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Influence of Sub-culturing on the Biometric Characteristics and Infectivity of Termite Mycopathogens

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ABSTRACT

Beauveria bassiana **(Bals.) Vuill., upto the fourth sub-culture and** ***Metarhizium anisopliae*** **(Metsch.) Sorokin var.** ***anisopliae*** **upto the third sub-culture recorded significantly higher biomass, mycelial growth, conidial count and viable conidia. However, subsequent sub-culturing of these mycopathogens showed significant reduction in all these biometric characteristics at various sub-cultures. Mortality data of worker (major) caste of** ***Odontotermes brunneus*** **(Hagen), with different sub-cultures showed significantly higher mortalities upto the fourth sub-culture in** ***B. bassiana*** **and till the third sub-culture in** ***M. anisopliae*****. The same trend was reflected in case of LT_{50} values in both the fungi. However, the virulence of these two mycopathogens was restored when the fungi were passed through their host** ***viz.*****, termites just once. Subsequent two passages through the termites did not markedly improve the virulence.**

KEY WORDS : *Beauveria bassiana, Metarhizium anisopliae*, sub-culturing, infectivity, termites

Mass multiplication of mycopathogens is a prerequisite for their field use in controlling pests. A cheap media for mass production and knowledge about how long one can go on sub-culturing the mycopathogens, without hampering the infectivity, is of paramount importance. The present study was undertaken to know the effect of continuous sub-culturing in carrot medium on the biometric characteristics and infectivity.

MATERIALS AND METHODS

Workers major of *Odontotermes brunneus* (Hagen) were infected with *Beauveria bassiana* and *Metarhizium anisopliae* and the fungi were isolated from the cadavers and grown on standard mycological media to get the pure culture of the respective fungi. Further sub-culturing upto seven sub-cultures was done using carrot medium (40 g of carrot pieces with 60 ml of water taken in 250 ml conical flasks). Culturing of fungi was done at 25°C and cultures were incubated for 10 days. The biomass production of mycopathogens was studied as per Hall and Bell (1961), while the mycelial growth was measured as per Easwaramoorthy and Jayaraj (1977). The conidial coun in respect of the two fungal pathogens, at each sub-culture, was recorded with a Neubauer haemocytometer (Jones, 1962). The criterion for conidial germination was the development of a germ tube equal to the diameter of the conidia. The per cent conidial germination was studied as per Walstad *et al.* (1970).

The conidial suspension prepared from the fungal cultures at each sub-culture was tested for its infectivity against the workers major of *O. brunneus* at a conidial load of 10^7 conidia ml^{-1} at 25 ± 2°C. Insects were directly sprayed with an atomizer. Soft wood pieces and freshly collected fungal comb pieces

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Table 1. Influence of successive sub-culturing on the growth and sporulation of *B. bassiana* (Bb) and *M. anisopliae* (Ma) in carrot medium

Sub-culture	Biomass (mg)		Mycelial growth (mm)		Conidial count (x 10^7 ml^{-1})		Viable Conidia %	
	Bb	Ma	Bb	Ma	Bb	Ma	Bb	Ma
First	68.00^{a}	64.00^{a}	42.00^{a}	39.70^{a}	2.54^{a}	1.66^{a}	90.60^{a}	87.28^{a}
Second	66.00^{ab}	63.00^{ab}	40.63^{ab}	36.63^{ab}	2.48^{ab}	1.62^{a}	89.00^{a}	84.58^{a}
Third	65.00^{ab}	60.00^{ab}	38.88^{ab}	35.38^{ab}	2.39^{ab}	1.46^{ab}	88.58^{ab}	82.13^{ab}
Fourth	62.00^{ab}	57.00^{bc}	38.25^{abc}	33.29^{bc}	2.24^{ab}	1.43^{b}	88.13^{ab}	78.45^{bc}
Fifth	59.00^{bc}	51.00^{cd}	37.20^{bcd}	30.17^{cd}	2.02^{bc}	1.09^{c}	86.00^{bc}	75.98^{cd}
Sixth	54.00^{cd}	50.00d	37.13^{bcde}	28.34^{d}	1.80^{cd}	1.04^{d}	84.15^{c}	73.78^{dc}
Seventh	49.00^{d}	46.00^{d}	35.38^{cde}	27.00^{d}	1.69^{d}	1.01^{d}	80.23^{d}	72.05^{d}

Mean separation in a column by DMRT at 5% level

were given as fod to the treated insects. Each treatment with hundred insects was replicated four times. The LT_{50} values were determined for each fungus by taking mortality counts upto seven days at an interval of 6h.

