"Studies on the effect of chloroform extract of *Ocimum canum* (Family Lamiaceae) on the III and V instar larvae of silkmoth *Bombyx mori* (Family Bombycidae)"

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Abstract

Sericulture is an agro-based cottage industry in India, which aims at increased production of silk. In recent years several technologies have been developed to improve the quality of silk. Labour saving devices linked with improvement in the quality and saving the feed of the larvae are also being worked out. Mulberry is the sole food plant for the silkworm *Bombyx mori* L. The fresh and nutritive quality of mulberry leaf plays an important role in the development of worm stabilizing the cocoon production and silk productivity. In order to improve the quality of silk and quantity of cocoons it is necessary to enrich the nutrient quality of mulberry leaves. This can be achieved by the supplementation of mulberry leaves with extra nutrients. The use of plant extracts in sericulture can serve as an alternate source to get more of cocoons, silk and will enrich the economy. In the present study of it was observed that the leaf extract of *Ocimum canum* proved to be very effective regarding growth and maturation of silkmoth *Bombyx mori* L.

KEYWORD: *Ocimum canum*, *Bombyx mori*, maturation, Phytoecdysones.

Introduction

Silk is a natural protein fiber, some forms of which can be woven into textiles. The best-known type of silk is obtained from cocoons made by the larvae of the mulberry silkworm in India, reared in captivity (sericulture). The shimmering appearance, for which silk is prized, comes from the fibers. Silk is the most elegant textile in the world with unparalleled grandeur, natural sheen, and inherent affinity for dyes, high absorbance, light weight, soft touch and high durability and known as the "Queen of Textiles" the world over. On the other hand, it stands for livelihood opportunity for millions owing to high employment oriented, low capital intensive and remunerative nature of its production. The very nature of this industry with its rural based on-farm and off-farm activities and enormous employment generation potential has attracted the attention of the planners and policy makers to recognize the industry among one of the most appropriate avenues for socio-economic development of a largely agrarian economy like India.

India and China together have a lion's share of total silk production in the world. Among the producers of silk, except Brazil, all other countries are in Asia alone. China has a share of 80.06 percent in the world production of silk, followed by India, which has a share of 13.77 per cent. China produces international grade raw silk and hence has been one of the major stake holder in the international silk market. India is considered to be the second largest producer of silk, and imports nearly 7000 MT annually to suffice the domestic demand. Out of the annual raw silk production, there exists a huge domestic demand as the consumption rate is highly elastic. This has resulted in creating a larger gap in production. The domestic consumption has been estimated to reach 25000 MT annually in the coming years. Brazil, Thailand and Uzbekistan are the other major

mulberry raw silk producing countries accounting for 1.42 per cent, 1.34 per cent and 0.89 per cent respectively. Japan and South Korea were once the major producers of silk but have started abandoning sericulture due to industrialization. However, they continue to consume a substantial quantity of silk.

Advent of recent techniques in sericulture industry made it possible to practice sericulture in an intensive scale **(R.K. Dutta, 1984).** With the advanced researches in sericulture, Seri-culturists turned the sericulture industry leading to greater profits than most of the agricultural crops. Madhya Pradesh has been practicing sericulture and producing cocoons of good quantity.

Shiv kumar *et al.* (1995) state that sericulture farmers can be benefited by the commercial use of ethanolic extract of *Ocimum* as a phytoecdysone, to minimize the maturation period of larval stage of *Bombyx mori.* The application of plant extract at 72 hrs of 5^{th} instar significantly accelerated the maturation of larva**.** According to **Maribashetty *et al*. (1997)** extracted ecdysone from C*henopodium giganticum* showed broad spectrum hormonal activity in the early maturation of larva. **Trivedy *et al*. (2003)** observed similar results for early and uniform maturation of *Bombyx mori* L. by administering phytoecdysteroid extracted from plant of family Caryophyllaceae, anytime after 72-75 hours in 5^{th} instar.

