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Compatibility of Entomopathogenic Fungi with Neonicotinoids for the Management of Brown Plant Hopper of Rice, *Nilaparvata lugens* stal. (Delphacidae: Hemiptera)

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ABSTRACT

Background: Rice is the staple food of Indians and it is attacked by more than 100 species of insects. Among all, brown planthopper (BPH), *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae) is one of the major sucking insects, which can cause appreciable damage by sucking sap on other hand transmitting viral diseases. Farming community using several insecticides for managing the BPH. However, continuous use of these insecticides causes health hazards and environmental pollution and also leads to development of insecticide resistance. Keeping this view in mind, the present investigation has been planned with combined use of fungal formulations and commonly used neonicotinoid insecticides in rice eco system for managing BPH.

Methods: This experiment was carried out in two parts, one is studied the compatibility between neonicotinoids (Thiamethoxam 25 SG and Dinotefuran 20 SG) and entomopathogenic fungi (*Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* =*Verticillium lecanii*) by following the standard poison food technique. Second one is after studied the compatibility between neonicotinoids and entomopathogenic fungal agents, inhibitory studies were conducted under glasshouse conditions.

Result: The study revealed that two tested insecticides affected the radial growth of fungi partially at all the three concentrations (0.5RC, RC and 1.5RC). At recommended concentration (RC), dinotefuran 20 SG @0.4 g/l was found to be slightly harmful to all three fungi (20% - 35% reduction of the growth of the fungus) and thiamethoxam 25 SG @ 0.25g/l appeared to be slightly harmful to *B. bassiana* and *M. anisopliae* and moderately harmful to *L. lecanii* (35-50% reduction of the growth of the fungus). Thiamethoxam 25SG alone recorded 65.0% mortality of BPH and when mixed with *B. bassiana* and *M. anisopliae* gave 86.25% and 81.25% mortality of BPH respectively under glass house conditions. Similarly, Dinotefuron 20 SG alone could gave 98.75% mortality of BPH.

Key words: Brown planthopper, Compatibility, Dinotefuran, Entomopathogenic fungi, Neonicotinoids, Thiamethoxam.

INTRODUCTION

Rice is the staple food of Indians and it is cultivated in an area of 43.39 million hectares in India with an annual production of 104.32 million tonnes and with 2.4 tonnes per hectare average yield (MOSPI, 2018). Insect pests are one of the major problems in rice production, wherein more than 100 species of insects attack and damage the crop. Among all, brown planthopper (BPH), *Nilaparvatalugens* (Stal) (Hemiptera: Delphacidae) is one of the important sucking insects, which can cause appreciable damage by sap sucking and transmitting the viruses such as ragged stunt and grassy stunt (Jena *et al.,* 2006). Due to infestation plants turn yellow and dry up rapidly. At early infestation, round and yellow patches appear, which soon turn brownish due to drying up of the plants which is called as 'hopper burn' and could result in yield loss ranging from 10-75% (Park *et al.,* 2008). Insecticides are the major means of managing the BPH. However, continuous use of insecticides causes health hazards and environmental pollution, besides development of insecticide resistance among the populations of BPH (Jhansi Lakshmi *et al.,* 2010). Hence, it is very difficult to manage the insects by insecticides alone, but on the other hand for this for effective management of insects, combined use of chemical pesticides with bio pesticides such as bacteria, fungi and viruses would bring down the incidence of BPH effectively. Keeping this view in mind, the present investigation has been planned with combined use of fungal formulations and commonly used neonicotinoid insecticides like thiamethoxam 25 SG and dinotefuran 20 SG for managing BPH.

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MATERIALS AND METHODS

Experiment was carried out to evaluate neonicotinoid insecticides

and entomopathogenic fungi (*Beauveria bassiana*, *Metarhiziumanisopliae* and *Lecanicilliumlecanii*) both in the laboratory and glasshouse at the Indian Institute of Rice Research, Hyderabad.

