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Research Article

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Studies on Effect of Temperature and Relative Humidity on Aspergillosis in Silkworm, *Bombyx mori* L.

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The growth and multiplication of *Aspergillus flavus* Link and *A. tamarii* Kita were observed *in vitro* under variable temperatures of 22 – 31°C. The matte weight of mycelium and number of conidia/ml were significantly higher ($P < 0.01$) at the higher temperature than the lower temperature in both the species of *Aspergillus*. *In vivo* the mortality in silkworm, *Bombyx mori* L. with the infection of *Aspergillus* species was significantly ($P < 0.01$) different at different temperature and relative humidity conditions.

Key words: *Bombyx mori*, Aspergillosis, Temperature, Relative humidity

Introduction

Aspergillosis, an important disease in the young age silkworm, *Bombyx mori* L., is reported to be caused by several species of *Aspergillus* fungi, which can grow saprophytically in the silkworm rearing environment like on soil surface and rearing appliances (Aoki, 1971; Ayuzawa *et al.*, 1972). The conidia of *Aspergillus* under favorable conditions of temperature and relative humidity may adhere to silkworm during rearing and become the source of infection. Both temperature and relative humidity have major role on the growth of mycelium, sporulation and germination of spores. May *et al.* (1931) and Gould (1938) reported the temperature between 30–33°C for growth of *Aspergillus flavus* and 20°C for *A. tamarii*. *Aspergillus* grows well and sporulate abundantly at the temperature range of 23 – 26°C (Thom and Raper, 1945). Tandon and Chauhan (1955), Chinnaswamy (1983) and Peter (1988) observed mycelia dry weight and radial mycelial growth of *Aspergillus* fungi at different temperature conditions. Chinnaswamy (1983) noticed that Aspergillosis occurred in all seasons with varied level of infection from season to season. Kawakami (1982b) observed that the temperature and relative humidity prevailing in young silkworm rearing houses were also quite favorable for the development of *Aspergillus* and spread of infection in silkworm. aspergillosis is more common in young silkworms and information on influence of environmental factors on spread of this disease are limited. Hence, the present study was made to determine the multiplication and growth of *Aspergillus flavus* Link and *A. tamarii* Kita and the infection of the disease in silkworm under variable temperature and relative humidity conditions.

Materials and Methods

The conidial suspension of *Aspergillus flavus* and *A. tamarii* was prepared in double distilled water separately from their cultures maintained in laboratory of Silkworm Pathology, Central Sericultural Research and Training Institute, Mysore, India after identification of fungi from Division of Plant Pathology, Indian Agriculture Research Institute, New Delhi, India. The density of conidia (1×10^7) was counted using haemocytometer. The conidial suspension (1.0 ml) of both *Aspergillus* species were inoculated separately to 25 ml of Samsinokavas modified liquid culture media in sterilized conical flasks. The cultures were incubated at 22, 25, 28 and 31°C temperature in incubators for 7 days. Three replications were maintained for each treatment. The total mycelia weight and the number of conidia/ml of *Aspergillus flavus* and *A. tamarii* were counted and recorded separately for each treatment

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and replication.

One mililiter conidial suspension containing 1×10^7 conidia/ml was sprayed using a glass automiser once over 100 newly hatched bivoltine silkworms (NB4D2) and newly moulted 2nd instar and two ml over newly moulted 3rd instar. After infection, the silkworm was reared at 22, 25, 28 and 31°C with 70, 80 and 90% relative humidity at each temperature. To maintain the required temperature and relative humidity, electric heaters with thermostat and aerosol humidifiers were used. The rearing practices like feeding, bed cleaning, etc., were followed as advocated by Krishanaswami (1979). For each treatment, three replications were maintained for the infection of *Aspergillus flavus* and *A. tamarii* separately. The experiment was conducted for 1st, 2nd and 3rd instars and repeated three times. Observations were made for 10 days on mortality of silkworms with the infection of *Aspergillus* and data were converted in square root transformation and statistically analyzed using Completely Randomized Design (Snedecor and Cochran, 1967).

