# BIOEFFICACY OF CAPSULE FORMULATIONS OF *BEAUVERIA* AND *METARHIZIUM* IN MANAGING BANANA WEEVILS

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**(2018-11-015)**

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*by*

**DEVI BALAKRISHNAN**

**(2018-11-015)**

**THESIS**

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**KERALA, INDIA**

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## DECLARATION

I, hereby declare that this thesis entitled **"Bioefficacy of capsule formulations of *Beauveria* and *Metarhizium* in managing 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.

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Date: (2018 -11-015)

# CERTIFICATE

Certified that this thesis entitled **"Bioefficacy of capsule formulations of *Beauveria* and *Metarhizium* in managing banana weevils"** is a record of bonafide research work done independently by Ms. Devi Balakrishnan under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the advisory committee of Ms. Devi Balakrishnan (2018-11-015), a candidate for the degree of **Master of science in Agriculture** with major in Agricultural Entomology, agree that this thesis entitled "Bioefficacy of capsule formulations of *Beauveria* and *Metarhizium* in managing banana weevils" may be submitted by Ms. Devi Balakrishnan, in partial fulfilment of the requirement for the degree.

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Devi Balakrishnan

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| | |
|---|---|
| ˚C | Degree Celcius |
| % | Percentage |
| @ | At the rate of |
| CD | Critical Difference |
| *et al.* | And others |
| Fig. | Figure |
| g | Gram |
| $g^{-1}$ | Per gram |
| h | Hours |
| ha | Hectare |
| HPMC | Hydroxy Propyl Methyl Cellulose |
| KAU | Kerala Agricultural University |
| kg | Kilogram |
| L | Litre |
| $L^{-1}$ | Per litre |
| mL | Millilitre |
| $mL^{-1}$ | Per milliliter |
| NS | Non Significant |
| RBD | Randomised Block Design |
| Sp. or spp | Species (singular or plural) |
| *viz.* | Namely |

# *Introduction*

# 1. INTRODUCTION

Microbial pesticides are safe alternatives to chemical pesticides, research of which gains more attention in the epoch of organic farming. Entomopathogenic fungi have an imperative position among the microbial bioagents, due to the easiness in their mass production as well as their contact mode of action, unlike bacteria and viruses. Lack of suitable formulations with adequate shelf life is the main obstruction which holds them at a lower level in the global pesticide market. The share of biopesticides in world's total pesticide market is only 1.3 per cent (Bailey, 2010). Research on development of improved formulations is one of the prime areas to enhance the adoption of microbial pesticides. Appropriate formulations that can be delivered precisely to the target, would enhance the field performance of many potential entomopathogens. Slow progress in research on formulation technology and delivery mechanisms is one of the major hurdles in this field.

Capsule is a novel formulation encompassing high field efficacy, controlled release of active ingredients, easiness in handling, transport and application. It is a stable formulation wherein the bioagent is encapsulated in coatings and thus protected from extreme environmental conditions such as UV radiation, rain and temperature (Burges and Jones, 1998). Capsules have more residual stability than spray formulations and need less space for storage and transport. Chances of contamination, which is the main hindrance with other formulations such as wettable powders and liquid suspensions can be rectified, as they are encapsulated in a protective covering.

Formulations are to be delivered precisely in the targeted locale, which is more essential in the case of pests that have a concealed habitat, as with the case of borers and internal tissue feeders infesting various crops. This is more important in the case of crops such as banana which have a waxy plant surface that refrain them from absorbing the insecticide that is sprayed on them leading to inadequate adherence, penetration and sufficient contact with the insects. Though the insecticides including biopesticides are effective against the banana weevils, lack of proper delivery systems adversely affects the field performance.

One of the major havoc to banana farmers in terms of economic loss is the incidence of the banana pseudostem weevil, *Odoiporus longicollis* Oliver (Coleoptera: Curculionidae) and the rhizome weevil, *Cosmopolites sordidus* Germer (Coleoptera: Curculionidae). Pseudostem weevil causes 10 to 90 per cent yield loss depending up on the growth stage of the crop and management efficiency (Padmanaban and Sathiamoorthy, 2001), whereas rhizome weevil causes an yield loss of 40 to 100 per cent as reported by Akello *et al.* (2008). It is the concealed nature of these pests that deters conventional plant protection methods from reaching them.

*O.longicollis* deposits their egg in the air chambers of pseudostem. Thereafter the emerging grub bores into the inner layers and reaches the central core feeding its, way exhibiting bore hole symptoms. A fungal bioagent can cause infection if it comes in contact with the grubs, preferably the first two instars. Likewise, *C. sordius* oviposits on the peripheral layer of rhizome and the emerging grubs bore into the inner portion causing tunnels within. A fungal biopesticides, should precisely target the rhizome so that it can cause infection in the larval stage of the pest. Capsule is the ideal formulation that can deliver the pathogen into the leaf sheath, leaf axil, bore holes, soil or even into the rhizome targeting these pests.

Reports across the globe reveal the efficacy of the entompathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin in managing the banana weevils (Filho *et al.*(1991) ; Nakinga and Moore (2000) ; Beegum and Anitha, (2006); Irulandi *et al.*, (2012); Remya and Reji (2020).

The present study entitled "Bioefficacy of capsule formulations of *Beauveria* and *Metarhizium* in managing banana weevils" was undertaken with the following objectives

- Standardisation of dosage and method of application of biocapsules for prophylactic treatment of banana weevils
- Standardization of dose of *Beauveria* capsules for curative treatment of pseudostem weevil
- Standardization of dosage of *Metarhizium* capsules for curative treatment of rhizome weevil

# *Review of Literature*

## 2. REVIEW OF LITERATURE

Microbial biopesticides have emerged as green alternatives to chemical pesticides owing to the concern towards safer food. It opens a new avenue towards sustainable agriculture. Report on "Microbial biopesticides market - global industry growth, trends and forecasts, 2017-2022" states that microbials hold a prime share in the global biopesticide market. Safety to human beings and animals and specificity to target organisms are the advantages of microbials (Nawaz *et al.*, 2016). Among the various groups of microorganisms, entomopathogenic fungi exhibit an exceptional port of entry that enables them to act like a contact insecticide, unlike the bacteria and the viruses that need to be ingested.

### 2.1 ENTOMOPATHOGENIC FUNGI IN PEST MANAGEMENT

Entomopathogenic fungi have immense potential as biopesticides owing to their easiness in mass production. Fungi belonging to over 100 genera contribute a key share of commercially exploited microbials (St. Leger and Wang, 2010). There are approximately 1.5 to 5 million species under this group. They mainly belong to the orders Entomophthorales and Hypocreales (formerly called Hyphomycetes) which includes the genera such as *Entomophthora*, *Septobasidium* , *Neozygites* , *Aspergillus* , *Beauveria* , *Fusarium* , *Hirsutella* , *Metarhizium* , *Nomuraea* , *Paecilomyces* , *Lecanicillium* , *Erynia.*, *Furia* , *Pandora*, *Zoophthora* etc. The most commonly used species in pest management comprises *Metarhizum anisopliae* (Metsch.) Sorokin, *Beauveria bassiana* (Balsamo) Vuillemin, *Lecanicillium lecanii* (Zimmermann) Zare and Gams, *Hirsutella thompsonii* (Fisher) and *Isaria fumosorosea* (Wize) Brown and Smith (Perez *et al*., 2014).

### 2.1.1 Efficacy of entomopathogenic fungi and their formulations against banana weevils

Although about 19 insect pests were found associated with banana in India from planting to harvesting (Padmanaban *et al*., 2002), the banana pseudostem weevil, *Odoiporus longicollis* Oliver (Coleoptera: Curculionidae) and rhizome weevil, *Cosmopolites sordidus* Germer (Coleoptera: Curculionidae) has been recognized as its major key pests.

#### *2.1.1.1 Pseudostem weevil*

Pseudostem weevil is a monophagous pest of banana and plantains limiting the production and productivity, posing serious threat to banana production (Visalakshi *et al*., 1989 Valmayor *et al*., 1994). Anitha (2000) conducted a survey regarding the severity of pseudostem weevil in southern districts of Kerala and reported that the highest incidence occurred in Thiruvananthapuram district and variety Nendran was the most susceptible one. The severity of the loss was greater when infestation occurred at the early vegetative stage (five months old).

*B. bassiana* was reported to be effective in the management of pseudostem weevil in banana (Kung, 1955). Infectivity of *M. anisopliae* to *O.longicollis* was reported by Wang and Yen (1972). They reported that the mortality of weevils varied with the method of application of the microbial agent.

Anitha (2000) reported that *M. anisopliae* at a spore concentration of $15 \times 10^{5}$ spores $mL^{-1}$ caused mortality of adults from seventh day onwards reaching up to 96.01 per cent on the $14^{th}$ day after treatment (DAT). The study also revealed that the application of spore suspension ($15 \times 10^{5}$ spores $mL^{-1}$) @ 1 L plant $^{-1}$ as leaf axil filling as soon as the oviposition punctures appeared resulted in 74.41 per cent mortality of the grubs. Fungi such as *Fusarium solani* (Mart.) Sacc. and *Mucor heimalis* f. sp. *heimalis* Wehmer were also reported to be infective to pseudostem weevil (Padamanaban, 2001).

In a field experiment conducted by Beegum and Anitha (2006), it was observed that prophylactic treatment by leaf axil filling of spore suspension of *B. bassiana* @ 1.8 × $10^{7}$ spores $mL^{-1}$ gave 52.42 per cent mortality of grubs which was more or less equal to leaf axil application of chlorpyriphos 20 EC 0.05 % (69.82 per cent). When *M. anisopliae* spore suspension @ 1.6 ×$10^{6}$ spores $mL^{-1}$ was used for leaf axil filling, prophylactically, the mortality noted was only 34.83 per cent. In the case of curative management, they reported less efficacy of *B. bassiana*. Leaf axil filling with spore suspension @ 1.8 × $10^{7}$ spores $mL^{-1}$ (500 mL $plant^{-1}$) caused 46.66 per cent mortality to grubs, which was inferior to the application of chlorpyriphos 20 EC 0.05 % (72.5 per cent).

In a laboratory study, Padmanaban *et al.* (2009) reported that spraying the conidial suspension of *B. bassiana* (isolate 17–6) as well as immersion of weevils in the spore suspension recorded 100 per cent mortality of *O. longicollis* and *C. sordidus* within six days. They also reported that placing split pseudostem trap swabbed with maize and rice chaffy grain formulation of *B. bassiana* (17–6) @ 15 g $trap^{-1}$ in the field seven month after planting resulted in 77.23 per cent mortality in the former and 72.47 per cent in the latter.

Efficacy of *B. bassiana* in managing pseudostem weevil was further reported by Irulandi *et al.* (2012). Wettable powder formulation of the fungus, when applied in pseudostem pieces @ 25 g $trap^{-1}$ and placed in the open field @100 number $ha^{-1}$, caused 75.36 per cent mortality of adults within 96 hours after treatment (HAT).

Prabhavathi (2012) explained the efficacy of *B.bassiana* to cause mortality in pseudostem weevil, through endophytic association with banana plants. In a pot culture study carried out using two month old tissue cultured Cavendish variety, various methods of application were tested like root dipping, rhizome dipping and stem injection with conidial suspension (@ 1.5 × $10^{9}$ conidia $mL^{-1}$ and found that highest colonization was there in rhizome dipping method where there was 73.33 per cent mortality of weevils.

Sivakumar (2017) demonstrated that *Metrahizium majus* (isolate no. CPCRIMKY-1) 2%, when applied in the leaf axils, two months after planting

(MAP) resulted in 80 per cent mortality of the grubs one week after treatment (WAT).

Efficacy of capsule formulations of chitosan based *B. bassiana* against pseudostem weevil was assessed by Remya and Reji (2018) in a pot culture experiment. They reported that prophylactic treatment of plants with these biocapsules resulted in 91.67 per cent reduction in population, whereas for curative treatment it was talc based capsules that exhibited superiority with 100 per cent reduction in population which was equivalent to that of leaf axil filling with chlorpyriphos 20 EC @ 0.05%.

Alegesan *et al*. (2019), reported that *B. bassiana* isolate KH3 (MN165867) can be exploited as a potential biocontrol agent against *O. longicollis* @ 1 $\times 10^{8}$ conidia $mL^{-1}$ resulting in more than 90 per cent mortality within 12-18 DAT.

### *2.1.1.2 Rhizome weevil*

Banana rhizome weevil is an important pest which causes yield reduction when the infestation is continued for a long period (Ostmark, 1974; Gowen, 1995). In established fields, damage can lead to plant loss through premature death, toppling and snapping, delayed maturation and reduced bunch weights (Rukazambuga *et al*., 1998, 2002). In case of severe infestation, rhizome weevil can cause yield loss of 40 to 100 per cent (Akello *et al*., 2008).

*M. anisopliae* and *B. bassiana* were reported to be effective to third instar larvae of *C. sordidus* under laboratory conditions (Busoli *et al.*, 1989). Infectivity of *B. bassiana* to rhizome weevil was demonstrated by Kaaya *et al.* (1993). They reported 98 to 100 per cent mortality of third instar larvae within nine DAT to the dry fungal spores. In a laboratory study carried out by Carballo (1998), it was reported that 15% oil formulation of *B. bassiana* @ 1 $\times 10^{8}$ spores $mL^{-1}$ caused 97 per cent mortality of *C. sordidius.*

Nakinga and Moore (2000) reported that *B. bassiana* formulated in maize @ $2 \times 10^{15}$ conidia ha $^{-1}$ and in soil @ $2 \times 10^{14}$ conidia ha $^{-1}$ were effective than oil formulation, in controlling rhizome weevil, resulting in 60-78 per cent and 50 per cent mortality of weevils respectively within eight WAT. The infectivity of the oil formulation was even lower, reducing the weevil population for the first few weeks after application, but followed by a quick population buildup which might be due to uneven coverage caused by clogging of the sprayer.

In a field experiment conducted by Godonou *et al.* (2000), application of oil palm kernel cake (OPKC) based formulation of conidial powder of *B. bassiana*, isolate IMI330194 ($10^{9}$ g OPKC $^{-1}$) , it was revealed that these were effective against rhizome weevil causing 77 per cent mortality to adults after 28 days of release of weevils.

According to Khan and Gangaprasad (2001), 20 per cent mortality of adults of rhizome weevil was observed in adult dip bioassay using *B. bassiana* @ 4.57 x $10^{7}$ spores mL$^{-1}$, 40 DAT. Another study conducted by Fancelli *et al.* (2013), stated that *B. bassiana* isolate CNPMF 218 was effective against rhizome weevil causing 96 per cent mortality under laboratory conditions, within eight days of inoculation. Akello *et al*. (2008) reported 46 to 86.7 per cent reduction of rhizome weevils in banana plants inoculated with *B. bassiana* strain G 41 by dipping rhizomes in 300 mL of *B. bassiana* suspension (4.57 x $10^{7}$ conidia mL$^{-1}$ ) for 2 h .

