Asian Journal of Chemistry Vol. 21, No. 1 (2009), 36-46

Effect of Pollarding on Foliar Chemistry of *Terminalia arjuna* and Rearing Performance of Tasar Silkworm

SHRUTI RAI†‡*, K.K. AGGARWAL* and C.R. BABU†
School of Biotechnology, Guru Gobind Singh Indraprastha University
Kashmere Gate, Delhi-110 006, India
Tel/Fax: (91)(11)24360060; E-mail: kkagg36@yahoo.com

Temporal and spatial distribution of plant's foliage generate microclimatic conditions that influence the chemistry of foliage, which in turn regulate the herbivory and foraging behaviours of insects. These aspects have not been studied with respect to tasar silkworm and its host plant, *Terminalia arjuna*. Pollarding reduces all the foliar constituents of *T. arjuna*, particularly the defense chemicals thus confirming the role of canopy in foliar chemistry. Pollarding of trees simulates low light conditions in the canopy of *T. arjuna* with respect to variation in foliar constituents. Thus in terms of antinutrients, pollarding has beneficial effect for tasar silkworm rearing.

Key Words: Pollarding, Foliar, *Terminalia arjuna*, Tasar silkworm.

INTRODUCTION

Sericulture is a cottage and/or agro forestry and forestry-based industry and provides sustainable livelihoods to rural communities. There are many factors that contribute to the low silk productivity in India, particularly non-mulberry silk. The non-mulberry silkworms are yet to be domesticated. Their host plants are forest species and their sericulture is practiced by tribal communities for a short period of the year (July-August and October-November), as one of the alternative livelihood[1]. The interactions between silkworms and their host plants constitute an example of beneficial herbivory. Five instars have been recognized in the life cycle of tasar silkworm, *Antheraea mylitta*, besides pupa and adult moth. All the larval instars feed on host trees, which could either be planted or found in forest communities[1].

†Centre for Environmental Management of Degraded Ecosystems, School of Environmental Studies, University of Delhi, Delhi-110 007, India.

‡Present address: Room No. 547, Ministry of Environment and Forests, Paryavaran Bhawan, CGO Complex, New Delhi-110 003, India; E-mail: shrutisrai@rediffmail.com

Plants and their insect herbivores are constantly at chemical war, as the plants have to overcome herbivore pressure and herbivores have to overcome host plant's chemical defenses. These interactions led to the evolutionary trends in chemical traits that influence survival, growth and reproduction of insect herbivores. The herbivores in turn evolve their own defense responses to overcome host plant's chemical defenses. The consequence of these continued interactions is the extensive diversity in traits that are involved in defense strategies against each other leading to evolution of races, subspecies and species in both plants and insects and to the regulation of community structure[2-5].

The quality of leaves of host plants as food for insects may play an important role in keeping forest consumption at low levels[6]. Several leaf traits such as nitrogen content[7], water content[8], carbohydrate and phenolic content[9] influence the food value of leaves for insects. Leaf protein content is an important source of nitrogen for herbivores and hence essential for growth and development of insect herbivores. These aspects of plant-insect herbivory are yet to be investigated with respect to host plants and their silkworm herbivores.

Terminalia arjuna is extensively grown throughout India. It is cultivated for rearing tasar silkworm in tribal areas of many states in India. In some areas it is pollarded every year not only to produce young shoots bearing new foliage but also to harvest cocoons at ease without climbing the tree. *T. arjuna*-tasar silkworm herbivory is little understood from the point of view of micro-environmental factors in response to pollarding that regulate foliar chemistry.

EXPERIMENTAL

Selection of pollarded and unpollarded *T. arjuna* plants: Plantations of different accessions of *Terminalia arjuna* are maintained at the Central Tasar Research and Training Institute (CTRTI), Ranchi, Jharkhand, India. These plantations were used for carrying out different *in situ* experiments as well as the source of material for *in vitro* experiments. In some plants no pollarding was done while in others pollarding was done annually. To assess the effect of pollarding on foliar chemistry and rearing performance of tasar silkworm, two sets of plants *i.e.* pollarded and unpollarded were selected. For each set, young, semi-mature and mature leaves from 5 plants were used for analyzing the foliar chemistry. The height of pollarded trees was 2.43-2.74 m in comparison to unpollarded trees of 3.96-4.26 m.

