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Competency in Mother Moth Examination Methods of *Antheraea mylitta* D. in the Detection of Pebrine Spores and Quality Evaluation

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ABSTRACT

Pebrine disease in tasar silkworm is considered to be most serious because of its chronic pathogenicity. Prophylactic method of mother moth examination proves to be the foolproof technique for pebrine identification. So, the study was conducted to investigate pebrine spores detection competency and evaluation of their quality in existing mother moth examinations. Four existing mother moth examination methods namely Prick and See, Conventional, Fuziwara and Delayed Mother Moth Examination (DMME) by Fuziwara were selected for the study. The maximum pebrine and hatching percentages were noticed in 4 days DMME with 23.2 and 93.1%, respectively. While, minimum values were observed in prick and see with 4.3% and 84.3, respectively. No significant variations in Cocoon weight, Shell weight and S.R% was observed in cocoons obtained from the different mother moth testing methods. Least percentage of effective rate of rearing (ERR) was observed in Prick and See method (58.75%) followed by Conventional method (61.25%) and Fuziwara (63.15%). High percentage of ERR was observed in DMME which ranged from 64.53% (1st day after egg laying) to 67.28% (4th day after egg laying). Least percentage of improvement of ERR% over Prick and See was observed in Conventional (2.50%) followed by Fuziwara (4.40%). High percentage of ERR over Prick and See was observed in DMME which ranged from 5.78% (1st day) to 8.53% (4th day). Consequently, among the tested mother moth examinations, DMME by Fuziwara after delaying four days from the egg laying has given best results. This method can be implemented in the research institutions for the maintenance of germplasm and breeders stock.

Key words: *Antheraea mylitta* D., pebrine, mother moth examination, disease incidence, rearing performance, germplasm, breeders stock

INTRODUCTION

Silkworm diseases form major constraint in realizing full crop yield (Kumar and Nik, 2011). Pebrine disease in tasar silkworm is caused by *Nosema* species (Microsporidia) and contributes crop loss of 20-25% (Singh *et al.*, 2011). Pebrine is considered to be most serious because of its chronic pathogenicity which leads to perpetuation of the disease through trans-ovarial dissemination, causing acute microsporidiosis in the consequent generation (Singh *et al.*, 2004). Thus, the disease not only affects the particular tasar silkworm stock, but also leads to cross contamination and secondary contamination, causing endemic backlash.

The chemical based disinfectants, drug formulations and use of botanicals are possible alternatives to treat infectious diseases (Kumar *et al.*, 2012; Jazani *et al.*, 2009; Chanda *et al.*, 2011). But, for prevention/control of pebrine disease prophylactic and curative measures by using chemicals is not economic, eco-friendly and also have many limitations to be effective in open and outdoor rearing (Kumar *et al.*, 2011). As yet, prophylactic method of mother moth examination proves to be the perfect technique, being practiced in all the tasar cultural countries (Kumar and Basavaraja, 1995). Prick and See, Conventional, Modified Fuziwara and Delayed Mother Moth Examination by Modified Fuziwara are different types of mother moth examination methods practiced to detect pebrine disease in tasar silkworm (Singh *et al.*, 2011).

There may be certain relaxation in the criteria for production of laying for commercial crops, since commercial cocoons are not used for silkworm seed production. However, for germplasm maintenance, stock maintenance and rearings for research purposes absolutely disease-free, especially pebrine free layings are required. The efficacy of the method depends on two factors; (1) the technical procedure employed for extracting and concentrating the spores from the host body tissues and (2) The number of spores present in the body of the moth. While the technical procedures for homogenization and extraction of spores have been reasonably refined now, the efficacy of spores under moderate or low infection levels is for satisfactory. The general detection level is considered to be approximately 10^5 spores/single moth (Singh *et al.*, 2004).

In view of this fact, attempts to enhance the sporulation of the parasite to improve the chances of detection have been made by using different methods. The rate of spore production of *Nosema mylittansis* is greatly enhanced in relation to the age of moth. More number of mature spores is formed, as the moths become older (Singh *et al.*, 2004). In contrast to the research carried out on diseases in *Bombyx mori* L. not much work has been published so far on pathological aspects of tasar silkworm (Kumar *et al.*, 2011; Reddy *et al.*, 2008, 2009a, b, 2010). Till the date no information is available on the efficacy of the existing various mother moth examinations being practiced in tasar culture.

Hence, in the present study, an attempt was made to find out proficiency of various mother moth examination methods being practiced in tasar culture for the detection of pebrine spores and also evaluated their quality.

MATERIALS AND METHODS

Pilot Project Centre, Kuschira, Dumka, Jharkhand was selected as study area based on availability of scientific personals, facilities, manpower, grain age capacity, availability of rearers, and climatic condition. The experiment was conducted during June, 2010 to Sep, 2011.

