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Anti-protozoan activity of different locally available chemicals against dreadful pebrine disease (*Nosema* spp.) of silkworm (*Bombyx mori* L)

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Abstract

Pebrine disease of silkworm caused by *Nosema* spp. is most dangerous disease because of its transovarian mode of transmission, fast secondary spread and recurrence and has posed a great challenge for its control throughout Globe especially in India. The disease ruined the silk industry in France and Italy as early as in 1845. Disease outbreak can cause 100% crop loss for years if not managed properly. Keeping in view the magnitude and severity of disease some locally available chemicals with known anti protozoan activity were evaluated at different concentrations for management of this dreadful disease. The overall results obtained after completion of 3 years trial have shown significant potential of all the test chemicals for management of the disease, out of three test chemicals Sodium bicarbonate at 0.6% was found most effective as compared to metronidazole and carbendazim in all the parameters under study.

Keywords: Silkworm, disease, chemicals, management

Introduction

Sericulture in India is much older than Europe thus question of silkworm disease is no new one in Indian sericulture. Jammu & Kashmir state is one of the pioneer states for the production of univoltine/bivoltine silk of international standard. Since mulberry silkworm has been domesticated from time immemorial and continuous domestication has made it susceptible to many dreadful diseases like pebrine, muscardine, grasserie and flacherie. Average silkworm cocoon crop loss due to various diseases is to the tune of 15-47% (Tayal *et al.*, 2017) [8]. Among these diseases, the pebrine caused by *Nosema sp.* is one of the most dreadful diseases having potential to wipe out the silk industry. In the state of Jammu & Kashmir for hundreds of years, silkworms and its seeds were free from any infestation especially pebrine. But unfortunately, in due course of time silkworms in our state were also attacked by the dreadful disease the “pebrine” scientifically known as Microsporidiosis. It is due to the outbreak of Microsporidiosis that Kashmir lost its only productive univoltine silkworm race “Kashmir Race” in 1878 (Kamili and Masoodi, 2000) [6]. Keeping in view the magnitude of this disease and the loss it causes to the farmers as well as importance of sericulture in the current times, it was seriously thought to work out a sound, ecofriendly and cost effective method of managing this dreadful disease.

Material and Methods

The evaluation of different chemicals was conducted on Silkworm seed of race SKUA-R 6 laid by the moths infected with pebrine disease (*Nosema*). The test chemicals included Sodium bicarbonate @ 0.2%, 0.4% and 0.6% concentrations, metronidazole @ 0.2%, 0.4% and 0.6% concentrations and carbendazim @ 0.25%, 0.50% and 1.0% concentrations with three Controls (Control I- untreated but inoculated, Control II-Distilled water treated but inoculated and Control III- Untreated healthy). Moths of the patronized seeds were subjected to microscopic examination individually for the presence of pebrine infection. Seed layings with uniform spore load were selected for the study. The diseased seed was kept under hibernation as per the standard procedure (Kamili and Masoodi, 2000 and Rajan *et al.*, 2005) [6, 7] and were released from the cold storage for incubation. During the incubation a portion of seeds of infected layings were examined microscopically to ensure that the seeds under incubation are having full spore load of pebrine (*Nosema*). The rearing of patronized silkworms was carried out by following the standard methods of rearing (Krishnaswamy *et al.*, 1973) [5].

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After hatching and brushing, the samples of first age and second age larvae were again examined microscopically to confirm and ensure further that the first age silkworms and second age silkworms under rearing are infected with pebrine infection. The rearing and management study was conducted *in vivo*. The patronized worms were reared in mass upto 2nd age. The 3rd age larvae were counted and divided into three replications with 200 worms per replicate.

Preparation of chemicals

The test chemicals were in different physical form and were also tested in different concentrations, metronidazole was in tablet form whereas sodium bicarbonate and carbendazim were in powdered form. The tablets of metronidazole were crushed to make the powder of the chemical. The concentration of three different chemicals under study was prepared by dissolving the given chemicals in 1 litre of distilled water. On the basis of active ingredient of chemicals the required concentrations were obtained. The sodium bicarbonate was used at 0.2, 0.4 and 0.6%. concentrations and prepared by dissolving 2 grams, 4 grams and 6 grams of sodium bicarbonate separately in 1 litre of distilled water. The metronidazole was used at 0.2, 0.4 and 0.6%. The required concentrations of metronidazole were prepared by dissolving metronidazole tablets in 1 litre of distilled water. The carbendazim was used at 0.25, 0.5, and 1.0%. The three concentrations of carbendazim were obtained by dissolving different quantities in 1 litre of distilled water.

