BIOEFFICACY OF ENTOMOPATHOGENIC FUNGI AGAINST RICE BUG, *Leptocorisa oratorius* Fab. (HEMIPTERA: ALYDIDAE)

By
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(2014-11-147)

THESIS

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DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
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DECLARATION

I hereby declare that the thesis entitled **"Bioefficacy of entomopathogenic fungi against rice bug, *Leptocorisa oratorius* Fab. (Hemiptera: Alydidae)"** is a bona-fide record of research work done by me during the course 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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Certified that thesis entitled **"Bioefficacy of entomopathogenic fungi against rice bug, *Leptocorisa oratorius* Fab. (Hemiptera: Alydidae)"** is a bona-fide record of research work done independently by **Ms. Neenu Chandran (2014-11-147)** 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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Introduction

1. INTRODUCTION

Rice, *Oryza sativa* L., forms the staple diet for over 3.5 billion people across the globe and is cultivated in 114 countries. Asia accounts for about 90 per cent of the world's rice production and over 75 per cent of the world's supply is consumed by people in Asian countries, and thus, rice is of immense importance to food security of Asia.

Rice is equally important from a national perspective, as more than 65 per cent of Indian population depends on rice as their staple food. Even though India is the second largest producer of rice in the world with 95 Mt per year, nearly 20 per cent of it is lost due to pests and diseases. As enhancing the area under rice is becoming increasingly difficult, preventing the losses due to pests and diseases is of utmost significance. Reducing the loss due to pests and diseases is even more critical to Kerala, which is a net importer of rice and thus suffers huge drain to its economy on this account alone.

About 187 species of insects have been recorded on rice (Yunus and Ho,1980) though a few are considered as serious pests. The rice earhead bug, *Leptocorisa* spp. are one of the major pests of paddy and is distributed across almost all the rice growing tracts in the country. Different species of rice bugs have been reported from different parts of the country. Among these, *Leptocorisa oratorious* Fab. is the most common species seen in south India, though *L. acuta* Thunb. and *L. chinensis* Dallas have also been reported.

The adult bug is pale brown to greenish-brown, slender and cylindrical in shape. Total development period of *L. oratorious* is around 26 days under field conditions, with an adult longevity of 83 and 37 days for females and males respectively (Hosamani *et al.* 2009). The damage is caused by both the nymphs and adults sucking sap from the grains during the milky grain stage. When the bugs feed during the flowering stage, the grains become empty or partially filled. Attack during later stages such as the milky grain stage and hard dough stage often affects the seed weight and quality. Losses due to the rice bug infestation generally varies from 15-20 per cent (Nigam and Verma, 1985) and often extend up to 98 per cent in severe cases (Bhadauria and Singh, 2009).

Because of the severity of the earhead bug damage in many parts of the world, several approaches have been tried to check the bug menace. However, inspite of these efforts, management of rice bug mostly involves the use of broad spectrum synthetic insecticides such as malathion and carbaryl, leading to contamination of environment as well as accumulation of toxic residues in the harvested produce. Effective, ecofriendly and economic options remain to be developed against rice bug.

Biocontrol would be one of the most attractive and acceptable option against rice bug, as with any other pest. Several studies have indicated that entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vullemin and *Metarhizium anisopliae* (Metsch) Sorokin could be effective against rice bug. However, a viable solution has not emerged till date. It was in such a background that the AICRP on BCCP & W, Vellanikkara succeeded in isolating a fungus from rice bug itself. It was later identified as *B. bassiana* (MTCC 12047). The local isolate, along with *M. anisopliae* were found to be promising in the laboratory studies (NBAIR, 2015a).

It was in this context that the present study was conducted with the objective of evaluating bioefficacy of entomopathogenic fungi against rice bug, *Leptocorisa oratorius* Fab.

Review of literature

2. REVIEW OF LITERATURE

The earhead bugs (*Leptocorisa* spp.) are major pests of rice throughout the world. Management of rice bug primarily involves the use of chemical insecticides. With public concern over the use of chemical in agriculture and their impact on the environment continuing to mount, safer approaches like the biological control are gaining greater significance. A number of natural enemies have been evaluated against rice bug. Among these the enomopathogenic fungi, particularly *Beauveria bassiana* (Balsamo) Vullemin, *Metarhizium anisopliae* (Metsch) Sorokin and *Lecanicillium lecanii* (Zimm.) Zare and Games have been considered as the most promising. The studies evaluating the above fungi against rice bug are reviewed in this chapter.

2.1 *Beauveria bassiana* (Balsamo)Vullemin

Beauveria bassiana, the white muscardine fungus is one of the most ubiquitous and extensively studied entomopathogenic fungus (Wraight and Carruthers, 1999). *B. bassiana* was first reported by Agostino Bassi from silk moth larva (*Bombyx mori* L.) in 1835. It belongs to the family Cordycipitaceae and order Hypocerales. It is pathogenic to insects of a number of orders such as Coleoptera, Hemiptera, Lepidoptera, Diptera and Dermaptera (Toledo *et al.,* 2008).

2.2 Bioefficacy of *Beauveria bassiana* against rice bug

Several studies have been carried out to evaluate the efficacy of *B. bassiana* against rice bug. Tuan (2014a), for instance, studied the virulence of three isolates of *B. bassiana*, namely, AJ III^{-1}, BPH (isolated from *Nilaparvata lugens* Stal.) and GLH 9 (isolated from *Nephotettix virescens*) against the rice bug, *Leptocorisa oratorius* under laboratory conditions. The isolate GLH 9 exhibited greater virulence compared to the other two strains. GLH 9 recorded the highest LC_{50} value of 4.65 x

10^9conidia/ml against third instar nymphs and the lowest LC_{50} value of 1.3 x 10^9 conidia/ml against fifth instar nymph. A similar trend was also noticed in median lethal time, with LT_{50} for third instar being 7.95 days as against 3.37 days for fifth instar and 2.89 days for adults. Based on these results, the study concluded that early instar nymphs of rice bug were more resistant to fungal infection than late instar nymphs or adults.

Similarly, Loc and Chi (2005) conducted greenhouse and field experiments to evaluate the efficacy of selected isolates of entomopathogenic fungi against rice earhead bug, *L. acuta*. Treatment with *B. bassiana* isolates produced highest mortality of 57.50 to 77.70 per cent under green house conditions and 45.3 to 74.9 per cent under field conditions ten days after treatment.

Kalita *et al.* (2009) observed that application of *B. bassiana* at the boot leaf stage of rice was effective in reducing grain damage by gundhi bug. Plots treated with the fungus suffered only 5.15 per cent grain damage as against a significantly higher 13.06 per cent in untreated control.

Reduction in rice bug population following treatment with *B. bassiana* was reported by Girish and Balikai (2015), who recorded a markedly lower bug population of 2.29 bugs per hill in plots treated with *B. bassiana* (10^7 conidia ml^{-1}) as against 7.42 bugs per hill in untreated control.

Singh *et al.* (2015) also documented a similar reduction in bug population with a mean value of 0.59 bugs/hill in plots treated with *B. bassiana* while untreated control had a mean number of 1.75 bugs/hill.

2.3 Bioefficacy of *Beauveria bassiana* against other insect pests

Beauveria bassiana has also been evaluated against pests in a number of crops, underlining the efficacy of the fungus against a wide range of insect taxa.

Hazarika and Puzari (1997) reported that *B.bassiana* effectively controlled the egg, larval and pupal stages of rice hispa, *Dicladispa armigera* (Olivier) during summer, autumn and spring seasons. Field experiments showed that egg stage was more susceptible than other stages. Infection of eggs ranged from16.95 to 45.15 per cent, compared to 1.67 to 40.63 per cent of adult bugs depending on the seasons.

Laboratory assays by Wraight *et al.* (2000) in Texas demonstrated the capacity of *B. bassiana* to infect silver leaf whitefly (*Bemisia argentifolii* Bellows & Perring) even at very low relative humidity. Nymphs of the whitefly suffered 35 per cent infection at relative humidity as low as 25 per cent, upon being treated with *B. bassiana* at the rate of 0.6 - 1.4 x 10^3 conidia/mm^2 of leaf.

Lecuona *et al.* (2001) evaluated four strains of *B. bassiana* obtained from Argentina against triatomine bug, *Triatoma infestans* Klung. The lowest LD_{50} values for nymphs were recorded by the strains Bb 10 and Bb 65, while for adults, the strains were Bb 1, Bb 10, and Bb 65. The highest mean mortality was obtained with strain Bb 10 at 26^0C.

Application of *B. bassiana* at the rate of $1x10^6$ conidia/ml reduced the population of stink bug (*Nezara viridula* L.) by 23 per cent after 25 days (El-Zoghby, 2003).

Samuels and Coracini (2004) screened six isolates of *B. bassiana* at the rate of $5x10^8$ conidia per ml against 4th instar nymphs and adult females of chinch bug, *Blissus antillus* Leonard. *B. bassiana* ARSEF 792 was reported as the most virulent isolate to nymphs and adults, causing 53 and 78 per cent infection respectively. The LT_{50} values for both nymphs and adults were recorded as 7.8 and 5.0 days, respectively.

Application of either of the *B. bassiana* isolates, LRC 28 and RSB at the rate of $5x10^{12}$ conidia per ml, were found to be as effective as a single application of cypermethrin in suppressing rice stink bug, *Oebalus pugnax* (F.) population 7-8 days after application (Patel *et al.*, 2006).

The effect of endophytic *B. bassiana* in banana plants against the banana weevil, *Cosmopolites sordidus* (Germar) was examined by a screen house study at Uganda. Root dipping of tissue cultured banana plants in *B. bassiana* suspension ($1.5x10^7$ conidia/ml) for 2 h resulted in 23.5 to 88.9 per cent insect mycosis as well as 42.0 to 86.7 per cent reduction in plant damage (Akello *et al.,* 2008).

Application of three isolates of *B. bassiana,* namely, GHA, ERL 1170 and ERL 5672 against adult brown marmorated stink bug, *Halyomorpha halys* Stal. caused significantly higher mortality of 65 to 100 per cent when compared to *M. anisopliae* isolates (ERL 1540 and ERL 1171) with 40 to 88 per cent mortality 12 days after treatment (Gouli *et al.*, 2012).

Reddy *et al.* (2013) conducted a field trial with three different entomopathogenic fungi *viz., B. bassiana, M. anisopliae* and *L. lecanii* at the rate of 5g/l having $1x10^8$ CFU along with standard check, acephate 75 SP (1.5 g/l) against brown plant hopper, *Nilaparvata lugens*. Five days after treatment, acephate was found to be the most effective treatment with 19.30 hoppers/hill compared to the entomopathogens *B. bassiana, M. anisopliae* and *L. lecanii* with 55.60, 54.40 and 63.70 hoppers per hill, respectively. However, ten days after second spray, the insect population in plots treated with *B. bassiana* and *M. anisopliae* registered 17.20 and 18.80 hoppers per hill and were found to be at par with acephate.

Field evaluation of *B. bassiana* against major insect pests of paddy during Kharif season of 2011 at Allahabad by Singh *et al.* (2015) revealed that *B. bassiana* at the rate of 5 kg ha^{-1} was the most effective against the kharif grasshopper, *Hieroglyphus banian* (F.) (0.66 insect/hill), yellow stem borer, *Scripophaga incertulas* Wlk (7.19 % hill damage) and rice hispa beetle, *Dicladispa armigera* (1.35 insect/hill) followed by *B. bassiana* @ 2.5 kg/ha, in comparison to untreated control which recorded higher numbers of kharif grasshoppers (1.10 insect/hill), yellow stem borer (8.56 % hill damage) and rice hispa beetles (1.81 insect/hill).

2.4 *Metarhizium anisopliae* (Metsch) Sorokin

Metarhizium anisopliae, an anamorphic fungus which belongs to the Phylum Ascomycota, is the most extensively studied species of the genus *Metarhizium*. The first use of *M. anisopliae* as a microbial agent against insects was in 1879, by Elie Metschnikoff to control the wheat grain beetle, *Anisoplia austriaca* Reitt. The disease caused by the fungus was named as green muscardine disease because of the green colour of its spores (Schabel 1976). *M. anisopliae* has been reported from a wide range of insect hosts. Veen (1968) recorded 204 instances of natural infestation by *M. anisopliae* in insect species belonging to seven orders. Most of the listed host insects of *M. anisopliae* belonged to the order Coleoptera. According to Goettel *et al.* (1990) the host range of *M. anisopliae* contained Orthoptera, Dermaptera, Isoptera, Hemiptera, Diptera, Coleoptera, Hymenoptera, Siphonaptera and Lepidoptera among class Insecta as well as arthropods belonging to Amphipoda and Acari.

2.5 Bioefficacy of *Metarhizium anisopliae* against rice bug

Treatment of the different developmental stages of *L. oratorius* with *M. anisopliae* at 1 x 10^8 conidia ml^{-1} resulted in 97, 80, and 70 per cent mortality of early and late instar nymphs as well as adult bugs respectively, eight days after exposure. Egg stage was resistant to fungal infection. It was also revealed that *L. oratorius* transmitted *M. anisopliae* to healthy individuals, leading to the spread of infection (Burdeos, 1994).

The pathogenicity of fourteen *M. anisopliae* isolates against *L. oratorius* was evaluated under greenhouse conditions. The LC_{50} and LT_{50} values of most virulent strains, namely, TIAONG (1.39 x 10^6 conidia/ml), DRC (3.5 x 10^6 conidia/ml) and VISCA (6.42 x 10^6 conidia/ml) were 4, 5.7 and 5.7 days respectively. Mortality of adult bugs exposed to *M. anisopliae* at the rate of 1 x 10^8 conidia ml^{-1} commenced on the third day after exposure and resulted in 90, 80, and 73 per cent mortality after 8 days for TIAONG, DRC and VISCA, respectively (Burdeos and Gabriel, 1995).

Field application of the conidial suspension of *M. anisopliae* at the rate of 1 x 10^{12} conidia ha^{-1} at milky grain stage reduced 91 per cent of *L. oratorius* population two weeks after spraying, in contrast to 83 and 37 per cent population reduction in insecticide treated and untreated plots respectively. *M. anisopliae* treated plots recorded the highest grain yield, followed by chemically treated plots with 14 and 13 per cent yield advantage over the untreated plots respectively (Estoy and Gabriel, 1996).

