COMPATIBILITY AND SYNERGISM OF THE ENTOMOPATHOGENIC FUNGUS *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno WITH OTHER CROP PROTECTANTS

by

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(2017-11-066)

THESIS

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DECLARATION

I, hereby declare that the thesis entitled "Compatibility and synergism of the entomopathogenic fungus *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno with other crop protectants" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled "Compatibility and synergism of the entomopathogenic fungus *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno with other crop protectants" is a record of research work done independently by Ms. Keerthana K. (2017-11-066) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
@	At the rate of
<	Less than
>	Greater than
°C	Degree celsius
µg	microgram
ANOVA	Analysis of variance
CD	Critical Difference
cfu	Colony- forming units
DAI	Days After Inoculation
DAS	Days After Spraying
EC	Emulsifiable Concentrate
et al	And other co workers
g	Gram
h	Hour
kg	Kilogram
L	Litre
min	Minutes
mL	Millilitre
No.	Number
ppm	Parts per million
SC	Suspension Concentrate
Sl.	Serial
SL	Soluble Liquid
sp. or spp.	Species (singular and plural)
viz.,	Namely
WG	Wettable Granules
WP	Wettable Powder

Introduction

1. INTRODUCTION

Biological control in particular when accomplished by entomopathogenic fungi is a technique that reduces the population density of insect pests in Biointensive Pest Management (BIPM) programmes. Globally, research on biopesticides is catching momentum in recent years to seek environment friendly alternatives to hazardous chemical insecticides. Of these, entomopathogenic fungi are 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 suppression of pest population. There are over 100 genera of entomopathogenic fungi with approximately 750 species, reported from different insects. With this perspective, 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 worldwide for pest management (Rabindra and Ramanujam, 2007).

Genus *Lecanicillium* is known for its pathogenicity to wide range of sucking pests. *L. lecanii* is a promising species infective to whiteflies, aphids, scales, and mealy bugs. Inadmissibly, they are mostly non-infective to true bugs. An attempt to overcome this problem encompassed the identification of an indigenous isolate *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno, (ITCC Accession No. LsVs1 7714), from soils of Vellayani. It was found to be more virulent and adaptable to the environmental conditions prevailing in Kerala (Rani *et al.*, 2014, 2015). Its infectivity to rice bugs was proven by Sankar and Rani (2018).

An average yield loss of 18.5 per cent is caused by rice bug, *Leptocorisa acuta* F. (Pathak and Khan, 1994). Farmers fail to contain this devastating pest even after repeated application of highly persistent insecticides that decimate the natural beneficial fauna and pollute the water bodies.

L. saksenae can fairly play a pivotal role in managing rice bug without the problems of resurgence and resistance.

In commercial rice production, biocontrol agents cannot totally replace synthetic chemical pesticides. Furthermore, there are strong evidences that indicate the harmful effects of chemical pesticides on the survival of entomopathogenic fungi. Successful integration of a potent and indigenous isolate such as *L. saksenae* in the IPM package for the management of rice bug, warrants compatibility and synergistic studies with other biorationals and chemical insecticides. Investigations in this line would enable proper selection and scheduling of pest management tools, thereby accruing the benefits of compatible and synergic combinations as well as in minimizing the deleterious effects of non-compatible combinations. Therefore, the present investigation was focused on the following aspects.

- Determination of botanical, chemical and microbial insecticides as well as fungicides that are compatible with *L. saksenae*
- Assessment of synergistic effect of *L. saksenae* with suitable combinations of other biorationals
- Evaluation of field efficacy of effective combinations in managing rice bugs
- Impact of *L. saksenae* and its combination with other compatible components on the natural enemy population in rice ecosystem

Review of Literature

2. REVIEW OF LITERATURE

Fungal entomopathogens are important microbial pesticides used against variety of insect pests. Unlike other entomopathogens, entomopathogenic fungi infect insects through direct contact *via* penetration through the host cuticle. The common entomopathogenic fungi such as *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii, Nomuraea rileyi, Isaria fumosorosea* etc. have been extensively exploited for the management of a variety of insect pests (Hasan, 2014).

Genus *Lecanicillium* is widely used for managing the sucking pests of several crops. Pathogenicity of *Lecanicillium muscarium* (Petch) Zare and Gams to *B. tabaci,* and *Trialeurodes vaporariorum* (Westwood) was ascertained by Cuthbertson *et al.* (2005) and Scorsetti *et al.* (2008). *L. lecanii* was found to be infective to *Aphis gossypii* Glover, *Aphis craccivora* Koch. *Bemisia tabaci* (Gennadius), *Lecanium* sp. and *T. vaporariorum* (Lokesh, 2014 and Nithya, 2015).

Lecanicillium saksenae (Kushwaha) Kurihara and Sukarno (ITCC Accession No. LsVs1 7714) an indigenous entomopathogenic fungus isolated from soils of Vellayani, Kerala was found to be more adaptable to the environmental conditions prevailing in Kerala and was amenable to mass multiplication. Investigations carried out during the past four years proved its infectivity to an array of sucking pests including rice bug, the major sucking pest of rice. (Rani *et al.*, 2014; 2015). Apart from a wider host range compared to *L. lecanii*, it was observed to be more virulent based on the lethal dose and lethal time. Its pathogenicity could be established by detecting the cuticle degrading enzymes and toxins (Jasmy, 2016) and its field efficacy was proved in rice ecosystem, against the rice bug *Leptocorisa acuta* (Thunberg) (Sankar and Rani, 2018).

2.1 COMPATIBILITY OF ENTOMOPATHONENIC FUNGI WITH OTHER CROP PROTECTANTS

Integration of entomopathogenic fungi in IPM programme needs evaluation of its compatibility with other strategies that are likely to be used in combination to contain the pest complex. *In vitro* compatibility tests on entomopathogenic fungi with various components such as botanical, chemical and microbial insecticides as well as fungicides enables to assess their toxicological impact on the organism. It also forms a key to formulate tank mix formulations that can encounter when more than one type of pests attacking the same crop.

2.1.1 Compatibility of Entomopathogenic Fungi with Neem-based Botanicals

Compatibility of fungi is usually assessed based on their radial growth and sporulation using poisoned media technique and more confirmatively through germination studies.

2.1.1.1 Radial Growth

Several researchers studied the effect of neem based botanicals on entomopathogenic fungi. The impact varies with the plant part used as well as the method of extraction.

In vitro studies conducted by Depieri *et al.,* (2005) showed that aqueous extract of neem seed @ 1 to 2 % exhibited significant inhibition in growth of *B. bassiana* by 9.8 to 12.3 per cent. Ambethgar *et al.* (2009) observed Neem seed kernel extract (NSKE) 5 % inhibited the mycelia growth of *B. bassiana* by 22.22 per cent and *M. anisopliae* by 48.52 per cent. Deb *et al.* (2017) demonstrated that there was 21.39 to 66.67 per cent inhibition in the growth of *B. bassiana*, when grown in medium poisoned with 3 to 7% of aqueous NSKE. Kelwatkar *et al.* (2017) observed 38.28 per cent inhibition of *M. anisopliae* by NSKE 2.5 %.

Neem oil 2 % was reported to be inhibitive to the growth of *B. bassiana* and *M. anisopliae* by 36 per cent (Hirose *et al.,* 2001). Mohan *et al.* (2007) studied the

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effect of neem oil 0.3 % on the mycelial growth of 30 isolates of *B. bassiana* and concluded that it inhibited the growth of mycelia in seven isolates, while it enhanced the growth in nine other isolates and in the remaining 14, growth was similar to that in control. Potato Dextrose Agar (PDA) amended with 0.2 % neem oil, inhibited the growth of *M. anisopliae* during the first week, but later it did not. Rani (2016) reported that neem oil emulsion 0.5 % to 2 % significantly inhibited the mycelial growth of *L. saksenae.*

Neem leaf extract in alcohol (0.2 %) was found to exhibit a concentration dependent inhibition in *B. bassiana* (Gupta *et al.,* 1999). The alcohol extract exhibited more inhibition compared to water extract. Depieri *et al.* (2005) noticed that three days after inoculation, colonies of *B. bassiana* displayed only 1.8 to 4.8 per cent reduction in growth in media poisoned with neem leaf aqueous extract at 1.5 % to 15 %, respectively. However, the vegetative development of colonies in both concentrations recovered by the sixth day after inoculation with negligible reduction (0.3 %) in growth. Deb *et al.* (2017) observed that aqueous neem leaf extract at 3 %, 5 % and 7% resulted in 16.94, 39.17 and 68.33 per cent inhibition in *B. bassiana,* indicating a concentration dependent inhibition

2.1.1.2 Sporulation

Aqueous extract of neem seed kernels 4 % caused 41.5 per cent inhibition in the conidiogenesis of *B. bassiana* (Depieri *et al.,* 2005). Reduction in conidiogenesis of *B. bassiana* was 35 per cent when grown in medium poisoned with neem seed kernel extract 1 % (Islam *et al.,* 2010a).

Hirose *et al.* (2001) reported that neem oil 2 % caused more than 50 per cent reduction in conidiation of *M. anisopliae* and *B. bassiana.* Marques *et al.* (2004) found no change in the sporulation of *B. bassiana, M. anisopliae* and *Paecilomyces farinosus* (Holmskiold) Brown and Smith with 5 % neem oil. Visalakshy *et al.* (2006) reported an enhanced sporulation in *P. farinosus* and *L. lecanii* when grown in media amended with 0.2 % neem oil. Illathur *et al.* (2018)

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reported that 1 to 3 % neem oil inhibited conidiation of *L. lecanii* by 17.27 to 25.47 per cent.

2.1.1.3 Germination

Aqueous extracts of neem seed kernel (2 %) and neem leaves (1.5 %) did not affect the viability of conidia of *B. bassiana* (Depieri *et al.,* 2005). Reduction in conidial viability noted was only 0.2 to 1.3 per cent with neem seed kernel 2 % and neem leaf extract 1.5 %. Neem seed kernel extracts up to 1 % concentration reduced the germination of *B. bassiana* only by 12 per cent (Islam *et al.,* 2010a).

Emulsible neem oil below 5 % did not cause significant fungitoxic effects on germination of *B. bassiana* (Rodriguez - Lagunes *et al.,* 1997). According to Hirose *et al.* (2001) conidial germination was affected when the media was amended with 2 % neem oil. There was significant reduction in germination of *M. anisopliae* (45 per cent) and *B. bassiana* (17 per cent).

Significant inhibition was spotted in the germination of *B. bassiana* spores by the commercial formulation of neem leaves (Nimkol-L) at concentrations that are equal to or greater than 5 % a.i. (Castiglioni *et al.,* 2003). Zorzetti *et al.* (2012) reported that ethanolic extracts of neem leaves had no adverse effect in the germination of *B. bassiana* conidia.

2.1.2 Compatibility of Entomopathogenic Fungi with Insecticides

Compatibility of the entomopathogenic fungi with old and new generation insecticides tested in this study are reviewed here under.

2.1.2.1 Radial Growth

Medium poisoned with dimethoate 30% EC 0.015 % inhibited the mycelial growth of *B. bassiana* only by 20 per cent and therefore the insecticide was considered to be harmless by Kachot *et al.* (2018). It was reported by Kakati *et al.* (2018) that dimethoate 30% EC 0.05 % exhibited 21.25 and 27.81 per cent

inhibition of *L. lecanii* and *B. bassiana*, whereas in *M. anisopliae* inhibition was up to 61.07 per cent.

Chlorpyriphos 20% EC 0.015 % completely inhibited the growth of *B. bassiana* (Oliveira *et al.*, 2003). Sub-normal (0.006 %) and normal doses (0.06 %) of chlorpyriphos 20% EC caused 27.33 per cent and 48.33 per cent inhibition in the growth of *B. bassiana* (Ambethgar *et al.* 2009). Strong suppression of *B. bassiana* and *M. anisopliae* by chlorpyriphos 20% EC 0.05 % was reported by Khan *et al.* (2012). The insecticide was also found to be non-compatible to *Paecilomyces lilacinus* (Thom) Samson with 80 per cent inhibition (Mathew and Louis, 2019).

Imidacloprid 200 SL 0.0045% caused 0.56 to 1.28 per cent inhibition in growth of *B. bassiana* isolates (Rajanikanth *et al.,* 2010). Imidacloprid 17.8% SL was found to inhibit the growth of *Lecanicillium longisporum* (Petch) Zare and Gams. There was 1.16 to 3.37 per cent inhibition of growth in medium poisoned with 0.003 to 0.006 % of the formulation (Panahi *et al.,* 2012).

Thiamethoxam 250 WG 0.16 % was found to be compatible with *L. lecanii* in terms of mycelial growth (Filho *et al.,* 2001). Thiamethoxam 25% WG 0.005 % caused 21 per cent reduction in mycelial growth of *B. bassiana* and five per cent reduction in the growth of *M. anisopliae,* whereas in *Paecilomyces* sp., vegetative growth was stimulated (Neves *et al.,* 2001).

Chlorantraniliprole 18.5% SC 0.006 % had a stimulative effect on growth of *B. bassiana, M. anisopliae* and *L. lecanii* (Vijayasree, 2013). Chlorantraniliprole 18.5% SC at recommended dose caused 35.09 per cent reduction and at half dose caused 30.82 per cent reduction in the growth of *M. anisopliae.* In *B. bassiana* the inhibition was 30.49 and 28.23 per cent with recommended and half doses, respectively (Joshi *et al.,* 2018).

Flubendiamide 39.35% SC 0.005 % caused 13.25 to 39 per cent inhibition in the growth of *L. lecanii, B. bassiana* and *M. anisopliae* (Vijayasree, 2013).

2.1.2.2 Sporulation

Dimethoate 30% EC at half the field dose (0.02 %) and field dose (0.05 %) was found to inhibit sporulation of *B. bassiana* by 7 per cent and 34.1 per cent respectively (Raj *et al.,* 2011). Neeraja and Manjula (2014) reported that dimethoate 30% EC 0.05 % inhibited the conidial yield of *N. rileyi* by 91.66 per cent.

Chlorpyriphos 20% EC was found to have detrimental effect on sporulation of *B. bassiana* at 0.1 - 1 % concentration (Usha *et al.*, 2014). Total inhibition in sporulation was noticed in *Beauveria brongniartii* (Saccardo) Petch, *B. bassiana and M. anisopliae* when grown in media poisoned with chlorpyriphos 20% EC 0.04 % (Prabhu *et al.,* 2007).

Imidacloprid 200 WDG, caused 7.34 per cent reduction in sporulation of *B. bassiana,* whereas it was increased by 11.22 per cent and 28.37 per cent in *M. anisopliae* and *Paecilomyces* sp. (Neves *et al.*, 2001). Gonzalez and Pena (2017) observed different strains of *B. bassiana* exhibited varying responses to imidacloprid 17.8% SL 0.06 %. The strain BbDc34 produced more conidia whereas conidiation was inhibited in BbYs35.

Thiamethoxam 25% WG 0.005% exhibited 12.37 per cent reduction in sporulation of *B. bassiana* and 22.32 per cent reduction in *M. anisopliae*. The same concentration increased conidiation in *Paecilomyces* sp. by 43.43 per cent (Neves *et al.,* 2001). Enhanced sporulation (45 per cent increase) was observed when *L. lecanii* was inoculated into medium poisoned with thiamethoxam 250 WG 0.02% (Filho *et al.,* 2001). Thiamethoxam 250 WG 0.0025 % had significantly reduced the conidiation in *M. anisopliae* (CG 168) while in *B. bassiana* there was no inhibition (Silva *et al.,* 2013).

2.1.2.3 Germination

Dimethoate 30% EC 0.02 to 0.05 %, caused 9 to 26 per cent reduction in conidial germination of *B. bassiana* (Raj *et al.*, 2011). Khan *et al.* (2012) reported that dimethoate 0.05 % caused 90 per cent inhibition in germination of spores of *M. anisopliae* and *B. bassiana*

Chlorpyriphos 20% EC 0.03% exhibited complete reduction in germination of *B. bassiana* (Oliveira *et al.*, 2003). Total inhibition in germination was noted in *B. bassiana* strain 11 and 13 whereas only 69.38 per cent reduction was observed in strain 5A. (Rajanikanth *et al.*, 2010). Chlorpyriphos 20% EC 0.05 % inhibited the germination completely in *B. bassiana* and *M. anisopliae* (Khan *et al.*, 2012).

Imidacloprid 17.8% SL 0.003 - 0.006 % inhibited the germination of *L. longisporum* spores by 81 to 90.5 per cent (Panahi *et al.*, 2012). Usha *et al.* (2014) stated that imidacloprid 17.8% SL at recommended dose (0.006%) caused 7.5 per cent reduction in germination of *B. bassiana* isolate B 55 whereas no reduction was observed at $1/5^{th}$ and $1/10^{th}$ of recommended doses.

Thiamethoxam 25% WG 0.005% did not cause any inhibition in germination of *B. bassiana* and *M. anisopliae,* whereas a significant reduction was noticed in case of *Paecilomyces* sp. (Neves *et al.*, 2001). Silva *et al.* (2013) reported that thiamethoxam 250 WG 0.02 % affected the germination of *M. anisopliae* at 20 h after incubation, while there was no significant reduction after 48 h.

In chlorantraniliprole 18.5% SC poisoned medium there was 87.67 and 91 per cent germination of *B. bassiana* at recommended and half the recommended doses respectively, whereas in *M. anisopliae* there was complete inhibition (Joshi *et al.*, 2018).

2.1.3 Compatibility of Entomopathogenic Fungi with Fungicides

Effect of old generation fungicides such as carbendazim 50% WP, mancozeb 75 WP, copper oxychloride 50% WP and the new generation fungicides, hexaconazole 5% EC and azoxystrobin 23% SC recently reported by various researchers is reviewed below.

2.1.3.1 Radial Growth

Carbendazim 50% WP 0.1 %, inhibited the growth rate of *L. lecanii* significantly (Krishnamoorthy and Visalakshi, 2007). At 0.05 %, it completely inhibited the growth of *B. bassiana* and *M. anisopliae,* whereas in *B. brongniartii* there was 33.1 per cent inhibition (Prabhu *et al.,* 2007). At 0.05 % and 0.1% there was total inhibition of *B. bassiana* (Deb *et al.* (2017). Joshi *et al.* (2018) also reported that carbendazim 50 WP was completely inhibitive to the growth of *B. bassiana* and *M. anisopliae* at 0.25 % and 0.125 % concentrations.

Mancozeb 75% WP 0.3 % exhibited 83.12 per cent growth inhibition in *L. lecanii* (Krishnamoorthy and Visalakshi, 2007). Gonzalez *et al.* (2012) observed that mancozeb 75% WP 10 mg kg^{-1} to 2000 mg kg^{-1} caused only 6.2 to 34.3 per cent inhibition in *L. lecanii*. Mancozeb 80 WP 0.08 % caused 65.2 per cent, 55.8 per cent and 30.8 per cent reduction in the mycelial growth of *B. brongniartii, B. bassiana* and *M. anisopliae,* respectively (Prabhu *et al.*, 2007). Mancozeb 75% WP 0.25 - 0.5 % caused 44.8 - 49.16 per cent inhibition in the growth of *B. bassiana* (Joshi *et al.,* 2018).

Copper oxychloride 50% WP 0.2% had fungistatic effect on *L. lecanii* with 79.24 per cent inhibition (Olan and Cortez, 2003). At 0.05 to 0.2 %, it inhibited the mycelial growth of *B. bassiana,* but it was found to be less toxic when paralleled with other copper-based fungicides (Martins *et al.*, 2012). Challa and Sanivada (2014) observed that *B. bassiana* isolates differed in their response to copper oxychloride 50% WP 0.3 %. Growth was completely inhibited in 8 isolates while in some isolates growth was stimulated.

Hexaconazole 0.1 % was found to inhibit the growth of *B. bassiana* and *M. anisopliae* by 80 per cent (Khan *et al.,* 2012). Joshi *et al.* (2018) reported complete inhibition in radial growth of *B. bassiana* and *M. anisopliae* when grown in medium poisoned with hexaconazole 5% EC 0.25 % and 0.125 %. Reddy *et al.* (2018) observed that in hexaconazole 10 ppm, growth of *B. bassiana* displayed a negative correlation with concentration up to two days. On the 10^{th} day the mean radial growth of 5.8 mm was observed. Above 100 ppm it caused complete inhibition of this fungus.

Azoxystrobin 23% SC 0.05% was reported to be deleterious to the vegetative growth of *B. bassiana* in laboratory assays conducted by Silva and Neves (2005). Its fungistatic effect was reported by Shah *et al.* (2009) in *B. bassiana* and *M. anisopliae* when grown in medium poisoned with 0.1% of the formulation. Silva *et al.* (2013) reported that it caused least inhibition in growth of *M. anisopliae.*

2.1.3.2 Sporulation

Carbendazim 50% WP at recommended dose, completely inhibited the growth and sporulation of *M. anisopliae*. (Rachappa *et al*., 2007). The fungicide at 0.05% completely inhibited sporulation in *B. bassiana, B. brongniartii* and *M. anisopliae* in poisoned medium (Prabhu *et al*., 2007).

Mancozeb at its field concentration almost inhibited the sporulation of *M. anisopliae* with a spore yield of 5.3 x 10^6 spores mL^{-1} (Rachappa *et al*., 2007). Mancozeb 80 WP 0.08 % completely inhibited the spore production in *B. brongniartii* however a significant reduction of 85.7 per cent and 69.5 per cent was noticed in *B. bassiana* and *M. anisopliae,* respectively (Prabhu *et al.,* 2007).

Copper oxychloride 50% WP caused 55 per cent reduction in sporulation of *B. bassiana* (Rachappa *et al.,* 2007). It was also reported to reduce the spore count in *B. bassiana* isolates B44, B56 and B57 regardless of the concentrations tested (0.1 %, 0.5 % and 1 %) (Usha *et al.,* 2014).

Hexaconazole 5 SC 0.2 % exhibited total inhibition in mycelial growth of *B. bassiana* and no conidiation was noticed since there was no growth (Raj *et al.*, 2011). Neeraja and Manjula (2014) observed complete inhibition in sporulation of *N. rileyi* when grown in medium poisoned with 0.1 % hexaconazole 5% EC.

In the case of azoxystrobin 23% SC there were contradictory reports in *M. anisolpae*. At 0.1 % it was found to reduce the conidiation by 50 per cent in *M. anisopliae* (Udayababu *et al.*, 2012), while Silva *et al.* (2013) reported that this formulation at the same concentration did not affected the sporulation of *M. anisopliae.*

2.1.3.3 Germination

Carbendazim 50% WP 0.2 % totally inhibited the conidial germination of *L. lecanii.* (Krishnamoorthy and Visalakshi, 2007). Challa and Sanivada (2014) also reported that there was 100 per cent inhibition in germination of *B. bassiana* with the fungicide. At 0.25 % and 0.125 % concentrations it completely repressed the germination of *B. bassiana* and *M. anisopliae* (Joshi *et al.*, 2018).

Mancozeb 75% WP at different concentrations significantly affected conidial germination of *L. muscarium.* At 0.001 - 0.1 % concentrations it resulted in 13 - 45 per cent reduction in germination (Ali *et al.*, 2013). Challa and Sanivada (2014) reported that at 0.3 % concentration it completely repressed the conidial germination in all the 30 isolates of *B. bassiana* tested. Whereas, Joshi *et al.* (2018) reported that at 0.25 to 0.5 % concentrations it exhibited 63.33 to 72.33 per cent germination of *B. bassiana.*

Copper oxychloride 50% WP 1 % completely inhibited the germination of *M. anisopliae* and *B. bassiana* (Majchrowicz and Poprawski, 1993). At 0.05 % it caused 69 per cent inhibition in the germination of *M. anisopliae* (Vidhate *et al.*, 2015).

Hexaconazole 5% EC at the recommended dose exhibited 100 per cent inhibition in germination of *B. bassiana.* (Raj *et al.,* 2011). Joshi *et al.* (2018) reported that at 0.25 - 0.125 % concentrations the fungicide completely suppressed the germination of *B. bassiana* and *M. anisopliae.* Khan *et al.* (2012) reported that there was complete inhibition of germination in *B. bassiana* and *M. anisopliae* when it was used at 0.1% concentration.

Azoxystrobin 23% SC 0.04 % inhibited germination of *I. fumusorosea* by 80 per cent (Alessandro *et al.,* 2011). It was reported to cause significant reduction in conidial germination of *M. anisopliae.* Silva *et al.* (2013) reported that the fungicide exhibited a delay in conidial germination of *M. anisopliae* isolate CG 168. None of the spores germinated at 24 h, but after 48 h there was 52 per cent germination.

2.1.4 Compatibility between Entomopathogenic Fungi

There is little information about the results of mixed application of entomopathogenic fungi in insects. Theoretically, combined application of different species of insect pathogens can increase the efficacy of pest control (Mahmoud, 2009).

Combined application of *B. bassiana* and *N. rileyi* did not yield a beneficial result in laboratory bioassays carried out in *Spodoptera litura* (Fabricius) larvae (Rao *et al.*, 2006). According to Mahmoud (2009) *B. bassiana* and *M. anisopliae* were mutually compatible in causing mortality of olive fruit fly, *Bactrocera oleae* (Gmelin). *L. lecanii* was inhibitive to either of the fungi, compared to sole treatments. Malekan *et al.* (2013) found that *B. bassiana* and *L. muscarium* are mutually compatible in the *in vitro* mortality testing carried out in greenhouse whitefly *T. vaporariorum.* Naveeda (2018) reported that the consortium of *M. anisopliae* and *B. bassiana* (10^8 spores mL^{-1}) resulted in higher mortality compared to their individual treatment in *Henosepilachna septima* (Fabricius), *Bactrocera cucurbitae* (Coquillett) and *Diaphania indica* Saund.

2.2 SYNERGISM OF ENTOMOPATHONENIC FUNGI WITH OTHER CROP PROTECTANTS

2.2.1 Entomopathogenic Fungi and Neem Based Insecticides

Synergistic action of entomopathogenic fungus and neem was studied by James (2003). He reported that an enhanced effect on mortality of *Bemisia argentifolii* Bellows and Perring when treated with combination treatment of neem 0.3 % (Neemix 4.5) and the entomopathogenic fungus *Paecilomyces fumosoroseus* (Wize) Brown and Smith (10^5 and 10^6 conidia mL^{-1}). According to Mohan *et al.* (2007), in the laboratory bioassay on *S. litura,* combination treatment with *B. bassiana* isolate (ITCC 4688) and neem oil (0.3%) had synergistic effect on mortality, while in the combination of *B. bassiana* ARSEF 1314 and neem oil, mortality was lower and LT_{50} value was higher, compared to treatment with the fungus alone. In combination treatment with the isolate BB1, there was a reduced effectiveness. The overall interaction of *B. bassiana* and neem was synergistic with the neem tolerant isolate but antagonistic with neem sensitive isolates. Islam *et al.* (2010b) demonstrated that combination of *B. bassiana* (10^7 conidia mL^{-1}) with neem (azadiractin 0.3 EC 0.5%), resulted in 27.6 per cent increase in nymphal mortality of *B. tabaci* seven days after treatment, compared to the individual treatments. Halder *et al.* (2018) indicated compatibility and synergistic action of *L. lecanii* (10^8 cfu g^{-1}) applied in combination with neem oil (5 %) in the ratio 1:1, where the mortality of mealy bug, *Phenacoccus solenopsis* (Tinsley) increased over the sole treatments.

2.2.2 Entomopathogenic Fungi and Insecticides

The application of entomopathogens along with pesticides can have enhanced effect on the insect pests. Pesticides can act as physiological stressors and thereby predispose insects to disease. Few studies have been reported regarding combined application of entomopathogenic fungi along with pesticides with the aim of increasing their efficacy (Inglis *et al.*, 1997; 2001).

According to Quintela and Mc Coy (1998), when bioassays were performed with different concentrations of either *B. bassiana* or *M. anisopliae* (10^5 spores mL^{-1}) and different concentrations of imidacloprid 21.4 F (100 ppm, 200 ppm) as a contact or oral treatment, an increase in mortality and mycosis was noticed in first instars of citrus root weevil *Diaprepus abbreviates* (Linnaeus), which denoted synergism. Dayakar *et al.* (2000) have also found that the combination of insecticides with *B. bassiana* exhibited 1.05 to 1.24 fold increase in virulence against *S. litura* over the sole treatments. Furlong and Groden (2001) investigated the interaction between *B. bassiana* and sub lethal doses of the insecticides imidacloprid 17.4 SC (1.5 ppm) and cyromazine 75 WP (17 ppm) under laboratory conditions in the larvae of Colorado potato beetle, *Leptinotarsa decemlineata* (Say). A synergistic action was noted when the second instars were fed with potato leaf discs treated with sub lethal doses of imidacloprid 17.4 SC along with *B. bassiana* conidia (10 to 60 conidia m^{-2}). Similar results were observed when larvae were sprayed directly with *B. bassiana* conidia and feeding leaf discs treated with imidacloprid 17.4 SC immediately after spraying.