Application of chemicals

The test chemicals were applied to the diseased worms orally through mulberry leaves. The test concentrations of the chemicals under study were sprayed on the mulberry leaf and treated leaves were fed to silkworms once during the 3rd age, twice during 4th age and on alternate days during the 5th age to check the efficacy of these chemicals towards pebrine infected larvae. For each treatment a separate untreated batch of the worms was kept as standard check Control-I, another batch of worms treated with distilled water was kept as control-II in order to study the residual effect of test chemicals on the commercial characters of the race under study.

Results and Discussion

The data pertaining to larval survival percentage (3rd, 4th and 5th age), effective rate of rearing (by number and weight), percentage of live cocoons, moth emergence percentage, and recovery of average Disease free layings (dfl) percentage was subjected to statistical analysis. The results obtained are given in the Table.

Larval survival percentage in 3rd age

Among the different chemical concentrations under study, Sodium bicarbonate at 0.6 percent concentration recorded the

significantly highest survival percentage of 93.97 percent, followed by 0.4 percent (93.77) and metronidazole at 0.6 percent exhibited survival percentage of 89.88 percent and metronidazole at 0.2 percent recorded survival percentage of 88.01 percent.

Larval survival percentage in 4th age

The highest (89.32%) larval survival percentage among the different concentrations was shown by sodium bicarbonate (at 0.6%), followed by 0.4 percent (88.01%) and metronidazole which recorded (89.88%) larval survival at 0.6 percent and carbendazim 1.00 percent exhibited larval survival (87.67%) at par with sodium bicarbonate 0.4%.

Larval survival percentage in 5th age

Among the different concentrations under the study the treatment sodium bicarbonate at 0.6 percent concentration exhibited highest larval survival (80.81%), followed by metronidazole which exhibited (79.21%) larval survival at 0.6 percent concentration and carbendazim recorded (77.17%) larval survival at 1.00 percent concentration.

Cocoon yield by number

Sodium bicarbonate at 0.6 percent concentrations exhibited highest cocoon yield (by number) of 8056 followed by metronidazole which recorded a value of 7901 of cocoon yield (by number) at 0.6 percent concentration and carbendazim exhibited a value of 7720 of cocoon yield (by number) at 1.00 percent concentration.

Percentage of live cocoons

The highest (95.37%) live cocoon percentage was recorded at 0.6 percent concentration of Sodium bicarbonate, followed by metronidazole which recorded (93.74%) live cocoon percentage at 0.6 percent concentration and carbendazim exhibited (93.16%) live cocoon percentage at 1.00 percent concentration.

Moth emergence percentage

Among the three chemicals evaluated in the study programme the highest moth emergence percentage (94.33) at 0.6 percent concentration was shown by the treatment Sodium bicarbonate, followed by metronidazole which recorded (93.36) moth emergence percentage at 0.6 percent concentration and carbendazim exhibited moth emergence percentage (93.13) at 1.00 percent concentration.

Percentage of average disease free layings (DFL'S)

The chemical Sodium bicarbonate recorded the highest recovery percentage of dfl's (96.23%) at 0.6 percent concentration, followed by metronidazole which showed recovery percentage of dfl's (95.53) at 0.6 percent concentration and carbendazim recorded (92.98%) recovery percentage of dfl's at 1.00 percent concentration.

Table 1: Effect of chemicals on survival Parameters of silkworm *Bombyx mori* L.

Chemicals	Concentrations (%)	Survival 3 rd age (%)	Survival 4 th age (%)	Survival 5 th age (%)	ERR (BY NO)	Live cocoons (%)	Moth emergence (%)	DFL (%)
Sodium bicarbonate	0.2	92.14	84.56	76.22	7700	91.47	89.71	92.87
	0.4	93.77	87.76	78.33	7830	94.33	92.46	94.90
	0.6	93.97	89.32	80.81	8056	95.37	94.33	96.23
Metronidazole	0.2	85.81	83.44	75.61	7542	90.22	91.50	90.36
	0.4	88.01	83.67	76.85	7630	92.07	91.90	91.89