Metarhizium anisopliae isolated from naturally infected insects were found to be pathogenic to rice bug. Three new isolates of *M. anisopliae viz.* OM3-BD, HG3-B and HG5-BD showed high efficacy against rice bug at the dose of 6 x 10^{12} conidia ha^{-1}. Under pot culture studies the above isolates caused mean mortality ranging from 74.7 to 87 per cent, ten days after treatment. Similar results, with 63.6 to 86.6 per cent mortality ten days after treatment were obtained in the field experiment also (Loc and Chi, 2005).

Herlinda *et al.* (2008) reported that isolates of *Metarhizium* sp. obtained from different parts of South Sumatra and other provinces in Indonesia induced 50-62 per cent mortality in nymphs of *L. oratorius*. The shortest LT_{50} of 5.75 days was recorded by an isolate obtained from *Tenebrio molitor* L., while the isolate from *Plutella xylostella* (L.) registered 7.46 days. The isolate obtained from *L. acuta* showed a higher nymphal mortality of 62 per cent, though with a higher LT_{50} value of 7.05 days. Different liquid formulations of the fungus, namely, maize media, rice media and Sabouraud dextrose broth (SDB) recorded nymphal mortality ranging from 86.67 -100.00 per cent. Cent per cent mortality was observed with maize media having 10^7 spores mL^{-1}. SDB having 10^3 spores mL^{-1} recorded 86.67 per cent mean mortality.

Girish and Balikai (2015) had evaluated selected botanicals, biopesticides and newer insecticides against *L. acuta* at University of Agricultural Sciences, Dharwad. They recorded average bug population of 1.79 bugs per hill in plots treated with *M. anisopliae* at the rate of 1 x 10^7 conidia mL^{-1}. The fungus was next best only to the chemicals thiamethoxam 25 WG 0.3g L^{-1} (0.75 bugs/hill), malathion 5% dust @ 20 kg ha^{-1} (1.13 bugs/hill), dimethoate 30 EC @ 1.75 ml L^{-1} (1.50 bugs/hill) and chlorpyriphos 20 EC @ 2ml L^{-1}(1.50 bugs/hill), and was significantly superior to untreated control with mean population of 7.42 bugs per hill.

Another study by Pelealu (2015) compared the pathogenicity of different isolates of *M. anisopliae* to *L. acuta.* He reported that the *M. anisopliae* isolate from North Minahasa district of Manado (MMTTO) caused 93.33 per cent mortality of *L. acuta* and was superior to MMITO (86.7%) and MMSAM (80.0%).

2.6 Bioefficacy of *Metarhizium anisopliae* against other insect pests

Laboratory evaluation of five isolates of *M. anisopliae* for the management of the groundnut bruchid, *Caryedon serratus* Olivier in Nigeria indicated that while the isolates tested were virulent to the beetle, there was variation in pathogenicity among the isolates. *M. anisopliae* isolate CPD 4 proved to be superior to other isolates and comparable to pirimiphos-methyl (Ekesi *et al.,* 2001).

Destefano *et al.* (2005) evaluated the effectiveness of *M. anisopliae* var. *anisopliae* strain E9, isolated from the pasture spittlebug, *Deois flavopicta* Stal, against larval, prepupal, pupal and adult stages of the South American fruit fly *Anastrepha fraterculus* (Weid.). Mortality was observed based on the adult emergence. Application of conidial suspension at the rate of 2.52 x 10^{10} conidia per gram of soil recorded the highest mortality of 86 per cent. The median lethal concentration (LC_{50}) was estimated as 8.44 x 10^9 conidia/g of soil.

Ansari *et al.* (2008) evaluated the potential of different strains of *M. anisopliae* against western flower thrips, *Frankliniella occidentalis* Pergande. The result showed that all strains, applied at the concentration of 1 x10^{10} conidia L^{-1} caused significantly higher mortality than fipronil. Among different *M. anisopliae* strains, V275 and ERL700 were consistently more effective and caused 85 to 90 and 91 to 96 per cent mortality of thrips larvae and pupae respectively. In comparison, untreated control registered a significantly lower mortality of 35 to 38 per cent, 11 days after inoculation.

In-vitro bioassay conducted to evaluate the bioefficacy of *M. anisopliae* against seven insect pests species showed variation in susceptibility among the insects to the fungus. The lowest LC_{50} value of 1.62x10^4 spores mL^{-1} was recorded in case of second instar larva of *Pericallia ricini* Fab. and the highest value of 1.75 x10^6, in case of third instar larva of *Helicoverpa armigera* (Hubner). Among the adult insects, *Aphis craccivora* (Koch) recorded lowest LC_{50} value (1.84 x 10^4), followed by *Oxycarenus hyalinipennis* (Costa), *Dysdercus cingulatus* (Fab.) and *Mylabris pustulata* (Thunb.) (Sahayaraj and Borgio, 2010).

Santi *et al.* (2011) observed that the addition of soyabean oil to *M. anisopliae* preparation (1x10^8 conidia ml^{-1}) enhanced the efficacy of the fungus, resulting in 100 per cent mortality of both nymphs and adult cotton stainer bugs, *Dysdercus peruvianus* (Guerin-Meneville) at six and seven days after exposure respectively.

Lezama-Gutierrez *et al.* (2012) tested two isolates of *M. anisopliae* (Ma 14 and Ma 65) against nymphs and adults of Asian citrus psyllid, *Diaphorina citri* Kuwayama under field conditions. The treatments were applied four times at a concentration of 2 x 10^{13} conidia/ha at fifteen days interval. Isolate Ma 65 was found to be the most effective treatment with a mean population of 1.00 nymph per bud compared to the 5.62 nymphs per bud in case of control, 48 days after treatment. Isolates of *M. anisopliae* reportedly caused 40 to 50 per cent mortality of the psyllid.

El-Salam *et al.* (2013) observed that *M. anisopliae* was as effective as the botanical insecticide nimbecidine against leaf miner, *Liriomyza trifolii* (Burg.) in faba bean. Both nimbecidine and *M. anisopliae* caused nearly 70 per cent reduction in the population of miner larvae after two applications.

Sumathi and Ramasubramanian (2013) evaluated biopesticides like neem oil (3%), *M. anisopliae* ($1x10^8$ spores mL^{-1}) and the newer insecticide profenophos (1000 ml ha^{-1}) against black bug. The study revealed that spraying with profenophos, *M. anisopliae* and neem oil resulted in 60.8, 47.1 and 41.2 per cent reduction in black bug population respectively.

A study was conducted at Tehran, Iran to compare the efficacy of three isolates of *M. anisopliae* var. *major viz.* M14, Iran 715c and Iran 437c to control sunn pest, *Eurygaster integriceps* Puton under laboratory conditions. The fungus induced mortality of 75, 88 and 90 per cent respectively when applied at the rate of 10^8 spores mL^{-1} (Sedighi *et al.*, 2013).

2.7 *Lecanicillium lecanii* (Zimm.) Zare and Games

The entomopathogenic fungus *Lecanicillium lecanii* (Zimm.) Zare and Games naturally infects a wide range of phytophagous hemipterans in general and is considered to be a potential microbial biocontrol agent. It was first reported in 1939 by Viegas, who observed the characteristic white halo formed by the fungus on the scale insect *Coccus viridis* (Green). The effectiveness of *L. lecanii* on *C. viridis* was studied and demonstrated for the first time in India by Santharam *et al.* (1977) and on banana aphid *Pentalonia nigronervosa* Coq. by Regupathy and Paramjothi (1977). It was found to be effective for the control of sap feeding pests like aphids, whiteflies, scale insects, thrips and mealy bugs (Evans and Samson, 1986).

2.8 Bioefficacy of *Lecanicillium lecanii* against sucking pests

Samsinakova and Kalalova (1976) reported that the use of conidial suspension ($4.1 x 10^7 L^{-1}$) of *L. lecanii* resulted in 80-100 per cent mortality of the scale insect, *Coccus hesperidium* L. in three weeks. Easwaramoorthy and Jayaraj (1978) found that application of *L. lecanii* ($16 x 10^6$ spores mL^{-1}) twice at fortnightly interval was highly effective against *C. viridis* under field conditions. Gour and Dabi (1988) reported that soil inoculation with fungal suspension of *L. lecanii* effectively controlled white grub (*Holotrichia consanguinea* Blanch) in pot culture experiments.

Schaaf *et al.* (1990) used wettable powder formulaton of *L. lecanii* for the control of green house whitefly, *Trialeurodes vaporariorum* (Westwood) on cucumber and tomato in greenhouse and found that the weekly spray with *L. lecanii* at 10^3 spores mL^{-1} resulted in 90 per cent reduction of population of whitefly.

Lecanicillium lecanii was found to be as efficient as buprofezin in controlling nymphs of silver leaf whitefly, *Bemisia tabaci* (Gennadius) infesting tomato. Phadke and Phadke (2000) conducted field experiments with a commercial formulation of *L. lecanii* (Verti-Guard^R) at the rate of 1g/L against brinjal whitefly, *B. tabaci* under greenhouse conditions for two consecutive years. The nymphal mortality of *B. tabaci* during the years 1998 and 1999 was recorded as 93 and 67 per cent respectively, five days after treatment.

Ghelani *et al.* (2006) tested *L. lecanii* ($2x10^8$ cfu/g) at 5.0 gL^{-1} against *A. gossypii* on Bt cotton under field conditions and reported 50 to 70 per cent mortality of the aphid.

Surveys for entomopathogenic fungi were carried out in greenhouses and open fields in various parts of Argentina. Pathogenicity tests for the fungal isolates obtained from whiteflies sampled on horticultural crops were conducted against nymphs of *T. vaporariorum*. The mortality of *T. vaporariorum* nymphs exposed to a conidial suspension of *L. lecanii* was 52.6 ± 8.3 per cent seven days post treatment and the median lethal time was 4.32 days. (Scorsetti *et al.*, 2008)

Banu *et al.* (2010) carried out a bioassay against nymphs and adults of *Paracoccus marginatus* William and Granara de Willink, with *M. anisopliae, V. lecanii* and *B. bassiana,* each at concentration of 1×10^8 conidia/ml. Cent per cent mortality of nymphs was recorded in all the three entomopathogenic fungi, whereas, in case of adults, *V. lecanii* recorded the highest mortality of 80 per cent followed by *M. anisopliae* and *B. bassiana* with 75 and 70 per cent mortality respectively seven days after treatment.

Subramaniam *et al.* (2010) obtained 30 and 60 per cent mortality of adults and nymphs of tea thrips, *Scirtothrips bispinosus* (Bagnall) respectively, when treated with *L. lecanii* isolated from same species.

Bioassay with *L. lecanii* against certain sap feeding insects of cotton *viz., A. gossypii, B. tabaci* and *Amrasca devastans* (Distant) showed a linear relation between per cent mortality and dose concentration. The per cent mortality increased from 73.33 to 100.00 per cent; 86.67 to 100.00 per cent and 60.00 to 93.33 per cent in case of *A. gossypii, B. tabaci* and *A. devastans,* respectively as the dose level increased from 0.15 to 0.25 per cent (Karthikeyan and Selvanarayanan, 2011).

A commercial formulation of *L. lecanii* (Verticel 1.15% WP) was evaluated against pest complex of transgenic *Bt* cotton at Agricultural Research Station, Dharwad. Verticel (7.5 kg ha^{-1}) was on par with acetamiprid 20 SP (100g ha^{-1}) in suppressing the population of thrips, aphids and leaf hoppers (Patil *et al.,* 2012).

Banu and Gopalakrishnan (2012) reported that oil based formulation of *L. lecanii* recorded a significantly higher mortality of 75 per cent in case of *Paracoccus marginatus* infesting cotton when sprayed twice at 15 days interval.

Suresh *et al.* (2012) evaluated *L. lecanii* against cowpea aphid, *A. craccivora* under field condition at three different concentrations *viz.*, 1×10^7, 1×10^8 and 1×10^9 spores mL^{-1}. The fungus recorded a mortality of 71.62 per cent at the highest dose of $1x10^9$ spores mL^{-1}.

Materials and methods

MATERIALS AND METHODS

The present study entitled "Bioefficacy of entomopathogenic fungi against rice bug, *Leptocorisa oratorius* Fab. (Hemiptera: Alydidae)" was undertaken in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara during the period August 2015 to July 2016. The study involved pot culture as well as field evaluation of entomopathogenic fungi against rice bug. Detailed description on the materials used and methods followed to conduct the experiment are discussed in this chapter.

3.1 PREPARATION OF FUNGAL CULTURE

The pure cultures of the entomopathogenic fungi *viz.* *Beauveria bassiana* (MTCC 12047), *Metarhizium anisopliae* and *Lecanicillium lecanii* were obtained from All India Coordinated Research Project on Biological Control of Crop Pests and Weeds, Vellanikkara. The fungi were multiplied on suitable media following standard procedures. *B. bassiana,* isolated from rice bug, was subcultured and maintained on the Sabouraud's maltose and yeast liquid medium, that of *M. anisopliae* in coconut water medium and *L. lecanii* in potato dextrose broth. These were incubated at room temperature for obtaining maximum growth and sporulation.

3.2 PREPARATION OF SPORE SUSPENSION

After 10-15 days of incubation, when profuse growth of sporulated mycelia of entomopathogenic fungi covered the broth surface, the conidia were harvested by blending entire mycelial mat along with the medium using a household mixer grinder. For the better dispersal of spores, few drops of surfactant, Tween 80 (0.02%) was added to the spore suspension. After thorough mixing, the suspension was filtered through a clean double layered muslin cloth. The number of spores per ml of spore suspension was enumerated using Neubauer Haemocytometer. The spore count in the suspension was estimated using the formula put forth by Lomer and Lomer (1996).

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

X = Average number of spores per small square

400 = Total number of small squares

10 = Depth factor

1000 = Conversion factor for mm^3 to cm^3

D = Dilution factor, and

Y = Number of small squares counted

Based on the number of spores, all the cultures were adjusted to 1x 10^9 spores per ml from which the lower concentrations required for the pathogenecity studies were prepared by serial dilution technique.

