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Effect of temperature on pathogenicity of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin against *Spilosoma obliqua* (Walker)

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ABSTRACT: Pathogenicity of *Beauveria bassiana* to *Spilosoma obliqua* larvae was higher at low temperature (20°C) compared to higher temperature (30°C). The LC_{50} values of *B. bassiana* at 20 and 30°C were 8.63 and 17.8×10^6 conidia ml^{-1} against second instar larvae, 16.4 and 62.3×10^6 against third instar, and 33.7 and 70.2×10^6 conidia ml^{-1} against fourth instar, respectively. The LT_{50} values at 20°C temperature were 155.4, 159.0 and 162.9 hours to second, third and fourth instar larvae, respectively, compared to 167.6, 168.9 and 169.8 hours, respectively, at 30°C temperature. The LC_{50} values of *Metarhizium anisopliae* against *S. obliqua* at 20°C temperature was 54.1, 66.2 and 73.8×10^6 conidia ml^{-1} against second, third and fourth instar larvae, respectively, which was 1.58, 1.14 and 1.08 fold lower than values obtained at 30°C temperature, i.e., 58.7, 75.1 and 80.1×10^6 conidia ml^{-1}, respectively. Similarly, the LT_{50} values at 20°C were 161.3, 162.9 and 169.5 hours against second, third and fourth instar larvae, respectively, which were 1.08, 1.08 and 1.05 fold lower than LT_{50} obtained at 30°C temperature, respectively.

KEY WORDS: *Beauveria bassiana*, *Metarhizium anisopliae*, pathogenicity, *Spilosoma obliqua*, temperature

INTRODUCTION

Beauveria bassiana (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin are well known insect pathogens, which kill the insect through invasion and profuse mycelial growth. However, host specificity of entomogenus fungi depends upon strain of entomogenous fungi, type of host, host instar, physiological state of the insect and abiotic factors like temperature, humidity, etc. Among these, temperature is important factor, which influences the efficacy of the entomogenous fungi. Poor efficacy of *B. bassiana* has been recoded at higher temperature. Selman *et al.* (1997) reported that *B. bassiana* isolates 247 and 373 caused higher mortality at 20°C and 24°C, respectively, to mature larvae of *Plutella xylostella*. Sivasankaran *et al.* (1998) reported higher susceptibility of third instar *Chilo infuscatellus* larvae to fungal infection at 25°C. Fargues and Luz (2000) reported that humidity and temperature influenced infection of entomogenus fungi against different stages of nymph of *Rhodnius prolixus* and the most favorable conditions for pathogenicity were 97 per cent RH and 20°C temperature. Liu *et al.* (1999) examined virulence of *B. bassiana* against green peach aphid, *Myzus persicae* at 28, 21, 16 and 11°C and showed that aphids had higher mortality at 28°C and 21°C than at 16 and 11°C at the concentration of 10^7 conidia ml^{-1}. In the light of above facts, an experiment was carried out to study the effect of temperature on pathogenicity of *B. bassiana* and *M. anisopliae* against *Spilosoma obliqua*.

MATERIALS AND METHODS

S. obliqua was collected from the Crop Research Centre (CRC), Pantnagar, and reared on castor leaves to obtain different instar larvae for the experiment. Two fungal pathogens, *viz.*, *B. bassiana* (MTCC984) and *M. anisopliae* (Pantnagar isolates) were used in the present investigation. The conidia used were harvested from eighteen-day-old cultures grown on SDA medium under aseptic condition using 100ml of sterile distilled water having 0.02 per cent Tween80 as wetting agent (Rombach *et al.,* 1986). The number of conidia in the stock solution was determined by haemocytometer (Improved Nuebauer Weber, England). The conidial concentrations, viz., $5x10^5$, $5x10^6$, $5x10^7$, $5x10^8$ and $5x10^9$ conidia ml^{-1} were prepared from stock solutions of both fungi. The prepared spore suspension was sprayed on second and third instar larvae of *S. obliqua*, separately. Ten larvae were taken for each treatment, which was replicated thrice. The treated larvae were incubated at two temperatures regimes, *viz.,* 20 ± 2°C and 30 ± 2°C at 95 ± 5 per cent RH. The mortality due to mycosis was recorded at one day interval and the cumulative mortality data of eight days was used for probit analysis.

RESULTS AND DISCUSSION

Effect of temperature on pathogenicity of *B. bassiana*

The results on pathogenicity tests indicated that *S. obliqua* larvae were more susceptible at lower temperature (20°C) compared to higher temperature (30°C). The LC_{50} values of *B. bassiana* at 20 and 30°C were $8.63x10^6$ and $17.8x10^6$conidia ml^{-1} against second instar larvae and $16.4x10^6$ and $62.3x10^6$ against third instar, respectively, while against fourth instar it was $33.7x10^6$ and $70.2x10^6$ conidia ml^{-1}, respectively. There was 2.06, 3.79 and 2.08 fold lower virulence of *B. bassiana* at 30°C temperature compared to that of 20°C. The LT_{50} values at 20°C temperature were 155.4, 159.0 and 162.9 hours to second, third and fourth instar larvae, respectively, compared to 167.6, 168.9 and 169.8 hours, respectively, at 30°C. Comparatively higher LC_{50} and LT_{50} values at 30°C indicated lower susceptibility of *S. obliqua* larvae to *B. bassiana* at higher temperatures and *vice-versa*.

