CHARACTERIZATION, EVALUATION AND FORMULATION OF *Beauveria bassiana* (Bals.) STRAINS AGAINST RICE BUG, *Leptocorisa* spp. (HEMIPTERA: ALYDIDAE)

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Characterization, evaluation and formulation of *Beauveria bassiana* (Bals.) strains against rice bug, *Leptocorisa* spp. (Hemiptera: Alydidae)"** is a *bonafide* record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that thesis entitled **"Characterization, evaluation and formulation of *Beauveria bassiana* (Bals.) strains against rice bug *Leptocorisa* spp.(Hemiptera: Alydidae)"** is a *bonafide* record of research work done independently by **Nasiya Beegum A. N. (2015-21-018)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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NASIYA BEEGUM A. N.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

Sl. No.	Symbol	Abbreviations
1	@	At the rate of
2	^{o}C	Degree Celsius
3	CD	Critical difference
4	cfu	Colony forming units
5	ITS	Internal transcribed spacer
6	PCR	Polymerase chain reaction
7	DNA	Deoxyribonucleic acid
8	rDNA	Ribosomal DNA
9	sp. or spp.	Species (Singular and Plural)
10	mg	Milligram
11	mg^{-1}	Per milligram
12	ha^{-1}	Per hectare
13	PEG	Polyethylene glycol
14	CMC	Carboxymethyl cellulose
15	rpm	Revolutions per minute
16	*viz.*	Namely

17	ha	Hectare
18	l	Litre
19	µ	Micro
20	bp	Base pairs
21	cm	Centimeter
22	DAI	Days after inoculation
23	DAS	Days after storage
24	DAT	Days after treatment
25	EPF	Entomopathogenic fungi
26	*et al.*	And others
27	Fig.	Figure
28	g	Gram
29	g^{-1}	Per gram
30	KAU	Kerala Agricultural University

Introduction

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops of the world and forms staple food for more than three fifths of humanity. Nearly ninety per cent of the area, production and consumption of rice happens to be in South and East Asian countries. In India, it is cultivated in an area of 43.7 million hectares, with an annual production of 106.29 million tonnes (Directorate of Economics and Statistics, 2018). The productivity of rice is greatly influenced by several factors, among which the insect pest menace is one of the most predominant.

The earhead bugs, *Leptocorisa* spp. are one of the most important sap sucking insect pest of rice in the tropics. Loss due to bug infestation ranges from 30 per cent (Tiwari, *et al.*, 2014) and often extends up to 98 per cent in severe cases (Bhadauria and Singh, 2009). Both nymphs and adults suck sap from developing rice grains during the milky grain stage, leading to discoloured, empty or half-filled grains. Nymphs are more destructive than adults.

Recommendations for the management of rice bug involves removal of grassy weeds, synchronized planting and use of broad spectrum insecticides. However, more often than not, cultural control measures are neglected and farmers resort to indiscriminate application of insecticides. Use of insecticides during grain stage is bound to result in pesticide residues in grains. Yet, viable, environment friendly and sustainable alternatives to insecticides for the management of rice bugs are yet to be developed. Biological control, one of the most attractive strategies in pest management, has hardly been successful in the management of the rice bug for want of effective natural enemies.

Entomopathogenic microbes, particularly fungi, have, been receiving increased attention of late as potential biocontrol agents of the rice earhead bug. *Beauveria bassiana* (Bals.), for instance, is an entomopathogenic fungus that grows naturally in soils throughout the world. Steinhaus (1956) reported that *B. bassiana* causes mycosis in 175 insect hosts from lepidopteran, coleopteran and

hemipteran orders. They are ideally suited as biopesticides owing to their broad host range as well as amenability for mass production and formulation.

The All India Co-ordinated Research Project on Biological Control of Crop Pests at Thrissur, Kerala isolated an entomopathogenic fungus (EPF) from rice bug which was later identified as *B. bassiana* (AICRP, 2015). Subsequent studies confirmed the potential of the above isolate as a natural enemy of the rice bug (Chandran, 2016) calling for further efforts towards the commercial exploitation of the isolate. Realization of the above goal however, demands characterization of the isolated fungus as a primary step. Entomopathogenic fungi, including *B. bassiana* display considerable heterogeneity, which necessitates any identification based on morphological characters to be supported by molecular characterization. Confirmation of the identity need to be followed by attempts at evaluation of the efficacy of the isolate as well as developing ecofriendly formulations that combines prolonged shelf life, ease of application and efficacy.

It is in this context that the present study entitled "Characterization, evaluation and formulation of *Beauveria bassiana* (Bals.) strain against rice bug, *Leptocorisa* spp. (Hemiptera: Alydidae)"was undertaken with the following objectives:

1. Collection, isolation and molecular characterization of local strains of *Beauveria bassiana* from rice growing tracts of Kerala
2. Evaluation of *B. bassiana* against earhead bug of rice, *Leptocorisa* spp.
3. Identification of cost effective mass production technology for the selected strain of *B. bassiana*
4. Formulation of the most effective strain of *B. bassiana*

Review of literature

2. REVIEW OF LITERATURE

The literature pertaining to the isolation and molecular characterization of *B. bassiana*, bioassay of rice bugs using *B. bassiana* and development of cost effective formulations of *B. bassiana* are briefly reviewed here.

2.1 *Beauveria bassiana* (Balsamo) Vuillemin

Beauveria bassiana (Balsamo) Vuillemin is an entomopathogenic fungus belonging to Division Ascomycota, Order Hypocreales and Family Cordycipitaceae. It causes white muscardine disease in various arthropod species. *Beauveria bassiana* was first reported by Agostino Bassi from silk worm larvae, *Bombyx mori* L. in 1835. The Genus *Beauveria* is famous for producing an array of biologically active secondary metabolites such as oosporin, bassianin, tenellin, beauvericin, bassianolides, beauveriolides *etc.* that have use in industrial, pharmaceutical and agricultural sectors (Xu *et al.*, 2009).

Petch (2006) was among the first to attempt classification of the genus *Beauveria* at species level. He classified it into eight different species *viz, B. effusa, B. densa, B. brongniartii, B. bassiana, B. globulifera, B. delacroixii, B. vexans* and *B. stephanodesis* based on conidial shape. Currently, six species of the genus are recognized, *viz. B. bassiana, B. brongniartii* (Sacc.), *B. caledonica* (Bissett and Widden), *B. bassiana* cf. Clade C, *B. vermiconia* (de Hoong and Rao) and *B. amorpha* (Sevim *et al.,* 2010). *Beauveria bassiana* is pathogenic to a wide range of insects belonging to Coleoptera, Hemiptera, Lepidoptera, Thysanoptera and Isoptera (Goettel *et al*., 2010).

2.2 Bioefficacy of *Beauveria bassiana* against insect pests

2.2.1 Bioefficacy of *Beauveria bassiana* against lepidopterous pests

Beauveria bassiana is one of the most extensively studied of all entomopathogenic fungi. It has been mainly used for biocontrol of lepidopteran pests with a number of reports on epizootic occurrences.

Beauveria bassiana remains one of the most widely used entomopathogens after *Bacillus thuringiensis*. It has a host range of over 700 species of insects, spread over most of the orders of the class Insecta (Meyling *et al.,* 2009). *B. bassiana* has been particularly successful against members of the Order Lepidoptera and to a lesser extent against hemipterans. Several studies have shown them to be as effective as synthetic insecticides.

Rao (1975) was among the first in the country to report infection of lepidopterans like the pink stem borer, *Sesamia inferens* (Wlk), rice stem borer, *Scirpophaga incertulas* (Wlk), rice leaf roller, *Parnara* spp., rice leaf folder, *Cnaphalocrosis medinalis* (Gn.) and stalk borer, *Chilo auricilius* Dudgn by *B. bassiana.*

Natural incidence of *B. bassiana* in larval populations of leaf folder, *Cnaphalocrocis medinalis* (Gn.) was also reported from Pondicherry by Ambethgar (1997). Easwaramoorthy (2003) isolated the white muscardine fungus from cadaver of internode borer of sugarcane, *Chilo sacchariphagous indicus* obtained during a field survey conducted at Coimbatore, Tamil Nadu.

Naik (2012) conducted a roving survey in Chittoor, Anantapur and Kurnool districts of Andhra Pradesh for local isolates of *B. bassiana* infecting lepidopteran caterpillars. Two strains, namely, SGb and MKb, obtained from *S. litura* and *H. armigera*, respectively, caused more than 50 per cent mortality of first, second and third instar larvae of *Spodoptera litura*. The mean mortality of I^{st}, II^{nd} and III^{rd} instar larvae of *S. litura* was 76.29 per cent for isolate SGb, which was followed by MKb strain with 63.70 per cent mortality, both applied at the rate of $1x10^{10}$spores ml^{-1}. Among the 13 strains of *B. bassiana* evaluated at the rate of 1×10^{8} spores ml^{-1}, six strains recorded more than 50 per cent mortality of I^{st} instar larvae, while three strains recorded more than 50 per cent mortality in II^{nd} instar larvae. However, all the thirteen strains caused less than 50 per cent mortality in III^{rd} instar larvae of *S. litura*. The strains of *B. bassiana* recorded less than 50 per cent mortality in I^{st}, II^{nd} and III^{rd} instar larvae of *S. litura*, when

applied at the rate of at 1 x 10^6 spores ml^{-1}. The native strain SGb recorded the least LC_{50} of 5.2 x 10^6 and $LC_{90}$of 1.1 x 10^{10} spores ml^{-1}, followed by native strain MKb.

Yadav *et al.* (2004), who evaluated a number of biopesticides against *Helicoverpa armigera* Hubner (Noctuidae: Lepidoptera) on chickpea, reported that *B. bassiana,* applied at the rate of 1 Kg ha^{-1} reduced the larval population from 2.63 per plant to 2.22, 1.54 and 1.43 per plant three, seven and fourteen days after spraying.

Rijal *et al.* (2008) evaluated the native strains of *M. anisopliae* and *B. bassiana*, against the third instar larvae of the pod borer, *Helicoverpa armigera* in chickpea, in terms of mortality, infection rate and LT_{50} under experimental conditions in Nepal. Out of the four *M. anisopliae* and two *B. bassiana* isolates evaluated, *M. anisopliae* M1 and *B. bassiana* B3, were reported as the most virulent. Ten days after treatment with M1 and B1 isolates at a concentration of 10^7spores ml^{-1}each, the larval mortality exceeded 85 per cent.

In yet another experiment, topical application of *B. bassiana* at four different concentrations (0.1, 0.125, 0.2 and 0.25 x 10^8spores ml^{-1}) on fourth instar larvae of *H. armigera* resulted in mortality of 76.7 per cent at the highest concentration of 0.25 x 10^8spores ml^{-1}(Prasad and Sayed, 2010). It was also reported that the mortality started two to three days after treatment. Infected larvae had morphological abnormalities as well as fragile skin.

An experiment was conducted at Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, for evaluating the efficacy of *B. bassiana* against pod borer complex of pulses. The treatments included wettable powder of *B. bassiana* at the rate of 1.0 and 1.5 Kg ha^{-1}, spinosad 45 SC at the rate of 73 g a. i. ha^{-1} and *B. thuringiensis* at the rate of 1.5 Kg ha^{-1}. *Beauveria bassiana* WP at the rate of 1.5 Kg ha^{-1} recorded significantly less grain damage by pod borer and also recorded more yield compared to all other treatments except spinosad (NBAIR, 2010).

Wraightand Carruthers (2010) screened 43 isolates of *B. bassiana* against diamond back moth (*Plutella xylostella* Linnaeus) (DBM), corn ear worm (*Helicoverpa zea* Boddie) (CEW), European corn borer (*Ostrinia nubilalis*) (ECB), fall armyworm (*S. frugiperda*) (FAW), beet armyworm (*Spodoptera exiigua* Hubner) (BAW), black cutworm (*Agrotis ipsilon* Hufnage) (BCW), cabbage worm (*Pieris rhapae*) (ICW) and cabbage looper (*Trichoplusia ni*) (CL). All the tested species were susceptible to *B. bassiana*. Beet armyworm and corn earworm were most susceptible to *B. bassiana* while fall armyworm was the least. Six days after spraying, mean lethal concentration (LC_{50}) of *B. bassiana* against CL, BCW, CEW, BAW, DBM, FAW, ECB and ICW were 125, 273, 4, 5, 7, 11, 98 and 12 conidia per mm^2, respectively.

Moorthi *et al.* (2011) evaluated *B. bassiana* isolates, *viz.*, Bb_{02}, Bb_{09} and Bb_{10} against tobacco caterpillar, *Spodoptera litura* using leaf spray method. Bb_{10} was found to be the most effective isolate, registering 80 per cent mortality four days after treatment and was followed by Bb_{09} and Bb_{02} with 66.67 and 73.33per cent mortality respectively.

Karabhantanal and Awaknavar (2012) who studied the effect of *B. bassiana* against *H. armigera* in tomato, reported that application of *B. bassiana*, at the rate of $1x\ 10^{11}$ conidiaml^{-1} resulted in 13.19 per cent larval mortality as well as eight per cent increase in yield over untreated control.

Susceptibility of second instar larvae of *Helicoverpa armigera* to seven genetically different strains of *B. bassiana* was tested under laboratory conditions by Swathi *et al.* (2017) at ICAR-IIHR, Bengaluru. Strain 4 induced cent per cent mortality, with an LC_{50} value of 2.02×10^5spores ml^{-1}. It was followed by strain 1 (92.5 % mortality) with LC_{50} value 52.48×10^5spores ml^{-1}.

2.2.2. Bioefficacy of *Beauveria bassiana* against non lepidopterous pests

Leite *et al.* (1987) carried out laboratory studies to determine the pathogenicity of *B. bassiana* against fifth instar nymphs of the

pentatomid, *Nezara viridula* on soybean. The entomopathogen caused 66.7 per cent mortality of the bug when sprayed at the rate of 10^7spores ml^{-1}.

Hazarika and Puzari (1995) evaluated *B. bassiana* against different stages of rice hispa, *Dicladispa armigera* (Olivier) in Sibsagar district, Assam and reported that the pest could be effectively controlled by *B. bassiana.* Egg stage was found to be more susceptible to *B. bassiana* than other stages. Infection of eggs ranged from16.95 to 45.15 per cent, while that of the adults was 1.67 to 40.63 per cent, depending on the season.

Ability of *B. bassiana* to infect silver leaf whitefly, *Bemisia argentifolii* (Bellows and Perring) even at very low relative humidity under laboratory conditions was demonstrated by Wraight *et al.* (2000) in Texas. Whitefly suffered 35 per cent infection at a relative humidity of 25 per cent when treated with *B. bassiana* at the rate of 0.6 to 1.4 x 10^3 conidia $mm^{-2}$of leaf.

Four strains of *B. bassiana*, *viz.* Bb 1, 10, 25 and 65 obtained from Argentina were evaluated against the triatomine bug, *Triatoma infestans* Klung and the LD_{50} values for both nymphs and adults of the pest were calculated by Leucona *et al.* (2001). Bb 65 and Bb 10 recorded the lowest LD_{50} values for nymphs (1.8 and 2.1 x 10^6 spores ml^{-1}respectively), while in the case of adults Bb 1 and Bb 10 (5.3 and 1.6 x 10^6 spores ml^{-1} respectively) registered the lowest LD_{50} values. The highest mean mortality of 97 per centof adult bugs was recorded by Bb 10 at a temperature of 26^0 C.

El-Zoghby (2003) reported that the application of *B. bassiana* at the rate of 1x10^6 conidia ml^{-1} reduced the population of stink bug, *Nezara viridula* L. in soybean by 23 per cent after 25 days. Abdel-Raheem *et al.* (2011) reported cent per cent mortality of *Nezara viridula* 11 days after the treatment with *B. bassiana* at the rate of 3 x 10^5spores ml^{-1}in soybean.

Patel *et al.* (2006) screened two *B. bassiana* isolates namely, LRC 28 and RSB at the rate of $5x10^{12}$ spores ml^{-1} against rice stink bug, *Oebalus pugnax* (F.) in small plot experiments. They reported that both isolates of *B. bassiana* were nearly as effective as chemical insecticide in suppressing the population of rice stink bug 7-8 days after treatment.

A screen house study at Uganda examined the effect of endophytic *B. bassiana* in banana plants against the banana rhizome weevil, *Cosmopolites sordidus* (Germar). It was observed that root dipping of tissue cultured banana plants in *B. bassiana* suspension ($1.5x10^7$ conidia ml^{-1}) for 2 h resulted in 42.0 to 86.7 per cent reduction in damage to plants (Akello *et al.*, 2008).

Karthikeyan and Jacob (2010) conducted a field trial at Regional Agricultural Research Station, Pattambi, Kerala to find out the efficacy of *B. bassiana* against rice blue beetle, *Leptisma pigmaea* Baly. Application of *B. bassiana*, at the rate of 10^7 spores per ml resulted in 61 to 72 per cent reduction in damage over control.

Suresh *et al.* (2010) conducted field experiments to evaluate the bioefficacy of *B. bassiana* along with that of selected insecticides such as chlorpyriphos, dichlorvos, endosulfan, imidacloprid, profenophos, neem oil and thiamethoxamat recommended concentrations against the cotton mealy bug, *Phenacoccus solenopsis* on cotton and the papaya mealybug, *Paracoccus marginatus* on papaya at Tamil Nadu Agricultural University, Coimbatore. Among the insecticides evaluated, chlorpyriphos recorded 100 per cent reduction followed by dichlorvos (90 per cent), imidacloprid (89.99 per cent), thiamethoxam (86.66 per cent) and profenophos (80 per cent), while neem oil recorded the lowest mortality of 63.33 per cent. *Beauveria bassiana* was found to be moderately effective and caused 77 per cent mortality, 10 days after treatment.

A field trial was conducted with three different entomopathogenic fungi *viz., L. lecanii, B. bassiana* and *M. anisopliae* at the rate of $1x10^8$ cfu ml^{-1} along

with standard check, acephate 75 SP (1.5 g/l) against brown plant hopper, *Nilaparvata lugens* by Reddy *et al.* (2013). Acephate was found to be the most effective treatment with mean population of 19.30 hoppers/hill. Treatments with *L. lecanii, B. bassiana* and *M. anisopliae* resulted in 63.70, 55.60 and 54.40 hoppers per hill respectively, five days after treatment. However, 10 days after second spray, the mean population of hoppers in plots treated with *M. anisopliae* and *B. bassiana* were 18.80 and 17.20 hoppers per hill, respectively and were on par with the hopper population in plots treated with acephate.

Beauveria bassiana has been known to produce a number of secondary metabolites such as beauvericin, bassianin, beauveriolides, bassianolide, tenellin and beauverolides (Jeffs and Khachatourians, 1997) which are toxic to insects.

Hamill *et al.* (1969) reported that beauvericin was a well-known mycotoxin produced by many fungi, such as *B. bassiana* and *Fusarium* spp. It is a cyclic peptide with repeating units of three molecules of N-methyl phenylalanine and three molecules of 2-hydroxy isovaleric acid and having empirical formula as $C_{45}H_{57}N_3O_9$. Beauvericin is soluble in organic solvents and the ionophoric characteristics of the molecules permits cation transport through the cell membrane. Gupta *et al.* (1995) extracted two analogues of beauvericin as beauvericin A and beauvericin B from *B. bassiana.*

Isarolides A, B and cyclodepsipeptides in *B. brongniartii* (Sacc.) were isolated by Briggs *et al.* (1966). Elsworth and Grove (1974) isolated two similar cyclotetradesipeptides, beauverolides H and I from the mycelium of *B. bassiana* from South Africa. Another cyclodepsipeptide, bassinolide was isolated by Suzuki *et al.* (1977) from mycelium of *B. bassiana.* Bassinolide was formed from four moles each of D-alpha hydroxyisovaleric acid and L-N methyl leucine.

Quesada-Moraga and Alain (2004) reported a toxic protein, bassiacridin refined from *B. bassiana* isolate collected from locust using chromatographic technique. Inoculation of 4^{th} instar nymphs of locust, *Locusta migratoria* with

the wholesome protein at the rate of 3.3 µg toxin per gram body weight triggered a mortality of 50 per cent.

2.2.3 Bioefficacy of *Beauveria bassiana* against rice bug

Loc and Chi (2005) reported a number of isolates of *B. bassiana* as effective against rice earhead bug, *Leptocorisa acuta* in Vietnam. Application of the fungus resulted in 45.3 to 74.9 per cent mortality of the bug ten days after spraying.

Kalita *et al.* (2009) evaluated *M. anisopliae* and *B. bassiana* against rice bug during 2007 - 2008 at the ICAR Research Complex, Sikkim.Sprays of *M. anisopliae* and *B. bassiana* at boot leaf stage of rice was effective in reducing the grain damage by 4.8 and 5.15 per cent respectively.

Similar results were also reported by Loc and Chi (2005), who evaluated 12 different isolates of *B. bassiana* against *L. acuta.* Mortality ranging from 57.5 to 77.7 per cent after 10 days of treatment was obtained, when the isolates were applied at a dose of 10^8spores ml^{-1}.

Reduction of nymphal population of the bug by 40 – 93 per cent was achieved using different isolates of *B. bassiana* by Herlinda *et al.* (2008). Nymphs showed loss of appetite and slow movement in the initial stages of infection.

Girish and Balikai (2015) also reported a decrease in gundhi bug population following spraying with *B. bassiana* at the Agricultural Research Station, Uttar Kannada, Karnataka. The mean population was 2.29 bugs per hill in plots treated with *B. bassiana* at the rate of 10^7 spores ml^{-1}as against 7.42 bugs per hill in untreated control plots.

Chandran (2016) conducted pot culture and field experiments with *B. bassiana, M. anisopliae* and *Lecanicillium lecanii* against rice bug. In the pot culture experiment, the fungi were assayed at four different concentrations from 10^5 to 10^8 spores ml^{-1}along with the insecticide malathion 500 g a.i. ha^{-1} and an

untreated control. Ten days after treatment, *B. bassiana* was the most effective among the three fungal pathogens tried, with mortality values ranging from 68.88 per cent at 10^5spores $ml^{-1}$to the highest value of 97.77 per cent at 10^8spores ml^{-1}. The most effective concentration of each of the entomopathogenic fungus identified in the pot culture experiment was evaluated along with malathion 500g ai ha^{-1} under field conditions. The results of field evaluation broadly agreed with the findings of the pot culture studies. Ten days after treatment, *B. bassiana* recorded a significant reduction in mean rice bug population which was found superior to both *M. anisopliae* and *L. lecanii* and was on par with malathion.

The efficacy of ten *B. bassiana* isolates from alfalfa weevil, *Hypera postica* (Gyllenhall) (Coleoptera: Curculionidae) as well as leaf beetle, *Gonioctena fornicata* (Bruggemann) (Coleoptera: Chrysomelidae), collected from Tokat province, Turkey was tested against *H. postica* larvae at four different concentrations of10^3, 10^5, 10^7 and 10^9spores ml^{-1}. The isolates GN-4, GN-23, HP-6 and HP-30 recorded more than 95 per cent mortality at a concentration of 10^7 spores ml^{-1}, five days after treatment, thus underscoring the potential of local isolates of *B. bassiana* as biocontrol agents against *H. postica* (Baysal *et al.*, 2018)

Sayed *et al.* (2019) conducted experiments to identify the bioefficacy of four genetically different isolates of *B. bassiana* (isolates 1,2,3 and 4) against rose aphid, *Macrosiphum rosae*L. Laboratory bioassay results indicated that isolate 1 was significantly superior (LC_{50} value of 6.46 x 10^4) to the other three (LC_{50} value of 1.46 x 10^5, 1.52 x 10^5 and 1.71 x 10^5, respectively). In field trials, isolate 1 at a concentration of 4.6 x 10^6spores ml^{-1} achieved the highest reduction in rose aphid infestation(6.97–8.7 aphid individuals per leaf).

The comparative efficacy of KAU isolate of *Lecanicillium saksenae* with NBAIR isolates of *L. lecanii*, *M. anisopliae* and *B. bassiana* against rice bug, *Leptocorisa acuta* was evaluated. Among the four biocontrol agents, *L. saksenae*

recorded a mean number of 1.75 bugs per sweep and was on par with *B. bassiana*(2.75bugs /sweep) (AICRP, 2019).

The importance of *B. bassiana* as an endophyte and as an antagonist to plant pathogenic fungi has become apparent only in the last two decades (Ownley *et al*., 2008). Renuka *et al.,* (2017) conducted glasshouse experiments to study the endophytic ability of six strains of *B. bassiana* in maize leaf and stem tissues. *B. bassiana* treated and untreated control plants were artificially infested with larvae of *Chilo partellus* to assess the stem borer damage. The isolate Bb-23 recorded the highest colonization in old stems (20.37 per cent), young stems (21.29 per cent) and in old leaves (27.78 per cent). Moreover, *B. bassiana* treated plants recorded significantly lower incidence of dead hearts (2.2-11.1%) and lower stem tunneling (2.7-4.3cm/plant) as compared to the untreated control plants with an average of 28.86 per cent dead hearts and 13.41 cm stem tunnels per plant.

2.3 Compatibility of *Beauveria bassiana* with pesticides

Compatibility of bioagents with other management measures, particularly chemical control is a major consideration in IPM since a natural enemy has to be often used in conjunction with pesticides due to economic and time constraints. Several workers have assessed the compatibility of *B. bassiana* with commonly used agrochemicals, which is reviewed here.

Alizadeh *et al.* (2007) evaluated the compatibility of *B. bassiana* with a number of commonly used pesticides such as flufenoxuron, imidacloprid, endosulfan, teflubenzuron, phosalone and amitraz. The effect of the above pesticides on vegetative growth, conidial germination and sporulation of the fungus were studied. All the insecticides tested, except flufenoxuron were compatible with *B. bassiana* and hence could be used along with *B. bassiana* in integrated pest management (IPM). Flufenoxuron, however, adversely affected the vegetative growth and sporulationof *B. bassiana.*

Compatibility of *B. bassiana* with dichlorvos 0.12 per cent, phosphamidon 0.005 per cent, cypermethrin 0.006 per cent, alphamethrin 0.008 per cent and

deltamethrin 0.002 per cent was studied by Puzari *et al.* (2006) using poisoned food technique. Phosphamidon, cypermethrin, alphamethrin and deltamethrin registered 50, 40, 48, and 46 per cent inhibition respectively while dichlorvos caused complete inhibition of the fungus.

Filho *et al.* (2001) conducted a similar experiment to assess the compatibility of *B. bassiana* with nine commonly used insecticides *viz.* acephate, carbosulfan, deltamethrin, diafenthiuron, endosulfan, fipronil,imidacloprid, monocrotophos and thiamethoxam. Results showed that thiamethoxam 0.005 per cent, acephate 0.15 per cent and diafenthiuron 0.1 per cent were compatible with *B. bassiana*, while total incompatibility was observed for carbosulfan 0.1 ppm.

Amutha *et al.* (2010) studied the compatibility of *B. bassiana* with twelve commonly used insecticides. Thirty days after inoculation, chlorpyriphos recorded 48.46 per cent inhibition in fungal growth over control, while spinosad, econeem, quinalphos, acetamiprid, endosulfan and thiodicarb recorded 50 to 79 per cent inhibition over control. Imidacloprid and triazophos recorded 80 to 90 per cent reduction and profenofos, indoxacarb and methyldemeton recorded more than 90 per cent inhibition as compared with control.

Dhar and Kaur in 2009 tested the compatibility of *B. bassiana* with acetamiprid (0.003 %) using poisoned food technique and documented an increase in the radial growth of *B. bassiana*, suggesting absence of any inhibitive effect. This, however, was contrary to the inhibitory effect of acetamiprid on radial growth reported earlier by Anderson and Roberts (1983).

Six strains of *B. bassiana* were tested for compatibility to four commonly used insecticides *viz.* chlorpyriphos, imidacloprid, spinosad and indoxacarb. Poisoned food technique was followed at field recommended doses of the insecticides (chlorpyriphos 0.05 %, imidacloprid 0.0045 %, spinosad 0.018 % and indoxacarb 0.0145 %). The results showed that all the strains were compatible with spinosad and imidacloprid. While no significant reduction was observed in terms of radial growth, significant reduction in sporulation and spore viability was

recorded for indoxacarb. Chlorpyriphos was found to be completely incompatible with all the strains (Rajanikanth *et al.*, 2010).

Joshi, *et al.* (2018) conducted an experiment to test the impact of chemical pesticides on *B. bassiana.* Six insecticides *viz.,* profenophos (4, 2, 1, 0.5, 0.25 %), indoxacarb (1.32, 0.66, 0.33, 0.165, 0.082 %), emamectin benzoate (0.5, 0.25, 0.125, 0.062, 0.031 %), novaluron (3.0, 1.5, 0.75, 0.375, 0.187 %), chlorantraniliprole (0.6, 0.3, 0.15, 0.075, 0.037 %) and lambda cyhalothrin (1.2, 0.6, 0.3, 0.15, 0.025 %) as well as four fungicides *viz.,* carbendazim (2, 1, 0.5, 0.25, 0.125 %), mancozeb (4, 2, 1, 0.5, 0.25 %), hexaconazole (0.5, 0.25, 0.125, 0.062, 0.031 %) and propiconazole (1, 0.5, 0.25, 0.125, 0.062 %)were evaluated at five different concentrations for their effect on sporulation and mycelial growth of the EPF. Profenophos was the most incompatible insecticide, causing cent per cent inhibition, whereas other insecticides like chlorantraniliprole, indoxacarb, emamectin benzoate, novaluron and lambda cyhalothrin were found to have little adverse effect on growth and sporulation of *B. bassiana.* Among the four fungicides evaluated, only mancozeb showed compatibility to some extent at the lower concentrations of 0.5 and 0.25 per cent (49.16 and 44.80 per cent inhibition) while carbendazim, propiconazole and hexaconazole showed complete inhibition of the fungus at all concentrations.

In general *B. bassiana* enjoys a high degree of compatibility with insecticides. However, the same cannot be said about fungicides. Most studies have reported near total inhibition of the fungus by most fungicides at all concentrations evaluated.

2.4 Molecular identification of entomopathogenic fungi

Molecular techniques serve as valuable tools for accurate identification of fungal species, in addition to identification using morphological characteristics (Bruns *et al.*, 1991).

Dhar, *et al*. (2019) isolated 13 entomopathogenic fungi from soils of Punjab in India. Molecular characterization of isolates were done using PCR amplification with specific ITS primer which led to the thirteen being confirmed as three isolates of *B. bassiana* and were named as BbR1, BbR2 and BbR3. Comparative RAPD-PCR amplification with 10various RAPD primers indicated that all the three isolates were closely related. Genetic relatedness dendrogram was developed using RAPD-PCR data by UPGMA.

Random Amplified Polymorphic DNA (RAPD) technology has been used widely to identify entomopathogenic fungi (Fungaro *et al.,* 1996; Jensen *et al.*; 2001 and Castrillo *et al.,* 2003). Restriction Fragment Length Polymorphism (RFLP) as well as Sequence Characterized Amplified Region (SCAR) were also used to identify various isolates of entomopathogenic fungi (Hegedus and Khachatourians, 1995, Li *et al.,* 2007; Castrillo *et al.* 2003).

Ramanujam *et al.* (2011) isolated and identified 31 *Lecanicillium* spp. using ITS 1, ITS 2 and 5.8 S gene of rRNA sequence. Schoch *et al.* (2012) attempted to sequence the Internal Transcribed Spacer (ITS) region using ITS primer sets.

Naik (2012), used RAPD technique with six random primers (OPE-04, OPA-15, OPAA-04, OPAA-06, OPAA-14 and UP-PCR) to show 100 per cent polymorphism. Five effective and promising *B. bassiana* strains (MKb, Kb, SGb, Hb and Bb) were characterized using rDNA-ITS sequences. All the five strains of *B. bassiana* showed 100 per cent homology with already submitted *B. bassiana* strain in NCBI GenBank.

2.5 Development of formulations of entomopathogenic fungi

The essential constituents of a mycoformulation are the living entity or technical ingredient, inert carrier material as well as adjuvants like humectants, stabilizing agents, wetting agents, spreading agents, binders and emulsifiers or a combination of these.

Wright and Chandler (1992) standardized protocol for the development of biorational mycoinsecticide formulation of *Beauveria bassiana* for the management of cotton boll weevil, *Anthonomus grandis.* The product contained boll weevil pheromone, sticker and Nufilm as UV protectant.

Dhembare and Siddique (2004) evaluated the mycoinsecticide formulations of *B. bassiana* in laboratory against gram pod borer, *Helicoverpa armigera* Hubner. They recorded the highest reduction in population of larvae at 72 h after treatment. They also observed that early instar larvae (1^{st} and 2^{nd} instar) were more susceptible to *B. bassiana* than later instars.

Ignoffo *et al.* (1979), developed formulations of *B. bassiana* using inert materials such as perlite or talc, starch, kaoline, bentonite *etc.* Puzari and Hazarika (1991) studied the efficacy of *B. bassina* added with various spreaders or stickers such as Tween 80 or Hamam and reported a very high percentage of mortality of *Dicladispa armigera.*

Ramarethinam *et al.* (2002) evaluated talc based formulation of *B. bassiana* called “Bio power” for its shelf life in three locations *viz.*, Ooty, Coimbatore and Chennai. Longer conidial viability and better survival ability of 11 months were recorded in Ooty followed by Coimbatore with 9 months.

2.5.1 Carrier material for formulating entomopathogenic fungus

Talc is an inorganic inert carrier, which enhances the viability and growth of the fungal spores by protecting them from desiccation and death of cells. Talc has good miscibility with water, storage stability and is convenient for spraying. Talc has chemical inertness, low moisture equilibrium, reduced moisture absorption, relative hydrophobicity and prevent the formation of hydrate bridges that helps longer shelf life.

The superiority of talc in maintaining the virulence of entomopathogenic fungi may be due to its high amount of carbohydrate and mineral composition. Brar *et al.* (2006) who suggested that a wettable powder should ideally consist of

1–10% dispersant, 50–80% technical powder, 3–5% surfactant and 15–45% filler by weight. Fillers are hydrophilic and usually contain silica which prevents cake formation. Among the dried formulations of biopesticides, much attention has been paid to WPs because of their good miscibility with water, longer shelf life and ease of application as sprays.