Plants are the richest source of organic chemicals on earth and phytochemicals have been reported to influence the life and performance of different insects **(Rajsekaragouda *et al*. 1997, Khyade, 2004).** Various extracts of medicinal plants have been tested by supplementation in the food of silkworm *Bombyx mori* (L) and were seen to influence the body weight, silk gland weight and the silk thread length **(Murugan *et al.* 1998).**

In the present study indigenous plant *Ocimum canum* of family Lamiaceae was selected to obtain its biologically active chemical compounds for the experiment. This plant was selected for the study because of its medicinal value, and abundance in the study area.

Materials and methods-

Collection of plant material-

Ocimum canum belongs to family Lamiaceae. Its taxonomic position is as follows-

***Ocimum canum*-**

Phylum - Magnoliophyta
Class - Magnoliopsida
Order- Lamiales
Family -Lamiaceae
Genus -*Ocimum*
Species -*canum* (Sims)

Commonly known as-Basil, Sweet basil

Ocimum canum belongs to family Lamiaceae. This annual plant is a native of African and Asian continent and grows to a height of 2 feet. It is also known as African

basil with a distinct mint flavor, with hairy leaves and scented flowers. *Ocimum canum* is used in medicinal preparations in various parts of the world specially for treating diabetes. The plant branches from the base and has an angled stem and oval pubescent leaves. Its leaves are tiny and fuzzy and have beautiful violet or white flowers, having a sweet scent resembling that of the clove. The leaves of *Ocimum canum* are opposite and toothed with small flowers, they are irregular and occur in crowded whorls. *Ocimum canum* has a small corrolla. This plant has an intense floral-fruity aroma.

For phytochemical analysis of plant the collected material after identification was used and a voucher specimen was procured in the herbarium sheet. The shade dried and powdered leaves were soxhlated in 90% alcohol, rectified spirit and water respectively.

The extract thus obtained was kept in a glass vial and stored in the refrigerator; percentage loss in weight was 96.2% and percentage yield was recorded as 6.3% in n-Hexane, 7.56% in chloroform and 5.38% in methanol.

Chemical analysis and identification of the compounds-

First the crude extract of plant was defatted in n-hexane and extracted with methanol and chloroform. The concentrated solution was allowed to stand when a green yellow deposit was obtained. The deposit was repeatedly crystallized from a mixture of chloroform and methanol, till a single spot was obtained by paper and thin layer chromatography.

Methylation: Purified fractions were separately dissolved in MeOH. In this process compound was washed by MeOH until it got converted into crystal or powder form. The material was tested chemically for glucose. For this Bendict's and Fehling's test were carried out. These tests were affirmative. The precipitate was greenish yellow (Bendict's test) and yellowish red (Fehling's).

Alkaline reagent test: To the test compound solution, few drops of NaOH solution were added. Intense yellow color was formed which turned to colorless in addition of few drops of dilute HCL, this indicated presence of flavonoids.

For further identification and structural elucidation of plant extract, the samples of were sent to SAIF, CDRI Lucknow for spectral analysis: where IR spectrum, UV spectrum, NMR and Mass spectrum was done.

IR Spectra

The IR spectra were recorded on Perkins - Elmer model 783 Spectrophotometer Graph.

UV Spectra

The UV spectra were recorded on Schimadzu U-160 Spectrophotometer and it was scanned in the range between 200-400 nm.

NMR

The H^1 NMR spectra and C^{12} NMR spectra were recorded on F + NMR Spectrophotometer (90 MHZ) in $CdCl_3$ using TMS as an internal standard and the chemical shifts were determined in ppm value (s) from TMS as internal standard Graph.

Mass Spectra

The mass spectra were recorded on a JEOL JMS 300 Spectrometer with accelerating potential of 3kv Graph.

