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Influence of phytoecdysteroids isolated from *Coix aquatica* on cocoon characteristics of silkworm *Bombyx mori* L.

Suryabhan Barge and Anilkumar Pardeshi

Abstract
Influence of phytoecdysteroids isolated from the plant, *Coix aquatic* on the cocoon characteristics of silkworm, *Bombyx mori* was studied. The experiments were performed by 0.5,1.0,1.5,2.0 and 2.5% concentration of phytoecdysteroid obtained from methanol solvent leaf extract of *Coix aquatica* and administered to 4th and 5th instars of silkworm with mulberry. The cocoon characteristics were influenced by various concentration of plant extract. The intensity of influence was depending on the time and dose exposure. The phytoecdysteroid at 2.0% concentration resulted increased cocoon weight. The average silk filament length, fibroin and sericin and denier were also increased with this supplementation of phytoecdysteroid over the control. In the present study the phytoecdysteroid of *Coix aquatica* have growth promoting effect and improved the cocoon characteristics of silk in *Bombyx mori.*

Keywords: plant extract, *Bombyx mori*, fibroin, sericin

Introduction
Sericulture is an agro based industry and a vital role in the improvement of rural economy of India. In India over near about three million people are employed in various field of sericulture. India is the second largest silk producer in the World after China. Germany is the largest consumer of Indian silk. Increase larval growth and cocoon quality and quantity would result better economics for this industry and meet the production needs. In recent years, many attempts have been made to improve the quality and quantity of silk through enhancing the leaves with nutrients, spraying with antibiotics, vitamins, hormones and hormone analogues, plant products or using extracts of plants.

Plants are the richest source of organic chemicals on earth and phytochemicals studies have been focused influence the life and performance of different insects (Rajasekaragouda *et al.*, 1997) [16]. Various extracts of medicinal plants have been tested by supplementation in the silkworm *Bombyx mori* and were seen to influence the body weight, silk gland weight and the silk thread length in *Bombyx mori* (Murugan *et al.*, 1998) [9]. Pardeshi and Bajad (2014a and b) [13, 14] Studied the nutritional supplementation of *Amaranthus hybridus* and *Xanthium indicum* plant extracts on economic performance of mulberry silkworm, *Bombyx mori* L. (Pardeshi and Barge 2017) [12] was studies Impact of oral supplementation of *Alternanthera sessilis* plant extract on the economic parameters of silkworm, *Bombyx mori* L. (V.K. Stanley Raja and S. Abraham Muthu Kumar 2016) [24]. was present studies investigation influence of chlorella and scenedesmus algal extracts on the economic traits like cocoon weight, shell weight, pupa weight, shell percentage, filament length, fibroin, sericin and denier of silk filament, of silkworm, *bombyx mori* L.in the present investigation the effect of blue green algae Spirulina on cocoon quantitative parameters cocoon character silkworm, (Venkatesh Kumar *et al*., 2009) [8]. The dietary nutritional management influences Botanical (*Ocimum sanctum*), on Commercial Parameters of the Silkworm, (Kuntamalla Sujatha *et al.*, 2015) [4]. According to (Fereshteh Amirmohammadi *et al.*, 2013) [3], Effects of methanol extract of *Pteridium aquillinum* (L) Kuhn (Dennstaedtiaceae), on some commercial and physiological parameters of were obtained on nutritional efficiency, and cocoon spinning silkworm, *Bombyx mori*.(Rudroju Shyamsundarachary *et al.*, 2016) [18], also found investigation on the Leaf and Seed Extracts of *Trichosanthes cucumerina* L. on Antibacterial Activity and gram positive and gram negative bacteria and influenced the larval and cocoon characteristics of *B. mori*, Commercial Parameters of Silkworm *Bombyx mori*.(Shah HA *et al*., 2017) [22], noticed show the present studies reported the effects of amoxocillin, oxytetracyclin and doxycyclin on

physiological parameters, growth and development of the larva and pupa of silkworm, *Bombyx mori.*

Coix L. is the most widely distributed oriental genus of a monoecious tribe Maydeae, family Poaceae, is a wild relative of cultivated maize (Koul and Paliwal 1964) [7]. It is of wide occurrence in most of south-east Asian countries and of great economic importance as food, forage and medicinal plant (Raut and Chamle, 2017) [17].

