Field evaluation of *Metarhizium anisopliae* liquid formulation (Bio-Magic®) against brown plant hopper, *Nilaparvata lugens* Stal on rice

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ABSTRACT

Field experiments were conducted to evaluate the bio-efficacy of *Metarhizium anisopliae* – based liquid formulations (Bio-Magic®1.50%) against Brown Plant Hopper (BPH), *Nilaparvatha lugens* Stal (Homoptera: Delphacidae) at the farmers' holdings of Theni district during *Summer* 2015 and *kharif* 2015. Bio-Magic was tested at three doses of 1500 mL, 2000 mL and 4000 mL ha^{-1} against BPH. The results revealed that Biomagic @ 4000 mL ha^{-1} (84.08 and 83.21 per cent) and 2000 mL ha^{-1} (82.76 and 81.62 %) statistically on par in terms of efficacy in suppressing the population of BPH after two rounds of application during both the seasons with increase in grain yield of 89.58 and 88.60 per cent over untreated check. The lower dose of Biomagic® 1.5 LF @ 1500 mL ha^{-1} ranked second in the order of efficacy, however it was better than the standard check (Quinolphos 25 EC @ 1500 mL ha^{-1}). All the three doses of Biomagic® tested were safer to the natural enemies and were on par with untreated check without any phytotoxicity effect.

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INTRODUCTION

Rice (*Oryza sativa* Linn.) is the most widely cultivated food crop in the world, occupying an area of about 161.10 million hectares with a production of 740.20 million tonnes (Anonymous, 2015a). It is the staple food of more than 60 per cent of the world's population in almost 112 countries. Asia accounts for 92 per cent of world's rice area and production respectively. Among the countries of rice cultivation in Asia, India has the largest area of 43.13 million ha, with production of 104.80 million tonnes which ranks second in production next to China and contributing 43 per cent of total food grain production and 46 per cent of total cereal production and continues to play a vital role in the national food grain supply. In Tamil Nadu the area under rice was 1.73 million hectares with a production of 7.12 million tonnes contributing about 5.20 per cent of the total rice production in India (Anonymous, 2015b). Among several production constraints, one of the main causes for the low productivity of rice is the damage caused by insect pests. There are about 20 major pests, which were recorded to damage rice crop right from nursery sowing to the harvest, causing 21 to 51 per cent yield loss. Among the pests, brown planthopper (BPH), *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae), is the monophagous pest of rice attacking leaf sheath and causes hopper burn symptoms, besides transmitting several viral diseases in a persistent manner without transovarial passage (Liu *et al.*, 2010).

Insecticidal control of BPH is generally achieved through pesticide usage for the past 20 years, which has resulted in several adverse ecological implications. Hence there is a dire need to develop alternative management strategies to mitigate these problems. Developments on methods of pest management in recent years indicated a great potential for harnessing biocontrol agents for the management of sucking pests of rice. Biological control is one of the viable tactics and environmentally benign strategy. Among the components of biocontrol, entomopathogens are more specific, which includes fungi, bacteria, viruses and entomopathogenic nematodes. Off these entomopathogenic fungi have the ability to suppress the pest population without harming the natural enemies (Shahid, 2012). Moreover, the fungal diseases were favoured by high humidity and high moisture, the microclimate available in the paddy fields would be most suitable for conidial germination and penetration and these fungi have a better prospect in the microbial control of insect pests of rice (Venkat Reddy *et al.*, 2013). The fungal pathogen, *Metarhizium anisopliae* is known to infect more than 200 species of insects belonging to the orders *viz.*, Coleopteran, Dermoptera, Homoptera, Lepidoptera and Orthoptera (Reddy *et al.*, 2013). Studies on the use of entomogenous fungi for controlling BPH in rice fields in Korea and the Philippines were reported by Aguda *et al.* (1988) and its control potential against rice pest in south India (Ramamohan Rao, 1989). Also researchers have explored the well-known *M. anisopliae* (Metschnikoff) Sorokin for BPH control (Song and Feng, 2011). It is necessary to restore this valuable tactic in management of BPH; many commercial products have been developed with entomopatogenic fungi. Therefore, the present investigation is undertaken to evaluate the potential of a biopesticide (Bio-Magic®) against BPH and its safety towards natural enemies and its phytotoxicity to rice plants, so that we can save the environment from the ill effects of pesticides at lower cost and the harvestable produce also will be pesticide free avoiding several health complications to human beings and the cattle wealth.

