Efficacy of chitin enriched formulations of *Lecanicillium* spp against sucking pests of rice *Oryza sativa* L.

by

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DECLARATION

I, hereby declare that the thesis entitled "Efficacy of chitin enriched formulations of *Lecanicillium* spp against sucking pests of rice *Oryza sativa* L." is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Hari Sankar S. S

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LIST OF ABBREVIATIONS AND SYMBOLS USED

@	At the rate of
%	Per cent
CD	Critical difference
ANOVA	Analysis of variance
DAT	Days after treatment
EPF	Entomopathogenic fungi
et al.	And others
Fig.	Figure
g	Gram
g^{-1}	Per gram
h	Hours
HAT	Hours after treatment
ha^{-1}	Per hectare
KAU	Kerala Agricultural University
L	Litre
LC	Lethal concentration
LT	Lethal time
mL	Millilitre
mL^{-1}	Per millilitre
NS	Non significant
NA	Not analyzed
sp. or spp	Species (singular and plural)
rpm	Revolutions per minute
viz.	Namely

Introduction

1. INTRODUCTION

Research on microbial pathogens of insects has increased considerably in recent years to evolve environment friendly alternatives to hazardous chemical insecticides. Entomopathogenic fungi (EPF) have an important position among the bioagents used for pest management, owing to their broad host range, pathogenicity route, and ability to control sucking pests as well.

Sucking pests are one of the major constraints in the production of rice. Average yield loss of 18.5 per cent is caused due to an array of insect pests of which twenty are major (Pathak and Khan, 1994). Apart from devitalizing the crop, some of them transmit viral diseases too. BPH *Nilaparvata lugens* Stal, green leaf hopper, *Nephotettix nigropictus* Stal and zig zag leafhopper *Recilia dorsalis* Motschulsky transmits dreadful viral diseases such as grassy stunt, ragged stunt, transitory yellowing, yellow dwarf, tungro and orange leaf virus (Abo and SY 1997). Hoppers generally dominate the vegetative phase of the crop, whereas, rice bugs take over the reproductive phase (Heinrichs, and Barrion, 2004).

These pests are normally contained by repeated application of highly persistent chemical insecticides that decimate the natural beneficial fauna and pollute the water bodies. Moreover, they have developed resistance to insecticides, leading to population levels beyond economic threshold. Entomopathogens rightfully play a pivotal role in managing these pests without the problems of resurgence and resistance. Of these, entomopathogenic fungi is the only group that can cause disease in sucking pests, owing to their unique mode of entry. Through penetration of cuticle and invasion of haemocoel, they find their way of infection and suppressing the pest population. In this context, microbial control using the fungal pathogens such as *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Lecanicillium lecanii* (Zimmermann) Zare and Gams, have been explored globally for their management (Rabindra and Ramanujam, 2007).

Fungi in the genus *Lecanicillium* are known for its pathogenicity to sucking pests infesting various crop plants. *L. lecanii* is a promising fungal bioagent primarily infesting whiteflies, aphids, scales, mealy bugs etc. Better performance of EPF can be achieved using more adaptable and virulent strains. *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno, (ITCC Accession No. LsVs 1 7714) is an indigenous virulent isolate from soils of Vellayani which was found infective to sucking pests and more adaptable under the climatic conditions prevailing in Kerala. Its virulence was characterized by accelerated speed of kill and infectivity to heteropteran bugs as well (Rani *et al.*, 2014., 2015).

For the upliftment of an indigenous isolate as an effective and safe bioagent, investigations on its pathogenicity and cross infectivity to natural enemies and host plants are inevitable. Improved formulations with extended shelf life and field persistence can enhance the performance of EPF under field conditions. Chitin enriched formulations of *L. lecanii* developed by Nithya (2015) was proved to be a promising bioformulation for sucking pest complex in vegetable ecosystem. *L. saksenae* was found to be more effective to sucking pests as per the preliminary field trials conducted by Rani e*t al.* (2014). In this context, a comparative evaluation of enriched formulations of *L. saksenae* and *L. lecanii* against sucking pests in rice ecosystem is crucial for extending their adoption as a biocontrol agent. Hence the present investigation was focused on the following aspects.

- Determination of the pathogenicity of *L. saksenae* and *L. lecanii* to the sucking pests of rice
- Determination of effective doses and lethal concentrations of the fungi
- Validation of its safety to non target organisms
- Evaluation of efficacy of the fungal bioformulations in managing rice bug
- Assessment on the impact of bioformulations on population of natural enemies in the rice ecosystem

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Review of Literature

2. REVIEW OF LITERATURE

Rice, the staple food crop of nearly half of the world population is cultivated and consumed majorly in Asia. India is the largest exporter of rice in the world, shipping 10 million tons in 2016 (IRRI, 2015). The country produces 106 million tonnes of rice and accounts for 22 per cent of world production (USDA, 2017). Apart from being a major food crop, it accounts to a major share in the agricultural exports and thereby foreign exchange of the country.

From seedling stage to harvest, the rice plant is attacked by one or the other sucking pests, hoppers in tillering and growth stages, while bugs in early reproductive stage (Heinrichs and Barrion 2004). Inspite of inflicting severe feeding damage in terms of hopper burn, many of them are notoriously famous vectors of several viral diseases of rice (Abo and SY 1997). Incessant and indiscriminate use of chemical pesticides in rice ecosystem has lead to resurgence of rice hoppers especially BPH, which was elevated to the position of major pest, owing to physiological adaptation and destruction of their natural enemy, *Cyrtorhinus lividipennis* Reuter (Srivastava and Dhaliwal, 2010).

Fungal entomopathogens are excellent microbial pesticides against sucking pest complex of rice ecosystem. Last three decades has seen tremendous strides in usage of entomopathogenic fungi (EPF) in pest management owing to their contact mode of action and wider host range. Nearly 750 species of fungi recorded to be pathogens of sucking pests. Among the common entomopathogens *Lecanicillium lecanii* (Zimmermann) Zare and Gams , *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Paecilomyces fumosoroseus* (Wize), *Hirsutella thompsonii* (Fisher) etc., *L. lecanii* has been extensively exploited worldwide for the management of sucking pests (Rabindra and Ramanujam, 2007).

2.1 PATHOGENICITY OF EPF TO RICE PESTS

2.1.1 *Leptocorisa* spp

Burdeos and Gabriel (1995) reported pathogenicity of 14 isolates of *M. anisopliae* to adult *Leptocorisa oratorius* F. The three promising isolates, Tiaong, DRC, and Visca at 10^8 spores mL^{-1} recorded 90, 80, and 73 per cent mortality respectively at 8 days after treatment (DAT), under screen house condition. Loc and Chi (2005) observed pathogenicity of 12 new isolates of *B. bassiana* and *M. anisopliae* to *L. acuta*, and their mortality ranged from 57.50 to 77.70 per cent, and 74.40 to 87.00 per cent 10 days after treatment with 10^7 conidia mL^{-1}. Herlinda *et al.*, (2008) reported 93 per cent mortality of *L. oratorius* when treated with an indigenous KBC isolate of *B. bassiana*. Malini (2015) found that NBAIR isolates of *B. bassiana* (Bb 5), *M. anisopliae* (Ma 4), four indigenous isolates of *B. bassiana* (Bb-m2, Bb-m3, Bb-m4, and Bb-m5) and one indigenous isolate of *M. anisopliae* (Ma-m1) were pathogenic to nymphs and adults of *L. acuta*. Discolouration and ovicidal action were reported on eggs of *L. acuta* treated with *B. bassiana* (Bb-5). The nymphs and adults upon treatment with the fungal spores exhibited arrested movement, reduced feeding, and preferred being solitary unlike untreated ones. There was reduction in copulation of treated adults.

2.1.2 *Nilaparvata lugens* (Stal)

Aguda *et al.*, (1984) observed that *M. anisopliae* and *B. bassiana* as the most frequently isolated entomopathogens from rice fields, collected from mycosed *N. lugens*, in Philippines. Li *et al.*, (2012) compared the efficacy of six isolates each of *M. anisopliae* and *B. bassiana* in a laboratory experiment and observed that the BPH derived isolates of *Metarhizium*, Mf82 and Ma20 recorded highest cumulative mortality of 82.10 and 65.40 per cent, respectively when BPH were topically treated with spores harvested from 15 day old cultures. Maketon *et al.*, (2015) screened 14

isolates against *N. lugens* and observed 88.89, 84.44, 82.22, 92.22 per cent mortality treated when with 10^8 spores mL^{-1} of *Metarhizium robertsii* J.F. Bisch., Rehner & Humber (CKM-048), *B. bassiana* (CKB-048), *Metarhizium flavoviridae* Gams and Rozsypal (CKM-083), and *Isaria fumosorosea* (Wize) (CKPF-095), respectively, under laboratory conditions.

2.1.3 Other Hoppers

Pathogenicity of *B. bassiana* against white backed planthopper *Sogatella furcifera* (Horvath), and green leafhopper *Nephotettix virescens* (Distant) was reported by Aguda *et al.*, (1984), and stated them to be potential biocontrol agents of hoppers in rice ecosystem. Rombach *et al.*, (1987) reported pathogenicity of *Metarhizium album* (Petch) to green leafhopper, *N. virescens*, and white hopper, *Cofana spectra* (Distant). Geng and Zhang (2004) observed high susceptibility of adults of *S. furcifera* to spores of *M. anisopliae* and recorded 85 and 100 per cent mortality of hoppers, for two spore doses of 10.5 and 116.3 spores mm^{-2}, 21 DAT. Maketon *et al.*, (2015), in a laboratory study to assess the efficacy of indigenous isolates against the hoppers found in paddy ecosystem found that, *B. bassiana* (CKB-048) caused 93.33 and 46.67 per cent mortality of *S. furcifera* and *N. virescens* while the corresponding mortality observed with *M. robertsii* (CKM-067) was 73.34 and 40.0 per cent respectively.

2.2 PATHOGENICITY OF *Lecanicillium* spp TO SUCKING PESTS

The genus *Lecanicillium*, previously named as *Verticillium* has been recently reclassified by Zare and Gams (2001), based on morphological and molecular characters. Mark *et al.*, (2008) opined the fungi in the genus *Lecanicillium* as important pathogens of insect and nematode pests and have the potential to be developed as a single best biocontrol agent against numerous insects, diseases and

nematode pests. Perusal of literature indicates the verity that this genus is more selective to homopteran pests rather than heteropterans.

2.2.1 *Lecanicillium lecanii* (Zimmermann) Zare and Gams

Infectivity of *L. lecanii* to *Trialeurodes vaporariorum* (Westwood), and *Aphis gossypii* Glover has been demonstrated by Hall (1982), wherein the spore suspensions from 14 day old cultures, controlled the established whitefly populations as well as the mobile nymphs that spread infestation to younger leaves. Kanagaratnam *et al.*, (1982) reported that weekly sprays of *L. lecanii* @ 10^7 spores mL^{-1} efficiently controlled the whitefly *T. vaporariorum* by five to 15 per cent, in glasshouse. *L. lecanii* was reported as a promising biocontrol agent against all the major cereal infesting aphids of the world by Feng *et al.*, (1990). The high virulence of *L. lecanii* to the aphids *Myzus persicae* (Sulzer), *A. gossypii* and *Brevicoryne brassicae* L. were ascertained by Alavo *et al.*, (2001). They observed 90 and 82 per cent mortality of *A. gossypii* for the two isolates, V24 and V18 when sprayed at 10^8 spores mL^{-1}, in laboratory experiments. Antifeedant and reproductive suppression properties of crude toxins isolated from *L. lecanii* were identified by Wang *et* al., (2007). Lokesh (2014) reported that *L. lecanii* is highly pathogenic to *A. gossypii* causing complete mortality 48 hours after treatment (HAT). The treated nymphs lost their mobility, stopped feeding and thread like mycelial outgrowths emerged from dead aphids. He also reported that it is pathogenic to *Bemisia tabaci* (Gennadius), *Scirtothrips dorsalis* Hood, and *Polyphagotarsonemus latus* Banks with more or less similar symptoms. Pathogenicity of *L. lecanii* to adults of *Amrasca biguttula biguttula* Ishida, *Aphis craccivora* Koch, *B. tabaci*, *Ferrisia virgata* Cockerell, *Tetranychus sp.* and *Lecanium sp.* was ascertained by Nithya (2015). She observed that the treated insects were lethargic, mobility was restricted and their body shrunk. The cadavers were stiff and mycelia emerged from the outlines/ margins of body and later spread as a white mat, covering the whole cadaver.

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2.2.2 *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno

L. saksenae was first described by Kushwaha (1980) as a keratin degrader and later Sukarno *et al.*, (2009) isolated it from the soil dwelling arthropods of Kalimantan province of Indonesia. Later, Pinto *et al.*, (2012) reported it to be an efficient degrader of herbicides and pesticides. Rani *et al.*, (2014, 2015) isolated an indigenous strain of *L. saksenae* (Accession no. ITCC LsVs 1 7714) and reported it to be a potent pathogen against a miscellany of homopterans *viz.*, *A. craccivora*, *A. gossypii*, *B. tabaci*, *A. biguttula biguttula* and *Coccidohystrix insolita* (Green). Incidentally the pathogenicity of *L. saksenae* to pod bug *Riptortus pedestris* F. as reported by them, created an opening for harnessing the potential of this isolate against heteropteran bugs, as well. Jasmy (2016) reported *L. saksenae* to be pathogenic to *A. craccivora*, *C. insolita*, *B. tabaci*, *A. biguttula biguttula*, and *L. acuta* causing 100 per cent mortality to all the test insects within 72 HAT when treated with a spore concentration of 10^7 spores mL^{-1}. She observed that the infected nymphs and adults of *C. insolita* became bare, by shedding waxy coating and turned brown at 24 HAT. Sluggish and arrested movements, coupled with reduced feeding were observed in other insects treated with *L. saksenae*.

2.2.3 *Lecanicillium attenuatum* Zare and Gams

L. attenuatum was reported to reduce the lifespan, fecundity and active reproductive period of cotton aphid *A. gossypii* (Kim, 2007). He observed a reduction in lifespan of first instar nymphs to be 10.80 and 8.40 days when treated with 10^4 and 10^8 spores mL^{-1} when compared to 12.2 days in control. The fecundity was reduced from 51 nymphs per female in control to 41, 26, and 22 nymphs per female in aphids treated with 10^4, 10^6 and 10^8 spores mL^{-1}. Kim *et al.*, (2008) established the higher virulence of aphid derived isolate of *L. attenuatum* (CNU-23) @ 10^6 spores mL^{-1}, to

the greenhouse aphid, *M. persicae*. They observed a mortality of 80 per cent under laboratory conditions and a higher mortality of 97 per cent, under field conditions.

2.2.4 *Lecanicillium muscarium* (Petch) Zare and Gams

Cuthbertson and Walter (2005) accounted an efficient control of *B. tabaci* in greenhouse conditions using *L. muscarium* @ 10^7 spores mL^{-1} where they noted 81 and 92 per cent mortality of the whiteflies on tomato and verbena plants respectively. The prospect of successful utilization of this fungus in Integrated Pest Management was ascertained by evaluating it for the management of *Thrips palmi* Karny where more than 60 per cent mortality was recorded (Cuthbertson *et al.*, 2005). Scorsetti *et al.*, (2008) observed better performance of *L. muscarium* compared to *L. lecanii* and *Lecanicillium longisporum* ((Petch) Zare and Gams the mortality recorded in *Trialeurodes vaporariorum* (Westwood) being 65.00, 52.60 and 35.00, per cent respectively, when treated @ 10^7 spore mL^{-1}. Saban *et al.*, (2010) studied the pathogenicity of six isolates of *L. muscarium* @ 10^7 spores mL^{-1} and observed 50.95 to 74.76 per cent mortality, seven days after treatment in *Ricania simulans* (Walker). High mortality (98 per cent) of hazelnut shield bug *Palomena prasina* (L.), was reported by Saruhan *et al.*, (2016) using spore suspension of *L. muscarium* (Isolate TR-07) @ 10^8 spores mL^{-1}.

2.2.5 *Lecanicillium longisporium* (Petch) Zare and Gams

Reduction in feeding as a result of mycosis by *L. longisporium* was observed by Roditakis *et al.*, (2008). The number of honey dew secretion events declined steadily two days after treatment which indicated reduction in feeding. Fadayivata *et al.*, (2014) reported the pathogenicity of *L. longisporium* LRC 190 strain against *Metopolophium dirhodum* (Walker) and *Sipha maydis* (Passerini), causing 94.36 and 77.14 per cent mortality respectively nine days after treatment with 10^8 spores mL^{-1}.

2.2.6 *Lecanicillium psalliotae* (Petch) Zare and Gams

Kheirabadi *et al.*, (2006) reported that two indigenous isolates of *L. psalliotae* IRAN 468 C and IRAN 518 C were pathogenic to *Rhipicephalus annulatus* (Say). The reproductive efficiency of females decreased by 78.15 per cent and egg hatching was reduced by 56.3 and 14 per cent respectively upon treatment with spore suspensions. Kumar (2015) reported the pathogenicity of *L. psalliotae* to cardamom thrips *Scirtothrips cardamomi* Ramk. At a spore dose of 1 x 10^7 conidia mL^{-1} the fungi caused 62.9 per cent mortality and the conidia penetrating the insect cuticle was clearly documented using scanning electron micrographs.

2.3 BIOSAFETY OF *Lecanicillium*

2.3.1 Cross Infectivity to Crop Plants

Cross infectivity of fungi in the genus *Lecanicillium* to crop plants should necessarily be validated, as the genus was previously included in the plant pathogenic genus *Verticillium*. Cuthbertson and Walter., (2005) reported no detrimental effect on tomato and verbena plants, when sprayed with 10^7spores mL^{-1}. Safety of *L. lecanii* and *Aspergillus parasiticus* Spear to various crops *viz.*, cotton (*Gossypium hirsutum* L.), wheat (*Triticum aestivum* L.), bean (*Phaseolus vulgaris* L.), corn (*Zea mays* L.), tomato (*Lycopersicum esculentum* L.), and pumpkin (*Cucurbita maxima* L.), through leaf and soil inoculation methods, was ascertained by Gurulingappa *et al.*, (2010). Endophytic colonization of *M. anisopliae*, *B. bassiana*, and *L. lecanii* in various host plants was reported and observed to cause no detrimental effects on the normal functioning of plant system, by Vidal and Jaber (2015). Jasmy (2016) ascertained the safety of *L. saksenae* to crop plants such as cowpea (*Vigna unguiculata* L.), bhindi (*Abelmoschus esculentus* L.), brinjal (*Solanum melongena* L.) and tomato (*L. esculentum*).

2.3.2 Safety to natural enemies

2.3.2.1 Predators

Rondon *et al.*, (1982) reported safety of 10^7 spores mL^{-1} of *L. lecanii* to the aphid predators *Cycloneda sanguinea* L., *Oxyptamus gastrostacus* Wiedemann and *Zelus* sp. *L. lecanii* spores @ 10^7 spores mL^{-1} was proven safe to predatory mite *Phytoseiulus persimilis* Athias-Henrio by Koike *et al.* (2005). Safety of predatory insects in rice ecosystem *viz.*, *C. lividipennis*, and spiders were reported by Reddy *et al.*, (2013). Toxins extracted from *L. lecanii* was found to have no detrimental effect on the whitefly predator coccinellid, *Delphastus catalinae* (Horn), and the toxins did not reduce the reproductive capacity or longevity of the beetles (Wang *et al.*, 2005). Suharsono and Prayogo (2014) observed that *L. lecanii* did not have any adverse effect on natural enemies *Coccinella* sp. and *Paederus* sp. Jasmy (2016) investigated the safety of *L. saksenae* to coccinellids, spiders, syrphids, and reported no symptoms of infection nor any abnormalities in their biology, when topically applied @ 10^7 spores mL^{-1}.

2.3.2.2 Parasitoids

The safety of *L. lecanii* spores @ 10^8 spores mL^{-1} to the aphid parasitoid *Aphidius colemani* Viereck, was confirmed by Kim *et al.* (2005). Malarvannan *et al.*, (2010) reported that spores of *L. lecanii* were safe to the egg parasitoids *Trichogramma chilonis* Ishii and *Trichogramma japonicum* Ashmead, at 10^7 spores mL^{-1}, and the treated females successfully parasitized their host *Corcyra cephalonica* Stainton. Jasmy (2016) reported safety of 10^7 spores mL^{-1} of *L. saksenae* to *Bracon brevicornis* F., *Goniozus nephantidis* Muesebeck, *T. japonicum* and *T. chilonis.*

2.4 DOSE MORTALITY RESPONSE OF SUCKING PESTS OF RICE TO EPF

2.4.1 *Leptocorisa* spp

A dose dependant mortality of second instar nymphs of *L. acuta* was reported by Herlinda *et al.*, (2008) when treated with *M. anisopliae* @ 10^3, 10^5, and 10^7 spores mL^{-1}. The mortality recorded was 90, 90, and 100 per cent, respectively. Malini (2015) observed a dose dependant trend in mortality of *L. acuta* nymphs, when treated with spore suspensions of *M. anisopliae* (Ma4) and *B. bassiana* (Bb5). As the spore concentration increased from 10^4 to 10^8 spores mL^{-1}, mortality increased from 37.50 to 100 per cent, at 10 DAT in the case of *B. bassiana* and the corresponding increase observed with *M.anisopliae* was from 30 to 100 per cent. Dose dependant mortality in *L. saksenae* was reported in *L. acuta* adults by Jasmy (2016). She observed that as the concentration increased from 10^6 to 10^8 spores mL^{-1} the death rate also increased from 80 to 100 per cent, at two DAT, under laboratory conditions.

2.4.2 *N. lugens*

Geng and Zhang (2004) observed a dose dependant mortality of nymphs and adults of *N. lugens* treated with *M. anisopliae* var *acridium* with doses 10^6, 10^7, and 10^8 spores mL^{-1}. The nymphal mortality recorded was 33, 51, and 68 per cent in increasing order of concentration, at 21 DAT, while for adults it was 81, 100 and 100 per cent respectively.

2.4.3. Other Hoppers

As spore concentration increased from 10^6 to 10^8, the mortality of nymphs of *S. furcifera* also increased from 42 to 86 per cent, and in adults, the higher spore dose of 10^8 spore mL^{-1} killed all the test insects on 18 DAT while the mortality recorded in lowest spore dose on 21 DAT was only 85 per cent (Geng and Zhang, 2004).

2.5 LETHAL CONCENTRATION AND LETHAL TIME OF EPF AGAINST SUCKING PESTS OF RICE

2.5.1 *Leptocorisa* spp

Burdeos and Gabriel (1995) did the bioassay of three promising isolates *viz.* Tiaong, DRC and Visca of *M. anisopliae* on *L.oratorius* and recorded an LC_{50} of 1.39, 3.54, and 6.74 x 10^6 spores mL^{-1} and LT_{50} of 4, 6, and 6 days respectively. Herlinda *et al.*, (2008) recorded a low LT_{50} of 1.14 days for 10^7 spores of an indigenous KBC isolate of *B. bassiana* and 1.26 days for 10^5 spores $mL^{-1}$of Mtm strain of *M. anisopliae* against *L. acuta.* Malini (2015) recorded an LC_{50} of 1.63, 3.55, 0.95, and 1.74 x 10^8 spores mL^{-1} for Bb-5, Bb-21 isolate of *B. bassiana*, *M. anisopliae* (Ma-4), and *Apergillus flavus* Link (Af-m1) against rice bug adults, five DAT, and an LT_{50} of 6.65, 6.92, 7.13, and 7.16 days respectively for 10^9 spores mL^{-1}, for the same set of fungi mentioned above.

2.5.2 *N. lugens*

Geng and Zhang (2004) calculated the LT_{50} of *M. anisopliae* var. *acridium* as 17.10, 12.57, and 9.14 days for adults and 21.00, 20.82, and 16.55 days for nymphs of BPH, using three different concentrations , 10^6, 10^7, and 10^8 spores mL^{-1}. Jin *et al.*, (2008) calculated the LC_{50} of two strains of *M. anisopliae* ARSEF 456 and ARSEF 576 to third instar nymphs of *N. lugens.* The LC_{50} values were 731 and 1124 conidia mm^{-2}, seven DAT while it was, 284 and 306 conidia mm^{-2}, 10 DAT. Li *et al.*, (2012) derived LT_{50} of two isolates of *M. anisopliae* obtained from *N. lugens viz.* Mf 82 and Ma 20. It was 4.9 and 6.7 days for adults of *N. lugens* when treated with $1x10^8$ spores mL^{-1}.

