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Standardization of mother moth examination for pebrine detection in samia species and diagnosis of pebrine disease in commercial seed production.

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Abstract

The Eri silkworm of the genus Samia is infected by protozoan disease pebrine caused by microsporidian *Nosema* species. The disease is transmitted to the next generation either through the eggs or consumption of contaminated leaves and it hinder eri seed production. So to prevent this disease strict mother moth examination is a must .Presently for diagnosis of pebrine disease, the mass mother moth examination of eri silk moth includes random selection of eri silk moths after laying the eggs followed by grinding, filtering, centrifuging and finally examination under microscope in 600 x magnification following the same procedure practising in *Antheraea assama / Bombyx mori* .As the size of *Bombyx mori* female mother moth is about 1cm to 1.5cm.,the size of the *Antheraea assama* female mother moth is about 3.5cm to 4 cm and that of *Samia* species is about 2.5cm to 2.8 cm. So, considering the size of the mother moth of *Samia* species and as per Research Committee suggestion to standardize the existing technology the new procedure evolved. The experiment has been conducted three times with slight alteration, addition, deletion, refinement of the existing procedure and finally fine tuning it as per requirement.

Keywords: pebrine, diagnosis, disease, seed production, mother moth.

1. Introduction

Microsporidiosis (commonly known as pebrine) is a dreadliest disease of all types of silkworm (Pasteur, 1870). It is causing serious damage to silkworm and directly responsible for the decline of the sericulture industry. Pebrine disease is caused by the microsporidian *Nosema* spp. and transmitted through eggs. So, female moth is the carrier of the disease. Infection takes place preorally and Transovarially. Pebrine disease is found throughout the year though the incidence of the disease is more during summer followed by Autumn and Spring in temperate countries.*Samia cynthia ricini(*Eri worm)is also affected by *Nosema spp.*Sometimes the crop failed due to this pebrine disease.Some pathological techniques with regard to Pebrine Nosema were carried out to control the disease. Studies has been done on various aspects of this dreaded disease in India by Mukherjee, (1919); Ghosh, (1949); Chowdhury, (1970); Geetha Bai *et al.,* (1985); (Jolly, 1986). Pioneer work on this disease outside India was done by Sato *et al.*,(1981) Hayasaka(1983), Hayasaka *et al.*, (1983,1987); Huang *et al.*,(1983); Li (1985); Kawarabata *et al.,* (1987) and Zhaoxi *et al.,* (1990). Since mother moth is the only carrier to its next generation so the only way is to check the disease is to identify the diseased/infected mother moth and discard it followed by through disinfection (Rajkhowa & Kumar,2012).

In *B.mori* after so many research work, Fuziwara centrifugal method is now widely accepted for detection of pebrine in mulberry silkmoth. This method is letter standardized in '90s in muga as per the size of the female muga moth. But in Eri no such

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recommended method is found. We are following the procedure of muga mother moth examination method only. So.there is essential for proper, systematic and scientific way of doing the activity of pebrine spore detection and standardize the existing procedures followed in other two types of silkmoth i.e. Muga and Mulberry as per the requirement and suitability of the Eri silk moth and achieve the desired results i.e to established an standardized procedure for detection pebrine spore in Eri silk moth which can be utilized as a practical guide. No standard and recommended method has been found or exist to detect the pebrine spores of Samia species.Hence, to enhance the Eri Silk production, production of disease free layings is a must for overall development of Eri silk industry. So, the present study was under taken to fulfill the goal in the field.

2. Materials and methods

The mass mother moth examination of eri silk moth includes random selection of eri female silk moths after laying the eggs followed by grinding, filtering ,centrifuging and finally examination under microscope in 600 x magnification.

New Producedure : The following procedure of Eri mother moth examination was standardized for seed production after conducting the following treatments under the experiment :

Table 1 :

No.of @ & moths tested (TREAT-1)	Quantity of K2CO3 soln. added (ml) in grinding (TREAT-2)	Conc.of K2CO3 considered for grinding (TREAT-3)	Rpm considered (TREAT-4)	Time considered for Rpm (TREAT-5)	REMARKS
2)	5ml,10ml,15ml,20ml,25ml, 30ml	0.7% 0.8% 0.9% 1.0% 1.1% 1.2%	2000rpm, 3000rpm, 4000rpm, 5000rpm, 6000rpm	(a) 2minutes (b) 3minutes (c) 4 minutes (d) 5 minutes	C R O S S E
3)	5 ml,10 ml,15 ml,20 ml,25 ml, 30ml	1.3% 1.4%	-do-	-do-	X A
4)	5 ml,10 ml,15 ml,20 ml,25 ml	1.5%	-do-	-do-	M
5)	5 ml,10 ml,15 ml,20 ml,25 ml, 30ml		-do-	-do-	I
6)	5 ml,10 ml,15 ml,20 ml,25 ml,30ml,		-do-	-do-	N
7)	5 ml,10 ml,15 ml,20 ml,25 ml, 30ml		-do-		A T I O N.

