am dearth of words to express my thanks to Athira who have helped me to overcome the hurdles in my research life.

I would like to express my whole hearted thanks to Nithya Chechi, Naveeda Chechi, Hari, Archana Chechi, Varsha Chechi, Tejaswi Chettan, Viswajyothi Chechi Anusree Chechi, Anju Chechi and Ishrath Chechi for their advices which have straightened my path of research.

I would like to acknowledge the help and support rendered by my juniors Keerthana, Divyasree, Ayisha, Lintu and Ajina.

I am also thankful to all the staff of College of Agriculture, Vellayani who have become a part of my thesis directly or indirectly.

I am most indebted to my Amma, Pappa and Reshma, for their affection, constant encouragement, moral support and their blessings and prayers in all the moments of my life which enabled me to complete this work.

Above all, I thank God Almighty who is the author of all inspirations.

Remya S

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

@	At the rate of
^{0}C	Degree Celsius
Ca	Calcium
CD	Critical Difference
CRD	Completely Randomised Design
cfu	colony forming units
DAS	Days After Storage
DAT	Days After Treatment
EPF	Entomopathogenic fungi
EPN	Entomopathogenic nematode
et al.	And others
Fig.	Figure
g	Gram
g^{-1}	Per gram
h	Hours
HAT	Hours After Treatment
HGT	Hard Gelatin Transparent
HGC	Hard Gelatin Coloured
HPMC	Hydroxy Propyl Methyl Cellulose
KAU	Kerala Agricultural University
L^{-1}	Per litre
LR	Laboratory Reagent
mg	Milligram
mL	Millilitre
mL^{-1}	Per millilitre
Na	Sodium
NS	Non Significant
sp. or spp.	Species (singular and plural)
s	Seconds
viz.	Namely

Introduction

1. INTRODUCTION

Microorganism based biopesticides represent an alternative path in crop protection because of their safety to human beings and other non-target organisms. Among the insect pathogenic microbes, entomopathogenic fungi have been investigated exhaustively for their potential use as biopesticides owing to their amenability to mass production. Although a myriad of fungi has been reported as effective in pest management, the share of biopesticides in worlds' total pesticide market is only 1.3 per cent (Bailey, 2010). One of the factors limiting its adoption is the lack of stable formulations. Formulation improvement is thus becoming the most important area to enhance and extend the activity of biopesticides. Development of appropriate formulations with improved stability and viability can enhance field performance of many potential entomopathogens.

The technology of bioformulation takes into consideration all the steps from production of a microbe to its subsequent action on the target pest. An ideal formulation is aimed at stabilising the organism during production, distribution and storage, and easiness in delivery to the target. A multitude of approaches are available to the formulator to attain the basic functions. It involves blending of active ingredients such as fungal spores with the inert materials like carriers and adjuvants in order to alter the physical characteristics of the active ingredients to a more desirable form. This in turn offers better protection of microbial agent from environmental conditions, release at controlled rates, as well as improved bioactivity and storage stability (Gasic and Tanovic, 2013).

The conventional wettable powder formulations of entompathogens fail to reach its expected potency due to reduced availability at the target site. To curb this downfall, it is the need of the hour to develop novel bioformulations for precise delivery.

Novel bioformulations comprise of capsules, microcapsules, gels, pellets, briquettes and tablets. The virtues of these novel formulations encompass high

field efficacy, controlled release of active ingredients, precise release, succinct storage and easiness in transport. Capsule is a stable formulation wherein the bioagent is encapsulated in coatings and thus protected from extreme environmental conditions of UV radiation, rain and temperature (Burges and Jones, 1998). Its residual stability is enhanced due to slow or controlled release of active ingredients. The chances of contamination being less, they have enhanced shelf life. Owing to their semisolid nature, gel formulations too offer enhanced viability to the organism that is formulated.

Banana, a major fruit crop of Kerala is susceptible to the banana weevils, pseudostem weevil, *Odoiporus longicollis* (Oliver) and the rhizome weevil, *Cosmopolites sordidus* (Germar) causing great havocs to banana farmers in terms of economic loss (Ostmark, 1974; Gailce *et al.* 2008). The complex life cycle of banana weevils and characters of the crop canopy deters plant protection agents from adhering to the waxy plant surface or penetrating the rhizome. Capsule and gel formulations can be effectively used in managing pseudostem weevil in banana by placing them in entry points of the weevil and in the rhizosphere for managing soil-dwelling pest, the rhizome weevil.

The present work, hence undertakes the task of developing protocols for capsule and gel formulations of the entomopathogenic fungi *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin that can offer new delivery systems for targetting banana weevils.

The study therefore emphasised on the following aspects of formulation

- Standardisation of coating and carrier materials for formulating capsules
- Standardisation of moisture content of the formulation
- Development of capsule formulations of *M. anisopliae* and *B. bassiana*
- Development of gel formulations of *M. anisopliae* and *B. bassiana*
- Shelf life of bio-capsules and gels
- Efficacy of superior formulations on banana weevils

Review of Literature

2. REVIEW OF LITERATURE

Biopesticides have emerged as green alternatives to chemical pesticides owing to the concern towards safer food. It opens a new avenue towards sustainable agriculture. As per the report on microbial biopesticides market - global industry growth, trends and forecasts, 2017-2022, microbials hold the largest share in the global biopesticide market. The advantages of microbials include safety to human beings and animals and specificity to target organisms (Nawaz *et al.,* 2016). Of the various microbials used in pest management, Entomopathogenic Fungi (EPF) have a unique mode of entry that enables them to function like a contact insecticide, unlike bacteria and viruses that need to be ingested.

2.1 ENTOMOPATHOGENIC FUNGI IN PEST MANAGEMENT

Entomopathogenic Fungi contributes a major share of microbials with approximately 1.5 to 5 million species belonging to over 100 genera (St. Leger and Wang, 2010). Commercially exploited and widely used EPF includes *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Beauveria bassiana* (Balsamo) Vuillemin, and *Lecanicillium lecanii* (Zimmermann) Zare and Gams.

Since 1960s, considerable number of mycoinsecticides and mycoacaricides have been registered worldwide (de Faria and Wraight, 2007), the most common formulation among them being Wettable Powder (WP). Inorder to ensure the delivery of target organism to the desired site in a precise manner, it should be suitably formulated. The literature pertaining to the various aspects of bioformulations are discussed below.

2.2 FORMULATIONS OF ENTOMOPATHOGENIC FUNGI

Bioformulations of EPF refer to blending of active ingredients such as fungal spores with inert materials like diluents and surfactants in order to alter the

16

physical characteristics of the active ingredients to a more desirable form. They include solid or dry formulations as well as liquid formulations.

Anderson and Roberts (1983) defined the essential constituents of liquid formulations as active ingredient (10-40 %), carrier liquid (35-65 %), suspensor ingredient (1 - 3 %), dispersant (1- 5 %) and surfactant (3 - 8 %).

Seaman (1990) defined various constituents in a dry formulation as active ingredient (50 - 80 %), carrier (15- 45 %), dispersant (1 - 10 %) and surfactant (3- 5 %). Based on particle size they were further classified as Wettable Powder (WP) (5 - 10 mm), Dusts (D) (10 mm), Water Dispersible Granules (WDG) (5-10 mm^3), Pellets (>10 mm^3) and Briquettes (>1 cm^3).

2.2.1 Shelf life of formulations

One of the critical obstacles in the commercialization of bio agents is the loss in viability of propagules over time. Acceptable formulations are very difficult to develop since it is difficult to maintain its viability and purity in storage (Woods, 2003). Microbial formulations should have a good microbial load of 10^7 cfu g^{-1} and shelf life of more than six months. Sustained availability of biocontrol agents can be ensured through formulation development protocols of products with better shelf life (Nakkeeran *et al.*, 2005).

Shelf life of fungi can be determined by evaluating various parameters like germination and the number of viable colonies. Spores which have germ tubes with twice the diameter of the spore or which exhibits conspicuous swelling are considered to be more viable (Herlinda, 2010). Faria *et al.* (2015) reported that debilitated conidia exhibiting slow-germination (requiring more than 16 h to germinate) are less virulent than vigorous conidia that exhibits fast germination (requiring less than 16 h to germinate). Increase in spore viability increases the chances of contact of a viable spore with the insect cuticle even after several days of application of the formulation.

The various factors governing shelf life of a microbial formulation includes carrier material, moisture content of the formulation and storage temperature which are being enumerated below.

2.2.1.1 Carrier materials used in formulations

The carrier materials used for formulating a microbe has a direct role on its shelf life. Several works have been carried out by various researchers to derive suitable carrier materials for solid and liquid formulations.

2.2.1.1.1 Solid carriers

Diatomaceous earth was reported to be a suitable carrier for formulating *Fusarium pallidoroseum* Cooke Sacc. (Faizal, 1992; Sunitha, 1997). Rani (2001) studied the suitability of carrier materials like diatomaceous earth, fine charcoal and semi-dry cadaver of groundnut aphid, *Aphis craccivora* Koch in maintaining spore viability of *F. pallidoroseum* and has reported that at both the storage temperatures, cadaver was the best carrier material followed by diatomaceous earth, leaf mold and charcoal in maintaining spore viability. Batta (2004) formulated *M. anisopliae* in oven ash, chalk powder, charcoal and wheat flour in the ratio 1:4 (w/w) and claimed that the formulations containing charcoal and oven ash had a conidial viability of 4.1 to 4.3 months at 20±1°C when compared to unformulated conidia with a viability of 0.9 months. Bagwan (2011) reported that organic carriers such as vermicompost, deoiled castor cake and farmyard manure were suitable in formulating *M. anisopliae* and *B. bassiana* with shelf life of 210, 190 and 160 days respectively. Abdel- Kader *et al.* (2012) reported that talc based formulation of *Trichoderma harzianum* Rifai maintained the viability upto three months while those with saw dust and chitosan retained the viability over five months of storage.

2.2.1.1.2 Liquid carriers

Stathers *et al.* (1993) revealed that the conidia of *M. anisopliae* retained viability in peanut oil, rapeseed oil and paraffin oil for 14 weeks. Lomer and Lomer (2001) observed comparatively higher germination of *M. anisopliae* and *B. bassiana* in formulations with diesel : sunflower oil in the ratio 7 : 3 ratio, than in diesel : groundnut oil in 7 : 3 ratio. Among the formulations of *M. anisopliae* based on refined oil and vegetable oils, groundnut oil maintained more than 90 per cent conidial viability even after 40 weeks of storage (Alves *et al.* 2002). Combination of coconut oil and soyabean oil in 50 : 50 ratio was found to increase the shelf life of *M. anisopliae* (Batta *et al.*, 2004). According to Banu and Gopalakrishnan (2012), sunflower oil retained the viability of *L. lecanii* spores compared to talc based formulations. Nithya and Rani (2017) developed chitin and chitosan enriched formulations of *L. lecanii* in groundnut oil and sunflower oil and reported that these had better viability and virulence compared to the talc based formulations, while in storage. They standardized the proportion of carrier and technical material in oil and talc formulations of *L. lecanii* as 65 : 35 and 80 : 20, respectively.

2.2.1.2 Moisture content of formulations

Moore *et al.* (1996) studied the influence of moisture on storage properties of *Metarhizium flavoviride* Gams and Rozypal and concluded that four to five per cent moisture is the optimum level based on germination rate (80 per cent) after one week. Derakshan *et al.* (2008) propounded that the viability of *L. lecanii* in talc formulations at five and 10 per cent moisture was on par, but significantly higher than that at 15 per cent. A lower moisture content of less than four per cent was the best for obtaining a dry consistency of tablets of *Beauveria*, *Metarhizium*, *Paecilomyces*, *Lecanicillium* and *Nomuraea* (Satyasayee *et al.*, 2008). Singh and Nautiyal (2012) claimed that *T. harzianum* formulated at eight per cent moisture in talc, exhibited maximum number of colony forming units, 10^{11}-10^{12} g^{-1}, compared to carrier materials like charcoal, cow dung, sawdust and vermiculite with moisture contents of 0, 2, 4, 6, 8, 10 and 12 per cent v/w.

2.2.1.3 Storage temperature

Blachere *et al.* (1973) observed that the viability of *Beauveria brongniartii* (Sacc.) Petch stored for four and seven weeks at room temperature was reduced by 85 per cent and 100 per cent, respectively. Nevertheless, when stored at 4°C, the spore survival was enhanced by 16 per cent, even after eight months. Daoust and Roberts (1983) claimed that a temperature decline from 37°C to 4°C increased the longevity of spores of *M. anisopliae* by over one year. According to Zhang *et al.* (1992), WP formulation of *B. bassiana* exhibited germination rate of more than 85 per cent after eight months of storage under refrigeration. Derakshan *et al.* (2008) opined that the number of viable colonies of *L. lecanii* was enhanced from 28.37 to 75.03 per cent at room temperature to 35.78 to 79.29 per cent under refrigeration. Devi and Hari (2009) claimed that in freshly prepared Suspension Concentrate of *B. bassiana*, the number of cfu was 1.1×10^{15} cfu g^{-1} at the time of production. After 24 months of storage under room temperature, there was a reduction in cfu to 3.0×10^{13} cfu g^{-1}. Das *et al.* (2013) claimed that in talc based WP formulation of *B. bassiana* stored at room temperature, refrigeration and deep freeze, there was a decline in viability with increase in storage temperature. They reported better viability under deep freeze with 2.02×10^{8} cfu g^{-1} number of cfu at the end of 10 months, while an equivalent viability (2.20×10^{8} mL^{-1}) was noted at the end of seven months when stored under refrigeration and six months, when stored at room temperature (2.02×10^{8} mL^{-1}). After 24 months of storage under room temperature, there was a decline in cfu value to 3.0×10^{13} cfu g^{-1}. According to Immediato *et al.* (2017), all conidial suspensions stored at room temperature and under refrigeration showed a reduction of about two logarithmic units of cfu mL^{-1}, from second month to twelfth month.

2.2.2 Conventional formulations

The basic conventional formulations are Wettable Powder, Dust, Granule and liquid formulations.

2.2.2.1 Wettable Powder

Faizal (1992) tested the efficacy of WP formulation of *F. pallidoroseum* and reported that diatomaceous earth based formulations were more effective in controlling *A. craccivora* than the talc based formulation. Olson and Oetting (1999) found that application of WP formulation of *B. bassiana* @ 2×10^{10} cfu g^{-1} resulted in reduction in the population of silver leaf whitefly, *Bemisia argentifolii* Bellows and Perring. Nugroho and Ibrahim (2007) observed that WP formulations of *M. anisopliae*, *B. bassiana* and *Isaria fumosoroseus* (Wize) Brown and Smith formulated in clay @ 1:4 with 1×10^{10} spores mL^{-1} was effective against broad mite, *Polyphagotarsonemus latus* (Banks) in chilli. They also reported that *B. bassiana* has an equal effect as that of the acaricide, amitraz (21.7 % a.i) in suppressing the mite population. Farsi *et al.* (2012) reported that WP formulation of *Lecanicillium musacarium* Zare and Gams blastospore could effectively control second instar nymphs of aphid, *A. gossypii* when compared to unformulated blastospores. Nilamudeen (2015) reported that the application of talc based WP of *B. bassiana* and *M. anisopliae* @ 20 g L^{-1} reduced the population of larvae of rice leaf roller, *Cnaphalocrocis medinalis* (Guenee) and nymphs and adults of rice bug, *Leptocorisa acuta*, Thunberg. Sankar (2018) demonstrated the efficacy of talc based formulation of *M. anisopliae* and *B. bassiana* @ 10^8 spores mL^{-1} in managing *L. acuta* from an initial count of seven and six per five hills to 2.66 and 3.33 per five hills, respectively.

2.2.2.2 Dust

Injections of conidial powder formulations of *B. Bassiana* into ant mounds using a probe, resulted in 52 to 60 per cent reduction in population of red imported fire ant, *Solenopsis invicta* Buren (Oi *et al.,* 1994). Ali (2016) revealed the effectiveness of dust formulation of *B. bassiana* over WP and emulsion, in maintaining conidial viability and virulence to tobacco caterpillar *Spodoptera littoralis* (Boisd.) larvae. Dust formulation of *M. anisopliae*, 10 per cent, with wheat flour as carrier could bring about 77 to 87 per cent mortality of

brown-banded cockroach, *Supella longipalpa* F. which is a vector of many human pathogens (Sharififard *et al.* 2014). Vinayaka *et al.* (2018) demonstrated the efficacy of *M. anisopliae* dust @ 10^8 spores mL $^{-1}$ (5 kg acre^{-1}) resulting in 83.33 per cent mortality of arecanut white grub, *Leucopholis lepidophora*, Blanchard.

2.2.2.3 Granules

Chiuo and Hou (1993) developed *B. bassiana* granules cultured on wine derivatives mixed with sand. The formulation contained 2×10^8 cfu g^{-1} and was effective in controlling Asian corn borer, *Ostrinia furnacalis* Guenee. Jaronski and Jackson (2008) demonstrated the efficacy of microsclerotial granules of *M. anisopliae* grown in media with high C : N ratio (30 - 50:1) against sugarbeet root maggot, *Tetanops myopaeformis* (Roder). They reported 100 per cent mortality of the pest within one week of treatment. Kim *et al.* (2014) reported that various isolates of *B. bassiana* produced as solid cultures in millet grains and applied as granules in rice in nursery stage were able to manage rice water weevil, *Lissorhoptrus oryzophilus* Kuschel effectively.

2.2.2.4 Liquid /oil formulations

Hernadez-Velazquez *et al.* (2003) reported that citroline oil formulation of *M. anisopliae* provided better control of Central American locust, *Schistocerca piceifrons piceifrons* Walker with lesser Median Lethal Time compared to vegetable oil formulation. Luz and Batagine (2005) developed formulations of *B. bassiana* based on 11 different vegetable oils and have found it to be effective against kissing bug, *Triatoma infestans* Klug. The efficacy of talc, lignite and liquid formulations of *M. anisopliae* was studied by Chelvi *et al.* (2011) and opined that liquid formulation of water-in-oil type was more effective for the control of sugarcane white grub, *Holotrichia serrata* F. Ritu *et al.* (2012) developed formulations of *M. anisopliae* using corn-oil, bentonite-based oil, gum and glycerin and claimed that 60 per cent bentonite based oil formulation was more effective in causing larval mortality of *Helicoverpa armigera* (Hubner). The formulation also maintained the viability of fungal spores and

offered easiness in application. Ummidi and Vadlamani (2013) reported that formulations of *B. bassiana* in sunflower oil, olive oil, castor oil and coconut oil exhibited higher mortalities of *Spodoptera litura* F. compared to unformulated conidia. Merril 10 SP (10 per cent mineral oil formulation of *M. anisopliae*) was effective in reducing the population of cattle tick, *Rhipicephalus microplus* Canestrini (Camargo *et al.*, 2014). Wraight *et al.* (2016) compared the efficacy of paraffine Oil Dispersion (OD) to clay-based WP formulation of *B. bassiana* against melon aphid, *A. gossypii* and deduced that OD is superior over WP as it increased the mortality of the pest by 27 per cent. Prithiva *et al.* (2017) evaluated the efficacy of oil formulations of *B. bassiana* and have unveiled its effectiveness on whitefly, *Bemisia tabaci* (Gennadius) in tomato, with a reduction in population of 45.86 per cent. Sankar (2017) reported that application of chitin enriched oil formulation of *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno has resulted in a decline in population of sucking pests in rice from 10 to 1.33 per five hills at 14 Days After Treatment (DAT). According to Nithya and Rani (2018), application of chitin enriched groundnut oil formulation of *L. lecanii* exhibited 96.74 to 98.93 per cent reduction in the population of cowpea aphid, *Aphis gossypii* (Glover).

2.2.3 Novel formulations

Novel formulations of EPF include capsules, microcapsules, gels, prills, pellets, tablets etc.

2.2.3.1 Capsule

Capsule is a stable formulation wherein the bioagent is encapsulated within coatings and thus protected from extreme environmental conditions like UV radiation, rain, temperature etc. and its residual stability is enhanced due to slow or controlled release (Burges and Jones, 1998).

2.2.3.1.1 Coating material of capsule

The ideal coating material standardized by Liu and Liu (2009) was Hydroxy Propyl Methyl Cellulose (HPMC) for a liquid phase coating of microencapsulated conidia of *M. anisopliae.* They reported an encapsulation efficiency of 78 per cent for HPMC, compared to chitosan and sodium alginate. Anandaraj (2016) developed hard gelatin coated bio-capsule of Plant Growth Promoting Rhizobacteria (PGPR) and claimed that the technology offers easiness in storage at normal temperature and delivery of the microbes under field conditions. Moreover, the technology was reported to be cost effective.

2.2.3.1.2 Carrier material for capsules

Dureja and Palmar (2009) developed capsule formulation of *Trichoderma* sp. by mixing dried, and powdered hyphae with organic or inorganic carriers with or without adjuvants that can be stored for two years under ambient conditions. Hiltpold *et al.* (2012) reported the efficacy of alginate based capsules of entomopathogenic nematodes, *Steinernema* spp. and *Heterorhabditis bacteriophora* and claimed that the damage caused by corn root worm, *Diabrotica virgifera virgifera* Dejean was reduced, when compared to water spray. Schoebitz *et al.* (2013) opined that sodium alginate is one of the most commonly used material for the encapsulation of microbials. *Trichoderma viride* Pers. formulated as capsules and tablets were found to be superior to powder formulations using talc and charcoal powder in terms of viability of the organism upto 260 days after storage (Baghel *et al.* 2014). Kim *et al.* (2015) developed calcium alginate capsules of entomopathogenic nematodes as a novel formulation for application in soil. It was observed that hardness of capsules was more in those which were pre-treated with Ca^{2+} at 4^0 C, whereas the population of nematodes was retained in those without pre-treatment.

2.2.3.2 Microcapsules

Microcapsules are generally prepared by physical, chemical and physico-chemical methods. Microcapsules based on microbes are developed by micro

encapsulation technique wherein microbial cells form the core which in turn is coated with a polymeric material which acts as the shell (Schoebitz *et al.,* 2013). Qureshi *et al.* (2014) developed microcapsules of *B. bassiana* coated with sodium humate using spray drying technique. These microcapsules were free flowing dark brown powder containing *B. bassiana* coated with sodium humate.

2.2.3.3 Gels

Gels have intermediate properties of solids and liquids and are suitable for solid formulations (Perrin, 2000).

The alginate based gel formulations were very effective in protecting *M. anisopliae* from inactivation by artificial solar radiation and high temperatures. (Pereira and Roberts, 1991). Entomopathogenic nematodes *S. glaseri* and *S. siamkayi*, formulated in alginate gel showed better retention of moisture, resulting in increased survival time of 24 and 22 weeks, respectively (Umamaheswari *et al.*, 2005). Divya *et al.* (2011) developed a formulation of *Heterorhabditis indica* Poinar, Karunakar and David, in hydrogel with enhanced rate of survival and higher degree of pathogenicity to *H. armigera*, compared to other formulations. Hussein *et al.* (2011) formulated agar gel of *S. feltiae* and found that it was superior to the aqueous formulation. Suspension of nematodes in one per cent agar gel was found to be effective in both laboratory and greenhouse tests.

2.2.3.4 Briquettes

Briquettes are solid blocks of several cubic centimeters designed for controlled release of organisms into water. Pellets and briquettes are made by agglomeration techniques. Briquettes containing *Bacillus thuringiensis* Berliner and *Bacillus sphaericus* ssp. *israeliensis* when placed in rain water was found to be effective against second instar larvae of *Anopheles gambiae* Meigen (Afraine *et al.,* 2016). Briquettes containing 10 per cent *B. thuringiensis* was found to kill *B. sphaericus* more rapidly when compared to pellet formulations of the same.

2.2.3.5 Prills

Prills are solidified granules that are formed when drops of liquid with organisms are dripped into a reacting liquid. Callaghan *et al.* (2002) developed prill formulation of *Serratia entomophila* against New Zealand grass grub, *Costelytra zealandica* (White). They demonstrated that, the release of bacteria was more in prills kept under high levels of soil moisture *i.e.,* 30 per cent, with a value of 4×10^4 cfu g^{-1}, while it was least in that with 15 per cent moisture (2×10^4 cfu g^{-1}). Lewis and Larkin (2008) developed alginate prill formulation of the biocontrol fungi *Cladorrhinum foecundissimum* Saccardo and Marchal and was found it to be effective in reducing damping-off disease in egg plant and pepper, when compared with formulations in rice bran.

2.2.3.6 Pellets

Pellets are solid masses of more than 10 mm^3 size, manufactured by mixing as a slurry or thick liquid which is then extruded under pressure like a long sausage and cut into a uniform shape (Burges and Jones, 1998).

Pelletized formulations of wheat bran or kaolin clay in an alginate gel containing conidia, chlamydospores or fermentor biomass of several isolates of the biocontrol fungi *Trichoderma* spp. and *Gliocladium virens* Miller, Giddens and Foster were prepared by Lewis and Papavizas (1985). The potentiality of alginate pellets of *B. bassiana* formulated with or without wheat bran was explored by Knudsen *et al.* (1990) in controlling cereal aphid, *Schizaphis graminum* (Rondani). It was observed that fungal sporulation was profuse in those pellets with wheat bran. Andersch (1992) developed a protocol for pellet formulation of *M. anisopliae* named as BIO 1020 with particle size 0.5-1 mm. The product was prepared by drying fungal cells and optimizing in fermenters. Schimazu *et al.* (1992) developed pellet formulation of *B. bassiana* using wheat bran for the control of Japanese pine sawyer beetle, *Monochamus alternatus* Hope, the host of pine wood nematode, *Burseaphelenchus xylophilus* (Steiner and Buhrer). Pellets of

Hirsutella rhossiliensis Pat. were formulated by Lackey *et al.* (1993) using one per cent alginate. They evaluated its efficacy in managing the cyst nematode, *Heterodera shachtii* Schmidt and found that the rate of transmission of fungal spores using dried pellets stored at 5°C for two weeks remained high, compared to that observed with storage at 22°C. *B. bassiana* alginate pellets coated with peanut oil reduced activity of red imported fire ant *S. invicta* (Bextine and Thorvilson, 2002).

2.2.3.7 Tablets

Tablets are compressed solid formulations containing microbes with or without excipients. de Medeiros *et al.* (2005) developed tablet formulation of *B. sphaericus* and found it to be effective as a larvicide against *Culex quinquifasciatus* Say, even without the addition of a UV protectant resulting in 100 per cent mortality. Tablets of *T. viride* formulated by Satyasayee *et al.* (2008) were found to be superior to powder based formulations in terms of contamination and greater viability. Baghel *et al.* (2014) formulated charcoal based tablets of *T. viride* at three different concentrations ranging from five to 15 per cent and observed that storability was maximum (77 to 97.67 per cent) in the highest concentration.

2.3 ENTOMOPATHOGENIC FUNGI IN THE MANAGEMENT OF BANANA WEEVILS

2.3.1 Pseudostem weevil

EPF such as *B. bassiana*, *Fusarium* solani (Mart.) Sacc. and *Mucor heimalis* f. sp. *heimalis* Wehmer were reported to be effective to manage pseudostem weevil in banana (Kung, 1955 and Padmanabhan and Sathiamoorthy, 2001). Pathogenicity of *B. bassiana* to *O. longicollis* was reported by Kung (1955) and that with *M. anisopliae* was reported by Wang and Yen (1972).

Mortality of the weevils was found to vary with the method of application of the microbial agent. Curative application of *M. anisopliae* @ 15 x 10^5 spores

mL^{-1} in the leaf axils was found to be equally effective as chlorpyriphos 20 EC 0.05 % causing 97.16 per cent mortality (Anitha, 2000). Godonou *et al.* (2000) observed that application of *B. bassiana* as Oil-Palm Kernel Cake based formulation recorded greater degree of mortality (42 per cent) than when it was applied as conidial powder. Leaf axil filling with the spore suspension of *B. bassiana* @ 1.8×10^7 mL^{-1} was reported to be effective in managing the grubs of the weevil (Beegum, 2005). Endophytic association of *B. basssiana* was found to highest in dipping method causing a mortality of 73.33 per cent, followed by injection method with 46.66 per cent mortality of weevils (Prabhavathi, 2012). Pseudostem weevil traps with 25 g WP formulation of *B. bassiana* recorded 56.75 per cent reduction in infestation (Irulandi *et al.*, 2012). Sivakumar (2017) has opined that the application of *Metarhizium majus* Bisch, Rehner and Humber @ two per cent as leaf axil filling in banana at two months after planting has resulted in 80 per cent mortality in the grubs of *O. longicollis* one week after treatment.