Collection of mother moths: Same day egg laid 7,000 mother moths were collected after 72 h of oviposition from the same lot for conducting the experiment and tested for pebrine detection. The testing was conducted by Prick and See, Conventional, and Fuziwara on the same day, having a population of 1000 moths per each method, except Delayed mother moth examination. In the case of delayed mother moth examination samples were stored at well ventilated room at temperature of 25-30°C and tested in 4 consecutive days containing 1000 mother moth samples per each day. The 100 Dfls obtained from different mother moth examination methods followed were given to the tasar model rearers, data was collected and analyzed statistically.

Prick and see method: Slides were kept serially and tooth picks or broom sticks of about 7 cm lengths were kept in a beaker. Placed a drop of 0.5% K_2CO_3 (or) 2% KOH on the slide with the help

of the stick/dropper. Mother moths were collected after 72 h of oviposition to the site of examination. Hold out the mother moth with the help of thumb, index and middle finger of one hand such that the dorsal portion of the abdomen faces up-wards. Pierced a tooth pick up to 3-4 cm in length with the other hand into the 3-4 inter segmental region and rotate inside the abdomen so as to smear it with body fluid. Took out the stick and mixed the body fluid with the previously dropped 0.5% of K_2CO_3 (or) 2% KOH on the slide. Covered the smear with cover slip and observed under microscope at 600 X magnification for the detection of pebrine spores.

Conventional method: In this process of examination, the lower middle portion of moth abdomen (4 to 7 segment) was cut with the help of a scissors and homogenized with the help of mortar and pestle by adding KOH (2%) and K_2CO_3 (0.5%) in equal volume. A drop of the homogenate was put on the clean glass slide and covered it with a clean cover slip and observed at 600 X magnification.

Modified Fuziwara method: Mother moths were collected after 72 h of oviposition (egg laying) in paper box of 9×9×4 cm measurement. Lower middle portion of individual mother moth (4 to 7 segments) was cut with the help of scissors and placed in a cavity of mortar. Five milliliter of 0.5% K_2CO_3 is added to it, for easy separation of Nosema spores from different tissues. Material was crushed thoroughly with pestle to homogenize the mixture. To filter the homogenized mixer, a bit of cotton was added in the mortar, pipetted out with the help of dropper and taken in 1.5 mL centrifuge tube. Filtered smear was centrifuged at 4000 rpm for 5 min. The supernatant was discarded and 5-6 drops of 2% KOH solution was added to sediment, which dissolved fat globules and made the observation easy. One drop of dissolved sediment was put on the glass slide, covered with cover slip and examined under the 600 x magnification of compound microscope.

Delayed mother moth examination by Modified Fuziwara method: In this method, mother moth testing was delayed from the egg laying and test was conducted daily from day 1 to 4 day by adopting modified fuziwara method as detailed above.

RESULTS

Disease incidence and hatching performance: Pebrine disease% in the lot was ranged in between 4.3 to 23.2%. Least percentage of pebrine was noticed with prick and see method (4.3%) followed by Conventional (8.9%) and Modified Fuziwara (13.5%). High percentage of pebrine was noticed with Delayed Mother Moth Examination which ranged from 13.6% (1st day after egg laying) to 23.2% (4th day after egg laying). Percentage of healthy moths in the lot was ranged in between 76.8 to 95.7% (Table 1). High percentage of healthy moths was observed in prick and see method (95.7%) followed by Conventional (91.1%) and Modified Fuziwara (86.5%). Least percentage of healthy moths was observed in Delayed Mother Moth Examination which ranged from 76.8% (4th day after egg laying) to 86.4% (1st day after egg laying). In the case of hatching % of pebrine free layings was ranged in between 84.3 to 93.1%. Least percentage of hatching was observed in prick and see method (84.3%) followed by Conventional (86.5%) and Modified Fuziwara (89.9%). High percentage of hatching was observed in Delayed Mother Moth Examination which ranged from 91.2% (1st day after egg laying) to 93.1% (4th day after egg laying).

Table 1: Pebrine disease % and hatching% of pebrine free layings in the same lot by adapting different types of mother moth examinations

Analysis	No. of moths tested	No. of pebrinised moths	Pebrine disease percentage	No. of healthy moths	Percentage of health moths	Hatching % of pebrinefreelayings
Mother moth examination method						
Prick and see	1000	43	4.3	957	95.7	84.3
Conventional	1000	89	8.9	911	91.1	86.5
Modified Fuziwara	1000	135	13.5	865	86.5	89.9
Delayed mother moth examination (Days after egg laying)						
1st day	1000	136	13.6	864	86.4	91.2
2nd day	1000	164	16.4	836	83.6	91.7
3rd day	1000	192	19.2	808	80.8	92.3
4th day	1000	232	23.2	768	76.8	93.1