Jaramillo *et al.* (2005) demonstrated that, the combined application of *M. anisopliae* (10^7 conidia mL^{-1}) with a lower dosage of imidacloprid 350 SC (30 µl L^{-1}) resulted in significantly higher mortality levels compared to sole applications of *M. anisopliae.* Purwar and Sachan (2006) observed a synergistic interaction between *M. anisopliae* (10^7 conidia g^{-1} sand) and imidacloprid 17.8% SL 1.05 g^{-1} causing increased mortality in *Spilarctia obliqua* (Walker) when applied in combination. They also noted a similar effect with the combination of *B. bassiana* (10^5 conidia mL^{-1}) and endosulfan 35 EC (2 ppm) where there was a 4.9 fold increase in toxicity resulting in high mortality of *S. obliqua* compared to sole treatment with insecticide. Zou *et al.* (2014) reported synergistic effect in the mortality of *B. tabaci* nymphs treated with *I. fumosorosea* (10^6 spores mL^{-1}) in combination with the technical grade of insecticides spirotetramat 0.125 %, imidacloprid 0.06 % and thiamethoxam 0.125 %. Yii *et al.* (2016) observed that combination of *M. anisopliae* @ 10^7 to 10^8 conidia g^{-1} bait and fipronil (technical

grade) 0.05 mg a.i. L^{-1} resulted in increased mortality and lower lethal time in *Coptotermus curvignathus* (Holmgren) compared to sole treatments. Santos *et al.* (2018) demonstrated that combination of spiromesifen 24 SC (1.56 ppm) and *Isaria javanica* (Friedrichs and Bally) Samson and Hywel-Jones (10^6spores mL^{-1}) resulted in additive mortality to *B. tabaci*. They also noticed that there was synergistic effect when *I. javanica* @ 5 x 10^6 spores mL^{-1} and spiromesifen 24 SC @ 12.5 ppm were combined.

2.2.3 Among Entomopathogenic Fungi

Mahmoud (2009) studied the interactive effect of combination spray of two entomopathogenic fungi. He observed that the combination spray of *B. bassiana* and *M. anisopliae* (10^8 spores mL^{-1}) caused 100 per cent mortality in olive fruit fly, *B. oleae* than when treated with either of *B. bassiana* and *M. anisopliae* (80 and 92 per cent, respectively). He also reported that the combinations *B. bassiana* + *L. lecanii* and *M. anisopliae* + *L. lecanii* resulted in reduced mortality compared to sole treatments. Malekan *et al.* (2013) found that *B. bassiana* and *L. muscarium* (10^6 spores mL^{-1}) are mutually compatible causing 89.43 per cent mortality in greenhouse whitefly *T. vaporariorum.* The sole treatments with *B. bassiana* and *L. muscarium* resulted in 70.46 and 86.63 per cent mortality in the insect. The study depicted that there was beneficial effect in using two fungi together compared to its single application.

2.3 SYNERGESTIC EFFECT OF ENTOMOPATHOGENIC FUNGI UNDER FIELD CONDITIONS

Very few studies have been conducted in the field to examine the combined effect of entomopathogenic fungi with insecticides or botanicals or with the combination of two or more species of entomopathogenic fungi.

Haroon *et al.* (2011) reported that, the *Metarhizium acridum* (Driver and Milner) Bisch, Rehner and Humber (50g ha^{-1}) + neem oil (0.8%) mixture resulted in more than 50 per cent mortality in the desert locust, *Anacridium melanorhodon*

(Walker) by 11th day. Area treated with *M. acridum* alone did not cause 50 per cent mortality even after 17 days in field trials to manage the pest.

Delgado *et al.* (1999) observed 55.6 per cent mortality in grasshoppers in the plots treated with the combination treatment of *B. bassiana* (10^{13} spores mL^{-1}) + diflubenzuron 2 F (0.03 %). After 14 days, population decreased by 38.1 per cent in plots treated with *B. bassiana* (10^{13} spores mL^{-1}) alone while it was 29.4 per cent in plots treated with diflubenzuron 2 F (0.03 %) alone. The effect of the diflubenzuron 2 F + *B. bassiana* mixture was reported to be additive and not synergistic. According to Togbe *et al.* (2014) there was no significant difference in the effectiveness among the sole application of neem oil (10 %) or *B. bassiana* Bb11 (10^{12} conidia mL^{-1}) or their combinations in controlling pests of cotton. This suggested the absence of a synergistic effect between neem oil and *B. bassiana.*

Materials and Methods

2. MATERIALS AND METHODS

The study entitled "Compatibility and synergism of the entomopathogenic fungus *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno with other crop protectants" 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 2017-19.

Maintenance of fungal cultures

L. saksenae, accession no ITCC LsVs 1-7714, an indigenous isolate from the soils of Vellayani reported by Rani *et al.* (2014) was utilized for the study. The other isolates used for this study were *Beauveria bassiana* (Balsamo) Vuillemin isolate Bb 5 and *Metarhizium anisopliae* (Metschnikoff) Sorokin Ma 4, originally sourced from National Bureau of Agricultural Insect Resources, Bengaluru. The cultures were periodically passed through their susceptible host insects such as rice bug, *Leptocorisa acuta* (Thunberg) for *L. saksenae,* pseudostem weevil, *Odoiporus longicollis* Oliver for *B. bassiana* and *M. anisopliae.* Pure cultures were maintained in Potato Dextrose Agar (PDA) slants.

Raising plants

The test insect rice bug, *L. acuta* was reared on the rice variety Prathyasa (MO-21). Sprouted seeds were sown in plastic pots of 23 cm x 21 cm containing potting mixture of clay, soil, and cow dung in the ratio 2:1:1. Planting was undertaken sequentially to ensure continuous availability of panicles, in the milky stage.

Rearing of rice bugs

Adult bugs were collected from the rice field and kept under observation for any latent infection. Potted rice plants with milky panicles mentioned above were kept for rearing rice bugs. Plants were caged with nylon net tied over a wooden frame. Ten pairs of healthy adults were transferred to the rearing cage. Egg masses were collected by clipping the leaves. Clippings were then placed in a Petri plate with a moistened filter paper and kept for five days in room temperature so as to ensure maximum emergence. Thereafter, the clippings with egg mass were transferred to the new plants and fixed at the base of panicle at milky stage using a stapler. Plants with milky panicles were replaced every two weeks to ensure an adequate supply of food. Third instar nymphs and adults of uniform age collected from the rearing cage were used for the laboratory studies (Valencia and Heinrichs, 1982)

3.1 COMPATIBILITY STUDIES

Compatibility of *L. saksenae* to neem-based botanicals, insecticides and fungicides were carried out under *in vitro* conditions using poisoned food technique (Nene and Thapliyal, 1997). Compatibility was assessed based on growth by measuring mean colony diameter (cm); sporulation by counting the spore using Neubauer Haemocytometer and germination by counting the germinated spores under 40x magnification in a compound microscope (Motic BA 210). Compatibility was further confirmed by calculating Biological Index (BI) proposed by Rossie-Zalaf *et al.* (2008). It was calculated using the formula

$$\mathbf{BI = [47 \times VG + 43 \times SP + 10 \times GER] / 100}$$

Where,

VG- Percentage of vegetative growth of the fungal colony in relation to control

SP- Percentage of sporulation in relation to control

GER- Percentage of conidial germination in relation to control

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BI values were grouped into three categories of toxicological classification *viz.* 0 to 41 = toxic ; 42 to 66 = moderately toxic ; >66 = compatible

Compatibility of *L. saksenae* with other entomopathogenic fungus were assessed based on mortality and the time taken for mortality of treated insects at 24 h interval till 7 days after spraying (DAS).

3.1.1 Compatibility of *L. saksenae* with neem-based botanicals

Neem based botanicals popular among the farmers were chosen for testing their compatibility with the entomopathogenic fungus *L. saksenae.* The neem-based botanicals tested were aqueous and solvent extracts of neem seed kernel, neem oil emulsion, as well as aqueous and solvent extracts of neem leaf. The treatments were as follows,

1. Neem seed kernel aqueous extract - 0.5%, 1%, 2%
2. Neem seed kernel solvent extract - 0.5%, 1%, 2%
3. Neem oil emulsion - 0.5%, 1%, 2%
4. Neem leaf aqueous extract - 1%, 2%, 4%
5. Neem leaf solvent extract - 1%, 2%, 4%

Details of the method of preparation of the above cited botanicals are mentioned below.

3.1.1.1 Preparation of Botanicals

3.1.1.1.1 Neem Seed Kernel Extract

Dried neem kernels were ground to a fine powder using a blender. In a 250 mL conical flask, 100 mL of sterile distilled water was taken and 20 g kernel powder was added to it. The mixture was kept overnight at room temperature. It was filtered through a double-layered muslin cloth followed by Whatmann No. 1

filter paper. The extract was stored in a glass vial at 4 °C in the refrigerator for further use.

3.1.1.1.2 Neem Seed Kernel Solvent Extract

Neem kernel powder (100 g) was taken in a conical flask, an equal quantity of hexane was added to it and kept for shaking for 24 h in a rotary shaker (ROTEK BOD incubator shaker ROSI-1). The oily supernatant was discarded. The procedure was repeated three to four times until the supernatant turned clear. After deoiling, kernel powder was collected and spread evenly on a paper for hexane to evaporate completely. Deoiled neem seed kernel powder (20 g) was taken in a separate conical flask to which 50 mL of methanol was added and kept for 24 h with periodic shaking in a rotary shaker. It was then filtered using a double-layered muslin cloth followed by Whatmann No. 1 filter paper. The procedure was repeated thrice with a fresh volume of methanol. The filtrates were then pooled in a china dish. The pooled filtrate was kept in a water bath at 50 °C to evaporate methanol completely (Plate 1 A). The final extract was stored at 4 °C in a refrigerator.

3.1.1.1.3 Neem Oil Emulsion

Thin slices of ordinary bar soap were added to the lukewarm water until it was saturated. One mL neem oil was thoroughly mixed with nine mL saturated soap solution by vigorously shaking to get 10 % neem oil emulsion.

3.1.1.1.4 Neem Leaf Aqueous Extract

Leaves without apparent symptoms of insect or disease attacks were collected from the College of Agriculture, Vellayani. Leaves were washed in running tap water to remove the dirt from it and dried under shade to remove the excess water. Then it was dried in a hot air oven at 50 °C for two days. Dried leaves were made to a coarse powder using a blender. Powdered leaves were stored in airtight containers for further use.

In a 250 mL conical flask, 20 g of dried leaf powder was taken. To this, 100 mL of distilled water was added and was subjected to shaking for 24 h in a rotary shaker. Contents were then filtered using a double-layered muslin cloth, followed by Whatmann No. 1 filter paper and the filtrate was collected and stored at 4 °C in a refrigerator.

3.1.1.1.5 Neem Leaf Solvent Extract

Dried neem leaf powder (25 g) prepared as mentioned in 3.1.1.1.4 was taken in a 250 mL conical flask to which 100 mL of 99 % methanol was added. The mixture was subjected to periodic shaking in a rotary shaker for 24 h. The contents were filtered using a double-layered muslin cloth followed by Whatmann No. 1 filter paper and the filtrate was collected. The procedure was repeated thrice with a fresh volume of methanol. The pooled filtrate was kept in a china dish in a water bath at 50 °C to evaporate the extractant (Plate 1 B). The final extract was stored in a glass vial at 4 °C in a refrigerator.

3.1.1.2 Growth Bioassay

Botanicals were prepared at double the desired concentrations for the study. Each of them was filtered using a bacterial proof syringe filter of 0.22 µm. Simultaneously, double strength PDA was also prepared. The medium was sterilized in a vertical autoclave at 121°C at 1.1 kg cm^{-2} for 20 min. After cooling, filtered botanicals were mixed with an equal quantity of double strength PDA under aseptic conditions in laminar air flow chamber. About 15 mL of poisoned medium was poured in Petri dishes and were kept for solidification. After solidification, Petri dishes were inoculated with five mm disc of seven day old actively growing culture of *L. saksenae* using a flame-sterilized cork borer. The Petri dishes were incubated at room temperature for the growth of inoculated fungus. PDA medium with sterile distilled water served as untreated control and that with 2 % methanol served as treatment control. The experiment was laid out in Completely Randomized Design (CRD) with each treatment replicated thrice. Observations

Pooled extract

Final extract

(A) Neem seed kernel solvent extract

Pooled extract

Final extract

(B) Neem leaf solvent extract

Plate 1. Solvent extracts of neem seed kernels and neem leaves

were recorded by measuring the radial growth of fungus on third, seventh, 10^{th} and 14^{th} days after inoculation (DAI).

3.1.1.3 Spore Enumeration

Spore count was enumerated from 20 day old culture. Conidia were dispersed in sterile water (10 mL) with 0.02 % Tween 20 by scraping off the mycelia by a sterilized L rod. The suspension was filtered using a double-layered muslin cloth. Ten µL suspension was then transferred into the haemocytometer using a micropipette for taking the spore count.

3.1.1.4 Conidial Germination

Spore suspension was prepared from a 20 day old culture and the spore count was adjusted to 10^5 conidia mL^{-1} by serial dilution method. Sterilized glass slides were evenly coated with a drop of molten poisoned PDA and were allowed to dry in a laminar airflow chamber. After drying 100 µL spore suspension was dropped on to the glass slide and was spread uniformly. The slides were then incubated in a Petri dish lined with moistened filter paper for 24 h at room temperature. After 24 h, the slides were observed under 40x magnification in a compound microscope to count 100 spores at random and the number of germinated spores were noted. Spores with germ tube more than the diameter of the spores were considered to be germinated. Counting was done thrice at random points on the slides and the average was taken.

3.1.2 Compatibility of *L. saksenae* with Insecticides

In vitro compatibility of *L. saksenae* was carried out using poisoned food technique. The treatments were as follows,

Table 1. Details of insecticides used in compatibility studies

S. No	Chemical name	Trade name	Concentrations
1	Dimethoate 30% EC	Tafgor	0.025%, 0.05%
2	Chlorpyriphos 20% EC	Mr. Bon	0.03%, 0.06%
3	Imidacloprid 17.8% SL	Tropical Magic	0.003%, 0.006%
4	Thiamethoxam 25% WG	Actara	0.0025%, 0.005%
5	Chlorantraniliprole 18.5% SC	Coragen	0.003%, 0.006%
6	Flubendiamide 39.35% SC	Fame	0.0025%, 0.005%

Compatibility was assessed by measuring the colony diameter, spore count and germination as described under 3.1.1.2, 3.1.1.3 and 3.1.1.4.

3.1.3 Compatibility of *L. saksenae* with Fungicides

In vitro compatibility of *L. saksenae* was carried out using the following fungicides.

Table 2. Details of fungicides used in compatibility studies

S. No	Chemical name	Trade name	Concentrations
1	Carbendazim 50% WP	Bavistin	0.1%, 0.2%
2	Mancozeb 75% WP	Zinthane M-45	0.15%, 0.3%
3	Copper oxychloride 50% WP	Fytran	0.1%, 0.2%
4	Hexaconazole 5% EC	Contaf	0.1%, 0.2%
5	Azoxystrobin 23% SC	Amistar	0.05%, 0.1%

Compatibility was assessed by measuring the colony diameter, spore count and germination as mentioned under 3.1.1.2, 3.1.1.3 and 3.1.1.4.

3.1.4 Compatibility of *L. saksenae* with Other Entomopathogenic Fungi

Compatibility of *L. saksenae* with other entomopathogenic fungi was assessed at their infective doses (conidia mL^{-1}) mentioned below.

1. *L. saksenae* @ (10^7) + *B. bassiana* @ (10^8)
2. *L. saksenae* @ (10^7) + *M. anisopliae* @ (10^8)
3. *L. saksenae* @ (10^7) + *B. bassiana* @ (10^8) + *M. anisopliae* @ (10^8)

4. *L. saksenae* @ (10^7)
5. *M. anisopliae* @ (10^8)
6. *B. bassiana* @ (10^8)
7. Control (water spray)

Spore suspensions were prepared by blending the 20 day old cultures, grown in Potato dextrose broth (PDB), in a blender for 10 s. The mixture was then filtered in a double-layered muslin cloth. Spore count was adjusted to respective concentrations using a Neubauer haemocytometer. The fungal spore suspensions prepared in sterile water were sprayed on to the test insects collected from laboratory culture using an atomiser. Mortality of bugs was recorded at 24 h interval for a period of seven days. The experiment was laid out in CRD with seven treatments and three replications. In each replication, 10 insects were maintained.

3.2 SYNERGISM of *L. saksenae*

To assess whether *L. saksenae* has any synergistic effect when combined with the compatible treatments selected from the experiment 3.1, an experiment was laid out in CRD. The synergistic effect was determined based on the mortality and feeding inhibition of the treated rice bugs. For each of the treatment combination, individual components were tested as treatment check. Observations were taken at 24 h interval, for a period of seven days commencing from the treatment application. Each treatment was replicated thrice, with 10 insects per replication.

3.2.1 Synergism of *L. saksenae* with Neem-based Botanicals

3.2.1.1 Assessment of Mortality

Compatible concentrations of each of neem-based botanicals were prepared as mentioned in 3.1. Both spore suspension and botanical were mixed thoroughly in the ratio 1:1 and kept for an hour. Rearing jars of size 15 cm diameter and 20 cm height were taken and nymphs and adults separately released into it. For each

treatment, 10 insects were maintained. Rice panicles with milky grains, staked in glass vials with sucrose solution were given as food. Approximately two mL mixture was applied uniformly in the container using an atomiser. Then, the container was covered with a clean muslin cloth and fastened with a rubber band. The mortality was noticed at 24 h intervals.

3.2.1.2 Assessment of Feeding Inhibition

Feeding inhibition was calculated by counting the grains, specifically

treatment. Percentage inhibition was calculated using the formula

$$\text{Feeding inhibition (\%)} = 100 - \left(\frac{\text{Number of damaged grains}}{\text{Total number of grains}} \right) \text{X } 100$$

3.2.2 Synergism of *L. saksenae* with insecticides

Compatible combinations from 3.1.3 were tested for the synergistic action as mentioned in 3.2.1.

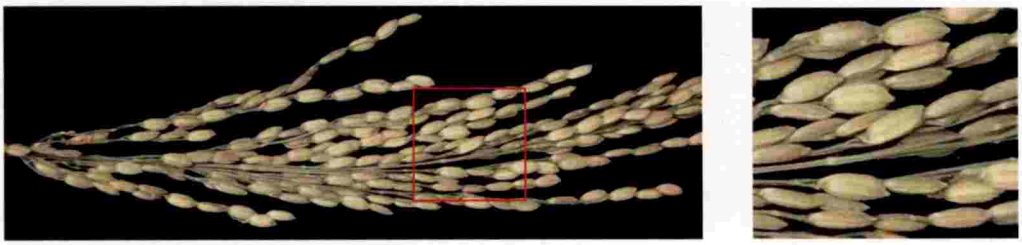
3.2.3 Synergism of *L. saksenae* with other entomopathogenic fungi

Compatible combinations from 3.1.5 were tested for the synergistic action as mentioned in 3.2.1

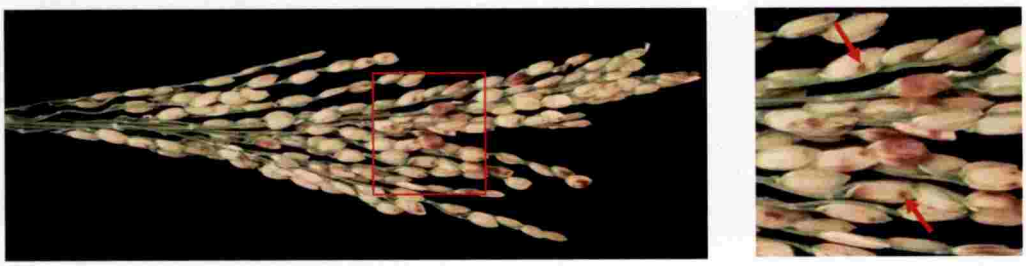
Effective treatments selected from the laboratory experiment were selected for testing their efficacy in the management of rice bug

3.3 FIELD EVALUATION

A field experiment was conducted at IFSRS, Karamana during January to May 2019. Medium duration rice variety Uma (MO 16) was selected for the study. Crop production techniques recommended in Package of Practices (2016) of KAU



(A) Healthy panicle



(B) Panicle infested with rice bug

Feeding punctures

Plate 2. Comparison of healthy and rice bug infested panicles

were followed except for plant protection. The experiment was laid out in an area of 200 m^2 in Randomised Block Design with six treatments and three replications. Unit plot size was 2 x 2 m. The treatments tested were

1. *L. saksenae* (10^7 spores mL^{-1}) + thiamethoxam 25% WG (0.0025%)
2. *L. saksenae* (10^7 spores mL^{-1}) and *B. bassiana* (10^8 spores mL^{-1})
3. *L. saksenae* (10^7 spores mL^{-1})
4. Thiamethoxam 25% WG (0.0025%)
5. *B. bassiana* (10^8 spores mL^{-1})

3.3.1 Spraying

Spraying operations were initiated when the rice bug population reached ETL (one to two bugs/hill). Each plot was separated using a shade net reaching over 50 cm above the crop canopy to restrict the movement of rice bugs after spraying. A pneumatic hand sprayer of three litres was used for the spraying operations. In each plot, 200 mL spray fluid was used. Separate sprayers were used for spore suspensions and chemical insecticides. Bioformulations were sprayed with coarse adjustment of the nozzle. The lance of sprayer was kept at a fixed height for uniform coverage. Spraying was undertaken in a spiral manner, starting from the border rows towards the centre to minimize the dispersal of the rice bugs. Sun pack screens were placed on all sides to prevent spray drift.

3.3.2 Assessment of Rice Bug Population

The population of rice bug was assessed based on the sweep and count data. Cumulative count of rice bug in five sweeps and five hills per plot were noted randomly. Sweeps were done by walking diagonally across the plot and avoiding border rows. One sweep comprise of a clockwise and anticlockwise movement of the wrist with hand outstretched and net held firmly.

Pre-treatment counts were taken a day before treatment and post-treatment count was taken after seven and 14 days. Spraying was repeated after 14 days.

3.3.3 Assessment of Natural Enemy Population

The population of natural enemies was assessed in the same way as mentioned in 3.3.2.

3.3.4 Yield data

Grain yield was recorded at harvest. The grains from treatment plots were harvested separately, sun-dried, winnowed, weighed and expressed in kg $plot^{-1}$.

3.3.5 Statistical analysis

The data obtained from the laboratory and field studies were subjected to analysis of variance (ANOVA) using WASP 2 software.

Results

4. RESULTS

The results of the study entitled "Compatibility and synergism of the entomopathogenic fungus *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno with other crop protectants" carried out during the period 2017-19 is detailed below.

4.1 COMPATIBILITY OF *L. saksenae*

4.1.1 With Neem- based Botanicals

The compatibility of *L. saksenae* to neem-based botanicals such as neem seed kernel aqueous extract - NSKE (A) (0.5 %, 1 %, 2 %), neem seed kernel solvent extract - NSKE (S) (0.5 %, 1 %, 2 %), neem oil emulsion - NOE (0.5 %, 1 %, 2 %), neem leaf aqueous extract - NLE (A) (1 %, 2 %, 4 %) and neem leaf solvent extract - NLE (S) (1 %, 2 %, 4 %) was assessed in terms of radial growth, sporulation and germination is presented below.

4.1.1.1 Radial growth

Radial growth of *L. saksenae* in terms of mean colony diameter, in PDA poisoned with botanicals is shown in Plate 3 (Table 3).

On the third day after inoculation (DAI), the lowest inhibition was noted in 0.5 % NOE and NLE (A) 1 %, which was on par with that of control (2.42 cm). The colony diameter observed were 2.45 cm and 2.37 cm respectively. The inhibition was lesser in NSKE (A) 0.5 % and 1 %, and NOE 2 %, which were on par with colony diameters 2.12 cm, 2.18 cm and 2.18 cm, respectively. There was more inhibition in NSKE (S) 0.5 % (1.93 cm) and NLE (S) 1 % (1.97 cm), which did not vary significantly. NOE 1 % and NLE (A) 2 % exhibited same growth of 1.77 cm and inhibited the growth more than the former treatments. Growth in NLE (S) 2 % (1.52 cm) and NSKE (S) 1 % (1.43 cm) was significantly lower. The treatments NSKE (S) 2 %, NLE (A), (S) 4 % recorded even lesser growth measuring

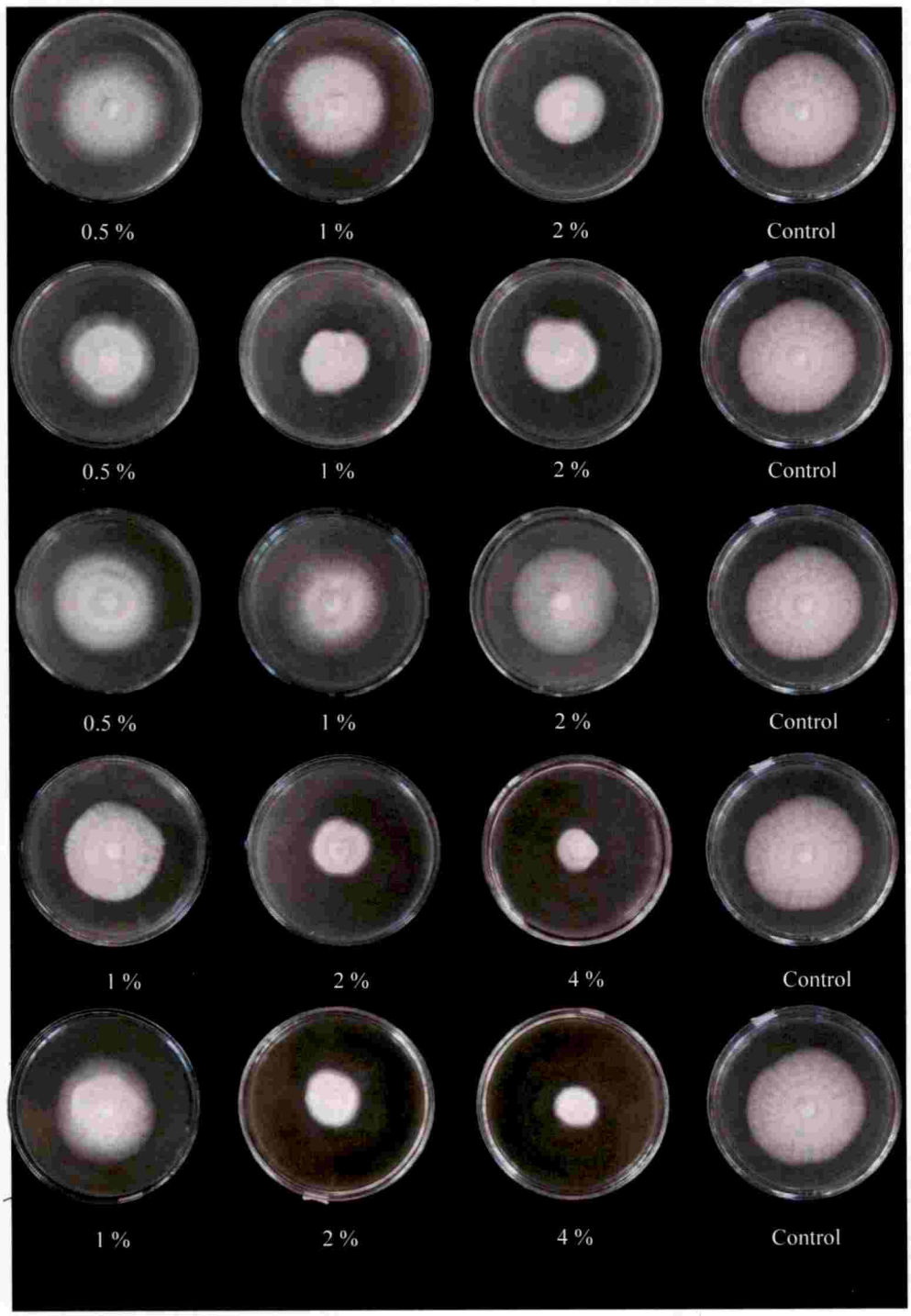


Plate 3. Radial growth of *L.saksenae* in PDA poisoned with neem based botanicals on 14th DAI

A-Aqueous S-Solvent DAI -Days After Inoculation

Table 3. Radial growth of *L. saksenae* in PDA poisoned with neem based botanicals

Botanicals	Concentrations (%)	Mean colony diameter (cm)			
		3 DAI	7 DAI	10 DAI	14 DAI
Neem Seed Kernel Extract (Aqueous)	0.5	2.12^{b} (1.46)	3.68^{b} (1.91)	4.37^{c} (2.09)	6.08^{ab} (2.46)
	1	2.18^{b} (1.48)	3.63^{bc} (1.90)	4.18^{cd} (2.04)	5.88^{bc} (2.42)
	2	0.60^{g} (0.78)	2.67^{e} (1.64)	3.47^{ef} (1.87)	4.87^{f} (2.219)
Neem Seed Kernel Extract (Solvent)	0.5	1.93^{c} (1.39)	3.18^{d} (1.78)	3.58^{e} (1.81)	5.07^{ef} (2.26)
	1	1.43^{ef} (1.19)	2.02^{h} (1.42)	2.80^{g} (1.67)	3.97^{g} (1.99)
	2	1.38^{f} (1.17)	2.23^{g} (1.49)	2.63^{g} (1.68)	3.57^{h} (1.88)
Neem Oil Emulsion	0.5	2.45^{a} (1.56)	3.98^{a} (2.00)	4.70^{b} (2.17)	6.27^{a} (2.50)
	1	1.77^{d} (1.32)	3.50^{c} (1.88)	4.00^{d} (2.00)	5.78^{c} (2.41)
	2	2.18^{b} (1.48)	3.57^{bc} (1.88)	4.17^{cd} (2.04)	4.88^{f} (2.21)
Neem Leaf Extract (Aqueous)	1	2.37^{a} (1.53)	3.70^{b} (1.92)	4.00^{d} (2.00)	5.32^{d} (2.31)
	2	1.77^{d} (1.33)	2.13^{gh} (1.46)	2.72^{g} (1.64)	3.32^{i} (1.82)
	4	1.33^{f} (1.16)	2.05^{h} (1.43)	2.38^{h} (1.55)	2.62^{k} (1.61)
Neem Leaf Extract (Solvent)	1	1.97^{c} (1.40)	3.23^{d} (1.80)	3.43^{ef} (1.85)	5.28^{de} (2.29)
	2	1.52^{e} (1.24)	2.77^{e} (1.66)	3.38^{f} (1.83)	4.15^{g} (2.03)
	4	1.33^{f} (1.15)	2.45^{f} (1.56)	2.65^{g} (1.63)	3.12^{j} (1.76)
Untreated Control		2.50^{a} (1.58)	4.05^{a} (2.01)	5.00^{a} (2.23)	6.17^{a} (2.48)
Treated control (Methanol)	2	2.42^{a} (1.56)	4.07^{a} (2.02)	5.10^{a} (2.25)	6.17^{a} (2.48)
CD (0.05)		(0.049)	(0.044)	(0.051)	(0.052)

DAI-Days after inoculation. Figures in the parentheses are square root transformed values. Values sharing same alphabets in superscript are statistically on par based on ANOVA

1.38 cm and 1.33 cm respectively. Highest inhibition in growth was noted in NSKE (A) 2 %, with colony diameter 0.60 cm.