3.3 POT EVALUATION OF ENTOMOPATHOGENIC FUNGI AGAINST RICE BUG

A pot culture experiment was conducted to evaluate the bioefficacy of different entomopathogenic fungi against *L. oratorius*, using the rice variety Uma. Twenty eight day old seedlings were transplanted to pots under open field condition. All the agronomic practices were followed as per the Package of Practice Recommendations: Crops (KAU, 2011). During the panicle initiation stage the potted plants were caged using nylon net (Plate 1). Fifteen field collected rice bugs including both nymphs and adults were released into each cage. The bugs were allowed 24 h for establishment on the caged rice plants before application of treatments.

The experiment was conducted in a Completely Randomized Design (CRD) with 14 treatments and three replications. The treatment details are listed in Table 1.

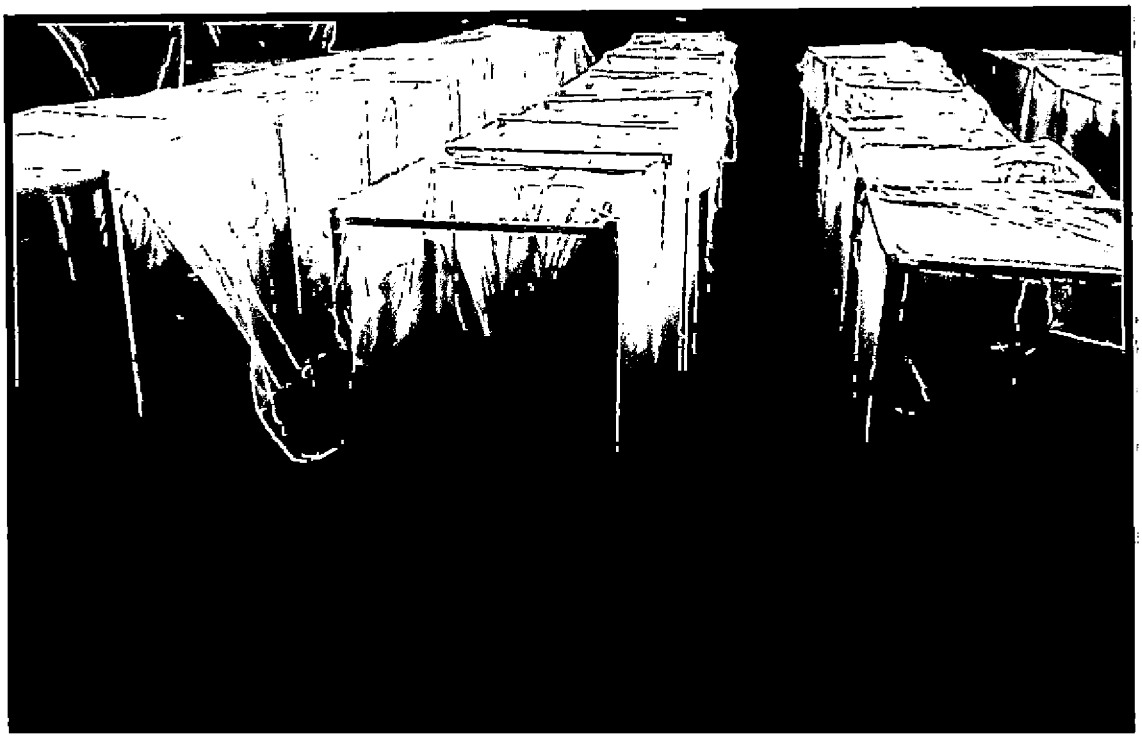


Plate 1. Layout of pot culture experiment

Plate 1. Layout of pot culture experiment

Table 1. Details of treatment under pot culture experiment

Sl. No.	Treatment	Concentration
T 1	*Beauveria bassiana*	1 x 10^5 spores mL^{-1}
T 2	*B. bassiana*	1 x 10^6 spores mL^{-1}
T 3	*B. bassiana*	1 x 10^7 spores mL^{-1}
T 4	*B. bassiana*	1 x 10^8 spores mL^{-1}
T 5	*Metarhizium anisopliae*	1 x 10^5 spores mL^{-1}
T 6	*M. anisopliae*	1 x 10^6 spores mL^{-1}
T 7	*M. anisopliae*	1 x 10^7 spores mL^{-1}
T 8	*M. anisopliae*	1 x 10^8 spores mL^{-1}
T 9	*Lecanicillium lecanii*	1 x 10^5 spores mL^{-1}
T 10	*L. lecanii*	1 x 10^6 spores mL^{-1}
T 11	*L. lecanii*	1 x 10^7 spores mL^{-1}
T 12	*L. lecanii*	1 x 10^8 spores mL^{-1}
T 13	Malathion 50EC	500 g ai ha^{-1}
T 14	Absolute control	-

Aqueous suspension of each fungus having the required spore concentration was prepared with 0.02 per cent Tween 80 solution. They were then sprayed on the caged rice plants. Spraying was carried out in the afternoon with a pneumatic hand sprayer of 1L capacity.

3.3.1 Observations

Rice bug population on caged plants was recorded at 24 h interval for 10 days after treatment application. Dead rice bugs were collected daily and placed in petridish containing moist filter paper to hasten the mycelial growth on infected cadavers. The cadavers which produced mycelial growth were used for proving pathogenicity.

3.3.2 Confirmation of pathogenicity

Pathogenicity was confirmed by proving Koch's postulates. The EPF infected rice bug cadavers were collected from the pots and kept in humid chamber for obtaining fungal growth. The fungus thus obtained was isolated. The pure culture was inoculated in potato dextrose agar media and kept for incubation at room temperature. After obtaining maximum growth and sporulation, live rice bugs were inoculated with the fungus. The mortality was observed and the symptom of infection compared with the original one obtained from the field.

3.3.3 Statistical analysis

The per cent mortality of rice bugs treated with different fungi was calculated and subjected to ANOVA test and means were compared with Least Significant Difference.

3.4 FIELD EVALUATION OF ENTOMOPATHOGENIC FUNGI AGAINST RICE BUG

The most effective concentration of each of the entomopathogenic fungus in the pot culture experiment was evaluated along with malathion 500g ai ha^{-1} as the insecticide check under field conditions during August and December 2015.

The experiment was laid out in a Randomized Block Design (RBD) comprising of five treatments with four replications. The study was carried out using the variety Uma, planted in plots of size 5x4 m^{2}. The details of treatments are given in Table 2.

Table 2. Details of treatment in field evaluation

Sl. No.	Treatment	Concentration
T 1	*Beauveria bassiana*	1 x 10^8 spores mL^{-1}
T 2	*Metarhizium anisopliae*	1 x 10^8 spores mL^{-1}
T 3	*Lecanicillium lecanii*	1 x 10^8 spores mL^{-1}
T 4	Malathion 50EC	500 g ai ha^{-1}
T 5	Absolute control	-

The field evaluation was conducted twice. The first experiment was during Rabi season of 2014 at paddy field of the Department of Agronomy, College of Horticulture, Vellanikkara (Plate 2) and the second during the summer season (January-May, 2016) at farmer's field in Muthuvara, Adat Grama Panchayat, Thrissur (Plate 3).

A nursery was raised and 28 days old rice seedlings were transplanted into the main field at a spacing of 20 x 10 cm. All the agronomic practices were undertaken as per the Package of Practices recommendations: Crops by Kerala Agricultural University (KAU, 2011).

Field cages of size 1m x 1m were placed in the field at the milky grain stage. Three cages were placed in each plot with one at the centre and two at opposite corners of the plot. Within each cage, five panicles were selected at random and tagged for recording grain damage. In the experiment conducted at farmer's field, the plants were not caged due to practical constraints.

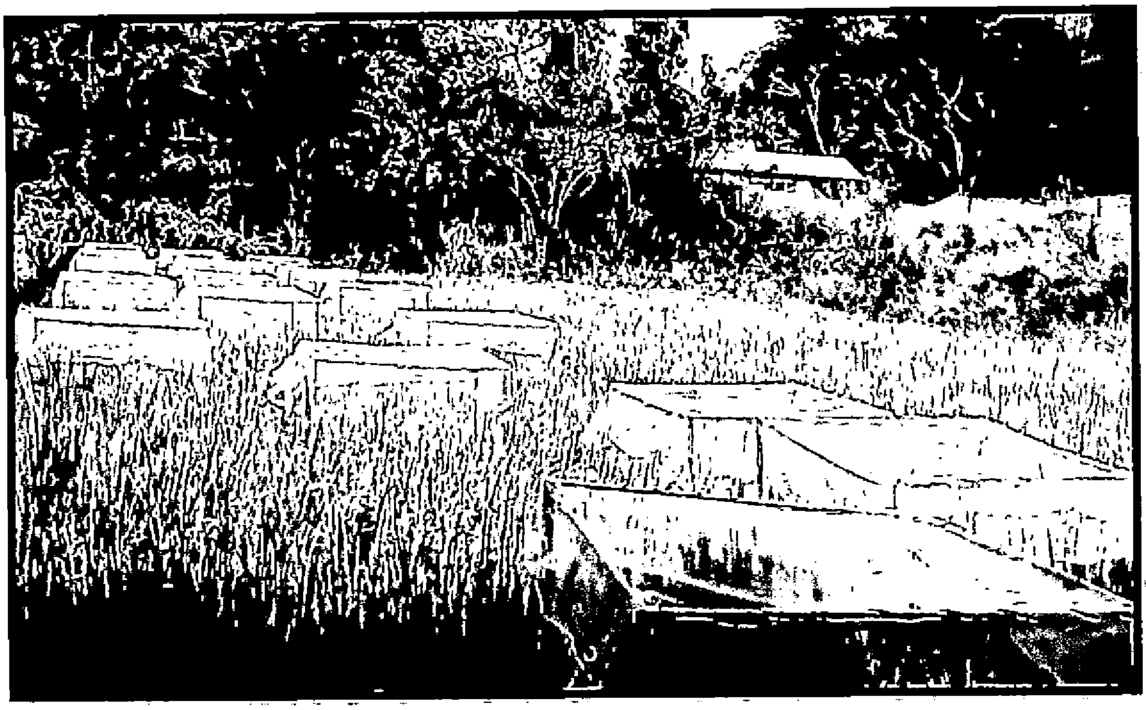


Plate 2. Layout of field experiment at Vellanikkara

Plate 3. Layout of field experiment at Muthuvara

Plate 2. Layout of field experiment at Vellanikkara

Plate 3. Layout of field experiment at Muthuvara

The fungal spore suspensions were prepared as stated in section 3.2. The treatments were imposed twice at 15 days interval by spraying on the crop. An untreated check was maintained to compare the results.

3.4.1 Observations

Observations on rice bug population as well as damage caused by rice bug were recorded before and after the application of treatments. The post treatment observations were recorded at five days interval.

3.4.1.1 Estimation of rice bug population

The rice bug population (nymphs and adults) as well as the number of damaged grains per panicle in each of the three cages per plot were recorded one day before spraying and again at five days interval after spraying. Mean data recorded from the three field cages fixed within each plot constituted one observation.

3.4.1.2 Observation on number of panicles

Number of hills as well as number of panicles per hill within each cage was recorded and mean of the values from three cages was taken as the observation for each replication.

3.4.1.3 Observation on damage caused by rice bug

Fresh panicles at milky grain stage were marked for recording observations. Total number of grains as well as number of infested grains (Plate 4) were recorded from the labeled panicles. The pre treatment count was taken a day before treatment and the post treatment counts were recorded at 5 and10 days after treatment.

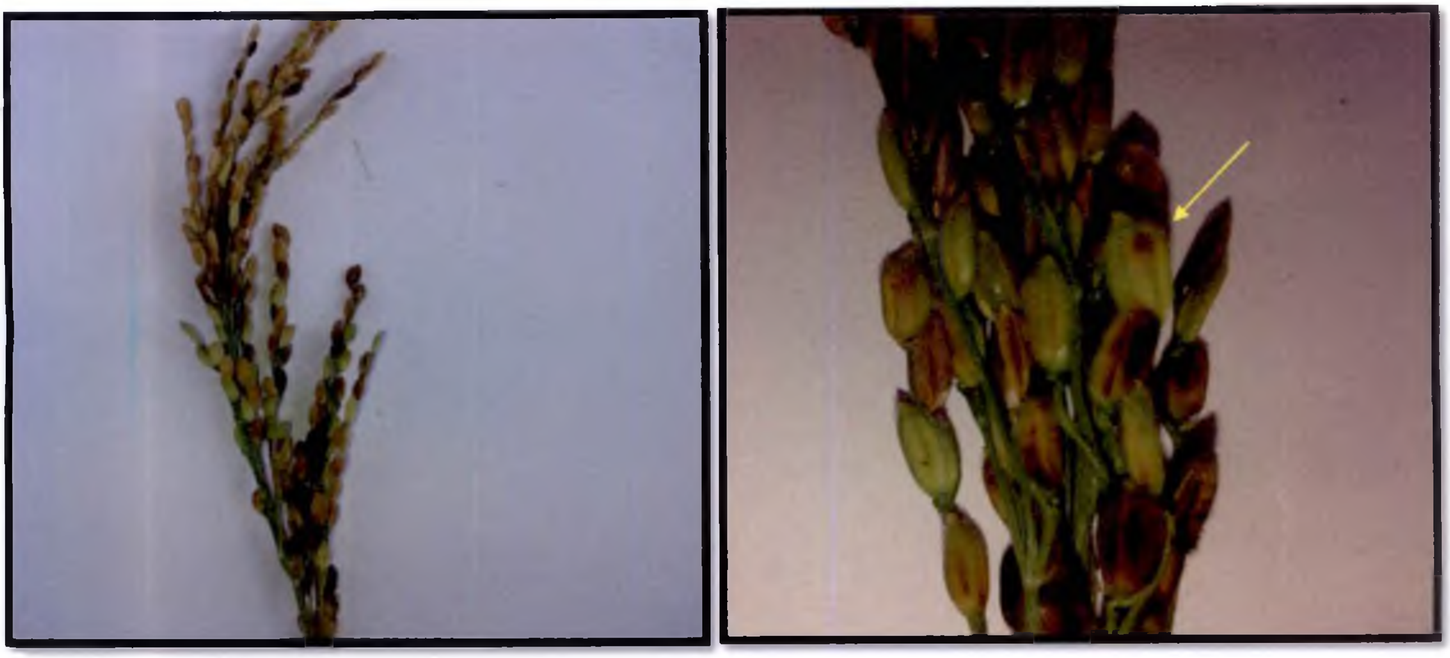


Plate 4. Grain damage by rice bug

3.4.1.2 Observations on grain yield

The grain yield from each cage was taken separately and mean from three cages was considered as the observation for each replication. Grain yield could not be collected from farmer's field for reasons beyond control.

