ENHANCEMENT OF COCOONING PROCESS IN *BOMBYX MORI* L. BY *SPINACIA OLERACEA* AND ITS EFFECT ON BIOLOGICAL AND ECONOMICAL CHARACTERS

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Abstract

One of the biggest problems encountered during silkworm rearing is the non-uniform larval maturation and synchronization of cocooning process at one time, which negatively affect the net cocoon production. The study was an attempt to use *Spinacia oleracea* (Spinach) as a source of phytoecdysteroid. Spinach can biosynthesize polypodine B and 20-hydroxyecdysone, which is the predominant insect - molting hormone. Mulberry leaves were dipped in two different concentrations of the aqueous extract of plant leaves (0.5 % and 1 %) and offered to larvae during different time periods of the fifth instar (24 hrs after 4th moult, 72, 120, 168 and every 48 hrs). The larval maturation events, cocooning process, as well as biological and economical characters for all tested larval groups were studied and compared the results with controls. The results showed that, the larval group fed every 48 hrs with treated mulberry leaves matured faster than all the other tested larval groups and succeeded in spinning cocoons by 80 % for both concentrations, whereas in control groups the cocoons ratio did not exceed 42 % at the same time. The effective rate of rearing was increased in almost all tested larval groups, compared to controls. The 72 hrs larval group showed the best cocoon characters. It may be concluded that, *Spinacia oleracea* leaf aqueous extract is able to hasten the larval maturation events and synchronize cocooning process when applied during the final larval instar. The labour, the rearing time and mulberry can be saved and Spinach is a cheaper source for commercial application in sericulture.

Key words: *Bombyx mori* , phytoecdysteroid , *Spinacia oleracea* (Spinach), cocooning process, biological and economical characters.

INTRODUCTION

Several efforts were made to evolve new technologies that are cast effective, labour saving and eco-friendly during silkworm rearing. Successful rearing is based on uniformity of worms maturation, spinning process and reduction of mortality rate. During silkworm rearing, farmers are forced to pick up and mount silkworms when they are ripe and the mounting process may be extended up to 2-3 days. This of course consumes a lot of time, labour and extra mulberry leaves and also increases costs beside marketing difficulties. Several scientists ascertained that feeding of exogenous supplements to the *Bombyx mori* L. larvae at certain stages of development has been shown to enhance growth and increase cocoon production.

Nair, *et al.* (2002and 2008) recommended the usage of exogenous ecdysteroids from plant sources, i.e. phytoecdysteroids (PEs) owing to its effectiveness, economic viability and easy availability from locally available plant materials. Phytoecdysteroids—structural analogues of molt hormones of insects, ecdysone (E) and 20-hydroxyecdysone (20E)—were first discovered in leaves of *Podocarpus nakai* in 1966. To date, the distribution pattern of phytoecdysteroids in plants has been studied sufficiently well, and their structural diversity has been established (Ufimtsev *et al.*, 2006

Phytoecdysteroids (PEs) are plants that contain insect steroid hormone analogues. Most PEs possess a cholest-7-en-6-ne carbon skeleton (C27), derived biosynthetically from cholesterol. These plants function as plant defenses against insects by acting as either feeding deterrents or through developmental disruption (Schmelz *et al.,* 2002).). Trivedy et al. (1998) extracted ecdysone from different plants, the presence of hormone was confirmed through thin layer chromatography and dose was quantified through HPLC.

The major objective of using PE in sericulture is to hasten the larval maturation events in the final larval instar and to synchronize the cocoon spinning process. This can save a lot of skilled labour which is required to pick up the ripe worms in time and substantial amount of mulberry leaf (Chou and Lu 1980). Masanori (1999) found that, the diet containing Spinach showed ecdysone-like effect on the silkworm larvae such as precocious molting, excessive larval molt and precocious

spinning depending on the fed stage. Though the effect was very similar to that exerted by the non-steroidal ecdysone agonist, tebufenozide.

The response of silkworm, *Bombyx mori* L. to minute quantities of these hormones or its analogues is beneficial. The intensity of influence was dependent on the time of application (Nair *et al.*, 2005). If administered when the silkworms are about to spin (onset of spinning), it will introduce simultaneous and uniform spinning and the mounting period can be shortened by about 40 to 50 per cent (Li *et al.*, 1992 and Rufai *et al.*, 2012).

