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Effect of Microsporidian Infection on Reproductive Potentiality on Mulberry Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) in Different Seasons

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Infection of pathogenic microsporidia, *Nosema bombycis* and *Nosema mylitta* (Chakrabarti and Manna, 2006) decreased egg production, fecundity, hatching % and increased sterile eggs in heavily infected mulberry silkmoth, *Bombyx mori* L. On an average a disease free moth laid upto 442.67 eggs with high hatching % (99.53) and less sterile eggs (0.47~2.00%). While an infected moth laid less number of eggs (7.00~412.00) with low hatching % (32.437~98.643) and high sterile eggs (2.143~129.571). Fecundity of disease free laying was highest (468.714) during season-1 then gradually decreased during season- 2 (414.000) to season- 3 (404.285). But fecundity of an infected laying was highest during season-2 and hatched eggs were lowest during season-2. Higher inoculums concentration of *N. mylitta* infected to 5th stage larva of mulberry silkworm drastically decreased the fecundity in season - 3 and lower inoculums concentration of *N. bombycis* decreased the fecundity in season-1 and 3. Season-3 was most effective season to decrease the fecundity and increase sterile eggs when both temperature and humidity were fluctuated from the optimum level.

Key words: Fecundity, Hatching %, *Nosema bombycis*, Pebrine disease, Silkworm.

Introduction

The infection of *Nosema* spp. to mulberry and non-mulberry silkworms are well established. Chakrabarti and Manna (2006) identified three *Nosema* spp. from three non-mulberry silkworms as *Nosema mylitta* from *Antheraea mylitta*, *N.ricini* from *Philosamia ricini* and *N.assamensis* from *A. assamensis* .The effect of *Nosema* spp. infection on the reproductive potentiality of these silkmoths are not effectively known. However, reports on reduced fecundity and longevity of adult corn borer due to *Nosema pyrausta* infection is in record (Zimmack and Brindley, 1957; Kramer,1959; Van Denburgh and Burbutis,1962; Windels *et al.*,1976; Hill and Gary,1979; Seigel *et al.*,1985; Baucer and Nordin ,1989). The pathogens develop more quickly at low temperature relative to development of the host and more slowly at high temperature and magnitude of spore production is strongly age specific and thus time dependent (Solter *et al.,* 1989). Kawarabata and Ishihara (1984) observed rapid increase in parasitised cells by 72 hours post inoculation and the rate of infection is reached about 80% or more by 10 days post inoculation. The increase in temperature in different seasons decrease the yield and Effective Rearing Rate (by number) does not favour good fecundity (Shivakumar *et al.*, 1997). Madana Mohanan *et al.* (2005) studied the effect of microsporidian infection in different seasons on reproductive potentiality of mulberry silkworm, *Bombyx mori L.* However, the present report is restricted to the comparative study of the effect of infections with different inoculums concentrations of *Nosema bombycis* and cross-infection by *N. mylitta* on the reproductive potentiality of *B. mori*.

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Material and Methods

Collection of mulberry silkworm eggs and preparation of host insects

Five disease free layings of *Bombyx mori* L. (Race-Nistari, Multivoltine) were collected from Central Sericultural Research and Training Institute, Berhampore, West Bengal, India on 28.11.2001 and brushed on 29.11.2001 in laboratory. In all these cases on an average 367 eggs per laying and 98% hatching were recorded. Larvae of *B. mori* were reared on a diet of fresh mulberry leaves (*Morus alba*, Var. S1). Larvae were allowed to grow till 4^{th} moult. After resuming 4^{th} moult, 5^{th} instar at '0' hour larvae were taken for experiment. A batch of selected larvae in three replications was reared as healthy control.

Collection of microsporidia from mulberry and tasar silkworm

Nosema spp. of mulberry and tasar silkworms were propagated in their respective primary host and purified from moths using percoll cushions (PVP coated silica particles, Sigma chemicals Co. USA) following Bhattachrya *et al.* (1994). A new improved haemocytometer with Thomazaiss counting slide (German Fine Optik) was used to count the spores under microscope for determining the inoculums concentration (Cantwell, 1970; Undeen, 1997).

