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Research Article

Pathogenicity and field efficacy of the entomopathogenic fungus, *Lecanicillium saksenae* Kushwaha, Kurihara and Sukarno in the management of rice bug, *Leptocorisa acuta* Thunberg

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ABSTRACT: A new indigenous isolate of *Lecanicillium* namely *L. saksenae* was tested for its efficacy against rice bug. Lecanicillium. *lecanii* was non pathogenic to rice bug. Peculiar pre mortality symptoms like ataxis, aggregation, convulsions were exhibited by test insects upon infection. *L. saksenae* was characterized by it's quick kill effect, causing 100 per cent mortality of adults and nymphs within 72h after treatment with high spore doses of 10^8 and 10^7 spores mL^{-1}. Furthermore, probit analysis done at 6 days of treatment, revealed the LC_{50} as 2.99 x 10^4 and 1.72 x 10^4 spores mL^{-1} for nymphs and adults respectively. LT_{50} (10^8spores mL^{-1}) was 17.58 and 18.58 hours for nymphs and adults respectively. Although the quick kill effect was not exhibited in field, the count of rice bugs in plots treated with oil formulated *L. saksenae* were significantly lower (1.33 bugs per plot). Moreover, all the bioformulations applied as treatments were evidently safe to natural enemies present in rice ecosystem. The yield recorded from plots treated with oil formulated *L. saksenae* was also higher (3.48 kg per plot of 2 x 2 m). This study projects the prospect of utilizing *L. saksenae*, and its oil formulations as an effective biocontrol agent against rice bug which is a major sucking pest of rice.

KEY WORDS: Entomopathogenic fungus, *Lecanicillium saksenae, Leptocorisa acuta*

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INTRODUCTION

Entomopathogenic fungi are vital components in pest control, especially in this era of organic farming and Good Agricultural Practices (GAP). Alarming reports of harmful levels of pesticide residues and increasing consumer preference to residue free foods call for need based research on microbial pathogens of insects and their virulent indigenous isolates. Despite their effectiveness, wide scale adoption of entomopathogenic fungi is hindered due to their increased time for kill and sensitivity to environmental vagaries. The genus *Lecanicillium* previously named as *Verticillium*, is well known for its pathogenicity to sucking pests such as aphids, mealy bugs, scales, whiteflies etc. Globally, *Lecanicillium lecanii* (Zimmermann) Zare and Gams is widely reported to be an entomopathogen to many homopteran sucking pests (Kim, 2007; Kim *et al.,* 2008; Mark *et al*., 2008; Scorsetti *et al.*, 2008; Malekan *et al.*, 2013). Its infectivity to heteropteran bugs had not been reported. An indigenous isolate of *L. saksenae* (Kushwaha) Kurihara and Sukarno, from soils of Vellayani, Kerala was reported to be more adaptable under the prevailing climatic conditions of Kerala and was observed to have a wider host range compared to other *Lecanicillium* species (Rani *et al*., 2014; Jasmy, 2016). The species was first described by Kushwaha in 1980 as a keratin degrading fungus isolated from Madhya Pradesh, India and later by Sukarno *et al*. (2009) from epiphytic and subterranean arthropods. The fungus is reported to be an efficient degrader of pesticides (Pinto *et al.,* 2012). Being an indigenous isolate of a comparatively infrequent species, detailed investigation on its bioefficacy and safety aspects is warranted. The new geographical isolate, LsVs1-7714 was found to be more adaptable under the prevailing climatic conditions of Kerala and was observed to have a wider host range compared to other *Lecanicillium* species. Therefore, an investigation was undertaken at College of Agriculture Vellayani, Kerala Agricultural University, to evaluate the prospects of utilizing this fungus against the major sucking pest of rice, the rice bug, *Leptocorisa acuta.* Pathogenicity and symptoms of mycosis of the new isolates were studied in detail and compared with the commonly used species *L. lecanii*. The dose mortality response, lethal time and lethal concentration values were ascertained. The laboratory results were validated under field conditions using spore suspensions as well as oil based formulations of the fungus

along with the other commonly used species *L. lecanii, Metarhizium anisopliae* and *Beauveria bassiana.* Safety of these entomopathogens to the common natural enemies in the rice ecosystem was also studied.