Foliar chemistry of leaves of different age groups of pollarded and unpollarded plants: The variability in protein, carbohydrate, tannin contents and per cent protease inhibition activity was assessed among leaves of different age groups collected from pollarded and unpollarded plants fully exposed to sunlight. The procedures followed are given below:

Soluble proteins: The quantitative variation in leaf soluble proteins was assessed following the procedure outlined by Bradford[10]. 5 g of fresh leaves harvested from plants were homogenized in 150 mM *tris*-HCl buffer pH 8.0 in ratio 1:5 (w/v) using pestle and mortar. The homogenate was centrifuged at 12000 rpm for 10 min at 4 °C. The content of the supernatant was processed for the estimation of protein using bovine serum albumin (BSA) as a standard. Protein concentration was expressed as mg/g fresh weight of leaf tissue.

Carbohydrate content: Total neutral sugar content was determined by the phenol sulfuric acid method described by Dubois *et al.*[11] using D-glucose as a standard. 5 g of fresh leaves were homogenized in 150 mM *tris*-Cl buffer pH 8.0 in ratio 1:5 (w/v) with the help of pestle and mortar. 10 and 20 µL of the sample was taken in separate test tubes and volume was made up to 1 mL with double distilled water. Blank was set with water. D-glucose was used as standard. 1 mL of 4 % aqueous phenol was added to each tube. After incubating the samples and standards for 5 min at room temperature, 5 mL of concentrated H_2SO_4 was added into each tube. The mixture was incubated at 25-30 °C for 20 min and optical density was taken at 490 nm on Bausch and Lomb Spectronic 20 spectrophotometer. Carbohydrate content was finally expressed as mg/g fresh weight of leaves.

Tannin content: Total tannin content was determined by Folin-Denis method, following the procedure described by Swain and Hillis[12]. 500 mg of fresh leaves were chopped into uniform sized small pieces and suspended in 10 mL of 80 % methanol for 24 h. 10 µL of supernatant was decanted into a test tube. 0.25 mL of Folin-Denis reagent and 0.5 mL of Na_2CO_3 solutions were added to 10 µL of supernatant and diluted to 5 mL using double distilled water. Tannic acid was used as standard. The samples and standards were incubated for 0.5 h at room temperature. Optical density was taken at 760 nm. The total tannin content was finally expressed as mg/g fresh weight of leaves.

Protease inhibitor activity: The protease inhibitor activity in crude proteins of different leaf samples was assayed by using ready-made gelatin coated X-Ray films[13]. Small rectangular ready-made gelatin coated strips of the X-Omat XK-5 X-ray films (Kodak Chemical Corporation) were used as substrate for assessing the activity of protease inhibitor.

The protease inhibitor activity was further confirmed spectrophotometrically by checking the suppression of bovine trypsin using the substrate tosyl-arginyl-methyl ester hydrochloride (TAME), following the procedure outlined by Birk[14]. 25 µL of trypsin (1 mg/mL) was prepared in 1 mm HCl. Trypsin was pre-incubated with known amount of leaf inhibitor extract in 1 mL *tris* buffer (46 mM) containing 11.5 mM $CaCl_2$ (pH 8.1). The substrate

solution (1.04 M TAME in 46 mM *tris*-HCl buffer, pH 8.1) was prepared and the reactions were carried out in a Gilford response UV-Vis spectrophotometer, set at 247 nm wavelength.

Rearing performance of tasar silkworm: To assess the variability in rearing performance of tasar silkworm, semi-mature leaves from pollarded and unpollarded plants were used for rearing tasar silkworm under indoor conditions.

Pre-weighed twigs bearing semi-mature leaves were placed in plastic boxes (15 cm × 7 cm × 8 cm). Ten newly hatched larvae (zero day old) were carefully brushed on the leaves. Three replicates for each treatment, *i.e.* pollarded and unpollarded were maintained. All the plastic boxes containing larvae and its food were kept inside a room having 27-32 °C temperature, 65-80 % relative humidity and photoperiod of 11 h L : 13 h D. After 24 h of feeding starting from zero day, the number of larvae survived and fresh weight of all the survived larvae were recorded for each replicate of the treatment. The left over plant material were oven dried at 80 °C for 24 h and then weighed. This procedure was repeated everyday with fresh lot of twigs bearing specific leaf types till the larvae attained V instar, except that the amount of food provided increased with the increase in age of larvae and the experiment was terminated after 30 d. From the total weight of survived larvae, the weight of individual larva was calculated. The dry weights of larvae were calculated using standard curve prepared by plotting oven dry weight (dried at 80 °C for 24 h) of larvae against the number of larvae survived at the end of the phase. The larvae used for the standard curve was derived from a separate replicate of each treatment.