Guerri-Agullo *et al.* (2010) described the efficacy of *B. bassina* solid formulations on red palm weevil, *Rhynchophorus ferrugineus* (Oliver) (Coleoptera: Curculionidae). The formulation composed of a highly pathogenic strain of *B. bassina* isolated from *R. ferrugineus.* The formulation was evaluated three times in 2009 in two locations (Catral and El-Hondo), at three months interval. The authors reported, that it had caused 70 – 85 per cent mortality of *R. ferrugineus*.

2.5.2 Effect of adjuvants on formulation of *Beauveria bassiana*

Humectants such as diethylene glycol and glycerol each at 0.1 per cent was added to the spore suspension of the fungus *Lecanicillium lecanii* by Easwaramoorthy and Jayaraj (1977) for increasing the efficacy of the pathogen against the coffee green bug (*Coccus viridis* Green). The mortality rate of the bug increased from 47.5 to 62.6 per cent by using *V. Lecanii* formulated with glycerol 0.1 per cent and to 92.6 per cent by addingTween-20 at 0.05 per cent to the spore suspension.

Swathi *et al.* (2018) conducted an *in vitro* study for evaluating the compatibility of 12 commonly used additives (tween-20, tween-40, tween-60, tween-80, triton-x, glycerol, kaolite - silica gel, sunflower oil, neem oil, pongamia oil and CMC) at three different concentrations (0.1, 0.5 and 1.00 %) with *B. bassiana* using poisoned food technique. Among the various additives examined highest radial growth was observed in CMC 1 per cent (81.29 mm) which was followed by Kaolite 0.5 per cent (73.83 mm) and glycerol 0.5 per cent (52.93 mm).

Gatarayiha *et al.* (2009) conducted greenhouse experiments on tomato, eggplant, cucumber and green bean to study the effect of Break thru® (poly ether – poly – methyl siloxane – copolymer, a silicon surfactant) and an oil emulsion, of *B. bassiana* against spotted spider mite, *Tetranychus urticae* Koch. Two sprays were performed at one week interval. Leaf damage was reduced from 70 per cent in the untreated control to 40 per cent in treatments involving Break thru®.

2.5.3 Storability of *Beauveria bassiana*

Feasibility of storage without losing viability is a major concern in formulations of bioagents and has attracted attention of several workers. Thus, Prior*et al.,* (1988) had observed that oil suspension of *B. bassiana* could be stored for 40 days in the refrigerator. Puzari *et al.* (1991) studied the shelf life of *B. bassiana* oil formulation in terms of virulence. At room temperature, the virulence (90.97 per cent) did not change up to 90 days, after which the spore germination reduced significantly to 82.20 per cent after 120 days of storage. With increase in storage time, the infectivity and viability decreased significantly. Complete loss of viability was observed after 180 days.

Das *et al,* (2013) compared the shelf life of talc based formulation of *B. bassiana* stored at room temperature (25^0 C), with that stored under refrigerated conditions (4°C) as well as under deep freeze conditions (–4°C). They reported that at room temperature the viability of spores was retained for up to 180 days with 2.022×10^8 spores per gram, with 48 per cent pathogenicity. Under refrigerated condition spore density remained the same but pathogenicity was higher at 69.45 per cent and it lasted for a longer period of 210 days. Under deep refrigeration, the viability lasted for 300 days with same spore concentration.

A study was conducted by Gerding-Gonzalez *et al.*, (2007) to evaluate optimum concentration of chitin. Chitin was added to sodium alginate pellet formulation of *B. bassiana* at0, 0.5, 1, 2, 3 and 4 per cent (w/v) concentration and was incubated for 21 days. Three times improvement in conidia production was

observed 21 days after incubation, in case of two per cent chitin in sodium alginate pellet.

Srikanth *et al.* (2018) evaluated conidial viability of selected formulations (lignite, talc, dry yeast and vermicast) of the *Beauveria brongniartii* by media planting method and bioassay against *Holotrichia serrata* F. (Coleoptera: Scarabaeidae). Talc formulation had the highest shelf life with colony forming unit of 2.4 – 4.2 x 10^6 cfu g^{-1} nine months after storage. The study indicated possibility of lignite and talc as carrier materials.

Materials and methods

3. MATERIALS AND METHODS

The present study on "Characterization, evaluation and formulation of *Beauveria bassiana* (Bals,) strains against rice bug *Leptocorisa* spp. (Hemiptera: Alydidae)" was conducted at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, Thrissur and RARS Pattambi, during the year 2016-2019. The details of the materials used and the methods followed for the study are described below.

3.1 SURVEYAND COLLECTION OF RICE BUG CADAVERS

Surveys were conducted in the rice growing tracts in Alappuzha, Ernakulam, Thrissur and Palakkad districts of Kerala for collection of entomopathogenic fungi infected rice bugs. Roving surveys were carried out during the period from June 2016 to September 2016 (Kharif season) and November 2016 to February 2017 (Rabi season) coinciding with the milky grain stage of the crop. Rice bug cadavers were collected from the field and brought to laboratory for isolation of fungus. The details of the locations surveyed and seasons during which the survey was conducted are presented below (Table 1).

3.2 ISOLATION AND MAINTANANCE OF FUNGI FROM RICE BUG CADAVERS

Rice bug cadavers were obtained only from one location *viz.,* Pattambi. The rice bug cadavers collected from rice fields were kept separately in moist chambers for the development of fungal growth (Plate 1). Cadavers with fungal growth were selected for the isolation of fungi as per standard procedures (Zimmermann, 1986). They were surface sterilized using one per cent sodium hypochlorite followed by repeated washing in sterile water under aseptic conditions. The cadavers were then covered in sterilized filter paper and air dried. The dried cadavers were subsequently placed in Petri dishes containing potato dextrose agar (PDA) medium for the development of mycelia (Plate 2).

Table 1. Details of locations surveyed for entomopathogenic fungi of rice bug, *Leptocorisa* spp.

Sl. No	Location	G.P.S.	Varieties	Season	Area (ha)
Palakkad					
1	Vadakencherry Manikkapadam	Lat: 10.6198 Lng: 76.4962	Jyothi, Kanchana, Sheryas	Kharif 2016	30.00
2	Muthalamada	Lat: 10.61199 Lng: 76.74045	Jyothi, Njavara, Sheryas	Kharif 2016	28.00
3	Vadakencherry Cherukannambra	Lat: 10.60387 Lng: 76.47299	Jyothi, Kanchana	Rabi 2017	8.00
4	Vadakencherry Chundakkad	Lat: 10.60524 Lng: 76.47324	Jyothi, Kanchana	Rabi 2017	3.60
5	Pattambi	Lat: 10.81254 Lng: 76.18887	Jyothi	Rabi 2017	3.84
Ernakulam					
1	Vyttila	Lat: 9.98106 Lng: 76.32477	Vyttila varieties	Kharif 2016	0.75
2	Varappuzha	Lat: 10.07434 Lng: 76.27136	Pokkali, Vyttila varieties	Kharif 2016	27.00
Thrissur					
1	Alagappanagar	Lat: 10.43397 Lng: 76.27253	Jyothi	Kharif 2016	24.00
2	Mannuthy	Lat: 10.53598 Lng: 76.26835	Uma	Kharif 2016	0.60
3	Nenmanikkara	Lat: 10.43692 Lng: 76.25343	Jyothi	Rabi 2017	1.40
4	Mulankunnathukavu	Lat: 10.61575 Lng: 76.2364	Jyothi	Rabi 2017	11.20
Alappuzha					
1	Moncompu	Lat: 9.43718 Lng: 76.42782	Uma,local accessions	Kharif 2016	9.00
2	Kuttanad - Thekkekkara	Lat: 9.43137 Lng: 76.4296	Uma	Kharif 2016	3.50
3	Moncompu	Lat: 9.43718 Lng: 76.42782	Uma, local accessions	Rabi 2017	9.00

Plate 1. Rice bugs infected by *B. bassiana*

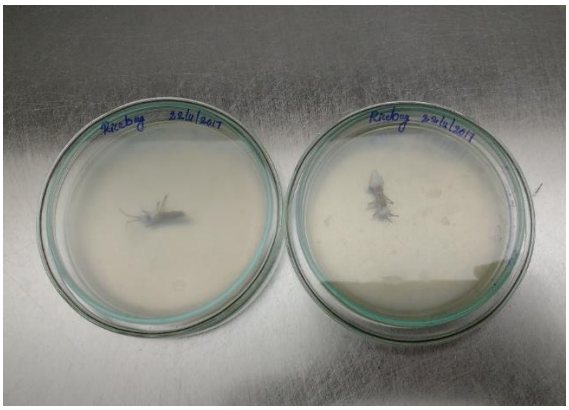


Plate 2. Isolation of *B. bassiana* from rice bug cadaver

Plate 3. Growth of entomopathogenic fungi obtained from Pattambi and Vellanikkara in PDA broth, 15 days after inoculation

Streptomycin, an antibiotic, was added to PDA medium at the rate of 0.16g per 200 ml to prevent bacterial contamination before pouring the medium into Petri dishes. Subculturing was done after the development of mycelia. The pure cultures of the fungi were maintained on PDA slants.

3.3 IDENTIFICATION OF ENTOMOPATHOGENIC FUNGI

3.3.1 Morphological identification

Petri dishes containing PDA media were inoculated with the isolated fungi. The plates were maintained at room temperature and observed for mycelial colour and growth. The shape of the conidia was observed under a phase contrast microscope (Leica DM500) at 100x magnification. The size of the conidia was calculated by measuring the dimensions of ten conidia under a phase contrast microscope equipped with Leica Application Suite (LAS) image analysing software (Leica DM500) and the mean was worked out.

3.3.2 Molecular identification

Molecular characterization of fungi were done by sequence analysis of the ribosomal spacer (ITS_1 and ITS_4). DNA isolation was done with the facilities available at AINPAO, Vellanikkara, Thrissur and sequencing was carried out at Sci-genome, Kochi. The protocol for DNA isolation and sequencing was as follows.

3.3.2.1 DNA isolation

The DNA isolation was done by following CTAB method with suitable modifications. The protocol followed is outlined below.

Crush the mycelia with micro pestle (sterilized)

↓

Add 1 ml CTAB extraction buffer and vortex

↓

Incubate at 65^0C for 90 min

↓

Cool and add equal amount of chloroform : isoamyl alcohol (24:1) with gentle mixing

↓

Centrifuge at 10,000 rpm for 10 min

↓

Take aqueous layer and add equal amount of chloroform isoamyl alcohol

↓

Centrifuge again at 10,000 rpm for 10 min

↓

Take top aqueous layer, add equal amount of chilled isopropanol and mix gently

↓

Add 30 µl, 3 M sodium acetate incubate at $^{-}20^{0}$Cfor 1 h

↓

Centrifuge at 13,000 rpm for 10 min

↓

Remove supernatant and add one ml of 70 per cent ethanol, centrifuge at 13,000 rpm for 10 min

↓

Air dry to remove ethanol

↓

Suspend the pellet in 25 µl distilled water

3.3.2.2 Analysis of purity of isolated DNA

Nanodrop (JENWAY Genova Nano, ver.1.55.3) was used to evaluate the purity of DNA. Maximum absorbance of nucleic acid was at 260 nm whereas for proteins it was280 nm. Purity of isolated DNA was calculated by finding absorbance at 260 nm divided by 280 nm. A value between 1.8 and 2.0 denoted that the DNA was pure and free from protein.

The quantity of the isolated DNA was checked using agarose gel electrophoresis. Five µl of DNA sample was mixed with two µl of gel loading buffer and loaded in 0.8 per cent agarose gel for agarose gel electrophoresis.

3.3.2.3 Preparation of agarose gel and agarose gel electrophoresis

The protocol followed for the preparation of agarose gel and agarose gel electrophoresis is outlined below.

Seal the gel casting tray with tape to form a mould, check the level and set the horizontal section of bench

↓

Boil 0.8 per cent agarose solution and allow it to cool to 42 to 45^0 C

↓

Add 0.5 µg/ml of ethidium bromide to the agarose gel solution

↓

Pour the gel to the casting tray to a height of 3 to 5 mm with comb in position in such a way that the teeth are 0.5 to one mm above the plate

↓

Allow gel to solidify, remove the comb and tape and place the tray with gel in electrophoresis tank

↓

Add 1x TAE buffer to the tank to cover the gel to a depth of 1 mm

↓

Mix the DNA sample with gel loading dye in the ratio 5 : 2 µl and load the mixture into the well of gel

↓

Run the gel at a voltage of 70 volts

↓

Turn off the power supply, when the loading dye migrates about $2/3^{rd}$ of the gel

The gel was visualized under UV transilluminator and the image was documented using gel documentation system (Invitrogen Life Technologies E-Gel imager).

3.3.2.4 PCR analysis

Polymerized Chain Reaction (PCR) was carried out in a 20 µl reaction volume which contained 10 µl PCR mix, H_2O – 8 µl, DNA 0.8 µl and 0.6 µl

reverse and forward primer each. Details of the primers used for amplification are given below (Table 2).

Table 2. Details of primers used for PCR amplification

Target	Primer name	Direction	Sequence (5' →3')
ITS	ITS-1F	Forward	TCCGTAGGTGAATGAACCTTGCGG
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC

The polymerized chain reaction was carried out in a PCR thermal cycler (Veriti Thermocycler (Applied Biosystems)) as per the following details.

Initial denaturation - 94^0C for 4 minutes
Denaturation - 94^0C for 45 minutes
Primer annealing - 54^0C for 1 minute } Number of cycles – 36
Primer extension - 72^0C for 2 minutes
Final extension - 72^0Cfor 8 minutes
Incubation - 4^0C for infinity

3.3.2.5 Agarose Gel Electrophoresis of PCR product

The PCR products were analysed in 1.2 per cent agarose gel containing 0.5 µg/ml ethidium bromide. Five µl PCR product mixed with 2µl loading dye was loaded into the wells of the gel and electrophoresis was done at 75v power supply.

3.4 BIOASSAY OF FIELD COLLECTED ISOLATES OF ENTOMOPATHOGENIC FUNGI

The efficacy of field collected entomopathogenic fungi was assessed through a set of experiments that involved laboratory, pot culture and field evaluations.

3.4.1.1 Maintenance of rice bug population

For conducting bioassay, rice bugs were reared in cages covered with nylon net and containing rice plants at milky grain stage (plate 4). A group of 80 – 100 adult bugs of either sex of *L. oratorius* were collected from the paddy fields and were released into the cages. The bugs were allowed to mate and oviposit.

The shiny black eggs laid on leaf lamina were collected from the cage by excising the leaf portion containing eggs, placed in a plastic box lined with moist tissue paper and were covered with muslin cloth. Upon emergence, the rice bug nymphs were transferred to cages containing rice plants at milky grain stage.

3.4.1.2 Mass multiplication of fungus and preparation of spore suspension

The fungal cultures were mass multiplied in the Sabouraud Maltose Agar and Yeast (SMAY) liquid medium at 25 ^{0}C for maximum growth and sporulation. Fifteen to twenty days after incubation, when the fungal mycelia were observed to cover the broth surface (Plate 3). The mycelial mats were retrieved from the bottles and were ground using a household mixer grinder for harvesting the conidia. After grinding, the mixture was filtered through a clean muslin cloth into a beaker and a few drops of Tween 80 (0.02 per cent) was added for better dispersal of the spores. The spore count in the filtrate was assessed using Neubauer Haemocytometer and the total spore load in the suspension was estimated using the formula by Lomer and Lomer (1996).

$$\text{Number of spores} = \frac{X \times 400 \times 10 \times 1000 \times D}{Y}$$

where X is the average number of spores per small square, the value 400 represents the total number of small squares, the value 10 represents the depth factor, D is the dilution factor, 1000 denotes conversion factor for mm^3 to cm^3 and Y is the number of small squares counted.

The spore count was adjusted to obtain concentrations of 1×10^7, 1×10^8 and 1×10^9 spores ml^{-1} by serial dilution technique.

3.4.1.3 Bioassay of isolates of entomopathogenic fungi in laboratory

Laboratory bioassay of the two isolates of entomopathogenic fungi obtained during the survey and which were subsequently identified as *Beauveria bassiana* and *Choanephora cucurbitarum*was conducted to assess the bioefficacy of the isolates against nymphs and adults of *L. oratorius.* The experiment was conducted in a completely randomized design with seven treatments and three replications. Each fungus was evaluated at three different concentrations. The treatment details are furnished in Table 3.

Table 3. Treatments for the laboratory evaluation of entomopathogenic fungi of rice bug, *Leptocorisa* spp.

Sl. No.	Treatment	Spore concentration
T 1	*Choanephora cucurbitarum*	1×10^7 spores ml^{-1}
T2	*C. cucurbitarum*	1×10^8 spores ml^{-1}
T3	*C. cucurbitarum*	1×10^9 spores ml^{-1}
T4	*Beauveria bassiana* VKA isolate	1×10^7 spores ml^{-1}
T5	*B. bassiana* VKA isolate	1×10^8 spores ml^{-1}
T6	*B. bassiana* VKA isolate	1×10^9 spores ml^{-1}
T7	Untreated control	-

Plate 4. Laboratory rearing of rice bug, *Leptocorisa oratorius*

Plate 5. Laboratory bioassay of nymphs of *Leptocorisa oratorius*

Plate 6. Laboratory bioassay of adults of *Leptocorisa oratorius*

Panicles of variety Jyothi at milky grain stage were clipped off and their cut ends were covered with moist cotton to prevent drying up. They were then placed in plastic jars of 17 x 10 cm dimension.Rice bug nymphs of uniform age were released at the rate of twenty nymphs per bottle into plastic bottles containing rice panicles. The jars along with the bugs were uniformly sprayed with respective treatments. Mortality of rice bugs was recorded at 24 h interval for seven days. From the data collected, per cent mortality of rice bug nymphs was calculated and was corrected using Abbot's formula (Plate 5, 6).

3.4.1.4 Observations

Mortality of treated bugs as well as nymphs was recorded at 24 h interval for upto seven days after treatment. The values were corrected for natural mortality by applying Abbott's formula (1925). Cadavers were collected daily and placed in Petri plates containing moist filter paper to confirm mycosis.

3.4.1.5 Statistical analysis

Mean per cent mortality of rice bugs treated with entomopathogenic fungi was calculated and subjected to ANOVA.

3.4.2 Pot culture evaluation of entomopathogenic fungi against rice bug

The bioefficacy of the two entomopathogenic fungi was confirmed through a pot culture experiment, in a completely randomized design with seven treatments and three replications. The fungi were evaluated at two concentrations each, which were found to be the most effective in the laboratory bioassay.

Thirty days old rice seedlings (variety Jyothi) were transplanted into pots and were maintained under open conditions. During panicle initiation, the potted plants were caged using locally fabricated nylon net cages with PVC pipe frames. A mixed population of laboratory reared nymphs and adults of rice bugs were released into each cage at the rate of 20 insects per cage (Plate 7). Following the release of bugs in the cages, the rice plants in the cages along with bugs were sprayed with the respective treatments (Table 4).

Table 4. Treatments for the pot culture evaluation of entomopathogenic fungi of rice bug, *Leptocorisa* spp.

Sl. No.	Treatment	Spore concentration
T 1	*Beauveria bassiana* VKA 01 strain	1 x 10^8 spores ml^{-1}
T2	*B. bassiana* VKA 01 strain	1 x 10^9 spores ml^{-1}
T3	*C. cucurbitarum*	1 x 10^8 spores ml^{-1}
T4	*C. cucurbitarum*	1 x 10^9 spores ml^{-1}
T5	*B. bassiana* (NBAIR strain)	1 x 10^8 spores ml^{-1}
T6	Malathion 50 EC	500 g ai ha^{-1}
T7	Untreated control	-

A strain of *B. bassiana* obtained from NBAIR Bengaluru served as a bio agent check and malathion 50 EC 500 g ai ha^{-1} formed the chemical check (Plate 8).

3.4.2.1 Observations

3.4.2.1.1 Estimation of rice bug population

Mortality of rice bugs were recorded at 3, 5, 7, 10, 15 and 20 days after treatment. The infected cadavers were collected during the course of recording observations and placed in Petri plates containing moist filter paper to confirm pathogenicity.

3.4.2.1.2 Observations on damage caused by rice earhead bug

Freshly emerged panicles of milky grain stage were marked for taking observations on damage by rice bugs. The panicles were harvested separately at maturity and total number of grains as well as the number of chaffy grains on each of the labeled panicles were recorded. A total of twelve observations were recorded from each treatment.

Plate 7. Release of rice bugs in to the cages

Plate 8. Layout of pot culture experiment

3.4.2.2 Statistical analysis

The mean per cent mortality of rice bugs in different treatments was calculated and subjected to ANOVA.

3.4.3 Field evaluation of entomopathogenic fungi against rice bug

Field evaluation of the most effective entomopathogenic fungus against rice bug, identified in the previous experiment, was carried out in two districts, *viz.* Thrissur and Palakkad. The experiments were laid out in an exploded block design comprising of five treatments including control. Variety Jyothi was used for the experiment and the plot size was 5 x 8 m^2. The details of treatments are given in Table 5. As in the pot culture, *B. bassiana* (NBAIR strain) formed a check

Table 5. Treatments for the field evaluation of the most promising isolate of entomopathogenic fungus against rice bug, *Leptocorisa* spp.

Sl. No.	Treatment	Spore concentration
T 1	*Beauveria bassiana* (VKA 01 isolate)	1 x 10^8 spores ml^{-1}
T2	*B. bassiana* (NBAIR strain)	1 x 10^8 spores ml^{-1}
T3	Malathion 50 EC	500 g ai ha^{-1}
T4	Azadirachtin	0.005 %
T5	Untreated control	-

The field experiment in Palakkad district was conducted from September 2017 to January 2018 at the Regional Agricultural Research Station, Pattambi (Plate 9).

The rice seedling nursery was prepared during the month of September. Seedlings were transplanted twenty days after sowing, at a spacing of 20 x 10 cm. All the agronomic practices were carried out as per the Package of Practices Recommendations by Kerala Agricultural University (KAU, 2016).

The field experiment in Thrissur district was conducted during July to September 2018 in farmer's field at Varadium, Avanoor Grama Panchayath, Thrissur (Plate 10).

At both locations, treatments were applied as and when the bug population crossed the economic threshold level of one to two bugs per hill. Care was taken to avoid spray drift by placing shade nets in between the treatments. The treatments were applied during evening hours.

Both VKA 01 isolates and NBAIR strains of *B. bassiana* were applied at a spore concentrations of 10^8 spores ml^{-1} each, which were identified as the most effective treatment in pot culture studies. Malathion 50 EC at the rate of 500 g ai ha^{-1} and azadirachtin 0.005 per cent served as checks. Prior to use, fungal cultures were sieved through sterile muslin cloth and the filtrate was mixed with 0.02 per cent Tween 80 for easy dispersal of spores. The treatments were applied using a pneumatic knapsack sprayer. An untreated check was also maintained.

3.4.3.1 Observations

3.4.3.1.1 Estimation of rice bug population

The rice earhead bug population per m^2 (nymphs and adults) were counted one day before spraying and again at five days intervals till 20 days after treatment. Observations were recorded from twelve randomly selected 1 m^2 quadrants within each plot.

3.4.3.1.2 Observations on damage caused by rice earhead bug

Freshly emerged panicles of milky grain stage were marked for taking observations on damage by rice bugs (Plate 12). The panicles were harvested separately at maturity and total number of grains as well as the number of chaffy grains on each of the labeled panicles were recorded. A total of twelve observations were recorded from each plot.

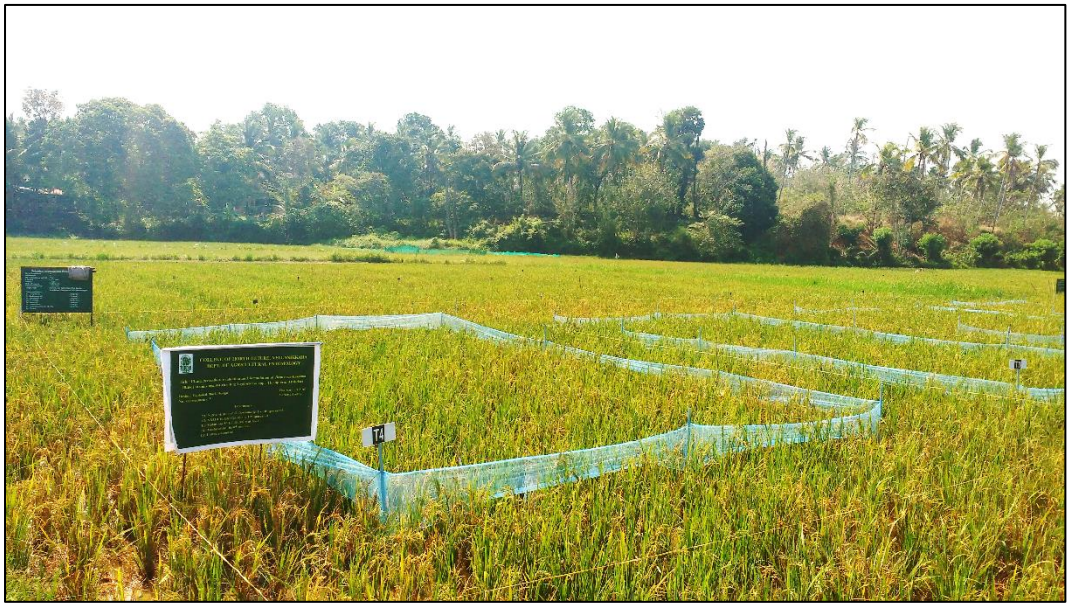


Plate 9. Layout of field experiment at Pattambi, Palakkad

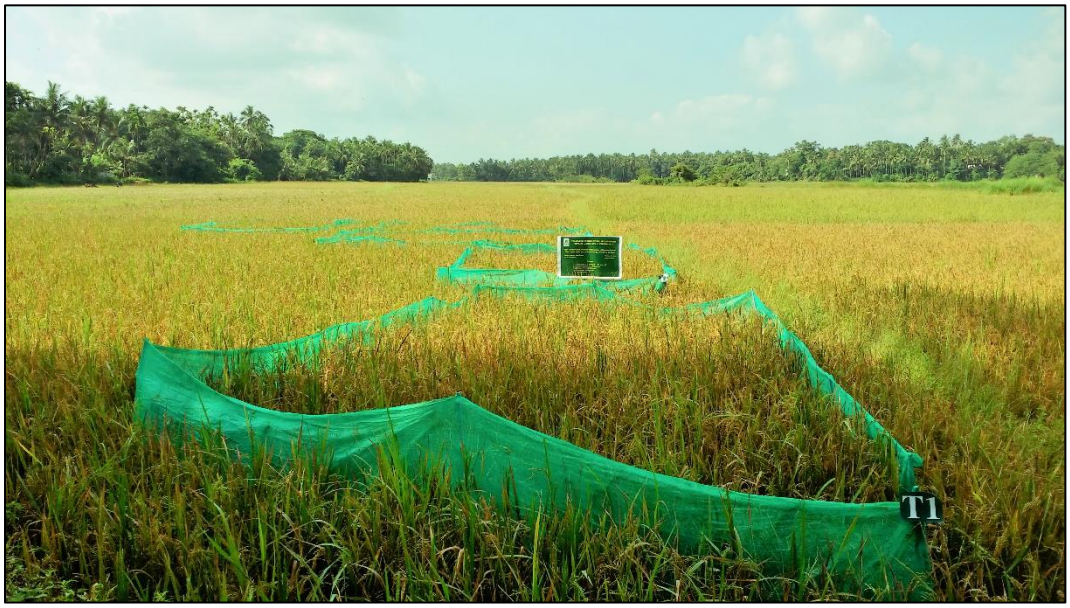


Plate 10. Layout of field experiment at Varadium, Thrissur

Plate 11. Harvesting using quadrant of size 1 m^2

Plate 12. Grain damage by rice bug

3.4.3.1.3 Observations on grain yield

Yield from twelve quadrants of 1 m^2 area in each plot was recorded. Paddy in each quadrant was harvested separately using a quadrant of size 1 m^2 area (Plate 11) and the mean yield per square meter was worked out.

3.4.3.3 Statistical analysis

Data on the number of rice bugs were subjected to square root transformation and covariance analysis. The per cent damage due to rice bugs as well as yield data was subjected to ANOVA and the means were separated by using Least Significant Difference (LSD).

3.5 ASSESSING COMPATIBILITY WITH INSECTICIDES AND FUNGICIDES

The compatibility of VKA 01 strain of *B. bassiana* with commonly used insecticides and fungicides was assessed through the poisoned food technique (Falck, 1907). Treatment details of the experiment are furnished in Table 6.

Table 6. Treatments for assessing compatibility of *Beauveria bassiana* VKA 01 strain with selected insecticides and fungicides

Treatment	Insecticide/fungicide	Dose
T1	Flubendiamide 20 WDG	25g ai ha^{-1}
T2	Lambda cyhalothrin 5 EC	50g ai ha^{-1}
T3	Malathion 50 EC	500g ai ha^{-1}
T4	Acephate 75 SP	750g ai ha^{-1}
T5	Azadirachtin 0.005 %	0.005 %
T6	Propiconazole 25 EC	0.025 %
T7	Copper hydroxide 77 WP	0.2 %
T8	Control	

The insecticides and fungicides used for testing were first UV sterilized by placing them in a laminar air flow chamber. The required quantity of each chemical, corresponding to the recommended dose, was then added to PDA medium aseptically and was mixed thoroughly. The media mixed with each of the selected pesticide were poured in to separate sterile Petri dishes. A one cm disc of *B. bassiana* VKA 01 isolate from actively growing culture was cut out with the help of a cork borer. The fungal disc was transferred to the center of each of the above Petri plates using a sterile inoculation needle. Uncontaminated PDA medium inoculated with fungus served as control. The Petri plates were kept at room temperature for incubation till the fungal growth in the control plate completely covered the Petri plate. Each treatment was replicated thrice.

3.5.1 Observations

Radial growth of the fungus in each treatment was measured using a ruler. Per cent inhibition of *B. bassiana* VKA 01 strain growth in the poisoned media was calculated using the formula suggested by Vincent, 1927.

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where, C = diameter of fungal growth in control (cm)

T = diameter of fungal growth in treatment (cm)

3.6 DEVELOPMENT OF FORMULATIONS OF *Beauveria bassiana*

The VKA isolate of *B. bassiana* was formulated in to talc based, oil based and aqueous formulations.

3.6.1.1 Preparation of spore concentrate

Spores of the VKA isolate of *B. bassiana* was harvested from SMAY medium following the procedure of Kim *et al.* (2007) with suitable modifications. The conidia of *B. bassiana* VKA 01 isolate were separated by filtrate through sterilized Whatman No.1 filter paper. The filtrate was centrifuged at 10,000 rpm

for 30 min. The resultant conidial pellet was washed with sterile distilled water and resuspended in 10 ml oil for oil based formulation and in sterile distilled water for water based formulation. The above concentrated spore suspensions were used for the preparation of formulations.

3.6.1.2 Carrier materials

Vegetable oil such as sunflower oil, rice bran oil, coconut oil, seasame oil and palm oil were used as carrier material for the preparation of oil based formulation, while distilled water and talc were used as carrier materials for aqueous and solid formulations respectively. All the above materials were sterilized by autoclaving before use.

3.6.1.3 Additives used in formulations

Polyethylene glycol, tween 80, CMC and glycerol were evaluated as for oil and aqueous based formulation while chitin and chitosan were evaluated for talc based formulation (Table 7). All the adjuvants except CMC were sterilized by autoclaving while CMC was subjected to UV sterilization.

Table 7. Additives evaluated for development of formulations of VKA strain of *Beauveria bassiana*

Sl. No.	Additives	Utility	Source
1	Glycerol	Nutrient carrier, humectant, plasticizer, emulsifier, osmotic protectant, adhesive	Merck Life Science Private Limited
2	Tween 80	Emulsifier, stabilizer	Merck Life Science Private Limited
3	Poly Ethylene Glycol (PEG)	Emulsifier, stabilizer	Merck Specialities Private Limited
4	Carboxy Methyl Cellulose (CMC)	Thickner, binder, sticker, viscocity modifier, stabilizer, water retention agent, lubricant	Loba chemie Private Limited
5	Chitin crude	Nutrient carrier, inhibitory to pathogenic fungus	MATSYAFED
6	Chitosan crude	Nutrient carrier, inhibitory to pathogenic fungus	MATSYAFED

3.6.1.4 Flow chart for the preparation of formulations

The sequence of experimentation to develop *B. bassiana* VKA 01 strain formulation is given below in the form of a flow chart.

Mass production of *B. bassiana* VKA 01 strain

↓

Standardization of carrier material

↓

Standardization of optimum concentration of additives

↓

Bioassay of formulation against rice bug

↓

Shelf life evaluation of the best formulation

3.6.2 Development of oil based formulation of *Beauveria bassiana* VKA 01 strain

3.6.2.1 Selection of most suitable oil carrier

Five commonly available vegetable oils (Table 8) were evaluated to identify the most suited carrier for oil based formulation, through inhibition zone technique (Brown and Kothari, 1975) in a completely randomized design (CRD). Each treatment was replicated thrice. Spores of *B. bassiana* VKA 01 isolate identified as the most promising isolate through bioassay studies were uniformly spread over sterile PDA plates using a sterile L rod. A sterile filter paper disc of one cm diameter dipped in one of the five vegetable oils was placed at the center of the Petri plate inoculated with spores.

Table 8. Vegetable oils and brands evaluated as carrier for oil based formulation of *Beauveria bassiana* VKA 01 strain

S. no.	Oil selected	Brand
1	Sunflower oil	Gold winner
2	Palm oil	Ruchi gold
3	Sesame oil	Edayam
4	Coconut oil	Kera
5	Rice bran oil	Pulari

The agar plates containing the oil discs were incubated for a week at room temperature and observed for zone of inhibition, if any.

3.6.2.2 Observations

After the inocubation period, the plates were then visually assessed for zone of inhibition around the filter paper disc. The oil with least inhibition was selected as the most suitable carrier.

