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Influence of Temperature on the Growth, Sporulation and Infectivity of Mycopathogens Against Termites

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ABSTRACT

***Beauveria bassiana* (Bals.) Vuill and *Metarhizium anisopliae* var. *anisopliae* (Metsch.) Sorkin when grown at temperatures of 25 and 30°C recorded maximum biomass, mycelial growth, conidial count and conidia. At these temperature they were highly infective against *Odontotermes brunneus* Hagen. At temperatures below 25°C and above 30°C there was marked and significant reduction in the biometric characteristics and infectivity.**

KEY WORDS : *Beauveria bassiana, Metarhizium anisopliae Odontotermes, brunneus* infectivity, temperature effects

Temperature is the key factor in the development and activity of all organisms. For mycopathogens, the optimum temperature is usually between 20 and 25°C (Roberts and Campbell, 1977). The necessity to maintain optimum temperature during the culturing process for better efficiency of the entomopathogenic fungi is well - documented in literature. The present study was conducted to know the optimum temperature for the growth of *Beanuveria bassiana* (Bals.) Vuill and *Metarhizium anisopliae* var. *anisopliae* (Metch) and their infectivity to *Odontotermes brunneus* Hagen.

MATERIALS AND METHODS

Pure fungal colonies of *B. bassiana* and *M. anisopliae* were cultured on Sabouraud's dextrose agar enriched with one per cent yeast extract (SDAY) and Emerson's yeast phosphate soluble starch agar (YPSs agar), respectively, at 25°C in the laboratory. From the actively growing colonies of the two mycopathogens, a 10 mm disc of fungal mat was taken with the help of a sterilized cork borer and inoculated in the culture media taken in Petri dishes. The fungi were allowed to grow for 10 days in BOD incubators maintained at temperatures of 10, 15, 20, 25, 30 and 35 ±1°C. At each temperature level four replications were maintained. Conidial count was recorded with a Neubauer haemocytometer (Jones, 1962), and the conidial germination was recorded as per the procedure by Walstad *et al.* (1970).

In order to test the infectivity of each of the fungal colony grown at a particular temperature, the conidia were harvested freshly by washing from surface of the culture plates using 75 ml of 0.02 per cent Tween 80^R. (Rombach *et al.*, 1986). The harvested coni dial suspensions were filtered through a double layer muslin and centrifuged (3000 rpm) for 20 min and then resuspended in 25ml of 0.02 per cent Tween 80^R. Conidial concentration in the filtrates was determined with the help of an improved Neubauer haemocytometer and conidial viability was determined as suggested by Gillespie (1986). A conidial concentration of 10^7 was standardised using sterile distilled water containing 0.02 per cent Tween 80^R (Roberts and Yendol, 1971).

Termites to be treated were taken in a Petri dish and sprayed with 3 ml of conidial

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suspension directly with an atomizer. Treated termites were maintained on their natural food i.e. fresh fungus comb pieces and soft wood at 25 ± 2°C in the laboratory. Hundred insects were used for each treatment and there were four replications. LT_{50} values were determined by taking the mortality count at 6 h interval in each treatment.

RESULTS AND DISCUSSION

Growth, sporulation (conidial production) and infectivity of *B. bassiana* and *M. anisopliae* showed considerable variations when incubated at different temperatures. There was significant increase in the biometric growth characteristics of these two fungi as the temperature increased. Conidial count in *B. bassiana* increased upto 25°C only. In the case of *M.anisopliae*, mycelial growth increased upto 25°C, while conidial count and viable conidia increased significantly upto 15 and 20°C, respectively. (Table 1).

Table 1. Influence of temperature on the growth and sporulation of *B. bassiana* (Bb) and *M. anisopliae* (Ma)

Temperature	Mycelial growth (mm)		Conidial count ($\times 10^7$ ml^{-1})		Viable conidia (%)	
	Bb	Ma	Bb	Ma	Bb	Ma
10	17.0^c	11.5^d	0.90^d	1.20^b	65.0^d	51.0^c
15	27.0^b	24.0^c	1.90^c	2.45^{ab}	85.0^b	72.0^b
20	34.0^a	30.0^b	2.53^b	3.75^a	91.5^a	83.0^a
25	39.0^a	38.0^a	3.10^a	4.50^a	94.0^a	88.0^a
30	34.0^a	36.0^a	2.95^a	4.40^a	92.1^a	86.0^a
35	20.0^c	7.0^d	0.75^d	1.00^b	48.0^c	41.0^d

Mean separation in vertical columns by DMRT at 5% level

B. bassiana and *M.anisopliae* cultured at 25°C caused the highest mortality. However, they were on par with mortalities caused by fungi cultured at 20°C and 30°C. Significant reduction in the infectivity was noticed at 35°C (Fig.1) in both the fungi. The LT_{50} values for the two fungi (Table 2 & 3) decreased as the temperature increased and recorded lowest values at 25°C, indicating that 25°C was the optimum temperature for growth and infectivity of these fungi. A temperature of 10°C and 35°C proved to be unfavourable for the successful growth and infectivity of these fungi.