On the seventh DAI, there was no inhibition in NOE 0.5 % (3.98 cm) compared to untreated control (4.05 cm). The inhibition was lowest in NSKE (A) 0.5 % and NLE (A) 1 % with mean colony diameter 3.68 cm and 3.70 cm. This was followed by NSKE (A) 1 % (3.63 cm) and NOE 2 % (3.57 cm) which did not vary significantly. Mean colony diameter noted was lesser in NOE 1 % (3.50 cm). The treatments NSKE (S) 0.5 % NLE (S) 1 % recorded even lesser growth of 3.18 cm and 3.23 cm respectively, which were on par with each other. Inhibition was more in treatments NSKE (A) 2 % and NLE (S) 2 % (2.67 cm and 2.77 cm, respectively). NLE (S) 4 %, NSKE (S) 2 % and NLE (A) 2 % inhibited the fungus greatly (2.45 cm, 2.23 cm and 2.13 cm, respectively). NSKE (S) 1 % and NLE (A) 4 % were the most inhibitive treatments.

The trend observed was more or less the same on the 10th DAI. Among the treatments inhibition was least in NOE 0.5 % with mean colony diameter 4.70 cm. This was followed by NSKE (A) 0.5 % (4.37 cm). This was followed by NSKE (A) 1 % (4.18 cm) and NOE 2 % (4.17 cm) which did not vary significantly from each other. NOE 1 % and NLE (A) 1 % exhibited same growth of 4 cm and inhibited the growth more than the former treatments. This was followed by NSK(S) 0.5 % (3.58 cm). The inhibition was lesser in NSKE (A) 1 %, and NOE 2 %, which were on par with colony diameter measuring 4.18 cm and 3.47 cm, respectively. Inhibition was more in treatments NSKE (A) 2 % and NLE (S) 1 % and were on par (3.47 cm and 3.43 cm, respectively). The growth in NLE (S) 2 % was 3.38 cm. NSKE (S) 1 %, 2 %, NLE (A) 2 % and NLE (S) 4 % were on par, the mean colony diameter being 2.80 cm, 2.63 cm, 2.72 cm and 2.65 cm, respectively. The highest inhibition was observed in NLE (A) 4 % (2.38 cm).

The trend observed was exactly the same on 14th DAI. The lower dose of NOE (0.5 %) exhibited a stimulative growth, with mean colony diameter being 6.27 cm. The inhibition noted was least in NSKE (A) 0.5 % (6.08 cm) which was followed by NSKE (A) 1 %, NOE 1 % , NLE (A) 1 %, NLE (S) 1 % , NSKE (S)

0.5 % varied significantly from each other, with colony diameter being 5.88 cm, 5.78 cm, 5.32 cm, 5.28 cm and 5.07 cm respectively. Inhibition was more in treatments NOE 2 % and NSKE (A) 2 % were on par (4.88 cm and 4.87 cm, respectively). The treatments NLE (S) 2 % and NSKE (S) 1 % recorded even lesser growth measuring 4.15 cm and 3.97 cm respectively. NSKE (S) 2 %, NLE (A) 2 % and NLE (S) 4 % inhibited the growth more (3.57 cm, 3.32 cm and 3.12 cm, respectively). The highest inhibition was noted in NLE (A) 4 % (2.62 cm).

4.1.1.2 Sporulation

Analysis of data on spore count taken on 20th day, revealed that all the treatments affected the sporulation of the fungus. (Table 4). The lowest inhibition was noticed in NLE (A) 1 % with spore count being 4.41 x 10^7 mL^{-1}. Sporulation was even lesser in NSKE (S) 0.5 % and 1 %, which were statistically similar (4.05 x 10^7 mL^{-1} and 3.68 x 10^7 mL^{-1}, respectively). This was sequentially followed by NSKE (A) 0.5 %, NKSE (A) 1 %, NLE (A) 2 %, NSKE (S) 2 %, NOE 0.5 % and NSKE (A) 2 %, which were statistically different from each other. The sporulation noted were 3.26 x 10^7 mL^{-1}, 2.65 x 10^7 mL^{-1}, 2.39 x 10^7 mL^{-1}, 2.30 x 10^7 mL^{-1}, 1.87 x 10^7 mL^{-1} and 1.77 x 10^7 mL^{-1} , respectively. The highest inhibition in sporulation was exhibited by the treatments NOE 1 % and 2 %, NLE (A) 4 %, NLE 1 %, 2 % and 4 % with spore count being 0.78 x 10^7 mL^{-1}, 0.52 x 10^7 mL^{-1}, 0.74 x 10^7 mL^{-1}, 0.69 x 10^7 mL^{-1}, 0.85 x 10^7 mL^{-1} and 0.65 x 10^7 mL^{-1}, respectively.

In general, spore count decreased with increase in concentration in all the botanicals tested. The least inhibition in sporulation was observed in aqueous extract of NLE 1 %. Solvent extracts was more inhibitive in NLE while in NSKE it was aqueous extracts that were found to be more inhibitive. NLE (S) was inhibitory at all concentrations, while NLE (A) was inhibitory only at 4 %. Except at lower dose, NOE was found to inhibit sporulation.

4.1.1.3 Germination

Germination exhibited by neem based botanicals is presented in Table 4. NSKE (A) 0.5 % (97.67 per cent) did not inhibit the conidial germination of *L. saksenae.* All the other treatments affected the germination. Among the treatments, lowest inhibition was exhibited by NSKE (A) 1 % (97.33 per cent). NSKE (A) 2 % and NLE (A) 1 % exhibited 95.33 per cent germination. This was followed by NLE (A) 2 % and 4 % and NOE 0.5 % which were statistically different from each other with 94.67 and 91.34 and 87 per cent germination, respectively. The treatments NOE 1 % and NSKE (S) 0.5 % recorded even lesser germination with 82.33 and 82 per cent, respectively. NSKE (S) 1 % (79.67 per cent) and NOE 2 % (77.67 per cent) were on par. Inhibition was even higher in NSKE (S) 2 % and NLE (S) 1 % (75.67 per cent and 75.33 per cent, respectively). This was followed by NLE (S) 2 % with germination of 57.33 per cent. Inhibition was highest in NLE (S) 4 % (37.33 per cent).

In general, a concentration dependent decrease in germination was observed among all the neem-based botanicals. Solvent extracts were found to be more inhibitive compared to aqueous extract. On comparison of recommended field doses (NSKE 2 %, NOE 2 % and NLE 4 %), it was NLE (S) that was most inhibitive followed by NSKE (S), NOE, NLE (A) and NSKE (A). Least germination was observed in NLE (S) 4 %.

4.1.1.4 Compatibility Status

Biological Index (BI) of *L. saksenae* that indicates its compatibility status with neem based botanicals is presented in Table 5.

Lower dose (0.5 %) of both the aqueous and solvent extracts of neem seed kernel, aqueous extract of neem leaf (1 %) as well as neem oil emulsion (0.5 %) were compatible with *L. saksenae*. BI values calculated ranged from 67 to 75.

Table 4. Spore count and germination of *L. saksenae* in PDA poisoned with neem based botanicals

Botanicals	Concentrations (%)	Spore count at 20th DAI (10^7 spores mL^{-1})	Germination after 24h (%)
Neem Seed Kernel Extract (Aqueous)	0.5	3.26^{cd} (1.80)	97.67^{a} (81.87)
	1	2.65^{de} (1.62)	97.33^{ab} (80.73)
	2	1.77^{g} (1.32)	95.33^{bc} (77.54)
Neem Seed Kernel Extract (Solvent)	0.5	4.05^{bc} (2.00)	82.00^{f} (64.90)
	1	3.68^{bc} (1.90)	79.67^{fg} (63.21)
	2	2.30^{efg} (1.51)	75.67^{g} (60.44)
Neem Oil Emulsion	0.5	1.87^{fg} (1.37)	87.00^{e} (68.90)
	1	0.78^{h} (0.88)	82.33^{f} (65.18)
	2	0.52^{h} (0.72)	77.67^{fg} (61.83)
Neem Leaf Extract (Aqueous)	1	4.41^{b} (2.10)	95.33^{bc} (77.54)
	2	2.39^{ef} (1.54)	94.67^{c} (76.83)
	4	0.74^{h} (0.85)	91.34^{d} (72.91)
Neem Leaf Extract (Solvent)	1	0.69^{h} (0.80)	75.33^{g} (60.27)
	2	0.85^{h} (0.92)	57.33^{h} (49.23)
	4	0.65^{h} (0.81)	37.33^{i} (37.60)
Untreated Control		7.57^{a} (2.76)	99.0^{a} (84.27)
Treated control (Methanol)	2	7.55^{a} (2.77)	99.0^{a} (84.27)
CD (0.05)		(0.217)	(3.638)

DAI-Days after inoculation. Figures in the parentheses are square root transformed values for spore count and angular transformed values for germination. Values sharing same alphabets in superscript are statistically on par based on ANOVA

Table 5. Compatibility status of *L. saksenae* with neem based botanicals

Botanicals	Concentrations (%)	VR (%)	SP (%)	GER (%)	BI	Status
Neem Seed Kernel Extract (Aqueous)	0.5	98.64	43.11	98.65	75	Compatible
	1	95.40	34.99	98.32	70	Compatible
	2	78.91	23.25	96.30	57	Moderately toxic
Neem Seed Kernel Extract (Solvent)	0.5	82.16	50.48	82.83	69	Compatible
	1	64.32	64.02	80.47	66	Moderately toxic
	2	57.83	62.52	76.43	62	Moderately toxic
Neem Oil Emulsion	0.5	101.62	24.81	87.88	67	Compatible
	1	93.78	10.29	83.16	57	Moderately toxic
	2	79.18	6.82	78.45	48	Moderately toxic
Neem Leaf Extract (Aqueous)	1	86.21	58.21	96.30	75	Compatible
	2	53.78	31.52	95.62	48	Moderately toxic
	4	42.43	9.76	92.26	33	Toxic
Neem Leaf Extract (Solvent)	1	85.67	9.09	76.09	52	Moderately toxic
	2	67.29	11.10	57.91	42	Moderately toxic
	4	50.54	8.64	37.71	31	Toxic

VR - Vegetative growth in relation to control, SP - Sporulation in relation to control GER - Germination in relation to control, BI - Biological Index.
Compatible (> 66), Moderately toxic (42 - 66) and Toxic (BI < 42).

Among these the lowest BI was recorded for NOE (0.5 %) and the highest was recorded on NSKE (A) 0.5 %. Comparison of BI value of the medium doses revealed that only NSKE (A) 1 % was compatible with *L. saksenae* (BI=70). The other treatments with BI values ranging from 42 to 66 were moderately toxic.

Higher dose of all the botanicals tested *viz.* aqueous and solvent extracts of neem seed kernel and neem oil emulsion were moderately toxic to *L. saksenae* with BI value 48 to 62. Higher dose of aqueous and solvent extracts of neem leaf was toxic to *L. saksenae* with BI values 33 and 31, respectively.

4.1.2 With Insecticides

Compatibility of *L. saksenae* to old generation insecticides such as dimethoate 30% EC, and chlorpyriphos 20% EC and new generation insecticides such as imidacloprid 17.8% SL, thiamethoxam 25% WG, chlorantraniliprole 18.5% SC and flubendiamide 39.35% SC, assessed in terms of radial growth, sporulation and germination is presented below. The compatibility was assessed both at recommended as well as at their half doses.

4.1.2.1 Radial Growth

Radial growth of the fungus in terms of mean colony diameter, recorded on the 14th DAI in PDA poisoned with insecticides is presented in Plates 4A and B. Growth of the fungus expressed as mean colony diameter is presented in Table 6.

4.1.2.1.1 At Half the Recommended Dose

Analysis of mean colony diameter on third DAI revealed that, in poisoned media highest growth was observed in the new generation insecticide flubendiamide 39.35% SC 0.0025 % (1.53 cm), which was lower than the mycelial growth in untreated medium (1.58 cm). The mean radial growth in thiamethoxam 25% WG 0.0025 % and chlorantraniliprole 18.5% SC 0.003 % poisoned media ranked second and did not vary significantly among them (1.45 cm and 1.42 cm

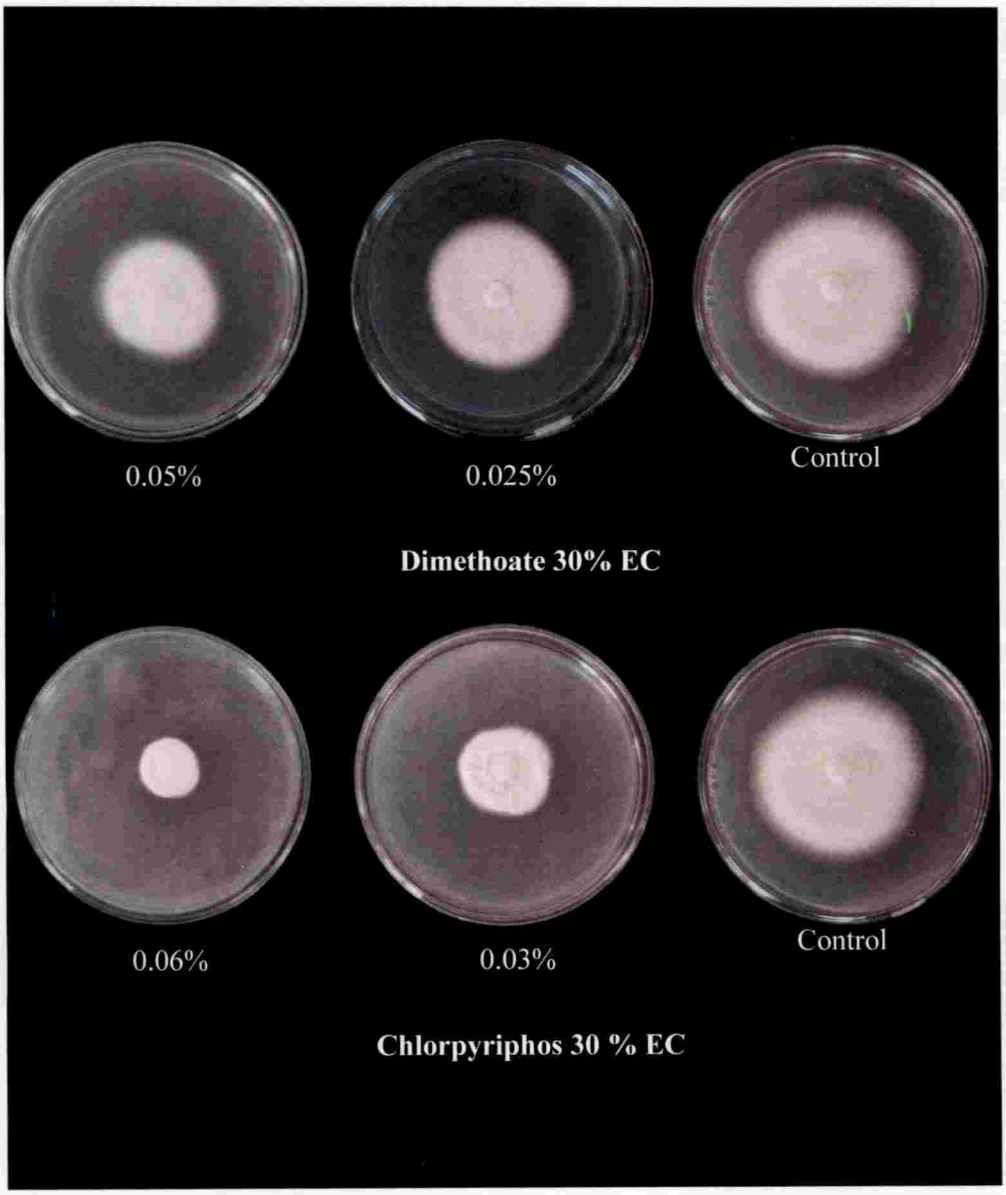


Plate 4 A. Radial growth of *L.saksenae* on PDA poisoned with old generation insecticides on 14th DAI

DAI – Days After Inoculation

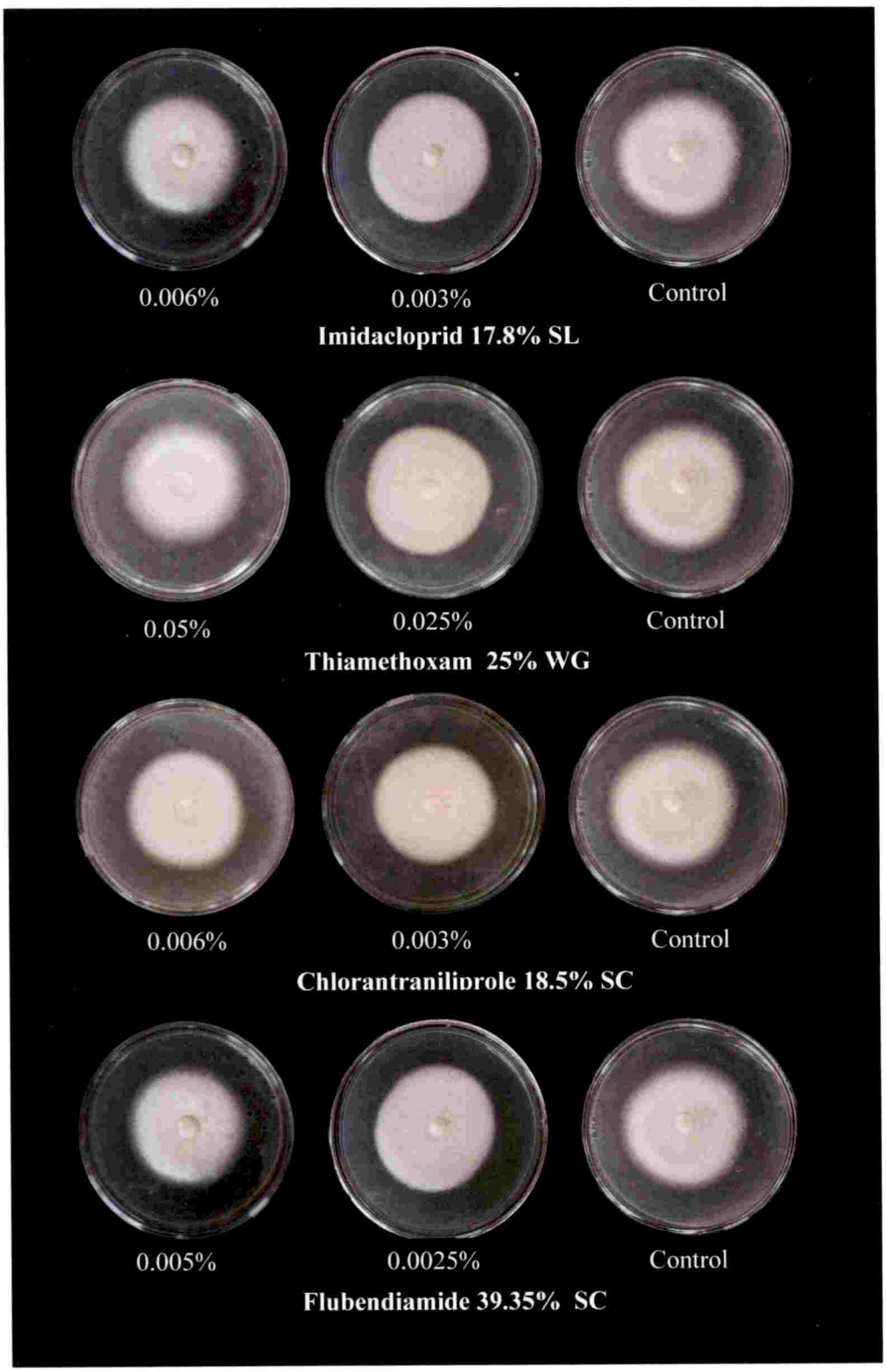


Plate 4 B. Radial growth of *L.saksenae* in PDA poisoned with new generation insecticides on 14th DAI

DAI – Days After Inoculation

Table 6. Radial growth of *L. saksenae* in PDA poisoned with insecticides

Insecticides	Concentrations (%)	Mean colony diameter (cm)			
		3 DAI	7 DAI	10 DAI	14 DAI
Dimethoate 30% EC	0.025	1.25^{e} (1.12)	3.02^{d} (1.74)	4.10^{e} (2.03)	5.33^{e} (2.30)
	0.05	1.03^{f} (1.01)	2.53^{e} (1.60)	3.42^{f} (1.84)	4.55^{f} (2.14)
Chlorpyriphos 20% EC	0.03	0.93^{f} (0.97)	1.83^{f} (1.36)	2.47^{g} (1.57)	3.25^{g} (1.81)
	0.06	0.80^{g} (0.89)	1.35^{g} (1.16)	1.78^{h} (1.34)	2.32^{h} (1.52)
Imidacloprid 17.8% SL	0.003	1.35^{cde} (1.17)	3.22^{bc} (1.79)	4.50^{abc} (2.12)	5.75^{cd} (2.40)
	0.006	1.37^{cde} (1.16)	3.22^{bc} (1.79)	4.48^{abcd} (2.11)	5.87^{bc} (2.42)
Thiamethoxam 25% WG	0.0025	1.45^{bc} (1.21)	3.25^{ab} (1.81)	4.57^{ab} (2.14)	5.88^{bc} (2.42)
	0.005	1.32^{de} (1.14)	3.22^{bc} (1.79)	4.33^{cd} (2.08)	5.78^{bc} (2.41)
Chlorantraniliprole 18.5% SC	0.003	1.42^{bc} (1.19)	3.18^{bc} (1.78)	4.40^{bcd} (2.10)	5.78^{bc} (2.41)
	0.006	1.30^{de} (1.14)	3.12^{cd} (1.76)	4.28^{de} (2.06)	5.62^{bc} (2.37)
Flubendiamide 39.35% SC	0.0025	1.53^{ab} (1.23)	3.19^{bc} (1.78)	4.55^{ab} (2.14)	5.92^{b} (2.43)
	0.005	1.38^{cd} (1.18)	3.25^{ab} (1.81)	4.38^{bcd} (2.09)	5.88^{bc} (2.42)
Control		1.58^{a} (1.25)	3.38^{a} (1.83)	4.66^{a} (2.16)	6.10^{a} (2.47)
CD (0.05)		(0.053)	(0.037)	(0.051)	(0.031)

DAI-Days after inoculation.

Figures in the parentheses are square root transformed values.

Values sharing same alphabets in superscript are statistically on par based on ANOVA

respectively). Mycelial growth observed in imidacloprid 17.8% SL 0.003 % and the old generation insecticide dimethoate 30% EC 0.025 % exhibited were statistically different (1.35 cm and 1.25 cm). It was chlorpyriphos 20% EC 0.03 % that exhibited least growth (0.93 cm).

On the seventh DAI, among the poisoned media thiamethoxam 25% WG 0.0025 % exhibited highest growth (3.25 cm) which was lower than that of unpoisoned medium (3.38 cm). This was followed by imidacloprid 17.8% SL 0.003 %, flubendiamide 39.35% SC 0.0025 %, and chlorantraniliprole 18.5% SC 0.003 % which did not vary significantly. The radial growths obtained were 3.22 cm, 3.19 cm and 3.18 cm respectively. The medium poisoned with dimethoate 30% EC 0.025 % displayed a significantly less radial growth (3.02 cm). Chlorpyriphos 20% EC 0.03 % exhibited least growth (1.83 cm) among the treatments.

At 10th DAI, among the treatments thiamethoxam 25% WG 0.0025 % (4.57 cm) and flubendiamide 39.35% SC 0.0025 % (4.55 cm) exhibited highest mycelial growth, which were lower than that in unpoisoned medium (4.66 cm). This was followed by imidacloprid 17.8% SL 0.003 %, (4.5 cm) chlorantraniliprole 18.5% SC 0.003 % (4.40 cm) and dimethoate 30% EC 0.025 % (4.10 cm), which were significantly different from each other. Least growth (1.83 cm) was exhibited by chlorpyriphos 20% EC 0.03 %.

On 14th DAI, among the poisoned media highest growth was noticed in flubendiamide 39.35% SC 0.0025 % (5.92 cm) followed by thiamethoxam 25% WG 0.0025 % (5.88 cm) and chlorantraniliprole 18.5% SC 0.003 % (5.78 cm). Growth in imidacloprid 17.8% SL 0.003 % and dimethoate 30% EC 0.025 %, was 5.75 cm and 5.33 cm, respectively which varied significantly. Least growth (3.25 cm) was noticed in medium poisoned with chlorpyriphos 20% EC 0.03 %. All the treatments depicted significantly less radial growth than that of unpoisoned medium (6.1 cm)

At half dose, flubendiamide 39.35% SC was least inhibitory followed by thiamethoxam 25% WG and chlorantraniliprole 18.5% SC. Imidacloprid 17.8% SL

was comparatively inhibitive. Among the old generation insecticides chlorpyriphos 20% EC was more inhibitive.

4.1.2.1.1 At Recommended Dose

Three DAI, growth of the fungus observed in all the poisoned media was significantly lower than that in unpoisoned medium (1.58 cm). Among the poisoned media, flubendiamide 39.35% SC 0.005 % (1.38 cm) exhibited highest mycelial growth followed by imidacloprid 17.8% SL 0.006 % (1.37 cm). Growth observed in thiamethoxam 25% WG 0.005 % (1.32 cm) and chlorantraniliprole 18.5% SC 0.006 % (1.30) were on par with each other. Growth in dimethoate 30% EC 0.025 % (1.03 cm) was significantly less and least growth was noticed in chlorpyriphos 20% EC 0.03 % (0.8 cm).

Seven DAI, among the poisoned media, highest radial growth (3.25 cm) was noticed in flubendiamide 39.35% SC 0.005 %. The corresponding growth in unpoisoned medium was 3.38 cm. This was followed by those in thiamethoxam 25% WG 0.005 % and imidacloprid 17.8% SL 0.006 % (3.22 cm each), which did not vary significantly from each other. Growth in chlorantraniliprole 18.5% SC 0.006 % was 3.12 cm, which was statistically lower. Growth in dimethoate 30% EC 0.025 % was significantly less (2.53 cm) and least in chlorpyriphos 20% EC 0.03 % (1.35 cm).