2.6 COMPARATIVE VIRULENCE OF INDIGENOUS STRAINS AND EXOTIC STRAINS

Doberski (1981) tested pathogenicity of different strains of *B. bassiana* against elm bark beetle *Scolytus scolytus* (F.) and reported higher virulence of native European strains of *B. bassiana* JD-1 and JD-2 with LT_{50} of 6.2 and 6.3 days respectively than exotic strain from United States of America JD-8, with LT_{50} 10.3 days. A study conducted by Gindin *et al.*, (2000) compared the pathogenicity of 15 indigenous strains of *L. lecanii* @ 10^7 spores mL^{-1}, from Israel with 18 exotic strains from Russsia, Georgia, Kazakhstan, Cyprus and Turkey on *Bemisia argentifolii* (Gennadius) and found that some of the indigenous strains were superior with a mean LT_{50} of 3.2 to 4 days, while that of exotic strains ranged from four to seven days or more. Wang *et al.*, (2004) conducted bioassays of six strains of *L. lecanii* against whitefly *B. tabaci* and concluded that two indigenous and one exotic isolates were superior to other strains. Higher virulence of native strains from Benin was also reported by Cherry *et al.*, (2005) while working with *M. anisopliae* and *B. bassiana* against *Callosobruchus maculatus* (F.) in stored cowpea. The indigenous isolates of *M. anisopliae* and *B. bassiana* from Benin, West Africa were more virulent than the exotic strains from Kenya, France, Ivory Coast, and Brazil. The LT_{50} values of indigenous strains were 3.10 and 3.27 days respectively, which was significantly lower than those with exotic isolates (4.60 and 4.61). Kulkarni *et al.*, (2008) studied the efficacy of indigenous strains of *M. anisopliae* from Pune, India, against gram pod borer *Helicoverpa armigera* (Hub.). The field study revealed that per cent pod damage in *M. anisopliae* @ 5 x 10^{12} conidia ha^{-1} treated plots were statistically similar with that of endosulfan treated at 350 g ai ha^{-1}. The per cent efficacy computed for endosulfan and *M. anisopliae* treated plots were on par, implying the superiority of indigenous strains, and its applicability in integrated pest management.

2.7 FIELD EFFICACY OF EPF AGAINST SUCKING PESTS OF RICE

In a study conducted by Rombach *et al.*, (1986 a) to compare the efficacy of five EPF *viz.* conidial suspensions @ 4.5 x 10^{12} conidia ha^{-1} of *M. anisopliae*, *M. flavoviride B. bassiana* (BbE), *B. bassiana* (Bb 252) and *Hirsutella citriformis* (Speare), they observed that *M. anisopliae* was more effective and caused 91 to 100 per cent mortality of *N. lugens* three weeks after application, followed by, *M. flavoviride* causing 60.3 to 100 per cent mortality. In another study conducted by them (Rombach *et al.*, 1986 b) it was repored that *M. anisopliae*, *B. bassiana* and *Paecilomyces lilacinus* (Thom) were equally effective to black stink bug *Scotinophara coarctata* (F.) when applied @ 2.5 x 10^{12} conidia ha^{-1} bringing about significant reduction in population of *S. coarctata* when compared to untreated control. Loc and Chi (2005) observed 86.6 and 89.4 per cent mortality of *L. acuta* when treated with a field dose of 6 x 10^{12} spores mL^{-1} of Ometar (Bioformulation of *M. anisopliae* in Thailand) and OM_3-BD (isolate of *M. anisopliae*). Grain damage of 4.89 and 5.15 per cent, due to *L. acuta* feeding was recorded in plots treated with *M. anisopliae* (Biomagic®) and *B. bassiana* (Biopower®), when compared to 13.06 per cent damage in untreated plots. Reddy *et al.*, (2013) observed that $2x10^8$ spores mL^{-1} of *M. anisopliae* and *B. bassiana* were equally effective as Acephate 75 %, in managing BPH population, while *L. lecanii* was inferior. The reduction in population recorded was 77.00, 78.90, 88.20 and 41.2 per cent, respectively 10 days after second spraying. Millet based granular formulation of *B. bassiana* developed by Lee *et al.*, (2015) showed 87.7 per cent efficacy in reduction of *N. lugens* when compared to 94.6 per cent efficacy in Etofenprox 0.5 G application, 15 days after field application. Malini (2015) reported that plots treated with 10^{10} spores mL^{-1} of *M. anisopliae* were statistically on par with that of chlorantraniliprole @ 30 g ai ha^{-1} and Acephate @ 250 g ai ha^{-1} in terms of extent of grain damage caused by *L. acuta*. *M. anisopliae* treated plots recorded 53.21 and 54.22 per cent reduction at 21 DAT in the first and second field trials.

Materials and Methods

3. MATERIALS AND METHODS

The study entitled "Efficacy of chitin enriched formulations of *Lecanicillium* spp against sucking pests of rice *Oryza sativa* L. was carried out in the Biocontrol Laboratory for Crop Pest Management, Department of Agricultural Entomology, College of Agriculture, Vellayani and Integrated Farming Systems Research Station (IFSRS), Karamana, Thiruvananthapuram, during 2015-17.

3.1 PATHOGENICITY STUDIES

Infectivity of *Lecanicillium* spp *viz.*, *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno and *Lecanicillium lecanii* (Zimmermann) Zare and Gams to the major sucking pests of rice was carried out under laboratory conditions. The following sucking pests which were found to be predominant in rice fields of Thiruvananthapuram district were selected for the study.

Bugs

1. Rice bug, *Leptocorisa acuta* (Thunberg) [Hemiptera: Alydidae]
2. Black bug, *Scotinophara coarctata* (F.) [Hemiptera: Pentatomidae]
3. Shield bug, *Menida versicolor* (Gmelin) [Hemiptera: Pentatomidae]

Hoppers

1. Brown planthopper, *Nilaparvata lugens* (Stal) [Hemiptera: Delphacidae]
2. Green leafhopper, *Nephotettix nigropictus* (Stal) [Hemiptera: Cicadellidae]
3. White leafhopper, *Cofana spectra* (Distant) [Hemiptera: Cicadellidae]
4. White winged planthopper *Nisia nervosa* (Motschulsky) [Hemiptera: Meenoplidae]

3.1.1 Maintenance of Fungal Cultures

Two *Lecanicillium* species maintained in the Biocontrol Laboratory for Crop Pest Management, Department of Agricultural Entomology, College of Agriculture, Vellayani, Thiruvananthapuram were utilized for the study. *L. lecanii* isolate No. Vl 8, was originally sourced from National Bureau of Agricultural Insect Resources, Bengaluru, and *L. saksenae* with Accession no: ITCC LsVs 1 7714 was the indigenous strain isolated from soils of Vellayani by Rani *et al.*, (2014). The cultures of these species were revived by periodically passing them through one of their susceptible host insects and maintained by subculturing. *L. lecanii* was periodically passed through *Aphis craccivora* Koch and *L. saksenae* was passed through *L. acuta*. Pure cultures were then maintained on Sabouraud Dextrose Agar (SDA) slants. Mass multiplication was done using chitosan amended Sabouraud Dextrose broth (SDB).

3.1.2 Mass Multiplication

Medium for maintaining fungal cultures was prepared by dispensing 20 g of dextrose and 10 g of peptone in 1 L of water. Hundred mL of the medium was poured to 250 mL conical flasks. Crude chitosan, procured from Matsyafed, Neendakara was added to each flask at the rate of 5 g $flask^{-1}$. The flasks were then plugged with cotton and sterilized at 121° C and 1.1 kg cm^{-2} for 20 minutes in a horizontal autoclave. The medium was cooled to room temperature and inoculated aseptically with 5 mL of spore suspensions of the pure cultures mentioned in para 3.1.1.

3.1.3 Maintenance of Plants

The test insects for pathogenicity studies were reared on rice plants of the variety Prathyasa (MO- 21). Seeds were procured from Department of Agronomy, College of Agriculture, Vellayani. Sprouted seeds were sown to plastic pots of 20 x

25 cm, filled with soil collected from rice field. Rice plants were planted sequentially to ensure continuous supply of seedlings and panicles.

3.1.4 Rearing Test Insects

3.1.4.1 Rice bug

Bugs collected from rice fields were brought to the laboratory and observed for any latent infections. Active and healthy adults were released into a rearing cage with potted rice plants bearing panicles in milky stage. Rearing cage was of size 165 x 120 x 120 cm made of aluminium frame and nylon mesh. Older pots were replaced periodically with new pots with plants in the milky stage. Inspection window provided on the sides of the cage facilitated collection of bugs using a hand net.

3.1.4.2 Hoppers

Hoppers *viz., N. lugens, N. nigropictus, N. nervosa* and *C. spectra* collected from rice fields were brought into laboratory and observed for any signs of latent infection. One month old rice plants (para 3.1.3) were used for rearing hoppers. The potted plants covered with cages made of OHP sheet served as rearing cages. The top side of the rearing cage was covered with a muslin cloth tied around it. "V" shaped slits made on the sides of the cylindrical cage facilitated collection of hoppers using an aspirator. Healthy, uninfected adult hoppers were released at the rate of 20 per cage and their progeny served as stock cultures. Adults and nymphs of uniform age were used for pathogenicity studies.

3.1.5 Pathogenicity Tests

Pathogenicity of *L. saksenae* and *L. lecanii* was tested in egg, nymphs and adult stages of the major pest *L. acuta*, and in the adults of other bugs and hoppers mentioned in 3.1. Spore suspension was prepared by blending 14 day old cultures of

the fungi, in a blender for 10 seconds and filtered through double layered muslin. Spore count was adjusted to 10^7 spores mL^{-1} using Neubauer haemocytometer.

3.1.5.1 L. acuta

Eggs, nymphs and adults of *L. acuta* were tested for susceptibility to the fungi *L. lecanii* and *L. saksenae*.

3.1.5.1.1 Eggs

Egg masses collected from stock culture were treated with spore suspensions of the test fungi by leaf dip method. The treated eggs were air dried and kept in a moist chamber made of Petri plates lined with moist tissue paper. Observations were recorded on symptoms of mycosis and nymph emergence. Newly emerged nymphs were transferred to rearing jars provided with fresh panicle as food source. Panicles were kept afresh by keeping the cut end of their stalks immersed in water taken in a vial. The treated egg masses were observed for any symptoms of mycosis. Egg masses treated with sterile water served as control.

3.1.5.1.2 Nymphs

Third instar nymphs of *L. acuta* were collected from stock culture in polythene bags and ten nymphs were transferred to each of the plastic jars of size 15 cm diameter and 20 cm height, lined with tissue paper. Spore suspensions prepared as mentioned in 3.1.5 were sprayed topically using an atomizer. One minute after treatment, the treated nymphs were transferred to rearing jars, the top of which was covered with a muslin cloth,. Panicles in milky stage, staked in plastic cups filled with clay served as food source. They were replaced as and when required to ensure adequate supply of milky grains. Nymphs sprayed with sterile water served as control. The treated insects were observed at 24 h interval for a period of 21 days for any signs of mycosis and mortality.

3.1.5.1.3 Adults

One day old adults collected from the stock culture were subjected to treatment as mentioned in 3.1.5.1.2 (Plate 1 A). Ten adults constituted the sample for pathogenicity studies. Observations on mycosis and mortality were taken for a period of 21 days.

3.1.5.2 S. coarctata

Stink bugs were collected from the field for pathogenicity tests. They were kept under observation to rule out any latent infection. Healthy adults were treated as in 3.1.5.1.2

3.1.5.3 M. versicolor

Field collected bugs were screened for any latent infection and healthy adults were subjected to treatment as described in 3.1.4.2.1

3.1.5.4 Hoppers

Uniform aged adults (two day old) of *N. lugens*, *N. nigropictus*, *N. nervosa*, and *C. spectra*, collected from the stock culture were used for studies. Thirty each of nymphs and adults were transferred into a plastic jar lined with tissue paper. Spore suspension was topically applied using an atomizer. Particle size of droplets was adjusted to minimum to ensure that nymphs and adults were not drowned. The treated insects were transferred to rearing jars, the top of which was covered with muslin cloth tied around. One month old rice plants, planted in plastic cups kept inside the rearing jars served as food source for the hoppers. The treated hoppers were observed for signs of mycosis and mortality for a period of 21 days.

3.2 CROSS INFECTIVITY TO CROP PLANTS

Cross infectivity of *L. lecanii* and *L. saksenae* to rice plant was tested by leaf and soil inoculation methods.

3.2.1 Maintenance of Rice Plants

One month old healthy seedlings as maintained in para 3.1.3 were used for the study.

3.2.2 Preparation of Spray Solution

Fourteen day old fungal cultures were blended in a mixer grinder for 10 seconds, mycelia was filtered off and filtrate was centrifuged in a Rotek centrifuge at 5000 rpm for 20 minutes to obtain spore pellets. These pellets were dispensed in required quantities of the supernatant to make the final spore concentration to 10^9 spores mL^{-1} (100 times the infective dose).

3.2.3 Leaf Inoculation

A circular area of the leaf was marked with ball point pen. The marked area was rubbed with a sandpaper to cause abrasion. Cotton pads dipped in spore suspensions were placed around the rubbed area. The entire plant was covered using a polythene cover with pin pricks (Plate 1 C). Leaves treated with sterile water served as control. Plants were observed for a period of one month for any signs of infection. Each treatment was replicated five times with one plant per replication. In each replication, treatments were applied on five leaves selected at random.

3.2.4 Soil Inoculation

One month old plants free of any disease symptoms were used for the study. Each pot was drenched with 200 mL of spore suspension. Each treatment was replicated five times with one plant per replication. Observations were recorded on symptoms of infection for a period of one month. Pots drenched with 200 mL sterile water served as control.

3.3 SAFETY TO NATURAL ENEMIES

Safety of the *L. saksenae* and *L. lecanii* to the following natural enemies in rice ecosystem was tested

1. Coccinellids, *Micraspis discolor* (F.) and *Coccinella transversalis* F. [Coccinellidae: Coleoptera]
2. Mirid bug, *Cyrtorhinus lividipennis* Reuter [Miridae: Hemiptera]
3. Carabid beetle, *Ophionea nigrofasciata* Schmidt-Gobel [Carabidae: Coleoptera]
4. Spiders, *Tetragnatha maxillosa* (Thorell) [Tetragnathidae: Araneae], *Oxyopes shweta* Tikader [Oxyopidae: Araneae]

3.3.1 Rearing of Natural Enemies

3.3.1.1 Coccinellids

Adult beetles of *M. discolor* and *C. transversalis*, collected from rice fields were reared separately in rearing jars. Aphid infested cowpea pods were given as food, and were replaced periodically to ensure adequate and fresh supply of food. Upon egg laying, the egg masses were clipped off and transferred to another rearing jar for maintaining the stock culture.

3.3.1.2 Mirids

Adults of *C. lividipennis* collected from rice fields were brought to the lab and transferred to plastic jars. They were kept under observation to rule out any field infection.

3.3.1.3 Carabids

Field collected beetles of *O. nigrofasciata* were observed for any signs of infection and used for safety studies.

3.3.1.4 Spiders

Spiders were collected from field, using a sweep net and kept under observation to rule out any latent infection. They were confined in rearing jars and fed with adult hoppers.

3.3.2 Safety Tests

The spray solution was prepared as mentioned in 3.2.2. The spore concentration used was 100 times the infective dose.

3.3.2.1 Coccinellids

3.3.2.1.1 Adults

One day old adults of each species (10 numbers each) collected separately from the stock culture were topically sprayed with each of the spore suspensions as described in 3.1.4.1. Treated insects were transferred to rearing jars and aphid infested cowpea pods were given as food. Beetles sprayed with sterile water served as control. Observations on mortality and symptoms of mycosis were recorded until 100 per cent mortality was observed in control or till the insects completed their life cycle, which ever was earliest.

3.3.2.1.2 Grubs

Five day old grubs collected from stock culture were subjected to safety tests and kept under observation as described in para 3.3.2.1.1

3.3.2.1.3 Eggs

Egg masses clipped off from leaves were dipped in spore suspension, air dried and incubated in moist chamber as described in 3.1.5.1.1. Observations were recorded for symptoms of mycosis till hatching. Egg masses dipped in sterile water served as control.

3.3.2.2 Mirid bugs

Spore suspensions were sprayed on the inner surface of rearing jar using an atomizer. The mirid bugs were then released into the treated jars the top of which was closed with a wet muslin cloth. Care was taken not to drown the bugs. After 10 minutes, 10 mirid bugs each were transferred to three rearing jars and nymphs of BPH were provided as food. Observations were taken as per 3.3.2.1.1.

3.3.2.3 Carabid beetle

Healthy beetles of *O. nigrofasciata* were subjected to treatment and observations were recorded as described in 3.3.2.1.1.

3.3.2.4 Spiders

Adult spiders of uniform age were collected from stock culture and transferred to plastic jars. Spore suspensions of each fungus were applied topically using an atomizer. Excess fluid was drained off and 10 spiders each were transferred to three separate rearing jars with 20 day old rice plant in a plastic cup (Plate 1 B). BPH were

(A) Pathogenicity test to rice bug

(B) Safety test to spiders

(C) Leaf inoculation method

Plate 1. Experimental setup for pathogenicity, safety and cross infectivity studies

provided as food. They were kept under observations till 100 per cent mortality was observed in control.

The eggs of *T. maxilllosa* and *O. shweta* were collected from stock culture and were transferred to 9 cm plastic Petri plates and sprayed with spore suspension. Eggs sprayed with sterile water served as control. Treated eggs were incubated and data on mortality and spiderling emergence were observed until no further emergence from control was observed.

3.4 DETERMINATION OF EFFECTIVE DOSE

Effective doses of the fungi were estimated by studying the dose mortality response of test insects treated with varying concentrations of spore suspension ranging from 10^3 to 10^8 spores mL^{-1}, employing the same procedure for pathogenicity studies. Observations on mortality were recorded at 24 h interval for a period of seven days.

3.4.1 Preparation of Spore Suspensions and Conduct of the Experiment

Spore suspensions were prepared as described in 3.1.5. Test insects procured from the stock culture (3.1.4), were treated with spore suspensions of different concentrations, as per the procedure mentioned in 3.1.5.1.2. Each treatment was replicated thrice with 10 insects per treatment. A set of insects treated with sterile water served as control. Observations were recorded on mortality at an interval of 24 h for a period of seven days.

3.5 DETERMINATION OF LETHAL CONCENTRATIONS AND LETHAL TIME

For accurate comparison of virulence of the two species, the mortality data recorded in para 3.4 was made use of for calculating the lethal concentration values (LC_{50} and LC_{90}). Another set of *L. acuta* treated with varying spore concentrations were observed at 12 h interval for their mortality to calculate the lethal time (LT_{50}).

3.6 DETERMINATION OF FIELD EFFICACY OF CHITIN ENRICHED BIOFORMULATIONS

In order to assess the efficacy of the chitin enriched fungal bioformulations against the major sucking pest of rice, *L. acuta*, a field trial was conducted at Integrated Farming Systems Research Station, Karamana, during November 2016 to March 2017. Medium duration rice variety Uma (Mo16) was selected for the experiment. Leaving behind plant protection, the crop production techniques followed KAU (2011) recommendations. The experiment was laid out in an area of 200 m^2 following Randomized Block Design with eight treatments and three replications. The plot size was 2 x 2 m.

Treatments

T1 - Chitin enriched formulation of *L saksenae* (10^7 spores mL^{-1}) @10mL L^{-1}

T2 - Chitin enriched formulation of *L. lecanii* (10^7 spores mL^{-1}) @10mL L^{-1}

T3 - Spore suspension of *L. saksenae* @10^7 spores mL^{-1}

T4 - Spore suspension of *L. lecanii* @10^7 spores mL^{-1}

T5 - Talc based formulation of *M. anisopliae* (10^8 spores mL^{-1}) @ 20 g L^{-1} - Biocontrol check 1

T6 - Talc based formulation of *B. bassiana* (10^8 spores mL^{-1}) @ 20 g L^{-1} - Biocontrol check 2

T7 - Malathion 0.1% - Chemical check

T8 - Untreated check

Doses of T1, T2, T3 and T4 were fixed based on the data from experiment 3.4 conducted to determine effective dose. Spraying was undertaken when the rice bug population in the field reached its economic threshold level (ETL), two bugs $hill^{-1}$. Stem borer and leaf folder infestations were managed using eggs of *Trichogramma japonicum* Ashmead and *Trichogramma chilonis* Ishii respectively, @

five cards per hectare, of each species, commencing from 30 days after transplanting till harvest.

3.6.1 Preparation of Bioformulations

3.6.1.1 Oil Formulations

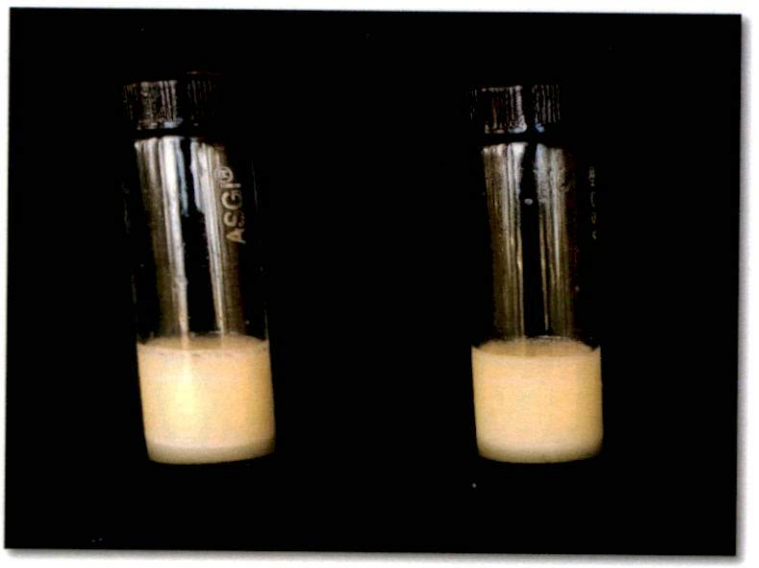
Chitin enriched formulations were prepared as per protocol developed by Nithya (2015) with slight modifications.

L. lecanii and *L. saksenae* were cultured in roux bottle on chitosan (5% w/v) amended SDB. Fourteen day old cultures were blended for 15 seconds in a blender. It was then filtered through double layered muslin cloth and the filtrate was transferred to 250 mL sterile polypropylene centrifuge bottles. The filtrate was then centrifuged at 5000 rpm for 20 minutes in a Rotek centrifuge. The supernatant was carefully decanted without disturbing the spore pellet deposited at the bottom (Plate 2 A). The pellet was washed twice in a small quantity of sterile water to remove any traces of mycelia. The washed pellet was then dispensed in minimal quantity of sterile water to get a spore concentrate. This was serially diluted to obtain a spore count of 10^8 (Plate 2 B), to ensure a spore concentration of 10^7 spores mL^{-1} in the spray solution to be applied in the field.

Oil formulation was prepared by mixing spore and carrier material in the ratio 35:65 (Plate 2 C). The carrier material comprised of groundnut oil enriched with 0.1 per cent colloidal chitin and adjuvant combination (AC1). Required quantities of the various components were transferred into a reagent bottle, sterilized and cooled to room temperature. UV protectant 1 per cent was added as a tank mix.

(A) Spore pellet

(B) Spore concentrate



(C) Chitin enriched oil formulations

Plate 2. Stages of preparation of chitin enriched oil formulations

3.6.1.1.1 Preparation of Colloidal Chitin

Chitin flakes procured from Matsyafed, Neendakara was treated with concentrated hydrochloric acid in ice cold conditions under constant stirring for thirty minutes. The mixture was filtered through a layer of glass wool and the filtrate was collected in constantly agitated ice cold water. The milky suspension thus obtained was filtered through Whatman™ No. 1 filter paper. The colloidal chitin that deposited on the sides of the funnel was washed several times until pH reached neutrality. This was collected, dried and stored under refrigeration.

