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Research Article

EFFECT OF PHYTO ECDYSTEROIDS ISOLATED FROM DIPLOCLISIA GLAUCESCENS (BLUME) DIELS AND COSCINIUM FENESTRATUM (GAERTN.) COLEBR. AND JUVENILE HORMONE ANALOGUE ISOLATED FROM CULLEN CORYLIFOLIUM (L.) MEDIK. ON ECONOMIC PARAMETERS OF BOMBYX MORI L. UNDER FIELD CONDITION

Sreejit, C.M*[1]., Sasidharan K[2]., Chinchu Bose[3]., Thomas Mathew, P[4] and Banerji, A[3]

[1]Research Department of Botany, Sree Narayana Mangalam College Maliankara, Maliankara PO, Ernakulam, Kerala, India- 683516
[2]General Secretary, Kerala Sericulture Farmers Association, Nedungode Kalam, Kuthannur, Palakkad, Kerala
[3]School of Biotechnology, Amrita Viswa Vidyapeetam, Amritapuri, Kollam, Kerala, India- 690525
[4]Research Department of Botany, Union Christian College Aluva, Ernakulam, India-683102

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A B S T R A C T

In insects, moults and metamorphosis are initiated and coordinated by the interplay of juvenile hormone (JH) and moulting hormone (MH). External application of plants extracts containing phytoecdysteroids (PE) is found to reduce the maturation process in silkworms and synchronise it. Field level experiments were conducted to test the effects of PE isolated from the indigenous plants *Diploclisia glaucescens* (Blume) Diels (DG) and *Coscinium fenestratum* (Gaertn.) Colebr. (CS) on bivoltine double hybrids $FC_1 \times FC_2$ –a popular variety of silkworms. Synchronisation and significant reduction in maturation time by 12 h of silkworms was obtained on application of PE. A convenient method of using both JH analogue and PE extracts together on a single crop was also developed. Silk worm (*Bombyx mori* L.) positively responds to exogenous JH analogues and mimics when applied in minute quantities at appropriate time and thereby enhances the commercial traits. On application of JH analogue- Backuchiol - rich extract from another indigenous plant *Cullen corylifolium* (L.) Medik. (CC) produced an increase of 15-20% with regard to cocoon weight and shell weight. This technology is simple, affordable and could be scaled to industrial level.

INTRODUCTION

The process of metamorphosis is characteristic to larval growth and development in insects and is controlled by circulating hormones like Juvenile Hormone (JH) and Moulting Hormone (MH) (Wigglesworth, 1985). Ecdysteroids (MH) regulate the time and onset of moulting, while JH determines whether it is larval to larval or from larval to pupal (Sehnal, 1989; Riddiford, 1994). When the threshold JH level to retain the larval features diminishes in the haemolymph below the normal level, the larvae prepare to metamorphose into pupa (Nair *et al*., 2003). In China, various plant extracts rich in ecdysteroids are used in the last instar stage of *Bombyx mori* L. for fastening and synchronisation of the maturation process (Chow and Lu, 1980). Such studies were totally lacking in India till last decade, but since then some serious attempts have been made in this regard and some plants containing ecdysteroids have been identified (Banerji *et al*., 1971). Considerable amount of ecdysteroids were isolated from DG and CS collected from Wayanad District, Kerala as a part of our bioprospection study. Phytoecdysteroid rich extracts from these plants were used in sericulture for the first time. Reduction in mounting time and decline in economic parameters due to phyto ecdysteroid use were recorded. Judicious use of plant extracts towards the end of last instar stage reduces the labour involved in mounting and also help sericulture farmers rescue the crop in case of acute leaf shortage.

The silkworm *Bombyx mori* L. positively responds to exogenous application of JH analogues, when applied in minute quantities, at appropriate time by enhancing commercial traits such as cocoon weight, shell weight and length of the silk filament (Akai *et al*., 1985). Effect of JH analogue along with PE produces synchronisation in maturation as well as increase in cocoon and silk parameters. JH analogue - Backuchiol- rich extract isolated from CC has been used along with PE on the commonly cultivated bivoltine double hybrids (FC_1 x FC_2) silkworm variety. The extraction

Corresponding author:* **Sreejit, C.M
Research Department of Botany, Sree Narayana Mangalam College Maliankara, Maliankara PO, Ernakulam, Kerala, India- 683516

technology is simple and could be put to industrial level so that import of JH analogues and PE from outside to meet the needs of sericulture farmers in India could be avoided.

MATERIAL AND METHODS

Isolation

Isolation of ecdysterone from DG and CS

Diploclisia glaucescens (Blume) Diels is a liana species belonging to Menispermaceae family common in forest areas of Kerala state and is used by tribal folks in their indigenous medicinal system as a remedy for back pain.

