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Laboratory evaluation of four fungal pathogens against the teak defoliator, *Hyblaea puera* (Cramer) (Lepidoptera: Hyblaeidae)

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ABSTRACT: Effect of four entomopathogenic fungi, *Beauveria bassiana*, *B. brongniartii, Paecilomyces fumosoroseus* and *Metarhizium anisopliae*, isolated from different host insects, was tested in the laboratory against the teak defoliator, *Hyblaea puera* (Cramer) (Lepidoptera: Hyblaeidae) to assess their virulence and efficiency. Five different concentrations, $2x10^6$, $4x10^6$, $6x10^6$, $8x10^6$ and $1x10^7$ conidia ml^{-1} were directly and indirectly applied onto third instar larvae of *H. puera*. All the four fungi tested under both application methods were pathogenic to *H. puera* with higher efficiency in direct application than indirect application. At 9 days after exposure, the mean per cent mortality obtained was 100, 93.02, 93.02 and 66.74%, respectively, for *B. bassiana*, *B. brongniartii, P. fumosoroseus* and *M. anisopliae* at the highest concentration of 1 x 10^7 conidia ml-1 in direct application. Mortality of the larvae varied according to the concentration of conidia, method of application and fungi. The lowest LC_{50} (1.89 x 10^6 conidia ml^{-1}) and LT_{50} (91.18 hr) values obtained for *B. bassiana* in direct application showed that *B. bassiana* was more pathogenic to *H. puera* than the other three fungi.

KEY WORDS: *Beauveria bassiana, B. brongniartii,* entomopathogenic fungi*, Hyblaea puera, Metarhizium anisopliae, Paecilomyces fumosoroseus*

INTRODUCTION

The teak defoliator, *Hyblaea puera* (Cramer) (Lepidoptera: Hyblaeidae) is recognized as one of the most important pests of teak. In India, its outbreaks occur almost every year over extensive areas. In Kerala, repeated outbreaks of the pest are common in each year, with two to three total defoliations during the early part of the growth season (Beeson, 1941; Nair *et al.*, 1985). Every year, high intensity outbreaks of *H. puera* occur immediately after the pre–monsoon showers in late February or early March in Kerala and can be witnessed up to July. Defoliation by this pest has been assumed to cause heavy loss of about 44% of the potential volume increment in 4–8 year old teak plantations (Nair *et al*., 1996). Application of commercial preparations of *Bacillus thuringiensis* has been found effective against *H. puera* in some experimental plots and private plantations in India (Senguttuvan *et al*., 2000; Loganathan and David, 2000). In Thailand, *B. thuringiensis* has been applied using fogging machines and aircraft, particularly for high value plantations (Hutacharern *et al*., 1993). Since *B. thuringiensis* is the only commercially available control agent, other alternatives should also be investigated. Entomopathogenic fungi are important microbial control agents against agricultural insect pests. In forestry, during the rainy season, temperature and humidity conditions in the plantations and nursery are suitable for the application of entomopathogenic fungi in the field. In the present study, pathogenicity of four entomopathogenic fungi, *viz., Beauveria bassiana, B. brongniartii, Paecilomyces fumosoroseus* and *Metarhizium anisopliae* against *H. puera* larvae was tested under laboratory conditions.

MATERIALS AND METHODS

Fungal pathogens

Four pathogens, *viz., Beauveria bassiana* (host–Lepidoptera: Hesperiidae), *Paecilomyces fumosoroseus* (host – Lepidoptera: Pyralidae) both collected from Nilambur, *B. brongniartii* (host – Coleoptera: Chrysomelidae) collected from Vazhani and *Metarhizium anisopliae* (host – Hemiptera: Pentatomidae) collected from Konni forest range of Kerala were screened in the laboratory against third instar larvae of *H. puera.*

Mass production

In order to maintain the virulence of the fungi and to select the appropriate doses for bioassay, laboratory reared *H. puera* larvae were inoculated with the conidial suspension of all the four fungi separately. Reisolated conidia from the larvae were used for subculturing the fungi on Potato Dextrose Agar–Yeast Extract (PDA–Y) media. The fungi were mass cultured at 25 ± 1°C, 80 ±2% RH, and 12: 12 (L: D) photoperiod. Conidial suspension of each fungus was prepared from 14–day–old cultures using sterile distilled water added with wetting agent Tween 20 (0.1%). The stock solution was counted using Improved Neubauer Hemocytometer and five different concentrations, $2x10^6$, $4x10^6$, $6x10^6$, $8x10^6$ and $1x10^7$ conidia ml^{-1}, were prepared and used in all the bioassays.