2.3.2 Rhizome weevil

The pathogenicity of *M. anisopliae* and *B. bassiana* to *C. sordidus* was reported by Busoli *et al.* (1989). Four isolates of *B. bassiana* and one isolate of *M. anisopliae* were found to be pathogenic to third instar larvae of *C. sordidus*, under laboratory conditions, causing 98 to 100 per cent mortality by nine days of exposure to dry fungal spores (Kaaya *et al.*, 1993). A field trial was conducted using *B. bassiana* by adult dip bioassay (4.57×10^7 spores mL^{-1} for 10 days) and found that it resulted in 20 per cent mortality to *C. sordidus* in 40 days (Khan and Gangaprasad, 2001). Akello *et al.* (2008) reported that after 15 weeks of colonisation, presence of *B. bassiana* as an endophyte in banana tissues greatly reduced banana weevil population. Fancelli *et al.* (2013) reported that *B. bassiana* strain CNPMF 218 caused 20 per cent mortality of *C. sordidus* adults leading to 40 per cent reduction in population size after 12 months of treatment. A field trial conducted by Joseph (2014) proved the efficacy of talc based *M. anisopliae* @ 30 g L^{-1} on grubs of *C. sordidus,* resulting in least number of tunnels (0.63) and reduction in the number of grubs from four to 0.29. Curative soil drenching with

talc formulations of *M. anisopliae* and *B. bassiana* @ 30 g L^{-1} were equally effective in reducing the number of immature stages of rhizome weevil, from six to 1.16 and 1.82, respectively (Varsha, 2017).

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled 'Novel bioformulations of entomopathogenic fungi and their efficacy against banana weevils' was carried out at the Biocontrol Laboratory for Crop Pest Management, Department of Agricultural Entomology, College of Agriculture, Vellayani and the field trial was conducted at Instructional Farm, College of Agriculture, Vellayani. The entomopathogenic fungi used for the study were *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ma4) and *Beauveria bassiana* (Balsamo) Vuillemin (Bb5), sourced from National Bureau of Agricultural Insect Resources (NBAIR) and maintained in the Biocontrol Laboratory, College of Agriculture, Vellayani.

3.1 MAINTENANCE OF ENTOMOPATHOGENIC FUNGI

Cultures of entomopathogenic fungi used for the study were revived periodically by passing through susceptible hosts to maintain virulence. The hosts used for reviving the cultures were banana weevils. Pure cultures and sub cultures were maintained in Potato Dextrose Agar (PDA) slants. These were then mass produced in Potato Dextrose Broth (PDB) and used for preparing the formulations.

3.2 DEVELOPMENT OF CAPSULE FORMULATIONS

An ideal capsule formulation should be stable while in storage and disintegrate easily while in field. Therefore it is mandatory to standardize the coating material that can encapsulate the active ingredient. Apart from the coating material, carrier material selected should be in such a way that it retains the size and shape of the capsule and at the same time retains the viability of the organism formulated. With a view to standardize an ideal coating material, carrier material and moisture content, preliminary trials were carried out as follows.

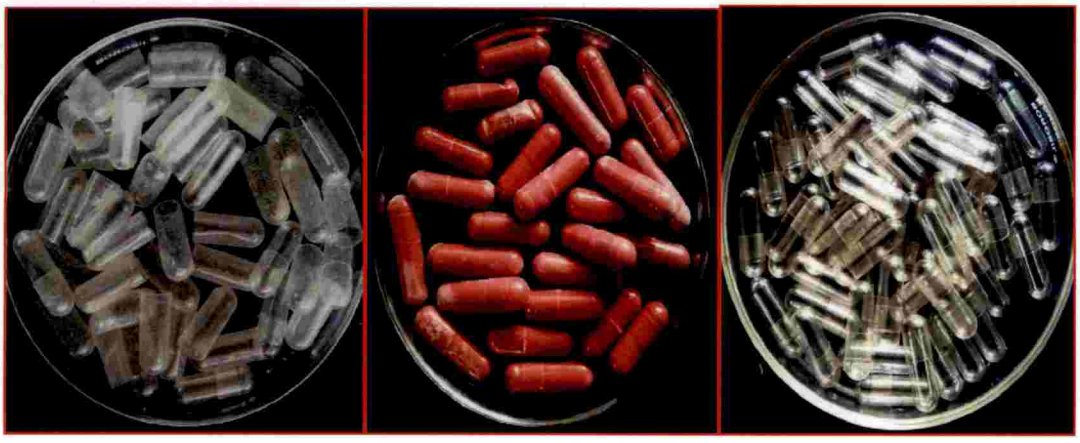
3.2.1 Standardisation of coating and carrier material

The coating materials tested were Hard Gelatin Transparent (HGT), Hard Gelatin Coloured (HGC) and Hydroxy Propyl Methyl Cellulose (HPMC) (Plate 1). The carrier materials tested were talc, chitin, chitosan, sodium alginate and calcium alginate.

The capsules used for the study were of size '00' coming under three categories, HGT, HGC and HPMC which can accommodate 500 - 700 mg of the content depending upon the molecular weight of the material to be enclosed. To conduct the disintegration studies, fixed quantity of each of the carrier materials, talc (Industrial Grade), chitin (crude), chitosan (crude), sodium alginate (LR grade) and calcium alginate (LR grade) were filled, without incorporating the entomopathogens, using a capsule filling device. The quantity used per capsule was 300 mg in the case of talc based ones, 500 mg for chitin based capsules, 350 mg for chitosan, 350 mg for sodium alginate capsules and 400 mg for calcium alginate capsules.

3.2.1.1 Disintegration under ambient conditions

The capsules filled with carrier materials alone were kept under ambient conditions in Petri plates of 9 cm diameter, lined with filter paper, to observe the extent of disintegration. During the experimental period, the RH prevailed in four different ranges *ie*., 50-60, 60-70, 70-75 and 75-80 per cent with corresponding temperature ranges of 33°C, 33-33.5°C, 32-33°C, 31.5-32°C. Inorder to study the disintegration at higher humidity levels that can be anticipated in the field, the treatments were placed in a humid chamber which was created by filling the base of an enclosed container with moist cotton. The humidity was thereby adjusted to 80-85 per cent by trial and error method and the corresponding temperature was noted (27.5°5 to 30°C), using a thermohygrometer fitted inside the chamber. Observations were recorded at 24 h interval on the time taken for disintegration and extent of disintegration of capsules. The experiment was conducted in CRD with 15 treatments, each replicated thrice.



1a. HGT 1b. HGC 1c.HPMC

Plate 1. Capsule coatings used for the study

3.2.1.2 Disintegration at varying soil moisture levels

In order to manage the soil dwelling pests by directly placing the capsules in the planting pit, as in the case of rhizome weevil of banana it is necessary to study the time taken for disintegration at varying levels of soil moisture. Therefore, HGT, HGC and HPMC capsules formulated using five different carrier materials mentioned in para 3.2.1 were studied at five different moisture levels *viz.,* 5, 15, 20, 25 and 30 per cent. The capsules were placed in plastic Petri plates of 9 cm diameter, filled with 30 g of red soil, the moisture content of which was adjusted to the desired level by adding measured volume of water by trial and error. The moisture level was determined using a Moisture analyser (AXIS, Model ATS 60). Observations were recorded on the time taken for disintegration of quarter, half, three-fourth and full capsules. The experiment was conducted in CRD with 15 treatments, each replicated thrice. The treatments were as follows:

T1 – HGT capsule with talc as carrier

T2 – HGT capsule with chitin as carrier

T3 – HGT capsule with chitosan as carrier

T4 – HGT capsule with sodium alginate as carrier

T5 – HGT capsule with calcium alginate as carrier

T6 – HGC capsule with talc as carrier

T7 – HGC capsule with chitin as carrier

T8 – HGC capsule with chitosan as carrier

T9 – HGC capsule with sodium alginate as carrier

T10 – HGC capsule with calcium alginate as carrier

T11 – HPMC capsule with talc as carrier

T12 – HPMC capsule with chitin as carrier

T13 – HPMC capsule with chitosan as carrier

T14 – HPMC capsule with sodium alginate as carrier

T15 – HPMC capsule with calcium alginate as carrier

3.2.1.3 Disintegration in plant

It is necessary to assess the performance of the capsule when placed directly in plants as in the case of banana, where the delivery mechanism is as important as the selection and dose of the insecticide. Capsule degradation time was therefore assessed by placing them in vulnerable points of entry of pseudostem weevil such as leaf sheath, leaf axils and bore holes of seven month old plants of variety Nendran. The atmospheric temperature and humidity that prevailed during this experimental period was collected from the Department of Agricultural Meteorology, College of Agriculture, Vellayani. The experiment was conducted in CRD with 15 treatments detailed as in 3.2.1.2. Each treatment was replicated thrice.

3.2.1.3.1 When placed between sheaths

The capsules mentioned in para 3.2.1.2 were placed between the two outer sheaths of banana pseudostem and were observed for the time taken for disintegration of quarter, half, three-fourth and full capsules. The number of treatments, number of replications and experimental design was the same as that in 3.2.1.2.

3.2.1.3.2 When placed in leaf axils

The experiment was carried out in the same way as described in para 3.2.1.3.1 by placing one capsule each in four of the leaf axils surrounding the spindle leaf.

3.2.1.3.3 When placed in bore holes

`The experiment was carried out in the same way as described in para 3.2.1.3.1 by placing capsules into the bore holes of infested plants.

3.2.2 Standardization of moisture content of the carrier material for capsules

Moisture content of the capsule should be set in such a way that the coating is stable in storage, but disintegrate easily while in use. At the same time

the moisture content of the formulation should be ideal to retain the viability of the fungi that is being formulated. It may vary according to the carrier material used for formulation. Hence the experiment was carried out using two different carrier materials, talc and chitosan which were encapsulated in the best coating material standardized as in para 3.2.1.The treatments were as follows.

T1 – Capsule with talc at 5 per cent moisture content

T2 – Capsule with chitosan at 5 per cent moisture content

T3 – Capsule with talc at 8 per cent moisture content

T4 – Capsule with chitosan at 8 per cent moisture content

T5 – Capsule with talc at 10 per cent moisture content

T6 – Capsule with chitosan at 10 per cent moisture content

T7 – Capsule with talc at 15 per cent moisture content

T8 – Capsule with chitosan at 15 per cent moisture content

Each treatment was replicated thrice.

3.2.2.1 *Disintegration studies of capsules with EPF*

Fourteen day old culture broth of *M. anisopliae* and *B. bassiana*, were blended in a mixer and filtered using a strainer. The filtered spore suspension was mixed with carriers, talc/chitosan in the ratio 1:3 and the moisture content of the formulations were adjusted to 5, 8, 10 and 15 per cent as described in 3.2.1.2. The formulation thus prepared was filled within empty capsules with HGT. The capsules were tested for their stability under room temperature as per the procedure described under 3.2.1.1. The treatments detailed below were replicated thrice.

T1 – HGT with *B. bassiana* incorporated in talc with 5 per cent moisture content

T2 – HGT with *B. bassiana* incorporated in talc with 8 per cent moisture content

T3 – HGT with *B. bassiana* incorporated in talc with 10 per cent moisture content

T4 – HGT with *B. bassiana* incorporated in talc with 15 per cent moisture content

T5 – HGT with *B. bassiana* incorporated in chitosan with 5 per cent moisture content

T6 – HGT with *B. bassiana* incorporated in chitosan with 8 per cent moisture content

T7 – HGT with *B. bassiana* incorporated in chitosan with 10 per cent moisture content

T8 – HGT with *B. bassiana* incorporated in chitosan with 15 per cent moisture content

T9 – HGT with *M. anisopliae* incorporated in talc with 5 per cent moisture content

T10 – HGT with *M. anisopliae* incorporated in talc with 8 per cent moisture content

T11 – HGT with *M. anisopliae* incorporated in talc with 10 per cent moisture content

T12 – HGT with *M. anisopliae* incorporated in talc with 15 per cent moisture content

T13– HGT with *M. anisopliae* incorporated in chitosan with 5 per cent moisture content

T14 – HGT with *M. anisopliae* incorporated in chitosan with 8 per cent moisture content

T15 – HGT with *M. anisopliae* incorporated in chitosan with 10 per cent moisture content

T16 – HGT with *M. anisopliae* incorporated in chitosan with 15 per cent moisture content

The ideal moisture content noted from this experiment was utilized for preparation of the bio-capsules incorporating *M. anisopliae* and *B. bassiana* spores.

3.2.2.2 *Viability of the capsules*

Initially, the carrier materials were mixed well with spore suspension @ 10^8 spores mL^{-1} and to study the effect of carrier materials on the viability of spores, the number of colony forming units (cfu) were recorded at weekly intervals for a period of one month. It was estimated by serial dilution and agar plating technique using Rose Bengal Agar (RBA) as suggested by (Aneja, 2003). The number of viable colonies was estimated using the formula

$$\text{Viable cells } mL^{-1} = \frac{\text{Mean plate count} \times \text{Dilution factor}}{\text{Volume of sample plated (mL)}}$$

The tabulated data were subjected to statistical analysis using WASP 1 (Web Assisted Statistical Package) software.

3.2.3 Development of capsule formulations of *Metarhizium* and *Beauveria*

The coating material, the carrier material and the moisture content standardized from Experiments 3.2.1 and 3.2.2 were made use of in preparing the bio-capsules of *M. anisopliae* and *B. bassiana*.

3.2.3.1 Preparation of spore suspension

M. anisopliae and *B. bassiana* were inoculated in PDB and allowed for sporulation. After 14 days, the fungal cultures were blended in a mixer and strained using a strainer to obtain spore suspension.

3.2.3.2 Preparation of primary powder

The resultant spore suspension was centrifuged in a Rotek centrifuge at 4000 rpm for 20 minutes to obtain spore pellet. The spore pellet was washed gently with sterile distilled water to remove mycelial mat adhering to the same.

The spore pellet was mixed with equal quantity of chitosan to obtain primary powder @ 10^{10} spores g^{-1}.

3.2.3.3 Spore germination assay

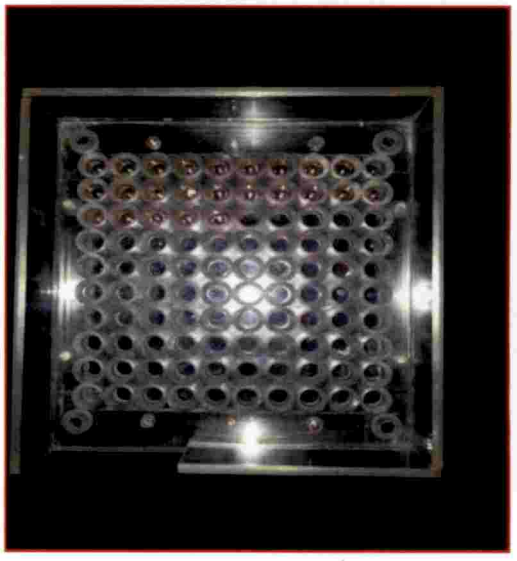
Inorder to test the viability of spores before formulation, germination assay was carried out by 'Hanging drop technique' as described by Pimpalgaonkar and Chandel (2014). A stock solution of primary powder was prepared by dissolving one gram of the same in one mL of sterile water. The sample (100 µL) was placed on a coverslip and it was then inverted onto the concave depression of cavity slide to produce a 'hanging drop'. The slide was fixed with lactophenol-cotton blue stain and was observed under high power (45 x) of a compound microscope (Motic, Model: BA210LED). Spore suspension prepared from 14-day old culture broth served as the control. Observations were recorded on total number of spores and number of spores germinated till 100 per cent germination was noted. A spore was considered to be germinated when the length of the germ tube was twice the diameter of the spore as described by Herlinda (2010). Germination percentage was calculated using the formula,

$$\text{Germination percentage} = \frac{\text{No. of spores germinated}}{\text{Total no. of spores}} \times 100$$

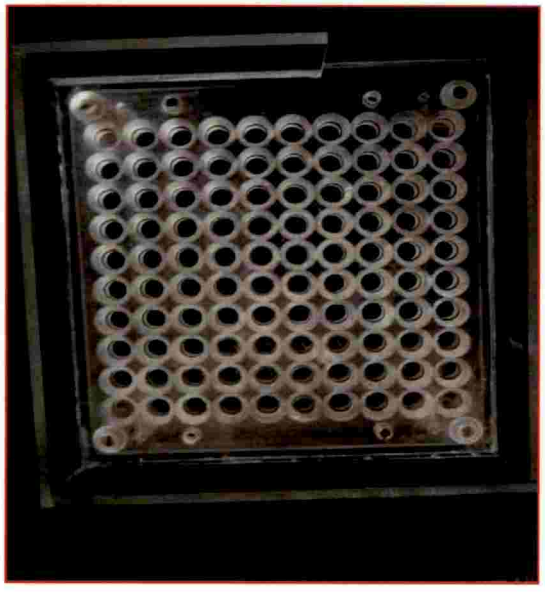
3.2.3.4 Filling the bio-capsules

The primary powder was mixed with selected carriers in the ratio 1:20 to form the filling material (spores and carrier material). The empty capsules of superior coating material were filled with the material using hand operated capsule filling device. It consisted of several parts, a unit of double trays, in which the lower tray is having 100 holes for holding upper portion of the split capsules, a single tray with 100 holes that can hold the other halves of the split capsules, a cuboidal tray to spread the capsules, a stand with tray, a powder guard, a brush and a trampling tool. Various steps involved in preparing and filling the empty capsules are illustrated in Plates 2 and 3.

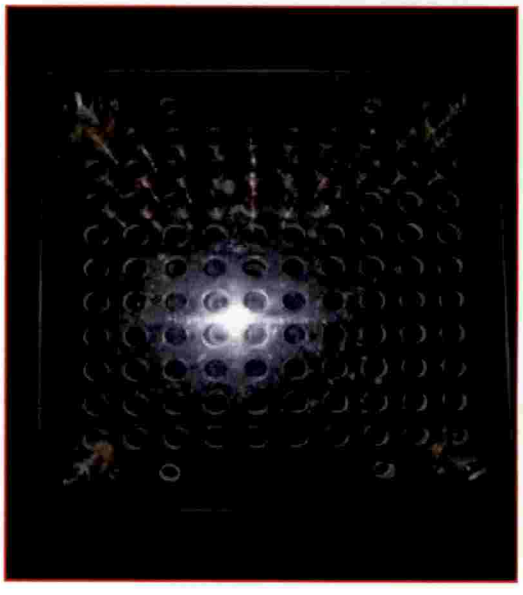
Step 1. Single tray above the a double tray with capsules



Step 2. Cuboidal tray above double tray



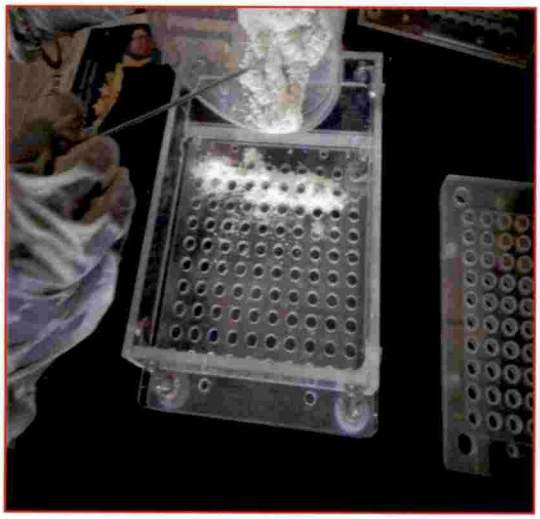
Step 3. Cuboidal tray kept above a tray with lower part of capsules spread



Step 4 Upper and lower trays presssed to lock the capsules

Plate 2. Steps involved in filling capsules

Contd…..



Step 5. Placement powder guard

Step 6. Spreading filler material

Step 7. Pressing the whole assembly

Step 8. Separation of capsules from middle tray

Plate 3. Steps involved in filling capsules

For filling the capsules, initially the upper portion of capsules were spread onto double tray by placing cuboidal tray over it. Then, single tray with 100 holes was placed above this and the unit was pressed lightly. The lower portion of capsules were spread onto tray with a stand using cuboidal tray and the filler material was transferred to this portion using a powder guard and brush, and then pressed using a trampling tool. Then the unit containing upper portion was kept above the tray with lower portion of capsules and was pressed mechanically to lock the capsules. The fully formed capsules were then collected from the single tray (Plate 4).

3.2.3.5 Assessment of shelf life of Metarhizium and Beauveria capsules based on viability

Shelf life of formulations was assessed based on viability observed as the number of colony forming units.

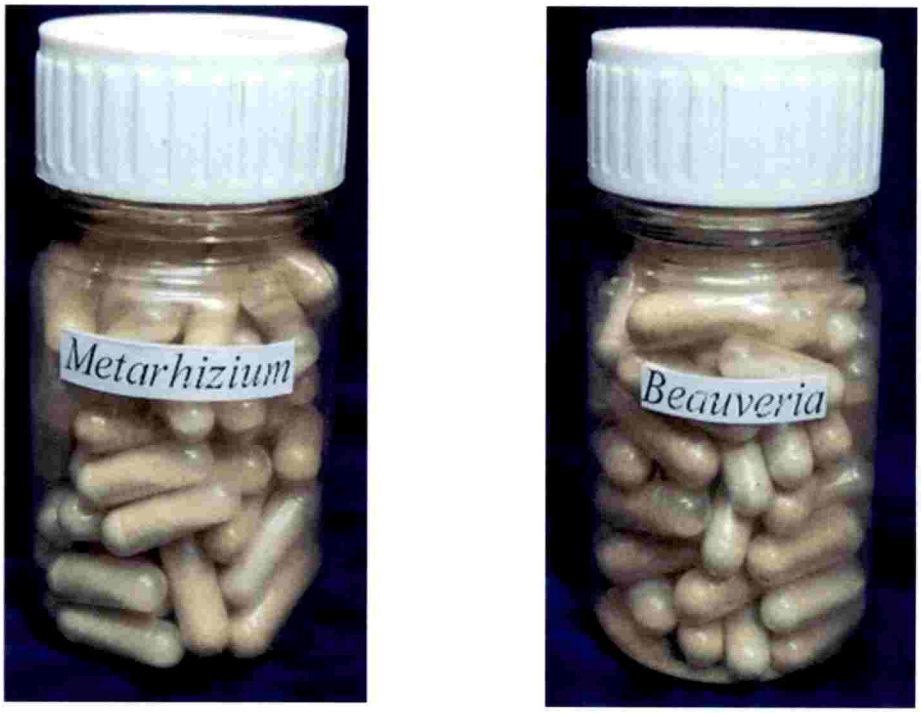
3.2.3.5.1 Estimation of cfu

One gram of formulation was dissolved in nine mL of sterile distilled water and was serially diluted to 10^{-5} concentration. Number of cfu present in the formulations stored at room temperature as well as refrigeration was estimated by agar plating technique at fortnightly intervals for three months. Based on the observations on cfu, the superior treatments were selected for evaluation against banana weevils.

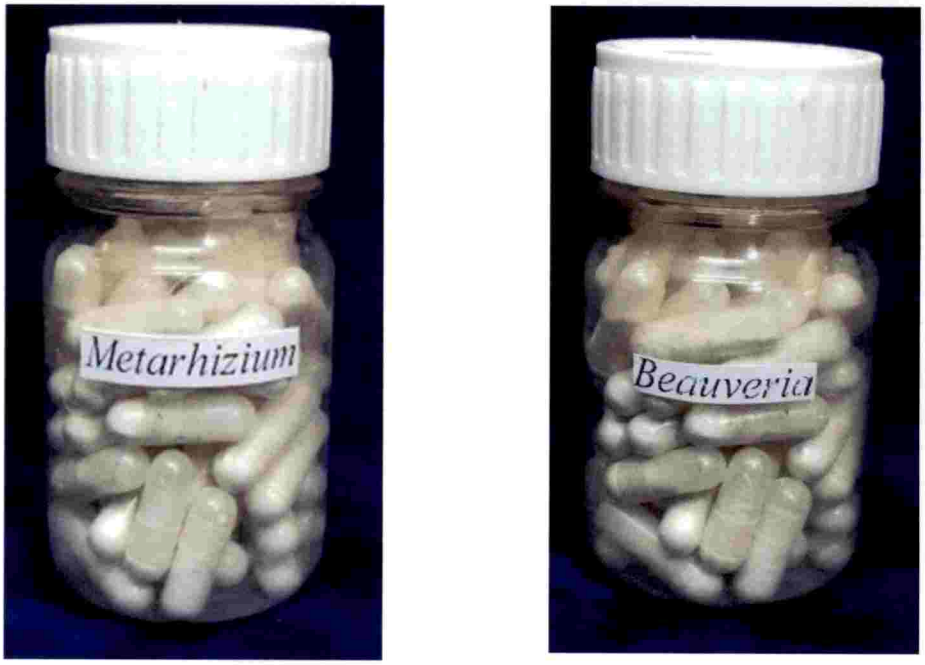
The tabulated data was analysed employing the technique for split-plot design using MSTAT software (Gomez and Gomez, 1984).

3.3 DEVELOPMENT OF GEL FORMULATIONS

As with the case of other formulations, the carrier material (gel base) that can be used for formulating a gel of microbial organism should be in such a way that it does not react with the active ingredient and at the same time retain its viability. The gel consistency is a physical property of a substance, determined by



4a. Capsules formulated in chitosan



4b. Capsules formulated in talc

Plate 4. Capsules of *Metarhizium* and *Beauveria*

the moisture content and storage temperature. With this view, an experiment was laid out in CRD with eight treatments, each replicated thrice, to determine the gel consistency. Different gel bases selected for the preparation of gel formulation includes chitosan, gelatin, agar and alginate. The following proportions were tested as below so as to derive a suitable gel consistency.

T1 – Chitosan and spore pellet in 5:1 ratio

T2 – Chitosan and spore pellet in 10:1 ratio

T3 – Gelatin and spore suspension in 1:1 ratio

T4 – Gelatin and spore suspension in 2:1 ratio

T5 – Agar and spore suspension in 1:1 ratio

T6 – Agar and spore suspension in 2:1 ratio

T7 – Gelatin and mycelial mat entrapped in alginate matrix in 1: 1 ratio

T8 – Gelatin and mycelial mat entrapped in alginate matrix in 2:1 ratio

The spore pellet and spore suspension were prepared as per the procedure in 3.2.3.2 and 3.2.3.1 was used for the preparation of gel formulations.

3.3.1. Preparation of Gel formulations

3.3.1.1 Chitosan based Gels

Spore suspensions of *M. anisopliae and B. bassiana* were prepared from 14-day old cultures. The spore suspensions were centrifuged as described in para 3.2.3.2 to obtain spore pellet which was then added to 30 per cent chitosan gel (unsterilized) procured from Pelican Biotech and Chemical labs, Alappuzha, in various proportions mentioned in para 3.3 and the ideal ratio derived (1:10), by trial and error method was selected. The final formulation contained 10^8 spores mL^{-1}.

3.3.1.2 Gelatin based Gels

Gelatin of LR grade (unsterilized) was used to prepare the gel base using the procedure described in para 3.3.1.1. The ideal ratio, 2:1 was selected to attain the appropriate consistency. The final formulation had a spore count of 10^8 spores mL^{-1}.

3.3.1.3 Agar based Gels

Spore suspensions from 14-day old cultures of *M. anisopliae* and *B. bassiana* were added to molten solution of two per cent agar (LR grade). For this two gram agar was dissolved in 100 mL of sterile distilled water.

3.3.1.4 Alginate based gels

Gel formulations of *M. anisopliae* and *B. bassiana* in alginate were developed as per the procedure described by Pereira *et al.* (1991) with slight modifications. Sodium alginate (five gram) was dissolved in 10 mL of 100 per cent ethanol and 500 mL of culture was immediately poured to this. Then, equal amount of mycelial paste was added to this. The fungal culture was mixed with equal volume of a four per cent (w/v) solution of calcium chloride ($CaCl_2.2H_20$) to form an insoluble precipitate entrapping the mycelia. This precipitate was blended for 40 s to further break the alginate-mycelium particles. The liquid phase of this mixture was filtered out to obtain the fungal mat trapped in a matrix of calcium alginate. Gelatin and fungal mat were mixed in the ratio 2:1. The final formulation contained 10^8 spores mL^{-1}.

3.3.2 Assessment of viability of Gel formulations

3.3.2.1 Spore germination rate in different gel bases

Spore germination assay was carried out till 100 per cent germination was noted (three days) by 'Hanging drop technique' as per the procedure described in para 3.2.3.3 and the treatments were ranked based on the germination percentage.