Inoculation of microsporidia of mulberry and tasar to mulberry silkworm

Mulberry silkworm, *Bombyx mori* (Race- Nistari, multivoltine) were reared in indoor under laboratory condition on a diet of fresh mulberry leaves during 29.11.2001 to 02.01.2002 at 25~28°C, 65~72% R.H and 12L+12D photoperiodic condition. Larvae were fed on fresh mulberry leaves smeared with *Nosema bombycis* and *N. mylitta*. Briefly, the procedure was involved dipping a leaf dish (28.27 cm^2) in 200 μl. of spore suspension, drying and then allowing the larvae to feed on the dish for a period of 6 hours. A batch of 60 larvae was fed to 10 leaf dishes. The healthy control groups were fed with the fresh mulberry leaves washing in distilled water.

Second season rearing

For the second season, procedure was involved same as in case of previous rearing, inoculation, purification etc. Eggs were hatched during 14.02.2002 to 11.03.2002 and rearing was conducted in between 28.5~34.5°C and 55~81% R.H.

Third season rearing

For the third season, procedure was involved as in case of previous rearing, inoculation, purification etc. Eggs were hatched during 10.05.2002 to 01.06.2002 and rearing was conducted in between 20~40.5°C and 64~90.5% R.H.

Recording of data

After the cocoon formation, infected mulberry moths were allowed for coupling and gravid females were allowed for egg laying. The laid eggs were categorized into 3 groups, hatched, sterile and blue eggs. Then the fecundity (number of eggs per laying), hatching % and sterility % were calculated from the following formula:

Hatching % = (Number of hatched eggs × 100) / Total number of eggs laid by a female

Sterility % = (Number of sterile eggs × 100) / Total number of eggs laid by a female

All the data are statistically analyzed by using ANOVA.

Results

The average number of eggs laid by a gravid female (Fecundity) was highest (468.714) during season-1 then gradually decreased during season- 2 (455.000) to season - 3 (404.285) in control batches.Fecundity was always less in all infected batches (7- 412) than control. Higher inoculums concentration (1.52×10^8 spores/ml) (T-0) of *N. mylitta* infected to 5^{th} stage larvae drastically decreased the range of the fecundity (7.0) particularly in season - 3 and similarity lower inoculums concentration (1.52×10^6 spores/ml) (M-2) of *N. bombycis* drastically decreased the fecundity in season-1 (54.714) and season-3 (81.428) (Table 1).

The significant differences are observed among the treatments ($P<0.01$), seasons ($P<0.05$) as well as interaction between treatments and seasons ($P<0.01$). *Nosema mylitta* was found most virulent to decrease the fecundity than *N. bombycis*. The mean value of treatments T-0 (115.93), T-1 (167.29) and T-2 (273.83) having a significant difference of mean, have a significant difference among the inoculums concentrations per ml. of T-0, T-1 and T-2 of which, T-0 shows maximum decrease of the fecundity. Further, the value of treatments M-0 (238.102), M-1 (308.246) and M-2 (138.269) having a difference of mean, have a significant difference among inoculums concentrations M-0, M-1 and M-2, where M-2 shows maximum decrease of the fecundity. There is a significant ($P<0.01$) difference among the season-1 (218.992), season-2 (231.735) and season-3 (225.167) also, of which season-1 was most effective for decrease of fecundity. The significant difference ($P<0.01$) is observed in interaction of treatments and seasons. This indicates the significant difference in impact of treatments in various seasons (Table 1).

Infection of *Nosema* and formation of sterile eggs

The number of sterile eggs was increased with the decreasing inoculums concentrations of *N. mylitta* cross-infected to mulberry silk worm while, the number of ster-

Table 1. Infection of mulberry larvae (5th instar) with different concentrations of *Nosema* sp. (M = *Nosema bombycis* N., T = *N.mylitta* Chakrabarti and Manna, S1, S2 and S3 = Season 1, 2 and 3., Inoculums concentrations 0, 1 and 2 = 1.52×10^8, 1.52×10^7 and 1.52×10^6 spores/ml. ,CON = Healthy control, Wt. = Weight in g)