Table 2: Rearing performance of the Dfls raised by adapting different types of mother moth examinations

Analysis	No. of Dfls.	Cocoon wt. (g)	Shell wt. (g)	S.R%	E.R.R %moth	Improvement of E.R.R% over
						Prick and see method
Name of mother examination						
Prick and see	800	11.10	1.285	11.58	58.75	0.00
Conventional	800	11.17	1.267	11.34	61.25	2.50
Modified Fuziwara	800	11.15	1.280	11.48	63.15	4.40
Delayed mother moth examination (Days after egg laying)						
1st day	800	11.13	1.280	11.50	64.53	5.78
2nd day	800	11.09	1.283	11.57	65.78	7.03
3rd day	800	11.30	1.309	11.58	66.15	7.40
4th day	800	11.28	1.303	11.55	67.28	8.53

Rearing performance: Cocoon weight in the lots was ranged in between 11.09 to 11.30 g. High cocoon weight was observed in Delayed Mother Moth Examination (DMME) method (3rd day) 11.30 g followed by DMME, 4th day (11.28 g), Conventional (11.17 g), Modified Fuziwara (11.15 g) and DMME, 1st day 11.13 g. Low cocoon weight was observed in DMME, 2nd day with 11.09 g. Shell weight in the lots was ranged in between 1.267 to 1.309 g (Table 2). High shell weight was observed in DMME method (3rd day) 1.309 g, followed by DMME, 4th day (1.303 g), Prick and see (1.285 g) and DMME, 2nd day 1.283 g. Low shell weight was observed in Conventional method with 1.267 g. S.R% in the lots was ranged in between 11.34% (Conventional method) to 11.58% (Prick and see, DMME-3rd day).

E.R.R % in the lot was ranged in between 58.75 to 67.28%. Least percentage of E.R.R was observed in Prick and See method (58.75%) followed by Conventional method (61.25%) and Modified Fuziwara (63.15%). High percentage of E.R.R was observed in Delayed Mother Moth Examination which ranged from 64.53% (1st day after egg laying) to 67.28% (4th day after egg laying). In the case of improvement of E.R.R% over Prick and See method in the lot was ranged in between 2.50 to 8.53%. Least percentage of improvement was observed in Conventional method (2.50%) followed by Modified Fuziwara (4.40%). High percentage of E.R.R over Prick and See method was observed in Delayed Mother Moth Examination which ranged from 5.78% (1st day after egg laying) to 8.53% (4th day after egg laying).

DISCUSSION

High percentage of pebrine incidence was noticed in Delayed Mother Moth Examination (DMME) as the number of days of delay in testing mother moths are increased from the date of egg laying. These observations have provided an important clue to improve the chances of detection of the disease in infected specimen, if the examination of moths are delayed for a certain period of time after emergence. It is quite responsible to believe that as the moths become old and weak, the nutritional status of the body tissues gets reduced, promoting the parasitic organism to decelerate the cyclic development through production of the short coiled type pyriform spores and switch over to the production of more mature long coiled type spores, so that, consequent to the death and decay of the host, they will be safely preserved in the environment to be ingested by another susceptible host (Sasidharan *et al.*, 2003). The rate of spore production is generally enhanced in relation to the age of the moths and more number of matured spores are formed, as the moths become older (Singh *et al.*, 2007). Least percentage of pebrine was observed in prick and see method followed by Conventional and Modified Fuziwara which indicated the sensitivity of the methods for the identification of pebrine spores are in the order of prick and see > Conventional > Modified Fuziwara > Delayed Mother Moth Examination. High percentage of healthy moths was observed in prick and see method followed by Conventional, Modified Fuziwara and Delayed Mother Moth Examination that means the test was conducted in the same lot but when methods of testing was differed the results were also differed. It can be assumed that higher the number of healthy moths lower the sensitivity and lower the number of healthy moths higher the sensitivity to identify microsporidia spore. These results are contrast to the Sharan *et al.* (1992) when they have conducted pebrine test in pupal stage, there was no difference in the percentage of infection in between conventional and centrifugal methods. This may be due to the difference in testing stage of the tasar silkworm.

High percentage of hatching was observed in Delayed Mother Moth Examination (DMME) which enlightened that, the pebrinised layings were less because of its sensitivity to identify the pebrine spores. The effectiveness of this method however, depends not only on the technical procedure employed for concentrating the spores but also and even more on the intensity of the spores in the body of the moth itself (Sasidharan *et al.*, 2003). In contrast to this, the least percentage of hatching was observed in Prick and See which notified that pebrinised layings might be there because of poor sensitivity of the methods could not able to identify the diseased layings. The presence of disease in the eggs causes irregularity in hatching and death that influences hatching percentage. No significant variations in cocoon weight, shell weight and S.R% was observed in cocoons obtained from the different mother moth testing methods whereas significant variation was observed in case of E.R.R%. The testing methods could able to influence the cocoon yield but not the cocoon parameters. E.R.R percentage improvement over the prick and see method is in the order of Conventional > Modified Fuziwara > Delayed Mother Moth Examination. In especially DMME, from day 1 to day 4 the percentage improvement is in the order of day 1 > day 2 > day 3 > day 4.

