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# Effect of juvenile hormone (JH) analogues containing plant extracts of *Bougainvillea glabra* on larval morphometric parameters of silkworm *Bombyx mori*

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**Abstract**
The study reports on the effects of a phytojuvenoid derived from the plant *Bougainvillea glabra*, which was orally administered to third, fourth and fifth instar silkworm larvae to enhance their growth and development. The different concentrations of isolated phytojuvenoid from the plant extract of *Bougainvillea glabra* fed to the III, IV and V silkworm larvae supplemented with mulberry leaves at 6%, 12%, 18%, 24%, and 30%. The phytojuvenoid produced a beneficial effect on the larval growth index. The extent of growth improvement depended on both the duration and the dosage of exposure. Among the concentrations tested, the 24% treatment of concentration was the most effective in enhancing larval growth performance. The larval weight and relative growth rate of the final instar larvae become significantly increases compared with the control group. Overall, the bioactive phytojuvenoid extract of *Bougainvillea glabra* exhibited a growth-promoting effect on the morphometric study of the silkworm, *Bombyx mori* L.

**Keywords:** *Bombyx mori*, JH analogues, larval parameter

## Introduction

Sericulture farming are requiring low budget investment and offers more returns to farmers. It is an excellence farming option for promoting new livelihoods and supporting the rural economy in India. Sericulture is an important part of the rural economy in India, as it provides jobs and income to many people in villages. Silk is produced by the larvae of *Bombyx mori* L., which is considered as valuable insect in agro-based industry. The growth and development of silkworms depend completely on mulberry leaves and its nutrient compatibility.

India ranks as the world's second-largest producer of silk, following China. Among the countries that import Indian silk, Germany stands out as the biggest consumer. The silk production analysis revealed that the sericulture has better prospects for growth in the developing countries. Increase larval growth development would result better economics into cocoon quality and quantity for silkworm rearing and meet the production needs.

Hormones are vital for the growth and development of insects, especially during metamorphosis. In the silkworm *Bombyx mori*, the juvenile hormone keeps the insect in its larval stage, while the molting hormone also known as ecdysone, triggers shedding of the old skin and advancement to the next stage. A proper balance between these hormones ensures healthy development and high-quality silk production.

In recent decades, numerous initiatives have been undertaken to enhance larval growth and development, as well as to improve the quality and yield of silk produced by the silkworm *Bombyx mori* through nutritional supplementation of antibiotics, vitamins, hormones, hormone analogues like 20-hydroxyecdysone and juvenile hormone analogues, as well as various botanical extracts. Khyade *et al*., (2016) [20] reported the terpenes and terpene (volatile organic compounds and aromatic compounds) containing plant of. P. sylvestris shows the juvenoid activity of insects of silkworm, were topically applied to larvae of silkworm and helps to improve larval and cocoon parameter and silk filament of silkworm *Bombyx mori* L. Riddiford and Ashburner (1990) [26] investigated the impact of juvenile hormone mimics on the larval development and metamorphosis of Drosophila melanogaster and found that an excess of juvenile hormone disrupted the formation of adult imaginal structures. Similarly, peptide derivatives of juvenile hormone analogues exhibited significant hormonal activity, with L-isoleucyl-L-alanyl-p-aminobenzoic acid ethyl ester identified as the most active compound, showing effects comparable to juvabione on pyrrhocorid bugs (Zaoral and Slama, 1970) [35]. Dong *et al*., (2017) [08] investigated the impact of various artificial diet with the mulberry on the growth and vitality of silkworms, *Bombyx mori*. Manjunatha *et al*., 2017 [32] recorded the toxicological effect of Azadirachta indica, Zingiber officinale, Lantana camara and Acorus calamus extracts on larval and economic parameter of cocoon which increased the larval weight after treatment of Zingiber officinale on silkworms, the stimulatory effect of ginger found in protein synthesis in silk gland of silkworm. Deshmukh and Khyade (2013) [07] found effective rearing rate, larval consumption index after dietary supplementation of Aloe vera and its formulation on the V instar larvae and economic parameters of cocoon in silkworm *Bombyx mori*.