3.4.2 Statistical analysis

Data on the mean number of rice bugs were subjected to square root transformation and covariance analysis was computed using MSTATC (Gomez and Gomez, 1984). The per cent reduction of rice bugs over pre treatment count was worked out for each observation and the means were separated by using Least Significant Difference (LSD).

The per cent damage due to rice bug corresponding to each treatment was subjected to ANOVA and2 the means were separated by using Least Significant Difference (LSD).

The yield data was subjected to ANOVA and the means were separated by using Least Significant Difference (LSD).

Results

4. RESULTS

The results of the study "Bioefficacy of entomopathogenic fungi on rice bug *Leptocorisa oratorius* Fab." carried out at Department of Agricultural Entomology, College of Horticulture, Vellanikkara from August 2015 to July 2016 is presented here.

4.1 POT CULTURE EVALUATION OF ENTOMOPATHOGENIC FUNGI AGAINST RICE BUG

The mean rice bug population following application of treatments is given as Annexure 1. The per cent mean mortality is presented in Table 3.

Mortality of rice bugs was observed only in cages treated with malathion 50 EC (500g ai ha^{-1}) for the first two days after treatment, which recorded 91.11 per cent mortality in the first 24 h itself. No further increase was observed till ninth day after treatment.

Among the entomopathogens evaluated, *B. bassiana* induced mortality by third day after treatment. The fungus, applied at the rate of $1x10^8$ spores mL^{-1} recorded 4.44 per cent mortality three days after treatment. *B. bassiana* applied at concentrations of $1x10^5$, $1x10^7$ and $1x10^8$ spores mL^{-1} recorded mean mortality of 2.22, 4.44 and 6.66 respectively, four days after treatment. All the three treatments were on par with each other and were significantly superior to control.

Mortality was observed in seven out of twelve treatments involving entomopathogenic fungi five days after treatment. The mean mortality of 91.11 per cent registered in case of the insecticide malathion continued to be significantly superior to other treatments. It was followed by *B. bassiana* applied at the rate of $1x10^8$ spores mL^{-1} with a mean mortality of 22.22 per cent. *B. bassaiana* at both $1x10^6$ and $1x10^7$ spores mL^{-1} as well as *L. lecanii* at $1x10^5$ spores mL^{-1} recorded 11.11 per cent mortality each. Treatment with *L. lecanii* ($1x10^7$ spores mL^{-1}) as well as *M.*

anisopliae (1×10^8 spores mL^{-1}) resulted in 6.66 per cent mortality each. The lowest mean mortality value of 4.44 per cent was observed in case of *B. bassiana* (1×10^5 spores mL^{-1}) as well as untreated control.

B. bassiana at all the concentrations, *L. lecanii* at three out of the four concentrations and *M. anisopliae* at its highest concentration evaluated, caused mortality of bugs six days after treatment. The highest mortality of 51.11 per cent was recorded by *B. bassiana* applied at the rate of 1×10^8 spores mL^{-1}. This was followed by *B. bassiana* applied at 1×10^7 and 1×10^6 spores mL^{-1}, with an identical 17.77 per cent kill. *L. lecanii* at 10^5 spores mL^{-1}, *M. anisopliae* at 1×10^8 spores mL^{-1} and control induced average mortality of 11.11 per cent each. *B. bassiana* at 1×10^5 spores mL^{-1}, *L. lecanii* at 10^7 and 10^8 spores mL^{-1} as well as control recorded mean mortality of 8.88, 6.66, 4.44 and 11.11 per cent respectively.

All the three entomopathogenic fungi at all concentrations tried, had caused mortality of rice bug seven days after treatment. The highest mean mortality of 86.66 per cent was caused by *B. bassiana* at the rate of 1×10^8 spores mL^{-1}, which was on par with malathion applied at 500g ai per ha^{-1}. Both *B. bassiana* at 10^8 spores mL^{-1} and malathion were significantly superior to all other treatments. Lower concentrations of *B. bassiana viz.* 10^6, 10^7 and 10^5 spores mL^{-1} caused an average of 48.88, 46.66 and 28.88 per cent mortality respectively. *M. anisopliae* induced mean mortality ranging from 8.88 (10^5 spores mL^{-1}) to 26.66 per cent (10^8 spores mL^{-1}), while *L. lecanii* recorded the lowest mortality values ranging from 4.44 (10^5 spores mL^{-1}) to 15.55 (10^8 spores mL^{-1}) per cent.

Eight days after treatment, *B. bassiana* applied at 10^8 spores mL^{-1} emerged as the most effective treatment with 95.55 per cent mortality (Plate 5). This was followed by malathion 500g ai ha^{-1} with 93.33 per cent morality. Both the above treatments were on par with each other and were significantly superior to all other treatments. *B. bassiana* at the rate of 10^7 spores mL^{-1} was the third best treatment

Plate 5. Rice bugs infected with *Beauveria bassiana*

with 73.33 per cent mortality and was followed by *B. bassiana* at 10^6 and 10^5 spores mL^{-1} (62.22 and 53.33 per cent mortality respectively). Among the different concentrations of *M. anisopliae* evaluated, application at the rate of 10^8 spores mL^{-1} resulted in 48.88 per cent mortality (Plate 6) which was on par with *B. bassiana* applied at 10^6 and 10^5 spores mL^{-1}. *L. lecanii* at both 10^8 and 10^7 spores mL^{-1} registered a similar mortality of 42.22 per cent. The lowest mortality of 28.88 per cent was also recorded by *L. lecanii* at 10^5 spores mL^{-1}.

All the entomopathogenic fungi induced mortality above 40 per cent nine days after treatment. The highest average mortality of 95.55 per cent recorded in case of *B. bassiana* at the rate of $1x10^8$ spores mL^{-1} was followed by malathion with 93.33 per cent. Both were on par with *B. bassiana* at the rate of 10^7 spores mL^{-1} (88.88 per cent). *B. bassiana* at 10^7 spores mL^{-1} as well as *M. anisopliae* at the rate of 10^8 spores mL^{-1} recorded 73.33 and 68.88 per cent mean mortality respectively and were on par with each other as well as with *B. bassiana* at 10^5 spores mL^{-1} and *M. anisopliae* at 10^7 spores mL^{-1}, both inducing 62.22 per cent mean mortality. Lower doses of *M. anisopliae* recorded mortality ranging from 44.44 (10^5 spores mL^{-1}) to 53.33 (10^6 spores mL^{-1}) per cent. *L.lecanii* was found to be the least effective among the three fungi evaluated, with mortality ranging from 53.33 per cent (10^8 spores mL^{-1}) to 40.00 per cent, recorded in case of treatments with both 10^5 and 10^6 spores mL^{-1}.

Mortality induced by *B. bassiana* at 10^8 spores mL^{-1} increased to the highest value of 97.77 per cent on the 10^{th} day after treatment. This was followed by malathion with 95.55 per cent and *B. bassiana* at 10^7 spores mL^{-1} with 93.33 per cent mortality respectively, all being on par with each other. Above three treatments were followed by *B. bassiana* at 10^6 and 10^5 spores mL^{-1}. *M. aniospliae* registered mean mortality value of 55.55 and 71.11 per cent at 10^5 and 10^8 spores mL^{-1} respectively. *L. lecanii* recorded average mortality ranging from 40.00 (10^5 spores mL^{-1}) to 62.22 (10^8 spores mL^{-1}) per cent.

Plate 5. Rice bugs infected with *Beauveria bassiana*

Table 3. Mortality of rice bug due to entomopathogenic fungi

Treatment	Mean mortality of rice bug (%)						
	4 DAT	5 DAT	6 DAT	7 DAT	8 DAT	9 DAT	10 DAT
Beauveria bassiana @ 1x 10^5 spores/ml	2.22	4.44	8.88 (17.11)	28.88 (32.48)	53.33 (46.95)	62.22 (52.22)	68.88 (56.19)
B. bassiana @ 1x 10^6 spores/ml	0.00	11.11	17.77 (23.88)	48.88 (44.36)	62.22 (52.41)	73.33 (59.42)	82.22 (69.02)
B. bassiana @ 1x 10^7 spores/ml	4.44	11.11	17.77 (24.20)	46.66 (43.04)	73.33 (59.35)	88.88 (73.76)	93.33 (80.65)
B. bassiana @ 1x 10^8 spores/ml	6.66	22.22	51.11 (45.75)	86.66 (72.25)	95.55 (82.36)	95.55 (82.36)	97.77 (84.51)
Metarhizium *anisopliae* @ 1x 10^5 spores/ml	0.00	0.00	0.00	8.88 (17.11)	42.22 (40.36)	53.33 (46.91)	55.55 (48.19)
M. anisopliae @ 1x 10^6 spores/ml	0.00	0.00	0.00	24.44 (29.46)	31.11 (33.87)	44.44 (41.75)	55.55 (48.24)
M. anisopliae @ 1x 10^7 spores/ml	0.00	0.00	0.00	15.55 (22.69)	37.77 (37.80)	62.22 (52.19)	64.44 (53.51)
M. anisopliae @ 1x 10^8 spores/ml	0.00	6.66	11.11 (18.83)	26.66 (30.97)	48.88 (44.40)	68.88 (56.12)	71.11 (57.51)
Lecanicillium lecanii @ 1x 10^5 spores/ml	0.00	11.11	11.11 (19.26)	8.88 (17.11)	28.88 (32.29)	40.00 (39.08)	40.00 (39.08)
L. lecanii @ 1x 10^6 spores/ml	0.00	0.00	0.00	4.44 (10.22)	37.77 (37.56)	40.00 (39.07)	40.00 (39.07)
L. lecanii @ 1x 10^7 spores/ml	0.00	6.66	6.67 (12.37)	8.88 (14.08)	42.22 (39.69)	42.22 (40.12)	48.88 (44.35)
L. lecanii @ 1x 10^8 spores/ml	0.00	0.00	4.44 (10.22)	15.55 (23.13)	42.22 (40.51)	53.33 (47.07)	62.22 (52.09)
Malathion 50 EC (500g a.i/ha)	91.11	91.11	91.11 (75.91)	91.11 (75.91)	93.33 (80.65)	93.33 (80.65)	95.55 (82.36)
Absolute control	0.00	4.44	11.11 (18.83)	13.33 (20.33)	28.88 (32.29)	35.55 (36.29)	35.55 (36.29)
CD (0.05)			15.10	14.25	15.60	15.72	14.71
CV			33.28	30.40	25.69	20.39	15.40

Values given in the parentheses are arcsine transformed values

* Mean of 3 observations

DAT – Days after treatment

Plate 6. Rice bugs infected with *Metarhizium anisopliae*

4.2 FIELD EVALUATION

4.2.1 Effect of different entomopathogenic fungi on rice bug populations

Effect of various treatments on the mean rice bug population is presented as Annexure II. Since the pre treatment count showed significant variation, covariance analysis was carried out and the analysed data is presented in Table 4.

Mean rice bug population was the lowest in plots treated with malathion 50 EC (500g ai ha^{-1}) throughout the period of observation. Five days after treatment, the mean population was 3.07 bugs per m^2 and was superior to all other treatments. *B. bassiana, M, anisopliae* and *L. lecanii* recorded, on an average, 11.62, 12.54, 11.83 bugs per m^2 respectively and were on par with control (12.67 bugs/m^2).

Malathion recorded the lowest mean bug population of 3.95 bugs per m^2 10 days after treatment, showing a slight increase over the value at five days after treatment. Plots treated with *B. bassiana* had 6.29 bugs per m^2 which was on par with the population in plots treated with malathion. Plots treated with *M. anisopliae* and *L. lecanii* showed a slight increase in bug population (13.50 and 12.30 bugs/m^2 respectively) and were on par with control registering 13.91 bugs per m^2.

Average number of bugs increased across all the treatments 15 days after treatment. While malathion recorded the lowest mean population of 4.27 bugs per m^2. *B. bassiana* treated plots had 8.68 bugs per m^2. Both the treatments were on par with each other and were significantly superior to the remaining treatments. *M. anisopliae* (14.64 bugs/m^2) and *L. lecanii* (15.67 bugs/m^2) were the least effective treatments and were on par with untreated control having 15.01 bugs per m^2.

Five days after second spraying, the lowest mean bug population of 9.43 per m^2 was recorded in case of *B. bassiana.* It was followed by *M. anisoplaie* (14.27 bugs/m^2) and malathion (14.56 bugs/m^2). However, there was no significant difference between any of the treatments.

Table 4. Effect of entomopathogenic fungi on rice bug population

Sl.No.	Treatments	Mean* rice bug population (No./m^2)		
		5 DAT	10 DAT	15 DAT
T 1	*Beauveria bassiana* @ $1x10^8$ spores mL^{-1}	11.62	6.29	8.68
T 2	*Metarhizium anisopliae* @ $1x10^8$ spores mL^{-1}	12.54	13.50	14.64
T 3	*Lecanicillium lecanii*@ $1x10^8$ spores mL^{-1}	11.83	12.30	15.67
T 4	Malathion 50EC @ 500g a,i. ha^{-1}	3.07	3.95	4.27
T 5	Absolute control	12.67	13.91	15.01
	CD (0.05)	4.96	6.13	7.84
	CV	26.56	33.84	37.04

Values given in the table are adjusted treatment mean
* Mean of 12 observations

4.2.2 Effect of entomopathogenic fungi on grain damage caused by rice bug

Results on mean grain damage (Table 5) revealed that as in case of bug population, malathion continued to record the lowest grain damage among all the treatments. Plots treated with malathion recorded the lowest mean damage of 8.39 per cent, followed by *B. bassiana* treated plots with 10.46 per cent grain damage five days after treatment. However, there was no significant difference between all the treatments. Ten days after treatment, malathion recorded an average grain damage of 8.67 per cent and was followed by *B. bassiana* with 13.08 per cent, both being at par. *M. anisopliae* and *L. lecanii* recorded mean grain damage of 24.01 and 22.05 per cent respectively and were on par with 27.09 per cent grain damage in control.