The specimens of DG were collected from Kalpetta, ($11^0$59'43" North, $76^0$09'43"East), Wayanad District, Kerala in the month of March 2011. The identity was established by comparing the characteristics as described in floras; a voucher specimen has been submitted in the M. S. Swaminathan Herbarium, Wayanad, Kerala (MSSH-0105). 100 g of leaves were put to sequential extraction with solvents of increasing polarity starting from Petroleum Ether (PEt) and the ecdysterone positive fractions were obtained in Ethyl Methyl Ketone (MEK) and Methanol (MeOH) fractions. MEK and MeOH extracts were put to column chromatography and pure white crystals of ecdysterone were obtained from ecdysterone positive column fractions.

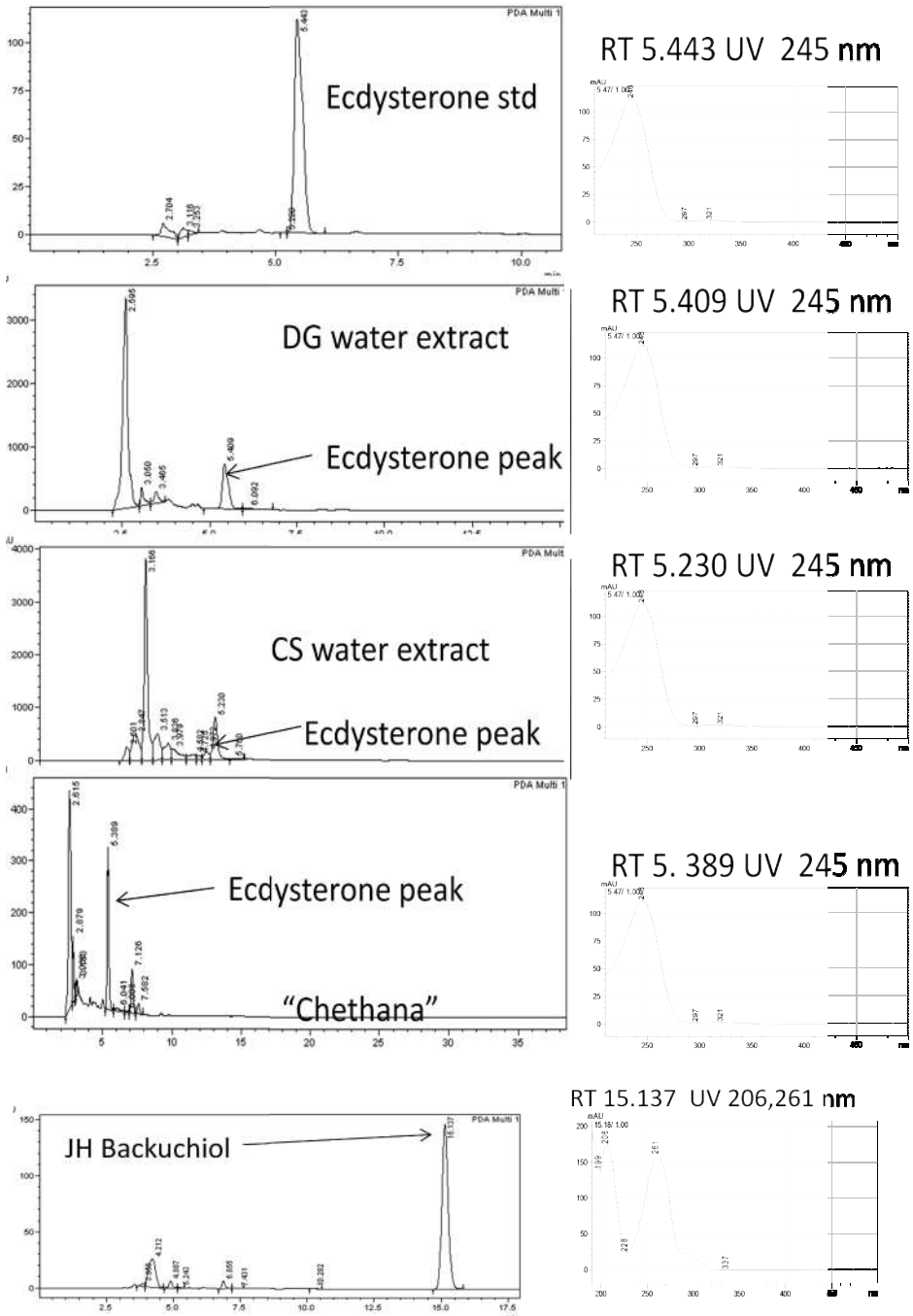

Fig 1 HPLC Profile of Ecdysterone standard, ecdysterone rich water extracts and JH. Analogue Backuchiol. DG= *Diploclisia glaucescens* (Blume) Diels., CS= *Coscinium fenestratum* (Gaertn.) Colebr., RT= Retention Time. HPLC Analyses were performed on a Shimadzu-SPD-M20A HPLC, equipped with DAD detector, with Phenomenex luna 5u C_{18} (2) 100A, size 250 × 4.60 nm column. All the compounds were detected at 254 nm at room temperature with an elutent flow rate of 1.2 ml/min and an injection volume of 10 µL. The mobile phase consisted of MeOH (A) and water (B) in 1: 1 ratio.