Various plant sources were identified which contained moderate to high amounts of PEs and used them in sericulture to manage the silkworm rearing during the last stage of larval development (Wong *et al.*,1979; Chou and Lu, 1980 and Masanori 1999).

The present technology is an attempt to induce uniform spinning or to advance maturation events in silkworm by applying *Spinacia oleracea* (Spinach) leaf aqueous extract. As well as study the comparative response of silkworm to the application of Spinach at 24 , 72 , 120, 168 and every 48 hrs after the fourth molt.

MATERIALS AND METHODS

Disease-free eggs of univoltine local hybrid, were used in this investigation. Silkworms were reared under standard recommended conditions at 26 ± 2 ° C temperature, 75 ± 5 % relative humidity (Krishanswami *et al.*, 1973). Kokuzo-27 mulberry variety leaves were fed to silkworms four times a day as recommended. After the fourth ecdysis, larvae were divided into groups of 30 larvae and two control groups reared under the same conditions. *Spinacia oleracea* leaves were washed, shade dried, milled in a blender and then filtered by using a refinery. According to Vedhamathi (2004), two amounts of spinach fine powder were weighted (0.5 and 1 g) added to 100 ml distilled water, left overnight then they were filtered through layers of Whatman filter paper to obtain clear extract. The mulberry leaves , with known weight, were dipped in *Spinacia oleracea* two separate amounts of solutions (0.5 % and 1 %) just one time and then the larvae were allowed to feed on normal leaves during the fifth stage. The first treatment was after 24 hrs after the fourth molt, 2nd treatment was 72 hrs, 3rd treatment was 120 hrs and 4th treatment was 168 hrs after the fourth molt. There was a specific group which was allowed to

feed on treated leaves every 48 hrs during the fifth larval stage (48 hrs *). Each treatment were replicated four times of 30 larvae.

For control groups, one larval group was fed on leaves without any treatments and the other group fed on leaves treated only with distilled water only to verify that the effect was due to Spinach only.

Data collection: Larval weight was recorded after the fourth molt before the first feeding for all groups and on the eighth day. The duration of the fifth instar was calculated in the treated larvae. When the larvae started ripening, the rearing beds were examined every 6 hours and the ripe worms were collected, counted and transferred to mounting frames for cocoon building. After cocooning, the cocoon weight, cocoon shell weight, shell percentage and pupal weight were recorded for both sexes. The data were subjected to statistical analysis system version 9.1 program proc. GLM (SAS Institute 2003).

RESULTS AND DISCUSSION

The study of insect-plant interactions is currently one of the most actively investigated areas in chemical ecology, partly owing to its interesting perspectives for the development of new biopesticides with plant origins. These interactions involve numerous secondary plant metabolites that may interfere with behavior, growth and/or development of insects (Rharrabe *et al.*, 2010). Insect molting hormones, phytoecdysteroids, have been reported to occur in over 100 plant families. Plants, unlike insects, are capable of the biosynthesis of ecdysteroids from mevalonic acid, and in several cases the biosynthesis of phytoecdysteroids was also demonstrated to proceed via sterols. Phytoecdysteroids are secondary metabolites produced by many plants. They represent analogues of insect steroid hormones (ecdysteroids) that control insect growth, development, and reproduction (Lafont 1997).

Spinacia oleracea (Spinach) biosynthesizes polypodine B and 20-hydroxyecdysone, which is the predominant insect-molting hormone found in plant species (Adler and Grebenok, 1995). Li, *et al.* (2002) developed a research program on the PE biosynthetic pathway and its regulation in Spinach, one of the few accumulating crop species. They performed labelling experiments with excised Spinach leaves at different ontogenetic stages (or sub-cellular fractions of them) using both very early (mevalonic acid) or late (3-oxo-ketodiol, 2-deoxy-ecdysone, 2-

deoxy-20E, ecdysone) 20E precursors. The latter are classically used with Arthropods. Bakrim, *et al.* (2006) confirmed earlier findings that young leaves are unable to produce PEs and accumulate those produced by older ones (Grebenok and Adler 1991).

