

## MUGA HEAL-*TERMINALIA CHEBULA* BASED BIOFORMULATION AS AN ANTI-FLACHERIE AGENT AND A SILK FIBER ENHANCER

BALAGOPALAN UNNI\*, PALLAV DOWARAH\*, SAWLANG BORSINGH WANN\*  
AND PARUCHURI GANGADHAR RAO\*

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*Muga silkworms, Antheraea assamensis are reared outdoor only and hence the worms are exposed to the adverse climatic conditions, pests and predators. In silkworms, the disease which affects large population of silkworms usually during the hot and humid summer climatic conditions, results in decrease in their silk producing capacity. Muga sericulture farmers face setback in production of good quality cocoons due to unfavorable hot humid climatic conditions and diseases which accounts for 20-70% mortality of silkworms during summer. Control measures are not feasible as muga silkworm rearing is practised outdoors and the use of chemical formulations for controlling the disease is detrimental to the silkworms' health and also not economical. Thus the use of bioformulation "Muga heal" prepared from Terminalia chebula fruits is the only alternative. Hence, the technology was standardized, selected and recommended to enhance the health of the larvae and production of quality silk fiber. The preparation of the bioformulation is simple and can easily be adopted by sericulture farmers. The bioformulation can be applied in all seasons in muga sericulture to boost the silk production. The technology is suitable for the healthy growth of larvae and improves cocooning of muga silkworm, Antheraea assamensis with uniform shape, size, improves quality of silk having more reeling filament length and unbreakable length*

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### Introduction

The climate of North East India is suitable for growth of the non-mulberry silkworm, *Antheraea assamensis* Helfer (muga silkworm) that produces the golden yellowish color silk which is found nowhere else in the world. Muga silkworm, *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae), the economic insect is unique, prerogative and Geographical Indicator to the North Eastern region of India.

The golden yellow yarn produced by this insect is lustrous, highly durable, strongest and toughest of all natural silks having multifarious utilities for which its demand is increasing in world fabric market. The silkworm is

polyphagous, multivoltine (5-6 crops per year), semi-domesticated in nature. The worms are reared outdoor on plants. On maturity, the worms crawl down at the end of completion of five larval instars, are collected by rearers, and allowed to spin cocoons inside rearing house. Muga silkworm feeds primarily on "Som" (*Persea bombycina* King ex Kost.) and "Soalu" (*Litsea monopetela* Pers.). The other food plants include "Diglotti" (*Litsea salicifolia* Roxb. ex. Wall.) and "Mejankori" (*Litsea citrata* Blume) are of secondary importance. *Antheraea assamensis* is indigenous to North Eastern region of India and is reared only under outdoor condition on standing host trees, unlike other silkworms which can be raised easily under indoor conditions.

Very recently, emphasis has been focused by sericulture research workers to increase the shell weight of this silkworm through different approaches as it is the

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\* Biotechnology Division, CSIR-North-East Institute of Science & Technology, Jorhat 785 006, Assam (Correspondence to BG Unni, e-mail: bgunni@rii.jorhat.res.in)

primary factor contributing the actual silk return from its cocoons. With the increase in feeding efficiency of leaves, silk yield also increases. The current information appeared in one of the national dailies shows that the production of Muga silk in 2007-2008 and 2008-2009 was about 105 metric tones while in 2009-2010 it was about 90 metric tones. Because of its outdoor rearing the muga silkworms, *A. assamensis* are exposed to the adverse climatic conditions, pests, predators and bacterial infections. In muga silkworms the disease which affects large population of silkworms usually during the hot and humid summer climatic conditions results in high mortality, which affects the silk production and even those surviving there is decrease in their silk producing capacity. The diseases associated with pathogenic bacteria come under the general term “flacherie” which refers to the flaccid condition exhibited by infected silkworms due to different ailments. A potent bacterial strain of *Pseudomonas aeruginosa* Strain AC-3 has been identified in the Biotechnology Laboratory of CSIR-North East Institute of Science & Technology, Jorhat, as the organism which causes the flacherie disease of muga silkworm<sup>1</sup>. About 20-30% loss of muga silkworms has been estimated<sup>2</sup> to be accountable for infection of this bacterial strain, which mainly occur during the later part i.e. 4<sup>th</sup>-5<sup>th</sup> larval instars. A protein from the bacteria exhibiting toxic affect on the health of the silkworm larvae was also purified and characterized. Recurrence of death due to various diseases caused by different agents including bacteria is an integral phenomenon and a burning problem of the muga silkworm rearers for successful crop harvest.

