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ORIGINAL ARTICLE

Effect of different anti-protozoan drugs against pebrine disease of silkworm *Bombyx mori*. L with special reference to its impact on commercial cocoon characteristics

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ABSTRACT
Microsporidiosis also known as Pebrine disease caused by *Nosema sp.* is a dreadful disease of silkworm (*Bombyxmori. L)*and has ruined the silk industry in France and Italy as early as in 1845. The disease has the potential to cause 100 per cent crop loss and can result in enormous economic loss to the farmers if not managed properly. Recently, the incidence of Microsporidiosis occurrence in Kashmir Division has become more complex ranging from 9 to 18.98 %. Keeping in view the magnitude of this disease as well as value of sericulture in the current times it was seriously thought to work out a sound and cost-effective method of managing this dreadful disease without affecting commercial cocoon characteristics of silkworm. The present findings revealed that out of three chemicals screened for their efficacy against the disease Sodium bicarbonate @ 0.8% was found most effective as compared to Ornidazole and Rifampicinin all commercial cocoon parameters under study.
Key Words: *Bomyx mori, Disease, Chemicals, commercial cocoon characteristics, Sodium bicarbonate, Sericulture, Microsporidiosis, Pebrine disease, pebrinised*

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INTRODUCTION

In India, Sericulture is not only a tradition but also a living culture. Indian silk has enthralled all categories of consumers across the world with its vast repertoire of motifs, techniques and brilliant hues. Silk reigns supreme as an object of desire, famous for its fabric of high fashion, its finery and distinct colors. Jammu& Kashmir state has been one of the trailblazer states for the production of univoltine/bivoltine silk of international standard but the mulberry silkworm, *Bombyx mori* L., since times immemorial has been the prey to various dreadful diseases such as pebrine, muscardine, grasserie and flacherie. In India, the average silkworm cocoon crop loss due to various diseases is to the tune of 15-47% [13]. 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 the UT of Jammu and Kashmir 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 [9]. Since then J&K state became dependent on silkworm seeds imported from different sericultural countries and states. The disease has become more complex now because of the perpetual incidence of different types of microsporidian infection in silkworms [12]. Recently, the incidence of Microsporidiosis occurrence in Kashmir Division has been reported to be 18.98%, 13.46%, 9.00% in South, North & Central zones of Kashmir, respectively [3-6].
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, and cost-effective method of managing this dreadful disease. Though some anti- protozoan chemicals have been tried earlier against the pebrine disease but the significant or total control over the disease has not been reported [1, 11], thus putting the silk industry of the state under the threat. This led to the conception of

idea of studying effect of different chemicals on the commercial cocoon characteristics of silkworm for the management of the Pebrine disease.

MATERIAL AND METHODS

Experimental Material

Silkworm seed of race SKUA-R 6 laid by the moths infected with pebrine disease (*Nosema*) was taken for the study programme. The chemicals used for the study programme were Sodium bicarbonate (0.6%, 0.7% and 0.8%), Ornidazole (0.75%, 1.0% and 1.25%) and Rifampicin (2.75%, 3.0% and 3.25%) with three Controls (Control I- untreated but inoculated, Control II-Distilled water treated but inoculated and Control III- Untreated healthy).

Method of study

Moths of the pebrinised seeds were thoroughly examined under microscope individually for the presence of pebrine infection. The seeds of those moths which exhibited 100 percent spore load were involved in the study. The diseased seeds were kept under hibernation as per the standard procedure [6, 8] 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 pebrinised silkworms was carried out by following the standard methods of rearing carried out by Krishnaswamy *et al.*, [7]. After hatching and brushing, the samples of first age and second age larvae were again examined microscopically to further confirm and ensure that the first age silkworms and second age silkworms under rearing were infected with pebrine infection. The rearing and management study was conducted *in vivo*. The pebrinised 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 ornidazole and rifampicin were in tablet form whereas sodium bicarbonate was in powdered form. The tablets of ornidazole and rifampicin were crushed to make the powder of these chemicals. The concentration of three different chemicals under study were 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 concentration of sodium bicarbonatewas prepared by dissolving 6 grams, 7 grams and 8 grams of sodium bicarbonate separately in 1 litre of distilled water. Similarly, concentration of ornidazole was prepared by dissolving ornidazole tablets 9.5, 11.5 and 13.5 separately in 1 litre of distilled water and finally the three concentrations of rifampicin were obtained by dissolving 5, 6 and 7 tablets separately in 1 litre of distilled water.