The constituents of 100 g raw Spinach as cited from USDA Nutrient database contain; carbohydrates=3.6g ; sugars= 0.4g; dietary fiber= 2.2g; fat = 0.4g; protein= 2.2g; vit.A equiv.= 469µg; vit. A= 9400 IU ; beta carotene = 5626 µg; lutein and zeaxanthin= 12198 µg; vit B9= 194 µg; vit.C= 28mg; vit.E= 2 mg; vit K=483 µg; calcium= 99mg and iron= 2.7mg.

In the present study, the data represented the cocooning percentages among each group under investigation were observed and tabulated in table (1). The highest percentage during the first day of spinning process was recorded for larval group fed on treated leaves every 48 hrs for both concentrations 0.5 and 1 %, they were 80 and 76, respectively. As well as the larval spinning process was ended by about 24hrs compared to the other tested groups and controls, except 72 hrs – 1% group. It may be concluded that, to hasten larval spinning process, it preferred to apply Spinach with 0.5 % every 48 hrs after the fourth molt.

Table 1. Cocooning percentage during spinning process

	First day	2nd day	3rd day	4th day
Control - 1	42 %	92 %	100 %	
Control - 2	40 %	86 %	100 %	
24 hrs - 0.5%	49 %	97 %	100 %	
24 hrs - 1%	10 %	79 %	88 %	100 %
72 hrs - 0.5	62 %	97 %	100 %	
72 hrs – 1 %	54 %	100 %		
120 hrs – 0.5 %	63 %	96 %	100 %	
120 hrs – 1 %	52 %	93 %	100 %	
168 hrs – 0.5 %	53 %	97 %	100 %	
168 hrs – 1 %	51 %	97 %	100 %	
Every 48 hrs - 0.5%	80 %	100 %		
Every 48 hrs - 1%	76 %	100 %		

This sort of reduction in Spinach treatment reduces labour involvement in picking of the ripe worms and saves mulberry leaves. The same conclusion was ascertained by Nair, *et al.* (2005) while investigated the differential response of

silkworms to phytoecdysteroids (PE) from plant source (*Radyx achyranthes*), and that PE extracted from *Sesuvium portulacastrum* (Nair *et al.*, 2002) and that from plant family Caryophyllacea (Trivedy *et al.*, 2003). This difference in the larval and mounting duration is because of a physiological role played by the exogenous ecdysteroid on the insect development system. The feeding larvae always contain a baseline level (low titer) of ecdysone but reaches to pupation inducing peak before pupation (Sehnal, 1989). By giving an extra dose of plant based ecdysteroid at the critical time, the pupation inducing peak of ecdysteroids content in silkworm is advanced and thereby change the larval behavior as such (Nair *et al.*, 2005).

The fifth instar larval duration was significantly affected as represented in Table (2), the effect was largely dependent on the time of application. When applied to 24 hrs and every 48 hrs old fifth larvae, the larval duration was shortened significantly from other tested groups as well as control groups 1 and 2 (30.667, 30.333 and 31.666 days, respectively). While the other treatments were not significantly .

larval weight was significantly increased in 72 hrs group compared to control groups 1 and 2 (2.55, 2.394 and 1.96 g, respectively). The larval group fed upon treated leaves every 48 hrs was not significantly differ with control 1, it recorded (2.544 and 2.394 g, respectively), while the control 2 group significantly recorded the lowest weight compared with the other tested groups.

Effective rate of rearing (ERR %) was not significantly affected among all tested groups comparing with controls 1 and 2. The highest percentage was 99.289 % recoded for 48 hrs groups.

Table 2. Larval parameters for 0.5 % Spinach concentration

	Cont.1	Cont. 2	24 hrs	72 hrs	120 hrs	168 hrs	Every 48 hrs*	LSD
Larval duration (day)	31.666 ab	31.666 ab	30.667 c	31.000 abc	31.000 abc	32.000 a	30.333 c	1.119
Larval wt (g)	2.394 ab	1.960 C	2.500 ab	2.550 a	2.327 ab	2.282 b	2.544 ab	0.262
ERR %	96.666 a	96.666 A	98.889 a	97.777 a	98.889 a	98.889 a	99.289 a	6.726

The same trend was observed in treatments with Spinach 0.1% (Table3). While all the other tested groups were not significantly affected upon feeding on treated leaves. ERR% was 100% in both 48 and 168 hrs and the percentages were not significantly affected in all tested groups. These results are confirmed by Nair *et*

al. (2010) as he mentioned that the PE had a significant positive effect on survival rate in the larvae treated in winter and summer. The positive effects of aqueous extract of Spinach leaves was also demonstrated by (Rehab Taha, 2012) when studied its effect on *B. mori* eggs.

