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Influence of Weather Factors on Incidence and Intensity of Microsporidiosis in Silkworm (*Bombyx mori* L.)

[1]V.K. Rahmathulla, [1]C.M. Kishor Kumar, [2]B.S. Angadi and [2]V. Sivaprasad
[1]P3 Basic Seed Farm, National Silkworm Seed Organization, Central Silk Board, Ring Road, Srirampura, Karnataka, Mysore, 570 008, India
[2]National Silkworm Seed Organization, BTM lay out, Madivala, Bangalore, Karnataka, 560 068, India

Corresponding Author: V.K. Rahmathulla, P3 Basic Seed Farm, National Silkworm Seed Organization, Central Silk Board, Ring Road, Srirampura, Karnataka, Mysore, 570 008, India Tel: 919449621740

ABSTRACT

Change in climate involving temperature variation influences the incidence of microsporidiosis in silkworm. As microsporidian disease in silkworm is epidemic, transovarially transmitted to progeny eggs and prevention of the disease is very vital in parental silkworm seed multiplication. The impact of different climatic conditions viz., summer, rainy and winter on the incidence of microsporidian disease in silkworm was studied at a sericulture Basic Seed Farm, at Mysore, Karnataka, India as a part of the regular monitoring and control of the disease carried out for a period 3 years (2008-10). The results indicated that the disease incidence was higher during the beginning of winter season (October-November) followed by rainy season (July-September) and the incidence was least in summer season (March-May). Correlation analysis showed that the incidence was correlated positively with humidity and negatively with temperature recorded from the study location.

Key words: Microsporidiosis, climatic factors, *Bombyx mori* L., correlation analysis

INTRODUCTION

Assessment of the effects of global climate changes in agriculture and allied fields might help to properly anticipate and adapt farming technologies to maximize agricultural production and later this situation necessitates finding out the impact of climate change on crop production (Srivani *et al*., 2007). Sericulture is the science that deals with the production of silk by rearing silkworm. Silkworm diseases are major constraint in realizing full potentiality of crop yield in sericulure (Kumar and Naik, 2011). Pebrine is one of the most destructive diseases of silkworm (*Bombyx mori* L.) and had been a major cause for the decline of French silk industry in the latter half of the 19th century. The disease is caused by highly virulent parasitic microsporidian *Nosema bombycis* Nageli, the epizootic has become increasingly complex as different strain and species have been observed from the infected silkworm as well as other lepidopteran insects (Ananthalakshmi *et al*., 1994; Kishore *et al*., 1994; Sharma *et al*., 2003; Bhat and Nataraju, 2004; Selvakumar *et al*., 2005). *Nosema* species also infects other silk producing insects like Eri, Tropical tasar and Chinese tasar and Muga silkworm. Pebrine is considered to be the most serious disease in non-mulberry silkworm and contribute 20-25% crop loss (Kumar *et al*., 2011) and the sporadic disease differs from other silkworm diseases as it is transmitted both horizontally and vertically. The common route of infection of pebrine disease is through the diseased mother moth (transovarial transmission). The disease is transmitted to some extent by transovum transmission through the external contamination of eggs by pebrine spores or through contaminated leaves fed to silkworm. The adverse effect of environmental conditions on microsporidian spores especially sunlight have been documented and intensity and amount of bright sunlight available for inactivation of spores depends on the season (Patil, 1993). Seasonal occurrence and influence of temperature on microsporidian infection in silkworm and other insects were studied (Noamani *et al.*, 1971; Iwano, 1987). Weather is one of the important parameter that influences insect disease epidemics. Hence, understanding of weather and climatic conditions is required to provide base line information for developing simple and reliable pest and disease prediction systems (Selvaraj *et al.*, 2011).

Besides this, quality cocoon and silk production is very closely related to nutritional factors. Dietary efficiency of silkworm plays a major role in converting the mulberry leaves consumed by larva to healthy pupa, cocoon and ultimately to silk (Seidavi, 2009). Maintenance and multiplication of the basic stocks of different races/breeds in sericulture is very important from the point of quality seed production. If recommended practices are not followed, the race characters will show variation affecting the cocoon and silk quality (Kumar *et al.*, 1999; Reddy *et al.*, 2010). For increasing the production of quality bivoltine silk one and most essential requirement is assurance of production of high quality bivoltine seed cocoon and pebrine disease free eggs, which contribute high hybrid vigor in commercial cocoon production (Ashfaq *et al.*, 2000; Hussain and Javed, 2002).