RESULTS AND DISCUSSION

Higher biomass, mycelial growth, conidial count and viable conidia were recorded upto the fourth sub-culture in *B. bassiana* and upto the third sub-culture in *M. anisopliae*. In the subsequent sub-cultures, there was significant reduction in these biometric characteristics (Table 1). The same trend was reflected in the mortalities and LT_{50} values (virulence) caused by these fungi (Table 2,3). Considerable revival in the virulence was observed when the fungi were passed through

Table 2. Probit analysis of time-mortality response of *O. brunneus* workers major to *B. bassiana* in successive sub-cultures and after passing through host

Sub-cultures/passage through host	Chi^{2}* (3)	Regression equation	LT_{50}** (h)	Fiducial limits (95%) (h)
I	0.61	Y = 4.74129x -18.42297	87.13	84.16 - 90.21
II	0.82	Y = 4.70392x -18.38506	93.62	90.40 - 96.96
III	0.75	Y = 4.97709x -19.91625	101.43	98.13 - 104.84
IV	1.21	Y = 3.19504x -11.11049	110.24	104.70 - 116.07
V	3.59	Y = 5.53893x -23.18676	122.70	119.10 - 126.40
VI	1.11	Y = 4.82135x -19.72723	134.48	129.97 - 139.16
VII	0.89	Y = 5.15598x -21.51880	139.09	134.72 - 143.60
Passage through host after V sub-culture	0.60	Y = 4.46766x -16.93325	81.15	78.22 - 84.20
Passage through host after VI sub-culture	1.58	Y = 3.27873x -11.14990	84.26	80.13 - 88.60
Passage through host after VII sub-culture	1.28	Y = 2.76169x - 8.58327	86.36	81.34 - 91.96

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

** at 4×10^{7} conidia ml^{-1}

Table 3. Probit analysis of time-mortality response of *O. brunneus* workers major to *M. anisopliae* in successive sub-cultures and after passing through host.

Sub-cultures/passage through host	Chi^{2*} (3)	Regression equation	LT_{50}** (h)	Fiducial limits (95%) (h)
I	1.47	Y = 3.59256x -12.85521	93.33	89.15 - 97.71
II	1.19	Y = 3.07944x -10.36328	97.49	92.41 - 102.84
III	1.97	Y = 4.70087x -18.66576	108.22	104.49 - 112.08
IV	1.59	Y = 4.77538x -19.19794	116.74	112.78 - 120.83
V	1.03	Y = 4.46821x -17.78144	125.47	120.93 - 130.18
VI	2.52	Y = 5.65584x -24.01428	134.88	131.01 - 138.86
VII	1.13	Y = 5.06771x -21.08696	140.49	136.00 - 145.14
Passage through host after V sub-culture	0.69	Y = 4.69986x -18.29467	90.45	87.34 - 93.68
Passage through host after VI sub-culture	0.79	Y = 4.78611x -18.78431	93.20	90.05 - 96.46
Passage through host after VII sub-culture	1.08	Y = 4.56631x -17.76872	96.88	93.44 - 100.43

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

** at 4×10^7 conidia ml^{-1}

their host (termites) after the fifth sub-culture. However, subsequent passages through the host did not improve the infectivity significantly.

Reduction in virulence due to sub-culturing was reported in *B. bassiana* (Schaerffenberg, 1964). Sundarababu (1980) reported significant reduction in growth, sporulation and virulence beyond fifth sub-culture in *M. anisopliae* var. *major* in carrot medium. On several artificial media, a sudden deterioration of the calories might occur and valuable fungus cultures could easily be lost (Rombach and Gillespie, 1988). However, the extent and rapidity of the decrease in virulence of the fungus depended upon the culture medium (Ignoffo, 1981). The possible reason for the loss in virulence might be due to the lack of certain nutritional components required for enzyme activity or toxin production in the growth medium. West and Briggs (1968) observed a decline in toxin production after repeated sub-culturing of *B. bassiana* on artificial medium. The saprophytic cycle of development of the fungus was inferior to the parasitic cycle in terms of virulence (Lappa and Goral, 1976). Evlakhova (1966) attributed the loss of virulence to lack of chitin components, while Bunzli and Buttiker (1959) attributed it to lack of nitrogenous matter. Deficiency of these substances in the carrot medium employed in the present study for sub-culturing might be the reason for the reduction in growth, sporulation and virulence. Carrot was found to lack certain specific nutrients including chitin components etc. (Gopalan *et al.*, 1971). Samsinakova and Kalalova (1983) suggested optimisation of the culture media by additives for increasing the virulence of *B. bassiana.*

The phenomenon of restoration of virulence, as recorded in the present study, in the attenuated conidia of *B. bassiana* by passing through *Leptinotarsa decemlineata* Say.,

was reported by Schaerffenberg (1964). Similar observations have been documented in certain other muscardine fungi (Fargues, 1981; Ignoffo, 1981; Kmitowa, 1982; Sundarababu *et al.*, 1983).

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