On the 10th DAI, unpoisoned medium exhibited radial growth 4.67 cm, which was significantly higher than all the other treatments. Imidacloprid 17.8% SL 0.003 % exhibited highest radial growth (4.48 cm) followed by flubendiamide 39.35% SC 0.0025 %, thiamethoxam 25% WG 0.005 % and chlorantraniliprole 18.5% SC 0.006 %, which vary significantly. The radial growths obtained were 4.38 cm, 4.33 cm and 4.28 cm respectively. The medium poisoned with dimethoate 30% EC 0.025 % displayed a significantly less radial growth (3.42 cm). Least growth (1.83 cm) was noticed in chlorpyriphos 20% EC 0.03 %.

At 14 DAI, all the treatments varied significantly with respect to unpoisoned medium (6.1 cm). Flubendiamide 39.35% SC 0.005 %, imidacloprid 17.8% SL 0.006 % thiamethoxam 25% WG 0.005 % and chlorantraniliprole 18.5% SC 0.006 % depicted highest growth (5.88 cm, 5.87 cm, 5.78 cm and 5.62 cm) respectively and were on par with each other. The medium poisoned with dimethoate 30% EC 0.05 % observed a significantly less radial growth (4.55 cm). Chlorpyriphos 20% EC 0.06 % exhibited least growth (2.32 cm) among the treatments.

At recommended dose, flubendiamide 39.35% SC depicted highest growth compared to imidacloprid 17.8% SL, thiamethoxam 25% WG and chlorantraniliprole 18.5% SC, sequentially. Least growth was noticed in chlorpyriphos 20% EC among the old generation insecticides.

4.1.2.2 Sporulation

4.1.2.2.1 At Half the Recommended Dose

Spore count of *L. saksenae* (10^7 mL^{-1}) in PDA poisoned with different insecticides is indicated in Table 7. The highest sporulation (1.35 x 10^7 mL^{-1}) was observed in chlorantraniliprole 18.5% SC 0.003 % which was on par with that in unpoisoned medium (1.35 x 10^7 mL^{-1}). Sporulation observed in flubendiamide 39.35% SC 0.0025 % (1.33 x 10^7 mL^{-1}) and dimethoate 30% EC 0.025 % (1.31 x 10^7 mL^{-1}), was higher and did not vary significantly among each other. Spore count in thiamethoxam 25% WG 0.0025 % (1.18 x 10^7 mL^{-1}) and imidacloprid 17.8% SL 0.003 % (1 x 10^7 mL^{-1}) were significantly lower and differed from each other. Spore count in chlorpyriphos 20 EC 0.03 % (0.52 x 10^7 mL^{-1}) was the least.

4.1.2.2.2 At Recommended Dose

Among the insecticides tested, highest sporulation (1.32 x 10^7 mL^{-1}) was observed in chlorantraniliprole 18.5% SC 0.006 %. Sporulation in flubendiamide 39.35% SC 0.005 % was significantly lower (0.85 x 10^7 mL^{-1}). In dimethoate 30% EC 0.05 %, spore count was 0.66 x 10^7 mL^{-1} which was still lower. Sporulation in

media poisoned with imidacloprid 17.8% SL 0.006 % (0.62 x 10^7 mL^{-1}) and thiamethoxam 25% WG 0.005 % (0.56 x 10^7 mL^{-1}) ranked next and were on par with each other. Least sporulation was noticed in chlorpyriphos 20% EC 0.06 % (0.29 x 10^7 mL^{-1}).

Among the treatments, chlorantraniliprole 18.5% SC exhibited highest sporulation in recommended dose followed by its half dose and flubendiamide 39.35% SC. Thiamethoxam 25% WG and imidacloprid 17.8% SL affected sporulation. Irrespective of the doses. Chlorpyriphos 20% EC exhibited the least spore count.

Considering both mycelial growth and sporulation it was flubendiamide that was least inhibitive. Though imidacloprid 17.8% SL and thiamethoxam 25% WG did not inhibit the mycelial growth it affected sporulation significantly. Chlorantraniliprole 18.5% SC was found to be inhibitive than flubendiamide 39.35% SC and thiamethoxam 25% WG with respect to their mycelial growth but it did not affect sporulation. Dimethoate 30% EC though affected the mycelial growth of the fungus it has less inhibition in sporulation.

4.1.2.3 Germination

4.1.2.2.1 At Half the Recommended Dose

Germination of *L. saksenae* was significantly affected by all the insecticides (Table 7). The highest germination was noted in imidacloprid 17.8% SL 0.003 % and flubendiamide 39.35% SC 0.0025 %, which were on par with each other. Germination percentage observed was 85.67 and 61.00 respectively. This was followed by thiamethoxam 25% WG 0.0025 % (40.67 per cent), chlorantraniliprole 18.5% SC 0.003 % (34.67 per cent) and chlorpyriphos 20% EC 0.03 % (31.19 per cent). Dimethoate 30% EC 0.025 % exhibited the least germination of 22.33 per cent.

4.1.2.2.2 At Recommended Dose

At recommended dose, all the insecticides affected conidial germination. The highest germination (44.67 per cent) was observed in imidacloprid 17.8% SL 0.006 %. Chlorantraniliprole 18.5% SC 0.006 % and flubendiamide 39.35% SC 0.005 % ranked second (40 per cent and 36 per cent respectively), which were on par with each other. The treatments thiamethoxam 25% WG 0.005 % (31 per cent), dimethoate 30% EC 0.05 % (18 per cent) significantly affected germination. Least germination (7.33 per cent) was noticed in chlorpyriphos 20% EC 0.06 %. Corresponding germination observed in unpoisoned medium was 98.33 per cent.

4.1.2.4 Compatibility Status

Compatibility status of *L. saksenae* with different chemical insecticides is indicated in Table 8.

All the new generation insecticides were found to be compatible based on their biological index, both at half and recommended doses. BI index varied from 67 in thiamethoxam 25% WG 0.005 % to 94 in flubendiamide 39.35% SC 0.0025 %.

The old generation insecticide dimethoate 30% EC was compatible at lower concentration (0.025 %) with BI value 85. Chlorpyriphos 20% EC was moderately toxic at lower concentration (BI= 44) and toxic at recommended dose (BI= 27).

4.1.3 With Fungicides

Compatibility of *L. saksenae* to old generation fungicides such as carbendazim 50% WP, mancozeb 75% WP and copper oxychloride 50% WP and new generation fungicides such as azoxystrobin 23% SC and hexaconazole 5% EC, assessed in terms of radial growth, sporulation and germination is presented below. The compatibility was assessed both at recommended as well as at their half doses.

Table 7. Spore count and germination of *L. saksenae* in PDA poisoned with insecticides

Insecticides	Concentrations (%)	Spore count at 20th DAI (10^7 spores mL^{-1})	Germination after 24h (%)
Dimethoate 30% EC	0.025	1.31^{ab} (1.14)	22.33^{f} (28.15)
	0.05	0.66^{e} (0.80)	18.00^{f} (24.85)
Chlorpyriphos 20% EC	0.03	0.52^{f} (0.72)	31.19^{e} (33.59)
	0.06	0.29^{g} (0.53)	7.33^{g} (15.65)
Imidacloprid 17.8% SL	0.003	1.00^{c} (1.01)	85.67^{b} (51.96)
	0.006	0.62^{ef} (0.79)	44.67^{c} (41.89)
Thiamethoxam 25% WG	0.0025	1.18^{b} (1.09)	40.67^{cd} (39.60)
	0.005	0.56^{ef} (0.74)	31.00^{e} (33.80)
Chlorantraniliprole 18.5% SC	0.003	1.35^{a} (1.16)	34.67^{de} (36.06)
	0.006	1.32^{ab} (1.15)	40.00^{cd} (39.22)
Flubendiamide 39.35% SC	0.0025	1.33^{ab} (1.16)	61.00^{b} (51.38)
	0.005	0.85^{d} (0.92)	36.00^{cde} (36.87)
Control		1.35^{a} (1.17)	98.33^{a} (83.87)
CD (0.05)		(0.740)	(5.232)

DAI-Days after inoculation. Figures in the parentheses are square root transformed values for spore count and angular transformed values for germination. Values sharing same alphabets in superscript are statistically on par based on ANOVA.

Table 8. Compatibility status of *L. saksenae* with chemical insecticides

Insecticides	Concentrations (%)	VR (%)	SP (%)	GER (%)	BI	Status
Dimethoate 30% EC	0.025	87.70	96.32	22.71	85	Compatible
	0.05	73.77	48.31	18.31	57	Moderately toxic
Chlorpyriphos 20% EC	0.03	53.28	37.71	31.19	44	Moderately toxic
	0.06	37.30	21.28	7.46	27	Toxic
Imidacloprid 17.8% SL	0.003	94.67	74.19	87.12	85	Compatible
	0.006	96.31	45.8	45.43	70	Compatible
Thiamethoxam 25% WG	0.0025	96.72	86.63	31.53	86	Compatible
	0.005	95.49	40.77	41.36	67	Compatible
Chlorantraniliprole 18.5% SC	0.003	94.67	96.57	35.26	90	Compatible
	0.006	92.21	99.14	40.68	90	Compatible
Flubendiamide 39.35% SC	0.0025	96.72	97.73	62.04	94	Compatible
	0.005	97.54	62.67	36.61	76	Compatible

VR - Vegetative growth in relation to control, SP - Sporulation in relation to control, GER - Germination in relation to control, BI - Biological Index.

Compatible (> 66), Moderately toxic (42 - 66), and Toxic (< 42)

4.1.3.1 Radial Growth

Radial growth of the fungus in terms of mean colony diameter, recorded on 14th DAI in PDA poisoned with fungicides is presented in Plates 5A and B.

4.1.3.1.1 At Half the Recommended Dose

Mean colony diameter of *L. saksenae* in PDA poisoned with different fungicides is indicated in Table 9. On the third DAI, there was significant reduction in radial growth in all the poisoned media. The radial growth observed in copper oxychloride 50% WP was 0.93 cm which was significantly higher than that observed in medium treated with azoxystrobin 23% SC 0.1 % (0.68 cm). There was no mycelial growth in the media poisoned with carbendazim 50% WP, mancozeb 75% WP and hexaconazole 5% EC, both at half and recommended doses. Growth of fungus in unpoisoned medium (1.37 cm) was significantly higher than that observed in treatments.

Similar trend was noticed in the radial growth at seventh, 10th and 14th DAI. Highest growth was observed in copper oxychloride 50% WP on seventh, 10th and 14th DAI with mean colony diameter of 1.83 cm, 2.62 cm and 3.22 cm, respectively. This was followed by growth observed in azoxystrobin 23% SC with 1.33 cm, 1.78 cm and 2.25 cm respectively on seventh, 10 and 14 DAI. The corresponding growth observed in untreated medium was 3.15 cm, 4.32 cm and 5.27 cm, respectively.

4.1.3.1.2 At Recommended Dose

On the third DAI, highest mean radial growth (0.93 cm) was sighted in copper oxychloride 50% WP 0.2 %. Azoxystrobin 23% SC 0.1 % (0.73 cm) ranked second. No growth was observed in media poisoned with carbendazim 50% WP, mancozeb 75% WP and hexaconazole 5% EC.

Similar trend was noticed on seventh, 10th and 14th DAI. Highest growth was observed in copper oxychloride 50% WP 0.2 % on seventh, 10th and 14th with the mean colony diameter of 1.9 cm, 2.67 cm and 3.33 cm. Azoxystrobin 23% SC

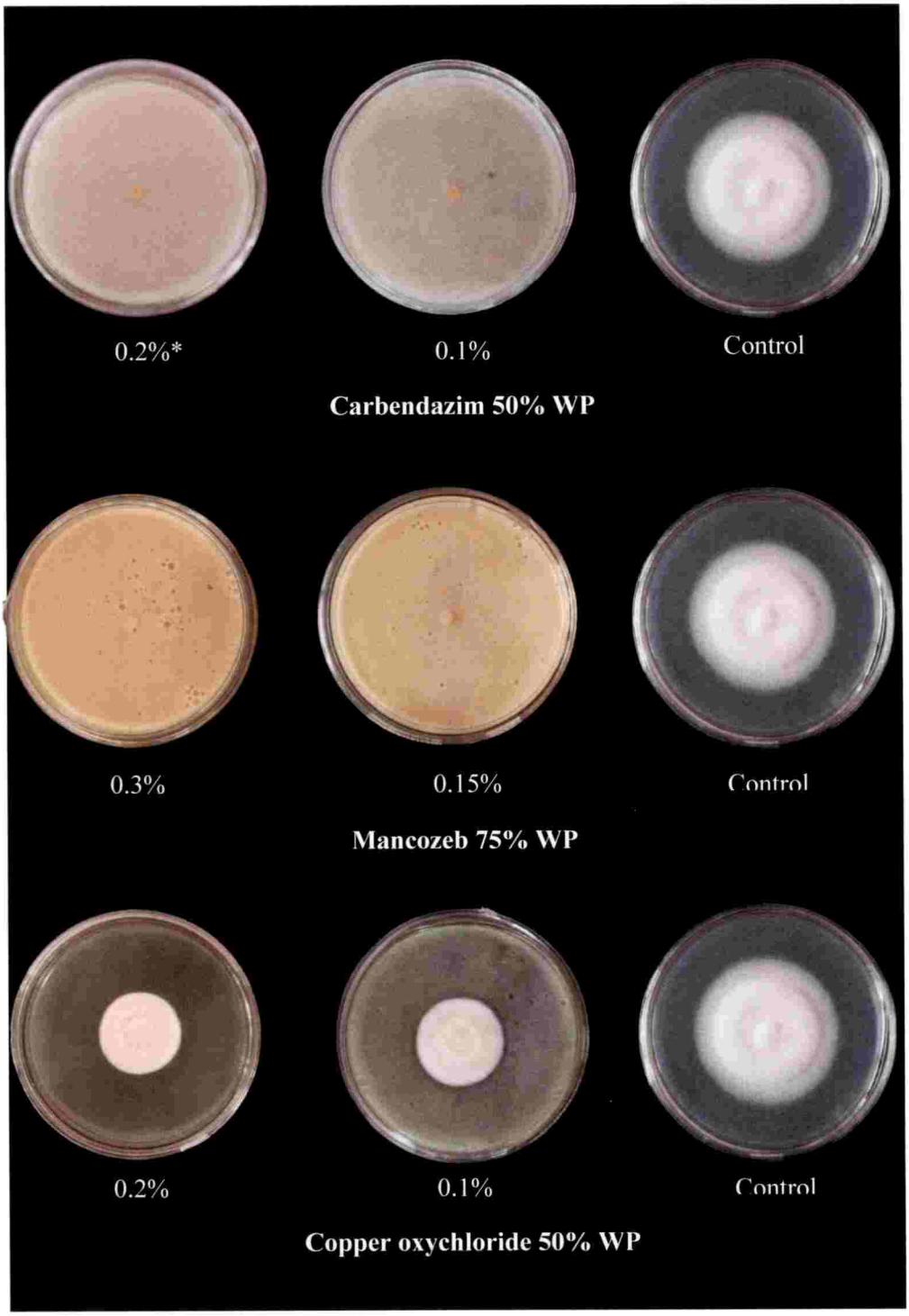


Plate 5A . Radial growth of *L.saksenae* in PDA poisoned with old generation fungicides on 14th DAI

DAI – Days After Inoculation

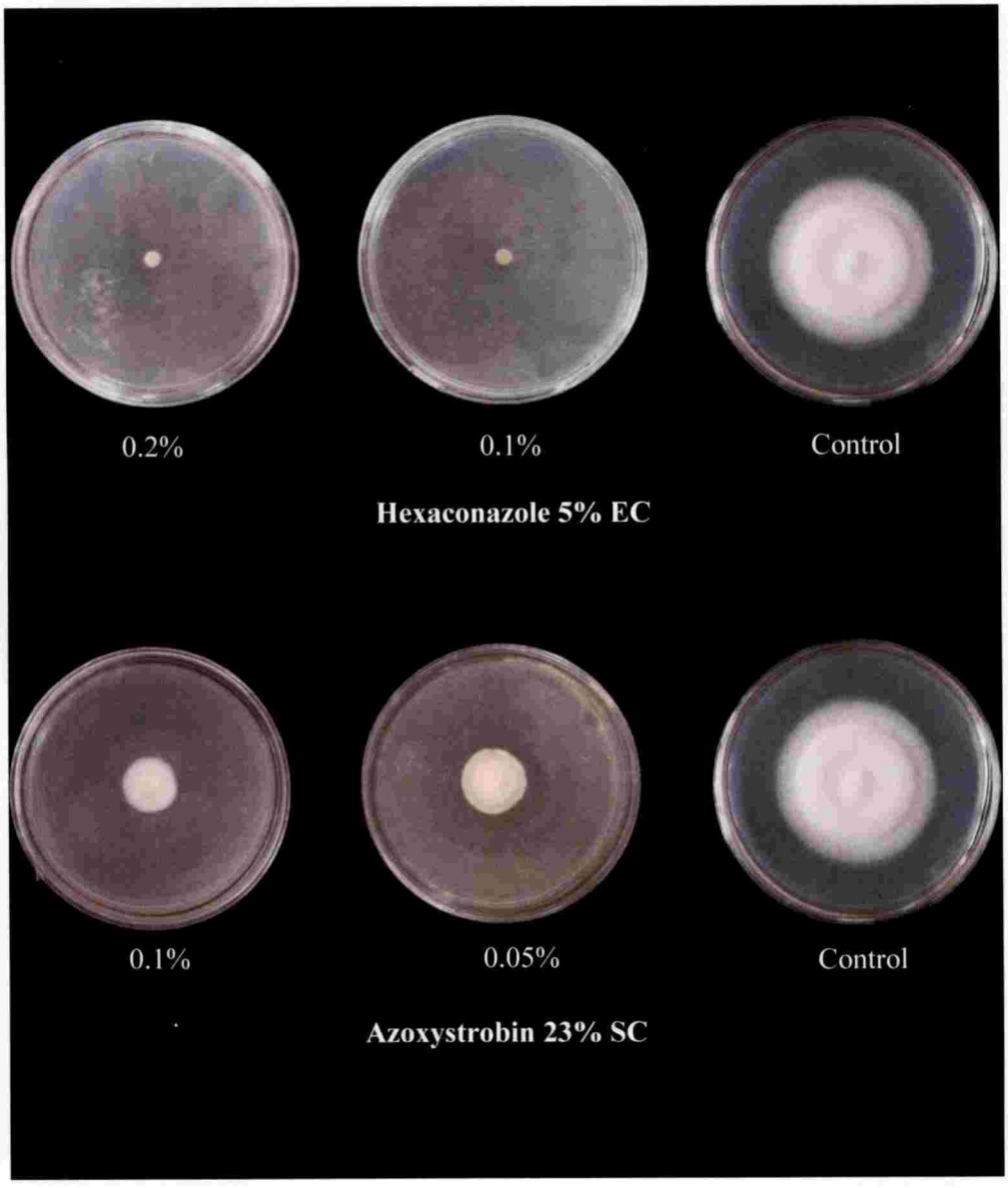


Plate 5 B . Radial growth of *L.saksenae* in PDA poisoned with new generation fungicides on 14th DAI

DAI – Days After Inoculation

0.1 % ranked second with growth of 1.48 cm, 2.13 cm and 2.53 on seventh, 10^{th} and 14^{th}, respectively.

All the treatments exhibited significantly less growth than the unpoisoned medium. Carbendazim 50% WP, mancozeb 75% WP and hexaconazole 5% EC totally inhibited the mycelial growth of *L. saksenae* at both the doses. Growth observed in all other treatments were significantly low when compared to unpoisoned medium. The old generation fungicide copper oxychloride 50% WP was least inhibitive followed by the new generation fungicide azoxystrobin 23% SC.

4.1.3.2 Sporulation

Spore count of *L. saksenae* in fungicide poisoned medium enumerated on the 20^{th} DAI is indicated in Table 10.

Carbendazim 50% WP, mancozeb 75% WP and hexaconazole 5% EC totally affected the sporulating capacity of *L. saksenae*, both at half and recommended doses.

At half the recommended dose, the old generation fungicide, copper oxychloride 50% WP recorded the highest spore count (4.22×10^7 mL^{-1}). The spore count in azoxystrobin 23% SC was significantly lower (1.94×10^7 mL^{-1}). Corresponding spore load in untreated medium was 5.92×10^7 mL^{-1}.

At recommended dose the trend observed was exactly the same. Copper oxychloride 50% WP recorded the highest spore count (3.61×10^7 mL^{-1}) followed by azoxystrobin 23% SC (2.14×10^7 mL^{-1}).

4.1.3.3 Germination

Germination of *L. saksenae* in medium poisoned with fungicides is indicated in Table 10.

None of the spores germinated when inoculated in medium poisoned with carbendazim 50% WP, mancozeb 75% WP and hexaconazole 5% EC after 24h

Table 9. Radial growth of *L. saksenae* in PDA poisoned with fungicides

Fungicides	Concentrations (%)	Mean colony diameter (cm)			
		3 DAI	7 DAI	10 DAI	14 DAI
Carbendazim 50% WP	0.10	0.00^{d} (0.70)	0.00^{d} (0.70)	0.00^{e} (0.70)	0.00^{e} (0.70)
	0.20	0.00^{d} (0.70)	0.00^{d} (0.70)	0.00^{e} (0.70)	0.00^{e} (0.70)
Mancozeb 75% WP	0.15	0.00^{d} (0.70)	0.00^{d} (0.70)	0.00^{e} (0.70)	0.00^{e} (0.70)
	0.30	0.00^{d} (0.70)	0.00^{d} (0.70)	0.00^{e} (0.70)	0.00^{e} (0.70)
Copper oxychloride 50% WP	0.10	0.93^{b} (1.19)	1.83^{b} (1.54)	2.62^{b} (1.77)	3.22^{b} (1.93)
	0.20	0.93^{b} (1.20)	1.90^{b} (1.55)	2.67^{b} (1.78)	3.33^{c} (1.95)
Hexaconazole 5% EC	0.10	0.00^{d} (0.70)	0.00^{d} (0.70)	0.00^{e} (0.70)	0.00^{e} (0.70)
	0.20	0.00^{d} (0.70)	0.00^{d} (0.70)	0.00^{e} (0.70)	0.00^{e} (0.70)
Azoxystrobin 23% SC	0.05	0.68^{c} (1.09)	1.33^{c} (1.36)	1.78^{d} (1.52)	2.25^{d} (1.66)
	0.1	0.73^{bc} (1.10)	1.48^{c} (1.40)	2.13^{c} (1.62)	2.53^{c} (1.73)
Control		1.37^{a} (1.36)	3.15^{a} (1.91)	4.32^{a} (2.19)	5.27^{a} (2.40)
CD (0.05)		(0.091)	(0.055)	(0.059)	(0.072)

DAI-Days after inoculation. Figures in the parentheses are square root transformed values. Values sharing same alphabets in superscript are statistically on par based on ANOVA

at both their doses.

In other fungicides tested at their lower dose, highest germination (12.33 per cent) was recorded in copper oxychloride 50% WP. Germination in azoxystrobin 23% SC was negligible (0.67 per cent).

At higher dose the germination observed with copper oxychloride 50% WP was 4.17 per cent. None of the spores germinated in azoxystrobin 23% SC.

4.1.3.4 Compatibility Status

Compatibility status of *L. saksenae* with different chemical fungicides is indicated in Table 11.

None of the fungicides were found compatible. Carbendazim 50% WP, mancozeb 75% WP, hexaconazole 5% EC and azoxystrobin 23% SC were toxic at both the concentrations while copper oxychloride 50% WP was moderately toxic at half and recommended doses. The BI values were 57 and 64 respectively.

4.1.4 With Other Entomopathogenic Fungi

Compatibility of *L. saksenae* 10^7 spores mL^{-1} with *B. bassiana* 10^8 spores mL^{-1} and *M. anisopliae* 10^8 spores mL^{-1} assessed in terms of mortality of rice bugs is presented in Tables 12 and 13.

4.1.4.1 Mortality of Rice Bugs

4.1.4.1.1 Nymphs

Bugs treated with the combination spray of *L. saksenae* and *B. bassiana* exhibited 80 per cent mortality on the first day after treatment. The corresponding mortality observed with *L. saksenae* alone was significantly higher (86.67 per cent). There was no mortality in bugs treated with *B. bassiana*. When *L. saksenae* and *M. anisopliae* were sprayed in combination the mortality was 36.67 per cent. Whereas nymphs treated with *M. anisopliae* alone did not die. When all the three

Table 10. Spore count and germination of *L. saksenae* in PDA poisoned with fungicides

Fungicides	Concentrations (%)	Spore count at 20th DAI (10^7 spores mL^{-1})	Germination after 24h (%)
Carbendazim 50% WP	0.10	0.00^{e} (0.70)	0.00^{e} (0.28)
	0.20	0.00^{e} (0.70)	0.00^{e} (0.28)
Mancozeb 75% WP	0.15	0.00^{e} (0.70)	0.00^{e} (0.28)
	0.30	0.00^{e} (0.70)	0.00^{e} (0.28)
Copper oxychloride 50% WP	0.10	4.22^{b} (2.18)	12.33^{b} (20.55)
	0.20	3.61^{c} (2.02)	4.17^{c} (11.55)
Hexaconazole 5% EC	0.10	0.00^{e} (0.70)	0.00^{e} (0.28)
	0.20	0.00^{e} (0.70)	0.00^{e} (0.28)
Azoxystrobin 23% SC	0.05	1.94^{d} (1.59)	0.67^{d} (4.61)
	0.1	2.14^{d} (1.63)	0.00^{e} (0.28)
Control		5.92^{a} (2.54)	100.00^{a} (89.72)
CD (0.05)		(0.102)	(1.609)

DAI-Days after inoculation. Figures in the parentheses are square root transformed values for spore count and angular transformed values for germination.
Values sharing same alphabets in superscript are statistically on par based on ANOVA.

fungi were combined at their infective doses, the mortality exhibited was 43.33 per cent which was on par with the combination *L. saksenae* + *M. anisopliae*. In bugs treated with sterile water there was no mortality after 24 h.

More or less a similar trend was noticed on the second day after spraying. *L. saksenae* + *B. bassiana* exhibited a death rate of 83.33 per cent whereas *L. saksenae* alone resulted in a significantly higher mortality of 93.33 per cent. Mortality observed in *L. saksenae* + *M. anisopliae* (46.67 per cent) and *L. saksenae* + *B. bassiana* + *M. anisopliae* (50.00 per cent) were on par. No mortality was observed in nymphs treated with either *B. bassiana* or *M. anisopliae*.

On the third day, 93.33 per cent mortality was observed in combination spray of *L. saksenae* + *B. bassiana* which was on par with that observed with *L. saksenae* (96.67 per cent). There was no mortality of nymphs treated with *B. bassiana* alone. When *L. saksenae* + *M. anisopliae* were combined, mortality was 56.67 per cent, while in the case of *L. saksenae* + *B. bassiana* + *M. anisopliae*, it was 53.33 per cent, which were on par with each other. No insects were found dead when treated with *M. anisopliae* alone.

Fourth DAS, 100 per cent mortality was attained in combination spray of *L. saksenae* + *B. bassiana* as well as treatment with *L. saksenae* alone. Whereas, in the treatment *B. bassiana* alone, there was no mortality at all. The combination sprays of *L. saksenae* + *M. anisopliae* and *L. saksenae* + *B. bassiana* + *M. anisopliae* resulted in 56.67 and 73.33 per cent mortality, which were significantly different from each other. No mortality was recorded in nymphs treated with *M. anisopliae*.

Cumulative mortality of insects treated with *B. bassiana* increased to 43.33 per cent on fifth day. In the combination spray of *L. saksenae* + *M. anisopliae* the death rate increased to 63.33 per cent which was significantly higher from that observed in bugs treated with *B. bassiana* alone. *L. saksenae* + *B. bassiana* + *M. anisopliae* combination resulted 76.67 per cent mortality which was on par with the treatment *L. saksenae* + *M. anisopliae*. Bugs treated with conidial suspension

Table 11. Compatibility status of *L. saksenae* with fungicides

Fungicides	Concentrations (%)	VR (%)	SP (%)	GER (%)	BI	Status
Carbendazim 50% WP	0.10	0.00	0.00	0.00	0	Toxic
	0.20	0.00	0.00	0.00	0	Toxic
Mancozeb 75% WP	0.15	0.00	0.00	0.00	0	Toxic
	0.30	0.00	0.00	0.00	0	Toxic
Copper oxychloride 50% WP	0.10	59.85	79.56	12.76	64	Moderately toxic
	0.20	61.90	63.69	4.34	57	Moderately toxic
Hexaconazole 5% EC	0.10	0.00	0.00	0.00	0	Toxic
	0.20	0.00	0.00	0.00	0	Toxic
Azoxystrobin 23% SC	0.05	41.82	20.80	0.51	29	Toxic
	0.1	47.03	24.45	0.00	33	Toxic

VR - Vegetative growth in relation to control, SP - Sporulation in relation to control, GER - Germination in relation to control, BI - Biological Index.