Information about the relation to environmental conditions conducive for the microsporidian disease incidence and its intensity is scanty. The impact of environmental conditions and their fluctuation in relation to microsporidian disease incidence and spread of disease is not quantitatively studied. Therefore, the present study was initiated to characterize environmental conditions conducive for the microsporidiosis in silkworm.

MATERIALS AND METHODS

Study location: The study area located in Mysore, India situated at 12^{0}18'N 76^{0}39'E 12.30^{0}N 76.65^{0}E and has an average altitude of 770 m (2,526 ft). It is in the southern region of the state of Karnataka and spreads across an area of 128.42 km^2 (50 m^2). The summer is from March to middle of June, monsoon is from middle of June to October and the winter from November to February. The highest temperature recorded in Mysore during summer was 38.5°C (101^{0}F) and in winter, lowest of temperatures 9.6°C (49^{0}F) have been recorded. The average annual rainfall received by the city was 798.2 mm.

Study of microsporidiosis incidence in silkworm: As a part of the regular pebrine disease-monitoring programme at Sericulture Basic Seed Farm, Mysore, the study was undertaken during the period (2008-2010) for analyzing the seasonal incidence of microsporidiosis in silkworm. The microscopic examination for pebrine disease was done for every crop from egg stage to adult moth. In this method, individual mother moth or larvae collected in a mortar and crushed with help of a pestle or a group of five moths were crushed by using mixer by adding 0.6% K_2CO_3 (1:4). The samples were filtered through cotton, collected in 2 or 10 mL tubes and centrifuged at 6000 rpm for 10 min. The supernatant was decanted and a few drops of K_2CO_3 solution were added to the sediment and thoroughly mixed in a cyclomixer. Two smears from each sample were examined under a binocular microscope at 600 X magnifications. Microsporidian spores were detected and identified by observation on luster, shape, size and by their particular brownian movement. Besides, the intensity of spores in a microscopic field also assessed. The climatic data like maximum and minimum temperature, humidity and rainfall of every month from the study location were also recorded. Analyzed the influence of different climatic conditions such as summer, rainy and winter on microsporidian disease incidence in silkworm seed production.

Statistical analysis: A correlation study was done to analyze the relationship between disease incidence and weather factors. A multiple linear regression analysis was accomplished, in order to investigate the simultaneous influence of the climatic factors.

RESULTS AND DISCUSSION

Microsporidian infection in silkworm: The investigation on the incidence of microsporidiosis in silkworm during the silkworm crop period occurring in different seasons was analysed (2008-10). The results showed that during the year 2008, the infection was recorded very low (January-February and March- April months) and succeeding months (May-June) infection was not at all recorded (Table 1). After onset of monsoon, the infection was reappeared and further increased and reached maximum during the month of October-November (13.96%).

Similar, observations were recorded in 2009 and results showed that infection was recorded low (5.49 and 0.43%, respectively for December-January and February-March). During summer months, (April-June) infection was not recorded. The infection was again noticed during rainy season and it was reached peak stage during the period of October-November (11.49%).

The observation was in similar line during the year 2010 and the results revealed that as usual the infection was started with a low intensity during January (3.20%) and further disease incidence was reduced during the months of February-March (1.67%). Summer it was recorded significantly very low (0.08% for April-May and 0.06% for Jun-July) and the infection were increased during the succeeding months and highest was recorded during October-November period (20.7%).

