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Effect of phytoecdysteroid (β-ecdysone) on synchronization of maturation in silkworm *Bombyx mori* L

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Abstract
Spinach (*Spinacia oleracea*) belonging to family Chennopodiaceae was reported to be having insect moulting hormone β-ecdysone or 20-hydroxyecdysone. The Leaves and soft shoots of this plant were collected, washed, dried and later on powdered for obtaining plant extract by using methanol. (Hot extraction by Soxhlet apparatus) The effect of this 20-hydroxyecdysone was later on tested on silkworm *Bombyx mori* L. for synchronization of maturation by using silkworm double hybrid ($CSR6 \times CSR_{26}$) × ($CSR_2 \times CSR_{27}$). In the present study fully grown larvae of 5th instar were fed with mulberry leaves sprayed with different concentrations of this plant extract at 48 h, 72 h, 120 h and at the onset of maturation during 5th instar. The control batches (without any treatment) and a group receiving mounting hormone (branded hormone SAMPOORNA), were maintained in similar conditions. The time and number of spinning worms during mounting process and other parameters were recorded in all the batches. Silkworms took 36-40 hours in normal course for completion of mounting completed the same in 18-24 hours when fed with mulberry leaves sprayed with this plant extract. The bivoltine silkworm hybrid immediately started spinning within 6-10 hours when fed with plant extract sprayed leaves at the end of 5th age when nearly 3-5% worms had started spinning. The mounting process was hastened and finished within 24 hours in plant extract sprayed batches and also in branded hormone sprayed batches. Both were statistically at par as compared to control (without any treatment) which took 36-40 hours for completion of mounting.

Keywords: Sampoorna, β-ecdysone, synchronization, maturation, *Bombyx mori* L

Introduction
Sericulture is an agro based rural industry which helps in economic development of people living below poverty level particularly in rural areas. Silk is one of the most elegant textiles in the world with unparalleled qualities like grandeur, natural sheen, and inherent affinity for dyes, high absorbance, light weight, soft touch, and high durability which makes it the "Queen of Textiles". Although India has many advantages for becoming number one in the world raw silk production however inspite of these advantages the productivity and production does not increase to the expected level, due to many reasons, these include imbalance in synchronization of larval maturation, poor leaf quality, spread of diseases and even some lacunae in management of the rearing. Among these one of the contributory factor for low productivity/ production is mounting of immature worms which again can be attributed to prolonged maturation. The delay in mounting is due to non-synchronization in maturation of worms at the time of spinning. It has been observed that silkworms in a population do not mature simultaneously and the maturation process continues for 2-3 days in tropical zones and 3-4 days in temperate zones under field conditions (Nair *et al.*, 2002) [4]. Due to this prolonged maturation self-mounting of worms become difficult as mounting material required for seri position can't be applied at a time on these worms. Under such situation farmers are forced to pick up the matured silkworms one by one continuously for mounting and allow others to continue feeding till these mature. This results in mortality of worms in rearing beds. The maturation of worms is attributed to a regulatory mechanism controlled by a hormone called ecdysone (20-hydroxyecdsone) present in the silkworm. The non-uniform maturation is due to the fact that hormone dose not reach to the optimum titre essentially required for spinning in all the worms simultaneously (Trivedy *et al.*, 2006) [10]. The problem of non-synchronization can be solved by managing early maturation in population (Zhuang *et al.*, 1992) [11] by application of ecdysteriod for achieving uniformity in spinning and thereby uniformity in

commercial cocoons. Studies have established that judicious administration of ecdysteroids known as moulting hormone (MH) accelerates the maturation in larvae, synchronize the spinning activities and shorten the mounting period in silkworm (*Bombyx mori* L) (Nair *et al.*, 2002) [4]. The exogenous dose of ecdysteroid on the other hand hastens these activities. It is known that some plants synthesize ecdysteroid as a defence mechanism and it does occur in these plants in large quantities (Schmelz *et al.*, 2000) [7]. The phytoecdysteroid have been seen to be 20 times more active than Zoo- ecdysteroids (Nair *et al.*, 2002) [4]. Ecdysteriod in.
the context of sericulture can be any phyto-sterol structurally closer to the original insect ecdysteroid, i. e20-hydroxycedysone, which can induce a response in silkworm equal to that of the natural ecdysteriod and could be used for synchronizing the maturation activity and to achieve uniform spinning in silkworms within a period of 18-24 hours after application to mulberry leaf and feeding same to silkworm just when 3-5% worms mature for spinning, instead of the usual 3-4 days under field conditions so as to save substantial amount of mulberry leaf and labour. It can also advance the maturation activities and hasten the cocoon spinning process under stress conditions when there is unforeseen shortage of mulberry leaf during facultative period of fifth age (last three days). Rufaie *et al.* (2012) [6] has reported that reasonable concentration of 20-hydroxyecdysone (β-ecdysone) was found in 3 locally available plant extracts of *Taxus wallichiana* Zucc. (Himalayan Yew), *Cupressus tularosa* Linn (Cupreous) and *Datura stramonium* Linn. (Datura). These were later used for synchronization of maturation in Silkworm *Bombyx mori* L.