Results

In vitro multiplication and growth of *Aspergillus* at different temperature

Mean values of number of conidia/ml and matte weight of *Aspergillus flavus* and *A. tamarii* under different temperatures are presented in Table 1. Results showed that multiplication of conidia and development of mycelia in culture medium were slow at the lower temperature of 22°C as the number of conidia/ml 1.25×10^5 (11.736) and 4.00×10^5 (12.899) and matte weight of mycelium (0.08 g and 0.10 g) were lowest in both *Aspergillus flavus* and *A. tamarii,* respectively. The multiplication and growth were faster as the temperature increased and it was maximum at 4.32×10^7 (17.504) conidia/ml and 0.51 g in *Aspergillus flavus* and 5.12×10^7 (17.728) conidia/ml and 0.51 g in *A. tamarii* at 31°C. The density of conidia/ml and mycelial weight were significantly different ($p < 0.01$) at different temperature.

Infection of Aspergillus species in silkworm at variable temperature and relative humidity

Results shown in Table 2 indicate that the mortality in 1st, 2nd and 3rd instars of silkworm was lowest with infection of *Aspergillus flavus* (29.44, 26.22 and 10.88%) and with infection of *A. tamarii* (20.77, 18.33 and 4.55%) at 22°C. The mortality percentage was increased as temperature rose and it was highest due to *Aspergillus flavus* (75.33, 70.00 and 59.11%) and due to *A.tamarii* (70.55, 68.33 and 47.00%) at 31°C.

Similarly the average mortality in 1st, 2nd and 3rd instars of silkworm was lower at low relative humidity of 70% (25.66 to 40.08% with infection of *Aspergillus flavus* and 16.58 to 35.25% with infection of *A. tamarii*). The mortality was higher at high relative humidity of 90% (38.91 to 62.50% and 31.42 to 55.25% with infection of *Aspergillus flavus* and *A. tamarii* respectively).

The interaction of temperature and relative humidity on aspergillosis in silkworm has shown that the mortality was lowest (8.00 to 24.33% due to *Aspergillus flavus* and 2.66 to 16.00% due to *A. tamarii*) at low temperature with low relative humidity (T1 × RH1). The aspergillosis in silkworm increased with the increase of relative humidity at constant temperature as well as with increase of temperature with constant relative humidity. The highest mortality (65.33 to 90.00% due to *Aspergillus flavus* and 58.00 to 85.33% due to *A. tamarii*) in all tested instars of silkworm was recorded at high temperature 31°C and high relative humidity 90% combination *i.e.*, T4 × RH3.

The mortality in silkworm at different temperature, relative humidity and interaction of temperature and relative humidity was comparatively lower with the infection of *Aspergillus tamarii* than *A. flavus* in each instar (Table 2 and Fig. 1.), indicating the low virulence of *A. tamarii.*

Table 1. *In vitro* multiplication and growth of *Aspergillus* species under different temperatures

Temperature (°C)	Aspergillus flavus		Aspergillus tamarri	
	No. of conidia (ml)	Matte weight (g)	No. of conidia (ml)	Matte weight (g)
22°C	1.25×10^5 (11.736)	0.08	4.00×10^5 (12.899)	0.10
25°C	1.25×10^7 (16.118)	0.31	4.10×10^7 (17.504)	0.31
28°C	2.51×10^7 (17.082)	0.34	4.88×10^7 (17.728)	0.33
31°C	4.32×10^7 (17.504)	0.51	5.12×10^7 (17.728)	0.51
CD 5%	0.22	0.002	0.05	0.04
MS	21.02**	16.98**	0.09**	0.09**

**, Significant at 1% ($P < 0.01$).

Values in parenthesis are log transformation.

Table 2. Mortality in silkworm, *Bombyx mori* L. rearing after inoculation of newly hatched and 1st and 2nd instar just after moult with *Aspergillus* (1×10^6)