In the field evaluation studies of *B. bassiana* and *M. anisopliae* against *C. sordidus,* Joseph (2014) proved that talc based formulation of both these fungi @ 30 g L$^{-1}$ resulted in least population of adults in the soil, 0.0 in the former and 0.63 in the latter. Varsha (2017) recorded that talc based formulation of both *M. anisopliae* and *B. bassiana* were effective in controlling rhizome weevil when applied @ 30 g L$^{-1}$ by soil drenching.

Remya (2018) assessed the bioefficacy of Metarhizium capsules to rhizome weevil under laboratory conditions and found that these can be effectively used against rhizome weevil. She has also reported the efficacy of *M. anisopliae* formulated as gels. Gels made of chitosan placed in the planting pit two weeks after the release of weevil exhibited 61 per cent reduction when applied prophylactically and 36 per cent reduction when applied curatively. In a pot culture experiment conducted by Remya and Reji (2020), prophylactic application of Metarhizium capsules in the planting pit at @ four plant-$^{1}$, was found to cause 47 to 58 per cent reduction in infestation of rhizome weevil, while in curative treatment, there was 50 to 58.33 per cent reduction in infestation.

## 2.2 FORMULATIONS OF ENTOMOPATHOGENIC FUNGI

Formulation is a mixture of an active ingredient with inert or inactive carrier materials. Burges and Jones (1998) stated that bioformulation comprises of the aids to preserve organisms and also the mechanisms to deliver them to their targets. The active ingredient is mostly a viable organism, which can be either a live microbe or spore whose survival during storage is very essential for its successful development (Auld *et al*., 2003). Additives in a formulation protect the organism from harsh environmental conditions (Schliser *et al*., 2004). Of the innumerable number of mycoinsecticides and mycoacaricides registered worldwide, Wettable Powder is the most common among them (de Faria and Wraight, 2007).

### 2.2.1 Types of bioformulations

Currently, biopesticides are formulated mainly in solid carriers like talc, peat, lignite, clay, etc. However, these conventional formulations suffer from major setbacks such as bulkiness in storage, shorter shelf life, high contamination and low field performance.

Bioformulations 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 further 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 $cm^3$). Capsules, microcapsules, gels, prills, pellets, tablets etc. are some of the novel formulations of entomopathogenic fungi. Woods (2003) emphasized the need of development of a microbial formulation that aims at preserving the organism with good physical properties and delivering them to the target area without hampering its pathogenicity.

### *2.2.1.1 Conventional formulations*

The basic conventional formulations are Wettable Powder, Dust, Granule and liquid formulations. These formulations developed and evaluated using *B. bassiana* and *M.anisopliae* are reviewed here under.

#### *2.2.1.1.1 Wettable Powder*

Olson and Oetting (1999) found that application of WP formulation of *B. bassiana* @ $2\times10^{10}$ 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* and *B. bassiana* formulated in clay @ 1:4 with $1\times10^{10}$ 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. Nilamudeen (2015) reported that, application of talc based WP of *B. bassiana* and *M. anisopliae* @ 20 g $L^{-1}$ reduced larval population of larvae of rice leaf roller, *Cnaphalocrocis medinalis* (Guenee) as well as the nymphs and adults of rice bug, *Leptocorisa acuta* Thunberg. Sankar and Reji (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 population of seven and six per five hills to 2.66 and 3.33 per five hills, respectively.

*2.2.1.1.2 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 %, with wheat flour as carrier resulted in 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$^{-1}$) that caused 83.33 per cent mortality of arecanut white grub, *Leucopholis lepidophora*, Blanchard.

*2.2.1.1.3 Granules*

Chiuo and Hou (1993) developed *B. bassiana* granules cultured on wine derivatives mixed with sand. The formulation containing $2\times10^8$ cfu g$^{-1}$ 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 WAT. Kim *et al*. (2014) reported that various isolates of *B. bassiana* produced as solid cultures in millet grains, when applied as granules paddy nurseries, could effectively manage rice water weevil, *Lissorhoptrus oryzophilus* Kuschel effectively.

*2.2.1.1.4 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 where the mortality was 97 per cent compared to that with the conidial suspension (52.5 per cent). Luz and Batagine (2005) developed formulations of *B. bassiana* based on different vegetable oils which was 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) .They opined that water in oil type liquid formulation 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 % 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 % 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.

#### *2.2.1.2 Novel Formulations*

Formulations that can be delivered precisely to the target, would enhance the field performance of many potential entomopathogens. Suitable formulations targeting precise delivery is the need of the hour especially in the case of pests which have a concealed nature of growth and development. Some of the novel bioformulations based on entomopathogenic fungi are reviewed below.

*2.2.1.3 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).

Remya and Reji (2018) standardized the coating material and carrier material for capsule formulations of entomopathogenic fungi. They concluded that gelatin capsules with talc or chitosan as the carrier material is ideal for the preparation of biocapsules of entomopathogenic fungi based on the disintegration studies in plants and soil in relation with moisture, temperature and relative humidity, Hydroxy Propyl Methyl Cellulose (HPMC) coating and chitosan as the carrier is the better combination for controlled release of inoculam.

*2.2.1.4 Microcapsules*

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

*2.2.1.5 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). Remya (2018) prepared gel formulations of *B. bassiana* and *M. anisopliae* using chitosan, gelatin, agar and alginate. Based on the spore germination rate, shelf life of gels and extent of contamination, chitosan based gel was ranked as the superior one. In a pot culture study, she observed that prophylactic application of chitosan based gel of *M. anisopliae* in the planting pit @ 10 g plant$^{-1}$ resulted in 61.11 per cent reduction in *C. sordidus* grubs, whereas only 36.11 per cent reduction was there in curative application.

*2.2.1.6 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).

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). *B. bassiana* alginate pellets coated with peanut oil reduced activity of red imported fire ant *S. invicta* (Bextine and Thorvilson, 2002).

Though novel formulations like prills, briquettes and tablets are available with entomopathogenic bacteria and plant disease control fungus such as *Trichoderma* spp such formulations are yet to be developed in entomopathogenic fungi.

## *Materials and Methods*

# 3. MATERIALS AND METHODS

The current research work on "Bioefficacy of capsule formulations of *Beauveria* and *Metarhizium* in managing banana weevils" was carried out at the Biocontrol laboratory for crop pest Management, Department of Agricultural Entomology, College of Agriculture, Vellayani, Instructional Farm, College of Agriculture, Vellayani and farmers field at Peringamala, Venganoor Panchayath, Thiruvananthapuram, during the year 2018-2020.

## 3.1 MAINTENANCE OF FUNGAL CULTURES

The cultures maintained in the Biocontrol Laboratory, Department of Agricultural Entomology, College of Agriculture, Vellayani, originally sourced from National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru were utilized for the study. The isolate of *Beauveria bassiana* (Balsamo) Vuillemin used was Bb5 and that of *Metarhizium anisopliae* (Metschnikoff) Sorokin was Ma4, whose virulence was maintained by periodically passing it through the host insects the banana pseudostem weevil, *Odoiporus longicollis* Oliver and the rhizome weevil C*osmopolites sordidus* Germer, respectively. Pure and sub cultures of these fungi were maintained in Potato Dextrose Agar (PDA) slants. Mass production was carried out in by static liquid fermentation in Potato Dextrose Broth (PDB), which was used for the preparation of capsules.

## 3.2 PREPARATION OF CAPSULES

Capsules of *B. bassiana* and *M. anisopliae* were prepared using 14 day old cultures grown in PDB. The protocol used was that developed by Remya and Reji, 2018.

### 3.2.1 Mass culturing the fungus

Mass culturing the fungus was done in Potato Dextrose Broth (PDB).

### 3.2.2 Preparation of spore suspension

Fourteen day old sporulating cultures were blended in a mixer by adding a drop of tween 20 and filtered using a double layered muslin cloth (Plate 1a).

### 3.2.3 Preparation of primary powder

Spore suspension after straining was centrifuged in a Rotek centrifuge for 20 min at 4000 rpm to obtain spore pellet (Plate 1d). Using 1 mL of sterile distilled water the spore pellet was washed gently to remove the mycelial mat adhering to it. Primary powder was prepared by mixing spore pellet and chitosan (crude) in the ratio 1:1 to obtain $10^{10}$spores $g^{-1}$.

### 3.2.4 Filling the capsules

Filling material was prepared by mixing the primary powder with chitosan in the ratio 1:20. The empty capsules of Hydroxy Propyl Methyl Cellulose (HPMC) were filled using a hand operated capsule filling device (Plate 2 and 3), which yield 100 capsules in one set.

## 3.3 FIELD EFFICACY OF BIOCAPSULES

Field experiments were conducted to study the efficacy of capsules in prophylactic as well as curative methods of management of banana weevils, along with the standardization of dose as well as method of application of capsules. Three sets of experiments were laid out for this purpose.

Package of Practices recommendations (Organic) of Kerala Agricultural University (KAU, 2017) was followed throughout the crop period, except for plant protection. Periodic removal of older leaves and leaf sheaths were followed as a cultural control measure in all the treatment as well as control plants.

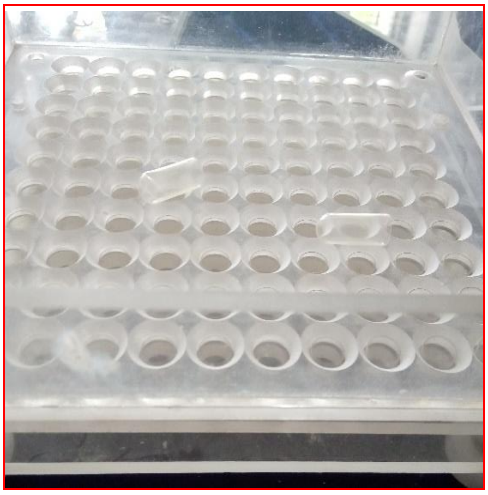
a. Straining the blended culture

b. Spore suspension in centrifuge bottles

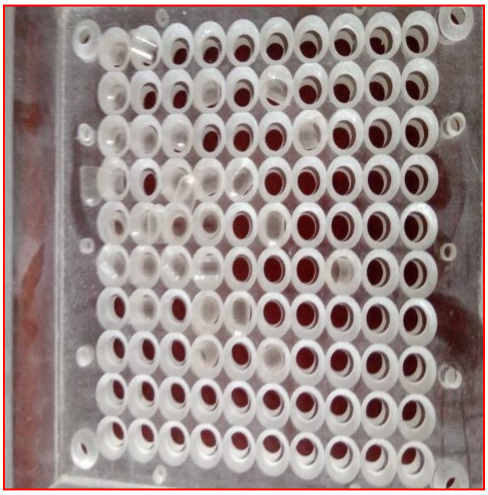
c. Centrifugation at 4000 rpm, 20 min

d. Spore pellet at the bottom of the can

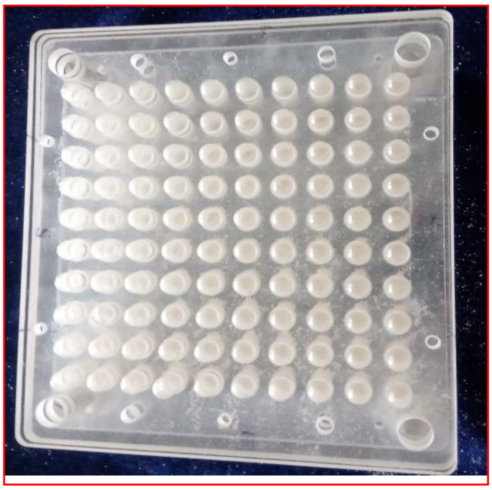
Plate 1. Preparation of spore pellet



a. Cuboidal tray (lower portion)



b. Cuboidal tray (upper portion)



c. Upper and lower trays pressed to lock the capsules

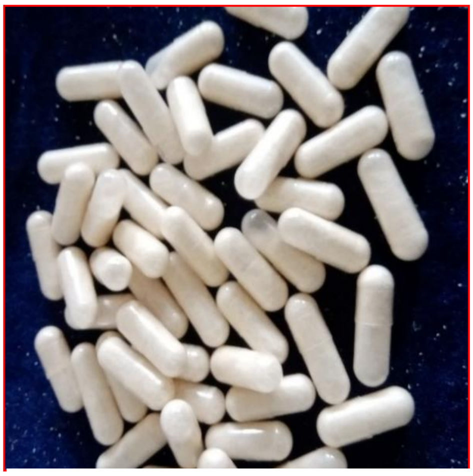
d. Placement of powder guard

Plate 2. Capsule filling trays

a. Spreading the filler material

b. Assemblage of filling trays

c. Formation of capsules inside the tray



d. Capsules ready for packing

Plate 3. Filling the empty capsules

### 3.3.1 Standardization of dose and method of application of biocapsules for prophylactic treatment of banana weevils

The experiment was carried out in the Instructional Farm Vellayani during 2019-20 (Plate 4a). Banana suckers of variety Nendran procured from Instructional farm, College of Agriculture, Vellayani were used for the experiment.

For prophylactic treatment, same set of plants were used for both the weevils. For pseudostem weevil treatments were initiated from the fourth month of planting using Beauveria capsules, whereas that for the rhizome weevil Metarhizium capsules were tested which was initiated from the planting day onwards. Beauveria capsules were placed in the leaf sheath at a height of 1 to 1.5m from the base of the plant, where more number of ovipostional punctures are expected.

The experimental plot was laid out in RBD with six treatments and four replications. The treatments were as follows

T1- Four Beauveria capsules one each in outer four leaf axils at monthly intervals starting from fourth month till bunch emergence + Four Metarhizium capsules one each on four sides in the planting pits at monthly intervals from planting to bunch emergence .

T2 - Four Beauveria capsules placed one each in outer four leaf axils at bimonthly intervals starting from fourth month till bunch emergence + Four Metarhizium capsules placed one each on four sides in the planting pits at bimonthly intervals, from planting to bunch emergence

T3- Four Beauveria capsules placed inside the outer leaf sheath, starting from fourth month till bunch emergence at monthly intervals + Plugging the rhizome with four Metarhizium capsules at paring, followed by pit application at monthly intervals.

T4 - Four Beauveria capsules placed inside the outer leaf sheath, starting from fourth month till bunch emergence at bimonthly intervals + Plugging the rhizome with four Metarhizium capsules at paring followed by pit application at bimonthly intervals.

T5 - Chemical check - chlorpyriphos 20 EC @ 150 g a.i $ha^{-1}$

T6 - Untreated check

#### ***3.3.1.1 Method of application of capsules for pseudostem weevil***

Capsules of *B. bassiana* and *M. anisopliae* prepared as described in Para 3.2 were used in the experiment. Methods of application of capsules were as described below.

##### *3.3.1.1.1 Leaf axil application*

Beauveria capsules were placed one each in the outer four leaf axils (Plate 5a) selected at North, South, East and Western side of the plant and a total of three applications was done in the case of monthly treatments and two applications in the case of alternate months.

##### *3.3.1.1.2 Leaf sheath application*

The capsules were inserted in between the outermost two leaf sheaths (Plate 5b) at a height of 30 cm below the leaf axils, with one each on the four sides of the plant. A total of three applications were done in the case of monthly treatment and two applications in case of bimonthly applications.