The three years of data clearly indicated that during October-November months recorded maximum infection and highest values of high and low humidity of each day of the study location coincide with minimum temperature experienced to contribute the multiplication of pathogen. Intensity of pebrine spore in microscopic field also recorded more during favorable season (October -November). During summer, one or two spore per smear was recorded. A large number of factors like temperature, humidity and biotic components of the substrate in which they are found influenced the survival of microsporidia in an environment (Kramer, 1976). Pebrine spores will remain viable for a maximum period of 225 days, in wet soil and a minimum of 135 days in wet compost under tropical condition (Patil, 1993). The climate change can affect the response of insects

Table 1: Microsporidian infection in silkworm during crop period (%)

2008	Jan.-Feb.	Feb.-Mar.	Mar.-Apr.	May-June	Jul.-Aug.	Sept.-Oct.	Oct.-Nov.	Average
	1.70	0	01.71	0	2.65	5.70	13.96	3.67
2009	Dec.-Jan.	Feb.-Mar.	April	May-June	Aug.- Sept.	Oct.-Nov.		Average
	5.49	0.43	0	0	10.2	11.49		04.60
2010	January	Feb.-Mar.	April-May	June-July	Aug.-Sept.	Oct.-Nov.	Dec.-Jan.	Average
	3.20	1.67	0.08	0.06	0.10	20.7	1.80	3.94

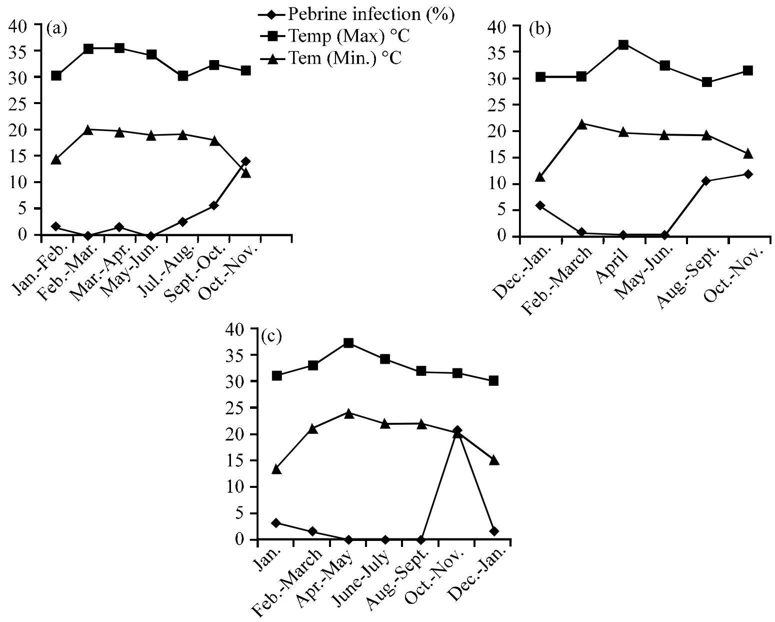

Fig. 1(a-c): Relation between temperature and microsporidian infection in silkworm for (a) 2008, (b) 2009 and (c) 2010

to the host plants and pathogens to host insect, although it is difficult to predict the impact of climate changes on various insects and pathogens. The overall response is dependant on the impact of climate change on the insect-tree, host-natural enemy-pathogen relationship (Khan *et al*., 2003).

Variation in weather factors and disease incidence: During the year 2008 highest temperature about 35°C was recorded in the months of February- April. Succeeding months of May-June, due to the onset of monsoon temperature falls to 29-30°C and after a slight increase again reached at the level of 30°C during September-October months (Fig. 1). During, 2008 minimum temperature of the day was recorded lowest in the month of October-November (12°C) and February -March months highest minimum temperature was recorded (20°C). During the year 2009 February-March period does not experienced severe summer and later April month was recorded highest temperature. Minimum temperature of the day was recorded lowest during the period of December-January (11°C).Highest minimum temperature was recorded during February-March period. However, in 2010, temperature was further increased during the summer season and highest temperature was recorded (37°C). Figure 1 also showed the graphical representation of microsporidian infection in different months and it indicated that infection was low in summer season.

In 2008, maximum humidity was recorded during the rainy season and it was recorded highest during September-October period (99%). In 2009, highest humidity was recorded (96%) in the month of October-November and in 2010, similar condition was repeated (Fig. 2). Minimum humidity was recorded lowest during the winter and summer season and lowest was recorded 30% in 2008 and 2010 (February-March). The graphical representation also showed that there was relation between humidity and microsporidian infection. During rainy season (June-November) maximum humidity and highest infection of microsporidian disease was recorded (Fig. 2).