3.6.2.3 Effect of selected oils on growth of *Beauveria bassiana* VKA 01 strain spores

The two vegetable oils identified as most promising in the previous experiment were further evaluated for their effect on shelf life of spores. For this the spores were stored in selected carrier oils for fifteen days and cfu were counted. Initial concentration of colony forming units (cfu) were maintained as 2.45×10^8. Treatments were kept in 15 ml autoclavable glass bottles at room temperature.

Enumeration of viable spores after storage was carried out following plate count technique (Miles *et al.*,1938). Each sample was serially diluted to get concentrations of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} spores ml^{-1}. Hundred µl from the last referred sample was uniformly spread over sterile PDA

plate using a sterile L rod. This was incubated at room temperature till spore germination.

3.6.2.4 Observations

Fifteen days after storage, spread plate was done in PDA media and the number of colonies developed were counted and number of colony forming units per ml was calculated as follows.

$$\text{Number of cfu ml}^{-1} = \frac{\text{Number of colonies}}{\text{Amount plated x dilution}}$$

3.6.2.5 Standardization of adjuvants for oil based formulation

Identification of the most suited oil base was followed by efforts to standardize the adjuvants required for the development of oil based formulation. The four additives were evaluated at three different concentrations each in a Completely Randomized Design with 13 treatments and three replications in each sample (Plate 13). The details of the treatments are given in Table 9.

Each adjuvant was added to the carrier oil selected in the previous experiment, after visually assessing the miscibility. All the additives were added under laminar air flow chamber to maintain aseptic conditions. Initial concentration of colony forming units (cfu) was maintained as 2.5×10^8. Ten ml of each treatments was taken in separate 15 ml autoclavable glass bottles and were maintained at room temperature.

3.6.2.6 Observations

One month after storage, 100 µl from each bottle was drawn using a micropipette and was uniformly spread over sterile PDA plates using a sterile L rod. This was incubated at room temperature till spore germination. The number of colonies developed were counted and number of colony forming units per ml was calculated as previously described.

Plate 13. Oil formulations of *Beauveria bassiana* VKA 01 strain with adjuvants

Plate 14. Aqueous based formulations of *Beauveria bassiana* VKA 01 strain with adjuvants

Table 9. Treatments for evaluation of adjuvants as additives for oil based formulation of *Beauveria bassiana* VKA 01 strain.

Sl. No.	Treatment
T1	Palm oil + *B. bassiana* VKA 01 spore + Tween 80 (0.5 %)
T2	Palm oil + *B. bassiana* VKA 01 spore + Tween 80 (1 %)
T3	Palm oil + *B. bassiana* VKA 01 spore + Tween 80 (2 %)
T4	Palm oil + *B. bassiana* VKA 01 spore + Glycerol (1 %)
T5	Palm oil + *B. bassiana* VKA 01 spore + Glycerol (3 %)
T6	Palm oil + *B. bassiana* VKA 01 spore + Glycerol (5 %)
T7	Palm oil + *B. bassiana* VKA 01 spore + PEG (0.5 %)
T8	Palm oil + *B. bassiana* VKA 01 spore + PEG (1 %)
T9	Palm oil + *B. bassiana* VKA 01 spore + PEG (2 %)
T10	Palm oil + *B. bassiana* VKA 01 spore + CMC (0.5 %)
T11	Palm oil + *B. bassiana* VKA 01 spore + CMC (1 %)
T12	Palm oil + *B. bassiana* VKA 01 spore + CMC (2 %)
T13	Palm oil + *B. bassiana* VKA 01 spore

3.6.3 Development of aqueous based formulation of *Beauveria bassiana* VKA 01 strain

Spores of *B. bassiana* VKA 01 strain were harvested from SMAY media, concentrated and resuspended in 10 ml sterile distilled water as already described. This concentrated spore suspension was used for the preparation of formulations.

3.6.3.1 Standardization of adjuvants for aqueous formulations of *Beauveria bassiana* VKA 01 strain

Four adjuvants, *viz.* tween 80, glycerol, PEG and CMC were evaluated at three different concentrations in a completely randomized design (CRD) with 13 treatments and three replications (Plate 14). The details of the adjuvants are furnished in Table 10.

Table 10. Treatments for evaluation of adjuvants as additives for aqueous based formulation of *Beauveria bassiana* VKA strain

Sl. No.	Treatment combinations
T1	Water + *B. bassiana* VKA 01 spore + Tween 80 (0.5 %)
T2	Water + *B. bassiana* VKA 01 spore + Tween 80 (1 %)
T3	Water + *B. bassiana* VKA 01 spore + Tween 80 (2 %)
T4	Water + *B. bassiana* VKA 01 spore + Glycerol (1 %)
T5	Water + *B. bassiana* VKA 01 spore + Glycerol (3 %)
T6	Water + *B. bassiana* VKA 01 spore + Glycerol (5 %)
T7	Water + *B. bassiana* VKA 01 spore + PEG (0.5 %)
T8	Water + *B. bassiana* VKA 01 spore + PEG (1 %)
T9	Water + *B. bassiana* VKA 01 spore + PEG (2 %)
T10	Water + *B. bassiana* VKA 01 spore + CMC (0.5 %)
T11	Water + *B. bassiana* VKA 01 spore + CMC (1 %)
T12	Water + *B. bassiana* VKA 01 spore + CMC (2 %)
T13	Water + *B. bassiana* VKA 01 spore (Control)

Each adjuvants was added to the spore suspension, after visually assessing the miscibility. All the additives were added under laminar air flow chamber to maintain aseptic condition. Initial concentration of colony forming units (cfu) was maintained as 2.46 x 10^8. Ten ml of treatments was taken in separate 15 ml autoclavable glass bottles at room temperature.

3.6.3.2 Observations

One month after storage, 100 µl from each bottle was drawn using a micropipette and was uniformly spread over sterile PDA plates using a sterile L rod. This was incubated at room temperature till spore germination. The number of colonies developed were counted and number of colony forming units per ml was calculated as previously described.

3.6.4 Development of talc based formulation of *Beauveria bassiana* VKA 01 isolate

The fungal cultures were mass multiplied in the Sabouraud Maltose Agar and Yeast (SMAY) liquid medium. These cultures were incubated at 25 ^{0}C for maximum growth and sporulation. Fifteen to twenty days after incubation, the fungal mycelia were observed to cover the broth surface (Plate 3). The mycelial mats were then retrieved from the bottles and were ground using a household mixer grinder for harvesting the conidia.

Talc powder were sterilized by heating in autoclave. After cooling, the talc was mixed with the homogenized fungal mat in 1:1 ratio (w/v) under laminar air flow chamber to maintain aseptic conditions.

3.6.4.1 Standardization of adjuvants for talc based formulation

Two adjuvants, namely chitin and chitosan were evaluated at three different concentrations each (Table 11). Fifty gram of talc based formulations were packed and stored in sterile polyethylene covers (65 microns) at room temperature. The initial cfu for *B. bassiana* VKA 01 strain was 2.34 x 10^8 spores

ml^{-1}. The moisture content was checked using a moisture meter and maintained at 12 per cent at the time of packing.

Table 11. Treatments for evaluation of adjuvants as additives for talc based formulation of *Beauveria bassiana* VKA 01 strain

Sl. No.	Treatment combinations
T1	Talc + *B. bassiana* VKA 01 strain spores + Chitin (3 %)
T2	Talc + *B. bassiana* VKA 01 strain spores + Chitin (5 %)
T3	Talc + *B. bassiana* VKA 01 strain spores + Chitin (7 %)
T4	Talc + *B. bassiana* VKA 01 strain spores + Chitosan (0.5 %)
T5	Talc + *B. bassiana* VKA 01 strain spores + Chitosan (1 %)
T6	Talc + *B. bassiana* VKA 01 strain spores + Chitosan (2 %)
T7	Talc + *B. bassiana* VKA 01 strain spores

3.6.4.2 Observations

Colony forming units were counted one month after storage. Colony forming units was assessed by taking one gram sample from each pack and serially diluting it to get concentrations of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} spores ml^{-1}. Hundred µl from the last referred sample was uniformly spread over sterile PDA plate using a sterile L rod. This was incubated at room temperature till spore germination. The number of colonies developed were counted and number of colony forming units per ml was calculated.

3.6.5 Evaluation of formulations of *Beauveria bassiana* VKA 01 strain against rice bugs under field conditions

The efficacy of *B. bassiana* VKA 01 strain formulations developed against rice bugs was evaluated through a field experiment conducted at the Regional Agricultural Research Station, Pattambi from September, 2017 to January 2018.

Plate 15. Talc formulations of *B. bassiana* VKA 01 strain with adjuvants

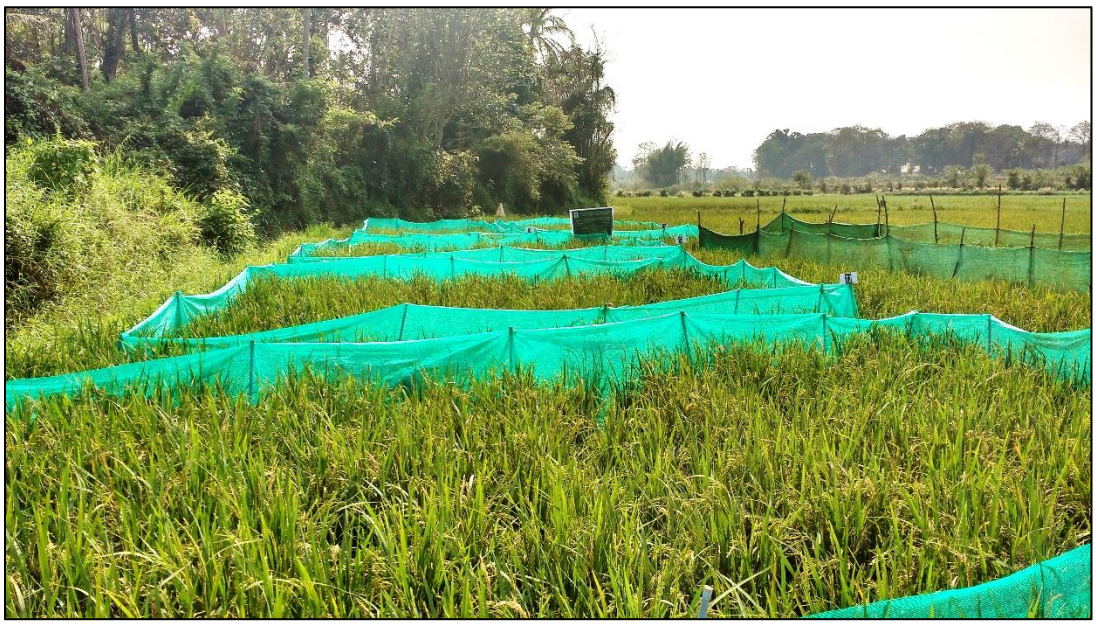


Plate 16. Layout of field experiment for evaluation of formulations

The experiments were laid out in an exploded block design comprising of five treatments including control. Variety Jyothi was used for the experiment and the plot size was 5 x 8 m^2. The details of treatments are given below in Table 12.

Table 12. Treatments for field evaluation of formulations of *Beauveria bassiana* VKA 01 strain against rice bug, *Leptocorisa* spp.

Sl. No.	Treatment	Spore concentration
T 1	Talc formulation	1 x 10^8 spores ml^{-1}
T2	Oil based formulation	1 x 10^8 spores ml^{-1}
T3	Water based formulation	1 x 10^8 spores ml^{-1}
T4	Malathion 50 EC	500 g ai ha^{-1}
T5	Control	-

The crop was raised as already described. Treatments were applied as and when the bug population crossed the economic threshold level of one to two bugs per hill. Pre count of rice bugs (nymphs and adults) in each plot was recorded prior to spraying. Post treatment bug population per m^2 was recorded 5, 10, 15 and 20 days after treatment. Care was taken to avoid spray drift by placing shade nets in between the treatments. The treatments were applied during evening hours using a pneumatic sprayer. An untreated check was also maintained.

3.6.5.1 Estimation of rice bug population

The rice earhead bug population per m^2 (nymphs and adults) were counted one day before spraying and again at five days interval till 20^{th} days after treatment. Observations were recorded from twelve randomly selected 1 m^2 quadrants (*insitu* count) within each plot.

3.6.5.2 Observations on damage caused by rice earhead bug

Freshly emerged panicles of milky grain stage were marked for taking observations. Total number of grains as well as number of damaged chaffy grains were recorded from the labeled panicles. Twelve observations were recorded from each plot.

3.6.5.3 Yield

Yield per m^2 was calculated from each treatment. Grains were harvested separately for each treatment. Harvesting was done using a quadrant of size 1 m^2 area. From each treatment 12 quadrants were separately harvested and average was calculated.

3.6.6 Shelf life evaluation

Shelf life studies of the most promising oil based, aqueous and talc based formulations of *B. bassiana* VKA 01 strain identified in the previous study was carried out under ambient conditions (Plate 18).

Ten milliliter each of the oil based formulation of *B. bassiana* VKA 01 strain containing1.33 x 10^9 cfu ml^{-1} was taken and stored in 15 ml autoclavable glass bottle with aluminium lid. Oil containing spores alone was used as control. Samples were drawn from formulations at 30 days interval up to 12 months and evaluated for viability (cfu m^{-1}) of the entomopathogen as described in previous sections. The data were subjected to statistical analysis.

Similarly,10 ml of the aqueous formulation of *B. bassiana* VKA 01 strain containing 1.66 x 10^9 spores ml^{-1} was taken and stored in 15 ml autoclavable glass bottle. *B. bassiana* VKA 01 strain alone in sterile distilled water were maintained as control. Samples were drawn from formulations at 30 days interval up to 12 months and evaluated for viability (cfu m^{-1}) of the entomopathogen as already described.

Plate 17. Shelf life evaluation of talc based formulation of *Beauveria bassiana* VKA 01 strain

Plate 18. *Beauveria bassiana* 01 strain oil and aqua based formulation for storage

The talc based formulation of *B. bassiana* VKA 01 strain identified as superior to all other combinations in previous experiment was evaluated for shelf life under room temperature. Fifty grams of talc based formulations of *B. bassiana* VKA 01 strain containing 1.66 x 10^9 cfu ml^{-1} was taken. Talc containing *B. bassiana* VKA 01 strain culture mix without any additives was kept as control. The formulations were stored in polyethylene covers (65 microns). Samples were drawn from formulations at 30 days interval for up to 12 months and evaluated for viability (cfu ml^{-1}) of the entomopathogen (Plate 17) as already described in previous sessions. These data were analyzed adopting CRD with three replications.

Results

4. RESULTS

The results of the study "Characterization, evaluation and formulation of *Beauveria bassiana* (Bals,) strains against rice bug *Leptocorisa* spp. (Hemiptera: Alydidae)" carried out at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, Thrissur, during the year 2016-2019 are presented here.

4.1 SURVEY FOR ENTOMOPATHOGENIC FUNGI ON RICE BUG, *Leptocorisa* spp.

Roving surveys were conducted during 2016 – 17 in rice growing tracts of Alappuzha, Ernakulam, Thrissur and Palakkad districts of Kerala to collect rice bugs infected by entomopathogenic fungi. A total of 14 locations across four districts were surveyed during the Kharif and Rabi seasons (Virippu and Mundakan seasons respectively) of 2016-17. Locations surveyed included five fields in Palakkad, four in Thrissur, three in Alappuzha and two in Ernakulam. The surveys were done during the milky grain stage of the paddy crop. The details of locations surveyed and the cadavers collected are presented in Table 13.

4.1.1 Survey in Palakkad district

Five locations were surveyed in Palakkad district. Manikkapadam (30 ha) and Muthalamada (28 ha) were surveyed during Kharif season of 2016-17, while, Cherukannambra (8 ha), Chundakkad (3.6 ha) and Pattambi (3.84 ha) were surveyed during Rabi season. Two mycosed cadavers of rice bugs were obtained from rice fields at RARS Pattambi.

4.1.2 Survey in Ernakulam district

In Ernakulam district, two locations, namely, Vyttila (0.75 ha) and Varappuzha (27 ha) were surveyed during the milky grain stage of the Kharif crop. No rice bug cadavers were obtained from either of the locations.

Table 13. Survey for entomopathogenic fungi infecting rice bug, *Leptocorisa* spp. in rice growing tracts of Kerala

S. No	Location	Latitude and longitude	Varieties	Season	Area (ha)	Strains obtained
Palakkad						
1	Vadakencherry Manikkapadam	Lat:10.6198 Lng:76.496	Jyothi, Kanchana, Sheryas	Kharif	30.0	0
2	Muthalamada	Lat:10.6119 Lng:76.740	Jyothi, Njavara, Sheryas	Kharif	28.0	0
3	Vadakencherry Cherukannambra	Lat:10.6038 Lng:76.473	Jyothi, Kanchana	Rabi	8.0	0
4	Vadakencherry Chundakkad	Lat:10.6052 Lng:76.473	Jyothi, Kanchana	Rabi	3.6	0
5	Pattambi	Lat:10.8125 Lng:76.188	Jyothi	Rabi	3.84	1
Ernakulam						
1	Vyttila	Lat:9.9811 Lng:76.324	Vyttila varieties	Kharif	0.75	0
2	Varappuzha	Lat:10.0743 Lng:76.271	Pokkali, Vyttila varieties	Kharif	27.0	0
Thrissur						
1	Alagappanagar	Lat:10.4339 Lng:76.272	Jyothi	Kharif	24.0	0
2	Mannuthy	Lat:10.5359 Lng:76.2683	Uma	Kharif	0.6	0
3	Nenmanikkara	Lat:10.4369 Lng:76.2534	Jyothi	Rabi	1.4	0
4	Mulankunnathukavu	Lat:10.6157 Lng:76.2364	Jyothi	Rabi	11.2	0
Alappuzha						
1	Moncompu	Lat:9.4371 Lng:76.4278	Uma, local accession	Kharif	9.0	0
2	Kuttanad Thekkekkara	Lat:9.4314 Lng:76.4296	Uma	Kharif	3.5	0
3	Moncompu	Lat:9.4371 Lng:76.4278	Uma, local accession	Rabi	9.0	0

4.1.3 Survey in Thrissur district

Four locations were surveyed in Thrissur district, with two locations *i.e*., Alagappanagar (24 ha) and Mannuthy (0.6 ha) being covered during Kharif season and the remaining two *viz.* Nenmanikkara (1.4 ha) and Mulankunnathukavu (11.2 ha) being surveyed during Rabi season. Cadavers of rice bug infected by fungi were not obtained from any of these locations.

4.1.4 Survey in Alappuzha district

In Alappuzha district, two locations, Moncompu (9 ha) and Kuttanad, Thekkekkara (3.5 ha) were surveyed during Kharif. In Moncompu, survey was repeated during Rabi season as well. However infected rice bug cadavers were not obtained.

4.2 ISOLATION OF ENTOMOPATHOGENIC FUNGI FROM RICE BUG CADAVERS

Entomopathogenic fungi were isolated from the two mycosed rice bug cadavers obtained from rice fields of RARS Pattambi following standard procedures and were maintained as pure cultures. They were subsequently used for identification of the isolated fungus (Plate 19, 20).

4.3 IDENTIFICATION OF ENTOMOPATHOGENIC FUNGI

4.3.1 Morphological characters

4.3.1.1 Colony characters

Observations on initial and final colour of the colonies and their growth pattern were recorded. The fungal isolate from both the cadavers from Pattambi produced identical white coloured hyphal mats with slightly raised foldings which turned greyish at the center after 12-13 days in PDA media (Plate21). The isolate obtained from AICRP on BCCP Vellanikkara (VKA 01 strain) produced white coloured uniform growth with cottony appearance in PDA media (Plate 22).

Plate 19. Rice bug cadavers obtained from RARS Pattambi

Plate 20. Rice bug cadavers preserved in AICRP on BCCP

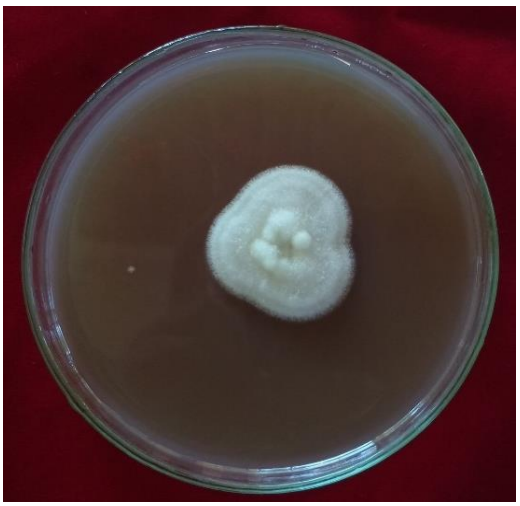


Plate 21. Colony of Pattambi isolate

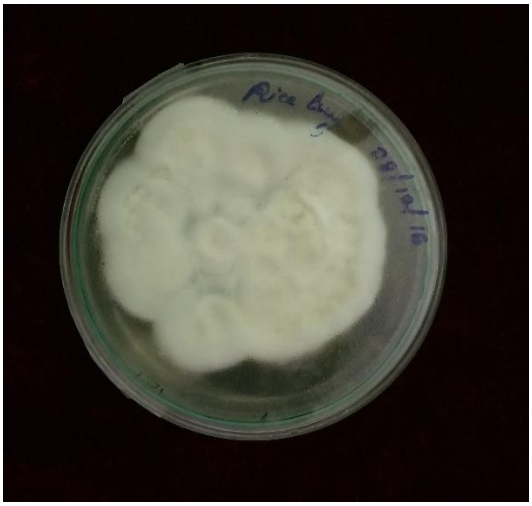


Plate 22. Colony of Vellanikkara isolate

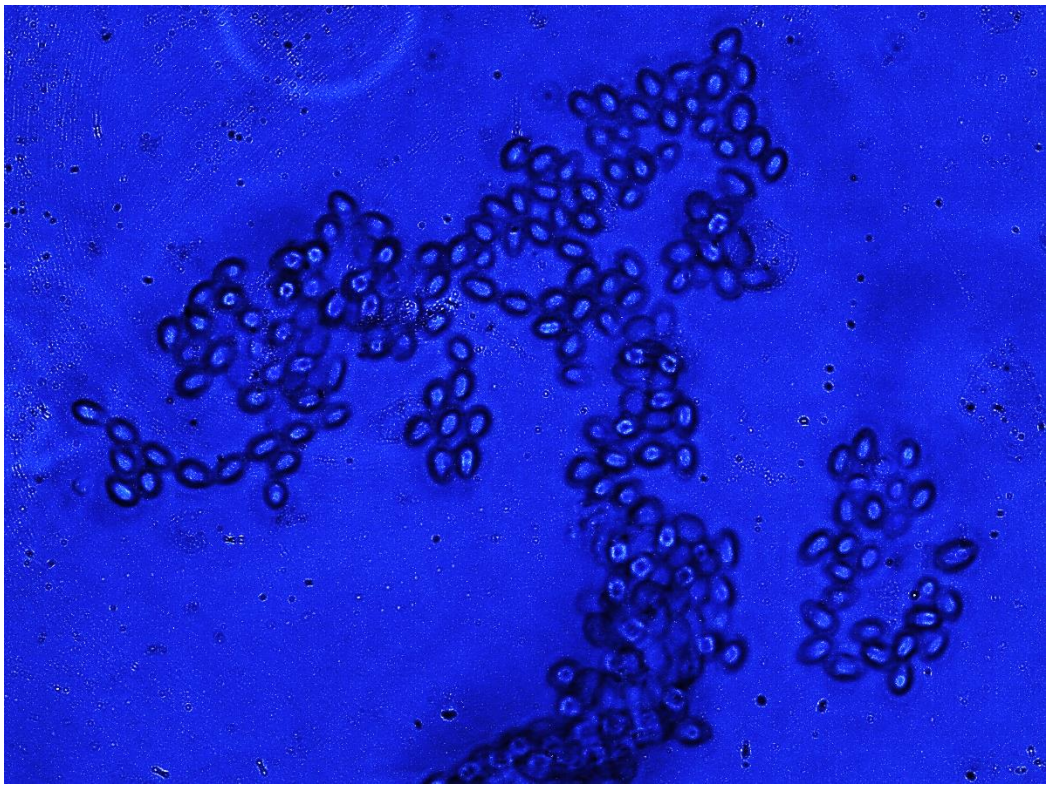


Plate 23. Spores of Pattambi isolate (45X)

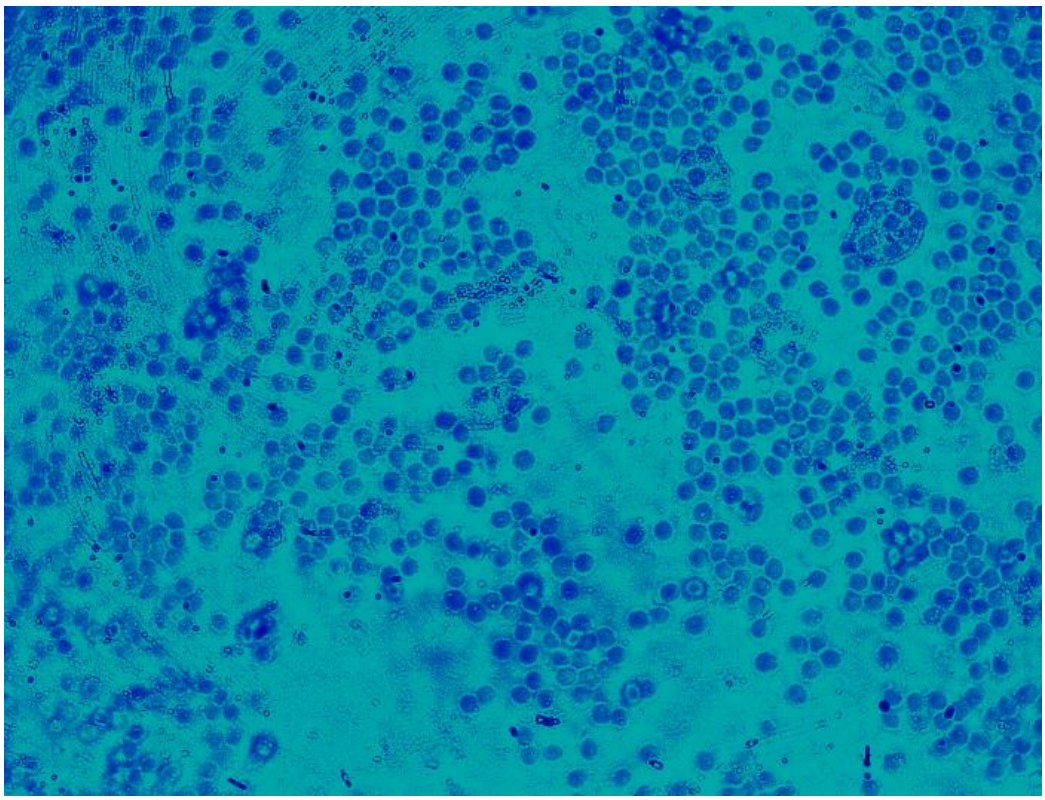


Plate 24. Spores of Vellanikkara isolate (45X)

4.3.1.2 Size and shape of spores

Length and width of the spores of each isolate were measured using a microscope (Leica) equipped with LAS image analyzing software. The length and width of spores varied among the isolates (Table 14). The isolate from Pattambi recorded mean length and width of 4.33 µm and 3.32 µm respectively, which were greater than the mean length and width of 1.90 µm and 1.91 µm respectively in case of Vellanikkara isolate.

The spores of Vellanikkara isolate were globose in shape while the fungus isolated from Pattambi produced spindle shaped spores (Plates 23, 24).

Table 14. Evaluation of colony characters of fungal isolates from *Leptocorisa* spp.

Sl. No.	Fungal isolates	Initial colour of hypha	Final colour of hypha	Spore dimension (µm)	Conidial shape	Growth pattern
1	Pattambi	White	Greyish	Length:4.33 Width:3.32	Spindle	Slightly raised with folding
2	Vellanikkara	White	White	Length:1.91 Width: 1.90	Globose	Cottony appearance

4.3.2 Molecular characterization of fungal isolates from *Leptocorisa* spp.

The fungi isolated from rice bug cadavers at Pattambi were subjected to molecular characterization along with VKA 01 strain obtained from Vellanikkara. The ITS (Internal Transcribed Spacer) region was amplified using the ITS primers (F: TCCGTAGGTGAACCTGCGG, R: TCCTCCGCTTATTGATATGC) and the PCR product had one intact band at 600 bp when resolved in one per cent agarose gel (Plate 25).

4.3.2.1 ITS sequence of isolate of entomopathogenic fungus from Pattambi

The ITS sequencing of the Pattambi isolate yielded a 467 base pair (bp) sequence as given below.

GAGTTTTATTTGGGAGGCCCCAACAAAGTCCAAGTCGCAAGAGCTTTC
CTTTATATTAAAAAAAAGTTCAGGCTAGATGAACAGATTCAGGCCTTA
CTCAATTTAAAAGGTCGCCATTGCTAGCTTCCTTCATGACCATTCAAAA
AAAAATTTTGAATGAGGGTTGTTTTTGATACTGAAACAGGCGTGCTCA
CTGGAATACCAATGAGCGCAAGATGCGTTCAAAGACTCGATGATTCAC
TGAATTTGCAATTCACACTAGTTATCGCAATTTGCTACGTTCTTCATCG
ATGCAAAAGCCAAGAGATCCATTGTTAAAAGTTGTTTTATAGATATTA
CTACCTATGTTACATTTTATAATCTGATCAATTGAAAGTATATAAACAG
GGTACCAAGCCTAAGCTTGACCATAGCTCGGTTAACATTCCTCATGCC
TACCCATATAGCACAAGAACATCCCTCAAACGCCA

The above sequence when subjected to BLAST (Basic Local Alignment Search tool) analysis showed cent per cent similarity with *Choanephora cucurbitarum* strain GFP023 with the accession number MF942131.1. The isolate was assigned accession number MN061053 by NCBI (National Center for Biotechnology Information).

4.3.2.2 ITS sequence of isolate of entomopathogenic fugus from Vellanikkara

The ITS sequencing of the *B. bassiana* Vellanikkara isolate yielded a 520 base pair (bp) sequence as given below.