##### *3.3.1.1.3. Leaf axil filling of Chlorpyriphos*

In the treated control plants, leaf axil filling of chlorpyriphos 20 EC @ 2mL $L^{-1}$ was carried out, by dripping the spray fluid into the axils using an adjusted nozzle

4a. Experiment for prophylactic treatment


4b. Experiment for curative treatment

Plate 4. Layout of experimental plots

of a knapsack sprayer. Only one application was done for chemical. The quantity of insecticide solution administered in one plant was 200 mL.

***3.3.1.2 Method of application of capsules for rhizome weevil***

*3.3.1.2.1 Plugging rhizomes*

Healthy rhizomes of sword suckers were subjected to the usual paring process during which Metarhizium capsules were plugged into the precise holes made with a sterile cork borer on four sides of the parred rhizomes (Plate 6), to ensure proper contact with the target insect.

*3.3.1.2.2. Pit application*

Metarhizium capsules were placed in the rhizosphere, one each on all the four sides of the rhizome at the time of planting and thereafter on the four sides of the pit at intervals mentioned in the treatments (Plate 5c).

*3.3.1.2.3. Leaf axil filling of chlorpyriphos*

Leaf axil filling was carried out as mentioned in 3.3.1.1.3

***3.3.1.3 Observations***

*3.3.1.3.1 Pseudostem weevil*

To assess the incidence of pseudostem weevil, number of bore holes was observed during the period of study at fortnightly intervals, starting from two weeks after treatment application. Number of grubs, pupae and adults present in the pseudostem was recorded at the time of destructive sampling done at seven months after planting (MAP)

*3.3.1.3.2. Rhizome weevil*

In order to assess the effect of Metarhizium capsules on rhizome weevil infestation, destructive sampling was done at seven MAP. For this plants were uprooted, cut horizontally and counted the number of tunnels, adults, grubs and pupae

*3.3.1.3.3 Incidence of other pests and diseases*

Incidence of foliage and sap feeding insects and diseases were observed throughout the crop period and recorded.

**3.3.2 Standardization of dosage of Beauveria capsules for curative treatment of pseudostem weevil**

The capsules were tested under field conditions for their curative efficacy in managing pseudostem weevil. A farmer’s plot was selected at Peringamala, Venganoor panchayth Thiruvananthapuram district for the experiment. The selected field and nearby area were highly infested by the weevil. Naturally infested plants of uniform intensity were selected and tagged for the experiment (Plate 4b). The experiment was laid out in RBD with four treatments and four replications, with two plants per replication. Periodic removal of older leaves and leaf sheaths were followed as a cultural control measure in all plants in order to reduce the infestation.

The capsules were placed in the leaf axils and bore holes at varying intervals mentioned below starting from fifth month till bunch emergence. The following were the treatment details

T1 - Bb capsules @ one each into bore holes and outermost four leaf axils of pseudostem, at weekly intervals.

a. Leaf axil application

b. Leaf sheath application

c. Pit application

Plate 5. Method of application of capsules

a. Holes made using a sterile cork borer

b. Insertion of capsules into hole

c. Rhizomes plugged with capsules

Plate 6. Plugging of rhizomes with capsules

T2 - Bb capsules @ one each into bore holes and outermost four leaf axils of pseudostem, at fortnightly intervals.

T3 - Chemical check - chlorpyriphos 20 EC @ 150g a.i $ha^{-1}$

T4 - Untreated check

#### *3.3.2.1 Leaf axil treatment*

Beauveria capsules were applied as mentioned in 3.3.1.1.1.

#### *3.3.2.2 Bore hole application*

Treatments were given to naturally infested plants. Plants with ooze out or oviposition punctures on the pseudostem were treated. Capsules were inserted into the area showing the symptom at intervals mentioned in the treatments.

#### *3.3.2.3. Leaf axil treatment with chlorpyriphos*

Treatment was carried out as explained in 3.3.1.1.3.

#### *3.3.2.4 Observations*

Treated plants were observed for presence of oviposition punctures or bore holes at fortnightly intervals. Gelly like ooze was also counted as a bore hole. Number of grubs, pupae and adults were counted at the time of harvest.

### 3.3.3 Standardization of dosage of Metarhizium capsules for curative treatment of rhizome weevil

A farmer’s plot was selected at Peringamala, Venganoor Panchayath, Thiruvananthapuram district for the experiment. The selected field and nearby area was endemic to rhizome weevil. Metarhizium capsules were placed in the planting pit one each on four sides at periodic intervals mentioned as in the treatments listed below. The experiment was laid out in RBD with five treatments and three replications, with two plants per replication.

Treatments

T1 - Four Ma capsules in the planting pit at fortnightly interval.

T2 - Four Ma capsules in the planting pit at monthly intervals

T3 - Four Ma capsules in the planting pit at bimonthly intervals

T4 - Fipronil 0.3 G @ 75 g a.i $ha^{-1}$

T5 - Untreated check

***3.3.3.1 Method of application of capsules***

Pit application of Metarhizium capsules were applied from fourth month of planting on four sides of the pit at intervals mentioned in the treatments.

***3.3.3.2. Method of application of Fipronil***

Fipronil 0.3 G was applied to planting pits @ 10 g $plant^{-1}$. Single application has been done in the experiment.

*3.3.3.3. Observations*

Number of tunnels and population of the pests were assessed at the time of harvest by uprooting the plants and splitting open the rhizomes.

3.4 STATISTICAL ANALYSIS

The data obtained from field experiments were subjected to analysis of variance (ANOVA) using WASP 1 (Web Assisted Statistical Package) software.

# *Results*

# 4. RESULTS

The results of the work entitled "Bioefficacy of capsule formulations of *Beauveria* and *Metarhizium* in managing banana weevils" carried out during 2018-2020 in the Department of Agricultural Entomology, College of Agriculture, Vellayani is presented below. The work was conducted as three main experiments *viz.*, standardization of dosage and method of application of capsules for prophylactic treatment of banana weevils, standardization of dosage of Beauveria capsules for curative treatment of pseudostem weevil and Standardization of dosage of Metarhizium capsules for curative treatment of rhizome weevil.

## 4.1 STANDARDIZATION OF DOSAGE AND METHOD OF APPLICATION OF BIOCAPSULES FOR PROPHYLACTIC TREATMENT OF BANANA WEEVILS

Method of application of capsules and the total number of capsules needed plant $^{-1}$ for prophylactic treatment was assessed by applying the treatments on the fourth month after planting (MAP). Four different methods of application were tested. The two methods targeted against pseudostem weevil were leaf axil application and leaf sheath application, while those targeted for rhizome weevil were pit application and plugging the rhizome. For testing the prophylactic effect, the treatments for both the weevils were applied in the same set of plants.

### 4.1.1 Efficacy of prophylactic treatment of Beauveria capsules on incidence of pseudostem weevils

Efficacy of the capsules was assessed based on the presence of bore holes made by the weevil on the pseudostem as well as the population of various stages of the pest after prophylactic treatment, which was carried out from the fourth month of planting till bunch emergence.

Table 1. Effect of prophylactic application of biocapsules in the management of banana weevils

| Treatments | Total no. of capsules plant$^{-1}$ | *Mean no. of bore holes observed after treatment application | | | | |
|---|---|---|---|---|---|---|
| | | 4 MAP | | 5 MAP | | 6 MAP |
| | | 1st fortnight (precount) | 2nd fortnight | 1st fortnight | 2nd fortnight | 1st fortnight |
| Leaf axil application of Bb + Pit application of Ma at (monthly intervals) | 12 Bb + 24 Ma | 0 | 0 (0.70) | 0 (0.70) | 0 (0.70) | 0.5(0.96)$^{cd}$ |
| Leaf axil application of Bb + Pit application of Ma at (bi monthly intervals) | 8 Bb + 12 Ma | `0 | 0 (0.70) | 0.25(0.83) | 0.75(1.05) | 1.5(1.40)$^{b}$ |
| Leaf sheath application of Bb at monthly intervals + Plugging rhizomes at paring (Followed by monthly application in the pit) | 12 Bb + 28 Ma | 0 | 0 (0.70) | 0 (0.70) | 0 (0.70) | 0 (0.70)$^{d}$ |
| Leaf sheath application of Bb at bimonthly intervals + Plugging rhizomes at paring (Followed by bimonthly application in the pit) | 8 Bb + 16 Ma | 0 | 0 (0.70) | 0 (0.70) | 0.25(0.83) | 0.75(1.05)$^{bcd}$ |
| Chlorpyriphos 20 EC (2mL L$^{-1}$) (Leaf axil filling) | | 0 | 0 (0.70) | 0 (0.70) | 0.25(0.83) | 1.0 (1.18)$^{bc}$ |
| Untreated control | | 0 | 0.25(0.83 ) | 1.0 (1.18) | 1.75(1.40) | 3.25 (1.89)$^{a}$ |
| CD (0.05) | | NA | NS | NS | NS | (0.413) |

Bb- *Beauveria bassiana* capsules @ 4 plant $^{-1}$ Ma – *Metarhizium anisopliae* capsules @ 4 plant $^{-1}$ NA- Not Analysed
NS – Not Significant MAP-Months After Planting *Mean of four replications.Values in the parentheses are square root transformed

#### *4.1.1.1 Presence of bore holes after prophylactic treatment*

Incidence of the pest was assessed based on the number of bore holes that appeared on the pseudostem, noted at fortnightly intervals, the data of which are presented in Table 1

During the fourth month after planting (MAP), *i.e.* two weeks after prophylactic treatment, none of the plants exhibited the symptom except the untreated plants. On the fifth month (first fortnight after treatment), the second method of application T2 *i.e* leaf axil + pit application at bimonthly intervals exhibited symptoms with 0.25 number of bore holes, while all the plants in other methods appeared symptom free. During the second fortnight of fifth month T2 (0.75 bore holes) and T4 (0.25 bore holes) exhibited symptoms. T1 (leaf axil + pit application at monthly intervals) and T3 (leaf sheath application + plugging the rhizome followed by pit application at monthly intervals) were free from symptoms of infestation.

Significant variation among the treatments was observed during the second fortnight of sixth month. T3 exhibited superiority with no symptoms. T1 ranked second with a mean number of 0.5 bore hole and T4 the third, with 0.75 bore hole. Plants treated with chemical check chlorpyriphos 20 EC 0.05 % had 1.00 bore hole. T2 was inferior among treatments with a mean of 1.5 bore holes. The highest number of bore holes was noted in the untreated plants (3.25).

While comparing the number of capsules applied plant$^{-1}$ and the damage symptom it is evident that plants in which 12 capsules were placed exhibited less symptom than those applied with eight capsules, as the number of bore holes observed varied significantly among these (0 and 0.75 respectively). Monthly application of capsules either in the leaf axils or in between leaf sheaths had statistically similar effects.

Table 2. Effect of prophylactic application of biocapsules on the population of pseudostem weevil

| Treatments | Total no. of capsules plant $^{-1}$ | *Mean population assessed at destructive sampling (7 MAP) | | |
|---|---|---|---|---|
| | | No. of grubs | No. of pupae | No. of adults |
| Leaf axil application of Bb + Pit application of Ma at (monthly intervals) | 12 Bb + 24 Ma | 0.75(1.11)$^{cd}$ | 0.25(0.85) | 0.75(1.11)$^{c}$ |
| Leaf axil application of Bb + Pit application of Ma at ( bi monthly intervals) | 8 Bb + 12 Ma | 1.75(1.49)$^{ab}$ | 1 (1.20) | 2 (1.58)$^{b}$ |
| Leaf sheath application of Bb at monthly intervals + Plugging rhizomes at paring (Followed by monthly application in the pit) | 12 Bb + 28 Ma | 0.25(0.85)$^{d}$ | 0.25(0.85) | 0.25(0.85)$^{d}$ |
| Leaf sheath application of Bb at bimonthly intervals + Plugging rhizomes at paring (Followed by bimonthly application in the pit) | 8 Bb + 16 Ma | 1.25(1.31)$^{bc}$ | 1 (1.20) | 1.75(1.49)$^{b}$ |
| Chlorpyriphos 20 EC (2mL L$^{-1}$) (Leaf axil filling) | - | 0.50 (1)$^{cd}$ | 0.75(1.11) | 0.75(1.11)$^{c}$ |
| Untreated control | - | 2.75(1.80)$^{a}$ | 1.50(1.41) | 2.75(1.80)$^{a}$ |
| CD (0.05) | | (0.332) | NS | (0.183) |

Bb- *Beauveria bassiana* capsules @ 4 plant $^{-1}$ Ma – *Metarhizium anisopliae* capsules @ 4 plant $^{-1}$

NS – Not Significant MAP – Months After Planting

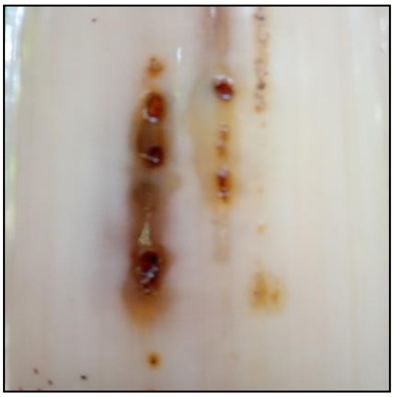
* Mean of four replications Values in the parentheses are square root transformed

a. Leaf axil + pit application @ monthly intervals

b. Leaf axil + pit application @ bimonthly intervals

c. Leaf sheath + plugging rhizome + pit application @ monthly intervals

d. Leaf sheath + plugging rhizome + pit application @ bimonthly intervals



e. Leaf axil filling with chlorpyriphos

f. Untreated check

Plate 7. Bore holes observed after treatment application in prophylactic treatment of pseudostem weevil

### 4.1.2 Efficacy of prophylactic treatment of Metarhizium capsules on incidence of rhizome weevil

Efficacy of the capsules was assessed based on the presence of tunnels made by the weevil on the rhizomes as well as the population of various stages of the pest after prophylactic treatment that was carried out before and after planting.

#### *4.1.2.1 Presence of tunnels in the rhizome at destructive sampling (seven MAP)*

Number of tunnels formed in the rhizome at the time of destructive sampling is presented in Table 3.

Plants treated with capsules as per the third method (T3 - leaf sheath application + plugging the rhizomes at paring + pit application at monthly intervals) indicated its superiority over the other treatments as there were no tunneling in the rhizomes when cut open. T4 (leaf sheath application + plugging the rhizomes at paring + pit application at bimonthly intervals) ranked second, with 0.50 number of tunnels. T1 (leaf axil application + pit application at monthly intervals) ranked third with mean number of 1.0 tunnel per rhizome, which was on par with that observed in plants treated with chlorpyriphos 20 EC 0.05% (0.75). In T2 the number of tunnels observed was 1.25, whereas it was highest in the untreated plants (2.0).

#### *4.1.2.2. Population of rhizome weevils at destructive sampling (seven MAP)*

Effect of biocapsules on the population of rhizome weevil is presented in Table 3. Based on the population of grubs, T3 was found to be the best treatment as the rhizomes of these plants did not show the presence of any grubs. This was followed by T1 and T4 with a mean number of 0.5 grubs each. T2 with mean number of 1.0 grubs rhizome$^{-1}$ which ranked third was inferior to chlorpyriphos 20 EC treatment 0.05% (0.75). Highest number of grubs was noted in untreated plants (2.25).

