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Pathogenicity of certain indigenous isolates of entomopathogenic fungi against rice leaf folder, *Cnaphalocrocis medinalis* (Guenee)

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ABSTRACT: The pathogenicity of 42 local isolates of entomopathogenic fungi including *Beauveria bassiana* (22), *Metarhizium anisopliae* (three), *M. flavoviride* (one), *Nomuraea rileyi* (four), *Paecilomyces* sp. (one), *Aspergillus* spp. (five), *Fusarium* spp. (three), *Zoophthora radicans* (two) and *Entomophthora* sp. (one) originating from a range of insect species were evaluated against third instar larvae of *Cnaphalocrocis medinalis*. All the isolates tested were pathogenic to the pest at varying degrees. In single-dose (1×10^7 conidia / ml) time-mortality assay, only five isolates of *B. bassiana*, *viz.*, BbCm KKL 1100, BbCm TVR 0101, BbCm ADT 0101 (isolated from *C. medinalis*), BbMp KKL 1195 (isolated from *Marasmia patnalis*) and BbOn KKL 0597 (isolated from *Oxya nitidula*), were superior to all other isolates with BbCm KKL 1100 having the lowest LT_{50} value of 7.81days. In the multiple dose assays (ranging from 1×10^4 to 1×10^9 conidia / ml), the Karaikal isolate of *B. bassiana* (BbCm KKL 1100 isolated from *C. medinalis*) was found to be the most virulent. The LC_{50} value estimated at 13 days post-inoculation from three independent bioassays for this isolate was 2.8×10^3conidia / ml. This was closely followed by BbOn KKL 0597 (isolated from *Oxya nitidula*) with a LC_{50} of 2.2×10^4 conidia / ml. Based on the time-dose mortality factor, the *B. bassiana* isolate BbCm KKL 1100 has been selected as a potential microbial agent for further investigations in field conditions.

KEY WORDS: *Cnaphalocrocis medinalis*, entomopathogenic fungi, pathogenicity

INTRODUCTION

The rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae) is a major pest of the rice growing countries of Asia (Khan and Joshi, 1990) and other rice growing tracts of the world necessitating a serious consideration for efficient management of this pest. Over the years, control of rice leaf folders has been almost exclusively based on use of chemical insecticides. There are long-term concerns about insecticide exposure risks, health and environmental hazards, residue persistence, development of resistance and elimination of natural enemies. Besides, some of the insecticides reported effective earlier such as carbofuron and quinalphos, are also now being reported to cause leaf folder resurgence (Nadarajan and Skaria, 1988; Devanesan *et al.*, 1995). Efforts at

breeding rice varieties resistant to leaf folder and conservation of beneficial natural enemies have met with only limited success, and leaf folder threat still remains unabated. Therefore, effective alternative control measures are necessary if long-term suppression of leaf folders is to be attained. Biological control, including the use of entomopathogenic fungi, offers a sound alternative management strategy against several insect pests.

In rice ecosystem, spontaneous epizootics of entomomycoses are very common (Ramamohan Rao, 1989; Narayanasamy, 1993). In nature, larval populations of rice leaf folder are susceptible to several species of entomopathogenic fungi including *Beauveria bassiana* (Bals.) Vuill. (Ambethgar, 1997) and *Zoophthora radicans* (Brefeld) Batko in rice fields (Ambethgar, 2002). Preliminary pathogenicity tests using single isolates of *B. bassiana* and *Metarhizium anisopliae* (Rama Mohan Rao, 1989) against *C. medinalis* larvae were found promising in earlier investigations. Keeping this in view, the present experiments were conducted to evaluate the isolates of indigenously isolated entomopathogenic fungi from diverse ecosystems in order to select the most virulent isolates for the control of rice leaf folder.