Compatible (> 66), Moderately toxic (42 - 66), and Toxic (< 42

Table 12. Effect of *L. saksenae* and other entomopathogenic fungi on mortality of rice bug nymphs under laboratory conditions

Treatments (spores mL^{-1})	Cumulative mortality at 24 h interval (%)						
	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS	6 DAS	7 DAS
L. saksenae (10^7) + *B. bassiana* (10^8)	80.00^{b} (63.44)	83.33^{b} (66.15)	93.33^{a} (77.40)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)
L. saksenae (10^7) + *M. anisopliae* (10^8)	36.67^{c} (37.23)	46.67^{c} (43.07)	56.67^{b} (48.93)	56.67^{c} (48.93)	63.33^{b} (52.77)	76.67^{b} (61.22)	83.33^{b} (66.15)
L. saksenae (10^7) + *B. bassiana* (10^8) + *M. anisopliae* (10^8)	43.33^{c} (41.15)	50.00^{c} (45.00)	53.33^{b} (47.01)	73.33^{b} (59.71)	76.67^{b} (61.71)	80.00^{b} (67.55)	83.33^{b} (69.78)
L. saksenae @ 10^7	86.67^{a} (68.85)	93.33^{a} (77.40)	96.67^{a} (83.25)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)
B. bassiana @ 10^8	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{c} (0.91)	0.00^{d} (0.91)	43.33^{c} (41.15)	43.33^{c} (41.15)	43.33^{c} (41.15)
M. anisopliae @ 10^8	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{c} (0.91)	0.00^{d} (0.91)	3.33^{cd} (6.75)	3.33^{d} (6.75)	6.67^{d} (12.60)
Control (water spray)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{c} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)
C.D (0.05)	(4.457)	(9.415)	(12.040)	(8.391)	(11.260)	(14.276)	(13.679)

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

Table 13. Effect of *L. saksenae* and other entomopathogenic fungi on mortality of rice bug adults under laboratory conditions

Treatments (spores mL^{-1})	Cumulative mortality at 24 h interval (%)						
	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS	6 DAS	7 DAS
L. saksenae (10^7) + *B. bassiana* (10^8)	83.33^a (66.15)	83.33^a (66.15)	83.33^b (66.15)	96.67^a (83.25)	100.00^a (89.09)	100.0^a (89.09)	100.00^a (89.09)
L. saksenae (10^7) + *M. anisopliae* (10^8)	23.33^b (28.78)	43.33^b (41.07)	53.33^c (46.93)	56.67^b (48.93)	63.33^b (52.77)	73.33^b (59.00)	80.00^b (63.93)
L. saksenae (10^7) + *B. bassiana* (10^8) + *M. anisopliae* (10^8)	20.00^b (26.56)	36.67^b (36.93)	43.33^c (41.15)	46.67^b (43.07)	56.67^{bc} (48.93)	70.00^b (56.96)	73.3^b (59.0)
L. saksenae @ 10^7	76.67^a (61.71)	83.33^a (66.15)	93.33^a (77.40)	96.67^a (83.25)	100.00^a (89.09)	100.0^a (89.09)	100.00^a (89.09)
B. bassiana @ 10^8	0.00^c (0.91)	0.00^c (0.91)	0.00^d (0.91)	0.00^c (0.91)	46.67^c (43.07)	66.67^b (54.78)	70.00^b (56.78)
M. anisopliae @ 10^8	0.00^c (0.91)	0.00^c (0.91)	0.00^d (0.91)	0.00^c (0.91)	0.00^d (0.91)	0.00^c (0.91)	3.33^c (6.75)
Control (water spray)	0.00^c (0.91)	0.00^c (0.91)	0.00^d (0.91)	0.00^c (0.91)	0.00^d (0.91)	0.00^c (0.91)	0.00^c (0.91)
C.D (0.05)	(4.468)	(9.658)	(8.019)	(10.721)	(7.722)	(5.412)	(8.672)

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

The same trend was observed on sixth and seventh days. Mortality exhibited by combination spray of *L. saksenae* +*B. bassiana* + *M. anisopliae* and *L. saksenae* + *M. anisopliae* were 80 per cent and 76.67 per cent respectively on sixth day after spraying which did not vary significantly among each other. The bugs treated with *B. bassiana* and *M. anisopliae* alone exhibited a mortality of 43.33 and 3.33 per cent respectively. On the seventh day, 83.33 per cent mortality was observed in treatments *L. saksenae* + *M. anisopliae* and *L. saksenae* + *B. bassiana* + *M. anisopliae*. The mortality observed with *B. bassiana* was 43.33.The mortality exhibited in the treatment *M. anisopliae* was negligible (6.67 per cent).

No mortality was observed in bugs treated with plain water till the end of seven days.

Though the treatment *L. saksenae* + *B. bassiana* was less effective when compared to *L. saksenae* alone during the first two days, third day onwards they were equally effective causing 100 per cent mortality on the fourth day. In case of the combination treatments *L. saksenae* + *M. anisopliae* and *L. saksenae* + *B. bassiana* + *M. anisopliae*, lesser mortality was observed. It was 83.33 at the end of the experimental period which was significantly lower when compared to *L. saksenae* + *B. bassiana* (100). Time taken for 100 per cent mortality was 4 days in *L. saksenae* and *L. saksenae* +*B. bassiana*, whereas other treatments did not yield the same level of death.

4.1.4.1.2 Adults

Analysis of data revealed that one day after spraying, the highest mortality was observed in *L. saksenae* + *B. bassiana* (83.33 per cent) which was on par with that observed with *L. saksenae* alone (66.67 per). There was no mortality in insects treated with *B. bassiana* alone. In combination sprays, *L. saksenae* + *M. anisopliae* and *L. saksenae* + *B. bassiana* + *M. anisopliae* the mortality did not vary significantly. It was 23.33 per cent and 20 per cent, respectively. No insects were found dead when treated with *M. anisopliae*.

More or less a similar trend was noticed up to four DAS wherein, highest mortality was exhibited by the treatments *L. saksenae* + *B. bassiana* and *L. saksenae* alone (96.67 each). The combination spray of *L. saksenae* + *M. anisopliae* resulted in 56.67 per cent mortality on the fourth day while *L. saksenae* + *B. bassiana* + *M. anisopliae* resulted in 46.67 per cent, which did not vary significantly from each other. No mortality was observed in treatments *B. bassiana* and *M. anisopliae*, alone.

On fifth DAS, 100 per cent mortality was observed in *L. saksenae* and *L. saksenae* + *B. bassiana*. The corresponding mortality observed in *B. bassiana* alone was 46.67 per cent. The combination spray of *L. saksenae* + *M. anisopliae* exhibited 63.33 per cent mortality, while *L. saksenae* + *B. bassiana* + *M. anisopliae* resulted in 56.67 per cent mortality. Insects treated with *M. anisopliae* alone did not exhibited death.

On the sixth day, 73.33 and 70 per cent mortality was recorded with *L. saksenae* + *M. anisopliae* and *L. saksenae* + *B. bassiana* + *M. anisopliae*. Significantly lower mortality was recorded in bugs treated with *B. bassiana* (66.67 per cent). Bugs treated with *M. anisopliae* did not die.

At the end of the experimental period (seventh day), *L. saksenae* + *M. anisopliae* and *L. saksenae* + *B. bassiana* + *M. anisopliae* resulted in 80 and 73.33 per cent mortality respectively, which were on par with each other. *B. bassiana* treated insects exhibited 70 per cent mortality. Death rate in *M. anisopliae* was negligible (3.33 per cent).

Bugs treated with plain water did not die till the end of the experiment.

The treatment *L. saksenae* + *B. bassiana* resulted in higher mortality during initial days compared to *L. saksenae* alone. By fifth DAS both the treatments caused 100 percent mortality of adults. Combination treatments, *L. saksenae* + *M. anisopliae* and *L. saksenae* + *B. bassiana* + *M. anisopliae*, were less effective.

From the *in vitro* compatibility studies based on radial growth, sporulation and germination, it could be inferred that botanicals such as aqueous and solvent extracts of neem seed kernels at 0.5 %, aqueous extracts of neem leaves and seed kernels at 1 % and neem oil emulsion at 0.5 % concentration were compatible with *L. saksenae*. Among the insecticides, all the new generation insecticides such as imidacloprid 17.8% SL, thiamethoxam 25% WG, chlorantraniliprole 18.5% SC and flubendiamide 39.35% SC at its recommended as well as half the recommended doses were compatible with *L. saksenae.* No fungicides tested were compatible with *L. saksenae.* Based on the mortality and time taken for mortality, the entomopathogenic fungus, *B. bassiana* was found to be compatible with *L. saksenae*, while *M. anisopliae* was found to have an inhibitive effect on *L. saksenae*

4.2 SYNERGISM STUDIES of *L. saksenae*

Botanical, chemical and microbial insecticides which were found to be compatible and superior were selected for further evaluation of their synergistic effect, with *L. saksenae* based on the mortality and feeding inhibition in rice bugs.

4.2.1 With Neem Based Botanicals

The neem based botanicals selected for synergism study were NSKE (A) 0.5 % and 1 %, NSKE (S) 0.5 %, NLE (A) 1 % and NOE 0.5 %.

4.2.1.1 Mortality of Rice Bugs Treated with L. saksenae, Neem based Botanicals and their Combinations

Cumulative mortality of nymphs and adults recorded at 24 h interval is presented in Table 14 and 15.

4.2.1.1.1 Nymphs

Analysis of data revealed that, on the first day after spraying, the highest

mortality of 83.33 per cent was observed in nymphs treated with *L. saksenae* as well as with the combinations *L. saksenae* + NSKE (A) 0.5 %, 1 % and *L. saksenae* + NSKE (S) 0.5 %. (83.33, 80 and 80 per cent). The treatment *L. saksenae* + NLE (A) 1 % and *L. saksenae* + NOE 0.5 % ranked second with 70.and 66.67 per cent mortality, respectively. There was no mortality in NSKE (A) 0.5 %, 1 %, NSKE (S) 0.5 %, NLE (A) 1 % and NOE 0.5 %.

Second day after spraying nymphs, highest mortality (86.67 per cent) was observed in *L. saksenae* which was on par with *L. saksenae* + NSKE (A) 0.5 % (83.33 per cent). The treatments *L. saksenae* + NSKE (A) 1 % and *L. saksenae* + NSKE (S) 0.5 % ranked second with 80 per cent mortality. This was followed by *L. saksenae* + NLE (A) 1 % (73.33 per cent), *L. saksenae* + NOE 0.5 % (66.67 per cent) and NSKE (A) 1 % (3.33 per cent), which varied each other. There was no mortality in NSKE (A) 0.5 %, NSKE (S) 0.5 %, NLE (A) 1 % and NOE 0.5 % treated bugs.

The trend observed was the same on the third DAS. The highest mortality was exhibited by *L. saksenae* (96.67 per cent). This was followed by the mortality recorded in *L. saksenae* + NSKE (A) 0.5 % (83.33 per cent), *L. saksenae* + NSKE (A) 1 %, *L. saksenae* + NSKE (S) 0.5 % (80 per cent each) and *L. saksenae* + NOE 0.5 % (70 per cent) which did not vary significantly. NOE 0.5 % (13.33 per cent) and NLE (A) 1 % (10 per cent) were on par. Mortality exhibited by NSKE (A) 0.5 %, NSKE (A) 1 % and NSKE (S) 0.5 % was negligible (6.67 per cent).

On the fourth DAS, 100 per cent mortality was observed in nymphs treated with *L. saksenae*, while *L. saksenae* + NSKE (A) 0.5 % recorded 96.67 per cent, which did not vary each other. The bugs treated with all the combination sprays of *L. saksenae* were on par with each other. Mortality exhibited by the treatments were *L. saksenae* + NSKE (S) 0.5 % (86.67 per cent), *L. saksenae* + NLE (A) 1 % cent (83.33 per cent), *L. saksenae* + NSKE (A) 1 % (80 per cent) and *L. saksenae* + NOE 0.5 % (76.67 per cent). Bugs treated with botanicals alone exhibited lesser

Table 14. Effect of *L. saksenae* and botanicals on mortality of rice bug nymphs under laboratory conditions

Treatments	Cumulative mortality at 24 h interval (%)						
	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS	6 DAS	7 DAS
*L. saksenae** + NSKE (A) 0.5%	83.33^{a} (66.15)	83.33^{a} (66.15)	83.33^{b} (66.15)	96.67^{a} (83.25)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)
L. saksenae *+ NSKE (A) 1%	80.00^{a} (63.44)	80.00^{ab} (63.44)	80.00^{b} (63.44)	80.00^{b} (63.44)	83.33^{bc} (66.15)	86.67^{b} (68.85)	93.33^{bc} (77.40)
L. saksenae *+ NSKE (S) 0.5%	80.00^{a} (63.44)	80.00^{ab} (63.44)	80.00^{b} (63.44)	86.67^{b} (68.85)	86.67^{bc} (68.85)	90.00^{b} (74.69)	96.67^{ab} (83.25)
*L. saksenae** + NLE (A) 1 %	70.00^{b} (56.78)	73.33^{bc} (59.00)	76.67^{b} (61.22)	83.33^{b} (66.15)	86.67^{bc} (68.85)	90.00^{b} (74.69)	90.00^{bc} (74.69)
*L. saksenae** + NOE 0.5%	66.67^{b} (54.78)	66.67^{c} (54.78)	70.00^{b} (56.78)	76.67^{b} (61.22)	76.67^{c} (61.22)	83.33^{b} (66.15)	90.00^{bc} (74.69)
*L. saksenae**	83.33^{a} (66.15)	86.67^{a} (68.85)	96.67^{a} (83.25)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)
NSKE (A) 0.5%	0.00^{c} (0.91)	0.00^{d} (0.91)	6.67^{cd} (12.60)	13.33^{c} (18.01)	20.00^{d} (26.56)	30.00^{c} (33.00)	36.67^{d} (37.15)
NSKE (A) 1%	0.00^{c} (0.91)	3.33^{d} (6.75)	6.67^{cd} (12.60)	13.33^{c} (21.15)	20.00^{d} (26.56)	23.33^{c} (28.78)	26.67^{d} (31.00)
NSKE (S) 0.5%	0.00^{c} (0.91)	0.00^{d} (0.91)	6.67^{cd} (9.46)	20.00^{c} (26.07)	26.67^{d} (31.00)	30.00^{c} (33.00)	43.33^{d} (41.15)
NLE (A) 1%	0.00^{c} (0.91)	0.00^{d} (0.91)	10.00^{c} (15.31)	23.33^{c} (28.07)	30.00^{d} (33.00)	33.33^{c} (35.00)	43.33^{d} (41.15)
NOE 0.5%	0.00^{c} (0.91)	0.00^{d} (0.91)	13.33^{c} (21.15)	16.67^{c} (23.85)	26.67^{d} (31.00)	36.67^{c} (37.15)	40.00^{d} (38.85)
Control (water spray)	0.00^{c} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{e} (0.91)	0.00^{d} (0.91)	0.00^{e} (0.91)
CD (0.05)	(3.643)	(6.401)	(13.734)	(11.812)	(9.072)	(11.391)	(10.434)

*10^{7} spores mL^{-1} A-aqueous S-solvent DAS-Days after spraying No. of insects per replication: 10 Figures in the parentheses are angular transformed values. Values sharing same alphabets in superscript are statistically on par based on ANOVA

Table 15. Effect of *L. saksenae* and botanicals on mortality of rice bug adults under laboratory conditions

Treatments	Cumulative mortality at 24 h interval (%)						
	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS	6 DAS	7 DAS
*L. saksenae** + NSKE (A) 0.5%	60.00^{b} (50.76)	70.00^{b} (56.76)	80.00^{a} (63.44)	90.00^{ab} (74.69)	96.67^{ab} (83.25)	100.00^{a} (89.09)	100.00^{a} (89.09)
L. saksenae * + NSKE (A) 1%	56.67^{b} (48.85)	60.00^{b} (50.76)	60.00^{bc} (50.76)	76.67^{bc} (61.21)	80.00^{c} (63.93)	80.00^{bc} (63.93)	93.33^{b} (77.40)
L. saksenae * + NSKE (S) 0.5%	43.33^{c} (41.15)	46.67^{c} (43.07)	50.00^{c} (45.00)	56.67^{c} (48.85)	56.67^{d} (48.85)	63.33^{cd} (52.77)	83.33^{bc} (54.78)
L. saksenae * + NLE (A) 1 %	60.00^{b} (50.76)	63.33^{b} (52.77)	70.00^{ab} (57.00)	76.67^{bc} (61.71)	83.33^{bc} (69.78)	90.00^{b} (74.69)	90.00^{bc} (74.69)
L. saksenae * + NOE 0.5%	43.33^{c} (41.07)	43.33^{c} (41.07)	46.67^{c} (42.99)	53.33^{c} (46.93)	56.67^{d} (48.85)	63.33^{cd} (52.77)	80.00^{c} (57.00)
*L. saksenae**	66.67^{a} (54.78)	76.67^{a} (61.22)	86.67^{a} (68.85)	93.33^{a} (77.40)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)
NSKE (A) 0.5%	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	10.00^{de} (15.31)	20.00^{ef} (26.56)	23.33^{e} (28.78)	23.33^{e} (28.78)
NSKE (A) 1%	0.00^{d} (0.91)	3.33^{d} (6.75)	6.67^{d} (9.46)	13.33^{d} (21.15)	20.00^{ef} (26.56)	23.33^{e} (28.78)	26.67^{de} (31.00)
NSKE (S) 0.5%	0.00^{d} (0.91)	0.00^{d} (0.91)	3.33^{d} (6.75)	3.33^{de} (6.75)	6.67^{g} (9.46)	10.00^{f} (15.31)	30.00^{de} (32.71)
NLE (A) 1%	0.00^{d} (0.91)	0.00^{d} (0.91)	6.67^{d} (9.46)	13.33^{d} (18.01)	26.67^{e} (31.00)	43.33^{de} (41.07)	43.33^{d} (41.07)
NOE 0.5%	0.00^{d} (0.91)	0.00^{d} (0.91)	3.33^{d} (6.75)	6.67^{de} (9.46)	10.00^{fg} (15.31)	10.00^{f} (15.31)	26.67^{de} (31.00)
Control (water spray)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{e} (0.91)	0.00^{g} (0.91)	0.00^{g} (0.91)	0.00^{f} (0.91)
CD (0.05)	(3.274)	(6.274)	(11.914)	(15.402)	(14.402)	(12.599)	(11.299)

10^{7} spores mL^{-1} A-aqueous S-solvent DAS-Days after spraying No. of insects per replication: 10 Figures in the parentheses are angular transformed values. Values sharing same alphabets in superscript are statistically on par based on ANOVA

mortality than the combination spray of *L. saksenae* and botanicals. Mortality noted in NLE (A) 1 %, NSKE (S) 0.5 %, NOE 0.5 %, and NSKE (A) 0.5 % and 1 % were 23.33 per cent, 20 per cent, 16.67 per cent and 13.33 per cent each respectively.

By the fifth day, *L. saksenae* + NSKE (A) also recorded 100 per cent mortality. All other combination sprays except *L. saksenae* + NOE 0.5 % were on par, mortality ranging from 83.33 to 86.67 per cent. Nymphs treated with *L. saksenae* + NOE 0.5 % exhibited significantly lower mortality (76.67 per cent). Mortality recorded in treatment with botanical sprays ranged from 20 to 30 per cent which were on par with each other. On the sixth day, combination spray of *L. saksenae*+ NSKE (S) 0.5 % and *L. saksenae* + NLE (A) 1 % exhibited 90 per mortality in nymphs, while other combinations such as *L. saksenae* + NSKE (A) 1 % and *L. saksenae* + NOE 0.5 % recorded 86.67 per cent and 83.33 per cent mortality respectively and were on par with each other. Mortality noticed in bugs treated with botanical sprays were 36.67 per cent, 33.33 per cent, 30 per cent, 30 per cent and 23.33 per cent respectively, with NOE 0.5 %, NLE (A) 1 %, NSKE 0.5 % both aqueous and solvent and NSKE (A) 1 %.

At the end of the experiment, (seventh day) *L. saksenae* + NSKE (S) 0.5 % exhibited 96.67 per cent mortality of nymphs. This was followed by *L. saksenae* + NSKE (A) 1 % (93.33 per cent), *L. saksenae* + NLE (A) 1 % and *L. saksenae* + NOE 0.5 % (90 per cent each) which were on par. The botanicals such as NSKE (S) 0.5 %, NLE (A) 1 %, NOE 0.5 %, NSKE (A) 0.5 % and NSKE (A) 1 % resulted in mortality ranging from 40 to 26.67 per cent, which did not vary significantly among each other.

Mortality exhibited by *L. saksenae* as well as *L. saksenae* + NSKE (A) 0.5 % were on par except for the third day. The time taken to achieve 100 per cent mortality increased by one day in combination spray with NSKE (A) 0.5 % compared to that *L. saksenae.* Combination sprays with other botanicals exhibited significantly reduced mortality from the second day onwards and could not achieve 100 per cent mortality within seven days.

4.2.1.1.2 Adults

In the case of adults, on the first DAS, the highest mortality observed was in those treated with *L. saksenae* (66.67 per cent). This was followed by the treatments, *L. saksenae* + NSKE (A) 0.5 %, *L. saksenae* + NLE (A) 1 % (60 per cent each) and *L. saksenae* + NSKE (A) 1 % (56.67 per cent), which were on par with each other. Bugs when sprayed with combination spray of *L. saksenae* + NSKE (S) 0.5 % recorded mortality of only 43.33 per cent. No bugs were found dead when treated with botanicals alone.

On the second day also, the highest mortality (76.67 per cent) was observed in bugs treated with *L. saksenae* and in *L. saksenae* + NSKE (A) 0.5 %. The combination sprays of *L. saksenae* + NSKE (A) 1 % and *L. saksenae* + NLE (A) 1 % and ranked second with 60 and 63.33 per cent mortality, respectively. This was followed by *L. saksenae* + NSKE (S) 0.5 % and *L. saksenae* + NOE 0.5 % which recorded mortality of 46.67 per cent and 43.33 per cent respectively, and did not vary significantly. NSKE (A) 1 % exhibited only 3.33 per cent mortality, which varied significantly from all other treatments. Bugs treated with botanicals such as NSKE (A) 0.5 %, NSKE (S) 1 %, NLE (A) 1 % and NOE 0.5 % did not die.

In the case of adults, on the third DAS, mortality recorded in *L. saksenae* and *L. saksenae* + NSKE (A) 0.5 % was on par with mortality of 86.67 and 80 per cent respectively. Mortality exhibited by *L. saksenae* + NLE (A) 1 %, *L. saksenae* + NSKE (A) 1 %, and *L. saksenae* + NSKE (S) 0.5 % varied significantly with 70, 60 and 50 per cent mortality, respectively. The treatments such as NLE (A) 1 %, NSKE (A) 1 % (6.67 per cent each), NSKE (S) 0.5 % and NOE 0.5 % (3.33 per cent each) were statistically on par. No bugs were found dead when treated with NSKE (A) 0.5 %.

On the fourth DAS, the highest mortality (93.33 per cent) was recorded in bugs treated with *L. saksenae* which was followed by *L. saksenae* + NSKE (A) 0.5 %. (76.67 per cent). The combination sprays such as *L. saksenae* + NLE (A) 1 % and *L. saksenae* + NSKE (A) 1 % resulted in same mortality (76.67 per cent), while

L. saksenae + NSKE (S) 0.5 % and *L. saksenae* + NOE 0.5 % exhibited 56.67 per cent and 53.33 per cent mortality, which did not vary significantly. Both NSKE (A) 1 % and NLE (A) 1 % exhibited mortality of 13.33 per cent. This was followed by NSKE (A) 0.5 % (10 per cent), NOE 0.5 % (6.67 per cent) and NSKE (S) 0.5 % (3.33 per cent), which were statistically similar.

In adults, *L. saksenae* treatment resulted in 100 per cent mortality on the fifth day, while combination sprays exhibited varying range of mortality. *L. saksenae* + NSKE (A) 0.5 % recorded 96.67 per cent mortality, while *L. saksenae* + NLE (A) 1 %, *L. saksenae* + NSKE (A) 1 % and *L. saksenae* + NSKE (S) 0.5 % resulted in 83.33 and 80 per cent death, respectively. 80 per cent which varied significantly. Bugs treated with *L. saksenae* + NOE 0.5 % and *L. saksenae* + NSKE (S) 0.5 % resulted in same mortality (56.67 per cent). The botanical NLE (A) 1 % exhibited 26.67 per cent mortality whereas both 0.5 % and 1 % of NSKE (A) resulted in death rate of 20 per cent. This was followed by NOE 0.5 % (10 per cent) and NSKE (S) 0.5 % (6.67 per cent).

Hundred per cent mortality was observed in *L. saksenae* + NSKE (A) 0.5 %, on the sixth day. This was sequentially followed by the treatments such as *L. saksenae* + NLE (A) 1 % (90 per cent), *L. saksenae* + NSKE (A) 1 % (80 per cent), *L. saksenae* + NSKE (S) 0.5 % and *L. saksenae* + NOE 0.5 % (63.33 per cent each). The botanicals NLE (A) 1 % exhibited death rate of 43.33 per cent where as other botanicals such as NSKE (A) 0.5 %, 1 %,(23.33 per cent each),NSKE (S) 0.5 % and NOE 0.5 % (23.33 per cent) were significantly lower.

On the seventh day, mortality observed in *L. saksenae* + NSKE (A) 1 % was 93.33 per cent. The combination sprays such as *L. saksenae* + NLE (A) 1 % (90 per cent) mortality and *L. saksenae* + NSKE (S) 0.5 % (83.33 per cent mortality) were on par with each other, whereas *L. saksenae* + NOE 0.5 % resulted in 80 per cent mortality, which was significantly lower. Bugs treated with NLE (A) 1 %, exhibited mortality of 43.33 per cent. The treatments NSKE (S) 0.5 %, NSKE (A) 1 % and NOE 0.5 % were on par and exhibited mortality of 30, 26.67 and 26.67 per cent respectively. Least death rate (23.33 per cent) was observed in NSKE (A) 0.5 %.

None of the bugs died when treated with plain water during the entire period of study.

Mortality exhibited by *L. saksenae* was noticed to be the best among the treatments with 66.67 per cent mortality even on the first day after treatment. Combination spray with NSKE (A) 0.5 % exhibited mortality that was on par with *L. saksenae* on third sixth and seventh day, and on the other days significantly lower mortality was noted. Time taken to achieve 100 per cent mortality also increased by one day in combination spray of *L. saksenae* with NSKE (A) 0.5 %. Combination sprays with other botanicals exhibited significantly less mortality and could not achieve 100 per cent mortality within seven days.

4.2.1.2 Feeding Inhibition

Feeding inhibition of rice bugs was assessed by comparing the percentage of damaged grains in the treatments with that in the untreated plants (Table 16). Damage noticed in *L. saksenae* + NSKE (S) 0.5 % was the lowest (10.86 per cent) whereas it was highest in NSKE (A) 0.5 % (45.56). The corresponding damage in untreated plants was 90.09 per cent.

The lowest damage (10.86 per cent) was noticed in *L. saksenae* + NSKE (S) 0.5 %, which was on par with those observed in *L. saksenae* + NSKE (A) 1 %, *L. saksenae, L. saksenae* + NSKE (A) 0.5 %, *L. saksenae* + NLE (A) 1 % and *L. saksenae* + NOE 0.5 %. The percentage inhibition calculated was 11.52, 12.18, 12.37, 14.07 and 14.85, respectively.

Percentage of inhibition in feeding ranged from 49.44 - 67.68 in plants treated with botanicals. Among the botanicals, least damage was noticed in NOE 0.5 % (29.13 per cent) which was significantly lower than those observed in other botanicals and higher than those observed with *L. saksenae* and with botanicals. The damage calculated in NSKE (S) 0.5 % and NSKE (A) 1 % were on par, values being 36.86 and 37.93, respectively. Highest damage was noticed in NLE (A) 1 % (45.15 per cent) and NSKE (A) 0.5 % (45.56 per cent) respectively.

