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Influence of Chemical-Induced Liberation of Pebrine Spores in Tasar Silkworm Mother Moth Examination

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Abstract

Tasar silkworm suffers from various diseases especially Pebrine, Virosis, Bacteriosis and muscardine. Approximately 30-35% of crop losses can be attributed to Pebrine disease, with sporadic instances leading to complete crop failure. The primarily infection of Pebrine is by vertical transmission with the pathogen transferring the infection directly from parent to offspring. To ensure the perpetuation of Pebrine-free generations of silkworms, disease-free layings are produced through the examination of the mother moth. It is necessary to improve the visibility of spores by complete liberation, staining, cleaning of spores and dissolution/removal of fat bodies, debris of body tissues, *etc*. Certain chemicals are selected and tested the results revealed that, Sarcosyl, Formalin and Ethanol treated samples field was very clear and no debris was observed. Sarcosyl and Ethanol treated samples have shown 212,000 and 54,000 more number of pebrine spores cm^{-3} liberated when compared with K_2CO_3 (control) at 0.5% chemical concentration. Sarcosyl treated samples has shown 122,000 more number of pebrine spores cm^{-3} liberated when compared with K_2CO_3 (control) at 1% chemical concentration. Citric acid (300,000), Ethanol (100,000) and Sarcosyl (500,000) obtained more number of liberated pebrine spores cm^{-3} when compared with K_2CO_3 (control) at 2% chemical concentration. Sarcosyl (160,000), Ethanol (70,000) and Formalin (100,000) chemical treated samples obtained more number of liberated pebrine spores cm^{-3} when compared with K_2CO_3 (control) at 5% concentration. Sarcosyl, Formalin, Ethanol and Citric acid chemicals have shown better performance when compared with other tested chemicals and K_2CO_3 (control). Sarcosyl stands first position followed by Ethanol, Formalin and Citric acid chemicals in more number of liberation of pebrine spores cm^{-3}.

Keywords: *Antheraea mylitta* D., Pebrine, Mother moth examination, Sarcosyl

Introduction

The Tasar silkworm (*Antheraea mylitta* D.), an insect of significant economic importance, is predominantly cultivated in outdoor environments using host trees such as *Terminalia arjuna* (Arjuna), *Terminalia tomentosa* (Asan) and *Shorea robusta* (Sal) (Kiran Kumar *et al.*, 2017). This species, however, is susceptible to several diseases, notably Pebrine (caused by the Microsporidia family Nosematidae), Virosis (caused by the *Antheraea mylitta* Cytoplasmic Polyhedrosis virus, a reovirus), Bacteriosis (various bacterial infections) and Muscardine (a fungal disease). Reports indicate that these diseases collectively contribute to significant crop loss, with estimates ranging from 20% to 40% in silkworm populations in India (Sahay *et al.*, 2000).

Among these diseases, Pebrine is particularly damaging, accounting for approximately 30-35% of crop losses and leading to intermittent instances of complete crop failure (Sahay *et al.*, 2000; Chakrabarty *et al.*, 2012). Pebrine

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stands out as a primary constraint in the production of raw silk, severely impacting both the quantity and quality of silk produced (Singh *et al.*, 2018). This parasitic infection, primarily transmitted through vertical transmission from parent to offspring, can lead to the death of young silkworms, especially during the 3rd instar stage and can cause reduced cocoon production. If the infection manifests during the 4th instar, it may progress to the adult stage, resulting in the production of infected eggs, which further exacerbates the problem and threatens egg supply.

To combat Pebrine and ensure the production of disease-free silkworm generations, systematic examination of mother moths is crucial (Singh *et al.*, 2021). Despite rigorous microscopic examination and the elimination of infected egg layings, Pebrine infection persists. This ongoing issue may stem from inadequate identification of infective spore stages, the presence of vegetative stages of the Nosema life cycle during microscopic examination and incomplete spore liberation from infected tissues. Additional factors such as fat bodies, tissue debris and other contaminants also contribute to the challenge.