Unfortunately, till date, no report is so far available for containing any diseases of muga silkworm. As it is a highly sensitive organism towards chemical treatments, use of bio-formulations of plant, bacterial or animal origin is considered as the best alternative for conducting experiments on management of the diseases of muga silkworm. Therefore, attempts of first kind were made by us with the traditional folk medicinal plants extracts in controlling the flacherie of the muga silkworm and a bioformulation has been developed. A comparative study of some folk medicinal plants was carried that can enhance production of high yield quality silk fiber and for healthy growth of larvae of muga silkworm, *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae). Out of all the folk medicinal plants tested the dried fruits of *Terminalia chebula* fruits were found to be the best and most suitable.

### Materials and Methods

The bacterial isolate AC-3 of *Pseudomonas aeruginosa* which was found to be most pathogenic to

muga silkworm<sup>3</sup> was maintained in nutrient agar slants. For testing antibacterial activity strain was cultured in nutrient broth and 24 hour cultured broth was used for assay.

**Plant extract Preparation:** Dried fruits of *Terminalia chebula* were purchased from the local market (Figure 1 a and b) and were cleaned with water and alcohol, soaked in water for 24 hours. The outer pulp of the fruits was removed and sun dried. The pulp was ground to powder in a mixture grinder. The dry fruits powder of *Terminalia chebula* was soaked in water for 48 hours. The extract was filtered through muslin cloth and the filtrate was used for spraying purpose<sup>3</sup>. When the larvae entered into the fifth instar, a 20% solution of the extract were directly sprayed on the som plants; *Persea bombycina*. Simultaneously a homogenous solution of 0.5% Gallic acid was prepared and sprayed directly on the separate som plants and the larvae were allowed to feed on the sprayed leaves from fifth instar from 0 - day till spinning.

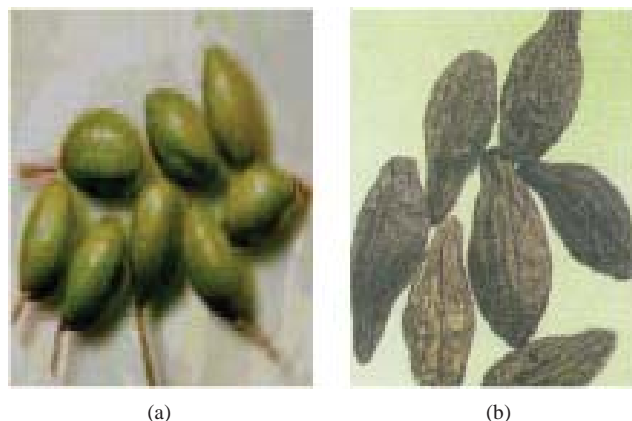


Fig. 1. a. Raw and b. dried fruits of *T. chebula*

**Phyto-chemical screening:** A portion of the concentrated methanolic extract of *T. chebula* containing antibacterial compounds having activity against *P. aeruginosa* Strain AC-3 were subjected to Thin layer chromatographic analysis in silica gel G coated plates and tried with different solvents. The presence of alkaloids, flavonoid and terpene etc. were identified after using specific spray reagents<sup>4</sup>.

**In vitro assay of *T. chebula* and its bioactive components at different concentration:** The antibacterial activity of *Terminalia chebula* and its standard bioactive components and combination of above was tested against the isolated *P. aeruginosa* strain AC-3 by conventional agar cup assay method. 100µl of plant extract and compound mixture were used for assay. The plates were observed after 24 hours incubation at 30°C. Antibacterial activity was

expressed as inhibition zone (mm) produced by the plant extract and the compound mixture.

