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

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Abstract

Plants are the richest source of organic chemicals on earth and phytochemicals have been reported to influence the life and performance of different insects**.** Various extract of medicinal plants have been tested by supplementation in the silkworm *Bombyx mori* (L) and were seen to influence the body weight, silk gland weight and the silk thread length in *Bombyx mori***.** Almost all insects are host specific and select their most preferred food in order to extract the maximum benefit out of it, although most of them eat a great many varieties**.** Different food plants may influence differently the food intake, efficiency of digestion, conversion of food to body biomass and finally the growth and development of insect critically reviewed these aspects of nutrition and ecology of insect and concluded that nutritional indices as well as growth and development of insect varied on different host plants. Generally varieties of the some species may exert variable effect on the relative survival of herbivore insect. In recent years, attempts have been made in sericulture to study the effect of temperature**,** relative humidity**,** ecological factors**,** egg magnetization**,** cocoon refrigeration**,** cocoon magnetization**,** 20-hydroxyecdysone hormone and phytoecdysteroid hormone**,** on the performance of silkworm. The phytochemicals could benefit sericulture by improving the silk yield of *B. mori* and its commercial production**.** Man has benefited from the silk, produced by silkworm and subsequently researchers have always been trying to unveil the factors that can be manipulated to the benefit of the silkworm rearers**.** Spraying the leaf extract of *Ocimum basilicum*, are gaining importance because of their wide spectrum of biological action, novel mode of action and eco-friendly nature**.** In the present study of research work it was observed that the leaf extract of *Ocimum basilicum* proved to be a very effective on growth and maturation of silkmoth *Bombyx mori* L.

KEYWORD: *Ocimum basilicum*, *Bombyx mori*, Maturation, Phytoecdysones.

Introduction

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 continues to be the second largest producer of silk in the World. Among the four varieties of silk produced, as in 2013-14, Mulberry accounts for 79% (18715 MT), Tasar 7.3 % (1729 MT), Eri 13.2% (3116 MT) and Muga 0.5% (119 MT) of the total raw silk production of 23,679 MT. This is against the production of 23060 MT Silk by end of XI Plan (2011-12) in the country.

Production of raw silk in India was 26,480 MT in 2013-14 of which, mulberry raw silk output aggregated to 19,476 MT (74%). The remaining 7,004 MT (26%) was Vanya silks. Mulberry sericulture is mainly practiced in five states namely, Karnataka, Andhra Pradesh, West Bengal, Tamil Nadu and Jammu & Kashmir which jointly account for about 96% of the total mulberry silk production in the country. Raw silk is of two kinds, viz., mulberry and non-mulberry. The distinction arises from the rearing of silk worms either upon mulberry leaves or on other plants. Mulberry silk is produced mainly in Karnataka, West Bengal, Jammu & Kashmir, Tamil Nadu and Andhra Pradesh although some other States have made some progress in this direction under their development plans. These five states collectively account for 97% of the total area under mulberry cultivation and 95% of raw silk production in the country. Now, as a result of growing realization, sericulture is gaining ground in non-traditional areas too.

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 **(Shivkumar *et al.*1995).** 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 extract of medicinal plants have been tested by supplementation in the silkworm *Bombyx mori* (L) and were seen to influence the body weight, silk gland weight and the silk thread length in *Bombyx mori* **(Murugan *et al.* 1998).** Dietary supplementation of the leaf, flower and pod extracts of *Moringa oleifera* **(Rajeswari and Isaiarasu, 2004).**

In the present study indigenous plants *Ocimum basilicum* of family Lamiaceae were selected to obtain their biologically active chemical compounds for the experiment. These plants were selected for the study because medicinal values, and their abundance in the study area.

Material and methods-

Collection of plant material-

Ocimum basilicum belongs to family Lamiaceae. Its taxonomic position and introduction is as under-

***Ocimum basilicum*-**

Phylum - Magnoliophyta

Class - Magnoliopsida

Order- Lamiales

Family -Lamiaceae

Genus -*Ocimum*

Species -*basilicum* (Linn.)

Commonly known as- Sweet basil, french basil, Indian basil.