Fifteen days after treatment, plots treated with malathion suffered 9.06 per cent mean grain damage and was significantly superior to all other treatments. Plots treated with *B. bassiana* had 19.31 per cent mean grain damage and was superior to *M. anisopliae* (24.61%) and *L. lecanii* (25.15%).

Malathion recorded the lowest grain damage of 9.36 per cent five days after treatment five days after the second spray and was significantly superior to the remaining treatments. *B. bassiana* with 20.98 per cent mean grain damage was the next best treatment. *M. anisopliae, L. lecanii* and control, with mean damage of 29.40, 36.96 and 32.04 per cent respectively.

4.3.3 Effect of different entomopathogenic fungi on yield

Data on mean grain yield obtained from different treatments are provided in Table 6. The grain yield varied from 0.44 kg/m^2 obtained from plots treated with *M. anisopliae* to 0.49kg/m^2 in case of plots treated with malathion. No significant difference was observed between any of the treatments.

Table 5. Effect of entomopathogenic fungi on grain damage

Sl. No.	Treatments	Grain damage (%)			
		Before spraying	After spraying		
			5 DAT	10 DAT	15 DAT
T 1	*Beauveria bassiana* @ $1x10^8$ spores mL^{-1}	7.48 (16.84)	10.46 (17.81)	13.08 (22.85)	19.31 (26.00)
T 2	*Metarhizium anisopliae* @ $1x10^8$ spores mL^{-1}	10.29 (17.77)	11.42 (18.45)	24.01 (32.99)	24.61 (29.68)
T 3	*Lecanicillium lecanii*@ $1x10^8$ spores mL^{-1}	7.33 (16.07)	12.38 (18.67)	22.05 (29.62)	25.15 (30.08)
T 4	Malathion 50EC @ 500g a,i. ha^{-1}	7.93 (14.73)	8.39 (17.01)	8.67 (17.40)	9.06 (17.45)
T 5	Absolute control	8.03 (15.22)	13.02 (18.99)	27.39 (32.86)	29.58 (32.88)
	CD (0.05)	NS	NS	6.18	3.63
	CV	20.17	22.46	14.79	8.66

Values given in the parentheses are arcsine transformed values

* Mean of 20 observations

Table 6. Effect of entomopathogenic fungi on yield

Sl. No.	Treatments	Mean* grain yield (kg/m²)
T 1	*Beauveria bassiana* @ 1x10^8 spores mL^{-1}	0.47
T 2	*Metarhizium anisopliae* @ 1x10^8 spores mL^{-1}	0.44
T 3	*Lecanicillium lecanii*@ 1x10^8 spores mL^{-1}	0.45
T 4	Malathion 50EC @ 500g a,i. ha^{-1}	0.49
T 5	Absolute control	0.45
	CD (0.05)	NS

* Mean of 4 observations

4.3 FIELD EVALUATION AT FARMER'S FIELD

Field evaluation of different entomopathogenic fungi against rice bug *L. oratorius* was also carried out in a farmer's field at Muthuvara in Thrissur from January to May 2016.

4.3.1 Effect of entomopathogenic fungi on rice bug population

4.3.1.1 Effect of entomopathogenic fungi on rice bug population after first spray

Table 7 presents the mean insect population before and after first round of treatment.

The results revealed that there was a reduction in bug population across all the treatments at five days after treatment. The lowest mean population of 5.00 bugs per m^2 was observed in plots treated with malathion, which was significantly superior to all other treatments. This was followed by *B. bassiana* with 13.75 bugs/m^2 which was superior to both *M. anisopliae* (23.00 bugs/m^2) and *L. lecanii* (23.50 bugs/m^2) as well as control (24.75 bugs/m^2).

An appreciable reduction in average bug population was observed in case of plots treated with malathion as well as *B. bassiana* ten days after treatment. The lowest mean population of 3.50 bugs per m^2 was observed in plots treated with malathion, followed by 7.50 bugs per m^2 in plots treated with *B. bassiana,* which were on par with each other. However, the mean population of rice bugs showed an increase in plots treated with both *M. anisopliae* and *L. lecanii* as well as in control.

The mean rice bug population in plots treated with *B.bassiana* (8.50 per m^2) was at par with that of the insecticide treated plots (5.50 per m^2) 15 days after treatment. *M. anisopliae* and *L.lecanii* recorded average bug population of 24.50 and 26.50 bugs/m^2 respectively and were on par with control (25.75 bugs/m^2).

Table 7. Effect of entomopathogenic fungi on rice bug population

Sl. No.	Treatments	Mean rice bug population (No./m^2)			
		Pre treatment count	5 DAT	10 DAT	15 DAT
T 1	*Beauveria bassiana* @ 1x10^8 spores mL^{-1}	39.00	13.75	7.50	8.50
T 2	*Metarhizium anisopliae* @ 1x10^8 spores mL^{-1}	36.75	23.00	29.50	24.50
T 3	*Lecanicillium lecanii*@ 1x10^8 spores mL^{-1}	39.50	23.50	27.50	26.50
T 4	Malathion 50EC @ 500g a,i. ha^{-1}	37.50	5.00	3.50	5.50
T 5	Absolute control	37.25	24.75	31.25	25.75
	CD (0.05)	NS	7.948	4.618	6.004
	CV	9.848	28.657	15.099	21.469

* Mean of 4 observations

4.3.1.2 Effect of entomopathogenic fungi on rice bug population after second spray

The treatments were applied a second time 15 days after the first round of application. The population count obtained was subjected to covariance analysis considering the significant variation between different treatments with respect to bug population following the first round of application. The results are presented in Table 8.

The lowest bug population of 5.74 bugs per m^2 five days after second spray was recorded in plots treated with malathion 500g ai per ha^{-1}, followed by 5.86 bugs per m^2 in those treated with *B. bassiana.* Both the above treatments were on par with each other and were significantly superior to the remaining treatments. Both *M. anisopliae* and *L. lecanii* recorded higher mean population of 15.61 and 13.52 bugs per m^2 respectively and were on par with each other. Both the treatments were significantly superior to control (22.74 bugs per m^2).

Ten days after second spray, *B. bassiana* at the rate of $1x10^8$ spores mL^{-1} recorded the lowest mean population of 6.51 bugs per m^2 (Plate 7), followed by malathion with 7.77 bugs per m^2. Both the treatments were on par with each other and were significantly superior to others. *M. anisopliae* was the next best treatment with 13.92 bugs per m^2. *L. lecanii* and control had 23.92 and 28.77 bugs per m^2 respectively. Both the above treatments registered a significant increase in population from the values at five days after second treatment.

Plate 7. Rice bugs infected with *Beauveria bassiana* in field at Muthuvara

Table 8. Effect of second spray of entomopathogenic fungi on rice bug population

Sl. No.	Treatments	Mean rice bug population (No./m²)	
		5 DAT	10 DAT
T 1	*Beauveria bassiana* @ 1x10^8 spores mL^{-1}	5.86	6.51
T 2	*Metarhizium anisopliae* @ 1x10^8 spores mL^{-1}	15.61	13.92
T 3	*Lecanicillium lecanii*@ 1x10^8 spores mL^{-1}	13.52	23.92
T 4	Malathion 50EC @ 500g a,i. ha^{-1}	5.74	7.77
T 5	Absolute control	22.74	28.77
	CD (0.05)	2.76	4.74
	CV	7.16	9.84

Values given in the table are adjusted treatment mean
* Mean of 4 observations

4.3.2 Effect of entomopathogenic fungi on damage caused by rice bugs

4.3.2.1 Effect of first spray of entomopathogenic fungi on damage caused by rice bugs

The total number of grains per panicle and number of damaged grains per panicle at different post treatment intervals were recorded. Based on the above observations per cent damage was calculated and has been presented in Table 9.

Plots treated with malathion recorded the lowest mean damage of 13.46 per cent, followed by *B. bassiana* treated plots with 16.67 per cent grain damage. Highest damage of 17.20 per cent was recorded in plots treated with *M. anisopliae,* while *L. lecanii* and control recorded mean grain damage of 16.96 and 16.80 per cent respectively. However, there was no significant difference between the treatments.

Ten days after treatment, damage level was found to have increased substantially in plots treated with *M. anisopliae, L. lecanii* and control. However, plots treated with malathion and *B. bassiana* recorded significantly lower mean damage of 15.18 and 15.80 per cent respectively. Plots treated with *M. anisopliae* (26.35%) and *L. lecanii* (26.70%) suffered highest average grain damage and were on par each other as well as with the untreated control (26.76%).

At 15 days after treatment, mean grain damage was lowest in plots treated with malathion (14.64%) and was followed those treated with by *B. bassiana* (18.22%), both being on par with each other. Plots treated with *M. anisopliae* and *L. lecanii* suffered 25.12 and 26.96 per cent damage respectively, which was on par with control (29.55%).

4.3.2.2 Effect of second spray of entomopathogenic fungi on grain damage caused by rice bug

A second spray of the treatments was carried out 15 days after first application. Per cent grain damage recorded at 15 days after first spray was taken as

Table 9. Effect of entomopathogenic fungi on grain damage due to rice bug

Sl. No.	Treatments	Grain damage (%)			
		Before treatment application	5 DAT	10 DAT	15 DAT
T 1	*Beauveria bassiana* @ $1x10^8$ spores mL^{-1}	13.89 (3.73)	16.67 (3.93)	15.80 (4.31)	18.22 (4.26)
T 2	*Metarhizium anisopliae* @ $1x10^8$ spores mL^{-1}	13.25 (3.65)	17.20 (4.06)	26.35 (5.18)	25.72 (5.07)
T 3	*Lecanicillium lecanii*@ $1x10^8$ spores mL^{-1}	13.78 (3.72)	16.96 (4.03)	26.70 (5.24)	26.96 (5.18)
T 4	Malathion 50EC @ 500g a,i. ha^{-1}	14.60 (3.46)	13.46 (3.58)	15.18 (4.01)	14.64 (3.82)
T 5	Absolute control	13.17 (3.614)	16.80 (4.11)	26.76 (5.21)	29.55 (5.41)
	CD (0.05)	NS	NS	0.65	0.60
	CV	9.62	5.99	8.81	8.13

Values given in the parentheses are SQRT transformed values
* Mean of 20 observations

pre treatment observation. Since the values exhibited significant variation among treatments, the data was subjected to covariance analysis and the adjusted treatment means were used for comparison.

Table 10 presents the per cent grain damage observed after the second spray.

Five days after the second round of spray, *B. bassiana* with 12.21 per cent mean grain damage was found to be significantly superior to other fungi and was statistically on par with malathion which recorded the lowest damage of 11.08 per cent. *M. anisopliae* and *L. lecanii* with mean damage of 36.73 and 34.41 per cent respectively were on par with control plot with 37.8 per cent mean grain damage.

Plots treated with *B. bassiana* had the lowest average grain damage of 14.78 per cent, marginally lower than 14.84 per cent in case of plots treated with malathion ten days after treatment. Both were on par with each other. Remaining treatments, *viz.* control, *L. lecanii* and *M. anisopliae* recorded significantly higher grain damage of 27.32, 28.59 and 33.07 per cent respectively.

Table 10. Effect of second spray of entomopathogenic fungi on grain damage due to rice bug

Sl. No.	Treatments	Grain damage (%)	
		5 DAT	10 DAT
T 1	*Beauveria bassiana* @ 1×10^8 spores mL^{-1}	12.21	14.78
T 2	*Metarhizium anisopliae* @ 1×10^8 spores mL^{-1}	36.73	27.32
T 3	*Lecanicillium lecanii*@ 1×10^8 spores mL^{-1}	34.41	28.59
T 4	Malathion 50EC @ 500g a,i. ha^{-1}	11.02	14.84
T 5	Absolute control	37.80	33.07
	CD (0.05)	5.47	6.76
	CV	0.51	0.35

Values given in the table are adjusted treatment mean
* Mean of 20 observations

Discussion

5. DISCUSSION

A study entitled "Bioefficacy of entomopathogenic fungi against rice bug, *Leptocorisa oratorius* Fab. (Hemiptera: Alydidae)" was conducted at Department of Agricultural Entomology, College of Horticulture, Vellanikkara from August 2015 to July 2016. The results obtained during the study are discussed here.

5.1 POT CULTURE EVALUATION OF ENTOMOPATHOGENIC FUNGI AGAINST RICE BUG

The preliminary evaluation of the three entomopathogenic fungi *viz. B. bassiana* local isolate, *M. anisopliae* and *L. lecanii* comprised of a pot culture experiment. The fungi were evaluated at four different concentrations ranging from 10^5 to 10^8 spores mL^{-1}. These treatments were compared with a commonly used insecticide malathion and an untreated control.

Cages treated with malathion (500g a.i. ha^{-1}) recorded 91 per cent mean mortality of the released bugs within a day. Malathion treatment went on to record 93.33 per cent kill by the eighth day and 95.55 per cent by the tenth day. On the other hand, no mortality was observed in cages treated with entomopathogenic fungi for up to 72 h after treatment. Among the fungal pathogens, the local isolate of *B. bassiana* applied at the rate of $1x10^8$ spores mL^{-1} alone induced mortality within three days after treatment. Average mortality ranging from 4.44 to 22.22 per cent was observed in *B. bassiana* treated pots five days after treatment.

Mortality of bugs in cages treated with both *M. anisopliae* as well as *L. lecanii* was first observed on fifth day as well. However, significant levels of kill by the two entomopathogens at all doses evaluated occurred only by the eighth day. Once induced, infection by all the three fungi progressed fairly consistently and in a dose dependent manner.