There has been no attempt so far to study the effect of *Coix aquatica* plant extract on the silkworm *B. mori*. The present study is an attempt to evaluate the influence of various concentration of plant extract on the growth and economic performance of silkworm *Bombyx mori* L.

Materials and Methods

Animal collection

The silkworm breed selected for the experiment was Indian bivoltine hybrid (CSR_2 X CSR_4) Disease free laying seeds of the silkworm, *Bombyx mori* were obtained from district sericulture office, Aurangabad. After hatching larvae were isolated from stock culture and feeding them with appropriate quantity of fresh mulberry leaves. The IV and V instars larvae were utilized for the experiment. After third instar, the larvae were acclimatized to the laboratory condition and divided in to six experimental groups including control. During this period larvae were fed four times a day and maintain necessary disinfection condition.

Plant collection

To observe the influence of phytoecdysteroids isolated from *Coix aquatica* on cocoon characteristics of silk in *Bombyx mori*, the experiments were performed with various concentration of phytoecdysteroid administered to the IV and V instar larvae. For the extraction of phyto ecdysteroid, the plant, *Coix aquatica* was identified and authenticated by the department of Botany, Deogiri College, Aurangabad. The leaves of plants were collected, washed thoroughly with distilled water and shed dried. The dried leaves were powdered with the help of mechanical device. Further 50 gm powdered, thus obtained was subjected to extraction through soxhlet apparatus with 500 ml methanol solvent for 24 hrs. After 24 hrs, given extract was filtered and filtrate was evaporated completely. Evaporated extract as phytoecdysteroid material dissolved in distilled water and diluted to 0.5, 1.0, 1.5, 2.0 and 2.5% concentration for further experiment. Fresh mulberry leaves were sprayed with each concentration and then dried in air for 10 minutes. Treated leaves of various concentrations were fed to IV and V instar larvae, four feeding per day the silkworm larvae fed mulberry leaves sprayed with distilled water and served as control. The feeding was maintained up to the cocoon stage of the silkworm. Cocoon weight, shell weight, papal weight, filament length and cocoon shell ratio were determined for all doses. Results were presented as means ±S.D.

Cocoon parameters

Cocoon weight: Five days after spinning 10 cocoons were harvested and weighed.

Pupa weight: After taking weight of cocoon the pupae were removed outside and weighed.

Cocoon shell weight: After taking weight of pupae the empty cocoon shell were weighed.

Cocoon shell ratio: Shell ratio is calculated by the formula,

$$\text{Shell ratio \%} = \frac{\text{cocoon shell wt.}}{\text{cocoon wt.}} \times 100$$

$$\text{Denier of the filament} = \frac{\text{Single cocoon filament weight (gm)}}{\text{Single cocoon filament length (m)}} \times 9000$$

Sericin and fibroin contents of cocoon

The sericin and fibroin content of the cocoon was estimated by Muthukrishnan *et al*., (1978) [10]. After the completion of the spinning the cocoons were collected, opened to remove the pupae. The shells were dry at 80^0 C and weighed. The cocoon shells were treated with 0.5% KOH for 6 hrs. and thoroughly washed in hot water. The following formulae were used to estimate the sericin and fibroin content and the percentage of sericin and fibroin in the total cocoon shell was then calculated.

Sericin (gm) = initial dry wt. of shell - dry wt. of shell after alkali treatment

Fibroin (gm) =dry wt. of shell - Sericin contain

$$\text{Fibroin \%} = \frac{\text{wt.of fibroin}}{\text{wt.of shell}} \times 100$$

Sericin % = 100 – fibroin %

Results and Discussion

The data on the effect of various concentration of *Coix aquatica* plant extract on cocoon characteristics of silkworm *Bombyx mori* are presented in Table I. The dietary supplementation of phytoecdysteroids isolated from *C. aquatic* with various concentrations (0.5, 1.0, 1.5, 2.0%) to the IV and V^{th} silkworm larvae resulted in an increase in their cocoon characteristics. The sericin and fibroin contents were also found to be increased.