Factor	Mortality (%)					
	Aspergillus flavus			*Aspergillus tamrii*		
	1st instar	2nd instar	3rd instar	1st instar	2nd instar	3rd instar
Temperature						
T1 (22°C)	29.44 (5.45)	26.22 (5.14)	10.88 (3.34)	20.77 (4.59)	18.33 (4.31)	4.55 (2.21)
T2 (25°C)	42.66 (6.52)	41.55 (6.45)	23.33 (4.82)	31.77 (5.65)	30.77 (5.56)	12.33 (3.55)
T3 (28°C)	58.00 (7.61)	55.33 (7.43)	36.33 (6.02)	56.33 (7.50)	52.88 (7.27)	32.11 (5.64)
T4 (31°C)	75.33 (8.68)	70.00 (8.36)	59.11 (7.71)	70.55 (8.40)	68.33 (8.26)	47.00 (6.87)
RH						
RH1 (70%)	40.08 (6.29)	37.25 (6.04)	25.66 (4.83)	35.25 (5.81)	33.00 (5.61)	16.58 (3.81)
RH2 (80%)	51.50 (7.09)	48.08 (6.86)	32.66 (5.52)	44.08 (6.51)	42.66 (6.39)	24.00 (4.58)
RH3 (90%)	62.50 (7.82)	59.50 (7.63)	38.91 (6.08)	55.25 (7.28)	52.08 (7.06)	31.42 (5.30)
Interaction						
T1 × RH1	24.33 (4.98)	20.00 (4.52)	8.00 (2.91)	16.00 (4.06)	13.66 (3.76)	2.66 (1.77)
T1 × RH2	30.00 (5.52)	26.66 (5.21)	10.33 (3.23)	20.66 (4.60)	18.00 (4.30)	4.00 (2.11)
T1 × RH3	34.00 (5.87)	32.00 (5.70)	14.66 (3.89)	25.66 (5.11)	23.33 (4.88)	7.00 (2.73)
T2 × RH1	32.33 (5.69)	33.00 (5.78)	15.33 (3.94)	24.00 (4.95)	22.33 (4.77)	8.33 (2.97)
T2 × RH2	40.00 (6.36)	38.00 (6.20)	25.00 (5.05)	31.66 (5.67)	33.33 (5.81)	12.00 (3.53)
T2 × RH3	56.33 (7.51)	53.66 (7.38)	29.66 (5.49)	39.66 (6.33)	36.66 (6.10)	16.66 (4.14)
T3 × RH1	44.00 (6.66)	40.00 (6.36)	25.33 (5.08)	44.33 (6.69)	42.00 (6.52)	20.00 (4.52)
T3 × RH2	60.33 (7.78)	58.33 (7.67)	37.66 (6.18)	54.33 (7.40)	50.66 (7.15)	32.33 (5.73)
T3 × RH3	70.33 (8.39)	67.66 (8.25)	46.00 (6.81)	70.33 (8.42)	66.00 (8.15)	44.00 (6.67)
T4 × RH1	60.00 (7.78)	56.00 (7.51)	54.33 (7.38)	56.66 (7.56)	54.00 (7.38)	35.33 (5.98)
T4 × RH2	76.66 (8.74)	69.33 (8.35)	58.00 (7.65)	69.66 (8.37)	68.66 (8.31)	47.66 (6.94)
T4 × RH3	90.00 (9.51)	84.66 (9.23)	65.33 (8.11)	85.33 (9.26)	82.33 (9.10)	58.00 (7.65)
CD at 5%						
Temperature	(0.28)	(0.29)	(0.30)	(0.22)	(0.22)	(0.18)
RH (%)	(0.24)	(0.25)	(0.26)	(0.19)	(0.20)	(0.16)
Temp × RH	(0.49)	(0.50)	(0.53)	(0.38)	(0.38)	(0.31)

Values in parentheses are Square Root Transformation (SQRT).