Based on the data recorded in each replicate of each treatment, the different rearing performance parameters of different instars were calculated using the formulae outlined by Waldbauer[15]. Table-1 gives the duration of each instar stage in the development of tasar silkworm. The different rearing performance parameters selected together with the formulae used for their estimation are given in Table-2.

TABLE-1
DURATION OF DIFFERENT PHASES (INSTARS) IN THE LIFE CYCLE OF TASAR SILKWORM (EXPRESSED IN TERMS OF NUMBER OF DAYS) WITH SINGLE MOLTING DAY AFTER EACH INSTAR

Instar	Number of days
1st	5
2nd	3
3rd	6
4th	5
5th	9

TABLE-2
DEFINITIONS AND METHODS OF ESTIMATION OF DIFFERENT REARING PERFORMANCE PARAMETERS USED [Ref. 15]

Parameters	Formulae used for estimation
Mass gain (Growth)	Average weight of individuals at day 1of instar - Average weight of individuals at the end of instar duration
Per cent Mortality	No. of individuals died at the end of each instar duration × 100
Leaves Consumed	Amount of leaf added during instar duration - Amount of left over leaf during instar duration

RESULTS AND DISCUSSION

Variability in Foliar Characteristic of leaves of different age groups of pollarded and unpollarded plants

Soluble protein content: The soluble protein concentration in mature leaves was almost similar among pollarded and unpollarded plants and was lower as compared to young and semi-mature leaves of pollarded and unpollarded plants. The pollarded plants showed lower protein content in the leaves of all age group as compared to that of unpollarded plants. The range of variation in soluble proteins for young, semi-mature and mature leaves was 8.7-9.1, 8.0-8.1 and 6.6-7.0, respectively, across the plant types (Fig. 1A). The differences in means between leaves of different age groups and between plant types were statistically significant at $p < 0.05$ (Table-3).

Soluble carbohydrate content: The carbohydrate content increased with increase in age of leaves and the values were highest in mature leaves and lowest in young leaves of pollarded and unpollarded plants. The range of variation in carbohydrate content for young, semi-mature and mature leaves was 40.0-50.2, 56.6-62.1 and 65.6-75.0, respectively, across the plant types. Leaves of all age groups from pollarded plants showed lower value as compared to that of unpollarded plants (Fig. 1B). The difference in means between leaves of different age groups and between plant types were statistically significant at $p < 0.05$ (Table-3).

Tannin content: The patterns of variability in tannin content were just the reverse of those observed for carbohydrate content of pollarded and unpollarded plants. The range of variation in tannins for young, semi-mature and mature leaves was 6.1-7.3, 4.8-5.9 and 4.5-5.7, respectively, across the plant types. The pollarded and unpollarded plants showed lowest tannin content in mature leaves and highest in young leaves. The leaves from pollarded plants showed lower tannin content as compared to unpollarded plants (Fig. 1C). The differences in means between plant types were statistically significant at $p < 0.05$ (Table-3).

TABLE-3
TWO WAY ANOVA FOR DIFFERENT FOLIAR CONSTITUENTS IN LEAVES OF DIFFERENT AGE GROUPS FROM POLLARDED AND UN-POLLARDED PLANTS