Treatment	Season-1	Season-2	Season-3	Mean	SE
T0	316.714 (313.586)	31.857 (28.187)	7.000 (6.027)	118.523 (115.933)	99.354
T1	142.428 (141.883)	172.857 (161.674)	201.571 (198.304)	172.285 (167.287)	17.075
T2	412.000 (411.883)	160.857 (142.942)	269.285 (266.654)	280.714 (273.826)	72.723
M0	193.428 (190.495)	214.000 (211.920)	315.571 (311.892)	241.000 (238.102)	37.755
M1	270.142 (267.944)	342.285 (336.630)	323.000 (320.163)	311.809 (308.246)	21.564
M2	54.714 (44.217)	294.285 (290.861)	81.428 (79.727)	143.476 (138.269)	75.798
CON	468.714 (462.992)	455.000 (449.928)	404.285 (393.398)	442.666 (435.397)	19.594
MEAN	265.449 (218.992)	238.734 (231.735)	228.877 (225.167)		

(Data in parenthesis are retransformed value)

Table 2. Infection of mulberry larvae with different concentrations of *Nosema* sp. on and rate of formation of sterile eggs (M = *Nosema bombycis* N., T = *N.mylitta* Chakrabarti and Manna, S1, S2 and S3 = Season 1, 2 and 3., Inoculums concentrations 0, 1 and 2 = 1.52×10^8 , 1.52×10^7 and 1.52×10^6 spores/ml. ,CON = Healthy control, Wt. = Weight in g)

Treatment	Season-1	Season-2	Season-3	Mean	SE
T0	23.000 (20.829)	8.000 (4.466)	2.143 (1.415)	11.048 (8.903)	6.211
T1	33.571 (32.628)	12.000 (11.341)	5.286 (4.201)	16.952 (16.056)	8.533
T2	65.000 (62.895)	32.714 (31.415)	9.714 (9.005)	35.810 (28.438)	16.034
M0	129.571 (128.128)	80.429 (76.387)	50.714 (41.916)	86.905 (82.144)	22.993
M1	12.286 (10.656)	7.857 (7.436)	3.571 (2.788)	7.905 (6.960)	2.516
M2	6.000 (4.848)	4.143 (3.575)	22.857 (21.455)	11.000 (9.960)	5.953
CON	2.429 (2.040)	2.000 (1.712)	1.571 (1.339)	2.000 (1.697)	0.247
MEAN	38.837 (37.432)	21.020 (16.905)	13.694 (11.731)		

(Data in parenthesis are retransformed value)

ile eggs was decreased with decreasing inoculums concentrations of *N. bombycis* infected to mulberry silk worm (Table 2). Further seasonal effect is also clear when study is concentrated on sterile eggs. Number of sterile eggs shows decreasing in trend from season-1, season- 2 and season-3 gradually in all the treated and control batches. Maximum range of sterile eggs (50.714~129.571) were observed when higher inoculums concentration (1.52×10^8 spore/ml) of *N.bombycis* infected to mulberry silkworm in all the seasons while, maximum range of sterile eggs (9.714~65.000) were observed in infected silkworm in all the seasons when lower concentration (1.52×10^6 spore/ml)

Table 3. Effect of different concentrations of *Nosema* spp. on hatching % of mulberry larva (M = *Nosema bombycis* N., T = *N.mylitta* Chakrabarti and Manna, S1, S2 and S3 = Season 1, 2 and 3., Inoculums concentrations 0, 1 and 2 = 1.52×10^8, 1.52×10^7 and 1.52×10^6 spores/ml. ,CON = Healthy control, Wt. = Weight in g)

Treatment	Season-1	Season-2	Season-3	Mean	SE
T0	92.938 (92.934)	79.832 (79.299)	75.244 (74.161)	82.671 (82.131)	5.301
T1	76.393 (76.223)	92.647 (92.553)	97.434 (97.425)	88.825 (88.734)	6.367
T2	84.452 (83.889)	71.535 (69.555)	96.271 (96.243)	84.086 (74.784)	7.143
M0	32.437 (31.548)	62.842 (62.188)	84.704 (84.512)	59.994 (59.416)	15.155
M1	95.515 (95.503)	97.585 (97.563)	98.875 (98.868)	97.325 (97.311)	0.978
M2	87.371 (86.805)	98.643 (98.648)	70.160 (68.601)	85.391 (84.685)	8.282
CON	99.487 (99.381)	99.540 (99.536)	99.580 (99.575)	99.535 (99.497)	0.027
MEAN	81.228 (80.913)	86.089 (82.001)	88.895 (88.484)		

(Data in parenthesis are retransformed value)

of *N. mylitta* inoculated to 5th stage '0' hr. of mulberry silkworm (Table 2).