Recent research has focused on enhancing the detection and control of Pebrine through improved methodologies (Bhattacharya *et al.*, 1995; Datta *et al.*, 1998; Samson *et al.*, 1998; Patil and Sharadamma, 1999). One promising approach involves chemical-induced liberation of Pebrine spores from infected moths or eggs. Similar experiments were conducted by Thangavelu *et al.* (1995) and Singh *et al.* (2018). By disrupting the spores' protective mechanisms, these chemicals may enhance visibility and facilitate better detection and management. Studies have investigated various chemical treatments to increase spore release, improve staining techniques and address the removal of fat bodies and tissue debris (Singh *et al.*, 2002; Aneja, 2003; Singh *et al.*, 2005). These advancements aim to refine the current diagnostic and control strategies, addressing the persistent challenge of Pebrine in Tasar silkworm production.

Hence, it is necessary to improve the visibility of spores by complete liberation, staining, cleaning of spores and dissolution/ removal of fat bodies, debris of body tissues, *etc*.

Materials and Methods

Tasar silkworm moths were procured from RTRS, Warangal and experiment was conducted in Sri Krishnadevaraya University, Anantapur during 2021-22.

Selection of the Chemicals

Based on the literature, the following chemicals (Table 1), which are efficient in liberation and clean of spores, removal and dissolution of unwanted materials in the smear were selected and collected for the study.

Preparation of Chemicals Stock Solution

The selected chemicals such as sds, sulphuric acid, acetic acid, hydrochloric acid, ethanol, citric acid, sodium acetate, sodium silicate, Formalin, sodium hydroxide, EDTA + potassium hydroxide, poly Ethylene glycol and Sarcosyl were prepared 10% concentration stock solution by following method.

Table 1: Selected chemicals for the study

Sl. No.	Name of the Chemical	Sl. No.	Name of the Chemical
1	Poly ethylene glycol	8	Ethanol
2	EDTA + Potassium hydroxide	9	Hydrochloric acid
3	Sodium hydroxide	10	Acetic acid
4	Formaline	11	Sulphuric acid
5	Sodium silicate	12	SDS
6	Sodium acetate	13	Sarcosyl
7	Citric acid		

Preparation of Solid State Chemicals Stock Solution

Calculation in Percentage (weight/volume)

$$10\% \text{ of } 100 \text{ mL of chemical} = \frac{100 \text{ mL of the chemical} \times 10 \text{ g}}{100}$$

The obtained amount of chemical is then dissolved in 100 mL of distilled water.

Preparation of Liquid State Chemicals Stock Solution

Calculation in Percentage (volume/volume)

$$10\% \text{ of } 100 \text{ mL of chemical} = \frac{100 \text{ mL of the chemical} \times 10 \text{ mL}}{100}$$

The obtained volume of that chemical (10 mL) is dissolved in 90 mL of distilled water.

Serial Dilution

9 mL of distilled water was taken in a measuring cylinder and then added 1 mL of any particular solution that is to be diluted. 10% concentration of the required solution was prepared by performing this step and total volume of 100 mL of required stock solutions were prepared for each of the selected chemicals. 1 mL of solution from a particular stock solution of any chemical was taken in a test tube with a pipette and final volume was made upto 10 mL by adding distilled water and thus further diluted solution was made and the solution obtained was diluted by factor 10. This step was followed with all the other selected chemicals but the volume of distilled water taken was in proportion according to the required concentration of the particular solution to be diluted.

Selection of the Different Chemical Concentrations for the Study

0.5%, 1%, 2%, 3%, 4%, 5%, 7.5% and 10% chemical concentrations were selected for the study.

Identification of Pebrinized Moth and its Collection (Anonymous, 2023)

• Infected moths typically exhibit deformities such as crumpled wings and a reduced number of scales on their abdomens.

• The scales on the wings and abdominal area are easily detachable.

• Reduced efficacy in mating and egg-laying activities.

The moths with above characters were collected in a paper box for their examination (Figure 1).

Figure 1: Moths with crupled wings and scale less abdomen

Tasar Moth Examination

1. Pebrinized moths were collected.

2. The tissue from 8-6 segments of the moth was cut and then divided into two equal parts by weight.

3. The two samples were taken in different mortars and leveled as A and B. Both the samples were crushed identically with different pestles as follows:

Sample A: Chemical solution to be tested.

Sample B: K_2CO_3 (Control)

4. Both the smears were observed under student microscope at 600X magnification and the observations were recorded.

Counting of Pebrine Spores

• 1.5 mL of all the samples were taken in different ependorf tubes and well labeled and then subjected to centrifuge at 5000 rpm for 10 min.

• The supernatant was then discarded and 1mL of distilled water was added to each ependorf tube and then vortexed for 3 min. for proper mixing of the pallet with distilled water.