3.6.1.2 Talc Based Formulations

M. anisopliae (Ma4) and *B. bassiana* (Bb5) cultures maintained in the Biocontrol Laboratory, Department of Agricultural Entomology, College of Agriculture, Vellayani were cultured on potato dextrose broth. Fourteen day old cultures were whirred in a blender for 15 seconds and the resultant mixture was mixed with talc, three times the quantity of blended mix so as to ensure a spore concentration of 10^9 spores mL^{-1}(to ensure a spore concentration of 10^8 in the spray solution to be applied in the field). This was air dried for three days and pulverized to a fine powder and stored in sealed plastic bags.

3.6.1.3 Spore Suspensions of Fungi

Spore suspensions of *L. lecanii* and *L. saksenae* for field application were prepared as per procedure described in 3.1.4.1.

3.6.2 Preparation of Spray Solutions

Spray solutions of oil formulations were prepared at 1 per cent concentration (10 mL^{-1}) and that of talc based formulations were prepared at 2 per cent (20g L^{-1}). The solutions prepared from talc based formulation were sieved through a muslin cloth before use.

3.6.3 Spraying

Spraying operations were initiated when the population of the bugs reached ETL. A pneumatic hand sprayer of one litre capacity was used for spraying. The quantity of spray fluid was 250 mL $plot^{-1}$. Sunpack screens were placed on all sides of the plot to prevent drift. The bioformulations were sprayed with coarse droplet adjustment of the nozzle. While spraying the spray lance was kept at fixed height from the canopy in all plots to ensure uniform deposition. Spraying was carried out in a spiral fashion, commencing from the border rows towards the centre to minimize the dispersal of rice bug from one plot to another. A separate sprayer was maintained for insecticide spraying.

3.6.4 Assessment of Rice Bug Population

Population was assessed based on sweep and count data. Cumulative count of bugs in five sweeps and five random hills $plot^{-1}$ was recorded (Smitha, 2004). Sweeps were done taking diagonal walk across the plot, avoiding border rows. A sweep comprised of a clockwise and an anticlockwise movement of the wrist, with hand outstretched and net firmly.

Pretreatment counts were taken before treatment and post treatment counts were taken at intervals of three, seven, 10 and 14 days. Spraying was repeated after 14 days when population regained ETL.

3.6.5 Assessment of Natural Enemy Population

Population of natural enemies was assessed in the same way as that of rice bug, based on counts from five sweeps $plot^{-1}$. Observations were recorded separately on the number of predatory spiders, predatory insects (coccinellids, mirids, and reduviids) and parasitoids.

Gross yield, net yield and straw yield were recorded at the time of harvest.

3.6.6 Statistical Analysis

The data obtained from laboratory and field experiment were subjected to analysis of variance (ANOVA) using WASP 1 software and the treatment differences were compared. Probit analysis was carried out using SPSS Ver. 21 to work out the LC_{50}, LC_{90}, and LT_{50},with fiducial limits fixed at 95 per cent.

Results

4. RESULTS

Efficacy of chitin enriched bioformulations of the indigenous isolate *Lecanicillium saksenae* (ITCC Accession No. LsVs 1 7714) and the NBAIR isolate *L. lecanii* (Vl 8) on sucking pests of rice and their impact on natural enemies of rice ecosystem are depicted below.

4.1. PATHOGENICITY OF *L. saksenae* AND *L. lecanii*

The response of sucking pests namely rice bug, *Leptocorisa acuta* (Thunberg), black bug, *Scotinophara coarctata* (F.), shield bug, *Menida versicolor* (Gmelin), brown planthopper, *Nilaparvata lugens* (Stal), green leafhopper, *Nephotettix nigropictus* (Stal), white leafhopper, *Cofana spectra* (Distant) and white winged planthopper, *Nisia nervosa* (Motschulsky) when tested for their susceptibility to *L. saksenae* and *L. lecanii* is presented in Table 1.

Disease symptoms of pests which showed response is detailed below

4.1.1. *Leptocorisa acuta*

4.1.1.1 L. saksenae

Eggs of *L. acuta* treated with spore suspension of *L. saksenae* @ 10^{7} spores mL^{-1} did not take infection. Mycosis was absent and the eggs hatched normally as in the case of untreated eggs.

Following 6-8 h post treatment, the nymphs and adults exhibited restlessness. They were constantly combing the dorsal part of their body, wings and antennae with forelegs, as if to remove an extraneous substance. Post 10-14 h of treatment, the bugs were unable to cling onto the sides of the rearing jars and were seen frequently slipping down to the bottom of the jar. Typical symptoms of ataxis commenced after this and the bugs got aggregated. At 20-24 hours after treatment (HAT), the bugs were seen lying on their back, showing convulsions, wherein, the same jerking type of

Table 1. Pathogenicity of *Lecanicillium* spp to sucking pests of rice

Sl. No	Pest		Taxonomic position	Response	
	Common name	Scientific name		*L. saksenae*	*L. lecanii*
1	Rice bug	*Leptocorisa acuta*	Hemiptera Alydidae	+ve	-ve
2	Black bug	*Scotinophara coarctata*	Hemiptera Pentatomidae	-ve	-ve
3	Shield bug	*Menida versicolor*	Hemiptera Pentatomidae	-ve	-ve
4	Brown planthopper	*Nilaparvata lugens*	Hemiptera Delphacidae	+ve	+ve
5	Green leafhopper	*Nephotettix nigropictus*	Hemiptera Cicadellidae	+ve	+ve
6	White leafhopper	*Cofana spectra*	Hemiptera Cicadellidae	+ve	+ve
7	White winged planthopper	*Nisia nervosa*	Hemiptera Meenoplidae	+ve	+ve

movement repeated, with occasional twitching of legs and arching of abdomen. The convulsions faded as the bug slipped into paralysis and death.

Mortality occurred 1 h after exhibiting convulsions. Complete mortality of nymphs and adults was recorded at 72 HAT. Cadavers exhibited growth of white mycelia, initially emerging from the legs, antennal bases, inter segmental membranes of the abdominal region and around the compound eyes, gradually spreading over the entire body, five days after death (Plate 3 A and B).

4.1.1.2 L. lecanii

L. lecanii was found to be non infective to the eggs of *L. acuta*. They hatched normally, fed actively and attained maturity during the experimental period.

Infectivity trials of *L. lecanii* showed similar results on nymphs and adults. There was no abnormal behavior. Mortality was not recorded during the period under observation. Cadavers placed in moist chamber did not develop any mycelial growth.

4.1.2 *Scotinophara coarctata*

Both *L. saksenae* and *L. lecanii* were non infective to the black bug.

4.1.3 *Menida versicolor*

The shield bug was resistant to infections from *L. saksenae* and *L. lecanii*.

4.1.4 *Nilaparvata lugens*

4.1.4.1 L. saksenae

Nymphs and adults were susceptible to *L. saksenae*. Hoppers became inactive at 24 HAT and were found idle on the bottom of the rearing jars. Both the nymphs as well as adults started dying at this point of time. White fluffy mycelia emerged from the cadaver four days post mortality (Plate 3 C). The mycelia initially emerged from

the intersegmental membrane of abdomen, around the compound eyes and later covered the entire body into a cottony mass. All the nymphs and adults were killed at 120 HAT.

4.1.4.2 L. lecanii

Symptoms and mortality were similar to that of *L. saksenae*. There was variation in the time taken for mortality. Hundred per cent mortality of nymphs occurred at 168 HAT. Cadavers were covered with white mycelia four days after mortality (Plate 4 A).

4.1.5 *Nephotettix nigropictus*

4.1.5.1 L. saksenae

Symptoms of mycosis observed in *Nephotettix*, were similar in all respects to that in *N. lugens*. The time taken for mortality varied. Mycelial growth was visible on fifth day of mortality (Plate 3 D). Hundred per cent mortality of nymphs and adults was observed at 72 and 96 HAT, respectively

4.1.5.2 L. lecanii

Spores of *L. lecanii* were infective to both nymphs and adults of *N. nigropictus*. Hundred per cent mortality of nymphs and adults occurred at 144 HAT and 168 HAT. Symptoms of mycosis resembled that of *L. saksenae*, but there was no mycelial growth covering the wings as found in *L. saksenae* and white mycelial growth was obtained five days after treatment (Plate 4 B).

4.1.6 *Cofana spectra*

4.1.6.1 L. saksenae

Hoppers treated with *L. saksenae* became inactive few hours post treatment. Symptoms of mycosis resembled that of *N. lugens*. Complete mortality of adults

occurred within 96 HAT. The treated insects lost their creamy white colour and became tanned and failed to develop mycelia even after 20 days post mortality. The cadaver resembled empty shell (Plate 3 E).

4.1.6.2 L. lecanii

Spores of *L. lecanii* were infective to *C. spectra* and symptoms were similar to that of *N. lugens*. Complete mortality occurred at 168 HAT. The treated insects lost their lustrous white colour and did not develop any mycelia even after 20 days post mortality.

4.1.7 *Nisia nervosa*

4.1.7.1 L. saksenae

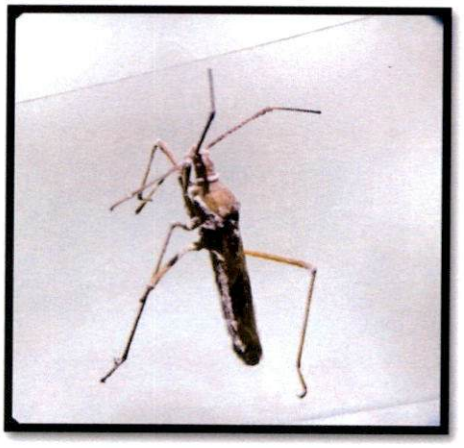
Prior to death, the white dusty coating on the body of the adult hoppers were shed off. Cadavers lost their natural creamy white colour and later turned brown. The mycelia did not cover the wings as in *N. lugens* (Plate 3 F). Nymphs and adults were killed at 72 HAT.

4.1.7.2 L. lecanii

Symptoms of mycosis were similar to that of *L. saksenae*. The time of kill was delayed much. Mycelial growth was observed four days after mortality. Emergence was initially from the inter segmental regions of abdomen, joints of legs which later spread over the entire body, in a similar way found in *N. lugens* (Plate 4 C).

4.2 CROSS INFECTIVITY TO RICE PLANTS

The spore suspensions of the test fungi when inoculated @ 10^9 spores mL^{-1} by leaf and soil inoculation methods, did not produce any noticeable symptoms for a



(A) Mycelial emergence from *Leptocorisa acuta*

(B) Cadaver of *Leptocorisa acuta* covered with mycelia

(C) *Nilaparvata lugens*

(D) *Nephotettix nigropictus*

(E) *Cofana spectra*

(F) *Nisia nervosa*

Plate 3. Symptoms of mycosis of *Lecanicillium saksenae* on sucking pests of rice

(A) *Nilaparvata lugens*

(B) *Nephotettix nigropictus*

(C) *Nisia nervosa*

Plate 4. Symptoms of mycosis of *Lecanicillium lecanii* on sucking pests of rice

period of one month and the crop completed its growth without any noticeable changes.

4.3 SAFETY TO NATURAL ENEMIES

Spore suspensions of *L. saksenae* and *L. lecanii* @ 10^9 spores mL^{-1} when sprayed on natural enemies *viz.*, eggs, grubs, and adults of the coccinellids, *Micraspis discolor* (F.) and *Coccinella transversalis* F., the adults of the mirid, *Cyrtorhinus lividipennis* Reuter, the carabid, *Ophionea nigrofasciata* Schmidt-Gobel, and the spiders *Tetragnatha maxillosa* (Thorell) and *Oxyopes shweta* Tikader, did not cause any mycosis nor any mortality.

4.4 EFFECTIVE DOSES OF THE FUNGI

Infectivity of the indigenous isolate *L. saksenae* and the exotic isolate *L. lecanii* in terms of effective dose was compared based on the dose - mortality response of various stages of test insects treated with spore suspensions.

4.4.1 *Lecanicillium saksenae*

4.4.1.1 L. acuta Nymphs

Dose-mortality response of third instar nymphs treated with varying spore concentrations of *L. saksenae* is indicated in Table 2.

At 24 HAT, the highest mortality of 93.33 per cent observed with 10^8 spores mL^{-1}, was statistically on par with the mortality percentage observed with 10^7 (86.67). The corresponding mortality observed with 10^6 spores mL^{-1} was 60 per cent and that with 10^5 spores mL^{-1} was 10 per cent. Lower doses of 10^4 and 10^3 caused negligible rate ranging from 0 and 3.33 per cent.

At 48 HAT, both 10^8 and 10^7 spores mL^{-1} were on par, recording 100 and 93.33 per cent mortality respectively. Seventy per cent mortality was observed with

Table 2. Dose - mortality response of *L. saksenae* to *L. acuta* nymphs

Treatments (Spores mL^{-1})	Mortality at 24 h interval (%)						
	24	**48**	**72**	**96**	**120**	**144**	**168**
10^8	93.33^a (77.40)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)
10^7	86.67^a (68.850)	93.33^a (77.40)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)
10^6	60.00^b (50.86)	70.00^b (56.99)	73.33^b (59.00)	83.33^b (66.15)	96.67^b (68.85)	100^a (89.09)	100^a (89.09)
10^5	10.00^c (18.44)	13.33^c (21.15)	23.33^c (28.78)	36.67^c (37.23)	46.67^c (41.15)	63.33^b (47.01)	76.67^b (53.07)
10^4	0^d (2.87)	10.00^{cd} (15.31)	16.67^c (23.85)	16.67^d (23.85)	26.67^d (23.85)	33.33^c (28.78)	43.33^c (35.22)
10^3	3.33^d (6.75)	3.33^{de} (6.75)	3.33^d (6.75)	10.00^d (15.31)	13.33^d (21.15)	23.33^c (26.07)	30.00^c (30.99)
Control	0^d (0.91)	0^e (0.91)	0^d (0.91)	0^e (0.91)	0^e (0.91)	0^d (0.91)	0^d (0.91)
C.D (0.05)	10.701	13.862	8.210	9.997	9.970	8.098	7.123

Figures in parentheses are angular transformed values. No. of insects per replication: 10. Values sharing same alphabets in superscript are statistically on par based on ANOVA

10^6 spores mL^{-1}. The mortality observed with 10^5 spores mL^{-1} was 13.33 and those with 10^4 and 10^3 were 10 and 3.33 respectively.

Complete mortality was recorded with 10^8 and 10^7spores mL^{-1} at 72 HAT. Treatment with 10^6 spores mL^{-1} resulted in 73.33 per cent mortality of nymphs. The lower doses 10^5 and10^4 caused only 23.33 and 16.67 per cent respectively. The lowest dose 10^3 recorded least mortality of 3.33 per cent.

At 96 HAT, 83.33 per cent death rate was observed with 10^6 spores mL^{-1}. The lower doses 10^5, 10^4 and 10^3 recorded less than 50 per cent 36.67, 16.67 and 10 respectively).

Similar trend was noted on the fifth day (120 HAT), the lower doses 10^5, 10^4 and 10^3 recorded lower death rates of 46.67, 26.67 and 13.33 respectively.

On the sixth day (144 HAT), 10^6 spores mL^{-1} caused 100 per cent mortality while 63.33 per cent was recorded with 10^5 spores mL^{-1}, the mortality was less than 50, with lower doses of 10^4 and 10^3 spores mL^{-1} (33.33 and 23.33 per cent, respectively).

At the end of the experimental period, (seventh day- 168 HAT), 10^5 spores mL^{-1} caused 76.67 per cent death. Corresponding death rate was 43.33 and 30.00 with 10^4 and 10^3 spores mL^{-1}, respectively.

None of the untreated insects died during the experimental period

4.4.1.2 L. acuta Adults

The dose - mortality response of *L. saksenae* to adults of *L. acuta* is furnished in Table 3. At 24 HAT, 10^7 spores mL^{-1} caused 70 per cent mortality which was statistically on par with that of 10^8 spores mL^{-1}(63.33 per cent). While 36.67 per cent mortality was recorded with 10^6 spores mL^{-1}, none of the treated insects died with 10^5, 10^4, and 10^3 spores mL^{-1}

Table 3. Dose - mortality response of *L. saksenae* to *L. acuta* adults

Treatments (Spores mL^{-1})	Mortality at 24 h interval (%)						
	24	**48**	**72**	**96**	**120**	**144**	**168**
10^8	63.33^a (52.85)	96.67^a (83.25)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)
10^7	70.00^a (56.99)	83.33^b (66.15)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)
10^6	36.67^b (24.44)	53.33^c (46.93)	66.67^b (54.78)	73.33^b (65.56)	86.67^b (68.85)	100^a (89.09)	100^a (89.09)
10^5	0^c (0.91)	6.66^d (12.60)	6.67^c (12.60)	26.67^c (30.99)	43.33^c (41.15)	53.33^b (47.01)	63.33^b (53.07)
10^4	0^c (0.91)	0^e (0.91)	3.33^c (6.75)	10.00^{cd} (15.31)	16.67^d (21.15)	23.33^c (28.78)	33.33^c (35.22)
10^3	0^c (0.91)	0^e (0.91)	3.33^c (6.75)	6.67^d (12.60)	10.00^d (15.31)	20.00^c (26.07)	26.67^c (30.99)
Control	0^c (0.91)	0^e (0.91)	0^c (0.91)	0^d (0.91)	0^e (0.91)	0^d (0.91)	0^d (0.91)
C.D (0.05)	14.965	10.215	11.824	17.687	9.970	8.098	7.123

Figures in parentheses are angular transformed values. No. of insects per replication: 10. Values sharing same alphabets in superscript are statistically on par based on ANOVA

After 48 h, the highest spore load (10^8) killed 96.67 per cent of test insects, while 10^7 and 10^6 were the succeeding superior treatments, with 83.33 and 53.33 per cent mortality, respectively. A negligible death rate of 6.66 per cent was recorded in treatment 10^5, while no mortality was recorded at all in subsequent lower doses 10^4 and 10^3 spore mL^{-1}.

All the test insects were killed in treatments 10^8 and 10^7, at 72 HAT. When 66.67 per cent insects were killed in 10^6 spores mL^{-1}, the lower doses 10^5, 10^4 and 10^3 caused significantly lower mortality (6.67,3.33and 3.33 per cent respectively).

At 96 HAT, 73.33 per cent death rate was observed with 10^6 spores mL^{-1}. The lower doses 10^5, 10^4, and 10^3 recorded less than 50 per cent (26.67, 10 and 6.67 respectively).

Similar trend was noted on 120 HAT where the lower doses 10^5,10^4 and 10^3 recorded lower death rates of 43.33, 16.67 and 10 respectively.

At 144 HAT 100 per cent death was noted with spore load of 10^6 also. Corresponding death rate noted with lower doses of 10^5, 10^4, and 10^3 were 53.33, 23.33 and 20 per cent, respectively.

At the end of the experimental period (168 HAT), 10^5 spores mL^{-1} caused 63.33 per cent death. A negligible death rate of 33.33 and 26.67 was noted with 10^4 and 10^3 spores mL^{-1}.

Untreated insects did not die during the experimental period

4.4.1.3 N. lugens Nymphs

Dose - mortality response of *L. saksenae* to nymphs of *N. lugens* is indicated in Table 4. Highest spore concentration of 10^8 spores mL^{-1} caused 36.67 per cent mortality, which was statistically on par with 10^7 and 10^6 spores mL^{-1} (30 and 26.67

Table 4. Dose - mortality of *L. saksenae* to *N. lugens* nymphs

Treatments (Spores mL^{-1})	Mortality at 24 h interval (%)						
	24	**48**	**72**	**96**	**120**	**144**	**168**
10^8	36.67^a (37.15)	66.67^a (54.99)	80.00^a (63.93)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)
10^7	30.00^a (33.00)	50.00^b (45.00)	70.00^{ab} (56.99)	86.67^b (68.85)	100^a (89.09)	100^a (89.09)	100^a (89.09)
10^6	26.67^a (30.29)	50.00^b (45.00)	63.33^b (53.07)	80.00^b (63.93)	86.67^b (68.85)	100^a (89.09)	100^a (89.09)
10^5	10.00^b (18.44)	10.00^c (18.44)	30.00^c (33.00)	40.00^c (39.14)	43.33^c (41.07)	53.33^b (46.93)	60^b (50.86)
10^4	0^c (0.91)	10.00^c (18.44)	13.33^d (21.15)	23.33^d (28.78)	33.33^c (35.22)	40.00^c (39.24)	53.33^{bc} (46.93)
10^3	0^c (0.91)	0^d (0.91)	10.00^d (18.44)	23.33^d (28.78)	33.33^c (35.22)	43.33^c (41.15)	46.67^c (43.07)
Control	0^c (0.91)	0^d (0.91)	0^e (0.91)	0^e (0.91)	0^d (0.91)	0^d (0.91)	0^d (0.91)
C.D 0.05%	9.385	6.166	10.386	7.862	6.364	4.926	4.998

Figures in parentheses are angular transformed values. No. of insects per replication: 10. Values sharing same alphabets in superscript are statistically on par based on ANOVA

per cent respectively). The next superior treatment was 10^5 spores mL^{-1} with 10.0 per cent mortality. There was no mortality with lower doses, 10^4 and 10^3 spores mL^{-1}.

Mortality recorded at 48 HAT was 66.67 per cent when treated with 10^8 spores mL^{-1}. Fifty per cent mortality was observed with 10^7 and 10^6 spores mL^{-1}. A percentage mortality of 10 was observed on both 10^5 and 10^4 spores mL^{-1} which were statistically on par with each other. No mortality was observed with the lowest dose.

At 72 HAT, 10^8 spores mL^{-1} recorded 80 per cent mortality, which was statistically superior to all other treatments, while 70 and 63.33 per cent mortalities were observed for 10^7 and 10^6 spores mL^{-1}, respectively. In all other doses the percentage mortality was less than 50 (30, 13.33 and 10 with 10^5, 10^4 and 10^3 respectively).

Complete mortality of *N. lugens* was observed on the fourth day (96 HAT) when treated with 10^8 spores mL^{-1}, which was superior to all other treatments. A mortality of 86.67, and 80 per cent was observed in treatments with $10^7$and 10^6 spores mL^{-1} and were on par. The lower doses 10^5, 10^4 and 10^3 resulted in 40, 23.33 and 23.33 per cent mortality, respectively. There was no death of untreated insects till the experiment was terminated.

4.4.1.4 N. lugens Adults

Observations on adults of *N. lugens* (Table 5) treated with *L. saksenae* spores revealed that, the mortality was very low at 24 HAT. It was 26.67 per cent with 10^8 spores mL^{-1}, followed by 13.33 and 6.66 per cent in 10^7 and 10^6. No mortality was recorded for lower doses of 10^5,10^4 and 10^3 spores mL^{-1}.

At 48 HAT, 10^8 and 10^7 spores mL^{-1} was statistically on par with 70 and 60 per cent mortality respectively. Adults treated with 10^6 spores mL^{-1} resulted in 46.67 per cent mortality which was statistically similar to the mortality recorded with 10^7 spores mL^{-1}. Lower dose, 10^5 spores yielded, 16.67 per cent mortality. Lower dose

Table 5. Dose - mortality of *L. saksenae* to *N. lugens* adults

Treatments (Spores mL^{-1})	Mortality at 24 h interval (%)						
	24	**48**	**72**	**96**	**120**	**144**	**168**
10^8	26.67^{a} (30.78)	70.00^{a} (56.99)	83.33^{a} (66.15)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)
10^7	13.33^{ab} (18.01)	60.00^{a} (51.15)	83.33^{a} (69.78)	93.33^{a} (77.40)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)
10^6	6.66^{bc} (12.60)	46.67^{a} (43.07)	66.67^{ab} (55.78)	90.00^{a} (74.69)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)
10^5	0^{c} (0.90)	16.67^{b} (23.85)	33.33^{bc} (22.45)	50.00^{b} (45.00)	53.33^{b} (46.93)	63.33^{b} (52.77)	66.67^{b} (54.99)
10^4	0^{c} (0.90)	6.67^{bc} (12.60)	10^{cd} (15.31)	20.00^{bc} (26.07)	33.33^{bc} (34.92)	43.33^{bc} (41.07)	46.67^{bc} (42.99)
10^3	0^{c} (0.90)	0^{c} (0.90)	6.67^{d} (9.46)	13.33^{cd} (13.68)	20.00^{c} (21.45)	23.33^{c} (23.37)	33.33^{c} (34.64)
Control	0^{c} (0.90)	0^{c} (0.90)	0^{d} (0.91)	0^{d} (0.91)	0^{d} (0.91)	0^{d} (0.91)	0^{d} (0.91)
C.D (0.05)	12.820	17.030	23.383	18.943	16.094	17.907	12.009

Figures in parentheses are angular transformed values. No. of insects per replication: 10. Values sharing same alphabets in superscript are statistically on par based on ANOVA

of 10^4, showed negligible rate of 6.67, while there was no death in the lowest concentration of 10^3 spores mL^{-1}.