Table 16. Effect of *L. saksenae* and neem based botanicals on feeding behaviour of rice bug

Treatments	Grain damage (%)	Feeding inhibition (%)	Inhibition over control (%)- 7 DAT
*L. saksenae** + NSKE (A) 0.5%	12.37^{e}	87.63	86.26
*L. saksenae** + NSKE (A) 1%	11.52^{e}	88.48	87.22
*L. saksenae** + NSKE (S) 0.5%	10.86^{e}	89.14	87.94
*L. saksenae** + NLE (A) 1%	14.07^{e}	85.93	84.38
*L. saksenae** + NOE 0.5%	14.85^{e}	85.15	83.53
*L. saksenae**	12.18^{e}	87.82	86.47
NSKE (A) 0.5%	45.56^{b}	54.44	49.44
NSKE (A) 1%	37.93^{c}	62.07	57.90
NSKE (S) 0.5%	36.86^{c}	63.14	59.09
NLE (A) 1%	45.15^{b}	54.85	49.89
NOE 0.5%	29.13^{d}	70.87	67.68
Control (water spray)	90.09^{a}	9.91	-
CD (0.05)	4.115		

*10^{7} spores mL^{-1} A-Aqueous S-Solvent DAT- Days After Treatment

Figures in the parentheses are square root transformed values. Values sharing same alphabets in superscript are statistically on par based on ANOVA

Both *L. saksenae* as well as combination sprays exhibited highest feeding inhibition and were on par making it evident that feeding inhibition was due to the effect of *L. saksenae.*

4.2.2 With Insecticides

Compatible insecticides with high BI values, such as flubendiamide 39.35% SC 0.0025 % and chlorantraniliprole 18.5% SC 0.003 % and 0.006 % and thiamethoxam 25% WG 0.0025 % were selected for synergism studies.

4.2.1.1 Mortality of Rice Bugs Treated with L. saksenae, Insecticides and their Combinations

Cumulative mortality of nymphs and adults under laboratory conditions, recorded at 24 h interval is presented in Table 17 and 18.

4.2.1.1.1 Nymphs

Analysis of data revealed that on the first DAS, there was 83.33 per cent mortality in the treatment combinations *L. saksenae* + thiamethoxam 25% WG 0.025 % and *L. saksenae* + flubendiamide 39.35% SC 0.0025 %. The mortality of nymphs sprayed with conidial suspension of *L. saksenae* was also 83.33 per cent. Thiamethoxam 25% WG 0.025 % resulted in 73.33 per cent mortality, while there was no mortality in bugs treated with flubendiamide 39.35% SC 0.0025 %. Mortality observed with the combination *L. saksenae* + chlorantraniliprole 18.5% SC 0.003 % and *L. saksenae* + chlorantraniliprole 18.5% SC 0.006 % was 80 and 76.67 per cent, respectively. Insects treated with chlorantraniliprole 18.5% SC 0.003 % and 0.006 % did not die.

On the second DAS, the combination spray of *L. saksenae* + thiamethoxam 25% WG 0.0025 % and *L. saksenae* + flubendiamide 39.35% SC 0.0025 % resulted in 83.33 per cent mortality of nymphs. While *L. saksenae* exhibited the highest mortality of 86.67 per cent. This was followed by thiamethoxam 25% WG 0.0025 %

Table 17. Effect of *L. saksenae* and insecticides on mortality of rice bug nymphs under laboratory conditions

Treatments	Cumulative mortality at 24 h interval (%)						
	I DAS	2 DAS	3 DAS	4 DAS	5 DAS	6 DAS	7 DAS
L. saksenae * + flubendiamide 39.35% SC 0.0025%	83.33^{a} (66.15)	83.33^{ab} (66.15)	86.67^{b} (68.85)	93.33^{ab} (77.40)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)
L. saksenae * + chlorantraniliprole 18.5% SC 0.003%	80.00^{ab} (63.44)	80.00^{bc} (63.44)	90.00^{b} (71.56)	93.33^{ab} (77.40)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)
L. saksenae * + chlorantraniliprole 18.5% SC 0.006%	76.67^{ab} (61.22)	76.67^{bc} (63.44)	83.33^{bc} (66.15)	90.00^{b} (74.69)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)
L. saksenae * + thiamethoxam 25% WG 0.0025%	83.33^{a} (66.15)	83.33^{ab} (66.15)	86.67^{b} (68.85)	90.00^{b} (74.69)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)
L. saksenae *	83.33^{a} (66.15)	86.67^{a} (68.85)	96.67^{a} (83.25)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)
Flubendiamide 39.35% SC- 0.0025%	0.00^{c} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	3.33^{d} (6.75)
Chlorantraniliprole 18.5% SC-0.003%	0.00^{c} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	6.67^{c} (12.60)	13.33^{c} (21.15)	13.33^{c} (21.15)
Chlorantraniliprole 18.5% SC-0.006%	0.00^{c} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	3.33^{d} (6.75)	6.67^{d} (9.46)
Thiamethoxam 25% WG-0.0025%	73.33^{b} (59.00)	73.33^{c} (59.00)	73.33^{c} (59.00)	73.33^{c} (59.00)	76.67^{b} (61.22)	80.00^{b} (63.44)	83.33^{b} (66.15)
Control (water spray)	0.00^{c} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)	0.00^{d} (0.91)
CD (0.05)	5.266	5.266	10.505	12.782	5.824	6.006	10.303

* 10^{7} spores mL^{-1} DAS-Days after spraying No. of insects per replication: 10 Figures in the parentheses are angular transformed values. Values sharing same alphabets in superscript are statistically on par based on ANOVA

Table 18. Effect of *L. saksenae* and insecticides on mortality of rice bug adults under laboratory conditions

Treatment	Cumulative mortality at 24 h interval (%)						
	I DAS	2 DAS	3 DAS	4 DAS	5 DAS	6 DAS	7 DAS
L. saksenae * + flubendiamide 39.35% SC 0.0025%	60.00^{b} (50.85)	73.33^{bc} (59.00)	80.00^{a} (63.93)	93.33^{a} (77.40)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)
L. saksenae * + chlorantraniliprole 18.5% SC 0.003%	63.33^{ab} (52.77)	80.00^{a} (63.44)	86.67^{a} (68.85)	93.33^{a} (77.40)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)
L. saksenae * + chlorantraniliprole 18.5% SC 0.006%	63.33^{ab} (52.77)	70.00^{c} (56.78)	80.00^{a} (63.93)	83.33^{a} (66.15)	96.67^{a} (83.25)	100.00^{a} (89.09)	100.00^{a} (89.09)
L. saksenae * + thiamethoxam 25% WG 0.0025%	70.00^{a} (56.78)	80.00^{a} (63.44)	83.33^{a} (66.15)	93.33^{a} (77.40)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)
L. saksenae *	66.67^{ab} (54.78)	76.67^{ab} (61.22)	86.67^{a} (68.85)	93.33^{a} (77.40)	100.00^{a} (89.09)	100.00^{a} (89.09)	100.00^{a} (89.09)
Flubendiamide 39.35% SC- 0.0025%	0.00^{c} (0.91)	0.00^{e} (0.91)	0.00^{c} (0.91)	0.00^{c} (0.91)	0.00^{c} (0.91)	0.00^{c} (0.91)	0.00^{c} (0.91)
Chlorantraniliprole 18.5% SC-0.003%	0.00^{c} (0.91)	0.00e (0.91)	0.00^{c} (0.91)	0.00^{c} (0.91)	3.33^{c} (6.75)	3.33^{c} (6.75)	3.33^{c} (6.75)
Chlorantraniliprole 18.5% SC-0.006%	0.00^{c} (0.91)	0.00^{e} (0.91)	0.00^{c} (0.91)	0.00^{c} (0.91)	0.00^{c} (0.91)	3.33^{c} (6.75)	6.67^{c} (12.60)
Thiamethoxam 25% WG-0.0025%	66.67^{ab} (54.78)	66.67^{d} (54.78)	66.67^{b} (54.78)	66.67^{b} (54.78)	70.00^{b} (56.78)	80.00^{b} (63.93)	83.33^{b} (66.15)
Control (water spray)	0.00^{c} (0.91)	0.00^{e} (0.91)	0.00^{c} (0.91)	0.00^{c} (0.91)	0.00^{c} (0.91)	0.00^{c} (0.91)	0.00^{c} (0.91)
CD (0.05)	(4.530)	(3.478)	(7.373)	(11.345)	(7.706)	(7.706)	(9.983)

* 10^{7} spores mL^{-1} DAS-Days after spraying No. of insects per replication: 10 Figures in the parentheses are angular transformed values. Values sharing same alphabets in superscript are statistically on par based on ANOVA

(73.33 per cent) mortality, which was statistically lower. The combination spray of *L. saksenae* + chlorantraniliprole 18.5% SC 0.003 % and *L. saksenae* + chlorantraniliprole 18.5% SC 0.006 % exhibited statistically similar death rate of 80 and 76.67 respectively. Flubendiamide 39.35% SC and chlorantraniliprole 18.5% SC did not cause any mortality of nymphs.

On the third day, combination sprays of *L. saksenae* + chlorantraniliprole 18.5% SC 0.003 % (90 per cent), *L. saksenae* + flubendiamide 39.35% SC 0.0025 % and *L. saksenae* + thiamethoxam 25% WG 0.0025 % resulted in 86.67 per cent mortality which was statistically on par with each other. *L. saksenae* exhibited the highest mortality of 96.67 per cent, which was significantly higher than other treatment. This was followed by *L. saksenae* + chlorantraniliprole 18.5% SC 0.006 % which exhibited mortality of 83.33 per cent. The treatment *L. saksenae* + thiamethoxam 25% WG 0.0025 % exhibited 86.67 per cent mortality while thiamethoxam 25% WG 0.0025 % alone caused 73.33 per cent which was significantly less from the combination treatment. No mortality was observed in nymphs treated with chlorantraniliprole 18.5% SC 0.003 %, 0.006 % and flubendiamide 39.35% SC 0.0025 %.

At four DAS, there was 100 per cent mortality in insects treated with *L. saksenae*. The combination sprays *L. saksenae* + chlorantraniliprole 18.5% SC 0.003 % and *L. saksenae* + flubendiamide 39.35% SC 0.0025 % exhibited 93.33 per cent each while a significantly lower mortality was observed on *L. saksenae* + chlorantraniliprole 18.5% SC 0.006 % and *L. saksenae* + thiamethoxam 25% WG 0.0025 % (90 per cent each). The nymphs treated with thiamethoxam 25% WG 0.0025 % exhibited 73.33 per cent mortality, whereas no bugs died when treated with chlorantraniliprole 18.5% SC 0.003 %, 0.006 %, as well as flubendiamide 39.35% SC 0.0025 %.

By fifth DAS, *L. saksenae* as well as all the insecticide combinations with *L. saksenae* achieved 100 per cent mortality. The treatment thiamethoxam 25% WG 0.0025 % ranked second with 76.67 per cent mortality. Chlorantraniliprole 18.5%

SC 0.003 % exhibited 6.67 per cent mortality, while no mortality was noted in chlorantraniliprole 18.5% SC 0.006 % and flubendiamide 39.35% SC 0.0025 %.

On the first day, mortality exhibited by *L. saksenae* and combination sprays of *L. saksenae* + thiamethoxam 25% WG 0.0025 % and *L. saksenae* + flubendiamide 39.35% SC 0.0025 % were on par. On second and third days mortality exhibited in combination sprays were lower when compared to treatment with *L. saksenae* alone. When *L. saksenae* took four days for 100 per cent mortality, its effective combinations took five days.

4.2.2.1.2 Adults

On the first day, the highest mortality observed was 70 per cent, in the treatment *L. saksenae* + thiamethoxam 25% WG 0.0025 % which was statistically higher than that observed with *L. saksenae* @ 10^7 spores mL^{-1} as well as thiamethoxam 25% WG 0.0025 % (66.67 per cent each). The combination spray of *L. saksenae* + chlorantraniliprole 18.5% SC 0.003 % and *L. saksenae* + 0.006 %, exhibited 63.33 per cent death, whereas *L. saksenae* + flubendiamide 39.35% SC 0.0025 % exhibited 60 per cent, which was significantly lower. None of the bugs died in the treatments such as chlorantraniliprole 18.5% SC 0.003 %, 0.006 %, and flubendiamide 39.35% SC 0.0025 %.

On the second day, the combination sprays of *L. saksenae* + thiamethoxam 25% WG 0.0025 % and *L. saksenae* + chlorantraniliprole 18.5% SC 0.003 % exhibited 80 per cent mortality. The treatment with spore suspension of *L. saksenae* exhibited 76.67 per cent mortality which was statistically higher than the death rate observed in *L. saksenae* + flubendiamide 39.35% SC 0.0025 % (73.33 per cent). The treatment *L. saksenae* + chlorantraniliprole 18.5% SC 0.006 % exhibited 70 per cent mortality which was significantly lower than the former treatments. Thiamethoxam 25% WG 0.0025 % exhibited 66.67 per cent mean mortality. There was no death of bugs treated chlorantraniliprole 18.5% SC 0.003 %, 0.006 % and flubendiamide 39.35% SC 0.0025 %.

Third DAS, the effect of *L. saksenae* was found to be on par with that of its combination with insecticides. In *L. saksenae* as well as *L. saksenae* + chlorantraniliprole 18.5% SC 0.003 % there was 86.67 per cent mortality, while *L. saksenae* + thiamethoxam 25% WG 0.0025 %, *L. saksenae* + chlorantraniliprole 18.5% SC 0.006 % and *L. saksenae* + flubendiamide 39.35% SC 0.0025 % exhibited statistically similar mortality of 83.33, 80 and 80 per cent mortality, respectively. A lower mortality was 66.67 per cent was noted in thiamethoxam 25% WG 0.0025 %. None of the bugs died when treated with chlorantraniliprole 18.5% SC 0.003 %, 0.006 % and flubendiamide 39.35% SC 0.0025 %.

Four DAS, when the adults were treated, *L. saksenae* and all the combination sprays except *L. saksenae* + chlorantraniliprole 18.5% SC 0.006 % exhibited a death rate of 93.33 per cent. *L. saksenae* + chlorantraniliprole 18.5% SC 0.006 % exhibited 83.33 per cent mortality which did not vary statistically. Only 66.67 per cent mortality was noticed in the treatment thiamethoxam 25% WG 0.0025 % whereas bugs treated with the insecticides such as chlorantraniliprole 18.5% SC 0.003 %, 0.006 %, and flubendiamide 39.35% SC 0.0025 % did not die.

By fifth day, mortality in *L. saksenae* and its combinations were on par. *L. saksenae* + thiamethoxam 25% WG 0.0025 %, *L. saksenae* + chlorantraniliprole 18.5% SC 0.003 % and *L. saksenae* + flubendiamide 39.35% SC 0.0025 % exhibited 100 per cent mortality, while *L. saksenae* + chlorantraniliprole 18.5% SC 0.006 % exhibited 96.67 per cent mortality. Mortality noticed in thiamethoxam 25% WG 0.0025 % was 70 per cent. Negligible mortality was noted in chlorantraniliprole 18.5% SC 0.003 % (3.33 per cent). No bugs were found dead when sprayed with chlorantraniliprole 18.5% SC 0.006 % and flubendiamide 39.35% SC 0.0025 %.

A similar trend was noticed on sixth and seventh days after spraying in nymphs as well as adults. Bugs treated with plain water did not exhibited any mortality till the end of the experiment period.

In adults on the first day itself, the combination treatment of *L. saksenae* with thiamethoxam 25% WG 0.0025 % killed 70 per cent of the bugs, while a lower mortality was observed when treated with *L. saksenae* (66.67 per cent) But by the third day, both *L. saksenae* as well as the combinations exhibited similar range of mortality and could achieve 100 per cent mortality within five days.

4.2.2.2 Feeding Inhibition

Feeding inhibition calculated based on percentage of grains damaged is presented in Table 19.

Damage noticed was the lower in *L. saksenae* (12.01 per cent) and its combination treatments (13.61-17.88 per cent) whereas it was highest in flubendiamide 39.35% SC 0.0025 % (84.91). The corresponding damage in untreated plants was 87.98 per cent.

Analysis of data on percentage damage revealed that *L. saksenae* as well as its combination sprays exhibited less damage. The percentage damage were 12.01, 13.61, 14.66, 16.60 and 17.88 in *L. saksenae*, *L. saksenae* + chlorantraniliprole 18.5% SC 0.006 %, *L. saksenae* + chlorantraniliprole 18.5% SC 0.003 %, *L. saksenae* + thiamethoxam 25% WG 0.0025 % and *L. saksenae* + flubendiamide 39.35% SC 0.0025 %, respectively. Damage in thiamethoxam 25% WG 0.0025 % was 43.42 per cent, which was statistically higher than other combinations, while it was 80.55 in chlorantraniliprole 18.5% SC 0.006 %, which varied significantly. The damage observed in chlorantraniliprole 18.5% SC 0.003 % and flubendiamide 39.35% SC 0.0025 % were 83.11 and 84.91 per cent respectively which did not vary each other significantly.

4.2.3 Synergism of *L. saksenae* with *B. bassiana*

Synergistic effect of *L. saksenae* at 10^7spores mL^{-1} with *B. bassiana* at 10^8 spores mL^{-1}, assessed based on the mortality and feeding inhibition of rice bug, both nymphs and adults is presented in Tables 20 – 22.

4.2.3.1 Mortality of Rice Bugs

4.2.3.1.1 Nymphs

Cumulative mortality of nymphs treated with *L. saksenae* and *B. bassiana* at their effective doses is indicated in Table 20.

On the first DAS, mortality recorded in *L. saksenae* + *B. bassiana* was 80 per cent, which was statistically lower than that treated with *L. saksenae* (85) alone. No bugs died when sprayed with *B. bassiana* and plain water (control). On the second DAS, same trend was noticed. The death rate *L. saksenae* + *B. bassiana* increased to 85 per cent while bugs treated with *L. saksenae* (90 per cent).

Third DAS, the effect of *L. saksenae* and *L. saksenae* + *B. bassiana* was statistically same (95 per cent). By the fourth day, both the treatments attained 100 per cent mortality while it was negligible (2.5 per cent) in bugs treated with *B. bassiana* and nil in water spray. At the end of the experiment (7^{th} day), bugs treated with *B. bassiana* recorded 70 per cent mortality.

4.2.3.1.2 Adults

Mortality of rice bug adults with *L. saksenae* and *B. bassiana* is indicated in Table 21.

On the first DAS, *L. saksenae* + *B. bassiana* exhibited highest mortality of 75 per cent followed by *L. saksenae* (65 per cent). Both the treatments varied significantly with each other. No mortality was observed in case of *B. bassiana* and control.

On the second DAS, *L. saksenae* in combination with *B. bassiana* exhibited 82.50 per cent death and *L. saksenae* alone recorded 80 per cent mortality, which were on par with each other. Bugs treated with *B. bassiana* and water did not die.

Third DAS, *L. saksenae* + *B. bassiana* exhibited 85 per cent mortality while *L. saksenae* recorded significantly higher mortality (92.50 per cent).

Table 19. Effect of *L. saksenae* and insecticides on feeding behaviour of rice bug

Treatments	Grain damage (%)	Feeding inhibition (%)	Inhibition over control (%) 7 DAT
L. saksenae * + flubendiamide 39.35% SC 0.0025%	17.88[d]	82.12	79.69
L. saksenae * + chlorantraniliprole 18.5% SC 0.003%	14.66[d]	85.34	83.33
L. saksenae * + chlorantraniliprole 18.5% SC 0.006%	13.61[d]	86.39	84.48
L. saksenae * + thiamethoxam 25% WG 0.0025%	16.60[d]	83.40	81.16
L. saksenae *	12.01[d]	87.99	86.32
Flubendiamide 39.35% SC- 0.0025%	84.91[ab]	15.09	3.50
Chlorantraniliprole 18.5% SC-0.003%	83.11[ab]	16.89	5.51
Chlorantraniliprole 18.5% SC-0.006%	80.55[b]	19.45	8.36
Thiamethoxam 25% WG-0.0025%	43.42[c]	56.58	50.53
Control	87.98[a]	12.02	-
CD (0.05)	6.423		

*10^7 spores mL^{-1} DAT- Days After Treatment

Figures in the parentheses are square root transformed values.

Values sharing same alphabets in superscript are statistically on par based on ANOVA

No insects were found dead in *B. bassiana* and in control.

On the fourth day, the mortality observed in *L. saksenae* and *L. saksenae* + *B. bassiana* was same (95per cent). Hundred per cent mortality was exhibited by both the treatments on the fifth day. No bugs were found dead in control throughout the study.

4.2.3.2 Feeding Inhibition

Percentage of damaged grains by rice bug treated with *L. saksenae* and *B. bassiana* is indicated in Table 22. Least grain damage (12.21 per cent) was observed in bugs when treated with *L. saksenae* + *B. bassiana*, which was on par with *L. saksenae* (14.32 per cent).

Feeding inhibition was highest in *L. saksenae* + *B. bassiana* (85.39 per cent) and *L. saksenae* (83.02 per cent) which was on par, whereas it was least in *B. bassiana* (79.71 per cent).

From the synergism studies under laboratory conditions, in the botanicals, *L. saksenae* + NSKE (A) 0.5 % exhibited a better performance compared to combinations with other botanicals, but the effect was either on par or lesser when compared to *L. saksenae* with respect to mortality and feeding inhibition of rice bugs. When the insecticides were combined, *L. saksenae* + thiamethoxam 25% WG 0.0025 % exhibited increased mortality in adults than *L. saksenae,* but were comparable. Mortality in combination treatment with *B. bassiana* as well as *L. saksenae* were almost equivalent. The time taken to attain 100 per cent mortality increased by one day in combination treatments with botanicals, while it was same when combined with thiamethoxam 25% WG 0.0025 % (in adults) and *B. bassiana.* From this, it is evident that the any of the botanicals, insecticide and entomopathogenic fungi had a synergism with *L. saksenae*, but their effect was increased when combined with *L. saksenae* in managing rice bug.

Table 20. Effect of *L. saksenae* and *B. bassiana* on mortality of rice bug nymphs under laboratory conditions

Treatments (spores mL^{-1})	Cumulative mortality at 24 h interval (%)						
	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS	6 DAS	7 DAS
L. saksenae (10^7) + *B. bassiana* (10^8)	80.00^b (63.44)	85.00^b (67.50)	95.00^a (80.32)	100.00^a (89.09)	100.00^a (89.09)	100.00^a (89.09)	100.00^a (89.09)
L. saksenae (10^7)	85.00^a (67.50)	90.00^a (75.94)	95.00^a (80.32)	100.00^a (89.09)	100.00^a (89.09)	100.00^a (89.09)	100.00^a (89.09)
B. bassiana (10^8)	0.00^c (0.91)	0.00^c (0.91)	0.00^b (0.91)	2.50^b (5.28)	50.00^b (53.77)	65.00^b (53.77)	70.00^b (56.78)
Control (water spray)	0.00^c (0.91)	0.00^c (0.91)	0.00^b (0.91)	0.00^b (0.91)	0.00^c (0.91)	0.00^c (0.91)	0.00^c (0.91)
CD (0.05)	(3.611)	(7.654)	(11.021)	(11.699)	(6.276)	(6.276)	(4.258)

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

Table 21. Effect of *L. saksenae* and *B. bassiana* on mortality of rice bug adults under laboratory conditions

Treatments (spores mL^{-1})	Cumulative mortality at 24 h interval (%)						
	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS	6 DAS	7 DAS
L. saksenae (10^8) + *B. bassiana* (10^8)	65.00^b (53.77)	82.50^a (65.46)	85.00^b (67.50)	95.00^a (80.32)	100.00^a (89.09)	100.00^a (89.09)	100.00^a (89.09)
L. saksenae (10^7)	75.00^a (60.11)	80.00^a (63.80)	92.50^a (75.94)	95.00^a (80.32)	100.00^a (89.09)	100.00^a (89.09)	100.00^a (89.09)
B. bassiana (10^8)	0.00^c (0.91)	0.00^b (0.91)	0.00^c (0.91)	0.00^b (0.91)	45.00^b (42.12)	45.00^b (42.12)	47.50^b (43.56)
Control (water spray)	0.00^c (0.91)	0.00^b (0.91)	0.00^c (0.91)	0.00^b (0.91)	0.00^c (0.91)	0.00^c (0.91)	0.00^c (0.91)
CD (0.05)	(3.986)	(5.616)	(7.654)	(11.021)	(5.134)	(2.678)	(1.697)

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

Table 22. Effect of *L. saksenae* and *B.bassiana* on feeding behaviour of rice bug

Treatments (spores mL^{-1})	Grain damage (%)	Feeding inhibition (%)	Inhibition over control-7 DAT (%)
L. saksenae (10^7)+ *B. bassiana* (10^8)	12.21[c]	87.79	85.39
L. saksenae(10^7)	14.32[c]	85.68	83.02
B. bassiana (10^8)	17.14[b]	82.86	79.71
Control (water spray)	83.76[a]	16.24	-
CD (0.05)	2.767		

DAT- days after treatment Figures in the parentheses are square root transformed values. Values sharing same alphabets in superscript are statistically on par based on ANOVA

4. 3 EFFICACY OF *L. saksenae* AND ITS COMBINATION WITH CHEMICAL AND MICROBIAL INSECTICIDES IN THE MANAGEMENT OF RICE BUG

Efficacy of *L. saksenae* at 10^7 spores mL^{-1} and its combination with thiamethoxam 25% WG 0.0025 % and *B. bassiana* at 10^8 spores mL^{-1} in the management of rice bug assessed in term of population is presented in Table 23.

4.3.1 Effect on Rice Bug Population

4.3.1.1 First Spraying

The population of the bugs did not vary significantly among the various plots before treatment. Seven days after spraying, effect of *L. saksenae* + *B. bassiana* and *L. saksenae* was on par, mean population being 10.50 and 10.75 per plot, respectively. The plots treated with the combination spray of *L. saksenae* + thiamethoxam 25% WG 0.0025 % exhibited a significantly higher population (12.25 per plot). This was followed by population in plots treated with *B. bassiana* (13.50 per plot). The highest population was recorded in thiamethoxam 25% WG @ 0.0025 % (16.25 per plot) and in the untreated control (16.50 per plot).

Fourteenth DAS, a decrease in population was seen in all the treatments, the most effective being *L. saksenae* (5.20 bugs per plot) followed by *L. saksenae* + *B. bassiana* (5.25 per plot). The mean population per plot was 6.75 in plots treated with *B. bassiana* which was significantly higher than the former treatments. The population of bugs in thiamethoxam 25% WG treated plots was significantly higher (7 per plots). The least reduction in population was recorded in *L. saksenae* + thiamethoxam 25% WG (7.5 per plot).

4.3.1.1 Second Spraying

Seven days after spraying, *L. saksenae* was revealed to be the most effective treatment in controlling the population of rice bug (3.00 per plot). This was

Plate 6. Layout of the field experiment

Table 23. Effect of *L. saksenae* and its combination with thiamethoxam and *B. bassiana* on population of rice bug

Treatments	No of rice bugs per plot*				
	First spraying			Second spraying	
	Precount	7 DAS	14 DAS	7 DAS	14 DAS
L. saksenae @ 10^7 spores mL^{-1} + *B. bassiana* @ 10^8 spores mL^{-1}	12.75 (3.56)	10.50^{c} (3.24)	5.25^{bc} (2.29)	4.25^{ab} (2.03)	2.75^{bc} (1.66)
L. saksenae @ 10^7 spores mL^{-1} + Thiamethoxam 25% WG 0.0025%	13.50 (3.66)	12.25^{b} (3.49)	7.50^{ab} (2.73)	4.25^{ab} (2.05)	3.5^{b} (1.86)
L. saksenae @ 10^7 spores mL^{-1}	12.75 (3.57)	10.75^{c} (3.27)	5.20^{c} (2.28)	3.00^{b} (1.54)	2.25^{c} (1.50)
B. bassiana @ 10^8 spores mL^{-1}	12.50 (3.54)	13.50^{b} (3.67)	6.75^{b} (2.59)	4.00^{ab} (1.97)	3.00^{bc} (1.72)
Thiamethoxam 25 % WG 0.0025%	13.00 (3.60)	16.25^{a} (4.03)	7.00^{b} (2.63)	5.25^{a} (2.29)	5.25^{a} (2.29)
Untreated control	13.50 (3.68)	16.50^{a} (4.06)	9.00^{a} (3.00)	6.25^{a} (2.49)	5.25^{a} (2.29)
CD (0.05)	NS	(0.379)	(0.309)	(0.538)	(0.291)

* Plot size 2x2 m Mean of four replications. DAS-Days after spray. NS -Non Significant
Figures in the parentheses are square root transformed values.
Values sharing same alphabets in superscript are statistically on par based on ANOVA.

followed by the treatments such as *L. saksenae* + thiamethoxam 25% WG (4.25), *L. saksenae* + *B. bassiana* (4.25) and *B. bassiana* (4.20), which were on par with each other. The least effective treatment was thiamethoxam 25% WG 0.0025 % which exhibited a population of 5.25 bugs per plot.

Similar trend was noticed on 14th day, population being lowest in plots treated with *L. saksenae* (2.25 bugs per plot). Effect of *L. saksenae* + *B. bassiana* (2.75 per plot) and *B. bassiana* (3.00 per plot) were on par. Population recorded in *L. saksenae* + thiamethoxam 25% WG was 3.5 per plot and it was 5.25 per plot when treated with thiamethoxam 25% WG alone.