ACCCTTCTGTGACCTACCTATCGTTGCTTCGGCGGACTCGCCCCAGCCC
GGACGCGGACTGGACCAGCCCCGCCGGGGACCTCAAACTCTTGTATTC
CAGCATCTTCTGAATACGCCGCAAGGCAAAACAAATGAATAACTTTCA
ACAACGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGC
GATAAGTAATGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACG
CACATTGCGCCCGCCAGCATTCTGGCGGGCTGCCGTTCGAGCGTCATT
TCAACCCTCGACCTCCCCTGGGGGAGGTCGGCGTTGGGGACCGGCAGC

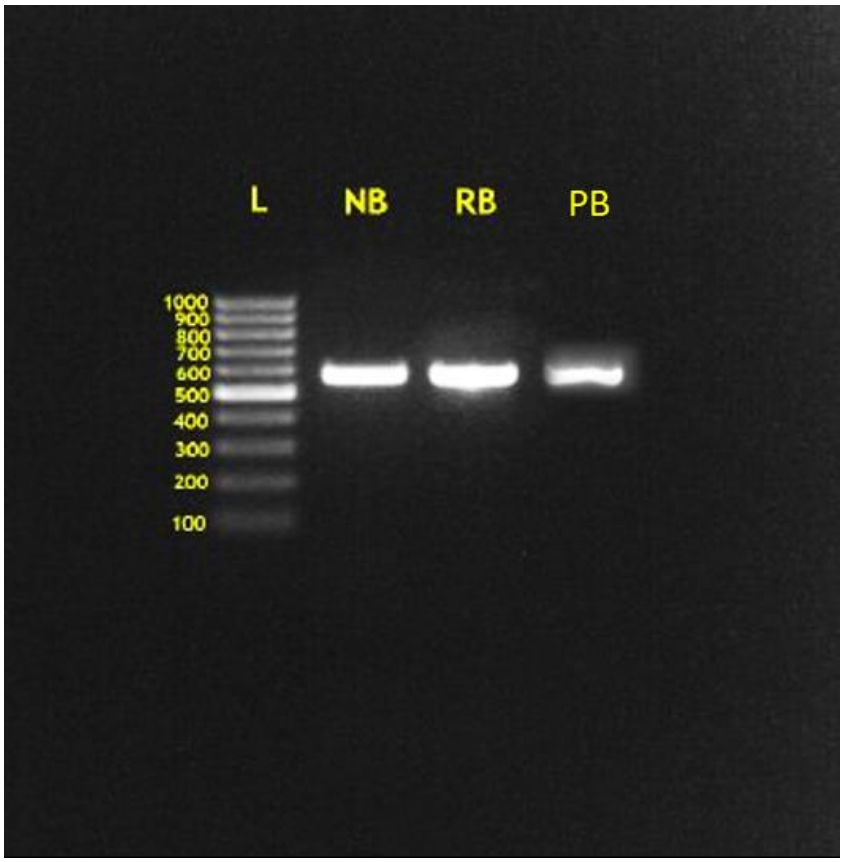


Plate 25. PCR gel image

(NB – *B. bassiana* NBAIR isolate, RB - Vellanikkara isolate, PB – Pattambi isolate)

Sequence View for Process ID: RBPAT001-18

Specimen Details Current

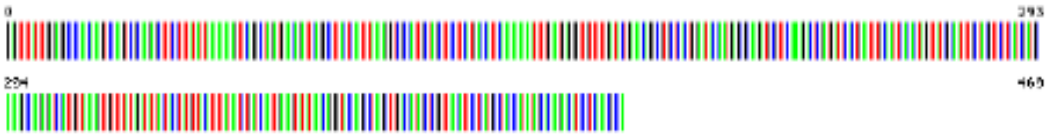
Sample ID:	RBPAT
Process ID:	RBPAT001-18
Project:	RBPAT
Tax Names:	Zygomycota, Zygomycetes, Mucorales, Choanephoraceae, Choanephora
Taxon:	Choanephora
Rank Name:	genus
Sampling Protocol:	N/A
BIN URI:	N/A
Kingdom:	Fungi

Marker Summary

Marker Code	Sequence Length	GC	Ambiguous	Trace Count
ITS	470	38.1%	0%	2

ITS

Illustrative Barcode



Nucleotide Sequence

GAGTTTTATTTGGGAGGCCCCAACAAAGTCCAAGTCGCAAGAGCTTTCCTTTATATTAAAAAAAAGTTCAGGCTAGATGAACAGATTCAGGCCTTACTCAAT
TTAAAAGGTCGCCATTGCTAGCTTCCTTCATGACCATTCAAAAAAAAATTTTGAATGAGGGTTGTTTTTGATACTGAAACAGGCGTGCTCACTGGAATACCA
ATGAGCGCAAGATGCGTTCAAAGACTCGATGATTCACTGAATTTGCAATTCACACTAGTTATCGCAATTTGCTACGTTCTTCATCGATGCAAAGCCAAGAG
ATCCATTGTTAAAGTTGTTTTATAGATATTACTACCTATGTTACATTTTATAATCTGATCAATTGAAAGTATATAAACAGGGTACCAAGCCTAAGCTTGAC
CATAGCTCGGTTAACATTCCTCATGCCTACCCATATAGCACAAGAACATCCCTCAAACGCCA

Sequence Metadata

Genbank Accession:	N/A
Translation Matrix:	N/A (Non-coding marker)
Last Updated:	2018-08-04 03:20:07.428914
Sequence Runsite:	Kerala Agricultural University

Plate 26. DNA barcode of Pattambi isolate generated by BOLD systems v4

Sequence View for Process ID: RBVKA001-18

Specimen Details Current

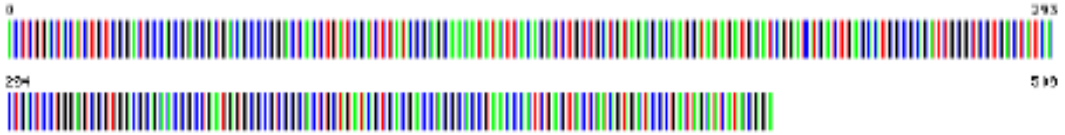
Sample ID:	RBVKA
Process ID:	RBVKA001-18
Project:	RBVKA
Tax Names:	Ascomycota, Sordariomycetes, Hypocreales, Cordycipitaceae, Beauveria, Beauveria bassiana
Taxon:	Beauveria bassiana
Rank Name:	species
Sampling Protocol:	N/A
BIN URI:	N/A
Kingdom:	Fungi

Marker Summary

Marker Code	Sequence Length	GC	Ambiguous	Trace Count
ITS	510	56.9%	0%	2

ITS

Illustrative Barcode



Nucleotide Sequence

ACCCTTCTGTGACCTACCTATCGTTGCTTCGGCGGACTCGCCCCAGCCCGGACGCGGACTGGACCAGCCCCGCCGGGGACCTCAAACTCTTGTATTCCAGCA
TCTTCTGAATACGCCGCAAGGCAAAACAAATGAATAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG
AATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCTGCCGTTCGAGCGTCATTTCAACCCTCGACCTCCC
CTGGGGGAGGTCGGCGTTGGGGACCGGCAGCACACCGCGGCCCTGAAATGGAGTGGCGGCCCGTCCGCGGCGACCTCTGCGTAGTAATACAGCTCGCACCGG
AACCCCGACGCGGCCACGCCGTAAAACACCCAACTTCTGAACGTTGACCTCGAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA

Sequence Metadata

Genbank Accession:	N/A
Translation Matrix:	N/A (Non-coding marker)
Last Updated:	2018-10-06 02:25:53.787059
Sequence Runsite:	Kerala Agricultural University

Plate 27. DNA barcode of Vellanikkara isolate generated by BOLD systems v4

ACACCGCGGCCCTGAAATGGAGTGGCGGCCCGTCCGCGGCGACCTCTG
CGTAGTAATACAGCTCGCACCGGAACCCCGACGCGGCCACGCCGTAA
AACACCCAACTTCTGAACGTTGACCTCGAATCAGGTAGGACTACCCGC
TGAACTTAAGCATATCAATAAGCGGAGGAA

The above sequence when subjected to BLAST (Basic Local Alignment Search tool) analysis showed cent per cent similarity with *B. bassiana* isolate Bb2 having accession number KX376474.1 and was assigned accession number MN062772 by NCBI.

An account was opened in BOLD systems v4 database and new projects were created for the two fungal samples isolated from rice bug cadavers. The sample from Vellanikkara was named "RBVKA" (Plate 26, 27). Specimen data *viz.*, specimen taxonomy, specimen identifier, specimen details, collection details were given and an auto generated process ID 'RBVKA001-18' was obtained. Then primer details, fasta sequence and trace files (.ab1) were uploaded to database and corresponding barcode generated. The process was repeated in case of the isolate from Pattambi as well, resulting in the name, "RBPTB" and auto generated process ID 'RBPTB001-18'.

4.4 EVALUATION OF ENTOMOPATHOGENIC FUNGI AGAINST RICE BUG

4.4.1 Bioassay of fungal isolates against nymphs of rice bug in laboratory

Bioefficacy of *Beauveria bassiana* Vellanikkara isolate and (hereafter referred to as *B. bassiana* VKA 01 strain) *Choanephora cucurbitarum* against rice bug nymphs were evaluated at three different concentrations *viz.* 10^7,10^8 and 10^9sporesml^{-1}under laboratory conditions. Rice bug nymphs of uniform age were released at the rate of twenty nymphs per bottle into plastic bottles containing rice panicles of milky grain stage. The jars along with the bugs were uniformly sprayed with respective treatments. Mortality of rice bugs was recorded at 24 h interval for seven days. From the data collected, per cent mortality of rice bug nymphs was calculated and was corrected using Abbot's formula (Table 15).

Table 15. Bioefficacy of local isolates of entomopathogenic fungi against nymphs of rice bugsin laboratory

Treatment	Mortality (%)						
	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT
T1: *Choanephora cucurbitarum* @ 10^{7} spores ml^{-1}	3.39^{a}	10.35^{bc}	26.67^{cd}	28.07^{c}	30.36^{d}	36.53^{b}	46.14^{b}
T2: *C. cucurbitarum* @ 10^{8} spores ml^{-1}	3.39^{a}	5.17^{cd}	14.99^{de}	45.61^{b}	51.79^{c}	51.91^{b}	53.84^{b}
T3: *C. cucurbitarum* @ 10^{9} spores ml^{-1}	5.09^{a}	8.62^{bcd}	33.33^{c}	40.35^{c}	33.93^{c}	38.45^{b}	40.37^{b}
T4: *Beauveria bassiana* VKA 01 strain @ 10^{7} spores ml^{-1}	5.09^{a}	17.24^{ab}	74.99^{b}	82.46^{ab}	83.93^{b}	92.31^{a}	96.15^{a}
T5: *B. bassiana* VKA 01 strain @ 10^{8} spores ml^{-1}	3.39^{a}	22.41^{a}	88.33^{ab}	94.74^{a}	96.43^{a}	98.08^{a}	100.00^{a}
T6: *B. bassiana* VKA 01 strain @ 10^{9} spores ml^{-1}	3.39^{a}	24.14^{a}	89.99^{a}	94.74^{a}	98.21^{a}	100.00^{a}	100.00^{a}

DAT – Days after treatment, Mean values in each column followed by a common letter are not significantly different by DMRT (p = 0.05)

Twenty four hours after treatment, both *B. bassiana* VKA 01 strain and *C. cucurbitarum* recorded 3.39 to 5.09 per cent mortality at the different concentrations evaluated and were at par with each other. *Choanephora cucurbitarum* at concentrations of $10^7$and 10^8sporesml^{-1}as well as *B. bassiana* VKA 01 strain applied at $10^8$and 10^9 spores ml^{-1}recorded 3.39 per cent mortality. Both *C. cucurbitarum* at $10^9$and *B. bassiana* VKA 01 strain at 10^7spores ml^{-1} recorded the highest mortality of 5.09 per cent.

Choanephora cucurbitarum, applied at the rate of 10^7, 10^8 and 10^9 spores ml^{-1}recorded 10.35, 5.17 and 8.62 per cent mortality respectively, two days after treatment. Application of *B. bassiana* VKA 01 strain at the rate of 10^7, 10^8 and 10^9spores ml^{-1} resulted in 17.24, 22.41 and 24.14 per cent mortality respectively. The above three treatments were on par with each other while *B. bassiana*VKA 01 strain applied atthe rate of 10^8 and 10^9ml^{-1} were significantly superior to all the treatments involving *C. cucurbitarum*.

Three days after treatment, application of *C. cucurbitarum* at the rate of 10^7, 10^8 and 10^9sporesml^{-1}registered 26.6, 14.99 and 33.33 per cent mortality respectively. Corresponding values *B. bassiana* VKA 01 strain were 74.99, 88.33 and89.99 per cent respectively. *Beauveria bassiana* VKA 01 strain applied at both10^8 and 10^9sporesml^{-1}were on par with each other, while the former was on par with *B. bassiana* VKA 01 strain applied at the rate of 10^7 spores ml^{-1}as well.

Four days after spraying, *C. cucurbitarum* applied at the rate of 10^7, 10^8 and 10^9 spores ml^{-1}had caused28.07, 45.61 and 40.35 per cent mortality respectively. *Beauveria bassiana* VKA 01 strain, applied at the rate of $10^8$and 10^9 spores ml^{-1}recorded the highest mortality of 94.74 per cent each, which was followed by the same, applied at the rate of10^7spores ml^{-1} with 82.46 per cent mortality.

Choanephora cucurbitarum applied at the rate of 10^7, 10^8 and 10^9 spores ml^{-1} induced30.36, 51.79 and 33.93per cent mortality respectively, five days after treatment. Significantly superior mortality values were registered by *B. bassiana*

VKA 01 strain at all the concentrations evaluated. The entomopathogen, applied at the rate of10^9spores ml^{-1} recorded highest mortality of 98.21 per cent which was at par with the 96.43 per cent mortality recorded when it was applied at the rate of10^8spores ml^{-1}. The fungus, applied at the rate of 10^7 spores ml^{-1}recorded 83.93 per cent mortality.

Six days after treatment, *C. cucurbitarum* applied at the rate of 10^7, 10^8 and 10^9 spores ml^{-1}caused 36.53, 51.91 and 38.45 per cent mortality and were at par. In comparison, *B. bassiana* VKA 01 strain, sprayed at the rate of10^9spores ml^{-1} registered cent per cent mortality, followed by *B. bassiana* VKA 01 strain applied at the rate of 10^8 and 10^7 spores $ml^{-1}$with 98.08 and 92.31 per cent mortality. All the three treatments involving *B. bassiana* VKA 01 strain were on par with each other and were significantly superior to those of *C. cucurbitarum.*

Application of *C. cucurbitarum* caused 46.14, 53.84 and 40.37 per cent mortality of rice bugs at concentrations of 10^7, 10^8 and 10^9 spores ml^{-1} respectively seven days after treatment, the treatments being on par with each other.*Beauveria bassiana* VKA 01 strain, applied at the rate of 10^8 as well as 10^9 spores ml-1 resulted in cent per cent mortality seven days after treatment. The lowest concentration of *B. bassiana* VKA 01 strain, evaluated, i.e., 10^7 spores ml^{-1} registered 96.15 per cent kill of bug population. All the three treatments involving *B. bassiana* VKA 01 strain were on par with each other and were significantly superior to those involving *C. cucurbitarum.*

4.4.2 Bioassay of fungal isolates against adults of rice bug in laboratory

Bioefficacy of *B. bassiana* VKA 01 strain and *C. cucurbitarum* against adults of rice bug was evaluated at three different concentrations *viz.* 10^7,10^8 and 10^9 spores ml^{-1} under laboratory conditions. Rice bug adults of uniform age were released at the rate of twenty bugs per jars into plastic jars containing rice panicles (variety Jyothi) of milky grain. The jars, along with the bugs inside were uniformly sprayed with respective treatments. Mortality of rice bugs was recorded for seven days at 24 h interval. From the data collected, per cent mortality of rice

bug adult was calculated and was corrected using Abbot's formula. The results are presented in Table 16.

Twenty four hours after treatment, *C. cucurbitarum*, applied at the rate of 10^7, 10^8 and 10^9 spores ml^{-1} recorded mortality ranging from 3.39to 6.78 per cent, while *B. bassiana* VKA 01 strain applied at the same concentrations killed 5.09, 6.78 and 8.48 per cent of adult bugs exposed to the treatments. All the treatments evaluated were on par with each other.

Two days after spraying, *C. cucurbitarum* applied at the rate of 10^7, 10^8 and 10^9spores ml^{-1} recorded 8.62, 10.35 and 5.18 per cent mortality respectively. *Beauveria bassiana* VKA 01 strain, applied at the same rates, on the other hand, registered mortality values of 25.86, 20.69 and 32.76 per cent respectively. All the treatments of *B. bassiana* VKA 01 strain were on par with each other and were significantly superior to *C. cucurbitarum*.

Three days after treatment, 10.71, 8.93 and 8.93 per cent of bugs treated with*C. cucurbitarum* at the rate of 10^7, 10^8 and 10^9 spores ml^{-1} respectively had suffered mortality. In case of *B. bassiana*VKA 01 strain the highest mortality of 73.21 per cent was observed at the concentration of 10^8 spores ml^{-1}. This was followed by *B. bassiana* VKA 01 strain at the rate of 10^9 spores ml^{-1} with 64.29 per cent mortality. Both were at par with each other and were significantly superior to other treatments. *Beauveria bassiana* VKA 01 strain, applied at the rate of 10^7 spores ml^{-1}had caused 51.79 per cent mortality.

Four days after spraying, all the treatments involving *C. cucurbitarum* recorded an identical value of 10.89 per cent mortality.*Beauveria bassiana* VKA 01 strain induced the highest mortality of 90.91per cent when applied at the rate of 10^8 spores ml^{-1}. At the concentration of 10^9 spores ml^{-1}, the fungus caused 89.09 per cent mortality. Both the above values were on par with each other and were superior to the mortality of 76.36 per cent registered at the concentration of 10^7 spores ml^{-1}.

Table 16. Bioefficacy of field collected entomopathogenic fungus against adults of rice bugsin the laboratory

Treatments	Mortality (%)						
	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT
T1: *Choanephora cucurbitarum* @ 10^{7} spores ml^{-1}	6.78^{a}	8.62^{b}	10.71^{c}	10.89^{c}	14.55^{c}	14.81^{c}	18.52^{c}
T2: *C. cucurbitarum* @ 10^{8} spores ml^{-1}	5.09^{a}	10.35^{b}	8.93^{c}	10.89^{c}	16.36^{c}	16.67^{c}	18.52^{c}
T3: *C. cucurbitarum* @ 10^{9} spores ml^{-1}	3.39^{a}	5.18^{b}	8.93^{c}	10.89^{c}	12.73^{c}	18.52^{c}	22.23^{c}
T4: *Beauveria bassiana* VKA 01 strain @ spores ml^{-1}	5.09^{a}	25.86^{a}	51.79^{b}	76.36^{b}	85.45^{b}	87.04^{b}	90.74^{b}
T5: *B. bassiana* VKA 01 strain @ 10^{8} spores ml^{-1}	6.78^{a}	20.69^{a}	73.21^{a}	90.91^{a}	94.55^{a}	96.30^{a}	98.15^{a}
T6: *B. bassiana* VKA 01 strain @ 10^{9} spores ml^{-1}	8.48^{a}	32.76^{a}	64.29^{ab}	89.09^{a}	96.36^{a}	98.15^{a}	98.15^{a}

DAT – Days after treatment, Mean values in each column followed by a common letter are not significantly different by DMRT (p = 0.05)

Choanephora cucurbitarum applied at the rate of 10^7, 10^8 and 10^9spores ml^{-1}recorded 14.55, 16.36 and 12.73 per cent mortality respectively, five days after spraying *Beauveria bassiana* VKA 01 strain, in comparison recorded mortality of 96.36, 94.55 and 85.45 per cent respectively for the corresponding spore concentrations. Treatments involving the two higher concentrations of *B. bassiana* VKA 01 strain were significantly superior to the remaining four treatments.

Six days after treatment, *C. cucurbitarum* applied at the rate of 10^7, 10^8 and 10^9 spores ml^{-1}recorded 14.81, 16.67 and 18.52 per cent mortality and were at par with each other.*Beauveria bassiana* VKA 01 strain sprayed at the rate of 10^9 spores ml^{-1} recorded highest mortality of 98.15 per cent and was followed *B. bassiana* VKA 01 strain applied at a dose of 10^8 spores ml^{-1} with 96.30 per cent mortality. The above two treatments were significantly superior to *B. bassiana* VKA 01 strain sprayed at the rate of 10^7 spores ml^{-1} (87.04 % mortality) as well as all concentrations of *C. cucurbitarum.*

Choanephora cucurbitarum sprayed at the rate of 10^7, 10^8 and 10^9spores ml^{-1}recorded 18.52, 18.52 and 22.23 per cent mortality of rice bugs respectively seven days after treatment. In comparison, *B. bassiana* VKA 01 strain applied at the rate of10^7, 10^8 and 10^9spores ml^{-1}had registered90.74, 98.15 and 98.15 per cent mortality respectively. *Beauveria bassiana* VKA 01 strain at the rate of 10^8 and 10^9 spores ml^{-1} were on par with each other and were significantly superior to the remaining treatments.

Beauveria bassiana VKA 01 strain recorded more than 90 per cent mortality of both nymphs and adults of rice bugs seven days after treatment at all the concentrations evaluated. However, the highest mortality registered by *Choanephora cucurbitarum* was 53.84 per cent in case of nymphs and 22.23 per cent in case of adults of rice bug, seven days after treatment.

4.4.3 Bioassay of fungal isolates against rice bug in pot culture

Bioefficacy of the two most effective concentrations each (*viz.* 10^8 and10^9spores ml^{-1}) of the two isolates as identified in the laboratory bioassay were evaluated in a pot culture experiment. Mixed population of 20 rice bugs, released on rice plants at milky grain stage were treated with respective concentrations of *B. bassiana* VKA 01 strain and *C. cucurbitarum* isolates. *Beauveria bassiana* NBAIR strain at the rate of 10^8spores ml^{-1} served as check, while malathion 500 g ai ha^{-1} formed the insecticide check. The number of dead rice bugs was recorded 3,5,7,10,15 and 20 days after treatment. From the data collected, per cent mortality of rice bugs was calculated (Table 17).

Three days after spraying, malathion 500 g ai ha^{-1} recorded cent per cent mortality and was significantly superior to all other treatments. The plots treated with *C. cucurbitarum* at the rate of 10^8 and 10^9spores ml^{-1} recorded 0.57 and 1.14 per cent mortality and were at par with each other. However, *B. bassiana* VKA 01 strain, applied at the rate of 10^8spores ml^{-1} recorded 3.43 per cent mortality and was followed by *B. bassiana* NBAIR strain at the rate of 10^8spores ml^{-1} with 2.29 per cent mortality and *B. bassiana* VKA 01 strain applied at the rate of 10^9 spores per ml with 1.72 per cent mortality, both of which were at par. *Choanephora cucurbitarum,* applied at the rate of 10^8 and 10^9spores ml^{-1}recorded 0.57 and 1.14 per cent mortality respectively and were on par with each other.

Beauveria bassiana VKA 01 strain, applied at the rate of $10^8$and 10^9spores ml^{-1}recordedmean mortality of 26.32and 25.15per cent respectively five days after treatment, followed by *B. bassiana* NBAIR strain with 21.64 per cent mortality. All the three treatments were on par with each other and were significantly superior to those involving *C. cucurbitarum*, Pots treated with *C. cucurbitarum* at the rate of 10^8 and10^9 spores ml^{-1} recorded 5.26 and 2.92 per cent mortality and were on par with each other.

Beauveria bassiana VKA 01 strain at all the concentrations evaluated caused more than fifty per cent mortality of bugs seven days after treatment. The

Table 17. Bioefficacy of local isolates of entomopathogenic fungi against rice bugin pot culture

Sl. No.	Treatment	Mortality (%)					
		3 DAT	5 DAT	7 DAT	10 DAT	15 DAT	20 DAT
1	*Beauveria bassiana*VKA 01 strain @ 10^8 spores ml^{-1}	3.43^{b}	26.32^{b}	51.53^{b}	85.71^{b}	98.11^{a}	99.36^{a}
2	*B. bassiana*VKA 01 strain @ 10^9 spores ml^{-1}	1.72^{bcd}	25.15^{b}	50.92^{b}	88.20^{b}	98.11^{a}	99.36^{a}
3	*Choanephora cucurbitarum* @ 10^8 spores ml^{-1}	0.57^{cd}	5.26^{c}	8.59^{d}	16.77^{d}	21.38^{c}	26.92^{c}
4	*C. cucurbitarum* @ 10^9 spores ml^{-1}	1.14^{cd}	2.92^{c}	5.52^{de}	14.91^{d}	23.27^{c}	30.77^{b}
5	*B. bassiana* NBAIR strain @ 10^8 spores ml^{-1}	2.29^{c}	21.64^{b}	42.95^{c}	76.40^{c}	88.05^{b}	98.08^{a}
6	Malathion 50 EC @ 500 g ai ha^{-1}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}

DAT – Days after treatment, Mean values in each column followed by a common letter are not significantly different by DMRT (p = 0.05)

highest mortality of 51.53 per cent was recorded by *B. bassiana* VKA 01 strain applied at the rate of 10^8spores ml^{-1}. This was followed by *B. bassiana* VKA 01 strain applied at the rate of 10^9spores $ml^{-1}$with 50.92per cent kill, both being at par. *Beauveria bassiana* NBAIR strain recorded 42.95 per cent mortality. Pots treated with *C. cucurbitarum* at the rate of 10^8 and 10^9spores ml^{-1} recorded significantly lower mortality values of 8.59 and 5.52 per cent respectively as compared to *B. bassiana* VKA 01 strain.

Ten days after treatment, pots treated with *B. bassiana* VKA 01 strain applied at the rate of $10^8$and 10^9 spores ml^{-1}emerged as the most effective treatments with 85.71 and 88.20 per cent mortality respectively. Both the above treatments were on par with each other and were followed by *B. bassiana* NBAIR strain with 76.40 per cent mortality. The above treatments were significantly superior to all the treatments of *C. cucurbitarum*, with 16.77 and 14.91 per cent mortality when applied at the rate of 10^8 and 10^9spores ml^{-1}respectively.

Pots treated with *B. bassiana* VKA 01 strain, applied at the rate of 10^8 and 10^9 spores ml^{-1} induced the highest mortality of 98.11 per cent each, and were on par with the mortality recorded by malathion. This was followed by *B. bassiana* NBAIR strain with a significantly lower 88.05 per cent mortality. *C. cucurbitarum,* applied at the rate of 10^8 and 10^9 spores ml^{-1}, recorded 21.38 and 23.27 per cent mortality respectively, which was significantly lower than the treatments involving *B. bassiana* VKA 01 strain.

Twenty days after spraying, pots treated with *B. bassiana* VKA 01 strain, applied at the rate of 10^8 and 10^9spores ml^{-1} induced identical values of 99.36 per cent mortality, followed by *B. bassiana* NBAIR strain 10^8spores ml^{-1} with 98.08 per cent mortality. Above three treatments were on par with the insecticide, malathion and also were significantly superior to *C. cucurbitarum*, with 26.92 and 30.77 per cent mortality, when applied at the rate of 10^8 and 10^9spores ml^{-1}.

4.4.3.1 Effect of entomopathogenic fungi on grain damage by rice bug

Pots treated with malathion had the lowest mean grain damage of 2.05 per cent and was significantly superior to all other treatments (Table 18). *Beauveria bassiana* VKA 01 strain, applied at the rate of 10^8 and 10^9spores ml^{-1} recorded 33.34 and 38.94 per cent mean chaffy grains per panicle respectively andwas followed by *B. bassiana* NBAIR strain (42.27 %). *Choanephora cucurbitarum* applied at the rate of10^8 and 10^9spores ml^{-1}suffered 51.27 and 49.64 per cent grain damage respectively. The above mentioned four treatments were at par with each other. The highest grain damage was recorded in untreated control (68.16 %).

Table 18. Effect of isolates of entomopathogenic fungi on grain damage by rice bug

Sl. No.	Treatment	Chaffy grains per panicle (%)
1	*Beauveria bassiana* VKA 01 strain @ 10^8 spores ml^{-1}	33.34^{c}
2	*B. bassiana* VKA 01 strain @ 10^9 spores ml^{-1}	38.94^{bc}
3	*Choanephora cucurbitarum* @ 10^8 spores ml^{-1}	51.27^{b}
4	*C. cucurbitarum* @ 10^9 spores ml^{-1}	49.64^{b}
5	*B. bassiana* NBAIR strain @ 10^8 spores ml^{-1}	42.27^{bc}
6	Malathion 50 EC @ 500g aiha^{-1}	2.05^{d}
7	Control	68.16^{a}

Mean values in each column followed by a common alphabets are not significantly different by DMRT (p = 0.05)

4.4.4 FIELD EVALUATION OF *Beauveria bassiana* VKA 01 STRAIN AGAINST RICE BUG

Beauveria bassiana VKA 01 strain at the rate of 10^8spores ml^{-1},identified as the best treatment in pot culture experiment was evaluated under field conditions in two districts, namely, Thrissur and Palakkad. The fungal isolate was compared with Malathion 50 EC @ 500 g ai ha^{-1}, a botanical pesticide

(Azadirachtin 0.005 %), *B. bassiana* NBAIR strain at the rate of 10^8 spores ml^{-1} and untreated control.

4.4.4.1 Field evaluation at Palakkad

Field evaluation of VKA 01 strain of *B. bassiana* against rice bug *Leptocorisa* spp. was carried out at RARS Pattambi in Palakkad.

4.4.4.1.1 Effect of *Beauveria bassiana* VKA 01 strain on rice bug population

The effect of various treatments on the mean rice bug population is presented in Table 19. Five days after treatment, the mean population of bugs in malathion treated plot was 0.5 bugs per m^2, which was significantly superior to all other treatments. It was followed by *B. bassiana* VKA 01 strain, with 15.42 bugs per m^2, which in turn, was significantly superior to *B. bassiana* NBAIR strain with 18.33 bugs per m^2. Azadirachtin treated plot had a significantly higher number of 21.33 bugs per m^2 and was on par with untreated control having 23.83 bugs per m^2.

Ten days after treatment, malathion recorded the lowest mean bug population of 6.25 bugs per m^2 which was on par with *B. bassiana* VKA 01 strain treated plots which had 9.25 bugs per m^2.*Beauveria bassiana* NBAIR strain recorded 11.67 bugs per m^2. A mean bug population of 19.34 bugs per $m^2$was recorded in azadirachtin treated plots, followed by untreated control (20.17 per m^2). Both were on par with each other and had significantly higher mean bug population than preceding treatments.

Fifteen days after spraying, the average number of bugs in plots treated with malathion, *B. bassiana* VKA 01 strain and *B. bassiana* NBAIR strain, were 9.83, 10.23 and 12.33 per m^2, respectively, all the three being on par with each other. However, plots treated with azadirachtin as well as untreated control plot continued to harbour significantly higher number of 18.67 and 19.75 bugs per m^2 respectively.

Table 19. Effect of *Beauveria bassiana* VKA 01 strain on rice bug population at Pattambi, Palakkad

Sl. No.	Treatment	Population of rice bugs (no. per m^2)				
		Precount	5 DAT	10 DAT	15 DAT	20 DAT
1	*B. bassiana* VKA 01 strain @ 10^8 spores ml^{-1}	26.83[a]	15.42[c]	9.25[c]	10.23[b]	11.63[b]
2	*B. bassiana* NBAIR strain @ 10^8spores ml^{-1}	27.08[a]	18.33[b]	11.67[b]	12.33[b]	12.92[b]
3	Malathion 50 EC @ 500 g ai ha^{-1}	27.42[a]	0.58[d]	6.25[c]	9.83[b]	10.75[b]
4	Azadirachtin 0.005 per cent	28.92[a]	21.33[a]	19.34[a]	18.67[a]	15.92[a]
5	Untreated control	28.58[a]	23.83[a]	20.17[a]	19.75[a]	14.67[a]

DAT – days after treatment. Mean values in each column followed by a common letter are not significantly different by DMRT (p = 0.05)

Twenty days after spraying, malathion once again recorded the lowest mean bug population of 10.75 bugs per m^2 which, however was on par with *B. Bassiana* VKA 01 strain, applied at the rate of 10^8 spores ml^{-1} (11.63 bugs per m^2) and *B. bassiana* NBAIR strain applied at the rate of 10^8 spores ml^{-1} (12.92 bugs per m^2). Azadirachtin treated plot had a significantly higher 15.92 bugs per m^2 and was on par with untreated control having 14.67 bugs per m^2.

4.4.4.1.2 Effect of *Beauveria bassiana* VKA 01 strain on grain damage by rice bug

Mean grain yield obtained from different treatments are presented in Table 20. Highest grain yield of 529.17 g per m^2 was recorded in plots treated with malathion. Plots treated with *B. bassiana* VKA 01 strain and NBAIR strain recorded 521.83 and 486.67 g per m^2, respectively and which were on par with the yield recorded in insecticide treated plots. Plots treated with azadirachtin, with a mean yield of 437.50 g per m^2 was on par with untreated control which yielded 435.00 g per m^2.

Table 20. Effect of *Beauveria bassiana*VKA 01 strain on grain damage by rice bug and yield at Palakkad

Sl no.	Treatment	Mean chaffy grains/panicle (%)	Yield (g/m^2)
1	*Beauveria bassiana* VKA 01 strain @ 10^8 spores ml^{-1}	12.03^{b}	521.83^{ab}
2	*B. bassiana* NBAIR strain @ 10^8 spores ml^{-1}	13.72^{b}	486.67^{b}
3	Malathion 50 EC @ 500 g ai ha^{-1}	8.37^{c}	529.17^{a}
4	Azadirachtin 0.005 per cent	16.68^{a}	437.50^{c}
5	Untreated control	17.78^{a}	435.00^{c}

Mean values in each column followed by a common letter are not significantly different by DMRT (p = 0.05)

4.4.4.2 Field evaluation at Thrissur

Field evaluation of VKA 01 strain of *B. bassiana* against rice bug *Leptocorisa* spp. was also carried out at Varadium in Thrissur from July to September 2018. The results are presented in Table 21.

4.4.4.2.1 Effect of *Beauveria bassiana* VKA 01 strain on rice bug population

Plots treated with malathion 50 EC at the rate of 500 g ai ha^{-1} had the lowest mean population of 2.5 bugs m^2, five days after spraying and was significantly superior to all other treatments. Plots treated with *B. bassiana* VKA 01 strain and *B. bassiana* NBAIR strain had an average of 15.75 and 15.00 bugs/m2, respectively and were on par with each other.

The above treatments were significantly superior to both azadirachtin (19.25 bugs/m^2) and untreated control (19.42 bugs/m^2), the latter two again being on par with each other.

Plots treated with *B. bassiana* VKA 01 strain and *B. bassiana* NBAIR strain recorded mean population of 10.67 and11.34 bugs perm^2 ten days after spraying and were on par with the mean bug population of 11.08 per m^2 in malathion treated plot. All the three treatments were significantly superior to azadirachtin and untreated control with mean population of 18.34 and 19.92 bugs per m^2 respectively.

Fifteen days after spraying, *B. bassiana* VKA 01 strain, *B. bassiana* NBAIR isolate and malathion 50 EC once again registered lower bug populations of 11.25, 12.67 and 13.33 bugs per m^2, respectively. While they were on par with each other, they were also significantly superior to azadirachtin which had 17.84 bugs per m^2. Untreated control had the highest mean bug population (19.25 bugs per m^2) and was on par with azadirachtin.

Twenty days after treatment, all treatments had comparable bug populations ranging from 12.34 to 15.17 bugs per m^2. Plots treated with *B.*

Table 21. Effect of *Beauveria bassiana* VKA 01 strain on rice bug population at Thrissur

Sl. No.	Treatment	Population of rice bugs (no. per m^2)				
		Precount	5 DAT	10 DAT	15 DAT	20 DAT
1	*B. bassiana*VKA 01 strain@ 10^8 spores ml^{-1}	18.92 a	15.75 b	10.67 b	11.25 b	12.34 a
2	*B. bassiana* NBAIR strain @ 10^8spores ml^{-1}	18.58 a	15.00 b	11.34 b	12.67 b	13.00 a
3	Malathion 50 EC @ 500 g ai ha^{-1}	18.42 a	2.50 c	11.08 b	13.33 b	13.75 a
4	Azadirachtin 0.005 per cent	19.67 a	19.25 a	18.34 a	17.84 a	14.50 a
5	Untreated control	19.00 a	19.42 a	19.92 a	19.25 a	15.17 a

DAT – days after treatment. Mean values in each column followed by a common letter are not significantly different by DMRT (p = 0.0

bassiana VKA 01 strain and NBAIR strain had 12.34 and 13 bugs per m^2 respectively, while plot treated with malathion recorded 13.75 bugs per m^2 which was followed by azadirachtin and untreated control with 14.5 and 15.17 bugs per m^2,respectively.

4.4.4.2.2 Effect of *Beauveria bassiana* VKA 01 strain on grain damage by rice bug

Plots treated with malathion had the lowest mean grain damage of 6.13 per cent and was significantly superior to other treatments (Table 22). This was followed by *B. bassiana* VKA 01 strain and NBAIR strain with 12.27 and 13.58 per cent chaffy grains respectively, both being on par with each other. The above three treatments had significantly lower mean grain damage compared to plots treated with azadirachtin having 17.2 per cent chaffy grains and untreated control with 18.14per cent grain damage.