• Then one 10 µL of the sample were taken in a haemocytometer and then subjected to counting of spores.

• All the observations were noted down and tabulated in table 2, 3 and 4.

• No. of Spore = average of all five readings × 10^4 spores cm^{-3} formula was used to calculate the total number of spores present in the taken sample.

Results and Discussion

Visual observations of field treated with different chemicals are represented in table 2. No significant difference was noticed when the samples were treated with Poly ethylene glycol, EDTA + Potassium hydroxide, Sodium hydroxide, Sodium silicate, Sodium acetate, Citric acid, Acetic acid and SDS. Formalin and Ethanol treated samples the field was very clear at the same time fluorescent shining was more in pebrine spores. In the case of Sarcosly treated samples, clear field was observed to good extent and the amount of liberation of spores were very much high.

Performance of the different chemicals at 0.5% and 1% is depicted in table 3. The results of 0.5% chemical concentration revealed that, the number of liberated pebrine spores cm^{-3} ranged from 110,000 to 638,000. The least number of liberated pebrine spores cm^{-3} was observed in Acetic acid with 110,000 where as high number of liberated pebrine spores cm^{-3} was observed in Sarcosyl with 638,000. It is observed that, in all most all chemicals 200,000 pebrine spores cm^{-3} were liberated, except in Acetic acid, Sodium acetate and Hydrochloric acid. In the case of control, the number of liberated pebrine spores cm^{-3} was 426,000. Ethanol (54,000) and Sarcosyl (212,000) obtained more number of liberated pebrine spores cm^{-3} when compared with K_2CO_3 (control).

The results of 1% chemical concentration revealed that, the number of liberated pebrine spores cm^{-3} ranged from 198,000 to 812,000. The least number of liberated pebrine spores cm^{-3} was observed in Hydrochloric acid with 198,000 where as high number of liberated pebrine spores cm^{-3} was observed in Sarcosyl with 812,000. It is observed that, in all most all chemicals 200,000 pebrine spores cm^{-3} were liberated, except in Hydrochloric acid. In the case of control, the number of liberated pebrine spores cm^{-3} was 690,000. Sarcosyl (122,000) obtained more number of liberated pebrine spores cm^{-3} when compared with K_2CO_3 (control).

Performance of the different chemicals at 2% and 5% is depicted in table 4. The results of 2% chemical concentration revealed that, the number of liberated pebrine spores cm^{-3} ranged from 160,000 to 1,400,000. The least number of liberated pebrine spores cm^{-3} was observed in Acetic acid with 160,000 where as high number of liberated pebrine spores cm^{-3} was observed in Sarcosyl with 1,400,000. It is observed that, in all most all chemicals 500,000 pebrine spores cm^{-3} were liberated, except in Acetic acid, Sulphuric acid, Sodium acetate, Formalin, EDTA + Potassium hydroxide and Hydrochloric acid. In the case of control, the number of liberated pebrine spores cm^{-3} was 900,000. Citric acid (300,000) Ethanol (100,000) and Sarcosyl (500,000) obtained more number of liberated pebrine spores cm^{-3} when compared with K_2CO_3 (control).

The results of 5% chemical concentration revealed that, the number of liberated pebrine spores cm^{-3} ranged from 179,000 to 480,000. The least number of liberated pebrine spores cm^{-3} was observed in Sodium hydroxide with 179,000 where as high number of liberated pebrine spores cm^{-3} was observed in Sarcosyl with 480,000. It is observed that, in all most all chemicals 200,000 pebrine spores cm^{-3} were liberated, except in Sodium hydroxide, Citric acid and Hydrochloric acid. In the case of control, the number of liberated pebrine spores cm^{-3} was 320,000. Formalin (100,000), Ethanol (70,000) and Sarcosyl (160,000) obtained more number of liberated pebrine spores cm^{-3} when compared with K_2CO_3 (control).

Ethanol (54,000) and Sarcosyl (212,000) obtained more number of liberated pebrine spores cm^{-3} when compared with other tested chemicals and K_2CO_3 (control) at 0.5% chemical concentration. The results of 1% chemical concentration revealed that, Sarcosyl (122,000) performed

well and obtained more number of liberated pebrine spores cm^{-3} than all tested chemicals. Citric acid (300000), Ethanol (100,000) and Sarcosyl (500,000) obtained more number of liberated pebrine spores cm^{-3} when compared with other tested chemicals and K_2CO_3 (control) at 2% chemical concentration. The results of 5% chemical concentration revealed that, Formalin (100,000), Ethanol (70,000) and Sarcosyl (160,000) obtained more number of liberated pebrine spores cm^{-3} when compared with other tested chemicals and K_2CO_3 (control).