After 72 h, both 10^8 and 10^7 spores mL^{-1} caused similar mortality of 83.33 per cent, followed by 66.67 and 33.33 per cent, with 10^6 and 10^5 spores mL^{-1} respectively. Lower spore doses, 10^4 caused only 10 per cent mortality while there was no death in 10^3 spores mL^{-1}, as well as in untreated insects.

Hundred per cent mortality was achieved after 96 h in highest spore load of 10^8 spores mL^{-1}. Statistically similar results were observed for 10^7 and 10^6 spores mL^{-1} with mortality percentage of 93.33 and 90, respectively. Half of the population died with 10^5 spores mL^{-1}, whereas lower doses recorded only less than 20 per cent mortality. Both 10^7 and 10^6 spores mL^{-1} recorded complete mortality of the test insects 120 HAT, while only 53.33, 33.33 and 20 per cent mortality was observed for lower doses in their order of decreasing concentration from 10^5 spores mL^{-1} to 10^3 spores mL^{-1}. None of the insects died in the untreated lot.

4.4.1.5 N. nigropictus Nymphs

Dose-mortality response of *Nephotettix* nymphs to *L. saksenae* is presented in Table 6. At 24 HAT, treatment with 10^8 spores mL^{-1}recorded 93.33 per cent mortality which was followed by the mortality caused by 10^7 (83.33 per cent). Though a lesser rate of 66.67 per cent was noted with 10^6 spores mL^{-1}, as observed in other hoppers, lower doses of 10^5,10^4 and 10^3 caused negligible (10 per cent) or no mortality at all.

All the treated insects died at 48 HAT, with the highest spore dose 10^8 which was statistically on par with 96.67 per cent death rate observed with 10^7 spore mL^{-1}. The spore dose, 10^6 yielded 80 per cent mortality, but the lower doses were not effective, the mortality being 13.33, 6.67 and 3.33 respectively with 10^5,10^4 and 10^3 spores mL^{-1}.

Table 6. Dose - mortality response of *L. saksenae* to *N. nigropictus* nymphs

Treatments (Spores mL^{-1})	Mortality at 24 h interval (%)						
	24	**48**	**72**	**96**	**120**	**144**	**168**
10^8	93.33^a (80.54)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)
10^7	83.33^b (66.15)	96.67^a (83.25)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)
10^6	66.67^c (54.78)	80.00^b (63.93)	90.00^b (74.70)	96.67^a (83.25)	100^a (89.09)	100^a (89.09)	100^a (89.09)
10^5	10.00^d (18.44)	13.33^c (21.15)	16.67^c (23.85)	26.67^b (30.99)	43.33^b (41.15)	60.00^b (50.86)	70.00^b (56.99)
10^4	0^e (0.91)	6.67^{cd} (12.60)	10.00^c (18.44)	20.00^{bc} (26.07)	30.00^c (33.00)	36.67^c (37.23)	46.67^c (43.07)
10^3	0^e (0.91)	3.33^d (6.75)	6.67^c (12.60)	13.33^c (21.15)	16.67^d (23.85)	26.67^d (30.99)	30^d (35.22)
Control	0^e (0.91)	0^d (0.91)	0^d (0.91)	0^d (0.91)	0^d (0.91)	0^e (0.91)	0^e (0.91)
C.D (0.05)	10.541	12.978	11.397	9.216	5.661	5.195	5.263

Figures in parentheses are angular transformed values. No. of insects per replication: 10. Values sharing same alphabets in superscript are statistically on par based on ANOVA

By the third day (72 HAT), all the insects treated with 10^7 spores were also dead. Spore dose of 10^6 could result in 90 per cent mortality, but still lower doses were found to be insufficient to yield a fairly high death rate. It was 16.67, 10 and 6.67 when treated with doses of 10^5, 10^4, and 10^3, respectively.

At 120 HAT, 10^6 spores mL^{-1} also recorded 100 per cent mortality. The death rate was too low in 10^5, 10^4, 10^3, with 43.33, 30, and 16.67 per cent mortalities respectively.

4.4.1.6 N. nigropictus Adults

The mortality of *N. nigropictus* adults treated with different doses of *L. saksenae* is compiled in Table 7, shows that the highest dose of 10^8 spores mL^{-1} was effective, causing 80 per cent mortality of adult hoppers 24 HAT, which was closely followed by the mortalities observed with 10^7, 10^6 spores mL^{-1} (73.33 and 56.67 per cent respectively). The doses 10^5, 10^4 and 10^3 were found to be insufficient causing negligible death (6.67) or no death at all.

At 48 HAT, 90 per cent death was recorded with 10^8 spores mL^{-1}, closely followed by 86.67 per cent mortality caused by 10^7 spores mL^{-1}. The dose 10^6 spores mL^{-1} caused 70 per cent death. As noted in all the previous cases the doses, 10^5, 10^4 and 10^3 caused insignificant death rates of 6.67 and 3.33 or no death of the treated insects.

Highest spore concentration of 10^8 spores mL^{-1} was found to be superior (100 per cent mortality) than 10^7 spores mL^{-1} (93.33 per cent) and 10^6 spores mL^{-1}, (80 per cent). Significantly low mortality was recorded with spore concentrations of 10^5, 10^4, and, 10^3 with 13.33, 10 and 3.33 per cent mortalities, respectively.

Second highest spore concentration of 10^7 spores mL^{-1} caused 100 per cent and mortality at 96 HAT, while 10^6 spores mL^{-1} resulted in 90 per cent mortality. The mortality trend for lower spore doses remained similar to previous days with 10^5,

Table 7. Dose - mortality response of *L. saksenae* to *N. nigropictus* adults

Treatments (Spores mL^{-1})	Mortality at 24 h interval (%)						
	24	**48**	**72**	**96**	**120**	**144**	**168**
10^8	80.00^{a} (63.93)	90.00^{a} (74.69)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)
10^7	73.33^{ab} (59.00)	86.67^{ab} (68.85)	93.33^{b} (77.40)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)
10^6	56.67^{b} (48.85)	70.00^{b} (56.99)	80.00^{c} (63.93)	90.00^{b} (74.69)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)
10^5	6.67^{b} (9.46)	6.67^{c} (9.46)	13.33^{d} (21.15)	23.33^{c} (28.78)	30.00^{b} (33.22)	50.00^{b} (45.00)	60.00^{b} (50.86)
10^4	0^{c} (0.91)	3.33^{c} (6.75)	10.00^{d} (18.44)	16.67^{cd} (23.85)	23.33^{b} (28.78)	26.67^{c} (30.99)	33.33^{c} (35.22)
10^3	0^{c} (0.91)	0^{c} (0.91)	3.33^{e} (6.75)	10.00^{d} (15.31)	10.00^{c} (15.30)	13.33^{d} (21.15)	20.00^{d} (26.07)
Control	0^{c} (0.91)	0^{c} (0.91)	0^{e} (0.91)	0^{e} (0.91)	0^{d} (0.91)	0^{e} (0.91)	0^{e} (0.91)
C.D (0.05)	11.463	15.600	11.101	12.914	9.044	5.535	6.673

Figures in parentheses are angular transformed values. No. of insects per replication: 10. Values sharing same alphabets in superscript are statistically on par based on ANOVA

10^4,10^3, recording very low mortality to the tune of 23.33, 16.67, and 10 per cent, respectively.

At 120 HAT, 10^6 spores mL^{-1} caused 100 cent per cent mortality of test insects. Lower doses still remained ineffective with 30, 23.33 and 10 per cent mortality with the concentrations 10^5, 10^4 and 10^3 spores mL^{-1}, respectively.

4.4.1.7 N. nervosa Nymphs

Dose - mortality response of *L. saksenae* to nymphs of *N. nervosa* is indicated in Table 8. At 24 HAT, highest spore dose of 10^8 killed 93.33 per cent of nymphs while 10^7 and 10^6 spores mL^{-1} killed 80 and 73.33 per cent respectively. As with the case of other hoppers 10^5, 10^4 and 10^3 caused insignificant death rate (6.67) or no death at all.

Hundred per cent mortality was caused by 10^8 spores mL^{-1} at 48 HAT, which was statistically on par with the mortalities caused by both 10^7 and 10^6 (89.33 and 86.67 respectively). Least mortality of 6.67 was recorded for 10^5 and 10^4 spores mL^{-1} and 3.33 per cent mortality for the lowest dose.

At 72 HAT, 10^7 and 10^6 spores mL^{-1} caused mortality of all test insects, while 10^5 and 10^4 spores mL^{-1} recorded 20 per cent each. Only 10 per cent mortality was recorded in lowest spore dose of 10^3 spores mL^{-1}.

4.4.1.8 N. nervosa Adults

Mortality of adult hoppers of *N. nervosa* to varying spore concentrations of *L. saksenae* is furnished in Table 9. At 24 HAT, highest mortality was recorded in 10^8 spores mL^{-1} (83.33) while, 10^7 and 10^6 spores mL^{-1} recorded 76.67 and 66.67 per cent mortality, respectively. No mortality was recorded in lower spore concentrations.

Table 8. Dose - mortality response of *L. saksenae* to *N. nervosa* nymphs

Treatments (Spores mL^{-1})	Mortality at 24 h interval (%)						
	24	**48**	**72**	**96**	**120**	**144**	**168**
10^8	93.33^a (80.54)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)
10^7	80.00^{ab} (67.77)	89.33^a (80.54)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)
10^6	73.33^b (59.22)	86.67^a (71.99)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)	100^a (89.09)
10^5	6.67^c (9.46)	6.67^b (9.46)	20.00^b (26.56)	33.33^b (35.22)	46.67^b (43.07)	53.33^b (46.93)	63.33^b (52.77)
10^4	0^c (0.91)	6.67^b (9.46)	20.00^b (26.07)	26.67^c (21.15)	36.67^c (37.15)	43.33^b (41.07)	50.00^c (45.00)
10^3	0^c (0.91)	3.33^b (6.75)	10^c (18.44)	13.33^c (18.01)	20.00^d (26.56)	26.67^c (30.99)	33.33^d (35.22)
Control	0^c (0.91)	0^b (0.91)	0^d (0.91)	0^d (0.91)	0^e (0.91)	0^d (0.91)	0^e (0.91)
C.D (0.05)	19.563	20.725	4.897	10.541	5.010	6.795	5.019

Figures in parentheses are angular transformed values. No. of insects per replication: 10. Values sharing same alphabets in superscript are statistically on par based on ANOVA

Table 9. Dose - mortality response of *L. saksenae* to *N. nervosa* adults

Treatments (Spores mL^{-1})	Mortality at 24 h interval (%)						
	24	**48**	**72**	**96**	**120**	**144**	**168**
10^8	83.33^{a} (69.78)	93.33^{a} (77.40)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)
10^7	76.67^{ab} (61.22)	90.00^{a} (74.69)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)
10^6	66.67^{b} (54.78)	76.67^{a} (61.22)	86.67^{b} (68.85)	93.33^{a} (77.40)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)
10^5	0^{c} (0.91)	6.67^{b} (9.46)	6.67^{cd} (9.46)	20.00^{b} (26.07)	40.00^{b} (38.85)	53.33^{b} (46.93)	66.67^{b} (55.08)
10^4	0^{c} (0.91)	0^{b} (0.91)	13.33^{c} (21.15)	20.00^{bc} (22.23)	30.00^{bc} (33.00)	40.00^{bc} (39.14)	43.33^{c} (41.07)
10^3	0^{c} (0.91)	3.33^{b} (6.75)	6.67^{cd} (12.60)	13.33^{bc} (21.15)	20.00^{c} (26.56)	26.67^{c} (30.99)	36.67^{c} (37.23)
Control	0^{c} (0.91)	0^{b} (0.91)	0^{d} (0.91)	3.33^{c} (6.75)	3.33^{d} (6.75)	3.33^{d} (6.75)	3.33^{d} (6.75)
C.D (0.05)	11.803	16.364	12.662	17.046	11.257	11.500	11.108

Figures in parentheses are angular transformed values. No. of insects per replication: 10. Values sharing same alphabets in superscript are statistically on par based on ANOVA

At 48 HAT, the death rate was statistically similar in insects treated with 10^8, 10^7, and 10^6 spores mL^{-1} (93.33, 90, and 76.67 respectively). Among the lower spore doses, only 10^5 and10^3 recorded mortality of 6.67 and 3.33 per cent, respectively.

Both 10^8 and 10^7 spores mL^{-1} caused 100 per cent mortality at 72 HAT, while a mortality of 86.67 was recorded with 10^6 spores mL^{-1}. Lower spore doses of 10^5, 10^4, and 10^3 caused lesser mortality of 6.67, 13.33, and 6.67 per cent, respectively.

At 96 HAT, the mortality recorded with 10^6 spores mL^{-1} was 93.33, which was statistically on par with the two superior treatments, 10^8 and 10^7. Twenty per cent mortality was observed for both 10^5 and 10^4 spores mL^{-1}, while 10^3 spores mL^{-1} recorded 13.33 per cent mortality.

Complete mortality was recorded for 10^6 spores at 120 HAT, while lower spore doses of 10^5, 10^4, and 10^3 caused mortality of 40, 30, and 20 per cent respectively

4.4.2. *Lecanicillium lecanii*

4.4.2.1 N. lugens nymphs

The dose - mortality response of *L. lecanii* to nymphs of *N. lugens* is presented in Table 10.

For the first 72 HAT the mortality recorded was minimal with highest mortality recorded in 10^8 spores mL^{-1}(40 per cent), while other treatments recorded further low mortality ranging from 3.33 to 30 percent.

At 96 HAT, the higher spore doses, 10^8 and 10^7 spores mL^{-1} resulted in 66.67 per cent mortality, each. At this point of time, the mortality recorded with other doses was negligible (6.67 to 13.33 per cent).

Table 10. Dose - mortality response of *L. lecanii* to *N lugens* nymphs

Treatments (Spores mL^{-1})	Mortality at 24 h interval (%)						
	24	**48**	**72**	**96**	**120**	**144**	**168**
10^8	3.33	10.00	40.00	66.67^a (55.08)	86.67^a (72.49)	96.67^a (83.25)	100^a (89.09)
10^7	0	10.00	30.00	66.67^a (54.99)	83.33^a (69.78)	93.33^a (77.40)	100^a (89.09)
10^6	3.33	6.67	10.00	13.33^b (21.15)	43.33^b (41.07)	70.00^b (56.99)	86.67^b (71.99)
10^5	3.33	6.67	10.00	13.33^b (21.15)	23.33^{bc} (28.78)	43.33^c (41.15)	60.00^c (50.86)
10^4	0	3.33	3.33	6.67^b (12.60)	10.00^c (21.15)	16.67^d (23.85)	23.33^d (28.78)
10^3	0	3.33	6.67	10.00^b (18.44)	13.33^c (18.44)	16.67^d (23.85)	20.00^d (26.56)
Control	0	0	0	0^c (0.91)	0^d (0.91)	0^e (0.91)	0^e (0.91)
C.D (0.05)	NA	NA	NA	11.219	17.126	11.462	10.860

Figures in parentheses are angular transformed values. NA- Not analyzed. No. of insects per replication: 10. Values sharing same alphabets in superscript are statistically on par based on ANOVA

By the end of 120 h (fifth day), maximum mortality (86.67 and 83.33per cent) was noted with the higher doses (10^8 and 10^7spores mL^{-1}), which were statistically on par. In all other treatments, the death rate was less than 50 (10 to 43.33) per cent.

On the sixth day (144 HAT), the superior treatments were 10^8 and 10^7spores mL^{-1} with 96.67 and 93.33 per cent death respectively. This was followed by 10^6 spores mL^{-1} which recorded 70 per cent mortality. The lower doses resulted in lower death rates ranging from 16.67 to 43.33.

Higher doses 10^8 and 10^7 spores mL^{-1} recorded complete mortality of nymphs. Lower spore dose of 10^6 spores mL^{-1}recorded 86.67 per cent and that of 10^5 resulted in 60 per cent death. A lesser death rate was observed with 10^4 and 10^3spores mL^{-1} (23.33 and 20 respectively).

4.4.2.2 N. lugens Adults

Mortality response of *N. lugens* adults to *L. lecanii* (Table 11) exhibits a similar pattern as that of nymphs to varying doses of spore. For the first 48 h of treatment, less than 10 per cent mortality was observed in all treatments. Spore load of 10^8 recorded 36.67 per cent mortality, while 10^7 recorded 30 per cent, 72 HAT. The mortality for further lower doses (10^6 and below) were very low to the tune of less than or equal to 6.67 per cent.

Significant level mortality of test insects occured only after 96 HAT with 10^8 spores mL^{-1} recording maximum mortality of 50 per cent, which was statistically on par with that of 10^7 spores mL^{-1} (46.67). Mortality observed with 10^6 and 10^5spores $mL^{-1}$was only 30 and 10. Lower doses of 10^4 and 10^3 recorded significantly lower mortality of 3.33 and 6.67 per cent respectively.

Both 10^8 and 10^7 spores mL^{-1} recorded same mortality of 60 per cent at 120 HAT, while 10^6 and 10^5 spores mL^{-1} caused 36.67 and 23.33 per cent mortality, respectively.

Table 11. Dose - mortality of *L. lecanii* to *N lugens* adults

Treatments (Spores mL^{-1})	Mortality at 24 h interval (%)						
	24	**48**	**72**	**96**	**120**	**144**	**168**
10^8	3.33	6.67	36.67	50.00^{a} (45.00)	60.00^{a} (50.86)	83.33^{a} (61.71)	100^{a} (89.09)
10^7	0	3.33	30.00	46.67^{a} (42.99)	60.00^{a} (50.86)	70.00^{ab} (56.99)	76.67^{b} (61.22)
10^6	0	6.67	6.67	30.00^{ab} (33.00)	36.67^{b} (37.15)	53.33^{bc} (47.00)	63.33^{bc} (53.07)
10^5	3.33	3.33	6.67	10.00^{bc} (15.31)	23.33^{bc} (28.78)	40.00^{c} (39.14)	50.00^{c} (45.00)
10^4	0	0	3.33	3.33^{c} (9.46)	10.00^{cd} (18.44)	16.67^{d} (23.85)	26.67^{d} (30.99)
10^3	3.33	3.33	3.33	6.67^{c} (6.75)	10.00^{d} (15.31)	13.33^{d} (21.15)	20.00^{d} (26.07)
Control	0	0	3.33	3.33^{c} (6.75)	3.33^{d} (6.75)	3.33^{e} (6.75)	3.33^{e} (6.75)
C.D (0.05)	NA	NA	NA	18.127	13.326	12.809	11.631

Figures in parentheses are angular transformed values. NA- Not analyzed. No. of insects per replication: 10. Values sharing same alphabets in superscript are statistically on par based on ANOVA

At 144 HAT, 10^8 spores mL^{-1} caused a statistically superior mortality of 83.33 per cent, while 70 and 53.33 per cent mortality was recorded for 10^7 and 10^6 spores mL^{-1}, respectively.

All the test insects were killed with 10^8 spores mL^{-1} at 168 HAT, while 76.67 and 63.33 per cent mortality was recorded for 10^7 and 10^6 spores mL^{-1}. Lower spore doses of 10^5, 10^4, 10^3 caused 50, 26.67 and 20 per cent mortality respectively.

4.4.2.3 N. nigropictus Nymphs

The dose - mortality response of *L. lecanii* to nymphs of *N. nigropictus* is compiled in Table 12. There did not occur any significant death of the treated insects till the end of the third day (72 HAT).

At 96 HAT, highest spore load of 10^8 spores mL^{-1} caused 73.33 per cent mortality, which was on par with that of 10^7 spores mL^{-1} (56.67). The doses 10^6 to 10^3 spores mL^{-1} was found to be insufficient to cause even 50 per cent mortality.

Hundred per cent of the treated insects died with both 10^8 and 10^7 spores mL^{-1} on the sixth day (144 HAT), while only 50 per cent of the population was killed by the dose10^6 spores mL^{-1}. Lower spore doses 10^5, 10^4 and 10^3, caused 33.33, 36.67, and 16.67 per cent mortality, respectively. A similar trend was observed at 168 HAT

4.4.2.4 N. nigropictus Adults

Observation on the adults, treated with different spore concentrations of *L. lecanii* is compiled in Table 13.Until 72 HAT, significant mortality of test insects was not recorded in any of the treatments. Even with the highest spore concentration of 10^8 spores mL^{-1}, only less than 50 per cent mortality was recorded. It ranged from 3.33 to 46.67 per cent.

Table 12. Dose - mortality response of *L. lecanii* to *N. nigropictus* nymphs

Treatments (Spores mL^{-1})	Mortality after 24 h interval (%)						
	24	**48**	**72**	**96**	**120**	**144**	**168**
10^8	10.00	13.33	43.33	73.33^{a} (59.00)	96.67^{a} (83.25)	100^{a} (89.09)	100^{a} (89.09)
10^7	10.00	13.33	33.33	56.67^{a} (49.22)	83.33^{a} (69.78)	100^{a} (89.09)	100^{a} (89.09)
10^6	3.33	6.67	10.00	23.33^{b} (28.780	40.00^{b} (39.14)	50.00^{b} (45.00)	63.33^{b} (53.07)
10^5	6.67	10.00	10.00	16.67^{b} (23.85)	26.67^{bc} (30.99)	33.33^{bc} (35.00)	46.67^{b} (43.07)
10^4	0	3.33	10.00	13.33^{bc} (21.15)	16.67^{bcd} (23.85)	36.67^{cd} (23.85)	46.67^{b} (42.99)
10^3	0	3.33	6.67	10^{bc} (18.44)	13.33^{cd} (21.15)	16.67^{cd} (22.23)	23.33^{c} (28.78)
Control	0	0	0	6.67^{c} (9.46)	6.67^{d} (9.46)	6.67^{d} (9.46)	6.67^{d} (9.46)
C.D (0.05)	NA	NA	NA	14.088	17.593	17.636	13.476

Figures in parentheses are angular transformed values. NA- Not analyzed. No. of insects per replication: 10. Values sharing same alphabets in superscript are statistically on par based on ANOVA

Table 13. Dose - mortality response of *L. lecanii* to *N. nigropictus* adults

Treatments (Spores mL^{-1})	Mortality at 24 h interval (%)						
	24	**48**	**72**	**96**	**120**	**144**	**168**
10^8	3.33	20.00	46.67	66.67^{a} (55.78)	86.67^{a} (72.49)	100^{a} (89.09)	100^{a} (89.09)
10^7	0	6.67	20.00	40.00^{b} (39.24)	63.33^{b} (53.07)	86.67^{b} (71.99)	100^{a} (89.09)
10^6	0	3.33	3.33	20.00^{bc} (26.07)	40.00^{b} (39.14)	50.00^{c} (45.00)	66.67^{b} (54.99)
10^5	0	0	3.33	10.00^{cd} (18.44)	16.67^{c} (23.85)	36.67^{cd} (37.23)	40.00^{c} (39.24)
10^4	0	3.33	3.33	6.67^{de} (9.46)	16.67^{c} (23.85)	23.33^{de} (28.78)	33.33^{c} (35.22)
10^3	0	0	3.33	3.33^{de} (6.75)	6.67^{cd} (12.60)	13.33^{e} (21.15)	20.00^{d} (26.56)
Control	0	0	0	0^{e} (0.91)	0^{d} (0.91)	0^{f} (0.91)	0^{e} (0.91)
C.D (0.05)	NA	NA	NA	15.833	15.269	11.495	5.359

Figures in parentheses are angular transformed values. NA- Not analyzed. No. of insects per replication: 10. Values sharing same alphabets in superscript are statistically on par based on ANOVA

At 96 HAT, the highest dose recorded 66.67 per cent mortality, whereas all other doses resulted in lesser mortality ranging from 3.33 to 40 per cent. The lowest dose did not cause death to the treated insects.

At 120 HAT, 86.67 per cent mortality was observed in 10^8 spores mL^{-1}, while10^7 spores mL^{-1} recorded 63.33 per cent mortality. Forty per cent mortality was caused by 10^6 spores and even lower mortality was recorded in lower doses of 10^5, 10^4, and 10^3 spores mL^{-1} (16.67, 16.67, 6.67 per cent, respectively).