**Muga silkworm and its rearing:** The disease free eggs of *Antheraea assamensis* were obtained from Central Muga Eri Research and Training Institute (CMER&TI), Central Silk Board, Lahdoigarh, Jorhat, Assam, India. The eggs were disinfected by dipping in formalin solution (2%) for 10 minutes. Newly emerged larvae were transferred within 12 hours to the host plants covered with net. As soon as the silkworms moulted out from fourth instar and entered fifth larval instar, the day is considered as 0 (zero) day. Spraying of the formulations was done from this day, daily once or twice on alternate days. Twenty larvae were released into each set and weight gained on each day was recorded till spinning. Other parameters like, cocoon weight, shell weight etc. was also recorded and statistically analyzed.

**Experiment under field conditions:** Similar to experiments conducted under laboratory condition, experiment with *A. assamensis* was done with three sets consisting of 10 dfls (disease free layings) larvae. In one set the larvae were kept as control, in the second set larvae were fed with leaves sprayed with *Terminalia chebula* extract and in third set, larvae were allowed to feed on leaves sprayed with 0.5% gallic acid till spinning. The sets were replicated thrice with 500 worms for each replication and repeated thrice during January – February, April-May and July-August seasons of muga rearing (Figure 2 a, b). The survivability of larvae and other rearing performance parameters fed on the two different formulations and control was noted and statistically analyzed.

**Post cocoon analysis:** Muga and eri cocoons from all the experimental sets were collected. Parameters like cocoon weight, pupal weight, shell weight etc. were recorded. Silk fiber analysis was carried out according<sup>5-6</sup>



(a) Muga heal extract



(b) Application on muga host plants

to the parameters like total filament length, non-breakable filament length etc. were recorded.

## Results and Discussion

**Phyto-chemical screening:** The preliminary analysis of the spots detected on the TLC plates shows the presence of alkaloids, flavonoid and terpene etc. after using specific spray reagents. The separation that was done in the solvents system toluene: ethyl acetate: formic acid showed the presence of gallic acid, ellagic acid and tannic acid and this was confirmed by running the standard compound along with it and  $R_f$  values (Figure 3).

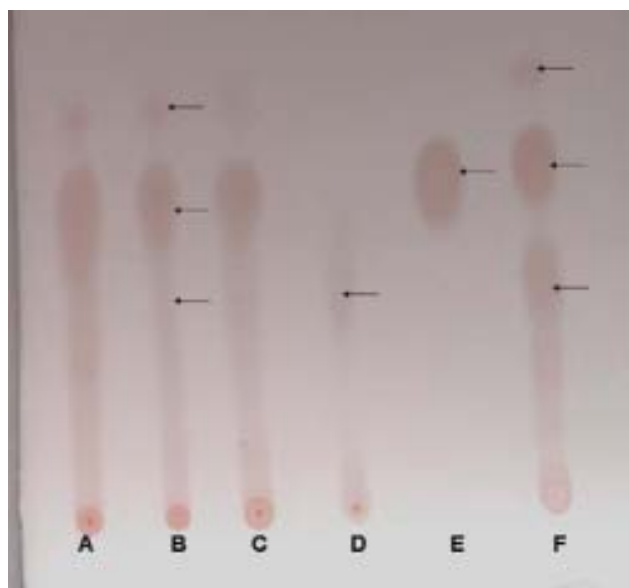


Fig. 3. Methanolic extract of (A = combination of *T. chebula*, gallic acid, ellagic acid and tannic acid; B = *T. chebula*; C = combination of *T. chebula*, and ellagic acid; D = Ellagic acid; E = Gallic acid; F = Tannic acid)

### Study on spraying of the bioformulation on *P. bombycina* :

Significant variation was observed in amount of weight gained by *A. assamensis* larvae when fed with treated leaves (Figure 4). There was gradual increase on the weight of the larvae from 0 day of fifth instar, but variation was profound in treated ones over control larvae. SDS-PAGE of proteins derived from silk glands of

## T. chebula

*A. assamensis* larvae detected more deeply stained protein bands in treated larvae compared to non-treated ones