Foliar constituents	Source of variation	SS	df	MS	F
Protein	Between different age groups of leaves	23.87	1	23.87	122.95**
	Between plant types	3.30	1	3.30	17.03**
	Interaction	0.46	1	0.46	2.38NS
	Within	6.98	36	0.19	
	Total	34.62	39		
Carbohydrate	Between different age groups of leaves	1196.83	1	1196.83	24.03**
	Between plant types	544.64	1	544.64	10.93**
	Interaction	37.63	1	37.63	0.75NS
	Within	1792.36	36	49.78	
	Total	3571.48	39		
Tannin	Between different age groups of leaves	0.81	1	0.81	1.42NS
	Between plant types	7.63	1	7.63	13.29**
	Interaction	0.0001	1	0.0001	0.0002NS
	Within	20.65	36	0.57	
	Total	29.10	39		
Per cent Protease Inhibition Activity	Between different age groups of leaves	148.22	1	148.22	6.52**
	Between plant types	2640.62	1	2640.62	116.22**
	Interaction	11.02	1	11.02	0.48NS
	Within	817.9	36	22.71	
	Total	3617.77	39		

**Highly significant at $p < 0.05$; NS = Non significant at $p < 0.05$.

Per cent protease inhibition activity: The variability patterns in per cent protease inhibition activity were similar to those observed for tannin content. The range of variation in protease inhibition activity for young, semi-mature and mature leaves were 78.1-88.2, 58.2-72.1 and 55.5-69.3, respectively, across the plant types (Fig. 1D). The per cent protease inhibition activity of mature and young leaves of pollarded and unpollarded plants was lowest and highest, respectively. The leaves of all age groups of pollarded plants showed lower values as compared to those of unpollarded plants. The difference in means between leaves of different age groups and between plant types were statistically significant at $p < 0.05$ (Table-3).

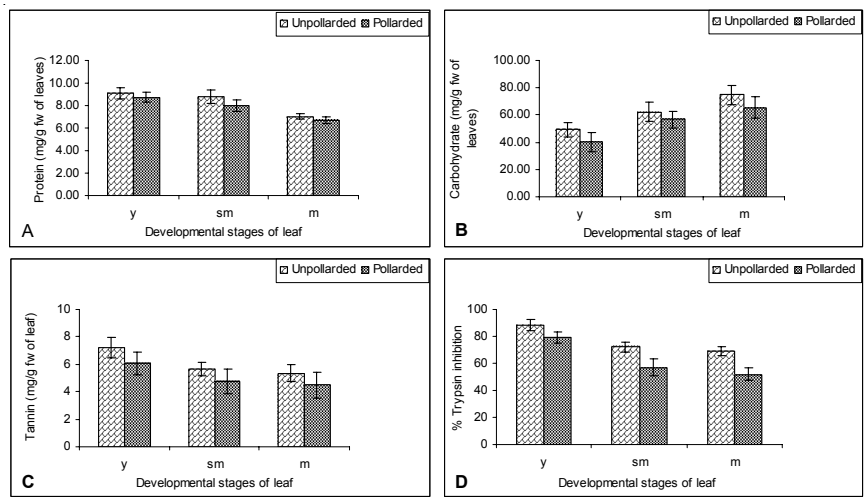

Fig. 1. Histograms showing variability in (A) protein content; (B) carbohydrate content; (C) tannin Content and (D) per cent protease inhibition activity among leaves of different age groups from pollarded and unpollarded plants

Variability in rearing performance of tasar silkworm on semi-mature leaves from pollarded and unpollarded trees

Mass gain: The mass gain values increased with increased in age of instars (except for 5th instar) under both treatments; the highest values were observed for 4th instars fed on leaves of pollarded and unpollarded plants and lowest values were noted for 1st instars fed on leaves of both pollarded and unpollarded plants. All instars showed higher mass gain values when fed on leaves of pollarded plants as compared to those fed on leaves of unpollarded plants. The range of variation in mass gain for 1st, 2nd, 3rd, 4th and 5th instars was 0.014-0.024, 0.05-0.051, 0.025-0.080, 0.2-0.33 and 0.23-0.24, respectively, across the plant types (Fig. 2A). The 'H' value was statistically significant only for between instars at $p < 0.05$ (Table-4).

Per cent mortality: The percent mortality was lowest for 1st instars fed on leaves of both pollarded and unpollarded plants and highest for 5th instars fed on leaves of both types of plants. All the instars fed on leaves of pollarded plants showed higher per cent mortality as compared to those fed on leaves of unpollarded plants. The range of variation in percent mortality for 1st, 2nd, 3rd, 4th and 5th instars was 0-6.6, 16.6-6.6, 46.6-53.3, 73.3-76.6 and 83.3-86.6, respectively, across the plant types (Fig. 2B). The 'H' value was statistically significant only for between instars at $p < 0.05$ (Table-4).