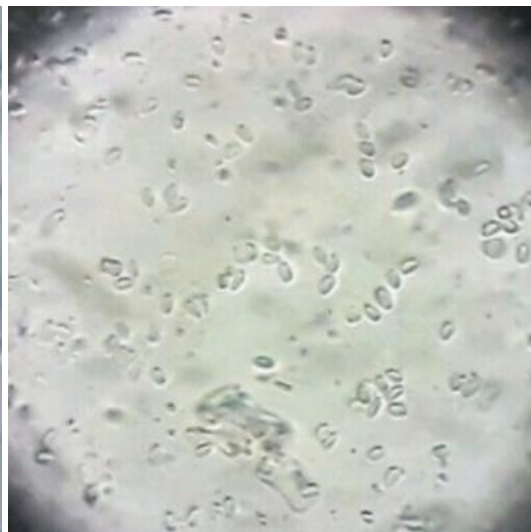
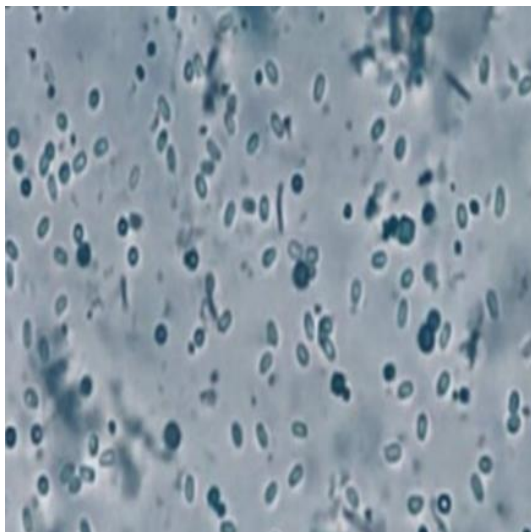
	0.6	89.88	85.12	79.21	7901	93.74	93.36	95.53
Carbendazim	0.25	85.31	81.32	74.91	7450	89.37	90.01	91.09
	0.50	87.09	82.03	75.88	7578	90.40	92.22	92.11
	1.00	87.67	83.29	77.17	7720	93.16	93.13	92.98
Control(untreated)		72.33	56.22	37.11	970	0.00	0.00	0.00
CD=0.05		1.10	1.67	1.98	113	0.97	0.64	0.76



A) Silkworm Rearing at Experimental site



b) Application of Chemicals on Mulberry leaves



c) Microscopic Examination of Seed and Larvae for presence of Pebrine spores

Discussion

The silkworm (*Bombyx mori* L.) has become prey to many pathogenic micro-organisms due to prolonged domestications. Among different diseases Pebrine disease is considered to be most dangerous disease because of Transovarial (Han *et al* 1988) [4]. The pathogen has also varied Host ranges and has been reported in different insects (Chanderashakeran *et al* 2017) [3]. The micro spordia is highly dangerous and can wipe-out whole silk industry if not addressed intime. Many strains of Nosema has been identified to attack the silkworm. Among them NIK-5D,Nik-Pr,NIK-1Cc,NIK1 So and NIK 1Dp are prominent to attack with different damage magnitudes (Bhat and Kamilli 2009) [2]. The highest (96.53%) anti target pathogen activity was recorded in Sodium bicarbonate at 0.6% followed by metronidazole (95.23) 0.6% and carbendazim (92.98) at 1.0% respectively. The effect of chemicals also increased with increase in concentrations. The residual effect of chemicals was also tested however no side effect of test chemicals was found on commercial cocoon characters and heath of worms.

Conclusion

The present study has revealed that all the three chemicals have significant anti-protozoan activity, However, Sodium bicarbonate at 0.6 percent concentration has been found most effective in the control of pebrine disease of silkworms. Thus, the management of pebrine disease with the Sodium bicarbonate seems to be farmer friendly, also because of its low cost and easy availability. The other two chemicals namely metronidazole and carbendazim have also been found effective against pebrine disease of silkworms, but Sodium bicarbonate (0.6% concentration) surpassed these two in efficacy. Studies also reveal that with the increase in concentration of the chemicals the overall effect on results has also increased. From the studies it is clear that Sodium bicarbonate (0.6 percent) which is highly cost effective and readily available could be recommended for controlling the dreadful pebrine disease in bivoltine silkworm (*Bombyx mori* L.) under temperate climatic conditions and farmers can also harvest a good cocoon crop with good returns.

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