Table 3. Efficacy of prophylactic application of biocapsules on population of rhizome weevil at seven months after planting

| Treatments | Total no. of capsules plant $^{-1}$ | *Mean no. of tunnels | *Mean population per plant at destructive sampling | |
|---|---|---|---|---|
| | | | No. of grubs | No. of adults |
| Leaf axil application of Bb + Pit application of Ma at (monthly intervals) | 12 Bb + 24 Ma | 1(1.18)$^{bc}$ | 0.50(0.96)$^{bc}$ | 1(1.18)$^{abc}$ |
| Leaf axil application of Bb + Pit application of Ma at ( bi monthly intervals) | 8 Bb + 12 Ma | 1.25(1.31)$^{b}$ | 1 (1.22)$^{b}$ | 1.25(1.31)$^{ab}$ |
| Leaf sheath application of Bb at monthly intervals + Plugging rhizomes at paring (Followed by monthly application in the pit) | 12 Bb + 28 Ma | 0(0.70)$^{d}$ | 0(0.70)$^{c}$ | 0(0.70)$^{c}$ |
| Leaf sheath application of Bb at bimonthly intervals + Plugging rhizomes at paring (Followed by bimonthly application in the pit) | 8 Bb + 16 Ma | 0.5(0.96)$^{cd}$ | 0.50(0.96)$^{bc}$ | 0.75(1.09)$^{bc}$ |
| Chlorpyriphos 20 EC (2mL L-$^{1}$) (Leaf axil filling) | | 0.75(1.09)$^{bc}$ | 0.75(1.09)$^{b}$ | 0.75(1.09)$^{bc}$ |
| Untreated control | | 2(1.72)$^{a}$ | 2.25(1.65)$^{a}$ | 2.5 (1.64)$^{a}$ |
| CD (0.05) | | (0.207) | (0.302) | (0.534) |

Bb- *Beauveria bassiana* capsules @ 4 plant $^{-1}$ Ma – *Metarhizium anisopliae* capsules @ 4 plant.$^{-1}$ NA- Not Analysed

NS – Not Significant MAP- Months After Planting *Mean of 4 replications Values in the parentheses are square root transformed

a. Leaf axil + pit application @ monthly intervals

b. Leaf axil + pit application @ bimonthly intervals

c. Leaf sheath + plugging rhizome + pit application @ monthly intervals

d. Leaf sheath + plugging rhizome + pit application @ bimonthly intervals

e. Leaf axil filling with chlorpyriphos

f. Untreated check

Plate 8. Tunnels observed in prophylactic treatment of rhizome weevil at the time of destructive sampling

Considering the population of adults also T3 was superior as the rhizomes were free from weevils. Plants treated with the third method (T3) were free from adult weevils when split open. T4 was the next superior method of application which was in parity with the chemical check (0.75 adults). T1 ranked third with an average of 1.00 weevil plant$^{-1}$ followed by T2 (1.25). Untreated plants recorded highest number of weevils (2.5).

Percentage reduction in the population of grubs and adults is indicated in Fig. 2. There was 100 per cent reduction in the population of grubs and adults in T3. T4 was the next superior treatment with reduction to the tune of 77.77 and 70 per cent. T1 and chemical treatment was observed with a reduction of 77.77, 60 and 66.66 and 70 respectively.

While comparing the number of capsules applied plant$^{-1}$ and the damage symptom, it is evident that plants in which rhizomes were plugged at paring followed by pit application at monthly intervals with a total of 28 capsules plant$^{-1}$, exhibited less symptom than those applied with 16 capsules applied in the same way at bimonthly intervals. The number of tunnels and population observed varied significantly among these (0 and 0.50 respectively).

#### 4.1.3. Incidence of other pests and diseases

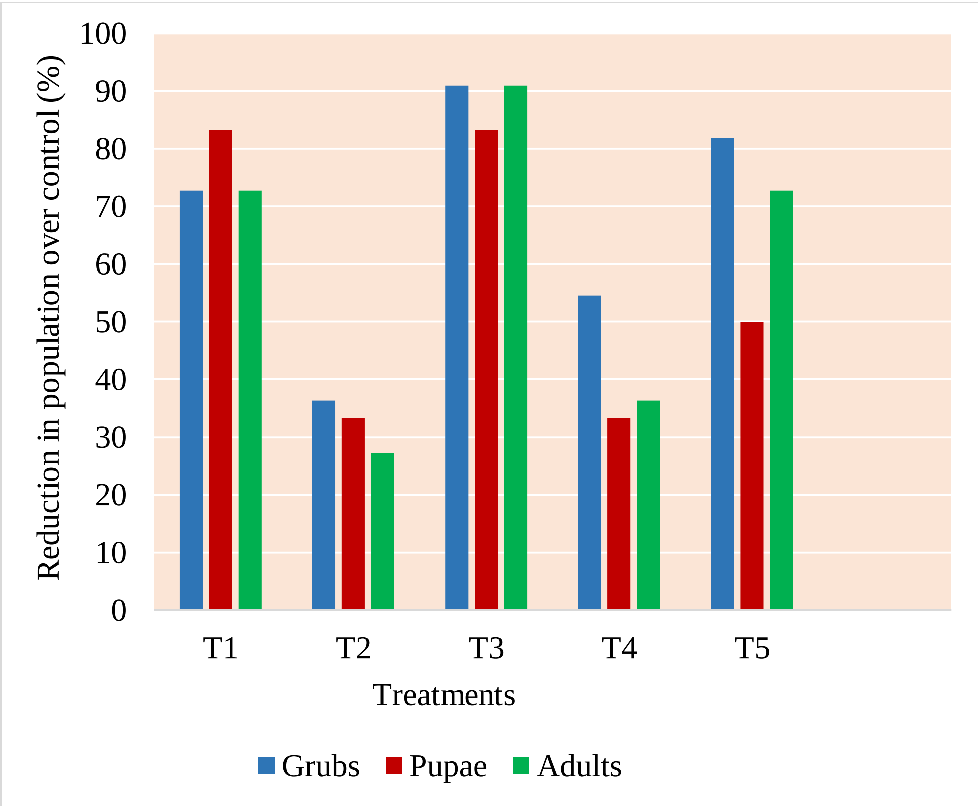
During the experimental period low incidence of defoliators such as *Spodoptera litura* F. and banana skipper, *Erionota thrax* L were also noted. Along with the defoliators sap feeding feeding insects such as banana lace wing bug, *Stephanitis typicus* (Distant), rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin were also noted. Minor incidence of Sigatoka leaf spot caused by the fungus *Pseudocercospora musicola* (Zimm.) was also noted.

Table 4. Effect of Beauveria capsules in curative management of pseudostem weevil

| Treatments | Total no. of capsules $plant^{-1}$ | * Mean no. of bore holes observed after treatment application | | | | | |
|---|---|---|---|---|---|---|---|
| | | 5 MAP | | 6 MAP | | 7 MAP | |
| | | 1st fortnight | 2nd fortnight | 1st fortnight | 2nd fortnight | 1st fortnight | 2nd fortnight |
| Leaf axil application @ 4 $plant^{-1}$ + bore hole filling (at weekly intervals) | 62 | 1.50 (1.18) | 0.75 $(1.18)^{b}$ | 0.25 $(0.96)^{b}$ | 0.75 $(1.05)^{b}$ | 0.75 $(1.09)^{b}$ | 0.75 $(1.09)^{c}$ |
| Leaf axil application @ 4 $plant^{-1}$ + bore hole filling (at fortnightly intervals) | 38 | 2.00 (1.39) | 1.75 $(1.47)^{b}$ | 1.00 $(1.18)^{b}$ | 1.0 $(1.18)^{b}$ | 1.25 $(1.27)^{b}$ | 1.50 $(1.40)^{b}$ |
| Chlorpyriphos 20 EC (2mL $L^{-1}$) (Leaf axil filling) | - | 2.00 (1.39) | 1.50 $(1.38)^{b}$ | 1.00 $(1.18)^{b}$ | 1.00 $(1.18)^{b}$ | 0.750 $(1.05)^{b}$ | 1.00 $(1.18)^{c}$ |
| Untreated control | - | 2.25 (1.49) | 3.50 $(1.98)^{a}$ | 4.50 $(2.22)^{a}$ | 5.75 $(2.48)^{a}$ | 6.00 $(2.54)^{a}$ | 7.50 $(2.81)^{a}$ |
| CD(0.05) | | NS | (0.339) | (0.486) | (0.549) | (0.535) | (1.852) |

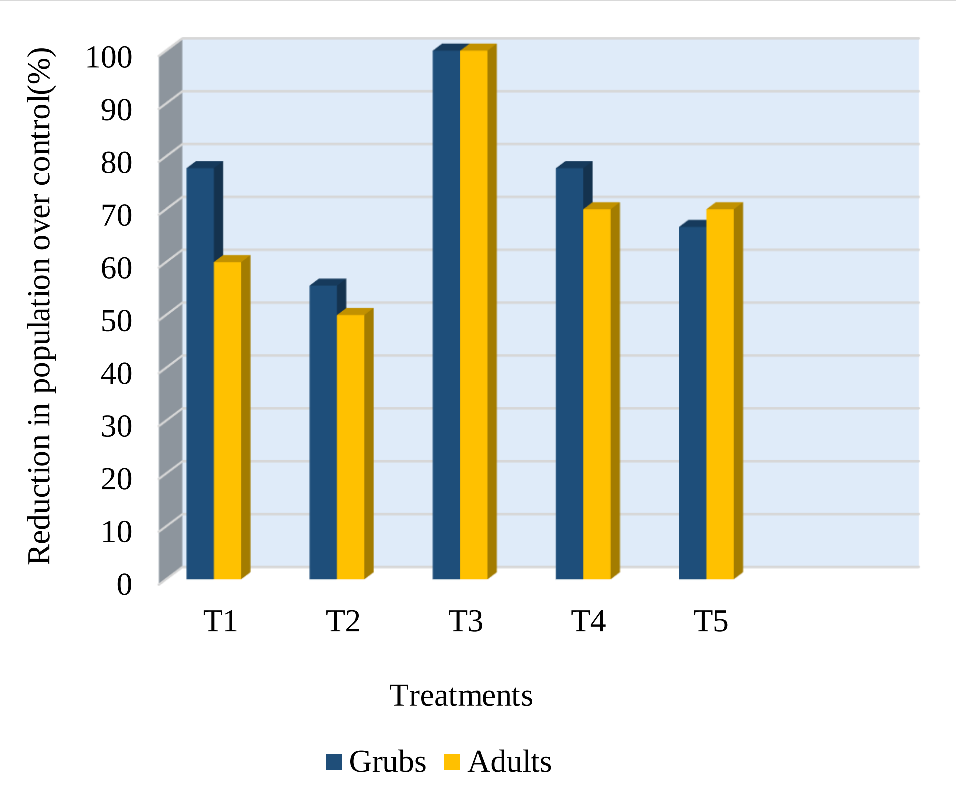
MAP – Months After Planting NS – Not Significant *Mean of four replications

Values in the parentheses are square root transformed




T1 - Leaf axil application of Bb + Pit application of Ma, at monthly intervals
T2 - Leaf axil application of Bb + Pit application of Ma, at bi monthly intervals
T3 - Leaf sheath application of Bb at monthly intervals + Plugging rhizomes at paring followed by monthly application in the pit
T4 - Leaf sheath application of Bb at bimonthly intervals + Plugging rhizomes at paring followed by bimonthly application in the pit
T5 - Chlorpyriphos 20 EC (Leaf axil filling) @ 2 mL $L^{-1}$

Fig.1 Effect of Beauveria capsules on population of pseudostem weevil (Prophylactic treatment)




T1 - Leaf axil application of Bb + Pit application of Ma, at monthly intervals
T2 - Leaf axil application of Bb + Pit application of Ma, at bimonthly intervals
T3 - Leaf sheath application of Bb at monthly intervals + Plugging rhizomes at paring followed by monthly application in the pit
T4 - Leaf sheath application of Bb at bimonthly intervals + Plugging rhizomes at paring followed by bimonthly application in the pit
T5 - Chlorpyriphos 20 EC (Leaf axil filling) @ 2 mL $L^{-1}$

Fig.2 Effect of Metarhizium capsules on population of rhizome weevil (Prophylactic treatment)

## 4.2 STANDARDIZATION OF DOSAGE OF BEAUVERIA CAPSULES FOR CURATIVE TREATMENT OF PSEUDOSTEM WEEVIL

Total number of capsules needed plant$^{-1}$ during the entire crop growth period for curative management of pesudostem weevil was assessed by applying the treatments on naturally infested plants of uniform infestation. Method of application was leaf axil application at varying time intervals *viz.* weekly and fortnightly. Efficacy of these treatments assessed based on the severity of symptom expression as well as the population of the pest at the time of harvest is presented below.

### *4.2.1 Presence of bore holes after curative treatment*

**N**umber of bore holes present on the pseudostem noted at fortnightly intervals is presented in Table 4.

During five MAP *i.e.* two weeks after the first treatment, all the treated plants exhibited symptoms. Least number of bore holes was noted in T1 (leaf axil application at weekly intervals) with a mean number of 1.5. T2 (leaf axil application at fortnightly intervals) ranked next with an average of 2.0 holes and was on par with those observed in plants treated with chlorpyriphos 20 0.05 % EC. Highest symptom expression was observed in the untreated plants (2.25).

At six MAP, T1 was found to be the best treatment with least number of bore holes (0.75) which was statistically on par with chemical treatment and T2, where the mean number of holes observed was 1.0 each. Highest population was noted in the untreated plants (5.75).

. During the seventh month, T1 was found to be the best method of application of capsules, where the mean number of holes observed was 0.75. This was statistically similar to the symptom expressed in chemical treatment (T3) (1.0).

T2 was the next effective method with a mean number of 1.5 bore holes. The symptom expressed was highest in the untreated plants (7.50).

While comparing the number of capsules applied plant$^{-1}$ and the damage symptom, it is evident that plants in which 62 capsules were placed exhibited less symptom than those applied with 38 capsules, as the number of bore holes observed varied significantly among these (0.75 and 1.50 respectively).

### *4.2.2. Population of pseudostem weevil after curative treatment*

The number of grubs, pupae and adults present inside the pseudostem were counted at the time of harvest, by splitting open the pseudostem longitudinally. Table 5 reveals the population of the pest after curative treatment.

Lowest population of grubs (2.0) was noted in T1 (leaf axil application at weekly intervals) which was on par with those observed in plants treated with chlorpyriphos 20 EC 0.05 % (1.87). Mean number of grubs noted in T2 was 3.12, while the highest population was noted in the untreated plants (4.12).

Based on the population of pupae, the most effective treatment was T1 where the mean number was 1.12, which was on par with chemical check (1.37). Mean number of pupae observed in T2 was 1.87. Population in the untreated plants was significantly high when compared to the treated plants (2.87).