3.3.2.2 Estimation of number of colony forming units

One gram of gel formulation was dissolved in nine mL of sterile distilled water and was serially diluted to 10^{-5} concentration. Number of cfu of test organisms and contaminants were estimated by serial dilution agar plating technique using RBA at fortnightly intervals for three months as per the procedure described in 3.2.2.2.

The tabulated data was analysed employing the technique for split-plot design using MSTAT software (Gomez and Gomez, 1984).

3.3.2.3 Assessment of time taken for dispersal of propagule from the gel

The promising treatments from the above experiment was placed in PDA and the time taken for the dispersal of fungal propagule was assessed based on the extent of spread of gel in the media as well as the number of colony forming units observed after 24 h interval, for a period of one week.

Based on the observations on germination percentage and the number of cfu, the superior treatments were selected for further evaluation under field conditions.

3.4 EFFICACY OF CAPSULES AND GELS ON BANANA WEEVILS

3.4.1 Pathogenicity studies

A preliminary evaluation on pathogenicity of unformulated *M. anisopliae* and *B. bassiana* were tested on adults and grubs of pseudostem weevil and rhizome weevil before formulating them. For this purpose, stock cultures of *Odoiporus longicollis* (Oliver) and *Cosmopolites sordidus* (Germar) were maintained in the laboratory.

3.4.1.1 Maintenance of stock cultures

3.4.1.1.1 Pseudostem weevil

The grubs collected from the field were released into fresh pseudostem pieces (Variety Nendran) of 10 cm length by making holes with a glass rod on the cut surface. They were then kept in cylindrical glass troughs of size 10×15 cm, the

top portion of which was covered with a muslin cloth. Fresh pseudostem pieces were provided once in two days. The grubs were observed for pupation by splitting open the pseudostem once in two days. The pupae were collected and kept in separate glass troughs for adult emergence. The adults were provided with pseudostem pieces and the eggs laid within the air chambers were observed for the emergence of grubs. The larval period was completed within 30-35 days. Second instar grubs and adults thus obtained were used for testing pathogenicity.

3.4.1.1.2 Rhizome weevil

The grubs and adults collected from the field were released onto fresh rhizome bits kept separately within cylindrical glass troughs of size 10×15 cm, the top portion of which was covered with a muslin cloth. The rhizome pieces were changed once in a week. Mid-instar grubs and adults thus maintained were used for testing pathogenicity.

3.4.1.2 Pathogenicity of M. anisopliae to O. longicollis

3.4.1.2.1 Adults

The spore suspension from 14-day old cultures @ 10^8 spores mL^{-1}, predetermined using a Neubaur's haemocytometer, was sprayed onto the grubs and adults of pseudostem weevil and rhizome weevil. Treatment with sterile distilled water served as the control. Each treatment was replicated thrice with three insects per replication.The treated insects were observed for symptoms of mycosis and mortality.

3.4.1.2.2 Grubs

The second instar grubs of *O. longicollis* were subjected to pathogenicity test in the same manner as described in para 3.4.1.2.1.

3.4.1.3 Pathogenicity of B. bassiana to O.longicollis

Pathogenicity tests were conducted on adults and grubs *O. longicollis* with spore suspension of *B. bassiana* @10^8 spores mL^{-1} as described in para 3.4.1.2.1.

3.4.1.4 Pathogenicity of M. anisopliae to C. sordidus

The adults and grubs of *C. sordidus* were subjected to pathogenicity test with *M. anisopliae* @ 10^8 spores mL^{-1} as described in para 3.4.1.2.1.

3.4.1.5 Pathogenicity of M. anisopliae to O. longicollis

The adults and grubs of *O. longicollis* were treated with spore suspension of *M. anisopliae* @ 10^8 spores mL^{-1} as described in para 3.4.1.2.1.

3.4.2 Laboratory evaluation of the formulations

A preliminary trial was conducted in the laboratory to assess the efficacy of capsule formulations and gel formulations.

3.4.2.1 Capsule formulations against O. longicollis

The promising capsule formulations were kept within holes made on the pseudostem pieces of 15 cm length (four holes per pseudostem) using a glass rod and third instar grubs of *O. longicollis* @ one $hole^{-1}$ were released into the pseudostem. The pseudostem pieces were split open every day and examined for symptoms of morbidity and mortality of the grubs, for a period of five days.

3.4.2.2 Capsule formulations against C. sordidus

The promising capsule formulations were kept @ four in the soil (red soil) around the rhizome and third instar grubs were artificially released into the rhizome. The capsules were observed daily for the rate of disintegration and symptoms of morbidity and mortality were noted by cutting open the rhizome and keeping them back intact. The observations were taken for a period of five days.

3.4.2.3 Gel formulations against C. sordidus

The promising gel formulation was applied on the rhizome region @ 10 g $rhizome^{-1}$. This was followed by the release of second instar grubs. The grubs were observed daily for the symptoms of morbidity and mortality, for a period of three days, as in para 3.4.2.2.

3.4.3 Pot culture studies

The promising capsule formulations and gel formulations were evaluated for their efficacy in managing *O. longicollis* and *C. sordidus* by artificially infesting five month old pseudostem and rhizome of Nendran variety of banana, raised in grow bags of size 55 × 34 × 22 cm (Plate 5). The crop was raised as per the recommendations of Package of Practices, Kerala Agricultural University, 2016, except for plant protection.

3.4.3.1 Capsule formulations against O. longicollis

After releasing pseudostem weevil, the pseudostem was lined with nylon net to confine the weevils (Plate 5a).

The effective treatments selected from the above set of experiments were chosen for field evaluation of formulations. The experiment was conducted with six treatments and three replications with one plant per replication.

The following were the set of treatments

T1: Capsules of test organism with talc enclosed in HGT coating

T2: Capsules of test organism with chitosan enclosed in HGT coating

T3: Spore suspension of test organism (10^8 spores mL^{-1})

T4: Chlorpyriphos 20 EC 0.05 per cent

T5: No weevils released

T6: Control

Two sets of experiments were conducted as mentioned below, to study the effect of capsules.

3.4.3.1.1 Prophylactic treatment

The efficacy of capsule formulations was tested against *O. longicollis* by placing the capsules in four leaf axils around the spindle leaf @ four plant $^{-1}$ and the rest of the treatments (spore suspension @ two L $plant^{-1}$, chlorpyriphos 20 EC

5a. Pseudostem lined with net

5b. Grow bag covered with net

Plate 5. Layout of the experimental field

0.05 per cent @ one L plant^{-1}) were applied by pouring the solutions into the central leaf axils of banana. Second instar grubs were artificially released into the pseudostem through leaf axils two weeks after the treatment @ four plant^{-1}. Observations were taken two weeks after releasing the grubs by destructive sampling of banana.

3.4.3.1.2 Curative treatment

Two days after artificially infesting the plants with second instar grubs, the treatments were applied to plants in the same manner as mentioned in 3.4.3.1. Observations were recorded two weeks after treatment application by destructive sampling.

3.4.3.1.3 Destructive sampling

The efficacy of capsule formulations on pseudostem weevil was assessed by destructive sampling. The degree of infestation was recorded by giving scores based on the number of bore holes, (Plate 6) as described by Anitha (2000). The number of grubs and pupae present within the pseudostem were also noted.

Scoring index

0 - Plants with no symptoms

1 - Plants with 1 to 5 bore holes on the pseudostem

2 - Plants with 6 to 10 bore holes on the pseudostem

3 - Plants with more than 10 bore holes on the pseudostem

4 - Plants with pseudostem about to break or already broken

3.4.3.2 Efficacy of capsule formulations against C. sordidus

The effective treatments selected from the above set of experiments were chosen for field evaluation of formulations. The experiment was conducted with six treatments, each replicated thrice. The treatments described in 3.4.2.1 were evaluated both as prophylactic and curative treatments.

3.4.3.2.1 Prophylactic treatment

6a. Plants with 1-5 bore holes

6b. Plants with 6-10 bore holes

6c. Plants with more than 10 bore holes

6d. Plants with broken pseudostem

Plate 6. Scoring indices for pseudostem weevil damage

For testing the efficacy of capsules against *C. sordidus*, capsules were placed in soil around the rhizome @ four plant^{-1} and the rest of the treatments (spore suspension @ two L plant^{-1}, Chlorpyriphos 0.05 per cent 20 EC @ one L plant^{-1}) were drenched around the rhizome. Second instar grubs of the weevils were artificially infested onto the plants two weeks after the treatment @ four plant^{-1} near the rhizome region. Care was taken to prevent escape of the released weevil, by lining the inner portion of growbags with nylon net before filling and planting suckers. After releasing the weevils too, the exposed soil surface in the grow bag was also covered with a nylon net as shown in Plate 5b. Observations were taken two weeks after releasing the weevils by destructive sampling.

3.4.3.2.2 Curative treatment

Two days after artificial infestation with weevils, the treatments were applied in the same manner as mentioned in 3.4.3.1. Observations were recorded two weeks after treatment application, by destructive sampling.

3.4.3.2.3 Destructive sampling

For assessing the efficacy of capsules on rhizome weevil, destructive sampling of suckers was done. Plants were uprooted slowly, the suckers were cut horizontally and the number of lesions on the sucker was noted. The extent of damage on rhizomes was assessed based on scoring method (Plate 7) developed by Vilardebo (1973). Then the rhizome was cut into small pieces to take the count of grubs and pupae, if present.

0 - No damage

10 - Traces of galleries

20 - Galleries on approximately 1/4 of rhizome

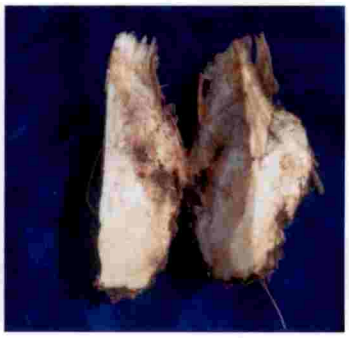
30 - Galleries on approximately 1/3 of rhizome

40 - Galleries on approximately 1/2 of rhizome

60 - Galleries on approximately 3/4 of rhizome

7a. No damage

7b. Traces of galleries



7c. Galleries on 1/4 of rhizome

7d. Galleries on 1/3 of rhizome

7e.Galleries on 1/2 of rhizome

7f. Galleries on 3/4 of rhizome

7g. Galleries on the whole of rhizome

Plate 7. Scoring indices for rhizome damage

100 - Galleries on approximately whole of rhizome

3.4.3.2 Efficacy of gel formulations on banana rhizome weevil

The promising gel formulations were evaluated for their efficacy in managing *C. sordidus* in pot culture by artificially infesting the rhizome of Nendran variety of banana. The effective treatments selected from the preliminary set of experiments were chosen for field evaluation of formulations. Plants were raised as per the recommendations of Package of Practices, Kerala Agricultural University, 2016, except for plant protection. The experiment was conducted with five treatments, each replicated four times.

The following were the set of treatments

T1: Gel formulation incorporated with test organism

T2: Spore suspension of test organism (10^8spores mL^{-1})

T3: Chlorpyriphos 20 EC 0.05 per cent

T4: No weevils released

T5: Control

Two sets of experiments were conducted as mentioned below:

3.4.3.2.1 Prophylactic treatment

For testing the efficacy of gel formulations against *C. sordidus*, gel was applied in rhizome @ 10 g $plant^{-1}$ and the rest of the treatments (spore suspension @ two L $plant^{-1}$, Chlorpyriphos 20 EC 0.05% @ 1 L $plant^{-1}$) were drenched around the rhizome. Second instar grubs of the weevils were artificially infested onto the plants two weeks after the treatment @ four $plant^{-1}$ near the rhizome region. Observations were recorded two weeks after releasing weevils by destructive sampling.

3.4.3.2.2 Curative treatment

55

Two days after artificial infestation with weevils, the treatments were applied in the same manner as mentioned in 3.5.1.

3.4.3.2.3 Destructive sampling

The efficacy of gel formulations on rhizome weevil was assessed by destructive sampling of the plant which was done as per the procedure described in 3.4.3.2.3.

The tabulated data were subjected to statistical analysis using WASP 1 (Web Assisted Statistical Package) software.

Results

4. RESULTS

The results of the work entitled "Novel bioformulations of entomopathogenic fungi and their efficacy against banana weevils" carried out during 2016-18 in the Department of Agricultural Entomology, College of Agriculture, Vellayani is presented below. The work was conducted under three main modules *viz.*, Development of capsule formulations of *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Beauveria bassiana* (Balsamo) Vuillemin, development of Gel formulations of *M. anisopliae* and *B. bassiana* and assessment of their efficacy in managing the banana weevils, *Odoiporus longicollis* (Oliver) and *Cosmopolites sordidus* (Germar).

4.1 CAPSULE FORMULATONS

4.1.1 Standardization of coating and carrier materials for encapsulation

The coating material was determined based on their stability under ambient conditions as well as their disintegration when filled with different types of carrier *viz.*, talc, chitin, chitosan, sodium alginate and calcium alginate. They were evaluated for their ability to disintegrate when placed in soil as well as in plant. The time taken for disintegration of the capsules at varying soil moisture levels and the corresponding level of disintegration were noted.

4.1.1.1 Disintegration under ambient conditions

Stability of the capsules was assessed under varying atmospheric conditions of RH and temperature ranges that could be anticipated during different periods of a year. The stability of capsules assessed under varying atmospheric conditions is presented in Table 1.

Upto 75 per cent RH with corresponding temperature range of 32 to 33.5°C, all the coatings were stable irrespective of the carrier materials used till the end of 24 h. When the RH was 75-80 per cent with corresponding temperature

Table 1. Effect of coating material and carrier material on disintegration of capsules at room temperature

Sl. No.	Capsule coating and carrier material	Time taken for disintegration (h)		
		RH – 70-75% Temp – 32-33^{0}C	RH - 75-80% Temp– 31.5-32^0 C	RH- 80-85% Temp – 27.5-30^{0}C
Hard Gelatin (Transparent) coating (HGT)				
1	Talc	*	*	>48
2	Chitin	*	*	>48
3	Chitosan	*	> 48	> 48
4	Sodium alginate	*	*	> 48
5	Calcium alginate	*	*	> 48
6	Empty capsules	*	*	> 48
Hard Gelatin (Coloured) coating (HGC)				
7	Talc	*	*	> 48
8	Chitin	*	*	*
9	Chitosan	*	*	*
10	Sodium alginate	*	*	*
11	Calcium alginate	*	*	*
12	Empty capsules	*	*	> 48
Hydroxy Propyl Methyl Cellulose coating (HPMC)				
13	Talc	*	*	> 48
14	Chitin	*	*	> 48
15	Chitosan	*	*	> 48
16	Sodium alginate	*	*	> 48
17	Calcium alginate	*	*	> 48
18	Empty capsules	*	*	> 48

*Stable

31.5 - 32°C, all the coating materials were stable till 24 h. But after 48 h, the HGT (Hard Gelatin Transparent) coated chitosan filled capsules started disintegrating. After 48 h, at higher levels of RH (80-85 per cent) and temperature (27.5 – 30°C), all the coating materials started disintegration irrespective of their carrier materials, except HGC (Hard Gelatin Coloured) capsules filled with chitin, chitosan, sodium alginate and calcium alginate.

It is therefore inferred that all the three coatings were stable under ambient conditions of storage, but under high RH of more than 80 per cent, the coatings disintegrated faster.

4.1.1.2 Disintegration at varying soil moisture levels

The ability of the capsules to disintegrate when placed under varying levels of soil moisture *viz.*, 5, 15, 20, 25 and 30 per cent, were assessed based on the time taken for disintegration.

All the capsule coatings filled with different carrier materials retained stability when kept in soil at 5 and 15 per cent moisture (Permanent Wilting Point).

4.1.1.2.1 Soil moisture -20 per cent (Available Water Holding Capacity)

When the soil was just wet and unirrigated, after 12 h, it was observed that half of the surface area of talc filled and chitosan filled HGT capsules disintegrated (Table 2). Talc and chitosan filled HGC capsules and chitin and chitosan filled HPMC (Hydroxy Propyl Methyl Cellulose) capsules also disintegrated at this point of time. HGT capsules filled with chitin were comparatively resistant to moisture content. Only quarter of it disintegrated at this point of time. Half of the surface area of all the empty capsules disintegrated after 12 h, irrespective of the coating material.

After 24 h, three quarter of talc filled HGT, chitosan filled HGT, talc filled HGC, chitosan filled HGC, calcium alginate filled HGC, chitin filled HPMC and

Table 2. Effect of coating material and carrier material on disintegration of capsules at 20% soil moisture

Sl. No.	Capsule coating and carrier material	Time taken for disintegration (h) RH – 72-75% and temperature – 33.3-33.5°C			
		Quarter of the capsule	Half of the capsule	Three-quarter of the capsule	Full capsule
Hard Gelatin (Transparent) coating (HGT)					
1	Talc	-	12	24	48
2	Chitin	12	24	48	72
3	Chitosan	-	12	24	36
4	Sodium alginate	-	-	-	-
5	Calcium alginate	48	-	-	-
6	Empty capsules	-	12	24	36
Hard Gelatin (Coloured) coating (HGC)					
7	Talc	-	12	24	48
8	Chitin	48	72	144	168
9	Chitosan	-	12	24	48
10	Sodium alginate	-	-	-	-
11	Calcium alginate	-	12	24	48
12	Empty capsules	-	12	24	36
Hydroxy Propyl Methyl Cellulose coating (HPMC)					
13	Talc	-	24	48	96
14	Chitin	-	12	24	48
15	Chitosan	-	12	24	48
16	Sodium alginate	-	-	-	-
17	Calcium alginate	48	-	-	-
18	Empty capsules	-	12	24	36

chitosan filled HPMC capsules disintegrated. At this point of time, chitin filled HGT and talc filled HPMC were found to be tolerant to 20 per cent moisture level, their disintegration level being half. Empty capsules, irrespective of coating material exhibited three-quarter disintegration of their surface area.

By the end of 36 h, chitosan filled HGT capsules completely disintegrated. At this point, empty capsules also disintegrated completely.

At the end of 48 h, quarter portion of calcium alginate filled HGT capsules, chitin filled HGC capsules and calcium alginate filled HPMC capsules disintegrated. By this time, only three quarter of chitin filled HGT capsules and talc filled HPMC capsules disintegrated. Complete disintegration was noticed with talc filled HGT and HGC; chitosan filled HGC and HPMC; calcium alginate filled HGC and chitin filled HPMC capsules.

After 72 h, half portion of the surface area of HGC and full portion of chitin filled HGT capsules disintegrated.

By 96 h, talc filled HPMC capsule completely disintegrated. After 144 h, three quarter of chitin filled HGC capsules disintegrated and it took 168 h (one week) for complete disintegration of chitin filled HGC capsules.

None of the three capsule coatings filled with sodium alginate undergo disintegration.

4.1.1.2.2 Soil moisture - 25 per cent (Available Water Holding Capacity)

When the moisture content of soil in which the capsules were placed was increased by 5 per cent, the results obtained were more or less same as that in 20 per cent soil moisture (Table 3).

When the soil was subjected to light irrigation, after 12 h, it was observed that half of the surface area of talc filled HGT capsules disintegrated. Talc and chitosan filled HGC capsules and chitin and chitosan filled HPMC capsules also disintegrated at this moment. Half of the surface area of all the empty capsules

Table 3. Effect of coating material and carrier material on disintegration of capsules at 25% soil moisture

Sl. No.	Capsule coating and carrier material	Time taken for disintegration (h) RH – 72-75%, Temperature – 33.3-33.5°C			
		Quarter of the capsule	Half of the capsule	Three-quarter of the capsule	Full capsule
Hard Gelatin (Transparent) coating (HGT)					
1	Talc	-	12	24	48
2	Chitin	12	24	48	72
3	Chitosan	-	12	24	36
4	Sodium alginate	-	-	-	-
5	Calcium alginate	48	-	-	-
6	Empty capsules	-	12	24	36
Hard Gelatin (Coloured) coating (HGC)					
7	Talc	-	12	24	48
8	Chitin	48	72	144	168
9	Chitosan	-	12	24	36
10	Sodium alginate	-	-	-	-
11	Calcium alginate	-	12	24	48
12	Empty capsules	-	12	24	36
Hydroxy Propyl Methyl Cellulose coating (HPMC)					
13	Talc	-	24	48	96
14	Chitin	-	12	24	48
15	Chitosan	-	12	24	48
16	Sodium alginate	-	-	-	-
17	Calcium alginate	48	-	-	-
18	Empty capsules	-	12	24	36

disintegrated after 12 h, irrespective of the coating material. After 24 h half portion of chitin filled HGT and talc filled HPMC were found to disintegrate. By the end of 36 h, chitosan filled HGT and HGC capsules and empty capsules completely disintegrated. At the end of 48 h, complete disintegration was noticed with talc filled HGT and HGC; chitosan filled HPMC, calcium alginate filled HGC and chitin filled HPMC capsules. After 72 h, half portion of the surface area of HGC and full portion of chitin filled HGT capsules disintegrated. By 96 h, talc filled HPMC capsules completely disintegrated. It took 168 h (one week) for complete disintegration of chitin filled HGC capsules.

4.1.1.2.3 Soil moisture - 30 per cent (Field Capacity)

At 30 per cent soil moisture (Table 4), after 12 h, it was noticed that quarter portion of talc filled HGC, half of talc filled HGT capsules, chitin filled HGT capsules, chitosan filled HGT capsules, chitin filled HPMC capsules, chitosan filled HPMC capsules and all the empty capsules got disintegrated.

After 24 h, half of talc filled HGC capsules and talc filled HPMC capsules underwent disintegration. It was also noticed that three-quarter of talc filled HGT capsules, chitin filled HGT capsules, chitin filled HPMC capsules, chitosan filled HPMC capsules, sodium alginate filled HPMC capsules and empty HPMC capsules have been disintegrated. Moreover, chitosan filled HGT capsules and empty capsules of HGT and HPMC have encountered complete disintegration at this point of time.

After 36 h, three-quarter of talc filled HGC capsules and full portion of empty HGC capsules got disintegrated.

After 48 h, quarter portion of calcium alginate filled HGT capsules, chitin filled HGT capsules, calcium alginate filled HGT capsules got disintegrated. It was also observed that half of chitosan filled HGC capsules and calcium alginate filled HPMC capsules also disintegrated at this instant. Three-quarter of talc filled HPMC capsules were also susceptible to disintegration within 48 h whereas

Table 4. Effect of coating material and carrier material on disintegration of capsules at 30% soil moisture

Sl. No.	Capsule coating and carrier material	Time taken for disintegration (h) RH – 72-75%, Temperature – 33.3-33.5°C			
		Quarter of the capsule	Half of the capsule	Three-quarter of the capsule	Full capsule
Hard Gelatin (Transparent) coating (HGT)					
1	Talc	-	12	24	48
2	Chitin	-	12	24	48
3	Chitosan	-	12	-	24
4	Sodium alginate	-	-	-	-
5	Calcium alginate	48	-	-	-
6	Empty capsules	-	12	-	24
Hard Gelatin (Coloured) coating (HGC)					
7	Talc	12	24	36	48
8	Chitin	48	-	84	-
9	Chitosan	24	48	-	60
10	Sodium alginate	-	-	-	-
11	Calcium alginate	48	-	-	-
12	Empty capsules	-	12	24	36
Hydroxy Propyl Methyl Cellulose coating (HPMC)					
13	Talc	-	24	48	60
14	Chitin	-	12	24	48
15	Chitosan	-	12	24	48
16	Sodium alginate	-	-	24	-
17	Calcium alginate	-	48	-	-
18	Empty capsules	-	12	-	24

complete disintegration was noticed in the case of talc filled HGT capsule, chitin filled HGT capsule, talc filled HGC capsule and chitin filled HPMC capsule.

It took 60 h for the complete disintegration of sodium alginate filled HGC capsule and talc filled HPMC capsule.

At the elapse of 84 h, three-quarter of chitin filled HGC capsule got disintegrated.

It was observed that most of the capsule coatings within different carrier materials got disintegrated entirely after 48 h. Chitosan filled HGT and HPMC capsules disintegrated fully after 24 h itself. But, it took 60 h for the entire disintegration of chitosan filled HGC capsules and talc filled HPMC capsules. Calcium alginate filled HGT and HGC capsules behaved in the same way as when placed in soil with 20 and 25 per cent moisture contents. But, three-quarter of sodium alginate filled HPMC capsules have underwent disintegration after 24 h at 30 per cent soil moisture, whereas it remained as such without disintegration under 20 – 25 per cent moisture (Plate 8).

All the capsule coatings with different carrier materials, except the coating with HPMC showed a tendency to disintegrate after 12 h of placement, under ambient conditions. It can be deduced that HGT is the best coating material based on stability and disintegration criterion. Chitosan is the best carrier as it was observed that under soil moisture range of 20 to 30 per cent which is normally prevalent in an irrigated area, they got fully disintegrated after 24-36 h.

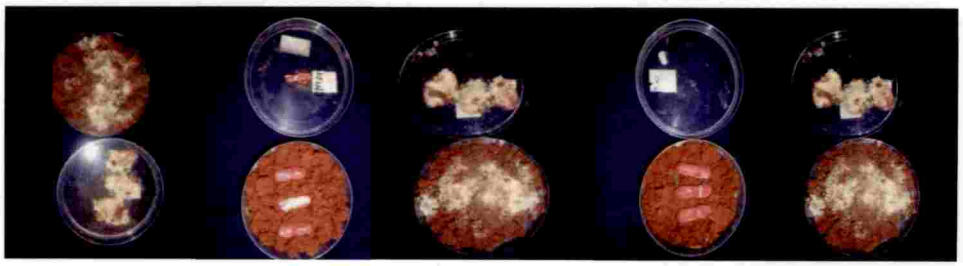
Sodium alginate filled capsules were susceptible to surface contamination and did not disintegrate except that within HPMC coating, of which three-quarter portion disintegrated after 24 h. Thereafter, they did not undergo further disintegration.

4.1.1.3 Disintegration in plant

HGT, HGC and HPMC capsules after filling with selected carrier materials were placed in banana and observed for the time taken for disintegration.

Talc chitin chitosan Ca alginate Na alginate

8a. HGT capsules filled with various carriers at 48 h



Talc Chitin Chitosan Ca alginate Na alginate

8b. HGC capsules filled with various carriers at 48 h

Talc Chitin Chitosan Ca alginate Na alginate

8c. HPMC capsules filled with various carriers at 48 h

Plate 8. Disintegration of capsules placed in soil at 30 per cent moisture

4.1.1.3.1 When placed between the sheaths

The time taken for disintegration of capsules and carrier materials when placed between sheaths of banana is given in Table 5.

When the capsules were tested for their degradability in banana, on a normal sunny day, when there was no rainfall or irrigation (RH – 70 %, Temperature - 33°C), the capsules remained intact for three days. But when the atmospheric humidity was high (RH – 77 %) due to receipt of rain fall (6.76 mm), the disintegration was faster in leaf axil or in between the sheaths. They started disintegrating within 24 h, with complete disintegration after 48 h (Plate 9).

4.1.1.3.2. When placed between the leaf axils

The time taken for the disintegration of HGT, HGC and HPMC capsules when placed between the leaf axils of banana is depicted in Table 6.

The results obtained were similar to those observed when the capsules were placed between the pseudostem sheath. HGT, HGC, HPMC capsules with different carrier materials except HGT with talc got disintegrated wholly after 24 h. But, talc filled HGT capsules and all empty capsules got disintegrated completely after 48 h (Plate 10).

4.1.1.3.3 When placed within the bore holes

When the different types of filled capsules along with selected carrier materials were placed into the bore holes of pseudostem, after 12 h, three - quarter of all capsules disintegrated and by 24 h, there was total disintegration (Table 7).

Therefore, it is deduced that placement of capsules in leaf axils or within pseudostem sheath can be adopted for prophylactic control, as there is slow release while placement in bore holes can be considered for curative application, as the disintegration is faster. From the above experiments, it is concluded that HGT is the ideal coating as it was stable at ambient conditions, at the same time disintegrating completely in field conditions. Chitosan and talc are the ideal carrier materials as they hastens disintegration in soil and plant, respectively.