TABLE-4
ANALYSIS OF VARIANCE IN DIFFERENT NUTRITIONAL INDICES ESTIMATED FOR INSTARS FEEDING ON LEAVES OF POLLARDED AND UN-POLLARDED PLANTS UNDER INDOOR REARING CONDITIONS USING SCHEIRER-RAY-HARE EXTENSION OF KRUSKAL-WALLIS TEST WITH 'H' VALUE AS 'χ^2' VALUE FOR THE TEST OF SIGNIFICANCE

Rearing parameters	Source of Variation	SS	df	MS	H
Mass Gain	Between leaf samples	130.66	1	130.66	2.40 NS
	Between instars	922.33	3	307.44	16.97**
	Interaction	99	3	33	1.82 NS
	Within	97.33	16	6.08	
	Total	1249.33	23		
% Mortality	Between leaf samples	13.5	1	13.5	0.26 NS
	Between instars	1069.25	3	356.41	21.04**
	Interaction	0.083	3	0.027	0.001 NS
	Within	86	16	5.37	
	Total	1168.83	23		
Leaves consumed	Between leaf samples	6	1	6	0.09 NS
	Between instars	1314	3	438	21.04**
	Interaction	33.33	3	11.11	0.53NS
	Within	82.66	16	5.16	
	Total	1436	23		

**Highly significant at $p < 0.05$; NS = Non-significant at $p < 0.05$.

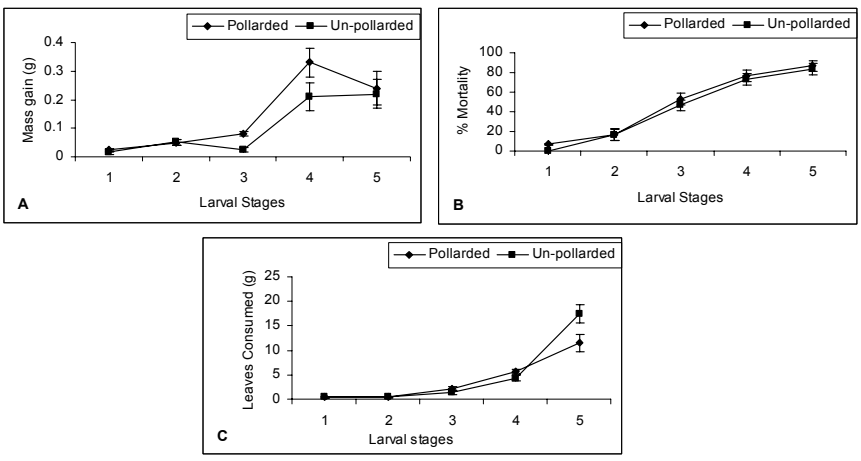

Fig. 2. Line graph showing rearing performance measured during indoor rearing as (A) mass gain; (B) % mortality and (C) leaves consumed on leaves from pollarded and unpollarded trees

Leaves consumed: The early instars showed lowest leaves consumed values as compared to that of late instars (4th and 5th) under both the treatments. 3rd and 4th instars fed on leaves of pollarded plants showed higher values as compared to those fed on leaves of unpollarded plants. The range of variation in leaves consumed for 1st, 2nd, 3rd, 4th and 5th instars was 0.38-0.40, 0.40-0.45, 1.32-2.05, 4.30-5.70 and 11.50-17.40, respectively, across the plant types (Fig. 2C). The 'H' value was statistically significant only for between instars at $p < 0.05$ (Table-4).

In plant-insect herbivore interactions, a number of defense and counter defense strategies are evolved by both the host plant and insect herbivore[16,17]. The architecture of individual trees and the spatial distribution of individual trees in a pure stand or a community, together with the temporal and spatial distribution of their foliage generate microclimatic conditions that influence the chemistry of foliage, which in turn regulate the herbivory and foraging behaviours of insects[9].

The change in chemical constituent of plant leads to alteration in resistance following herbivory. These chemicals include secondary metabolites such as phenolics, terpenes, alkaloids and glucosinolates, and a variety of defense related proteins. Some of them act independently and other combines with proteins and carbohydrates and act as antinutrients. Tannins and protease inhibitors are chemical defenses that are widely used by plants against their herbivores.

