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Seasonal Impact of Microsporidian Infection on the Reproductive Potential of Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae)

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Impact of microsporidian infection and season on reproductive potential of *Bombyx mori* L. was investigated in the laboratory. Microsporidian infection significantly ($P < 0.01$) reduced fecundity and hatching and increased sterility and mortality of eggs. Among the microsporidia, *Nosema* sp. 2 infected silkworm produced eggs with least fecundity and hatching percent as well as highest dead and sterile eggs followed by *Nosema* sp. 1 and *N. bombycis*. Microsporidia, in general, significantly reduced fecundity and hatching percent of eggs and increased number of dead and sterile eggs in all the three seasons except *N. bombycis* in July - August (S3) and *Nosema* sp. 2 in January - February (S1). Since, seed production is the anchor sheet of mulberry sericulture, coefficient of egg lying is considered as an important aspect and the industry quite often facing shortage of disease free layings. The present study indicates that *B. mori* is more susceptible to microsporidia during S2 followed by S3 and S1 and *Nosema* sp2. is most virulent followed by *Nosema* sp1. and *N. bombycis*.

Key words: *Bombyx mori*, Microsporidia, Season, Reproductive potential

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Introduction

Pebrine is a microsporidian disease of silkworm, which shows both horizontal and vertical transmission. 'Ovipositioning' is one of the important aspects of reproduction and its behavior in insects is nearly as diverse as the species themselves (Engelmann, 1970). Physiology of egg laying and its influencing factors are of much significance in economically important insects like silkworm. The oviposition behaviour of insects is peculiarly suited to the conditions in which the species occur, habitat or the substrata on which they oviposit and it depends on a complex array of information that the species obtain from its environment (Engelmann, 1970). Besides, in unfavourable condition, parasitization and weak condition of the insect, produces less number of eggs with low hatching and higher sterility. Insect larvae sustaining sublethal infection may be characterized by prolonged developmental periods, suppressed pupal weight, reduced fecundity and shortened adult longevity (Thomson, 1958; Wilson, 1983; Bauer and Nordin, 1988b, 1989). Pebrine is a very dreaded disease of silkworm, which shows both horizontal and vertical transmission. Effect of pebrine infection on food utilization efficiency and reproductive potential of *Antheraea mylitta* D. has been studied (Rath *et al.*, 2001, 2003). But, information on the effect of microsporidian infection as well as season on reproductive potential of mulberry silkworm, *Bombyx mori* L. is scanty. So, we decided to undertake a systematic study on the impact of microsporidian infection and season on the reproductive potential of *B. mori*.

Materials and Methods

A popular multivoltine silkworm, Nistari (marked) fed on mulberry (*Morus alba* cultivar S_1) leaves was selected for the study. Three microsporidia *viz.*, *Nosema bombycis, Nosema* sp. 1 and *Nosema* sp. 2 isolated respectively from *B. mori*, *A. mylitta* and *Diacrisia obliqua* W. were taken for the investigation. Spores were isolated from the respective adult and purified by isodensity equilibrium centrifugation using percoll (Sato and Watanabe, 1980). Newly hatched larvae were counted using magnifying lens and fine brush and separated into batches of fifty. Two hundred fifty µl of spore suspension (1×10^6/ml) of each microsporidium in endotoxin free (ETF) water was smeared on leaf disc (5.3 cm dia.) individually and fed to one batch of 50 larvae. A total of 200 larvae were inoculated for each microsporidium. A healthy control was also reared in identical condition, where the larvae were given the leaf disc dipped in ETF water. Then, the larvae were reared separately on normal mulberry leaves in wooden trays (35 cm × 25 cm × 5 cm) up to spinning. The cocoons from the microsporidian inoculated and healthy lots were used for grainage operation. The obtained eggs were surface sterilized in 2% formalin and used for further rearing. During grainage, data on mating success and egg laying behaviour were recorded. In addition, fecundity, egg hatching, dead and sterile eggs were also recorded. The experiment was conducted in three seasons, one favorable, January - February (S1) and two unfavourable, April - May (S2) and July - August (S3) with distinct average temperature [T1 (18.62 ± 1.7°C); T2 (29.46 ± 2.6°C) and T3 (29.38 ± 2.6°C)] and relative humidity [H1 (82.70 ± 3.4%); H2 (78.6 ± 3.3%) and H3 (87.39 ± 2.6%)]. The data were subjected to ANOVA and means were compared for significant difference.