Inoculation

Both direct (topical) and indirect (given along with fresh teak leaf disc) application methods were tested. In direct application, a standard atomizer, which expelled approximately 100µl of the suspension on one squeeze was used. Ten healthy third instar larvae of *H. puera* were placed in a sterile Petri–dish (10 cm diam) and 100 µl of the inoculum was sprayed onto the larvae using the atomizer. The treated larvae were allowed to remain in the Petri–dish for 2 min and then transferred to sterile rearing tubes filled with semi–synthetic diet (Mathew *et al*., 1990). Larvae sprayed with sterile water served as control. In indirect application, 10 µl of each dose was applied onto tender fresh teak leaf discs (5×5 mm). A single larva was placed in each rearing tube and allowed to feed on the leaf disc. Larvae fed on leaf discs treated with water served as control. The larvae that consumed the whole leaf disc within 2 hr were transferred to rearing tubes containing semi–synthetic diet. Mortality of the larvae was counted at 24 hr interval until complete death or pupation. Three replicates with ten larvae each were maintained for each dose. Treated insects were incubated at 25 ± 1°C, 80 ± 2% RH, and 12: 12 (L: D) photoperiod.

Reisolation

Dead larvae were incubated for 3–4 days and randomly selected insects were surface sterilized in sodium hypochlorite (1%) for 1–2 min and washed in three serial changes of sterile water to eliminate saprobe growth. The larvae were then plated on PDA–Y media. Fungi were examined under the microscope to confirm the presence of the test fungus.

Data analyses

The mortality data after correction for natural mortality using Abbott's formula (Abbott, 1925) was used for further analyses. The data from direct and indirect applications were pooled and subjected to probit analysis and LC_{50}, LD_{50} and LT_{50} were determined. POLO (© Le Ora Software, 1987), the software based on Finney (1971), was used for analyzing replication–wise data. The percentage mortality values were angular transformed and used for one–way analysis of variance (ANOVA) to find the significance in variation between fungus and dose. Duncan's Multiple Range Test (DMRT) (SPSS version 10) was performed on the transformed values to test the significance in the mean per cent mortality.

RESULTS AND DISCUSSION

The time–concentration–mortality trends observed in third instar larvae of *H. puera* under direct and indirect application methods are shown in Figs. 1 and 2. In direct application, mortality of *H. puera* larvae was not observed until second day for the treatments with *B. brongniartii* and *M. anisopliae*, whereas mortality of the larvae occurred on the first day itself in the case of *B. bassiana* at the concentration of $8x10^6$ conidia ml^{-1}. In the case of *P. fumosoroseus*, mortality was observed only on the third day. In direct application, on 9^{th} day after exposure, per cent larval mortality was 60.1–100 for *B. bassiana*, 43.3–93.3 for *B. brongniartii*, 33.3–93.3 for *P. fumosoroseus* and 26.7–66.66 for *M. anisoplia*e for the concentrations ranging from $2x\ 10^6$ to $1\ x\ 10^7$ conidia ml^{-1} (Fig.1).

In indirect application, mortality of larvae did not occur until third day with *P. fumosoroseus* and *M. anisopliae,* whereas mortality of the larvae occurred on the second day with *B. bassiana and B. brongniartii* for the highest dose of $1\ x\ 10^7$ conidia/ larva. In indirect application, on 9^{th} day after exposure to treated leaves per cent larval mortality was 50–83.3 for *B. bassiana*, 16.6–50 for *B. brongniartii,* 13.3–53.3 for *P. fumosoroseus* and 10–46.6 for *M. anisopliae* for the doses ranging from $2x10^6$ to $1x10^7$ conidia/ larva (Fig. 2).

The highest mortality of 23.3% occurred with *B. bassiana* on 4^{th} and 5^{th} day in direct and indirect application, respectively, for the highest dose ($1x10^7$ conidia ml^{-1}). The mortality in the untreated control (3.3%) was observed only in the bioassays with *B. bassiana*. Larval death mostly occurred during 4–8 and 5–10 days with direct and indirect applications, respectively, in all the fungi. In both direct and indirect application, larval mortality increased with increase in conidial concentration. The infected larvae became sluggish and stopped feeding.