Efficacy of the treatments on the adult population was same as those reflected in the population of grubs and pupae. T1 exhibited superiority with a mean number of 1.87, which was on par with the chemical treatment (2.25). Mean number of adults observed in T2 was 3.25. Highest population was recorded in the untreated plants (4.87).

Fig. 3 represents the percentage reduction in the pest population over control. Reduction in population was highest in T1 (51.51, 60.8 and 61.5 per cent grubs, pupae and adults respectively). Chemical treatment was also found to be

a. Leaf axil application at weekly intervals

b. Leaf axil application at fortnightly intervals

c. Leaf axil filling with chlorpyriphos

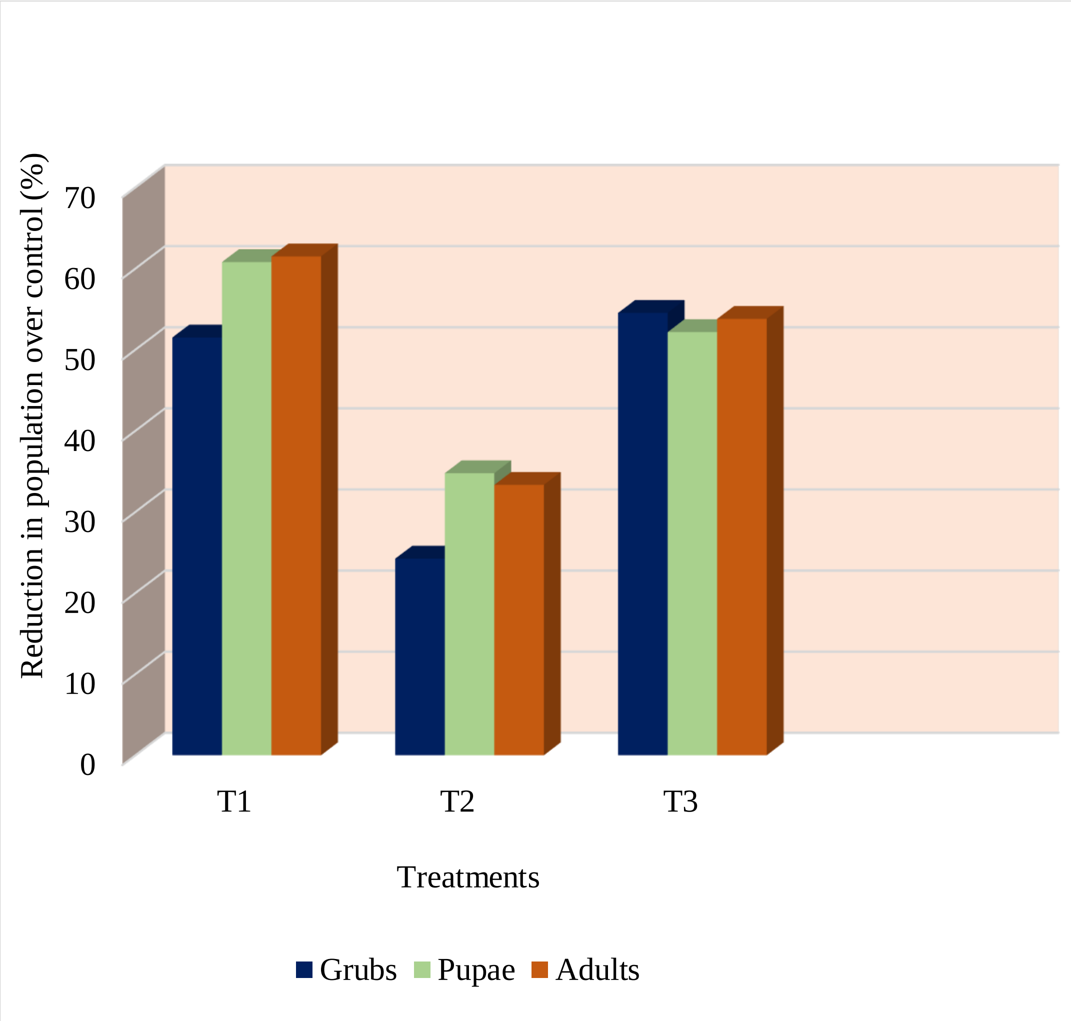
d. Untreated check

Plate 9. Bore holes observed after the treatment application in curative treatment of pseudostem weevil

Table 5. Effect of curative treatment with Beauveria capsules on population of pseudostem weevil

| Treatments | Total no. of capsules $plant^{-1}$ | *Mean population of pest per plant at harvest | | |
|---|---|---|---|---|
| | | Grubs | Pupae | Adults |
| Leaf axil application @ 4 $plant^{-1}$ + bore hole application (at weekly intervals) | 62 | 2 (1.40)[b] | 1.12(1.03)[b] | 1.87(1.36)[c] |
| Leaf axil application @ 4 $plant^{-1}$ + bore hole application (at fortnightly intervals) | 38 | 3.12(1.76)[a] | 1.87(1.35)[ab] | 3.25(1.79)[b] |
| Chlorpyriphos 20 EC (2mL $L^{-1}$) (Leaf axil filling) | - | 1.87(1.36)[b] | 1.37(1.16)[b] | 2.25(1.49)[c] |
| Untreated control | - | 4.12(2.02)[a] | 2.87(1.69)[a] | 4.87(2.20)[a] |
| CD (0.05) | | (0.265) | (0.361) | (0.255) |

* Mean of four replications Values in the parentheses are square root transformed




T1 - Leaf axil application @ 4 plant$^{-1}$ + bore hole filling at weekly intervals

T2 - Leaf axil application @ 4 plant$^{-1}$ + bore hole filling at fortnightly intervals

T3 - Chlorpyriphos 20 EC (Leaf axil filling) @ 2mL L$^{-1}$

Fig.3 Effect of Beauveria capsules on population of pseudostem weevil at the time of harvest (Curative treatment) found to be superior with a reduction of 54.54, 52.17, 53.8 per cent respectively.Least reduction was in T2 with 24.24,34.78 and 33.33 percentage reduction of grubs, pupae and adults repectively.

While comparing the number of capsules applied plant$^{-1}$ and the population assessed it is evident that plants inoculated with 62 capsules were placed exhibited least population (2.0, 1.12, 1.87 per cent) than those applied with 38 capsules (3.12, 1.87, 3.25 per cent) as the number of grubs, pupa and adults observed varied significantly among these treatments.

*4.2.3. Incidence of other pests and diseases*

During the experimental period high incidence of defoliator *S. litura* F. was noted. Sap feeding insects such as *S. typicus, A. rugioperculatus* and banana spittle bug, *Phymatostetha deschamps* Lethierry were also noted. Minor incidence of Sigatoka leaf spot caused by the fungus *P. musicola* (Zimm.) was also noted.

***4.2.4. Yield***

Table 6 represents the yield obtained plant$^{-1}$ after the curative treatment of pseudostem weevil infestation. Highest yield was recorded in T1 *i.e.* Leaf axil application @ four Beauveria capsules plant $^{-1}$ + bore hole application (at weekly intervals). The mean yield recorded was 8.37 kg plant $^{-1}$ followed by that in chemical treatment (8.12 kg). Mean yield of T2 was 7.87 kg. The lowest yield was recorded in the untreated plants (7.12 kg).

4.3 STANDARDIZATION OF DOSAGE OF METARHIZIUM CAPSULES FOR CURATIVE TREATMENT OF RHIZOME WEEVIL.

Effect of biocapsules for curative treatment of rhizome weevil was assessed by applying the treatment in an endemic area. Dosage was assessed by the application of capsules in the pit at varying intervals *viz.* fortnightly, monthly and bimonthly.

Table 6. Effect of curative treatment of pseudostem weevil with Beauveria capsules on the yield of banana

| Treatments | Total no. of capsules plant $^{-1}$ | *Mean yield plant$^{-1}$ (kg) |
|---|---|---|
| Leaf axil application @ 4 plant $^{-1}$ + bore hole application (at weekly intervals) | 62 | 8.37 |
| Leaf axil application @ 4 plant $^{-1}$ + bore hole application (at fortnightly intervals) | 38 | 7.87 |
| Chlorpyriphos 20 EC (2mL L$^{-1}$) (Leaf axil filling) | - | 8.12 |
| Untreated control | - | 7.12 |
| CD (0.05) | | NS |

NS- Non Significant * Mean of four replications

#### *4.3.1. Presence of tunnels in the rhizome at the time of harvest*

Table 7 represents the results of the field experiment conducted in farmer's field.

Number of tunnels in rhizomes observed at the time of harvest was least in T1 (pit application at fortnightly intervals) which was statistically on par with the results obtained in Fipronil 0.3 G (10 g plant $^{-1}$) treated plants. The mean number observed was 2.33 each. T2 (pit application at monthly intervals), ranked second with 3.33 number of tunnels. T3 (pit application at bimonthly intervals) recorded a mean number of 4.00. Number of tunnels observed in the untreated plants was the highest (5.50).

#### *4.3.2. Population of rhizome weevils at the time of harvest*

Population of adults and grubs observed in the rhizome at the time of harvest revealed that, T1 (pit application at fortnightly intervals) was the best treatment, where the number of grubs recorded was 1.16. It was on par with chemical treatment with a mean number of 1.16 grubs per rhizome. T2 (pit application at monthly intervals) ranked second, with a mean number of 2.00 grubs per rhizome. T3 (pit application at bimonthly intervals) was the least effective method (2.83). Population of the grubs was highest in the untreated plants (3.5).

Considering the population of adults, fortnightly application of capsules (T1) was found to be superior which was statistically on par with chemical treatment, Fipronil 0.3 G *i.e.* 1.5 and 1.66 respectively. T2 ranked second with mean number of 2.667, followed by T3 with a number of 3.5. Highest population of adults (4.00) was noted in the untreated plants.

Fig. 4 represents the percentage reduction of pest population over control. In the case of T1, there was 66.65 per cent reduction in the population of grubs and 62.5 per cent reduction in adults. In T3, percentage reduction of grubs

Table 7. Effect of Metarhizium capsules on the curative management of banana rhizome weevil

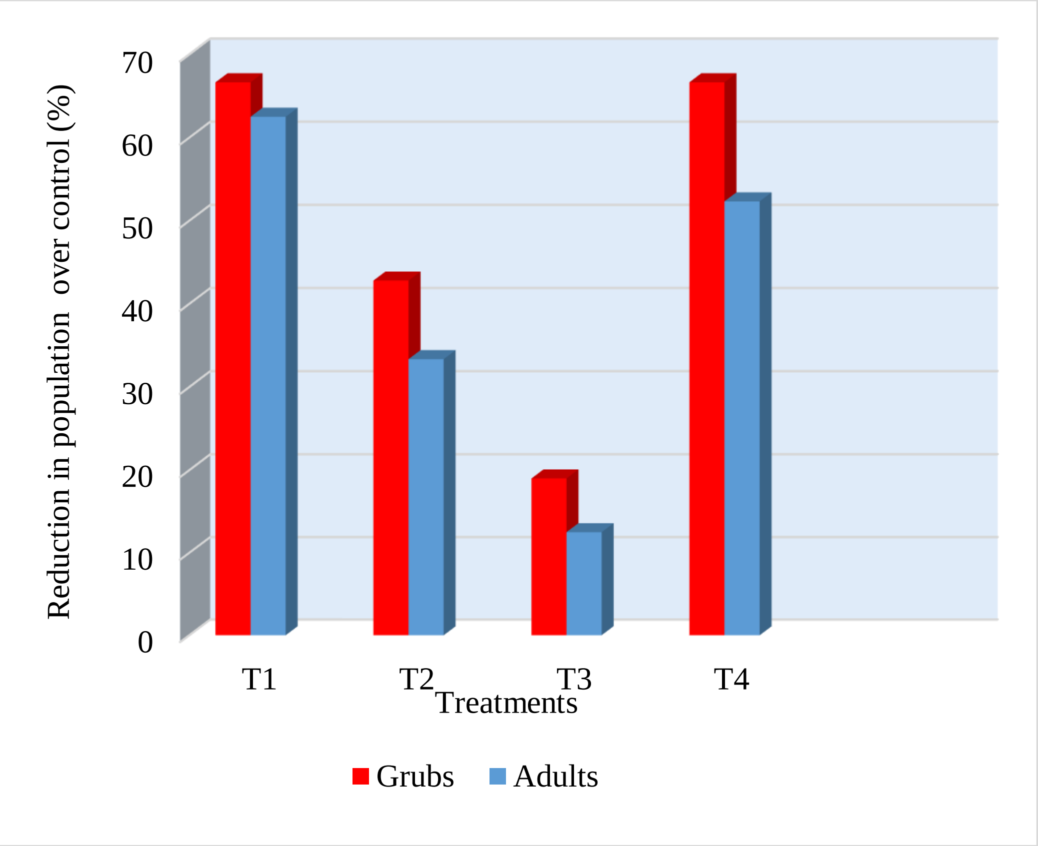
| Treatments | Total no. of capsules plant$^{-1}$ | No. of tunnels | *Mean population of rhizome weevil at harvest | |
|---|---|---|---|---|
| | | | Grubs | Adults |
| Four capsules plant$^{-1}$ in the planting pit at fortnightly interval | 28 | 2.33(1.50)$^{c}$ | 1.16(1.07)$^{c}$ | 1.50(1.21)$^{c}$ |
| Four capsules plant $^{-1}$ in the planting pit at monthly interval | 12 | 3.33(1.82)$^{bc}$ | 2(1.40)$^{b}$ | 2.66 (1.63)$^{b}$ |
| Four capsules plant $^{-1}$ in the planting pit at bimonthly interval | 8 | 4(1.99)$^{ab}$ | 2.83(1.68)$^{a}$ | 3.50(1.86)$^{ab}$ |
| Fipronil 0.3 G @ 10 g plant-$^{1}$ (Soil application) | | 2.33(1.50)$^{c}$ | 1.16(1.07)$^{c}$ | 1.66(1.27)$^{c}$ |
| Untreated control | | 5.50(2.34)$^{a}$ | 3.50(1.86)$^{a}$ | 4 (1.99)$^{a}$ |
| CD (0.05) | | (0.388) | (0.260) | (0.338) |

*Mean of three replications Values in the parentheses are square root transformed

Table 8. Effect of curative treatment of rhizome weevil with Metarhizium capsules on the yield of banana

| Treatments | Total no. of capsules plant $^{-1}$ | *Mean yield plant$^{-1}$ (kg) |
|---|---|---|
| Four capsules plant$^{-1}$ in the planting pit at fortnightly interval | 28 | 8.83$^{a}$ |
| Four capsules plant $^{-1}$ in the planting pit at monthly interval | 12 | 8$_{a}$ |
| Four capsules plant $^{-1}$ in the planting pit at bimonthly interval | 8 | 6.83$^{b}$ |
| Fipronil 0.3 G @ 10 g plant$^{1}$ ( Soil application) | - | 8.33$^{a}$ |
| Untreated control | - | 6.66$^{b}$ |
| CD (0.05) | | 0.926 |

* Mean of three replications




T1 - Four capsules plant$^{-1}$ in the planting pit at fortnightly interval

T2 - Four capsules plant$^{-1}$ in the planting pit at monthly interval

T3 - Four capsules plant$^{-1}$ in the planting pit at bimonthly interval

T4 - Fipronil 0.3 G (Soil application) @ 10 g plant-$^{1}$

Fig.4 Effect of Metarhzium capsules on the population of rhizome weevil (Curative treatment) was 42.8 and that of adults was 33.35. Least reduction was observed in T2 with 19 and 12.5 percent reduction of grubs and adults respectively. In chemical treatment, reduction noted was 66.65 and 58.32 respectively in the case of grubs and adults.