Application of chemicals

The different concentrations of the chemicals under study were sprayed on the mulberry leaves which was fed to silkworms of all the treatments once during the 3rd age. During the 4th age the treated leaves were given twice to silkworms under study while as during the 5th age the treatment was given on the alternate days 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 effect of chemicals under study on the commercial cocoon characters of the treated SKUAR-6 germplasm Bank of the College of Temperate Sericulture was used for comparison.

RESULTS AND DISCUSSION

The data pertaining to5thinstar larval duration (days), Average weight of ten matured larvae (g), Total larval duration (days/hours), Average single cocoon weight (g), Average single shell weight (g), Shell ratio percentage, Average cocoon filament length (m) was recorded and subjected to statistical analysis. The results obtained are summarized in Table -1.

5thinstar larval duration (h)

The results revealed non-significant impact of all three treatments on the 5thinstar larval duration. Among the various concentrations used under study the maximum 5thinstar larval duration (167 h) was recorded at 0.75, 2.75 and 3.0 per cent concentration of Ornidazole and Rifampicin, respectively. The minimum 5thinstat larval duration (164 h) at 0.8 per cent concentration was recorded by Sodium bicarbonate. However, statistically no significant difference was recorded among the treatments under study and the control batch (untreated healthy).

Table 1: Effect of three chemicals on different commercial cocoon parameters of silkworm

Chemicals	Conc. (%)	5th instar larval duration (days)	Average weight of ten matured larvae (g)	Total larval duration (hours)	Average single cocoon weight (g)	Average single shell weight (cg)	Shell ratio percentage	Average cocoon filament length (m)
Sodium bicarbonate	0.6	165	50.16	611	2.32	0.38	18.10	18.10
	0.7	165	50.20	609	2.33	0.39	18.45	18.45
	0.8	164	51.46	604	2.39	0.43	19.66	19.66
Ornidazole	0.75	167	49.33	614	2.28	0.35	17.98	17.98
	1.0	166	50.27	611	2.33	0.36	18.03	18.03
	1.25	166	51.0	609	2.37	0.39	19.41	19.41
Rifampicin	2.75	167	49.33	612	2.34	0.35	17.69	17.69
	3.0	167	50.13	610	2.35	0.35	18.37	18.37
	3.25	165	50.93	605	2.36	0.38	19.14	19.14
Control Treatments: C.D (p≤0.05)	Untreated healthy	165	50.57	604	2.34	0.42	18.70	1194
	Treatment	NS	NS	NS	0.02	0.02	NS	NS
	T x C	NS	NS	NS	0.03	0.03	NS	32.25
	Control vs rest	NS	2.01	NS	1.56	0.09	1.55	170.41

Average Weight of ten matured larvae (g)

The highest average weight of larvae 51.46 g among the treatments under study was recorded by sodium bicarbonate followed by Ornidazole(51.0g) and Rifampicin (50.93g). The treatments Ornidazole and Rifampicin were statistically at par but Sodium bicarbonate surpassed both the chemicals in efficacy.

Total larval duration (hours)

Among the three treatments under study, Sodium bicarbonate recorded total larval duration of (608 h) followed by Rifampicin (609 h) and Ornidazole (611 h). The maximum total larval duration (611 h) among the concentrations under study was recorded by Sodium bicarbonate at 0.6 per cent concentration and minimum (604 h) at 0.8 per cent concentration. In the treatment Ornidazole the highest total larval duration 614 h was observed at 0.75 per cent concentration and lowest (609 h) at 1.25 per cent concentration. Whereas, Rifampicin recorded the highest value of 612 h of total larval duration at 2.75 per cent concentration and minimum of 605 h at 3.25 per cent concentration.

