STUDIES ON SOME ENTOMOGENOUS FUNGI TO CONTROL BROWN PLANT HOPPER IN RICE

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ABSTRACT

All of isolates of fungi Beauveria bassiana and Metarhizium anisopliae were collected and purified from different sites infected effectively on BPH including Omon (Vietnam), Hyderabad, Pant Nagar, and Tamil Nadu (India). The pathogenicity tests with different isolates of Beauveria bassiana and Metarhizium anisopliae against third instar nymphs and adults of brown planthopper (BPH) were done in glass house. The fungal source collected at local site was more virulent to BPH than exotic ones. The most effective concentration of B. bassiana in controlling BPH is $6x10^{12}$ spores/ha. B. bassiana and M. anisopliae gave good results to control brown planthopper at 7-day after spraying treatment and they were also more effective than the combined treatments and Rotenone. Rice bran medium was the most suitable for mass production of B. bassiana, because it produced much more dried biomass product than other media. Under favorable conditions for developing BPH, fungal applied treatments could give significantly higher yield as compared to insecticidal applied treatments, but under unfavorable conditions for developing BPH, rice yields among treatments were not significantly different.

Key words: fungi, brown planthopper, *Beauveria bassiana, Metarhizium anisopliae*

INTRODUCTION

More than 700 species of fungi, mostly Deuteromycetes and Entomophorales from about 90 genera are pathogenic to insects (Rombach et al. 1986). A complex of fungal pathogens has been identified from pests of rice (Rombach et al. 1986). Moreover, the fungal diseases were favored by high humidity, the microclimate available in the rice fields would be most suitable for the disease development and these fungi have a better prospect in the microbial control of insect pests of rice.

On rice crop, previously published studies on the use of *B. bassiana* fungus for controlling brown plant hopper (BPH) , white back plant hopper (WBPH) and green leaf hopper (GLH) were reported (Aguda et al., 1984). Entomogenous fungi to control BPH,

Nilaparvata lugens Stal. and rice black bug, *Scotinophora coarctata* were used in rice fields in Korea and the Philippines (Rombach et al. 1986; Aguda et al. 1987; Aguda et al., 1988). The control potential of certain entomopathogenic fungi of rice pest has been studied in the south of India (Ramamohan Rao, 1989).

The present study has been taken up with the following objectives

OBJECTIVES

1- To isolate the fungi from naturally infected insects at Omon , Cantho.

2- Pathogenicity of entomogenous fungi against brown plant hopper.
3- Compatibility of entomogenous fungi with botanicals, pesticides and their combinations for the control of brown plant hopper on rice.
4- Mass production of the entomogenous fungi and its use for brown plant hopper management.

MATERIALS AND METHODS

Materials

Equipment for fungal study in laboratory, tools for testing and rearing brown plant hopper in the greenhouse and the necessary things (fertilizers, pesticides, fungi...) and experimental field for testing effectiveness of entomogenuos fungi on brown plant hopper.

Methods

Collection, isolation and purification of the fungus

The insects were found either sticking to the leaf sheath or floating on standing water, being overgrown by a chalky white mass of conidia. The cadavers were collected in sterile glass tube for isolating the causal organism in the laboratory. The fungus culture was purified by single conidium culture on PDA medium subsequently subculturing was done.

The pathogenicity tests

The pathogenicity tests with different isolates of *Beauveria bassiana* and *Metarhizium anisopliae* against third instar nymphs and adults of brown planthopper (BPH) were done in glass house. The conidial concentration was 10^7 conidia ml^{-1} in the prepared suspension with 0.02 percent Tween 80 (R) surfactant (Rombach et al. 1986).

Suitability of different media for mass production of B. bassiana

The suitability of different media for mass production of *B. bassiana* was tested to find out a good and cheap medium for *B. bassiana* culturing, followings the standard microbiological methods

Field efficacy of B. bassiana and M. anisopliae and their integration with botanical products or insecticide

The experiment was laid out in a randomized complete block design with three replications, the plot size was 3x3m. Twelve hills for transplanting rice or wooden frame (60x60cm) for direct seeding rice were enclosed in a nylon cage (60x60x150 cm) in the center of each plot, under suitable conditions for BPH development, we additionally used BPH susceptible variety DS20 and enlarged plot area to 6x5m for testing efficacy of fungi on BPH. The BPHs were released into each cage. Count of living BPH was taken at one day before treatment and at 1, 3, 7, 14 and 21 days after treatment. The reduction of hoppers was also calculated. Grain yield was obtained to evaluate the efficiency of the treatment.