The cocoon weight of the control group was 0.956±0.05gm.while after supplementation of phytoecdysteroids containing plant extract of *Coix aquatica*, the cocoon weight was gradually increased (2.178±0.10gm) at 2.0%concentration of plant extract containing phytoecdysteroid while at2.5% concentration, cocoon weight was reduced (1.461±0.09 gm). Similarly, pupa weight (1.668±0.08), shell weight (0.510±0.006), shell ratio (23.415±1.30) were also increased in moderate concentration of experimental group at 2.0% concentration.

The maximum filament length (901±48 meter), filament weight (0.289±0.004gm.) and denier (2.886±0.10) were also gradually increased at 2.0% of plant extract containing phytoecdysteroid. The sericin and fibroin content were also recorded in control and experimental group. The maximum fibroin percent were estimated in the present experiment was89.47±1.80 at the concentration of 1.0% phytoecdysteroid feed to silkworm larvae.

Table: Influence of phytoecdysteroid from *Coix aquatica* plant extracts on economic parameters of silkworm, *Bombyx mori*

Group	Treatment	Cocoon wt.(g)	Pupa Wt.(g)	Initial dry wt. of shell	Shell ratio (%)	Filament Length (m)	Filament Wt. (g)	Denier	Sericin (%)	Fibroin (%)
I	control	0.956 ±0.05	0.707 ±0.04	0.149 ±0.004	15.585 ±1.25	721 ±53	0.169 ±0.004	2.109 ±0.09	25.51 ±1.72	73.82 ±2.10
II	0.5%	0.961 ±0.06	0.768 ±0.05	0.153 ±0.004	15.920 ±1.24	794 ±32	0.197 ±0.009	2.232 ±0.10	20.27 ±1.61	79.73 ±2.10
III	1.0%	1.338 ±0.07	1.050 ±0.06	0.228 ±0.005	17.040 ±1.25	831 ±38	0.209 ±0.005	2.263 ±0.11	18.75 ±1.52	89.47 ±1.80
IV	1.5%	1.868 ±0.08	1.521 ±0.07	0.347 ±0.005	18.576 ±1.28	865 ±39	0.231 ±0.005	2.403 ±0.12	13.55 ±1.33	86.45 ±2.40
V	2.0%	2.178 ±0.10	1.668 ±0.08	0.510 ±0.006	23.415 ±1.30	901 ±48	0.289 ±0.004	2.886 ±0.10	21.57 ±1.81	78.43 ±1.88
VI	2.5%	1.461 ±0.09	1.138 ±0.06	0.323 ±0.005	22.108 ±1.28	891 ±43	0.261 ±0.004	2.636 ±0.09	19.51 ±1.56	80.49 ±2.70

Values are mean ± Standard deviation of three observations.