Nosema bombycis is found most virulent to increase the sterile eggs than *Nosema mylitta* in *B. mori*. The mean value of treatments T-0 (8.90), T-1 (16.06) and T-2 (28.44) having a significant difference of mean, have a significant difference among the concentration of pathogen per ml. of T-0, T-1 and T-2 of which, T-0 shows better result to increase the sterile eggs. Further, the value of treatments M-0 (82.14), M-1 (6.96) and M-2 (9.96) having a difference of mean, have a significant difference among M-0, M-1 and M-2, where M-0 shows better result to increase the sterile eggs. There is a significant ($p < 0.01$) difference among the season-1 (37.43), season-2 (16.91) and season-3 (11.73) also, of which season-1, is most effective for increase of the sterile eggs. The significant difference ($p < 0.01$) is observed in impact on interaction of treatments and different seasons (Table 2).

Effect of different concentrations of *Nosema* spp. on hatching % of *B. mori*

The hatching% was always higher in control batches (99.48 ~ 99.58%) than infected batches (32.43 ~ 98.87%). Higher concentration (1.52×10^8 spores/ml) of *N. bombycis* (M-0, 32.43%) in Season-1 and lower concentration (1.52×10^6 spores/ml) of *N. mylitta*. (T-2, 71.54%) in Season-2 were maximally affected to decrease hatching % (Table 3). *N. bombycis* is found slightly more virulent to decrease hatching % of eggs than *Nosema mylitta*. The mean value of treatments T-0 (82.13%), T-1 (88.73%) and T-2 (74.78%) having a significant difference of mean, have a significant difference among the dose of pathogen concentration per ml. of T-0, T-1 and T-2 of which, T-2 shows better result to decrease hatching % of eggs. Further, the value of treatments M-0 (59.42), M-1 (97.31) and M-2 (84.69) having a difference of mean have a significant difference among M-0, M-1 and M-2, where M-2 shows better result to decrease the hatching %. Differences of means in different seasons are non-significant i.e., performances of three seasons are at par. The significant difference ($p < 0.01$) is observed in interaction of treatments and seasons (Table 3).

Discussion

The microsporidia, *Nosema bombycis* and *N. mylitta*, in the present study affect the reproductive potentiality by reducing fecundity and hatching % and increase the sterile eggs production in *Bombyx mori.* The inoculums concentrations of *N. mylitta* inoculated to *B. mori* drastically decrease the hatching % and the range of fecundity and increase the sterile eggs production. Similar observations on *Nosema pyrausta* causing reduction in fecundity with increase of sterile eggs as well as reduced hatched eggs in female moths are available (Zimmack and Brindley, 1957; Kramer, 1959; Van Denburgh and Burbutis, 1962; Windels *et al.,* 1976; Hill and Gary, 1979; Seigel *et al*., 1985).

Kramer (1959) found, as did Van Denburgh and Burbutis (1962) that oviposition and fecundity were adversely affected when the protozoan *N. pyrausta* infects female moths. Zimmack *et al*. (1954) found that infected moths of field collected European corn borer larvae laid less egg masses and eggs than did apparently healthy moths. Zimmack and Brindley (1957) observed that the percentage of infected larvae survived to adults were lower and those adults laid fewer egg masses and eggs and exhibited reduced longevity. In highly infected *A. mylitta* recorded significant decrease of egg production, fecundity, hatching and increase eggs retension (Jolly and Sen, 1972; Rath *et al.,* 2001). Madana Mohanan *et al*. (2004) made detail study with three pathogens *N. bombycis, Nosema* sp. I and *Nosema* sp. II collecting from *Bombyx mori, Antheraea mylitta* and *Diacrasia oblique* (Bihar hair caterpillar) with single dose of spore suspension,1×10^{6} spores/ml on hatched mulberry larvae in one favourable season, January-February and two unfavourable seasons, April - May and July - August, where as the present findings are restricted with two microsporidian *N. bombycis* and *N. mylitta* collecting from *B. mori* and *A. mylitta* infected with three different doses of spore suspension 1.52×10^{6}, 1.52×10^{6} and 1.52×10^{6} spores/ml to 5th stage mulberry larvae in two favourable seasons December-January and February - March and single unfavourable season May-June. It is observed in the present findings that *N. mylitta* reduced fecundity maximally in season-2 and season-3 in *B. mori* when moths are highly infected with higher inoculums concentration. But *N. bombycis* while infected with lower concentration effected maximum in season-1 and season-3. The difference of concentration required for disease development may be due to their wild / virulence in nature (Madana Mohanan *et al*., 2005). Fluctuation of temperature and relative humidity from the optimum in season -2 and season -3 level results in decreased ovulation and fecundity and increased retention of eggs in *B. mori* supports the findings of Mathur *et al.* (1995).