While comparing the number of capsules applied plant$^{-1}$ and the damage symptom it is evident that the plants in which 28 capsules were placed exhibited less symptom which was on par with chemical treatment than those applied with 12 capsules and eight capsules.

### *4.3.3. Yield*

Table 8 represents the yield obtained plant$^{-1}$ after the curative treatment of rhizome weevil. Highest yield was recorded in T1 (placement of Metarhizium capsules @ four plant $^{-1}$ in the planting pit at fortnightly intervals) with a mean yield of 8.83 kg plant$^{-1}$ .This was statistically on par with the yield obtained in Fipronil 0.3 G (10 g plant$^{-1}$) treatment (8.83 kg plant$^{-1}$) and T2 (placement of capsules @ four plant $^{-1}$ in the planting pit at monthly interval) (8.00 kg plant$^{-1}$). Mean yield of T3 (placement of capsules @ four plant$^{-1}$ in the planting pit at bimonthly intervals) was 6.83 kg plant$^{-1}$, which was statistically on par with that of the untreated plants (6.66 kg plant$^{-1}$).

# *Discussion*

## 5. DISCUSSION

Microbial pesticides based on entomopathogenic fungi are the most commercially exploited biopesticides in the era of organic farming and sustainable development. Biological products are highly target-specific and desirable, but acceptable formulations are very difficult to be developed as it is not easy to maintain their viability during storage. Conventional commercial formulations of the entomopathogens often fail to reach its expected potency due to improper delivery at the target site. Capsule is a stable formulation, wherein the bioagent is encapsulated in coatings and thus protected from extreme environmental conditions such as UV radiation, rain and temperature. Capsules have more residual stability than spray formulations. It makes delivery effective in the case of leaf axil or bore hole application or soil application for the weevil pests of banana. The results of the research work entitled "Bioefficacy of capsule formulations of *Beauveria* and *Metarhizium* in managing banana weevils" are discussed below.

The banana variety selected for the study was Nendran as it was reported to be the most susceptible variety to pseudostem weevil in Kerala (Visalakshi *et al*., 1989; Jayasree, 1992 and Charles *et al*., 1996). It is the lower crude fibre content and higher succulent nature of the pseudostem that makes the variety highly susceptibile to the pseudostem weevil, *Odoiporus longicollis* Oliver (Lalitha *et al*., 2000). Banana weevils being notorious due to their concealed nature of feeding and development, often cause crop loss, as the farmers fail to take adequate and timely control measures. It is difficult to manage them once they get established inside the plant (Padmanaban *et al.,* 2001). Therefore, preventive measure to avoid infestation is a very important management tactic to contain banana weevils. Though several insecticides, both chemical and biological are effective when tested under laboratory conditions, it is the lack of precise delivery mechanism that renders the crop unsaved from these pests.

Laboratory studies conducted by the previous researcher, Remya (2018) revealed that it was *Metarhizium anisopliae* (Metschnikoff) that was more infective to the rhizome weevil, *Cosmopolites sordidus* Germer and *Beauveria bassiana* (Balsamo) Vuillemin to *O. longicollis.* Furthermore, pot culture studies carried out by her in banana, showed promising results on these biocapsules based on entomopathogenic fungi. The biocapsules developed by Remya and Reji (2020) was 100 per cent organic and biodegradable as well. The coating material standardised by them was cellulose (HPMC-Hydroxy Propyl Methyl Cellulose) and the best carrier material selected for slow release was chitosan, the natural biopolymer obtained from shell of crustaceans. The fungi used were the NBAIR isolates *B. bassiana* (Bb5) and *M. anisopliae* (Ma4), as several previous research works focused on these entomopathogenic fungi for the management of banana weevils. The spore load maintained in the capsules was $10^{10}$, which is 100 times the normal infective dose worked out by previous researchers. The infective dose of *B. bassiana* determined for pseudostem weevil was $1.8 \times 10^7$ spores $mL^{-1}$ as reported by Beegum (2005) and $10^8$ conidia $mL^{-1}$ as per the report of Alagesan *et al.* (2019). Likewise, for *M. anisopliae* it was $7.89 \times 10^7$ spores $mL^{-1}$ for rhizome weevil (Brences and Carballo, 1994); $5 \times 10^7$ spores $mL^{-1}$ (Gangaprasad, 2001) and $10^8$ spores $mL^{-1}$ (Varsha, 2017).

Though the existing formulations available in the market have a spore load of $10^8$, capsule being a smart formulation which is concise and precise, the spore load was increased several times with a view to reduce bulkiness as well as to ensure sufficient quantity of inoculam in the targeted area.

With this back ground, experiments were laid out separately to evaluate the efficacy of capsules for prophylactic as well as curative treatment. There were three sets of experiments laid out in the field (1) Efficacy of biocapsules (both Beauveria and Metarhizium) applied as prophylactic treatment against both the weevils (2)

Efficacy of Beauveria capsules in curative treatment of pseudostem weevil (3) Efficacy of Metarhizium capsules in curative treatment of rhizome weevil.

In contrast to the spraying method, here the capsules were applied into the exact location of attack such as bore holes, leaf axils and in the outer most leaf sheath, targeting the eggs, early instars and adults of pseudostem weevil, and inside the rhizome by plugging them before planting the rhizomes and in the planting pit thereafter targeting the adults, eggs and early instar grubs.

For the prophylactic treatment, two different methods of application and two time intervals were tried to standardize the dosage and method of application of these capsules. The methods tested were leaf sheath application and leaf axil application. The time intervals selected were monthly and bimonthly, from fourth month of planting, till bunch emergence. The number of capsules used per plant varied with the method of application. It was 12 for leaf sheath and leaf axil application when applied monthly, while was eight when placed bimonthly.

Results of the first experiment revealed that placement of four Beauveria capsules, one each on the four sides of the outer leaf sheaths, at a height of 1.5 m from the ground, at monthly intervals was more effective than placing them on the leaf axils. There were no symptoms of infestation in these plants. Destructive sampling of plants done during the seventh month revealed that there was 90.9 per cent reduction in the population of grubs and adults and 83.33 per cent reduction in the pupal population. When the same dose and application method was practiced, at a longer time interval *i,e.* on alternate months, the efficacy was much reduced. The reduction in population of grubs, pupae and adults noticed was only 54.54, 33.33 and 36.36 per cent, respectively.

The superiority observed may be attributed to the fact that the chances of fungal conidia to come in contact with the eggs or first instar of the weevil is more in the outer leaf sheath as stated in the studies carried out by Dutt and Maiti (1971), who reported that mating of weevils occurs in the outer leaf sheath. Likewise, Jayasree (1992), while studying the biology of *O.longicollis*, observed that the eggs were laid in the air chamber through the ovipositional slits made on the outer epidermal layer of the leaf sheath.

The other method, *i.e.* application of one capsule each in the outer four leaf axils was also effective, when applied every month. Here the reduction in population observed was 72.2 per cent in the case of grubs and adults and 83.33 per cent in pupae. However, the efficacy was less when applied on alternate months, where the percentage reduction was 36.36, 33.33 and 27.27 in the case of grubs, pupae and adults respectively. The positive results obtained here can be because of the fact that, the site of oviposition can also be the axil so that the conidia dispersed from the capsule has more chance to come into contact with the eggs or first instar or even the female weevil at the time of oviposition. Anitha (2000), while studying the bioecology of pseudostem weevil, concluded that the preferred site of oviposition by these weevils is at a height of 1 to 1.5 m from the ground level and that the ovipositional slits were more concentrated near the leaf axil. During a survey conducted in southern districts of Kerala, in the variety, Nendran. Sivakumar *et al*. (2017) reported that oviposition can happen in the petioles too, as symptoms of infestation were noticed in the petioles.

Less efficacy of both the methods, applied at a longer time interval *i.e.* alternate months, clearly indicated the role of quantity of inoculam of the fungus that is needed to cause infection to the weevil. Entomopathogenic fungi are well known for their dose dependant ability to cause morbidity or mortality in insects that they come in contact. Inoculam level is an important factor which affects the performance of a pathogen. In general, higher doses give higher mortality. Even then, variations in the effective dose that can cause symptoms, may vary with the pests or even the host plant or habitat. The defense mechanism of the pest is yet another predisposing factor in causing disease or death. Virulence of a pathogen is generally determined in terms of quantity of the inoculam that comes in contact with the host insect.

The dose - dependent efficacy of *B. bassiana* observed in this study is an indication of dose dependent morbidity or mortality of the various stages of the weevil and is in conformity with the findings of several researchers who tested varying doses of this fungus on crop pests. Anderson *et al* (1988) observed 65.8 per cent reduction of the larval populations of grubs of colarado potato beetle *Leptinotarsa decimlineata* (Say), when sprayed at a dosage of 5 x $10^{13}$ CFU $ha^{-1}$, while with a lower dose of 5 x $10^{12}$ CFU $ha^{-1}$, the percentage reduction was 39.5. Similarly, Sivasankaran *et al*. (1990) observed that second and third instar larvae of sugarcane early shoot borer , *Chilo infuscatellus* Snellen were more susceptible to higher concentrations of *B. bassiana* ($10^7$ spores $mL^{-1}$) causing 68.53 to 75.93 per cent mortality, whereas it was only 51.47 per cent at a lower dose of $10^5$ spores $mL^{-1.}$ Reports of Manjula and Padmavatamma (1999) on *Helicoverpa armigera* (Hubner), revealed that at a higher concentration of $10^9$spores $mL^{-1}$, the mortality was 84 per cent and at the lower doses of $10^5$ and below the death rate was 66.13 and less. Dose dependant nature of *B. bassiana* is evident in many other works such as those reported by Rachana *et al.* (2008) in *Spodoptera litura* F. and *H. armigera* and Mehinto *et al*. (2014) in *Maruca vitrata* F.

In a field experiment conducted by Beegum (2005), it was reported that prophylactic treatment by leaf axil filling of *B. bassiana* spore suspension @ 1.8 × $10^{7}$ $mL^{-1}$ resulted in 52.48 per cent mortality of grubs which was found to be equally effective as leaf axil filling of chlorpyriphos 20 EC 0.03 % and Neem Azal 0.4 %. She also reported that leaf axil filling with spore suspension of *M.anisopliae* was effective, causing 34.83 per cent mortality to grubs. Remya (2018) while studying the prophylactic effect of biocapsules against banana weevils in a pot culture study revealed that, leaf axil application of Beauveria capsules @ four $plant^{-1}$ resulted in 91.67 per cent reduction in the pest population over control which was on par with chemical treatment chlorpyriphos 20 EC 0.05 %.

In the experiment to test the efficacy of capsules for curative treatment of pseudostem weevil, combined application in the outer four leaf axils as well as into bore holes if present, was attempted at two time intervals, weekly and fortnightly. Population of weevils assessed at the time of harvest revealed that there was 51.5, 60.8 and 61.5 per cent reduction in the population of grubs, pupae and adults respectively. However, the same method when practiced at fortnightly intervals, the reduction in population was 24.24, 34.78 and 33.33 of grubs, pupae and adults, respectively.

Efficacy of Beauveria capsules in managing pseudostem weevil was earlier reported by Remya (2018). She has reported that curative application of Beauveria capusles in the leaf axils or leaf sheath @ four plant$^{-1}$, was effective in managing the pest, causing 91.67 per cent reduction in population when compared to the untreated plants, two weeks after treatment. She also reported that the treatment was as effective as insecticide treatment with chlorpyriphos 20 EC 0.05 %. In her preliminary studies, she could observe that capsules will remain intact up to three days if there is no rainfall (RH - 70 % and Temperature - 33 ºC) and it would disperse the contents readily within a day when RH is 77 %, due to the receipt of rainfall. She also reported that placement of capsules in the bore holes resulted in faster disintegration and therefore concluded that either leaf sheath or leaf axil application can be considered for prophylactic control, while bore hole application is better for curative control.

Several other studies pertaining to the curative treatment of banana weevils with *B. bassiana* spore suspension carried out by earlier workers also indicated the efficacy of this fungus. In the field experiment carried out by Beegum and Anitha (2006), there was 46.66 per cent mortality of *O.longicollis* grubs, when the spore suspension was applied in the leaf axils @ $1.8 \times 10^7$ spores mL$^{-1}$ (500 mL plant $^{-1}$). They also reported that, the first and second instar grubs were more susceptible to infection. In another study using traps, Irulandi *et al.* (2012) reported that *B. bassiana* when applied in pseudostem pieces @ 25 g trap$^{-1}$ and placed in open field @100 ha$^{-1}$, caused 75.36 per cent mortality in adults within 96 hours after treatment (HAT).

The population reduction indicated in all these studies including the present one, can be even due to the successful transmission of the pathogen from the diseased to the healthy insects. Such horizontal transmission of the fungus had been demonstrated by Schoeman (1999), who reported symptoms as well as death of untreated weevils which were reared along with the treated ones. Similar results of horizontal transmission of *B. bassiana* on banana rhizome weevil had been reported by Tinzaara *et al*. (2007).

The efficacy can also be attributed to the endophytic nature of *B. bassiana.* Akello *et al.* (2007) established the endophytic association of *B.bassiana* in banana due to which he claimed 42 to 86.7 per cent reduction in the population of banana rhizome weevil. Its endophytic activity was further reported from many plants from which recovery varied with the plant parts examined (Ownley *et al*., 2009). According to Prabhavathi (2012), rhizome dip in spore suspension of $10^{10}$ and $10^{9}$ spores $mL^{-1}$ resulted in 75.08 and 72.05 per cent mortality of adults, respectively. Divyasree (2019), while studying the endophytic association of entomopathogenic fungi with rice and cowpea, observed 52.4 per cent reduction in the chaffy grains in plants inoculated with *B.bassiana.*

However, some strains and isolates of *B.bassiana* were reported to be non infective to *O.longicollis*. The culture from National Research Centre for Banana (NRCB) as well as the isolate from College of Agriculture, Vellayani, KAU (ITCC 6063), did not cause any mortality to grubs when evaluated under laboratory conditions (Sivakumar, 2017). He could observe only a negligible death rate in adults (6.67 per cent) with KAU isolate, while there was no death at all with NRCB culture, even after 10 days. Such variation among the isolates and strains of the same species is a common phenomenon in entomopathogenic fungi.