Larval mortality on 9^{th} day in direct and indirect application was used for the probit analysis. There was a good linear relationship between probit transformed mortality data and log10 transformed concentrations. $\chi 2$ test for heterogeneity about the regression line of each bioassay was not significant at the level of $p > 0.05$ (Table 1). Because of

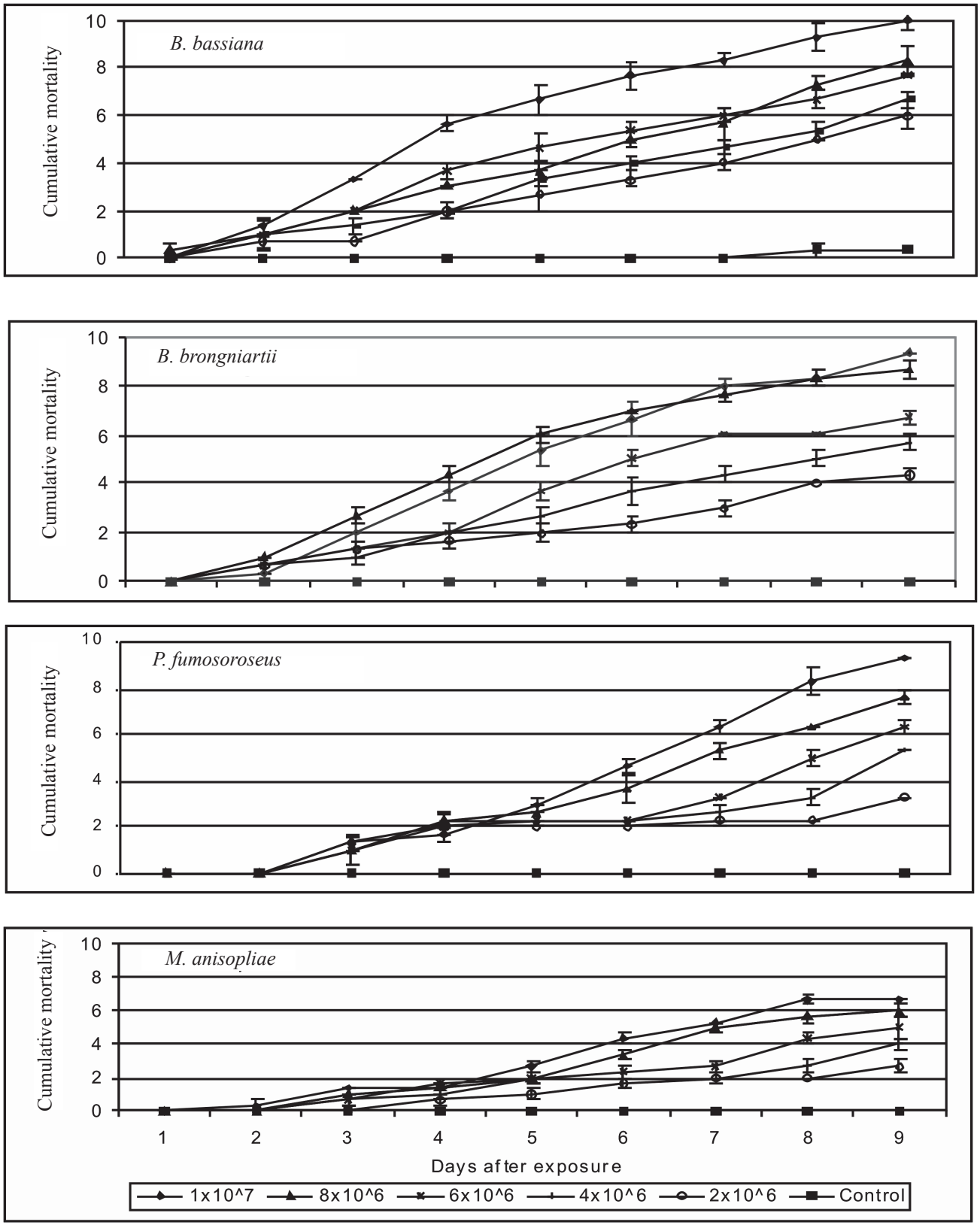

Fig. 1. Cumulative mortality of third instar *H. puera* larvae in direct application at different conidial concentrations (conidia ml^{-1}) of four fungi