MATERIALS AND METHODS

Field experiments were conducted for two seasons in the farmer's holdings at Veerapandi village, Theni block of Theni district during *summer* 2015 and *kharif* 2015 to assess the bio-efficacy of Bio-Magic® 1.50% LF formulation (*M. anisopliae*) against BPH. The field experiments were carried out in plots of 4 x 5 m size in randomized block design (RBD) with six treatments (T_1 - Bio-Magic® 1.50% LF @ 1500 mL ha^{-1}, T_2 - Bio-Magic® 1.50% LF @ 2000 mL ha^{-1}, T_3 - Bio-Magic® 1.50% LF @ 4000 mL ha^{-1}, T_4- Neem oil based EC containing Azadiractin 0.03% @ 2000 mL ha^{-1}, T_5 - Quinalphos 25% EC @ 1500 mL ha^{-1}, T_6 - Untreated check) and four replications using popular variety and farmer's choice Jaya (*summer* 2015) and Akshaya (*kharif* 2015). Routine Agronomic practices were adapted as per recommended cultivation practices equally for all treatments except plant protection measures. Two rounds of foliar sprays were applied at 15 days interval commencing from 30 days after transplanting during dawn and dusk timings, using aspee pneumatic knapsack sprayer with cone nozzle using 500 L ha^{-1} as spray fluid based on the ETL.

The population of nymphs and adults of BPH, coccinellids (*Cheilomenes sexmaculata* F. and *Coccinella transversalis* F.) and spiders (*Lycosa pseudoannulate* B. and *Oxyopes javanus* T.) were recorded on five randomly selected hills per replication for each treatment on 7th and 14th days after I spray where as for second spray the population was recorded on 7th, 14th and 21st days after spray apart from pretreatment counts and the population was expressed as number of BPH / hill and number / 5 hills (natural enemies).

Separate field experiment was conducted to study the phytotoxic effect; Bio-Magic® 1.50% LF at doses of 1500, 2000 and 4000 mL ha^{-1} was sprayed over rice plants and compared with untreated check. There were five replications and two applications at 15 days interval during 25 and 30 days after transplanting. Doses were sprayed to run off point using a pneumatic knapsack sprayer with hydraulic cone nozzle using 500-700 L ha^{-1} as spray fluid. The visual observations on phytotoxic symptoms like leaf injury on tips and leaf surface, vein clearing, wilting, necrosis, epinasty and hyponasty were recorded on pre-treatment and 1, 3, 7, 10 and

14 days after 1st, 2nd spray. The leaf injury on tips and leaf surface was observed based on 1-10 rating scale. Rice plants showing 1-10 per cent phytotoxicity symptom was categorized as 1, 2 (11-20%), 3 (21-30%), 4 (31-40%), 5 (41-50%), 6 (51-60%), 7 (61-70%), 8 (71-80%), 9 (81-90%), 10 (91-100%). The yield was recorded at the time of harvest, separately in each treatment and yield data were computed as q ha^{-1}.

Statistical analysis

The data on field study were subjected to ANOVA. Before analysis, the data on population were subjected to square root transformation. In order to know the interaction between treatments, data were subject to factorial RBD and the treatment means were separated by LSD (Least Significant Difference). The yield data were subjected to square root transformation and the means were compared by LSD to single out the best treatment using software AGRESS.

RESULTS AND DISCUSSION

Bio-Magic® on population of BPH

The pre treatment observation on population of BPH ranged from 10.75 to 11.50 and 13.50 to 14.25 nos. per hill in both the field experiments I (*Summer* 2015) and II (*Kharif* 2015) (Table 1). Among the treatments, The mean data of both spray revealed that Bio-Magic® 1.50% LF @ 4000 mL ha^{-1} was effective in reducing the population of *N. lugens,* recording the lowest population of 3.00 and 4.00 nos./ hill, with a per cent reduction of 84.08 and 83.21 over untreated check, which was on par with Bio-Magic® 1.50% LF @ 2000 mL ha^{-1} followed by Bio-Magic® 1.50% LF @ 1500 mL ha^{-1} which was on par with standard check quinalphos 25% EC @ 1500 mL ha^{-1} and were found superior to Neem oil based EC containing Azadiractin 0.03% when compared to untreated check (Table 1).

