Research Article

Studies on the Response of Different Silkworm (*Bombyx mori* L.*)* Breeds to Chloroform Exposure at Different Time Durations

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Abstract

Digestive juice samples serve as strong source of enzyme for measuring the amylase activity of different breeds of silkworm, *Bombyx mori* L. However, the methodologies for obtaining the digestive juice are still a point of debate among the researchers. It was observed that when the worms were subjected to exposure of chloroform vapours for 20-30 seconds (sub effective timing), it did not result in the vomiting of the gut juice, though some tendency to vomit (nausea) was noticed after 23 seconds in some breeds. SKUAST-28 recorded the sub effective timing of 26 seconds, while the shortest sub effective timing was recorded in case of NB_4D_2 (23 seconds). From 30-40 second (effective timing), all the races were found to vomit sufficient quantity of gut juice without any deleterious effect on the silkworm larvae. Exposure timing above 40 seconds was found lethal to the silkworms as it resulted in their death. When the larvae of SKUAST-28 were exposed to chloroform vapours for 45 seconds, they died

Keywords: *Amylase, Breed, Bombyx mori, Chloroform and Gut juice*

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Introduction

Sericulture is a farm-based, labour intensive and commercially attractive economic activity falling under the cottage and small-scale sector. It particularly suits rural based farmers, entrepreneurs and artisans as it requires low investment but with a potential for relatively higher returns. An analysis of the trends in the international silk production suggests that sericulture has better prospects for growth in the developing countries rather than in the advanced countries. India is the second largest producer of raw silk after China with 14.57 per cent share in global raw silk production and the biggest consumer of raw silk and silk fabrics in the world. Silkworm, *Bombyx mori* L. is an important Lepidopteran insect and is utilized for the commercial production of natural silk fibre. Silkworm domesticated over centuries has become very delicate and susceptible to various diseases caused by microsporodia, bacteria, viruses and fungi (Doreswamy *et al*., 2004) and in spite of strict adherence to rearing techniques, it is difficult to obtain desirable cocoon yield if diseases affect the silkworm. Diseases in silkworm are the major constraints in achieving high silk productivity. Although variety of reasons including poor quality of leaf coupled with its insufficient availability are responsible for this dismal scenario, nevertheless the chief contributing factor has been identified as the voluminous silkworm mortality suffered by the farmers during rearing period which in turn is the outcome of poor digestibility of larvae triggered by the impeded activity of digestive enzyme called “amylase”. To work on this aspect collection of digestive juice (as a source of enzyme) is a prerequisite. The present study was, therefore, carried out to standardise timing and method for collection of digestive juice from these delicate insects without causing any harm to them.

Materials and Methods

In the present study an attempt was made to standardize and validate a method for getting the digestive juice from the silkworm, *Bombyx mori* L. Towards this endeavour six breeds of the mulberry silkworm, *Bombyx mori* L. viz., Pure Mysore, Nistari, SKAU-R-6, SKUAST-28, NB_4D_2 and SH_6 were selected for the study. Disease free layings of these races were incubated under laboratory conditions at 25^{o}C and relative humidity of 75 per cent and then allowed to hatch. Rearing of all the silkworm breeds under study (Plate 1) was carried out as per the standard package of practices (Raja, 2000). The experiment was laid out in a completely randomized block design with four replications for each treatment. Each replication comprised of 200 silkworms of uniform age and size retained after third moult.

Figure 1 Rearing of silkworm (*Bombyx mori* L)

From each replication and treatment, ten larvae were randomly selected and starved for four hours. Then the worms were subjected to brief exposure to chloroform vapours resulting in the vomiting of gut juice. The silkworms were exposed to chloroform vapours for different time durations in order to induce vomiting of gut juice. The required amount of the vomited gut juice was collected in pre-cooled eppendorf tubes. Digestive juice samples were centrifuged @ 10,000r/10min (Abraham *et al.*, 1992). The supernatant was transferred to new tubes and kept at -20^{o}C for analysis (Plate 2).

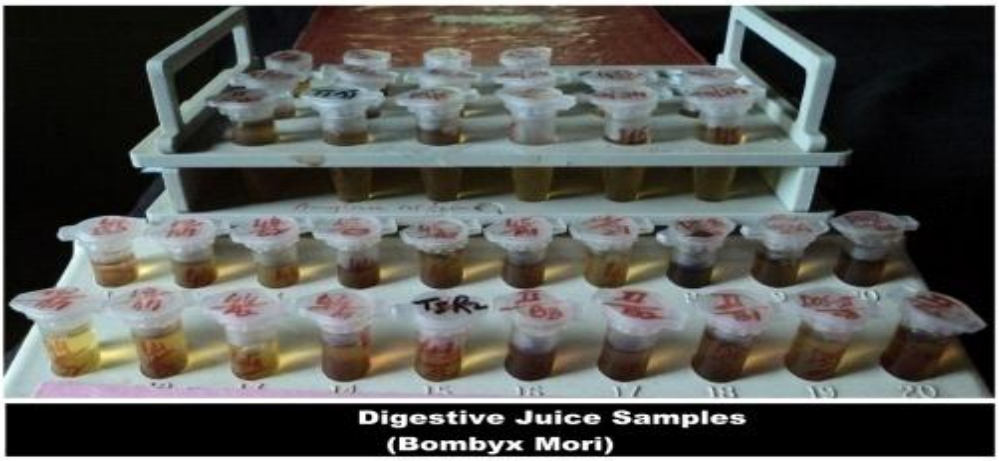

Figure 2 Digestive juice samples of silkworm (*Bombyx mori* L.)