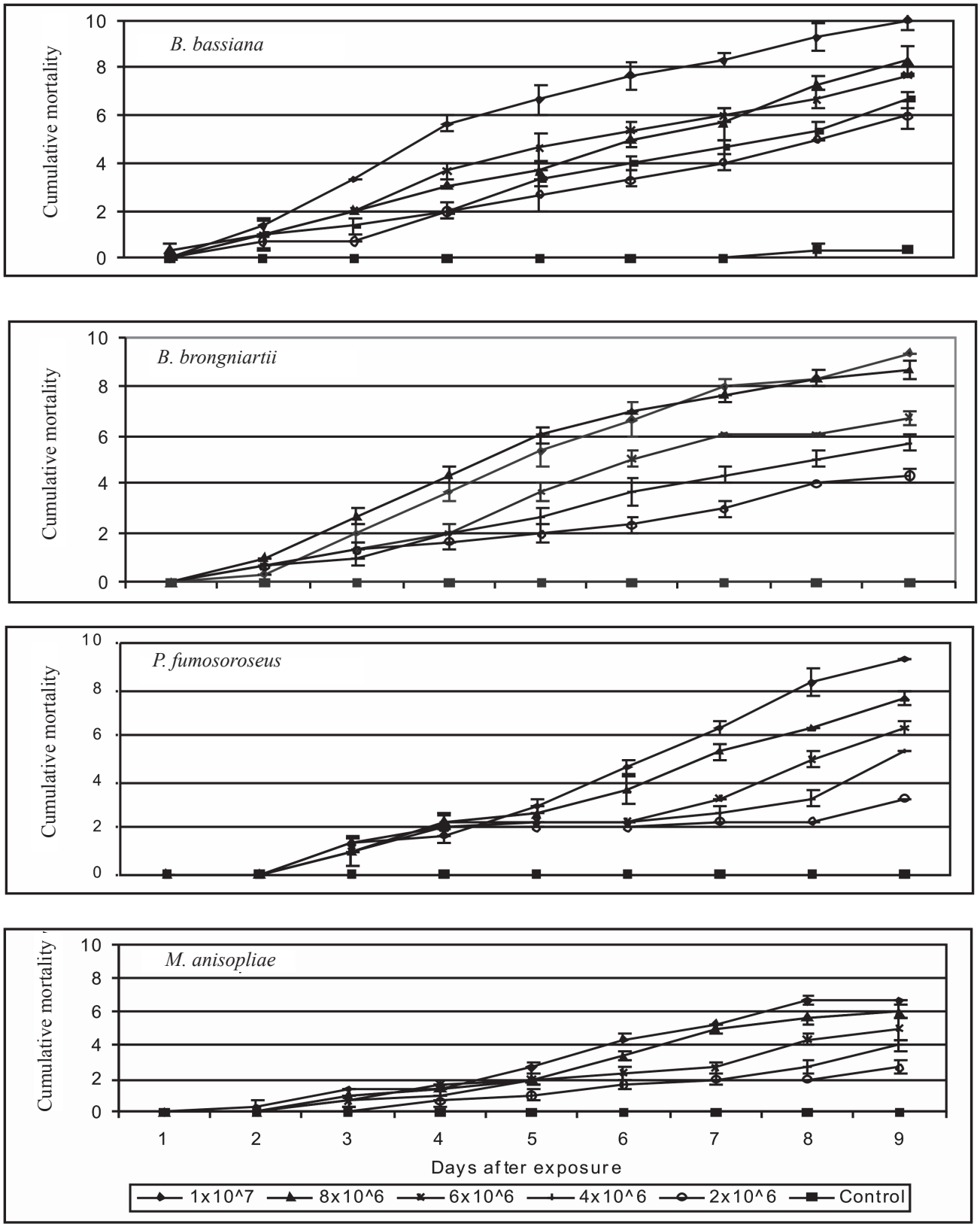

Fig. 2. Cumulative mortality of third instar *H. puera* larvae in indirect application at different conidial concentrations (conidia/ larva) of four fun

Table 1. LC_{50}/ LD_{50} values for direct and indirect application of four fungi to *H. puera*