Average single cocoon weight (g)

Among the different concentrations under study, the highest single cocoon weight (2.39 g) was observed in the treatment Sodium bicarbonate at 0.8 per cent concentration and the lowest (2.28 g) was recorded at 0.75 concentration of Ornidazole. Sodium bicarbonate at 0.8 per cent concentration recorded the highest (2.39 g) single cocoon weight and lowest single cocoon weight (2.32 g) at 0.6 per cent concentration. The treatment Ornidazole revealed the highest single cocoon weight (2.37 g) at 1.25 per cent concentration and lowest (2.28 g) at 0.75 per cent concentration. Rifampicin recorded the maximum single cocoon weight (2.36 g) at 3.25 per cent concentration and the minimum (2.34 g) at 2.75 per concentration.

Average single shell weight (cg)

Among the different concentrations under study, the highest single shell weight (0.43 cg) was observed at 0.8 per cent concentration of Sodium bicarbonate and the lowest (0.35 cg) was recorded at 0.75, 2.75 and 3.0 per cent concentration of the treatments Ornidazole and Rifampicin, respectively. Ornidazole revealed the highest single shell weight (0.39 cg) at 1.25 per cent concentration and lowest (0.35cg) at 0.75 per cent concentration. However, the treatment Rifampicin recorded maximum single cocoon weight of 0.38 cg at 3.25 per cent concentration and minimum (0.35 cg) at 2.75 and 3.0 per cent concentrations. All the three treatments were found at par with control batch (untreated healthy). The overall results depicted the effectiveness of chemicals increases with the increase in the concentration.

Shell ratio percentage

Among the different treatments, the highest shell percentage (19.66%) was recorded in the treatment sodium bicarbonate at 0.8 per cent concentration and lowest (17.69%) at 2.75 per cent concentration in the treatment Rifampicin. Among the different concentrations under study, Sodium bicarbonate recorded the highest shell percentage (19.66%) at 0.8 per cent concentration and lowest (18.10%) at 0.6 per cent concentration. However, the treatment Ornidazole revealed the highest shell percentage (19.41%) at 1.25 per cent concentration and lowest (17.98%) at 0.75 per cent concentration. The treatment Rifampicin has

shown the maximum shell percentage (19.14%) at 3.25 per cent concentration and the lowest (17.69%) at 2.75 per concentration.

Average cocoon filament length (m)

Among the different concentrations, the highest silk filament length (1205 m) was shown at 0.8 per cent concentration of Sodium bicarbonate and the lowest (1127 m) at 0.75 per cent concentration by Ornidazole. In the treatment Sodium bicarbonate the highest silk filament length (1205 m) was observed at 0.8 per cent concentration and lowest (1179) at 0.6 per cent concentration. However, in Ornidazole the maximum silk filament length (1197 m) was recorded at 3.25 per cent concentration and lowest (1144 m) at 3.0 per cent concentration. The treatment Rifampicin recorded the highest (1198 m) silk filament length at 3.25 per cent concentration and lowest (1156 m) at 2.75 per cent concentration. The overall results depicted that the effectiveness of chemicals enhance with the increase in the concentration.

The Prolonged domestication and continuous rearing has exposed this silworm (*Bomyxmori.* L) to various pathogenic micro-organisms. . Phylogenetic analysis suggests classification into four genera: *Nosema, Vairimorpha, Cystosporogenes*, and *Endoreticulatus*. Some strains classified into genera *Nosema* and *Vairimorpha e*xhibits high genetic homology with *N. bombycis* and *Vairimorpha sp.* NIS-M11, which cause pébrine disease (Yuji Imura *et al*., 2020). Thus, conventional pébrine inspection facilitates the elimination of microsporidia, thereby preventing the spread of this disease The most dangerous out of these is a parasite attacking the silkworm which belongs to a group of primitive obligate eukaryotic intracellular parasite i.e., Microsporidia and genus *Nosema*. This microsporidian is highly dangerous and can wipe out the silk industry if not addressed. Earlier, it has also been reported to attack the silkworms [2] race in Kashmir causing pebrine disease of different magnitudes. Several microsporidia other than *Nosema bombycis* like *Nosema apis, Varimorpha* sp*., Pleistophora* sp*., Thelohania* sp. etc. are also reported to cause pebrine disease in different insects [4]. Microsporidian sp. can cause pebrine, a dreadful disease and lead to destructive disorder in Muga silkworm, *Antheraea assamensis Helfer b*y vertical and horizontal transmission [5]. The dreadful disease 'Pebrine' has been responsible for ruining the silk industry in France and Itlay [6]. Records reveal that the disastrous effect of the pebrine disease was even felt in Kashmir as the productive indigenous univoltine breed "Kashmir Race" was lost due to outbreak of pebrine disease some 131 years back [8].