MATERIALS AND METHODS

Rearing of *C. medinalis* larvae

The stock culture of *C. medinalis* larvae was maintained under glasshouse conditions at Rice Breeding Station, Tamil Nadu Agricultural University, Coimbatore, by adopting the methods devised at IRRI (Waldbauer and Marciano, 1979). For this, both male and female moths of *C. medinalis* were collected from the field and released into oviposition cages (45 x 45 x 60 cm^3) containing susceptible rice plants of cv IR 50. Ten per cent honey solution was provided in the cages as food source for adults. The early third instar larvae (6-7 day old) collected from the culture were used for all the experiments.

Source of fungal isolates

The fungal isolates tested in this study were: (a) isolated from a range of rice insects collected in the survey conducted in Tamil Nadu and Pondicherry states; (b) obtained from Experimental Research Farm, Agriculture Department, Karaikal, (c) Regional Research Station, TNAU, Vridhachalam; (d) Sugarcane Breeding Institute, Coimbatore, and (e) Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute, New Delhi. The details on the entomopathogenic fungi, isolate code, the insects from which various isolates were derived and location of collection are presented in Table 1.

Culturing fungi and preparation of conidial suspension

All fungal isolates were initially passed through the larvae of *C. medinalis* and the type-isolates were reisolated on Sabouraud Dextrose Agar medium with 0.25 percent w/v yeast extract (SDAY). After re-isolation from the cadavers, the isolates were purified by sub-culturing in SDAY slants. All the isolates were maintained at 26°C ± 2°C in a B.O.D incubator. The conidia were harvested from 14-day-old sporulated cultures by rinsing the surface of the Petriplates using 50ml of sterile distilled water containing 0.02 per cent Tween 80®. Prior to bioassays, the viability of the conidia was determined. For each isolate, a small drop of about 0.1 ml of the conidial suspension was spread separately on microscope slides smeared with SDAY. The slides were incubated at 26°C for 36-48 h to allow maximum germination under these conditions. Germination percentage was recorded by direct examination at 200 X under a phase contrast microscope. The initial conidial population in the stock suspensions was assessed using an improved Neubauer haemocytometer under a phase contrast microscope. From the stock suspension, desired concentrations of conidia, *viz.* 1 x 10^4, 10^5, 10^6, 10^7, 10^8 and 10^9 per ml were prepared by serial dilution with aqueous solution of Tween 80® (0.02 %).

Screening assays

The screening experiments with the different isolates of entomopathogenic fungi had two major components: an initial single-dose screening assay, followed by multiple-dose mortality assays

Table 1. Fungal isolates screened against third instar larvae of *C. medinalis*

Isolate code no.	Insect host	Location
Beauveria bassiana		
BbCm KKL 1194	*Cnaphalocrocis medinalis*	Karaikal
BbCm KKL 1095	*Cnaphalocrocis medinalis*	Karaikal
BbMp KKL 1195	*Marasmia patnalis*	Karaikal
BbCm CBE 1100	*Cnaphalocrocis medinalis*	Coimbatore
BbCm CBE 1200	*Cnaphalocrocis medinalis*	Coimbatore
BbCm KKL 1100	*Cnaphalocrocis medinalis*	Karaikal
BbCm CBE 0101	*Cnaphalocrocis medinalis*	Coimbatore
BbCm ADT 0101	*Cnaphalocrocis medinalis*	Aduthurai
BbCm TVR 0101	*Cnaphalocrocis medinalis*	Tiruvarur
BbCm TIR 0101	*Cnaphalocrocis medinalis*	Tirur
BbCm CDL 0201	*Cnaphalocrocis medinalis*	Cuddalore
BbCm ASD 0201	*Cnaphalocrocis medinalis*	Ambasamuthram
BbSi TIR 0201	*Scirpophaga incertulas*	Tirur
BbHa SMD 0299*	*Helicoverpa armigera*	Semmedu
BbSl SMD 0299*	*Spodoptera litura*	Semmedu
Bb Scl TIR 0101	*Scotinophara lurida*	Tirur
BbLa CBE 0201	*Leptocorisa acuta*	Coimbatore
BbOn KKL 0597	*Oxya nitidula*	Karaikal
BbOn CBE 1200	*Oxya nitidula*	Coimbatore
Bb SBI 0101*	Unknown	Coimbatore
Bb ITCC 4512*	Unknown	IARI, New Delhi
BbPf VRI 1198*	*Plocaederus ferrugineus*	RRS, Vridhachalam
Metarhizium anisopliae		
MaHb CBE 1200	*Hieroglyphus banian*	Coimbatore
MaOn CBE D201	*Oxya nitidula*	Coimbatore
Ma ITCC 4514**	Unknown	New Delhi
Metarhizium flavoviride		
Mf ITCC 4984**	Unknown	New Delhi
Nomuraea rileyi		
NrHa SMD 0199**	*Helicoverpa armigera*	Semmedu
NrSl SMD 0299**	*Spodoptera litura*	Semmedu