Fungi other than *B. bassiana* were also reported to be effective against *O.longicollis*. Curative treatment of pseudostem weevil using *M.anisopliae* was earlier suggested by Anitha (2000). She reported that the fungus at a spore concentration of $15 \times 10^{5}$ spores $mL^{-1}$ caused mortality from seventh day onwards reaching up to 96.01 per cent on the $14^{th}$ day after treatment (DAT). She had also reported that its efficacy was equivalent to chlorpyriphos 20 EC 0.05 %. In her study, other entomopathogenic fungi such as *Fusarium solani* (Martius) Saccardo and *Mucor heimalis* Wehmeron were also reported to be effective against pseudostem weevil. In the studies conducted by Sivakumar (2017), *Metrahizium majus* (isolate no. CPCRIMKY-1) 2%, when applied in the leaf axils, two months after planting (MAP) was found to cause 80 per cent mortality of the grubs one week after treatment.

In the experiment to evaluate the efficacy of Metarhizium capsules in prophylactic treatment of rhizome weevil , two different methods of application and two time intervals were tried to standardize the dosage and method of application. The methods tested were pit application and plugging of capsules in the rhizome at paring, followed by pit application. The time intervals selected were monthly and bimonthly. The number of capsules used per plant varied with the method of application. It was 28 and 16 in the treatments where capsules are plugged into the rhizome followed by pit application at monthly and bimonthly intervals whereas 24 and 12 when it was applied in the pit at monthly and bimonthly intervals, respectively.

Results of this experiment revealed that, placement of four capsules by plugging into the rhizome at the time of paring on four sides, followed by placement of four capsules in the pit at monthly intervals was more effective than all other treatments, as there were no symptoms of infestation in these plants. Destructive sampling of the plants done during the seventh MAP revealed that, there was 100 per cent reduction in the population of grubs and adults. When the same method of application was practiced at bimonthly intervals, the efficacy was reduced. The reduction in population noted was 77.77 and 70 per cent of grubs and adults respectively. This might be because of the fact that the chances of fungal conidia to come in contact with the weevil at the time of oviposition, or with the eggs and developing grubs that develop due to infestation after planting or to the grubs that hide inside the rhizome even after paring, application of capsules in the pit at monthly intervals would have maintained the presence of the inoculam in the soil near to the rhizome. Presence of *C. sordidus* eggs in the rhizome near to the soil level was reported in the studies carried out by Viswanath (1976) and Arleu (1982).

Capsules ensure slow release of inoculam, compared to direct application by drenching talc based *M. anisopliae* @ 30g $L^{-1}$ as suggested by Varsha (2017) to manage rhizome weevil. Disintegration studies of these capsules carried out by

Remya (2018) revealed that the time taken for release of the fungal conidia is directly proportional to the moisture content in the plant as well as the totality of whether, especially the atmospheric humidity. She stated that the capsules remain intact up to a moisture level of 15 %, and that complete disintegration happens after 48 h, when the soil moisture increases to 20 % and more.

Therefore, it is suggested that for curative application of capsules, soil moisture should be ensured between 20 and 30, where 30 % is the field capacity. On the other hand for prophylactic management, as slow release is preferred, the normal prevailing moisture level shall be maintained.

Chitosan, the carrier material used in the capsules, might have played an additional role in bringing down the pest population. Presence of extracellular chitosan, would trigger the chitinase secretion by the fungus, thereby enhancing the infection process in insects. Furthermore, chitosan itself would trigger the enzyme chitinase in insects, which would enhance the degradation of insect cuticle, thereby facilitating the infection process of the pathogen. Archana (2017) studied the efficacy of chitosan in pest control and reported that natural biopolymers such as chitin and chitosan can be exploited for the holistic management of crop plants as it has the ability to regulate the population of pests. She reported that chitosan 7% caused feeding inhibition in the pumpkin caterpillar, *Diaphania indica* Saunders and brinjal leaf beetle, *Henosepilachna vigintioctopunctata* F. to an extent of 46.23 and 100 per cent respectively, on the first day. Efficacy of chitosan in reducing the population of soil inhabiting pests such as the root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood was further stated by Archana and Reji (2019). They observed that, chitosan 7 % could reduce the hatchability of *M. incognita* within four days by 66.67 per cent and cause 90 per cent mortality of juveniles within one day of treatment.

Pit application alone was also effective when applied every month. Here the reduction in population observed was 77.77 per cent in the case of grubs and 60 per cent in adults. This may be due to the close contact of capsules with the ovipositional sites of the weevil as mentioned above. However, the efficacy was less when applied on alternate months, owing to the reduction in quantity of the inoculam to cause morbidity or mortality, The percentage reduction noticed was 55.55 and 50 of grubs and adults respectively.

Pot culture studies carried out by Varsha (2017) revealed that prophylactic drenching of talc formulation and spore suspension of *M. anisopliae* @ $10^8$ spores $mL^{-1}$ recorded least rhizome damage (14.16 per cent) followed by talc formulation of *B. bassiana* (31.66 per cent). In another study conducted by Remya (2018), prophylactic application of Metarhizium capsules in the soil around the plant @ four plant $^{-1}$ resulted in 47.22 per cent reduction in the rhizome weevil population, compared to untreated plants.

The dose - dependent efficacy of *M. anisopliae* observed in this study, is in conformity with the findings of Butt (1992) who observed that, with an increase in spore concentration for *M. anisopliae* isolate V90 from $10^6$ to $10^7$ conidia $mL^{-1}$ decreased the $LT_{50}$ of cabbage stem flea beetle *Psylliode schrysocephala* L. from 11 to five days. Similarly, Arthurs (2001) reported that both infective dose and incubation temperature of *M. anisopliae* var. *acridum* significantly affected the mortality of desert locust *Schistocerca gregaria* Forskal.

For curative treatment of rhizome weevil, placement of four Metarhizium capsules in the planting pit at varying intervals *viz.* fortnightly, monthly and bimonthly intervals were carried out. The number of capsules used per plant varied with the intervals. It was 28, 12 and eight when applied fortnightly, monthly and bimonthly intervals.

Results of this experiment revealed that placement of four capsules in the planting pit at fortnightly intervals (seven applications), were as effective as one time soil application of Fipronil 0.3 G @ 10 g $plant^{-1}$. Population of weevils assessed at the time of harvest revealed that, there were 66.65 and 62.5 per cent reduction in the population of grubs and adults and 66.65 and 58.325 per cent in the case of chemical treatment respectively. When the same dose and method of application was practiced, at monthly intervals, the reduction in the population was only 42.8 in grubs and 33.35 in adults. While application on alternate months could not yield satisfactory results. There was only 19 and 12.5 per cent reduction of grubs and adults respectively, in these plants.

Efficacy of *M. anisopliae* to rhizome weevil noted in this study is in concurrence with that of Joseph (2014) who proved the efficacy of talc based *M. anisopliae* to the grubs @ 30 g $L^{-1}$ under field conditions, with least number of tunnels (0.63). Varsha (2017) recorded that talc based formulation of both *M. anisopliae* and *B. bassiana* were effective in controlling rhizome weevil when applied @ 30 g $L^{-1}$ by soil drenching. Remya (2018) observed that gel formulations of *M. anisopliae* when placed in pit provided 30 per cent reduction in curative control.

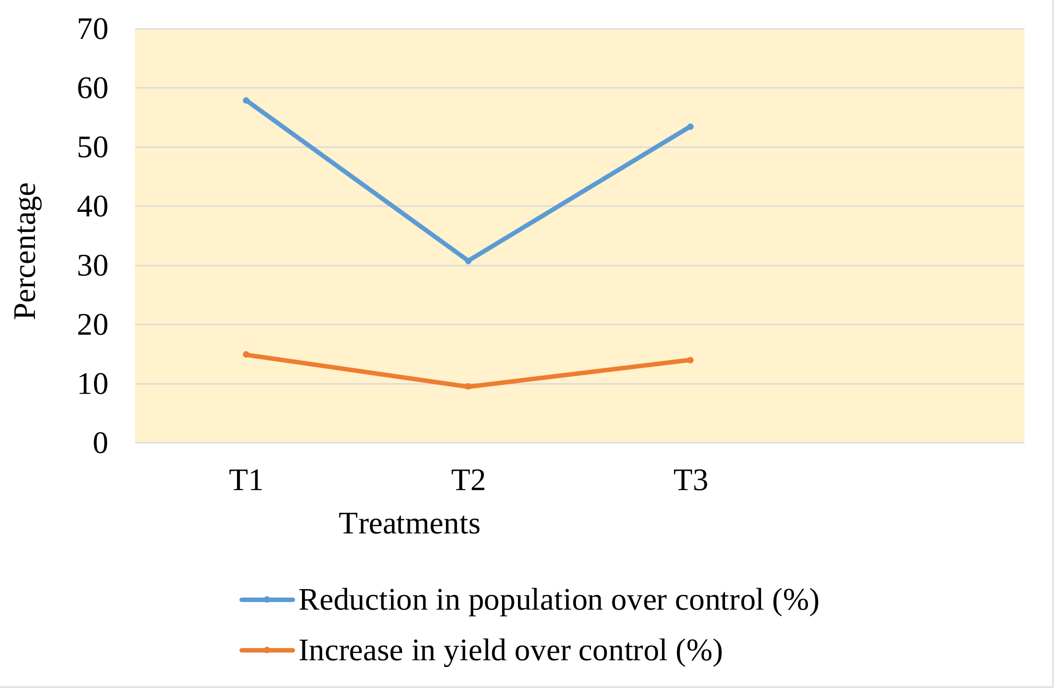
Fungi other than *M. anisopliae* were also reported to be effective against rhizome weevil. In an experiment conducted by Filho *et al*. (1994), 100 per cent mortality was claimed, when *B. bassiana* was applied at $1.8 \times 10^9$ spores $mL^{-1}$, while it was only 20 per cent with $5\times 10^6$ spores $mL^{-1}$. Similar results were also noted in the study carried out by Carballo (1998) who reported that 15 % oil formulation of *B. bassiana*, caused 97 per cent mortality of rhizome weevil when the concentration was $1 \times 10^8$ spores $mL^{-1}$, whereas the mortality decreased from 97 per cent to 10 per cent when the dose of *B. bassiana* was reduced to $5 \times 10^7$ spores $mL^{-1}$. Nakinga and Moore (2000) reported that *B. bassiana* formulated in maize and soil @ $2\times 10^{15}$ conidia $ha^{-1}$ and $2 \times 10^{14}$ conidia $ha^{-1}$ respectively was effective in controlling rhizome weevil resulting in 60 to 78 per cent and 50 per cent mortality within eight weeks after treatment

Although the efficacy of any plant protection operation is measured in terms of reduction in population or damage or symptom expression, ultimately it is appraised on the basis of yield. Observations recorded at the time of harvest revealed that the mean yield per plant was significantly high in curative treatment with Beauveria capsules applied weekly, to manage pseudostem weevil. The mean yield recorded per plant was 8.37 kg in those plants treated at weekly intervals, while in those treated at fortnightly intervals it was significantly low (7.87 kg $plant^{-1}$). The percentage increase in yield over the untreated plant was 14.9 in the former, while it was 9.51 per cent in the latter. In chemical treatment with single application of chlorpyriphos 20 EC 0.05% it was 14 per cent. The increase in yield may be directly attributed to the reduction in damage caused by the pest and indirectly attributed to its endophytic association. In the plants treated with Beauveria capsules at weekly intervals percentage increase in yield was more (14.9), where the reduction in pest population was also high (57.93 per cent) as seen in Fig. 5. This clearly indicates the correlation between yield and weevil population. Several references substantiate the increased plant growth due to endophyitic association of *B.bassiana* with crop plants. Ericcson (2007) reported that there was 26.3 per cent increase in the stock and foliage weight of maize, when inoculated with *B. bassiana* through foliar spraying.  Likewise, Akello *et al*. (2008) reported that banana rhizomes dipped in the conidial solution of *B.bassiana* showed an increase in plant height, leaf length and width, fresh and dry shoot weight of the plants.

Yield recorded in the plants after the curative treatment of rhizome weevils with Metarhizium capsules indicated that the mean yield per plant was significantly high (8.33 kg), when treated fortnightly and equivalent to single application of Fipronil 0.03% G. The yield recorded in monthly and bimonthly treatments were significantly lower, mean yield per plant being 8 kg and 6.83 kg, respectively. The yield increase may be attributed to the effectiveness of these capsules against rhizome weevil. Fig.6 indicates the percentage increase in yield (25.99) was more in the plants treated with Metarhizium capsules at fortnightly intervals with highest reduction in pest population (64.5 per cent).

There are some studies which show the effect of *M. anisopliae* in the yield of plants. Kabaluk and Ericcson (2007) revealed that corn seeds treated with the conidia of *M. anisopliae* (strain F52) increased the crop stand density (78 per cent) and fresh weight of shoots (9.6 t $ha^{-1}$) by controlling the incidence of wire worm, *Melanotus communis* Gyllenhal. Similarly, Divyasree (2019) reported that there was 39.89 per cent increase in grain yield, when rice plants were inoculated by seed soaking with *M. anisopliae* (Ma4).

Production cost calculated was Rs. 0.5 capsule$^{-1}$. Therefore, for prophylactic management of both the weevils, cost incurred for three applications (3 x 4 =12

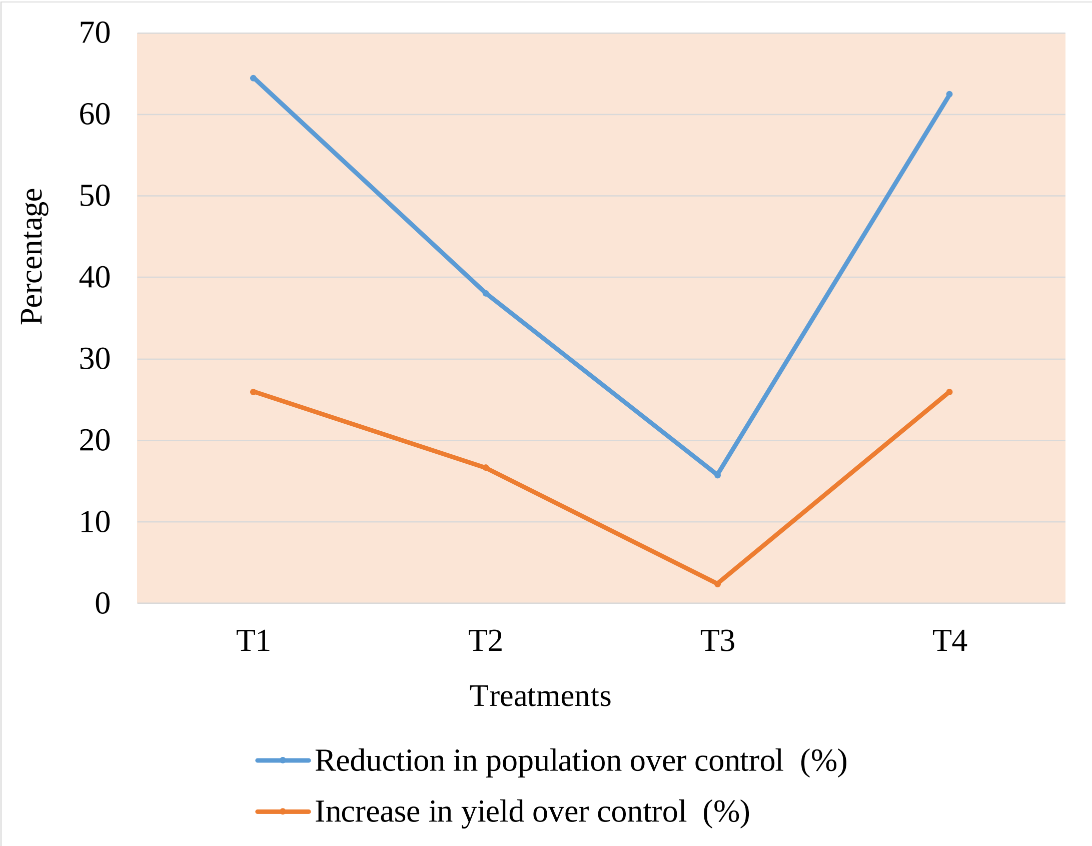



T1 - Leaf axil application @ 4 plant$^{-1}$ + bore hole application (at weekly intervals)

T2 - Leaf axil application @ 4 plant$^{-1}$ + bore hole application (at fortnightly intervals)

T3 - Chlorpyriphos 20 EC (2 mL L$^{-1}$) (leaf axil filling)

Fig.5 Effect of population of pseudostem weevil on yield - Curative treatment




T1 - Four capsules plant$^{-1}$ in the planting pit, at fortnightly interval

T2 - Four capsules plant$^{-1}$ in the planting pit, at monthly interval

T3 - Four capsules plant$^{-1}$ in the planting pit, at monthly interval

T4 - Fipronil 0.3 G (soil application) @ 10 g plant $^{-1}$

Fig.6 Effect of population of rhizome weevil on yield- Curative treatment

Beauveria capsules) and seven applications (7 x 4 Metarhizium capsules) is Rs. 20 per plant. (Rs. 6 for Beauveria capsules + Rs. 14 Metarhizium capsules), while the cost of single application of chlorpyriphos in the leaf axil (200 mL plant $^{-1}$) is Rs. 0.24 and for single soil application of Fipronil (10g plant$^{-1}$) it is Rs.1. For curative treatment of pseudostem weevil, cost incurred for 11 applications of Beauveria capsules is Rs. 31 and for rhizome weevil, cost incurred for seven applications of Metarhizium capsules is Rs. 14.