MATERIALS AND METHODS

Bioassay

Mother culture of *Lecanicillium saksenae* (ITCC accession number LsVs1 7714) was isolated and characterized at molecular level by Rani *et al*. (2015) was sourced from Biocontrol Laboratory for Crop Pest Management, Department of Agricultural Entomology, College of Agriculture Vellyani, Kerala Agricultural University and *L. lecanii* (Vl 8) was originally sourced from NBAIR, Bengaluru. The fungi were sub cultured on Sabouraud Dextrose Broth amended with chitosan (5% w/v). Fourteen-day old cultures were blended, sieved through double layered muslin cloth and spore count was estimated using Neubauer hemocytometer. Spore suspensions containing 10^7 spores ml^{-1} was used to test pathogenicity and spore suspensions having 10^3 to 10^8 spores mL^{-1} were used for bioassay.

Bugs collected from rice fields were brought to the laboratory and observed for any latent infections. Active and healthy adults were released into a rearing cage with potted rice plants bearing panicles in milky stage. Rearing cage was of size 165 x 120 x 120 cm made of aluminium frame and nylon mesh. Older pots were replaced periodically with new pots with plants in the milky stage. Inspection window provided on the sides of the cage facilitated collection of bugs using a hand net. Laboratory culture of rice bugs ensured adequate number of uniformly aged test insects. Eggs, third instar nymphs and a day old adults were tested for pathogenicity. Each experiment was replicated thrice with ten insects per replication. Egg masses were dipped in spore suspensions, air dried and incubated in Petri plates lined with moistened tissue paper. Egg mass dipped in sterile water served as control, nymphal emergence was observed, and emerged nymphs were transferred to rearing jars with fresh milky stage panicles of rice. Adults and nymphs collected separately in plastic jars, were sprayed with the spore suspensions using an atomizer and transferred to rearing jars after one minute. Rearing jars were provided with milky panicles and top was covered with muslin cloth. Sterile water spray served as control. Symptoms of mycosis and mortality were observed at 24 hour interval for assessing dose- mortality and $LC_{50.}$ Another set of experiment was set up with three spore doses 10^8,10^7 and 10^6 with four replications and mortality was recorded at 12 hour interval for generating LT_{50} values.

Field evaluation

In order to assess the efficacy of *Lecanicillium saksenae* in comparison with other entomopathogenic fungi against the major sucking pest of rice, *Leptocorisa acuta***,** a field trial was conducted by raising the medium duration rice variety Uma (Mo16). The crop was raised as per KAU (2011) package of practice recommendations. The experiment was laid out in an area of 200 m^2 following Randomized Block Design with eight treatments and three replications. The plot size was 2 x 2 m.

Treatments

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

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

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

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

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

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

T7 - Malathion 0.1% - Chemical check

T8 - Untreated check

Spraying was undertaken when the rice bug population in the field reached its Economic Threshold Level (ETL) i.e., two bugs $hill^{-1}$. Stem borer and leaf folder infestations were managed using trichocards of *Trichogramma japonicum* Ashmead and *T. chilonis* Ishii respectively, @ five cards per hectare. For preparation of bioformulations the protocol developed by Nithya and Rani (2017) was followed. Talc based products were prepared by mixing 14 day old cultures with talc in the ratio 1:3 and air drying them. Spraying was carried out in a spiral fashion, commencing from the border rows towards the centre and sunpack screens were used to prevent spray drift.

Population was assessed based on sweep and count data. Cumulative count of bugs and natural enemies *viz*., predators, parasitoids and spiders, in five sweeps and five random hills $plot^{-1}$ was recorded (Smitha, 2004). Pretreatment counts were taken before treatment and post treatment counts were taken at intervals of three, seven, 10 and 14 days. Spraying was repeated after 14 days when population regained ETL.