The plants are the most abundant natural source of juvenile hormone analogues for voracious plant feeder insects. The plant derived fraction containing juvenoids act to temporarily halt the progression of insect metamorphosis due to biochemical reactions including chitin synthesis in the body of larval stages of insects. Accordingly, 10 mL concentrations of acetone extract from Vitis vinifera, Alstonia scholaris, Santalum album, Lantana camara, Syzygium cumini, and Tectona grandis were applied topically to *Bombyx mori* larvae. The treatment resulted in a noticeable decrease in chitin deposition within the larval body wall. (Khyade *et al*., 2012b) [21]

The juvenile hormone or Juvenile Hormone analogues and their juvenoid activity prolonged the larval metamorphic life and helps to enhanced quality and quantity of silk. (Ajami and Riddiford, 1973 [1]; Grenier and Grenier, 1983 [13]; Khyade *et al*., 2002 [18]; Dyana et.al.,2015[09]; Kamimura and Kiguchi, 1980 [16]; Baishya and Hazarika, 1996 [03]; Calvez *et al*., 1976 [06] and Jadhav and Kallapur, 1989 [14]; Kale and Pardeshi (2025) [15]. Naphade *et al*. (2024) [30] studied mulberry leaves enriched with a plant extract of Phyllanthus emblica. The results showed improvements in cocoon weight, filament length, and filament weight of the silkworm, *Bombyx mori* L. Mohamed et.al., 2023 [22] evaluated the effect of fortified mulberry leaves with herbal plant extracts of Cucurbita moschata and Ipomoea batatas on the growth of silkworms and their weight of cocoon, pupa, shell, and shell ratio of silkworm *Bombyx mori*. Gobena and Bhaskar (2015) [12] determined the impact of fortification of $M_5$ mulberry leaves with the extracts of Parthenium hysterophorus, Phyllanthus niruri and Psoralea coryleifolia on growth and commercial qualities of silk of *Bombyx mori* (PM×CSR2). Srivastava and Upadhyay (2013;2015; 2017) [27, 28, 29] reported influences of phytojuvenoid on protein contents of the silkworm larvae and improved productivity of cocoon, silk filament and the life stages of silkworm. There has been no attempt so far to study the impact of juvenile hormone analogues isolated from the plant *Bougainvillea glabra* on the silkworm, *Bombyx mori*. The aim of this contribution is to evaluated the impact of plant extract containing phytojuvenoid on larval morphometric performance of silkworm, *Bombyx mori*.

## Material and Methods

### Animal Collection

The silkworm breed selected for the experiment was Indian bivoltine hybrid ($FC_2$ X $FC_2$) disease free laying of the silkworm, *Bombyx mori* were obtained from silk rearing farm, Dongargaon (Chhatrapati Sambhajinagar) after hatching larvae were isolated from stock culture and feeding them with appropriate quantity of fresh mulberry leaves. The third, fourth and fifth instar larvae were utilized for the experiment. After third instar, the larvae were acclimatized and divided into six experimental groups including control. During this period larvae were fed four times a day and maintain necessary disinfection condition.

### Plant Collection

The plant *Bougainvillea glabra* was identified and authenticated by the Department of Botany, Deogiri College, Aurangabad. Healthy leaves were collected, washed thoroughly with distilled water, and shade dried. The dried leaves were then powdered using a mechanical grinder. the plant powdered, 50 grams were taken and extraction using by using Soxhlet apparatus with 500 ml of ethanol for 24 hours. After extraction, the ethanolic extract was filtered and the filtrate was completely evaporated. The obtained extract was dissolved in distilled water and diluted to prepare different concentrations 6%, 12%, 18%, 24%, and 30% for further experiments. Fresh mulberry leaves were sprayed with each concentration of the extract and dried for 10 minutes. These treated leaves were then fed to III, IV, and V instar larvae of *Bombyx mori*, four times feeding per day. A control group was maintained by feeding larvae mulberry leaves sprayed with distilled water. Feeding were continued until the cocoon stage of the silkworm. Larval growth parameters were recorded for all treatment groups, and the results were expressed as mean ± standard deviation (S.D.).