Effect of temperature on pathogenicity of *M. anisopliae*

The LC_{50} values of *M. anisopliae* against *S. obliqua* at 20°C temperature were 54.1 $x10^6$, 66.2 $x10^6$ and $73.8x10^6$ conidia ml^{-1} against second, third and fourth instar larvae, respectively, which were 1.58, 1.14 and 1.08 fold lower than values obtained at 30°C, i.e., $58.7x10^6$, $75.1x10^6$ and $80.1x10^6$ conidia ml^{-1}, respectively. Similarly, the LT_{50} values at 20°C were 161.3, 162.9 and 169.5 hours against second, third and fourth instar larvae, respectively, which were 1.08, 1.08 and 1.05 folds lower than LT_{50} obtained at 30°C temperature, respectively. Lower LC_{50} and LT_{50} value of *M. anisopliae* at 20°C indicated that *M. anisopliae* was comparatively more virulent at 20°C than 30°C temperature.

Results on the pathogenicity tests revealed that both fungi were more virulent to *S. obliqua* larvae at 20°C than at 30°C . Roberts and Campbell (1994) reported that the optimum temperature for growth of entomogenous fungus ranged from 20 to 30°C. At higher temperature, there may be a possibility of lower germination of conidia. According to Martin *et al.* (2000), *B. bassiana* was unable to germinate at higher temperature, so could not control the Colorado potato beetle. The fungal infection was severe in *Chilo infuscatellus* at 25°C and was more virulent at lower temperature (21°C) than higher temperature (28°C) to aphid (Liu *et al.*, 1999), which supported the present finding that the virulence of fungus may be influenced by

Table 1. Dosage mortality response of *S. obliqua* to *B. bassiana* and *M. anisopliae* at 20° and 30°C temperature

Instar	χ^2 value	Regression equation (y= a + bx)	LC_{50} value (x 10^6 conidia ml^{-1})	Fiducial limit (x 10^8- 10^4 conidia ml^{-1})	Relative virulence
At 20°C *B. bassiana*					
II	0.70	Y = 3.6870 + 0.1893x	8.63	1.400 - 50.319	-
III	1.12	Y = 3.6405 + 0.1884x	16.4	2.226 - 1.211	1.90
IV	0.44	Y = 3.6902 + 0.2006x	33.7	6.751 - 1.685	3.90
At 20°C *M. anisopliae*					
II	0.31	Y = 3.4122 + 0.2053x	54.1	5.441 - 5.393	-
III	0.69	Y = 2.2289 + 0.3543x	66.7	2.491 - 1.761	1.23
IV	0.05	Y = 3.7804 + 0.1549x	73.8	1.529 - 3.570	1.36
At 30°C *B. bassiana*					
II	0.02	Y = 3.7430 + 0.1690x	17.8	3.205 - 9.973	-
III	0.08	Y = 3.1369 + 0.2390x	62.3	4.510 - 8.620	3.50
IV	0.20	Y = 3.4597 + 6.2055x	70.2	70.37 - 7.011	3.94
At 30°C *M. anisopliae*					
II	0.50	Y = 3.7366 + 0.1626x	58.7	10.38 - 3.326	-
III	0.11	Y = 3.6526 + 0.1710x	75.1	44.730 - 4.825	1.28
IV	1.40	Y = 3.3765 + 0.2045x	80.1	80.720 - 7.210	1.36

Table 2. Time mortality response of *S. obliqua* to *B. bassiana* and *M. anisopliae* at 20° and 30°C temperature

Instar	χ^2 value	Regression equation (y= a + bx)	LC_{50} value (x 10^6 conidia ml^{-1})	Fiducial limit (x 10^6 - 10^6 conidia ml^{-1})	Relative virulence
Instar	χ^2 value	Regression equation(y = bx + a)	LC_{50} (h)	Fiducial limit (h)	Relative virulence (fold)
At 20°C *B. bassiana*					
II	0.95	Y = 7.1762 x - 4.1528	155.4	182.8 - 138.1	-
III	0.13	Y = 3.1752 x - 1.9979	159.0	200.1 - 140.1	1.02
IV	1.03	Y = 3.3240 x - 2.3539	162.9	206.9 - 141.9	1.04
At 20°C *M. anisopliae*					
II	0.21	Y = 3.4109 x - 1.7664	161.3	202.6 - 140.9	-
III	0.91	Y = 3.0341 x - 1.7395	162.9	192.5 - 145.8	1.00
IV	0.45	Y = 2.8710 x - 1.3947	169.5	235.9 - 142.8	1.05
At 30°C *B. bassiana*					
II	0.30	Y = 3.2524 x - 2.2358	167.6	218.8 - 145.5	-
III	0.80	Y = 2.9832 x - 1.6466	168.9	228.3 - 144.4	1.01
IV	0.40	Y = 2.7712 x --1.4247	169.8	234.7 - 140.8	1.02
At 30°C *M. anisopliae*					
II	0.19	Y = 3.1751 x - 1.4726	170.2	222.5 - 146.7	-
III	0.27	Y = 3.5964 x - 3.1130	177.5	225.6 - 154.3	1.04
IV	0.85	Y = 3.2547 x - 2.3174	178.2	247.9 -153.6	1.05

temperature. *B. bassiana* showed higher virulence to *S. obliqua* larvae compared to *M. anisopliae* at both the temperature regimes.

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