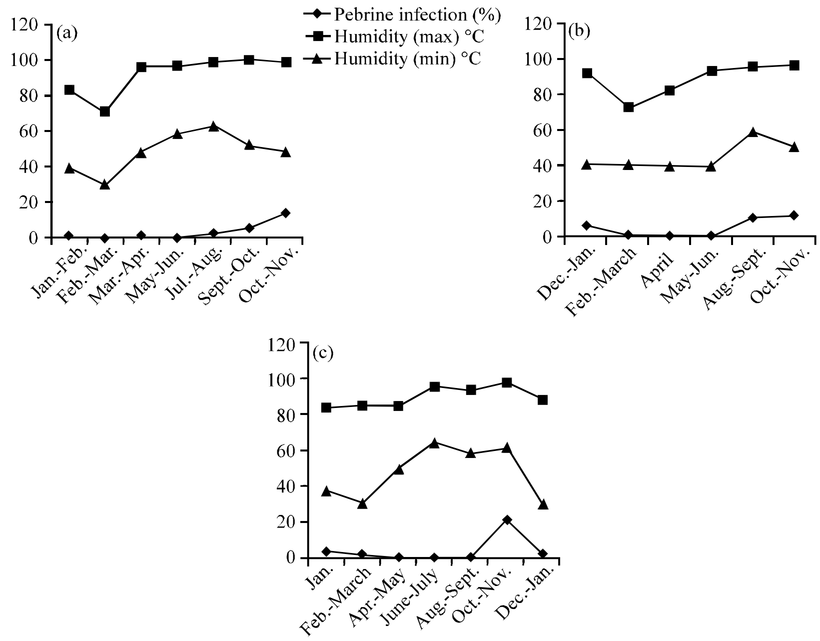

Fig. 2(a-c): Relation between humidity and microsporidian infection in silkworm for (a) 2008, (b) 2009 and (c) 2010

Table 2: Association of climatic factors with pebrine infection for 3 years

Years	Max. temperature	Max. humidity	Min. temperature	Min. humidity	Rainfall
2008	-0.429**	0.460**	-0.784**	0.229*	0.236*
2009	-0.537**	0.714**	-0.439**	0.856**	0.137
2010	-0.300*	0.510**	-0.065	0.305*	0.520**
Pooled	-0.422**	0.561**	-0.429**	0.463**	0.297**

**,* Significant at 1% and at 5% level, respectively

Association weather factors with microsporidian infection: The incidence of microsporidiosis in silkworm showed great sensibility to weather variations occurring over the period studied. Climate change resulting in increased temperature could influence pest and disease incidence in several complex ways. Although, different workers reported that climate changes and temperature effects might tend to depress disease incidence in insects.

A negative correlation was observed between the percentage of microsporidian infection in silkworm and maximum temperature recorded from the study location during different months of 3 years (r = -0.429 in 2008, r = -0.537 in 2009 and r = -0.300 in 2010). The correlation coefficient data indicated that when temperature rises up during hot months of February-April the infection was recorded very low or nil (Table 2). Many factors are known to influence the rate of growth and development of insects and its predators, parasites and pathogens. Among the temperature has a profound influence as it governs certain biological aspects of insects (Ali and Rizvi, 2008). Similarly, a significant negative correlation was recorded between percentage of infection and minimum temperature. Correlation studies were also worked out between pebrine infection and maximum humidity, minimum humidity and rainfall recorded during different months for a period of three years. All correlation coefficient (r) data showed that there was a correlation between the two

Table 3: Regression equation showing relation between microsporidiosis and climatic factors of the study location

