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NUTRIENTS FROM SOIL TO HOST PLANT LEAVES AND ITS IMPACT ON COMMERCIAL TRAITS OF TROPICAL TASAR SILKWORM

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ABSTRACT

Synthesis of silk involves a cascade of biochemical pathways involving numerous enzymatic reactions involving conformation transition of the liquid silk protein to silk fibre which is induced by several factors including changes in the cationic concentration and agro-climatic conditions. Tropical tasar silkworm produces *Vanya* silk of commercial importance. A study was carried out in two locations of Mayurbhanj district in Odisha having difference in altitude (Kendujuani, 340 - 440 mASL and Chandua 40 - 60 mASL) as well as agronomic conditions involving host plants (Asan and Arjun) during autumn season to determine the impact of soil macronutrients on tasar commercial traits. Physiochemical properties of the soil and leaf samples were analysed using standard analytical methods and tools. Results indicated acidic soil at both Kendujuani (pH 5.438±0.091) and Chandua (pH 5.337±0.092) with EC values of 0.076±0.13 dS/m and 0.081±0.012 dS/m respectively. Available soil and leaf nutrients (N, P, K, OC) were found to have higher value in Kendujuani ($P < 0.01$) in Asan plant in comparison to Chandua as revealed by ANOVA. The values were comparatively less in Arjun in comparison to Asan, however, the trend of soil and leaf macronutrients over two places were similar to that of Asan. The leaf biochemical parameters viz. total proteins, total carbohydrates and chlorophyll content were also significantly high ($P < 0.01$) in Kendujuani with higher phenol content in Chandua. Commercial traits of cocoon *viz.* cocoon weight, shell weight, cocoon volume, shell ratio and silk filament length had significantly higher values at Kendujuani ($P < 0.05$ to < 0.001). In both the sites there was no nutrient replenishment, however, there was a difference in pattern of canopy and leaf fall. In Kendujuani, the natural nutrient cycle might have contributed to rich nutrient conditions in terms of macronutrients. This study highlights the importance of soil nutrients on the expression of commercial traits of tasar silkworm.

***Keywords*:** *Antheraea mylitta*, Leaf macronutrient, Soil macronutrient, Tasar silk.

Introduction

Mayurbhanj district in Odisha is known for tasar cultivation since time immemorial in both economic plantations as well as forest based plantations known as *Adapahi* (Hota *et al*., 2014). However, the production and production is not uniform as the quantum of cocoons as well as the productivity traits varies from place to place. The geography of district shows conspicuous physiographic variations and is mainly represented by high hills, isolated hillocks, vast undulating plains and also alluvial tract represented with rivers and tributaries. Tropical tasar silkworm produces *Vanya* silk of commercial importance and the productivity of the cocoons largely depends on the agro-climatic condition, nutrient status of the soil and other environmental factors. Moreover the formation of the silk fibre is also induced by the macronutrients and the micronutrients present in the soil which determines the economic parameters of the silk produced. Wide range of variations in the soil nutrients of tasar producing regions of Mayurbhanj are also reported (Pandiaraj *et al*., 2020).

Nutrients are stored in the soil in the form of organic and inorganic molecules and their concentration is usually low. The environmental conditions and amount of different non-available forms decides the rate of conversion and removal of various nutrients from soil solution and also determines the amount of nutrients that need to be converted into soluble or available form in the soil which is taken up by the plants. The nutrients are present in their ionic forms which enter into the plant through various biochemical processes and hence are classified as macro and micronutrients on the basis of their need in the plant and their function thereof. According to the availability of nutrients, food plants influence differently on ingestion of food, digestion, assimilation and conversion of food materials to body mass and ultimately on the growth and development of insects (Bhattacharya and Pant, 1976). For various physiological processes, growth, and development tasar silkworm require nutrients which are derived from the host plant leaves which in turn come from the soil (Reddy *et al*., 2010). The nutrient contents of the leaf is directly proportional to the growth and development of the silkworm larvae and hence had a huge impact on sericulture as industry (Benchamin and Jolly, 1986).

The study of soil parameters and the amount of nutrients uptake by the host plants will help to bridge the difference between rearing of tasar silkworm and desired economic character of the cocoons. Therefore, the study was taken to analyze major soil nutrients, leaf biochemical parameters and cocoon productive traits in two diverse tasar producing zones of Mayurbhanj district in Odisha.

Material and Methods

Study sites and sampling

To determine the amount of nutrient available in the soil as well as in the host plant leaves, the soil samples and the leaf samples were collected from two locations of Mayurbhanj district in Odisha having wide difference in altitude (Kendujuani, 21.647^{0}N, 86.122^{0}E, 440 mASL and Chandua 21.906^{0}N, 86.825^{0}E, 60 mASL) as well as agronomic conditions involving two host plants (Asan and Arjun) during autumn season coinciding the commercial crop rearing over two consecutive years. Soil samples were collected randomly following standard procedure from different points throughout the plantation and *Adpahis* of forest area. Medium size Asan and Arjun leaves were collected from the same locations, kept inside water proof pouches, transported to laboratory in ice-pack and subsequently stored in -20^0 C deep freezer till further analysis.