The data obtained from bioassay and field were subjected to Analysis of Variance (ANOVA) using WASP 1 software and probit analysis was carried out using SPSS Ver. 21 with the fiducial limits fixed at 95 per cent.

RESULTS AND DISCUSSION

Bioassay

Pathogenicity trials revealed that the spores of *Lecanicillium saksenae* were infective to rice bugs and the time to kill was very short, while *L. lecanii* was non pathogenic. However, mycosis was absent in eggs and they emerged normally as in case of untreated eggs for both the fungi. Adults and nymphs infected with *L. saksenae* showed distinct symptoms prior to death. Post 6-8 hours of treatment, they were restless and constantly combing the dorsal part of their body, wings and antennae with legs as if to remove an extraneous matter. The bugs lost clinging capacity, 10-14 hours after treatment and were seen constantly slipping from the sides of the rearing jar. The fallen bugs exhibited aggregation. Post 20-24 hours of treatment the bugs were on their backs, convoluting with repeated jerking movements of legs combined with abdominal arching. The convulsions gradually faded as the bugs slipped into paralysis and death. Cadavers exhibited growth of white mycelia, initially emerging from the legs, antennal bases, inter segmental membranes of the abdominal region and around the compound eyes gradually spreading over the entire body, five days after death (Fig. 1). The pathogen was isolated from the treated dead bugs and identity confirmed.

Bioassay revealed that the spore doses @ 10^8 and 10^7 spores mL^{-1} were equally effective against the nymphs (Table 1) killing all the test insects within 72 hours of treatment. Lower spore doses of 10^4 and 10^3 spores mL^{-1} were least infective in causing nymphal mortality.

Similar results were observed for adults as well (Table 2), wherein 10^8 and 10^7 spores mL^{-1} caused 100 per cent mortality within 72 HAT (Hours after treatment).The LC_{50} value of spore suspension of *L. saksenae*, 6 days after treatment to adults of *L. acuta* was worked out to be 2.99 x 10^4 spores mL^{-1}, while for nymphs it was 1.72 x 10^4 spores mL^{-1}. The LC_{90} values computed were 8.19 x 10^5 spores mL^{-1} and 5.35 x 10^5 spores mL^{-1} for adults and nymphs respectively. The LT_{50} value computed for *L. saksenae* against *L. acuta* is furnished in Table 3. The LT_{50} for 10^8 spores was 17.58 and 18.58 hours for nymphs and adults respectively. For 10^7 spores mL^{-1}, the LT_{50} recorded were 19.97 hours and 19.91 hours for adults and nymphs, respectively, while the highest LT_{50} was recorded for 10^6 spores mL^{-1} with 40.39 hours and 44.03 hours in adults and nymphs, respectively.

Field efficacy

Observations on the mean population of rice bug per five random hills and five sweeps (Table 4) revealed the efficacy of oil formulation of *L. saksenae*, the count being 3.00 bugs $plot^{-1}$ seven days after first spraying and 1.33 bugs $plot^{-1}$ fourteen days after second spraying. The control obtained was in parity with one of the biocontrol check using *M. anisopliae*, population being, 4.33 bugs $plot^{-1}$ on seventh day after first spraying and 1.67 bugs per plot on fourteenth day after second spraying. Plots treated with spore suspensions of *L. saksenae* recorded 4.33 and 2.00 bugs $plot^{-1}$, ranking second while bug count in untreated check was the highest among all, recording upto 5.33 bugs per plot fourteen days after second spray.