Coscinium fenestratum (Gaertn.) Colebr. is an another liana species belonging to Menispermaceae family seen in forest areas of Kerala state, which is highly endangered due to over exploitation and is used by tribal folks in their indigenous medicinal system for hyperpressure and diabetes. The CS specimens were collected from Boys Town, Mananthavady, Wayanad District, Kerala in the month of July 2012.(11°84'18" N, 75°92'09" E). The leaves were collected and air dried. Botanical voucher specimen has been deposited in M. S. Swaminathan Herbarium, Wayanad, Kerala (MSSH-0104). 100 grams of leaves were put to sequential extraction with solvents of increasing polarity starting from PEt and the ecdysterone positive fractions was obtained in the MEK fraction. MEK extract was put to column chromatography and pure white crystals of ecdysterone were obtained from ecdysterone positive column fractions.

The ecdysterone isolated from both DG and CS was characterised by TLC, HPLC, UV spectroscopy, IR spectroscopy and LC-MS and was found to be more than 98 % pure and was comparable with the standard (Sigma).

Isolation of backuchiol rich plant extract

Seeds of *Cullen corylifolium* were purchased from a local ayurvedic crude drug supplier shop at Kollam District, Kerala in the month of July 2012.

A voucher specimen was has been submitted in the herbarium M.S.Swaminathan Herbarium, Wayanad, Kerala (MSSH-0106). Dried seeds (100 gm) were powdered and extracted with PEt in a Soxhlet extractor. After 24 hours solvent was removed under reduced pressure using a rotary evaporator at 35^0C to obtain a 9 % extract. On TLC, bakuchiol appeared as an olive green spot (PEt: Ethyl acetate: 7: 3; vanillin-Hydrochloric acid (HCl) spray). PEt extract was purified further using column chromatography with acidic alumina (Merck). Psorlane and Isopsorlane fractions were removed and backuchiol rich extract was obtained which was confirmed with HPLC.

Rearing of silk worms

The present study was conducted in the month of November 2012 in the well maintained field of a farmer who had a track record of more than twenty years in sericulture. Bivoltine double hybrids (FC_1 x FC_2) were brought in the second instar stage from Chawki rearing centre, and grown in field conditions. Temperature was maintained at 20 $\pm$ 3 °C and humidity conditions were adjusted to 75$\pm$ 5% relative humidity. 12 hours day/night period was available during the study period. Mulberry leaves of Victory 1 (V1) genotype from a periodically watered garden was fed liberally three times a day.

Ecdysterone (MH) preparation

A stock solution (10 mg ecdysterone in 100 ml water) was diluted to 10, 20 and 30 ppm concentrations respectively.

JH analogue preparation

Backuchiol rich extract (10 mg) was prepared as an emulsion using minimum quantity of acetone and Tween® 20 in 100 ml water. From this stock solution 1.25, 2.5, 5 and 10 ppm concentrations were made.

Preparation of water extracts and positive controls

Dried leaves of both DG and CS (10 g) were boorishly powdered and refluxed with 100 ml water each for 1 h. The extracts were made up to a constant volume (75 ml). 1 ml each of the extracts was taken for HPLC. The HPLC profiles of the extracts were monitored for ecdysterone absorbance (Figure 1). The HPLC profile of the pure ecdysterone solution (10 mg in 100 ml water) gave an absorbance of 100 milli absorbance units (mAU) for ecdysterone peak. Both DG and CS water extracts showed an absorbance of 800 mAU for ecdysterone peaks. 15 ml of DG and CS water extracts were made up to 100 ml as stock solutions. A commercially available 'phytoecdysteroid' preparation by the name '*Chethana*' was also taken as positive control. The HPLC profile of 1 ml of that (1:1dilution with water) gave an absorbance of 300 mAU for the ecdysterone peak. Two concentrations (15 ppm and 30 ppm) were made from both DG and CS extract stock solutions for application on silkworm larvae. Ecdysterone positive control was diluted to the same level but '*Chethana*' was used as such. Medium control and general control were also kept for comparison.

