

Assessment of bioactivity in *Bombyx mori* (L.) fed on mulberry leaves fortified with giloy and ashwagandha extracts

Kumari Swarnlata¹, Lekh Raj¹, SM Mahboob Hassan²

¹ Department of Zoology, Patna University, Patna, Bihar, India

² Department of Zoology, Patna Science College, Patna University, Patna, Bihar, India

Abstract

Silkworm is a lepidopteran insect which produces the very important protein called silk, the “Queen of Textile” through the process called sericulture. *B. mori* (L.) completes its life cycle through passing different stages of development following 1st, 2nd, 3rd, 4th & 5th instar, 5th instar is the final instar stage and it is the mature stage of larval period. Yellow coloured ripped 5th instar forms a cocoon from which adult *B. mori* comes out. Soon after adults start mating and after 24hrs of mating females start laying eggs which further hatched into 1st instar larvae and cycle goes on. In this study fortified mulberry leaves with Giloy and Ashwagandha extract were taken to observe the different biological activities of *B. mori*. Here Giloy and ashwagandha extracts were fed to *B. mori* which significantly enhanced various aspects of silkworm development & productivity. Silkworm, *B. mori* were reared and exhibited faster growth with notable shorter larval & pupal duration in comparison to control mulberry leaves. In addition, *B. mori* showed high moth emergence rate, increased fecundity & hatching % at 5% Giloy extract treatment in comparison to control and other extract treatments.

Keywords: Lepidopteran, *B. Mori* (L.), giloy, ashwagandha, moth emergence, hatching %

Introduction

Sericulture is “agro- based” rural industry which combines the features of agriculture & village. It is the process in which rearing of silkworm is done to produce silk by including the operations which are required to produce silk fiber (Krishnaswami *et al.*, 1973,1978). Silk is a lustrous, shiny, smooth protein fiber, also called the “Queen of Textile”. Silk is not only marked with textile industry but also plays very important role in medicine, biotechnology, food supplements, biomaterials, surgical sutures, artificial blood vessels, tire lining, parachutes, electrical insulating material, oil, protein & artificial vitamins & even its waste materials can be used as artificial diet for animals & as green manure for crop production (Ishfaq & Akram,1999).

India is an agricultural country after village industries like handloom & khadi. Sericulture plays a very important role as it provides full or partial employment to about 6.5 million people in India. This sector also encourages women to get employed. It plays a very important role in enhancing the economy of India & also provides employment to the farmers & attracts profile seeking entrepreneurs as this farming requires low investment with relatively higher returns and also provides production of higher quality textiles.

After its homeland China, India ranks 2nd largest producer of silk (Vijay Prakash & Pandit, 2005). In 2016-2017 it was recorded that raw silk produced in India was 30.348 MT out of which 21.273 MT was contributed by *B. mori* (L.) Which makes it the 2nd largest producer in the world after China. The Indian Sericulture market size reached INR 451.6 billion in 2022. Further, IMARC group expects the market to reach INR 1,194.5 billion by 2028 exhibiting the growth rate (CAGR) of 17.7% during 2023-2028. The Indian Sericulture market size reached INR 612.7 billion in 2024.

IMARC group expects that the market will reach INR 2,217.5 billion by 2033, exhibiting a growth rate (CAGR) of 14.6% during 2025-2033. India is not only 2nd largest producer of silk but also largest consumer of raw silk & silk produced fabrics and products. Member secretary of Central Silk Board, P. Sivakumar said that India's Sericulture from fabric-centric is now moving towards other sectors including developing pharmaceutical applications. He also said that production of raw silk grew from 1,242 MT at CSBs establishment in 1948 (6% of global share) to 39,000 MT, now giving India 42% of world production.

The lepidopteran insect, *B. mori* is the common mulberry silkworm which is domesticated easily. *B. mori* undergoes complete metamorphosis, which means its life cycle passes through four stages including egg, larva, pupa & adult. The sole food of *B. mori* is mulberry leaves. They feed only on mulberry leaves to make a cocoon as its protective layer. In 1976, Anonymous reported that Beta Sitosterol along with some sterols and water-soluble substance plays an important role as the major factor which is responsible for the feeding stimulation & boosts the biting power of *B. mori* (L.).