The local isolate of *B. bassiana* was consistently superior among the three fungal pathogens tried, with mean mortality values ranging from 68.88 per cent at 10^5 spores mL^{-1} to the highest value of 97.77 per cent at 10^8 spores mL^{-1} ten days after treatment. Treatments involving *B. bassiana* were significantly superior to control as well as other entomopathogenic fungi from eighth day onwards. The average mortality induced by the fungus at $1x10^8$ spores mL^{-1} was at par with the insecticide from eighth day onwards. Equally impressive results were observed at the concentration of $1x10^7$ spores mL^{-1} as well.

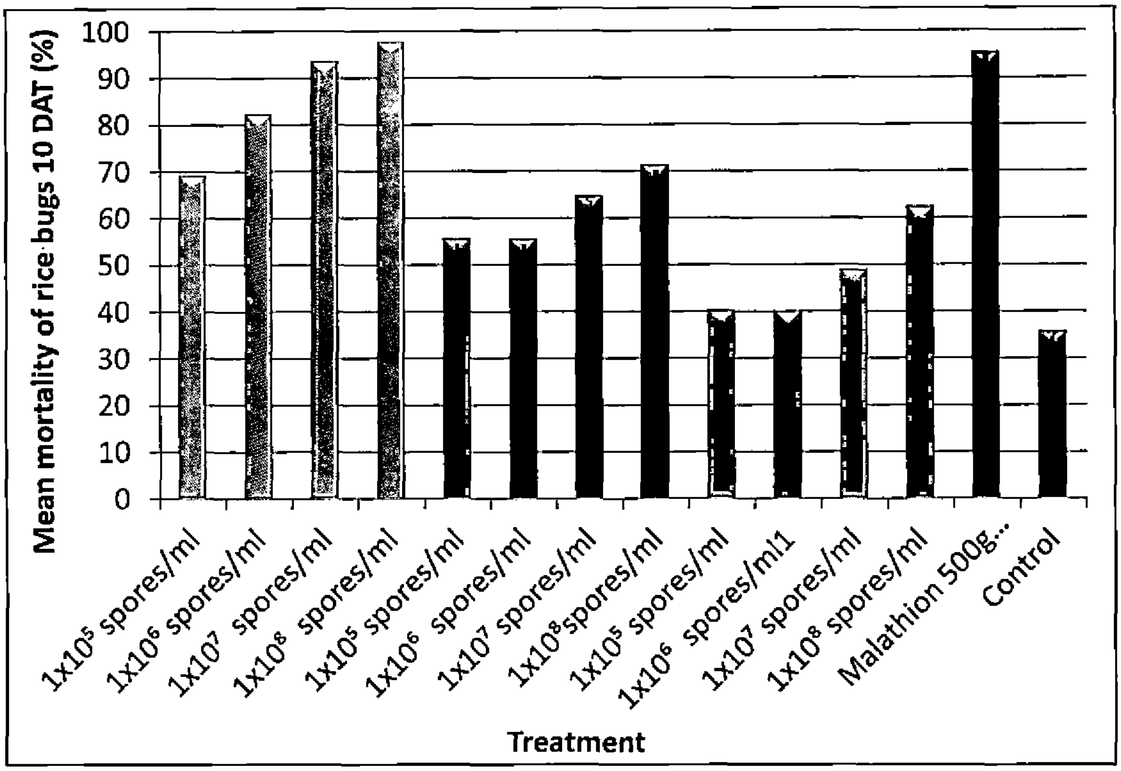
Treatment with *M. anisopliae* at the highest concentration of $1x10^8$ spores mL^{-} induced mortality from fifth day onwards. Induction of kill was delayed in case of lower concentrations. As in case of *B. bassiana,* there was an increase in mortality at all concentrations from seventh day onwards. The highest mean mortality of 71.11 per cent was recorded at concentration of $1x10^8$ spores mL^{-1} ten days after treatment which was comparable to the mortality achieved by *B. bassiana* at $1x10^6$ spores mL^{-1}.

Treatment with different concentrations of *L. lecanii* was similar to that of *M. anisopliae* in that mortality was induced by the sixth day. However, the mortality was at par with untreated control in all instances except at the highest concentration on the tenth day of observation.

The pot culture study confirmed the pathogenicity of all the three entomopathogenic fungi to rice bug. Equally apparent was their ability to cause marked reduction of the rice bug population, albeit to varied extents.

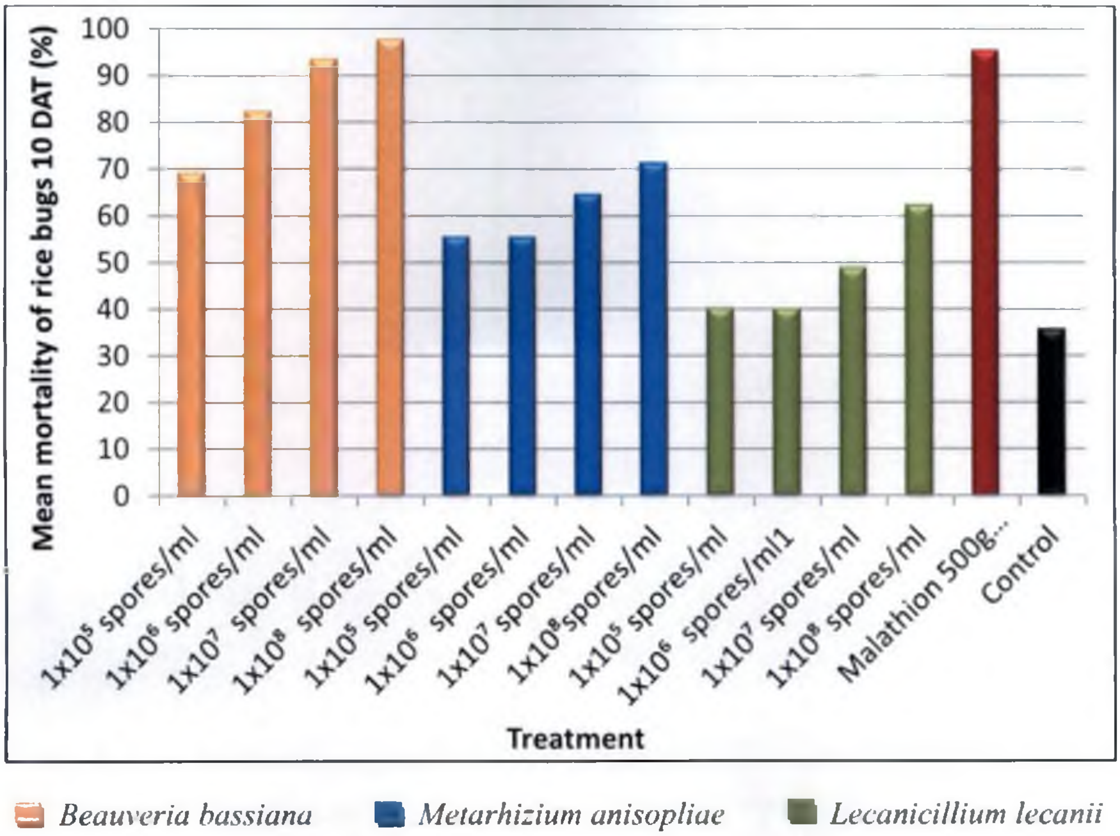
Beauveria bassiana at $1x10^8$ spores mL^{-1}, caused the highest mean mortality of 97.77 per cent ten days after treatment, ahead of malathion with 95.55 per cent mortality. Even at lower concentrations of 10^7 and 10^6 spores mL^{-1} the fungus could induce mortality of 83.22 and 93.33 per cent by tenth day which was comparable to that by malathion (Fig. 1).

Fig. 1 Mortality of rice bug by entomopathogenic fungi in pot culture



Beauveria bassiana *Metarhizium anisopliae* *Lecanicillium lecanii*

Fig. 1 Mortality of rice bug by entomopathogenic fungi in pot culture



Among *M. anisopliae* and *L. lecanii*, only *M. anisopliae* could induce a significant level of mortality (71.11%) by the tenth day. The results were indicative of the potential that the local isolate of *B. bassiana* has as a biocontrol agent of rice bug. The above findings draw support from Baharally (2014) who evaluated the efficacy of *B. bassiana* against rice bug under laboratory conditions. *B. bassiana*, at the rate of $6x10^3$ conidia per ml had registered bug mortality of 4.00, 39.10 and 51.85 per cent at 24, 48 and 72 hours after exposure respectively.

Ability to efficiently regulate pest populations apart, time taken for effecting such a regulation is also significant. The insecticide malathion had caused a mean mortality of 91.11 per cent within 24 h. This, however, was along expected lines. Malathion is a broad spectrum organophosphate compound with contact and stomach action and has been recommended for the management of rice bug. In comparison, all the three entomopathogenic fungi required a minimum of 72 h to successfully infect the bugs. This again is in agreement with known facts about infection by entomopathogenic fungi. For instance, the above observations were in line with the findings of Getzin (1961) who reported that a minimum of 48h was required for infection by entomopathogenic fungi to begin. Similarly, Yadav and Neeraj (2012) noticed that *B. bassiana* took three to seven days to kill an insect.

Among the three fungal pathogens, *B. bassiana* required the shortest time of five days to induce mortality at all the concentrations evaluated, whereas, both *M. anisopliae* and *L. lecanii* required longer period for inducing mortality. A possible reason for the faster infection by *B. bassiana* could be that it has been isolated from rice bug itself. As a result it could be more virulent and better adapted to the defenses of the host insect.

All the three fungi evaluated in present study have been screened against rice bug previously by several workers. Herlinda *et al.* (2008), for instance, reported that isolates of *Metarhizium* sp., applied at the rate of $1x10^7$ spores mL^{-1} produced bug mortality of 90.00 to 100 per cent. Application at 10^3 and 10^5 spores mL^{-1} resulted in 86.67 to 90.00 per cent mortality respectively. According to Loc and Chi (2005), who evaluated local isolates of *B. bassiana* and *M. anisopliae* under green house conditions for three consecutive years, *M. anisopliae* at the rate of 10^7 spores mL^{-1} registered higher mortality of 75.3 to 87.00 and was more pathogenic to rice bug than *B. bassiana* (10^7 spores mL^{-1}) with 59 to 77.7 per cent mortality.

The findings of the present study indicate that *B. bassiana* was the most effective among the entomopathogenic fungus evaluated and thus would seem to be at variance with the earlier reports cited above. However, it is noteworthy that both the above reports had found the local isolates of the entomopathogens to be the most effective ones in the respective studies. The present study too had used the local isolate of *B. bassiana* along with strains of *M.anisopliae* and *L. lecanii* procured from other sources, which could explain the better performance of *B. bassiana* as compared to the other fungi evaluated. Thus the study is also a pointer to the importance of locally adapted strains or populations of natural enemies in biological control.

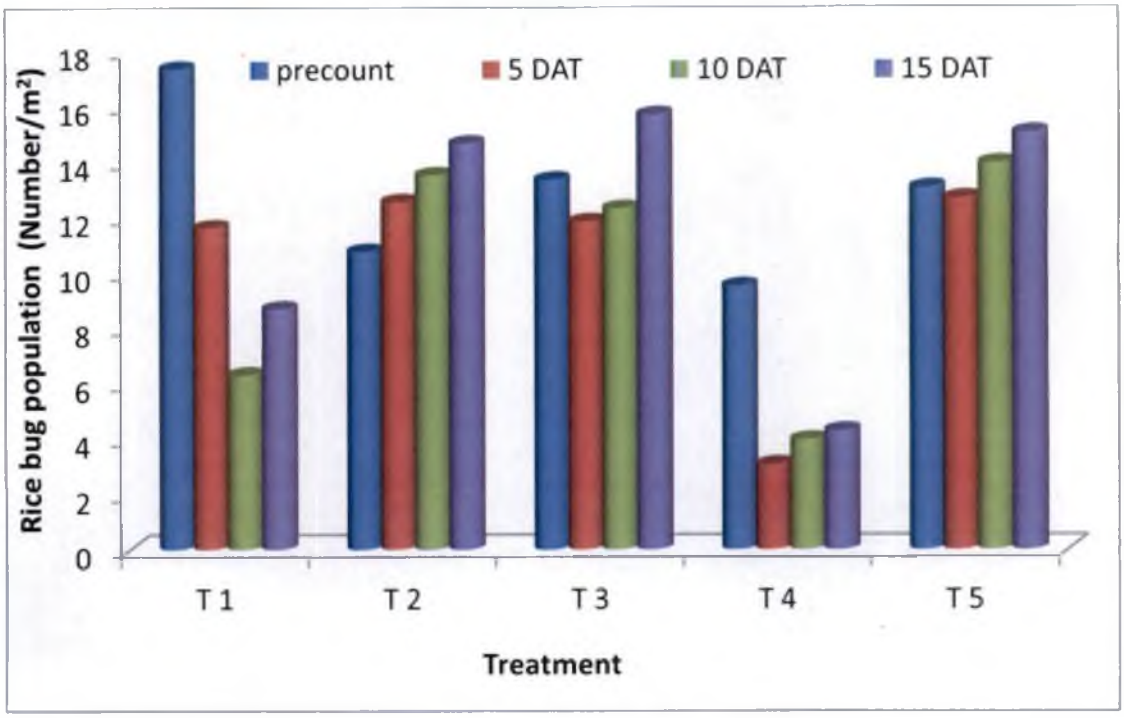
5.2 FIELD EVALUATION

The most effective concentration of each of the entomopathogenic fungus, identified through the pot culture study was evaluated under field experiment.

5.2.1 Effect of different entomopathogenic fungi on rice bug population

The results of field evaluation broadly agreed with the findings of the pot culture studies. Malathion registered the lowest mean bug population ranging from 3.07 to 4.27 per m^2. Plots treated with malathion had significantly lower population of bugs five days after treatment. In comparison, plots treated with entomopathogenic fungi registered no significant difference in mean bug population either among themselves or with control, five days after treatment (Fig. 2).