Bio-Magic®1.50% LF is a biological insecticide based on selective strain of *Metarhizium anisopliae* which is a naturally occurring fungus in which spores are suspended in liquid formulation suitable for spraying like a chemical insecticide. Spores come in contact with cuticle of insect pests and germinate and grow directly through the spiracle in to the inner body of the host and drain the nutrients and infected hosts eventually die. The findings on the consistent efficacy of Bio-Magic®1.50% LF (*M. anisopliae*) against BPH are in concurrence with the reports of Vothi (2005) from Vietnam, who reported that the registered bioinsectcides, OMETAR have been produced from entomophaggous fungi, *M. anisopleae* found to be effective against insect pests and could reduce production costs. Kiran and Veeranna (2012) reported that the efficacy of *M. anisopliae* was similar to that of Thiomethoxam and Imidacloprid against BPH. Venkat Reddy *et al*. (2013) who found that *M. anisopliae* and *B. bassiana* was effective against BPH when increase in days after spray. Shoaib and Pandurang (2015) who also reported that *M. anisopliae* with conidial concentration 1 x 10^{10} and 1 x 10^{9} per mL was the most consistently effective and significantly superior over *Beauveria bassiana* and *Verticilium lecani* against BPH. Similarly the performance of *M. anisopliae* against BPH was also reported by several authors *viz.*, Krutmuang (2011), Maoye *et al* (2012), Li Mao-Ye *et al*. (2012).

Bio-Magic® against predator populations

The pre treatment population of coccinellid varied from 3.25 to 4.00 and 3.75 to 4.50 per 5 hills in the first and second season field experiment, respectively (Table 2). Mean number of coccinellid predators was high in plots sprayed with Bio-Magic® 1.50% LF @ 1500 mL ha^{-1} resulting 5.94 and 6.50 nos./5 hills which was on par with Bio-Magic® 1.50% LF @ 2000 mL ha^{-1} (5.75 and 6.38 nos./ 5 hills) and Bio-Magic® 1.50% LF @ 4000 mL ha^{-1} (5.63 and 6.19 nos./ 5 hills). Neem oil based EC containing Azadiractin 0.03% was found moderately safe to coccinellids by recording 4.50 and 5.06 nos./ 5 hills and quinalphos 25% EC @ 1500 mL ha^{-1} was toxic to coccinellids recording the lowest mean population of 2.94 and 3.56 nos./ 5 hills,

Table 1. Efficacy of *Metarhizium anisopliae* 1.50% LF commercial product against BPH on rice incidence (population/hill) during summer 2015 and *kharif* 2015

Treatments	BPH population / hill*									
	Summer-2015					*Kharif*-2015				
	Pre count	I Spray	II Spray	mean	% redaction over untreated check	Pre count	I Spray	II Spray	mean	% redaction over untreated check
T1 Bio-Magic 1.50% LF @ 1500 mL/ha	11.50	5.50[b]	4.00[b]	4.75[b]	74.80	14.25	6.75[b]	4.92[b]	5.83[b]	73.51
T2 Bio-Magic 1.50% LF @ 2000 mL/ha	11.00	4.25[a]	2.25[a]	3.25[a]	82.76	13.75	5.50[a]	3.25[a]	4.38[a]	79.37
T3 Bio-Magic 1.50% LF @ 4000 mL/ha	11.25	4.00[a]	2.00[a]	3.00[a]	84.08	14.00	5.25[a]	2.75[a]	4.00[a]	81.50
T4 Neem Oil based EC containing	11.50	7.88[c]	7.08[c]	7.48[c]	60.32	14.25	9.13[c]	8.08[c]	8.60[c]	60.92
Azadiractin 0.03% @ 2000 mL/ha	11.00	5.88[b]	4.25[b]	5.06[b]	73.16	13.75	7.13[b]	5.25[b]	6.19[b]	70.85
T5 Quinalphos 25% EC @ 1500 mL/ha	10.75	15.63[d]	22.08[d]	18.85[d]	-	13.50	17.88[d]	23.83[d]	20.85[d]	-
T6 Untreated check										
SED ±	NS	0.0897	0.1146	0.0992	-	NS	0.0801	0.0992	0.0877	-
CD (p=0.05)	NS	0.1999	0.2554	0.2211	-	NS	0.1785	0.2210	0.1954	-