Family Moraceae, mulberry belongs to genus *Morus*, are of 35 species which is broadly classified into two types: (a) *Dolichostylae*, which have long style & (b) *Macromorus*, which have long style. As data reported by Gopal, 1994 [3] states that there are more than 1000 varieties of mulberry which are being cultivated and classified into three types: (1) *M. bombycina* (K.) (2) *M. alba* (L.) (3) *M. latifolia* (L.). In 1992 Kobayashi reported that through the breeding varietal improvement of mulberry through breeding not only gives superior leaf yield for feeding mulberry silkworm but also it helps in better raw silk formation & its adaptability to the climate & resistance against the disease.

Mahmood *et al.*, 1987^[15] stated that silkworms of different species may have compositional differences which further might lead to variations on *B. mori* (*L.*) growth and silk production. Nutrient containing mulberry leaves plays a very important role in the growth rate of *B. mori* larvae & subsequently also helps in silk production. Younger larvae of silkworm require more moisture for proper digestion but mature larvae of silkworm can easily feed on less moisture mulberry leaves (Koyuncu F. & Sabhat A *et al.*, 2004, 2016). The quality of mulberry leaves alongside other factors like climate & rearing techniques plays an important role in successful cocoon production and high quality of silk production (Matsumura *et al.*, 1958)^[18]. For increasing the nutritional value & healthy production of mulberry silkworm, mulberry leaves are fortified with carbohydrates (Narayanan & Iyenger *et al.*, 1967)^[20], protein (Goudar & Kaliwal, 1999)^[4], amino acid (Subburathinam & Krishna, 1992)^[24], cyanobacteria (Sujatha & Krishna, 2013)^[13] & combination of extracts of medicinal or botanical plants (Sujatha & Rao, 2003; Takhilque, 2012; Kuntamalla & Purushotham, 2004)^[13, 19, 14]. In 2007 Kanafi *et al.*, reported that the secret of growth & development of *B. mori* lies in wealthy nutrition. In 2007 onwards Rajabi *et al.*,^[21] also reported that mulberry leaves fortification has a positive effect on nutritional supplementation on the commercial traits of silkworm. It has been also reported that many herbals, medicinal botanicals like Tulsi, also vera, turmeric, ashwagandha etc can improve the silk yield in mulberry silkworm.

Giloy (*Tinospora cordifolia*) belongs from the family "Menispermaceae" has many common names like Guduchi, Heart- leaf Moonseed, Amrita etc is indigenous to the tropical region of Indian subcontinent. In ayurveda, it is a very famous medicinal herb & is used for the treatment of various disorders. It contains many phytochemicals like alkaloids, saponins, phytosterols, tannins, amino acids, phenols, glycosides, proteins, flavonoids, terpenoids, carbohydrates etc. Giloy is also called the "Queen of Herbs" as it is an immune booster herb. Ashwagandha (*Withania somnifera*) is also one of the most important & magical herbs in ayurveda. commonly known as Winter Cherry & it belongs from the family "Solanaceae". Found in India, the Middle east & some parts of Africa. Particularly the root part of this plant is used as herbal medicines and as dietary supplements. Ashwagandha contains phenolics, alkaloids, tannin, saponin, glycosides, starch etc. Both Giloy and Ashwagandha contain high medicinal value & phytochemicals therefore in this study mulberry leaves are fortified with Giloy & Ashwagandha extracts to observe the biological development of *B. mori*.

Materials & Methods

Study Location

The study of research was conducted from November 2023 to February 2024 at the Department of Zoology, Patna Science College, Patna University, Patna (Bihar). This study was done to observe the effect of mulberry leaves fortified with Giloy and Ashwagandha on the biology of *B. mori* (*L.*).

Experiment Design

The experimental design was set with one control and six

treatments i.e. 3 sets of mulberry leaves fortified with Giloy extract treatment and 3 sets of mulberry leaves fortified with Ashwagandha extract at different concentrations (Table 01). Experimental block design of 60 dlf larvae was set up in each replication. The rearing was done with some modification according to environmental conditions by following the rearing method of Krishnaswami S. (1973, 1978).

