



EFFECTS OF INSECTICIDE AND METARHIZIUM ANISOPLIAE COMBINATIONS AGAINST LEPTOCORISA ACUTA

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ABSTRACT

This study evaluated the effects of combining the entomopathogen green muscardine fungus *Metarhizium anisopliae* (Metchnikoff) with three compatible chemical insecticides (imidacloprid, thiamethoxam, and pymetrozine) against the rice gundhi bug *Leptocorisa acuta* (Thunberg) in rice under field conditions. The results revealed that among the various treatments, imidacloprid exhibited the most promising results, leading to a bioefficacy of 71.13% followed by imidacloprid (half-dose) + *M. anisopliae* combination, which achieved a bioefficacy of 63.95% in the field over two consecutive seasons. The highest reduction in *L. acuta* population over control was observed in the imidacloprid (69.73%) followed by *M. anisopliae* + pymetrozine (half-dose) (65.79%). In terms of rice grain yield, imidacloprid again outperformed other treatments with a yield of 41.98 q ha⁻¹ with a benefit-cost ratio of 2.57 followed by the imidacloprid (half-dose) + *M. anisopliae* combination, which yielded 41.61 q ha⁻¹ with B:C ratio of 2.42.

Key words: Insecticides, entomopathogen, imidacloprid, thiamethoxam, pymetrozine, *Metarhizium anisopliae*, benefit-cost ratio, mortality, recommended dose, rice, combination

The rice gundhi bug *Leptocorisa acuta* (Thunberg), is a notorious insect pest in rice-producing countries. Both the nymphs and adults cause damage to rice by sucking out the contents of developing grains from the flowering stage to the soft dough stage, causing unfilled or empty grains and discoloration (Hill, 2008). Loss due to bug infestation ranges from 30% (Tiwari et al., 2014) and often extends up to 98% in severe cases (Bhadauria and Singh, 2009). The extensive use of insecticides can accelerate their resistance and result in a resurgence of herbivore pests (Lou et al., 2022). To prevent paddy from being infested by pests, various methods, such as cultural controls, biological controls, and chemical controls, have been implemented (Fahad et al., 2015). Among these methods, chemical control has been the primary approach for managing sucking pests (Ko et al., 2015). However, concerns about the impact on natural predators, pollinators, environmental pollution, and human health have led to increased regulations on insecticide usage and a push to reduce their application.

Microbial control, a safe and highly effective method in integrated pest management (Singh et al., 2019), features entomopathogenic fungi, such as

green muscardine fungus *Metarhizium anisopliae* (Metchnikoff), as environmentally friendly biological agents with low resistance risk (Zimmermann, 2007; Knols et al., 2010). Entomopathogenic fungi are often considered slow acting, taking more time than conventional methods to achieve sufficient insect mortality (Nawaz et al., 2022). Combining these fungi with chemical insecticides enhances pest control efficacy, particularly against resistant pests (Tang et al., 2019), however, this slower action can be overcome by integrating them with faster-acting insecticides, leading to reduced costs and extended effectiveness (Bitsadze et al., 2013; Shariffard et al., 2011). Despite the potential of these combinations, limited data exists on insecticide-entomopathogenic fungi combinations against *L. acuta*. The present study aims to address this gap by evaluating insecticide compatibility with *M. anisopliae* and its toxicity to *L. acuta*.

MATERIALS AND METHODS

The study assessed the effectiveness of the entomopathogenic fungus *M. anisopliae* alone and in combination with three compatible insecticides