Thus in the present study, although temperatures from 10-35°C supported growth and sporulation of the fungi, the optimum was 25°C. However, temperature upto 30°C did not have significant effect on the biometric characteristics (Walstad *et al.*, 1970). Hussey and Tinsley (1981) reported that *B. bassiana* could be cultured at any temperature between 8 and 30°C, the optimum temperature being 24°C. Sanzhimitupova and Kalvish (1979) reported that there was no conidial formation at 35°C in *B. bassiana*. However, in the present study, growth and sporulation to a certain extent did occur. It is also reported that the maximum, minimum and optimal temperature required for *B. bassiana* vary with the isolate or strain or geographical origin of the isolate. In addition to the phenomenon of selection, recombination and mutation, fungi may exhibit variation in their temperature requirements (Yendol and Hamlen, 1973). Similarly, a strain of *M. anisopliae* caused no infection below 10°C (Doberski, 1981) and the susceptibility of *H. zea* larvae to *Nomuraea rileyi* Samson was lower at 15°C than at 20 and 30°C (Mohammed *et al.*, 1977).

M. anisopliae is one of the most promising candidate pathogens for control of subterranean pests. The optimum temperature for

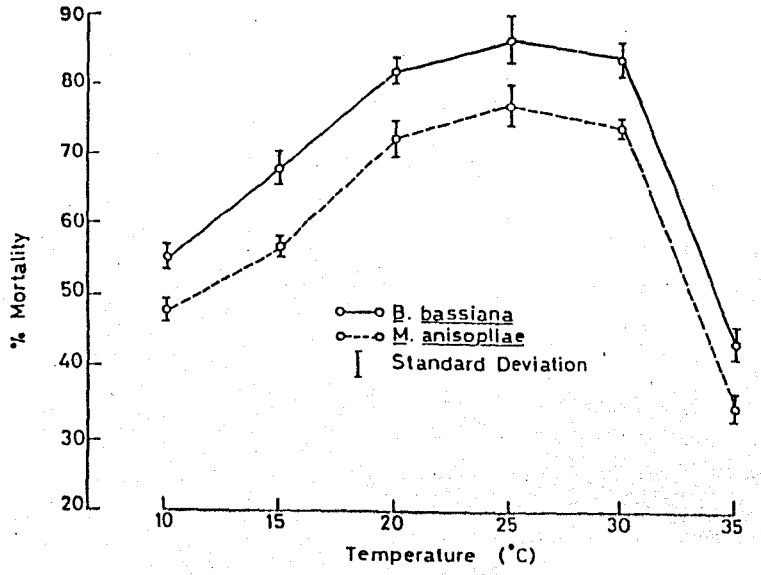

Fig.1. Influence of temperature on the infectivity of mycopathogens

Table 2. Probit analysis of time-mortality response of *O. brunneus* workers major to *B. bassiana* incubated at different temperatures

Temperature (°C)	Chi^2 (3)	Regression equation	LT^*_{50} (h)	Fiducial limits (95%) (h)	Mortality (%)
10	0.50	Y = 4.75200x - 18.90314	107.18	103.52 - 110.95	56.00^d
15	2.05	Y = 5.25566x - 21.31718	101.71	98.57 - 104.95	69.50^c
20	1.57	Y = 4.95539x - 19.67506	95.37	92.25 - 98.59	83.75^{ab}
25	1.19	Y = 4.11344x - 15.21766	82.22	78.99 - 85.58	86.25^a
30	1.71	Y = 4.18061x - 15.72729	90.77	87.26 - 94.41	79.25^b
35	0.91	Y = 5.15457x - 21.07362	114.37	110.78 - 118.09	44.50^e

* at 4 x 10^7 Conidia ml^{-1}

Table 3. Probit analysis of time-mortality response of *O. brunneus* workers major to *B. bassiana* incubated at different temperatures

Temperature (°C)	Chi^2 (3)	Regression equation	LT^*_{50} (h)	Fiducial limits (95%) (h)	Mortality (%)
10	1.43	Y = 4.66839x - 18.65701	116.80	112.76 - 120.49	46.75^d
15	0.99	Y = 3.91274x - 14.74423	111.20	106.62 - 115.92	59.25^c
20	3.21	Y = 4.88403x - 19.47666	102.69	99.29 - 106.22	72.75^{ab}
25	1.06	Y = 4.21691x - 15.86286	88.59	85.20 - 92.12	76.25^a
30	1.96	Y = 4.38781x - 16.87377	96.63	93.07 - 100.32	70.50^b
35	0.62	Y = 4.63057x - 18.55164	121.93	117.67 - 126.34	42.75^d

* at 4 x 10^7 Conidia ml^{-1}

germination and growth is about 25°C (Latch and Kain, 1983). The effect of temperature on the stability and survival of fungal conidia often depends on soil humidity. The half life of conidia of *B. bassiana* ranged from 14 days at 25°C to 276 days at 10°C (Lingg and

Donaldson, 1981). Conidia held at -15°C exhibited little or no loss in viability regardless of water content, relative humidity or pH. However, conidia were not recoverable after 10 days from soil held at 55°C.

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