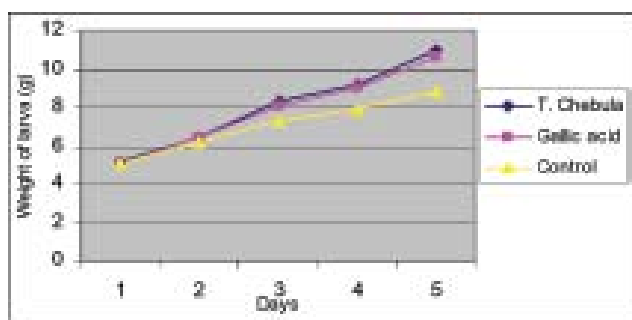


Fig. 4. Diagram showing amount of weight gained of *A. assamensis* larvae fed with leaves treated with different solutions.

Profound effect of the *T. chebula* formulation and gallic acid was observed on effective rate of rearing (*i.e.* number of worms survived out of total worms released). Significantly higher numbers of mature worms survived (more than 75-80%) throughout the seasons which were fed with *T. chebula* formulation and gallic acid treated plants, compared to 40-46% survival on non-treated plants (Fig-5).

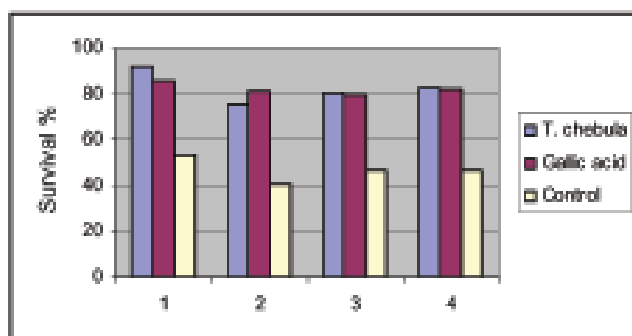


Fig-5. Survival percentage of muga silkworms treated with different formulations during different seasons (1=January – February, 2= April-May and 3= July-August, 4= average of the seasons).

Average cocoon weight for *T. chebula* formulation and gallic acid treated plants were 6.832-7.687 g which was significantly higher compared to control (5.323 g). Similarly, the shell weight and fiber content was significantly more in the cocoons obtained from *T. chebula* formulation and gallic acid treated plants (0.703g and 0.667 g, respectively of shell weight and 0.501-0.522 g, respectively for fiber) over the non-treated ones (0.483 g and 0.35 g). Moreover, the total filament length and non-breakable filament length was also statistically significant for treated plants over the control plants. From the field experiment that was performed in CSIR-NEIST, Jorhat and rearing performance that was done in CMER&TI, CSB, Lahdoigarh in large scale, it was found that survivability

of larvae is higher in *T. chebula* treated larvae with that of control. The post cocoon analysis of treated and control cocoons also showed the differences in fiber production and quality. It was observed that larvae fed with *T. chebula* sprayed leaves showed highest yield in cocoon weight and silk filament length. The results were further confirmed through large scale demonstrations at farmers' level. Flavonoids and related phenolic compounds act as strong feeding deterrents to many insects, they may sometimes be stimulatory to other<sup>7</sup>. Polyphenolic acid, Chlorogenic acid was reported to have strong growth promoting action in *Bombyx mori* silkworm<sup>8</sup>. Similarly, gallic acid also enhances moulting and accelerates the growth of *Bombyx mori* silkworms<sup>9</sup>. Presence of high amount of gallic acid in *T. chebula* fruits have, therefore, has strong stimulatory effect on enhancing feeding of leaves and thus accelerated the growth of *A. assamensis* silkworms resulting with higher silk production. From the experiment, it can be inferred that, spraying *Terminalia chebula* fruit based bioformulation on the leaves and branches of some plants, *Persea bombycina*, the principal food plant of *A. assamensis* can be effectively applied for dual purpose of reducing the intensity of flacherie disease on *A. assamensis*, caused by *P. aeruginosa* AC-3 strain and for enhanced cocoon production with uniform shape size and improved quality of silk. The technology Award for Life Sciences-2011 was awarded to CSIR-North East Institute of Science and Technology (CSIR-NEIST), Jorhat for developing *Terminalia chebula* based bioformulation (Muga Heal).

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