RESULTS AND DISCUSSION

Laboratory and Greenhouse experiments

Comparison of efficacy of fungal sources on BPH

All of isolates of fungi were collected and purified from different sites infected effectively on BPH. Moreover, the local (Omon) isolate was more virulent than the exotic ones, this means that the local isolate showed more its toxicity than the exotic one, BPH nymphs were more susceptible than BPH adults (table1)

Table 1. Efficacy of *B.bassiana* sources in controlling BPH (CLRRI Greenhouse, Dry season 1998)

No	Fungal sources	Efficacy (%) at 7 days after spraying	
		Nymphs	Adults
1	Omon	85.8 a	79.5 a
2	Hyderabad	68.3 d	61.5 c
3	Pant Nagar	80.8 b	77.8 a
4	Tamil Nadu	75.8 c	70.8 b

Means followed by a common letter are not significantly different at the 5% level by DMRT

Determination of the most effective concentration of B. bassiana in controlling BPH

Different concentrations of *B. bassiana* could infect and damage to BPH, ranging from $6 \cdot 10^{12} – 10 \cdot 10^{12}$ spores. ha^{-1} . These concentrations addressed better control BPH as compared to others (table 2) with mortality percentage from 60.3-70.7%. Therefore, the most suitable concentration for controlling BPH is $6x10^{12}$ spores ha^{-1} (Table 2).

Table 2. Efficacy of BPH control of *B. bassiana* at different concentrations at 7-day after spraying (CLRRI Greenhouse, Dry season 1998)

Treatments	7 days after spraying
B. bassiana $2x10^{12}$ spores/ha	6.9 c
B. bassiana $4x10^{12}$ spores/ha	49.9 b
B. bassiana $6x10^{12}$ spores/ha	67.3 a
B. bassiana $8x10^{12}$ spores/ha	67.4 a
B. bassiana $10x10^{12}$ spores/ha	70.7 a
CV (%)	6.5
$LSD_{0.05}$	8.3

Means followed by a common letter are not significantly different at the 5% level by DMRT

Efficacy of BPH control of B. bassiana and M. anisopliae and their combinations with insecticides

B. bassiana and *M. anisopliae* gave good results to control brown planthopper at 7-day after spraying traetment and they were also more effective than the combined treatments and Rotenone. However, these two entomogenous fungi were less effective than Trebon (Table 3).

Table 3. Efficacy of BPH control of *B. bassiana* and *M. anisopliae* and their combinations with insecticides based on BPH mortality percentage (CLRRI Greenhouse, Dry season 1999)

Treatments	**Days after spraying**		
	1	3	7
1. *Beauveria bassiana*	0.0 e	0.0 e	76.2 b
2. *Metarhizium anisopliae*	0.0 e	0.0 e	73.6 bc
3. *B.bassiana* + Trebon	33.8 c	41.3 c	77.6 b
4. *B.bassiana* + Rotenone	25.0 d	32.5 d	69.6 cd
5. *M.anisopliae* + Trebon	35.0 c	42.5 c	68.4 d
6. *M.anisopliae* + Rotenone	25.0 d	32.5 d	68.3 d
7. Trebon	87.5 a	95.0 a	97.3 a
8. Rotenone	66.3 b	62.3 b	69.7 cd
9. Control (Untreated)	0.0 e	0.0 e	0.0 e
CV(%)	12.6	10.1	5.02
$LSD_{.05}$	5.56	5.07	5.06

Means followed by a common letter are not significantly different at the 5% level by DMRT

Suitability of different media for mass production of B. bassiana

The experimental result showed that rice bran medium was the most suitable for mass production of *B. bassiana*, because it produced much more dried biomass product than other media (Table 4)

Table 4. Suitability of different media for mass production of *B. bassiana* (CLRRI Laboratory, Dry season, 1999)

Treatments	Dried biomass weight (gram) (14 days after incubation)
Rice bran	2.38 a
Milled rice	1.25 b
Rice bran + Rice straw	1.19 b
Corn powder	1.04 b
Potato + Dextrose	0.49 c
Sweet potato	0.43 c
Rice straw	0.16 d
Corn cob	0.14 d
$LSD_{.05}$	0.23
CV (%)	18.3

Means followed by a common letter are not significantly different at the 5% level by DMRT

Field experiments

Determination of the most effective concentration of B. bassiana in controlling BPH

Like greenhouse results, the most suitable concentration in controlling BPH is $6x10^{12}$ spores ha^{-1}, it could last 14 days after spraying, moreover, yield was not significantly different among treatments due to late spraying of *B. bassiana* (Table 5).