NrPu KKL 0295	*Pseudaletia unipuncta*	Karaikal
NrPu KKL 1196	*Pseudaletia unipuncta*	Karaikal
Paecilomyces sp.		
PfSI TIR 0201	*Scotinophara lurida*	Tirur
Aspergillus spp.		
AfCm KKL 1101	*Cnaphalocrocis medinalis*	Karaikal
AfCm TIR 0201	*Cnaphalocrocis medinalis*	Tirur
AfOn CBE 1200	*Oxya nitidula*	Coimbatore
AfOn KKL 0101	*Oxya nitidula*	Karaikal
AfOn KKL 0201	*Oxya nitidula*	Karaikal
Fusarium spp.		
FpCm CBE 0201	*Cnaphalocrocis medinalis*	Coimbatore
FpCm KKL 1101	*Cnaphalocrocis medinalis*	Karaikal
FpCm ADT 1201	*Cnaphalocrocis medinalis*	Aduthurai
Zoophthora radicans		
ZrCm KKL 1194	*Cnaphalocrocis medinalis*	Karaikal
ZrCm KKL 1201	*Cnaphalocrocis medinalis*	Karaikal
Entomophthora sp.		
EcSi TIR *0201*	*Scirpophaga incertulas*	Tirur

*Isolates originated from other than rice ecosystem; **isolates other than rice ecosystem

(Theunis and Aloali, 1998). Third instar (6-7 day-old) larvae of *C. medinalis* were bioassayed for their susceptibility to the different isolates. Each fungal isolate was assayed by dip-ping the larvae in batches of thirty in respective concentration of spore suspension for 10 seconds as described by Negasi *et al.* (1998).

Single-dose screening assay

The single-dose screening assay was carried out using the 42 isolates of entomopathogenic fungi (Table 1). A standard dose of 1 x 10^8 conidia/ml in 0.05 per cent Tween 80® was prepared for each isolate. Thirty greenhouse reared third instar *C. medinalis* larvae per isolate were surface sterilized with sodium hypo-chlorite solution (0.1%) and inoculated by immersing them in 10 ml of conidial suspension for 10 seconds. Control larvae were immersed in blank Tween 80® (0.05%). Following inoculation, the larvae were placed for 5 min on filter paper to drain excess water to avoid development of the saprophytic fungi under the condition of excess dampness on the larvae during post-inoculation periods. The treated larvae were carefully transferred to mylar-film rearing cages containing 45 day-old single rice plant of cv IR 50 raised in clay pots (10 cm diam 15 cm ht) with 2.5 cm standing water. The upper open end of cages was wrapped using cotton cloth pieces. The larvae were monitored daily for food consumption, development and mortality owing to mycoses. To determine LT_{50} of the fungal isolates, the larval mortality count was recorded at 24 h interval until thirteenth day of treatment or till the larvae entered encysting pupal stage. From the tenth day data, percentage of larval mortality due to observable mycosis was calculated and sample mean of the time to death was calculated

for each of the assay. Dead larvae were removed to prevent extra contamination spreading through fungal inoculum. The moribund cadavers were incubated for 48h in a moist chamber and monitored for hyphal emergence and external sporu-lation. In case of fungus emergence, mycelia from ten randomly selected cadavers per isolate were sampled and cultured on SDAY plates for confirming the identity. Based on the death rate, mean percent mortality was worked out for different intervals.

