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IMPACT OF FOLIAR SUPPLEMENTATION OF NITROGEN ON PROTEIN CONTENT OF *TERMINALIA ARJUNA* LEAF AND TASAR SILKWORM *ANTHERAEA MYLITTA* D

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ABSTRACT

The quantity and quality of silk produced by silkworm depend upon the food quality mainly; the protein content of the leaf which in turn depends upon the nitrogen content of the leaf. Three doses of urea, 1.5%, 2.0% & 2.5% were used as foliar spray on *Terminalia arjuna* plants with plain water as control. Leaf from the host plants were sampled before spray and at the 7th, 14th and 21st days after spray for protein estimation. Samples of silkworm larvae were collected at their different age *viz*., at beginning of the IV instar, end of the IV instar, beginning of the V instar and at the end of the V instar. The larvae at the end of IV instar showed maximum (2.327 µg/ml) haemolymph protein. The haemolymph protein of larvae at the beginning of IV instar was 1.693µg/ml and that at the beginning of V instar was 2.018µg/ml. Both these values were significantly lesser than that of at the end of IV instar (2.327µg/ml). The haemolymph protein in the larvae at the end of V instar (1.812µg/ml) was further lesser than that in the larvae at the beginning of V instar (2.018µg/ml). Maximum protein was recorded in the haemolymph of larvae at the end of their IV instar which decreases during the V instar indicating its utilization in silk formation during the V instar. Thus it may be concluded that foliar spray of nitrogen on *T. arjuna* plants not only improves the protein content of leaf but also increases the haemolymph protein of the larvae reared on treated plants.

Keywords: *Terminalia arjuna*, *Antheraea mylitta*, protein, haemolymph.

Introduction

The quantity and quality of silk produced is directly dependent on the leaf quality, which influences the healthy growth of silkworm larvae and thereby affects the cocoon production. Various studies in the past and present on silkworm nutrition have established that it is the quality of leaf that ultimately reflects on growth and development of the silkworm as well as on overall silk production. A high nutritive value in the leaf increases the resistance of silkworm as well as cocoon production and raw silk quality. Several studies have been carried out for improvement in tasar crops through application of major and micronutrients (Sinha *et al.*, 1999, 2002 & 2006). Unlike other insects, the silkworm usually takes almost all the nutritional constituents required for its growth and reproduction from a limited number of host plants. It is well established that the silkworm requires certain essential sugars, proteins, amino-acids, fatty acids and vitamins for its normal growth, and reproduction. Such studies have extensively been done in case of mulberry silkworm, *Bombyx mori* L. (Sengupta *et al.*, 1972). The silkworm larva being the only feeding stage requires sufficient nutrients in the form of carbohydrates (glycogen, trehalose *etc.*), nitrogenous compounds (proteins, amino acids glycerol *etc*.).

Nitrogen is important constituent of chlorophyll, amino-acids, proteins, protoplasts, nucleotides, phosphatides and alkaloids, and is a part of many vitamins, enzymes and hormones. Hence, it is one of the most essential nutrients for plant growth and development.

Silk is mainly made up of two proteins, Fibroin & Sericin and nitrogen is one of the main constituents of protein. Thus it is also the one of the main constituents of silk. Nitrogen is also the main constituents of haemolymph, the extracellular circulating fluid that fills the body cavity or haemocoel. Haemolymph circulates freely within the body bathing different tissues (Arnold, 1979; Jones, 1979). It is physically isolated from direct contact with body tissues by a thin permeable membrane which lines the haemocoel (Ashhurst and Richards, 1979). Haemolymph is like the blood of higher animals and comprises of two main components *viz*., plasma and corpuscles or haemocytes (Richards and Davies, 1977). Haemocytes comprise of many mesodermal cells which are nucleated, comparable to leukocytes of other vertebrates (Arnold, 1979). They act as storage reservoir for many materials essential for a variety of insects.

The present study was undertaken to understand the role of foliar spray of nitrogen on tasar silkworm growth through bio-metabolite activities in the body which reflect in the form quality improvement of cocoon.

Materials and Methods

The farm and laboratory of the Central Tasar Research and Training Institute, Ranchi, are located at Latitude- 23º.21N, Longitude- 85 º.20E and an altitude of 652 mMSL, annual rainfall 1400 mm, soil of the farm is laterite with pH ranged 5.3 to 6.0, in the state of Jharkhand, India. Tasar silkworm host plant, Arjun, *Terminalia arjuna* plantation having 6' x 6' feet spacing and maintained through package and practices followed as per recommendation of institute. The bivoltine larvae of the Daba ecorace of Tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae) were used in this study. Experimental rearing was conducted during second crop (October-November).