Material and Methods

This study was carried out at College of Temperate Sericulture, Mirgund and Indian Institute of Integrative Medicine (Regional Research Laboratory) Sanatnagar Srinagar during spring 2017.

Collection of locally available plant material for obtaining plant extract containing Ecdysone (20-hydroxyecdysone)

The following plant material belonging to Chennopodiaceae family was collected for extraction.

S. No.	Botanical Name	Family	Local Name
1	*Spinacia oleracea* L.	Chennop-odiaceae	Spinach

Preparation of plant extract

The plant material i. e. leaf & tender branches of *Spinacia oleracea* L (Spinach) were taken and washed with distilled water and then shade dried till brisk. The dried plant material was powdered in a grinder for making fine powder for getting extract. The powdered material was placed in a porous paper and immersed in a solvent made from methanol and water in the ratio of 3:2. The same was boiled in a soxhlet apparatus for eight hours and extract was collected in a conical flask. The extract was than separated from residue by filtration by passing it through whatman's filter paper (No 40) and subsequent centrifugation was done. The extract was concentrated at 60ºC temperature by using a rotary evaporator and again dissolved in methanol and water solvent and partitioned against hexane phase so as to remove non- polar compounds (Hoffman & Hetru, 1983) [2]. This thick vicious extract was then taken for using it in the experiment. It has already been reported that the extract of *Spinacia oleracea* (Spinach) contains abundant quantity of Ecdysteroid (20-Hydroxyecdysone) which is similar to the structure of moulting hormone in silkworm *Bombyx mori* L. (Ahmad bakarim *et al.* 2008) [1].

Administration of Phytoecdysteroid (PE)

The plant extract of *Spinacia oleracea* L (Spinach) was re-dissolved in methanol to prepare 10 per cent stock solution (w/v) i. e. (10g in 100ml) which was used to prepare further dilution of 1:100 (1%) and 1:50 (2%) (v/v) concentration of the extract in distilled water (Jeyapual *et al.*, 2003) for further use during silkworm rearing. During 5th instar a uniform quantity of plant extract i. e. 20ml from each concentration containing Phytoecdysteroid (20-hydroxyecdysone) was sprayed on 100 g of mulberry leaf with an atomizer for *peros* administration to 100 larvae in a replication. As silkworm continued to grow in size during Vth instar the quantum of mulberry leaf to be fed to these silkworms was increased during fifth instar period. The quantity of plant extract to be sprayed on mulberry leaves for feeding silkworm was also increased accordingly. Extract containing Phytoecdysteroid (PE) was administered to silkworms along with mulberry leaf at different duration i. e. on 2nd day (48h), 3rd day (72h), 5th day (120h) and at the onset of spinning during Vth instar. In addition to that a branded commercial formulation (Sampoorna) containing same hormone was also used as per its recommended concentration as positive Control. An absolute Control was also maintained in which only distilled water was used side by side for evaluating the performance of plant extract containing 20- hydroxyecdysone

Material of Study

Mulberry genotype

Ichinose (During Chawki rearing)
Goshoerami (During late age rearing)

Silkworm hybrid

Double hybrid (CSR6X CSR26) × (CSR2XCSR27)
The rearing of silkworms was done as per the package of practices formulated by the college (Anonymous, 2003), and the design of experiment was CRD.

Results and Discussion

Phytoecdysteroid for synchronization of maturation

In the present study when plant extract containing phytoecdysteroid (20-hydroxyecdysone) was administered person in the required concentrations at the onset of spinning when 3-5 per cent larvae were ripe in a batch, it resulted in maturation of 51. 22 and 59. 75 per cent larvae within 12 hour of treatment as against 25. 89 per cent observed in control-II(without treatment) within the same period. Although the Control-I (Hormone) was a purified product still then the crude plant extract containing phytoecdysteroid (20-hydroxycedysone) was equally effective for synchronization of maturation. All the remaining larvae matured quickly within 24 hours and all the larvae were transferred to mountages for seri position. However in case of Control-II
full maturation took more time and finished within 36 hours as compared to treated batches which were treated at the onset of spinning. The mounting duration was brought down from 36 hours (under normal condition) to 24 hours by the application of plant extract. Thus there was an average gain of 12 hours as a result of this treatment. It is therefore clear that when silkworms are treated at the onset of spinning with plant

extract containing 20-hydroxyecdysone, the mounting period can be shortened by about 40 to 50 per cent. The present study showed a clear difference in mounting duration between the Control-II and the treated ones. More than 80 per cent larvae matured in about 18 h after treatment, where as in Control-II, only 30-40 per cent larvae matured within same period when application was done at the onset of spinning.