Multiple-dose bioassay

The most promising isolates of fungi identified in the virulence screen were assessed further in a series of multi-ple dose mortality assays. The bioassays were carried out three times using six different conidial concentrations con-taining 1 x 10^4, 10^5, 10^6, 10^7, 10^8 and 10^9 viable conidia/ml in Tween 80^a (0.05%) as surfactant. Thirty third instar *C. medinalis* larvae per concentration were directly dipped in 10ml of conidial suspension for 10 seconds. Three replications were maintained for each concentration. The control larvae numbering 30 were immersed in blank Tween 80^a(0.05%) only. Following inoculation, the larvae were placed on filter paper to permit excess conidial suspension to drop off before they were transferred to the rearing cages. Larval mortality was recorded at 24 h intervals until the thirteenth day of treatment or until attaining encysting of larvae. Dead larvae were removed and incubated for 48 h in a moist chamber to check for mycoses. The tenth day mortality data were con-sidered for the probit analysis.

Statistical analysis

All the analyses were carried out in SPS Advanced Statistical Program version 6.1. Time-dosage mortality data were subjected to probit analysis as prescribed by Finney (1962). Larval mortality in control was corrected using Abbott's correction (Abbott, 1925). Angular values of mortality were then subjected to analysis of variance (ANOVA) and means were compared by Duncan's multiple-range test (Gomez and Gomez, 1964). The LT_{50} and LC_{50} values were computed with the corresponding 95% confidence limits (Finney, 1962). Regression analysis of probit mortality log conidia concentration was calculated from the number of live larvae at the beginning and end of each treatment. Before pooling the replicate, a chi^2 parallelism test was done. The regression equation was submitted to a chi^2 goodness of fit test.

RESULTS AND DISCUSSION

Selection of an isolate with desirable characteristics is an essential part of a successful microbial control programme. The knowledge on the efficacy of entomopathogenic fungi against rice leaffolders is limited (Aguda and Rombach, 1987; Narayanasamy, 1993). In the present study, germination of all fungal isolates ranged from 89 to 94 per cent. Successful microbial control with fungal entomopathogens is dependent on fungal strains with a high rate of infection (Burges and Thomson, 1971). Isolates of fungal entomopathogens from target insects, or more closely related to them, are not necessarily the most virulent microbial agents (Prior, 1990), and for this reason isolates from heterogeneous insect hosts were also evaluated.

Single-dose screening assay

In the initial single-dose screening assays, all the 42 isolates tested were able to infect *C. medinalis* larvae, but found to exhibit a wide variation in their infectivity. The mean mortality induced by various fungal isolates at standard conidial concentration of 1 x 10^7 conidia/ml on day 7 and 10 are mentioned in Table 2. The resulting LT_{50} value of respective isolate against *C. medinalis* is shown with acceptable c^2 values ($P< 0.05$) in Table 3.

Among the fungi tested, *B. bassiana* isolates showed greater virulence than isolates of rest of the fungi. With the exception of BbOn KKL 0597, which was isolated from *Oxya nitidula* (rice grasshopper), all other isolates derived from hosts other than *C. medinalis* showed relatively low potency in terms of LT_{50} to *C. medinalis* as evident from standard-dose screening assay. This suggests

Table 2. Mean mortality caused by different isolates in single dose screening assay against third instar *C. medinalis* larvae