i.e. imidacloprid, thiamethoxam, and pymetrozine, against the *L. acuta*. The research was conducted at the instructional farm of the college of post graduate studies in agricultural sciences (CPGS-AS), central agricultural university (Imphal), Umiam, Meghalaya, India, (25°40'52.32"N and 91°54'41.04"E), spanning two consecutive kharif seasons from 2021 to 2022. All the chemicals were procured from the local pesticide shop and biocontrol agent *M. anisopliae* (Um-met) (accession no: OL375172) was supplied by biopesticide production unit, CGPS-AS, central agricultural university, Umiam, Meghalaya. The treatments were administered in October and November, coinciding with moderate *L. acuta* infestations. Each trial consisted of eight treatments, each replicated four times. To ensure effective application, tween® 80 (0.5%) was used as a surfactant in all treatments. Among the treatments, four involved the sole application of three chemicals as well as *M. anisopliae* at their recommended dosages, while the other three combined *M. anisopliae* with these chemicals at half the recommended dosages. No pest management practice was used in the control plot. The susceptible rice variety CAUS 107 was chosen for the trial, with each plot measuring 4 m² and a 1 m buffer zone between experimental plots where no spraying occurred. The different treatments were applied once during the panicle initiation stage, at a dilution rate of 500 litres per hectare by using a knapsack sprayer in the evening. Pest population monitoring began one day before the initial spray and continued for a month, focusing on ten randomly selected plants in each treatment. Population data were recorded at 3, 7, 14, 21, and 28 days after the first spray, with both nymphs and adults counted to assess treatment efficacy, at the end of the season, yield data was also observed to estimate the benefit-cost ratio. The relative effectiveness of each treatment was evaluated using the Henderson and Tilton's formula (Henderson and Tilton 1955). The field experiments data were analysed using a two-way analysis of variance (ANOVA) with a randomized complete block design (RCBD). To compare means and determine significant differences, Tukey's honestly significant difference (HSD) test was employed at a significance level of $p < 0.05$. All the analyses were conducted using statistical package for the social sciences (SPSS) version 22.0.

RESULTS AND DISCUSSION

The impact of *M. anisopliae* and various insecticides, both individually and in a 1:1 combination, on *L. Acuta* mortality at different time points in the years 2021 and 2022 is presented in Table 1. In every spray application

during both seasons, all treatments were found to be superior over the control (Water). The *L. acuta* population did not show significant variation among the treatments the day before spraying ($F_{7,3} = 1.837$, $P = 0.133$). However, three days spraying (DAS), there was a significant reduction in *L. acuta* populations, with notable differences among the treatments compared to the control ($F_{7,3} = 391.22$, $p < 0.05$). The imidacloprid 17.8% SL treatment exhibited the lowest population of *L. acuta* (0.39/ plant), followed by the combined application of *M. anisopliae* + pymetrozine 50% WG (0.53/ plant) and *M. anisopliae* + imidacloprid 17.8% SL (0.60/ plant). At 7 DAS, *L. acuta* populations remained significantly lower in the imidacloprid 17.8% SL-treated plots (0.53/ plant) compared to other treatments ($F_{7,3} = 183.21$, $p < 0.05$). This trend continued through 14, 21, and 28 DAS, with the imidacloprid 17.8% SL treatment consistently showing the lowest *L. acuta* populations (0.68, 0.85, and 1.03/ plant, respectively), followed by the combined application of *M. anisopliae* + pymetrozine 50% WG (0.81, 0.88, and 1.00/ plant). The highest reduction in *L. acuta* population over control was observed in the imidacloprid 17.8% SL treatment (69.73%), followed by *M. anisopliae* + pymetrozine 50% WG (65.79%), *M. anisopliae* + imidacloprid 17.8% SL (64.04%), *M. anisopliae* + thiamethoxam 25% WG (61.84%), pymetrozine 50% WG (57.46%), and thiamethoxam 25% WG (50.00%).

The efficacy of *M. anisopliae* alone was the lowest at 42.33% after 28 DAS. Sole application of imidacloprid 17.8% SL (71.13%) was found to be the most effective treatment, which was statistically comparable with combined application of *M. anisopliae* + imidacloprid 17.8% SL (63.95%) ($F_{7,3} = 222.94$, $P = 0.06$) but significantly outperforming the control ($F_{7,3} = 222.94$, $p < 0.05$). The second-best result was obtained with the combined application of *M. anisopliae* + imidacloprid 17.8% SL (63.95%), followed by *M. anisopliae* + thiamethoxam 25% WG (62.97%) and *M. anisopliae* + pymetrozine 50% WG (62.68%), all of which were significantly different from the control ($F_{7,3} = 222.94$, $P < 0.05$). In contrast, the sole applications of thiamethoxam 25% WG (49.06%) and pymetrozine 50% WG (47.04%) exhibited lower bioefficacy compared to their respective combinations with *M. anisopliae*. These findings are consistent with prior research by Rath et al. (2015) and Gupta and Kumar (2017), who reported that Imidacloprid 17.8% SL at a rate of 300 gm/ ha was effective in managing the pest population. Similar results were presented by Tigga et al. (2018) and Morya and Kumar (2019),

who found that imidacloprid 17.8% SL was highly effective in reducing the number of *L. Acuta* (0.70/ hill). Additionally, imidacloprid 17.8% SL at a lower rate of 25g ai/ha (1.28) was also significant in reducing *L. acuta* populations, as observed by Ashokappa (2015), and Ghoghari et al. (2019).