Table 1: Synchronization in maturation at the end of 5th age

Percentage of Maturation				
S. No	**Hours**	**Control-I (Sampoorna Hormone)**	**Plant Extract (Phytoecdysone)**	**Control-II (Distilled Water)**
1	6	10. 56	15. 43	8. 31
2	12	59. 75	51. 22	25. 89
3	18	90. 55	83. 91	39. 89
4	24	100	100	79. 80
5	30			90. 40
6	36			100
7	42			
8	48			
9	54			
10	60			

The present study is almost in agreement with the finding of Philip *et al.* (2007) [5] who has also reported that 80 per cent larvae were ready for mounting in about 16 h after treatment. Where as in control only 15- 30 per cent larvae matured by that time.

A similar result was reported by using phytoecdysteroid extracted from *Sesuvivum portulacastrum* on silkworm hybrids (Nair *et al.*, 2002) [4] and that from caryophyllaeae family of plants on pure silkworm breeds (Trivedy*et al.*, 2003c) [9]. This difference in the larval and mounting duration is because of a physiological role played by feeding ecdysteroid exogenously along with mulberry leaf on the insect development system. The feeding larvae always contain a baseline level or low titer of ecdysone but reach to highest peak i.e pupation inducing peak before pupation (Sehnal, 1989) [8]. By giving an extract dose of plant based ecdysteroid at the critical time, the pupation inducing peak of ecdysteroid content in silkworm is advanced and maturation of worms is achieved within a stipulated period of time and thereby change the larval behavior as such. Due to its advantages, the formulations containing phytoecdysteroid has been accepted quickly by the farmers in some parts of the country and it has given them the desired result in the form of synchronized maturation which results in culmination of rearing process quickly. The study opens up possibility of utilization of the plant extract in bringing about the most desired effect of simultaneous maturation in commercial silkworm rearing carried out in field.

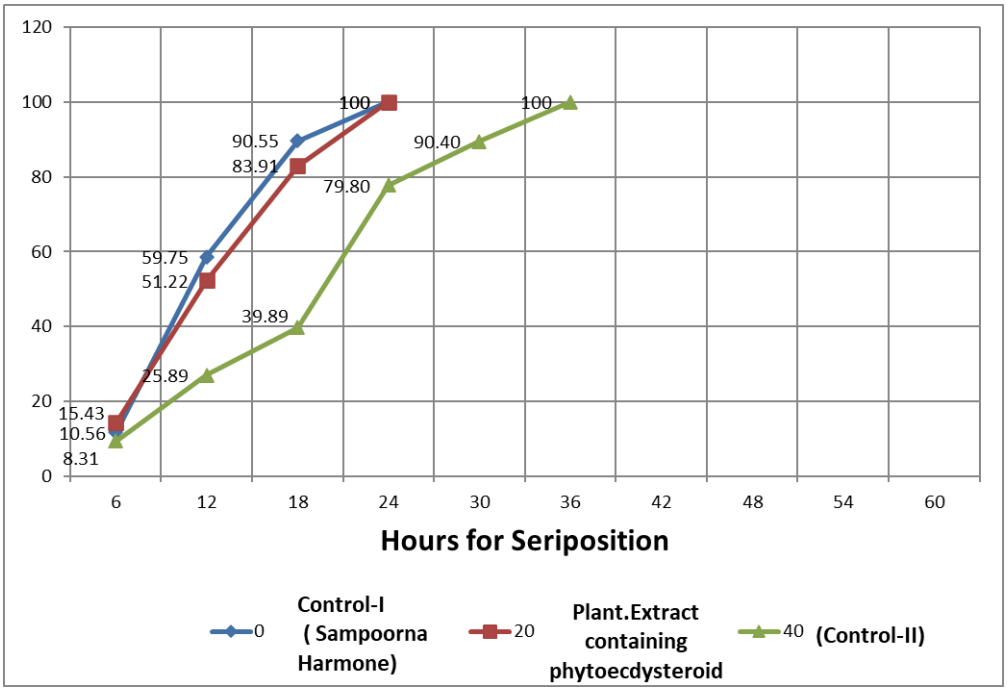

Fig 1: Hours for Seriposition

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