Talc based Chitin based Chitosan based Ca alginate Na alginate

9a. HGT capsules

Talc based Chitin based Chitosan based Ca alginate Na alginate

9b. HGC capsules

Talc based Chitin based Chitosan based Ca alginate Na alginate

Plate 9c. HPMC capsules

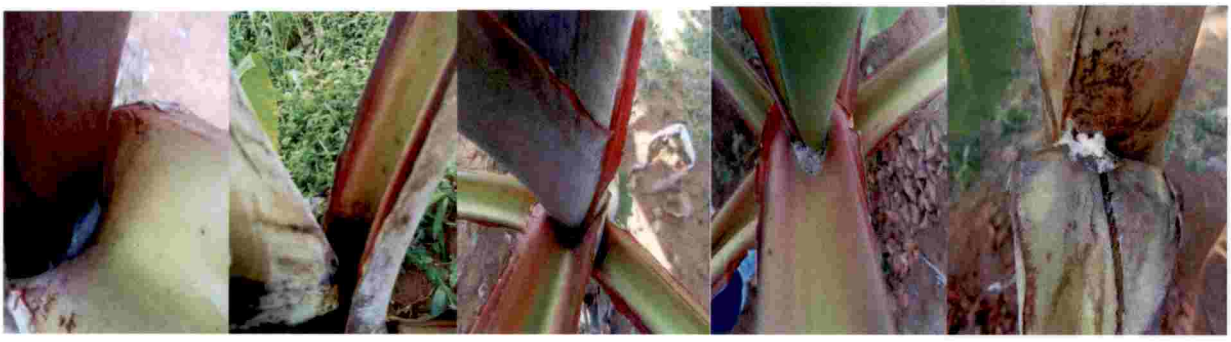
Plate 9. Disintegration of different capsules placed between sheath at 48 h

Talc based Chitin based Chitosan based Ca alginate Na alginate

10a. HGT capsules

Talc based Chitin based Chitosan based Ca alginate Na alginate

10b. HGC capsules



Talc based Chitin based Chitosan based Ca alginate Na alginate

10c. HPMC capsules

Plate 10. Disintegration of different capsules placed in leaf axil at 48 h

Table 5. Effect of coating material and carrier material on disintegration of capsules when placed between the sheaths of banana

Sl. No.	Capsule coating and carrier material	Time taken for disintegration (h) RH – 77%, Temperature – 32.8°C, Rainfall – 6.76 mm			
		Quarter of the capsule	Half of the capsule	Three-quarter of the capsule	Full capsule
Hard Gelatin (Transparent) coating (HGT)					
1	Talc	-	-	12	24
2	Chitin	-	-	24	48
3	Chitosan	-	-	24	48
4	Sodium alginate	-	-	-	48
5	Calcium alginate	-	-	24	48
6	Empty capsules	-	-	12	24
Hard Gelatin (Coloured) coating (HGC)					
7	Talc	-	-	24	48
8	Chitin	-	-	24	48
9	Chitosan	-	-	24	48
10	Sodium alginate	-	-	-	48
11	Calcium alginate	-	-	24	48
12	Empty capsules	-	-	12	24
Hydroxy Propyl Methyl Cellulose coating (HPMC)					
13	Talc	-	-	24	48
14	Chitin	-	-	24	48
15	Chitosan	-	-	-	48
16	Sodium alginate	-	-	-	48
17	Calcium alginate	-	-	-	48
18	Empty capsules	-	-	12	24

Table 6. Effect of coating material and carrier material on disintegration of capsules when placed within the leaf axils of banana

Sl. No.	Capsule coating and carrier material	Time taken for disintegration (h) RH – 77%, Temperature – 32.8°C, Rainfall – 6.76 mm			
		Quarter of the capsule	Half of the capsule	Three-quarter of the capsule	Full capsule
Hard Gelatin (Transparent) coating (HGT)					
1	Talc	-	-	12	24
2	Chitin	-	-	24	48
3	Chitosan	-	-	24	48
4	Sodium alginate	-	-	-	48
5	Calcium alginate	-	-	24	48
6	Empty capsules	-	-	12	24
Hard Gelatin (Coloured) coating (HGC)					
7	Talc	-	-	24	48
8	Chitin	-	-	24	48
9	Chitosan	-	-	24	48
10	Sodium alginate	-	-	-	48
11	Calcium alginate	-	-	24	48
12	Empty capsules	-	-	12	24
Hydroxy Propyl Methyl Cellulose coating (HPMC)					
13	Talc	-	-	24	48
14	Chitin	-	-	24	48
15	Chitosan	-	-	-	48
16	Sodium alginate	-	-	-	48
17	Calcium alginate	-	-	-	48
18	Empty capsules	-	-	12	24

Table 7. Effect of coating material and carrier material on disintegration of capsules when placed within bore holes of pseudostem

Sl. No.	Capsule coating and carrier material	Time taken for disintegration (h) RH – 77%, Temperature – 32.8°C			
		Quarter of the capsule	Half of the capsule	Three-quarter of the capsule	Full capsule
Hard Gelatin (Transparent) coating (HGT)					
1	Talc	-	-	12	24
2	Chitin	-	-	12	24
3	Chitosan	-	-	12	24
4	Sodium alginate	-	-	-	24
5	Calcium alginate	-	-	12	24
6	Empty capsules	-	-	96	24
Hard Gelatin (Coloured) coating (HGC)					
7	Talc	-	-	12	24
8	Chitin	-	-	12	24
9	Chitosan	-	-	12	24
10	Sodium alginate	-	-	-	24
11	Calcium alginate	-	-	12	24
12	Empty capsules	-	-	96	24
Hydroxy Propyl Methyl Cellulose coating (HPMC)					
13	Talc	-	-	12	24
14	Chitin	-	-	12	24
15	Chitosan	-	-	-	24
16	Sodium alginate	-	-	-	24
17	Calcium alginate	-	-	-	24
18	Empty capsules	-	-	96	24

4.1.2 Standardization of moisture content of carrier material for capsules

The capsules prepared using the superior coating material *viz.,* HGT and the carrier materials *viz.,* chitosan and talc were tested for the optimum moisture content that can retain the keeping quality and at the same time maintain the viability of spore of the fungi used. The ideal moisture content for formulating *M. anisopliae* and *B. bassiana* was determined based on their tendency to disintegrate under ambient conditions of temperature and humidity as well as the number of colony forming units (cfu) mL^{-1}.

4.1.2.1 Disintegration at varying moisture levels

Disintegration of *M. anisopliae* and *B. bassiana* capsules, each formulated in talc and chitosan with HGT coating, tested at 5, 8, 10, 15 per cent moisture contents and under room temperature is detailed below.

4.1.2.1.1 M. anisopliae capsules

None of the *M. anisopliae* capsules (HGT) formulated in either talc or chitosan showed any sign of disintegration at five per cent moisture content. They were stable as such under normal room temperature (31.5 to 33°C) and atmospheric humidity (60 to 80 per cent). These capsules failed to disintegrate even at a higher atmospheric humidity (80 to 85 per cent).

At eight per cent moisture too the capsules, both HGT and HGC retained stability under ambient conditions. When atmospheric humidity ranged from 72 to 77 per cent (that is normally prevalent in field conditions), the capsules tend to disintegrate readily.

At 10 per cent moisture content all the capsules retained stability irrespective of coating and carrier materials.

When formulated at 15 per cent moisture content, it was found that HGT and HPMC coatings tend to disintegrate initially, but there was no further disintegration.

4.1.2.1.2 B. bassiana capsules

B. bassiana capsules behaved in a similar manner as those of *M. anisopliae*. Irrespective of coating and carrier materials, 8 to 10 per cent was observed to be the optimum moisture content for stability.

It is inferred that eight to 10 per cent is the ideal moisture range for formulating *Metarhizium* and *Beauveria* capsules, as the coating remained stable under ambient conditions enabling storage stability as well disintegration under field conditions.

4.1.3 Viability of spores in carrier materials under varying moisture levels

The HGT capsules of *M. anisopliae* and *B. bassiana* formulated in chitosan as well as talc were tested for their viability in terms of number of cfu mL^{-1}, at five, eight, 10 and 15 per cent moisture contents.

4.1.3.1 M. anisopliae capsules

4.1.3.1.1 Formulated in chitosan

The viability of *M. anisopliae* capsules formulated in chitosan expressed in terms of number of colony forming units is depicted in Table 8.

After one week of storage, there was no statistical difference in viability of the formulations prepared with 15, 10 and eight per cent moisture , their cfu values being 2.88×10^7 mL^{-1}, 2.77×10^7 mL^{-1} and 2.72×10^7 mL^{-1},respectively. The viability recorded at five per cent moisture content (2.48×10^7 cfu mL^{-1}) was statistically less.

After two weeks, it was noticed that the viability was statistically same in capsules formulated at 15 and 10 per cent moisture content, with cfu values 2.79×10^7 mL^{-1} and 2.73×10^7 mL^{-1}, respectively. This was followed by moisture regimes, eight and five per cent with cfu values 2.50×10^7 mL^{-1} and 2.35×10^7 mL^{-1}, respectively.

After three weeks, the formulations with 15, 10 and eight per cent were superior in viability (2.40×10^7 cfu mL^{-1}, 2.33×10^7 cfu mL^{-1} and 2.24×10^7 cfu mL^{-1}, respectively) which was succeeded by that in five per cent moisture content (1.79×10^7 cfu mL^{-1}) which was least viable.

After one month of storage, maximum viability was recorded in formulations with 15 and 10 per cent (2.35×10^7 cfu mL^{-1} and 2.29×10^7 cfu mL^{-1}, respectively), followed by moisture contents eight and five per cent, with cfu values 1.87×10^7 mL^{-1} and 1.69×10^7 mL^{-1}, respectively.

It is inferred that a moisture level of 10 per cent and more is essential to retain viability of the formulations.

4.1.3.1.2 Formulated in talc

The results indicating viability of *M. anisopliae* capsules formulated in talc are furnished in Table 9.

Moisture content of 15 per cent, maintained significantly higher spore viability (3.06×10^7cfu mL^{-1}), after one week, which was followed by 10, 8 and 5 per cent with cfu values 2.60×10^7, 2.49×10^7 and 2.43×10^7 mL^{-1} respectively. Observations recorded on the 15th DAS, did not vary significantly.

On the 21st day, maximum spore viability was registered in capsules formulated with 15 per cent moisture content (2.69×10^7 cfu mL^{-1}), which was followed by 10 and 8 per moisture contents (2.32×10^7 mL^{-1} and 2.18×10^7 mL^{-1} respectively). Least viability was exhibited by formulation with five per cent moisture content (1.80×10^7 cfu mL^{-1}).

After one month of storage, highest value of cfu was noticed with 15 per cent moisture content (2.65×10^7 mL^{-1}), which was followed by those with 10 and eight per cent (2.10×10^7 cfu mL^{-1}, each). Formulation with five per cent moisture content exhibited least viability (1.69×10^7 cfu mL^{-1}).

Table 8. Effect of moisture content on the viability of *M. anisopliae* capsules formulated in chitosan

Moisture content (%)	*Mean number of cfu (10^7 mL^{-1})			
	Storage period			
	7 DAS	15 DAS	21 DAS	30 DAS
5	2.48 (1.58)^{b}	2.35 (1.54)^{b}	1.79 (1.33)^{b}	1.69 (1.29)^{b}
8	2.72(1.63)^{ab}	2.50 (1.57)^{b}	2.24 (1.50)^{a}	1.87 (1.36)^{b}
10	2.77 (1.66)^{a}	2.73 (1.65)^{a}	2.33 (1.52)^{a}	2.29 (1.51)^{a}
15	2.88 (1.69)^{a}	2.79 (1.67)^{a}	2.40 (1.55)^{a}	2.35(1.54)^{a}
CD (0.05)	**0.072**	**0.077**	**0.082**	**0.097**

* Mean of four replications, DAS – Days After Storage, Figures in parantheses are values after $\sqrt{x+1}$ transformation

Table 9. Effect of moisture content on the viability of *M. anisopliae* capsules formulated in talc

Moisture content (%)	*Mean number of cfu (10^7 mL^{-1})			
	Storage period			
	7 DAS	15 DAS	21 DAS	30 DAS
5	2.43 (1.55)^{b}	2.32 (1.52)	1.80 (1.35)^{c}	1.69 (1.30)^{c}
8	2.49 (1.58)^{b}	2.44 (1.56)	2.18(1.48)^{b}	2.10 (1.45)^{b}
10	2.60 (1.60)^{b}	2.5 (1.58)	2.32(1.52)^{b}	2.10 (1.45)^{b}
15	3.06(1.75)^{a}	2.62 (1.62)	2.69 (1.63)^{a}	2.65 (1.63)^{a}
CD (0.05)	**0.114**	**NS**	**0.076**	**0.079**

* Mean of four replications, DAS – Days After Storage, Figures in parantheses are values after $\sqrt{x+1}$ transformation

4.1.3.2 B. bassiana capsules

4.1.3.2.1 Formulated in chitosan

On the 7th DAS and 21st DAS, the number of cfu recorded did not vary significantly (Table 10).

At 15 DAS, 15 per cent moisture levels exhibited maximum viability, with cfu value 2.74×10^7 mL^{-1}, which was closely followed by that in formulations of 10 per cent moisture content (2.62×10^7 mL^{-1}). The viability observed at eight and five per cent were on parity, with values 2.42×10^7 cfu mL^{-1} and 2.26×10^7 cfu mL^{-1}, respectively.

At the end of the experimental period (30 DAS), formulations with 15, 10 and eight per cent viability exhibited same degree of viability with cfu values, 2.27×10^7 mL^{-1}, 2.27×10^7 mL^{-1} and 2.23×10^7 mL^{-1}, respectively. Formulation with five per cent moisture content exhibited least viability (1.14×10^7 cfu mL^{-1}).

4.1.3.2.2 Formulated in talc

During the initial two weeks of storage, there was no statistical variation in the number of cfu observed in the capsules formulated at five, eight, 10 and 15 per cent moisture level (Table 11).

On the 21st day, formulations with 15 and 10 per cent moisture contents had maximum number of cfu, values being 1.39×10^7 mL^{-1} and 1.31×10^7 mL^{-1}, respectively. These formulations were succeeded by those with eight per cent moisture content (0.87×10^7 cfu mL^{-1}). The least number of cfu was observed at 5 per cent moisture content (0.63×10^7 mL^{-1}).

At the end of one month, it was seen that formulation with 15 per cent had maximum cfu (1.31×10^7 mL^{-1}), followed by that observed at 10 per cent moisture content (1.21×10^7 mL^{-1}). The spore viability observed with formulations with 10 and five per cent moisture content were on par with each other, with cfu values 0.76×10^7 mL^{-1} and 0.42×10^7 mL^{-1}, respectively.

Table 10. Effect of moisture content on viability of *B. bassiana* capsules formulated in chitosan

Moisture content (%)	*Mean number of cfu (10^7 mL^{-1})			
	Storage period			
	7 DAS	15 DAS	21 DAS	30 DAS
5	2.57 (1.60)	2.26 (1.51)^{b}	2.23 (1.49)	1.14 (1.07)^{b}
8	2.62 (1.62)	2.42 (1.54)^{b}	2.30 (1.52)	2.23 (1.49)^{a}
10	2.72 (1.65)	2.62 (1.62)^{ab}	2.30 (1.51)	2.27 (1.51)^{a}
15	2.86 (1.69)	2.74 (1.65)^{a}	2.41 (1.55)	2.27 (1.51)^{a}
CD (0.05)	**NS**	**0.088**	**NS**	**0.112**

* Mean of four replications, DAS – Days After Storage, Figures in parantheses are values after $\sqrt{x+1}$ transformation

Table 11. Effect of moisture content on viability of *B. bassiana* capsules formulated in talc

Moisture content (%)	*Mean number of cfu (10^7 mL^{-1})			
	Storage period			
	7 DAS	15 DAS	21 DAS	30 DAS
5	1.52 (1.23)	1.39 (1.18)	0.63 (0.79)^{c}	0.42 (0.64)^{c}
8	1.54 (1.24)	1.41 (1.24)	0.87 (0.93)^{b}	0.76 (0.87)^{c}
10	1.56 (1.25)	1.48 (1.22)	1.31 (1.16)^{a}	1.21 (1.06)^{b}
15	1.64 (1.29)	1.59 (1.27)	1.39 (1.18)^{a}	1.31 (1.15)^{a}
CD (0.05)	**NS**	**NS**	**0.041**	**0.047**

* Mean of four replications, DAS – Days After Storage, Figures in parantheses are values after $\sqrt{x+1}$ transformation

In general, for both the organisms, higher spore viability was observed at 10 and 15 per cent and least viability at five per cent. Therefore, 10 per cent was determined as the ideal moisture content for viability as 15 per cent affects the stability of coating.

4.1.4 Germination rate of spores in primary powder

The primary powders of the fungi to be formulated as capsules were ascertained for their spore germination rate before formulating them into capsules.

4.1.4.1 M. anisopliae

The germination rate of *M. anisopliae* spores in the aqueous suspension of primary powder was assessed for a period of five days in comparison with that of 14 day old pure culture (Fig. 1). It was observed that after 24 h, the rate of germination of primary powder was 39.22 per cent while that in pure culture was 48.06 per cent. After 72 h, the rate of germination was 51.06 per cent in primary powder and 100 percent in pure culture. The germination rate was 100 per cent in both the primary powder and pure culture, after 120 h.

4.1.4.2 B. bassiana

Rate of germination of *B. bassiana* spores in aqueous suspension of primary powder assessed for a period of five days is given in Fig. 2.

After 24 h, in the primary powder, 35.70 per cent of the spores germinated. The corresponding rate of germination in 14 day old pure culture was 48.06 per cent germination. After 72 h, primary powder exhibited 49.33 per cent germination, while in pure culture, it was 100 per cent. After 120 h, all the spores germinated in the primary powder.

4.1.5 Shelf life of capsules

Results of the shelf life studies based on carrier material, storage temperature and storage period are depicted below.

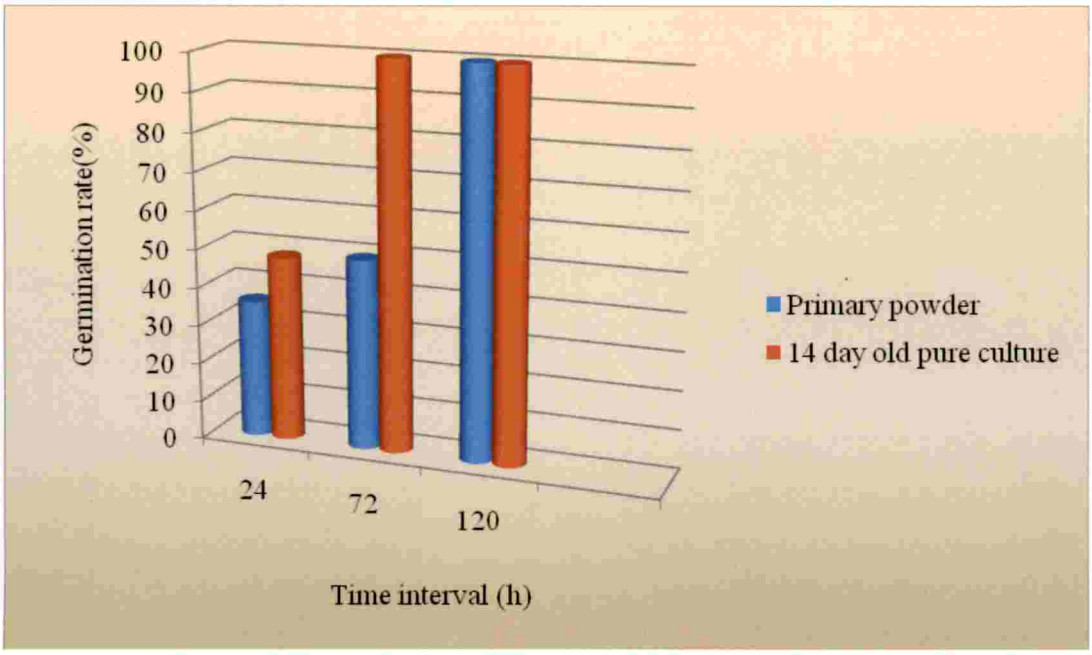


Fig. 1. Rate of germination of *M. anisopliae* spores in primary powder

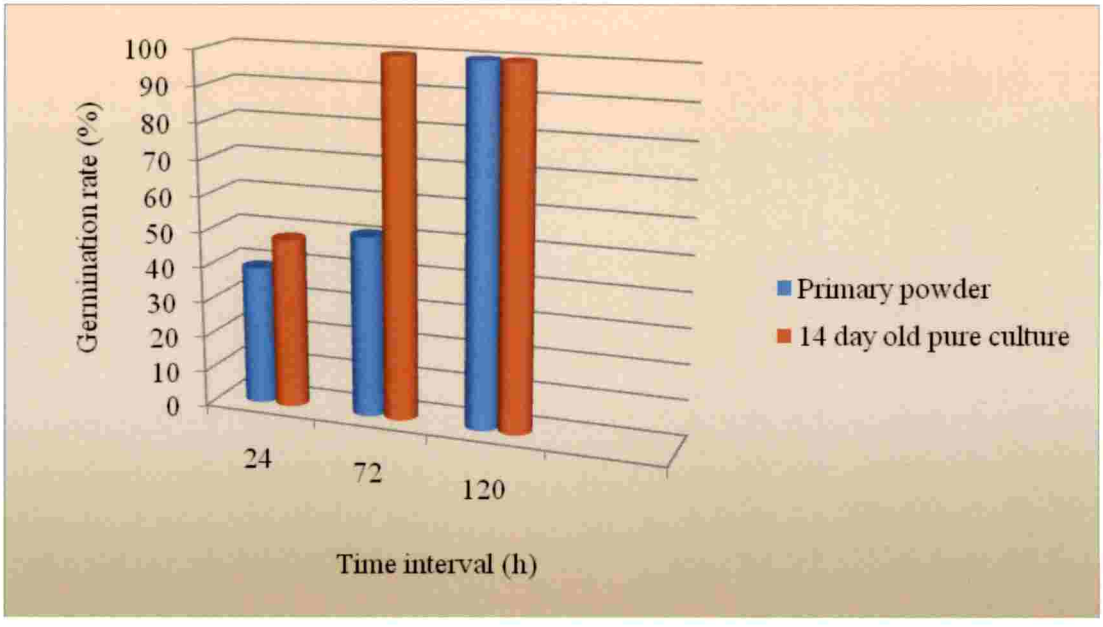


Fig. 2. Rate of germination of *B. bassiana* spores in primary powder

4.1.5.1 M. anisopliae capsules

Shelf life of the capsules in terms of viability is presented in Table 12.

4.1.5.1.1 Effect of carrier material

The mean number of cfu observed was significantly high in chitosan based capsules (2.51×10^7 mL^{-1}), while the corresponding value was 1.77×10^7 cfu mL^{-1} in talc based capsules.

4.1.5.1.2 Effect of storage temperature

Viability of capsules stored at both temperatures differed significantly. The mean number of cfu was significantly high in those stored under refrigeration. The mean value of cfu noted was 2.63×10^7 mL^{-1}, under refrigeration and 1.64×10^7 mL^{-1} at room temperature.

4.1.5.1.3 Effect of storage period

Mean number of viable colonies did not vary significantly till the end of three months of storage, with cfu values ranging from 2.79×10^7 mL^{-1} to 1.72×10^7 mL^{-1}.

4.1.5.1.4 Interaction effect of carrier material and storage temperature

Analysis of the data revealed that there was significant difference in viability based on storage temperature irrespective of carrier material. Chitosan based capsules stored under refrigeration (3.37×10^7 mL^{-1}) and talc based capsules under refrigeration (2.89×10^7 mL^{-1}) exhibited more number of cfu and were significantly higher to other treatment combinations. Capsules stored at room temperature recorded mean cfu value of 1.64×10^7 mL^{-1} each, which were on parity.

4.1.5.1.5 Interaction effect of storage period and storage temperature

The formulations stored under refrigerated condition exhibited more number of viable colonies all through the experimental period. The cfu values observed in formulations stored under refrigeration were on par with each other

Table 12. Effect of carrier material, storage temperature and storage period on shelf life of *Metarhizium* capsules

Treatments and combinations	Mean no. of cfu ($10^7 mL^{-1}$)
Carrier materials (A)	
Chitosan (A_1)	2.51
Talc (A_2)	1.77
CD (0.05)	**0.199**
Storage temperature (B)	
Room temperature (B_1)	1.64
Refrigeration (B_2)	2.63
CD (0.05)	**0.103**
Storage period - Days After Storage (DAS) (C)	
15 DAS (C_1)	2.79
30 DAS (C_2)	2.36
45 DAS (C_3)	2.16
60 DAS (C_4)	1.95
75 DAS (C_5)	1.88
90 DAS (C_6)	1.72
CD (0.05)	**1.770**
A × B Interaction	
A_1B_1	1.64
A_2B_1	1.64
A_1B_2	3.37
A_2B_2	2.89
CD (0.05)	**0.828**

C × B Interaction			
C_1A_1	2.47	C_1A_2	2.30
C_2A_1	1.86	C_2A_2	1.93
C_3A_1	1.59	C_3A_2	1.77
C_4A_1	1.39	C_4A_2	1.60
C_5A_1	1.33	C_5A_2	1.59
C_6A_1	1.21	C_6A_2	1.42
CD (0.05)	**0.250**		

C × A Interaction			
C_1B_1	3.28	C_1B_2	3.10
C_2B_1	2.78	C_2B_2	2.85
C_3B_1	2.48	C_3B_2	2.66
C_4B_1	2.31	C_4B_2	2.51
C_5B_1	2.17	C_5B_2	2.49
C_6B_1	2.02	C_6B_2	2.28
CD (0.05)	**0.351**		

upto 30th day, with cfu values ranging from 3.10×10^7 mL^{-1} to 2.85×10^7 mL^{-1}. Thereafter, there was a slight decline in the viability. The mean cfu count observed from 45th to 75th day remained statistically similar, with values ranging from 2.66×10^7 mL^{-1} to 2.49×10^7 mL^{-1}. The number of cfu observed on 90th day in capsules stored under refrigeration (2.28×10^7 mL^{-1}) was significantly lower.

The number of cfu observed on capsules stored at room temperature on the 15th day (2.47×10^7 mL^{-1}) was statistically equivalent to that observed on the 75th day under refrigeration. Thereafter the capsules stored under room temperature exhibited a gradual decline in viability over three months of storage, with values ranging from 1.86×10^7 mL^{-1} to 1.21×10^7 mL^{-1}.

4.1.5.1.6 Interaction effect of storage period and carrier materials

Viability of chitosan based capsules were superior to talc based ones throughout the storage period. A decline after 15 days of storage was observed in chitosan as well as talc based capsules stored at both the temperatures. Thereafter, the values remained steady till the end of two months, the number of cfu ranging from 2.78×10^7 mL^{-1} to 2.31×10^7 mL^{-1}.

The viability exhibited by chitosan based capsules on the 60th day was equivalent to that of talc based capsules on the 15th day (2.31×10^7 cfu mL^{-1} and 2.30×10^7 cfu mL^{-1}) and was maximum. Thereafter a gradual decline was noticed over the period of storage, the number of cfu ranging from 1.42×10^7 mL^{-1} to 1.93×10^7 mL^{-1}.

The results indicated that the shelf life of *Metarhizium* capsules can be maintained till three months of storage without significant loss in viability. However, the shelf life could be extended by two months when chitosan is used as the carrier material and storage can be undertaken in a refrigerator.

4.1.5.2 B. bassiana capsules

Table 13 shows the effect of carrier materials, storage temperature and storage period on shelf life of *B. bassiana* capsules.

Table 13. Effect of carrier material, storage temperature and storage period on shelf life of *Beauveria* capsules

Treatments and combinations			Mean no. of cfu (10^7 mL^{-1})
Carrier materials (A)			
Chitosan (A_1)			2.11
Talc (A_2)			2.02
CD (0.05)			**0.173**
Storage temperature (B)			
Room temperature (B_1)			1.66
Refrigeration (B_2)			2.48
CD (0.05)			**0.210**
Storage period – Days After Storage (DAS) (C)			
15 DAS (C_1)			2.36
30 DAS (C_2)			2.29
45 DAS (C_3)			2.01
60 DAS (C_4)			1.94
75 DAS (C_5)			1.97
90 DAS (C_6)			1.85
CD (0.05)			**1.552**
A × B Interaction			
A_1B_1			1.77
A_2B_1			1.54
A_1B_2			2.45
A_2B_2			2.50
CD (0.05)			**0.244**
C × B Interaction			
C_1B_1	2.00	C_1B_2	2.72
C_2B_1	1.96	C_2B_2	2.62
C_3B_1	1.57	C_3B_2	2.45
C_4B_1	1.42	C_4B_2	2.46
C_5B_1	1.57	C_5B_2	2.38
C_6B_1	1.45	C_6B_2	2.25
CD (0.05) 0.214			
C × A Interaction			
C_1A_1	2.48	C_1A_2	2.26
C_2A_1	2.42	C_2A_2	2.16
C_3A_1	1.95	C_3A_2	2.07
C_4A_1	1.85	C_4A_2	2.02
C_5A_1	2.04	C_5A_2	1.91
C_6A_1	1.95	C_6A_2	1.75
CD (0.05) 0.214			

4.1.5.2.1 Effect of carrier materials

Chitosan and talc based *Beauveria* capsules did not differ in terms of spore viability. The number of cfu observed in chitosan based capsules was 2.11×10^7 mL^{-1} and that in talc based capsules was 2.02×10^7 mL^{-1}.