Results

Analysis of variance revealed significant ($P < 0.01$) effect of treatments, seasons and their interactions. While comparing the overall treatment means it is found that each of the microsporidium significantly reduced fecundity and hatching percent of eggs and increased number of dead and sterile eggs. Among the microsporidia, *Nosema* sp. 2 resulted in least fecundity and hatching percent of eggs and highest dead and sterile eggs followed by *Nosema* sp. 1 (Table 1 and 2).

It was also found that microsporidia, in general, significantly reduced fecundity and hatching percent of eggs and increased number of dead and sterile eggs in each of the three seasons except *N. bombycis* in S3 and *Nosema* sp. 2 in S1. Fecundity was least in *Nosema* sp. 2 infected silkworms in various seasons, which was 27.2, 38.1 and 43.7% less than their respective healthy controls in S1, S2 and S3. During S3 silkworm produced significantly less number of eggs in healthy and *Nosema* sp. 2 infected silkworm. But, silkworms infected with *N. bombycis* pro-

Table 1. Impact of microsporidian infection and season on fecundity and hatching of a multivoltine silkworm breed Nistari (M)

Season	Fecundity (no.)				Egg hatching (%)			
	Nosema bombycis	*Nosema* sp. 1	*Nosema* sp. 2	Control	*Nosema bombycis*	*Nosema* sp. 1	*Nosema* sp. 2	Control
1	419.2	375.0	366.0	502.5	91.5	89.2	88.2	97.7
2	381.1	354.9	280.6	453.0	86.6	87.4	81.8	96.7
3	426.0	373.9	241.3	428.8	88.5	82.5	76.0	91.8
Mean	408.8	367.9	296.0	461.4	88.9	86.4	82.0	95.4
CD at 5%	Treatment (T) = 23.02; T × S = 39.86				Treatment (T) = 2.45; T × S = 4.24			

Table 2. Impact of microsporidian infection and season on dead and sterile eggs (%) of a multivoltine silkworm breed, Nistari (M)

Season	Dead eggs (%)				Sterile eggs (%)			
	Nosema bombycis	*Nosema* sp. 1	*Nosema* sp. 2	Control	*Nosema bombycis*	*Nosema* sp. 1	*Nosema* sp. 2	Control
1	3.95	4.25	2.87	0.89	4.52	6.55	8.96	1.44
2	6.10	5.98	7.77	1.27	7.28	6.63	10.46	2.02
3	2.94	6.93	10.92	3.33	3.14	10.60	13.05	3.01
Mean	4.33	5.72	7.19	1.83	4.98	7.93	10.82	2.16
CD at 5%	Treatment (T) = 1.26; T × S = 2.17				Treatment (T) = 1.70; T × S = 2.95			