Table 22. Effect of *Beauveria bassiana*VKA 01 strainon grain damage by rice bug and yield at Thrissur

Sl. No.	Treatment	Chaffy grains/panicle (%)	Yield (g/m^2)
1	*Beauveria bassiana* VKA 01 strain@ 10^8 spores ml^{-1}	12.27^b	459.13^b
2	*B. bassiana* NBAIR strain @ 10^8spores ml^{-1}	13.58^b	453.00^b
3	Malathion 50 EC @ 500 g ai ha^{-1}	6.13^c	547.67^a
4	Azadirachtin 0.005 per cent	17.20^a	391.83^c
5	Untreated control	18.14^a	373.17^d

Mean values in each column followed by a common letter are not significantly different by DMRT (p = 0.05)

4.4.4.2.3 Effect of *Beauveria bassiana* VKA 01 strain on yield

Mean grain yield obtained from different treatments are presented in Table 22. The grain yield varied from the lowest value of 373.13 g per m^2 in case of untreated plots to the highest value of 547.67 g m^{-2} in plots treated with malathion. *Beauveria bassiana* VKA 01 strain and NBAIR strain treated plots recorded yield of 459.13 and 453.00 g m^{-2} respectively and were on par with each other. Plots treated with azadirachtin, with a mean yield of 391.83 g m^{-2} and untreated control with 373.17 g grains m^{-2} were on par with each other but had significantly lower grain yield than other treatments.

4.5 *IN VITRO* EVALUATION ON COMPATIBILITY OF *Beauveria bassiana* VKA 01 STRAIN WITH SELECTED PESTICIDES

The VKA 01 strain of *Beauveria bassiana,* which was evaluated and was found effective against rice bug was tested for its compatibility with selected insecticides and fungicides through poisoned food technique. The results are presented in Table 23.

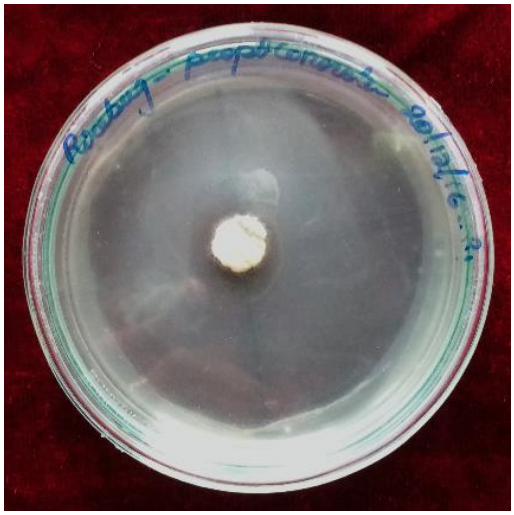
Table 23. Compatibility of *Beauveria bassiana* VKA 01 strain with selected pesticides

Sl. no.	Treatment	Per cent inhibition
1	Flubendiamide20 WDG @ 25 g a.i ha^{-1}	34.95 [d]
2	Lambda cyhalothrin5 EC @ 50 g a.i ha^{-1}	68.00 [b]
3	Malathion 50 EC @ 500 g a.i ha^{-1}	70.29 [b]
4	Acephate75 SP @ 750 g a.i ha^{-1}	31.29 [d]
5	Azadirachtin 0.005 per cent	48.19 [c]
6	Propiconazole25 EC @ 0.025 per cent	85.71 [a]
7	Copper hydroxide 77 WP @ 0.2 per cent	48.10 [c]

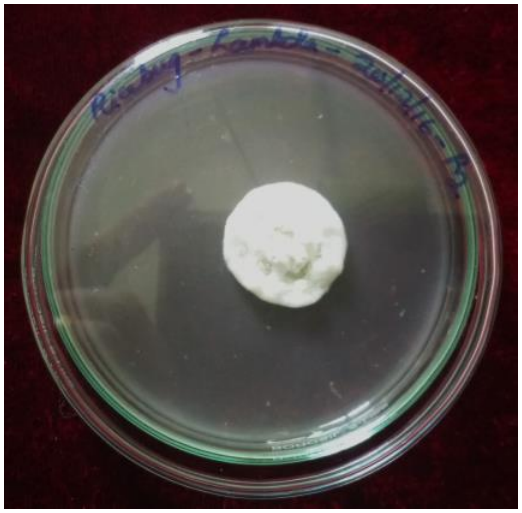
Mean values in each column followed by a common letter are not significantly different by DMRT (p = 0.05)

VKA -Control

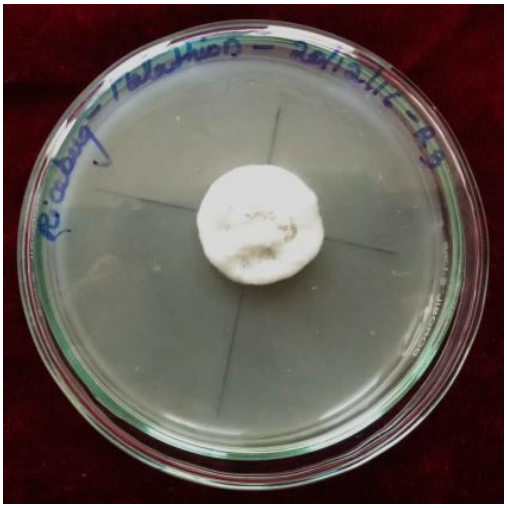
VKA - Flubendiamide



VKA - Propiconazole



VKA - Lambda cyhalothrin



VKA - Malathion

VKA - Copper hydroxide

Plate 28. Compatibility of *Beauveria bassiana* VKA 01 isolate with selected pesticides

All the pesticides evaluated showed significant reduction in the growth of the entomopathogen (Plate. 28). The fungicide propiconazole 25 EC at 0.025 per cent registered the highest inhibition of 85.71 per cent. This was followed by malathion as well as lambda cyhalothrin with significantly lower inhibition values of 70.29 and 68 per cent respectively, both being at par. Acephate and flubendiamide had significantly lower inhibitory effect compared to the above, with values of 31.29 and 34.95per cent respectively and were on par with each other. Both azadirachtin and copper hydroxiderecorded identical extent of inhibition by 48.19 and 48.10 per cent respectively.

4.6. STANDARDISATION OF FORMULATIONS OF *Beauveria bassiana* VKA 01 STRAIN

Standardisation of VKA 01 strain isolate of *B. bassiana* into talc, oil and liquid based formulations was carried out and the formulations were evaluated for their bioefficacy against rice bug as well as for their shelf life.

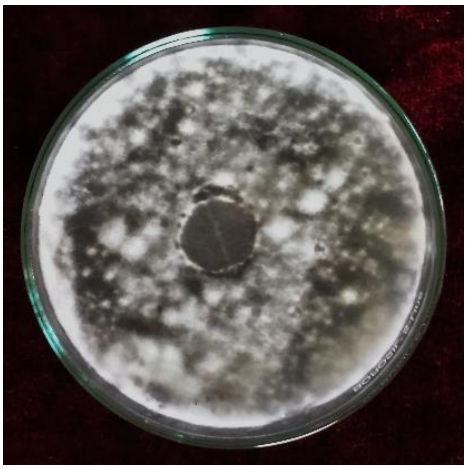
4.6.1 Standardisation of oil based formulation of *Beauveria bassiana* VKA 01 strain

4.6.1.1 Identification of the most suited oil base

The suitability of five commonly available vegetable oils *i.e.*, sesame oil, coconut oil, sunflower oil, rice bran oil and palm oil as carrier for *B. bassiana* VKA 01 strain in oil formulation was evaluated through inhibition zone technique. The zone of inhibition was visually assessed seven days after inoculation.

Rice bran oil and sunflower oil showed greater inhibition whereas coconut oil showed moderate level of inhibition (Plate 29). Neither palm oil nor sesame oil inhibited the fungus. Palm oil and sesame oil which were identified as most promising as carrier oils in the previous study were evaluated for the retention of viability of spores. Spores were suspended in each of the two selected vegetable oils at a concentration of 2.45 x 10^8spores ml^{-1} each. Fifteen days after storage,

Sunflower oil



Sesame oil

Palm oil

Coconut oil

Rice bran oil

Plate 29. Inhibition of *B. bassiana* VKA 01 strain by different vegetable oils

significantly higher number of viable spores were observed in case of palm oil with a final concentration of 1.56 x 10^8 spores ml^{-1} as against sesame oil having 7.3 x 10^7spores ml^{-1} (Table 24).

Table 24. Effect of selected oils on viability of *B. bassiana* VKA 01 strain spores

Sl. No.	Treatments	*B. bassiana* VKA 01 strain (cfu)
1	Palm oil + *B. bassiana* VKAspore	1.56 x 10^8
2	Sesame oil + *B. bassiana* VKAspore	7.3 x 10^7

4.6.1.2 Evaluation of adjuvants for oil based formulation

For identifying the most suited adjuvant as well as its optimum concentration, spores of *B. bassiana* VKA 01 strain were suspended in palm oil at a concentration of 2.5 x 10^8 spores ml^{-1}. Four adjuvants namely Tween 80, glycerol, poly ethylene glycol and carboxy methyl cellulose at three different concentrations each were mixed separately with the spores suspension and stored at room temperature. The number of colony forming units was assessed one month after storage.

Glycerol, at all the three concentrations evaluated, recorded significantly higher cfu than all other treatments (Table 25). At three per cent concentration, it recorded 2.06 x 10^8 spores ml^{-1}, which was significantly superior to the remaining treatments. This was followed by glycerol 5 per cent and glycerol 1per cent with 1.74 x 10^8 and 1.04 x 10^8 spores ml^{-1} respectively, which were again significantly superior to the remaining treatments.

CMC added at 1 per cent proved to be the next best treatment with a cfu count of 0.74 x 10^8 ml^{-1}. CMC at both 2 per cent and 0.5 per cent as well as PEG and Tween 80 at all the three concentrations evaluated had cfu count ranging from 0.28 to 0.37 ml^{-1}. Control, with no additives had the lowest cfu of 0.18 x 10^8spores ml^{-1}.

Table 25. Effect of adjuvants on viability of spores of *Beauveria bassiana* VKA 01 strain in oil formulation

Sl. No.	Treatment	*B. bassiana* cfu ($x10^8$ ml^{-1})
1	Palm oil + *B. bassiana* VKA 01 strain spore + Tween 80 0.5%	0.23 ^{fg}
2	Palm oil + *B. bassiana* VKA 01 strain spore + Tween 80 1%	0.35 ^{e}
3	Palm oil + *B. bassiana* VKA 01 strain spore + Tween 80 2%	0.28^{efg}
4	Palm oil + *B. bassiana* VKA 01 strain spore + glycerol 1%	1.04 ^{c}
5	Palm oil + *B. bassiana* VKA 01 strain spore + glycerol 3%	2.06 ^{a}
6	Palm oil + *B. bassiana* VKA isolate spore + glycerol 5%	1.74 ^{b}
7	Palm oil + *B. bassiana* VKA 01 strain spore + PEG 0.5%	0.34 ^{ef}
8	Palm oil + *B. bassiana* VKA 01 strain spore + PEG 1%	0.33 ^{ef}
9	Palm oil + *B. bassiana* VKA 01 strain spore + PEG 2%	0.31^{ef}
10	Palm oil + *B. bassiana* VKA 01 strain spore + CMC 0.5%	0.28^{efg}
11	Palm oil + *B. bassiana* VKA 01 strain spore + CMC 1%	0.74 ^{d}
12	Palm oil + *B. bassiana* VKA 01 strain spore + CMC 2%	0.37 ^{e}
13	Palm oil + *B. bassiana* VKA 01 strainspore (Control)	0.18^{fg}

Mean values in each column followed by a common letter are not significantly different by DMRT (p = 0.05)

4.6.2 Standardisation of aqueous formulation of *Beauveria bassiana* VKA 01 strain

Aqueous formulations of *B. bassiana* VKA 01 strain were prepared by mixing spores suspended in sterile water at a concentration of 2.5 x 10^8spores ml^{-1} separately with four different adjuvants *i.e.*, Tween 80, glycerol, PEG and CMC at three different concentrations each (Table26) . The spore suspensions of *B. bassiana* VKA 01 strain, mixed with respective adjuvants were stored at room

temperature for one month, following which the number of colony forming units were assessed.

Table 26. Effect of adjuvants on viability of spores of *Beauveria bassiana* VKA 01 strain in aqueous formulation

Sl. No.	Treatments	Final cfu ($x10^8$ ml^{-1})
1	Water + *B. bassiana* VKA 01 strain spore + Tween 80 0.5%	0.03 [d]
2	Water + *B. bassiana* VKA 01 strain spore + Tween 80 1%	0.04[d]
3	Water + *B. bassiana* VKA 01 strain spore + Tween 80 2%	0.09[d]
4	Water + *B. bassiana* VKA 01 strain spore + Glycerol 1%	0.08 [d]
5	Water + *B. bassiana* VKA 01 strain spore + Glycerol 3%	0.13 [d]
6	Water + *B. bassiana* VKA 01 strain spore + Glycerol 5%	0.10 [d]
7	Water + *B. bassiana* VKA 01 strain spore + PEG 0.5%	0.03 [d]
8	Water + *B. bassiana* VKA 01 strain spore + PEG 1%	0.02 [d]
9	Water + *B. bassiana* VKA 01 strain spore + PEG 2%	0.03[d]
10	Water + *B. bassiana* VKA 01 strain spore + CMC 0.5%	2.34 [a]
11	Water + *B. bassiana* VKA 01 strain spore + CMC 1%	1.93 [b]
12	Water + *B. bassiana* VKA 01 strainspore + CMC 2%	1.38 [c]
13	Water + *B. bassiana* VKA 01 strainspore (Control)	0.03 [d]

Mean values in each column followed by a common letter are not significantly different by DMRT (p = 0.05)

CMC at 0.5 per cent concentration recorded the highest cfu value of 2.34 x 10^8 spores ml^{-1}, which was significantly superior to other treatments. This was followed by CMC one per cent (1.93 X 10^8 per ml) and CMC two per cent (1.38 x 10^8 per ml), which were again significantly superior to other adjuvants. The

remaining treatments registered 0.02 to 0.13 x 10^8 cfu per ml and were on par with each other.

4.6.3 Standardization of talc based formulation of *Beauveria bassiana* VKA 01 strain

Sterilized talc powder (100 mesh size) was mixed with the homogenized fungal slurry at 1:1 ratio to obtain an initial cfu of 2.34 x 10^8 spores ml^{-1}. Two adjuvants, namely, chitin and chitosan at three concentrations each were added under laminar air flow chamber to maintain aseptic condition. The moisture content was maintained at 10 -12 per cent. The formulations were packed in sterile polyethylene covers of 65 microns and were stored at room temperature. The number of colony forming units was assessed one month after storage (Table 27).

Table 27. Effect of adjuvants on viability of spores of *Beauveria bassiana* VKA 01 strain in talc formulation

Sl. No.	Treatments (%)	Final cfu ($x10^8$ ml^{-1})
1	Talc + *B. bassiana* VKA 01 strain+ Chitin 3%	0.85 [b]
2	Talc + *B. bassiana* VKA 01 strain+ Chitin 5%	1.92 [a]
3	Talc + *B. bassiana* VKA 01 strain+ Chitin 7%	0.71 [b]
4	Talc + *B. bassiana* VKA 01 strain+ Chitosan 0.5%	0.12 [c]
5	Talc +*B. bassiana* VKA 01 strain+ Chitosan 1%	0.23 [c]
6	Talc +*B. bassiana* VKA 01 strain+Chitosan 2%	0.14 [c]
7	Talc + *B. bassiana* VKA 01 strain(control)	0.12 [c]

Mean values in each column followed by a common letter are not significantly different by DMRT (p = 0.05)

Talc mixed with five per cent chitin recorded the highest cfu of 1.92 x 10^8spores ml^{-1}one month after storage, which was significantly superior to the remaining treatments. The above treatment was followed by talc with chitin at three per cent (0.85 x 10^8 cfu ml^{-1}) and talc with chitin at seven per cent (0.71x 10^8 cfu ml^{-1}) both being on par with each other. All the three concentrations of chitosan *i.e.*, 0.5, 1.0 and 2 per cent registered significantly lower cfu values of 0.12, 0.23 and 0.14 x 10^8cfu ml^{-1}, respectively and were on par with control (0.12 x 10^8cfu ml^{-1}).

4.6.4 Evaluation of *Beauveria bassiana* VKA 01 strain formulations against rice bug in field

Field evaluation of the efficacy of the three formulations of *B. bassiana* VKA 01 strain against rice bug was carried out at RARS Pattambi, Palakkad. Each plot was sprayed with respective formulation containing 10^8 spores ml^{-1}. Treatments were applied as and when the bug population crossed the economic threshold level of one to two bugs per hill. Population of rice bugs (nymphs and adults) in each plot was recorded prior to spraying. Post treatment bug population per m^2 was recorded 5, 10, 15 and 20 days after treatment.The results are presented in Table 28.

Five days after treatment, plots sprayed with malathion 50 EC @ 500 g ai ha^{-1} recorded the lowest mean population of 3.92 bugs per m^2 and was significantly superior to the remaining treatments. Malathion was followed by talc as well as aqueous formulations with 13.83 and 15.58 bugs per m^2, respectively, both being on par with each other. Plot treated with oil based formulation had a mean bug population of 18.92 per m^2 and was on par with untreated control (19.32 bugs m^{-2}).

Plots treated with talc formulation had the lowest count of 9.08 bugs per m^2 ten days after treatment. This was followed by malathion as well as aqueous formulation with 9.32 and 9.75 bugs per m^2 respectively, all the three treatments being were on par with each other. The above treatments were significantly

Table 28. Efficacy of *Beauveria bassiana* VKA 01 strain formulations against rice bug in field

Sl. No.	Treatment	Population of rice bugs (no. per m^2)				
		Precount	5 DAT	10 DAT	15 DAT	20 DAT
1	Talc formulation @ 10^8 spores ml^{-1}	21.33^{a}	13.83^{b}	9.08^{b}	9.42^{c}	10.92^{ab}
2	Oil based formulation @ 10^8 spores ml^{-1}	22.67^{a}	18.92^{a}	16.92^{ab}	12.92^{bc}	12.79^{a}
3	Aqueous formulation @ 10^8 spores ml^{-1}	22.25^{a}	15.58^{b}	9.75^{b}	11.58^{bc}	13.75^{a}
4	Malathion 50 EC @ 500 g ai ha^{-1}	22.75^{a}	3.92^{c}	9.32^{b}	13.58^{b}	12.83^{a}
5	Control	21.60^{a}	19.32^{a}	18.58^{a}	17.92^{a}	14.25^{a}

DAT – days after treatment, Mean values in each column followed by a common letter are not significantly different by DMRT (p = 0.05)

superior to both oil formulation and untreated control (18.58 and 16.92 bugs per m^2), respectively.

Fifteen days after spraying, talc formulation once again had the lowest rice bug population of 9.42 bugs m^{-2} which, however was on par with bug populations in plots treated with aqueous as well as oil based formulations with mean bug population of 11.58 and 12.92 bugs m^{-2}, respectively. Pots treated with malathion 50 EC @ 500 g ai ha^{-1}recorded mean bug population of 13.58 bugs m^{-2}while untreated plot had 17.92 bugs m^{-2}.

The bug population was comparable among the different treatments twenty days after planting. Plots treated with talc formulation had the lowest number of 10.92 bugsm^{-2}, while the highest bug population of 14.25 bugs m^{-2} was recorded in control plot. Plots treated with oil based formulation and aqueous formulation had12.92 and 13.75 bugsm^{-2} respectively.

4.6.4.2 Effect of *Beauveria bassiana* VKA 01 strain on grain damage caused by rice bug

Comparison of the grain damage indicated that all the treatments were significantly superior to untreated control in terms of grain damage(Table 29).Malathion 50 EC @ 500 g ai ha^{-1} recorded the lowest extent of 10.08 per cent grain damage. The above treatment was on par with talc based formulation with 10.25per cent chaffy grains. *Beauveria bassiana* aqueous and oil based formulation recorded 14.50 and 14.84 per cent grain damage respectively and were on par with each other. Control plot recorded the highest value of 17.5 percent mean grain damage.

4.6.4.3 Effect of *Beauveria bassiana* VKA 01 strain formulations on grain yield

Mean grain yield obtained from different treatments are presented in Table 29. The grain yield varied from the highest value of 635.00 g per m^2 in case of

plots treated with talc formulation to 558.34 g per m^2in case of oil based formulation. Both talc based formulation as well as malathion (628.00 g m^{-2}) were on par with each other and were significantly superior to other treatments. Plots treated with aqueous formulation registered the next highest yield of 600.00 g m^{-2}. Untreated control (583.33 gm^{-2}) as well as oil based formulation (558.34 gm^{-2}) of *B. bassiana* had comparable grain yields.

Table 29. Effect of *Beauveria bassiana* VKA 01 strain formulations on grain yield

Sl. No.	Treatment	Chaffy grains/panicle (%)	Yield (g/m^2)
1	Talc formulation @ 10^8 spores ml^{-1}	10.25^c	635.83^a
2	Oil based formulation @ 10^8 spores ml^{-1}	14.84^b	558.34^c
3	Aqueous formulation @ 10^8 spores ml^{-1}	14.50^b	600.00^b
4	Malathion 50 EC @ 500 g ai ha^{-1}	10.08^c	628.00^a
5	Control	17.50^a	583.33^c

Mean values in each column followed by a common letter are not significantly different by DMRT (p = 0.05)

4.6.5 Evaluation of shelf life of *Beauveria bassiana* VKA 01 strain formulations

The results of the study on shelf life of different formulations of *B. bassiana* VKA 01 strain stored under ambient conditions at for up to 12 months are presented in Table 30.

One month after storage, the highest cfu value of 11.2 x 10^8cfu ml^{-1} was recorded in oil formulation with adjuvants. It was followed by chitin enriched talc formulation with 11 x 10^8 cfu ml^{-1}, talc formulation without adjuvants (9.63 x 10^8 cfu ml^{-1}) and aqueous formulation with 9.3 x 10^8 cfu ml^{-1}, all being on par with each other. The above formulations were significantly superior to both oil based formulation without adjuvants (6.8 x 10^8 cfu ml^{-1}) as well as aqueous formulation without adjuvants (4.6 x 10^4 cfu ml^{-1}).

Chitin enriched talc, with a spore count of 9.83 x 10^8cfu ml^{-1} as well as oil formulation with adjuvants, with a spore count of 9.3 x 10^8cfu ml^{-1} were significantly superior to the remaining treatments such as aqueous formulation with adjuvants (5.9 x 10^8cfu ml^{-1}) and talc formulation without adjuvants (5.2 x 10^8cfu ml^{-1}), two months after storage. The lowest count of 1.81 cfu ml^{-1} was recorded in aqueous formulation without adjuvants.

A similar trend was observed three months after storage as well. The viability of spores in chitin enriched talc formulation recorded the highest value of 8.8 x 10^8 cfu ml^{-1}. This was followed by oil formulation with adjuvants (8.1 x 10^8 cfu ml^{-1}) which was on par with the former. Both the above values were significantly superior to remaining treatments. Aqueous formulation with adjuvants recorded the next highest count of 2.4 x 10^8cfu ml^{-1} while talc formulation without adjuvants recorded 1.5 x 10^8 cfu ml^{-1}. Both oil as well as aqueous formulation without adjuvants did not have the prescribed cfu counts.

Four months after storage, only three treatments, namely, chitin enriched talc formulation, oil formulation with adjuvants and aqueous formulation with adjuvants had cfu counts above the legally prescribed standard of 10^8 cfu ml^{-1}. Among these, the chitin enriched talc (4.3 x 10^8 cfu ml^{-1}) and oil formulation (4.1 x 10^8 cfu ml^{-1}) had significantly higher spore count than aqueous formulation with adjuvants (1.3 x 10^8 cfu ml^{-1}) and were on par with each other.

Two formulations alone retained their viability five months after storage. Chitin enriched talc formulation with 2.5 x 10^8 cfu ml^{-1} was significantly superior to the remaining treatment, oil formulation with adjuvants, having 1.1 x 10^8 cfu ml^{-1} was observed.

At six months after storage, chitin enriched talc formulation alone recorded viability of 1.38 x 10^8 cfu ml^{-1}.None of the formulations had viability above the required levels seven months after storage.

Table 30. Effect of storage of *Beauveria bassiana* VKA 01 strain formulations on viability of spores

Sl. No.	Treatment combination	Initial count (ml^{-1})	Monthly cfu count ml^{-1}											
			1	2	3	4	5	6	7	8	9	10`	11	12
1	Oil formulation	1.33×10^9	11.2×10^8 $(9.03)^a$	9.3×10^8 $(8.97)^a$	8.1×10^8 $(8.92)^a$	4.1×10^8 $(8.61)^a$	1.1×10^8 $(8.03)^a$	4.1×10^7 $(7.61)^b$	6.25×10^5 $(5.74)^b$	4.5×10^4 $(4.66)^b$	3.2×10^3 $(3.50)^b$	0.00 $(0.00)^b$	0.00 $(0.00)^b$	0.00 $(0.00)^b$
2	Oil formulation without adjuvants	1.33×10^9	6.8×10^8 $(8.83)^b$	9.3×10^7 $(7.97)^d$	2.9×10^7 $(7.46)^d$	2.4×10^4 $(4.38)^d$	3.1×10^2 $(2.48)^d$	0.00 $(0.00)^e$	0.00 $(0.00)^e$	0.00 $(0.00)^e$	0.00 $(0.00)^c$	0.00 $(0.00)^b$	0.00 $(0.00)^b$	0.00 $(0.00)^b$
3	Aqueous formulation	1.66×10^9	9.3×10^8 $(8.97)^{ab}$	5.9×10^8 $(8.77)^{bc}$	2.4×10^8 $(8.37)^b$	1.3×10^8 $(8.09)^b$	1.1×10^7 $(6.85)^b$	9.1×10^4 $(4.95)^d$	6.3×10^2 $(2.80)^d$	7.3 $(0.92)^b$	0.00 $(0.00)^c$	0.00 $(0.00)^b$	0.00 $(0.00)^b$	0.00 $(0.00)^b$
4	Aqueous formulation without adjuvants	1.33×10^9	4.6×10^4 $(3.66^{)c}$	1.80 $(0.10)^e$	0.00 $(0.00)^e$	0.00 $(0.00)^e$	0.00 $(0.00)^e$	0.00 $(0.00)^e$	0.00 $(0.00)^e$	0.00 $(0.00)^e$	0.00 $(0.00)^c$	0.00 $(0.00)^b$	0.00 $(0.00)^b$	0.00 $(0.00)^b$
5	Talc formulation	1.66×10^9	11×10^8 $(9.04)^a$	9.8×10^8 $(8.89)^{ab}$	8.8×10^8 $(8.72)^a$	4.3×10^8 $(8.60)^a$	2.5×10^8 $(8.40)^a$	1.38×10^8 $(8.15)^a$	8.3×10^7 $(7.92)^a$	6.1×10^7 $(7.79)^a$	1.1×10^7 $(7.40)^a$	6.8×10^5 $(4.83)^a$	4.3×10^3 $(3.63)^a$	8.7 $(0.98)^a$
6	Talc formulation without adjuvants	1.66×10^9	9.6×10^8 $(8.94)^{ab}$	5.2×10^8 $(8.72)^c$	1.5×10^8 $(8.18)^c$	6.4×10^7 $(7.76)^c$	1.9×10^6 $(6.28)^c$	5.6×10^5 $(5.74)^c$	1.3×10^5 $(5.12)^c$	3.0×10^4 $(4.47)^c$	0.00 $(0.00)^c$	0.00 $(0.00)^b$	0.00 $(0.00)^b$	0.00 $(0.00)^b$

Figures in parentheses are logarithmic transformed values. Mean values in each column followed by a common letter are not significantly different by DMRT (p = 0.05)

Discussion

5. DISCUSSION

The present study entititled “Characterization, evaluation and formulation of *Beauveria bassiana* (Bals.) strains against rice bug *Leptocorisa* spp. (Hemiptera: Alydidae)” was conducted at the Department of Agricultural Entomology with the objectives of collection, isolation and molecular characterization of local strains of *B. bassiana*; evaluation against *Leptocorisa* spp. and identification of a cost effective formulation of the most effective strain of *B. bassiana.* The results generated in the present study are discussed here.

5.1 SURVEY FOR ENTOMOPATHOGENIC FUNGI INFECTING RICE BUG, *Leptocorisa* spp.

Surveys were conducted over two seasons during 2016-17 in the major rice growing tracts of Alappuzha, Ernakulam, Thrissur and Palakkad districts of Kerala to collect local isolates of *B. bassiana* infecting rice bug. Only two cadavers of rice bug infected by entomopathogenic fungi were obtained during the surveys and that too from only one location *i.e.,* Pattambi in Palakkad district.

As the number of isolates was very low, a fungal pathogen obtained from rice bug at Vellanikkara, Thrissur and maintained at AICRP on BCCP was also included in further studies.

5.2 ISOLATION AND IDENTIFICATION OF FUNGUS FROM RICE BUG CADAVERS

The fungi isolated from both the cadavers obtained from Pattambi had similar morphological characters. In both cases, the fungal colonies produced cottony white mycelia during the early stages of growth which later turned greyish at the centre. The mycelia were hyaline and aseptate with irregular branching. Sporangiospores were ellipsoid and brown to pale brown in colour, having a mean spore size of 3.32 x 4.33 µm.

The above observations indicated that both the bugs could have been infected by the same fungal species. This was subsequently confirmed when the

fungi isolated from both the cadavers were tentatively identified as *Choanephora* sp., based on cultural and morphological observations. The colony and spore characteristics observed in the present study matched the description of the above genus by George (2015). Abel – Mortal *et al.* (2010) had reported that *C. cucurbitarum* exhibited cottony white growth that turns yellowish brown in colour on ageing. The findings of the present study on spore characteristics were also in agreement with those of Yusuke *et al.* (2010), who reported the sporangiophores of *C. cucurbitarum* to be aseptate, hyaline, and smooth – walled. The spores were brownish, oval with striations and having a size of 8 - 13 x 9.12 - 29 µm.

*Choanephora cucurbitarum*is an air, soil as well as seed borne facultative plant pathogenic fungus that thrives best under humid conditions (George, 2015). Kown and Park (2002) also had reported *C. cucurbitarum* as a weak plant pathogen that mostly penetrated through wounds to cause infection.

The fungus isolated from rice bug cadavers obtained from Vellanikkara and maintained at AICRP on BCCP produced white mycelial growth having single celled spores on a zig –zag rachis. Micrometry studies revealed that the isolate possessed round to oval, colourless spores having a mean diameter of 1.9 µm. This was in agreement with the dimensions generally reported for *B. bassiana.* Safavi (2010) for instance, had recorded diameter of spores of *B. bassiana* to be between 1.85 ± 0.32 to 2.27 ± 0.22 µm. Similar observations were also made by Petch (2006) who reported that *B. bassiana* produced globose to ellipsoidal spores of size 1.7 to 5.5 µm.Based on the above cultural and morphological observations, the isolate was tentatively identified as *Beauveria sp.*

A number of workers have isolated *B. bassiana* from insects, including the rice bug, *Leptocorisa* spp. Loc and Chi (2005) had, for instance, evaluated 12 different isolates of *B. bassiana* against rice earhead bug, *Leptocorisa acuta*, and reported 57.5 to 77.7 per cent mortality of the bugs ten days after spraying.

The above identities of the two fungal isolates were confirmed through molecular characterization using Internal Transcribed Spacer (ITS) sequencing.

ITS sequencing of Vellanikkara isolate yielded 520 base pair (bp) sequences and Basic Local Alignment Search (BLAST) at National Center for Biotechnology Information (NCBI) identified the same as *B. bassiana*. Similarly the Pattambi isolate with 467 base pair sequences was identified as *Choanephora cucurbitarum.*

The isolate from Vellanikkara was then named as *Beauveria bassiana* VKA 01 strain. It is a well-known entomopathogenic fungus with a broad host range, including members of order Hemiptera, Lepidoptera and Coleoptera (Pendland and Boucias, 2008). Dhar *et al.* (2019) characterized 13 fungal isolates collected from soils of Punjab, having similarity with *B. bassiana.* Molecular characterization using PCR amplification with ITS primer confirmed only three isolates as *B. bassiana.*

5.3 EVALUATION OF NATIVE ISOLATES OF ENTOMOPATHOGENIC FUNGI AGAINST RICE BUG

The pathogenicity of the native isolates to both nymphs and adults of rice bug was first assessed through laboratory bioassay. The isolates wereseparately evaluated at three different concentrations *viz.* 10^7,10^8 and 10^9 spores ml^{-1}.

5.3.1 Bioassay of native isolates of entomopathogenic fungi against nymphs of rice bug in laboratory

Both *C. cucurbitarum* and *B. bassiana* VKA 01 strain could induce mortality within 24 h of treatment. Mortality varied from 3.39 to 5.09 per cent at all the three concentrations evaluated and were on par with each other.

Two days after treatment, there was appreciable increase in mortality in all the three treatments involving *B. bassiana*, VKA 01 strain. The values ranged from 17.24 to 24.14 per cent and were on par with each other. In comparison, nymphs treated with *C cucurbitarum* suffered significantly lower mortality that varied from 5.17 to 10.35 per cent (Fig. 1).

Beauveria bassiana VKA 01 strain was successful in inducing 74.99 to 89.99 per cent mortality of the nymphs at all the concentrations evaluated three days after spraying. The mortality recorded by *C. cucurbitarum* for the corresponding period was once again significantly lower at 14.99 to 33.33 per cent.

Both fungi continued to register increase in mortality of nymphs with time of exposure. Thus *B. bassiana* VKA 01 strain recorded mean mortality values from 82.46 to 94.74 per cent and 92.31 to 98.08 per cent four and six days after spraying respectively, while *C. cucurbitarum* induced significantly lower mortality values ranging from 30.36 to 51.79 per cent and 36.53 to 51.91 per cent for the same period of exposure.

Beauveria bassiana VKA 01 strain induced more than ninety per cent mortality of the rice bug nymphs at all the three concentrations six days after spraying, with cent per cent mortality being registered at both 10^8 and 10^9 spores ml^{-1} seven days after spraying.