M. anisopliae is very effective against hemipteran bugs (Maniania et al., 2022) and the early life stages such as nymphs are more susceptible than adults (Geng and Zhang et al., 2004). Optimal moisture conditions for the development of *M. anisopliae* on the eggs and nymphs of hemipteran pests are likely to occur during the rainy season (Santos et al., 2009) and Meghalaya, a distinctive region in northeastern India, supports a diverse flora and fauna due to its highly humid and tropical climate (Roy and Tomar, 2001). The combination of mycoinsecticides with chemical insecticides has been found to exhibit a synergistic action, increasing insect mortality, and reducing the time until death (Nawaz et al., 2022). For example, Jaramillo et al. (2005) observed increased mortality among subterranean burrower bugs (*Cyrtomenus bergi*) when they applied *M. anisopliae* in conjunction with Imidacloprid. Similar results were documented by Shakir et al. (2015), who noted that the mortality rate of *Cnaphalocrocis medinalis* was highest (61.91%) when Potassium silicate, *B. bassiana*, and Imidacloprid were combined, 20 days after application. These findings also partially correspond to those of Nyasani et al. (2015), who reported that using both *M. anisopliae* and Imidacloprid led to the greatest reduction in *Frankliniella occidentalis* population. Dash et al. (2020) additionally found that foliar spraying with imidacloprid 17.8% SL at a rate of 17.5 ml ai/ha, combined with multineem at 300 ppm at 55 and 70 days after spraying (DAS), was the most effective method, resulting in an 88.91% reduction in *L. acuta* populations. Imidacloprid 17.8% SL at 35g ai/ha also showed a substantial reduction of 85.14%, respectively.

In the cost economics analysis imidacloprid 17.8% SL stood out as the most profitable option, generating the highest gross returns and net profit of Rs. 85,629 and Rs. 61,671, respectively; next was *M. anisopliae* + imidacloprid 17.8% SL, which yielded gross returns and net profit of Rs. 84,889.50 and Rs. 60,049.50, respectively. Additionally, the plot treated with imidacloprid 17.8% SL demonstrated the highest cost-benefit ratio, achieving an impressive B:C ratio of 2.57. This treatment also resulted in a production yield of 41.98 q ha⁻¹. Following closely, the combined application of *M. anisopliae* + imidacloprid 17.8% SL achieved

a B:C ratio of 2.42 and a production yield of 41.61 q ha⁻¹ (Table 1). The most favourable results in terms of both yield and benefit cost ratio were observed in the treatment using imidacloprid 17.8% SL, with a yield of 41.98 q ha⁻¹ and a benefit cost ratio of 2.57. This suggests that Imidacloprid 17.8% SL is a valuable chemical for effectively managing *L. acuta*. This finding is consistent with Gupta et al. (2019), who also reported the highest yield in the imidacloprid treatment (46.800 q ha⁻¹) followed by triazophos (44.500 q ha⁻¹), with benefit cost ratios of 1:2.66 and 1:2.53, respectively. Similarly, Rath et al. (2015) observed that plots treated with imidacloprid 17.8% SL at a rate of 500 g a.i. ha⁻¹ recorded the highest grain yield at 5.18 t ha⁻¹, followed by thiamethoxam 25WG at 25 g a.i. ha⁻¹ (4.58 t ha⁻¹) and triazophos 40EC at 450 g a.i. ha⁻¹ (4.56 t ha⁻¹). Ashokappa, Prabhu, and Manjappa (2015) noted that the insecticides imidacloprid 17.8 SL at 0.25 ml/l, thiamethoxam 25 WG at 0.3 g/l, and malathion 5 D at 20 kg/ha resulted in the highest yields of 7049.26 kg ha⁻¹, 6461.11 kg ha⁻¹, and 6253.33 kg ha⁻¹, respectively. Girish and Balikai (2015) reported that thiamethoxam 25 WG at 0.3 g/lit led to the highest net profit of Rs. 65823.75, followed by malathion 5 D at 20 kg/ha with Rs. 62070.63.