All the treated insects died with highest spore concentration of 10^8 spores mL^{-1} at 144 HAT, while 10^7, and 10^6 spores mL^{-1} caused 86.67 and 50 per cent mortality respectively. A lower mortality of 36.67, 23.33 and 13.33 per cent, were caused by lower spore doses of 10^5, 10^4, and 10^3 spores mL^{-1}, respectively.

At 168 HAT, 10^7 spores mL^{-1} caused 100 per cent mortality. The corresponding mortality with 10^6 spores mL^{-1} was 66.67 per cent. Lower spore doses of 10^5, 10^4, and 10^3 spores mL^{-1}caused 40, 33.33 and 20 per cent mortality, in order of their decreasing spore concentration.

4.4.2.5 N. nervosa Nymphs

Observations on nymphs (Table 14) treated with *L. lecanii* revealed that the mortality was very low (3.33 per cent) or absent at 24 HAT.

Twenty per cent nymphal mortality was recorded with 10^8 spores mL^{-1}, at 48 HAT, while 10 per cent mortality was recorded for both 10^7 and 10^6 spores mL^{-1}. None of the insects were killed by lower doses.

The death rate was very less, even at the end of 72 h. It ranged from 6.67 to 40 per cent, in different concentrations.

Hundred per cent mortality of insects was observed at 120 HAT for 10^8 spores mL^{-1}, while mortality recorded for 10^7 and 10^6 spores mL^{-1} were statistically on par

Table 14. Dose - mortality of *L. lecanii* to *N. nervosa* nymphs

Treatments (Spores mL^{-1})	Mortality at 24 h interval (%)						
	24	**48**	**72**	**96**	**120**	**144**	**168**
10^8	3.33	20.00	40.00	70.00^{a} (56.99)	100^{a} (89.09)	100^{a} (89.09)	100^{a} (89.09)
10^7	0	10.00	23.33	40.00^{b} (39.14)	76.67^{b} (61.22)	90.00^{b} (74.69)	100^{a} (89.09)
10^6	3.33	10.00	20.67	50.00^{bc} (45.00)	63.33^{b} (52.77)	73.33^{c} (59.00)	86.67^{b} (71.99)
10^5	0	0	6.67	23.33^{d} (28.780	36.67^{c} (37.23)	43.33^{d} (41.15)	50.00^{c} (45.00)
10^4	0	0	20	26.67^{cd} (30.99)	33.33^{c} (35.22)	43.33^{d} (41.15)	50.00^{c} (45.00)
10^3	0	0	6.67	10.00^{e} (18.44)	10.00^{d} (14.20)	23.33^{e} (28.78)	33.33^{c} (35.22)
Control	0	0	6.67	6.67^{e} (12.60)	6.67^{d} (12.60)	6.67^{e} (12.60)	6.67^{d} (12.60)
C.D (0.05)	NA	NA	NA	0.789	9.527	11.951	13.247

Figures in parentheses are angular transformed values. NA- Not analyzed. No. of insects per replication: 10. Values sharing same alphabets in superscript are statistically on par based on ANOVA

(76.67 and 63.33). Lower spore doses of 10^5, 10^4, and 10^3 caused mortality of 36.67, 33.33 and 10 per cent respectively.

At 168 HAT, 10^7 spores mL^{-1}, caused complete mortality, which was followed by 86.67 per cent with10^6 spores mL^{-1}. Both, 10^5 and 10^4 spores mL^{-1} recorded 50 per cent mortality. Only 33.33 per cent nymphs were killed by 10^3 spores.

4.4.2.6 N. nervosa Adults

Mortality response of adult hoppers of *N. nervosa* to varying spore concentrations of *L. lecanii* is presented in Table 15. Observations at 24 HAT revealed that, there was no death or negligible (10 per cent) mortality with varying spore concentrations.

Similar trend pertained till 48 and 72 h as well, with the maximum mortality being 23.33 per cent (10^8 spores mL^{-1}).

Even at the end of 96 HAT, the percentage death recorded was less than 50, ranging from 46.67 with the highest dose to 3.33 per cent with 10^3 spores mL^{-1}.

After 168 h, highest spore concentration of 10^8 spores mL^{-1}, caused cent per cent mortality, while 10^7 and 10^6 spores mL^{-1} caused 90 and 73.33 per cent mortality respectively. Fifty per cent of the adult hoppers were killed by spore load of 10^5 spores mL^{-1}, while 10^4 and 10^3 spores mL^{-1} killed 36.67 and 20 per cent insects.

4.5 LETHAL CONCENTRATIONS AND LETHAL TIME

The data on dose - mortality response, subjected to Probit analysis revealed the lethal concentration to cause 50 and 90 per cent death (LC_{50} and LC_{90}) of treated insects. Lethal time was computed for the major sucking pest of rice, rice bug *L. acuta*, based on mortality data recorded t 12 h interval.

Table 15. Dose - mortality of *L. lecanii* to *N. nervosa* adults

Treatments (Spores mL^{-1})	Mortality at 24 h interval (%)						
	24	**48**	**72**	**96**	**120**	**144**	**168**
10^8	10.00	16.67	23.33	46.67^{a} (43.07)	66.67a (54.99)	86.67^{a} (71.99)	100^{a} (89.09)
10^7	0	3.33	20.00	36.67^{a} (37.15)	50.00^{ab} (45.00)	76.67^{ab} (61.22)	90.00^{b} (74.69)
10^6	0	10.00	23.33	36.67^{a} (37.15)	43.33^{bc} (41.07)	60.00^{b} (51.15)	73.33^{c} (59.71)
10^5	0	3.33	10.00	16.67^{b} (23.36)	26.67^{cd} (30.78)	30.00^{c} (33.00)	50.00^{d} (45.00)
10^4	0	0	6.67	13.33^{b} (21.15)	20.00^{d} (26.07)	30.00^{c} (33.00	36.67^{de} (37.23)
10^3	0	0	3.33	3.33^{c} (6.75)	6.67^{e} (12.60)	13.33^{c} (21.15)	20.00^{e} (26.56)
Control	0	0	0	0^{c} (0.91)	0^{e} (0.91)	0^{d} (0.91)	0^{f} (0.91)
C.D (0.05)	NA	NA	NA	11.481	12.853	14.039	12.054

Figures in parentheses are angular transformed values. NA- Not analyzed. No. of insects per replication: 10. Values sharing same alphabets in superscript are statistically on par based on ANOVA

4.5. 1 Lethal Concentrations

4.5.1.1 Lecanicillium saksenae

The LC $_{50}$ and LC_{90} values for the sucking pests of rice are furnished in Table 16.

4.5.1.1.1 L. acuta

The LC_{50} value of spore suspension of *L. saksenae*, 6 DAT to adults of *L. acuta* was worked out to be 2.99 x 10^4 spores mL^{-1}, while for nymphs it was 1.72 x 10^4 spores mL^{-1}. The LC_{90} values computed were 8.19 x 10^5 spores mL^{-1} and 5.35 x 10^5 spores mL^{-1} for adults and nymphs respectively.

4.5.1.1.2 N. lugens

The LC $_{50}$ for adults of *N. lugens* was 1.36 x 10^4 spores mL^{-1}, and that of nymphs was 1.68 x 10^4 spores mL^{-1}. The corresponding LC_{90} values were 4.71 x 10^5 and 7.00 x 10^5 spores mL^{-1}, respectively.

4.5.1.1.3 N. nigropictus

The LC_{50} of *L. saksenae* for adults and nymphs of *N. nigropictus* were 4.90 x 10^4 spores mL^{-1}, and 1.53 x 10^4 spores mL^{-1}, respectively and their corresponding LC_{90} values were 5.27 x 10^5 spores mL^{-1}, and 6.14 x 10^5 spores mL^{-1}.

4.5.1.1.4 N. nervosa

The LC_{50} value calculated against adults and nymphs of *N. nervosa* at 6 DAT were 1.63 x 10^4 and 1.50 x 10^4 spores mL^{-1}, and the LC_{90} values were 7.64 x 10^5 spores mL^{-1}, and 7.35 x 10^5 spores mL^{-1} for adults and nymphs respectively.

4.5.1.2 Lecanicillium lecanii

The LC_{50} and LC_{90}values computed for *L. lecanii* are presented in Table 17. 82

Table16. Lethal concentrations of *L. saksenae* to different sucking pests at six days after treatment

Test insect		LC_{50} (spores mL^{-1})	Fiducial limits (spores mL^{-1})		LC_{90} (spores mL^{-1})	Fiducial limits (spores mL^{-1})	
			Upper	Lower		Upper	Lower
L. acuta	Adults	2.99×10^4	1.06×10^5	7.98×10^3	8.19×10^5	1.76×10^7	2.00×10^5
	Nymphs	1.72×10^4	6.14×10^4	3.90×10^3	5.35×10^5	1.41×10^7	1.28×10^5
N. lugens	Adults	1.36×10^4	4.90×10^4	2.69×10^3	4.71×10^5	1.41×10^7	1.10×10^5
	Nymphs	1.68×10^4	6.25×10^4	3.29×10^3	7.00×10^5	2.21×10^7	1.56×10^5
N. nigropictus	Adults	4.90×10^4	1.48×10^5	1.25×10^4	5.27×10^5	1.53×10^7	1.67×10^5
	Nymphs	1.53×10^4	5.69×10^4	3.01×10^3	6.14×10^5	1.93×10^7	1.38×10^5
N. nervosa	Adults	1.63×10^4	6.21×10^4	3.04×10^3	7.64×10^5	2.61×10^7	1.65×10^5
	Nymphs	1.50×10^4	5.75×10^4	2.65×10^3	7.35×10^5	2.64×10^7	1.57×10^5

Table17. Lethal concentrations of *L. lecanii* to different sucking pests at six days after treatment

Test insect		LC_{50} (spores mL^{-1})	Fiducial limits (spores mL^{-1})		LC_{90} (spores mL^{-1})	Fiducial limits (spores mL^{-1})	
			Upper	Lower		Upper	Lower
N. lugens	Adults	6.13×10^5	6.19×10^6	8.17×10^4	5.34×10^8	1.40×10^8	3.03×10^7
	Nymphs	1.22×10^5	5.0×10^5	2.45×10^4	1.32×10^7	5.53×10^8	2.20×10^6
N. nigropictus	Adults	2.26×10^5	1.14×10^6	4.50×10^4	3.29×10^7	2.45×10^9	4.61×10^6
	Nymphs	1.13×10^5	5.49×10^5	2.09×10^4	1.63×10^7	1.17×10^9	2.37×10^6
N. nervosa	Adults	3.40×10^5	2.79×10^6	4.39×10^4	2.46×10^8	2.81×10^{11}	1.69×10^7
	Nymphs	4.49×10^4	3.34×10^5	4.72×10^3	1.33×10^7	2.23×10^{10}	1.17×10^6

4.5.1.2.1 N. lugens

L. lecanii recorded an LC_{50} of 6.13 x 10^5 spores mL^{-1} against adults of *N. lugens* and 1.22 x 10^5 spores mL^{-1} for nymphs. The corresponding LC_{90} values were 5.34 x 10^8 spores mL^{-1} and 1.32 x 10^7 spores mL^{-1} for adults and nymphs respectively.

4.5.1.2.2 N. nigropictus

The LC_{50} value was 2.26 x 10^5 spores mL^{-1} and 1.13 x 10^5 spores mL^{-1}, for adults and nymphs respectively, and the corresponding LC_{90} values were 3.29 x 10^7 spores mL^{-1} and 1.63 x 10^7 spores mL^{-1}

4.5.1.2.3 N. nervosa

For adults the LC_{50} value was 3.40 x 10^5 spores mL^{-1} while for nymphs it was 4.49 x 10^5 spores mL^{-1}. The corresponding LC_{90} values were 2.46 x 10^8 spores mL^{-1} and 1.33 x 10^7 spores mL^{-1} for adults and nymphs respectively.

4.5.2 Lethal Time

The LT_{50} value computed for *L. saksenae* against *L. acuta* furnished in Table 18, revealed that with the spore concentration of 10^8 spores mL^{-1}, nymphs recorded a LT_{50} of 17.58 h while for adults it was 18.58 h. For 10^7 spores mL^{-1}, the LT_{50} recorded were 19.97 h and 19.91 h for adults and nymphs, respectively, while the highest LT_{50} was recorded for 10^6 spores mL^{-1} with 40.39 h and 44.03 h in adults and nymphs, respectively.

4.6 FIELD EFFICACY OF CHITIN ENRICHED BIOFORMULATIONS

Field experiment was laid out (Plate 5) to evaluate the efficacy of chitin enriched bioformulations of *L. saksenae* and *L. lecanii* in managing rice bug as well as their impact on natural enemies in the rice ecosystem. The treatments tested were

Plate 5. Layout of field experiment

Table 18. Lethal time of different spore concentrations of *L. saksenae* to *L. acuta*

Treatments (Spores mL^{-1})	Mortality of rice bugs at 12 h interval (%)													
Adults	**12**	**24**	**36**	**48**	**60**	**72**	**84**	**96**	**108**	**120**	**132**	**144**	**156**	**168**
10^8	37.50	60.00	61.75	87.50	95.00	100	100	100	100	100	100	100	100	100
10^7	32.50	55.00	72.50	80.00	90.00	97.50	100	100	100	100	100	100	100	100
10^6	12.50	25.00	40.00	52.50	67.50	70.00	80.00	87.50	95.00	100	100	100	100	100
Nymphs														
10^8	25.00	75.00	85.00	92.50	100	100	100	100	100	100	100	100	100	100
10^7	22.50	65.00	75.00	90.00	97.50	100	100	100	100	100	100	100	100	100
10^6	7.50	30.00	42.50	50.00	57.50	65.00	72.50	77.50	85.00	90.00	97.50	100	100	100

Spore concentration (Spores mL^{-1})	LT_{50} Adults (Hours)	Fiducial limits (spores ml^{-1})		LT_{50} Nymphs (Hours)	Fiducial limits (spores ml^{-1})	
		Upper	Lower		Upper	Lower
10^8	18.58	25.87	8.95	17.58	23.20	10.46
10^7	19.97	27.24	10.90	19.91	26.00	12.54
10^6	40.39	51.41	29.28	44.03	54.95	32.27

chitin enriched oil formulations of *L. saksenae* and *L. lecanii;* Spore suspensions of *L. saksenae and L. lecanii* @ 10^7spores mL^{-1} (10 mL L^{-1}); and talc based formulations of *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin @ 10^8 spores mL^{-1} and the recommended dose of chemical insecticide Malathion 0.1%. Their impact on the population on rice bugs and natural enemies are illustrated below.

4.6.1 On population of *L .acuta*

The data on the population of *L. acuta* on the basis of number of bugs present in 5 sweeps and 5 random hills per plot are furnished in Table 19.

4.6.1.1 First Spraying

The population of bugs did not vary significantly among various plots before treatment. It did not vary significantly on the third day as well. Seven days after spraying (DAS), plots treated with chitin enriched oil formulation of *L. saksenae* @ 10^7 spores mL^{-1} was found to be the most effective treatment with a least population of 3.00 bugs. This was followed by the population (4.33) in talc based *M. anisopliae* 10^8 spores mL^{-1} and Malathion 0.1% (3.66) treated plots, which were statistically on par.

Population recorded in plots treated with spore suspension of *L. saksenae* @ 10^7 spores mL^{-1} was on par with that of talc based *B. bassiana* @ 10^8 spores mL^{-1} and ranked third with average population of 6.00 and 5.33 bugs respectively. Among the treated plots chitin enriched oil formulation of *L. lecanii* @ 10^7 spores mL^{-1} and spore suspension of *L. lecanii* @ 10^7 spores mL^{-1} recorded highest bug count of 8.00, and 7.33 respectively. In the control plot, the bug count was 10.00.

On the tenth day, plots treated with chitin enriched oil formulation of *L. saksenae* @ 10^7 spores mL^{-1} recorded a bug count of 4.67 while, talc based *M. anisopliae* @10^8 spores mL^{-1} recorded a population of 4.33 bugs. The population

Table19. Effect of bioformulations on population of *L. acuta*

Sl. No.	Treatments	No of rice bugs per 5 sweeps and 5 random hills*							
		Pretreatment	First spraying				Second spraying		
			3 DAT	7 DAT	10 DAT	14 DAT	3 DAT	7 DAT	10 DAT	14 DAT
1	Chitin enriched oil formulation *L. saksenae* @ 10^7 spores mL^{-1}	10.00 (3.11)	4.67 (2.15)	3.00^{d} (1.68)	4.67^{cd} (2.15)	3.67^{bc} (1.90)	4.67 (2.21)	2.67^{d} (1.64)	2.33^{c} (1.54)	1.33^{c} (1.27)
2	Chitin enriched oil formulation *L. lecanii* @ 10^7 spores mL^{-1}	7.00 (2.62)	6.67 (2.52)	8.00^{ab} (2.82)	7.00^{abc} (2.62)	6.33^{ab} (2.50)	9.67 (3.18)	8.67^{a} (2.91)	6.00^{ab} (2.54)	5.33^{a} (2.37)
3	Spore suspension of *L. saksenae* @ 10^7 spores mL^{-1}	4.33 (2.08)	6.33 (2.50)	6.00^{bc} (2.44)	5.00^{bcd} (2.21)	2.67^{c} (1.55)	5.00 (2.28)	4.00^{cd} (2.12)	3.33^{abc} (1.95)	2.00^{bc} (1.56)
4	Spore suspension of *L. lecanii* @ 10^7 spores mL^{-1}	4.67 (2.15)	8.00 (2.79)	7.33^{ab} (2.68)	7.33^{ab} (2.69)	6.00^{ab} (2.40)	8.00 (2.87)	6.67^{abc} (2.65)	6.33^{a} (2.60)	5.00^{ab} (2.34)
5	Talc based *M. anisopliae* 10^8 spores mL^{-1}	7.00 (2.52)	4.67 (2.13)	4.33^{cd} (2.07)	4.33^{d} (2.06)	2.33^{c} (1.41)	4.00 (1.65)	2.67^{d} (1.66)	2.33^{c} (1.54)	1.67^{c} (1.35)
6	Talc based *B. bassiana* @ 10^8 spores mL^{-1}	6.00 (2.41)	6.67 (2.58)	5.33^{bc} (2.30)	5.33^{bcd} (2.30)	5.67^{ab} (2.38)	4.67 (2.25)	3.33^{cd} (1.95)	3.33^{abc} (1.95)	2.67^{abc} (1.71)
7	Malathion 0.1%	7.00 (2.62)	5.33 (2.30)	3.66^{cd} (1.88)	6.00^{abcd} (2.45)	3.67^{bc} (1.90)	3.33 (1.90)	4.67^{bcd} (2.28)	2.67^{bc} (1.66)	2.67^{abc} (1.71)
8	Untreated Check	9.00 (2.99)	9.33 (3.05)	10.00^{a} (3.10)	8.33^{a} (2.89)	8.33^{a} (2.89)	8.33 (2.91)	8.67^{a} (2.91)	7.33^{a} (2.79)	5.33^{a} (2.37)
	CD (0.05)	N S	N S	0.598	0.497	0.810	N S	0.703	0.815	0.842

*Plot size 2 x 2 m. Mean of three replications. Figures in parentheses are square root transformed values. DAT - Days after treatment. NS - Non significant.

in spore suspensions of *L. saksenae* @ 10^7 spores mL^{-1} and talc based *B. bassiana* @ 10^8 spores mL^{-1} treated plots ranked third and were statistically similar (5.00 and 5.33 respectively). Count of bugs in plots treated with chitin enriched oil formulation of *L. lecanii* @ 10^7 spores mL^{-1} (7.0) and that with spore suspension of *L. lecanii* @ 10^7 spores mL^{-1} was 7.33. Plots treated with Malathion 0.1 per cent recorded a population of 6.0 while that recorded from untreated plot recorded the least count of 8.33 bugs.

After 14 days, chitin enriched oil formulations of *L. saksenae* @ 10^7 spores mL^{-1} recorded a population of 3.67 which was on par with the chemical check (3.67). The population recorded was least (2.33) in talc based *M. anisopliae* @ 10^8 spores mL^{-1} , and Malathion 0.1% ranked second (3.67). All other treatments *viz.* chitin enriched oil formulation of *L.* lecanii @ 10^7 spores mL^{-1}, spore suspension of *L. lecanii* @10^7 spores mL^{-1} and talc based *B. bassiana* @ 10^8 spores mL^{-1} recorded higher bug counts of 6.33, 6.0 and 5.67, respectively .The population of bugs in the untreated plot was 8.33.

4.6.1.2 Second Spraying

Observations recorded on the third day did not reveal any significant difference in the population of bugs.

Seventh day after spraying, it was found that chitin enriched oil formulations of *L. saksenae* @ 10^7 spores mL^{-1} recorded the least population of 2.67 which was on par with talc based *M. anisopliae* @ 10^8 spores mL^{-1} (2.67). Plots treated with spore suspension of *L. saksenae* @ 10^7 spores mL^{-1} ranked second and was on par with talc based *B. bassiana* @10^8 spores mL^{-1} (4 and 3.33 bugs $plot^{-1}$, respectively). Population in Malathion 0.1% treated plot was 4.67 bugs $plot^{-1}$. The treatment chitin enriched oil formulation of *L. lecanii* @ 10^7 spores mL^{-1} and spore suspension of *L. lecanii* recorded 8.67 and 6.67 bugs $plot^{-1}$. A bug count of 8.67 was recorded from untreated plot.

On tenth day chitin enriched formulation of *L. saksenae* 10^7 spores mL^{-1} recorded the lowest population of 2.33 bugs $plot^{-1}$ which was on par with talc based *M. anisopliae* @ 10^8 spores mL^{-1}, followed by that recorded in Malathion 0.1% (2.67). The count recorded from spore suspension of *L. saksenae* @ 10^7 spores mL^{-1} and talc based *B. bassiana* @ 10^8 spores mL^{-1} were statistically similar (3.33 each). Population in chitin enriched oil formulation of *L. lecanii* @ 10^7 spores mL^{-1} was higher (6.0) and those in spore suspension of *L. lecanii* @ 10^7 spores mL^{-1} and untreated plots were highest (6.33 and 7.33 respectively).

Fourteenth day after the second spraying, least population was observed with chitin enriched formulation of *L. saksenae* @ 10^7 spores mL^{-1} which was statistically on par with talc based *M. anisopliae* @ 10^8 spores mL^{-1} (1.33 and 1.67, respectively). The population recorded from plots treated with spore suspension of *L. saksenae* @ 10^7 spores mL^{-1} ranked second (2.0 bugs $plot^{-1}$). The treatments talc based *B. bassiana* @ 10^8 spores mL^{-1} and Malathion 0.1% ranked next with an average count of 2.67 each. The population of bugs in spore suspension of *L. lecanii* @ 10^7 spores mL^{-1} and chitin enriched oil formulation of *L. lecanii* @ 10^7 spores mL^{-1} was higher (5.33 and 5.0 respectively). The population of bugs was 5.33 in untreated plots.

4.6.2 On Population of Natural Enemies

4.6.2.1 Predatory Insects

The count of predators in the field, coccinellids, mirids and reduviids (Plate 6 A and B) per 5 sweeps $plot^{-1}$ is presented in Table 20.

After first spraying, pre count and post treatment counts on the third and seventh day did not vary among different plots. On the tenth day, variations were noted. Highest predator population was recorded in plots treated with chitin enriched oil formulation of *L. lecanii* @ 10^7 spores mL^{-1} (5.67) followed by chitin enriched oil

Plate 6. (A) Reduviid predators (unidentified) recorded in the experimental field

Coccinella transversalis

Micraspis sp.