Method	Fungi	Slope (±SE)[a]	χ^2 test[b]	LC_{50}/ LD_{50} with 95% FL[c] (conidia ml^{-1})
Direct	*B. bassiana*	1.81 (±0.49)	6.64	$1.89x10^6$ (6.35x105 – 2.81x106)
	B. brongniartii	2.15 (±0.46)	4.20	$2.83x10^6$ (1.75 – 3. 67x106)
	P. fumosoroseus	2.29 (±0.46)	4.08	$3.46x10^6$ (2.44 – 4.34x106)
	M. anisopliae	1.51 (±0.44)	1.21	$5.54x10^6$ (3.85 – 8.29x106)
Indirect	*B. bassiana*	1.33 (±0.45)	1.39	$2.44x10^6$ (5.29x105 – 3.72x106)
	B. brongniartii	1.43 (±0.47)	1.54	$1.20x10^7$ (7.97x106 –3.58x107)
	P. fumosoroseus	1.78 (±0.47)	1.73	$9.92x10^6$ (7.30x106 – 2.09x107)
	M. anisopliae	1.70 (±0.50)	1.01	$1.20x10^7$ (8.36x106 – 5.32x107)

[a] Standard error, [b] Goodness of fit test: table entries are not significant at 5 % level (df =11), [c] Fiducial limits

the favourable regression, t value 1.96 was used to calculate 95 per cent fiducial limits of the response doses.

All the four fungi tested by both the methods were pathogenic to *H. puera* under laboratory conditions. Difference in the virulence of the fungi to *H. puera* larvae was compared using LC_{50} and LT_{50} values under both application methods. In direct application, the least LC_{50} value was obtained for *B. bassiana* ($1.89x10^6$ conidia ml^{-1}) followed by *B. brongniartii* ($2.83x10^6$ conidia ml^{-1}) and LC_{50} value was high for *M. anisopliae* ($5.54x10^6$ conidia ml^{-1}) (Table 1). In the indirect application, the least LD_{50} value was obtained for *B. bassiana* ($2.44x10^6$ conidia/ larva) followed by *P. fumosoroseus* (9.92 $x10^6$ conidia/ larva) and the highest LD_{50} value was obtained for *B. brongniartii* and *M. anisopliae* (1.20 $x10^7$ conidia/ larva) (Table 1). LC_{50} values obtained for all the fungi in direct application were less compared to indirect application. The source of variation in susceptibility of *H. puera* to four fungi was determined by F test and found that the pathogens varied significantly ($p < 0.0001$) in their virulence in both the methods. The mean per cent mortality induced by the four fungi for varied doses was tested using DMRT followed by F test and found to be significantly different ($p < 0.05$) for the four fungi (Tables 2 and 3). In direct application, only the highest concentration, $1x10^7$ conidia ml^{-1} of *B. bassiana* was able to induce 100 per cent mortality of *H. puera.* The mean per cent mortality obtained was significantly different ($p < 0.05$) for each concentration within the fungus and between fungus, except for *B. brongniartii* and *P. fumosoroseus,* where 93.02 % mortality was obtained for the highest concentration of $1x10^7$ conidia ml^{-1} (Table 2).

In indirect application, the highest dose, $1x10^7$ conidia/ larva of *B. bassiana* alone induced 83.64% mortality, while the highest dose of other three fungi caused only around 50% mortality of the larvae. Mean per cent mortality obtained was significantly different ($p < 0.05$) for each dose within the fungi (Table 3).

The LT_{50} values obtained consistently decreased as the conidial concentration increased, but they differed between the fungus and method of application (Table 4). In direct application, the least LT50 value was obtained for *B. bassiana* (192.6–91.18 hr) followed by *B. brongniartii* (271.89 – 106.18 hr) and the highest LT50 value was obtained for *M. anisopliae* (368.44 – 161.54 hr) for the concentrations ranging from 2x106 to $1x10^7$ conidia ml^{-1}.

In indirect application, the least LT_{50} value was obtained for *B. bassiana* (216–130.55 hr) followed by *P. fumosoroseus* (385.57–236.41 hr) and the highest LT_{50} value was obtained for *B. brongniartii* (835.72–256.88 hr) for the doses ranging from $2x10_6$ to $1x10^7$ conidia/ larva (Table 4). The LT_{50} values obtained for the four fungi in direct application were less than those for indirect application. In all the bioassays, both LC_{50} and LT_{50} values were least for *B. bassiana*.