Table 3. Larval parameters for 1% Spinach concentration

	Cont.1	Cont. 2	24 hrs	72 hrs	120 hrs	168 hrs	Every 48 hrs*	LSD
Larval duration (day)	31.833 a	31.833 a	30.833 b	31.833 a	31.833 a	31.833 a	30.833 b	0.804
Larval wt (g)	2.394 ab	2.387 ab	2.019 c	2.582 a	2.296 abc	2.155 bc	2.418 ab	0.335
ERR %	96.666 a	96.666 a	92.666 a	98.889 a	96.666 a	100 a	100 a	9.069

The present study also examined the effect of Spinach aqueous extract administration on pupa and cocoon characters with 0.1(Table 4) and 5% (Table 5). This is extremely important because the benefits of hastened and synchronized maturation will be annulled if it adversely affect the cocoon traits.

Table 4. Pupal and Cocoon parameters for 0.5 % Spinach concentration

	Cont. 1	Cont. 2	24 hrs	72 hrs	120 hrs	168 hrs	48 hrs*	LSD
Pupation %	92.222 a	91.667 a	90 a	94.444 a	96.667 a	97.778 a	96.667 a	15.487
♂ Cocoon wt	1.038 a	1.037 a	1.004 a	1.087 a	1.041 a	1.041 a	0.983 a	0.171
♂ Cocoon sh wt	0.225 ab	0.203 b	0.219 ab	0.262 a	0.252 ab	0.246 ab	0.229 ab	0.053
♂ Cocoon sh %	21.851 ab	19.682 b	21.825 ab	24.045 a	24.028 a	23.680 a	23.340 a	3.388
♂ pupal wt	0.770 a	0.772 a	0.763 a	0.823 a	0.788 a	0.785 a	0.745 a	0.118
♀ Cocoon wt	1.340 ab	1.329 ab	1.282 ab	1.380 a	1.274 ab	1.289 ab	1.221 b	0.121
♀ Cocoon sh wt	0.238 a	0.256 a	0.260 a	0.274 a	0.242 a	0.255 a	0.235 a	0.041
♀ Cocoon sh %	17.812 a	19.495 a	19.975 a	20.379 a	19.654 a	19.804 a	19.306 a	2.943
♀ pupal wt	1.071 a	1.076 a	1.025 a	1.101 a	0.952 a	1.025 a	0.982 a	0.158

Table 5. Pupal and Cocoon parameters for 1 % Spinach concentration

	Cont. 1	Cont. 2	24 hrs	72 hrs	120 hrs	168 hrs	48 hrs*	LSD
Pupation %	92.222 a	91.667 a	86.666 a	94.444 a	92.222 a	94.444 a	100 a	24.025
♂ Cocoon wt	1.038 a	1.037 a	0.968 a	1.068 a	0.978 a	1.054 a	0.993 a	0.107
♂ Cocoon sh wt	0.225 a	0.203 a	0.221 a	0.249 a	0.227 a	0.248 a	0.227 a	0.047
♂ Cocoon sh %	21.851 a	19.683 a	22.926 a	23.543 a	23.152 a	23.427 a	22.961 a	3.923
♂ Pupal wt	0.770 a	0.772 a	0.743 a	0.809 a	0.745 a	0.797 a	0.755 a	0.077
♀ Cocoon wt	1.330 a	1.337 a	1.137 b	1.347 a	1.151 b	1.272 ab	1.220 ab	0.176
♀ Cocoon sh wt	0.238 a	0.256 a	0.237 a	0.265 a	0.228 a	0.251 a	0.241 a	0.058
♀ Cocoon sh %	17.810 a	19.495 a	19.827 a	19.999 a	19.852 a	19.812 a	19.908 a	3.233
♀ Pupal wt	1.068 a	1.065 a	0.890 b	1.076 a	0.915 b	1.012 ab	0.972 ab	0.135