Formalin, Ethanol, Citric acid and Sarcosyl chemicals have shown better performance when compared with other tested chemicals and K_2CO_3 (control). Sarcosyl stands first position followed by Ethanol, Formalin and Citric acid chemicals in more number of liberation of pebrine spores cm^{-3}.

Table 2: Visual observations of field treated with different chemicals

Sl. No.	Name of the Chemical	Observation
1	Poly ethylene glycol	No significant difference was observed.
2	EDTA + Potassium hydroxide	No significant difference was observed.
3	Sodium hydroxide	No significant difference was observed.
4	Formalin	Field was clear and aggregation of tissues was observed.
5	Sodium silicate	No significant difference was observed.
6	Sodium acetate	No effect was observed.
7	Citric acid	No significant difference was observed but the refractivity of spores was reduced.
8	Ethanol	Field was clear and did not affect its basic structure.
9	Hydrochloric acid	Clumps formation of cellular debris was observed.
10	Acetic acid	No significant difference was observed.
11	Sulphuric acid	Desiccation of all the other cells was observed along with inhibition of Brownian movement of spores.
12	SDS	No significant difference was observed.
13	Sarcosyl	Clearance of the field was observed to good extent and the amount of liberation of spores were very much high.
Control	K_2CO_3	The field was good with normal amount of liberation of spores.

Table 3: Performance of different chemicals at 0.5% and 1.0% concentrations

Sl. No.	Name of the Chemical	Chemical Concentration			
		0.50%		1.00%	
		Liberation of spores (No)	Spores more than control (No)	Liberation of spores (No)	Spores more than control (No)
1	Poly ethylene glycol	300,000	-126,000	490,000	-200,000
2	EDTA + Potassium hydroxide	280,000	-146,000	622,000	-68,000
3	Sodium hydroxide	360,000	-66,000	525,000	-165,000
4	Formalin	410,000	-16,000	673,000	-17,000
5	Sodium silicate	380,000	-46,000	333,000	-357,000
6	Sodium acetate	190,000	-236,000	390,000	-300,000
7	Citric acid	395,000	-31,000	411,000	-279,000
8	Ethanol	480,000	+54,000	656,000	-34,000
9	Hydrochloric acid	175,000	-251,000	198,000	-492,000
10	Acetic acid	110,000	-316,000	356,000	-334,000
11	Sulphuric acid	410,000	-16,000	473,000	-217,000
12	SDS	310,000	-116,000	580,000	-110,000
13	Sarcosyl	638,000	+212,000	812,000	+122,000
Control	K_2CO_3	426,000	0	690,000	0

Table 4: Performance of different chemicals at 2% and 5% concentrations

Sl. No.	Name of the Chemical	Chemical Concentration			
		2.00%		5.00%	
		Liberation of spores (No)	Spores more than control (No)	Liberation of spores (No)	Spores more than control (No)
1	Poly ethylene glycol	800,000	-100,000	300,000	-20,000
2	EDTA + Potassium hydroxide	490,000	-410,000	267,000	-53,000
3	Sodium hydroxide	500,000	-400,000	179,000	-141,000
4	Formalin	210,000	-690,000	420,000	+100,000
5	Sodium silicate	670,000	-230,000	230,000	-90,000
6	Sodium acetate	360,000	-540,000	273,000	-47,000
7	Citric acid	1,200,000	+300,000	197,000	-123,000
8	Ethanol	1,000,000	+100,000	390,000	+70,000
9	Hydrochloric acid	420,000	-480,000	180,000	-140,000
10	Acetic acid	160,000	-740,000	220,000	-100,000
11	Sulphuric acid	250,000	-650,000	290,000	-30,000
12	SDS	830,000	-70,000	310,000	-10,000
13	Sarcosyl	1,400,000	+500,000	480,000	+160,000
Control	K_2CO_3	900,000	0	320,000	0

Conclusion

In the case of Sarcosyl treated samples, 638,000 pebrine spores cm^{-3} were liberated where as in K_2CO_3 (control), the number of liberated pebrine spores cm^{-3} was 426,000 which is 212,000 pebrine spores cm^{-3} more than the control. More number of pebrine spores cm^{-3} leads to easy identification the pebrine spores in the homogenated samples. At the same time, Sarcosyl treated samples field was very clear and no debris were observed.