4.1.5.2.2 Effect of storage temperature

Spore viability exhibited by the capsules stored at both storage temperatures differed significantly with maximum spore viability under refrigerated storage (2.48×10^7 cfu mL^{-1}). The mean number of viable colonies observed in formulations stored at room temperature was 1.66×10^7 cfu mL^{-1}.

4.1.5.2.3 Effect of storage period

Viability of spores declined, but without significance in the capsules, throughout the observational period, the number of viable colonies ranging from 2.36×10^7 cfu mL^{-1} to 1.85×10^7 cfu mL^{-1}, from 15^{th} to 90^{th} DAS.

4.1.5.2.4 Interaction effect of carrier material and storage temperature

Refrigerated capsules exhibited higher viability compared to those stored at room temperature. Chitosan and talc based capsules had similar viability, the number of cfu observed was on par with each other at room temperature (1.77×10^7 mL^{-1} and 1.54×10^7 mL^{-1}). Under refrigeration too both the carriers exhibited similar viability (2.45×10^7 cfu mL^{-1} and 2.50×10^7 cfu mL^{-1}).

4.1.5.2.5 Interaction effect of storage period and storage temperature

The formulations stored under refrigeration had statistically higher number of viable colonies, ranging from 2.72×10^7 cfu mL^{-1} to 2.25×10^7 cfu mL^{-1}, respectively, from 15^{th} to 90^{th} DAS. The viability exhibited a narrow decline over the period of storage both under room temperature as well as under refrigeration, which was not in logarithmic units.

4.1.5.2.6 Interaction effect of storage period and carrier materials

Viability of chitosan based capsules remained on par till one month of storage (2.48×10^7 cfu mL^{-1} to 2.42×10^7 cfu mL^{-1}). Thereafter, a gradual decline

was noticed with statistically lower number at every succeeding fortnight till the end of the experimental period (1.85×10^7 cfu mL^{-1} to 2.04×10^7 cfu mL^{-1}).

In general, chitosan based capsules exhibited better retention of spore viability. Retention of viability was improved when stored under refrigeration.

4.2 GEL FORMULATIONS

Four different gel formulations *viz.*, chitosan based gel, gelatin based gel, agar based gel and alginate gels of *Metarhizium* and *Beauveria* (Plate 11), were assessed for their viability over a period of three months, the results of which are presented below.

4.2.1 Spore germination rate in different gel bases

The germination rate of spores embedded in different gel bases was assessed for a period of three days to get a preliminary idea on the bases used for formulation.

4.2.1.1 M. anisopliae gels

The rate of germination of spore suspensions prepared from *Metarhizium* gels is represented in Fig.3. After 24 h, the rate of germination did not vary significantly in chitosan based gel, gelatin based gel and alginate based gel. In chitosan based gel there was 85 per cent germination, while in gelatin base it was 83.33 per cent and in alginate base it was 81.67 per cent. The corresponding value in 14-day old pure culture was 47.68 per cent. After 72 h, all the spores germinated in gels (all types) as well as in pure culture.

4.2.1.2 B. bassiana gels

The rate of germination of spores in *B. bassiana* gel is presented in Fig. 4. After 24 h, the germination rates for chitosan based gel, gelatin based gel and alginate based gel were statistically same with 86.50 per cent, 83.63 per cent and 80.66 per cent germination rates, respectively. In the meantime, 14-day old pure

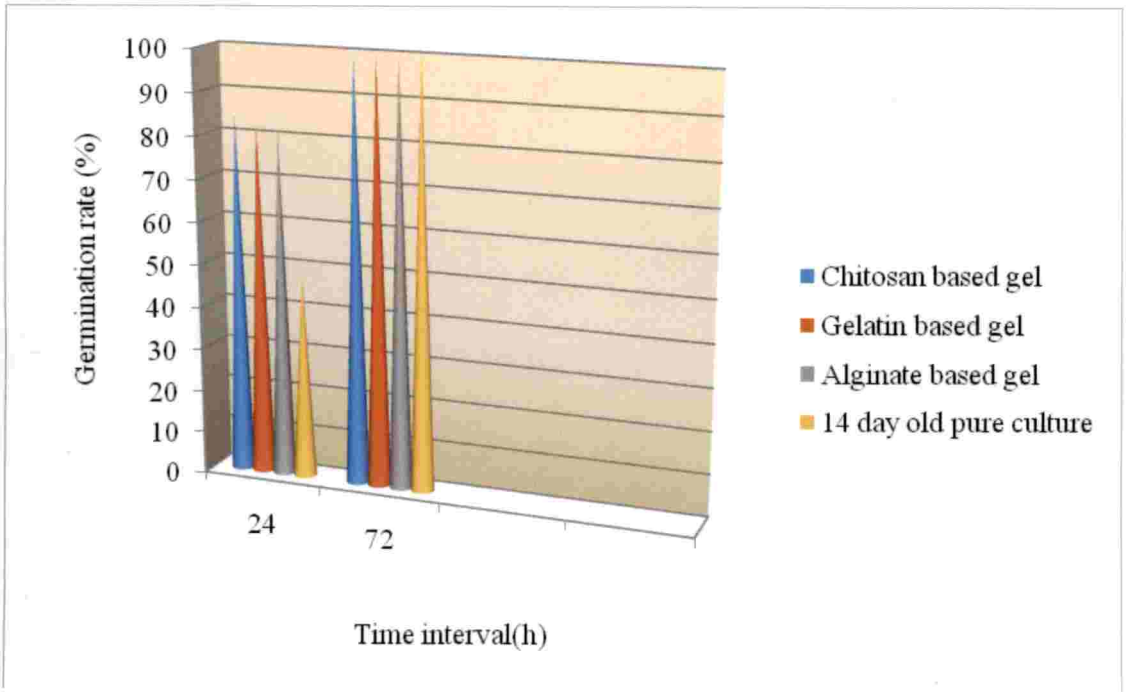


Fig. 3. Rate of germination of *B. bassiana* spores in gel bases

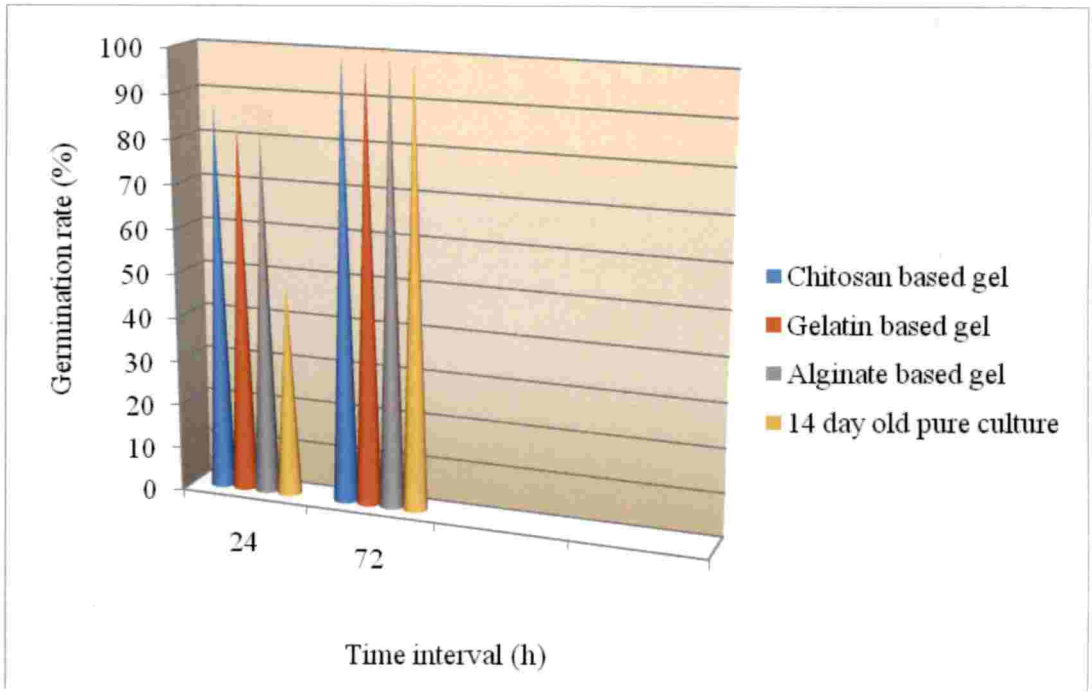


Fig. 4. Rate of germination of *M. anisopliae* spores in gel bases

Chitosan based Gelatin based Agar based Alginate based

11a. *Beauveria* gels

Chitosan based Gelatin based Agar based Alginate based

11b. *Metarhizium* gels

Plate 11. Gel formulations

culture exhibited 47.68 per cent germination. After 72 h, spore suspensions from all type of gels and pure culture exhibited 100 per cent germination.

4.2.2 Shelf life of gel formulations

The viability of gel formulations stored at room temperature and under refrigeration, in terms of number of viable colonies is presented in Tables 14 and 15.

4.2.2.1 M. anisopliae gels

4.2.2.1.1 Effect of gel base

All the three gel bases differed in their viability (Table 14), with significantly higher number of cfu (2.39×10^6 mL^{-1}) in chitosan based gel, followed by that in gelatin based gel (2.05×10^6 mL^{-1}). Alginate based gel contained least number of viable colonies (1.15×10^6 mL^{-1}).

4.2.2.1.2 Effect of storage temperature

Gels stored under refrigeration exhibited significantly higher number of viable colonies compared to those stored at ordinary temperature. The mean cfu values recorded were 2.30×10^6 mL^{-1} and 1.42×10^6 mL^{-1} respectively.

4.2.2.1.3 Effect of storage period

Number of viable colonies observed throughout the experimental period did not vary significantly over three months of storage. The mean cfu values ranged from 2.26×10^6 mL^{-1} on 15th day to 1.41×10^6 mL^{-1} on the 90th day.

4.2.2.1.4 Interaction effect of gel base and storage temperature

Irrespective of the gel base used, formulations stored under refrigeration had higher viability than those stored at room temperature. Highest viability was noted in chitosan based gels stored at lower temperature (3.28×10^6 cfu mL^{-1}). Gelatin based formulations stored at room temperature (1.51×10^6 cfu mL^{-1}) was equivalent to alginate based gel stored under refrigeration (1.40×10^6 cfu mL^{-1}),

Table 14. Effect of carrier material, storage temperature and storage period on shelf life of *Metarhizium* gels

Treatments	Mean no. of cfu (10^6 mL^{-1})
Carrier materials (A)	
Chitosan (A_1)	2.39
Gelatin (A_2)	2.05
Alginate (A3)	1.15
CD (0.05)	**0.137**
Storage temperature (B)	
Room temperature (B_1)	1.42
Refrigeration (B_2)	2.30
CD (0.05)	**0.113**
Storage period – Days After Storage (DAS) (C)	
15 DAS (C_1)	2.26
30 DAS (C_2)	2.10
45 DAS (C_3)	1.93
60 DAS (C_4)	1.80
75 DAS (C_5)	1.69
90 DAS (C_6)	1.41
CD (0.05)	**1.212**
A × B Interaction	
A_1B_1	1.86
A_2B_1	1.51
A_3B_1	0.91
A_1B_2	3.28
A_2B_2	2.25
A_3B_2	1.40
CD (0.05)	**0.195**

C × B Interaction	Mean no. of cfu (10^6 mL^{-1})	C × B Interaction	Mean no. of cfu (10^6 mL^{-1})
C_1B_1	1.80	C_1B_2	2.71
C_2B_1	1.69	C_2B_2	2.50
C_3B_1	1.45	C_3B_2	2.41
C_4B_1	1.30	C_4B_2	2.29
C_5B_1	1.25	C_5B_2	2.14
C_6B_1	1.05	C_6B_2	1.76
CD (0.05)	**0.298**		**0.298**

C × A Interaction	Mean no. of cfu (10^6 mL^{-1})	C × A Interaction		Mean no. of cfu (10^6 mL^{-1})	
C_1A_1	2.96	C_1A_2	2.46	C_1A_3	1.35
C_2A_1	2.68	C_2A_2	2.34	C_2A_3	1.27
C_3A_1	2.45	C_3A_2	2.15	C_3A_3	1.18
C_4A_1	2.30	C_4A_2	1.99	C_4A_3	1.10
C_5A_1	2.19	C_5A_2	1.86	C_5A_3	1.03
C_6A_1	1.75	C_6A_2	1.50	C_6A_3	0.97
CD (0.05)	**0.298**		**0.298**		**0.298**

indicating superiority of gelatin over alginate. The results reveal the superiority of chitosan as gel base and refrigeration as the storage temperature.

4.2.2.1.5 Interaction effect of storage temperature and storage period

Irrespective of the storage period, refrigeration retained better viability. The viability observed on 15th day after storage at room temperature was maximum (1.8 x 10^6 cfu mL^{-1}) and equivalent to that recorded on the 90th day with the gels stored at refrigeration (1.76 × 10^6 cfu mL^{-1}). This indicated an extension of shelf life by 60 days.

4.2.2.1.6 Interaction effect of gel base and storage period

The results revealed that, chitosan gel maintained higher viability throughout the storage period. The mean number cfu recorded on the 45th day (2.45× 10^6 mL^{-1}) was equivalent to that with gelatin base on the 15th day (2.46× 10^6 mL^{-1}). Similarly, its viability on 75th day (2.19× 10^6 cfu mL^{-1}) was in parity with that of gelatin base on the 45th day (2.15× 10^6 cfu mL^{-1}). This revealed the fact that chitosan as gel base which could extend the shelf life of the fungus by one month.

Alginate was inferior to other bases but rate of decrease in viability was negligible throughout the experimental period.

4.2.2.2 B. bassiana gels

Table 15 shows the impact of carrier material, storage temperature and storage conditions on the viability of *Beauveria* gels.

4.2.2.2.1 Effect of carrier materials

The mean number of cfu was highest in chitosan based gels (2.17 × 10^6 mL^{-1}), followed by gelatin based gels (1.25 × 10^6 mL^{-1}). The spore viability of alginate based gels recorded statistically the least (0.58 × 10^6 cfu mL^{-1}).

Table 15. Effect of carrier material, storage temperature and storage period on shelf life of *Beauveria* gels

Treatments	**Mean no. of cfu (10^6 mL^{-1})**
Carrier materials (A)	
Chitosan (A_1)	2.17
Gelatin (A_2)	1.25
Alginate (A3)	0.58
CD (0.05)	**0.385**
Storage temperature (B)	
Room temperature (B_1)	1.10
Refrigeration (B_2)	1.57
CD (0.05)	**0.315**
Storage period – Days After Storage (DAS) (C)	
15 DAS (C_1)	1.81
30 DAS (C_2)	1.66
45 DAS (C_3)	1.42
60 DAS (C_4)	1.16
75 DAS (C_5)	1.05
90 DAS (C_6)	0.97
CD (0.05)	**0.923**
A×B Interaction	
A_1B_1	2.10
A_2B_1	0.70
A_3B_1	0.51
A_1B_2	2.25
A_2B_2	1.81
A_3B_2	0.65
CD (0.05)	**0.547**

C×B Interaction	**Mean no. of cfu ($10^6$$mL^{-1}$)**	**C×B Interaction**	**Mean no.of cfu ($10^6$$mL^{-1}$)**
C_1B_1	1.65	C_1B_2	1.96
C_2B_1	1.54	C_2B_2	1.78
C_3B_1	1.20	C_3B_2	1.64
C_4B_1	0.86	C_4B_2	1.46
C_5B_1	0.74	C_5B_2	1.37
C_6B_1	0.63	C_6B_2	1.20
CD (0.05)	**0.464**		**0.464**

C×A Interaction	**Mean no. of cfu($10^6$$mL^{-1}$)**	**C×A Interaction**		**Mean no.ofcfu ($10^6$$mL^{-1}$)**	
C_1A_1	2.67	C_1A_2	1.76	C_1A_3	0.98
C_2A_1	2.55	C_2A_2	1.66	C_2A_3	0.76
C_3A_1	2.33	C_3A_2	1.35	C_3A_3	0.57
C_4A_1	1.98	C_4A_2	1.01	C_4A_3	0.49
C_5A_1	1.87	C_5A_2	0.90	C_5A_3	0.40
C_6A_1	1.63	C_6A_2	0.83	C_6A_3	0.28
CD (0.05)	**0.464**				**0.464**

4.2.2.2.2 Effect of storage temperature

Refrigerated formulations ranked high in terms of spore viability, with mean number of cfu as 1.57×10^6 mL^{-1} when compared to those formulations stored at room temperature (1.10×10^6 mL^{-1}).

4.2.2.2.3 Effect of storage conditions

The formulations retained viability of propagules till the end of the experimental period without significant decline in number of with cfu (1.81×10^6 mL^{-1} to 0.97×10^6 mL^{-1} over three months).

4.2.2.2.4 Interaction effect of carrier and storage temperature

Chitosan based gel was found to be superior at both the temperatures (2.25×10^6 cfu mL^{-1} and 2.10×10^6 cfu mL^{-1}). Gelatin based gels under refrigeration (1.81×10^6 cfu mL^{-1}) had an equivalent viability. Under room temperature it had an equivalent viability (0.70×10^6 cfu mL^{-1}) with that of alginate based gels under refrigeration (0.65×10^6 cfu mL^{-1}). Least number of cfu was in alginate base at room temperature (0.51×10^6 mL^{-1}).

4.2.2.2.5 Interaction effect of storage period and storage temperature

The number of viable colonies observed during the first 45 days of storage was significantly high both in formulations stored under refrigeration and room temperature which were on par. Thereafter the rate of reduction was lesser in refrigerated condition compared to that in storage.

4.2.2.2.6 Interaction effect of storage period and carrier material

Viability did not vary significantly for a period of three months of storage. Chitosan recorded highest number of viable colonies compared to gelatin and alginate. Viability of chitosan based gel at 60 days after storage (1.98×10^6 cfu mL^{-1}) was equivalent to that of gelatin based gel after 30 days (1.66×10^6 cfu mL^{-1}). It indicated the superiority of chitosan as gel base compared to gelatin and alginate whereby the shelf life is extended by 30 days. Maximum viability

recorded was 2.67 × 10^6 cfu mL^{-1} in chitosan on 15th day and the minimum was 0.28×10^6 cfu mL^{-1} in alginate based gels on the 90th day.

4.2.3 Extent of Contamination in gel formulations

The contaminants noticed were fungi such as *Aspergillus* and *Penicillium* and unidentified bacterial species. The number of contaminants in the formulations stored at room temperature and under refrigeration is presented in Table 16 and 17.

4.2.3.1 Metarhizium gels

4.2.3.1.1 At room temperature

After two weeks of storage (15th DAS), chitosan based gel contained least number of contaminants with cfu value 1.96 x 10^5 mL^{-1}. Both gelatin based and alginate based gels contained same degree of contamination with cfu values of 3.32×10^5 mL^{-1} and 2.86 × 10^5 mL^{-1}, respectively. The trend followed was same on 30th and 45th, 75th and 90th DAS (Table 16).

4.2.3.1.2 Under refrigeration

On 15th DAS, both gelatin based gel (1.12 × 10^5 cfu mL^{-1}) and alginate based gel (1.20 × 10^5 cfu mL^{-1}) contained least number of contaminants and were on par with each other. More number of contaminants was seen in chitosan based gel (1.86 × 10^5 cfu mL^{-1}). A more or less similar trend was noticed throughout the period of storage with minimum contamination in gelatin based gels (0.37 × 10^5 cfu mL^{-1}) and maximum in chitosan based gels (1.20 × 10^5 cfu mL^{-1}) on the 90th day.

4.2.3.2. Beauveria gels

4.2.3.2.1 At room temperature

The contaminants present in various gel formulations of *B. bassiana* are presented in Table 17. After one week of storage, gelatin based gel (2.30 × 10^5 cfu mL^{-1}) and alginate based gel (2.20 × 10^5 cfu mL^{-1}) had least contaminants which

Table 16. Extent of contamination in *M. anisopliae* gels

Sl. No.	Formulations	*Mean no. of cfu (10^5 mL^{-1})					
		Storage period					
		15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
At room temperature							
1	Chitosan based gel	1.96 $(1.40)^b$	1.93 $(1.39)^b$	1.20 $(1.09)^b$	1.76 (1.31)	1.40 $(1.19)^b$	1.10 $(1.05)^b$
2	Gelatin based gel	3.32 $(1.83)^a$	2.86 $(1.70)^a$	2.71 $(1.64)^a$	2.52 (1.59)	2.22 $(1.50)^a$	2.18 $(1.47)^a$
3	Alginate based gel	2.86 $(1.69)^a$	2.83 $(1.69)^a$	2.73 $(1.65)^a$	2.20 (1.48)	2.10 $(1.44)^a$	2.03 $(1.42)^a$
	CD (0.05)	**0.210**	**0.099**	**0.074**	**NS**	**0.133**	**0.148**
Under refrigeration							
1	Chitosan based gel	1.86 $(1.36)^a$	1.73 $(1.32)^a$	1.36 $(1.17)^a$	1.33 $(1.15)^a$	1.26 $(1.12)^a$	1.20 $(1.08)^b$
2	Gelatin based gel	1.12 $(1.07)^b$	1.01 $(1.01)^b$	0.76 $(0.87)^b$	0.65 $(0.80)^c$	0.57 $(0.75)^b$	0.37 $(0.61)^c$
3	Alginate based gel	1.20 $(1.08)^b$	0.85 $(0.92)^b$	0.84 $(0.91)^b$	0.83 $(0.92)^b$	0.82 $(0.90)^c$	0.79 $(0.90)^b$
	CD (0.05)	**0.111**	**0.131**	**0.100**	**0.087**	**0.093**	**0.256**

*Mean of five replications, DAS – Days After Storage, Figures in parantheses are values after $\sqrt{x+1}$ transformation

Table 17. Extent of contamination in *B. bassiana* gels

Sl. No.	Formulations	*Mean no. of cfu (10^6 mL^{-1})					
		Storage period					
		15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
At room temperature							
1	Chitosan based gel	3.73 $(1.93)^a$	3.70 $(1.93)^a$	3.56 $(1.89)^a$	2.86 $(1.69)^a$	2.63 $(1.62)^a$	2.36 $(1.54)^a$
2	Gelatin based gel	2.30 $(1.53)^b$	2.34 $(1.53)^b$	2.29 $(1.52)^b$	2.10 $(1.45)^b$	1.90 $(1.38)^b$	1.90 $(1.38)^b$
3	Alginate based gel	2.20 $(1.48)^b$	2.18 $(1.47)^b$	1.84 $(1.36)^c$	1.60 $(1.27)^c$	1.14 $(1.07)^c$	1.06 $(1.17)^c$
	CD (0.05)	**0.211**	**0.123**	**0.078**	**0.085**	**0.090**	**0.146**
Under refrigeration							
1	Chitosan based gel	1.83 $(1.35)^a$	1.66 $(1.28)^a$	1.26 $(1.11)^b$	1.16 $(1.06)^b$	0.70 $(0.84)^b$	0.66 $(0.81)^b$
2	Gelatin based gel	0.97 $(0.99)^b$	0.95 $(0.98)^b$	0.94 $(0.94)^c$	0.87 $(0.94)^c$	0.81 $(0.88)^b$	0.77 $(0.88)^b$
3	Alginate based gel	2.00 $(1.41)^a$	1.93 $(1.39)^a$	1.80 $(1.34)^a$	1.76 $(1.33)^a$	1.73 $(1.32)^a$	1.60 $(1.26)^a$
	CD (0.05)	**0.190**	**0.140**	**0.093**	**0.090**	**0.143**	**0.201**

*Mean of five replications, DAS – Days After Storage, Figures in parantheses are values after $\sqrt{x+1}$ transformation

were statistically identical. Maximum contamination was noticed in chitosan based gel (3.73×10^5 cfu mL^{-1}). The trend observed was same throughout the storage period. At the termination of the observational period too, the same trend was observed with alginate based gel with least contaminants (1.06×10^5 cfu mL^{-1}).

4.2.3.2.2 Under refrigeration

At 15th DAS, the number of contaminants was least in gelatin based gel with a cfu value of 0.97×10^5 mL^{-1}. The contaminants were more in chitosan based gel (1.83×10^5 cfu mL^{-1}) and alginate based gel (2×10^5 cfu mL^{-1}), which were statistically same. A similar trend was noticed on 30th, 45th, 60th and 75th days. At the termination of the experimental period (90th DAS), the same trend was observed with minimum contaminants in gelatin based and chitosan based gels which were statistically on par. Maximum contamination was noticed in alginate based gel with a cfu value of 1.60×10^5 mL^{-1}.

M. anisopliae and *B. bassiana* gels had maximum viability in chitosan base when stored at room temperature as well as under refrigeration. But, the extent of contamination was more in formulations stored at room temperature. At room temperature, alginate gel had minimum contaminants while it was alginate and gelatin based gels that were less contaminated at refrigeration.

4.2.4 Dispersal of fungal propagules from gel formulations

The time taken for dispersal of the fungal propagules of *Metarhizium* and *Beauveria* gels are presented below.

4.2.4.1 Metarhizium gels

After 2 h, the gel material started to disintegrate. Pin-head sized colonies appeared on the reverse side of the culture plate after 24 h. The number of cfu observed was 1.25×10^6 mL^{-1} after one week. In pure culture (14 day old), fungal growth started after 48 h. The cfu value observed was 1.59×10^7 mL^1.

4.2.4.2 Beauveria gels

The gel material disintegrated after two days and fungal growth appeared from fifth day onwards. Cfu observed was 1×10^6 mL^{-1}. In pure culture, the cfu value observed was 1.29×10^6 mL^{-1}, after three days.

The spores formulated in gel bases had a tendency to germinate earlier than that observed with the fungal disc of the pure culture.

4.3 EFFICACY OF CAPSULES AND GELS ON BANANA WEEVILS

4.3.1 Pathogenicity of the fungi

4.3.1.1 M. anisopliae to O. longicollis

4.3.1.1.1 Adults

Adults treated with the spore suspension of 14 day old culture, did not show any abnormal behavior or symptoms till eighth day. They appeared to be sluggish and exhibited reduced feeding on the ninth day and mortality was noticed on the 11^{th} day onwards. Symptoms of mycosis was seen on the 12^{th} day as white mycelial growth from the intersegmental regions which later on turned greenish. In the treatment with sterile distilled water, the insects exhibited normal behavior.

4.3.1.1.2 Grubs

The grubs exhibited reduced feeding after three days of treatment. The body turned stiff and mummified. Mortality was noticed on the fourth day onwards. White mycelial mat of the fungus was noticed all over the body on the fifth day, which later turned green.

4.3.1.2 B. bassiana to O. longicollis

4.3.1.2.1 Adults

The adults showed reduced feeding after one week of treatment. Mortality was observed on the eighth day. The intersegmental regions were covered with whitish mycelia on the ninth day.

4.3.1.2.2 Grubs

The grubs showed reduced feeding after two days of treatment, during which the body turned stiff and mummified. On the third day, the entire body was covered with whitish mycelia.

4.3.1.3 M. anisopliae to C. sordidus

4.3.1.3.1 Adults

The adults exhibited reduced feeding behaviour after 7th day of treatment. Mortality was observed on the 9th day onwards. The body was covered with white mycelial growth in the intersegmental region on the ninth day which later became greenish.

4.3.1.3.2 Grubs

The grubs became sluggish after two days of treatment, with reduced feeding symptoms. Mortality was observed from the third day onwards. The entire body was covered with whitish growth on the fourth day, which later became greenish.

4.3.1.4 B. bassiana to C. sordidus

4.3.1.4.1 Adults

There were no symptoms of mycosis or abnormal feeding behavior till ninth day. Thereafter, on the tenth day they exhibited less feeding behavior. Treated adults were found dead on the eleventh day after treatment. Afterwards, the intersegmental regions were covered with whitish mycelia.

4.3.1.4.2 Grubs

Avertness towards feeding and sluggishness in movement was noticed after fourth day. After death, the body became mummified and was covered with whitish mycelia.

The pathogenicity tests revealed that both the entomopathogens are infective against banana weevils, *M. anisopliae* had more affinity towards *C. sordidus* and *B. bassiana* towards *O. longicollis,* considering the time taken for exhibiting symptoms of mycosis.