Choanephora cucurbitarum, while continued to register increase in mortality six and seven days after spraying as well, registered values that were significantly lower than those recorded by *B. bassiana* VKA 01 strain for the corresponding periods. A discernable feature of *C. cucurbitarum* was that there was no consistent relationship between the spore concentration and nymphal mortality unlike in case of *B. bassiana.*

5.3.2 Bioassay of native isolates of entomopathogenic fungi against adults of rice bug in laboratory

The bioefficacy of the two fungal isolates, *B. bassiana* VKA 01 strain and *C. cucurbitarum* against adults of rice bug was also assessed in the laboratory (Fig. 2). Both the isolates recorded mortality ranging from 3.39 to 8.48 per cent at all the concentrations evaluated and were at par with each other one day after treatment. Two days after treatment, *B. bassiana* VKA 01 straininduced 20.69 to 32.76 per cent mortality at different spore concentrations, the values being on par

with each other. *C. cucurbitarum* on the other hand, had recorded significantly lower mortality of 5.18 to 10.35 per cent for the same period.

Three days after treatment, *B. bassiana* VKA 01 strain recorded the highest mortality of 73.21 per cent at the concentration of 10^8spores ml^{-1}. Mortality values were 64.29and 51.79 per cent at concentrations of 10^9 spores $ml^{-1}$and 10^7 spores ml^{-1}respectively. *Choanephora cucurbitarum*, on the other hand caused mortality ranging from 8.93 to 10.71per cent at varying concentrations, all the values being significantly lower than that of *B. bassiana* VKA 01 strain.

Beauveria bassiana VKA 01 strain could successfully infect 90.91 and 89.09 per cent of bugs treated at the rate of 10^8 and 10^9 spores ml^{-1} respectively four days after spraying, both being on par with each other. All the treatments involving *C. cucurbitarum* recorded identical but significantly lower values of 10.89 per cent mortality.

*Beauveria bassiana*VKA 01 strainwas successful in causing 85.45 to 96.36 per cent mortality five days after spraying and 87.04 to 98.15 per cent mortality six days after spraying. *Choanephora cucurbitarum* recorded consistently lower mortality values ranging from 11.734 to 14.55 and 14.81 to 18.52 per cent for corresponding period.

The highest mortality of 96.36 per cent was registered once again by *B. bassiana* VKA 01 strain, applied at the concentration of 10^9 spores ml^{-1}six days after treatment. At 10^7 and 10^8 spores ml^{-1} it caused 94.55 and 85.45 per cent mortality respectively. Treatments involving *B. bassiana* VKA 01 strain at both 10^8 and 10^9 spores ml^{-1} were on par with each other and were significantly superior to remaining treatments. On the other hand, *C. cucurbitarum* recorded only 14.81, 16.67 and 18.52 per cent mortality at at 10^7, 10^8 and 10^9 spores ml^{-1} respectively

Seven days after exposure, *B. bassiana* VKA 01 strain, induced the highest mortality of 98.15 per cent at both 10^8 and 10^9 spores ml^{-1}, followed by

significantly lower 90.74 per cent mortality at 10^7spores ml^{-1}. Corresponding values for *C. cucurbitarum* were 18.52 each and 22.23 per cent of bugs exposed.

A comparison of the bioefficacy of *B. bassiana* and *C. cucurbitarum* isolate against nymphs and adults of rice bug revealed that *B. Bassiana* VKA 01 strain is a better candidate for biocontrol of rice bug. Several authors have reported *B. bassiana* to be effective as biocontrol agent of rice bug. Baharally and Simon (2014), for instance, had evaluated the bioefficacy of *B. bassiana* against rice bugs under laboratory conditions. The entomopathogen, applied at the rate of 6 x 10^8 spores ml^{-1} recorded 4.0, 39.10 and 51.85 per cent mortality at 24, 48 and 72 h after treatment respectively, which is similar to the findings of the present study. On the other hand, *C. cucurbitarum* has been reported primarily as a plant pathogen and an opportunistic microbe that could attack insects only under favourable conditions (Wilson and Jose, 1965).

Girish and Balikai (2015) also reported the decrease in gundhi bug population following spraying with *B. bassiana.* The mean population was 2.29 bugs per hill in plots treated with *B. bassiana* at the rate of 10^7 spores ml^{-1}as against 7.42 bugs per hill in control plots without treatment application. Chandran (2016) also reported the superiority of *B. bassiana* over *M. anisopliae* and *L. lecanii* against rice bug.

The consistently low mortality caused by *C. cucurbitarum* in laboratory is in consonance with the above reports and is indicative of the low potential of the microbe as a biocontrol agent. Moreover, its pathogenicity to plants renders it unsuitable as a candidate for arthropod pest management.

Both the isolates revealed a similar pattern in disease progression, with mortality showing a linear relationship with time of exposure, irrespective of the spore concentration. This is along expected lines, since the increased exposure to the pathogen would lead to increased infection and *inter alia* increased mortality, as was also observed in the earlier cited study by Baharally and Simon (2014).

Fig. 1 Bioefficacy of local isolates of *Beauveria bassiana* and *Choanephora cucurbitarum* against nymphs of rice bug in laboratory

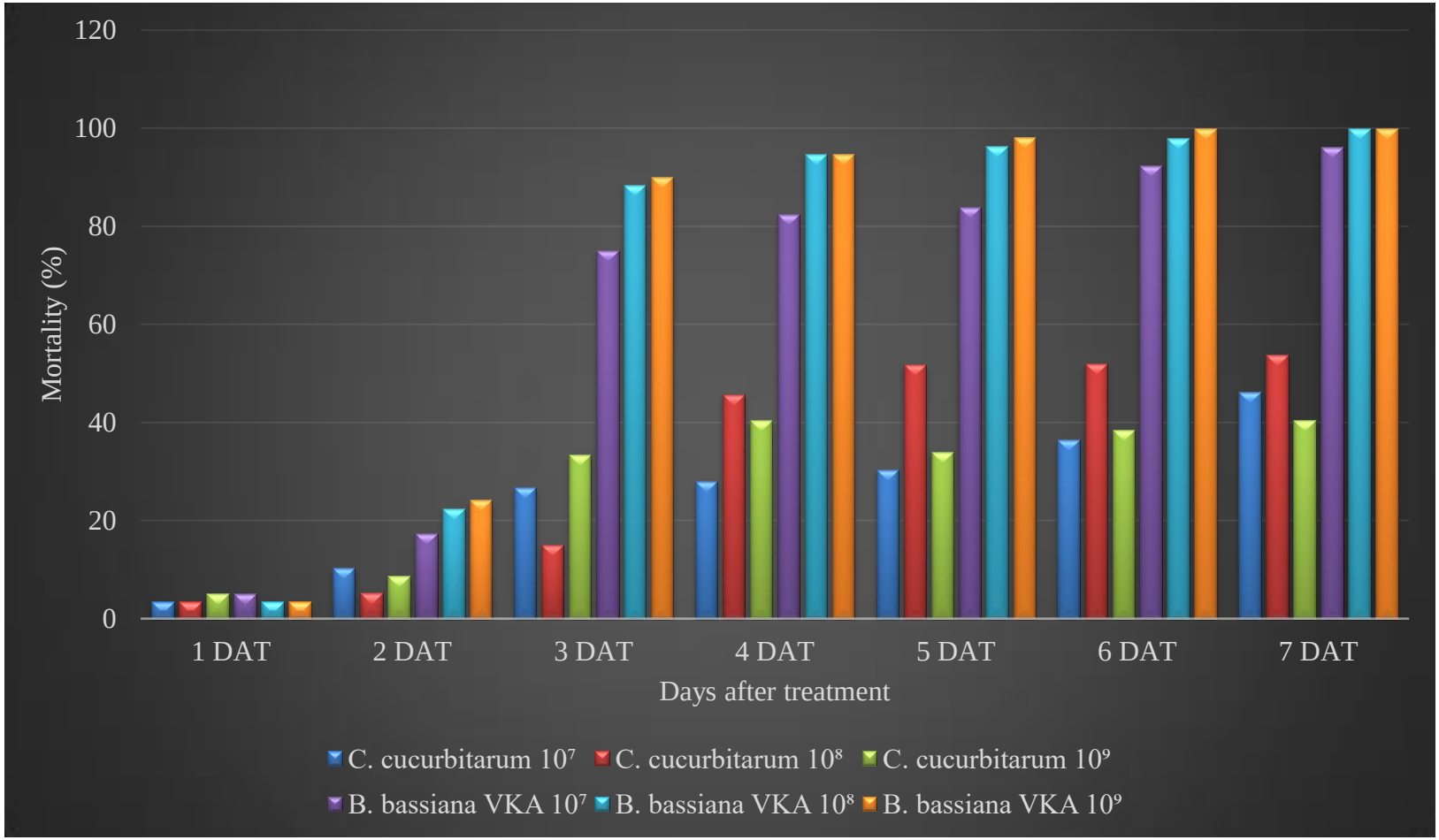
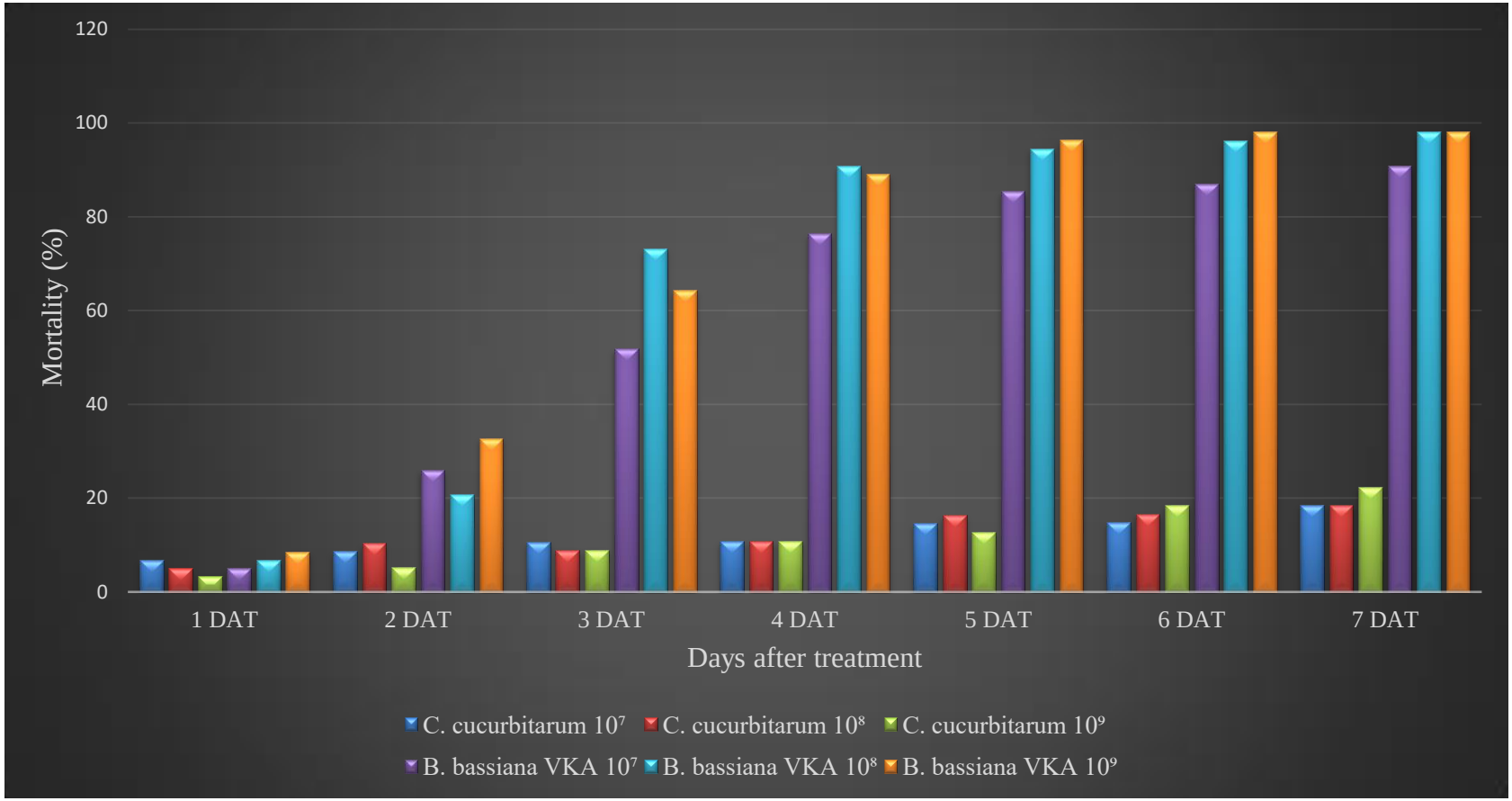


Fig. 2 Bioefficacy of local isolates of *Beauveria bassiana* and *Choanephora cucurbitarum* against adults of rice bug in laboratory



5.3.3 Bioassay of fungal isolates against rice bug in pot culture

The laboratory studies were followed by a pot culture evaluation of the native isolates of *B. bassiana* and *C. cucurbitarum* at the two most effective concentrations of 10^8 and 10^9 spores ml^{-1}, along with *B. bassiana* NBAIR strain (10^8 spores ml^{-1}) the insecticide malathion (500 g ai ha^{-1})and an untreated control (Fig. 3). The insecticide malathion recorded cent per cent mortality three days after spraying itself.

Beauveria bassiana VKA 01 strain applied at the rate of 10^8 spores ml^{-1} caused increasing mortality of 51.53, 85.71, 98.11 and 99.36 per cent 7, 10, 15 and 20 days after treatment and was on par with the same applied at 10^9spores ml^{-1}, with mortality values of 50.92, 88.20, 98.11 and 99.36 per cent for the corresponding days. Both the above treatments were significantly superior to the NBAIR strain, which recorded 42.95, 76.40, 88.05 and 98.08 per cent mortality throughout the study period except at 20 days after spraying, when it was on par with *B. bassiana* VKA 01 strain. *C. cucurbitarum*, applied at the rate of 10^8 spores ml^{-1}recorded 0.57, 5.26, 8.59, 16.77, 21.38 and 26.92 per cent mortality for 3, 7, 10, 15 and 20 days after treatment respectively. Similarly, *C. cucurbitarum*, applied at the rate of 10^9 spores ml^{-1}recorded 1.14, 2.92, 5.52, 14.91, 23.27 and 30.77 per cent mortality which was significantly lower than the treatments involving *B. bassiana* VKA 01 strain.

Evaluation of the effect of treatments on the grain damage by rice bugs revealed that plants treated with malathion had the lowest damage of 2.05 per cent and was significantly superior to all other treatments. All the three microbes evaluated were on par with each other in terms of grain damage, with values ranging from 33.34 per cent in case of *B. bassiana* VKA 01 strain applied at the rate of 10^8 spores ml^{-1}, to 49.64 per cent in case of *C. cucurbitarum* applied at the rate of 10^9 spores ml^{-1}. All the above treatments were also significantly superior to the untreated control with 68.16 per cent chaffy grains per panicle (Fig. 4).

Fig. 3 Bioefficacy of local isolates of *Beauveria bassiana* and *Choanephora cucurbitarum*against rice bug in pot culture

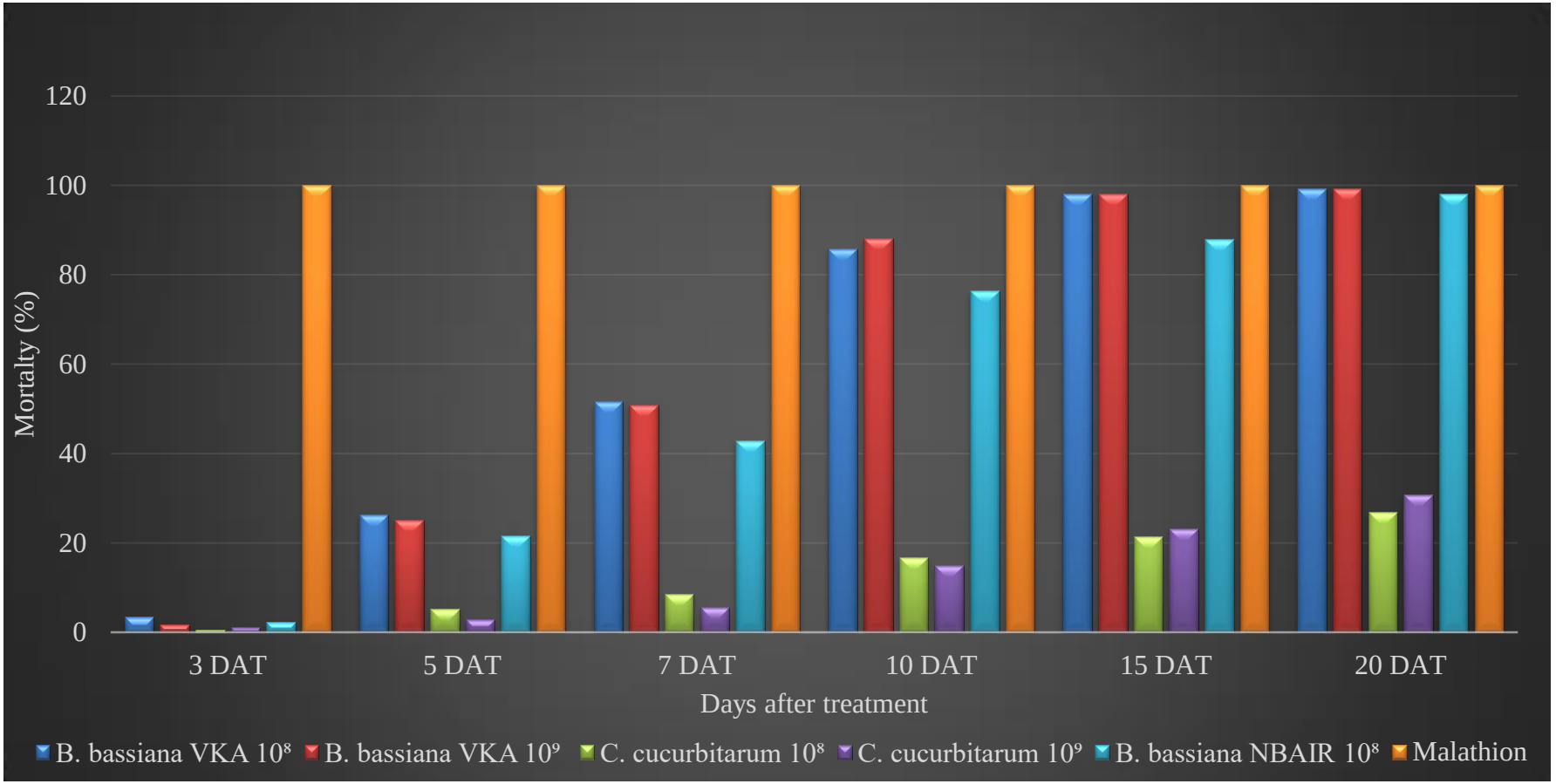
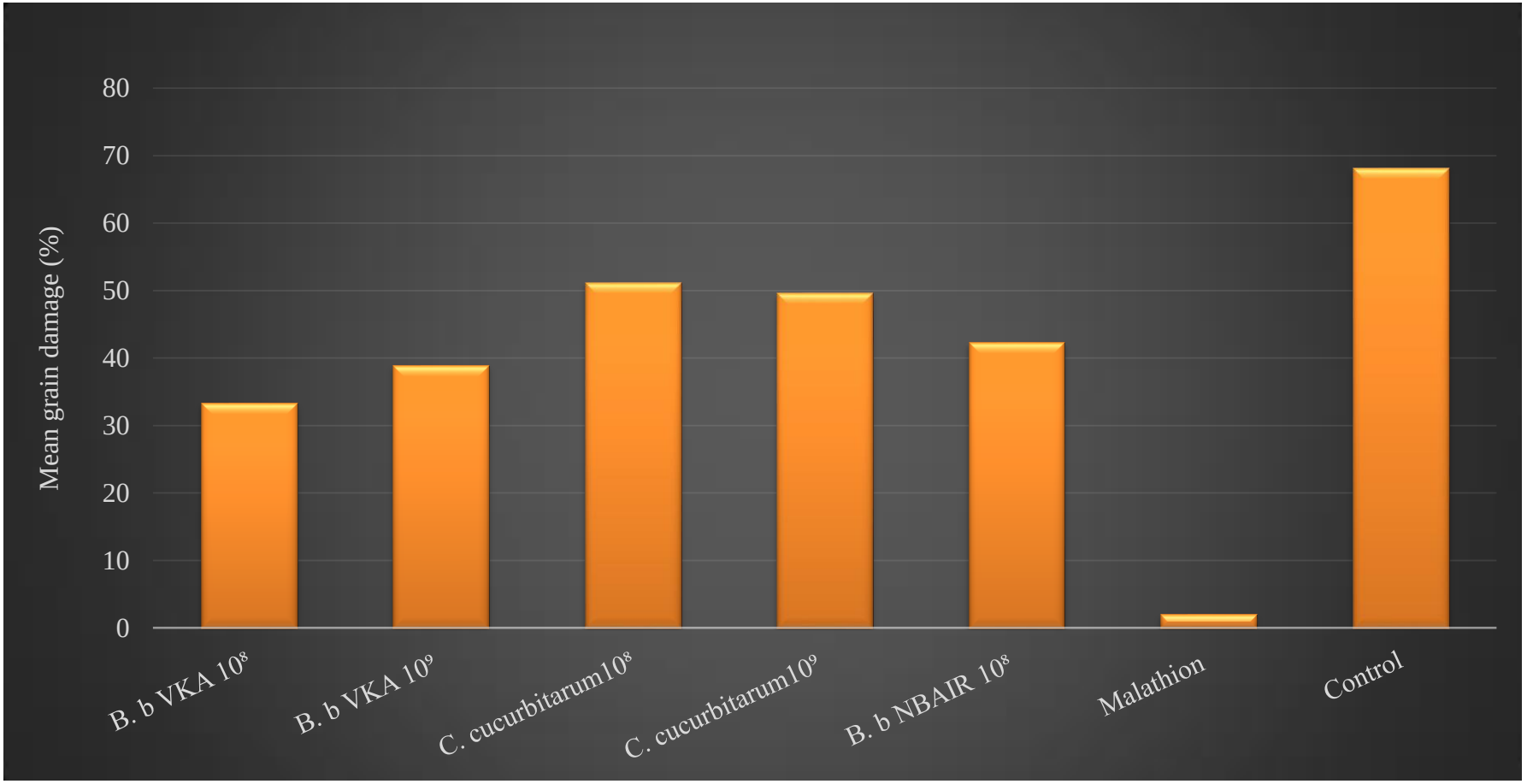


Fig. 4 Effect of native isolates entomopathogenic fungi on grain damage by rice bug in pot culture



The cent per cent mortality of bugs by malathion in the laboratory as well as pot culture studies, was along expected lines. Malathion is a broad spectrum organophosphate insecticide with contact and stomach action and is widely recommended for the management of rice bug. In comparison, the three entomopathogenic fungi caused very low levels of mortality three days after exposure. These findings are in line with that of Getzin (1961) who evaluated *B. bassiana* and noticed that a minimum of 48 h was required for infections to begin. Similarly, Yadav and Neeraj (2012) reported that, *B. bassiana* took three to seven days to infect an insect.

Among the fungal isolates, *B. bassiana* VKA 01 strain at both the concentrations evaluated was successful in causing significant mortality of over fifty per cent of bugs by seventh day and nearly cent per cent by fifteenth day. The findings of the pot culture experiment thus confirmed superiority of *B. bassiana* VKA 01 strain, applied at the rate of 10^8 spores ml^{-1} as the most effective treatment among the bioagents evaluated. *Beauveria bassiana* NBAIR strain also had recorded comparable mortality twenty days after treatment in the pot culture studies. However, the fungus had induced significantly low mortality levels consistently till fifteenth day.

Difference in virulence of isolates of entomopathogens have been frequently reported by several researchers. Thus, Loc and Chi (2005), who had evaluated the efficacy of new isolates of *B. bassiana* and *M. anisopliae* from naturally infected rice bugs against the rice bug *Leptocorisa acuta,* had reported mortality ranging from 57.5 to 77 per cent in case of *B. bassiana* and from 74.7 to 87 per cent in case of *M. anisopliae,* ten days after spraying. The differences between *B. bassiana* VKA 01 strain and NBAIR strain could thus be due to the natural variations in the virulence of isolates obtained from different locations.

Given the fact that quicker mortality of greater number of bugs is critical in case of a pest like rice bug, *B bassiana* VKA 01 strain could be a valuable tool in pest management of rice. Local isolates, especially from the same host, often

demonstrate greater efficacy in a given agro ecosystem due to their greater adaptability. The greater efficacy of *B. bassiana*, isolated from rice bug itself in the present study underscores the above generalization

5.3.4 EVALUATION OF *B. bassiana* VKA 01 STRAIN AGAINST RICE BUG UNDER FIELD CONDITIONS

The most effective concentration of *B. bassiana* VKA 01 strain identified in pot culture experiment was further evaluated along with *B. bassiana* NBAIR strain (10^8 spores ml^{-1}), the insecticide malathion (500 g ai ha^{-1}), azadirachtin 0.005.per cent and an untreated control under field conditions in two districts namely, Palakkad and Thrissur.

The results of field evaluation at Thrissur and Palakkad districts were broadly identical and agreed with the findings of the pot culture experiment. Malathion treated plots, as expected had the lowest population of 0.58 and 2.50 bugs per m^2atPalakkad and Thrissur, respectively and was significantly superior to other treatments, five days after treatment. While the next best treatment of *B. bassiana* VKA 01 strain, with 15.42 bugs per m^2 was superior to *B. bassiana* NBAIR strain with 18.33 bugs per m^2 at Palakkad, both the above treatments were on par with each other at trials in Thrissur, having identical bug populations of 15.75 and 15.00 per m^2respectively (Figs. 5 & 6). Azadirachtin and control plots had significantly higher population than other treatments at both the locations.

The local isolate (9.25 bugs/m^2) was on par with malathion (6.25 bugs/m^2) but continued to remain significantly superior to the NBAIR strain (11.67 bugs/m^2) 10 days after treatment at Palakkad. However, the two treatments remained on par with each other with values of 10.67 and 11.34 bugs per m^2 respectively, as also with malathion (11.08 bugs/m^2) at Thrissur.

Fig. 5 Effect of *Beauveria bassiana* VKA 01 strain on population of rice bug at Palakkad

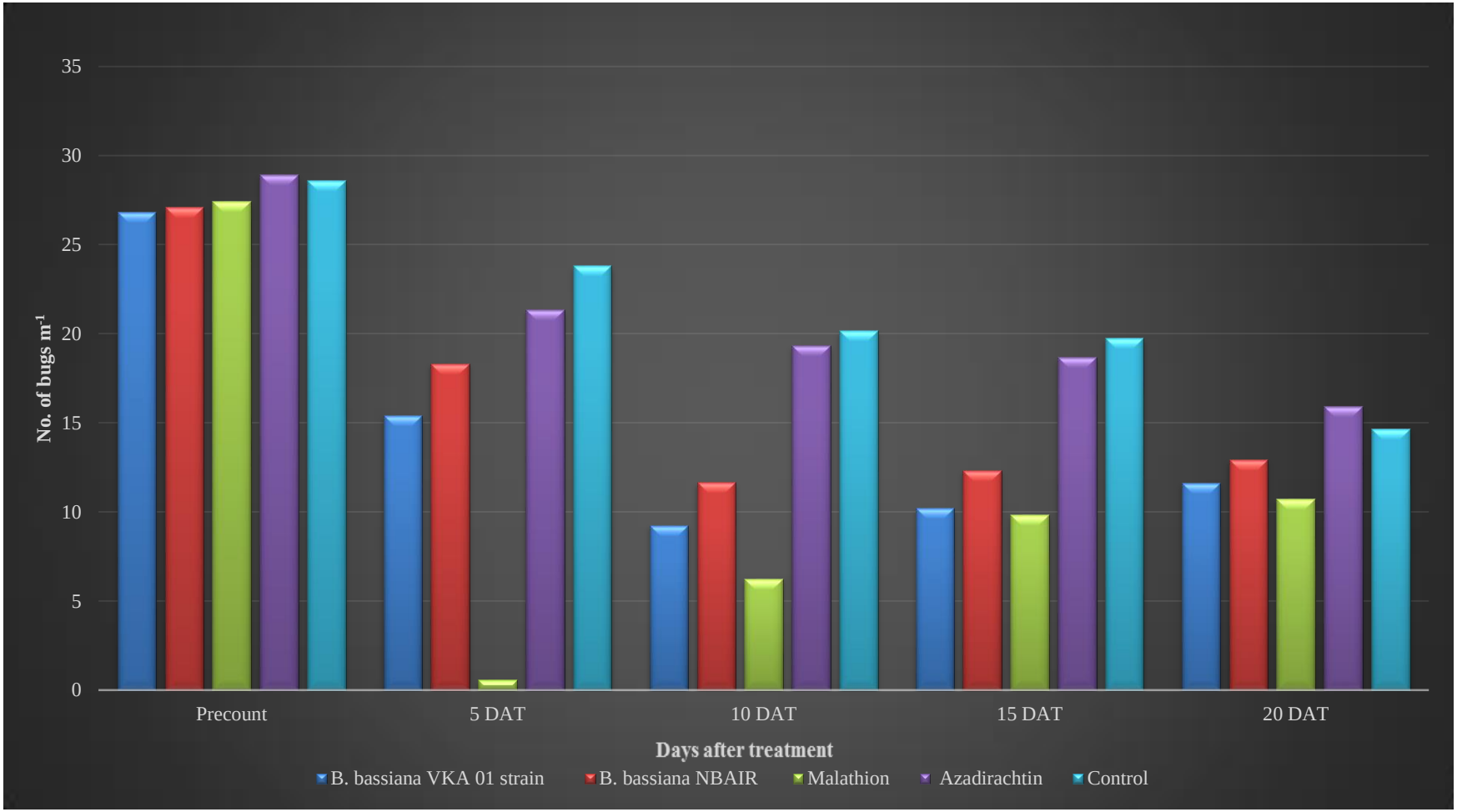


Fig. 6 Effect of *Beauveria bassiana* VKA 01 strain on population of rice bug at Thrissur

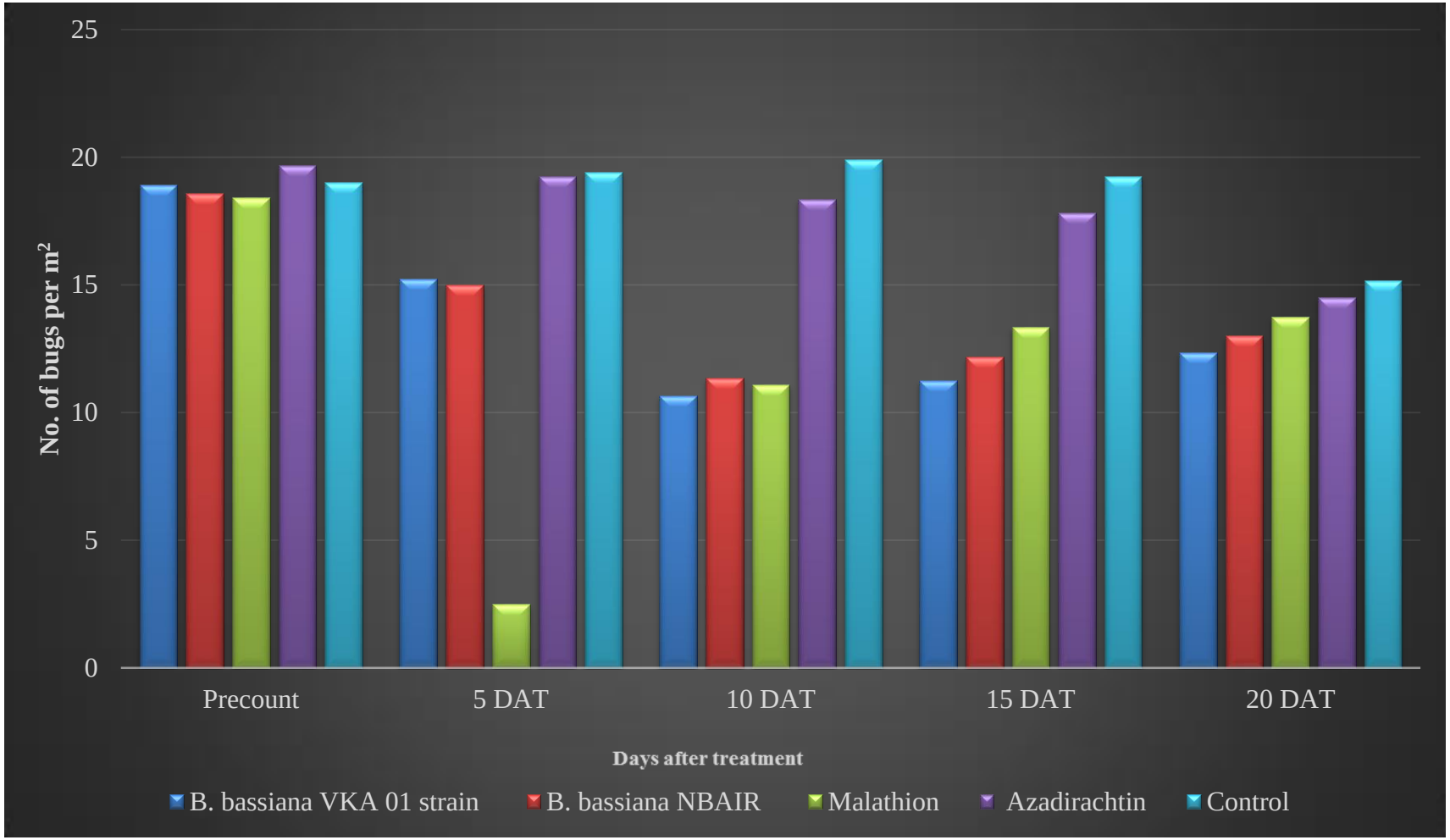
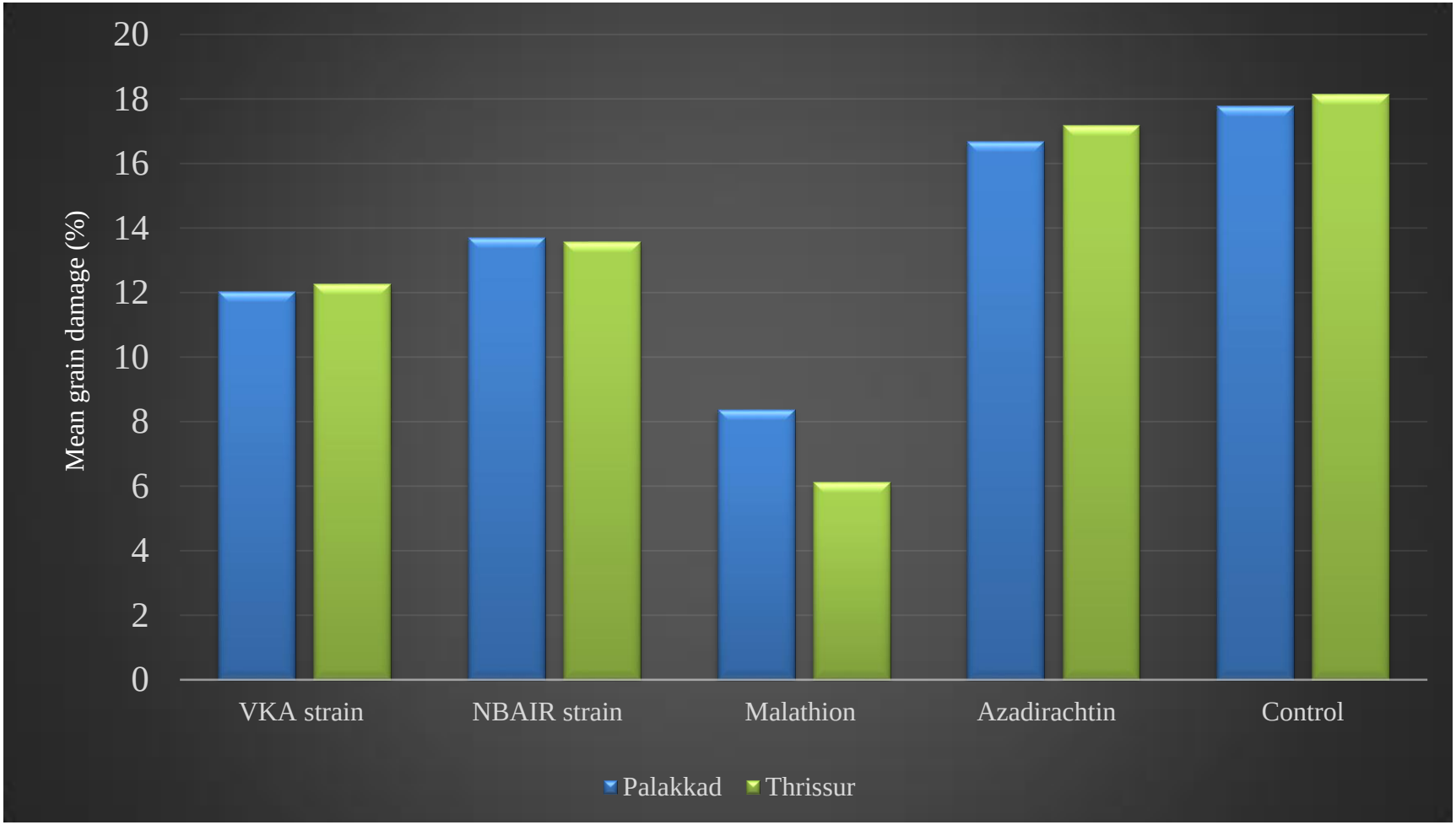


Fig. 7. Effect of *Beauveria bassiana* VKA 01 strain on grain damage by rice bug at Palakkad and Thrissur



Fifteen days after treatment, malathion, *B. bassiana* VKA 01 strain and *B. bassiana* NBAIR strain were on par with each other with bug population of 9.83, 10.23 and 12.33 bugs per m^2respectively at Palakkad. Similar results were obtained at Thrissur also, with corresponding number of 11.25, 12.67 and 13.33 bugs per m^2. The above three treatments were significantly superior to both azadirachtin and untreated control at both the locations.