Table 1: Experimental design of control and treated *B. mori* with different concentrations of Giloy and Ashwagandha extract on mulberry leaves.

01	Control	Mulberry leaves	<i>B. mori</i> larvae
02	1% Giloy extract	Mulberry leaves	<i>B. mori</i> larvae
03	3% Giloy extract	Mulberry leaves	<i>B. mori</i> larvae
04	5% Giloy extract	Mulberry leaves	<i>B. mori</i> larvae
05	1% Ashwagandha extract	Mulberry leaves	<i>B. mori</i> larvae
06	3% Ashwagandha extract	Mulberry leaves	<i>B. mori</i> larvae
07	5% Ashwagandha extract	Mulberry leaves	<i>B. mori</i> larvae

Treatment Details

Mulberry leaves were fortified with different concentrations of Giloy and Ashwagandha extracts and a treatment chart was prepared. T0 here stands for control group, T1 for 1% Giloy extract treatment, T2 for 3% Giloy extract treatment, T4 for 5% Giloy extract treatment, T5 for 1% Ashwagandha extract treatment, T6 for 3% Ashwagandha extract treatment, T7 for 5% Ashwagandha extract treatment (Table 02).

Table 2: Treatment Details of mulberry leaves fortified with Giloy and Ashwagandha on different concentrations of prepared extracts.

T0	Control
T1	1% Giloy extract
T2	3% Giloy extract
T3	5% Giloy extract
T4	1% Ashwagandha extract
T5	3% Ashwagandha extract
T6	5% Ashwagandha extract

Equipment for rearing of *Bombyx mori* (*L.*)

Plastic trays for housing the *B. mori* larvae were used. For supporting trays iron frames were used. For chopping of mulberry leaves soft wooden boards were used. For chopping the mulberry leaves an iron knife was used. For spacing & handling larvae of *B. mori* (*L.*) long thin sticks of bamboo are used. For brushing and Cleaning of 1st instar larvae, a clean soft feather was used. For bed cleaning of *B. mori* larvae cotton and nylon nets were used as shown in (fig. 04). Trays made from hay are used when the time of spinning of the cocoon starts (fig. 05). To maintain humidity and cover beds. In rearing beds pads are used to maintain optimum humidity. (fig. 02).

Collection & Rearing of *B. Mori* (*L.*) on the prepared Giloy & Ashwagandha extract

The disease-free laying of *B. mori* (*L.*) was collected from the Central Silk Board, NSSO, Purnia (Bihar) (fig. 01). Fresh Giloy leaves were collected from the botanical garden of the Department of Zoology, Patna Science College, Patna

University, Patna (Bihar). Ashwagandha roots were collected from the botanical garden of Sanjay Gandhi Park, Patna (Bihar). They were washed thoroughly with distilled water to remove the surface contaminants & were dried at room temperature for 2 to 3 days. Dried part of both the botanicals i.e. leaves of Giloy & root part of Ashwagandha were mixed separately. This prepared powder of both the botanicals were separately shocked in 100 ml of distilled water overnight and then filtered through the Whatman's filter paper & solvent was evaporated in a rotary vacuum evaporator. Separately each prepared extract was dried and the dried powder was dissolved in distilled water and diluted to form different concentrations i.e. 1%, 3% & 5% of Giloy and Ashwagandha extract for further experiment. The study was conducted at the Department of Zoology, Patna Science College, Patna University, Patna (Bihar). The rearing of *B. mori* was conducted as per the rearing method suggested by Krishnaswami S. (1973, 1978) with some modifications. Eggs were kept for hatching in the B.O.D incubator at maintained temperature and humidity i.e. 20° to 23°C & 60% to 65% respectively. For hatching of eggs, it is most important to maintain the temperature and humidity because