Hence, it is suggested that 0.5% Sarcosyl may be used in commercial grainages, private grainages and Research institutes for pebrine spore identification through the microscope.

References

Aneja, K.R., 2003. *Experiments in Microbiology, Plant Pathology and Biotechnology*. Fourth Edition. New Age International (P) Ltd., Daryaganj, New Delhi. p. 632.

Anonymous, 2023. Diseases and pests of mulberry silkworm. Central Tasar Research & Training Institute, Central Silk Board, Ranchi. Available at: http://silks.csb.gov.in/. Accessed on: January 29, 2023.

Bhattacharya, J., Krishnan, N., Chandra, A.K., Sen, S.K., Saratchandra, B., 1995. Labex: An effective bed disinfectant. *Indian Silk* 34(8), 35-36.

Chakrabarty, S., Manna, B., Saha, A.K., Bindroo, B.B., 2012. Comparative study on effect different types of *Nosema* sp. (Microsporidia: Nosematidae) on mulberry and vanya silkworms. *Journal of Biological Sciences* 12(1), 1-14. DOI: https://doi.org/10.3923/jbs.2012.1.14.

Datta, R.K., Baig, M., Nataraju, B., Balavenkatasubbaiah, M., Selvakumar, T., 1998. Vijeytha: An effective disinfectant. *Indian Silk* 36(9), 12-13.

Kiran Kumar, K.P., Singh, G.P., Sinha, A.K., 2017. Effect of eco-user friendly disease management on tasar cocoon crop improvement. *International Journal of Research in Biological Sciences* 7(1), 1-6.

Patil, C.S., Sharadamma, P., 1999. Sanjeevani: An effective bed disinfectant. *Indian Silk* 37, 13-14.

Sahay, D.N., Roy, D.K., Sahay, A., 2000. Diseases of tropical tasar silkworm, *Antheraea mylitta* D.: Symptoms and control measures. In: *Lessons on Tropical Tasar*. (Ed.) Thangavelu, K. Central Tasar Research and Training Institute, Piska-Nagri, Ranchi. pp. 61-79.

Samson, M.V., Singh, R.N., Sasidharan, T.O., 1998. Resham Jyoti - A wide spectrum bed disinfectant. *Indian Silk* 37(3), 9-10.

Singh, G.P., Xingmeng, L., Nataraju, B., Datta, R.K., 2002. Efficacy of Xiaotelin as a disinfectant against the pathogens of silkworm, *Bombyx mori* Linn. *Indian Journal of Sericulture* 41(1), 84-88.

Singh, G.P., Sahay, A., Roy, D.K., Sahay, D.N., 2005. Efficacy of disinfectants against cytoplasmic polyhedrosis virus and microspordia of tasar silkworm, *Antheraea mylitta* D. *International Journal of Industrial Entomology and Biomaterials* 10(1), 69-72.

Singh, G.P., Baig, M.M., Singh, J., Pandey, J.P., Sinha, A.K., Jena, K.B., 2018. Use of certain chemicals for easy and quick detection of Nosema mylittensis Spores infecting tropical tasar silkworm, *Antheraea mylitta* Drury (Saturniidae: Lepidoptera). *Journal of Entomology and Zoology Studies* 6(5), 2328-2331.

Singh, G.P., Baig, M.M., Bajpayi, C.M., 2021. Recent trends in tasar silkworm Antheraea mylitta Drury disease management. Chapter 8. In: *Methods in Silkworm Microbiology*. (Eds.) Gurtler, V. and

Subrahmanyam, G. *Methods in Microbiology*, Volume 49. Elsevier. pp. 175-200. DOI: https://doi.org/10.1016/bs.mim.2021.05.001.

Thangavelu, K., Bansal, A.K., Shukla, R.M., Saxena, N.N., Jayaswal, J., Goel, A.K., 1995. Evaluation of some chemicals for prevention of disease in tasar silkworm, *Antherea mylitta* Drury (Lepidoptera: Saturniidae). *Indian Journal of Sericulture* 34(2), 142-144.