Laboratory analyses

Physio-chemical properties of the soil and leaf samples were analysed using standard analytical methods and tools. Soil pH and electrical conductivity were measured using digital pH and conductivity meter. Nitrogen, Phosphorus, Potassium and Organic Carbon were assayed using standard methods. Protein concentrations of various samples were estimated by the method of Lowry *et al.* (1951). Carbohydrate concentration was measured according to the method of Yemm and Willis (1954). Total phenolics content was measured according to the method of Slinkard and Singleton (1977). Total chlorophyll content was measured according to the method of Anderson and Boardman (1964).

Tasar silkworm rearing and quantitative traits

Rearing of Daba (BV) race was conducted during Autumn season which is the second or commercial crop in two locations. Disease from layings of same brood was used for rearing after thorough microscopic examination for pebrine disease. The date of brushing was also same. Cocoon quantitative parameters viz. cocoon weight, shell weight, shell ratio and filament length was measured. Pupae were removed from cocoons and only shell was weighed for shell weight. Shell ratio was calculated as: Weight of cocoon shell/ Weight of live cocoon) × 100. Filament length of ten cocoons was estimated using standard method of post cocoon technology.

Statistical analysis

The data were analysed for mean and standard deviation with MS-Excel. The arithmetic means of two different groups were compared for significant difference using Student's t-test assuming equal variance. Significant difference for data involving places and host plants was determined using two-way Analysis of Variance (ANOVA).

Results

Study sites and rearing season

The study was conducted in two places in Mayurbhanj district, Kendujuani located towards western part of Similipal Biosphere Reserve and Chandua, located in alluvial plains at eastern part, thus widely differing in geographical terrain (Figure 1). The autumn season coinciding with the commercial crop or second crop is known for higher cocoon and shell weight, hence this period was considered for the study.

Fig. 1: Satellite map of Mayurbhanj district of Odisha, India showing the two study places A - Kendujuani, B – Chandua

Soil parameters

On analysis of the soil it was found that the Nitrogen, Phosphorus, Potassium, and Organic Carbon content in the soil present at Kendujuani recorded higher values in comparison to those at Chandua (Table 1). The pH was acidic in both the place but Chandua soil was significantly more acidic ($P < 0.05$) than Kendujuani. However, EC values were comparable for both the places. Available Nitrogen in Kendujuani was 132.1 ± 8.491 kg/hec, which was significantly higher ($P < 0.01$) than Chandua (120.7 ± 5.539 kg/hec). Available Phosphorous contents (kg/hec) in Kendujuani and Chandua were 10.267± 2.641 and 7.923± 1.154 respectively ($P < 0.01$). Similarly available Potassium content was also significantly high ($P < 0.05$) in Kendujuani (142.6± 23.838 kg/hec) than Chandua (117.5± 20.813 kg/hec). Soil organic carbon (g/kg) also was of identical trend (P < 001) (Table 1).

Leaf macronutrients and bio-chemicals

Leaf macronutrient content and biochemical parameters in Asan and Arjun leaves of two places are presented in Table 2. Available Nitrogen was found to be highest in Asan leaves at Kendujuani (1809±180 mg/Kg) and lowest value was recorded in Arjun leaves at Chandua (1575±196 mg/Kg). There was significant variation among places (F – 6.859, $P < 0.01$). The quantity of available Phosphorus was found to be highest in Asan leaves at Kendujuani (497±62 mg/Kg) and lowest value was recorded in Arjun leaves at Chandua (347±64 mg/Kg) . Moreover the leaves of both Asan and Arjun at Kendujuani showed higher value of available phosphorus. The variation was highly significant (F – 11.262, $P < 0.001$). The available potassium was found to be highest in Kendujuani in Asan leaves (938±139 mg/Kg) and lowest in Arjun leaves at Chandua (619±178 mg/kg) with significant variation for two places (F – 18.348, $P < 0.001$).

Table 1 : Soil pH, EC and macronutrients during Autumn season of rearing in Kendujuani and Chandua (Mean ± SD)

Soil Parameters	Kendujuani	Chandua	t value	P
Soil pH	5.438 ± 0.091	5.337 ± 0.092	2.495	< 0.05
Electrical conductivity (dS/M)	0.076 ± 0.013	0.081 ± 0.012	0.977	NS
Available Nitrogen content (kg/hec)	132.1 ± 8.491	120.7 ± 5.539	3.556	< 0.01
Available Phosphorous content (kg/hec)	10.267 ± 2.641	7.923± 1.154	2.572	< 0.01
Available potassium content (kg/hec)	142.6 ± 23.838	117.5 ± 20.813	2.501	< 0.05
Soil organic carbon (g/kg)	5.698 ± 0.354	4.601 ± 0.401	5.907	< 0.001