In the present study, the leaves of all age groups from unpollarded plants showed higher values in all the foliar traits as compared to those of pollarded plants. Differences in means between leaves of different age groups were statistically significant at $p < 0.05$ for all the traits except for tannin content.

Nitrogen content of leaf is a key nutrient that determines its food value, as it is an essential component of amino acids and proteins[7]. Soluble proteins were estimated and used as a measure of protein content of leaves. The pattern of variation of soluble proteins demonstrated that soluble protein increases with decreases in age of leaves and pollarding decreases the protein content. One of the important aspects of proteins in leaf feed is that the quality rather than the quantity which is critical for efficient utilization by herbivores[18,19]. For example, proteins often form complexes with tannins and polysaccharides, which cannot be hydrolyzed by proteases and hence the nutritional value of such protein complexes is extremely low[20].

Carbohydrates, particularly reducing sugars (monosaccharides and disaccharides) are essential nutrients for insect herbivory[21]. Polysaccharides include high molecular weight polymers, which form linkages with proteins and form lignins, suberin and cutin, all of which are involved in defense against pathogens and pests, including insect herbivores. The patterns of variability in carbohydrate content indicate that the carbohydrate content increases

with the age of leaves and unpollarded plants contain higher carbohydrate than the pollarded plants.

Tannins and protease inhibitors are antinutrients, in the sense that tannins combine with proteins and make them not available to the herbivores because these protein-tannin complexes cannot be attacked by proteases[9,20]. Similarly, the protease inhibitors inactivate the proteases of herbivores[22-24]. Pollarding decreases both tannins and protease inhibitor activity.

Limited information is available on food utilization aspects of insect herbivores such as *Bombyx mori*[25], *Heliothis zea*[26], *Manduca sexta*[8] and *Agrotis ipsilon*[27]. Although a few studies on the food selection by silkworm[28] and nutritional aspects of tasar silkworm has been carried out[29] the ecological significance of variability in foliar traits with respect to feeding behaviour and assimilation of nutrients by instars of tasar silk is not investigated from the point of management of sericulture. Different rearing experiments perfor-med using foliage of *T. arjuna* from pollarded and unpollarded plants are discussed with reference to foliar chemistry discussed above.

The basic patterns of variations in all rearing performance parameters *i.e.* mass gain, % mortality and leaves consumed of different instars feeding on semi-mature leaves from fully exposed pollarded and unpollarded plants demonstrated that there is marked diversity between pollarded and unpollarded plants in terms of rearing performance and most of the parameters shows higher values for pollarded as compared to unpollarded plants for all instars with a few exceptions. For example, the values of per cent mortality differed marginally between pollarded and unpollarded plants for all the instars except for 3rd instar. The mass gain was high for pollarded plants as compared to unpollarded plants for all instar except for 2nd instar; leaves consumed of pollarded plants was higher than of unpollarded except by 5th instar.

It is interesting to note that pollarding has reduced all the foliar constituents, particularly the defense chemicals thus confirming the role of canopy in foliar chemistry through regulation of light. Light is one of the key environmental factors that regulate the heat and moisture load in the air surrounding the plant, which in turn regulates the foliar morphology and chemistry. Higher photosynthates under high light condition lead to greater pools of carbohydrates. It has been reported by number of workers that a positive relationship exists between light and phenolic chemistry[30,31]. Consequently, canopy of *T. arjuna* is critical in generating chemical gradients that influence the silkworm larval feeding. Pollarding of trees simulates low light conditions in the canopy of *T. arjuna* with respect to variation in foliar constituents. Thus in terms of antinutrients, pollarding has beneficial effect for silkworm rearing. Consequently, pollarding of host plants may be a better cultural practice of host plants.

ACKNOWLEDGEMENTS

One of the authors, Shruti Rai thanks CSIR, New Delhi for the award of Senior Research Fellowship. Thanks are also to Mr. Dinesh Umrao and other scientists at Central Tasar Research & Training Institute (CTRTI), Ranchi for help during field studies. The authors also thank Dr. Arindam Bhattacharya, Department of Science & Technology and Dr. Ram Singh, University of Delhi for technical advice and scientific discussion.

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(*Received*: 19 July 2007; *Accepted*: 11 August 2008) AJC-6738