Design of experiments

Two experiments were done for testing the effect of PE and JH analogue on silkworms as given below. In the first experiment effect of PE was tested on *Bombyx mori* while in the second one both JH analogue and PE were used in the same batch of silkworms. This method incorporates the increase in economic parameters imparted by JH analogue as well as the fastening and synchronisation provided by the PE into one. A JH analogue rich extract from the plant CC was applied in ppm concentration on bivoltine di hybrids ($FC_1 \times FC_2$) in field conditions and its effects on economic parameters were analysed. However the time of use and concentration of JH analogue and PE should be given extra care for maximum benefits. Most JH analogues used in sericulture prolongs the larval period considerably, so any increase in larval period due to JH analogue application was also recorded.

Experiment No.1: PE extracts alone

Thirty larvae of bivoltine double hybrids (FC_1 x FC_2) of same size were taken out on the sixth day of fifth instar stage just before the onset of spinning and were grown in aerated trays of size (2 feet x 1 feet). Each concentration groups were taken in triplicate. Twenty ml of ecdysterone (PE) solution for each concentration were sprayed on to 20 g of fresh V1 mulberry leaves. There were total seven treatments of which, the first two were PE isolated from DG in pure form. In the next four, water extracts from DG and CS were used in two different concentrations. The seventh treatment was a commercially available formulation. Medium and absolute controls were maintained in parallel to compare the results. The sprayed leaves were allowed to dry up and were given as last feed. The medium control was treated with an equal quantity of solution without ecdysterone, while the absolute control was given leaves without any treatment.

Experiment No.2: JH analogue and PE extracts together

Fifty larvae of bivoltine double hybrids (FC_1 x FC_2) of similar size were taken out before feeding after the fourth moult and grown in aerated trays of size (3 feet x 2 feet). Each concentration groups were taken in triplicate. Twenty ml of JH

analogue for each concentration were sprayed just after 48 hours from the first feed in the fifth instar stage on the larvae directly using a garden sprayer. The sprayed larvae were allowed to dry up for half an hour and after that fed with fresh mulberry leaves. Medium and absolute controls were maintained in parallel to compare the results. The medium control was treated with an equal quantity of emulsion without JH analogue, while the absolute control was without any treatment.

Just before the onset of spinning twenty ml of 30 ppm PE solution were sprayed on to 20 g of fresh V1 mulberry leaves. The sprayed leaves were allowed to dry up and were given as last feed. Medium and absolute controls were maintained in parallel to compare the results. The medium control was given leaves sprayed with an equal quantity of solution without ecdysterone, while the absolute control was given leaves without any treatment.

RESULTS

PE rich extracts alone

The effects of water extracts containing ecdysteroids on the maturation and economic parameters are shown in Table 1 and Figure 2.

About 80 % larvae maturation was achieved by the end of 18 hours in both 15 and 30 ppm Ecdysterone concentrations. Both DG and CS water extracts also showed similar results in both concentrations. But '*Chethana*', the commercial preparation showed only 70 % maturation within this time. The medium control showed no significant difference from the general control and both exhibited only 50% maturation by this time. A similar result was reported by using plant extracts from *Sesuvium portulacastrum* on silkworm hybrids (Nair *et al.,* 2002) and that from plants of Caryophyllaceae family on pure silkworm breeds (Trivedy *et al*., 2003). Nair *et al*. (2005) in their study with phytoecdysteroids has recorded that within 18 h of the treatment, about 81% of the larvae matured whereas by the same time in the control only 37% of the larvae matured. Recently, Rufaie *et al*. (2012) during their study reported 58% maturation within 12 hours after phyto ecdysterone administration as against 27% observed in control. A small (10-12%) increase in hastening of spinning was shown by DG water extract above all the treatments. This may be due to the presence of a plethora of ecdysteroid related compounds reported from the plant (Bandara *et al*.,1989a; 1989b) which might had exerted positive synergistic effects. Survival rate was not affected by any of these ecdysterone treatments. An above 90 % survival rate was shown by all the treatments.