(C) *Cyrtorhinus lividipennis*

Plate 6.(B) Other predatory insects observed in the field

Table 20. Effect of bioformulations on predatory insects

Sl. No.	Treatments	No of predators per 5 sweeps plot^{-1}*								
		Pretreatment	First spraying				Second spraying			
			3 DAT	7 DAT	10 DAT	14 DAT	3 DAT	7 DAT	10 DAT	14 DAT
1	Chitin enriched oil formulation *L. saksenae* @ 10^7 spores mL^{-1}	2.67 (1.57)	2.67 (1.62)	1.33 (1.34)	4.33^{ab} (2.03)	1.33 (1.34)	2.67 (1.44)	3.00 (1.78)	3.00^{abc} (1.82)	3.33^{abc} (1.94)
2	Chitin enriched oil formulation *L. lecanii* @ 10^7 spores mL^{-1}	2.33 (1.52)	2.00 (1.33)	2.00 (1.55)	5.67^{a} (2.36)	2.67 (1.77)	3.33 (1.94)	4.67 (2.25)	2.67^{abc} (1.76)	2.67^{bc} (1.77)
3	Spore suspension of *L. saksenae* @ 10^7 spores mL^{-1}	4.33 (2.03)	3.33 (1.74)	2.67 (1.76)	3.00^{bc} (1.71)	2.33 (1.54)	2.67 (1.77)	3.33 (1.89)	2.67^{abc} (1.74)	1.33^{de} (1.29)
4	Spore suspension of *L. lecanii* @ 10^7 spores mL^{-1}	3.67 (1.90)	4.33 (2.65)	2.00 (1.32)	4.00^{ab} (1.97)	2.67 (1.66)	2.33 (1.66)	2.00 (1.55)	2.33^{bc} (1.64)	2.33^{cd} (1.677)
5	Talc based *M. anisopliae* @ 10^8 spores mL^{-1}	3.33 (1.74)	3.67 (1.86)	2.33 (1.54)	3.00^{bc} (1.68)	2.67 (1.71)	3.00 (1.85)	5.00 (2.28)	3.00^{ab} (1.84)	4.33^{ab} (2.19)
6	Talc based *B. bassiana* @ 10^8 spores mL^{-1}	3.67 (1.91)	3.00 (1.71)	1.67 (1.44)	3.00^{bc} (1.62)	2.00 (1.55)	4.00 (2.09)	3.00 (1.78)	4.33^{ab} (2.16)	3.67^{abc} (2.018)
7	Malathion 0.1%	5.00 (2.22)	2.00 (1.38)	0.67 (0.99)	1.67^{c} (1.27)	1.00 (1.17)	1.67 (1.38)	2.33 (1.66)	1.33^{c} (1.27)	1.00^{e} (1.23)
8	Untreated Check	4.67 (2.13)	3.00 (1.71)	5.00 (2.33)	4.67^{ab} (2.13)	5.00 (2.33)	3.67 (2.02)	4.33 (2.18)	4.67^{a} (2.28)	4.67^{a} (2.25)
	CD (0.05)	N.S	N.S	N.S	0.567	N.S	N.S	N.S	0.563	0.440

*Plot size 2 x 2 m. Mean of three replications. Figures in parentheses are square root transformed values. DAT - Days after treatment. NS - Non significant

formulation of *L. saksenae* @ 10^7 spores mL^{-1}, spore suspension of *L. lecanii* @ 10^7 spores mL^{-1}, and untreated control (4.33, 4.00, and 4.67 respectively) which were on par with each other. The plots treated with spore suspension of *L. saksenae* @ 10^7 spores mL^{-1}, talc based *M. anisopliae* @ 10^8 spores mL^{-1}, and talc based *B. bassiana* @ 10^8 spores mL^{-1}, recorded a predator count of 3.00 $plot^{-1}$. Least predator count of 1.67 was recorded in plots treated with Malathion 0.1% (1.67). The population did not vary significantly on 14th day after spraying.

After second spraying too, the population was on par on third and seventh day. On the tenth day after treatment, the predator count in Chitin enriched oil formulation of *L.* saksenae @ 10^7 spores $mL^{-1}$was on par with talc based *M. anisopliae* @ 10^8 spores mL^{-1} (3.00 each). Predator count in plots treated with talc based *B. bassiana* @ 10^8 spores mL^{-1} was 4.33, whereas the number of predators in by chitin enriched oil formulation of *L. saksenae* @ 10^7 spores mL^{-1}, chitin enriched oil formulation of *L. lecanii* @ 10^7 spores mL^{-1}, and spore suspension of *L. saksenae* @ 10^7 were on par (3.00, 2.67, and 2.67, respectively). The maximum predator count was observed in untreated plots (4.67). Minimum count was noticed in plots treated with spore suspension of *L. lecanii* @ 10^7 (2.33) which was on par with that observed in Malathion 0.1% treated plots (1.33).

Predator counts on 14 days after spraying in plots treated with chitin enriched oil formulation of *L. saksenae* @ 10^7 spores mL^{-1} and talc based *B. bassiana* @ 10^8 spores mL^{-1} were on par with average count of 3.33 and 3.67 respectively. Lower predator counts of 2.67and 2.33 were recorded from plots treated with chitin enriched oil formulation of *L. lecanii* @ 10^7 spores mL^{-1} and spore suspension of *L. lecanii* @ 10^7, while the least count was in Malathion 0.1% respectively (1.0). Highest predator count was recorded in untreated plots (4.67), followed by plots treated with talc based *M. anisopliae* @ 10^8 spores mL^{-1} (4.33).

4.6.2.2 Predatory Spiders

The population of predatory spiders viz. *Argiope* sp., *Tetragnatha* sp. and *Oxyopes* sp. (Plate 7) encountered in the rice field are furnished in Table 21. Analysis of data revealed that, all through the field trial, population did not vary much among the treatments. The count varied from 0.33 to 4.0 plot^{-1} .

4.6.2.3 Hymenopteran Parasitoids

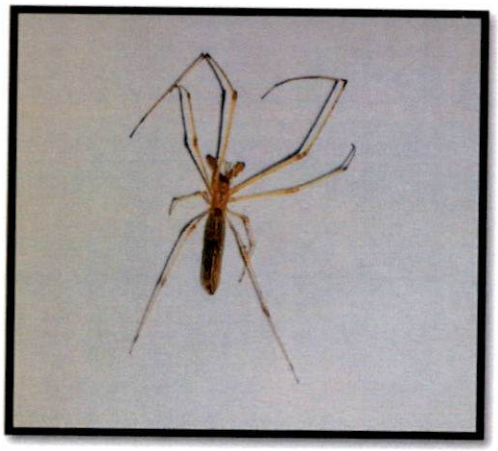
Only hymenopteran parasitoids were observed during the study. The common species encountered were *Bracon* sp. and *Xanthopimpla* sp. (Plate 8). Throughout the experimental period, their population did not vary significantly among the treatments, their counts ranging from 0 to 2.0 in various plots.

4.6.3 Yield

Data on grain yield expressed as gross yield (after threshing) and net yield (after threshing and winnowing) as well as on the straw yield are furnished in Table 23.

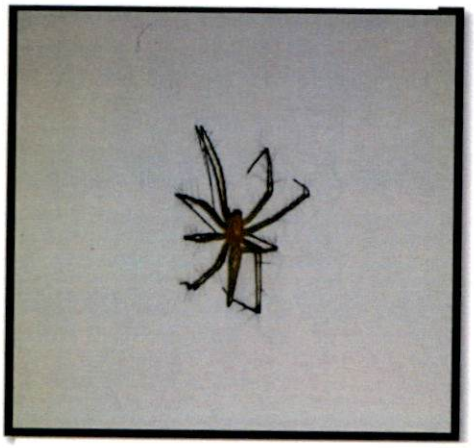
4.6.3.1 Gross Grain Yield

Highest gross yield of 3.55 kg plot^{-1} was recorded from plots treated with spore suspension of *L. saksenae* @ 10^7 spores mL^{-1} which was statistically on par with that of chitin enriched formulation of *L. saksenae* @ 10^7 spores mL^{-1} (3.48 kg plot $^{-1}$) which was followed by the grain yield of talc based *B. bassiana* @ 10^8 spores mL^{-1} (2.70 kg plot $^{-1}$) and chitin enriched oil formulation of *L. lecanii* @ 10^7 spores mL^{-1} (2.68 kg plot $^{-1}$). Yield obtained from spore suspension of *L. lecanii* @ 10^7 spores mL^{-1}, Malathion 0.1% and talc based *M. anisopliae* @ 10^8 spores mL^{-1} were on par (2.45, 2.43 and 2.40 kg plot^{1} respectively). Lowest gross yield was obtained from untreated plot (1.82 kg plot $^{-1}$).



Tetragnatha maxillosa

Tetragnatha sp.



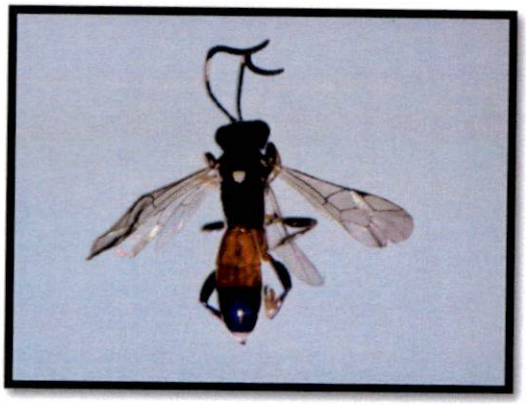
Oxyopes sp.

Oxyopes sp.

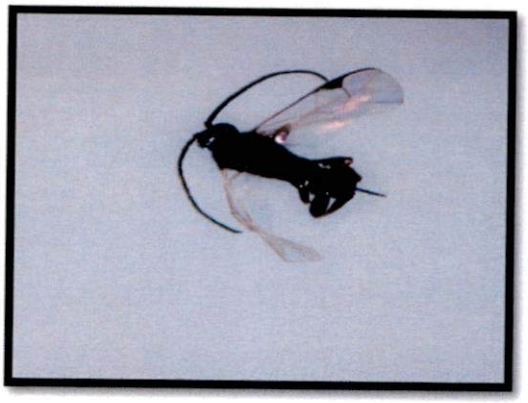
Oxyopes shwetha

Argiope sp.

Plate 7. Spiders recorded in the experimental field



(A) *Bracon* sp.



(B) *Opius* sp.

(C) *Xanthopimpla* sp.

(D) Scelionid wasp (unidentified)

Plate 8. Hymenopteran parasitoids recorded in the experimental field

Table 21. Effect of bioformulations on predatory spiders

Sl. No.	Treatments	No of spiders per 5 sweeps plot^{-1}*								
		Pretreatment	First spraying				Second spraying			
			3 DAT	7 DAT	10 DAT	14 DAT	3 DAT	7 DAT	10 DAT	14 DAT
1	Chitin enriched oil formulation *L. saksenae* @ 10^7 spores mL^{-1}	1.00 (1.17)	1.00 (1.22)	1.33 (1.34)	1.33 (1.13)	1.67 (1.46)	1.67 (1.27)	2.33 (1.52)	3.00 (1.71)	2.67 (1.60)
2	Chitin enriched oil formulation *L. lecanii* @ 10^7 spores mL^{-1}	1.00 (1.17)	0.67 (0.99)	2.00 (1.55)	1.67 (1.27)	1.00 (1.17)	1.33 (1.13)	2.67 (1.62)	2.67 (1.62)	2.33 (1.52)
3	Spore suspension of *L. saksenae* @ 10^7 spores mL^{-1}	0.67 (1.05)	1.00 (1.22)	1.67 (1.46)	2.00 (1.38)	2.33 (1.67)	1.67 (1.27)	3.00 (1.71)	4.00 (1.98)	3.33 (1.82)
4	Spore suspension of *L. lecanii* @ 10^7 spores mL^{-1}	0.33 (0.87)	0.67 (1.05)	1.00 (1.17)	1.33 (1.13)	1.33 (1.34)	1.33 (1.13)	2.00 (1.41)	2.67 (1.62)	2.67 (1.62)
5	Talc based *M. anisopliae* @ 10^8 spores mL^{-1}	0.67 (1.05)	0.67 (1.05)	1.00 (1.17)	2.00 (1.41)	2.33 (1.67)	1.67 (1.27)	2.33 (1.52)	3.00 (1.71)	3.00 (1.73)
6	Talc based *B. bassiana* @ 10^8 spores mL^{-1}	0.67 (0.99)	1.33 (1.34)	1.67 (1.44)	2.00 (1.38)	2.00 (1.55)	2.00 (1.38)	2.33 (1.52)	3.33 (1.82)	2.67 (1.62)
7	Malathion 0.1%	1.33 (1.34)	0.33 (0.87)	0.67 (0.99)	1.00 (1.00)	1.33 (1.34)	1.00 (1.00)	1.67 (1.27)	2.67 (1.62)	2.33 (1.52)
8	Untreated Check	1.00 (1.22)	1.33 (1.34)	1.67 (1.44)	2.00 (1.38)	1.67 (1.46)	2.33 (1.52)	3.00 (1.73)	4.00 (1.98)	3.00 (1.71)
	CD (0.05)	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

*Plot size 2 x 2 m. Mean of three replications. Figures in parentheses are square root transformed values. DAT - Days after treatment. NS - Non significant

Table 22. Effect of bioformulations on parasitoid population

Sl. No.	Treatments	No of parasitoids per 5 sweeps plot^{-1}*							
		Pretreatment	First spraying				Second spraying			
			3 DAT	7 DAT	10 DAT	14 DAT	3 DAT	7 DAT	10 DAT	14 DAT
1	Chitin enriched oil formulation *L. saksenae* @ 10^7 spores mL^{-1}	0.67 (1.05)	0.67 (1.05)	1.00 (1.22)	1.33 (1.34)	1.67 (1.46)	1.00 (1.22)	1.33 (1.34)	1.67 (1.46)	1.67 (1.46)
2	Chitin enriched oil formulation *L. lecanii* @ 10^7 spores mL^{-1}	0.33 (0.87)	0.67 (0.99)	0.67 (0.99)	1.67 (1.46)	0.33 (0.87)	0.67 (1.05)	0.67 (1.05)	1.00 (1.17)	0.67 (0.99)
3	Spore suspension of *L. saksenae* @ 10^7 spores mL^{-1}	1.00 (1.17)	0.33 (0.87)	0.33 (0.87)	0.67 (1.05)	0.67 (1.05)	1.00 (1.17)	1.00 (1.17)	1.67 (1.46)	1.33 (1.34)
4	Spore suspension of *L. lecanii* @ 10^7 spores mL^{-1}	0.67 (1.05)	0.67 (1.05)	1.00 (1.17)	1.00 (1.22)	0.67 (1.05)	0.67 (0.99)	1.00 (1.179)	1.33 (1.34)	1.00 (1.22)
5	Talc based *M. anisopliae* @ 10^8 spores mL^{-1}	0.33 (0.87)	0.67 (1.05)	1.00 (1.17)	1.00 (1.34)	0.67 (1.05)	1.00 (1.22)	1.33 (1.34)	1.00 (1.17)	1.67 (1.46)
6	Talc based *B. bassiana* @ 10^8 spores mL^{-1}	0.00 (0.70)	1.00 (1.22)	1.67 (1.44)	2.00 (1.17)	1.00 (1.17)	1.00 (1.22)	2.00 (1.55)	2.00 (1.55)	1.67 (1.46)
7	Malathion 0.1%	1.33 (1.34)	0.00 (0.70)	0.33 (0.87)	1.00 (1.55)	0.67 (1.05)	0.00 (0.70)	0.33 (0.87)	0.67 (1.05)	1.00 (1.22)
8	Untreated Check	1.00 (1.17)	1.33 (1.34)	1.00 (1.17)	2.00 (1.55)	1.33 (1.34)	1.67 (1.46)	1.67 (1.46)	2.00 (1.55)	2.00 (1.58)
	CD (0.05)	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

*Plot size 2 x 2 m. Mean of three replications Figures in parentheses are square root transformed values. DAT - Days after treatment. NS - Non significant

4.6.3.2 Net Yield

Plots treated with chitin enriched formulation of *L. saksenae* @ 10^7 spores mL^{-1} recorded highest net yield of 3.25 kg $plot^{-1}$, which was statistically on par with that of spore suspension of *L. saksenae* @ 10^7 spores mL^{-1} (3.09 kg $plot^{-1}$). Yield from chitin enriched oil formulation of *L. lecanii* @ 10^7 spores mL^{-1} treated plots ranked next (2.5 kg $plot^{-1}$), while those from Malathion 0.1%, talc based *M. anisopliae* @ 10^8 spores mL^{-1}, talc based *B. bassiana* @ 10^8 spores mL^{-1} and spore suspension of *L. lecanii* @ 10^7 spores mL^{-1} were on par with each other (2.20, 2.17, 2.13, and 2.05 kg $plot^{-1}$ respectively). Untreated plots recorded lowest economic yield of 1.63 kg $plot^{-1}$.

4.6.3.3 Straw Yield

Among the treatments, chitin enriched oil formulation of *L. lecanii* @ 10^7 spores mL^{-1}, spore suspension of *L. saksenae* @ 10^7 spores mL^{-1}, recorded highest straw yield of 9.67 kg $plot^{-1}$, while talc based *B. bassiana* @ 10^8 spores mL^{-1} recorded 9.17 kg plot 1. Malathion 0.1%, talc based *M. anisopliae* @ 10^8 spores mL^{-1}, and spore suspension of *L. lecanii* @ 10^7 spores mL^{-1} recorded 9.00, 7.83, and 7.33 kg $plot^{-1}$respectively. Lowest straw yields were obtained from plots treated with enriched formulation of *L. saksenae* @ 10^7 spores mL^{-1} (7.08) and untreated control (6.33).

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Table 23.Effect of bioformulations on yield of paddy

Sl. No.	Treatments	Yield (kg plot^{-1}) *		
		Gross	Net	Straw
1	Chitin enriched oil formulation *L. saksenae* @ 10^7 spores mL^{-1}	3.48^{a}	3.25^{a}	7.08^{cd}
2	Chitin enriched oil formulation *L. lecanii* @ 10^7 spores mL^{-1}	2.68^{b}	2.50^{bc}	9.67^{a}
3	Spore suspension of *L. saksenae* @ 10^7 spores mL^{-1}	3.55^{a}	3.09^{ab}	9.67^{a}
4	Spore suspension of *L. lecanii* @ 10^7 spores mL^{-1}	2.45^{bc}	2.05^{cd}	7.33^{bcd}
5	Talc based *M. anisopliae* @ 10^8 spores mL^{-1}	2.40^{bc}	2.17^{cd}	7.83^{abcd}
6	Talc based *B. bassiana* @ 10^8 spores mL^{-1}	2.70^{b}	2.13^{cd}	9.17^{ab}
7	Malathion 0.1%	2.43^{bc}	2.20^{cd}	9.00^{abc}
8	T8 Untreated Control	1.82^{c}	1.63^{d}	6.33^{d}
	C.D (0.05)	0.695	0.597	2.036

*Plot size 2 x2 m. Mean of three replications. Values sharing same alphabets in superscript are statistically on par based on ANOVA

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Discussion

5. DISCUSSION

Entomopathogenic fungi vary in their specificity and biological traits depending on their geographical origin. Bischoff *et al.*, (2009) pointed out that many isolates of entomopathogenic fungi (EPF) show geographical variations. Indigenous species or isolates can improve the field efficacy than exotic species. The genus *Lecanicillium* previously named as *Verticillium*, is well known for its pathogenicity to sucking pests such as aphids, mealy bugs, scales, whiteflies etc. Globally, *Lecanicillium lecanii* (Zimmermann) Zare and Gams is widely reported to be an entomopathogen to many homopteran sucking pests (Kim, 2007, Mark *et al.*, 2008, Kim *et al.*, 2008, Scorsetti *et al.*, 2008., Malekan *et al.*, 2013). Its infectivity to hetropteran bugs had not been reported.

An indigenous isolate of *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno, from soils of Vellayani, Kerala was reported to be pathogenic to heteropterans (Rani *et al.*, 2015, Jasmy, 2016). Being an indigenous isolate of a comparatively infrequent species, detailed investigation on its bioefficacy and safety aspects is warranted. Comparative efficacy of the indigenous isolate of *L. saksenae* which was first described by Kushwaha (1980) as a keratin degrader, with the NBAIR isolate of *L. lecanii* (Isolate Vl8, sourced from National Bureau of Agricultural Insect Resources), is discussed below. The efficacy has been evaluated based on their pathogenicity to various sucking pests, effective dose, LC_{50} and LC_{90} as well as their field efficacy in managing the major pest of rice, rice bug.

The qualitative trait referring to the inherent genetic capacity of a micro organism to cause disease that is mediated by specific virulence factors is termed as 'Pathogenicity'. It is the result of specific host-pathogen interaction, and hence it is imperative to have an insight into the pathogenicity of the fungal species to different host insects before exploiting them for pest management.

PATHOGENICITY AND BIOSAFETY OF *Lecanicillium* spp

In the present study it was revealed that *L. saksenae* is infective to rice bug *Leptocorisa acuta* (Thunberg), brown planthopper, *Nilaparvata lugens* (Stal), green leafhopper, *Nephotettix nigropictus* (Stal), white leafhopper, *Cofana spectra* (Distant) and white winged planthopper, *Nisia nervosa* (Motschulsky). While, *L. lecanii* , though pathogenic to the hopper pests, was non infective to rice bug.

Shinde *et al*., (2010) reviewed an extensive list of pest species susceptible to *L. lecanii.* Though the host range spanned across 21 families in eight orders including coccids, aleyrodids, aphids, there was only one report of infection to a hopper, *Idioscopus clypealis* (Lethierry) belonging to cicadellidae. Apart from this, there is no documented record on *L. lecanii* being pathogenic to hard bodied sucking pests.

The new isolate of *L. saksenae* being infective to rice bug becomes a rare instance of *Lecanicillium* infecting an Alydid pest. This offers ample scope in developing this fungus as a biopesticide for the management of sucking pest complex in rice ecosystem. Rani *et al.* (2015), on their preliminary studies on host range of this indigenous isolate has reported the infectivity to cowpea pod bug *Riptortus pedestris* (F.) Further, Jasmy, (2016) reported pathogenicity to *L. acuta.*

While observing the time taken for mortality of different insects at 10^7 spores mL^{-1} of *L. saksenae*, the susceptibility was in the order *L. acuta*, *N. nigropictus*, *N. nervosa* and *N. lugens* and that of *L. lecanii* as *N. nervosa*, *N. nigropictus* and *N. lugens*, with no infectivity to *L. acuta.* A critical introspection into the time taken to attain 100 per cent mortality revealed the superiority of *L. saksenae.* Complete mortality occurred within two to four days for nymphs and adults of *L. acuta, N. nigropictus* and *N. nervosa* treated with *L. saksenae* while for *N. lugens* it took five days. The time taken for attaining 100 per cent mortality was more in the case of *L. lecanii.* It took seven days to kill all nymphs and 90 per cent adults of *N. nervosa*, seven days for nymphs and adults of

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N. nigropictus and seven days for 100 per cent nymphs and 76.67 per cent adults of *N. lugens*.

Such variations in susceptibility of different sucking pests to the same fungus were mentioned earlier by Geng and Zhang (2004), where they observed a higher susceptibility of WBPH than BPH to *Metarhizium anisopliae* (Metschnikoff) Sorokin spores. *L. saksenae* and *L. lecanii* were non pathogenic to the pentatomids, black bug, *Scotinophara coarctata* (F.) and shield bug, *Menida versicolor* (Gmelin.). Contrary to the current finding, Saruhan *et al.*, (2016) reported high mortality of 98 per cent in the green shield bug, *Palomena prasina* L., a pest of hazelnut fruits, treated with spore suspension of *Lecanicillium muscarium* (Petch) Zare and Gams (Isolate TR-07) @ 10^8 spores mL^{-1}. This further authenticates the fact that EPF vary in their specificity and other biological traits from species to species and isolate to isolate.

Symptoms of mycosis *viz.* lethargy, cessation of feeding, mortality and fungal growth over the cadaver did not vary much among the two fungi, except with those observed in the case of *L .acuta* treated with *L. saksenae*. In the case of *L. acuta* treated with *L. saksenae,* restlessness, combing of body parts, loosing of clinging capacity, ataxis and aggregation were observed. Similar results of modified insect behaviour due to fungal infection were observed by Jensen *et al.*, (2001) in *Acyrthosiphon pisum* (Harris), treated with *Pandora neoaphidis* (Remaudiere and Hennebert) Humber. Combing of body parts and aggregation can be seen as an insect hygiene behaviour to remove the fungal conidia deposited on cuticle. Mutual grooming among nest mates of termite *Captotermes formosanus* Shiraki treated with conidia of *M. anisopliae,* has been identified as a disease defence mechanism (Yanaga and Shimizu, 2007). Similar pre death symptoms of mycosis were observed by Roditakis *et al.*, (2008) in aphid *Myzus persicae* (Sulzer) treated with *Lecanicillium longisporum* (Petch) Zare and Gams KV71 strain. The treated aphids exhibited non directional shaking movements (ataxis) with a high tendency for aggregation. The other symptoms of mycosis observed in *L. acuta,* were convulsions characterized by leg twitching

and abdominal arching. The same movements were repeated in cycles, the intensity of which faded slowly as the bug slipped into paralysis and death. Such abdominal arching and leg twitching in larval and adult stages of western flower thrips, *Frankliniella occidentalis* (Pergande) treated with *M. anisopliae* spores three to four days after treatment (DAT) were described earlier by Vestergaard *et al.*, (1995), and similar observations on legume flower thrips *Megalurothrips sjostedti* (Trybom) treated with *M. anisopliae* were reported by Ekesi and Maniania (2000). The emergence of mycelia from inter segmental membranes, bases of antennae, and leg joints as observed in this study when treated with *L. saksenae* and *L. lecanii* were earlier reported in the case of another species *L. longisporum* in *M. persicae*, by Roditakis *et al.*, (2008).