A significant reduction of eggs laid by infected females was observed during first gonotrophic cycle. However, this reduction was offset by an equally significant increase in egg production by infected females during second gonotrophic cycle. While no detrimental effects could be observed for physiological longevity and overall fecundity. Infected eggs showed 52% reduction in overall hatch. This difference is found to be highly significant ($P<0.01$). The reduction in hatch was manifested during the first three gonotrophic cycles only and the degree of hatch reduction actually attributed to the infection was reduced with each successive gonotrophic cycle (Geetha Bai and Mahadevappa, 1995). Present finding support the views in 1st gonotropic cycle and needs further investigation for comparision with the result of 2nd gonotropic cycle. Scientists recorded adverse effects of microsporidian infection on reproductive potentiality in insects (Steinhaus and Hughes, 1949; Yup-lian, 1995; Bansal *et al*., 1997).In the present observation less fecundity and more sterile eggs were recorded during Season - 2 and Season - 3 in control batches may be due to higher temperature (maximum 40°C) prevails during rearing period and higher temperature might have decreased ovulation, fecundity and increased retention of eggs varied with seasons (Madana Mohanan *et al*., 2005).Fecundity is higher in Season -1 due to lower temperature in control batches (Rath *et al*., 2001).Variation of ovulation, fecundity, sterile eggs and hatched eggs in different seasons support the views of Mathur *et al*. (1995).

In the present findings, higher concentration of *N. mylitta* (Chakrabarti and Manna, 2006) and lower concentration of *N. bombycis* are effected to increase sterile eggs and decrease fecundity due to the difference of virulence (wild in nature) of the two pathogens (Madana Mohanan *et al*., 2005).In the present finding reduced fecundity and egg hatching in microsporidian infected silkworm due to severe damage of fat body tissue and gonad tissue. The damage of muscular tissues following infection was possible reasons for the reduced fecundity in insects (Madana Mohanan *et al*., 2005; Hussaninein, 1951; Yup-lian, 1995). Gaugler and Brooks (1975) stated that fecundity reduction was correlated to extensive infection of adult fat body in corn earworm tranovarially infected with *N. heliothidis* and 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). Intensity of infection is more in female gonads than male gonads (Madana Mohanan *et al*., 2004) and microsporidian prevent cell differentiation in gonads (Syme and Green, 1972; Gordon *et al*., 1973). *Microsporidian itiiti* reduce the fecundity of *Listronotus bonariensis* (Malone, 1987). Similarly, Baucer and Nordin (1989) reported that sublethal doses 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 *Ambylospora* sp. (Andreadis and Hall, 1979). Reduced fecundity and fertility was observed in the present findings, similar in codling moth with *N.carpocapsae* under laboratory condition (Malone and Wigley, 1981). Mircsporidia used nutritive reserve used for reproduction; resulting fecundity (Thomson, 1958; Veber and Jasie, 1961 and Smirnoff and Chu, 1968) and fertility (Tanabe and Tamashiro, 1967) were reduced. More underdeveloped and non-chorinotaed eggs were laid by pebrine infected female moth of *A. mylitta* than disease free female (Rath *et al.,* 2001). Higher spore concentration was reported in gonads in *A. mylitta*, *A. assama* and *B. mori* (Bansal *et al.,* 1997). Reduction in successful mating in the present observation supports the view of Gaugler and Brooks (1975) and Mercer and Wigley (1987). Embryonic development ceased due to embryonic infection resulting more death and sterile eggs (Yup-lian, 1995). Infection in ovaries affected the process of oogenesis resulting sterile eggs even successful copulation (Mercer and Wigley, 1987). Similarly infection in duct and secretary epithelia of male reproductive organs affected pheromone production and transfer of spermatophore to spermatozoa, resulting mortality of spermatozoa. Therefore, it is concluded that not only seasons but also different dose and virulence/wild nature of the pathogens are responsible to reduce fecundity and hatching % and increase sterility of the eggs of adult infected by microsporidia.

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