Inhibitory studies in the laboratory (Poison food technique)

Standard poison food technique was followed to assess compatibility of the entomopathogenic fungi with various insecticides (Moorhouse *et al.,* 1992). Desired quantity of insecticides (*i.e*. thiamethoxam 25 SG and Dinotefuran 20 SG) based on field application rate (recommended concentration, half recommended concentration and 1.5 fold of recommended concentration) were added to the PDA medium (200 ml), subsequently it was autoclaved at 121°C (15 Psi) for 15-20 minutes in the conical flask before solidification (at 48°C) to get desired concentration and later they were mixed thoroughly. The medium was then poured equally into the petriplates. Each treatment was replicated four times. Small disc (5 mm dia.) of young fungal mycelium was cut with sterile cork borer and placed aseptically in the centre of plate containing the poisoned medium. Petri plates were incubated at 27±1°C, 80±5% relative humidity. Suitable check without poison was kept for comparison under the same conditions. Diameter of the fungal colony was measured at 2, 4, 6, 8 and 10 days after inoculation (DAI) and compared with standard check. The data were expressed as percentage growth inhibition of fungi by insecticide treated PDA (Hokkanen and Kotiluoto, 1992) and calculated by the following formula

$$X = \frac{Y\text{-}Z}{Y} \times 100$$

Where

X, Y, Z stand for percentage growth inhibition, radial growth of the fungus in untreated check and radial growth of the fungus in poisoned medium, respectively. The pesticides were further classified in evaluation categories of 1- 4 scoring index. 1 = harmless (<20% reduction), 2 = slightly harmful (20-35% reduction), 3 = moderately harmful (35-50% reduction), 4 = harmful (>50% reduction) in toxicity tests *in vitro* according to Hassan's classification scheme (Hassan, 1989).

Inhibitory studies under glasshouse conditions

The recommended dose of neonicotinoid insecticides were mixed with the recommended dose of effective fungal commercial formulations (5g/l) and sprayed on to the rice plants. Individuals of BPH were released on the sprayed plants and mortality was recorded at 24 hrs interval up to five days after spraying. The results were compared with those of neonicotinoid insecticides and fungal pathogens alone by recording data on % mortality.

RESULTS AND DISCUSSION

Effect of Thiamethoxam 25 SG on growth of entomopatho--genic fungi

Thiamethoxam showed moderately harmful effect on entomopathogenic fungi where radial growth of fungi was affected with increase in insecticide concentration (Table 1). In the present finding, thiamethoxam caused 19.19 to 39.15% reduction over control. Among the three fungi, *L. lecanii* was the most affected recording highest reduction of radial growth (39.15%) at 1.5 RC, 24.87% and 35.45% reduction at 0.5 RC and RC, respectively. According to Hassan's (1989) classification, thiamethoxam was slightly harmful to *L. lecanii* at 0.5 RC and moderately harmful to *L. lecanii* at both RC and 1.5 RC. With regard to *M. anisopliae*, Thiamethoxamwas slightly harmful at all three doses *i.e*. 0.5 RC, RC and 1.5 RC have significantly inhibited growth of *M. anisopliae* to an extent of 23.26 to 34.50% over control. Similar results were found with *B. bassiana* where significant inhibition of the radial growth was observed recording 19.19, 27.78 and 36.36% reduction over control at 0.5 RC, RC and 1.5 RC, respectively indicating thiamethoxam being harmless at 0.5 RC, slightly harmful at RC and moderately harmful at 1.5 RC. Baraiya (2003) also observed *B. bassiana* as highly compatible with thiamethoxam (0.005%) and imidacloprid (0.005%) recording less than 25% growth inhibition.

Table 1: Effect of thiamethoxam 25 SG on growth of entomopathogenic fungi.

Species of fungi	0.5 Recommended concentration		Recommended concentration		1.5 Recommended concentration		Untreated control radial growth (cm)	Mean
	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)		
B. bassiana	4.00	19.19	3.58	27.78	3.15	36.36	4.95	3.92[b]
M. anisopliae	4.95	23.26	4.50	30.23	4.23	34.50	6.45	5.03[a]
L. lecanii	3.55	24.87	3.05	35.45	2.88	39.15	4.73	3.55[c]
Mean	4.17[b]		3.71[c]		3.42[d]		5.38[a]	
CV (%)	5.56							
LSD (5%)								
Fungus	0.17							
Concentration	0.19							

Means with same letter are not significantly different at 5% level by DMRT.

RC- Recommended concentration.

Table 2: Effect of dinotefuran 20SG on growth of entomopathogenic fungi.

Species of fungi	0.5 Recommended concentration		Recommended concentration		1.5 Recommended concentration		Untreated control radial growth (cm)	Mean
	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)		
B. bassiana	3.85	26.32	3.80	27.27	3.78	27.75	5.23	4.16[b]
M. anisopliae	5.38	18.87	5.03	24.15	4.33	34.72	6.63	5.34[a]
L. lecanii	3.88	17.99	3.80	19.58	3.65	22.75	4.73	4.01[b]
Mean	4.37[b]		4.21[b]		3.92[c]		5.53[a]	
CV (%)	4.94							
LSD (5%)								
Fungus	0.16							
Concentration	0.19							

Means with same letter are not significantly different at 5% level by DMRT.
RC- Recommended concentration.