if there will be no maintenance then these eggs will not hatch and get into diapause. Upon hatching, silkworms were placed into rearing trays. Soft feathers were used to newly hatched larvae for picking and cleaning. Daily four time feeding of chopped mulberry leaves were provided to these newly hatched larvae i.e. 7.30am, 11.30pm, 3.30pm & at 08.00pm (fig. 02). This feeding was adjusted with larval growth & change of its instar. Uniformly prepared rearing bed was disinfected with formalin (2%), bleaching powder (0.03%), lime powder & vijetha powder (4kg/100 dfl) were used. During the time of moulting larvae were kept in undisturbed condition & were not fed at this time. After moulting beds were cleaned and vijetha was applied to prevent disease to *B. mori* (*L.*) (fig. 02). After each moult fresh feed was provided and quantity of food was adjusted according to the larval growth. Fully grown larval i.e. 5th instar larvae of *B. mori* which mature stage of *B. mori* larvae were transformed to mountages for spinning the cocoon. (fig. 03). Adults of *B. mori* emerged from the cocoon shells (fig. 04). Both male and female *B. mori* adults start mating just after the emergence (fig. 05). After mating females laid eggs.



Fig. 01.

Fig. 02.

Fig. 03

Fig. 04.

Fig. 05.

Fig. 06

Fig 1: Disease free layings of *B. mori*, out of which 1st instar is hatched out;

Fig 2: After bed cleaning nets were used and 4th instar larvae were fed with freshly chopped mulberry leaves;

Fig 3: Mature 5th instar larvae were placed on hay tray for spinning cocoons;

Fig 4: Adult *B. mori* (*L.*) comes out of cocoons shells;

Fig 5: Mating between male and female *B. mori*.;

Fig 6: After mating females laid eggs.

Data collection & Observation

The total larval period was recorded from the data of hatching of disease-free laying of *B. mori* (fig. 01) to the onset of spinning of 5th instar larvae (fig. 03) The total pupal period was noted from the spinning of 5th instar larvae (fig. 03 - 04) to the emergence of moth. After mating of male & female *B. mori* (fig. 05), female starts egg laying (fig. 06) & fecundity was recorded.

- Moth emergence percentage was calculated as the number of moth emerged / total number of cocoons $\times 100$

$$\text{Moth Emergence \%} = \frac{\text{No. of moth emerged}}{\text{Total no. of cocoons}} \times 100$$

- Fecundity was calculated by counting the no. of eggs laid by each female moth after mating.
- Hatching percentage was observed by counting empty egg shells immediately after bursting, here it is very important to notice late born larvae, unhatched and unfertilized eggs. For rearing and cocoon harvesting, hatching % is a very important characteristic. Hatching % is calculated as number of egg hatched/ number of eggs kept for bursting $\times 100$

$$\text{Hatching \%} = \frac{\text{No. of egg hatched}}{\text{No. of eggs kept for brushing}} \times 100$$

Table 3: Effect of feeding *B. mori* (*L.*) with fortified mulberry leaves with Giloy and Ashwagandha extract.

Treatment	Treatment Details	Larval duration (days)	Pupal duration (days)	Moth Emergence (%)	Fecundity	Hatching %
T0	Control	25.37	11.54	97.66 (81.39) *	442.34	85.33 (67.47) *
T1	1% Giloy extract	24.79	11.01	94.34 (76.65))	478.34	89.33 (70.92)
T2	3% Giloy extract	24.31	10.94	94.01 (75.93)	533.01	92.34 (73.41)
T3	5% Giloy extract	23.81	10.68	92.01 (74.47)	566.65	94.66 (76.63)
T4	1% Ashwagandha extract	25.11	11.30	95.34 (77.62)	462.66	89.67 (71.27)
T5	3% Ashwagandha extract	24.84	11.16	93.33 (75.06)	471.68	90.68 (72.76)
T6	5% Ashwagandha extract	24.62	11.08	84.68 (66.99)	517.34	91.34 (72.89)
	SE±	0.353	0.091	1.130	11.210	1.140
	C.D. at 5%	1.087	0.281	3.520	34.538	3.553
	C.V. (%)	2.48	1.43	2.594	3.91	2.733

*Figures in parentheses are angular transformed values.

Results & Discussion

▪ Larval Duration

In this study the Larval duration of *B. mori* (*L.*) at control was 25.37 at 1%, 3% & 5% Giloy extract treatment it was 24.79, 24.31 & 23.81 but at 1%, 3% & 5% Ashwagandha extract it was 25.11, 24.84 & 24.62 (days) respectively. Mala *et al.*, (2017) ^[16] reported that silkworm PM×CSR2 reared on mulberry leaves fortified with aqueous extract of Aloe vera at 100% concentration had shown the reduced larval duration i.e. 7.76 days compared to other treatments and control. G.C. Manjunath *et al.*, (2020) ^[17] also reported that PM×CSR2 silkworm breed showed shorter larval duration 171.24 (hrs) in Aloevera treated silkworm batch compared to control 173.9 (hrs.).