4.3.2 Effect on Natural Enemy Population

Natural enemy population recorded before and after treatment is furnished in Table 24. Predatory insects such as coccinellids, reduviids, spiders such as *Argiope sp., Oxyopes* sp. *and Tetragnatha sp*. hymenopteran parasitoids such as *Bracon* sp., *Isotima* sp., and *Xanthopimpla* sp. were encountered in the rice field. There was no significant variation in population among the treated and untreated plots. It ranged from 3 to 6.25 per plot in the treated plots and 4.74 to 6.5 per plot in the untreated plots.

4.3.3 Effect on Yield

The data on the grain yield per plot is indicated in Table 25. A significantly higher grain yield was noticed in the plots treated with *L. saksenae* (1.38 kg plot^{-1}), which was closely followed by that in the plots treated with *L. saksenae* + *B. bassiana* (1.32 kg plot^{-1}). The treatment, *L. saksenae* + thiamethoxam 25% WG 0.0025 % recorded grain yield of 1.18 kg plot^{-1}, which was significantly lower than the former treatments. The grain yield obtained in the plots treated with *B. bassiana* (1.12 kg plot^{-1}), thiamethoxam 25% WG 0.0025 % (1.15 kg plot^{-1}) and untreated control (1.02 kg plot^{-1}) did not vary significantly.

Table 24. Effect of *L. saksenae* and its combination with thiamethoxam and *B. bassiana* on population of natural enemies in rice ecosystem

Treatments	No of natural enemies per plot *				
	First spraying			Second spraying	
	Precount	7 DAS	14 DAS	7 DAS	14 DAS
L. saksenae @10^7 spores mL^{-1} + *B. bassiana* @ 10^8 spores mL^{-1}	5.25 (2.24)	4.25 (2.03)	5.00 (2.21)	4.25 (2.03)	5.75 (2.37)
L. saksenae @ 10^7 spores mL^{-1} + Thiamethoxam 25 % WG 0.0025%	3.50 (1.84)	3.00 (1.46)	4.75 (2.12)	5.25 (2.23)	6.25 (2.49)
L. saksenae @ 10^7 spores mL^{-1}	4.25 (2.05)	4.50 (2.10)	5.50 (2.34)	4.00 (1.96)	5.25 (2.29)
B. bassiana @ 10^8 spores mL^{-1}	4.50 (2.10)	4.75 (2.16)	5.00 (2.18)	4.50 (2.16)	5.25 (2.28)
Thiamethoxam 25 % WG 0.0025%	4.00 (1.96)	4.25 (2.05)	5.50 (2.34)	3.75 (1.92)	6.00 (2.44)
Untreated control	4.75 (2.13)	5.25 (2.27)	6.50 (2.52)	5.25 (2.27)	6.00 (2.44)
CD (0.05)	NS	NS	NS	NS	NS

* Plot size 2x2 m. Mean of four replications. DAS-Days after spray. NS- Non Significant
Figures in the parentheses are square root transformed values.
Values sharing same alphabets in superscript are statistically on par based on ANOVA.

Table 25. Effect of *L. saksenae* and its combination with thiamethoxam 25 % WG and *B. bassiana* on yield of paddy

Treatments	Grain Yield (kg plot^{-1})*
L. saksenae @ 10^7 spores mL^{-1}+ *B. bassiana* @ 10^8 spores mL^{-1}	1.32^{ab}
L. saksenae @ 10^7 spores mL^{-1} + Thiamethoxam 25% WG 0.0025%	1.18^{bc}
L. saksenae @ 10^7 spores mL^{-1}	1.38^{a}
B. bassiana @ 10^8 spores mL^{-1}	1.12^{c}
Thiamethoxam 25% WG 0.0025%	1.15^{c}
Untreated control	1.02^{c}
CD (0.05)	0.156

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

Discussion

5. DISCUSSION

Biological control, when particularly carried out with entomopathogenic fungi is a technique that can reduce the population density of pests in Integrated Pest Management (IPM) programme. Integration of any pest management tool in the IPM package, necessitates a thorough investigation on its compatibility with other components. Hence, the present investigation focused to study the compatibility of *Lecanicillium saksenae* with other crop protectants such as botanical, insecticides, fungicides as well as other entomopathogenic fungi. It also assessed the synergistic effect of the fungus with other components and their field efficacy in managing rice bug, *Leptocorisa acuta*, the major pest in rice.

5.1 COMPATIBILITY OF *Lecanicillium saksenae*

Among the botanicals used in plant protection, neem is known for its insecticidal and antifungal activity. Therefore it was inevitable to study its impact on *L. saksenae*. *In vitro* studies on compatibility of *L. saksenae* with botanicals revealed the non- inhibitive nature of lower concentration (0.5 %) of neem oil emulsion (NOE) in the mycelial growth of the fungus, while its higher concentrations (1 % and 2 %) were found to be moderately inhibitive. This finding is in accordance with the report of Rani (2016) which states the same trend of *L. saksenae* with neem oil emulsion.

Apart from the non-inhibitive nature of NOE, it was found to have a stimulative effect on growth of the fungus at lower concentrations. Parmar and Devakumar (1993) stated that neem at lower concentrations act as a growth regulator.

Compatibility studies of neem oil with other entomopathogenic fungi of the same genus revealed that inhibition of *Lecanicillium lecanii* by neem oil 1 % was negligible (Illathur *et al.,* 2018).

Other entomopathogenic fungi such as *Beauveria bassiana* was also found to be unaffected in growth by NOE below 5 % (Rodriguez-Lagunes *et al.,* 1997).

However, Depieri *et al.* (2005) reported that 0.5 % NOE reduced the growth of *B. bassiana* by 49.8 per cent. Inhibitive nature of NOE 0.2 % on the growth of *Metarhizium anisopliae* was demonstrated by Kumar *et al.* (2008) and its inhibition at 2 % concentration was reported by Kelwatkar *et al.* (2017). The disparity in results obtained in various studies might be due to the variation in the alkaloid content of the formulations used or due to varying content of the inhibitory principles which in turn is affected by the method of extraction.

In the case of aqueous extract of neem seed kernel NSKE (A) also, a less inhibitive nature was observed. Lower concentrations (0.5 %) did not affect the growth while higher concentrations (1 % and 2 %) inhibited its growth. Depieri *et al.* (2005) reported that NSKE (A) 1 % was found to affect the vegetative growth of *B. bassiana.*

In this study, solvent extract of neem seed kernel, NSKE (S) was inhibitive to *L. saksenae* compared to aqueous extracts. Reports by Gupta *et al.* (1999) substantiates this finding, wherein they states that NSKE (S) is more inhibitive than NSKE (A) to *B. bassiana.* So also, solvent extracts of *Annonna squamosa* were found to be more inhibitive than its aqueous extracts to *L. lecanii, Isaria fumosorosea* and *B. bassiana* (Sahayaraj *et al.,* 2011).

It was found that neem leaf extracts (NLE), both aqueous and solvent were inhibitive to *L. saksenae* at 1 %, but at 2 % and 4 % aqueous extracts were more inhibitive than solvent extracts. Gupta *et al.* (1999) explained that non-inhibitory action of solvent extracts of leaves might be due to the fact that solvents can elucidate more compounds from the leaves, of which some of them might contribute to the growth of the fungus. They also attributed this phenomenon to the degradation of active principles by the solvents, during the extraction process.

To derive a comparative effect of oil, kernel extracts and leaf of neem on mycelial growth of the fungus, effect of 2 % concentration was compared (Fig.1) It was observed that leaf extracts were more inhibitive (46.19 per cent) than seed kernel extracts (21.07 per cent) and oil emulsions (20.91 per cent). However, studies

of Depieri *et al.* (2005) revealed that in the case of *B.bassiana* leaf extracts were least inhibitive compared to seed kernels, at 2 % concentration. The variation observed may be due to the detoxification mechanism of the fungus, which varies from species to species. The disparity in results may also be due to the qualitative and quantitative differences in the composition of secondary metabolites which may vary with method of extraction as well as source of the botanicals.

The action mechanism of neem by-products on vegetative growth of fungi is speculated to be due to phytoallexines, sulfurade compounds, and triterpenoids in these products which are fungitoxic in action (Singh *et al.*, 1984; Bandopadhyay, 2002).

Analysis of data on sporulation revealed that in all the treatments there was a statistically significant reduction in spore count. However in the case of fungi sporulation is said to be significantly reduced only when there is a logarithmic reduction. Therefore, it was NSKE that was found to be non-inhibitive to *L. saksenae* with spore count to the tune of 10^7 as observed in the control. However, there was a concentration dependent reduction in sporulation. Rani (2016) reported similar effect of NSKE on sporulation of *L. saksenae,* with a concentration dependent inhibition without any logarithmic reduction in spore count.

Similar trend was also observed in the case of *L. lecanii, B. bassiana* and *M. anisopliae.* In *B. bassiana* 1 % NSKE (A) was less inhibitive than 2 % and 4 % concentrations (Depieri *et al.,* 2005). Ribeiro *et al.* (2012) also reported a concentration dependent inhibition in the sporulation of *B.bassiana* when poisoned with NSKE.

The finding that solvent extracts of seed kernels were more inhibitive to growth, while aqueous extract was more inhibitive to sporulation, may be due to interference of secondary metabolites in the botanicals with the media components, which create varying effects on its vegetative and reproductive growths. It may also be due to the fact that when the organism is under stress, it diverts the resources to produce more spores than its mycelia.

NOE inhibited sporulation significantly (75.30-93.13 per cent), but there was no logarithmic reduction at lower dose. On the other hand, it did not affect the mycelial growth of the fungus, as mentioned above. This is because of the fact that even if certain medium can support the mycelial growth, it cannot support sporulation. Moreover, more nutrients are required for sporulation than needed for its vegetative growth (Cochrane, 1958). Differential response of entomopathogenic fungus in growth and sporulation in medium poisoned with neem oil was earlier reported by Rani (2000) in the case of *Fusarium pallidoroseum* (Cooke) Sacc, a pathogen of pea aphid *Aphis craccivora.*

Effect of neem oil on *B.bassiana* was studied by Hirose *et al.* (2001). The study concluded that neem oil emulsion (2 %), caused 80 per cent inhibition in sporulation of the fungus. Further, Depieri *et al.* (2005) and Islam *et al.* (2010a) also reported high inhibitive nature of neem oil (0.5 to 1.5 %) to *B. bassiana*. They observed 80 per cent reduction in conidiogenesis of the fungus. In *L. lecanii,* neem oil at 1 % and 2 % concentrations, reduced sporulation by 17.27 per cent and 19.67 per cent, respectively (Illathur *et al.,* 2018).

NLE (S) was inhibitory to *L. saksenae* at all concentrations, while NLE (A) was inhibitory only at 4 %. Rani (2000) reported that sporulation of *F. pallidoroseum* enhanced with NLE (A) 5 %. Disparity in sporulating ability of these two fungi in the media poisoned with the same formulation is attributed to the variable response of these two species. Castiglioni *et al.* (2003) reported reduction in sporulation of *B. bassiana* by the commercial formulation of neem leaves (Nimkol -L), in concentrations that are equal or greater than 5 %.

Comparison of 2 % concentrations of oil, seed kernel and leaf extracts of neem on sporulation of *L. saksenae* shows that all the three formulations of neem affected the sporulation significantly, of which least inhibition was observed in neem leaf (68.43 per cent) compared to seed kernels (76.62 per cent) and oil emulsion (93.13 per cent). This finding is in accordance with that of Depieri *et al.* (2005) who reported that in *B.bassiana*, neem leaf extracts were less inhibitive (1.8 per cent) compared to seed kernels (28.8 per cent) and oil emulsion (78.7 per cent).

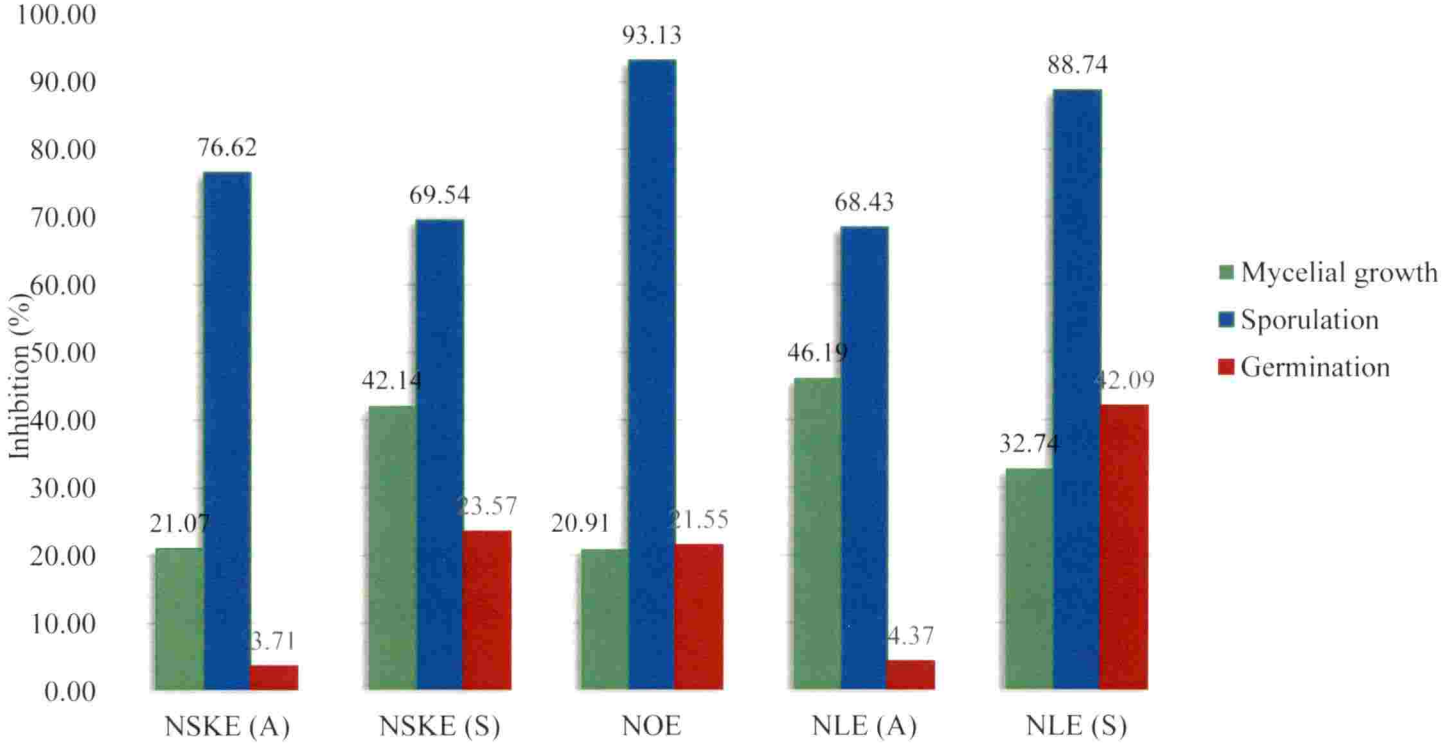


Fig 1. Inhibition in growth, sporulation and germination of *L.saksenae* by botanicals

As observed in the case of mycelial growth and sporulation, NSKE (A) 0.5 % did not affect the germination also (1.34 per cent). At higher concentrations 1 % and 2 %, there was only negligible inhibition (1.69 and 3.71 per cent). However, solvent extracts affected the germination to a lesser extent with inhibition of 17.17-23.57 per cent. Similar results were reported in *B. bassiana* with NSKE (A) 4 %, where the germination inhibition was negligible (0.2 per cent). (Depieri *et al.*, 2005).

Though sporulation of *L. saksenae* was affected by NOE, inhibition in germination was only 12.12-21.55 per cent. This finding is in accordance with that of Rodriguez-Lagunes *et al.* (1997) who reported that NOE did not affect the germination of *B. bassiana*, when tested at 5 % concentration.

Though NLE 1 % was moderately inhibitive to growth and highly inhibitive to sporulation, its inhibition on germination was less (3.71 to 4.37 per cent). NLE (S) 2 % and 4 % was more inhibitive (42.67 to 62.67 per cent). This is in contrary to the finding of Depieri *et al.* (2005) who observed only 1.3 per cent inhibition in germination of *B. bassiana* with NLE (A) 1.5 % concentration.

On comparison of 2 % concentration of the three formulations (Fig. 1), seed kernel extract that was found to be the less inhibitive to germination (3.71 per cent) of *L. saksenae*, than leaf extracts (4.37 per cent) and oil emulsion (21.55 per cent)

Concentration dependent reduction in growth, sporulation as well as germination of the fungus observed in this study is substantiated by the findings of Hirose *et al.* (2001), Castiglioni *et al.* (2003), Depieri *et al.* (2005), Ambethgar *et al.* (2009) Deb *et al.* (2017) and Illathur *et al.* (2018) in various entomopathogenic fungi.

Selection and scheduling of chemical insecticides and fungicides needs a thorough evaluation on its *in vitro* compatibility to the biocontrol agents which are simultaneously or sequentially used in the field. Hence the commonly used old generation as well as the new generation insecticides were tested for their compatibility with *L. saksenae.*

Experiment to assess the compatibility of chemical insecticides with *L. saksenae,* revealed that at half the recommended dose, the inhibition in growth was less than 10 per cent with all the new generation insecticides tested. At their recommended doses inhibition with flubendiamide 39.35% SC 0.005 %, imidacloprid 17.8% SL, thiamethoxam 25% WG 0.005 % and chlorantraniliprole 0.006 %, was less than 10 per cent. Whereas, in dimethoate 30% EC 0.05 % it was 25.41 per cent and in chlorpyriphos 20% EC 0.06 % it was the highest (61.97 per cent). These findings are in accordance with that of Filho *et al.* (2001) who reported that thiamethoxam 250 WG 0.02 % was not inhibitive to *L. lecanii.* Gurulingappa *et al.* (2011) reported its non-inhibitive action in *L. lecanii* even at higher concentration of 0.04 %. Non inhibition of flubendiamide 39.35% SC 0.002 % and chlorantraniliprole 18.5% SC 0.001 % to *L. lecanii* was observed in the studies conducted by Vijayasree (2013). Inhibitive nature of dimethoate 30% EC 0.05 % observed in this study is in concurrence with the findings of Kakati *et al.* (2018) who reported 21 per cent inhibition in *L. lecanii. L. saksenae* was found to be inhibited by imidacloprid 17.8% SL 0.003 %, moderately, Kakati *et al.* (2018) reported that imidacloprid 200 SC 0.05 % was not inhibitive to *L. lecanii.*

Strong inhibitory action observed in *L. saksenae* by chlorpyriphos might be due to the interference of chlorpyriphos in the uptake of carbon and nitrogen sources from the media which are essential for their growth and development as suggested by Pachamuthu *et al.* (1999). Findings of Saindane (2007) substantiated this inhibitive property of chlorpyriphos in *L. lecanii.*

Effect of insecticides on sporulation was almost similar to that observed in mycelial growth. At half the recommended dose, chlorantraniliprole 18.5% SC was not at all inhibitive, while the inhibition was less than 10 per cent in flubendiamide 39.35% SC and dimethoate 30% EC. Thiamethoxam 25% WG and imidacloprid 17.8% SL, were moderately inhibitive (12.59- 25. 93 per cent). Though dimethoate 30% EC inhibited mycelial growth, spore count was not that much reduced (2.96 per cent inhibition). Here also chlorpyriphos 20% EC was the most inhibitive insecticide (61.48 per cent).

A comparison of sporulation at recommended doses of insecticides is represented in Fig 2. Here also chlorantraniliprole 18.5% SC caused negligible (2.22 per cent) inhibition to sporulation, while flubendiamide 39.35% SC was moderately inhibitive (37.04 per cent). More than 50 per cent inhibition was noticed in dimethoate 30% EC, imidacloprid 17.8% SL, thiamethoxam 25% WG and chlorpyriphos 20% EC, the latter being highly inhibitive (78.52 per cent) These findings are in accordance with that of Rani (2018) who observed that chlorantraniliprole 18.5% SC, 0.006 % was less inhibitive to the sporulation of *L. saksenae*, flubendiamide 39.35% SC 0.005 % was moderately inhibitive and dimethoate 30% EC 0.05 % and chlorpyriphos 20% EC 0.06 % were highly inhibitive.

According to Neves *et al.* (2001), thiamethoxam 250 WG at 0.05 % caused 12.37 per cent reduction in sporulation of *B. bassiana* and 22.32 per cent reduction in *M. anisopliae*. Silva *et al.* (2013) observed that thiamethoxam 250 WG at 0.025 % had significantly reduced the conidiation in *M. anisopliae*.

Foregoing results on growth and sporulation of *L. saksenae* in media poisoned with insecticides, it concluded that with insecticides such as imidacloprid 17.8% SL and thiamethoxam 25% WG though there was no commendable reduction in mycelial growth, sporulation was adversely affected. This may be due to the alteration of C:N ratio of the medium at higher concentrations, which adversely affected the sporulation.

Germination assay revealed that at half the recommended dose imidacloprid 17.8% SL 0.006 % (12.88 per cent) was less inhibitive (37.96 per cent) followed by flubendiamide 39.35% SC 0.0025 %. Thiamethoxam 25% WG 0.0025 % and chlorantraniliprole 18.5% SC 0.006 % inhibited the germination by 58.64 and 64.74 per cent, respectively. While in chlorpyriphos 20% EC 0.03 % and dimethoate 30% EC 0.025 % inhibition rates were higher (68.28 and 77.29 per cent respectively).

At their recommended doses also germination was significantly affected by all the insecticides. It was 54.7 to 59.32 per cent in imidacloprid 17.8% SL 0.006

% and chlorantraniliprole 18.5% SC 0.006 % while in dimethoate 30EC at 0.05 % and chlorpyriphos 20% EC 0.06 % inhibition rates were too high (81.69 and 92.55 per cent, respectively (Fig. 2). Inhibition by imidacloprid 17.8% SL is supported by the findings of Panahi *et al.* (2012) who observed 13.37 per cent inhibition of *Lecanicillium longisporum*. Its inhibition even at lower concentration of 0.0008 % on *M. anisopliae* was reported by Schumacher and Poehling (2012). Inhibitory action of dimethoate and chlorpyriphos 0.05 % in conidial germination was supported by the findings of Khan *et al.* (2012) in *B. bassiana* and *M. anisopliae*.

Compatibility studies with fungicides revealed that all the fungicides tested exhibited significantly less growth. Copper oxychloride 50% WP was comparatively less inhibitive (38.89 per cent) and azoxystrobin 23% SC was moderately inhibitive (57.3 per cent) while carbendazim 50% WP, mancozeb 75% WP and hexaconazole 5% EC totally inhibited the growth of *L. saksenae* at both the doses. These findings are in accordance with that of Khalil (1985) who observed that copper oxychloride 50WP at 0.25 % caused only 29 per cent inhibition in growth of *L. lecanii.* However, Olan and Cortez (2003) observed highly inhibitive nature of copper oxychloride 50% WP in *L. lecanii* (79.28 per cent).

Inhibitive action of azoxystrobin 23% SC observed in this study is in accordance with the report of Kim *et al.* (2001) who reported its strong inhibition in *L. lecanii* (52 per cent). Inhibition of *L. saksenae* observed in this study is not in parity with the report of Gonzalez *et al.* (2012) who reported only 34.3 % reduction in mycelial growth of *L. lecanii.* This difference may be attributed to the variation in species.

Exactly same trend was noticed with sporulation and germination of the fungus at both the recommended and half doses. Sporulation in copper oxychloride 50% WP treated medium was least inhibited (28-39 per cent) followed by azoxystrobin 23% SC (63.85-67.22 per cent). There was total inhibition by carbendazim 50% WP, mancozeb 75% WP and hexaconazole 5% EC at both the doses. (Fig. 3).

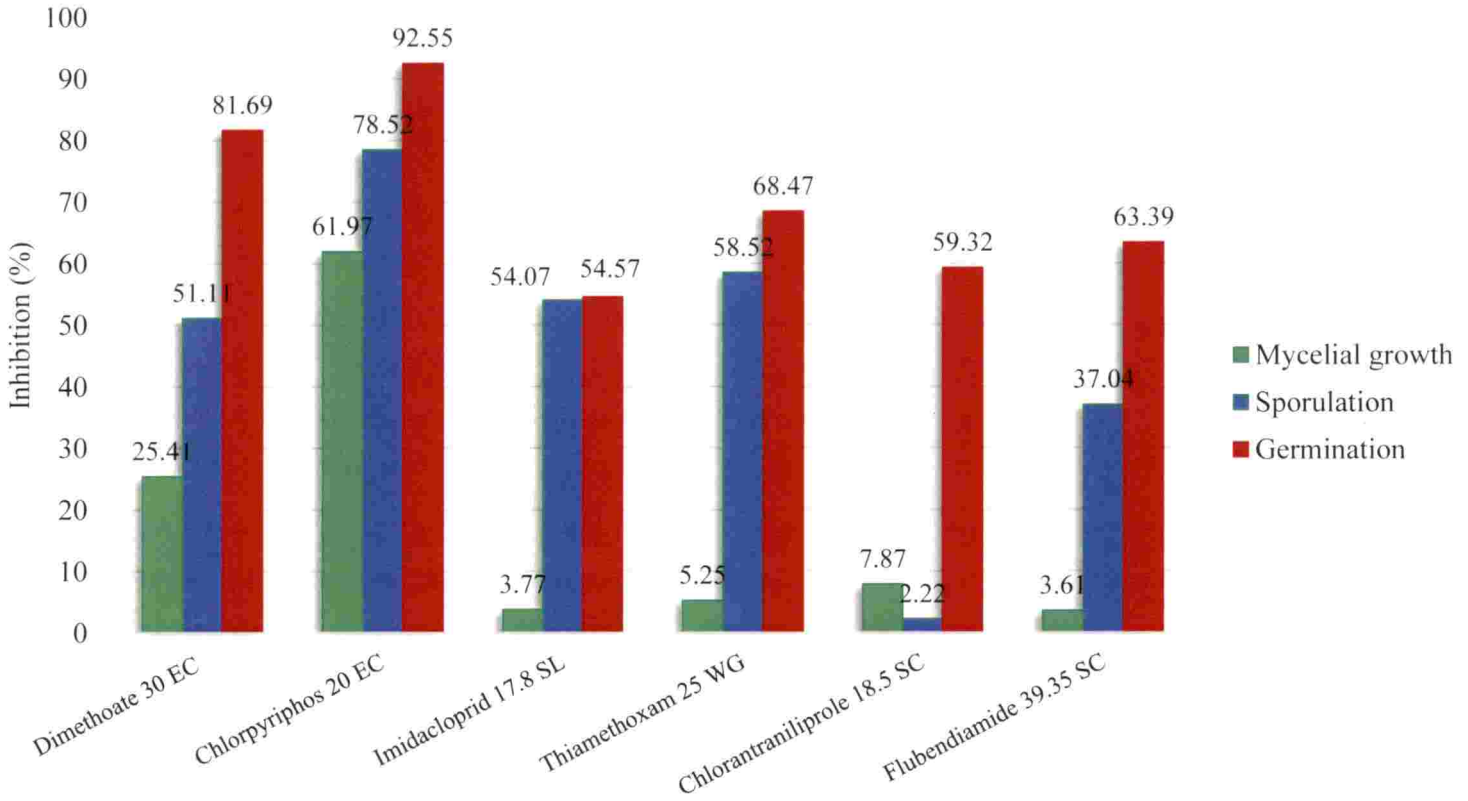


Fig 2. Inhibition in growth, sporulation and germination of *L.saksenae* with insecticides at their recommended doses

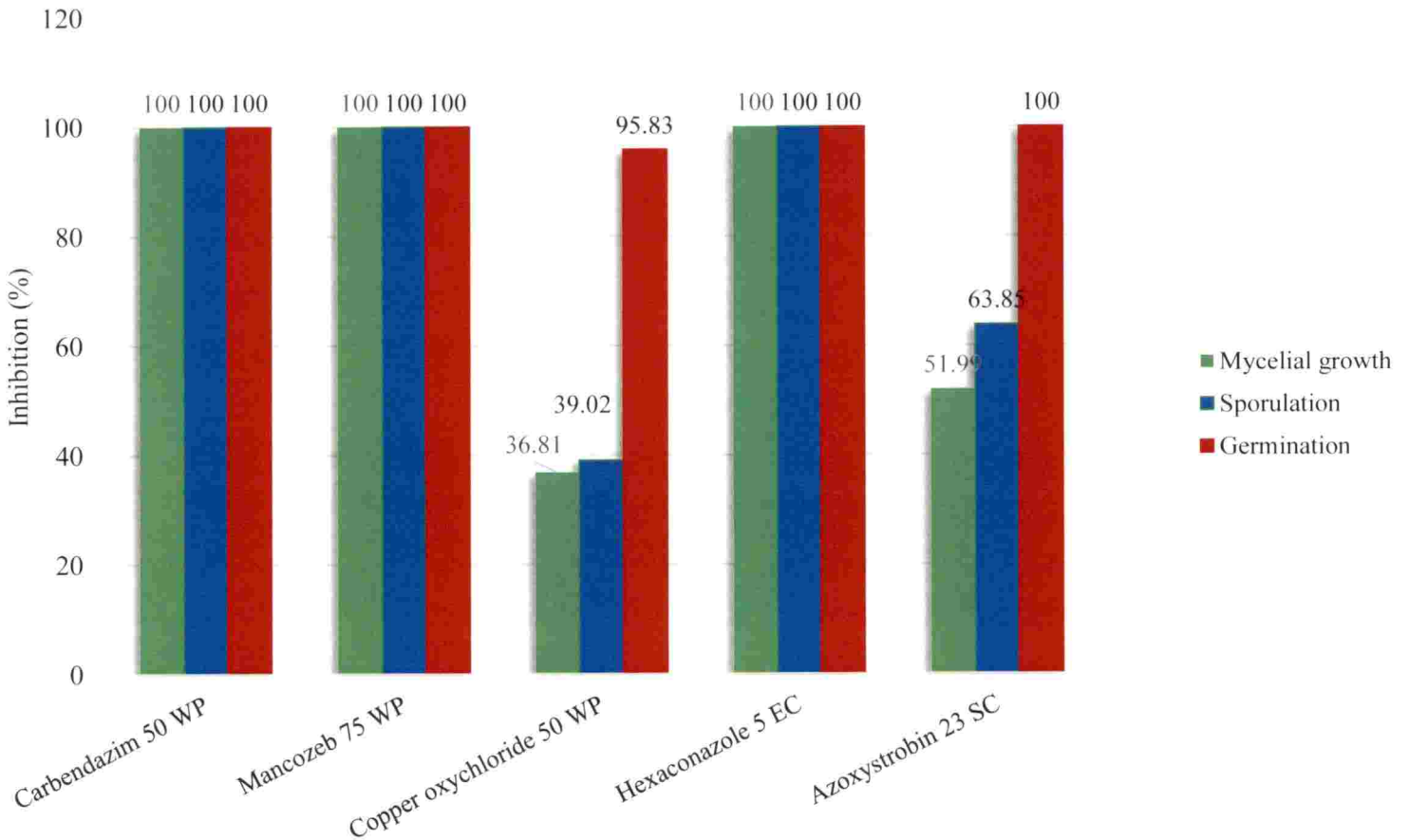


Fig 3. Inhibition in growth, sporulation and germination of *L.saksenae* with fungicides at their recommended doses

All the fungicides affected germination of the fungus drastically. Percentage inhibition was 87.67- 95. 83 in copper oxychloride 50% WP and 99.33-100 per cent in azoxystrobin 23% SC. In all other fungicides there was no germination at all.