Table 1 Result of application of Ecdysterone rich plant extracts (PE) on Bivoltine double hybrids (FC1 x FC2) at Sasi's farm, Kuthanoor, Palakkad Dist, Kerala

Dosage	Time of application	Maturation time		Cocoon number (Survival Rate)	Single cocoon wt	Single shell wt	Shell ratio	Filament length	denier
		Within 18 hrs (%)	After18 hrs (%)						
15 ppm EC	On 6th day of last instar when 5% larvae has started spinning	82	18	28.67	1.99	0.37	18.59	1085	2.9
30 ppm EC	On 6th day of last instar when 5% larvae has started spinning	84	16	29.33	1.84	0.33	17.93	892.5	2.6
15 ppm DG	On 6th day of last instar when 5% larvae has started spinning	94	06	29.33	1.93	0.35	18.13	1053.5	2.9
30 ppm DG	On 6th day of last instar when 5% larvae has started spinning	94	06	30	1.99	0.37	18.59	852.7	2.8
15 ppm CS	On 6th day of last instar when 5% larvae has started spinning	88	12	30	1.80	0.36	20.00	927.6	2.8
30 ppm CS	On 6th day of last instar when 5% larvae has started spinning	85	15	29.67	1.84	0.34	18.48	850.3	2.7
Chethana	On 6th day of last instar when 5% larvae has started spinning.	70	30	27.67	1.75	0.33	18.86	995.9	2.7
Med control	On 6th day of last instar when 5% larvae has started spinning	53	47	30	1.70	0.35	20.59	1127.2	3.2
Gen Control	On 6th day of last instar when 5% larvae has started spinning	53	47	30	1.85	0.36	19.46	818	2.7

EC=Ecdysterone, DG=*Diploclisia* leaf water extract, CS=*Coscinium* leaf water extract.

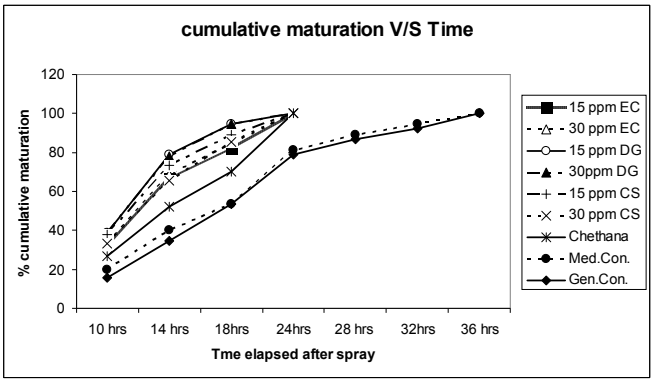

Fig 2 Cumulative maturation of larvae over time after MH Water Extract Spray. EC=Ecdysterone, DG= *Diploclisia* water extract, CS=*Coscinium* water extract.

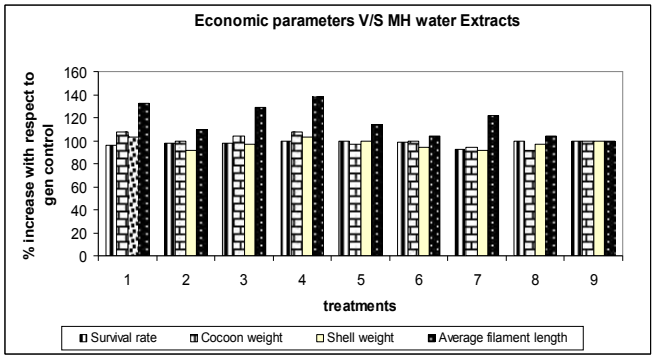

Fig 3 Economic parameter change above General Control due to MH Spray. 1=ecdysterone (EC) 15 ppm, 2= EC 30 ppm, 3=*Diploclisia* water extract (DG) 15 ppm, 4= DG 30 ppm, 5= *Coscinium* water extract (CS) 15 ppm, 6= CS 30 ppm, 7="*Chethana",* 8= Medium control, 9= General control.

These results agree with what has been reported early by Shivakumar *et al*., (1995; 1996). No significant mean difference was observed in the case of cocoon weight with respect to different doses of water extract treatments (Figure 3). The result of the present study was in line with the earlier works by Shivakumar *et al*., (1995; 1996). However in the case of shell weight, as recorded by Shivakumar *et al*., (1996) , a slight weight reduction was observed in the present study with respect and 30 ppm concentrations of PE, CS water extract and 'Chethana' treatments. Chow -Wei-Shan (1980) has reported that treatment of phytoecdysteroids in the later stages of *Bombyx mori* L. reduced shell weight which coincided with the tendency to curtail the feeding period of the last instar. With 15 ppm concentration level for PE no significant change of shell weight was observed in any of the treatments. Average filament length increased up to 33 % over general control. Results of Tukey HSD test for cocoon and shell weights showed that mean difference for all the seven PE and water extract treatments were not significant (Figure 6).

JH and PE extracts together

The effects of both JH and PE rich plant extracts together on the same batch of silkworm is summarised in Table 2.

alone and JH and PE together and with 2.5 ppm concentration the increase were 17% and 18% respectively (Figure 5).