Fungal isolate	Per cent mortality		Rank
	7 DAT	10 DAT	
Beauveria bassiana			
BbCm KKL 1194	55.55 (48.19)^{efg}	73.33 (58.93)$^{h-k}$	9
BbCm KKL 1095	63.33 (52.74)^{de}	77.77 (61.88)^{efg}	6
BbMp KKL 1195	65.55 (54.06)^{cd}	79.99 (63.48)^{e}	5
BbCm CBE 1100	53.33 (46.91)^{fgh}	71.11 (57.49)$^{j-m}$	11
BbCm CBE 1200	62.22 (52.07)^{def}	75.55 (60.37)$^{f-i}$	7
BbCm KKL 1100	83.33 (65.97)^{a}	95.55 (77.99)^{a}	1
BbCm CBE 0101	54.44 (47.55)^{efg}	72.22 (58.20)$^{j-l}$	10
BbCm ADT 0101	73.33 (58.93)^{bc}	86.66 (68.57)^{d}	3
BbCm TVR 0101	74.44 (59.64)^{b}	92.22 (73.87)^{b}	2
BbCm TIR 0101	62.22 (52.07)^{def}	75.55 (60.37)$^{f-i}$	7
BbCm CDL 0201	61.00 (51.35)^{def}	76.66 (61.11)$^{e-h}$	8
BbCm ASD 0201	51.11 (45.63)^{gh}	64.44 (53.39)^{opq}	12
BbSi TIR 0201	8.88 (17.27)^{nop}	52.22 (46.27)^{rs}	24
BbHa SMD 0299 *	53.33 (46.91)^{fgh}	70.00 (56.79)$^{k-n}$	11
BbSl SMD 0299 *	48.88 (44.36)^{ghi}	64.44 (53.39)^{opq}	13
BbScl TIR 0101	63.33 (52.74)^{de}	73.33 (58.90)$^{h-k}$	6
BbLa CBE 0201	48.88 (44.36)^{ghi}	68.88 (56.10)l^{mn}	13
BbOn KKL 0597	70.56 (56.80)^{bcd}	90.00 (71.56)^{c}	4
BbOn CBE 1200	55.55 (48.18)^{efg}	68.88 (56.10)^{lmn}	9
Bb SBI 0101	62.22 (52.07)^{def}	74.44 (59.64)$^{g-j}$	7
Bb ITCC 4512	65.55 (54.06)^{cd}	78.88 (62.66)^{ef}	5
BbPf VRI 1198	26.66 (31.06)^{k}	52.22 (46.27)^{rs}	17
Metarhizium anisopliae			
MaHb CBE 1200	3.33 (8.49)^{qr}	48.88 (44.36)st	27
MaOn CBE 0201	17.77 (24.91)^{lm}	63.33 (52.74)^{pq}	21
Ma ITCC 4514	2.22 (4.98)^{r}	37.77 (37.89)^{w}	28
Metarhizium flavoviride			
Mf ITCC 4984	21.11 (27.33)kl	45.55 (42.44)^{tu}	28

Nomuraea rileyi			
NrSl SMD 0199	35.55 (36.59)^{ij}	61.11 (51.42)st	16
NrHa SMD 0299	51.11 (45.63)^{gh}	66.66 (54.73)^{nop}	12
NrPu KKL 0299	51.11 (45.63)^{gh}	70.00 (56.79)$^{k-n}$	12
NrPu KKL 1196	22.22 (7.00)^{r}	55.55 (48.18)^{r}	28
Paecilomyces farinosus			
PfSl TIR 0201	44.44 (41.80)^{hij}	62.22 (52.08)^{q}	14
Aspergillus spp.			
AfCm KKL 1101	21.11 (27.33)st	45.55 (42.44)^{tu}	20
AfCm TIR 0201	5.55 (13.47)^{pq}	39.99 (39.22)^{vw}	26
AfOn CBE 1200	11.11 (19.42)^{no}	21.11 (28.63)^{y}	23
AfOn KKL 0101	25.55 (30.35)^{kl}	54.44 (47.54)^{r}	18
AfOn KKL 0201	14.44 (21.16)^{mn}	44.44 (41.80)u	22
Fusarium spp.			
FpCm CBE 0201	1.11 (3.50)^{r}	37.77 (37.91)^{w}	29
FpCm KKL 1101	41.10 (39.85)^{ij}	67.77 (55.43)^{mno}	15
FpCm ADT 1201	22.22 (28.10)^{kl}	48.88 (44.36)st	19
Zoophthora radicans			
ZrCm KKL 1194	48.88 (44.36)^{ghi}	68.88 (56.10)^{lmn}	13
ZrCm KKL 1201	48.88 (44.36)^{ghi}	67.77 (55.41)^{mno}	13
Entomophthora sp.			
EaSi TIR 0201	1.11 (3.50)^{r}	28.88 (32.50)^{x}	29