Table 2. Mean per cent mortality of *H. puera* caused by four fungi at different concentrations of conidial suspension (conidia ml^{-1}) in direct application

F*	Mean per cent mortality after 9 days (± SE)				
	Direct application				
	2x10^6	4x10^6	6x10^6	8x10^6	1x10^7
Bb	60 (50.76)^{efg}	66.74 (54.78±2)^{e}	76.82 (61.21±2.22)^{d}	83.64 (66.14±2.71)^{cd}	100 (90)^{a}
Bbr	43.31 (41.15±1.92)^{hi}	56.69 (48.84±1.92)^{efg}	66.74 (54.78±2.01)^{e}	86.99 (68.85±2.71)^{bc}	93.02 (74.67±3.11)^{b}
Pf	33.26 (35.21±2.01)^{ijk}	53.35 (46.92±1.92)^{fgh}	63.41 (52.77±2.01)^{ef}	76.82 (61.21±2.23)^{d}	93.02 (74.67±3.11)^{b}
Ma	26.52 (30.99±2.22)^{k}	39.86 (39.14±3.40)^{ij}	50 (45)^{gh}	60 (50.76)^{efg}	66.74 (54.78±2.01)^{e}

Mean values with different superscripts in each row and column are significantly different (DMRT, $p<0.05$); figures in parentheses are angular transformed values; F* – Fungi, *Bb – B. bassiana, Bbr – B. brongniartii, Pf – P. fumosoroseus, Ma – M. anisopliae.*

In direct application, *H. puera* was more susceptible to *B. bassiana* followed by *B. brongniartii* and least susceptible to *M. anisopliae* (Tables 1 and 4). In indirect application also, *H. puera* was more susceptible to *B. bassiana* followed by *P. fumosoroseus* and least susceptible to *B. brongniartii* (Tables 1 and 4). Susceptibility of the host to the pathogens was higher in direct application compared to indirect application.

The dead larvae became stiff and mummified. White fungal mycelia were observed on the intersegmental membranes on 3rd or 4th day in the direct application, whereas mycelial growth was observed on 5–7 days post inoculation in the indirect application and subsequently the entire body of the larva was covered with fluffy mycelial growth. In course of time, the larval body was covered

Table 3. Mean per cent mortality of *H. puera* caused by four fungi at different concentrations of conidial suspension (conidia/ larva) in indirect application

F*	Mean per cent mortality after 9 days (± SE)				
	Indirect application				
	2x10^6	4x10^6	6x10^6	8x10^6	1x10^7
Bb	50 (45)^{ef}	60 (50.76)^{cd}	66.74 (54.78±2.01)^{c}	76.82 (61.21±2.22)^{b}	83.64 (66.14±2.71)^{a}
Bbr	16.36 (23.85±2.71) ^{kl}	20 (26.56)^{k}	30 (33.21)^{j}	40 (39.23)^{ghi}	50 (45)^{ef}
Pf	13.01 (21.14±2.71)^{lm}	20 (26.56)^{k}	33.26 (35.21±2.01)^{ij}	43.31 (41.15±1.92)^{fgh}	53 (46.92±1.92)^{de}
Ma	10 (18.43)^{m}	20 (26.56)^{k}	30 (33.21)^{j}	36.60 (37.22±2.01)^{hij}	46.65 (43.07±1.92)^{efg}

Mean values with different superscripts in each row and column are significantly different (DMRT, $p<0.05$); figures in parentheses are angular transformed values; F* – Fungi, Bb – B. bassiana, Bbr – B. brongniartii, Pf – P. fumosoroseus, Ma – M. anisopliae

with characteristically coloured spores of each species – yellowish white in *B. bassiana* and *B. brongniartii*, pink in *P. fumosoroseus* and olive green in *M. anisopliae*. Reisolation of each fungus was carried out from randomly selected dead insects.

Complete pupation and adult emergence of *H. puera* occurred in all the controls, except for *B. bassiana* in which only 90 % pupation and adult emergence occurred. Pupation and adult emergence have a direct relationship with the concentration applied, virulence of the species and method of application. All survivors pupated at 6–7 days and adult emergence occurred at 17–18 days post– inoculation irrespective of the fungus and dose tested. All the pupae did not emerge and some emerged with abnormalities.

Of the two methods of application tested in the laboratory, direct application caused higher mortality of the larvae than indirect application. This is probably because the insect integument acts as the main pathway of infection for fungi. Mohammed Ali *et al*. (1991) while testing the efficiency of *B. bassiana* against larvae of *Atteva fabricella* and *P. fumosoroseus* against *Eligma narcissus* reported that direct application of the fungi was more effective than indirect application. In the present study, the lepidopteran isolate, *B. bassiana* was found to be more virulent against *H. puera*. This is probably because the host of the pathogen could be a decisive factor in the susceptibility of the test insect to the pathogen.