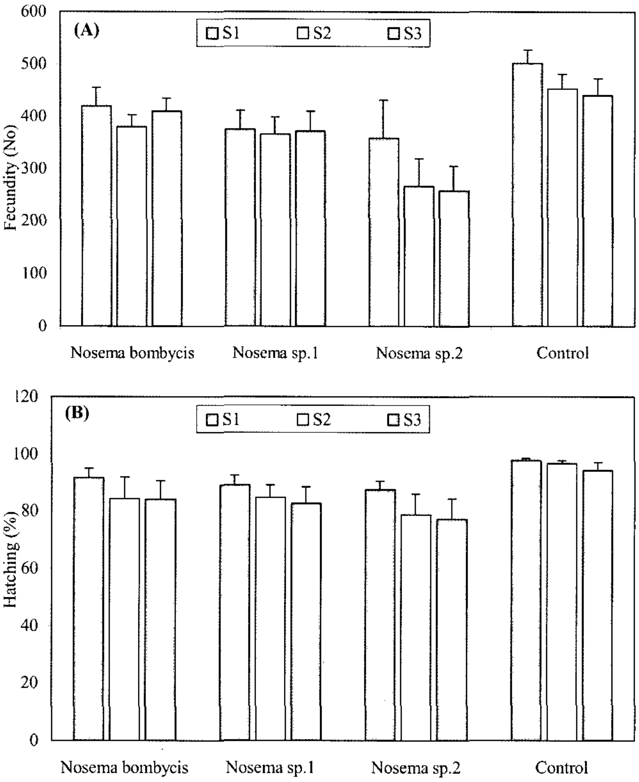

Fig. 1. Seasonal variation of (A) fecundity and (B) hatching of eggs of a multivoltine silkworm breed Nistari (M) infected with three microsporidia. Vertical bars represent SE of mean (n = 15). S1 = January - February, S2 = April - May, S3 = July - August.

duced significantly less number of eggs during S2 than other two seasons. Similarly, *Nosema* sp. 1 infected silkworms produced significantly less number of eggs during S2 (Fig. 1a). Highest hatching was also observed in eggs laid by healthy silkworm in all the seasons (Fig. 1b), which was significantly higher than microsporidian infected lots. Among the microsporidia, *Nosema* sp. 2 infected silkworm registered the least egg hatching (%), which was 9.5%, 14.9% and 15.8% less than healthy control in S1, S2 and S3 respectively (Table 1). Among the seasons, during S3 egg hatching found significantly less both in healthy and *Nosema* sp. 1 infected silkworms but *N. bombycis* infected silkworm showed significantly less egg hatching during S1 and S2. Egg hatching was significantly different in various seasons in silkworms infected with *Nosema* sp. 2 (Fig. 1b). During S2, 7.3% of the microsporidian infected adult females did not mate at all and 16.7% of mated females laid eggs in very irregularly. Similarly, in S3, 11.6% of infected females did not mate with the males and 23% of the properly mated females produced eggs in irregular manner. In general frequency of egg laying was drastically reduced in microsporidian infected females.

Nosema sp. 2 infected silkworm produced eggs with highest sterility and mortality. *Nosema* sp. 2 infected silkworms produced 7.5, 8.4 and 10.0% more sterile eggs than healthy silkworms in S1, S2 and S3 respectively. Percentage of sterile eggs was significantly different in *Nosema* sp. 2 infected silkworm during various seasons. In healthy silkworm, sterile eggs were significantly higher during S2 and S3 and in *Nosema* sp. 1 infected lots sterile eggs were significantly higher in S1 and S2. But in *N. bombycis* infected silkworms, sterility was significantly more during S1 and S3 (Table 2).

Percentage of dead eggs produced by microsporidian-infected silkworm was found significantly higher than healthy silkworm except *N. bombycis* infected silkworm in S3. Dead eggs in *Nosema* sp. 2 infected silkworm was 2.0, 6.5 and 7.6% more than the same produced by the healthy silkworm in S1, S2 and S3 respectively (Table 2). Dead eggs produced by silkworm infected with *Nosema* sp. 2 were significantly different in various seasons, whereas *Nosema* sp. 1 infected silkworms produced significantly less dead eggs during S1 than other two seasons. In healthy silkworm, it was significantly less during S1 and S2 but in *N. bombycis* infected silkworm, percentage of dead eggs was significantly less during S1 and S3 (Table 2).

Discussion

Mulberry silkworm is an important economic insect, which is commercially exploited for silk. Quality seed is the prime requirement for ultimate growth of sericulture industry. *N. bombycis*, a microsporidian parasite of *B. mori* causes the fatal disease pebrine. Reports are available on adverse effects of this parasite on insect tissues, reproductive potential and fertility (Steinhaus, 1949; Yuplian, 1995; Bansal *et al.*, 1997). Less fecundity and hatching of eggs during S2 and S3 may be due to higher temperature (> 29°C) during rearing, which might have decreased the ovulation and increased the retention of eggs. In *B. mori*, ovulation, fecundity and retention eggs varied with season (Mathur *et al.*, 1995), which is in conformity with the our result. Significant increase in total egg production and coefficient of egg laying with significantly low retention of eggs in low temperature was reported in *A. mylitta* (Rath *et al.*, 2001).