4.3.2 Efficacy of capsules and gels on banana weevils under laboratory conditions

4.3.2.1 Capsule formulations

Efficacy of *M .anisopliae* capsules on *C. sordidus* and *B. bassiana* capsules on *O. longicollis* under laboaratory conditions is presented below.

4.3.2.1.1 Chitosan based capsules of M. anisopliae

The capsule coating got disintegrated after 24 h when placed in moist soil. At 72 HAT, the grubs of *C. sordidus* showed reduced feeding and sluggishness. The grubs were observed to be dead at 96 HAT.

4.3.2.1.2 Talc based capsules of M. anisopliae

The capsule coating got fully disintegrated after 48 h when placed in moist soil. The grubs of *C. sordidus* exhibited symptoms of morbidity at 84 HAT and mortality at 96 HAT.

4.3.2.1.3 Chitosan based capsules of B. bassiana

B. bassiana capsule coatings disintegrated after 48 h of placement within banana leaf axil, and reduced the feeding activity of *O. longicollis* grubs. The infected grubs were found to be sluggish, 48 HAT. Mortality was observed on the third day after treatment (72 HAT).

4.3.2.1.4 Talc based capsules of B. bassiana

After 24 h of placing the capsules, it was observed that the coating got disintegrated entirely. Aversion in feeding was exhibited by grubs of *O. longicollis* at 72 HAT. Mortality was recorded at 84 HAT.

4.3.2.1.5 Chitosan based gels of M. anisopliae

The gel base underwent disintegration after 4 h of placement on the rhizome region. The grubs were reluctant to feed after 24 h. Mortality was noticed at 48 HAT.

The results obtained in laboratory evaluation of the formulations are in agreement with that observed in pathogenicity tests with regard to the time taken for mortality and symptoms of mycosis.

4.4 EFFICACY OF CAPSULES AND GELS ON BANANA WEEVILS

The efficacy of superior capsule formulations of *Beauveria* in the management of *O. longicollis* and efficacy of capsules and gel formulations of *Metarhizium* under field conditions is depicted below.

4.4.1 Efficacy of Capsule formulations of *Beauveria* on pseudostem weevil

4.4.1.1 Prophylactic treatment

Effect of *B. bassiana* capsules applied two weeks prior to the release of grubs, assessed based on damage index and pest population is presented in Table 18.

4.4.1.1.1 Damage index

Both the capsule formulations (chitosan based and talc based) were equally effective and were on par with treatment with spore suspension @ 10^8 spores mL^{-1} and chlorpyriphos 20 EC 0.05%. The damage index was one in all the treated plants, whereas it was maximum (4.5) in untreated plants.

4.4.1.1.2 Reduction in pest population

Both chitosan based and talc based capsules were found to be equally effective and statistically similar to the treatments with spore suspension @ 10^8 spores mL^{-1} as well as chlorpyriphos 20 EC 0.05%. When compared to untreated

control, there was a reduction of 91.67 per cent in pest population in each treatment. Percentage reduction noticed in plants treated with spore suspension @ 10^8 spores mL^{-1} and chlorpyriphos 20 EC 0.05% was 100 per cent each.

For prophylactic treatment, both capsule formulations were equally effective against *O. longicollis* as indicated by low damage indices of 1 and percentage reduction in pest population (91.67 per cent in prophylactic control and 91.67 - 100 per cent in curative control). The effect was on par with that of chemical treatment and spore suspension.

4.4.1.2 Curative treatment

The efficacy of *B. bassiana* capsules applied two weeks after the release of grubs, based on damage index and reduction in population over control, is presented in Table 19.

4.4.1.2.1 Damage index

Talc based and chitosan based capsules exhibited damage indices of 1.67 and 1.33 respectively and were similar to those with the treatments with spore suspension of *B. bassiana* @ 10^8 spores mL^{-1} (1.33) well as the treatment with chlorpyriphos 20 EC 0.05% (1.00).

4.4.1.2.2 Reduction in pest population

Talc based capsules were effective as chlorpyriphos 20 EC 0.05% as there was total destruction of the pest. Chitosan based capsules caused 91.67 per cent reduction in pest population while spore suspension @ 10^8 spores mL^{-1} caused 83.33 per cent reduction.

For curative treatment of pseudostem weevil, both the capsules were equally effective as those with spore suspension @ 10^8 spores mL^{-1} and chlorpyriphos 20 EC 0.05% based on damage index. But, percentage reduction in

Table 18. Effect of prophylactic application of *B. bassiana* capsules on pseudostem weevil

Sl.No	Treatments	Damage index	Reduction in pest population plant^{-1} over control (%)
		Mean of three replications	
1	Talc based capsules @ 4 capsules plant^{-1}	1 (1.23)^{b}	91.67
2	Chitosan based capsules @ 4 capsules plant^{-1}	1 (1.23)^{b}	91.67
3	Spore suspension @ 10^{8} spores mL^{-1}	1 (1.23)^{b}	100
4	Chlorpyriphos 20 EC 0.05%	1 (1.23)^{b}	100
5	Untreated control	4.5(1.86)^{a}	-
6	No release	0 (0.71)^{c}	-
	CD (0.05)	**0.196**	

Figures in parantheses are $\sqrt{x+1}$ transformed values

Table 19. Effect of curative application of *B. bassiana* capsules on pseudostem weevil

Sl. No	Treatments	Damage index	Reduction in pest population plant^{-1} over control (%)
		Mean of three replications	
1	Talc based capsules @ 4 capsules plant^{-1}	1.67(1.29)^{ab}	100
2	Chitosan based capsules @ 4 capsules plant^{-1}	1.33 (1.15)^{b}	91.67
3	Spore suspension @ 10^{8} spores plant^{-1}	1.33 (1.15)^{b}	83.33
4	Chlorpyriphos20 EC 0.05%	1.00 (1.23)^{b}	100
5	Untreated control	2.33 (1.52)^{a}	-
6	No release	0 (0.71)^{c}	-
	CD (0.05)	**0.244**	

Figures in parantheses are $\sqrt{x+1}$ transformed values

pest population was maximum in treatment with talc based capsules which was same as that with chemical treatment.

4.4.2 Efficacy of *M. anisopliae* capsules on rhizome weevil

4.4.2.1 Prophylactic treatment

The efficacy of *M. anisopliae* capsules applied two weeks before releasing the grubs assessed based on damage index, number of tunnels in the rhizome and reduction in pest population are presented in Table 20.

4.4.2.1.1 Damage index

Plants treated with chitosan based capsules suffered less damage (36.67) compared to those treated with talc based capsules (46.67). Treatment with spore suspension (damage index 43.33) was superior to talc based capsules (damage index 46.67), but inferior to chitosan based capsules which exhibited least damage (36.67).

4.4.2.1.2 Number of tunnels

The number of tunnels recorded in plants treated with both the capsules was statistically equal. It was 2.00 in chitosan based capsules and 2.66 in talc based, the former being on par with chemical treatment (1.00). The number of tunnels was 3.00 in plants treated with spore suspension @ 10^8 spores mL^{-1} which was on par with number observed in untreated plants (4.33).

4.4.2.1.3 Reduction in pest population

The treatments with both the capsules were equally effective in reducing the population of weevils. The percentage reduction in pest population observed in these plants were 55.56 per cent and 47.22 per cent in treatments with talc based and chitosan based capsules respectively. The reduction was 41.67 per cent in spore suspension @ 10^8 spores mL^{-1}. Maximum reduction (100 per cent) in population was observed in plants treated with chlorpyriphos 20 EC 0.05%.

Table 20. Effect of prophylactic application of *M. anisopliae* capsules on rhizome weevil

Sl. No	Treatments	*Damage index	Number of tunnels plant^{-1}	Reduction in pest population plant^{-1} over control (%)
		Mean of three replications		
1	Talc based capsules @ 4 capsules plant^{-1}	46.67 (6.86)^{ab}	2.66^{ab}	55.56
2	Chitosan based capsules @ 4 capsules plant^{-1}	36.67 (5.94)^{b}	2.00^{b}	47.22
3	Spore suspension @ 10^8 spores mL^{-1}	43.33 (6.56)^{b}	3.00^{ab}	41.67
4	Chlorpyriphos20 EC 0.05%	13.33 (3.67)^{c}	1.00^{b}	100
5	Untreated control	73.33 (8.53)^{a}	4.33^{a}	-
6	No release	0 (0.71)^{d}	0^{c}	-
	CD (0.05)	**1.918**	**2.253**	

Figures in parantheses are $\sqrt{x+1}$ transformed values

Prophylactic application of *Metarhizium* capsules was not much effective in managing pest though there was significant reduction in damage and pest population. There was 47.22 – 55.56 per cent reduction in pest population with the treatment with capsules.

4.4.2.2 Curative treatment

The efficacy of *M. anisopliae* capsules applied two weeks after releasing the grubs assessed based on damage index, number of tunnels in rhizome and reduction in pest population are presented in Table 21.

4.4.2.2.1 Damage index

Both chitosan based and talc based capsules recorded minimum damage index (DI) of 26.67 and 30, respectively, which were similar to that recorded with spore suspension @ 10^8 spores mL^{-1} (DI - 23.33) and chemical treatment (D1 - 16.67).

4.4.2.2.2 Number of tunnels

All the treatments were equally effective based on number of tunnels observed (0.67 each in treatments with chitosan based capsules, talc based capsules and spore suspension, 1 in chlorpyriphos 20 EC 0.05%). Maximum number of tunnels were noted in untreated plants (2.75).

4.4.2.2.3 Reduction in pest population

Plants treated with chitosan based and talc based capsules were equally effective exhibiting 50 and 58.33 per cent control. Treatments with spore suspension @ 10^8 spores mL^{-1} resulted in 91.67 per cent reduction in population over control and those treated with chlorpyriphos 20 EC 0.05% exhibited 100 per cent reduction in pest population.

The overall evaluation of *B. bassiana* and *M. anisopliae* capsules revealed that *Beauveria* capsules are highly effective to pseudostem weevil both as

Table 21. Effect of curative application of *M. anisopliae* capsules on rhizome weevil

Sl. No	Treatments	Damage index	Number of tunnels plant $^{-1}$	Reduction in pest population plant^{-1} over control (%)
		Mean of three replications		
1	Talc based capsules @ 4 capsules plant^{-1}	30 (5.47)^{b}	0.67^{b}	58.33
2	Chitosan based capsules @ 4 capsules plant^{-1}	26.67 (5.14)^{b}	0.67^{b}	50
3	Spore suspension @ 10^{8} spores mL^{-1}	23.33 (4.86)^{b}	0.67^{b}	91.67
4	Chlorpyriphos 20 EC 0.05%	16.67 (4.00)^{b}	1.00^{b}	100
5	Untreated control	86.67 (9.28)^{a}	2.75^{a}	-
6	No release	0 (0.71)^{c}	0	-
	CD (0.05)	**1.737**	**0.490**	

Figures in parantheses are $\sqrt{x+1}$ transformed values

prophylactic and curative control measures. Chitosan and talc based capsules of *Beauveria* were equally effective. The damage indices noted were on par with those of unformulated spores @ 10^8 spores mL^{-1} and chlorpyriphos 20 EC 0.05%, irrespective of the carrier material used (chitosan/talc) or the method of application (prophylactic/curative). The reduction in population was 91.67 per cent in prophylactic and 97.67 – 100 per cent in curative control.

Metarhizium capsules were moderately effective in managing rhizome weevil. Though there was significant reduction in damage when applied as prophylactic or curative as indicated by damage indices and number of tunnels, the percentage reduction in pest population was only to the tune of 47–55 per cent in prophylactic and 50 – 58 per cent in curative control.

4.4.3 Gel formulations

4.4.3.1 C. sordidus

4.4.3.1.1 Prophylactic treatment

The efficacy of chitosan based gel of *M. anisopliae* applied two weeks prior to the release of grubs assessed based on damage index number of tunnels in rhizome and reduction in pest population are presented in Table 22.

4.4.3.1.1.1 Damage index

There was significant reduction in the damage caused by weevils when plants were treated with gel formulations. The efficacy of chitosan based gel (damage index 46.67) was on par with that of spore suspension @ 10^8 spores mL^{-1} (damage index 33.33). Untreated plants exhibited greater damage index of 70.

4.4.3.1.1.2 Number of tunnels

Chitosan based gel formulation was equally effective as unformulated spores @ 10^8 spores mL^{-1} (2.00) and chlorpyriphos 20 EC 0.05% (1.00). Maximum number of tunnels was seen in untreated control (5.00).

Table 22. Effect of prophylactic application of *M. anisopliae* gels on rhizome weevil

Sl. No.	Treatments	Damage index	Number of tunnels	Reduction in pest population plant^{-1} over control (%)
		Mean of four replications		
1	Chitosan based gels @ 10 g plant^{-1}	46.67 (6.83)^{ab}	1.67^{b}	61.11
2	Spore suspension @ 10^8 spores mL^{-1}	33.33 (5.71)^{b}	2.00^{b}	100
3	Chlorpyriphos 20 EC 0.05%	20 (3.56)^{c}	1.00^{b}	100
4	Untreated control	70 (8.00)^{a}	5.00^{a}	-
5	No release	0 (0.71)^{d}	0	-
	CD (0.05)	**1.864**	**1.719**	

Figures in parantheses are $\sqrt{x+1}$ transformed values

4.4.3.1.1.3 Reduction in pest population over control

The plants treated with chitosan based gel exhibited 61.11 per cent reduction in population, while in treatment with spore suspension@ 10^8 spores mL^{-1} and chlorpyriphos 20 EC 0.05%, there was 100 per cent reduction in pest population.

4.4.3.1.2 Curative treatment

The efficacy of chitosan based gel of *M. anisopliae* applied two weeks after the release of grubs assessed based on damage index number of tunnels in rhizome and reduction in pest population are presented in Table 23.

4.4.3.1.2.1 Damage index

Based on damage index, chitosan based gel was not satisfactory as the value (30) was statistically higher than those observed with spore suspension @ 10^8 spores mL^{-1} (26.67) and chlorpyriphos 20 EC 0.05% (16.67).

4.4.3.1.2.2 Number of tunnels

Plants treated with chitosan based gel were found to contain the same number of tunnels as that with the treatments spore suspension @ 10^8 spores mL^{-1} and chlorpyriphos 20 EC 0.05% with 3.00, 2.66 and 1.66 number of tunnels respectively. Significantly more number of tunnels were seen in untreated plants (5.66).

4.4.3.1.2.3 Reduction in pest population over control

The percentage reduction in grubs was 36.11 per cent in plants treated with chitosan based gel, while 91.67 per cent reduction in treatment with spore suspension @ 10^8 spores mL^{-1}. The corresponding reduction in population when treated with chlorpyriphos 20 EC 0.05% was only 52.78 per cent.

Table 23. Effect of curative application of *M. anisopliae* gels on rhizome weevil

Sl. No.	Treatments	Damage index	Number of tunnels	Reduction in pest population plant^{-1} over control (%)
		Mean of four replications		
1	Chitosan based gel @ 10 g plant^{-1}	30 (5.47)^{b}	3.00^{b}	36.11
2	Spore suspension @ 10^{8} spores mL^{-1}	26.67(5.19)^{c}	2.66^{b}	91.67
3	Chlorpyriphos 20 EC 0.05%	16.67 (4.10)^{d}	1.66^{b}	52.78
4	Untreated control	70 (8.30)^{a}	5.66^{a}	-
5	No release	0 (0.71)^{e}	0	-
	CD (0.05)	**0.274**	**1.883**	

Figures in parantheses are $\sqrt{x+1}$ transformed values

Chitosan based gel lowered the level of damage significantly as evident with the lower damage indices observed. It could produce equivalent effect as chlorpyriphos 20 EC 0.05% as the extent of damage indicated by number of tunnels were on parity both in prophylactic and curative treatments.

Discussion

5. DISCUSSION

In the era of organic farming that targets sustainable development, microbials, especially entomopathogenic fungi are the most commercially exploited organisms. The conventional commercial formulations of the entomopathogens fail to reach its expected potency due to reduced availability at the target site. Development of novel bioformulations like capsule and gel would be a welcome step, to address these problems. The research work entitled "Novel bioformulations of entomopathogenic fungi and their efficacy against banana weevils'' was intended to develop protocols for developing capsule and gel formulations of entomopathogenic fungi, which is a first attempt of its type as per the purview of literature.

CAPSULE FORMULATIONS

Capsule formulations have an inner core containing active ingredient and carrier material, surrounded by a coating. Hence in the development of capsule formulations of microbes, standardization of the coating material, the carrier material, the moisture content of the ingredient that can retain stability as well as the viability is vital.

The common capsules used in pharmaceuticals are gelatin capsules where the coating is prepared from animal tissue and cellulose capsules prepared from plant tissue. Gelatin capsules can either be soft gelatin or hard gelatin. Soft gelatin capsules are encapsulated using sophisticated machines while hard gelatin and cellulose capsules can be prepared using simple apparatus. Hard gelatin capsule holds dry ingredients in powder form. The body is first filled with the mix of active ingredients and any excipients used, and then closed with the cap using either a manual or automatic press machine. This technology was made use of for preparing capsules of entomopathogenic fungi.

The first and foremost step was to standardise a suitable coating material that can remain stable under storage as well as disintegrate under field conditions when placed either in soil or in plants, for the management of soil inhabiting pests like rhizome weevil, *Cosmopolites sordidus* (Germar) and stem boring and plant inhabiting pests like pseudostem weevil, *Odoiporus longicollis* (Oliver).

The three coating materials tried were Hard Gelatin Transparent (HGT), Hard Gelatin Coloured (HGC), Hydroxy Propyl Methyl Cellulose (HPMC). Observations recorded on the time taken for disintegration and extent of disintegration revealed that all the three were stable under ambient conditions of storage (RH of 70 - 75 per cent and temperature 33 - 33.5°C), but under conditions of high RH (> 80 per cent) that may prevail in the open field conditions, the coatings disintegrated faster, taking > 48 h for HGT and HPMC. However, considering the degradability in plants, HPMC was superior. Coloured gelatin coatings were comparatively resistant as it took >72 h to disintegrate.

Earlier research works related to suitability of coating material and disintegration studies of capsules were seen carried out in the field of pharmaceuticals by Pina and Brojo (1996). They proved that the hard gelatin capsules disintegrate faster and released the contents readily. However, the study was based on the efficacy in relation to gastro intestinal tract of humans. Liu and Liu (2009) explored a liquid phase coating technique for the formulation of microencapsulated conidia of *Metarhizium anisopliae* (Metschnikoff) Sorokin MA126 and reported high (78 per cent) encapsulation efficiency with HPMC coating among other biopolymers such as chitosan and sodium alginate. The qualities of HPMC were highlighted by Charan (2015), who has reported that it has more physical strength, protection from moisture, protection from microbial contamination and compatibility with carrier materials, compared to starch based capsules. Hard gelatin coatings were successfully used for encapsulating PGPR by Ananadaraj (2016) and claimed that the technology offers easiness in storage and delivery of the microbes under field conditions. In addition to this, the technology was reported to be cost effective.

In this study the capsules filled with different carrier materials *viz.*, talc, chitin, chitosan, calcium alginate and sodium alginate when tested for their vulnerability to degrade under varying soil moisture levels of 5, 15, 20, 25 and 30 per cent, it was noted that none of them degraded up to a moisture level of 15 per cent. Thereafter, at 20 per cent moisture, *i.e.*, at available water holding capacity and RH of 72- 75 per cent, the time taken for complete disintegration of capsules varied with carrier material (36-96 h). At higher levels of soil moisture of 30 per cent *i.e.*, at field capacity the disintegration was quick (24-60 h). It was also observed that chitin and calcium alginate enclosed within different coatings were rather resistant to disintegration at 20, 25 and 30 per cent soil moisture. This may be attributed to the poor solubility of chitin in water which limits its application in the field of agriculture, as observed by Dutta and Dutta (2009). The delay in degradability of calcium and sodium alginates may be attributed to its insolubility in water and organic solvents. However, Hiltpold *et al.* (2012) reported the efficacy of alginate capsule formulations of entomopathogenic nematodes, *Steinernema* Weiser and *Heterorhabditis* sp. Poinar and claimed that the damage caused by corn root worm, *Diabrotica virgifera virgifera* was reduced, when compared to water spray.

Though all the coatings were equally stable under ambient conditions of storage, while considering the degradability in soil, as for soil inhabiting pests, HGT coated capsules with chitosan as carrier would be preferred as they disintegrated earlier and released the ingredients. Suitability of chitosan as a carrier material for biocontrol fungi was earlier suggested by Palma-Guerrero *et al.* (2007). They reported the degradability of chitosan by biocontrol fungi like *Beauveria bassiana* (Balsamo) Vuillemin, *Lecanicillium psalliotae* (Treschew) Zare and Gams, *Pochonia chlamydosporia* (Goddard) Zare and Gams, *Trichoderma harzianum* Rifai. Faster degradation of chitosan filled capsules in soil observed in this study may be attributed to the enzymatic action of soil-inhabiting fungi.

When the capsules were tested for their degradability in banana, on a normal sunny day, when there was no rainfall or irrigation (RH - 70 %, Temperature - 33°C), the capsules remained intact for three days, but when the atmospheric humidity was high (RH -77 %) due to receipt of rain fall (6.76 mm), the disintegration was faster in leaf axil or in between the sheaths. It started disintegrating within 24 h, with complete disintegration after 48 h. When placed into the bore holes, they disintegrated within 24 h, due to the innate moisture within bore holes. Considering all these aspects, it is deduced that placement of capsules in leaf axils or within pseudostem sheath can be adopted for prophylactic control, while placement in bore holes can be considered for curative application. Such degradation studies of capsules in relation to plant and soil is the first of its type in the field of agriculture.

A microbial formulation should be in such a way that, it should retain the viability of the microbe during storage. For this, moisture content of the formulation plays a vital role.

In this study with HGT capsules, prepared using chitosan and talc as carrier materials, it was noted that five per cent moisture content was unsuitable for encapsulation, irrespective of the carrier material used, as it could not support viability.

Fig. 5 represents the effect of moisture on number of viable colonies of *Metarhizium* and *Beauveria* capsules formulated in chitosan on the 30th Day After Storage (DAS). In the case of *Metarhizium* capsules formulated in chitosan, the number of viable colonies observed in formulations with 15 and 10 per cent moisture was statistically similar upto one month after storage. The cfu values observed in formulations with 15 per cent moisture content was 2.35× 10^7 cfu mL^{-1} on 30th DAS and in formulations with 10 per cent moisture content, the corresponding value was 2.29 x 10^7 mL^{-1}. But, at 15 per cent moisture, the coatings collapsed and did not retain storage stability. Therefore, 10 per cent was

determined as the ideal moisture content, as they could satisfy the conditions of storage and viability. The trend observed when formulated in talc was similar (Fig. 6).

In the case of *Beauveria* capsules formulated in chitosan, at 15 per cent moisture, the number of viable colonies was high 2.27 x 10^7 cfu mL^{-1} on 30^{th} DAS. At intermediate moisture levels of 10 and eight per cent, the cfu values were 2.27 x 10^7 mL^{-1} and 2.23 x 10^7 mL^{-1}, respectively. The trend observed in talc based capsules did not differ much from that observed for chitosan based capsules.

Derakshan *et al.* (2008) propounded that the viability of the entomopathogenic fungus *Lecanicillium lecanii* (Zimmermann) Zare and Gams in talc formulations at five and 10 per cent were on par and was significantly higher than that at 15 per cent. The percentage viability at room temperature under five per cent moisture was 28.37 after two months and 75.03 after six months and at 10 per cent moisture content, it was 27.59 after two months and 76.03 per cent after six months. Similarly, at 15 per cent moisture, the cfu values ranged between 21.86 and 71.56 per cent, after two months and six months, respectively. However, Posada-Florez (2008) reported that the 10 per cent moisture was high to maintain viability of *B. bassiana* spores and that five per cent can be considered as ideal moisture content. A lower moisture content of less than four per cent was used by Sathyasayee *et al.* (2008) for preparing tablets of *Beauveria*, *Metarhizium*, *Paecilomyces*, *Lecanicillium* and *Nomuraea*, to get a dry composition. Moore *et al.* (2010) have studied about the influence of temperature and moisture content on medium term storage for three to four months in *M. flavoviride* and concluded that four to five per cent moisture is optimum for storage. Singh and Nautiyal (2012) claimed that *T. harzianum* when formulated at eight per cent moisture content in talc, exhibited maximum number of colony forming units, 10^{11} to 10^{12} g^{-1}, when compared to carrier materials like charcoal,

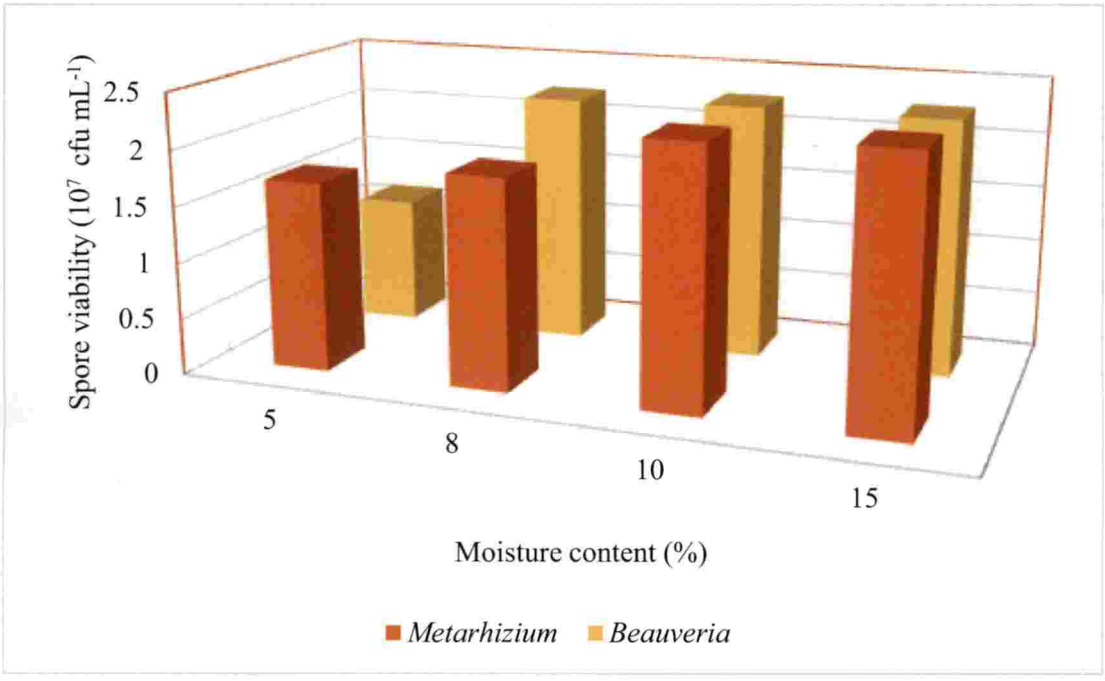


Fig. 5. Effect of moisture content on viability of chitosan based *Metarhizium* and *Beauveria* capsules at 30 DAS

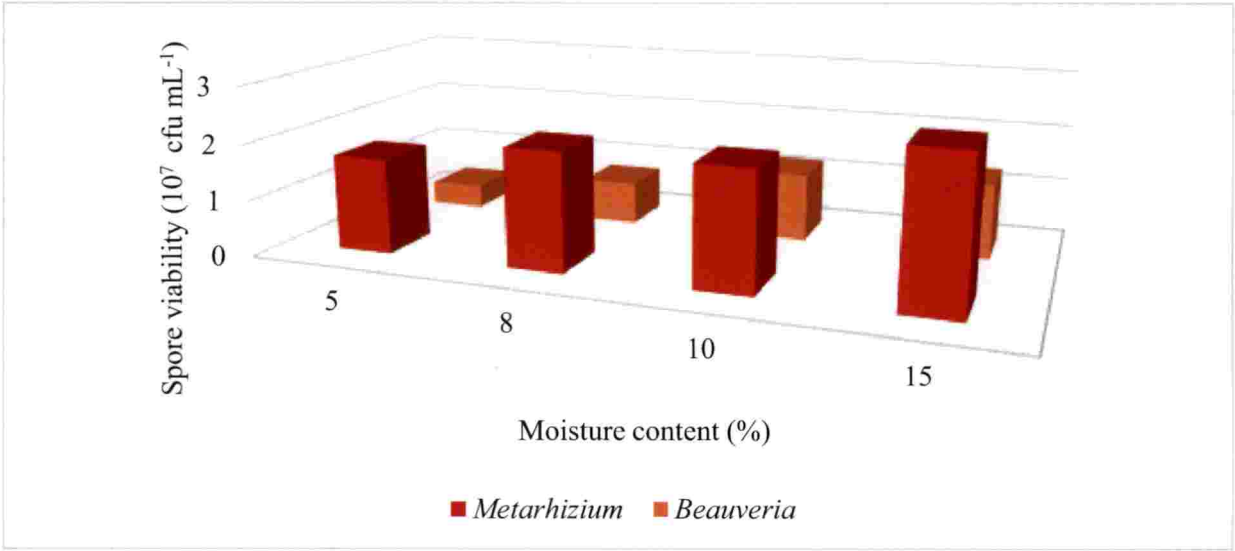


Fig. 6. Effect of moisture content on viability of talc based *Metarhizium* and *Beauveria* capsules at 30 DAS

cow dung, sawdust and vermiculite with moisture contents of 0, 2, 4, 6, 8, 10 and 12 per cent v/w.