Treatments with spray of urea and leaf sample collection

Commercial grade of Urea was dissolved in de-mineralized water with different concentration i.e. 1.5% (T1), 2.0% (T2), 2.5% (T3) urea and Control (water). The urea solution was sprayed on the Arjun plants during early hours of the day when the climate is cool and there is no direct sun light. There were four replications for each treatment. Twenty sample leave from each treatment/ replication were plucked and kept in pre-weighed paper envelops. The leaf samples were dried in hot air oven with periodically weighing them so as to arrive at constant weights. Estimation of nitrogen of Arjun leaf samples of treated and control lots were done with help of Kel-plus automatic nitrogen estimation system (Kjeldahl, 1883).

Silkworm Rearing and Haemolymph Samples collection treated and control larvae

Tasar silkworm of Daba BV race were mass reared on common Arjun plantation till 3rd instar using recommended package of rearing practices (Nagendra *et al.*, 2009). After 3rd and 4th moult out, day 0 larvae were separated from each instar and 50 larvae each instar of treatment/ replication were kept for collection of haemolyph sample for estimation of protein.

Haemolymph samples were collected from each treatment/ replication at four times i.e. at the beginning of 4th instar & before 4th moult of the larvae, beginning of 5th instar and before spinning of the larvae. Haemolymph from different age groups of larvae was collected into pre-chilled Eppendorf 1.5 ml tube containing 0.025% phenylthiourea (Dinesh Kumar *et al.*, 2013). It was centrifuged for 5 minute at 6000 rpm and supernatant was collected and stored at -80ºC till utilization. Protein essay was done by using Bradford method (1976).

Results and Discussion

The impact of foliar spray of urea in different concentrations on leaf at different intervals revealed that the highest nitrogen per cent was recorded on T2 (3.250 ±0.144) on day 15 followed by day 21 (3.040 ±0.023) and 90.59 % gain from day 0 to day 21 after foliar spray of urea against T1 on day 21 (2.933 ±0.103) and T3 on day 21 (2.818 ±0.082). In Treatment T3, gain in Nitrogen percentage (76.09 %) was higher than T1 (71.24 %) but lower than T2 (Table 1 and Figure 1). The significant differences were noticed on nitrogen concentration of leave of arjun plant on day 15 after foliar spray of Urea, whereas highly significant variation was observed in the nitrogen content of leave of different treatment and control. The increasing trend of nitrogen content in leave of different concentration of urea was observed up to day 15 there after it decreases.

The above findings are also supported by Lakshmi and Benarjee (2015) who evaluated fertilizers for ideal growth of *Terminalia arjuna* and enhancement of economic parameters of tropical Tasar silkworm, *Antheraea mylitta* Drury and results indicated that higher total soluble proteins, free amino acids, total soluble sugars, total reducing sugars and chlorophyll content were recorded than the control. The effect of graded levels of water soluble fertilizers on growth, yield of mulberry and cocoon quality was observed and improvement in protein was mainly attributed to the foliar spray where nutrients were rapidly absorbed by foliage and the plant metabolism/ assimilation might have been activated thus contributing for healthy green foliage resulting in synthesis of organic contents (Naveen *et al.*, 2019). The foliar spray of micronutrient on arjun leaves also enhances the quality and quantity (Sinha *et al.*, 1999, 2002 and 2006). Foliar spray of 1.5% urea was recommended on the basis of leaf yield and improvement cocoon parameter (Thangavelu *et al.*, 2000).

The result of protein content in the Haemolymph of different age group of 4th and 5th instar larvae fed on foliar sprayed leaves of different concentration of Urea in the arjun leaves is presented in figure1 and 2. The data reveals that in 4th instar, protein concentration increases with the increase of concentration of foliar spray of urea. The protein concentration in 4th instar observed to be maximum in T3 (2.692± 0.078 µg/ml) followed by T2 (2.640 ± 0.089 µg/ml) and significantly different among T1 and Control. Whereas percentage gain of protein content was maximum in T1 (41.00%) followed by T2 (28.54%) and lowest in control (18.77%). The similar observations was also reported by Dinesh Kumar el. al., 2012, 2013 who reported that protein concentration of haemolymph of 4th instar larvae in feeding is ranged from 2.97±0.02 µg/ml to 4.23± 0.01 µg/ml. Foliar application results indicated that total soluble proteins, free amino acids, total soluble sugars, total reducing sugars and chlorophyll content were recorded maximum than control (Lakshmi and Benarjee (2015). It can infer that our finding that the impact of foliar spray of urea increase the haemolymph protein in the Tasar silkworm which ultimate reflect in term of cocoon characteristics of *A. mylitta*.

In the 5th instar larvae, the protein level was observed to be higher during the start of the instar and there after it decreases during the end of instar in the all treatments of foliar sprayed leave fed larvae. The highest decrease (-19.31%) was recorded to be from 2.223± 0.149 µg/ml to 1.794± 0.067µg/ml followed by T1 (-15.50%) and decreases from 2.061± 0.012 µg/ml to 1.742± 0.001 µg/ml. Dinesh Kumar el. al. (2012, 2013) has also observed the same trend in the 5th instar larvae during early, middle and before spinning of cocoon, which support the same trend in the present findings.