Fig. 1. A. Mycelial emergence from *Leptocorisa acuta* and B. Cadaver infected with *Lecanicillium saksenae.*

Table 1. Dose - mortality response of *Lecanicillium saksenae* to *Leptocorisa acuta* nymphs

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

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

Table 2. Dose - mortality response of *Lecanicillium saksenae* to *Leptocorisa acuta* adults

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

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

Table 3. Lethal time of different spore concentrations of *Lecanicillium saksenae* to *Leptocorisa acuta*

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

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

Table 4. Field evaluation of *Lecanicillium* spp. against *Leptocorisa acuta*

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

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

Table 5. Effect of treatments on predatory insects

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

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

Population of insect predators (Table 5), *viz*. coccinellids, *Coccinella transversalis*, *Micraspis discolor*, mirids, *Cyrtorhinus lividipennis*, did not vary significantly on third, seventh and fourteenth day after first spray and third and seventh day after second spray among the treated and untreated plots, the average population ranging from 0.67 to 4.67 per plot. However, the population was significantly low (1.67, 1.33, and 1.00) in plots treated with Malathion 0.1% on the tenth day after first spay and tenth and fourteenth day after second spray. The count of predatory spiders *Tetragnatha* sp., and *Oxyopes* sp., and hymenopteran parasitoids., *Bracon* sp., *Xanthopimpla* sp., *Ophius* sp. did not vary significantly among the various plots throughout the experimental period.

The yield parameters (Table 6) were high in plots treated with oil formulation of *L. saksenae* and spore suspension of *L. saksenae.* The gross yield of (3.48 kg plot^{-1}) and net yield (3.25 kg plot^{-1}) recorded in plots (2 x 2 m) treated with oil formulation of *L. saksenae* was on par with those observed in plots treated with spore suspension of *L. saksenae,* gross yield being 3.55 and net yield 3.09 kg plot^{-1}, respectively.

The new isolate of *L. saksenae* being infective to rice bug becomes a rare instance of *Lecanicillium* infecting an Alydid pest. This offers ample scope for developing this fungus as a biopesticide for the management of sucking pest complex in rice ecosystem. Rani *et al.* (2014), on their preliminary studies on host range of this indigenous isolate has reported the infectivity to cowpea pod bug *Riptortus pedestris* (F.). Shinde *et al*. (2010) reviewed an extensive list of pest species susceptible to *L. lecanii.* Though the host range spanned across 21 families in eight orders including coccids, aleyrodids, aphids, there was only one report of infection to a heteropteran, *Idioscopus clypealis* (Lethierry) belonging to Cicadellidae. Apart from this, there is no documented record of *L. lecanii* being pathogenic to hard bodied sucking pests.

Symptoms of mycosis observed in the case of *L. acuta* treated with *L. saksenae* such as, restlessness, combing of body parts, loosing of clinging capacity, at axis and aggregation

Table 6. Effect of bioformulations on yield of paddy

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

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

were observed by Jensen *et al*. (2001) in *Acyrthosiphon pisum* (Harris), treated with *Pandora neoaphidis* (Remaudiere and Hennebert) Humber. Combing of body parts and aggregation can be seen as a defensive behaviour to remove the fungal conidia deposited on cuticle as well as the mechanism to prevent the pathogen spread. Mutual grooming among nest mates of termite, *Captotermes formosanus* Shiraki treated with conidia of *M. anisopliae,* has been identified as a disease defense mechanism (Yanaga and Shimizu, 2007). Pre death symptoms of mycosis exhibited by rice bugs were observed by Roditakis *et al*. (2008) in aphid *Myzus persicae* (Sulzer) treated with *Lecanicillium longisporum* (Petch) Zare and Gams KV71 strain. The treated aphids exhibited non directional shaking movements (ataxis) with a high tendency for aggregation. The other symptoms of mycosis observed in *L. acuta,* were convulsions characterized by leg twitching and abdominal arching. The same movements were repeated in cycles, the intensity of which faded slowly as the bug slipped into paralysis and death. Such abdominal arching and leg twitching in larval and adult stages of western flower thrips, *Frankliniella occidentalis* (Pergande) treated with *M. anisopliae* spores three to four days after treatment (DAT) were described earlier by Vestergaard *et al*. (1995) and similar observations on legume flower thrips *Megalurothrips sjostedti* (Trybom) treated with *M. anisopliae* were reported by Ekesi and Maniania (2000). The emergence of mycelia from inter segmental membranes, bases of antennae, and leg joints as observed in this study when treated with *L. saksenae* was earlier reported in the case of another species *L. longisporum* in *Myzus persicae* (Sulzer), by Roditakis *et al.* (2008).