Spore germination assay is inevitable to test the suitability of carrier material that is to be selected for formulating entomopathogens. Assay carried out using primary powder made of *M. anisopliae* spores and chitosan revealed that the rate of germination within 24 h, was lesser for the spores in primary powder (39.22 per cent) compared to those in pure culture suspension (48.06 per cent). However, the germination rate was equal in both after 120 h (100 per cent). This may be due to the fact that aqueous spores germinated faster as they are naked, while those incorporated in carrier material encountered a certain limit of dryness during the initial hours. Later on, as and when they absorbed moisture, they germinated. A more or less similar trend was observed with *Beauveria* spores in primary powder. This observation gives a positive indication on the storage stability of the proposed formulation which can eventually remain dormant in storage and can readily germinate in a spray solution.

Palma-Guerrero *et al.* (2007) studied the effect of chitosan on hyphal growth and spore germination of Entomopathogenic fungi (EPF) such as *B. bassiana* and *L. psaliotae,* other biocontrol fungi such as *P. chlamydosporia* and *T. harzianum* and demonstrated that chitosan at lower concentrations upto 1mg L^{-1} ,was non inhibitory to fungal spores, while higher concentrations exhibited slight inhibition .

One of the major challenges for the success of an entomopathogen in the field is to develop a formulation that can sustain its viability in storage. Assessment of shelf life is therefore of prime importance.

When the chitosan and talc based capsules were subjected to shelf life studies for a period of three months, it was seen that chitosan based *Metarhizium* capsules had better viability with a mean number of 2.51×10^7 mL^{-1} cfu while that of talc was 1.77×10^7 mL^{-1} . The number of viable colonies did not vary

significantly over three months storage time, mean number of cfu being 2.79 × 10^7 mL^{-1} cfu on 15th day and 1.72 × 10^7 mL^{-1} cfu on the 90th day. Storage under refrigeration was superior (2.63 × 10^7 mL^{-1}) to that under room temperature (1.64 × 10^7 mL^{-1}). The number of cfu observed in capsules stored at room temperature on the 15th day (2.47 × 10^7 mL^{-1}) was statistically equivalent to that observed on the 75th day under refrigeration (1.59 × 10^7 mL^{-1}), indicating that low temperature storage can extend the shelf life by 60 days .

In the case of *Beauveria* capsules the trend slightly varied in the case of carrier material. Here, both chitosan and talc were equally good in viability, the mean number of cfu being 2.11 × 10^7 mL^{-1} and 2.02 × 10^7 mL^{-1}. Though there was no significant decline in viability, here also storage under refrigeration indicated better viability. The number of cfu noted under refrigeration was 2.48 × 10^7 mL^{-1} and that under normal temperature was 1.66 × 10^7 mL^{-1}. The gradual decline observed in the number of viable colonies was negligible as there was no logarithmic reduction.

Though studies related to the suitability of carrier materials for capsule formulations of EPF are scarce, works related to standardisation of carrier material for the other EPF formulations are copious. Suitability of chitosan in developing formulations was excavated by St. Leger *et al.* (1986). They pointed out the ability of chitosan as a carbon source in enhancing the shelf life of *M. anisopliae*. Chitosan was reported as an ingredient that enhances spore viability in oil formulations of *L. lecanii* (Nithya and Rani, 2017) and *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno (Sankar, 2017).

Appropriateness of talc for carrying many entomopathogens have been tested by various researchers. Faizal (1992) observed that the spores of *Fusarium pallidoroseum* (Cooke) Sacc. formulated in talc and diatomaceous earth retained 75 per cent viability till four days of storage and thereafter, there was a significant decrease. A gradual decline in the number of viable colonies of *M. anisopliae* and *B. bassiana* in talc and gypsum was reported by Bagwan

(2011). He also reported the suitability of organic carriers such as vermicompost, deoiled castor cake and farmyard manure in formulating the fungi. When formulated in organic carriers, the shelf life of *M. anisopliae* and *B. bassiana* were 210, 190 and 160 days respectively, and in inorganic carriers, the shelf life was for 90 days. A shelf life of five months was reported by Abdel- Kader *et al.* (2012) when *T. harzianum* was formulated in talc and chitosan. Banu (2013) claimed that *L. lecanii* formulated in talc exhibited maximum viability of 58 to 97 per cent at room temperature and 70 to 98 per cent under refrigeration.

In this study, it was observed that capsule formulations can be stored under room temperature for a period of three months and that the shelf life could be extended by 60 days, under refrigeration. Similar observations were reported in the case of microbial formulations of several entomopathogenic fungi. Daoust and Roberts (1983) claimed that the temperature decline from 37°C to 4°C increased the longevity of spores of *M. anisopliae* by 18 to 24 months. Areggar (1992) demonstrated increased viability (98 per cent) of *B. brongniartii* up to two years under 2°C. Wettable powder formulation of *B. bassiana* as reported by Zhang *et al.* (1992), exhibited germination rate of more than 85 per cent after eight months of storage under refrigeration. Simkova (2009) reported enhanced germination of *B. bassiana* at low temperature storage, the germination rate being 97.67 per cent at 4°C after 90 days. Similarly Blanford *et al.* (2012) observed that there was no loss in viability of spores (100 per cent) of *B. bassiana* at 7°C when stored for 760 days, while those stored in room temperature exhibited only 30 per cent germination. Das *et al.* (2013) studied the effect of three storage temperatures *viz.*, room temperature, refrigeration and deep freeze, and reported that there was a decline in viability with storage period. Here, the number of viable colonies was 3.53×10^8 to 2.02×10^8 cfu g^{-1} at room temperature (from 30 DAS to 210 DAS), 3.56 to 2.20×10^8 under refrigeration (from 30 DAS to 210 DAS) and 3.41 to 2.02×10^8 (from 30 DAS to 300 DAS) under deep freeze, respectively.

GEL FORMULATIONS

Gel formulations consist of a base (carrier) into which the active ingredient is incorporated, and are semi-solid in nature contributing to increased viability of spores suspended in it.

The gel bases *viz*., gelatin, alginate and chitosan, tested for their suitability supported high germination of 80-87 per cent, compared to that of pure culture (48 per cent) after 24 h, owing to the fact that gels provide more humidity to the spores suspended in them, compared to the dry spores in the unformulated suspension. However, chitosan gel was found to be superior as the length of the germ tube was more, being twice the spore diameter. Increase in length of the germ tube is believed to be an indication of increase in viability as suggested by Soetoppo (2004) and Herlinda (2010). Shorter length of germ tube in alginate base may be due to its dry consistency.

Shelf life studies of *Metarhizium* gels revealed that chitosan gel supported maximum viability with a mean cfu value of 2.39 x 10^6 mL^{-1}, compared to gelatin (2.05 x10^6 mL^{-1}) and alginate based gels (1.15 x 10^6 mL^{-1}). The mean number cfu recorded on the 45th day by chitosan based gels (2.45 x 10^6 mL^{-1}) was equivalent to that with gelatin base on the 15th day (2.46 x 10^6 mL^{-1}). Likewise its viability on 75th day (2.19 x 10^6 mL^{-1}) was in parity with that of gelatin base on the 45th day (2.15 x 10^6 mL^{-1}). This is a clear evidence for chitosan being superior to gelatin in improving the shelf life by one month.

Storage under refrigeration significantly improved viability (2.30 x 10^6 mL^{-1}) compared to storage at room temperature (1.42 x 10^6 cfu mL^{-1}). The viability observed on 15th day after storage at room temperature was maximum (1.80 x10^6 cfu mL^{-1}) and equivalent to that recorded on the 90th day with the gels stored at refrigeration (1.76 ×10^6 cfu mL^{-1}), indicating an extension of shelf life by 60 days.

The trend did not differ in *Beauveria* gels. Superior viability of chitosan gel observed in this study might be due to its semi-solid consistency and

antimicrobial nature as suggested by Alberquenque (2010). Rodrigues *et al.* (2017) studied the viability of alginate formulations in pellets of *B. bassiana* under ambient conditions as well as under refrigerated storage for a period of six months and concluded that there was a reduction in viability from 97 to 46 per cent at room temperature, while it remained constant in refrigeration throughout the observational period.

The development of gel based formulations of EPF is the first of its kind. The work conducted by Umamaheswari *et al.* (2005) on alginate gels of *Steinernema siamkayi* and *Steinernema glaseri* proved that the shelf life of nematodes in these formulations could be enhanced by reducing nematode activity through physical trapping, metabolic inhibition, cold storage or by inhibition of anhydrobiosis. Ahmadi *et al.* (2015) have unearthed the fact that, in addition to the inherent properties of chitosan such as antibacterial, antifungal, biocompatible and biodegradable, it also exhibits cross-linking mechanism thus enabling sustained release profile for drug delivery systems. This same reason can be attributed to the effectiveness of chitosan based gels resulting in sustained release of microbes.

The inferiority of alginate gel noted in this study is well supported by the finding of Lee and Mooney (2012), who stated the property of alginate to form a thermally stable, rigid and brittle gel.

EFFICACY OF EPF AND ITS FORMULATIONS TO BANANA WEEVILS

Preliminary studies carried out in the laboratory unveiled that *M. anisopliae* was more infective to rhizome weevil and *B. bassiana* to pseudostem weevil, considering the time taken for mycosis and mortality. Furthermore, grubs were found to be more susceptible than adults, hard elytra of adults acted as a hindrance to the entry of fungal spores. This observation is in accordance with those of Anitha (2000), who reported the susceptibility of grubs

of pseudostem weevil, *O. longicollis* to *M. anisopliae* and Joseph (2014) and Varsha (2017) who reported the same on rhizome weevil, *C. sordidus*.

Pathogenicity of *B. bassiana* to *O. longicollis* noted in this study is in concurrence with those of Godonou *et al.* (2000), Beegum (2005), Padmanabhan *et al.* (2009), Shukla (2010), Irulandi *et al.* (2012) and Prabhavathi *et al.* (2014). Infectivity of *B. bassiana* to *C. sordidus* were earlier reported Khan *et al.* (2001), Akello *et al.* (2008), Fancelli *et al.* (2013) and Varsha (2017).

Beauveria capsules based on talc and chitosan when evaluated for their efficacy to manage pseudostem weevil, it was observed that when applied prophylactically in the four leaf axils, both the capsules were equally effective with damage indices (DI) of one each. The reduction in pest population over control was 91.67 per cent. For curative treatment, the damage indices were 1.67 and 1.33, respectively for both the capsules and were on par with spore suspension @ 10^8 spores mL^{-1}. It was also noticed that the percentage reduction in pest population was more in talc based capsules which was same as that with chemical treatment (100 per cent each).

Metarhizium capsules were moderately effective in managing rhizome weevil. Though there was significant reduction in damage when applied prophylactically or curatively, as indicated by damage indices and number of tunnels, the percentage reduction in pest population was only to the tune of 47 to 55 per cent in prophylactic and 50 to 58 per cent in curative control.

The foregoing results unveil that *Beauveria* capsules are highly effective to pseudostem weevil both as prophylactic and curative control measures and *Metarhizium* capsules were moderately effective in managing rhizome weevil.

Metrahizium gels when placed in planting pits, there was no 100 per cent reduction of pest population both in prophylactic and curative treatments, but it could lower the level of damage significantly as evident with the lower damage indices observed (46.67 in prophylactic control and 30 in curative control). It

could produce an equivalent effect of chlorpyriphos 20 EC 0.05% as the number of tunnels observed were on parity both in prophylactic and curative treatments. It is therefore inferred that *Metarhizium* gel is moderately effective for rhizome weevil.

The above finding on efficacy of these EPF on banana weevils has been previously proved in various studies. Anitha (2000), reported the efficacy of leaf axil filling of *M anisopliae* spore suspension @ 15 x 10^5 spores mL^{-1} in 1 L $plant^{-1}$ in reducing pseudostem weevil population. The efficacy of pseudostem trap with *B. bassiana* (25 g per trap) was evaluated by Irulandi (2002), who reported 56.75 per cent reduction in infestation of *O. longicollis.* As per the findings of Beegum (2005), leaf axil filling with *B. bassiana* was equally effective as chlorpyriphos 0.03 per cent. Prabhavathi (2012) obtained high mortality of 75 per cent when the fungus was applied as root and rhizome dip compared to injection (46 per cent). Joseph (2014) proved the efficacy of talc based *M. anisopliae* @ 30 g L^{-1} on grubs of *C. sordidus* under field condition resulting in least number of tunnels (0.63) and reduction in number of grubs from four to 0.29. Curative soil drenching with talc formulations of *M. anisopliae* and *B. bassiana* were equally effective in reducing the number of immature stages of rhizome weevil from six to 1.16 and 1.82 per rhizome respectively (Varsha, 2017).

Effective management of banana weevils using chemical or biological insecticides is either by pouring into the leaf axils or spraying for pseudostem weevil and by drenching the pits for rhizome weevil. These delivery mechanisms do not ensure proper management due to improper contact with the weevils that are concealed within. Capsule and gel formulations are better delivery options for controlled release of technical ingredient to the target. This study could standardize the protocol for developing capsule and gel formulations of EPF which is the first attempt of its kind. Investigations on shelf life of these formulations need to be done for a prolonged period. Standardization of the rate and time of application of these formulations in the open field conditions are also to be validated for consistent results.

Summary

6. SUMMARY

The present study entitled "Novel bioformulations of entomopathogenic fungi and their efficacy against banana weevils" was carried out at the Biocontrol Laboratory for Crop Pest Management and Instructional farm, College of Agriculture, Vellayani during the period 2016-18. The investigations were focused on developing protocol for capsule and gel formulations of the entomopathogenic fungi, *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin. The prime areas of research were to standardise the coating and carrier materials based on stability in storage and degradability in plants and soil; to determine the ideal moisture content for maintaining stability and viability of the formulations and to study the shelf life of the formulations under room temperature and refrigeration. Eventually, the bio-capsules and gels formulated were tested for their efficacy in managing the major pests of banana, the pseudostem weevil, *Odoiporus longicollis* (Oliver) and the rhizome weevil, *Cosmopolites sordidus* (Germar).

Experiment to standardise the coating and carrier materials for encapsulation of the fungi revealed that Hard Gelatin Transparent (HGT), Hard Gelatin Coloured (HGC) and Hydroxy Propyl Methyl Cellulose (HPMC) capsules were equally stable under ambient conditions of storage (room temperature 26-33° C and RH 60 - 80 per cent). At higher RH of 80 - 85 per cent, the capsules deformed after 48 h. HPMC coating took more time and HGC coating was comparatively resistant.

Degradation studies in the laboratory at varying soil moisture levels revealed that, in unirrigated soil with moisture content five to 15 per cent, HGT, HGC and HPMC capsules did not disintegrate for one week, irrespective of the carrier material used. In soil taken from an irrigated field with moisture content 20 to 25 per cent, the degradation time remained same. In irrigated soil with 30 per cent moisture which is equivalent to field capacity, HGT coated chitosan capsules disintegrated completely within 24 h of placement, while those based on chitin

took 48 h and calcium alginate 168 h for complete disintegration. But, sodium alginate filled capsules did not undergo disintegration till two weeks.

Disintegration studies in plants revealed that at a RH of 77 per cent and more, talc filled HGT capsules degraded faster with complete disintegration within 48 h. When placed in leaf axil or pseudostem sheath, talc filled HGT capsules disintegrated completely after 48 h. When placed into the bore holes made by the weevil, all the capsules underwent complete disintegration after 24 h. It is therefore deduced that the best coating material for encapsulation is HGT and the suitable carrier material for formulation can either be talc or chitosan and placing the capsules in leaf axils or within pseudostem sheath can be adopted for prophylactic treatment, expecting a slow release of the pathogen while, placement within bore holes can be suggested for curative control of pseudostem weevil.

Of the varying moisture levels ranging from five to 15 per cent, it was observed that five per cent moisture retained stability, but viability was comparatively less. In *Metarhizium* capsules formulated in chitosan with five per cent moisture, the number of cfu obtained at 10^{-5} dilution on the 30th day was 1.69×10^7 cfu mL^{-1}. At eight to 10 per cent moisture content, the corresponding values were 1.87×10^7 cfu mL^{-1} and 2.29×10^7 cfu mL^{-1}, respectively. At 15 per cent, the viability observed was 2.35×10^7 cfu mL^{-1}, but the capsules lost stability in storage at 15 per cent. Similar were the results obtained when formulated in talc. In *Beauveria* capsules formulated in chitosan, the viability observed on the 30th day was 1.14×10^7 cfu mL^{-1} with five per cent moisture content. In formulations with eight per cent and 10 per cent moisture levels, the corresponding values were 2.23×10^7 cfu mL^{-1} and 2.27×10^7 cfu mL^{-1}, respectively. At 15 per cent, the cfu value recorded was 2.27×10^7 mL^{-1}. In talc based capsules also the observation did not vary. Though 15 per cent moisture supported maximum viability, the ideal moisture content was determined as 10 per cent as it could retain both stability and viability without considerable reduction.

The foregoing results lead to the development of two types of capsules of each of the fungi with HGT coating, encapsulating the spores @ 10^8 spores g^{-1}, impregnated separately in chitosan or talc which served as carriers. The capsules were formulated with 10 per cent moisture content and were stored at room temperature as well as under refrigeration to observe their shelf life at fortnightly intervals.

Shelf life studies of capsules conducted for a period of three months revealed that, chitosan was more effective when compared to talc in retaining spore viability in *Metarhizium* capsules. The mean number of cfu observed in chitosan based capsules was 2.51×10^7 cfu mL^{-1} and in talc based capsules it was 1.77×10^7 cfu mL^{-1}. During the period of storage, there was no significant decline in the number of viable colonies. The number of viable colonies ranged from 2.79×10^7 cfu mL^{-1} to 1.72×10^7 cfu mL^{-1} for a period of three months. However, storage under refrigeration had better retention of viability both in chitosan and talc, cfu values being 3.37×10^7 mL^{-1} and 2.89×10^7 mL^{-1} respectively.

Analysis of the interaction of storage temperature and storage period revealed that the viability of capsules stored at room temperature on the 15^{th} day (2.47×10^7 mL^{-1}) was statistically equivalent to that observed on the 75^{th} day under refrigeration (2.49×10^7 mL^{-1}). Similarly, the viability exhibited by chitosan based capsules on the 60^{th} day (2.78×10^7 mL^{-1}) was equivalent to that of talc based capsules on the 15^{th} day (2.30×10^7 mL^{-1}) and was maximum. The results indicated that the shelf life of *Metarhizium* capsules can be maintained till three months of storage without significant loss in viability. Moreover, the shelf life could be extended by two months when chitosan was used as the carrier material and the formulation was under refrigerated storage.

In *Beauveria* capsules, the viability did not differ in both chitosan and talc based capsules. Here also, the number of viable colonies did not decline

statistically upto three months of storage, with cfu values ranging from 1.85 × 10^7 cfu mL^{-1} to 2.36 × 10^7 cfu mL^{-1}, respectively.

Analysis of the combined effect of carrier material and storage temperature revealed that chitosan and talc based *Beauveria* capsules had similar viability at room temperature (1.77 × 10^7 cfu mL^{-1} and 1.54 ×10^7 cfu mL^{-1} respectively) as well as under refrigeration (2.45 × 10^7 cfu mL^{-1} and 2.50 ×10^7 cfu mL^{-1} respectively). Invariably, formulations stored under refrigeration had statistically higher number of viable colonies (2.72 × 10^7 cfu mL^{-1} on 15th day to 2.25 × 10^7 cfu mL^{-1} on 90th day). The narrow decline observed over three months of storage at both the temperatures was however insignificant as it was not in logarithmic units. In general, chitosan based capsules exhibited better retention of spore viability. Retention of viability was improved when formulations were stored under refrigeration.

Shelf life studies of various gel formulations revealed that in *Metarhizium* gels, the mean number of cfu was highest in chitosan based gels (2.39 × 10^6 mL^{-1}), followed by gelatin based gels (2.05 × 10^6 mL^{-1}). Refrigerated formulations ranked high in terms of spore viability, with mean number of cfu 2.30 × 10^6 mL^{-1}, compared to those at room temperature (1.42 × 10^6 mL^{-1}). The number of viable colonies observed throughout the experimental period did not vary significantly over three months of storage, with mean number of cfu ranging from 2.26 x 10^6 mL^{-1} on 15th day to 1.41 x 10^6 mL^{-1} on the 90th day.

Analysis of the interaction effect between gel base/carrier material and storage temperature revealed that chitosan based gels stored at lower temperature supported highest number of viable colonies. (3.28 × 10^6 cfu mL^{-1}). Gelatin based formulations stored at room temperature (1.51 × 10^6 cfu mL^{-1}) was equivalent to alginate based gel stored under refrigeration (1.40 x 10^6 cfu mL^{-1}), indicating the superiority of gelatin over alginate.

Interaction effect between gel bases and storage period revealed the equivalent effect of chitosan based gel on 45th day (2.45 x 10^6 cfu mL^{-1}) to

gelatin base gel on the 15th day (2.46 x 10^6 cfu mL^{-1}). Similarly its viability on 75th day (2.19 x 10^6 cfu mL^{-1}) was in parity with that of gelatin base on 45th day (2.15 x 10^6 cfu mL^{-1}). This revealed the fact that chitosan when used for formulating gels could extend the shelf life of the organism by one month.

The individual effect of gel base, storage temperature and storage period on shelf life of *Beauveria* gels exhibited more or less a similar trend. The combined effect of gel base with each of the other factors such as temperature and storage period also remained same as that of *Metarhizium* gels. Chitosan based gel was found to be superior at both the temperatures (2.25 × 10^6 cfu mL^{-1}, under refrigeration and 2.10 × 10^6 cfu mL^{-1}, at room temperature). Gelatin based gels under refrigeration (1.81× 10^6 cfu mL^{-1}) had an equivalent viability. Under room temperature it had an equivalent viability (0.70 × 10^6 cfu mL^{-1}) with that of alginate based gels under refrigeration (0.65 × 10^6 cfu mL^{-1}). Least number of cfu was in alginate base at room temperature (0.51 × 10^6 mL^{-1}).

While analysing the interaction effect, it was noticed that the viability of chitosan based gel at 60 days after storage was equivalent to that of gelatin based gel after 30 days, indicating the superiority of chitosan as gel base compared to gelatin and alginate, whereby the shelf life was extended by 30 days.

Pathogenicity studies conducted on banana weevils under laboratory conditions using unformulated spore suspensions @ 10^8 spores mL^{-1} revealed that *M. anisopliae* was more infective to grubs of rhizome weevil and *B. bassiana* to those of pseudostem weevil, based on time taken for mycoses and mortality of treated insects. *Beauveria* capsules were therefore evaluated for their efficacy in managing pseudostem weevil and *Metarhizium* capsules and *Metarhizium* gels were tested for their efficacy against rhizome weevil.

Results of pot culture studies disclosed that for prophylactic treatment for pseudostem weevil, both chitosan and talc based *Beauveria* capsules were equally effective against *O. longicollis* as indicated by low damage indices of one each, which was on par that of chlorpyriphos 20 EC @ 0.05 %. The

corresponding index noticed in untreated control was 4.5. The reduction in pest population noticed with respect to control was 91.67 per cent each in chitosan and talc based *Beauveria* capsules.

For curative treatment of pseudostem weevil, both the capsules were equally effective as those with spore suspension and chlorpyriphos based on the damage index (DI). The DI noted was 1.33 in chitosan based *Beauveria* capsules while it was 1.67 in talc based capsules. But, the reduction in pest population was maximum in treatment with talc based capsules (100 per cent) which was same as that with chemical treatment. The results indicated that *Beauveria* capsules are highly effective to pseudostem weevil.

Prophylactic application of *Metarhizium* capsules was found to reduce the rhizome weevil damage significantly as indicated by a DI of 36.67 in chitosan based capsules and 46.67 in talc based capsules. The corresponding DI in chlorpyriphos 20 EC @ 0.05 % was 13.33 and that with spore suspension was 43.33. Eventhough there was significant reduction in the number of tunnels, the percentage reduction in pest population was only to the tune of 47 - 55 per cent.

Curative application of *Metarhizium* capsules had a moderate effect on rhizome weevil. Though the damage was reduced significantly (DI 26 ×- 30) with capsule placement, the reduction in pest population with respect to control was only 50 - 58 per cent.

Prophylactic application of chitosan based *Metarhizium* gel was able to lower the level of damage by rhizome weevils significantly as evidenced by lower DI 46.67. It could produce equivalent effect as chemical control, as the number of tunnels recorded was in parity both in prophylactic and curative treatments. The number of tunnels recorded in prophylactic treatment was 1.67, curative, 3 and for chemical control these values were 1 and 1.66, respectively.

The salient findings of the study are

- Hard Gelatin (Transparent) is the ideal coating material for capsule formulations based on storage and disintegration criteria.
- The filling material that is susceptible to disintegration in soil is chitosan and that in plant is talc.
- The ideal moisture content for the formulation is 10 per cent based on stability of the coating and viability of the organism.
- Chitosan can be the preferred carrier material for encapsulation of the fungi as it could support higher viability and extend the shelf life of capsules by two months.
- Refrigerated storage can extend the shelf life of capsules by two months.
- Chitosan was the superior gel base as it could support viability of the formulation and extend its shelf life by one month.
- *Beauveria* capsules applied prophylactically and curatively can effectively manage pseudostem weevil.
- *Metarhizium* capsules/gels applied in pits prophylactically and curatively can moderately control rhizome weevil.

References

7. REFERENCES

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.

Afraine, Y. A., Mweresa, N. G., Wanjala, C. L., Gilbreath, T. M., Zhou, G., Ming-Chieh, L., Githeko, A. K. and Yan, G. 2016. Evaluation of long-lasting microbial larvicide for malaria vector control in Kenya. *Malar J.* 15 (2): 577.

Ahmadi, F., Oveisi, Z., Samani, M. and Amoozgar, Z. 2015. Chitosan based hydrogels: characteristics and pharmaceutical applications. *Res. Pharma. Sci.* 10(1): 1-16.

Akello, J., Dubois, T., Coyne, D. and Gold, C. S. 2008. Colonisation and persistence of entomopathogenic fungus *Beauveria bassiana* in tissue culture banana. In: Ahmed, K. Z. (eds.) *Eighth African Crop Science Society Conference, El-Minia*, Egypt, 27-31 October 2007. pp. 857-861.

Alburquenque, C., Bucarey, S. A., Neira-carrillo, A., Urzua, B., Hermosilla, G., and Tapia, C. V. 2010. Antifungal activity of low molecular weight chitosan against clinical isolates of *Candida* spp. *Med. Mycol.* 48: 1018–1023.

Ali, S. S. 2016. Preparation and Evaluation of Three Laboratory Formulations of the Entomopathogenic Fungus *Beauveria bassiana*. *Egyptian J. Pest Control*. 26(1):107-110.

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.

Anandaraj, M. 2016. Microbial consortia in bio-capsule doubling the farmers income in spices. In: *Proceedings of the Seventh Indian Horticulture Congress*, 15-18 November 2016, New Delhi. The Horticultural Society of India, New Delhi, 81.

Andersch, W. 1992. Production of fungi as crop protection agents. In: *Pflanzenschutz-Nachrichten Bayer*. 45: 129-132.

Anderson, T. E. and Roberts, D. W. 1983. Compatibility of *Beauveria bassiana* isolates with insecticide formulations used in Colorado potato beetle (Coleoptera : Chrysomelidae) control. *J. Econ. Entomol.* 76(2): 1437-1441.

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

Anitha, N. 2000. Bioecology and integrated management of banana pseudostem weevil *Odoiporus longicollis* Oliv. Ph.D. (Ag) thesis, Kerala Agricultural University, Thrissur, 120p.