*Each value is the mean of four replications; NS: Non significant
In a column, means followed by common letter(s) are not significantly different by LSD (P= 0.05)

Table 2. Impact of *Metarhizium anisopliae* 1.50% LF commercial product on coccinellid populations (*Cheilomenes sexamaculata* and *Coccinella transversalis*) in rice ecosystem during summer 2015 and *kharif* 2015

Treatments	Population / 5 hills*							
	Summer-2015				*Kharif*-2015			
	Pre count	I Spray	II Spray	Mean	Pre count	I Spray	II Spray	Mean
T1 Bio-Magic 1.50% LF @ 1500 mL/ha	3.75	5.00^{a}	6.88^{a}	5.94^{a}	4.25	5.38^{a}	7.63^{a}	6.50^{a}
T2 Bio-Magic 1.50% LF @ 2000 mL/ha	3.25	4.75^{ab}	6.75^{a}	5.75^{a}	3.75	5.25^{a}	7.50^{a}	6.38^{a}
T3 Bio-Magic 1.50% LF @ 4000 mL/ha	3.50	4.63^{ab}	6.63^{a}	5.63^{a}	4.50	5.13^{a}	7.25^{a}	6.19^{a}
T4 Neem Oil based EC containing Azadiractin	3.75	4.00^{b}	5.00^{b}	4.50^{b}	4.25	4.38^{b}	5.75^{b}	5.06^{b}
0.03% @ 2000 mL/ha	4.00	3.13^{c}	2.75^{c}	2.94^{c}	4.50	3.50^{c}	3.63^{c}	3.56^{c}
T5 Quinalphos 25% EC @ 1500 mL/ha	3.75	5.25^{a}	7.13^{a}	6.19^{a}	4.00	5.50^{a}	7.75^{a}	6.63^{a}
T6 Untreated check								
SED ±	NS	0.1009	0.0921	0.0957	NS	0.0888	0.0849	0.0899
CD (p=0.05)	NS	0.2248	0.2053	0.2133	NS	0.1979	0.1891	0.2003

*Each value is the mean of four replications; NS: Non significant
In a column, means followed by common letter(s) are not significantly different by LSD (P= 0.05)

Table 3. Impact of *Metarhizium anisopliae* 1.50% LF commercial product on spider populations (*Lycosa pseudoannulate* and *Oxyopes javanus*) in rice ecosystem during summer 2015 and *kharif* 2015

Treatments	Population / 5 hills*							
	Summer-2015				*Kharif*-2015			
	Pre count	I Spray	II Spray	mean	Pre count	I Spray	II Spray	mean
T1 Bio-Magic 1.50% LF @ 1500 mL/ha	1.75	3.63[a]	5.88[a]	4.75[a]	2.50	4.50[a]	6.50[a]	5.50[a]
T2 Bio-Magic 1.50% LF @ 2000 mL/ha	2.00	3.50[a]	5.75[a]	4.63[a]	2.25	4.38[ab]	6.38[a]	5.38[a]
T3 Bio-Magic 1.50% LF @ 4000 mL/ha	2.00	3.38[ab]	5.63[a]	4.50[a]	2.50	4.25[ab]	6.25[a]	5.25[a]
T4 Neem Oil based EC containing Azadiractin 0.03% @ 2000 mL/ha	2.25	2.50[bc]	4.25[b]	3.38[b]	2.50	3.50[b]	4.88[b]	4.19[b]
T5 Quinalphos 25% EC @ 1500 mL/ha	2.50	1.88[c]	2.25[c]	2.06[c]	2.75	2.50[c]	3.00[c]	2.75[c]
T6 Untreated check	1.75	3.75[a]	5.88[a]	4.81[a]	2.50	4.63[a]	6.63[a]	5.63[a]
SED ±	NS	0.1230	0.1009	0.1101	NS	0.1081	0.0924	0.0993
CD (p=0.05)	NS	0.2740	0.2247	0.2453	NS	0.2409	0.2060	0.2212