Experimental Findings

The silkworms were exposed to chloroform vapours for different time durations in order to induce vomiting of gut juice. The required amount of the gut juice was used as a source of enzyme for measuring the amylase activity of different breeds of silkworm, *Bombyx mori* L. In the present study, it was observed that when the worms were subjected to exposure of chloroform vapours for 20-30 seconds (sub effective timing), it did not result in the vomiting of the gut juice, though some tendency to vomit (nausea) was noticed after 23 seconds in some breeds. SKUAST-28 recorded the sub effective timing of 26 seconds, while the shortest sub effective timing was recorded in case of NB_4D_2 (23 seconds). The sub effective timing recorded in other breeds include: Pure Mysore (24 sec), Nistari (26 sec), SH_6 (25 sec) and SKAU-R-6 (24 sec). From 30-40 second (effective timing), all the races were found to vomit sufficient quantity of gut juice without any deleterious effect on the silkworm larvae. Effective timing was recorded maximum in case of Nistari (34 seconds), followed by Pure Mysore and SKAU-R-6 (32 seconds), NB_4D_2, SH_6 and SKUAST-28 (31seconds). Exposure timing above 40 seconds was found lethal to the silkworms as it resulted in their death. When the larvae of SKUAST-28 were exposed to chloroform vapours for 45 seconds, they died. Lethal timings recorded in other breeds include: Pure Mysore (45 seconds), Nistari (46 seconds), NB_4D_2 (43 seconds), SH_6 (41 seconds) and SKAU-R-6 (44 seconds) (Table-1).

Table 1 Response of different silkworm breeds to chloroform exposure timing

Race	Sub effective timing (20-30 sec)	Effective timing (30-40 sec)	Lethal timing (>40 sec)
Pure Mysore	24	32	45
Nistari	26	34	46
NB_4D_2	23	31	43
SH_6	25	31	41
SKAU-R-6	24	32	44
SKUAST-28	26	31	45
$C.D_{(p\leq 0.05)}$	1.222	1.210	1.202

Discussion

Different breeds of the silkworm, *Bombyx mori* L. under study were exposed to chloroform fumes for different time durations so as to work out the effective timing at which we can get the required amount of enzyme source (digestive juice) and simultaneously there is no larval mortality at all. Different timings were evaluated and exposure timings were categorised on the basis of their effect on induction of vomiting as sub effective timing (20-30 seconds), effective timing (30-40 seconds) and lethal timing (>40 seconds) (Table-1). It was observed that initially when the larvae were exposed to chloroform fumes for 20-30 seconds, they did not vomit at all, though some sort of irritation was observed, which could be attributed to the fact that at this timing, chloroform started acting upon vomiting centre present in the insect brain, resulting in nausea (Omara and Sisodia, 1990). When the exposure to chloroform was continued (30-40 seconds), it resulted in the vomiting of the gut juice due to complete activation of the vomiting centre (Vennart and Mckee, 1955). When the exposure timing was continued (>40 seconds), it resulted in the death of the worms as the chloroform induced CNS depression and dehydration (Vennart and Mckee, 1955). The variations in the exposure timings observed among the breeds could be due to breed potential for tolerance to chloroform fumes. Literature supporting this study in respect of *Bombyx mori* could not be traced which indicates no such study has been carried out so far. However similar type of findings has been reported by Yang *et al.* (2010) and ME-Beauchamp *et al.* (2002) while evaluating the chloroform exposures estimation, hazard characterization and exposure response analysis in other animals like mice, dogs and pigs.

Conclusion

The present study provides important information that when the worms were subjected to exposure of chloroform vapours for 20-30 seconds (sub effective timing), it did not result in the vomiting of the gut juice, though some tendency to vomit (nausea) was noticed after 23 seconds in some breeds. SKUAST-28 recorded the sub effective timing of 26 seconds, while the shortest sub effective timing was recorded in case of NB_4D_2 (23 seconds). From 30-40 second (effective timing), all the races were found to vomit sufficient quantity of gut juice without any deleterious effect on the silkworm larvae. Exposure timing above 40 seconds was found lethal to the silkworms as it resulted in their death. When the larvae of SKUAST-28 were exposed to chloroform vapours for 45 seconds, they died.

Conflict of interest

There is no conflict of interest among the authors.

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