Fig. 2 Effect of entomopathogenic fungi on rice bug population in field experiment at Vellanikkara



T 1 – *Beauveria bassiana* at 1x10^8 spores/ml

T 2 – *Metarhizium anisopliae* at 1x10^8 spores/ml

T 3 – *Lecanicillium lecanii* at 1x10^8 spores/ml

T 4 – Malathion @ 500 g a.i. ha^{-1}

T 5 – Absolute control

However, by the tenth day after treatment, *B. bassiana*, with a mean bug population of 6.29 per m^2 was significantly superior to both *M. anisopliae* (13.50/m^2) and *L. lecanii* (12.30/m^2) and was on par with malathion (3.95/m^2). Both *M. anisopliae* and *L. lecanii* failed to record an appreciable reduction in bug population in the field, consistently being on par with control even ten days after treatment.

In the second field experiment at Muthuvara also, malathion continued to be the most effective treatment, with mean bug population of 5.00 and 3.50 per m^2 five and ten days after first round of spray respectively (Fig. 3).

Five days after treatment, *B. bassiana* was significantly superior to other entomopathogenic fungi. It was on par with malathion and significantly superior to other fungi both at 10 and 15 days after treatment.

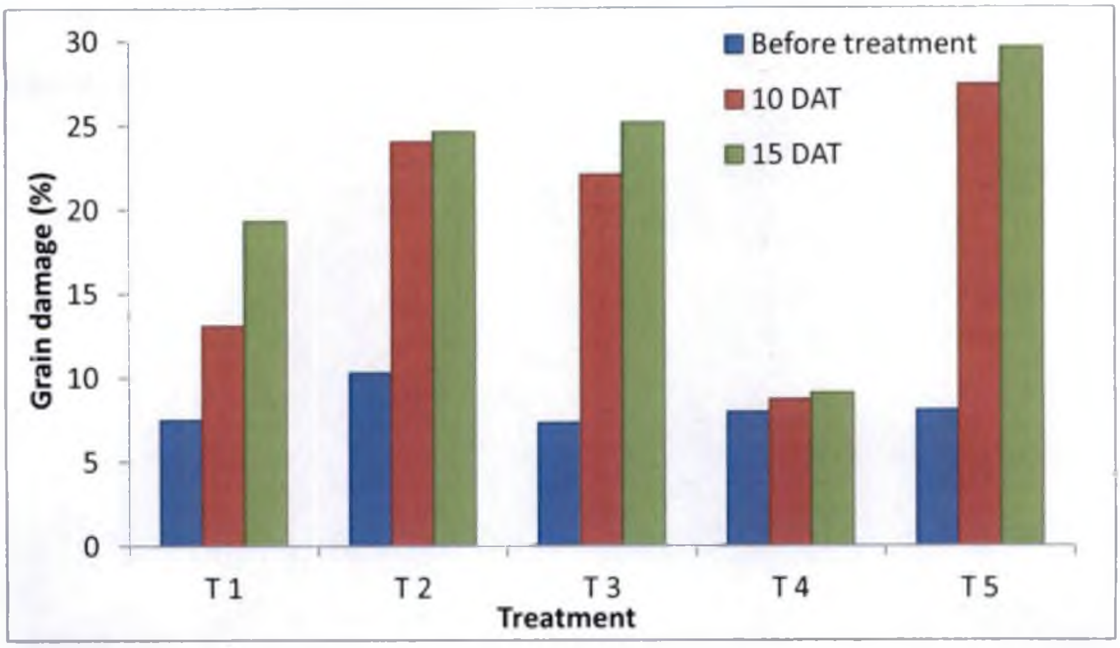
As in case of first treatment both *M. anisopliae* and *L. lecanii* failed to make any significant impact on the bug population and were on par with control.

Unlike in case of the first field evaluation, a second spray was possible in the second field experiment, where again malathion registered the lowest bug population of 5.74 per m^2 five days after treatment. However, the bug population showed an increase to 7.77 bugs per m^2 ten days after treatment.

Beauveria bassiana treated plots registered bug population values on par with plots treated with insecticide at both five and ten days after second spray. Ten days after treatment, the population in *B. bassiana* treated plots was lower than that of malathion treated plots. This was identical with the results obtained in pot culture studies, where *B. bassiana* caused greater reduction in bug population than malathion by tenth day after treatment.

The effectiveness of *B. bassiana* against rice bug under field conditions is in agreement with the findings of Tuan (2014b), who observed substantial reduction in rice bug population in plots treated with different concentrations of *B. bassiana*. *B.*

Fig. 3 Effect of entomopathogenic fungi on grain damage caused by rice bugs in field experiment at Vellanikkara



T 1 – *Beauveria bassiana* at 1×10^8 spores/ml

T 2 – *Metarhizium anisopliae* at 1×10^8 spores/ml

T 3 – *Lecanicillium lecanii* at 1×10^8 spores/ml

T 4 – Malathion @ 500 g a.i. ha^{-1}

T 5 – Absolute control

bassiana at the rate of 10^{13} conidia mL^{-1} was found to be at par with the insecticide malathion seven days after treatment. Ashokappa *et al.* (2015) reported that both *B. bassiana* and *M. ansopliae,* applied at the rate of $2gL^{-1}$ were found to be effective against rice bug under field conditions. Both the fungi recorded bug populations of 1.10 and 1.00 bugs per hill respectively seven days after treatment and were at par with each other.

A marginal reduction in rice bug population was observed in plots treated with *M. anisopliae* and *L. lecanii* after first spray, which was comparable to the untreated control. However, following the second round of application, *M. anisopliae* induced a consistent reduction in rice bug population to 17.15 and 16.75 bugs per m^2 five and ten days after second treatment respectively.

Metarhizium anisopliae, though effective at $1x10^8$ spores mL^{-1} in pot culture studies, did not cause any significant reduction in population of rice bug in field. This is at variance with previous reports. For instance, Girish and Balikai (2015) reported that *M. anisopliae* at the rate of $2gL^{-1}$ had reduced the rice bug population to 2.00 bugs per hill and was found superior to *B. bassiana* with a bug population of 2.50 bugs per hill 15 days after treatment. Similarly, Loc and Chi (2005) studied the efficacy of local isolates of *B. bassiana* and *M. anisopliae.* They found that *M. anisopliae* isolates were more pathogenic to *L. acuta* with 75.70 to 87.00 per cent mortality compared to the isolates of *B. bassiana* causing 59.3 to 77.7 per cent mortality ten days after treatment.

Among the three entomopathogenic fungi, *L. lecanii* was the least effective treatment against rice bug as was observed in pot culture studies. *L. lecanii* has been reported as more effective against aphids, whiteflies, mealybugs *etc.* by several authors (Ujjan and Shahzad, 2012; Chandrashekharaiah *et al.*, 2013; Banu and Gopalakrishnan, 2012). The present study confirms the above observations.

5.2.2 Effect of entomopathogenic fungi on damage caused by rice bug

The results on grain damage broadly followed the observation on bug population. In the field experiment at Vellanikkara, plots treated with malathion recorded the lowest average grain damage, ranging from 8.67 (10 DAT) to 9.36 per cent (15 DAT). The values were significantly superior to the remaining treatments (Fig. 4).

Beauveria bassiana recorded the lowest damage among the entomopathogenic fungi evaluated. The mean grain damage recorded were 13.08 and 19.31 per cent at 10 and 15 days after treatment respectively. Plots treated with *M. anisopliae* and *L. lecanii* recorded grain damage on par with that of untreated plots, suggesting that they were not effective in reducing the grain damage.

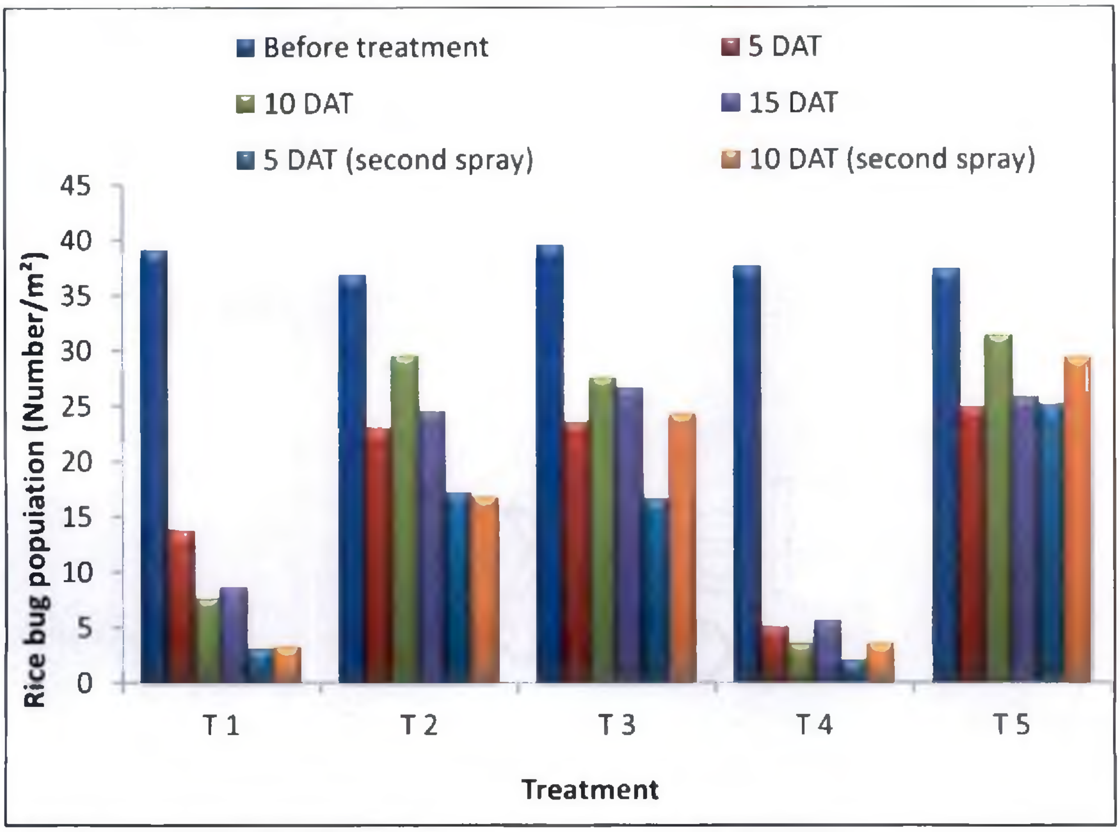
The above trend continued five days after second spray, with malathion, followed by *B. bassiana* continuing to be the most effective treatments.

Field evaluation at Muthuvara confirmed the finding of the first field evaluation (Fig. 5). Malathion recorded the lowest mean grain damage of 13.46, 15.18 and 14.64 per cent 5, 10 and 15 days after first round of treatment and was significantly superior to other treatments.

Plots treated with *B. bassiana* were next in terms of grain damage with 16.67, 15.8 and 18.22 per cent damage on an average at 5, 10 and 15 days after treatment respectively. It was significantly superior to both *M. anisopliae* and *L. lecanii*, both of which were on par with control.

Five days after imposing the treatment for a second time, malathion recorded the lowest grain damage of 11.02 per cent but was on par with *B. bassiana* with a marginally higher 12.21 per cent mean damage. Ten days after the second spray, plots treated with *B. bassiana* had lowest grain damage of 14.78 per cent as against 14.84 in malathion treated plots. The present findings were in line with that of Kalita *et al.*

Fig. 4 Effect of entomopathogenic fungi on rice bug population in field experiment at Muthuvara



T 1 – *Beauveria bassiana* at 1×10^8 spores/ml

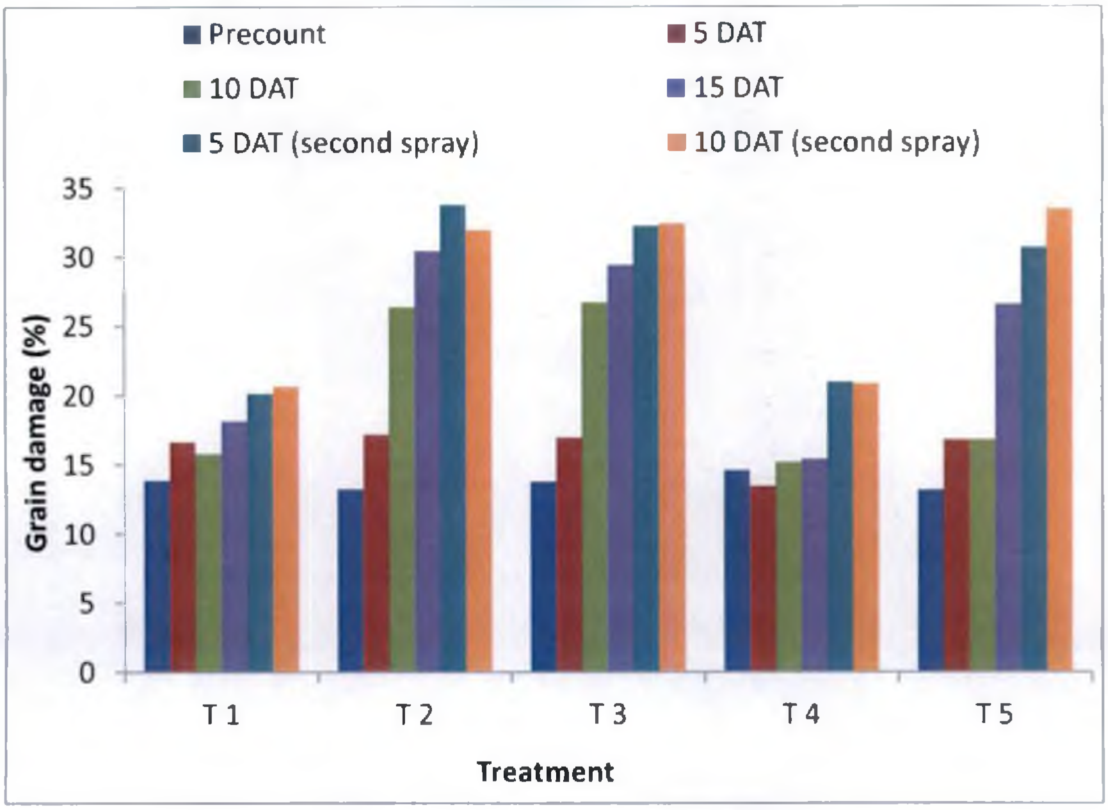
T 2 – *Metarhizium anisopliae* at 1×10^8 spores/ml

T 3 – *Lecanicillium lecanii* at 1×10^8 spores/ml

T 4 – Malathion @ 500 g a.i. ha^{-1}

T 5 – Absolute control

Fig. 5 Effect of entomopathogenic fungi on grain damage caused by rice bugs in field experiment at Muthuvara



T 1 – *Beauveria bassiana* at 1×10^8 spores/ml

T 2 – *Metarhizium anisopliae* at 1×10^8 spores/ml

T 3 – *Lecanicillium lecanii* at 1×10^8 spores/ml

T 4 – Malathion @ 500 g a.i. ha^{-1}

T 5 – Absolute control

(2009) who reported that *B. bassiana* was effective in reducing grain damage caused by rice bug. Treatment with the entomopathogen had registered 5.15 per cent grain damage, which was next best treatment to monocrotophos (2.39 per cent) and was significantly superior to the untreated control (13.06 per cent).