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AUTHOR CONTRIBUTION STATEMENT

Bimal Kumar Sahoo, Mahesh Pathak and Pranab Dutta conceived and designed the research, Bimal Kumar Sahoo conducted the experiment, Mahesh Pathak, N S Azad Thakur, Kennedy Ningthoujam, Raghubir K. Patidar provided the data analysis tools, Bimal Kumar Sahoo, Malsawmtluanga Hnialum, Hia Kalita and Sikha Haritha analysed the data, Bimal Kumar Sahoo, Mahesh Pathak and NSA Thakur wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

Table 1. Effect of insecticides and *Metarhizium anisopliae* alone and their 1:1 combination against *L. acuta*

Treatments	Concentrations (gm. or ml/ l)	Population of <i>L. acuta</i> (number per plant) (Pooled)										Yield					Economics			
		1 DBS	3 DAS	7 DAS	14 DAS	21 DAS	28 DAS	Mean population	Reduction over control	Bio- efficacy	Mean yield	% increase over control	Gross income (Rs/ ha)	Cost of cultivation (Rs/ ha)	Net profit (Rs/ ha)	Net gain over control (Rs/ ha)	BC ratio			
Control	-----	1.55± 0.29 ^a	1.74± 0.01 ^a	1.93± 0.05 ^a	2.20± 0.04 ^a	2.60± 0.03 ^a	2.95± 0.00 ^a	2.28	0.00	0.00 ^c	25.50	0.00	51994.50	16891.05	35103.45	0.00	1.20			
<i>Metarhizium anisopliae</i>	4.7x10 ⁸ cfu/gm @ 2.5 kg/ ha	1.30± 0.14 ^a	0.91± 0.02 ^b	1.01± 0.03 ^{bc}	1.16± 0.01 ^b	1.18± 0.03 ^b	1.30± 0.02 ^b	1.11	51.32	42.33 ^b	34.07	8.15	69495.15	24315.00	45180.15	10076.70	1.86			
Imidacloprid 17.8% SL	200 ml/ ha	1.63± 0.25 ^a	0.39± 0.02 ^c	0.53± 0.03 ^f	0.68± 0.03 ^e	0.85± 0.00 ^e	1.03± 0.03 ^{de}	0.69	69.73	71.13 ^a	41.98	33.26	85629.00	23958.00	61671.00	26567.55	2.57			
Pymetrozine 50% WG	300 g/ ha	1.23± 0.15 ^a	0.75± 0.00 ^e	0.88± 0.02 ^{cd}	0.96± 0.01 ^c	1.06± 0.02 ^c	1.18± 0.01 ^c	0.97	57.46	47.04 ^b	34.30	8.88	69964.35	22623.00	47341.35	12237.90	2.09			
Thiamethoxam 25% WG	100 g/ ha	1.55± 0.06 ^a	0.93± 0.03 ^b	1.05± 0.03 ^b	1.26± 0.03 ^b	1.20± 0.00 ^b	1.28± 0.03 ^b	1.14	50.00	49.06 ^b	36.09	14.58	73628.70	23701.00	49927.70	14824.25	2.11			
<i>Metarhizium anisopliae</i> + Imidacloprid 17.8% SL	4.7x10 ⁸ cfu/ gm @ 2.5 kg/ ha + 100 ml/ ha	1.55± 0.13 ^a	0.60± 0.02 ^d	0.73± 0.03 ^{de}	0.83± 0.01 ^d	0.93± 0.03 ^{de}	1.03± 0.03 ^{de}	0.82	64.04	63.95 ^a	41.61	32.11	84889.50	24840.00	60049.50	24946.05	2.42			
<i>Metarhizium anisopliae</i> + Pymetrozine 50% WG	4.7x10 ⁸ cfu/ gm @ 2.5 kg/ ha + 150 g/ ha	1.48± 0.41 ^a	0.53± 0.01 ^d	0.70± 0.02 ^e	0.81± 0.02 ^d	0.88± 0.01 ^e	1.00± 0.02 ^e	0.78	65.79	62.68 ^a	38.32	21.66	78172.80	25015.00	53157.80	18054.35	2.13			
<i>Metarhizium anisopliae</i> + Thiamethoxam 25% WG	4.7x10 ⁸ cfu/ gm @ 2.5 kg/ ha + 50 g/ ha	1.65± 0.26 ^a	0.60± 0.03 ^d	0.75± 0.03 ^{de}	0.89± 0.01 ^{cd}	1.00± 0.03 ^{cd}	1.10± 0.02 ^{cd}	0.87	61.84	62.97 ^a	39.63	25.82	80850.30	24656.00	56194.30	21090.85	2.28			
F stat		NS	391.22	183.21	444.50	1154.40	1045.42			222.94										
CD (p=0.05)		NS	0.06	0.09	0.07	0.06	0.06			4.36										
SE(m) ±		NS	0.02	0.03	0.02	0.02	0.02			1.51										
CV		NS	5.28	6.74	4.20	2.80	2.98			6.75										

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