### Larval Parameters (Morphometric study)

For the morphometric analysis, ten silkworm larvae were randomly selected from each treatment and control group. The body weight of each larva was measured using a precision electronic balance and recorded in grams. The mean larval weight for each group was then calculated and used for subsequent statistical analysis.

### Larval Growth Index

Newly emerged thirty, III instar larvae of seven day old and V instar larvae of sixteen days old were weighed (gm.) and the following formula was used to calculated as Growth Index (GI).

$$G.I = \frac{\text{Final Weight of V Instar larvae(gm)} - \text{Initial Weight of larvae(gm)}}{\text{Initial Weight of larvae(gm)}}$$

## Results and Discussion

The presents data on the effects of different concentrations of *Bougainvillea glabra* plant extract on the morphometric characteristics of *Bombyx mori* larvae. Dietary supplementation with varying concentrations (6%, 12%, 18%, 24%, and 30%) of the plant extract containing juvenoid compounds led to noticeable increases in both larval weight and growth index during the third, fourth, and fifth instar stages. In the control group, larvae began with an initial weight of 0.198 g, and this weight progressively increased with higher concentrations of phytojuvenoids in the diet.

In the present investigation, treatment with *Bougainvillea glabra* extract at concentrations of 6%, 12%, 18%, 24%, and 30% appeared to positively influence the growth of *Bombyx mori*, resulting in higher larval weight and an improved larval growth index compared to the control group. Several studies have similarly reported that bioactive compounds and varying concentrations of plant extracts or their natural formulations can enhance larval and cocoon characteristics, such as Ascorbic acid, folic acid, thiamin, vitamins, hormones and hormone anlogues, Alloe tonic etc., Etaberi et.al. (2004) [10]; Balasundaram *et al*. (2008) [04]; Khyade and Shendage (2012a) [19]; Pardeshi and Bajad (2014ab) [24 25]. Barge and Pardeshi (2017) [5] observed the growth promoting effect of nutritional supplementation of A. hybridus and X. indicum on larval growth and economic performance of silk in *Bombyx mori*. Insects development regulated by two hormone such as juvenile hormone and moulting hormone also role metamorphosis (Novak, 1966).[23] Srivastava *et al*., 1985 [33] reported the growth regulatory effect and juvenoid activity in the twenty types of flowering plant against insects of Dysdercus cingulatus. Khyade *et al*., 2016 [20] found extend life span after topical application of juvenoid activity of containing plant of Pine needles of Pinus sylvestris to the fifth instar larvae of silkworm, *Bombyx mori*. Garai *et al*., 2024 [11] study the effects of artificial juvenile mimic Pyriproxyfen and Serimore, and some phytojuvenoids plant extracted from Pinus roxburghii, Annona squamosa and Manihot esculenta and observed the economic parameters of silk, fibroin, sericin, filament weight, and maximum non-breakable filament length were increased.

The exogenous hormone analogues alter the growth pattern and development of larvae. This transformation is controlled by circulating hormones such as ecdysone, juvenile hormone, and prothoracicotropic hormone (Sakurai, *et al*., 1989) [31]. The exogenous supplementation of juvenile hormone mimics showed stimulatory impact on the larval and economic performance of silkworm, *Bombyx mori* (Akai *et al*., 1985) [02]. Exogenous supplementation juvenile hormone mimics showed stimulatory in larval development and economic performance of silkworm *Bombyx mori*. Tripathi (2024) [34] found the effect of bioactive phytojuvenoid compounds extracted from the leaves of Azadirachta indica and Pinus longifolia on the silk producing potential, weight of silk gland, cocoon, cocoon shell and shell ratio per cent of multivoltine mulberry silkworm, *Bombyx mori*. and resulted that the triple treatment of larvae with 25% concentration of phytojuvenoid (P. longifolia) is effective to improve the silk producing potential parameters of *Bombyx mori*.

In the present study, the larval growth performance was increased with oral administration of plant extract *Bougainvillea glabra* containing phytojuvenoid. It might be due to bioactive phytojuvenoid compounds and their inhibitory impact on the ecdysone which prolong the larval growth duration and helps better response as growth promoting and nutritive nature of the plant.