Mean separation in a column by DMRT at 5 % level

Table 3. Probit analysis of time-mortality response of third instar larvae of *C. medinalis* to some entomopathogenic fungal isolates @ 1 x 10^7 conidia mL^{-1}

Fungal isolates	χ^2	Regression equation	LT_{50} (days)	Fiducial limits	
				Lower	Upper
Beauveria bassiana					
BbCm KKL 1194	41.74	Y = 1.5423 + 7.5259x	7.40	7.06	7.76
BbCm KKL 1095	5.55	Y = 1.0791 + 4.9762x	6.14	5.61	6.71
BbMp KKL 1195	4.65	Y = 1.6565 + 4.3260x	5.93	5.33	6.59
BbCm CBE 1100	54.61	Y = 0.7662 + 6.4579x	7.81	7.40	8.25
BbCm CBE 1200	47.95	Y = 1.0206 + 6.7767x	7.73	7.34	8.14
BbCm KKL 1100	6.03	Y = 1.0149 + 8.4364x	5.16	4.84	5.51

BbCm CBE 0101	45.30	Y = 1.0654 + 6.8305x	7.73	7.34	8.13
BbCm ADT 0101	34.69	Y = 2.1288 + 8.3784x	7.10	6.79	7.41
BbCm TVR 0101	7.60	Y = 1.0162 + 5.3853x	5.49	4.97	6.07
BbCm TIR 0101	41.37	Y = 1.2722 + 7.0969x	7.65	7.28	8.04
BbCm CDL 0201	48.28	Y = 1.0236 + 6.8414x	7.59	7.22	7.99
BbCm ASD 0201	70.12	Y = 3.5390 + 9.0672x	8.74	8.38	9.12
BbSi TIR 0201	18.85	Y = 4.8100 + 9.7366x	10.17	9.71	10.66
BbHa SMD 0299	72.88	Y = 3.5662 + 9.1461x	8.64	8.29	9.00
BbSl SMD 0299	79.43	Y = 3.2747 + 8.7153x	8.90	8.52	9.30
BbScl TIR 0101	59.15	Y = 0.5949 + 6.3379x	7.63	7.23	8.06
BbLa CBE 0201	34.56	Y = 1.3065 + 6.9657x	8.04	7.64	8.47
BbOn KKL 0597	12.08	Y = 0.2489 + 7.0383x	5.57	5.18	5.99
BbOn CBE 1200	45.31	Y = 0.9922 + 6.6984x	7.84	7.44	8.27
Bb SBI 0101	85.37	Y = 3.6272 + 9.3279x	8.41	8.08	8.75
Bb ITCC 4512	53.25	Y = 1.2864 + 7.2090x	7.44	7.10	7.82
BbPf VRI 1198	35.67	Y = 4.5389 + 9.4814x	10.14	9.67	10.63
Metarhizium anisopliae					
MaHb CBE 1200	20.50	Y = 5.1978 + 9.8182x	10.93	10.40	11.48
MaOn CBE 0201	38.49	Y = 5.1351 + 10.3343x	9.56	9.19	9.96
Ma ITCC 4514	8.90	Y = 4.7903 + 9.1578x	11.72	11.10	12.43
Metarhizium flovoviride					
Mf ITCC 4984	41.19	Y = 4.3300 + 9.2035x	10.32	9.82	10.84
Nomuraea rileyi					
NrSl SMD 0199	28.10	Y = 1.3971 + 6.7277x	8.93	8.43	9.46
NrHa SMD 0299	41.27	Y = 0.9923 + 6.5974x	8.10	7.67	8.55
NrPu KKL 0299	47.81	Y = 0.8528 + 6.4548x	8.06	7.63	8.52
NrPu KKL 1196	21.92	Y = 5.4599 + 10.1885x	10.63	10.16	11.13
Paecilomyces sp.					
PfSl TIR 0201	63.26	Y = 3.5270 + 9.0112x	8.83	8.46	9.22
Aspergillus spp.					
AfCm KKL 1101	44.60	Y = 4.2321 + 9.0469x	10.48	9.95	11.03
AfCm TIR 0201	37.15	Y = 4.6598 + 9.1088x	11.49	10.86	12.16
AfOn CBE 1200	15.18	Y = 3.1319 + 7.2967x	13.00	11.88	14.26
AfOn KKL 0101	43.08	Y = 4.5298 + 9.4808x	10.12	9.65	10.60
AfOn KKL 0201	44.22	Y = 4.6201 + 9.0801x	11.47	10.83	12.13