Areggar, E. 1992. Conidia production of the fungus *Beauveria brongniartii* on barley and quality evaluation during storage at 2^0C. *J. Invertebr. Pathol.* 59:2-10.

Baghel, S., Dewangan, P., Koma, B. and Verma, K. P. 2014. Studies on *Trichoderma viride* formulations for enhancing its storability. *The Bioscan.* 9 (3): 1313-1316.

Bagwan, N. B. 2011. Mass multiplication and shelf-life of *Metarhizium anisopliae* and *Beauveria bassiana* in solid and liquid formulations. *Int. J. Plant Prot.* 4(1): 34-37.

Bailey, A. 2010. Biopesticides: Pest Management and Regulation. CABI International, Wallingford, 232p.

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 mealy bug, *Paracoccus marginatus* infesting cotton. *Indian J. Plant Prot.* 40(3): 182-186.

Batta, Y. A. 2004. Control of rice weevil (*Sitophilus oryzae* L., Coleoptera : Curculionidae) with various formulations of *Metarhizium anisopliae. Crop Prot.* 23(2): 103-108.

Beegum, S. M. K. 2005. Management of banana pseudostem weevil *Odoiporus longicollis* Oliv. using entomopathogenic fungi. Ph.D. (Ag) thesis, Kerala Agricultural University, Thrissur, 95 p.

Bextine, B. R. and Thorvilson, H. G. 2002. Field applications of bait-formulated *Beauveria bassiana* alginate pellets for biological control of the red imported fire ant (Hymenoptera : Formicidae). *Environmental Entomol.* 31(4): 746-752.

Blachere, H., Calvez, J. and Ferron, P. 1973. Etude de la formulation et de la conservation d'une preparation entomopathogene a base de blastospores de *Beauveria tenella* (Delacr. Siemaszko). *Ann. Zool. Ecol. Anim.* (Spanish) 5: 69-79.

Blanford, S., Jenkins, N. E., Christian, R., Chan, B. H. K., Nardini, L., Osae, M., Koekemoer. L., Coetzee, M., Read, A. F., and Thomas, M. B. 2012. *Malaria J.* 11(354): 1 – 14.

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.

Busoli, A. C., Fernandes, D. A. and Tayra, D. 1989. Control of banana weevil borer *Cosmopolites sordidus* by the fungi *Beauveria bassiana* and *Metarhizium anisopliae*. Anais – da – sociedade entomologica – do- Brazil (Spanish) 18: 33-41.

Callaghan, M. O., Gerard, E. M., Johnson, V. W., Townsend, R. J. and Jackson, T. A. 2002. Release of *Serratia entomophila* from prill formulations is affected by soil moisture. *Pasture Entomol.* 55(1): 291-297.

Camargo, M. G., Marciano, A. F., Perinotto, W. M. S., Quinelato, S., Golo, P. S., Angelo, I. C., Prata, M, C, A. and Bittencourt, V. R. E. P. 2014. Commercial formulation of *Metarhizium anisopliae* for the control of *Rhipicephalus microplus* in a pen study. *Vet. Parasitol.* 271–276.

Charan, M. 2015. What are the recent developments in capsules, tablets and tablet coating. http://Pharma info. net. [16 May 2017].

Chelvi, C. T., Thilagaraj, W. R. and Nalini, R. 2011. Field efficacy Of formulations of microbial insecticide *Metarhizium anisopliae* (Hyphocreales: Clavicipitaceae) for the control of sugarcane white grub *Holotrichia serrata* F. (Coleoptera : Scarabidae). *J. Biopestic.* 4 (2): 186-189.

Chiuo, W. C. and Hou, R. F. 1993. Infection of the Asian corn borer, *Ostrinia furnacalis* Guenee (Lep., Pyralidae), with entomopathogens under screen house conditions. 97(2): 151-156.

Daoust, R. A. and Roberts, D. W. 1983. Effect of formulation on the viability of *Metarhizium anisopliae* conidia. *J. Invertebr. Pathol.* 41(2): 151-160.

Das, P., Hazarika, L. K., Puzari, K. C. and Kalita, S. 2013. Influence of storage conditions on viability and infectivity of talc based WP formulation of *Beauveria bassiana* against rice hispa, *Dicladispa armigera* (Olivier). *J. Biol. Control.* 21(3): 229–233.

de Faria, M. R. and Wraight, S. P. 2007. Mycoinsecticides and Mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. *Biol. Control.* 97(2): 237 – 256.

de Medeiros, F. P. M., Santos, M. A. V. M., Regis, L., Rios, E. M. M. and Neto, P. J. R. 2005. Development of a *Bacillus sphaericus* tablet formulation and its evaluation as a larvicide in the biological control of *Culex quinquifasciatus*. *Mem. Inst. Oswaldo Cruz.* 100(4).

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

Devi, P. S. and Hari, P. P. 2009. *Beauveria bassiana* suspension concentrate – A mycoinsecticide for the management of *Helicoverpa armigera* (Hübner). *J. Biol. Control.* 23(4): 403–408.

Divya, K., Shankarm, M. and Prasad, J. S. 2011. Formulation technology of entomopathogenic nematode for the control of cotton boll worm, *Helicoverpa armigera*. *Biosci. Discovery*, 2(2): 147-80.

Dureja, P. and Palmar, B. S. 2009. Biopesticidal formulation with improved shelf life and the process of preparation. Patent No. 234480. Available: www. freepatentsonline.com. [10 March 2017].

Dutta, P. K. and Dutta, J. 2009. Perspectives for chitosan based antimicrobial films in food applications. *Food Chem.* 114(4): 1173-1182.

Faizal, M. H. 1992. Studies on the entomogenous fungus *Fusarium pallidoroseum* (Cooke) Sacc. associated with cowpea aphid, *Aphis craccivora* Koch M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 85p.

Fancelli, M., Dias, A. B., Junior, I. D., Jesus, S. C., Nascimento, A. S., Silva, S. O., Caldas, R. C. and Ledo, C. A. 2013. *Beauveria bassiana* strains for biological control of *Cosmopolites sordidus* (Germ.) (Coleoptera: Curculionidae) in plantain. *Hindwai Publ. Coop. Biomed. Res. Int.* 12: 7p.

Faria, M., Lopes, R. B., Souza, D. A. and Wraight, S. P. 2015. Conidial vigor vs. viability as predictors of virulence of entomopathogenic fungi. *J. Invertebr. Pathol.* 125(3): 68-75.

Farsi, M. J., Askary, H., Jahromi, T. K. H. and Pakdel, K. A. 2012. Bioassay of Wettable Powder formulation of *Lecanicillium muscarium* blastospore on *Aphis gossypii*. *Appl. Entomol. & Phytopathol.* 80(1): 51-60.

Gailce, L. J., Leelamathi, C. M., and Nirmal, J. 2008. Bionomics and management of the pseudostem weevil *Odoiporus longicollis* (Coleoptera: Curculionidae) in banana a review. *Agric. Rev.* 29 (3): 185 – 192.

Gasic and Tanovic, 2013. Biopesticide formulations, possibility of application and future trends. *Pestic. Phytomed.* 28(2): 97-102.

Godonou, I., Green, K. A., Lomer, C. J. and Afreh-Nuamah, K. 2000. Field evaluation of selected formulations of *Beauveria bassiana* for the management of the banana weevil (*Cosmopolites sordidus*) on plantain (*Musa* spp., AAB Group). *Biocontrol Sci. & Technol.* 10(6): 779-788.

Gomez, A. A. and Gomez, A. K. 1984. Statistical Procedure for Agricultural Research. An International Rice Research Institute book, New York.

Herlinda, S. 2010. Spore density and viability of entomopathogenic fungal isolates from Indonesia, and their virulence against *Aphis gossypii* Glover (Homoptera : Aphididae). *Trop. Life Sci. Res.* 21(1): 11–19.

Hernandez-Velazquez, V. M., Hunter, D. M., Barrientos-lozano, L., Lezama-Gutierrez, R. and Reyes-Villanueva, F. R. 2003. Susceptibility of *Schistocerca piceifrons* (Orthoptera: Acrididae) to *Metarhizium anisopliae* var. *acridum* (Deuteromycotina : Hyphomycetes) : laboratory and field trials. *J. Orthoptera Res.* 12(1): 89-92.

Hiltpold, I., Hibbard, B. E.,French, B. W. and Turlings, T. C. J. 2012. Capsules containing entomopathogenic nematodes as a Trojan horse approach to control the western corn rootworm. *Plant Soil.* 1-15.

Hussein, H., Adel, M. and Gelbic, I. 2011. Effectiveness of the entomopathogenic nematode *Steinernema feltiae* in agar gel formulations against larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say.) (Coleoptera: Chrysomelidae). *Open Life Sci.* 7(1):77-82.

Immediato, D., Latta, R., Camarda, A., Giangaspero, A., Capelli, G., Figueredo, L. A., Otranto, D. and Cafarchia, C. 2017. Storage of *Beauveria bassiana* Conidia Suspension: A Study Exploring the Potential Effects on Conidial

Viability and Virulence against *Dermanyssus gallinae* De Geer, 1778 Acari: Dermanyssidae. *Ann. Biol. Sci.* 5 (2): 69-76.

Irulandi, S., Aiyanathan, E. A. and Bhuvaneswari, S. B. 2012. Assessment of biopesticides and insecticide against pseudostem weevil *Odoiporus longicollis* Oliver in red banana. *J. Biopest.* 5 (1): 68-71.

Jaronski, S. T. and Jackson, M. A. 2008. Efficacy of *Metarhizium anisopliae* microsclerotial granules. *Biocontrol Sci. & Technol.*, 18(8): 849-863.

Joseph, A. R. 2014. Evaluation of entomopathogenic fungi for the management of coleopteran pests and characterization of pesticide tolerant strains. Ph.D. (Ag) thesis, Kerala Agricultural University, Thrissur, 335 p.

Kaaya, G. P., Reddy, K. V.S., Kokwaro, E. D. and Munyinji, D. M. 1993. Pathogenicity of *Beauveria bassiana*, *Metarizium anisopliae* and *Serratia marcescence* to banana weevil *Cosmopolites sordidus*. *Biocontrol Sci. Technol.* 3(1): 177-187.

KAU (Kerala Agricultural University). 2016. *Package of Practices Recommendations: Crops* (16th Ed.). Kerala Agricultural University, Thrissur, 310 p.

Khan, A. and Gangaprasad, G. 2001. Comparison of the effect of three entomopathogenic fungi in the management of banana bore weevil, *Cosmopolites sordidus* (Germer) (Coleoptera: Curculionidae). *Int. Pest Control.* 43(2): 208-213.

Kim, J. S., and Lee, S. J., Skinner, M., and Parker, B. L. 2014. A novel approach: *Beauveria bassiana* granules applied to nursery soil for management of rice water weevils in paddy fields. *Pest Manag. Sci.* 41(2): 125-127.

Kim, J., Jaffuel, G., and Turlings, T. C. J. 2015. Enhanced alginate capsule properties as a formulation of entomopathogenic nematodes. *Biocontrol.* 60(4): 527-535.

Knudsen, G. R., Johnson, J. B. and Eschen, D. J. 1990. Alginate pellet formulation of *Beauveria bassiana* (Fungi : Hyphomycetes) isolate pathogenic to cereal aphids. *J. Economic Entomol.* 83(6): 2225-2228.

Kung, K.S. 1955. Ecological studies on banana stem borer weevil *Odoiporus longicollis* Oliv. *J. Agric. Taiwan.* 11: 137-160.

Lackey, B. A., Muldoon, A. E. and Jaffee, B. A. 1993. Alginate pellet formulation of *Hirsutella rhossiliensis* for biological control of plant parasitic nematodes. *Biol. Control.* 3(2): 155-160.

Lee, K. Y. and Mooney, D. J. 2012. Alginate: Properties and biomedical applications. *Prog. Polym. Sci.* 37(1): 106-126.

Lewis, A. J. and Papavizas, C. G. 1985. Characteristics of alginate pellets formulated with *Trichoderma* and *Gliocladium* and their effect on the proliferation of fungi in the soil. *Plant Pathol.* 34: 571-577.

Lewis, J. A. and Larkin, R. P. 2008. Extruded granular formulation with biomass of biocontrol *Gliocladium virens* and *Trichoderma* spp. to reduce damping-off of egg plant Caused by *Rhizoctonia solani* and saprophytic growth of the pathogen in soil-less mix. *Biocontrol Sci. & Tecnol.* 7(1): 49-60.

Liu, C. P. and Liu, S. D. 2009. Formulation and characterization of the microencapsulated entomopathogenic fungus *Metarhizium anisopliae* MA 126. *J. Microencapsulation.* 26(5): 377-384.

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

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

Moore, D., Douro-kpindou, O. K., Jenkins, N. E. and Lomer, C. J. 1996. Effects of moisture content and temperature on storage of *Metarhizium flavoviride* conidia. *Biocontrol Sci. & Technol.* 6(1): 51-62.

Nakkeeran, S., Fernando, W.G.D. and Siddiqui, Z.A. 2005. Plant Growth Promoting Rhizobacteria formulations and its scope in commercialization for the management of pests and diseases. *PGPR: Biocontrol & Biofertilization*, Springer, The Netherlands, pp. 257-296.

Nawaz, M., Mabubu, J. I. and Hua, H. 2016. Current status and advancement of biopesticides: microbial and botanical pesticides. *J. Entomol. & Zool. Studies*. 4(2): 241-246.

Nilamudeen, M. 2015. Entomopathogenic fungi for the management of insect pests in rice ecosystem. Ph.D. (Ag) thesis, Kerala Agricultural University, Thrissur, 252 p.

Nithya, P. R. 2015. Improved formulation of *Lecanicillium lecanii* (Zimmermann) Zare and Gams and its evaluation against sucking pests. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 145p.

Nithya, P. R. and Rani, R. O. P. 2017. Evaluation of carrier materials for formulating entomopathogenic fungus *Lecanicillium lecanii* (Zimmermann) Zare and Gams. *J. Biol. Control*. 31(1): 50-55.

Nithya, P. R. and Rani, R. O. P. 2018. Boric acid to combat detrimental effect of UV in *Lecanicillium lecanii* (Zimmermann) Zare and Gams under field

conditions. In: *International Conference on Biological Control and Sustainable management*, 29-31 January 2018, Tamil Nadu, 329-331.

Nugroho, L. and Ibrahim, Y. 2007. Efficacy of laboratory prepared Wettable Powder formulation of entomopathogenous fungi *Beauveria bassiana, Metarhizium anisopliae* and *Paecilomyces fumosoroseus* against *Polyphagotarsonemus latus* (Bank) (Acari: Tarsonemidae) (Broad mite) on *Capsicum annum* (Chilli). *J. Biosci.* 1:1–11.

Oi, D. H., Pereira, R. M., Stimac, J. L. and Wood, L. A. 1994. Field applications of *Beauveria bassiana* for control of the red imported fire ant (Hymenoptera : Formicidae). 87(3): 623-630.

Olson, D. L. and Oetting, R. D. 1999. Compatibility of insect growth regulators and *Beauveria bassiana* (Balsamo) Vuillemin in Controlling Green Peach Aphid (Homoptera: Aphididae) on Greenhouse Chrysanthemums. *J. Entomol. Sci.* 34(3): 286-294.

Ostmark H. E. 1974. Economic insect pests of bananas. *Ann. Rev. Entomol.* 19:161-176.

Padmanabhan, B. 2009. Effect of mass multiplication media on sporulation, field efficacy and shelf life of *Beauveria bassiana* against rhizome weevil. Biological Control. 23(3): 1-4.

Padmanabhan, B. and Sathiamoorthy, S. 2001. *Musa pest fact.* 5: 8-11.

Palma-Gurrero, J., Jansson, H, B., Salinas, J., and Lopez-Lorka, L. V. 2007. Effect of chitosan on hyphal growth and spore germination of plant pathogenic and biocontrol fungi. *J. Appl. Microbiol.* 104: 541-553.

Posada-Florez, F. J. 2008. Production of *Beauveria bassiana* fungal spores on rice to control the coffee berry borer, *Hypothenemus hampei,* in Colombia. *J. Insect Sci.* 8(41): 1-14.

Pereira, R. M. and Roberts, D. W. 1991. Alginate and cornstarch mycelial formulations of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*. *J. Economic Entomol.* 84(6): 1657-1661.

Perrin, B. 2000. Formulation – Improving insecticides through encapsulation. *Pestic. Outlook.* 97(2): 68 -71.

Pimpalgaonkar, R. and Chandel, U. 2014. Efficacy of leaf exudate of *Jatropha curcas* L. on percentage spore germination inhibition of its selected phylloplane and rhizosphere fungi. *Indian J. Sci. Res.* 4 (1): 70-74.

Pina and Brojo, 1996. Enteric coating of hard gelatin capsules. *Inter. J. Pharmaceutics*. 133(1-2): 139-148.

Prabhavathi, M. K. 2012. Studies on the endophytic properties of entomopathogenic fungi, *Beauveria bassiana* (Balsamo) against banana pseudostem weevil, *Odoiporous longicollis* (Oliv.). M.Sc. (Ag) thesis, University of Agricultural Sciences, Bangalore, 112p.

Prithiva, J. N., Ganapathy, N. and Jeyarani, S. 2017. Efficacy of different formulations of *Beauveria bassiana* (Bb 112) against *Bemisia tabaci* on tomato. *J. Entomol. Zool. Studies*. 5(4): 1239-1243.

Qureshi, A. A., Vineela, V. and Devi, P. S. V. 2014. Sodium humate as a promising coating material for microencapsulation of *Beauveria bassiana* conidia. *Drying Technol.* 33(2): 162-168.

Rani, R. O. P. 2001. Production and evaluation of the fungus *Fusarium pallidoroseum* (Cooke) Sacc. as a biopesticide against pea aphid *Aphis craccivora* Koch PhD thesis, Kerala Agricultural University, Thrissur, 65 p.

Ritu, A., Anjali, C., Nidhi, T., Sheetal, P., and Deepak, B. 2012. Biopesticidal Formulation of *Beauveria bassiana* Effective against Larvae of *Helicoverpa armigera*. *J. Biofertil. Biopestic*. 3(3): 1-3.

Rodrigues, I. M. W., Filho, A. B., Giordano, I. B., Denadae, B. E., Fernandes, J. B., and Forim, M. R. 2017. Compatibility of polymers to fungi *Beauveria bassiana* and *Metarhizium anisopliae* and their formulated products stability. *Acta Scientiarum. Agron.* 39(4): 457-464.

Sankar, H. S. S. 2017. Efficacy of chitin enriched formulations of *Lecanicillium* spp against sucking pests of rice, *Oryza sativa* L. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 115p.

Sankar, H. S. S. 2018. Field efficacy of the entomopathogenic fungus *Lecanicillium saksenae* (Kushwaha) Kurihara & Sukarno against rice bug, *Leptocorisa acuta* Thunberg. In: *Proceedings of Innovations and Advancements in Agriculture and Plant Sciences*, 23 May 2018, Perambalur. 85.

Satyasayee, D., Reddy, P. N., Khan, P. A. and Koduru, U. D. 2008. Formulation of entomopathogenic fungus for use as a biopesticide. Patent No. WO 2009093257A2. Available: http://www.worldwide.espanet.com/WO 2009093257A2. html [08 Oct 2016].

Schimazu, M., Kushida, T., Tsuchiva, D. and Mitsubahii, W. 1992. Microbial control of *Monochamus alternates* Hope (Coleoptera – Cerambicidae) by

implanting wheat bran pellets with *Beauveria bassiana* in infected tree trunks. *J. Jpn. For. Soc.*, 74: 325-330.

Schoebitz, M., Lopez, M. D. and Roldan, A. 2013. Bioencapsulation of microbial inoculants for better soil–plant fertilization. A review. *Agron. Sustain. Dev.* 33(1): 751–765.

Seaman, D. (1990). Trends in the formulation of pesticides : An overview. *Pestic. Sci.* 29(4): 437.

Sharififard, M., Mossadegh, M. S., Vazirianzadeh, B. and Latifi, S. M. 2014. Evaluation of Conidia-Dust Formulation of the Entomopathogenic Fungus, *Metarhizium anisopliae* for Biocontrol of the Brown-Banded Cockroach, *Supella longipalpa* F. *J. Microbiol.* 7(6): 1-6.

Shukla, A. 2010. Insect pests of banana with special reference to weevil borers. *Int. J. Plant Prot.* 3(2): 387-393.

Simkova, J. 2009. Influence of different storage conditions on vitality and virulence of *Beauveria bassiana* spores. *J. Agrobiol.* 26(2): 75-81.

Singh, P. C. and Nautiyal, C. S. 2012. A novel method to prepare concentrated conidial biomass formulation of *Trichoderma harzianum* for seed application. *J. Appl. Microbiol.* 113(1): 1442-1450.

Sivakumar, T. 2017. Management of banana pseudostem weevil, *Odoiporus longicollis* (Olivier), using safe chemicals and bio-rational methods. Ph.D. (Ag) thesis, Kerala Agricultural University, Thrissur, 154p.

Soetoppo D. 2004. Efficacy of selected *Beauveria bassiana* (Bals.) Vuill. isolates in combination with a resistant cotton variety (PSB-Ct 9) against the

cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) Ph.D. (Ag) thesis, University of The Philippines Los Banos. 225 p.

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

St. Leger, R. J. and Wang, C. 2010. Genetic engineering of fungal biocontrol agents to achieve greater efficacy against insect pests. *Appl. Microbiol & Biotechnol.* 85(4): 901-7.

Sunitha, V. S. 1997. *Fusarium pallidoroseum* (Cooke) Sacc as a biocontrol agent for the pea aphid *Aphis craccivora* Koch M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 64 p.

Umamaheswari, R., Sivakumar, M. and Subramanian, S. 2005. Survival and infectivity of entomopathogenic nematode in alginate gel formulations against rice meal moth larva, *Corcyra cephalonica*, Stainton. *Nat. Product Radiance*, 5(2): 95-98.

Ummidi, V. R. S. and Vadlamani, P. 2013. Preparation and use of oil formulations of *Beauveria bassiana* and *Metarhizium anisopliae* against *Spodoptera litura* larvae. *Afr. J. Microbial Res.* 81(15): 1638-1644.

Varsha, V. 2017. Entomopathogenic fungi for the management of banana rhizome weevil (*Cosmopolites sordidus* Germer). M.Sc. (Ag) thesis, Kerala Agricultural University, 65p.

Vilardebo, A. 1973. The coefficient of infestation, criterion of evaluation of the degree of attack of the banana plantations by *Cosmopolites sordidus*, the banana black weevil. *Fruits*. 28: 417-426.

Vinayaka, J., Patil, R. R., and Prabhu, S. T. 2018. Field evaluation of EC formulations of *Metarhizium anisopliae* (Meschinikoff) Sorokin and few insecticides against arecanut white grub, *Leucopholis lepidophora,* Blanchard. *J. Entomol. Zool. Studies*. 6(2): 1034-1037.

Wang, B.T. and Yen, D.F. 1972. Studies on the entomogenous fungus, *Metarhizium anisopliae* Sorokin. *Memoirs of the College of Agriculture National Taiwan University.* 13(2): 31-46.

Woods, T.S. 2003. Pesticide formulations in AGR 185. In: *Encyclopedia of agrochemicals*, New York, pp.1-11.

Wraight, S. P., Filotas, M. J. and Sanderson, J. P. 2016. Comparative efficacy of emulsifiable-oil, wettable-powder, and unformulated-powder preparations of *Beauveria bassiana* against the melon aphid *Aphis gossypii. Biocontrol Sci. & Technol.* 26(7): 146-149.

Zhang, A. W., Liv, W. Z., Nong, X. Q., Deng, C. I., Dus, W. L. and Lang, B. 1992. A trial production of wettable powder of *Beauveria bassiana. Chin. J. Biol. Control*. 3: 118-120.

NOVEL BIOFORMULATIONS OF ENTOMOPATHOGENIC FUNGI AND THEIR EFFICACY AGAINST BANANA WEEVILS

by

REMYA S.
(2016-11-001)

Abstract of the thesis
Submitted in partial fulfilment of the
requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695522
KERALA, INDIA

2018

ABSTRACT

The study entitled 'Novel bioformulations of entomopathogenic fungi and their efficacy against banana weevils' was carried out at Dept. of Agrl. Entomology, College of Agriculture, Vellayani during 2016-2018, with the objective to develop novel formulations of entomopathogenic fungi, *Metarhizium anisopliae* (Metch.) Sorokin, *Beauveria bassiana* (Bals.) Vuillemin and to evaluate their efficacy in managing banana weevils. It was intended to develop capsule and gel formulations.

Experiment to standardize a coating material for developing capsules revealed that Hard Gelatin Transparent (HGT), Hard Gelatin Coloured (HGC) and Hydroxy Propyl Methyl Cellulose (HPMC), were equally stable under ambient conditions of storage (26 -33°C and RH 60-80%). On testing their ability to disintegrate under field conditions, it was noted that chitosan filled HGT capsules easily disintegrated at 20% soil moisture after 36 h and after 24 h at 30 % soil moisture. It took 144 h for all capsules to disintegrate in sheath or leaf axil and 24 h in bore holes. Talc and chitosan were superior, in maintaining storage stability as well as degradability.

A trial conducted to determine the moisture content of capsules, revealed that 10 % was the ideal moisture level content of the ingredient, to maintain storage stability as well as viability of the formulation. Viability noticed after three months was 2.29×10^7 cfu mL^{-1} and 2.27×10^7 cfu mL^{-1} in chitosan based capsules of *M. anisopliae* and *B. bassiana* and 2.10×10^7 cfu mL^{-1} and 0.76×10^7 cfu mL^{-1} in talc based capsules. Therefore, capsules were developed with HGT coating, with chitosan / talc as carrier at 10 % moisture content.

Shelf life studies revealed that chitosan was the best carrier material compared to talc in retaining viability of *Metarhizium* capsules, with a mean cfu of 2.51×10^7 mL^{-1} and 1.77×10^7 mL^{-1}. During a period of three months of storage, there was no decline in the number of viable colonies, cfu ranging from

1.72×10^7 mL^{-1} to 2.79×10^7 mL^{-1} . Storage under refrigeration had better retention of viability (2.63×10^7 cfu mL^{-1}) than at room temperature (1.64×10^7 cfu mL^{-1}). In *Beauveria* capsules, the viability did not differ in both chitosan and talc based capsules. Here also, the number of viable colonies did not decline statistically, till three months of storage, with mean cfu values ranging from 1.85 $\times 10^7$ mL^{-1} to 2.36×10^7 mL^{-1}. In general, shelf life of capsules could be extended by two months when chitosan was used as the carrier material. Low temperature storage could also improve the shelf life by two months.

Shelf life studies of gel formulations indicated that both *Metarhizium* and *Beauveria* gels exhibited high viability in chitosan at room temperature and under refrigeration. The mean number of viable colonies observed was 2.39×10^6 cfu mL^{-1} in chitosan based gels of *Metarhizium* and 2.17×10^6 cfu mL^{-1} in *Beauveria* gels. The number of viable colonies of *Metarhizium* and *Beauveria* observed throughout the experimental period did not vary significantly over three months of storage. The mean number of cfu being 2.26×10^7 mL^{-1} on the 15^{th} day and 1.41 x 10^6 mL^{-1} on the 90^{th} day . It was also inferred that chitosan when used for formulating gels could extend the shelf life of both organisms by one month.

Pathogenicity test disclosed the affinity of *M. anisopliae* to rhizome weevil and *B. bassiana* to pseudostem weevil. Pot culture studies to evaluate chitosan and talc based capsules of *B. bassiana* revealed that both the capsules of *Beauveria* were effective as chlorpyriphos 20 EC 0.05% for pseudostem weevil, in prophylactic and curative methods. The damage index (DI) was one each and reduction in pest population was 91.67 % in prophylactic control and 91.67-100 % in curative control. *Metarhizium* capsules reduced the damage caused by rhizome significantly, but the reduction in pest population was only to the tune of 47-55 % in prophylactic and 50- 58 % in curative control. Chitosan based gel of *Metarhizium* tested against rhizome weevil, reduced the damage significantly (DI 46.67 and 30, in prophylactic and curative methods). The pest population was reduced by 61.11 % in prophylactic and 36.11 % in curative methods.

The study could standardize the protocol for capsule and gel formulations of entomopathogenic fungi, retaining the viability and infectivity upto three months of storage. It is concluded that placement of *Beauveria* capsules in leaf axils prophylactically and curatively can effectively control pseudostem weevil and *Metarhizium* capsules and gels placed in the rhizosphere could manage the rhizome weevil moderately.