Standardization of Eri mother silk moth examination (Evoluved method)

Step1. Collection of materials

- After 3rd day of oviposition randomly selected female eri silk moths are taken for testing.
- But examination can be done in dry or dead moths also.
- For grinding 10 nos.of whole female eri silk moths

with 30 ml of K2CO3 solution is required

Step2: **Preparation of materials (Treatment)**

- After egg laying 10 numbers of eri whole moths are ground well with the help of marble made morter and pestle .
- After crushing 10 whole eri moths together add 30 ml of 1.3% or 1.4% K2CO3 solution to the grinded materials and made it a thick paste.
- Then materials is filtered through clean absorbent cotton with the help of a funnel and directly transferred it to the centrifugal tube'

Step3: **Centrifuge**

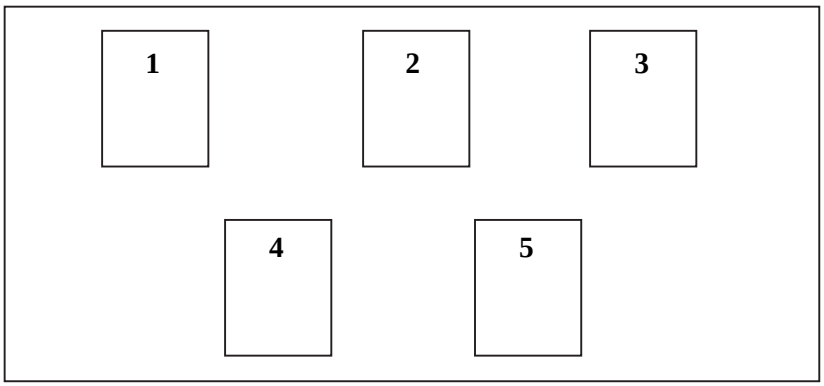
- Filtrate is collected and centrifuged at 2000 rpm for 4 minutes.
- After centrifugal the supernatant is discarded.

Step4:Cross examination

- Take smear from dispersed solution and prepare slide by covering with a cover slip and observe in the compound microscope preferably at 600 x magnification.
- If sediment is too thick add 1 drop of distilled before examination under microscope .
- Cross examination the individual slide to further confirm.
- Examine 5 fields per smear to detect pebrine spore.
- Detection of pebrine spore more than 5 (five) per smear should be rejected.
- In case of detection of more than 10% pebrine contaminated layings the whole lot should be rejected and burnt immediately.

Step5 : Disinfection and Hygiene

- All crushing set, examined slide etc. should be washed with 5% bleaching powder solution after examination.



Glass slide with smear arrangement

Table 2 : Ready reconer for intensity level of Eri pebrine

Sl No.	No. of spores per field	Grade	Remarks
01.	1-4	±	May be considered
02.	5-10	1+	Should be rejected.

Table 3 : Comparison between proposed technique and existing procedure

Proposed technique with suitable modification for restandardization of existing procedure*	Existing procedure
1. A slightly higher concentration of 1.3% K2CO3 is used for grinding of materials. Inclusion of slightly higher concentration of K2CO3 increases the pH, causing easier separation of spores.	1. 6% K2CO3 is used for grinding of materials.
2. Here, 10 nos. of eri whole moths are considered for grinding in 30 ml of 1.3-1.4% of K2CO3 as it is saturated /gets optimum level in marble made mortar pestle.	2. 20 nos. moths abdominal part considered .
3. 2000 rpm for 4 minutes is sufficient to liberate spore. Here, time is less consuming with less electricity consumption.	3. 3000-4000 rpm for 3 minutes .Here ,time is more consuming with more electricity consumption.
4. Here, dilution is not required.It is because 30 ml K2CO3 is sufficient enough to carry out the whole process and act as a time saver.	4. Here, dilution is required. Here again sediment is diluted with0.6% K2CO3. So, another extra activity is required here consuming more time.

* The experiment has been repeated during the months of April,15 and June'15and August'15 and found the same result.

3. Results & discussion

1. Under treatment 1(No.of @& moths to be treated) it is found that 10 nos.of female eri silk moths are suitable (from a lot 100 moths) for eri pebrine spore detection using marble made mortar and pestle for grinding.
2. After experimentation it is found that 1.3 to 1.4% K2CO3 solution found to be suitable for smear preparation in eri silk moths.
3. Centrifugal speed standardized at 2000 to 3000 rpm with 4 minutes.
4. For grinding 10 nos.of whole female eri silk moths with 30 ml of K2CO3 solution is required which produced about 12-13 ml of filtrate in the centrifuge tube (up to maximum level)

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