Fusarium spp.					
FpCm CBE 0201	8.14	Y = 4.8509 + 9.2165x	11.71	11.05	12.43
FpCm KKL 1101	61.21	Y = 5.1763 + 10.5043x	9.31	8.95	9.67
FpCm ADT 1201	46.38	Y = 4.3691 + 9.2597x	10.27	9.78	10.79
Zoophthora radicans					
ZrCm KKL 1194	54.40	Y = 0.8114 + 6.3941x	8.10	7.67	8.57
ZrCm KKL 1201	51.32	Y = 0.5826 + 6.0642x	8.32	7.85	8.84
Entomophthora sp.					
EaSi TIR 0201	23.10	Y = 4.0119 + 8.1625x	12.71	11.80	13.68

Table 4. Virulence of isolates to *C. medinalis* larvae as expressed by LT_{50} at 10^7 conidia mL^{-1}

High virulence (LT_{50} <7 days)	Moderate virulence(LT_{50} 7-9 days)	Low virulence (LT_{50} 9-11 days)	Avirulent isolates (LT_{50} > 11 days)
BbCm KKL 1100	BbCm KKL 1194	FpCm KKL 1101	AnOn KKL 0201
BbCm TVR 0101	BbCm CBE 1100	MaOn CBE 0201	AfCm TIR 0201
BbOn KKL 0597	BbCm CBE 1200	AfOn KKL 0101	FpCm CBE 0201
BbMp KKL 1195	BbCm CBE 0101	BbSi TIR 0201	*Ma* ITCC *4514*
BbCm KKL 1095	BbCm ADT 0101	BbPf VRI 1198	EaSi TIR 0201
	BbCm TIR 0101	FpCm ADT 1201	AfOn CBE 1200
	BbCm CDL 0201	Mf ITCC 4984	
	BbCm ASD 0201	AfCm KKL 1101	
	BbHa SMD 0299	NrPu KKL 1196	
	BbSI SMD 0299	MaHb CBE 1200	
	BbScl TIR 0101		
	BbLa CBE 0201		
	BbOn CBE 1200		
	Bb SBI 0101		
	Bb ITCC 4512		
	NrSI SMD 0199		
	NrHa SMD 0299		
	NrPu KKL 1095		
	Pf SC TIR 0201		
	ZrCm KKL 1194		
	ZrCm KKL 1201		

Table 5. Probit analyses of dose-mortality response of third instar larvae of *C. medinalis* to *Beauveria* isolates