Twenty days after spraying, malathion recorded the lowest mean bug population of 10.75 bugs per m^2 which was on par with *B. bassiana* VKA 01 strain (11.63 bugs per m^2) and *B. bassiana* NBAIR strain (12.92 bugs per m^2) at Palakkad, while at Thrissur, all the treatments had comparable bug populations ranging from 12.34 to 15.17 bugs per m^2.In terms of grain damage, plots treated with malathion suffered significantly lower mean grain damage at both Palakkad (8.37 %) and Thrissur (6.13 %). Both *B. bassiana* VKA 01 strain, with grain damage of 12.03 per cent and *B. bassiana* NBAIR strain, with grain damage of 13.72 per cent were on par with each other at Palakkad. The results were similar at Thrissur as well, with both the treatments registering identical damage levels of 12.27 and 13.58 per cent respectively (Fig. 7). The insecticide as well as the microbial bioagents were significantly superior to azadirachtin as well as untreated control at both Palakkad and Thrissur.

A similar trend was discernable in terms of yield as well, with malathion treated plots registering the highest yields of 529.17 and 547.67g per m^2 at Palakkad and Thrissur respectively. This was followed by *B. bassiana* VKA 01 strain with mean yields of 521.83 g m^{-2}at Palakkad and 459.13 g m^{-2}at Thrissur. These values, however, were on par with that of *B. bassiana* NBAIR strain registering 486.67 and 453.00 g m^{-2} yield at the respective locations (Fig. 8). The above treatments continued to be significantly superior to azadirachtin as well as untreated control at both the locations.

The results of the evaluation of bioefficacy of *B. bassiana* VKA 01 strain against rice bugs under field conditions are in agreement with the results of the

pot culture studies and affirms the potential of the isolate as a biocontrol agent of rice bug, *Leptocorisa* spp.

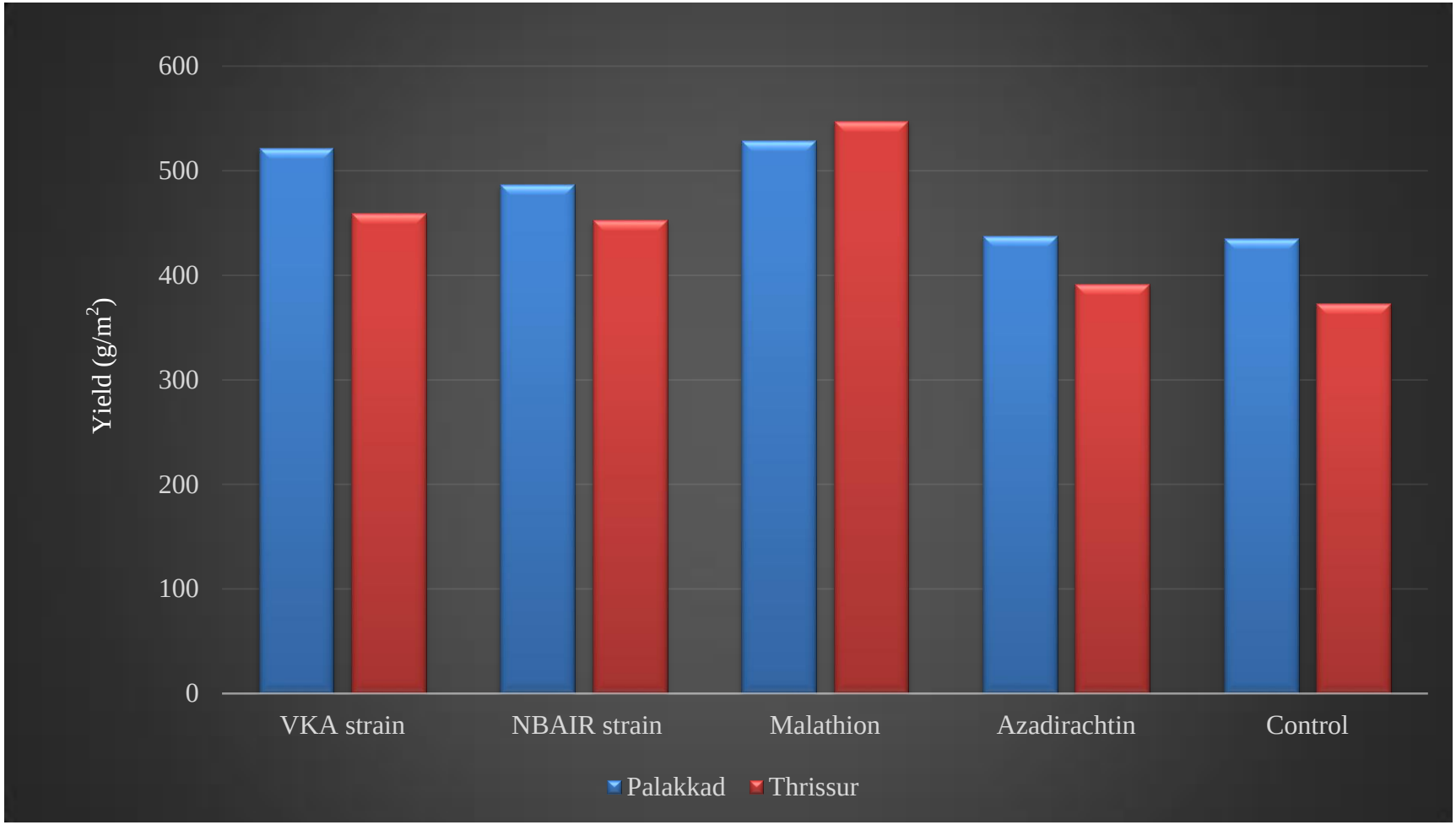
Several studies have reported the ability of *B. bassiana* to cause significant reduction in rice bug populations. Taun (2014), for example had reported substantial reduction in population of rice bugs in plots treated with different doses of the entomopathogen *B. bassiana*. *Beauveria bassiana* at the rate of 10^{13} conidia ml^{-1} was found to be at par with malathion having 1.39 and 1.15 bugs per linear meter respectively, seven days after treatment. Chandran (2016) also had demonstrated the efficacy of the VKA 01 strain of *B. bassiana* against rice bug. She reported that the fungus, applied at the rate of $1x10^{8}$ spores ml^{-1}effected mortality of the bug at levels on par with the insecticide, malathion.

The findings of the present study are entirely in agreement with the above observations. The plots treated with *B. bassiana* VKA 01 strain had bug populations comparable with the insecticide by the tenth day at both Palakkad and Thrissur.

The observations on effect of *B. bassiana* VKA 01 strain on the grain damage in the present study (Fig. 8) is also in agreement with the findings of Kalita *et al.* (2009) who reported that the application of entomopathogenic fungus *B. bassiana* was effective in reducing damage caused by rice bug. Treatments with *B. bassiana* had recorded 5.15 per cent mean grain damage, which was second only to that of monocrotophos (2.39 per cent) and was significantly superior to untreated control (13.06 per cent).

The pot culture as well as field experiments to evaluate the bioefficacy of the two fungal isolates against rice bugs demonstrated the potential of *B. bassiana* VKA 01 strain to effectively manage infestation by rice bug. *Beauveria bassiana* consistently proved to be as effective as insecticide malathion though the reduction in bug population was understandably not as pronounced till about a week after treatment.

Fig. 8. Effect of *Beauveria bassiana* VKA 01 strain on grain yield at Palakkad and Thrissur



5.4 COMPATIBILITY OF *Beauveria bassiana* VKA 01 STRAIN WITH SELECTED PESTICIDES

Beauveria bassiana VKA 01 strain, identified as the most effective local isolate in previous experiments was evaluated for its compatibility with selected insecticides and fungicides. Five insecticides and two fungicides commonly used for pest and disease management in rice were evaluated at their recommended doses for the extent of inhibition of the fungus using poisoned food technique.

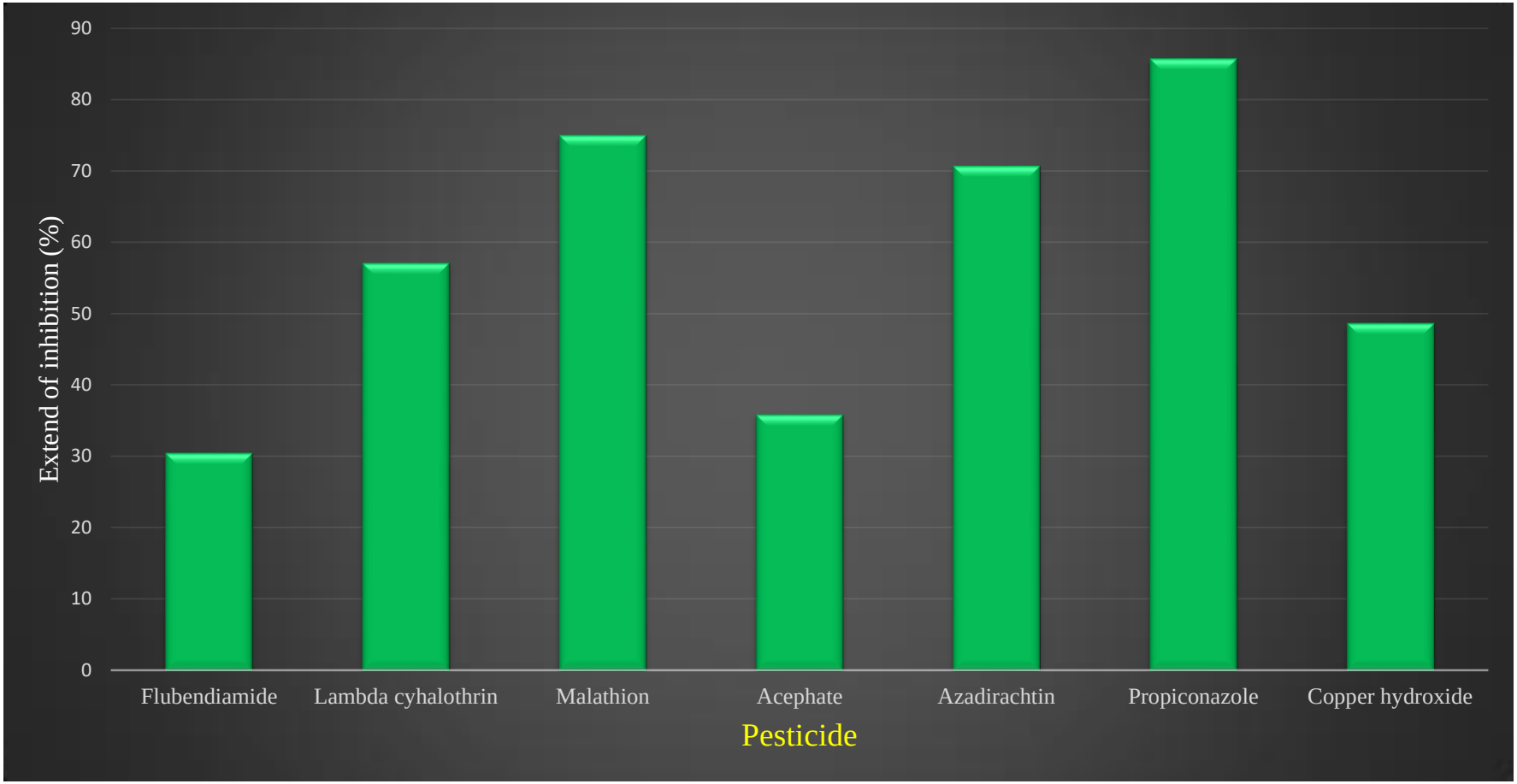
The relatively low level of inhibition of the entomopathogenic fungus by the two insecticides, namely, acephate and flubendiamide is along expected lines. Similarly, the highest degree of inhibition by the fungicide propiconazole is also only to be expected (Fig. 9). However, the significant degree of inhibition by the two insecticides, lambda cyhalothrin and malathion calls for further investigations, as they appear to be contrary to previous reports. Joshi *et al.* (2018) had observed that lambda cyhalothrin was compatible with *B. bassiana*at all the concentrations evaluated (1.2, 0.6, 0.3 0.15 and 0.075 per cent) with colony diameter of 5.56, 5.30, 6.00, 5.93 and 6.10 cm respectively. However the same study had also found propiconazole 25 per cent to be completely inhibitory to the linear growth of fungus at all the concentrations evaluated as was observed in the present case.

5.5 DEVELOPMENT OF FORMULATIONS OF *Beauveria bassiana* VKA 01 STRAIN

Based on the field evaluation, *B. bassiana* VKA 01 strain was formulated into talc based, oil based and aqueous formulations. Carrier materials such as sunflower oil, rice bran oil, coconut oil, sesame oil and palm oil for oil based formulation, distilled water for aqueous formulation and talc for solid formulation were evaluated.

Five commonly available vegetable oils were tested to identify the most suited carrier through inhibition zone technique (Brown and Kothari, 1975) in a completely randomized design (CRD).

Fig. 9. Effect of selected pesticides on growth of *Beauveria bassiana* VKA 01 strain



Based on the zone of inhibition, both sesame oil and palm oil were found to be equally compatible with the fungus, while rice bran oil, sunflower oil and coconut oil were inhibitory. Palm oil was previously reported as compatible to *B. bassiana* by Ekwenye (2006) also, through the same technique

The greater viability of *B. bassiana* spores in palm oil (1.5×10^8 cfu ml^{-1}), after 15 days of storage confirmed that palm oil was the most ideal carrier among the oils evaluated for formulation of *B. bassiana* (Fig. 10).

Identification of the most suitable oil base was followed by efforts to standardize the adjuvants required for the development of oil based formulation. Four different chemical adjuvants, namely glycerol, tween 80, carboxy methyl cellulose (CMC) and polyethylene glycol (PEG), were evaluated at three different concentrations by mixing them with palm oil and suspending the spores in the mixture for thirty days. The number of viable cfu was recorded after the storage period.

Glycerol was identified as the most ideal adjuvant for oil based formulation, with all the three concentrations proving to be significantly superior to other adjuvants evaluated along with. Glycerol, at three per cent registered the highest retention of conidial viability that was significantly superior to other treatments. At both the lower and higher concentrations (1 and 5 % respectively), however, it had significantly reduced the number of viable colonies than at the medium concentration of three per cent.

Glycerol a very versatile adjuvant that can act as a stabilizer (Jones and Burges, 1998), as well as a humectant (Burges, 1998) Kubicek and Druzhinina (2007) reported that glycerol delays the evaporation of liquid and favours spore germination. Similar observations about glycerol as a powerful depressor of water molecules were also made by Batta *et al.,* 2011. The suitability of glycerol as an adjuvant in oil formulations of *B. bassiana*, brought out in this study, is in agreement with the findings of Nithya (2015), who reported that glycerol

improved the viability *Lecanicillium lecanii* spores as well as the efficacy of the formulation.

Other adjuvants, except CMC at one per cent significantly reduced the number of viable colony forming units, when added to oil based formulations, to levels that were more or less comparable to untreated check.

The effectiveness of the combination of *B. bassiana* along with palm oil and three per cent glycerol could not be compared with previous reports due to the non availability of literature on such a formulation.

Carboxy methyl cellulose (CMC) at 0.5 per cent had recorded the highest value for viable cfu thirty days after storage and was followed by CMC at one per cent (Fig. 11) in the evaluation of adjuvants for aqueous formulations. Other adjuvants like tween 80, PEG and glycerol significantly reduced the cfu count and were comparable with untreated check.

CMC is a long chain, linear, water soluble polysaccharide derived from cellulose. Sharma *et al.* (1999) reported that, addition of CMC to *Beauveria brongniartii* formulation reduced the time required to cause 100 per cent mortality by more than a week. Similarly, Petlamul *et al.* (2017) had observed that CMC enhanced the ability of *B. bassiana* to release cellulolytic enzymes for degradation of cellulose and its utilization as carbon source for their growth.

The low retention of viability of spores upon addition of tween 80 to both oil based and aqueous formulations reported in the present study is contrary to previous reports. Easwaramoorthi and Jayaraj (1977) as well as Burges (1998) had reported the ability of tween 80 to rehydrate fungal spores. Luz and Betagin (2005), had further observed that tween 80 was relatively less toxic to *B. bassiana* spores. Tanuja *et al.* (2010) noted its ability to improve cell permeability and hasten spore germination. This was again confirmed by Mishra *et al.* (2013), who opined that tween 80 did not affect the viability of *B. bassiana* spores. The

findings of the present study may need further investigations in the light of the above reports.

Similarly, several authors have reported that PEG can be used as an adjuvant in biopesticide formulations. Hallsworth and Magan (1994) for instance, had reported that addition of PEG to a formulation can lead to accumulation of trehalose in conidia and thereby lead to increased resistance of spores to desiccation and heat. Derakhshan *et al.* (2008), likewise, had also reported of its effectiveness in enhancing viability of *M. anisopliae* spores.

Chitin enriched (5 %) talc formulation recorded higher cfu after 30 days after storage in case of solid formulation when compared to other adjuvants (Fig. 12).The above treatment was followed by talc with chitin at three per cent and talc with chitosan (7 %), both being at par.

Beauveria bassiana is a chitinolytic organism. Chitin is the major source of carbon for growth and multiplication of such chitinolytic microbes. Gerding-gonzalez *et al.* (2007) had reported that the use of two or three per cent chitin in wheat bran induced higher sporulation in alginate pellet formulations of *B. bassiana.* However, higher concentrations of chitin had led to declined conidia yield. Similarly, Sriram *et al.* (2010) had observed that addition of two to five per cent chitin in talc based formulations helped in maintaining high cfu of *Trichoderma harzianum* and also helped extend the shelf life of the formulation by two months. The findings of the present study are completely in agreement with the above reports.

Addition of chitin can also help improve fungal formulations by suppressing contaminants like *Penicillium* spp, which are unable to use chitin as a carbon source (Knudsen *et al.* 1990).

5.6 EVALUATION OF *Beauveria bassiana* VKA 01 STRAIN FORMULATIONS AGAINST RICE BUG UNDER FIELD CONDITIONS

Field evaluation of the best oil based, aqueous and talc based formulations of VKA 01 strain was carried out at RARS Pattambi, Palakkad. Five days after treatment, plots treated with malathion 50 EC @ 500 g ai ha^{-1} recorded the lowest mean population of 3.92 bugs per m^2 and was superior to all other treatments (Fig. 13). Malathion was followed by talc as well as aqueous formulations with 13.83 and 15.58 bugs per m^2 respectively, both being on par with each other. Plot treated with oil based formulation had a mean bug population of 18.92 per m^2 and was on par with untreated control (19.32 bugs/ m^2).

Plots treated with talc formulation had the lowest count of 9.08 bugs per m^2 ten days after treatment. This was followed by malathion as well as aqueous formulation with 9.32 and 9.75 bugs per m^2, respectively. All the three treatments were on par with each other and were significantly superior to both oil formulation as well as untreated control (18.58 and 16.92 bugs per m^2respectively). Fifteen days after spraying, talc formulation once again had the lowest rice bug population of 9.42 per m^2 which, though was on par with bug populations in plots treated with aqueous and oil based formulations with mean population of 11.58 and 12.92 bugs per m^2 respectively, was significantly superior to malathion (13.58 bugs/m^2) as well as untreated control.

Plots treated with talc formulation had the lowest number of 10.92 bugs per m^2 twenty days after application of treatments, while the highest population was recorded in control plot with 14.25 bugs per m^2. However, there was no significant differences between the various treatments.

All the treatments were significantly superior to untreated control in terms of grain damage. Malathion and the talc based formulation recorded significantly lower damage of 10.08 and 10.25 per cent chaffy grains respectively and were on par with each other. They were followed by aqueous formulation, which in turn was significantly superior to oil based formulation as well as untreated control.

Fig. 10. Effect of adjuvants on viability of *Beauveria bassiana* VKA 01 strain spores in oil based formulation

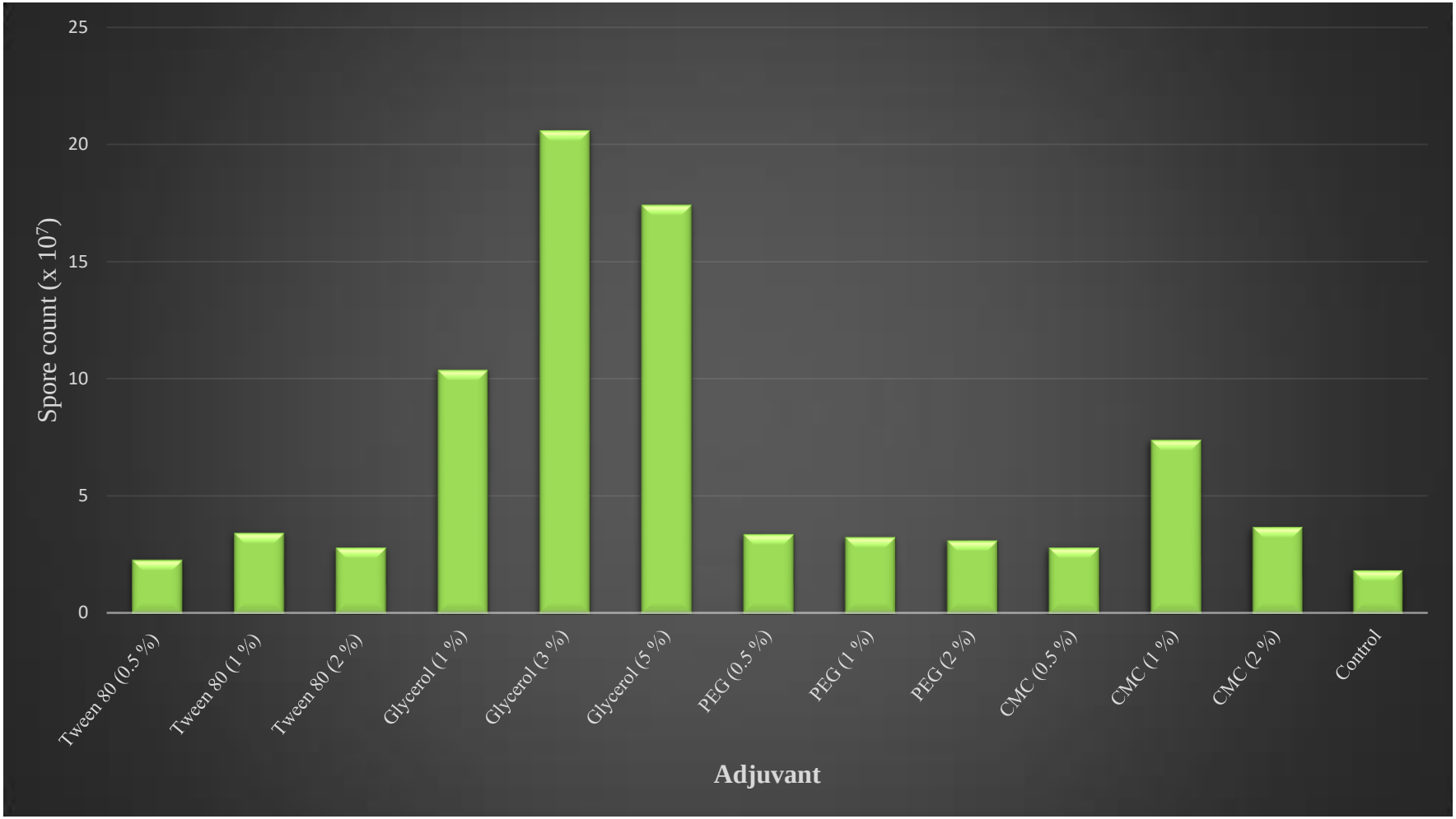


Fig. 11. Effect of adjuvants on viability of *Beauveria bassiana* VKA 01 strain spores in aqueous formulation

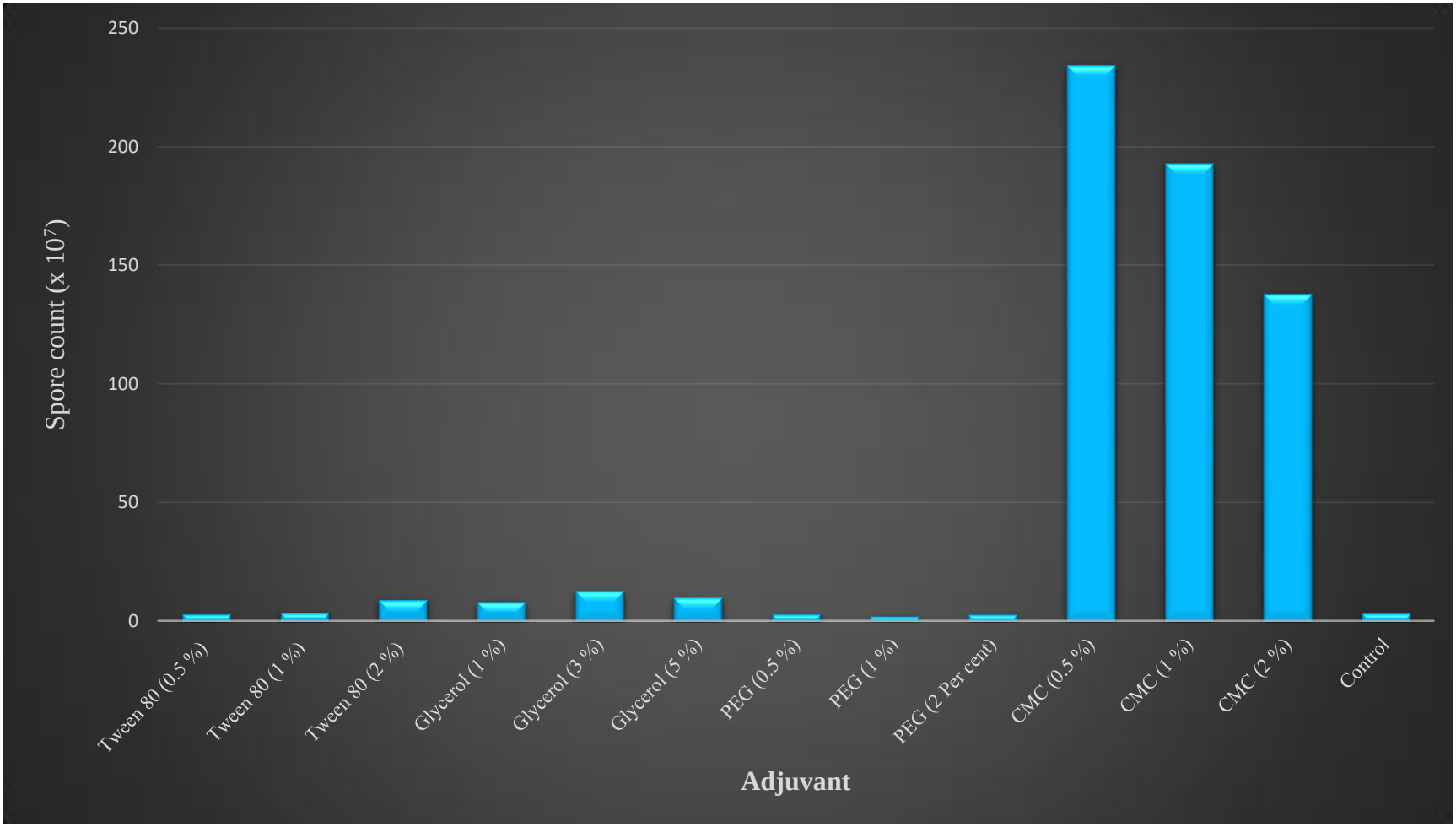
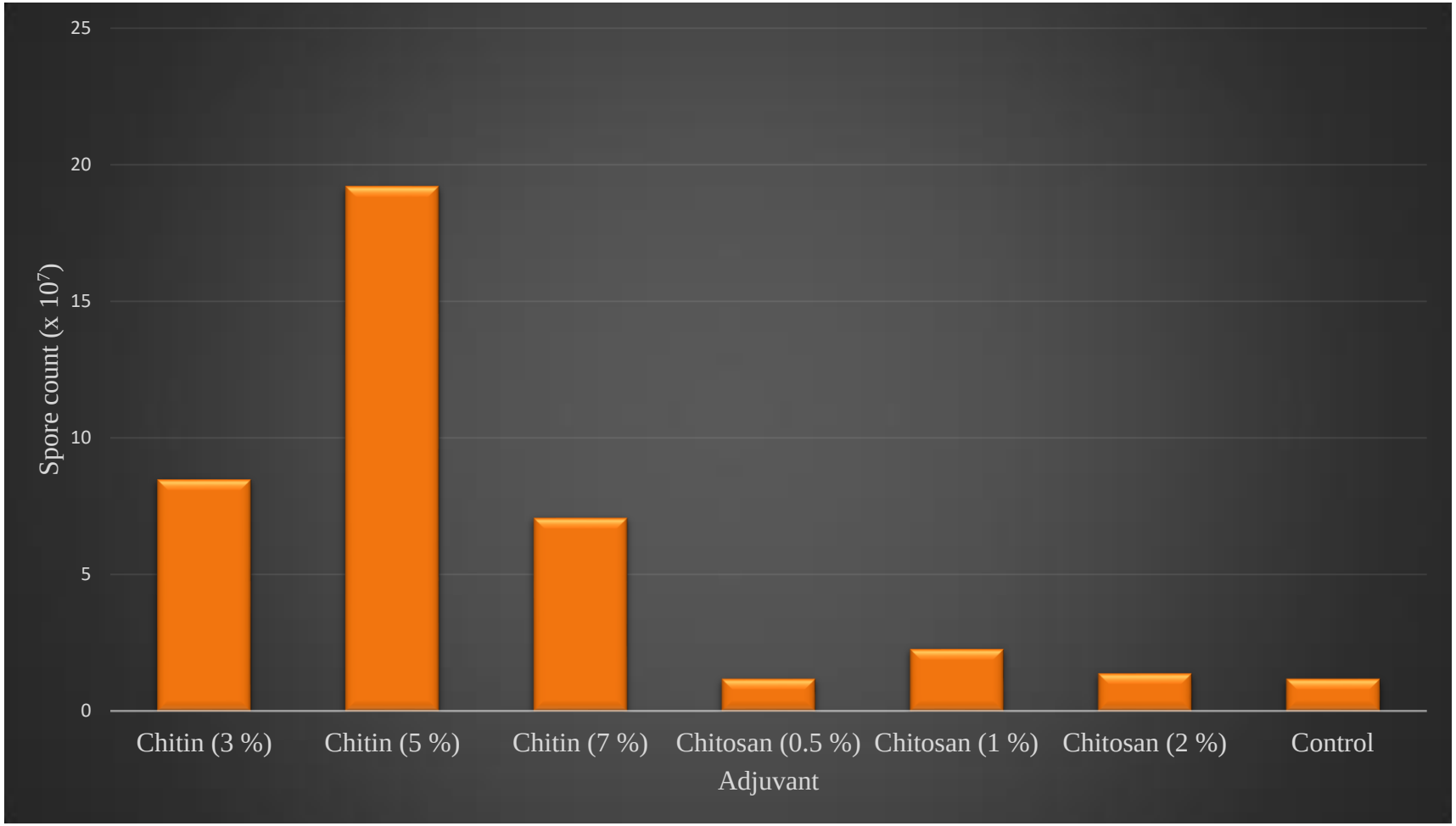


Fig. 12. Effect of adjuvants on viability of*Beauveria bassiana* VKA 01 strain spores in talc based formulation



The grain yield varied from 558.34 in plot treated with oil based formulation to 635.83 in plot treated with talc formulation. Both talc based formulation as well as malathion (628.00 g/m^2) were on par with each other and were superior to other treatments. Plots treated with aqueous formulation registered the next highest yield of 600.00 g/m^2. Untreated control (583.33 g) as well as oil based formulation (558.34 g) of *B. bassiana* had comparable grain yields (Fig. 14).