Safety of an entomopathogen is determined by a variety of factors. Highly specific fungi, with narrow host range putatively pose minimal threat to non target organisms and vice versa. Fungal strain, nutrition, physiological state of host, and defence mechanisms of host insect are some of the factors that determine the virulence of a strain. Fungi with narrow host range are highly virulent (Goettel *et al.*, 1995). Even if, *Lecanicillium* is one such genus with a narrow host range compared to the genera, *Beauveria* and *Metarhizium*, adverse effects on non target organisms such as crop plants, parasites and predators are to be ruled out.

The safety parameters of *L. saksenae* and *L. lecanii* were ascertained through cross infectivity trials on rice plant. Spore suspensions @ 10^9 spores mL^{-1} did not show any disease symptoms on rice plants, when inoculated through leaf and soil. Gurulingappa *et al.* (2010) reported safety of *Beauveria bassiana* (Balsamo) Vuillemin and *L. lecani* to the crop plants, cotton (*Gossypium hirsutum* L.), wheat (*Triticum aestivum* L.), bean (*Phaseolus vulgaris* L.), corn (*Zea mays* L.), tomato (*Lycopersicon esculentum* L.), and pumpkin (*Cucurbita maxima* L.). Vidal and Jaber (2015), revealed that *B. bassiana, M. anisopliae* and *L. lecanii* live as endophytes in various field crops without any ill effects on crop plants. Jasmy (2016) proved the safety of *L. saksenae* to crop plants such as cowpea

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(*Vigna unguiculata* L.), bhindi (*Abelmoschus esculentus* L.), brinjal (*Solanum melongena* L.) and tomato (*L. esculentum*).

In this study *L. saksenae* and *L. lecanii* were found to be non pathogenic to the natural enemies commonly seen in rice ecosystem. The spore suspension at a higher concentration of 10^9 spores mL^{-1} was found to be non infective to the coccinellids *viz.*, *Micraspis discolor* (F.) and *Coccinella transversalis* F., the mirid bug, *Cyrtorhinus lividipennis* Reuter, the carabid beetle, *Ophionea nigrofasciata* Schmidt-Gobel, and the predatory spiders *Tetragnatha maxillosa* (Thorell) and *Oxyopes shweta* Tikader. Safety of *L. saksenae* to the natural enemy complex of vegetable ecosystem was ascertained by Jasmy (2016). She found that topical application of spore suspension @ 10^9 spores mL^{-1} caused neither mycosis nor mortality to the predators *T. maxillosa* and *Oxyopus* sp. and *Xanthogramma scutellare* F. and the parasites *Bracon brevicornis* Wesmael, *Goniozus nephantidis* (Muesebeck), *Trichogramma japonicum* Ashmead and *Trichogramma chilonis* Ishii and suggested it as an ideal candidate in pest management programme.

Numerous investigations on the safety of the fungi in the genus *Lecanicillium,* to the predators and parasites has been carried out earlier too. Rondon *et al.*, (1982), found that *L. lecanii* was safe to the aphid predators *Cycloneda sanguinea* (L.), *Oxyptamus gastrostacus* (L.) and *Zelus* sp., while Wang *et al.* (2005) reported its safety to the coccinellid *Delphastus catalinae* (Horn). It was also found to be safe to the predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Koike *et al.*, 2005). Safety of *L .lecanii* to parasitoids was reported by Kim *et al.*, (2005), who found that the fungus at a concentration of 10^8spores mL^{-1} was non pathogenic to the aphid parasitoid *Aphidius colemani* Viereck. Zimmermann (2007) consolidated an extensive list of various natural enemies including bees, predatory mites, carabid beetles, parasitoids and pollinators to which *B. bassiana* and *Beauveria brogniartii* (Saccardo) Petch were safe. The European Food Safety Authority has approved the commercial formulation of *L. muscaruim*, Mycotal®, after scrutinizing its safety to non target

arthropods, soil vertebrates, honey bees and rats, on the basis of rigorous research conducted among its member countries (EFSA, 2010). Suharsono and Prayogo (2014) reported safety of *L. lecanii* @ 10^7 spores mL^{-1}, to the predators *Paederus* sp., *Oxyopes* sp., and *Coccinella* sp.

COMPARATIVE INFECTIVITY/VIRULENCE OF *Lecanicillium* spp

In general, mortality was observed to increase with the spore dose in all susceptible insects in all stages, with both the fungi (Fig. 1 A, B). However, in *L. saksenae* there was a sudden hike in mortality (20- 40 to 80- 100 per cent) when the concentration was increased from 10^5 to 10^7 spores mL^{-1}, whereas in *L. lecanii* the rate of increase was gradual (0- 40 to 40- 100 per cent).

The effective dose of *L. saksenae* against *L. acuta* could be fixed as 10^7 spores mL^{-1}, since there was no difference in mortality (100 per cent), observed with the highest concentration, 10^8 spores mL^{-1}, 48 - 72 hours after treatment (HAT). For other sucking pests too the effective dose remained same. The effective dose of *L. lecanii* (non infective to *L. acuta)* to the hoppers was also 10^7 spores mL^{-1}, since the extent of mortality recorded was more or less the same, even with the higher dose, 10^8 spores mL^{-1}. A status quo was found to be maintained with 10^8 and 10^7 spores mL^{-1} in the case of *L. saksenae* as well as *L. lecanii* but they differed in time taken for mortality.

When lower spore concentrations of *L. lecanii*, 10^5, 10^4, and 10^3 spores mL^{-1} caused significantly low or no mortality, the lower doses of *L. saksenae* caused 76.67 to 36.67 per cent mortality , indicating higher virulence of the indigenous strain.

The dose - dependent response in mortality observed in this study is in conformity with the findings of Vestergaard *et al.*, (1995) in flower thrips, *F. occidentalis* treated with different spore concentrations of *M. anisopliae.* They recorded 53.6, 85.7, 94.6 and 99.1 per cent mortality in adults , when treated with 10^5, 10^6, 10^7, and 10^8 spores mL^{-1}. A sudden spike in mortality from 53 to 99 per cent noted in Vestergaard's study is in conformity with the present one. Similar

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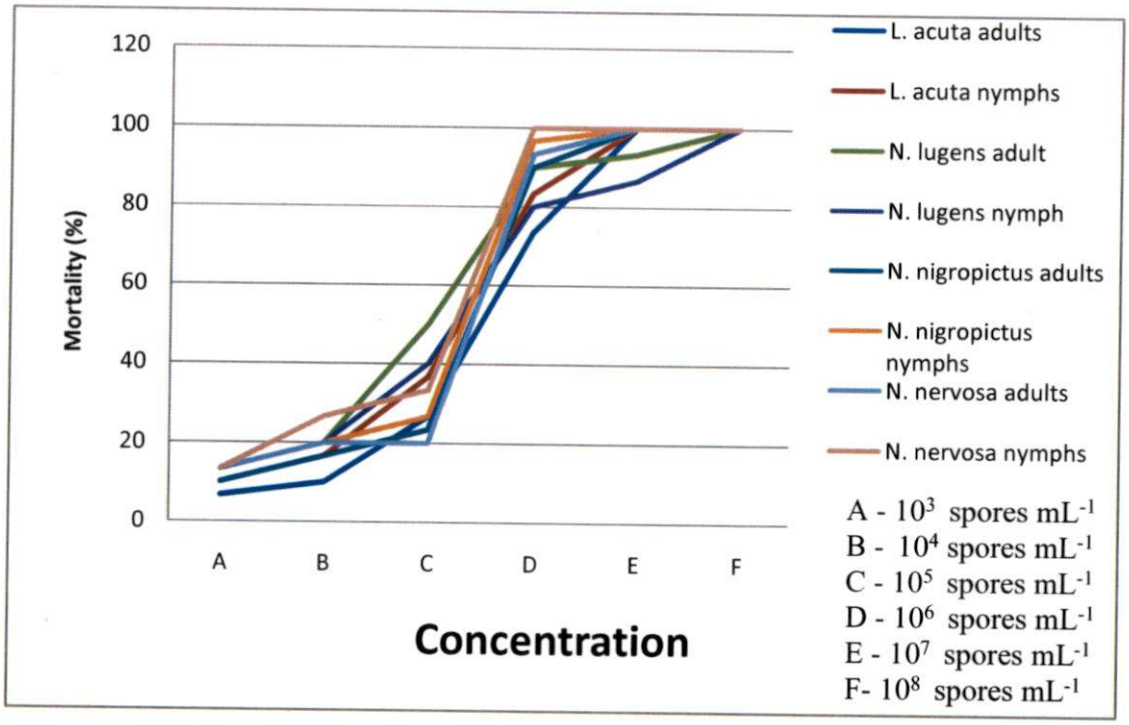
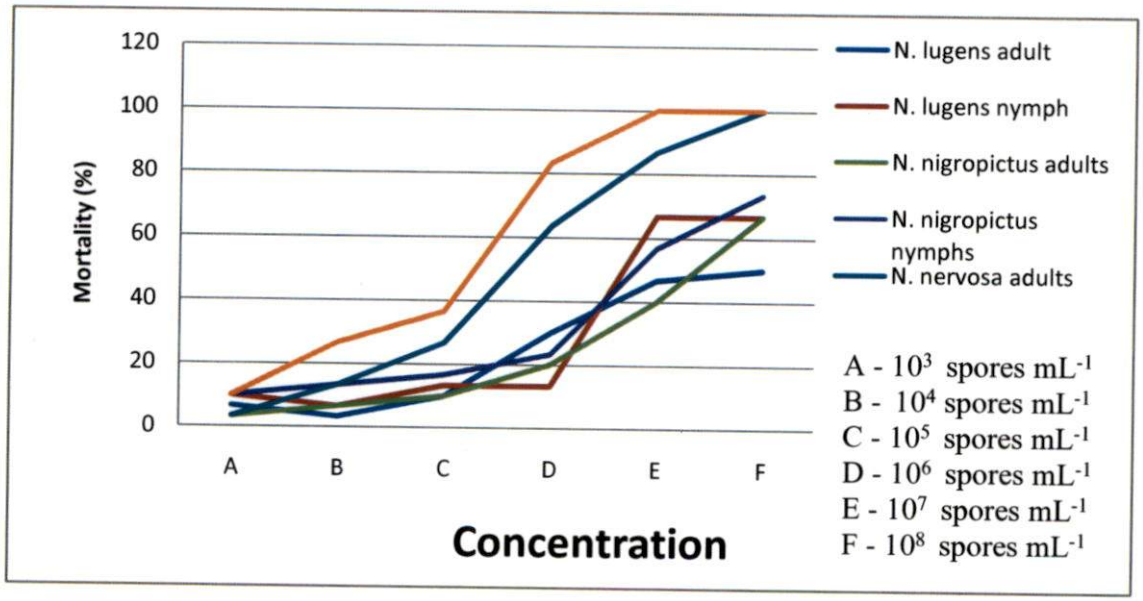


(A) *L. saksenae*



(B) *L. lecanii*

Figure 1. Dose - mortality response of sucking pests to *L. saksenae* and *L. lecanii* at 4 DAT

observations were also reported in a study conducted by Ekesi and Maniania (2000) with *M. anisopliae* (10^8, 10^7, and 10^6 spores mL^{-1}) on nymphs of legume flower thrips, *M. sjostedti,* three DAT. The mortality recorded was 15.2, 20.3, and 25.7 per cent in nymphs and 64.1, 72.7 and 100 per cent in adults, with 10^6, 10^7, and 10^8 spores mL^{-1}, respectively. Herlinda *et al.,* (2008) also documented the evidence for dose - mortality relationship in *M. anisopliae* spores against third instar nymphs of *L. acuta*. The mean mortality of nymphs increased from 90 to 100 per cent when the spore concentrations increased from 10^3 to 10^7.

On comparing the infectivity of *L. saksenae* to adults and nymphs of *L. acuta*, it was evident that nymphs were more susceptible, as 100 per cent mortality was achieved at 48 HAT while it took 72 h in the case of adults. This observation was more evident on comparing the mortality data for 10^6 spores mL^{-1}, wherein the mortality for nymphs were recorded as 60, 70, 73.33, 83.33, 96.67 per cent respectively and for adults it was 36.67, 53.33, 66.67, 73.33, 86.67 per cent respectively at 24 h interval. Stark evidence to differential infectivity of *L. saksenae* to different life stages of *N. nigropictus* is contrasted upon critical examination of Tables 6 and 7. When the highest spore concentration of 10^8 spores mL^{-1} recorded cent per cent kill of nymphs at 48 HAT, it took 72 h to achieve the same feat, in adult hoppers. Similar trends resurface while analysing the 100 per cent kill of 10^7 spores mL^{-1}, where it took 72 h in nymphs and 96 h in adults. This observation is made more authentic on comparison of the mortality observed with 10^5 spores mL^{-1}on the subsequent days. In nymphs, the mortality recorded were 10, 13.33, 16.67, 26.67, 43.33, 60 and 70 per cent while in adults it was 6.67, 6.67, 13.33, 23.33, 30, 50, and 60. Clearly the nymphs are more susceptible to fungal infections than adult hoppers.

Urquiza and Keyhani (2013), attributed the susceptibility of nymphs to the extensive sclerotization of the cuticle that takes place after final moult. The hard exoskeleton of adult insects, reduce their susceptibility to fungal pathogens when compared to the nymphs. Increased susceptibility of nymphs to *M. anisopliae* spores had also been described by Sedighi *et al.*, (2013). Hundred per cent

mortality was recorded in fifth instar nymphs of corn bug, *Eurygaster integriceps* Puton, while only 52.50 per cent of adults died, during 12 day observation period, when sprayed with spore suspensions of *M. anisopliae*.

Comparison of Tables 10 and 11, clearly states the susceptible nature of *N. lugens* nymphs to the spores of *L. lecanii,* than adults. A critical retrospect reveals higher percentage of nymphal mortality than adults for the same spore concentration. With 10^8 spores mL^{-1}, 66.67, 86.67, and 96.67 per cent mortality of nymphs was observed at 96, 120 and 144 HAT, while the adults recorded 50, 60, and 83.33 per cent mortality for the same time interval. The LC_{50} values computed at 144 HAT for both adults and nymphs, further authenticates the phenomenon. It is clear that the LC_{50} value for adult (Table 17) is 5 times high, indicating higher virulence of fungi towards nymphs (6.13×10^5 and 1.22×10^5 spores mL^{-1}) . Similar trends were noted in *N. nervosa* too. Fransen *et al*., (1987) reported similar findings with the younger instars of greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) where the nymphs exhibited more susceptibility to the spores of *Aschersonia aleyrodis* Webber than the adults. They observed that the adult whiteflies were resistant and took infections rarely.

Geng and Zhang (2004) reported higher susceptibility of adults of both BPH *N. lugens* and white backed planthopper (WBPH), *S. furcifera* to spores of *M. anisopliae* var. *acridum.* For two different spore doses of 10.5 and 116.3 spores mm^{-2} of treated surface, the mortality recorded was 81 and 100 per cent for adults of BPH, while it was 85 and 100 per cent for WBPH. The corresponding death rate in nymphs was 33 and 51 per cent for BPH and 42 and 75 per cent for WBPH. They concluded that the nymphs of *N. lugens* and *S. furcifera* were more resistant to fungal infections than adults. They speculate the shorter time of moulting in younger instars, and the smaller surface area of nymphs, result in reduced chances for cuticular interception of viable conidia.

Comparison of LC_{50} values of *L. saksenae* and *L. lecanii* on the sixth day after treatment (Tables 16 and 17) revealed that *L. lecanii* needed a tenfold increase in spore concentration ($1.13 - 6.13 \times 10^5$ spores mL^{-1}) than *L. saksenae*

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(1.50 - 4.90 x 10^4 spores mL^{-1}), in all the susceptible sucking pests, indicating higher virulence of the indigenous isolate. Concentration required to cause 90 per cent mortality further substantiated the improved virulence of *L. saksenae* (4.71 - 8.19 x 10^5 spores mL^{-1}), while for *L .lecanii* the value was hundred times higher (1.32 x 10^7 to 5.34 x 10^8 spores mL^{-1}). The Lethal Time for 50 per cent mortality (LT_{50}) for *L. saksenae* @ 10^8 spores mL^{-1} was 18.58 h and 17.58 h for adults and nymphs of rice bug.

Vestergaard *et al.*, (1995) calculated the LC_{50} of *M. anisopliae* to *F. occidentalis* as 5 x 10^6 and 3 x 10^5 conidia mL^{-1}, three and five days post-inoculation. Fadayivata *et al.*, (2014) reported the pathogenicity of *L. longisporum* strain LRC 190 to cereal aphids *Sipha maydis* Passerini and *Metopolophium dirhodum* (Walker) and calculated the LC_{50} as 5.9 x 10^5 and 3.2 x 10^6 conidia mL^{-1} and LT_{50} computed at 10^8 conidia mL^{-1} as 2.9 and 4.4 days, for *S. maydis* and *M. dirhodum*, respectively.

Lower LC_{50} values recorded with *L. saksenae* when compared to *L. lecanii* in the present study and *M. anisopliae* used in investigation of Vestergaard *et al.*, (1995) once again signifies increased virulence of *L. saksenae* to sucking pests. The LT_{50} values worked out by Vestergaard *et al.*, (1995) ranged from 1.7 days (40.80 h) with 10^8 conidia mL^{-1} to 7.0 days (168 h) with 10^5 conidia mL^{-1}. Less time taken by *L. saksenae* (LT_{50} = 17.58 to 18.58 h) against *L. acuta*, further proves its accelerated speed of kill.

A critical analysis across Tables 2-17 throws light into the superiority and virulence of *L. saksenae* over *L. lecanii* (Figure 2. A, B, C). Considering the major sucking pest during vegetative stage of rice BPH *N. lugens* as standard, the above statement is justified beyond doubt. There is an unmistakable difference in infectivity exhibited by *Lecanicillium* spp, characterized by higher virulence of one species over the other to the same test insects. Kirkland *et al.*, (2004) reported differential infectivity of entomopathogenic fungi *B. bassiana* and *M. anisopliae* to ticks *Amblyomma* spp. *B. bassiana* spores @ 10^8 spores mL^{-1} that caused 90 per cent mortality of *A. maculatum* and only 15 per cent in *A. americanum* over a 28

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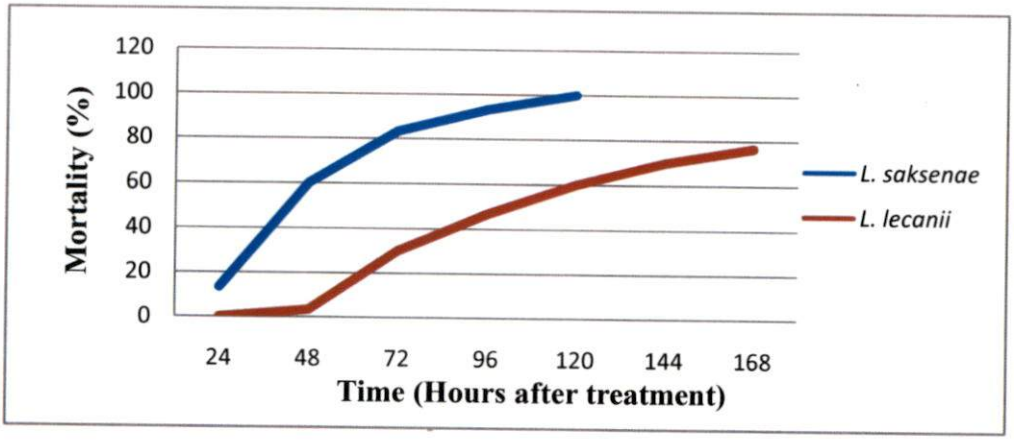
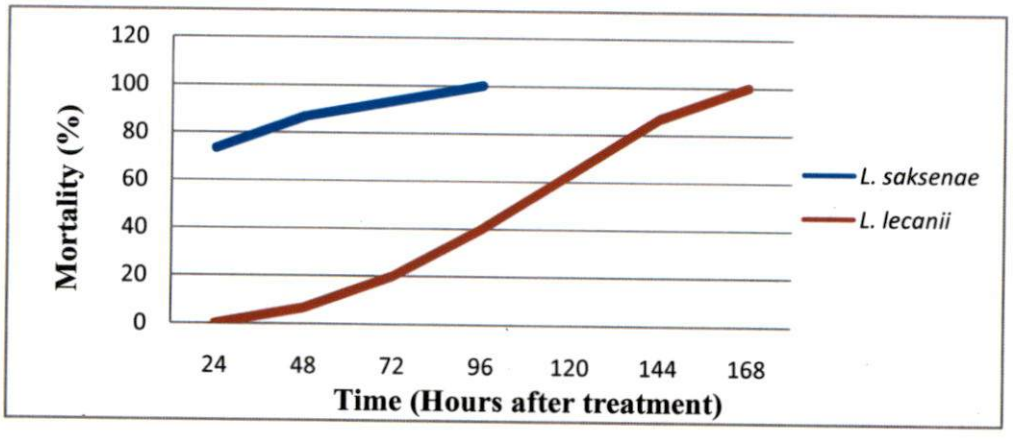
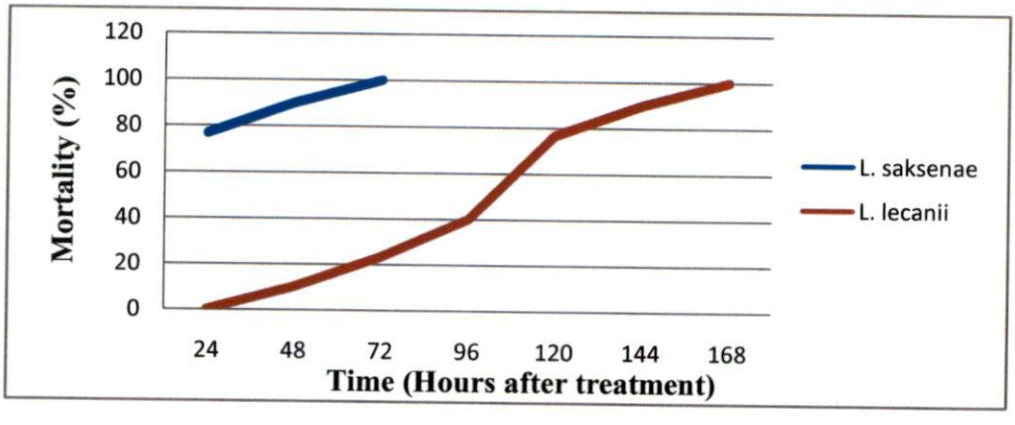


(A) *N. lugens* adults



(B) *N. nigropictus* adults



(C) *N. nervosa* adults

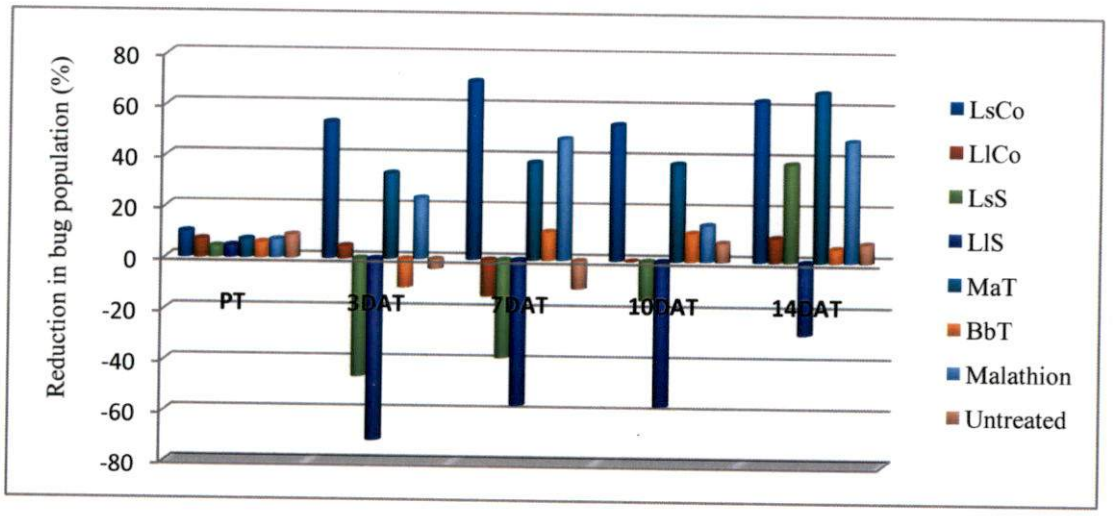
Figure 2. Comparative infectivity of *Lecanicillium* spp to insects @10^7 spores mL^{-1}

days observation period. Similarly *M. anisopliae* spores caused 60 and 15 per cent mortality to *A. maculatum* and *A. americanum*. Differential infectivity of *L. lecanii* was reported by Jackson *et al.*, (1985). Among the eighteen isolates, two isolates (A, B) were highly virulent, recording more than 90 per cent adult mortality in *Macrosiphoniella sanborni* Gillette, while isolates O, P, and R reported no mortality at all. In the present study, though *L. lecanii* was found to be infective to all the hoppers, a remarkable tendency of specificity has been observed in the case of the indigenous isolate *L. saksenae* against rice bug *L. acuta*.