Inhibitive nature of mancozeb 75% WP 0.15 %, noted in this study, has been earlier reported in *L. lecanii* by Khalil (1985). Further, Gonzalez *et al.* (2012) observed that mancozeb 75% WP 0.2 % inhibited the sporulation in *L. lecanii* by 70 per cent while total inhibition in germination was observed.

The present observation which revealed inhibitive action of carbendazim 50% WP is in agreement with that of Krishnamoorthy and Visalakshi (2007) who reported 100 per cent inhibition in growth and sporulation of *L. lecanii* with carbendazim 0.2 %. Total inhibition of *L. saksenae* by hexaconazole 5% EC, 0.2 % observed in this study is supported by the report of Reddy *et al.* (2018) who observed a complete inhibition by 0.1 % hexaconazole 5 SC in *L. lecanii*.

Complete inhibition of other entomopathogenic fungi such as *B. bassiana, B. brongniartii* and *M. anisopliae* by carbendazim 50WP, mancozeb 75% WP and hexaconazole 5% EC were reported by Prabhu *et al.* (2007), Deb *et al.* (2017) and Joshi *et al.* (2018).

The fungicidal activity of the strobilurins such as azoxystrobin relies on the ability of the active ingredient to disrupt energy production in fungal mitochondria and consequently prevents spore germination (Bartlett *et al.*, 2002). Singh *et al.* (2009) suggested that suppressive action of fungicides is due to their action on different metabolic sites within the fungus, which inhibits the energy production. In triazole fungicides such as hexaconazole, ergosterol biosynthesis is inhibited consequently preventing cell membrane formation and inhibition of mycelial growth, conidiation and conidial germination (Silva *et al.,* 2013).

Compatibility of entomopathogenic fungi is usually determined based on their radial growth and sporulation. Rossie-Zalaf *et al.* (2008) opined that germination is a critical factor that determines the survival of the conidia.

Biological Index (BI) takes into account the germination factor apart from its mycelial growth and sporulation. Therefore, in this study in order to get a confirmatory result, BI index was worked out using the growth, sporulation and germination parameters of *L. saksenae* in poisoned media.

Accordingly, botanicals such as lower concentrations of neem seed kernel aqueous extract, neem oil emulsion, neem leaf aqueous extracts and all the tested new generation insecticides at lower and higher doses with BI values greater than 66 were "compatible". Higher concentration of botanicals except neem leaf extract and chlorpyriphos 20% EC 0.03 %, dimethoate 30% EC 0.05 % and the fungicide copper oxychloride 50% WP 0.1% and 0.2 % with BI values 42-66, were "moderately toxic". Neem leaf extract at higher concentration (4%), recommended dose of chlorpyriphos 20% EC (0.06%) and the fungicides such as carbendazim 50% WP, mancozeb 75% WP and hexaconazole 5% EC with BI values less than 42, were "toxic" to *L. saksenae*.

One of the limitations of fungi as microbial control agents is that, each species and strains within a species are usually effective to a narrow range of hosts. Farmers prefer using a single organism for managing the pest complex in a crop. This issue to an extent can be sort out through the development of an appropriate co-formulation of two or more fungi or fungal strains with different host ranges and ecological tolerances (Wang *et al.,* 2002). Compatibility studies among microbes thus becomes inevitable. Compatibility among entomopathogens is generally assessed based on mortality test on the susceptible insects (Rao *et al.,* 2006; Mahmoud, 2009; Malekan *et al.,* 2013). On assessing the compatibility of *L. saksenae* with the commonly used entomopathogens such as *B. bassiana* and *M. anisopliae,* it was observed that *B. bassiana* when applied in combination with *L. saksenae*, did not reduce the death rate of rice bugs, indicating that they are non-inhibitive among themselves. However, the mortality observed in bugs treated with *L. saksenae* was on par with that observed in the combination treatment. In case of combination treatments, *L. saksenae* + *M. anisopliae* and *L. saksenae* + *B. bassiana* + *M. anisopliae*, lesser mortality was observed. It was 83.33 per cent at the end of

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the experimental period which was significantly lower when compared to *L. saksenae* + *B. bassiana* (100 per cent). Time taken for 100 per cent mortality was four days in *L. saksenae* and *L. saksenae* + *B. bassiana*, whereas other treatments did not yield the same level of death. Foregoing observations states that *B. bassiana* did not inhibit *L. saksenae*, while *M. anisopliae* was inhibitive.

Compatibility of *B. bassiana* with other entomopathogenic fungi were previously reported by Mahmoud (2009). He observed compatibility with *M. anisopliae*, while testing its effect on the mortality of olive fruit fly, *Bactrocera oleae*. He observed an increase in mortality with the combination spray, than in insects treated with either of the fungi.

Malekan *et al.* (2013) found that *Lecanicillium muscarium and B. bassiana* were mutually compatible based on *in vitro* mortality tests conducted in greenhouse whitefly, *Trialeurodes vaporariorum*.

However, Rao *et al.* (2006) reported that *B. bassiana* was not compatible with *Nomuraea rileyi*, when tested against *Spodoptera litura*.

5.2 SYNERGISM OF ENTOMOPATHOGENIC FUNGI

Several researchers have indicated that combinations of entomopathogenic fungi with botanicals or insecticides can have synergistic, antagonistic or additive effects on physiology and mortality on insects (Anderson *et al.,* 1989; Jaramillo *et al.*, 2005; Sharififard *et al.*, 2011; Ali *et al.*, 2018).

Bioassay studies conducted in this line revealed that *L. saksenae* @ 10^7 spores mL^{-1} as well as the combination spray of *L. saksenae* + NSKE (A) 0.5 % had equally impressive results on the mortality of rice bug, even on the first day after treatment. It was 83.33 per cent in both the treatments. Parity in mortality observed among these two treatments was only due to the additive interaction, when combined with the fungus. Bugs treated with botanicals did not die up to two days.

Time taken to achieve 100 per cent mortality was increased by one day in combination spray of *L. saksenae* + NSKE (A) 0.5 %, compared to that of

L. saksenae (four days). Combination sprays of *L. saksenae* with other botanicals or in treatment with botanicals alone exhibited significantly lower mortality and the death rate never reached 100 per cent till the end of the observation period, *i.e.* seven days. Therefore, *L. saksenae* is invariably superior to its combination treatments with botanicals or with botanicals alone. Furthermore, feeding inhibition observed in *L. saksenae* treated bugs did not vary from that observed in the combination treatment, making it evident that the inhibition was due to the effect of *L. saksenae* or its additive effect on botanicals.

Such additive effects of entomopathogenic fungi on neem based botanicals were reported by Halder *et al.* (2018) in the case of *L. lecanii* with neem oil in the ratio 1:1, where the combination resulted in increased mortality of *A. craccivora*.

Paecilomyces fumosoroseus @ 10^8 spores mL^{-1} (Wize) Brown and Smith in combination with azadirachtin 60µg mL^{-1} (Neemix 4.5) increased the mortality in *Bemisia argentifolii* (James, 2003). In the bioassays on *S. litura,* combination treatment with *B. bassiana* and neem was found to have synergistic effect on mortality (Mohan *et al.,* 2007).

When *L. saksenae* was sprayed singly and in combination with thiamethoxam 25% WG, and flubendiamide 39.35% SC, there was 83.33 per cent mortality in nymphs on the first day itself. When *L. saksenae* took four days for 100 per cent mortality, its effective combinations took five days. In adults, *L. saksenae* and each of its combination with thiamethoxam 25% WG, flubendiamide 39.35% SC and chlorantraniliprole 18.5% SC exhibited more or less same mortality. Time taken for 100 per cent death did not vary among these treatments. Mortality exhibited by the insecticides alone were always lower than that of the combination treatments, which revealed the knock down action on the bugs by the actions of toxins present in it. This observation substantiates the presence of toxic metabolites in *L. saksenae*. Jasmy (2016) isolated the toxin dipicolinic acid (0.044 g L^{-1}) and cuticle degrading enzymes chitinase, lipase and protease to the tune of 6.5 U mL^{-1}, 1.55 U mL^{-1} and 0.36 U mL^{-1}, respectively from *L. saksenae*

Bioassay studies conducted in citrus root weevil, *Diaprepus abbreviates* by Quintela and Mc Coy (1998) revealed that it was the combination treatments that performed better than the individual treatment with *B. bassiana* (5 x 10^5 conidia mL^{-1}) or *M. anisopliae* (5 x 10^5 conidia mL^{-1}) or imidacloprid (0.008 %). Jaramillo *et al.* (2005) reported that, the combined applications of *M. anisopliae* (1x 10^6 conidia mL^{-1}) with a lower dosage of imidacloprid 350 SC resulted in significantly higher mortality (>75 per cent) compared to sole applications.

When *L. saksenae* and *B. bassiana* were combined at their infective doses, even though the combination spray exhibited a lesser mortality during initial days, by the third and fifth day statistically similar mortality was noted in *L. saksenae* and its combinations. Feeding inhibition observed was also higher in combination sprays than that of sole treatments. Similar results on compatibility among entomopathogenic fungi was reported by Malekan *et al.* (2013), who found that *B. bassiana* and *L. muscarium* are mutually compatible in their *in vitro* mortality tests conducted in greenhouse whitefly, *T. vaporariorum.*

When *L. saksenae* was applied in combination with botanical or chemical insecticides, the latter may first weaken the defense mechanisms in insects, enabling easier invasion by the former. Entomopathogenic fungi thus take advantage to kill the insects synergistically and spread secondarily to cause epizootic, provided the pesticide does not affect viability and virulence of the fungi. Conversely, use of incompatible pesticide may cause inhibitory effects that often vary between fungal species and strains (Anderson *et al.,* 1989).

In vivo studies to examine the effective combinations as well as single treatments with *L. saksenae* (10^7 spores mL^{-1}) *B. bassiana* (10^8 spores mL^{-1}) and half the recommended dose of thiamethoxam 25% WG (0.0025 %), revealed that after two sprayings, population of rice bugs was lowest in plots treated with *L. saksenae* (2.25 bugs per plot) while a significantly higher population was noted in the treatments *L. saksenae* + *B. bassiana* (2.75 per plot) and *B. bassiana* (3.00 per plot), which were on par. The result indicates that *L. saksenae* is a potent bio

pesticide against rice bug. *L. saksenae* + *B. bassiana* could not exert the same level of control of rice bugs. *L. saksenae* is a specific pathogen with high speed of kill in rice bug, while *B. bassiana* is a broad spectrum microbe with less speed of kill. This study paves way to the possibility of utilizing these two pathogens in combination to address various pests of rice, coming under different insect orders.

Efficacy of *L. saksenae* in the management of rice bugs was demonstrated in the studies conducted by Sankar and Rani (2018). While evaluating the field efficacy of chitin enriched oil formulations of various entomopathogenic fungi, they observed that chitin enriched oil formulation of *L. saksenae* was more effective than its spore suspension @ 10^7 spores mL^{-1} in managing rice bugs. They have also reported that, compared to *B. bassiana* and *M. anisopliae, L. saksenae* is superior in bringing down the population of bugs.

In this study, population recorded in *L. saksenae* + thiamethoxam 25% WG (0.0025 %) was lower (3.5 per plot), compared to single application of half the dose of thiamethoxam 25% WG and untreated plots (5.25 per plot). On the contrary, Malini (2015), reported the superiority of thiamethoxam 25% WG 005 % in the management of rice bugs, compared to *B. bassiana* (Bb 5, Bb 21). Varied results of thiamethoxam, is due to the fact that in this study combined application was tested with half the dose.

Population of natural enemies were found to be unaffected neither by the application of biocontrol agents nor by spraying thiamethoxam 25% WG. It clearly indicates that *L. saksenae* as well as the combination treatments were safe to natural enemies. Safety of *L. saksenae* to natural enemies observed in this study, is in accordance with the findings of Sankar and Rani (2018) who reported that chitin enriched oil formulations, talc based formulation and spore suspensions of *L. saksenae* were safe to natural enemies found in rice agro ecosystem.

Observations on yield recorded at harvest, once again revealed the superiority of *L. saksenae* (1.38 kg $plot^{-1}$) in managing the bugs. Yield in the plots

treated with *L. saksenae* + *B. bassiana* was (1.32 kg plot^{-1}) ranked second. The treatment *L. saksenae* + thiamethoxam 25% WG 0.0025 % recorded a grain yield of 1.18 kg plot^{-1}, which was significantly lower than the former treatments. The grain yield obtained in the plots treated with *B. bassiana* (1.12 kg plot^{-1}), thiamethoxam 25% WG 0.0025 % (1.15 kg plot^{-1}) and untreated control (1.02 kg plot^{-1}) did not vary significantly. The findings of this study is in accordance with the results of Sankar and Rani (2018) who reported plots treated with chitin enriched oil formulations and spore suspensions of *L. saksenae* could yield more compared to that of plots treated with talc based formulations of *B. bassiana* and *L. lecanii.*

Therefore it may be concluded that in rice ecosystem during the milky stage *L. saksenae* alone can address the rice bug population. Moreover, in situation where lepidopteran pest is to be addressed along with rice bug, it may be contained using tank mix formulations of *L. saksenae* and *B.bassiana* or even *L. saksenae* with chlorantraniliprole 18.5% SC or flubendiamide 39.35% SC.

Summary

6. SUMMARY

The study entitled "Compatibility and synergism of the entomopathogenic fungus *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno with other crop protectants" was carried out at College of Agriculture, Vellayani and Integrated Farming System Research Station (IFSRS), Karamana during 2017-2019 with an objective to assess the compatibility of *L. saksenae* with botanical, insecticides, fungicides as well as other entomopathogenic fungi and to evaluate synergistic insecticidal effect.

In vitro studies on compatibility were carried out using poisoned food technique. Botanicals tested included aqueous and solvent extracts of neem seed kernels, neem leaves and neem oil emulsion. Comparison of radial growth of the fungus revealed that the lower concentration (0.5 %) of neem oil emulsion (NOE) did not inhibit the mycelial growth of the fungus. On the other hand it inhibited the sporulation (75.30 to 93.13 per cent) and germination (12.12 to 21.55 per cent) significantly. Neem seed kernel (NSKE (A)) 0.5 % did not inhibit the growth, sporulation or germination of the fungus, while at 1 % and 2 % concentrations there was 4.70 to 21.07 per cent inhibition in growth.

Lower concentration of Neem leaf extract (NLE (A)) 1 % inhibited the mycelial growth by 13.78 per cent, sporulation by 41.74 per cent and germination by 13.78 per cent. However at 2 % and 4 % concentrations there was 68.43 to 90.22 per cent inhibition in growth, 68.43 to 90.22 per cent inhibition in sporulation and 4.37 to 7.74 per cent inhibition in germination. Solvent extracts of leaves exhibited a significant and logarithmic reduction in the spore count (10^6 spores mL^{-1}).

On comparing botanicals at 2 % concentration, leaf extracts were found to be more inhibitive to mycelial growth than seed kernel extract and seed oil emulsion. All the three formulations affected sporulation significantly (68-91 per cent). Seed kernel extract was found to be the less inhibitive to germination (3.71 per cent), than leaf extracts (4.37 per cent) and oil emulsion (21.55 per cent).

Biological index (BI) worked out by considering all the three parameters such as growth, sporulation and germination indicated that NOE (0.5 %) NSKE (A) (0.5 %, 1 %), NSKE (S) (1 %, 2 %) and NLE (A) 1 % had "compatible" status (67-75) while, NOE (1 %, 2 %), NSKE (A) (1 % and 2 %), NSKE (S) (0.5 %, 1 %) and NLE (A) 1 % and NLE (S) (1 %, 2 %) were "moderately toxic" and NLE (A and S) (4 %) "toxic".

Among the insecticides tested, growth inhibition was less than 10 per cent in all the new generation insecticides tested *viz.* flubendiamide 39.35% SC, imidacloprid 17.8% SL, thiamethoxam 25% WG and chlorantraniliprole 18.5% SC. Old generation insecticide dimethoate 30% EC was moderately inhibitive (12.62 to 25.41 per cent) and chlorpyriphos 20% EC was highly inhibitive (46.72 to 61.97 per cent). Effect of insecticides on sporulation was almost similar to that observed in mycelial growth. There was only 2.22 per cent inhibition with chlorantraniliprole 18.5% SC 0.003 % while with flubendiamide 39.35% SC 0.0025 % there was 37.04 per cent inhibition. Imidacloprid 17.8% SL 0.003 %, thiamethoxam 25% WG 0.0025 %, dimethoate 30% EC 0.025 % and chlorpyriphos 20% EC 0.03 % were more inhibitory (3.77 per cent, 5.25 per cent, 25.41 per cent and 61.97 per cent respectively). Chlorpyriphos 20% EC exhibited highest inhibition in sporulation (78.52 per cent). Germination studies revealed that at their recommended doses germination was significantly affected by all the insecticides. It was 54.7 to 59.32 per cent in imidacloprid 17.8% SL 0.006 % and chlorantraniliprole 18.5% SC 0.006 %. In dimethoate 30% EC at 0.05 % and chlorpyriphos 20% EC 0.06 % inhibition rates were too high (81.69 and 92.55 per cent).

Based on BI values, it is concluded that the new generation insecticides at lower and recommended doses and the old generation insecticide dimethoate 30% EC at half the recommended dose were compatible to *L. saksenae*, BI values ranging from 67 to 94.

Fungicides were toxic to *L. saksenae* exhibiting strong inhibition on its growth, sporulation and germination. Copper oxychloride 50% WP and

azoxystrobin 23% SC were comparatively less inhibitive (38.89 and 57.30 per cent). Complete inhibition was noted in carbendazim 50% WP, mancozeb 75% WP and hexaconazole 5% EC. None of the fungicides was compatible with *L. saksenae,* BI value ranging from 0 to 64.

B. bassiana was found to be non-inhibitory to *L. saksenae* at their infective doses, based on the mortality of treated rice bugs. Sole treatment with *L. saksenae* as well as *L. saksenae* + *B. bassiana* caused 100 per cent mortality of nymphs and adults in four and five days, respectively. In the treatment, *L. saksenae* + *M. anisopliae* and *L. saksenae* + *B. bassiana* + *M. anisopliae* lower mortality was noticed and did not reach 100 per cent within seven days.

When the effective botanical insecticide selected from above experiments was applied singly as well is combination with *L. saksenae* on rice bugs reared under laboratory conditions, revealed that *L. saksenae* and its combination with NSKE (A) 0.5 % exhibited same level of mortality in nymphs (83.33 per cent), while in adults *L. saksenae* was superior (66.67 per cent). Time taken to achieve 100 per cent mortality in nymphs was four days in *L. saksenae*, while in combination spray of *L. saksenae* + NSKE (A) 0.5 % it was five days. None of the other combinations could achieve 100 per cent mortality within seven days.

Combined application of *L. saksenae* + thiamethoxam 25% WG 0.0025 % as well as *L. saksenae* + flubendiamide 39.35% SC 0.0025 % and *L. saksenae* was equally effective in bringing mortality to rice bug nymphs (83.33 per cent). Mortality exhibited was always lower in the treatments with insecticides alone compared to that of the combination treatments. Time taken for 100 per cent mortality was less (four days) in *L. saksenae* and it was five days in all the combination treatments.

When *L. saksenae* and *B. bassiana* were combined, during the initial days combination sprays exhibited lesser mortality (80 per cent) in nymphs compared to sole application of *L. saksenae* (85 per cent) on the first day. Third day onwards, the effect was the same and it took four days for attaining 100 per cent mortality.

Feeding inhibition in rice bugs treated with *L. saksenae* and its combinations with botanicals, insecticides and microbials were higher compared to the sole treatments. It ranged from 79.69 to 87.94 per cent.

Under field conditions, *L. saksenae* @ 10^7spores mL^{-1} + thiamethoxam 25% WG 0.0025 % and *L. saksenae* + *B. bassiana* @ 10^8 spores mL^{-1} recorded significantly higher population of 3.5 and 2.75 bugs per plot, compared to *L. saksenae* (2.25 bugs per plot). Yield recorded at harvest was high in plots (2 x 2 m) treated with *L. saksenae* (1.38 kg $plot^{-1}$) which was followed by combination spray of *L. saksenae* + *B. bassiana* (1.32 kg $plot^{-1}$). The population of natural enemies did not vary significantly among the treated and untreated plots.

It is concluded that *L. saksenae* was compatible with the botanicals such as neem seed oil emulsion 0.5 % and neem seed kernel extract 0.5 %. It was also compatible with the new generation insecticides flubendiamide 39.35% SC, chlorantraniliprole 18.5% SC, thiamethoxam 25% WG and imidacloprid 17.8% SL at both the doses. Among the microbials, *B. bassiana* was not inhibitive to *L. saksenae*. Fungicides such as carbendazim 50% WP, mancozeb 75% WP, copper oxychloride 50% WP, hexaconazole 5% EC and azoxystrobin 23% SC were inhibitory to *L. saksenae*. None of the botanicals, insecticides, fungicides or microbials had a synergistic effect with *L. saksenae*. In the management of rice bug, *L. saksenae* @ 10^7 spores mL^{-1} was superior to chemical or other microbial insecticides used singly or in combination. However, its combination with thiamethoxam 25% WG and *B. bassiana* were not found to be inhibitory. The natural enemy population in rice ecosystem was unaffected by *L. saksenae* or *B. bassiana* or thiamethoxam 25% WG 0.0025 % or their combinations.

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Abstract

COMPATIBILITY AND SYNERGISM OF THE ENTOMOPATHOGENIC FUNGUS *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno WITH OTHER CROP PROTECTANTS

by

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ABSTRACT

The study entitled "Compatibility and synergism of the entomopathogenic fungus *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno with other crop protectants" was carried out in College of Agriculture, Vellayani and Integrated Farming System Research Station, Karamana during 2017-2019 with the objective to assess the compatibility of *L. saksenae* with botanical insecticides, chemical insecticides, fungicides other entomopathogenic fungi and to evaluate synergistic insecticidal effect.

In vitro studies on compatibility was carried out using poisoned food technique, by assessing radial growth, sporulation and germination of *L. saksenae*. Botanicals tested were aqueous and solvent extracts of neem seed kernel, neem leaves and neem oil emulsion. Among them, lower dose of neem oil emulsion (NOE) 0.5% did not affect the mycelial growth of the fungus. It was 6.27 cm on 14^{th} day after inoculation which was on par with that of control (6.17 cm). Sporulation of the fungus was affected by all the botanicals, with least inhibition in lower dose of aqueous neem leaf extract (NLE (A)) 1% (4.41 x 10^7 spores mL^{-1}). The corresponding count in untreated medium was 7.57 x 10^7 spores mL^{-1}. Viability of the fungus was not affected by the lower dose of neem seed kernel extract (NSKE (A)) 0.5%, when compared to control (97.67 % and 99%). Biological index (BI) indicated that aqueous and solvent extracts of NSKE (0.5%), NSKE (A) 1%, NLE (A) 1% and NOE (0.5%) were compatible with *L. saksenae,* BI values being 75, 69, 70, 75 and 67, respectively.

Among the insecticides tested, flubendiamide 39.35 SC was least inhibitory to the growth of *L. saksenae*, both at half as well as recommended doses (5.92 cm and 5.88 cm), followed by half the recommended doses of thiamethoxam 25 WG (5.88 cm) and chlorantraniliprole 18.5 SC. (5.78cm). The corresponding growth in untreated medium was 6.10 cm. Sporulation in chlorantraniliprole at the recommended dose and control was same (1.35 x 10^7 spores mL^{-1}). Spore viability was affected by all the insecticides. Germination in imidacloprid was highest among the treatments (85.67 per cent).

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BI value indicated that imidacloprid, thiamethoxam, flubendiamide and chlorantraniliprole at recommended and half doses were compatible with *L. saksenae*. (67-94). None of the fungicides tested, were compatible with *L. saksenae.*

B. bassiana at 10^8 spores mL^{-1} was found to be compatible with *L. saksenae* at 10^7 spores mL^{-1} based on mortality of treated rice bugs. Treatments *L. saksenae* and *L. saksenae* + *B. bassiana* caused 100 per cent mortality on fourth and fifth day after spraying in nymphs and adults of rice bug.

Synergism studies revealed that, on the first day *L. saksenae* (10^7 spores mL^{-1}) and *L. saksenae* + NSKE (A) 0.5% were equally effective causing 83.33% mortality in nymphs, while in adults *L. saksenae* was superior to its combination (66.67 and 60%). *L. saksenae* + thiamethoxam 0.0025% exhibited significantly higher mortality in adults (70%) on the first day while in *L. saksenae* it was 66.67%. For nymphs, *L. saksenae* and *L. saksenae* + thiamethoxam 0.0025% was equally effective (83.33% mortality).

In both adults and nymphs, *L. saksenae* was more effective causing 75 per cent and 85 per cent mortality on the first day, while its combination with *B. bassiana* caused 65per cent and 80 per cent mortality.

L. saksenae and its combinations with botanicals, insecticides and microbials had higher feeding inhibition in rice bugs.

Under field conditions, *L. saksenae at* 10^7spores mL^{-1} + thiamethoxam 0.0025% and *L. saksenae* + *B. bassiana* at 10^8 spores mL^{-1} recorded significantly higher population of 3.5 and 2.75 bugs per plot, compared to that of *L. saksenae* (2.25 bugs per plot). Population of natural enemies did not vary significantly among the treated and untreated plots.

Yield recorded at harvest was higher in plots treated with *L. saksenae* (1.38 kg $plot^{-1}$) which was followed by combination spray of *L. saksenae* and *B.bassiana* (1.32 kg $plot^{-1}$)

It is concluded that *L. saksenae* is compatible with the botanicals such as neem seed oil emulsion 0.5% and neem seed kernel extract 0.5%. It was also compatible with the new generation insecticides flubendiamide, chlorantraniliprole, thiamethoxam and imidacloprid. Among the microbials, *B. bassiana* was not inhibitory. Fungicides such as carbendazim, mancozeb, copper oxychloride, hexaconazole and azoxystrobin were inhibitory to *L. saksenae*. None of the botanicals, insecticides, fungicides or microbials had synergistic effect with *L. saksenae*. In the management of rice bug *L. saksenae* was superior to its combination treatments and it did not affect the natural enemy population in rice ecosystem.

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