B. bassiana isolate	χ^2 (n^{-2})*	Regression equation (Slope)	LC_{50} conidia (mL^{-1})	Fiducial limits	
				Lower	Upper
BbCm KKL 1095	2.02	Y = 2.1184 + 3.8635x	$3.7 X10^5$	8.3×10^4	2.0×10^6
BbMp KKL 1195	1.64	Y = 2.2103 + 3.7850x	$2.8 X10^5$	6.3×10^4	1.6×10^6
BbCm KKL 1100	1.51	Y = 2.4947 + 3.9237x	$2.8 X10^3$	2.2×10^2	1.2×10^5
BbCm TVR 0101	0.93	Y = 1.5841 + 4.7870x	$1.4 X10^5$	4.2×10^4	6.0×10^5
BbOn KKL 0597	0.57	Y = 3.5485 + 2.6957x	$2.2 X10^4$	4.2×10^3	1.6×10^5

* All the lines are significantly a good fit ($P < 0.05$)

Table 6. Time-mortality relations of *B. bassiana* isolates against *C. medinalis* at different concentrations of conidia mL^{-1}

B. bassiana isolate	LT_{50} (in days)					
	10^4	10^5	10^6	10^7	10^8	10^9
BbCm KKL 1095	10.53	8.82	7.93	6.20	6.00	5.92
BbMp KKL 1195	11.98	9.70	7.78	6.98	6.13	6.00
BbCm KKL 1100	6.64	5.83	5.50	5.46	5.00	4.80
BbCm TVR 0101	6.99	5.97	5.90	5.78	5.49	5.36
BbOn KKL 0597	6.78	5.93	5.84	5.56	5.38	5.22

All lines are significantly good fits ($P<0.05$)

that neither the host of origin nor the phylogenetic relationship between potential hosts is a reliable indicator of the probable virulence of a specific fungal isolate to a specific host insect (Roberts and Yendol, 1971). The isolates derived from rice stemborer, *Scirpophaga incertulas* (BbSi TIR 0201), and cerambycid borer, *Plocaederus ferrugineus* (BbPf VRI 1198) displayed poor virulency than the rest of the *B. bassiana* isolates.

The isolates of *Metarhizium*, *Nomuraea*, *Paecilomyces*, *Aspergillus*, *Fusarium*, *Entomophthora* and *Zoophthora* showed significantly low virulence and recorded higher LT_{50} against larvae of *C. medinalis*. No mortality was noticed in the control until the termination of the experiment. Based on LT_{50} values, the virulency of the 42 fungal strains was classified into four categories as shown in Table 4. The isolates that had an LT_{50} value of less than 7 days were selected for second phase multiple-dose-mortality assays.

Multiple-dose mortality assays

The results on probit analysis of dose-mortality response of third instar *C. medinalis* larvae to selected *B. bassiana* isolates are presented in Table 5. The time at which the treated larvae died of mycosis varied considerably among the isolates tested. The earliest death induced by the highest concentration (1×10^9 conidia/ml) occurred on day 4.8 with BbCm KKL 1100; on day 5.3 with BbCm TVR 0101 and on day 5.5 with BbOn KKL 0595. The initial death induced by the lowest

concentration (1 x 10^4 conidia/ml) occurred on 6.6 day with BbCm KKL 1100. The lowest concentration of the isolate BbMp KKL 1195 took a long period of 11.9 days to reach 50 per cent mortality. The rate of increase in mortality was positively correlated with conidial concentration for all *B. bassiana* isolates.

The mortality rate also increased rapidly for the more virulent isolate BbCm KKL 1100. The isolates had longer LT_{50} especially at lower conidial concentrations (Table 6), but no distinct grouping of isolates was apparent in the higher conidial concentrations. However, due to the high virulence of BbCm KKL 1100 to *C. medinalis*, this isolate was selected for further field evaluation in the microbial control programme of rice leaf folder.

Furthermore, field application of the fungal isolate should allow final selection before *B. bassiana* is used as a microbial pest control agent. In addition, other factors such as mass production, conidial production ability, development of formulation and safety of the selected isolate must be studied both under laboratory and field conditions before being recommended as mycoinsecticide since conservation of natural enemies in the rice ecosystem is more meaningful.

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