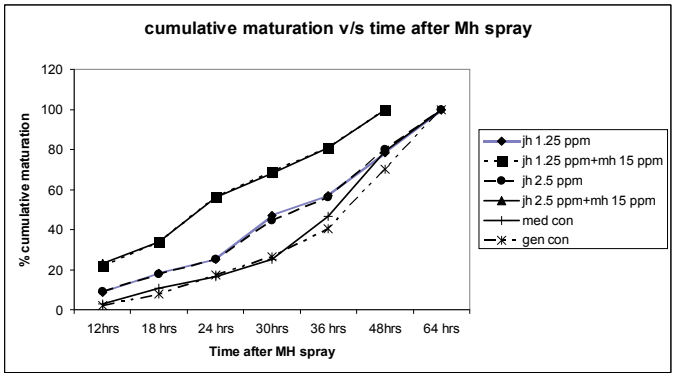

Fig 4 Cumulative maturation of larvae over time after MH spray. (jh= juvenile hormone analogue backuchiol rich extract, MH = ecdysterone).

Cocoon weight increase was constant for both JH alone and JH and PE together treatments by 10% for 1.25 ppm and 15% for 2.5 ppm concentrations. Shell weight increase showed a 15% increase for 1.25 ppm JH alone and 12% increase when used along with PE. With 2.5 ppm concentration both treatments gave a 15% increase each.

Table 2 Result of application of Backuchiol (JH analogue) + Ecdysterone (PE) on Bivoltine double hybrids (FC_1 x FC_2) at Sasi's farm, Kuthanoor, Palakkad Dist, Kerala

Backuchiol (JH analogue) + Ecdysterone (PE) Dosage	Time of application JH	Time of application PE	Length of Vth instar	Cumulative maturation%		Average Larval wt (10 Larvae)	Single cocoon wt	Single shell wt	Shell ratio	Filament length	denier
				Within 36 Hrs	After 36hrs						
1.25ppm	after 48 hrs in Vth Instar stage	On 6th day of last instar when 5% larvae has started spinning	168 hrs	81	19	43.46 (110%)	1.70 (110%)	0.37 (112%)	21.76	851.7 (90%)	2.8
1.25ppm	after 48 hrs in Vth Instar stage	----	168 hrs	57	43	43.84 (111%)	1.70 (110%)	0.38 (115%)	22.35	1088.5 (116%)	2.9
2.5ppm	after 48 hrs in Vth Instar stage	On 6th day of last instar when 5% larvae has started spinning	168 hrs	81	19	46.71 (118%)	1.78 (115%)	0.38 (115%)	21.34	1054.2 (112%)	2.8
2.5ppm	after 48 hrs in Vth Instar stage	-----	168 hrs	56	44	46.14 (117%)	1.78 (115%)	0.38 (115%)	21.34	1014.2 (108%)	3.1
Media Control	after 48 hrs in Vth Instar stage	On 6th day of last instar when 5% larvae has started spinning	168 hrs	46	56	40.27 (102%)	1.65 (105%)	0.34 (103%)	20.73	996.1 (106%)	2.6
General control	after 48 hrs in Vth Instar stage	On 6th day of last instar when 5% larvae has started spinning	168 hrs	40	60	39.36 (100%)	1.55 (100%)	0.33 (100%)	21.29	937.8 (100%)	2.7

About 80 % maturation was achieved by the larvae by the end of 36 hours while the control achieved only 40% maturation by this time (Figure 4). The medium control showed no significant difference from the general control. However there was an initial delay of about 18 hours in starting the spinning during this experiment due to a heavy downpour which occurred just after the PE treatment and the temperature stayed below 20^{0}C overnight.

When used together on the same batch of *Bombyx mori* L. both increase in economic parameters and hastening in maturation with synchronisation was attained. An 11% and 10% increase was obtained with respect to larvae weight with 1.25 ppm JH

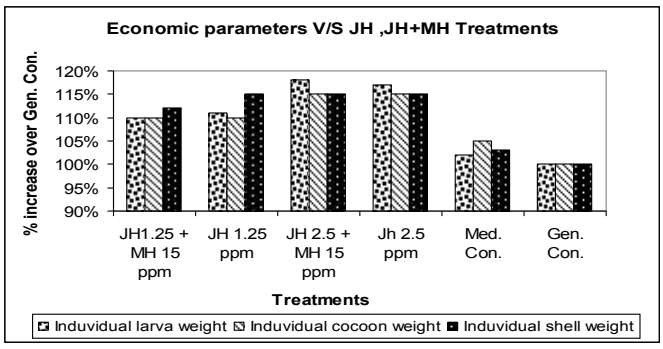

Fig 5 Economic parameter change above General Control due to JH and JH+ MH Spray. JH=Backuchiol, MH =ecdysterone.