Reduced fecundity and egg hatching in microsporidian-infected silkworm may be due to severe damage of fat body and gonadal tissues. In the present study, we also observed dissolution of fat body tissues in infected larvae. The damage of reproductive tissues and dissolution of muscular tissues following infection were the possible reasons for the reduced fecundity in insects (Hassanein, 1951; Yup-lian, 1995). Higher intensity of infection as well microsporidian multiplication was observed in the fat body of *B. mori* (Madana Mohanan, 2004). Similarly, fecundity reduction was correlated to extensive infection of adult fat body in corn earworm transovarially infected with *N. heliothidis* (Gaugler and Brooks, 1975). They explained that females are dependent on fat body for the protein reserves needed for egg production. Vitellogenin, a protein from the fat body is transported to the ovary for maturation of eggs (Bradley, 1983). Studies on tissue specificity and intensity of infection of these microsporidia in *B. mori* indicated that even though they were infective to both the gonads, intensity of infection was more in female gonad (Madana Mohanan *et al.*, 2004). Development of germ cells was interfered in microsporidian-infected gonads of the host, which ultimately prevented cell differentiation (Syme and Green, 1972; Gordon *et al.*, 1973). Fecundity reduction in *Listronotus bonariensis* infected with *Microsporidium itiiti* was correlated to shortening of the period of egg laying (Malone, 1987). Similarly, Baüer and Nordin (1989) reported that sublethal dosages of *N. fumiferanae* induced significant reduction of fecundity and total egg complement in spruce budworm. Significant reduction in hatching of eggs was reported in *Culex salinarius* transovarially infected with *Amblyospora* sp. (Andreadis and Hall, 1979).

Increased sterility and mortality of eggs in microsporidian-infected silkworm may be due to severe damage caused by the microsporidia in the reproductive tissues. Reduced fecundity and fertility was reported in codling moth infected with *N. carpocapsae* under laboratory conditions (Malone and Wigley, 1981). It was also reported that microsporidia deplete the nutritive reserves used for reproduction there by reduced fecundity (Thomson, 1958; Veber and Jasic, 1961; Smirnoff and Chu, 1968) and fertility (Tanabe and Tamashiro, 1967). The number of underdeveloped non-chorionated eggs was found more in pebrinised adults of *A. mylitta* than their disease-free counterparts (Rath *et al.*, 2001). Higher spore concentration was reported in gonads followed by fat body, gut and malpighian tubules in sericigenous insects *viz.*, *A. mylitta*, *A. assama* and *B. mori* (Bansal *et al.*, 1997).

In the present study, severely infected male and female adults did not mate properly. This could be the reason for high sterility in infected eggs. Similar reduction in mating success was also reported by many investigators (Gaugler and Brooks, 1975; Mercer and Wigley, 1987). Embryonic development ceased due to embryonic infection causing death of eggs and increased number of sterile and dead eggs (Yup-lian, 1995). Infection in ovaries might have been heavy enough to affect oogenesis rendering the female incapable of producing any fertile eggs, even if capable of copulation (Mercer and Wigley, 1987). Heavy infection in duct and secretary epithelia might have impaired cell and duct functions, possibly affecting the pheromone production, spermatophore transfer and mortality and survival of the spermatozoa. Splittstoesser and McEwen (1968) found that adult cabbage looper, *Trichoplusia ni*, which developed from larvae treated with sublethal dosages of *Thelohania* sp. were not affected in their mating frequency or fecundity, but fertility was greatly decreased.

Microsporidian dependent variation in fecundity, hatching, sterility and mortality of eggs may be due to variation in virulence of the microsporidia. Studies on lethal concentrations and host mortality with these microsporidia in *B. mori* revealed that *Nosema* sp. 2 is the most virulent followed by *Nosema* sp. 1 and *N. bombycis* (Madana Mohanan *et al.*, 2004). Present study helps to explain the adverse effect of microsporidia and high temperature on reproductive potential of *B. mori*.

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