## Conclusion

The study concluded that treating B. mori larvae with a moderate concentration of juvenile hormone (JH) analogue containing plant extracts from *Bougainvillea glabra* promoted larval growth performance.

**Table 1:** Effect of JH analogues containing plant extracts of *Bougainvillea glabra* on the larval growth performance of mulberry silkworm, *Bombyx mori* L.

| Group | Treatment Concentration Mg/ml | Larval wt. (gm) | | | Growth index | Cocoon weight (gm) |
|---|---|---|---|---|---|---|
| | | III$^{rd}$ Instar wt. | IV$^{th}$ Instar Wt. | V$^{th}$ Instar Wt. | | |
| I | Control | 0.251 ±0.048 | 0.394 ±0.062 | 0.990 ±0.581 | 4.000 ±0.780 | 1.627 ±0.815 |
| II | 6.0 | 0.357 ±0.074 | 0.461 ±0.066 | 1.232 ±0.613 | 5.222 ±0.395 | 1.659 ±0.456 |
| III | 12.0 | 0.371 ±0.078 | 0.489 ±0.053 | 1.416 ±0.484 | 6.151 ±0.318 | 1.705 ±0.790 |
| IV | 18.0 | 0.418 ±0.058 | 0.576 ±0.067 | 1.586 ±0.343 | 7.010 ±0.337 | 1.741 ±0.513 |
| V | 24.0 | 0.445 ±0.061 | 0.661 ±0.094 | 1.786 ±0.241 | 8.020 ±0.227 | 1.789 ±0.347 |
| VI | 30.0 | 0.512 ±0.073 | 0.747 ±0.064 | 2.023 ±0.245 | 9.217 ±0.471 | 1.883 ±0.546 |

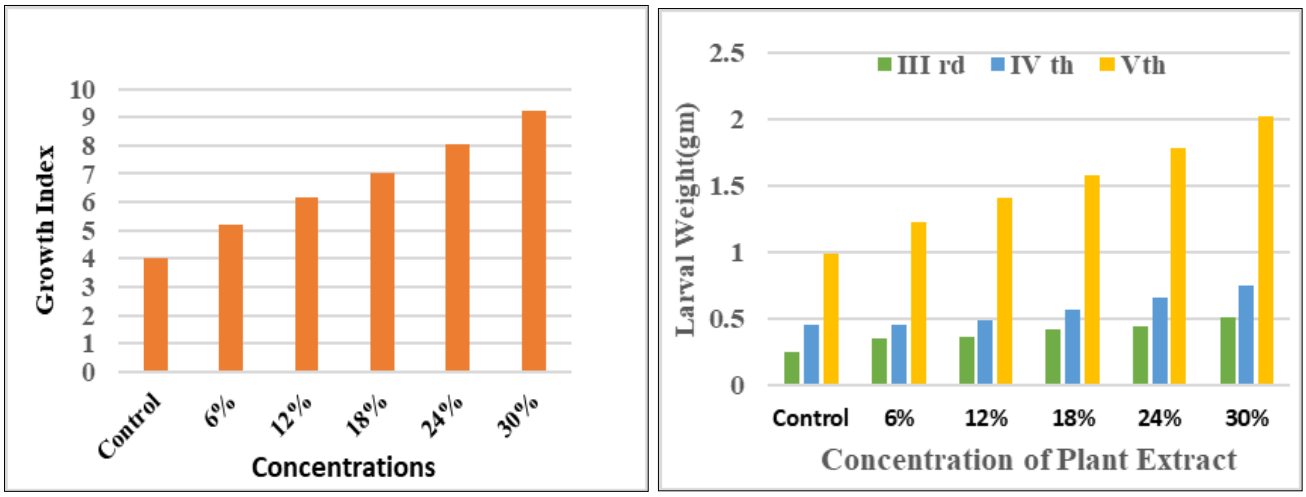


**Fig 1 & 2:** Effect of JH analogues containing plant extracts of *Bougainvillea glabra* on the larval growth index of mulberry silkworm, *Bombyx mori* L.

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