The increase in shell weight has been assumed to be converted into reelable silk as there was increase in average filament length except in 1.25 ppm JH and PE together treatment. An 8% to 16% increase was recorded for average filament length. The medium control did not show any significant increase over the general control. Results of Tukey HSD test for cocoon weight and shell weight showed that mean difference for all the four JH treatments were significant at p<05 level from general control for both cocoon and shell weight (Figure 6).

Fig 6 Tukey HSD Test Result. JH= Backuchiol rich plant extract, EC= ecdysteroid rich plant extract, DG= *Diploclisia* water extract, CS=*Coscinium* water extract.

MH		Cocoon weight	Shell weight
		Mean Difference	
Gen. Control	15 ppm EC	-0.13667	-0.01667
	30 ppm EC	0.014	0.02333
	15 ppm DG	-0.07533	0.00333
	30 ppm DG	-0.136	-0.01333
	15 PPM CS	0.05267	0.00133
	30 PPM CS	0.01333	0.01733
	CHETHANA	0.108	0.02533
	Med.Con.	0.154	0.00733

JH+MH		Cocoon weight	Shell weight
		Mean Difference	
Gen. Control	JH1.25 ppm + 15ppm MH	-.15367*	-.04067*
	JH 1.25 ppm	-.14833*	-.05133*
	JH 2.5 ppm + MH 15 ppm	-.22533*	-.04967*
	JH 2.5 ppm	-.23367*	-.05000*
	Med control	-.09500	-.01200

** Significant at P=.05 level.*

Similar type of increase in larvae weight has been reported on administration of JH analogues in previous studies (Akai *et al*., 1985; Trivedy *et al*., 1997). But different from those studies which invariably brought about an increase in larval period, here the increase in larva weight was achieved without any increase in larval period and they commenced spinning before 168 hours of fifth instar as in control. This result corroborates with that of Muroga *et a*l., (1975), Trivedy *et al*., (1996) and Nair *et al*., (2003) that increase in economic traits of silk worms on application of JH analogues need not necessarily be accompanied by an increase in larval period.

DISCUSSION

The result of the present study indicates that natural compounds mimicking juvenile hormone activity can be judiciously used in sericulture for the benefit of farmers. The problem of non uniform maturation in silkworms could be solved to a large extent by administering phytoecdysteroids isolated from DG and CS. Water extract of leaves of these plants were equally good in performance with that of the pure compound isolated. The extracts were standardised using HPLC for evaluating the level of ecdysteroids present in them. This technology can be industrially utilised for production of ecdysteroid rich formulations for synchronising maturation and spinning for the benefit of sericulture farmers. Mounting of larvae is a cumbersome process and includes a lot of care and labour. If the cocooning is not synchronised, the larvae which are late in cocooning may damage the already cocooned shells by urinating on it and changing its colour to brown and making it unfit for sale. Phytoecdysteroids induce speedy and synchronised maturation of larvae when applied at appropriate time. The economic parameters were not significantly affected by the PE application which otherwise would nullify the effect induced by ecdysteroids. Elevated level of ecdysteroids apparently shifts silk glands to their regression phase and stops protein synthesis. Since the PE application is done at the onset of spinning the protein synthesis would have attained its peak by this time and this might be the reason why the economic parameters are not much adversely affected by PE spray.

Backuchiol rich extract isolated from the seeds of CC showed hormetic effect when applied at appropriate time in ppm concentration level. Economic parameters of bivoltine double hybrids (FC_1 X FC_2) were increased by 10-15% without any increase in larval period. Increase in larval period in the fifth instar stage is crucial from Indian sericulture view point because more of larval period means more leaves and more labour. Here our extract shows an additional advantage since the increase in economic parameters was not at the cost of additional larval period. Hence whatever the increase, is directly transferred into the poor farmer's pocket as additional income. The potential of using both JH and PE together as a model for farmers was found excellent. The economic parameters increased in the same manner whether JH was sprayed alone or together with PE.

As a concluding note it can be assumed that the backuchiol and PE rich extracts isolated from the plants can be used to increase the silk production in our country. Plants from our own country could be used for the extraction so that import of juvenile hormone mimics and PE from outside could be reduced.

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References

Akai H., Kimura K., Kiguchi K. and Shibukawa A. (1985) Increase of silk production by repeated treatment with a juvenile hormone analogue. *J. Seric. Sci. Jpn.,* 54: 297-299.

Bandara B.M.R., Jayasinghe L., Karunaratne V., Wannigama G.P., Bokel M., Kraus W. and Sotheeswaran S. (1989a) Ecdysterone from stem of *Diploclisia glaucescens*. *Phytochemistry*, 28:1073-75.