Dose dependant mortality relationship was depicted very clearly by the varying spore doses of *L. saksenae.* A sudden hike in mortality from 20-40 to 80-100 per cent, was observed when the spore dose increased from 10^5 to 10^7 spores ml^{-1}. The effective dose of *L. saksenae* against *L. acuta* was fixed as 10^7 spores mL^{-1}, since there was no difference in mortality (100 per cent), observed with the highest concentration, 10^8 spores mL^{-1}, 48 - 72 hours after treatment (HAT). The dose - dependent response in mortality observed in this study is in conformity with the findings of Vestergaard *et al*., (1995) in flower thrips, *F. occidentalis* treated with different spore concentrations of *M. anisopliae.* They recorded 53.6, 85.7, 94.6 and 99.1 per cent mortality in adults, when treated with 10^5, 10^6, 10^7, and 10^8 spores mL^{-1}. A sudden spike in mortality from 53 to 99 per cent noted in Vestergaard's study is in conformity with the present one. On comparing the infectivity of *L. saksenae* to adults and nymphs of *L. acuta*, it was evident that nymphs were more susceptible, as 100 per cent mortality was achieved at 48 HAT while it took 72 h in the case of adults. Urquiza and Keyhani (2013), attributed the susceptibility of nymphs to the extensive sclerotization of the cuticle that takes place after final moult.

The LC_{50} of *L. saksenae* spores calculated 6 days after treatment (DAT) to adults and nymphs of rice bug were 2.99 x 10^4 and 1.72 x 10^4 spores ml^{-1} respectively. Fadayivata *et al*. (2014) reported the pathogenicity of *Lecanicillium longisporum* strain LRC 190 to cereal aphids *Sipha maydis* Passerini and *Metopolophium dirhodum* (Walker) and calculated the LC_{50} as 5.9 x $10^5$and 3.2 x 10^6 conidia mL^{-1} and LT_{50} computed at 10^8 conidia mL^{-1} as 2.9 and 4.4 days, for *S. maydis* and *M. dirhodum*, respectively.

Quick kill exhibited by *L. saksenae* in laboratory assays, was not witnessed in open field. This may be due to the fact that while preparing the oil formulations, only spores were utilized, eliminating the possible role of secondary metabolites like dipicolinic acid (DPA), reported from the same species by Jasmy (2016). The parity observed with the *M. anisopliae* was also due to the added advantage of blending the culture as a whole in the talc based product. This clearly indicates EPF performs

Fig 2. Field collected cadaver of rice bug mycosed with *L. saksenae.*

well when tank mix formulations of pure cultures are used rather than the pure formulations of spores. However, the meits of a basic formulation which includes better shelf life, easiness in application, less transport and marketing difficulties etc. makes formulation inevitable in the case of microbes. One interesting incident worth mentioning is about the persistence of *L. saksenae.* Few months after conclusion of the experiment, we collected rice bug cadavers mycosed with *L. saksenae* (Fig. 2), that continued to cause pathogenesis in spite of the hot and humid weather, while mycosed specimens of other fungi treated were not observed.

CONCLUSIONS

As opined by Kanaoka *et al.* (1978), mortality due to fungi is a combined effort of mechanical action of spores and chemical action of mycotoxins. The quick kill action of *L. saksenae* evident in this study, might be accomplished with the help of the DPA which is a proven insecticide compound (Assaf *et al.,* 2005). We suspect the presence of several such compounds acting hand in hand with the spores to give the Midas touch of "quick kill" characterized in *L. saksenae*. Further studies on identification and characterization of such compounds and molecular studies on genes coding for such toxins can open newer frontiers in biocontrol. The spectacular level of rice bug control achieved in this study, presents the oil formulations of *L. saksenae* as a promising alternative to insecticide in rice ecosystem. The adoption rate may be on frontline owing to it is safety to natural enemies as well. Real time multilocation and multi crop trials of oil formulations of *L. saksenae* can expand its applicability to vegetable ecosystem as well.

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