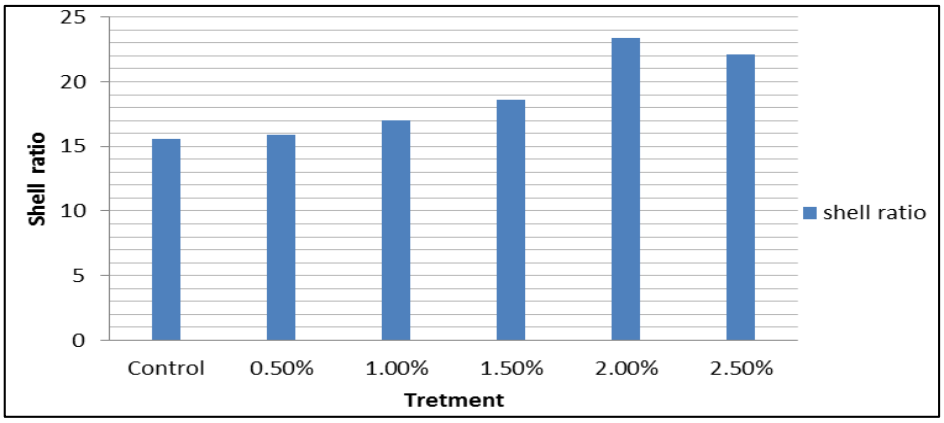

Fig 1: Influence of phytoecdysteroid from *Coix aquatica* on cocoon Shell ratio

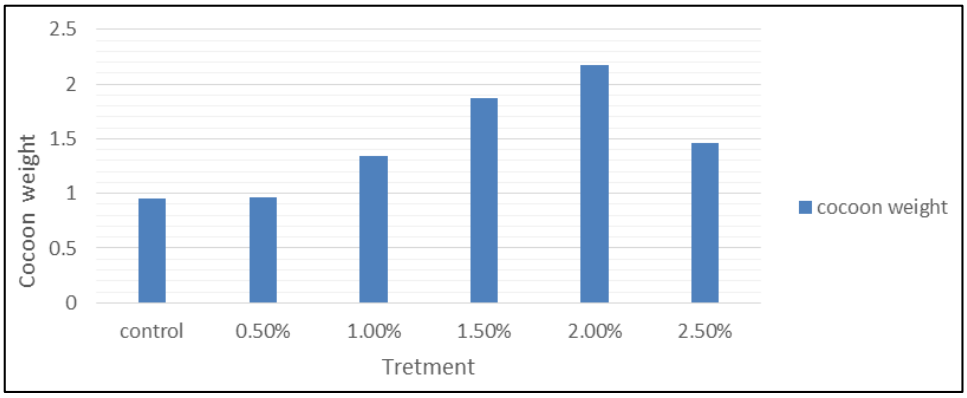

Fig 2: Influence of phytoecdysteroid from *Coix aquatica* on cocoon characterstic