Metarhizium anisopliae and *L. lecanii,* with damage ranging from 27 to 34 per cent, once again were on par with control, which registered 33 to 37 per cent damage. *L. lecanii* was found to be the least effective treatment against rice bug in field experiments and registered three times the pre count damage 15 days after treatment. Kalitha *et al.* (2009) for instance reported a higher per cent grain damage of 8.51 per cent in plots treated with *L. lecanii* and it was found to be the least effective treatment against rice bug, *L. acuta.* Ashokappa *et al.* (2015) also observed that malathion was superior over the entomopathogenic fungi with lowest per cent grain damage of 6.23 whereas, *B. bassiana* and *M. anisopliae* registered higher per cent grain damage of 10.87 and 10.02 per cent respectively.

The pot culture as well as field evaluation have demonstrated the efficacy of the local isolate of *Beauveria bassiana* to reduce the infestation by gundhi bug *L. oratorius* in rice. While its effect is not pronounced till about a week after treatment, it constantly proved to be as effective as the insecticide malathion. It had caused greater reduction in bug population and had led to lower grain damage in all three experiments, though the differences were not significant. From the above, it can be concluded that the local isolate of *B. bassiana* could be a highly potent biocontrol agent against the rice bug, *L. oratorius*. These findings are consistent with the results of simultaneous studies by AICRP on BCCP & W (NBAIR, 2015b) which also had demonstrated the efficacy of *B. bassiana.*

However, there was a time lag between the application of treatment and any appreciable reduction in bug population, suggesting that application of the fungus at milky grain stage, might therefore be unable to check the damage to the grains.

Advancing the timing of treatment by about ten days to panicle initiation stage, when rice bugs make their appearance in field, might prove to be a better strategy, against a pest that causes heavy damage in a relative short period. This, however, needs to be confirmed by further studies.

Summary

6. SUMMARY

A study entitled "Bioefficacy of entomopathogenic fungi against rice bug, *Leptocorisa oratorius* (Hemiptera: Alydidae)" was carried out in the Department of Agricultural Entomology, College of Horticulture, Vellanikara during 2015-2016. The study comprised of a pot culture as well as field evaluation of three entomopathogenic fungi against rice bug. The salient findings of the present study are summarized below

The pot culture experiment evaluated the efficacy of three entomopathogenic fungi *viz.* *Beauveria bassiana* (local isolate), *Metarhizium anisopliae* and *Lecanicillium lecanii* at four different concentrations ranging from $1x10^5$ to $1x10^8$ spores mL^{-1} against rice bug, *Leptocorisa oratorius*. These treatments were compared with a commonly used insecticide malathion (500g a.i. ha^{-1}) and an untreated control. Mortality of rice bugs were recorded at 24 h interval for 10 days.

Mortality of rice bugs occurred only in pots treated with malalthion for the first two days after treatment, which recorded 91.11 per cent mean mortality in the first 24 h itself. Application of *B. bassiana* at the rate of $1x10^8$ spores mL^{-1} registered 4.44 per cent mean mortality, three days after treatment. Among the entomopathogens evaluated, only *B. bassiana* induced mortality in the first four days.

All the three entomopathogenic fungi caused mortality of rice bug seven days after treatment. The highest mortality of 86.66 per cent was recorded with *B. bassiana* at the rate of $1x10^8$ spores mL^{-1} which was on par with malathion applied at 500g ai per ha^{-1}. Both were significantly superior to all other treatments seven days after treatment.

The mortality induced by *B. bassiana* at $1x10^8$ spores mL^{-1} increased to the highest value of 97.77 per cent on the 10^{th} day after treatment. This was followed by malathion at the rate of 500g ai ha^{-1} with 95.55 per cent mortality and *B. bassiana* at

$1x10^7$ spores mL^{-1} 10 days after treatment, all being at par with each other. *M. aniospliae* registered mean mortality of 55.55 and 71.11 per cent at $1x10^5$ and $1x10^8$ spores mL^{-1} respectively. *L. lecanii* recorded mortality ranging from 40.00 (10^5 spores mL^{-1}) to 62.22 (10^8 spores mL^{-1}) per cent.

The most effective concentration of each of the entomopathogenic fungus in the pot culture experiment was evaluated along with malathion 500g ai ha^{-1} under field conditions during Rabi (August - December) and summer (January - May) seasons. Observations on rice bug population as well as damage caused by rice bug were recorded before and after the treatments. The post treatment observations were recorded at five days interval.

Application of malathion at the rate of 500g a.i. ha^{-1} registered the lowest mean rice bug population of 4.96 bugs per m^2 and all other treatments were on par with each other five days after treatment. Substantial reduction in rice bug population was observed in plots treated with *B. bassiana* at the rate of $1x10^8$ spores mL^{-1} with 6.29 bugs per m^2 and it was found to be at par with malathion 500g ai ha^{-1} with 6.13 bugs per m^2 ten days after treatment. No significant reduction in rice bug population was observed in case of *M. anisopliae* and *L. lecanii* and both the treatments were on par with each other as well as with the untreated control.

In the second field experiment at Muthuvara also, malathion continued to be the most effective treatment, with mean bug population of 5.00 and 3.5 per m^2 five and ten days after first round of spray respectively, followed by *B. bassiana, which* was significantly superior to other entomopathogenic fungi and was on par with malathion. As in case of first treatment, both *M. anisopliae* and *L. lecanii* failed to make any significant impact on the bug population and were on par with control.

Beauveria bassiana treated plots registered bug population values on par with plots treated with insecticide at both five and ten days after second spray. Ten days after treatment the population in *B. bassiana* treated plots was lower that of malathion treated plots.

Similarly, plots treated with malathion had the lowest mean grain damage of 8.67 per cent and was significantly superior to all other treatments, five days after treatment. Both malathion and *B. bassiana,* which recorded lowest level of infestation among treatments were on par with each other and were significantly superior to other treatments 10 days after treatment. *M. anisopliae* and *L. lecanii* as well as untreated control showed more than hundred per cent increase in grain damage within the same period. During summer, a marginal increase in per cent grain damage of 15.80 and 15.18 was obtained in plots treated with *B. bassiana* and malathion respectively, ten days after treatment. Both these treatments were found to be superior over the other treatments and also at par with each other. All the entomopathogenic fungi, except *B. bassiana* registered a rapid increase in per cent grain damage.

The grain yield recorded during the first experiment varied from 0.44 kg/m^2 obtained from plots treated with *M. anisopliae* to 0.49 kg/m^2 in case of plots treated with malathion. No significant difference was observed between any of the treatments.

Both under pot culture experiment and field experiment, application of local isolate of *B. bassiana* at the rate of 1×10^8 spores mL^{-1} was found to be effective against rice bug, *L. oratorius* and at par with the commonly used insecticide, malathion. It had caused greater reduction in bug population and had led to lower grain damage in all three experiments, though the differences were not significant. All other entomopathogenic fungi *viz. M. anisopliae* and *L. lecanii* failed to manage rice bug population effectively.

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Appendix

Appendix I

Effect of entomopathogenic fungi on rice bug in pot culture

Treatment	Mean rice bug population (No./cage)									
	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT	8 DAT	9 DAT	10 DAT
Beauveria bassiana @ 1x 10^5 spores/ml	15.00	15.00	15.00	14.66	14.33	13.66	10.66	7.00	5.66	4.66
B. bassiana @ 1x 10^6 spores/ml	15.00	15.00	15.00	15.00	13.33	12.33	7.66	5.66	4.00	2.66
B. bassiana @ 1x 10^7 spores/ml	15.00	15.00	15.00	14.33	13.33	12.33	8.00	4.00	1.66	1.00
B. bassiana @ 1x 10^8 spores/ml	15.00	15.00	14.33	14.00	11.66	7.33	2.00	0.66	0.66	0.33
Metarhizium *anisopliae* @ 1x 10^5 spores/ml	15.00	15.00	15.00	15.00	15.00	15.00	13.66	8.66	7.00	6.66
M. anisopliae @ 1x 10^6 spores/ml	15.00	15.00	15.00	15.00	15.00	15.00	11.33	10.33	8.33	6.66
M. anisopliae @ 1x 10^7 spores/ml	15.00	15.00	15.00	15.00	15.00	15.00	12.66	9.33	5.66	5.33
M. anisopliae @ 1x 10^8 spores/ml	15.00	15.00	15.00	15.00	15.00	14.00	11.00	7.66	4.66	4.33
Lecanicillium lecanii @ 1x 10^5 spores/ml	15.00	15.00	15.00	15.00	15.00	13.33	13.66	10.66	9.00	9.00
L. lecanii @ 1x 10^6 spores/ml	15.00	15.00	15.00	15.00	15.00	15.00	14.33	9.33	9.00	9.00
L. lecanii @ 1x 10^7 spores/ml	15.00	15.00	15.00	15.00	15.00	14.00	13.66	8.66	8.66	7.66
L. lecanii @ 1x 10^8 spores/ml	15.00	15.00	15.00	15.00	15.00	15.00	12.66	8.66	7.66	5.66
Malathion 50 EC (500g a.i/ha)	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.00	1.00	0.66
Absolute control	15.00	15.00	15.00	15.00	14.33	13.33	13.00	10.66	9.66	9.66

Appendix II

Effect of entomopathogenic fungi on rice bug population in field at Vellanikkara

Treatments	Rice bug population (No./m^2)			
	Precount	Post treatment count		
		5 DAT	10 DAT	15 DAT
Beauveria bassiana @ $1x10^8$ spores mL^{-1}	17.33	14.42	10.00	12.79
Metarhizium anisopliae @ $1x10^8$ spores mL^{-1}	10.75	11.29	11.84	12.79
Lecanicillium lecanii @ $1x10^8$ spores mL^{-1}	13.33	12.17	12.75	16.17
Malathion @ 500g a.i. ha^{-1}	9.50	1.00	0.65	0.27
Absolute control	13.04	12.83	14.13	15.25

BIOEFFICACY OF ENTOMOPATHOGENIC FUNGI AGAINST RICE BUG, *Leptocorisa oratorius* Fab. (HEMIPTERA: ALYDIDAE)

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ABSTRACT OF THE THESIS

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ABSTRACT

A study titled "Bioefficacy of entomopathogenic fungi against rice bug, *Leptocorisa oratorius* Fab. (Alydidae: Hemiptera)" was carried out at Dept. of Agricultural Entomology to evaluate efficacy of three entomopathogenic fungi namely *Beauveria bassiana, Metarhizium anisopliae* and *Lecanicillium lecanii* for the biocontrol of rice bug. The preliminary evaluation comprised of pot culture experiment with the fungi being applied at four different concentrations ranging from 10^5 to 10^8 spores ml^{-1} along with insecticide malathion 500g a.i. ha^{-1} and an untreated control. Mortality of rice bugs were recorded at 24h interval for 10 days.

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 10^5 spores per ml to the highest value of 97.77 per cent at 10^8 spores per ml. This was followed by malathion with 95.55 per cent and *B. bassiana* at 10^7 spores per ml with 93.33 per cent mortality respectively, all being on par with each other. *M. aniospliae* and *L. lecanii* at the rate of 10^8 spores per ml registered the highest mean mortality of 71.11 and 62.22 per cent respectively.

The most effective concentrations of each of the entomopathogenic fungus identified in the pot culture experiment was evaluated along with malathion 500g ai ha^{-1} under field conditions during August-December at Vellanikkara and again during January-May in a Farmer field at Muthuvara. Observations on rice bug population as well as the damage caused by rice bug were recorded before treatment as well as at five and ten days after the treatment.

The results of field evaluation broadly agreed with the findings of the pot culture studies. Malathion registered the lowest mean bug population in both the experiments. 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.

As in case of bug population malathion continued to record the lowest grain damage among all the treatments. In the first field experiment, malathion recorded an average grain damage of 8.67 per cent at ten days treatment and was followed by *B. bassiana* with 13.08 per cent grain damage, both being at par. *M. anisopliae* and *L. lecanii* recorded mean grain damage of 24.01 and 22.05 per cent respectively and were on par with control (27.09 per cent grain damage). A similar trend was observed fifteen days after treatment as well.

Field evaluation at Muthuvara confirmed the finding of the first field evaluation. Malathion recorded the lowest grain damage of 13.46, 15.18 and 14.64 per cent 5, 10 and 15 days after first round of treatment and was significantly superior to other treatments. Plots treated with *B.bassiana* were next in terms of grain damage with 16.67, 15.8 and 18.22 per cent damage at 5, 10 and 15 days after treatment respectively. It was significantly superior to both *M. anisopliae* and *L. lecanii,* both of which were on par with control

The pot culture as well as field evaluation have demonstrated the efficacy of the local isolate of *Beauveria bassiana* to reduce the infestation by gundhi bug *L. oratorius* in rice. It constantly proved to be as effective as the insecticide malathion. It had caused greater reduction in bug population and had led to lower grain damage in all three experiments than the insecticide, though the differences were not significant. From the above, it can be concluded that the local isolate of *B. bassiana* could be a highly potent biocontrol agent against rice bug.

However, given the time lag between the application of treatment and any appreciable reduction in bug population, timing of the treatment could be critical in minimizing grain damage by the bug. Application of the fungus at milky grain stage could be insufficient to check the damage to the grains. Advancing the timing of treatment by about ten days to panicle initiation stage might prove to be a better strategy, against a pest that causes heavy damage in a relatively short period.