On the bases of spectral data obtained from SAIF CDRI Lucknow and on comparing the data with authentic markers available finally phytoecdysone, Ocimucinin and methylchavicol were determined in *Ocimum canum.*

Results and Discussion

The growth and maturation activities of silkmoth *B. mori* larvae were studied using the *Ocimum canum* leaf extracts. The experiment was conducted by 20 larvae of *Bombyx mori* in four replicates, with one control taking 100 gm of mulberry leaves in each tray with different concentrations of experimental leaf extracts. The growth of larvae was recorded after 24 hrs and 36 hrs. It was analyzed statistically and the parameters for each extract worked out were percentage of maturation, mean, S.D., S.E., and C.D.

For III instar *Bombyx mori* larvae among the five chloroform fraction of *Ocimum canum* the IV fraction was most effective as the mean maturation percentage after 24 hours and 36 hours were recorded to be 55.833 and 60.000 in 250 ppm concentration (Table No. 1). But for V instar larvae chloroform fraction V was recorded to be most effective showing mean maturation percentage after 24 and 36 hours as 64.166 and 69.166 respectively in 250 ppm concentration (Table No. 2). The present study shows that *Ocimum canum* leaf extracts improve the growth rate of *Bombyx mori* and bring early maturation in them.

According to **Jayapaul *et al.* (2003)** the increased rate of food absorption in silkworms was evident with the enrichment of mulberry leaves by leaf extracts of *Coffea arabica***. Sakia and Das (2005)** reported that the protein extract of muga pupa can be utilized as a supplement on the muga food plant as a growth stimulator in silkworm.

According to **Gururaja and Patil (1997)** *Amaranthus* grain powder is very rich in carbohydrates, protein, iron and a-carotene and it is found to be effective in increasing silk yield coupled with increased cocoon and shell weights. The application of leaf extract of *Acacia indica* and *Vitex negundo* at different concentrations on silkworm had significantly improved the shell weight and silk filament length **Sujatha and Rao (2004).** *Parthenium* root extract induced silkworms to feed more resulting in higher cocoon and pupal weight and better survival **Patil *et al.* (2005).** The conversion of ingested dry matter into shell was increased by 20% in silkworms due to the application of *Spirulina* solution **Kamalakannani *et al.* (2005).**

According to **Rojas *et al.* (1992), Radhika *et al.* (2011) Samatha *et al.* (2011)** extensive the effectiveness of the plant extract was due to combined action of chemical compounds such as alkaloids, flavonoids, triterpenoids, and other compounds of phenolic nature which are classified as antimicrobial compounds.

Karuppasamy *et al*. (2013) studied that mean larval weight of the final instar larvae increased as compared to the control due to turmeric powder extracts**.**

The results of the present investigation also follow the trend of previous researchers discussed, as the maturation time decreases with the use of experimental leaf extract and the treated larvae start producing silk earlier than the control. So there is a great possibility of using such leaf extracts as prophylactic measure for rearing silkworm and early production of silk.

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Table No. 1: Effect of *Ocimum canum* leaf extract Chloroform fraction IV on maturation of III instar larvae of *Bombyx mori*

Concentration (ppm)	No. of reared larvae	Maturation % observed in continuation after			
		24 hours		36 hours	
		No.	%	No.	%
50	20	11	55	12	60
100	20	11	55	12	60
150	20	12	60	13	65
200	20	12	60	13	65
250	20	13	65	14	70
Control	20	8	40	8	40
Mean		55.833		60.000	
S.D.		8.612		10.488	
S.E.		3.515		4.281	
C.D. at 5%		9.039		11.008	

Table No. 2: Effect of *Ocimum canum* leaf extract Chloroform fraction V on maturation of V instar larvae of *Bombyx mori*

Concentration (ppm)	No. of reared larvae	Maturation % observed in continuation After			
		24 hours		36 hours	
		No.	%	No.	%
50	20	12	60	14	70
100	20	13	65	14	70
150	20	13	65	15	75
200	20	14	70	15	75
250	20	15	75	15	75
Control	20	10	50	10	50
Mean		64.166		69.166	
S.D.		8.612		9.703	
S.E.		3.515		3.961	
C.D. at 5%		9.039		10.185	