In direct application on *H. puera, B. bassiana* was found to be more effective than *B. brongniartii, P. fumosoroseus* and *M. anisopliae* under controlled conditions with low LC_{50} and LT_{50} values. Rajak *et al*. (1993) reported that *B. bassiana* caused 96 % mortality in second instar larvae of the *H. puera*, at a concentration of $1x10^4$ conidia ml^{-1}. Sakchoowong (2002) reported that four conidial concentrations of *B. bassiana*, $2x10^5$, $2x10^6$, $2x10^7$ and $2x10^8$ conidia ml^{-1}, were able to produce 38.98, 47.13, 68.71 and 86.15% average mortality, respectively, while *M. anisopliae* produced only 25.92, 34.74, 41.48 and 49.70% mortality, respectively, with the above doses on third instar larvae of *H. puera* in Thailand. He also

Table 4. LT50 values for four fungi on *H. puera* at different concentrations of conidial suspension (conidia ml^{-1}) in direct and indirect application

Method	F*	LT_{50} (FL^a), hr				
		$2x10^6$	$4x10^6$	$6x10^6$	$8x10^6$	$1x10^7$
Direct	*Bb*	192.6 (165.50 – 243.11)	172.17 (148.77 – 211.22)	143.58 (126 – 167.55)	132.18 (116.48 – 151.59)	91.18 (81.15 – 100.75)
	Bbr	271.89 (208.41 – 468.46)	191.71 (163.79 – 244.28)	153.45 (135.96 – 177.82)	113.49 (103 – 124.22)	106.18 (94.35 –118.16)
	Pf	324.91 (245.60 – 694.14)	239.65 (195.27 – 353.17)	195.97 (170.88 – 242.66)	158.04 (143.20 – 177.84)	137.88 (127.20 – 149.51)
	Ma	368.44 (251.96– 981.72)	293.80 (226.91 – 526.21)	226.16 (190.86 – 307.08)	179.56 (160.46 – 209.97)	161.54 (144.68 – 185.38)
Indirect	*Bb*	216 (187.11 –276.74)	191.2 (171.59 –224.37)	169.29 (153.37 –192.09)	149.55 (136.21 –165.85)	130.55 118.29 – 144.05)
	Bbr	835.72 (382.84 – 47809.20)	735.72 (361.91 – 30283.31)	455.09 (291.85 – 2009.93)	327.59 (244.82 – 674.94)	256.88 (203.13 – 408.58)
	Pf	385.57 (269.46 – 1915.18)	381.02 (259.11 – 1061.05)	341.04 (252.17 –820.87)	286.41 (227.44 –484.69)	236.41 (200.66 – 321.24)
	Ma	555.66 (317.40 – 11912.9)	336.03 (247.31 – 726.90)	323.3 (248.88 – 824.43)	303.72 (238.94 – 579.95)	250.9 (210.41 – 358.73)

F* – Fungi, [a] Fiducial limits, *Bb – B. bassiana, Bbr – B. brongniartii, Pf – P. fumosoroseus, Ma – M. anisopliae*

observed that the LC_{50} value for *B. bassiana* ($1.4x10^6$ conidia ml^{-1}) was lower than that of *M. anisopliae* ($2.19x10^8$ conidia ml^{-1}). Barman and Nath (2002) reported that *B. bassiana* caused 86.29% mortality of *Herotia vitessoides* larvae with $1x10^7$ spores ml^{-1}. Similarly, *B. brongniartii* was reported to kill the larvae of *Hypsipyla robusta* (Kandaswamy, 1969).

Two key attributes of highly pathogenic isolates are their ability to kill the pest in a relatively short period of time and cause high mortality at relatively low doses (Butt, 2002). The LC_{50} and LT_{50} for all the four fungi varied with the pest and method of application with high effectiveness of topical application over oral application. The current study has shown the potential of lepidopteran–derived isolate of *B. bassiana* as a promising biocontrol agent against *H. puera*, at conidial concentrations and time lower than those required by *B. brongniartii, P. fumosoroseus* and *M. anisopliae*. Therefore, it may be worthwhile to define the environmental and other factors, *viz*., persistence and transmission influencing the infection of *H. puera* by *B. bassiana* and assess their effect on non–target species.

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