These results are in agreement with the earlier reports that the PE administration to silkworm for uniform maturation did not adversely affect cocoon characters (Nair et al., 2010, Trivedy et al., 2006 and Kumar et al., 2006). Phytoecdysteroids (PE) considered as a mediator in the regulation of silk protein synthesis, cocoon characters and influence on silkworm genome (Miao *et al.*, 2004). Fukuda (1942) was the first to propose that the increase of silk gland function during feeding period of the last larval instar is due to stimulation by ecdysteroid. Feeding on exogenous ecdysteroids (PE) increased the haemolymph ecdysteroid titer that apparently terminates feeding phase and shifts silk glands to their regression phase when they reach maximum protein synthesis (Akai and kiuchi, 1988).

From the obtained results, it may be concluded that, to hasten larval spinning process it preferred to apply Spinach with 0.5 % or 1 % every 48 hrs after the fourth molt, even the 72 hrs group showed the best pupal and cocoon parameters which were not significant. These results not in agreement with (Nair *et al.*, 2002, 2005, 2010), this is may be due to they use a pure extracted phytoecdysteroids.

CONCLUSION

The benefits of using *Spinacia oleracea* (Spinach) can be summarized in the following points; uniformity in spinning and shorten duration when use in appropriate time and dose. Save the rearing time because larval duration decreased and consequently mulberry leaves. Save the skilled labours to pick up the ripe worms in the time. It is able to stimulate silk protein, therefore, the silk production can be improved.

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تعزيز عملية التشرنق لدودة القز ***Bombyx mori*** L. بإستخدام نبات ***Spinacia oleracea*** و تأثير ذلك على الخصائص البيولوجية و الإقتصادية للحشره إعتمادا على وقت تطبيق و تركيز النبات

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واحدة من أكبر المشاكل التي نواجهها أثناء تربية دودة القز هو عدم نضوج اليرقات ودخولها مرحلة غزل الشرانق في وقت واحد مما يؤثر بالسلب على إنتاجية محصول الشرانق . هذه الدراسة محاوله لإستخدام نبات السبانخ و الذي يعرف بإحتوائه على مشابهات هرمون الإنسلاخ للحشرات .

تم غمر ورق التوت في تركيزين مختلفين من المستخلص المائي لأوراق السبانخ (٠,٥ % و ١ %) وتم تقديمه إلى اليرقات خلال فترات زمنيه مختلفه من العمر اليرقي الخامس فى خمسة معاملات مختلفة ، المعاملة الأولى (بعد ٢٤ ساعه من الإنسلاخ الرابع) والمعاملة الثانية (بعد ٧٢) والمعاملة الثالثة (بعد ١٢٠ ساعة)والمعاملة الرابعة (بعد ١٦٨ ساعة) والمعاملة الخامسة (كل ٤٨ ساعه) من الإنسلاخ الرابع ، وتم دراسة تأثير ذلك على سرعة نضوج اليرقات و عملية التشرنق و كذلك دراسة الخصائص البيولوجية و الإقتصاديه لكل المجموعات اليرقيه تحت الإختبار و مقارنة ذلك بمجموعات الكنترول .

أظهرت النتائج أن المجموعة اليرقية التي تغذت كل ٤٨ ساعة على ورق التوت المعامل نضجت أسرع من كل المعاملات الأخرى و نجحت في غزل الشرانق بنسبة ٨٠ % لكل من التركيزين مقارنة بالكنترول التي لم تتجاوز نسبة التعشيش فيها ٤٢ % في نفس الوقت . هذا بالإضافة إلى زيادة معدل كفاءة التربية في كل المجموعات اليرقية المعامله و تحت الإختبار مقارنة بالكنترول . وأظهرت المجموعة اليرقية التي تغذت على ورق معامل بعد ٧٢ ساعة من الانسلاخ أفضل النتائج .

ويمكن إستنتاج أن المستخلص المائي لأوراق نبات السبانخ قادر على الإسراع من نضوج اليرقات و تزامن عملية غزل الشرانق حينما يطبق خلال العمر اليرقي الخامس هذا بالإضافة إلى أنه مصدر رخيص و آمن و سهل التحضير و التطبيق على المستوي التجاري مما يوفر جهد و وقت التربية و أيضا ورق التوت .