Basil also known as French Basil or Sweet basil or Tulsi is an erect glabrous herb, 30-90 cm high, is indigenous to India. The leaves of basil have numerous oil glands with aromatic volatile oils. The herb bears clusters of small white lipped flowers in racemes. The freshly picked bright green leaves turn brownish green when dried and become brittle and curled. Basil grows as a perennial herb in tropical climates, and is planted as an annual herb in temperate regions, where it is sown directly from seed or is transplanted. Other member of basil family (Lamiaceae), grow well under competitive circumstances, basil prefers little competition for sun and water. Basil has been used as a folk remedy for an enormous number of ailments, including cancer, convulsion, deafness, diarrhea, epilepsy, gout, hiccup, impotency, insanity, nausea, sore throat, toothaches, and whooping cough. Basil has been reported in herbal publications as an insect repellent.

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 91.6% and percentage yield was counted and recorded in the tabular form. Yield percentage was recorded as 5.28% in n-Hexane, 6.56% in chloroform and 4.48% in methanol for *Ocimum basilicum.*

Chemical analysis and identification of the compounds-

First the crude extracts 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. Chemical test for sugar or glycoside like other compound: The material tested chemically for glucose. For these Bendict's and Fehling's test were carried out. These test were affirmative. The precipitate was greenish yellow (Bendict's test) and yellowish red (Fehling's).

Alkaline reagent test: To the test compound solution, add few drops of NaOH solution. Intense yellow color is formed which turn to colorless an addition of few drops of dilute HCL, this indicate presence of flavonoids.

Zinc Hydrolized test: To the test compound solution add a mixture of zinc dust and concentrated HCL give red color after few minutes.

For further identification and structural elucidation of plant extract, the purified samples of selected plant fraction 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.Graph.

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 and Basilimoside in *Ocimum basilicum.*

Result and discussion

The growth and maturation activities of silkmoth *B. mori* larvae were studied using the *Ocimum basilicum* 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 basilicum* the V fraction was most effective as the mean maturation percentage after 24 hours and 36 hours were recorded to be 58.33 and 61.66 in 250 ppm concentration (Table No. 1). But for V instar larvae chloroform fraction III was recorded to be most effective showing mean maturation percentage after 24 and 36 hours as 64.16 and 68.33 respectively in 250 ppm concentration (Table No. 2). The present study shows that *Ocimum basilicum* leaf extracts improve the growth rate of *Bombyx mori* and bring early maturation in them.

According to **A.Sampath *et al.* (2013)** the castor leaves supplemented with aqueous solution of Anabaena variabilis in 500 ppm concentration significantly increased the larval weight, pupation rate, cocoon yield, effective rate of rearing and reduced the larval mortality considerably as compared to control. The reduction in larval mortality might also be due to antibiotic activities of cyanobacterium that is evinced antibacterial, antiviral and antifungal effect on test insect **(Bloor and England, 1989).** The present findings on the effects of Anabaena variabilis on physiology of Eri silkworms are in close conformity with those results observed in *Bombyxmori* L., by **Venkataramana *et al.* (2003).** Further, **Jayaprakash *et al.* (2005)** reported that castor leaves feed supplemented with 300-400 ppm concentration of *Spirulina* aqueous extract to *Samia cynthia ricini* Boisd were effective in reducing larval mortality and increasing the larval weight, pupation rate, pupil weight and silk ratio. **Narayanaswamy and Ananthanarayana (2006)** also reported similar findings in case of *B. mori* L.

The nutritional supplement of soluble protein aqueous extracts from waste pupae on larval instars of *Antheraea assama* in different concentrations had good impact on the larval growth and cocoon parameters. The increased rate of food absorption in silkworms was evident with the enrichment of mulberry leaves by leaf extracts of *Coffea arabica* **(Jeyapaul *et al*. 2003).** The protein extract of muga pupa can be utilized as a supplement on the muga food plant as a growth stimulator in silkworm **(Saikia and Das, 2005).**

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).**

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 basilicum* leaf extract Chloroform fraction V 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	12	60	13	65
150	20	12	60	13	65
200	20	13	65	14	70
250	20	14	70	14	70
Control	20	8	40	8	40
Mean		58.333		61.666	
S.D.		10.327		11.254	
S.E.		4.216		4.594	
C.D. at 5%		10.840		11.812	

Table No. 2: Effect of *Ocimum basilicum* leaf extract Chloroform fraction III 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	13	65
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		68.333	
S.D.		8.612		9.831	
S.E.		3.515		4.013	
C.D. at 5%		9.039		10.319	