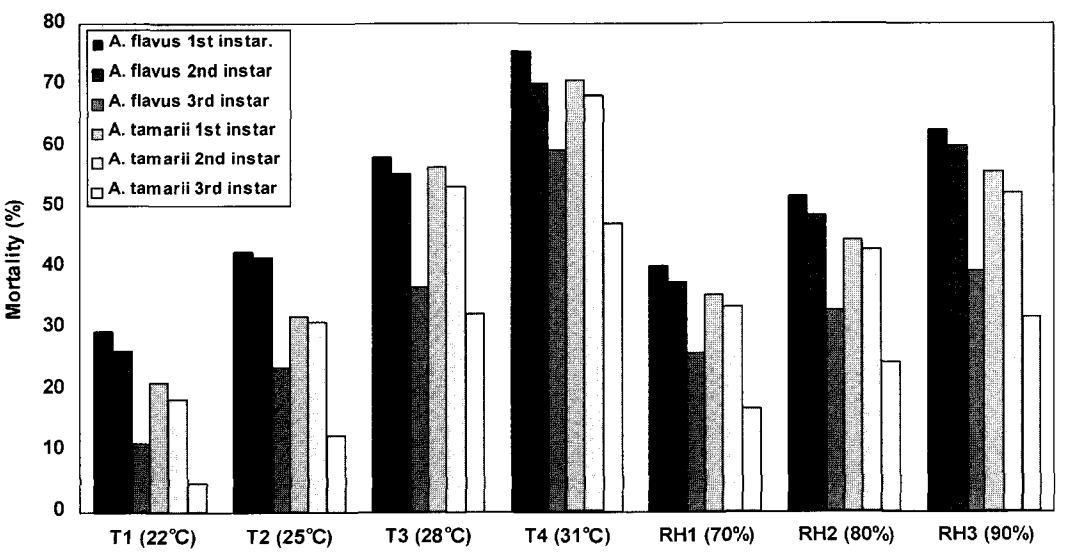

Fig. 1. Mortality due to infection of Aspergillus flavus nd A. tamarii at diifferent temperature and relative humidity in silkworm, *Bombyx mori*.

Table 3. Results of Anova test for mortality in silkworm rearing

Source of variation	Degree of freedom	Mean sum of square					
		Aspergillus flavus			*Aspergillus tamarii*		
		1st instar	2nd instar	3rd instar	1st instar	2nd instar	3rd instar
Temperature	3	17.35**	17.12**	30.83**	26.95**	27.88**	39.00**
RH	2	07.14**	07.58**	04.69**	06.45**	06.32**	06.61**
Temp. × RH	6	00.19 NS	00.16 NS	00.23 NS	00.08 NS	00.11 NS	00.23**
Error		00.08	00.09	00.10	00.05	00.05	00.03

*, significant at 5% ($P < 0.05$); **, significant at 1% ($P < 0.01$); and NS, non significant.

Similarly the mortality in silkworm larvae with the infection of *Aspergillus flavus* and *A. tamarii* was decreased from 1st to 3rd instars and this indicates the higher pathogenicity of the *Aspergillus* to young silkworms than the advanced silkworms (Table 2 and Fig. 1).

Discussion

In present study, the multiplication of conidia (Number of conidia/ml) and growth of mycelium (Matte weight) of *Aspergillus* and *A. tamarii* in culture medium were significantly different ($P < 0.01$) at different temperature and it was highest at the high temperature of 31°C. These findings are more or less in agreement with May *et al.* (1931) and Gould (1938) who reported that the growth of mycelium and sporulation of *Aspergillus flavus* was good at 30°C or above. However, they have reported good growth of *Aspergillus tamarii* at lower temperature of 20°C. Similarly Thom and Raper (1945) stated that majority of Aspergilli grow well and sporulate abundantly at 23 to 26°C. In a similar case Tondon and Chauhan (1955) recorded maximum radial mycelial growth at 30°C in case of *Aspergillus flavus* and Chinnaswamy (1983) and Peter (1988) in case of *Aspergillus tamarii.*

The significantly ($P < 0.01$) high mortality in silkworm with infection of *Aspergillus* at high temperature 28 and 31°C with relative humidity levels of 80 and 90% in present study (Table 3) is almost similar to the earlier work of Kawakami (1982b), where he stated that temperature 28 – 30°C and relative humidity 85 – 90% which is favorable for the growth of young silkworm also are favored for *Aspergillus* disease development. Further he described that as the *Aspergillus* fungi prefer high temperature and high relative humidity, the control may be more important in silkworm rearing in subtropics and tropics than in Japan. The significantly high ($p < 0.01$) mortality in silkworm larvae in each instar due to infection of *Aspergillus flavus* and *A. tamarii* at higher temperature is in agreement with the results of Chinnaswamy (1983) where he reported that disease development is slow at low temperature and more rapid at high temperature. The observation that the interaction of temperature and relative humidity on aspergillosis development in silkworm has shown significant difference ($p < 0.01$) in mortality in 3rd instar confirms the earlier work of Chinnaswamy (1983).

The higher mortality in 1st instar with the infection of both *Aspergillus flavus* and *A. tamarii* at all temperatures and relative humidities tested indicates higher pathogenicity of *Aspergillus* to 1st instar than later instars (Fig. 1), confirming the earlier reports of Aoki (1971), Aoki *et al.* (1972), Kawakami (1982b), Narayanaswamy and Govindan (1989) and Patil *et al.* (1996). Similarly the pathogenicity of *Aspergillus flavus* to different instars of eri silkworm is same as of *Bombyx mori* (Narayanaswamy *et al.*, 1988). *Aspergillus flavus* was more virulent than *A. tamarii* (Fig. 1). The variation in virulence in strains of *Aspergillus* species has been observed by Bhagyalakshmi (1994).

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