Talc based formulation of *B. bassiana* VKA 01 strain was superior to other formulations and was comparable to the insecticide malathion in terms of mean grain damage (Fig. 15) as well as mean yield. Fang *et al.* (2005) reported the increase in ability of *B. bassiana* to penetrate insect cuticle when formulated with chitin, resulting in improvement in virulence against insect. It could be that the presence of chitin in the formulation might have promoted the production of chitinases which are important cuticle degrading enzymes. This in turn could have led to greater chitinolytic activity, better penetration of insect cuticle and enhanced virulence of *B. bassiana* in the talc based formulation.

5.7 EVALUATION OF SHELF LIFE OF FORMULATIONS OF *Beauveria bassiana* VKA 01 STRAIN

The shelf life evaluation of oil based, talc based and aqueous formulations were conducted by assessing the viable spore count at a regular interval of 30 days for up to twelve months (Fig. 16).

One month after storage, the highest cfu value of 11.2 x 10^8cfu ml^{-1} was recorded in oil formulation with adjuvants. It was followed by chitin enriched talc formulation (11 x 10^8 cfu ml^{-1}), talc formulation without adjuvants (9.6 x 10^8 cfu ml^{-1}) and aqueous formulation (9.3 x 10^8 cfu ml^{-1}), with adjuvants (4.6 x 10^4 cfu ml^{-1}), all being on par with each other. The above formulations were significantly superior to both oil based formulation without adjuvants as well as aqueous formulation without adjuvants.

Fig. 13. Effect of formulations of *Beauveria bassiana* VKA 01 strain on rice bug population in field

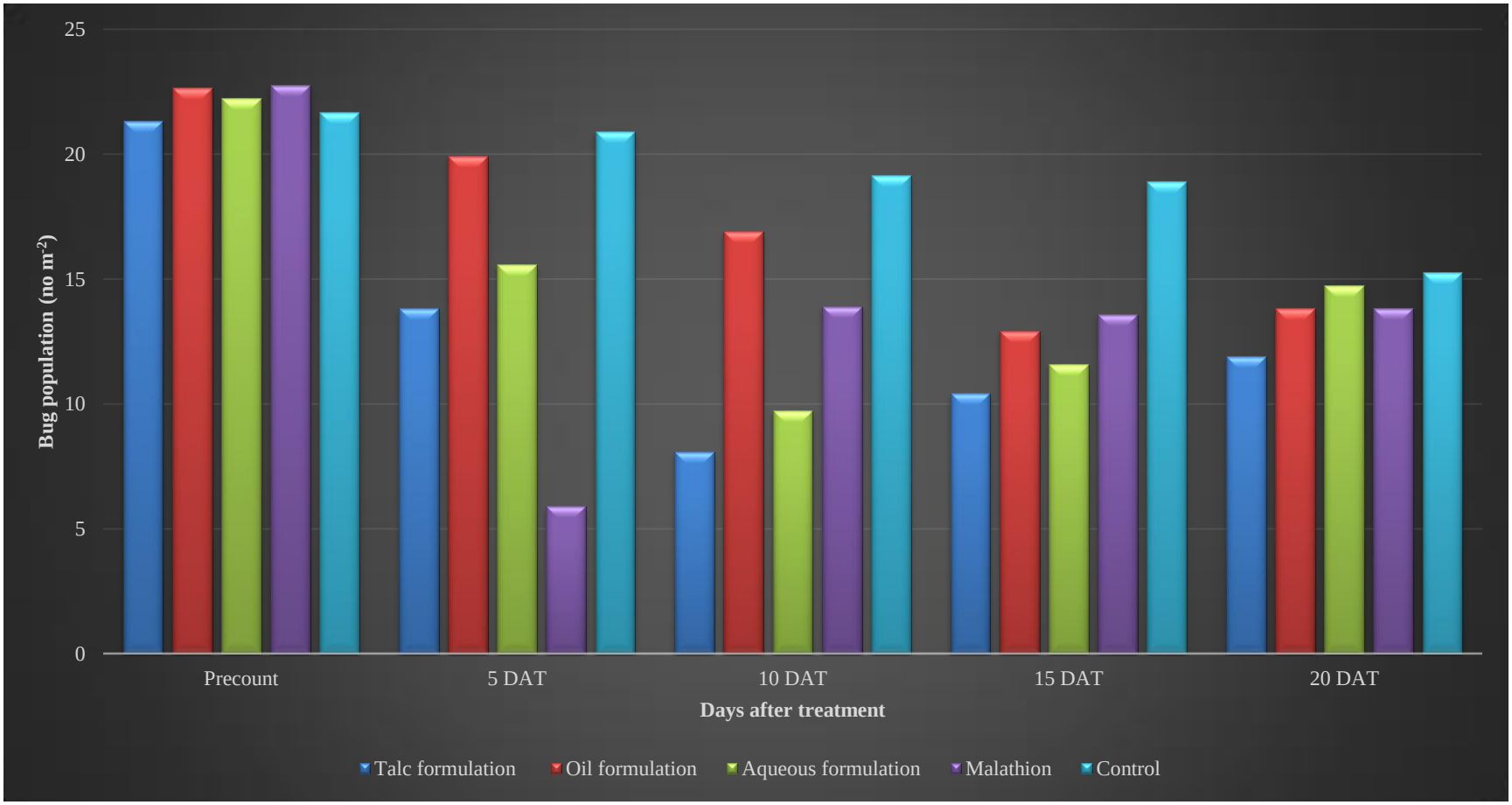


Fig. 14 Effect of *Beauveria bassiana*VKA01 strain formulations on grain yield

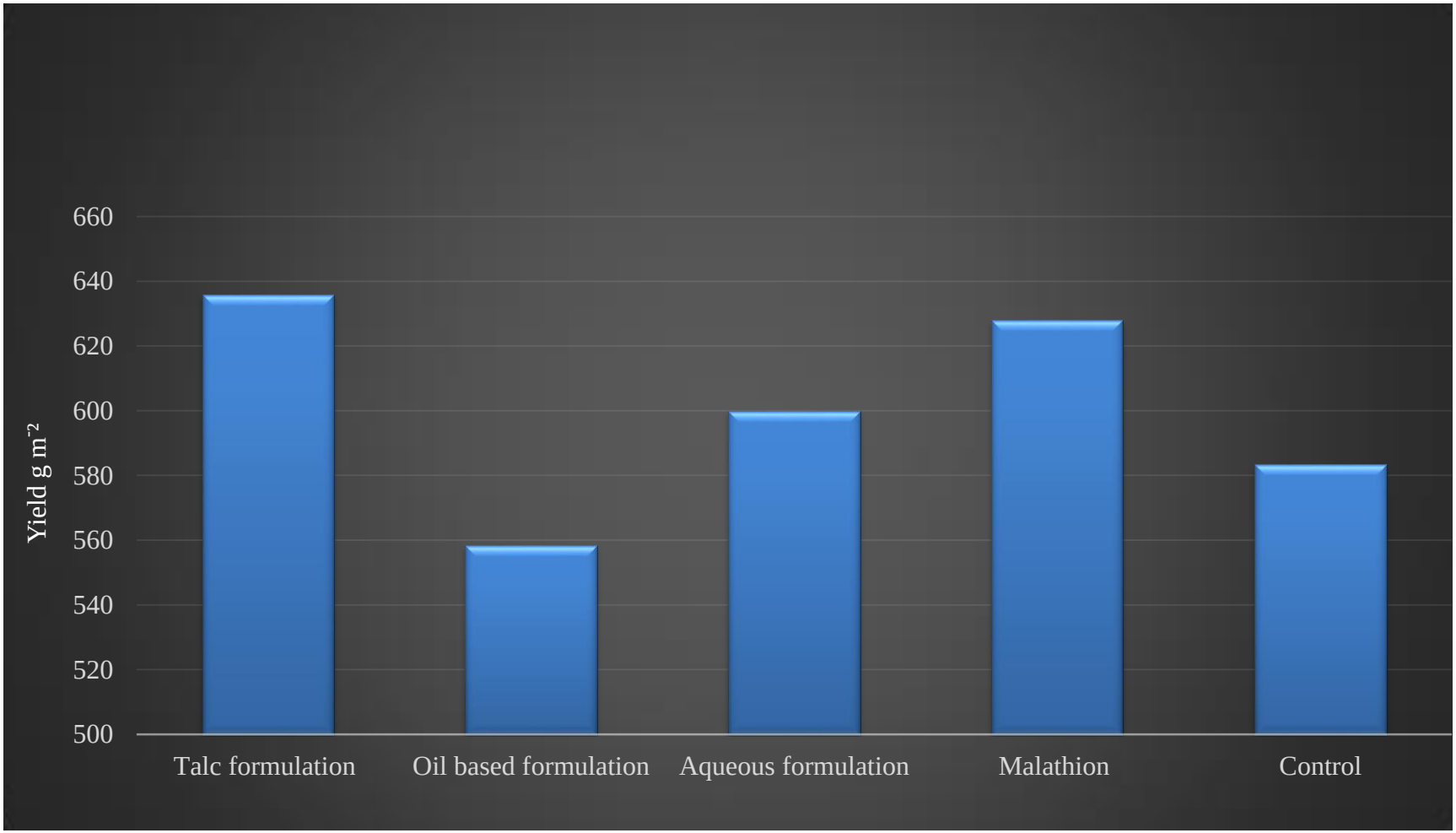
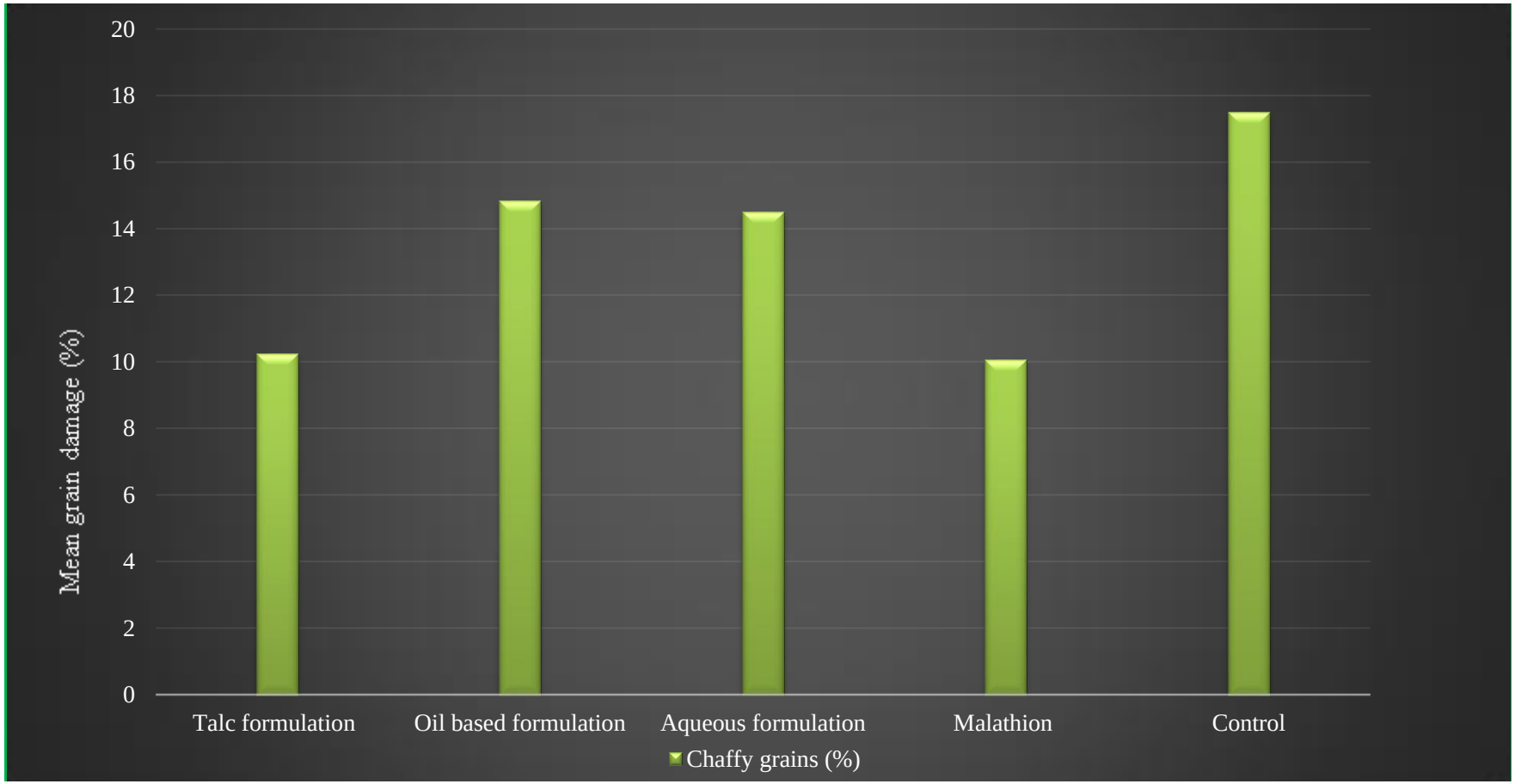


Fig. 15 Effect of *Beauveria bassiana* VKA01 strain formulations on grain damage



Chitin enriched talc, with a spore count of (9.8×10^8 cfu ml^{-1}) as well as oil formulation with adjuvants, with a spore count of (9.3×10^8 cfu ml^{-1}) were significantly superior to the remaining treatments such as aqueous formulation with adjuvants and talc formulation without adjuvants two months after storage.

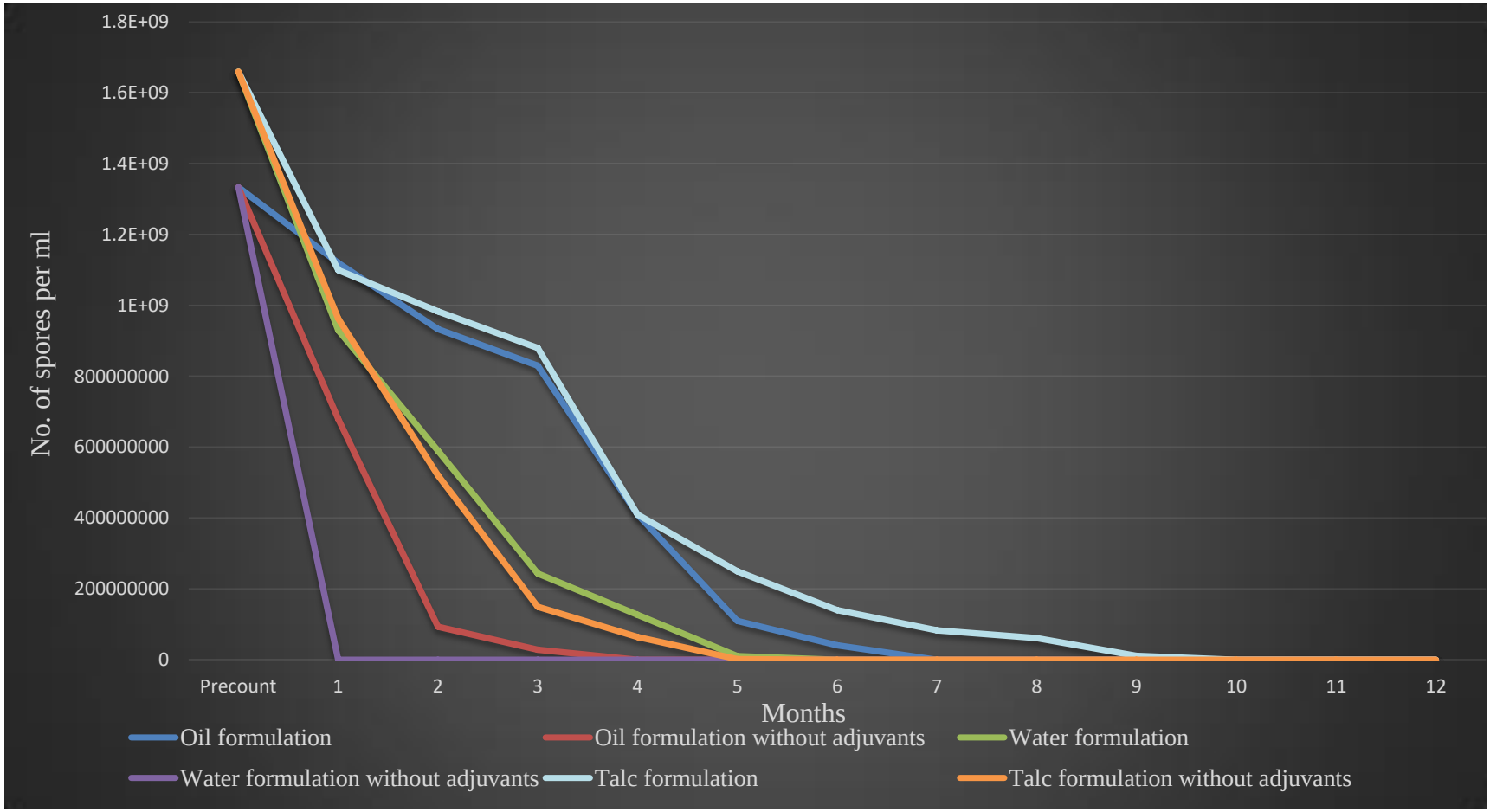
Similar trend was observed three months after storage as well. Chitin enriched talc, with a spore count of (8.8×10^8 cfu ml^{-1}) as well as oil formulation with adjuvants, with a spore count of (8.1×10^8 cfu ml^{-1}) were significantly superior to the remaining treatments such as aqueous formulation with adjuvants and talc formulation without adjuvants.

Four months after storage, only three treatments, namely, chitin enriched talc formulation (4.3×10^8cfu ml^{-1}), oil formulation with adjuvants (2.4×10^8cfu ml^{-1}) and aqueous formulation with adjuvants (1.3×10^8cfu ml^{-1}) had cfu counts above the legally prescribed standard of 10^8cfu ml^{-1}

At six months after storage, chitin enriched talc formulation alone recorded viability of 1.38×10^8cfu ml^{-1}.None of the formulations had viability above the required levels seven months after storage.

Several reports support the above results of the present study. Ramakrishnan *et al.* (1994), Raghuchander *et al.* (1995) and Rajendran *et al.*, (2007) observed that nearly twenty per cent of entomopathogenic fungal formulations available in market are wettable powders (WP) because of its ease of application. Talc powder reportedly retained the highest number of viable propagules for up to 180 days in case of *Trichoderma longibrachiatum* (Yashurant *et al.,* 2010; Sahid *et al.,* 2011). Kumar *et al.* (2013), who conducted the shelf life studies of different *Trichoderma viride* formulations had reported that talc based formulations yielded the highest extent of viable cfu.

Fig. 16. Shelf life of formulation of *Beauveria bassiana* VKA 01 strain



The present study thus has been successful in surveying, collecting, characterizing and identifying isolates of entomopathogenic fungi from rice bug, *Leptocorisa* spp. The isolates were further evaluated across a range of conditions in laboratory and field. The evaluation has established that a local isolate of *B. bassiana*, named as VKA 01 strain, could manage populations of rice bug as effectively as the recommended insecticide, malathion.

The entomopathogen was also successfully developed into different formulations which were subsequently evaluated for their efficacy as well as shelf life. A talc based formulation enriched with chitin was identified as the most promising one, with efficacy comparable to that of the insecticide malathion and having a shelf life of six months. The study has thus successfully transformed a potential bioagent into a viable technology and a valuable tool for integrated pest management.

Summary

7. SUMMARY

Rice (*Oryza sativa* L.) is one of the most important cereal crops of the world and forms staple food for more than three fifths of humanity. Nearly ninety per cent of the area, production and consumption of rice happens to be in South and East Asian countries. The productivity of rice is greatly influenced by several factors, among which the insect pest menace is the most predominant one. The earhead bug, *Leptocorisa* spp. is one of the most important sap sucking insect pests of rice in the tropics. Both nymphs and adults suck sap from developing rice grains during the milky grain stage, leading to discoloured, empty or half-filled grains. Nymphs are more destructive than the adults.

Entomopathogenic microbes, particularly fungi, have, of late, been receiving increased attention as potential biocontrol agents of the rice earhead bug. *Beauveria bassiana* (Bals.), for instance, is an entomopathogenic fungus that grows naturally in soils throughout the world. They are ideally suited as biopesticides owing to their amenability for mass production and formulation as well as ease of application.

The All India Co-ordinated Research Project on Biological Control of Crop Pests Centre at Thrissur, Kerala reportedly identified an entomopathogenic fungus (EPF) from rice bug which was later identified as *B. bassiana*. Subsequent studies conformed the potential of the above isolate as a natural enemy of the rice bug calling for further efforts towards possibility of commercial exploitation of the isolate. Realization of the above goal however, demanded characterization of the isolated fungus as a primary step. Entomopathogenic fungi, including *B. bassiana* display considerable heterogeneity which necessitates any identification based on morphological characters to be supported by molecular characterization.

Confirmation of the identity had to be followed by attempts at evaluation of the efficacy of the isolate as well as attempts at developing ecofriendly formulations that combines prolonged shelf life, ease of application and efficacy.

Surveys were conducted over two seasons during 2016-17 at major rice growing tracts of Alappuzha, Ernakulam, Thrissur and Palakkad districts of Kerala to collect entomopathogenic fungi infecting rice bug. Entomopathogenic fungus infected rice bug cadavers were obtained only from one location *i.e.,* Pattambi. As only one isolate was obtained, a precious fungal pathogen obtained from rice bug at Vellanikkara, Thrissur and maintained at AICRP on BCCP was also included in further studies.

The fungi isolated from the cadavers obtained from Pattambi had same morphological characters *viz.,* white coloured hyphal mat with slight raised folding which turned greyish at the center during the later stages. This indicated that both the bugs were infected by the same fungal species. The isolate obtained from Vellanikkara produced cottony white growth in PDA media. The spores produced from Pattambi isolate were spindle shaped whereas *B. bassiana* isolate from Vellanikkara (VKA 01 strain) produced globose spores

The ITS sequencing of Vellanikkara VKA 01 strain yielded 520 base pair (bp) sequences and Basic Local Alignment Search (BLAST) in National Center for Biotechnology Information (NCBI) confirming the identity as *B. bassiana*. Similarly in case of Pattambi isolate, 467 base pair sequences obtained showed that the fungus was *Choanephora cucurbitarum.*

The pathogenicity of the isolated native strains were assessed through laboratory bioassay. A noticeable reduction in population of nymphs and adults of rice bugs treated with *B. bassiana* VKA 01 strain was obtained three days after treatment. Among the two fungal isolates *B. bassiana* VKA 01 strain was found to be superior to *C. cucurbitarum.*

*Beauveria bassiana*VKA 01 strain recorded more than ninety per cent reduction in population of rice bug nymphs and adults five and seven days after treatment respectively. In comparison, *C. cucurbitarum* recorded significantly lower mortality values ranging from 18 – 22 per cent seven days after treatment.

The laboratory studies were followed by pot culture evaluation of the two native isolates *viz. B. bassiana* VKA 01 strain and *C. cucurbitarum* at three different concentrations each ranging from 10^7 to 10^9 spores ml^{-1}. These treatments were compared with *B. bassiana* NBAIR strain at the rate of 10^8spores ml^{-1}, the insecticide malathion and an untreated control. *Beauveria bassiana* VKA 01 strain caused more than eighty per cent mortality ten days after spraying and nearly cent per cent mortality by fifteenth day, while the highest mortality registered by *C. cucurbitarum* was 30.77 per cent, twenty days after treatment.

The most effective concentration of *B. bassiana* VKA 01 strain in pot culture experiment was evaluated under field experiment. The results of field evaluation at Thrissur and Palakkad district broadly agreed with the findings of the pot culture experiment. Plots treated with malathion had significantly lower population of bugs in both locations, five days after treatment.

However, ten days after treatment, *B. bassiana* VKA 01 strain, with a mean bug population of 9.25 bugs per m^2 was on par with malathion (6.25 bugs m^{-2}) and remained so till the conclusion of the experiment. Azadirachtin failed to record an appreciable reduction in rice bug population in the field, consistently being on par with control throughout the experiment at Palakkad district.

Similar results were also obtained in the second field experiment at Varadium (Thrissur). *Beauveria bassiana* VKA 01 strain recorded mortality values on par with malathion from ten days after spraying onwards.

In the field experiment at Pattambi (Palakkad) plots treated with malathion recorded the lowest mean grain damage of 8.37 per cent and was significantly superior to all other treatments. Plots treated with *B. bassiana* VKA 01 strain as well as *B. bassiana* NBAIR strain were next in terms of grain damage with 12.03 and 13.72 per cent and were on par with each other. Identical results were obtained in the field experiment at Varadium (Thrissur) as well. Plot treated with malathion recorded the lowest grain damage of 6.13 per cent, followed by *B. bassiana* VKA isolate (12.27 %)and *B. bassiana* NBAIR strain (13.58 %), both

being at par. Malathion as well as the two strains of *B. bassiana* were significantly superior to both azadirachtin and untreated control at both Palakkad and Thrissur.

Beauveria bassiana VKA 01 strain was evaluated for its compatibility with five insecticides and two fungicides, which are commonly used for pest and disease management in rice, following the poisoned food technique. Based on radial growth as well as per cent inhibition, the insecticides acephate and flubendiamide were found to be least inhibitory to *B. bassiana* VKA 01 strain with 31.29 and 34.95 per cent growth inhibition respectively. Propiconazole at 0.025 per cent concentration recorded the highest degree of 85.71 per cent inhibition.

Beauveria bassiana VKA 01 strain was formulated into talc based, oil based and aqueous formulations following standard procedures. Five commonly available vegetable oils such as sunflower oil, rice bran oil, coconut oil, sesame oil and palm oil were evaluated for oil based formulation through inhibition zone technique. Both palm oil and sesame oil proved to be least inhibitive to the fungus as against other oils tested.

Palm oil and sesame oil identified as the most compatible with *B. bassiana* VKA 01 strain in the previous study were evaluated for the storage life of spores. Palm oil registered the highest viable spore count of 1.56×10^8 spores ml^{-1} as against 7.3×10^7spores $ml^{-1}$for sesame oil.

Identification of the most suited oil base was followed by efforts to standardize the adjuvants required for the development of oil based formulation. Four different adjuvants, namely, glycerol, tween 80, carboxy methyl cellulose and polyethylene glycol were evaluated at three different concentrations each. Thirty days after storage, highest number of viable cfu were recorded in the combination involving three per cent glycerol (2.06×10^8cfuml^{-1}).

In case of aqueous formulation, the combination of *B. bassiana* spore in sterile distilled water and 0.5 per cent CMC recorded the highest number of viable

spores (2.34 x x10^8 ml^{-1}) thirty days after storage, followed by CMC (1 %) with significantly lower viable spore count of (1.93 x 10^8 ml^{-1}).

Chitin and chitosan, at three different concentrations each, were evaluated as adjutants for talc based formulation. Talc enriched with chitin (5 %) recorded the highest viable spore count of 1.92x10^8 ml^{-1}thirty days after storage.

The efficacy of the talc based, oil based and aqueous formulations of *B. bassiana* VKA 01 strain against rice bug was evaluated through a field experiment. While malathion, with a mean bug population of 3.92 per $m^2$was significantly superior to other treatments five days after application, both talc based and aqueous formulations registered values of 9.08 and 9.75 bugs m^{-2} which were on par with that recorded by malathion (9.32m^{-2}) in controlling rice bug population ten days after treatment, Fifteen days after treatment, the three formulations were on par with each other. While bug populations in plots treated with aqueous and oil based formulations, with values of 11.58 and 12.92bugsm^{-2}were on par with that in insecticide treated plot (13.58m^{-2}) plot treated with talc formulation registered 9.42 bugsm^{-2}which was significantly superior to that of malathion treated plot.

All the treatments were significantly superior to untreated control in terms of grain damage. Malathion and the talc based formulation recorded significantly lower damage of 10.08 and 10.25 per cent chaffy grains respectively and were on par with each other.

The grain yield varied from 558.34 in case of plots treated with oil based formulation to 635.83 in case of plots treated with talc formulation. Both talc based formulation as well as malathion (628.00 g/m^2) were on par with each other and were superior to other treatments. Plots treated with aqueous formulation registered the next highest yield of 600.00 g/m^2. Untreated control (583.33 g) as well as oil based formulation (558.34 g) of *B. bassiana* had comparable grain yields.

Talc based formulation of *B. bassiana* VKA 01 strain was superior to other formulations and was comparable to the insecticide malathion in terms of mean grain damage as well as mean yield.

The viable spore count in the three formulations was recorded at a regular interval of thirty days for up to twelve months to assess the storage life of the formulations. Two formulations, namely, chitin enriched talc formulation and oil formulation with adjutants, recording 2.5 x $10^8$and 1.1 x 10^8cfu ml^{-1} respectively alone retained spore viability above the CIB- RC prescribed limits five months after storage.

Six months after storage, chitin enriched talc formulation (1.38 x 10^8 spores ml^{-1}) alone recorded viability above the permissible limit while none of the formulations had viability above the required levels seven months after storage.

The study has successfully characterized, evaluated and formulated the *B. bassiana* VKA 01 strain, an entomopathogenic fungus isolated from rice bug, thus fulfilling the objectives for the study

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CHARACTERIZATION, EVALUATION AND FORMULATION OF *Beauveria bassiana* (Bals.) STRAINS AGAINST RICE BUG, *Leptocorisa* spp. (HEMIPTERA: ALYDIDAE)

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ABSTRACT OF THE THESIS

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Abstract

The rice bug, *Leptocorisa*spp. is one of the most important sap sucking insect pests of rice in the tropics. Both nymphs and adults suck sap from developing rice grains during the milky grain stage, leading to discoloured, empty or half-filled grains. Yield loss due to the bug infestation ranges from 10 to 35 per cent.

Management of the bug essentially involves spraying with insecticides, for want of safer options. Biological control of rice bug remains hampered by absence of ideal bioagents. The entomopathogenic fungus (EPF), *Beauveria bassiana* (Bals.) has been reported as infecting rice bug, from several parts of the world, including India. In this context, the present study was undertaken with the objectives of collection, isolation and molecular characterization of local strains of *B. bassiana,* evaluation against *Leptocorisa* spp., identification of a cost effective mass production technology for the selected strain of *B. bassiana*, and formulation of the most effective strain of *B. bassiana.*

Surveys were conducted over two seasons during 2016-17 at major rice growing tracts of Alappuzha, Ernakulam, Thrissur and Palakkad districts of Kerala to collect EPF infecting rice bug. However, EPF infected rice bug cadavers were obtained only from one location *i.e.*, Pattambi in Palghat district.As only one isolate was obtained, a potential fungal pathogen earlier obtained from rice bug at Vellanikkara, Thrissur and maintained at AICRP on BCCP was also included in further studies.

The above two isolates were identified through study of colony characters as well as through ITS sequencingand Basic Local Alignment Search Tool (BLAST). The isolate from Vellanikkara was identified as *Beauveria bassiana*, while the isolate from Pattambi was identified as *Choanephora cucurbitarum.*

The pathogenicity of the two isolates was assessed through laboratory as well as pot culture studies. In the laboratory, *B. bassiana*isolate recorded more than 90 per cent reduction in population of rice bug nymphs and adults five and

seven days after treatment respectively at all concentrations evaluated while *C. cucurbitarum* recorded only 18 to 53 per cent reduction in bug population.

Pot culture studies confirmed the superiority of *B. bassiana*isolate over *C. cucurbitarum* with *B. bassiana* treated pots registering over 99 per cent mortality by 20th day after treatment as against 27 to 31 per cent mortality in case of the latter.*B. bassiana*isolate was further evaluated at its most effective concentration, under field conditions in both Thrissur and Palakkad districts.*B. bassiana* proved to be on par with the insecticide malathion from 10thday onwards at both locations.

The compatibility of *B. bassiana* isolate with selected insecticides and fungicides was assessed using poisoned food technique. The results showed that the insecticide acephate was the most compatible with 31.29 per cent growth inhibition while the fungicide propiconazole was the least compatible with 85.71 per cent inhibition.

Methods for talc based, oil based and aqueous formulations of *B. bassiana*were standardized.Palm oil was identified as the most suited carrier for oil based formulation through inhibition zone technique as well as through assessment of viability of *B. bassiana* spores. Glycerol(3%), carboxy methyl cellulose (0.5%) and chitin (5%) formed the best adjuvants for oil based, aqueousand talc based formulations respectively, based on viable cfu count 30 days after storage.

All the three formulations were as effective as malathion in reducing rice bug populations in field trials. However, talc formulation was significantly superior to the remaining formulations in terms of yield as well as grain damage. Talc, oil and aqueous formulations had shelf life durations of six, five and four months respectively.