Table 5. Efficacy of controlling BPH of *B. bassiana* at different concentrations based on BPH mortality percentage (CLRRI, Winter-spring season 1997-1998)

Treatment	7 DAS	14 DAS	Yield (T/ha)
B. bassiana $2x10^{12}$ spores/ha	8.4 b	16.1 b	3.32 bc
B. bassiana $4x10^{12}$ spores/ha	8.3 b	24.7 b	3.45 abc
B. bassiana $6x10^{12}$ spores/ha	46.3 a	56.8 a	3.90 ab
B. bassiana $8x10^{12}$ spores/ha	52.2 a	60.8 a	4.02 a
B. bassiana $10x10^{12}$ spores/ha	50.1 a	64.6 a	3.92 a
Control (Untreated)	-	-	3.17 c
CV (%)	14.2	12.1	9.1
$LSD_{0.05}$	8.8	10.1	0.60

Means followed by a common letter are not significantly different at the 5% level by DMRT

Efficacy of BPH control of B. bassiana and M. anisopliae and their combinations with insecticides

Results of three consecutively seasons from 1998's dry season and 1999's dry season showed that:

Both fungi *B. bassiana* and *M. anisopliae* were effective in controlling BPH at 7 days after spraying and could last 21 days after spraying, with BPH mortality percentage ranging from 60 - 80%, Efficacy of BPH control of both fungi *B. bassiana* and *M. anisopliae* was relatively higher in dry season as compared with wet one (Table 6, 7, 8, 9).

Combinations of both fungi *B. bassiana* and *M. anisopliae* with insecticides such as Trebon, Bassa or Rotenone performed well in controlling BPH and were not significantly different with single fungal treatment, they could reach effectiveness to 80% (Table 6, 7, 8, 9).

Insecticides gave relatively high efficacy in controlling BPH ranging from 70 - 90% on the beginning days after spraying and were significantly different from fungal applied treatments from 7 days after spraying forward, moreover, their effective duration was gradually decreased later on, except Trebon could last longer and be effective highly to BPH (Table 6, 7, 8, 9).

Under favorable conditions for developing BPH, fungal applied treatments could give significantly higher yield as compared to insecticidal applied treatments, but under unfavorable conditions for developing BPH, rice yields among treatments were not significantly different (Table 6, 7, 8, 9).

Table 6. Efficacy of BPH control of *B. bassiana* and *M. anisopliae* and their combinations with insecticides based on BPH mortality percentage (CLRRI, Dry season, 1997 - 1998)

Treatments	Efficacy (%) *					Yield
	1 DAS	3 DAS	7 DAS	14 DAS	21 DAS	(T/ha)
B. bassiana	3.3 e	16.9 d	58.3 d	76.6 a	78.4 a	4.36 a
M. anisopliae	7.5 e	16.0 d	62.4 cd	79.6 a	80.2 a	4.21 ab
B. bassiana + Bassa	15.3 d	43.4 c	61.0 cd	73.9 a	79.6 a	4.22 ab
M. anisopliae + Bassa	20.6 c	43.7 c	69.5 b	74.6 a	79.6 a	4.17 ab
Bassa	74.4 a	78.7 a	76.7 a	53.7 c	39.0 b	3.88 b
Rotenone	33.9 b	53.0 b	66.6 bc	54.4 c	34.1 b	3.95 b
Rotenone + Bassa	35.5 b	56.8 b	68.1 b	63.4 b	39.2 b	4.08 ab
Control (Untreated)	–	–	–	–	–	3.13 c
CV (%)	10.2	10.4	5.5	7.2	12.4	5.3
$LSD_{0.05}$	4.3	7.0	5.6	7.5	11.7	0.36

**: data were transformed into arcsine for analyse*
Means followed by a common letter are not significantly different at the 5% level by DMRT

Table 7. Efficacy of BPH control of *B.bassiana* and *M.anisopliae* and their combinations with insecticides based on BPH mortality percentage (CLRRI, Wet season, 1998)

Treatments	Efficacy (%) *					Yield (T/ha)
	1 DAS	3 DAS	7 DAS	14 DAS	21 DAS	
B. bassiana	14.2 c	22.8 cd	70.4 b	72.7 ab	62.4 a	3.12 ab
M. anisopliae	9.3 c	41.7 bc	71.7 ab	77.2 a	63.3 a	3.15 ab
B. bassiana + Bassa	15.4 c	41.8 bc	73.4 ab	74.9 a	58.3 a	3.22 a
M. anisopliae + Bassa	37.6 b	63.2 ab	79.3 a	80.1 a	55.1 a	3.10 ab
Bassa	80.4 a	74.1 a	60.4 c	46.2 c	28.0 b	2.67 c
Rotenone	41.2 b	68.3 a	67.7 bc	54.4 bc	27.3 b	2.80 bc
Control (Untreated)	–	–	–	–	–	1.55 d
CV (%)	31.8	37.4	7.4	18.7	32.6	8.0
$LSD_{0.05}$	16.0	29.7	8.0	19.2	24.4	0.38