CONCLUSION

Among the tested mother moth examinations, Mother Moth Examination by Modified Fuziwara after delaying four days from the egg laying has given best results. This can be implemented in the Research Institutions for the maintenance of germplasm and breeders stock. In case of BSMTCS and Commercial Grainages it is recommended to conduct mother moth examination by Modified Fuziwara method, as the number of samples to be tested will be more number and due to the short period to supply the layings to the farmers.

REFERENCES

- Chanda, S., M. Kaneria and R. Nair, 2011. Antibacterial activity of *Psoralea corylifolia* L. seed and aerial parts with various extraction methods. Res. J. Microbiol., 60: 124-131.
- Jazani, N.H., M. Zartoshti, H. Babazadeh, N.A. Daiee, S. Zarrin and S. Hosseini, 2009. Antibacterial effects of Iranian fennel essential oil on isolates of *Acinetobacter baumannii*. Pak. J. Biol. Sci., 12: 738-741.
- Kumar, K.K.P. and S.S. Naik, 2011. Development of polyvoltinex bivoltine hybrids of mulberry silkworm, *Bombyx mori* L. tolerant to BmNPV. Int. J. Zool. Res., 7: 300-309.
- Kumar, K.K.P., A.K. Sinha, G.P. Singh and K.N. Madhusudhan, 2011. Efficacy of systemic fungicides for control of white muscardine in tasar silkworm, *Antheraea mylitta* D. Res. J. Microbiol., 6: 805-812.
- Kumar, K.K.P., G.P. Singh, A.K. Sinha, K.N. Madhusudhan and B.C. Prasad, 2012. Antiviral action of certain medicinal plants against AmCPV and their effect on cellular and biochemical changes in tasar silkworm, *Antheraea mylitta* D. Res. J. Med. Plant, 6: 92-99.
- Kumar, N.S. and H.K. Basavaraja, 1995. Disease management for successful silkworm crops. Indian Silk, 34: 13-17.
- Reddy, R.M., N. Suryanarayana and N.B.V. Prakash, 2008. Heterosis potential in selective parental F1 hybrids of divergent geographic ecotypes of tropical tasar silkworm, *Antheraea mylitta* D (Lepidoptera: Saturniidae). Acad. J. Entomol., 1: 32-35.
- Reddy, R.M., M.K. Sinha, G. Hansda and N.B.V. Prakash, 2009a. Application of parents by selection for basic and commercial seed efficiency in tropical tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). Acad. J. Entomol., 2: 56-61.
- Reddy, R.M., N. Suryanarayana, M.K. Sinha, N.S. Gahlot, G. Hansda, N.G. Ojha and N.B.V. Prakash, 2009b. Silk filament progression with backcross breeding generations in tropical tasar silkworm, *Antheraea mylitta* D. Int. J. Ind. Entomol., 19: 187-192.
- Reddy, R.M., M.K. Sinha and B.C. Prasad, 2010. Application of parental selection for productivity improvement in tropical tasar silkworm *Antheraea mylitta* Drury: A review. J. Entomol., 7: 129-140.
- Sasidharan, T.O., R.N. Singh, P.C. Shantha, B.P. Nair and V. Suryanarayana *et al.*, 2003. Improved technologies for silkworm seed production. Silkworm Seed Technology Laboratory, Bangalore, India, pp: 18-19
- Sharan, S.K., A.K. Bansal, R.M. Shukla and K. Thangavelu, 1992. A new method of detection of pebrine disease in tasar silk moth, *Antheraea mylitta* Drury (Saturniidae). J. Res. Lepidoptera, 31: 12-15.
- Singh, G.P., A.K. Sinha, P.K. Kumar and B.C. Prasad, 2011. Manual on Management of Diseases in Tasar Silkworm. Central Tasar Research and Training Institute, Ranchi, India, Pages: 96.
- Singh, R.N., P.C. Shantha, T.O. Shasidharan, A. Manjula and C.K. Kamble, 2004. Delayed mother moth testing for effective detection of pebrine. Indian Silk, 43: 7-9.
- Singh, R.N., A.G.K. Daniel, P.C. Santha, T.O. Sasidharan, M.V. Samson, A. Manjula and T.M. Veeraiah, 2007. Improved methods of pebrine detection in silkworm. Annual Report, Silkworm Seed Technology Laboratory, Bangalore, India.