Year	Max. temperature (X1)	Min. temperature (X2)	Max. humidity (X3)	Min. humidity (X4)	Rainfall (X5)
2008	Y = -34.228+0.942 X1	Y = -26.262+1.281 X1	Y = 35.149-0.206 X3	Y = 22.630-0.058 X4	Y = 24.709-0.009 X5
	R^2 = 0.180	R^2 = 0.615**	R^2 = 0.312*	R^2 = 0.379*	R^2 = 0.563**
2009	Y = -40.125+1.113 X1	Y = -17.370+2.012 X2	Y = 45.149-0.306 X3	Y = 49.670-1.458 X4	Y =12.546-0.101 X5
	R^2 = 0.281*	R^2 = 0.412**	R^2 = 0.282*	R^2 = 0.691**	R^2 =0.341*
2010	Y = -35.124+0.953 X1	Y = -6.104+1.492 X2	Y = 95.123-1.661 X3	Y = 32.542-0.321 X4	Y = 34.431-0.165 X5
	R^2 = 0.450**	R^2 = 0.171	R^2 = 0.320*	R^2 = 0.642**	R^2 = 0.551**

*,** Significant at 1% and at 5% level, respectively

variables. Significant ($p \leq 0.1$) positive correlation was observed between maximum humidity and microsporidian infection during the period (0.460 in 2008, 0.714 in 2009 and 0.510 in 2010). The analysis also revealed that a positive and highly significant correlation between percentage of infection and minimum humidity recorded (0.229 in 2008, 0.856 in 2009 and 0.306 in 2010). The rainfall data of the study location also significantly correlated with microsporidian infection (0.236 in 2008, 0.137 in 2009 and 0.520 in 2010). The results revealed that the due to continuous rain during the period (July-November) increased relative atmospheric humidity and created a favourable environmental condition for microsporidian multiplication and infection in silkworm batches reared. Mohanan *et al.* (2009) reported that maximum spore formulation in different tissues of silkworm recorded in low temperature condition (18.9±1°C) when compared to higher temperature condition (29.4±1.1°C). Microsporidian disease show more prevalence in the rainy and cooler seasons probably due to the persistence and survival of the pathogen in the environment for longer period and congenial condition for pathogen multiplication leading to easy spread (Baig, 1994). Slight variation in humidity during silkworm rearing favours the multiplication of microsporidian infection in silkworm when compared with slight variation in temperature (Chakrabarti and Manna, 2008). Femi-Ola and Aderibigbe (2009) estimated the bacterial population in the hindgut of wood eating termite and the results indicated that population was significantly higher during wet season when compared with dry season.

Regression model for the relation between weather factors and microsporidian infection: Linear regressions analysis revealed that measured environmental variables have significant effects on microsporidian infection ($p<0.05$) and confirming the results of correlation analysis. The importance of relative humidity and disease incidence for explaining significant portions of the independent variance for abundance of infections also emphasized. The stepwise regression analysis constructed to investigate the abiotic factors contributed the most to the variation of pebrine infection during the 3 years period (Table 3). Regression analysis showed that minimum humidity of each day recorded from the study location was significantly contributed to the incidence of the disease (37.90% in 2008, 69.10% in 2009 and 64.20% in 2010). Timing of the insect pest and disease appearance varies, depending on differences in temperature throughout the years, which makes the forecasting and management difficult. One way to promote our understanding of the phrenology of pathogen is to develop a population dynamics model that explicitly incorporates temperature-dependent development (Mahmood *et al.*, 2002). Similarly, maximum humidity also significantly and positively contributed for disease incidence (31.20% in 2008, 28.20% in 2009 and 32.00% in 2010). Analysis also showed that maximum temperature of the study location significantly and negatively contributed to the incidence of pebrine (18, 28 and

45% for 2008, 2009 and 2010, respectively). The forecast model can be used to predict the initiation and 'red alert' season of the microsporidian disease incidence in silkworm. This serves as a scale for the silkworm seed producers of both Government and private sectors to adopt effective crop protection measures at appropriate time.

CONCLUSIONS

The study results concluded that the microsporidian infection was recorded peak when minimum and maximum atmospheric relative humidity of the day was high during rainy season. However, low or zero level of disease infection was recorded during summer season where high temperature and low humidity was recorded. Research on epidemiological aspects to explain the dynamics of the disease under local conditions is of primary importance. Weather is one of the important parameter that influences disease epidemics in silkworm. Hence, understanding of weather and climatic conditions is required to provide base line information for developing simple and reliable disease prediction systems.

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