Table 3: Effect of thiamethoxam 25 SG in combination with entomopathogenic fungi on BPH.

Treatment	Mortality (%)				
	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS
Thiamethoxam 25 SG @ 0.25g/l	31.25	53.75	60.00	61.25	65.00
	(33.97)[a]	(47.13)[a]	(50.75)[b]	(51.48)[c]	(53.71)[c]
Thiamethoxam 25 SG @ 0.25g/l + *B. bassiana*@ 5g/l	33.75	57.50	76.25	83.75	86.25
	(35.50)[a]	(49.29)[a]	(60.81)[a]	(66.20)[a]	(68.21)[a]
Thiamethoxam 25 SG @ 0.25g/l + *M. anisopliae*@ 5g/l	32.50	55.00	73.75	78.75	81.25
	(34.74)[a]	(47.85)[a]	(59.16)[a]	(62.52)[ab]	(64.32)[ab]
Thiamethoxam 25 SG @ 0.25g/l + *L. lecanii*@ 5g/l	32.50	52.50	60.00	66.25	68.75
	(34.74)[a]	(46.41)[a]	(50.75)[b]	(54.46)[bc]	(55.99)[bc]
Control	0.00	0.00	0.00	2.50	2.50
	(0.00)[b]	(0.00)[b]	(0.00)[a]	(9.09)[d]	(9.09)[d]
CD	**6.15**	**5.45**	**6.27**	**8.45**	**8.58**
SE(m)	**2.02**	**1.79**	**2.06**	**2.78**	**2.82**

Means with same letter are not significantly different at 5% level by DMRT.
DAS- Days after spraying.

Effect of Dinotefuran 20 SG on growth of entomopatho--genic fungi

Mycelial growth of the fungi was inhibited concomitantly with increase in insecticide concentration. The vegetative growth of fungi was nominally affected by dinotefuran recording 17.99% - 34.72% reduction over control. Among the three fungi, *M. anisopliae* was most affected with 34.72% reduction in growth over control at 1.5 RC. Present findings (Table 2) clearly indicated that dinotefuran was harmless at 0.5 RC (18.87% reduction) and slightly harmful at RC (24.15% reduction) and 1.5 RC (34.72% reduction) to *M. anisopliae*. Similarly, dinotefuran was found slightly harmful to *B. bassiana* at all concentrations by recording 26.32, 27.27 and 27.75% reduction in the radial growth of the fungus over control at 0.5 RC, RC and 1.5 RC, respectively. Among three fungi, *L. lecanii* was least affected by dinotefuran which inhibited the radial growth of fungus up to 22.75% over control even at 1.5 RC. It indicates its safety at 0.5 RC (17.99% reduction) and RC (19.58% reduction) and slightly harmful effect at 1.5 RC (22.75%). This is further confirming the results of Khan *et al.* (2012) who stated that neonicotinoids (acetameprid, imidacloprid and thiamethoxam) were compatible and safer to *B. bassiana* and *M. anisopliae*.

The present results indicated that at recommended concentration of dinotefuran 20 SG, it was recorded 27.27% inhibited the growth of *B. bassiana* and 24.15% inhibited the growth of *M. anisopliae* and 19.58% inhibited the growth *L. lecanii*. Thiamethoxam 25 SG was recorded 27.78% inhibited the growth of *B. bassiana*, 30.23% inhibited the growth of *M. anisopliae* and 35.45% inhibited the growth of *L. lecanii*.

Effect of thiamethoxam 25 SG + entomopathogenic fungi on BPH

Results presented in the Table 3 indicated that thiamethoxam alone could cause 65.00% mortality of BPH under glasshouse conditions. Mortality by thiamethoxam was enhanced when combined with entomopathogenic fungi including *B. bassiana*, *M. anisopliae* and *L. lecanii.* It was

Table 4: Effect of dinotefuran 20 SG in combination with entomopathogenic fungi on BPH.