*Each value is the mean of four replications; NS: Non significant
In a column, means followed by common letter(s) are not significantly different by LSD (P= 0.05)

as against 6.19 and 6.63 nos./ 5 hills in untreated check. The same trend of toxicity was observed against spider population during both seasons. The population of spider ranged from 4.50 to 4.75 and 5.25 to 5.50 nos. per 5 hills due to all the three doses of Bio-Magic® and there was no significant difference among the doses of Bio-Magic®. Whereas neem oil based EC containing Azadiractin 0.03% and quinalphos 25% EC @ 1500 mL ha^{-1} recorded 3.38 and 4.19 nos.; 2.06 and 2.75 nos. / 5 hills as against population of 4.81 and 5.63 nos. / 5 hills in untreated check.

The safety of Bio-Magic® 1.50% LF against coccinellids and spiders found in this study was in conformity with the findings of Rachappa and Lingappa (2006) who reported that the *M. anisopliae* was found safe to natural enemies of BPH in rice field. Similar finding on safety of entomogenous fungi especially *M. anisopliae* to natural enemies of brown plant hopper was also reported by Kiran and Veeranna (2012), Venkat Reddy *et al.* (2013) and Kharbade *et al.* (2016).

Table 4. Efficacy of *Metarhizium anisopliae* 1.50% LF commercial product against BPH (paddy grain yield)

Treatments	**Yield (q / ha*)**				
	Summer -2015	**Kharif -2015**	**Mean**	**% yield increase over untreated check**	**CBR**
T1 Bio-Magic 1.50% LF @ 1500 mL/ha	52.64[b]	57.53[b]	55.09[b]	72.95	1.62
T2 Bio-Magic 1.50% LF @ 2000 mL/ha	58.00[a]	62.14[a]	60.07[a]	88.60	1.94
T3 Bio-Magic 1.50% LF @ 4000 mL/ha	58.23[a]	62.53[a]	60.38[a]	89.58	1.96
T4 Neem Oil based EC containing	44.19[c]	49.64[c]	46.92[c]	47.30	1.15
Azadiractin 0.03% @ 2000 mL/ha	51.82[b]	56.89[b]	54.36[b]	70.66	1.58
T5 Quinalphos 25% EC @ 1500 mL/ha	29.65[d]	34.05[d]	31.85[d]	-	-
T6 Untreated check					
SED ±	0.0300	0.0286	0.0293	-	-
CD (p=0.05)	0.0668	0.0637	0.0653	-	-

*Each value is the mean of four replications; In a column, means followed by common letter(s) are not significantly different by LSD (P= 0.05)

Phytotoxicity

The results of phytoxicity study revealed that Bio-Magic® 1.50% LF at doses of 1500, 2000 and 4000 mL ha^{-1} did not revealed any phytotoxic symptoms like leaf injury, wilting, vein clearing, necrosis, epinasty and hyponasty at any interval after treatment on leaves, tillers, ear heads and grains when compared to untreated check. This result is in concurrence with the reports of Kiran and Veeranna (2012) who also recorded that application of *M. anisoplea* did not cause any apparent phytotoxic effect.

Yield

The results on grain yield of the two seasons tested are presented in table 4. Among the treatments, Bio-Magic® 1.50% LF @ 4000 mL ha^{-1} and 2000 mL ha^{-1} recorded the highest grain yield which registered a per cent increase of 89.58 and 88.60 over untreated check, respectively. This was followed by Bio-Magic® 1.50% LF @ 1500 mL ha^{-1} and quinalphos 25% EC @ 1500 mL ha^{-1} with a respective increase of 72.95 and 70.66 per cent over untreated check. The treatment, Neem oil based EC containing Azadiractin 0.03% recorded the lowest yield with increase of 42.99 per cent over untreated check which recorded only 31.85 q ha^{-1}. Kiran and Veeranna (2012) also found similar results with use of *M. anisoplea* @ 2.5 kg ha^{-1} recorded significantly higher seed yield of paddy over *M. anisoplea* @ 2 kg /ha, Clothianidin and control. This was at par with Imidachloraprid.

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