▪ Pupal Duration

Short pupal duration attributes to their higher nutritional quality, which facilitates faster development & transition through the pupal stage, enhancing the efficiency of silk production & improving sericulture practices. In this study higher pupal duration was noticed at control i.e.11.54 & at 1%, 3% & 5% Giloy extract it was 11.01, 10.94 & 10.68 & at 1%, 3% & 5% Ashwagandha extract it was 11.30, 11.16 & 11.08 (days) respectively. At 5% Giloy extract pupal duration was recorded shorter in comparison to control & other concentrations of extracts treatment. Vallapu *et al.*, 2024 reported bivoltine double hybrid silkworm variety FC²×FC¹ when treated with tender mulberry leaves specially accelerates the growth of silkworm, reducing larval and pupal duration & also enhances the economical traits of silkworm.

▪ Moth Emergence

Moth Emergence is one of the important stages of *B. mori* life cycle, as it is a very critical stage because if the condition will be unfavourable then the pupal or cocoon stage does not transform & emergence of moth will be suspended. Many factors like nutrition of silkworm, temperature, humidity etc affects the moth emergence. Enriched with nutritional quality mulberry is a very important diet but the application of botanical extracts on silkworm also registered a significant result on emergence of moth. In this study lowest moth emergence was observed at control i.e. 97.66 % at 1%, 3% & 5% of Giloy extract it was noticed 94.34%, 94.01% & 92.01% & at 1%, 3% & 5% Ashwagandha extract it was 95.34%, 93.33% & 84.68% respectively. Similar result was by Sridevi *et al.*, 2004 ^[22] that the CSR2×CSR4 silkworm emergence was increased

when *Withania somnifera* & *Terminalia arjuna* administered to larvae of silkworm (98% moth emergence) followed by *Tinospora cordifolia* (94%), *Leptadenia reticulata* & *Tagetes erecta* (94%).

▪ Fecundity

Number of eggs laid by each female moth after mating is called fecundity. In this study fecundity observed at control was 422.34. At 1%, 3% & 5% Giloy extract fecundity was 478.34, 533.01 & 566.65 respectively and at 1%, 3% & 5% Ashwagandha extract fecundity was 462.66, 471.68 & 517.34 respectively. Rajesh Kumar & Gangwar in 2010 reported that food plants of different species influenced the larval growth, larval duration, cocoon & pupal weight, shell weight & fecundity.

▪ Hatching Percentage

In this study the hatching percentage at control was 85.33%, and at 1%, 3% & 5% the Giloy and Ashwagandha extract was 89.33%, 92.34% and 94.66%; 89.67%, 90.68 & 91.34%. Singh *et al.*, 2014 ^[23] reported that by using different concentrations of Aloe vera oil namely 0.25, 0.50, 0.75 & 1.0 ml as single, double & triple treatment, the triple treatment of 0.75 shows higher fecundity and egg hatchability and triple treatment of 1.0ml dose showed lower fecundity and egg hatchability of eggs. They suggested that if Aloe vera oil is applied carefully it may promote the production of high-quality cocoons on a commercial scale.

Conclusion

Plants based products are easily available, cost effective and most important these are environment friendly too. Mulberry leaves are fortified nutritional enrichment, which has the potential to develop an effective & sustainable alternative to uplift the health of silkworm in broad spectrum as well as it is a safe & cost-effective method for sericulture farmers for quality cocoon production. In this study Giloy and Ashwagandha plants produced used in the form of extract & were feeded to silkworm which significantly enhanced various aspects of silkworm development & productivity. Silkworm reared on different concentrations of Giloy and Ashwagandha treated mulberry leaves exhibited faster growth with notable shorter larval & pupal duration in comparison to control or no treatment mulberry leaves. In addition, this silkworm showed high moth emergence rate, increased fecundity & improved hatching (%).

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