The successful rearing of silkworms is associated with the larval and the cocoon characteristics such as larval duration, cocoon weight, shell weight, shell ratio and filament length etc. These parameters are directly influenced with the silkworm seed production, silk production as well as silk textiles. In other words, rearers, reelers, weavers and silk garment manufacturers are concerned with the performance of these parameters. Thus, it was very important to study the effect of these chemicals under investigation on the following commercial cocoon parameters of silkworms. In the present study, out of three chemicals used Sodium bicarbonate recorded the 5^{th}instar larval duration (165 h) and total larval duration (608 h) followed by Rifampicin (166 h) 5^{th}instar larval duration and (609 h) total larval duration and longest total larval duration (611 h) was recorded in the treatment Ornidazole. However, effect of all the treatments irrespective of their concentration were statistically at par. This clearly gives an indication that larval duration was not adversely affected by the use of the chemicals under study. In the present study maximum larval weight (50.61 g) was recorded in the treatment Sodium bicarbonate followed by Ornidazole (50.47 g) m and Rifampicin (50.13 g). These findings are in conformity with those of Liu-Shi Xian [9], Chandra *et al.* [3] and Zargar *et al.,* [15] who have found that Topsin-M and Bavistin at 1.0 per cent concentration recorded 35.408 g and 35.208 g weight of ten matured larvae. However, the preset findings surpass the results of these authors and Sodium bicarbonate at 0.8 per cent exhibited the weight of ten matured larvae as high as 50.61 g reflecting the good efficacy of the chemical. The highest cocoon weight (2.39 g) was recorded at 0.8 per cent concentration of sodium bicarbonate followed by (2.37 g) at 1.25 per cent concentration of Ornidazole. Rifampicin gave the cocoon weight of 2.36 g which was at par with the control. The results are supported by the findings of Zargar *et al.,* [15] who has found 1.538 g of single cocoon weight with the use of chemical Metrogyl at 0.2 per cent concentration. Weight of cocoon shell has high significance, as it is the shell that yields the silk after reeling. Hence, higher the weight of the shell, higher the silk yield from it [10]. The maximum shell weight (0.43 cg) was observed in the treatments Sodium bicarbonate at 0.8 per cent concentration followed by Ornidazole (0.39 cg) at 1.25 per cent concentration. In the present study, the maximum average mean shell percentage (18.74%) was recorded in the treatment Sodium bicarbonate followed by Ornidazole (18.47%) and rifampicin (18.40%). Silk filament is the end result of rearing and reeling. The longer the filament length, the better it is for weavers and textile producers. Thus it was important to observe the effect of the chemicals under study on the cocoon filament length. The present findings are in conformity with the study of Zargar *et al.,* [15] who found 744.56 m filament length with the use of chemical Codrinal at 0.4 per cent concentration.

However, the filament length under the influence of Sodium bicarbonate under the present study has been higher which confirms that the chemicals used in the present study have positive effect on the traits under study.

CONCLUSION

The present study has revealed that all the three chemicals have significant anti-protozoan activity, However, Sodium bicarbonate at 0.8 per cent concentration has been found most effective in the control of pebrine disease of silkworms without any deleterious effect on the performance of commercial cocoon traits like larval duration, weight of ten matured larvae, single cocoon weight, single shell weight, shell percentage and average cocoon filament length. . Thus, the management of pebrine disease with the Sodium bicarbonate seems to be cost effective with easy availability and farmer friendly. The other two chemicals namely Ornidazole and Rifampicin have also been found effective against pebrine disease of silkworms, but Sodium bicarbonate (0.8% concentration) surpassed these two in efficacy. Studies also reveal that with the increase in concentration of the chemicals the overall effectiveness on results has also increased. From the studies, it is clear that Sodium bicarbonate (0.8 per cent) which is highly cost effective and readily available could be recommended for controlling the dreadful pebrine disease in bivoltine silkworm (*Bombyxmori*L.) under temperate climatic conditions and farmers can also harvest a good cocoon crop with good returns, as it does not have any adverse impact on commercial cocoon characteristics.

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