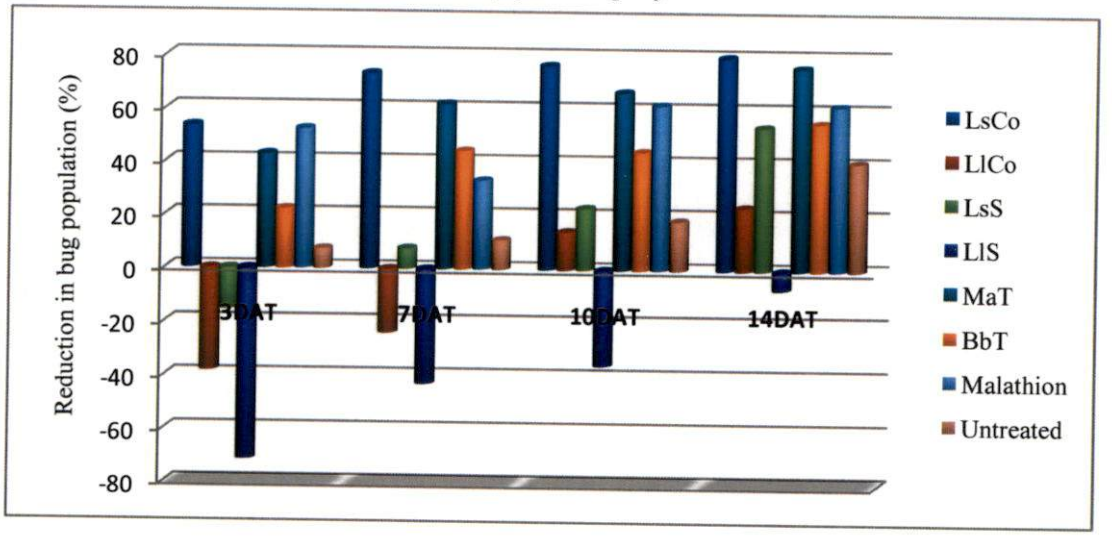
FIELD EFFICACY OF BIOFORMULATIONS OF *Lecanicillium*

Field evaluation is an indispensable tool to authentically validate the findings observed in laboratory experiments. The promising results obtained in laboratory assay may not replicate in field conditions due to various biotic and abiotic stresses present in open field condition. To compare the field efficacy of the bioformulations of *L. saksenae* and *L. lecanii* an experiment was laid out with seven treatments that included chitin enriched formulations of *L. saksenae* and *L. lecanii* @ 10 mL liter^{-1} (spores formulated in oil base @ 10^{8} spores mL^{-1}, spore suspensions (in sterile water) of *L. saksenae* and *L. lecanii* @ 10^{7} spores mL^{-1}. The talc based KAU product (culture broth : talc in the ratio 1:3) of *M. anisopliae* and *B. bassiana* @ 20 g L^{-1} served as biopesticide check and Malathion 0.1% , the chemical check. Two sprayings were given, when the bug counts reached ETL (2 bugs hill^{-1}). The efficacy was evaluated based on population of rice bugs per five sweeps and five random hills taken at third, seventh, tenth and fourteenth days after each spraying, (Figure 3. A, B) as well as the gross yield, net yield and straw yield recorded at harvest.

Among the bioformulations, chitin enriched formulation of *L. saksenae* recorded a minimum population of 1.33 bugs plot^{-1} at 14 days after spraying (DAS), which was on par with that recorded with the biocontrol check, *M. anisopliae* talc product (1.67 bugs plot^{-1}). Spore suspensions of *L. saksenae* which recorded 4.33 to 2.00 bugs plot^{-1}, ranked second. The knock down effect



(A) First spray



(B) Second spray

Figure 3. Effect of bioformulations on population of *L. acuta*

LsCo: Chitin enriched formulation of *L. saksenae* @10^7spores mL^{-1}
LlCo: Chitin enriched formulation of *L. lecanii* @10^7spores mL^{-1}
LsS: Spore suspensions of *L. saksenae* @10^7spores mL^{-1}
LlS: Spore suspension of *L. lecanii* @10^7spores mL^{-1}
MaT: Talc based *M. anisopliae* formulation @ 10^8spores mL^{-1}
BbT: Talc based *B. bassiana* formulation @ 10^8spores mL^{-1}
Malathion: Malathion 0.1%
Untreated check

noted in rice bug during the course of laboratory experiment could not be witnessed in the field, though it could effectively manage the population of bugs. This may be attributed to the fact that, the formulation which was purely based on spores has eliminated the role of metabolites present in the culture broth. *L. saksenae* as reported by Jasmy (2016), secretes a variety of primary metabolites *viz.* the cuticle degrading enzymes chitinase, lipase and protease as well as the secondary metabolites like dipicolinic acid which definitely might have played a vital role in bringing a knock down effect, had it been included in the formulation. This study thus urges the need to investigate in detail the biocide molecule present in *L. saksenae*.

L. saksenae has been reported as a keratin degrading fungus by Kushwaha in 1980 and also as a pesticide degrader by Pinto *et al.*, (2012). The parity observed with talc based product of *M. anisopliae* and chitin enriched formulation of *L. saksenae* may also be accounted with the role of metabolites that were included in the talc based product of *Metarhizium.* Malini (2015) reported the efficacy of the talc based product of *M.anisopliae* in managing rice bug population. She recorded a population of 17.33 bugs plot^{-1} at 21 DAT and the treatment ranked second, while blended cultures of *M. anisopliae* @ 10^{10} spores mL^{-1} ranked first with 16.33 bugs plot^{-1}. EPF cultures used as tank mix formulations will certainly be the best control strategy.

However, basic formulations have a differentially phased advantage over tank mix formulations in better shelf life, easiness in handling, marketing and storage. Prior *et al.* (1988) opined that the virulence of the fungal spores observed in laboratory are consistently maintained in field as well, by oil based formulations. Spores of *M. anisopliae* conidia were more virulent in oil formulation (Ibrahim *et al.*, 1999). Persistence of *M. anisopliae* spores was ensured in plant based oils (Inyang *et al.*, 2000).

Highest gross yield of 3.55 kg plot^{-1} (plot size: 2 x 2 m) recorded from plots treated with spore suspension of *L. saksenae* was statistically on par with that

of the bioformulation of *L. saksenae* (3.5 kg plot $^{-1}$). However a glance through the literature failed to unveil any studies which compared or evaluated the efficacy of *Lecanicillium* spp in the management of rice bug.

Natural enemy population observed separately as number of predatory insects (coccinellids, mirids, and reduviids), spiders, and hymenopteran parasitoids in five sweeps plot^{-1} revealed that the population in general, did not vary among the treated and untreated plots. However, on 10th day after first spraying and 10th and 14th day after second spraying, the population of predators was minimum in plots treated with Malathion 0.1%. The study clearly indicates that, bioformulations based on *L. saksenae* and *L. lecanii* as well as the bioformulations used as checks (*M. anisopliae* and *B. bassiana*) are safe to natural enemies found in the rice ecosystem *viz,* coccinellids, *C. transversalis*, *M. discolor*, mirid *C. lividipennis*, reduviids, spiders *Tetragnatha* sp., and *Oxyopes* sp., and hymenopteran parasitoids., *Bracon* sp., *Xanthopimpla* sp., *Ophius* sp. Clearly the bioformulations are safe to natural enemies found in rice ecosystem and this helps to promote their adoption as commercial products.

Reddy *et al.*, (2013) recorded highest spider count of 10.0, 11.4, and 10.5 per hill and 20.5, 23.5, and 21.8 mirids hill^{-1} in plots treated with 1 x 10^{8} CFU of *B. bassiana*, *L. lecanii*, and *M. anisopliae*, when compared to 2.5 spiders hill^{-1} and 4.0 mirids hill^{-1} in Acephate 75% SP treated plots, 10 days after second spray in rice field. Maketon *et al.*, (2015) tested the field efficacy of two indigenous strains of *B. bassiana* CKB-048 and *Metarhizium robertsii* J.F. Bisch., Rehner & Humber strain CKM-048, against *N .lugens*, and observed that the population of spiders *Lycosa pseudoannulata* (Boesenberg and Strand) and *T. maxillosa* and the mirid *C. lividipennis* did not vary significantly among the treatments reflecting the safety of entomopathogens to non target organisms.

Summary

6. SUMMARY

Alarming reports of harmful levels of pesticide residues and increasing consumer preference to residue free foods call for need based research on microbial pathogens of insects, their virulent indigenous isolates and economical and environmentally viable formulations. The study entitled "Efficacy of chitin enriched formulations of *Lecanicillium* spp against sucking pests of rice *Oryza sativa* L." was carried out at College of Agriculture, Vellayani and Integrated Farming Systems Research Station, Karamana, Thiruvananthapuram, during 2015-17. In this study a comparative performance of the indigenous isolate of *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno (Accession No. LsVs1 7714), and the NBAIR isolate of *Lecanicillium lecanii* (Zimmermann) Zare and Gams, in their efficacy in managing sucking pests of rice was assessed in terms of pathogenicity, virulence, cross infectivity to crop, safety to natural enemies and on the field efficacy.

Pathogenicity studies were conducted on *Leptocorisa acuta* (Thunberg), *Scotinophara coarctata* (F.) *Menida versicolor* (Gmelin) brown planthopper *Nilaparvata lugens* (Stal), green leafhopper *Nephotettix nigropictus* (Stal), white leafhopper *Cofana spectra* (Distant), and white winged planthopper *Nisia nervosa* (Motschulsky) using blent culture suspensions of both the fungi @ 10^7 spore mL^{-1}. The studies revealed that the indigenous isolate *L. saksenae* was infective to rice bug *L. acuta* and the hoppers, *N. lugens*, *N. nigropictus*, *N. nervosa* and *C. spectra,* whereas *L. lecanii* thoygh pathogenic to all the hoppers was not to major sucking pest of rice, *L. acuta.* The pentatomoid bugs *S. coarctata*, and *M. versicolor* did not take infection.

Symptoms exhibited by *L. acuta* when treated with *L. saksenae* were peculiar in terms of the behavioral changes observed. Post 14 hours of treatment, insects showed non directional movement, high tendency for aggregation, followed by convulsions characterized by abdominal arching and leg twitching, eventually leading

to paralysis and death. Time taken for 100 per cent mortality was 72 h in nymphs and adults. Symptoms developed in the treated insects were more or less similar in the case of hoppers which included lethargy, cessation of feeding and mycelial growth on cadavers, differing only in time taken for complete mortality. Complete mortality of insects treated with *L. saksenae* occurred within 72 to 120 hours after treatment (HAT), while for *L. lecanii* it was recorded at 144 to 168 HAT.

Successful adoption of an entomopathogen calls for validating their complete safety to non target organisms. Leaf and soil inoculation of spores @ 10^{9} spores mL^{-1} did not result in symptom development of potted rice seedlings. The natural enemies commonly sighted in rice ecosystem *viz*., the coccinellids *Micraspis discolor* (F.) and *Coccinella transversalis* F., the mirid bug, *Cyrtorhinus lividipennis* Reuter, the carabid beetle, *Ophionea nigrofasciata* Schmidt-Gobel, and the predatory spiders *Tetragnatha maxillosa* (Thorell) and *Oxyopes shweta* Tikader were found unaffected with topical application of spores @ 10^{9} spores mL^{-1}, proving the safety of both the indigenous and exotic fungi to non target organisms.

Experiment to work out the effective doses of the fungi, assessed the dose - mortality relationship between the fungi and susceptible insects. Mortality recorded at 24 h interval after topical application of six different spore concentrations ranging from 10^{3} to 10^{8} spores mL^{-1}, showed a dose dependant nature. Higher doses of *L. saksenae* @ 10^{8} and 10^{7} spores mL^{-1}, killed all the insects within 48 - 120 h, while with *L. lecanii* it took 120 - 168 h. Meanwhile, with both the fungi 10^{6} spores caused hundred per cent mortality beyond 72 HAT for *L. saksenae* while for *L. lecanii* the same spore concentration could not achieve cent per cent mortality throughout the experimental period. The lower concentrations 10^{5}, 10^{4}, and 10^{3} spores mL^{-1}, recorded very low or no mortality at all during the experimental period. Since the spore concentrations of 10^{8}, and 10^{7} were equally effective in attaining 100 per cent

mortality within the same time frame the effective dose was fixed as 10^7, with a view to save the inoculum.

The higher virulence of *L. saksenae* over *L. lecanii* was further substantiated by its lower Lethal Concentrations (LC 50 and LC 90). The LC_{50} of *L. saksenae* were 2.99 x 10^4 and 1.72 x 10^4 spores mL^{-1} for adults and nymphs of *L. acuta*, 1.36 x 10^4 and 1.68 x 10^4 spores mL^{-1} for adults and nymphs of *N. lugens*, 4.90 x 10^4 and 1.53 10^4 spores mL^{-1} for adults and nymphs of *N. nigropictus*, and 1.63 x 10^4 and 1.50 x 10^4 spores mL^{-1} for adults and nymphs of *N. nervosa*. In the case of *L. lecanii* the LC_{50} values calculated were , 6.13 x 10^5 and 1.22 x 10^5 spores mL^{-1} for adults and nymphs of *N. lugens*, 2.26 x 10^5 and 1.13 x 10^5 spores mL^{-1} for adults and nymphs of *N. nigropictus*, and 3.40 x 10^5 and 4.49 x 10^5 spores mL^{-1} for adults and nymphs of *N nervosa*. Similarly the LC_{90} of *L. saksenae* ranged from 4.71 - 8.19 x 10^5spores mL^{-1} and that of *L .lecanii* ranged from 1.32 x 10^7 to 5.34 x 10^8 spores mL^{-1}. Clearly, there is a tenfold increase in LC_{50} and hundred fold increase in LC_{90} of *L. lecanii* compared to *L. saksenae*. Less time taken by *L. saksenae* (LT_{50} = 17.58 to 18.58 h) against *L. acuta*, further proves its accelerated speed of kill.

Virulent geographical isolates, delivered as ideal formulations can improve field efficacy of microbes. Field trials to evaluate the efficacy of the bioformulations in managing rice bug as well as their impact on natural enemies was carried out in the rice ecosystem. The bioformulations tested, were chitin enriched oil formulation of *L. saksenae* @ 10^7 spores mL^{-1} (sprayed at 10mL per liter), chitin enriched oil formulation of *L. lecanii* @ 10^7 spores mL^{-1} (sprayed at 10 mL per liter), *L. saksenae* spore suspension @ and *L. lecanii* spore suspension @10^7 spores mL^{-1}. Talc based products of *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin @ 10^8 spores mL^{-1} (20 g per liter) served as biocontrol check and Malathion 0.1% served as chemical check. Observations on the mean population of rice bug per five random hills and five sweeps revealed the efficacy of chitin

enriched formulation of *L. saksenae*, the count being 3.00 bugs plot^{-1} seven days after first spraying and 1.33 plot^{-1} fourteen days after second spraying. The control obtained was in parity with one of the biocontrol check using *M. anisopliae* population being, 4.33 bugs plot^{-1} on seventh day after first spraying and 1.67 bugs per plot on fourteenth day after second spraying. Spore suspensions of *L. saksenae* which recorded 4.33 to 2.00 bugs plot^{-1}, ranked second. Quick kill exhibited by *L. saksenae* in laboratory assays, was not witnessed in open field owing to the fact that, only spores were taken for preparing the formulation, thus ruling out the suspected role of metabolites of *L. saksenae*, reported by Jasmy, 2016. The parity observed with the *M. anisopliae* was also due to the added advantage of blending the culture as a whole in the talc based product. This clearly indicates EPF performs well when tank mix formulations of pure cultures are used rather than the pure formulations of spores. However, the merits of a basic formulation which includes better shelf life, easiness in application, less transport and marketing difficulties etc. makes formulation inevitable in the case of microbes.

The yield parameters were high in plots treated with chitin enriched oil formulation of *L. saksenae* and spore suspension of *L. saksenae*. The gross yield of (3.48 kg plot^{-1}) and net yield (3.25 kg plot^{-1}) recorded in plots (2 x 2 m) treated with chitin enriched oil formulation of *L. saksenae* was on par with those observed in plots treated with spore suspension of *L. saksenae*, gross yield being 2.68 and net yield 2.50 kg plot^{-1}, respectively.

Population of insect predators, *viz.* coccinellids , *C. transversalis*, *M. discolor*, mirids, *C. lividipennis*, did not vary significantly on third, seventh and fourteenth day after first spray and third and seventh day after second spray among the treated and untreated plots, the average population ranging from 0.67 to 4.67 per plot. However, the population was significantly low (1.67, 1.33, and 1.00) in plots treated with Malathion 0.1% on the tenth day after first spay and tenth and fourteenth day after

second spray. The count of predatory spiders *Tetragnatha* sp., and *Oxyopes* sp., and hymenopteran parasitoids., *Bracon* sp., *Xanthopimpla* sp., *Ophius* sp. did not vary significantly among the various plots throughout the experimental period.

The salient findings of the investigation are

- The indigenous species *Lecanicillium saksenae* (Accession no. LsVs 1 7714) was infective to the major sucking pests of rice, the rice bug and BPH
- It was infective to various hoppers, but non infective to pentatomid bugs
- The exotic species, *L. lecanii* (NBAIR- Vl 8) was non infective to rice bug and pentatomids but infective to the hoppers
- *L. saksenae* was safe to natural enemies and non infective to rice plant
- *L. saksenae* caused 100 per cent mortality of rice bug within 48 hours of treatment
- Higher virulence of *L. saksenae* is substantiated by its lower LC_{50} and LC_{90} values
- Effective dose of *Lecanicillium* species is 10^7 spores mL^{-1}
- Chitin enriched formulation of *L. saksenae* @ 10^7 spores mL^{-1} applied at a dose of 10 mL l^{-1}, could effectively control the rice bug population, with a net yield of 8.125 tons ha^{-1}
- The bioformulations did not affect the natural enemy population, while Malathion reduced their population

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Efficacy of chitin enriched formulations of *Lecanicillium* spp against sucking pests of rice *Oryza sativa* L.

by

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Abstract of the thesis

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ABSTRACT

The current study entitled "Efficacy of chitin enriched formulations of *Lecanicillium* spp against sucking pests of rice *Oryza sativa* L. was carried out at College of Agriculture, Vellayani and Integrated Farming Systems Research Station, Karamana, Thiruvananthapuram, during 2015-17. The objective was to evaluate the efficacy of chitin enriched formulations of *Lecanicillium* spp against sucking pests of rice and to assess their impact on natural enemies. The species evaluated were the indigenous isolate *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno (Accession No. LsVs 1 7714) and the NBAIR Isolate of *Lecanicillium lecanii* (Zimmermann) Zare and Gams, (Vl 8).

The indigenous isolate, *L. saksenae* was found to be infective to rice bug *Leptocorisa acuta* (Thunberg), brown planthopper *Nilaparvata lugens* (Stal), green leafhopper *Nephotettix nigropictus* (Stal), white leafhopper *Cofana spectra* (Distant), and white winged planthopper *Nisia nervosa* (Motschulsky). The exotic species *L. lecanii* was infective to the hoppers but not to rice bug. The pentatomid bugs *Scotinophara coarctata* (F.) and *Menida versicolor* (Gmelin) were not susceptible to *Lecanicillium* spp. Symptoms of mycosis in hoppers which were similar in both the fungi included inactivity, cessation of feeding in dying insects and growth of white fluffy mycelia in the cadaver. The repeated tremors and convulsions were observed in rice bugs treated with *L. saksenae* within 14 h, which might be due to the action of toxic metabolites.

Cross infectivity studies to non target organisms revealed that *L. saksenae* was found to be safe to the predatory coccinellids *viz.*, *Micraspis discolor* (F.) and *Coccinella transversalis* F., the mirid bug, *Cyrtorhinus lividipennis* Reuter, the carabid beetle, *Ophionea nigrofasciata* Schmidt-Gobel, and the predatory spiders *Tetragnatha maxillosa* (Thorell) and *Oxyopes shweta* Tikader, when treated with

spore suspension of 10^9 spores mL^{-1}. Leaf and soil inoculation of the fungus @ 10^9 spores mL^{-1} did not show any cross infectivity to rice plants.

Laboratory experiment to determine the effective dose of the selected fungi revealed that, spore suspension @10^7 and 10^8 spores mL^{-1} of *L. saksenae,* caused 100 per cent mortality of *L. acuta* at 48 - 72 hours after treatment (HAT). Similar trend was observed in the case of the hoppers, *N. nigropictus, N. nervosa* and *C. spectra*, but for *N. lugens* it took 96 - 120 h. In the case of *L. lecanii*, @ 10^8 spores mL^{-1}, 100 per cent mortality of *N. lugens* adults was recorded at 168 HAT, while only 76.67 per cent died in 10^7 spores mL^{-1} during the same time period. The time taken was extended upto 168 h for other hoppers.

Comparison of median lethal concentration values of *L. saksenae* and *L. lecanii* on the sixth day after treatment, revealed that *L. lecanii* needed a tenfold increase in spore concentration (10^5 spores mL^{-1}), in all the susceptible insects, indicating higher virulence of the indigenous isolate. Lethal concentration to cause 90 percent mortality values further substantiates the improved virulence of *L. saksenae* (10^5spores mL^{-1}), while for *L .lecanii* the value was hundred times higher (10^7spores mL^{-1}). The LT_{50} of *L. saksenae* was 18.58 h and 17.58 h for adults and nymphs rice bug.

Field evaluation of the bioformulations revealed that, chitin enriched formulation of *L. saksenae* was superior in terms of population of rice bug (1.33 bugs per five sweeps and five random hills $plot^{-1}$) and yield (3.25 kg $plot^{-1}$, plot size 2 x 2 m). Though the talc formulation of *Metarhizium anisopliae* (Metschnikoff) Sorokin was equally effective in reducing the population (1.67) there was no significant increase in yield (2.17 kg $plot^{-1}$). Population of insect predators, *viz.* coccinellids, mirids, and reduviids did not vary significantly on third, seventh and fourteenth day after first spray and third and seventh day after second spray among the treated and untreated plots, the average population ranging from 0.67 to 4.67 per plot,. However,

the population was significantly low (1.67, 1.33, and 1.00) in plots treated with Malathion 0.1% on the tenth day after first spay and tenth and fourteenth day after second spray. The count of predatory spiders and hymenopteran parasitoids did not vary significantly among the various plots throughout the experimental period.

The study indicated that the indigenous isolate, *L. saksenae* is more virulent than the exotic isolate *L. lecanii*. Though *L. lecanii* could control the hopper pests in rice, it is non infective to the major pest, rice bug, which is effectively managed by chitin enriched formulations of *L. saksenae*. It is safe to natural enemies of rice ecosystem and non infective to rice plant. Hence, it can be an effective contender and suitable replacement to chemical pesticides for the management of sucking pests in rice.