**: data were transformed into arcsine for analyse*
Means followed by a common letter are not significantly different at the 5% level by DMRT

Table 8. Efficacy of BPH control of *B.bassiana* and its combinations with insecticides based on BPH mortality percentage (CLRRI, Dry season, 1998-1999)

Treatments	**Efficacy (%) ***				**Yield**
	1 NSP	3 NSP	7 NSP	14 NSP	(T/ha)
B. bassiana	8.7 c	10.5 b	72.4 b	69.1 ab	3.97 a
B. bassiana + Trebon	35.3 b	40.7 b	83.0 a	74.7 a	3.70 a
B. bassiana + Rotenone	31.2 b	39.2 c	79.9 ab	71.5 a	3.75 a
Trebon	70.8 a	84.6 a	79.7 ab	58.4 b	4.04 a
Rotenone	62.6 a	74.2 a	83.8 a	59.1 b	3.78 a
Control (Untreated)	-	-	-	-	3.08 b
CV (%)	12.9	16.1	8.2	9.8	8.6
$LSD_{0.05}$	10.13	15.1	10.2	12.3	0.58

**: data were transformed into arcsine for analyse*
Means followed by a common letter are not significantly different at the 5% level by DMRT

Table 9. Efficacy of BPH control of *M. anisopliae* and its combinations with insecticides based on BPH mortality percentage (CLRRI, Dry season, 1998-1999)

Treatments	Days after spraying				Yield
	1*	3*	7**	14**	(t/ha)
M. anisopliae	10.2 c	46.7 c	74.7 bc	76.1 b	3.65 a
M. anisopliae + Trebon	17.0 b	67.9 b	71.3 c	71.4 b	3.61 a
M. anisopliae + Rotenone	23.2 b	63.3 b	78.0 bc	73.6 b	3.72 a
Trebon	35.1 a	87.3 a	91.7 a	99.9 a	3.56 a
Rotenone	17.0 b	62.8 b	81.1 b	81.9 b	3.55 a
Control (Untreated)	-	-	-	-	3.23 b
CV(%)	17.2	13.3	5.3	7.9	6.8
$LSD_{0.05}$	6.6	16.6	7.9	11.1	0.45

**: data were transformed into arcsine for analyse*
***: data were transformed into $\sqrt{x+0.5}$ for analyse*
Means followed by a common letter are not significantly different at the 5% level by DMRT

CONCLUSIONS AND SUGGESTION

Conclusions

- The fungal source collected at local site was more virulent to BPH than exotic ones.
- The most effective concentration of *B. bassiana* in controlling BPH is $6x10^{12}$ spores/ha
- Efficacy of BPH control of both fungi *B. bassiana* and *M. anisopliae* was relatively high ranging from 60 - 80% and they could last rather long from 7 to 21 days after spraying.

- Combinations of fungi and insecticides with one-half recommended concentration in order to enhance their active duration and efficacy after spraying were obtained from the experiments

Suggestion

- The potential of these entomogenous fungi for controlling BPH needs to be continued studying on other rice insect pests
- The simple methods to produce fungal biomass has to be demonstrated in small scale areas before developing in large scale areas..

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TÓM TẮT

Tiềm năng sinh học của nấm ký sinh trong phòng trị rầy nâu trên lúa

Một trong những biện pháp phòng trừ sinh học là sử dụng các loại nấm gây bệnh cho côn trùng, trong đó có nấm phấn trắng *Beauveria bassiana* và nấm phấn xanh *Metarhizium anisopliae* đã được nghiên cứu. Nồng độ hữu hiệu của *Beauveria bassiana* là 6 x 10^{12} bào tử / ha có tác dụng trừ rầy nâu tốt nhất. Một số môi trường để nhân sinh khối nấm cũng được xác định. Cám gạo có hiệu qủa đáng tin cậy, với trọng lượng sinh khối cao nhất. Sử dụng chế phẩm nấm trắng *Beauveria bassiana* và nấm xanh *Metarhizum anisopliae* có hiệu lực 7 ngày sau khi phun, trên đồng ruộng và nhà lưới, tương đương với hiệu lực của Trebon và Rotenone.