Higher cost incurred for the cost of capsules can be compensated for its ecofriendly nature and safe to eat products. Apart from this, insecticide application needs hired labour @ Rs. 830/ day, which can be substituted with the family labour for easier and safer application of capsules. Furthermore, problems of pesticide residue, pest resistance and pest resurgence can also be overcome. While considering the premium price and higher demand of organic banana in the market, these fetch more income to the farmer. Joseph (2014) calculated a high B: C ratio for biopesticide treatment with talc based *B. bassiana* @ 30 g plant$^{-1}$ (1.57) than chemical treatment with chlorpyriphos 20 EC 0.03 % treatment (1.24).

The field experiments carried out in this work is a clear indication of the effectiveness of biocapsules in managing banana weevils.

# *Summary*

# 6. SUMMARY

The present study entitled "Bioefficacy of capsule formulations of *Beauveria* and *Metarhizium* in managing banana weevils" was carried out at the Biocontrol laboratory for crop pest Management, Department of Agricultural Entomology, College of Agriculture, Vellayani, Instructional Farm, College of Agriculture, Vellayani and in the farmer's field at Peringamala, Venganoor Panchayath, Thiruvananthapuram, during the year 2018-2020. The investigation was focused on standardizing the dosage and method of application of biocapsules of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin for the prophylactic and curative control of banana weevils *viz. Odoiporus longicollis* Oliver and C*osmopolites sordidus* Germer respectively.

Biocapsules of Beauveria and Metarhizium were prepared based on the protocol developed by Remya and Reji (2018). Capsules used for the study was prepared with HPMC as the coating material and chitosan as the carrier with a spore load of $10^{10}$ spores $mL^{-1}$. Preliminary studies conducted by Remya (2018) revealed that *B. bassiana* (Bb5) was more infective to pseudostem weevil and *M. anisopliae* (Ma4), to rhizome weevil. There were three field experiments for standardizing the dosage and method of application of capsules against the banana weevils, prophylactically and curatively.

For prophylactic management of pseudostem weevil, two methods of application *viz.* leaf sheath application and leaf axil application at two time intervals, monthly and bimonthly were tested from the fourth month of planting till bunch emergence. Capsules were applied three times in the case of monthly application and it was applied two times for bimonthly application, for chemical treatment with chlorpyriphos 20 EC 0.05% @ 2 mL $L^{-1}$ there was only one application. Among the different treatments, leaf sheath application of Beauveria capsules @ 4 plant $^{-1}$ at monthly intervals was found to be the best method with least symptom expression and population, at the time of destructive sampling. Percentage reduction in the pest population was highest here with 90.9 per cent reduction in the population of grubs and adults and 83.33 per cent reduction in the case of pupal population respectively. Leaf axil application of capsules @ 4 plant $^{-1}$ at monthly application was also effective with percentage reduction of population to the tune of 72.72 in the case of grubs and adults and 83.33 in pupae. Bimonthly application of capsules (both leaf sheath and leaf axil) was not that much effective in controlling the pseudostem weevil. Leaf axil application of chlorpyriphos 20 EC 0.05 % @ 2 mL $L^{-1}$ was also effective with 81.81, 50 and 72.72 per cent reduction in the population of grubs, pupae and adults. The number of capsules used plant$^{-1}$ was 12 for leaf sheath and leaf axil application when applied monthly and it was eight when placed bimonthly.

In the curative management of pseudostem weevil, leaf axil application and bore hole placement of capsules was tested at weekly and fortnightly intervals. Weekly application of capsules was found to be the best treatment which was comparable with the single application of chlorpyriphos 20 EC 0.05 % @ 2 mL $L^{-1}$.Reduction in the population of grubs, pupae and adults was to the tune of 51.51, 60.8, 61.5 per cent in the former and 54.54, 52.17, 53.8 in the latter. The number of capsules used plant$^{-1}$ was 62 for weekly application and 32 for fortnightly application. Yield plant $^{-1}$ recorded at the time of harvest revealed that there was 14.9 per cent increase in weekly application compared to that in control. Chemical treatment has also shown 14 per cent increase.

Two methods of application of Metarhizium capsules for the prophylactic treatment of rhizome weevil has been tested at two time intervals *i.e.* monthly and bimonthly. The methods tested were plugging of rhizome with capsules at the time of paring followed by pit application and pit application alone. Among the treatments tested, plugging of rhizome @ four capsules at paring followed by pit application @ four, at monthly intervals exhibited superiority. These plants were found to be free of infestation at the time of destructive sampling. The same method when applied bimonthly was also found to be effective with 77.77 per cent reduction in grubs and 70 per cent reduction in adults. This method was comparable with the pit application of capsules @ four at monthly intervals, where the population was 77.77 and 60 per cent respectively. The number of capsules used plant $^{-1}$ was 28 in plugging followed by pit application at monthly intervals while it was 16 when applied bimonthly. In the second method where pit application was alone followed number of capsules used were 24 (monthly) and 12(bimonthly).

Curative treatment of rhizome weevil has been done by placing the Metarhizium capsules in the pit at varying intervals *viz.* fortnightly, monthly and bimonthly. Chemical treatment of Fipronil 0.3 G @ 10 g plant$^{-1}$ was also tested as the chemical check. Placement of capsules @ four plant$^{-1}$ at fortnightly intervals was the best treatment which was comparable with the one time soil application of Fipronil 0.3 G 10 g plant$^{-1}$. Pest population observed at the time of harvest revealed that there was 66.65 and 62.5 per cent reduction in the case of grubs and adults in fortnightly application and 66.65 and 58.32 per cent in the case of chemical treatment. Monthly and bimonthly application of capsules was not that much effective and the population assessed at the time of harvest was more in these treatments. The number of capsules used was 28, 12 and eight in fortnightly, monthly and bimonthly application respectively. Percentage increase in yield was 25.99 at fortnightly application which was on par with that of chemical treatment. Increase in yield was 16.66 per cent in monthly application. Increase in yield over control was least (2.42 per cent) in bimonthly application.

The salient findings of the investigation are

- For the management of pseudostem weevil, prophylactic application of Beauveria capsules in the outer leaf sheath @ 4 plant$^{-1}$, at monthly intervals is effective and superior to single application of leaf axil filling of chlorpyriphos 20 EC 0.05 % @ 2 mL L$^{-1}$. Dosage of capsules plant$^{-1}$ for one crop season is 12.

- For curative treatment of pseudostem weevil, application of Beauveria capsules in the outer four leaf axils @ 4 plant$^{-1}$ + application in the bore holes if any, at weekly intervals is effective and equivalent to single application of leaf axil filling of chlorpyriphos 20 EC 0.05% @ 2 mL L$^{-1}$. Dosage of capsules plant$^{-1}$ for one crop season is 62.

- For the management of rhizome weevil, prophylactic treatment of rhizomes by plugging them with Metarhizum capsules during paring @ 4 rhizome$^{-1}$ + pit application of Metarhizium capsules at monthly intervals @ 4 is effective and superior to single application of leaf axil filling of chlorpyriphos 20 EC 0.05% @ 2 mL L$^{-1}$. Dosage of capsules plant$^{-1}$ for one crop season is 28.

- For curative treatment of rhizome weevil, pit application of Metarhizium capsules @ four at fortnightly intervals is equally effective as one time soil application of Fipronil 0.3 G 10 g plant$^{-1}$. Dosage of capsules plant$^{-1}$ for one crop season is 28.

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**BIOEFFICACY OF CAPSULE FORMULATIONS OF *BEAUVERIA* AND *METARHIZIUM* IN MANAGING BANANA WEEVILS**

*by*


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**Abstract of the thesis**

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**ABSTRACT**

The study entitled "Bioefficacy of capsule formulations of *Beauveria* and *Metarhizium* in managing banana weevils" was conducted at Instruction Farm, College of Agriculture, Vellayani, Thiruvananathapuram and in the farmers field at Venganoor panchayath, Peringamala, Thiruvananathapuram during 2018-2020. The objective of the study was to validate the efficacy of biocapsules of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin for the management of banana weevils. The study intended to standardize the dosage and method of application of the Beauveria capsules against the pseudostem weevil, *Odoiporus longicollis* Oliver and rhizome weevil, *Cosmopolites sordidus* Germer. The capsules were formulated at a higher spore load of $10^{10}$ with HPMC coating and chitosan as carrier following the protocol developed by Remya and Reji (2019).

There were three sets of field experiments (1) Efficacy of biocapsules (both Beauveria and Metarhizium) applied as prophylactic treatment against both the weevils (2) Efficacy of Beauveria capsules in curative treatment of pseudostem weevil (3) Efficacy of Metarhizium capsule in curative treatment of rhizome weevil. For prophylactic treatment of pseudostem weevil, two methods of application were tested (1) applying one Beauveria capsule each on all four sides of the outer leaf sheath and (2) applying one capsule each in the outer four leaf axils on all four sides of the plant. The time intervals tested were (1) monthly intervals and (2) bimonthly intervals, starting from fourth month till bunch emergence. Curative management was tested by leaf axil application + bore hole application at two time intervals, weekly and fortnightly. To test the prophylactic treatment against rhizome weevil two methods were tested (1) Plugging the rhizomes at paring on all four sides @ four capsules rhizome$^{-1}$, followed by pit application @ four plant$^{-1}$ and (2) pit application alone @ four, plant$^{-1}$. The time intervals tested

were monthly and bimonthly. For curative treatment, the methods tested were pit application at fortnightly, monthly and bimonthly intervals.

Results revealed that, placement of Beauveria capsules in the outer leaf sheath @ four, plant$^{-1}$ at monthly intervals starting from fourth month till bunch emergence was superior with least infestation. Destructive sampling of plants done in the seventh month revealed that there was 90.9 per cent reduction in the population of grubs and adults and 83.33 per cent reduction in the pupal population. The other method, outer leaf axil application @ four, plant$^{-1}$ at monthly intervals was also found to be effective with a reduction in the population of 72.2 per cent in the case of grubs and adults and 83.33 per cent in pupae. This was on par with single application of chlorpyriphos 20 EC 0.05 % @ 2 mL L$^{-1}$ as leaf axil filling, where the reduction in population of grubs, pupae and adults was to the tune of 81.81, 50 and 72.72 respectively. Both the methods when applied bimonthly were observed to be less effective.

For curative treatment of pseudostem weevil, weekly application of capsules was superior with 51.51, 60.8 and 61.5 per cent reduction in the population of grubs, pupae and adults respectively. This was equally effective as one time application of chlorpyriphos 20 EC 0.05 %@ 2 mL L$^{-1}$ as leaf axil filling, where the population observed was 54.54, 52.17 and 53.8 per cent of grubs, pupae and adults. Plants in which fortnightly application was done exhibited less control with 24.24, 34.78 and 33.33 per cent reduction of grubs, pupae and adults,respectively. Though there was a marginal increase in yield in the best method, statistically it did not vary from those of others.

In the case of rhizome weevil, for prophylactic treatment, placement of four Metarhizium capsules by plugging on the four sides, followed by pit application at monthly intervals was more effective than all other treatments. No tunneling was observed at the time of destructive sampling and the pest stages were totally absent (100 per cent control). When the same method was followed at bimonthly intervals, population reduction was 77.77 and 70 per cent of grubs and adults respectively.

Pit application was also effective when applied every month, with 77.77 and 60 per cent reduction of grubs and adults respectively.

For curative treatment of rhizome weevil, placement of four capsules in the planting pit at fortnightly intervals was equally effective as one time soil application of Fipronil 0.3 G @ 10g plant-$^{1}$. Population assessed at the time of harvest revealed that there was 66.65 and 62.5 per cent reduction in the population of grubs and adults, compared to 66.65 and 58.325 per cent in chemical treatment. Analysis of data on yield plant$^{-1}$ revealed that treatment with capsules at fortnightly and monthly interval were on par with that of chemical treatment and the treatment at bimonthly intervals was equivalent to the yield from untreated plants.

It is concluded that for prophylactic management of pseudostem weevil, Beauveria capsules placed in the outer leaf sheath at monthly intervals @ four plant$^{-1}$, starting from fourth month till bunch emergence is effective, while for curative treatment placement of capsules in the outer four leaf axils @ four plant$^{-1}$ and one each in the bore hole at weekly intervals is as effective as one time application of chlorpyriphos 20 EC 0.05 % @ 2 mL L$^{-1}$ treatment. For rhizome weevil, placement of Metarhizium capsules @ four plant$^{-1}$ by plugging the rhizome at the time of paring, followed by pit application at monthly intervals @ four plant$^{-1}$ is effective as prophylactic measure and for curative treatment, pit application of capsules at fortnightly intervals @ four plant$^{-1}$ is effective and equivalent to single application of Fipronil 0.3 G @ 10g plant-$^{1}$.

Therefore the biocapsules of Beauveria can be recommended for the management of pseudostem weevil and those of Metarhizium can be recommended for the management of rhizome weevil.