Bandara B.M.R., Jayasinghe L., Karunaratne V., Wannigama G.P., Bokel M., Kraus W. and Sotheeswaran S. (1989b) Diploclisin, a bidesmosidic triterpenoid saponin from *Diploclisia glaucescens*. *Phytochemistry,* 28: 2783–2785.

Banerji A., Chintalwar G.J., Joshi N.K. and Chadha M.S. (1971) Isolation of ecdysterone from Indian plants. *Phytochemistry*, 10:2225-2226.

Chow W.S. and Lu H.S. (1980) Growth regulation and silk production in Bombyx mori L. from phytogenous ecdysteroids. In: Progress in ecdysone Research. Hoffman J.A. (Ed.), Elsevier/North Holland Biomedical press, Amesterdam.

Muroga A., Nakajima M., Aomori S., Ozawa Y. and Nihmura M. (1975) Utilization of the synthetic juvenile hormone analog to the silkworm rearing on mulberry leaves. *J. Seri. Sci. Jpn.,* 44: 267-273.

Nair K.S., Trivedy K., Shyam R., Chintalwar G.J., Chinya P.K., Datta R.K., Chattopadhyay S. and Banerji A. (2002) Ecdysteroid from *Sesuvium portulacastrum* for synchronization of cocoon spinning in silkworm, *Bombyx mori* L. In: Advance in Indian Sericulture Research. Dandin S.B. and Gupta V.P. (Eds.) CSRTI, Mysore.

Nair K.S., Nair J.S., Trivedy K. and Vijayan V.A. (2003) Influence of backuchiol, a JH analogue from Bemchi (*Psoralia corylifolia*) on silk production in silkworm *Bombyx mori* L.(Bombycidae: Lepidoptera). *J. Appl. Sci. Environ. Mgt.*, 7(2): 31-38.

Nair K.S., Yun-Gen M. and Nirmalkumar S. (2005) Differential response of silkworm, Bombyx mori L. to phytoecdysteroid depending on the time of administration. *J. Appl. Sci. Environ. Mgt*., 9(3): 81 - 86.

Nair K.S., Babu C.M., Trivedy K. and Chinya P.K. (2010) Ecdysteroid extract from common catchfly, *Silene gallica* L. for rearing management of silkworm, *Bombyx mori* L. and stabilized cocoon crop. *J. Biopest.,* 3(1): 217 - 221.

Riddiford L.M. (1994) Cellular and molecular actions of juvenile hormone. I. General considerations and premetamorphic actions. *Adv. Insect. Physiol*., 24: 213-274.

Rufaie, Z.H., Munshi, N.A., Sharma, R. K., Ganie, N.A. and Malik, G.N., 2012. Effect of phytoecdysteroid (β-ecdysone) on synchronization of maturation in silkworm *Bombyx mori* L. *IJABR,* 2(2): 238-240.

Sehanl F. (1989) Hormonal role of ecdysteroids in insect larvae and during metamorphosis In: Ecdysone. Koolman J. (Ed.), Georg Thieme Verlag. Stuttgart.

Shivakumar G.R., Raman K.V.A., Reddy K.V.R., Magadum S.B., Datta R.K., Hussain S.S., Banerji A. and Chowdhary S.K. (1995) Effect of phytoecdysteroids on larval maturation and economic parametters of the silkworm *Bombyx mori* L.. *Indian J. Seric*., 34(1): 46-49.

Shivakumar G.R., Raman K.V.A., Magadum S.B., Datta R.K., Hussain S.S., Banerji A. and Chowdhary S.K. (1996) Effect of phytoecdysteroids on the spinning, cocoon and reeling parameters of the silkworm *Bombyx mori* L.. *Allelopathy J.,* 3(1):71-76.

Trivedy K., Porcheron P., Lafont R., Magadum S.B. and Datta R.K. (1996) Alteration of ecdysteroid titre and spinning by juvenile hormone analogue, R394 in silkworm *Bombyx mori*. *Indian J. Exp. Biol*., 34: 543-546.

Trivedy K., Nair K.S., Ahsan M.M. and Datta R.K. (1997) A juvenile hormone mimic modulated enhancement of silk productivity in silkworm *Bombyx mori* L., *Indian J. Seric.,* 36: 35-38.

Trivedy K., Dhar A., Kumar S.N., Nair K.S., Ramesh M. and Gopal N. (2003) Effect of phytoecdysteroid on pure breed performance of silkworm, *Bombyx mori* L. *Int. J. Indust. Entomol.,* 7(1): 29-36.

Wigglesworth V. B. (1985) Historical perspectives. In: Comprehensive insect physiology, biochemistry and pharmacology. Kerkut G. A. and Gilbert L. I. (Eds.), Oxford: Pergamon Press.

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