Decline in haemolymph protein in the older age (end of 5th instar) is due to protein utilization for silk synthesis and metabolic activities by the 5th instar larvae (Richards and Davies, 1979). The role of haemocytes, protein synthesis well documented by Ashhurst, and Richards, 1964; Sengupta, *et al.*, 1972. Arnold, 1979 and Pandey *et al.*, 2010. The hormonal changes during the 5th instar also limits the growth of body hence decrease of protein content during the end of

final instar larvae observed due to elevation of haemolymph ecdysone (Kumar *et al.*, 2008).

Hence, it can be concluded that the foliar spray of urea in the host plant of tasar silkworm *i.e.,* Arjun not only improves the plant health in terms of leaf nitrogen, but also resulted in increase of haemolymph protein of the larvae reared on such plants. Silkworm larvae of different age groups showed varying quantities of haemolymph protein, and maximum protein was recorded in the haemolymph of larvae at the end of their 4th instar indicating utilization of protein for synthesis of silk in silk glands of the silkworm larvae during the 5th instar.

Table 1: Nitrogen percentage in the leave of *T. arjuna* plants sprayed with different concentrations of urea.

Treatments	Nitrogen per cent in leave of different days after urea spray (Mean ± SE)				Gain (%)
	Day 0	Day 8	Day 15	Day 21	
T1 (1.5)	1.713 ± 0.036	1.950 ± 0.065	2.200 ± 0.091	2.933 ±0.103	71.24
T2 (2.0)	1.595 ± 0.041	2.718 ± 0.027	3.250 ±0.144	3.040 ±0.023	90.59
T3 (2.5)	1.600 ± 0.041	2.573 ± 0.013	2.725 ±0.075	2.818 ±0.082	76.09
Control	1.498 ± 0.037	1.548 ± 0.030	1.655 ±0.023	1.598 ±0.039	6.67
CD at 5%	**0.22**	**0.22**	**0.475**	**0.318**	

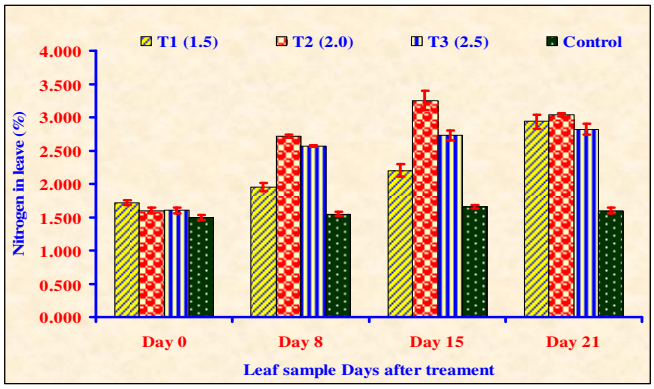

Fig. 1 : Nitrogen percentage in the leave of *T. arjuna* plants sprayed with different concentrations of urea.

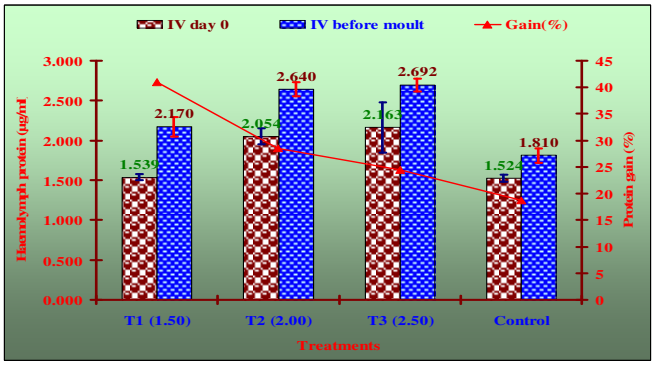

Fig. 2 : Haemolymph protein (µg/ml) in the 4th instar tasar silkworm larvae fed on the leaves of *T. arjuna* plants sprayed with different concentrations of urea

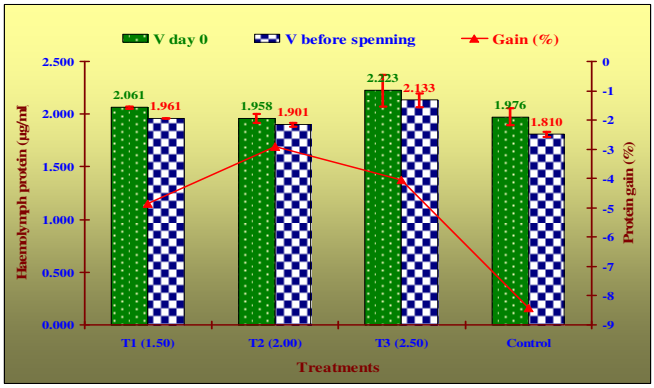

Fig. 3 : Haemolymph protein (µg/ml) in the 5th instar tasar silkworm larvae fed on the leaves of *T. arjuna* plants sprayed with different concentrations of urea

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