Treatment	Mortality (%)				
	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS
Dinotefuran 20SG @ 0.4g/l	86.25	95.00	98.75	98.75	98.75
	(68.21)[a]	(77.05)[a]	(83.55)[a]	(83.55)[a]	(83.55)[a]
Dinotefuran 20SG @ 0.4g/l +*B. bassiana*@ 5g/l	90.00	97.50	97.50	98.75	98.75
	(71.54)[a]	(80.87)[a]	(80.87)[a]	(83.55)[a]	(83.55)[a]
Dinotefuran 20SG @ 0.4g/l +*M. anisopliae*@ 5g/l	86.25	96.25	98.75	98.75	98.75
	(68.21)[a]	(78.80)[a]	(83.55)[a]	(83.55)[a]	(83.55)[a]
Dinotefuran 20SG @ 0.4g/l +*L. lecanii*@ 5g/l	92.50	97.50	98.75	98.75	98.75
	(74.08)[a]	(80.87)[a]	(83.55)[a]	(83.55)[a]	(83.55)[a]
Control	0.00	0.00	3.75	6.25	11.25
	(0.00)[b]	(0.00)[b]	(11.16)[b]	(14.47)[b]	(19.59)[b]
CD	9.96	12.72	10.79	8.98	8.91
SE(m)	3.28	4.18	3.55	2.95	2.93

Means with same letter are not significantly different at 5% level by DMRT.

DAS- Days after spraying.

clear from the studies that first two days after spraying, there was no significant difference in the mortality of BPH between thiamethoxam alone and thiamethoxam in combination with entomopathogenic fungi. However, from third day on wards, initiation of mycosis occurred and mortality was observed in entomopathogenic fungi combined treatments and later mycosis was increased. At three days after spraying, fungal mycosis was observed in thiamethoxam + entomopathogenic fungi combination treatments *i.e*. thiamethoxam + *B. bassiana* recording 76.25% mortality, followed by thiamethoxam + *M. anisopliae* recording 73.75% that were on par with each other and significantly superior over other treatments i.e. thiamethoxam and thiamethoxam + *L. lecanii* that have recorded 60.00% mortality. At 4 DAS, the % mortality increased in combination treatments, where thiamethoxam + *B. bassiana* resulted in maximum % mortality of 83.75%, thiamethoxam + *M. anisopliae* recorded 78.75% mortality which were on par with each other followed by thiamethoxam + *L. lecanii* recording 66.25% mortality that remained on par with thiamethoxam + *M. anisopliae* (78.75%). Thiamethoxam alone has recorded low 61.25% mortality, which was on par with thiamethoxam + *L. lecanii*. However, the treatments were significantly superior over control. Similar trend was observed at 5 DAS where thiamethoxam + *B. bassiana*, thiamethoxam + *M. anisopliae*, thiamethoxam + *L. lecanii*and thiamethoxam alone which resulted in 86.25, 81.25, 68.75 and 65.00 % mortality respectively and were significantly superior over control (2.50% mortality), *B. bassiana*with 8.75% mortality, *M. anisopliae*with 10.00% mortality and *L. lecanii* with 12.50% mortality (Reddy *et al.,* 2017).

The present results indicated that thiamethoxam at recommended dose was slightly harmful with 20-35% reduction in fungal growth (Table 4), when combined with fungi for management of BPH under glasshouse conditions, it could result in additive effect.

Effect of dinotefuran 20 SG with entomopathogenic fungi on BPH

Dinotefuran is a neonicotinyl insecticide belonging to neonicotinoid group and was evaluated for its efficacy against BPH alone as well as in combination with entomopathogenic fungi under glasshouse conditions. The findings indicated that (Table 4) dinotefuran alone caused complete mortality of BPH at three days after spraying. Dinotefuran was proved safe for entomopathogenic fungi like *B. bassiana*, *M. anisopliae*, *L. lecanii* without affecting growth and sporulation under laboratory conditions (Table 2). Even though complete mortality of BPH was observed with dinotefuran alone, mycosis was observed in combination treatments which means dinotefuran could not adversely affect the growth and sporulation of the fungi. Results indicated that there was no significant difference in mortality among dinotefuran alone and combination with entomopathogenic fungi. At 4 days after spraying and 5 days after spraying there was 98.75% mortality with dinotefuran alone and in combination with entomopathogenic fungi. All these treatments were on par with each other and significantly superior over control which has recorded 11.25% mortality at 5 days after spraying.

Present studies revealed that there is no need of mixing of any entomopathogenic fungi with dinotefuran as the insecticide itself could cause maximum (98.75%) mortality of BPH. However, if BPH develop resistance to dinotefuran in future, it can be used in combination with entomopathogenic fungi like *L. lecanii, B. bassiana* and *M. anisopliae*.

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