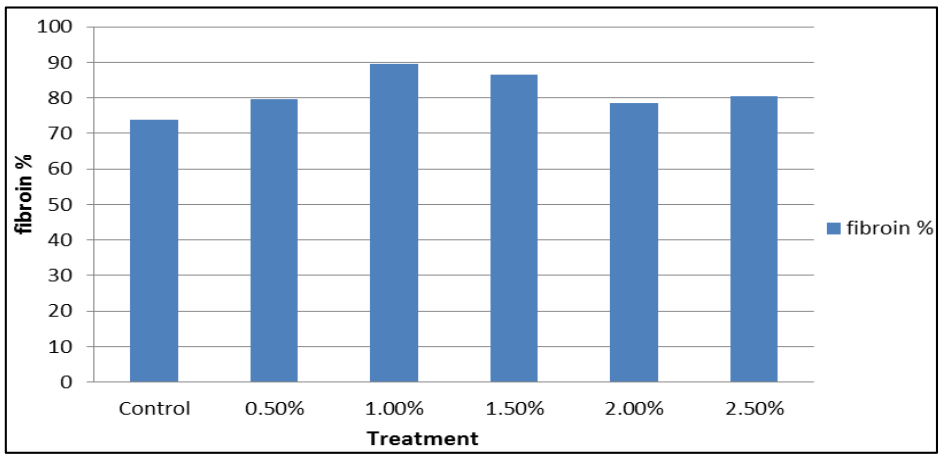

Fig 3: Influence of phyto ecdysteroid from *Coix aquatica* on fibroin %

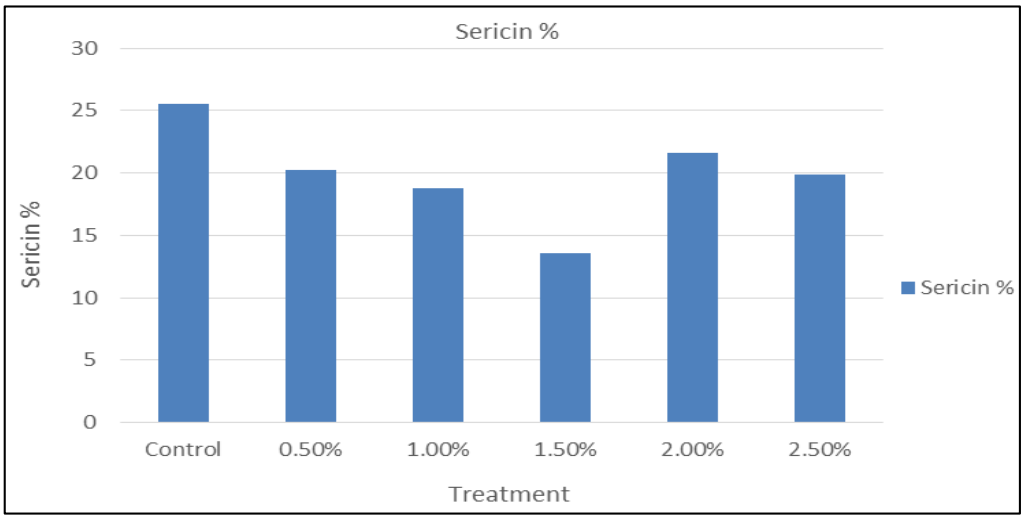

Fig 4: Influence of phyto ecdysteroid from *Coix aquatica* on Sericin %

In the present study the treatment of phyto ecdysteroid containing plant extract of *Coix aquatica* at the concentration of 0.5%, 1.0%, 1.5% and 2.0% may have beneficial effect on the growth of the silkworm *Bombyx mori* and also increased the cocoon weight, pupa weight, shell ratio, denier and filament length by enhancing feed efficacy than control whereas at 2.5% concentration, the overall performance of silkworm were comparatively reduced. Many researchers showed that the larval and cocoon characters improve by different concentration of plant extract and their natural formulation such as Ascorbic acid, folic acid, thiamin, vitamins, hormones, Alloe tonic etc. (Fereshteh A. *et al.*, 2013, Shah H, *et al*., 2017, Sarwa K *et al*., 2014, Khatak S *et al*., 2014, Karuppasamy K *et al*., 2013) [3, 22, 21, 6, 5].

The turmeric extract improved the larval growth and commercial parameters of silkworm, *Bombyx mori*. (Karuppasamy Karthikairaj *et al*., 2013) [5]. The green synthesis of silver nanoparticles exhibited the growth stimulant activity to improve silk yield in silkworm (Ganesh Prabu. *et al.*, 2012) [15]. Silkworm diet supplemented with Chlorella and Scenedesmus algal extracts improve the growth and economic traits (Stanley and Kumar, 2016) [24]. Feed efficacy and growth rate of silkworm larvae enhanced by Vitamin C (Balasundaram *et al*., 2013) [1].

Pardeshi and Bajad (2014 a and b) [13, 14] 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*. Barge and Pardeshi (2017 and 2018) [12, 2] found the *A. sessilis* and *Sida acuta* plant extract enhanced the growth and cocoon characteristics of silkworm, *Bombyx mori*. (Murugan *et al.*, 1998) [9] Noticed a strong correlation between the growth of silkworm and the silk production in the silkworm after the treatment of plant extracts. The supplementation of *Vigna unguiculata* aqueous extract with mulberry leaves at different concentration enhanced the quality and quantity of silk in *Bombyx mori* (Saravanan *et al*., 2011) [20].

Phytoecdysteroid, a chlolest -7 ene- 6- one- carbon skeleton (C_{27}) from a plant source, *Radix achyranthes* was administered to 5th instar silkworm per os at a rate of 2 µm per larva to different batches of silkworm at 48 h, 72 h and at the onset of cocoon spinning when a few larvae were ripe. The larval duration, larval mounting duration, cocoon characters and reeling traits were improved by phytoecdysteroid (Nair *et al*., 2005) [11]. (Sreejit *et al*., 2018) [23] applied minute quanitities of phytoecysteroids isolated from *Diploclisia glaucescens* and Juvenitle hormone from *Cullen corylifolium* which enhanced the commercial traits of silkworm, *Bombyx mori.*

The results of the study have confirmed that the application of phytoecdysteroids of *Coix aquatica* have explored the potential for improving the quality and quantity of silk in *Bombyx mori* at moderate range.

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