Table 2 : Leaf macronutrients and biochemical parameters during Autumn season of rearing in Kendujuani and Chandua (Mean ± SD)

Parameters	Place	Arjun	Asan	F value	P
Total leaf nitrogen content (mg/kg)	Kendujuani	1738 ±131	1809 ±180	6.859	0.01
	Chandua	1575 ±196	1643 ±264		
Total leaf phosphorus content (mg/kg)	Kendujuani	419 ±109	497 ±62	11.262	0.001
	Chandua	347 ±64	384 ±104		
Total leaf potassium content (mg/kg)	Kendujuani	776 ±120	938 ±139	18.345	0.001
	Chandua	619 ±178	741 ±132		
Total leaf proteins (mg/g)	Kendujuani	219 ±8.631	257 ±5.96	6.355	0.01
	Chandua	207 ±7.936	218 ±9.02		
Total carbohydrates (mg/g)	Kendujuani	4.447 ±0.24	2.91 ±0.194	7.395	0.01
	Chandua	1.959 ±0.366	2.08 ±0.173		
Total phenol content (mg/g)	Kendujuani	31.682 ±1.043	30.97±1.086	5.245	0.05
	Chandua	34.42 ±1.753	33.66 ±1.381		
Total chlorophyll content (mg/g)	Kendujuani	3.092 ±0.116	3.17 ±0.157	5.105	0.05
	Chandua	2.604 ±0.124	2.45 ±0.165		

During the second crop highest protein content in leaves was found in Asan leaves in Kendujuani (257 ± 5.96 mg/g) and lowest value was recorded in Arjun leaves at Chandua (207 ± 7.936 mg/g). Both Asan leaves in Chandua (218 ± 9.02 mg/g) and Arjun leaves at Kendujuani (219 ± 8.631 mg/g) showed intermediate results and at par with each other. There was significant variation observed (F – 6.355, $P < 0.05$). The total carbohydrate content was highest at Kendujuani in Arjun leaves (4.447 ± 0.24 mg/g). The results of the other three showed identical values but lowest value recorded in Arjun leaves at Chandua (1.959 ± 0.366 mg/g). Significant variation was observed over host plants and places ($P<0.001$). Total phenolics content was highest in Arjun leaves in Chandua (34.42 ± 1.753 mg/g) which was also at par with Asan leaves (33.66 ± 1.381mg/g) at the same place. Both Asan and Arjun leaves in Kendujuani showed significantly lower phenol content than Chandua. The variation among place and host plants was significant ($P < 0.05$). Both Asan and Arjun leaves at Kendujuani recorded higher values of total chlorophyll and were at par with 3.17 ± 0.157mg/g in Asan and 3.092 ± 0.116mg/g in Arjun leaves. Chlorophyll content in both host plants in Chandua was significantly less with lowest value recorded in Asan leaves (2.45 ± 0.165mg/g). There was a significant variation for host plants and places (p < 0.05.

Cocoon quantitative traits

The findings on the cocoon quantitative traits after the rearing of tasar silkworm on two host plants at both places are presented in Figure 2 to 5. At Kendujuani highest cocoon weight was recorded in females reared on Asan (16.45 ± 0.497 g) followed by Arjun (15.65 ± 0.58 g) while cocoons from Chandua from Arjun plants had lowest values (13.23 ± 0.405 g). ANOVA revealed significant variation between places as well as host plants ($P < 0.01$ & 0.05) (Figure 2). The shell weight also had the same trend as cocoon weight and there was significant variation among places ($P < 0.01$) and host plants ($P < 0.05$) (Figure 3). The shell ratio values were at par for all except the Arjun based male cocoons at both the places which had significantly lower values ($P < 0.05$) (Figure 4). Highest silk filament length was recorded at Asan and Arjun based female cocoons at Kendujuani with values of 855 ± 51 and 837 ± 45 m respectively. Arjun based male cocoons at Chandua had lowest value (605 ± 59 m). Variation was significant for place as well as host plant ($P < 0.05$) (Figure 5).

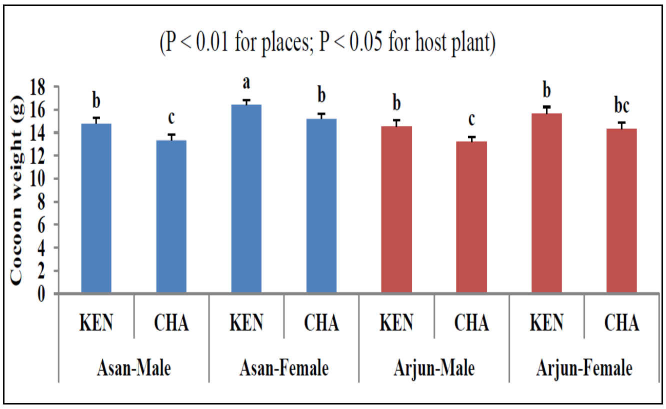

Fig. 2: Cocoon weight (g) in male and female cocoons reared on two different plants in Kendujuani (KEN) and Chandua (CHA)

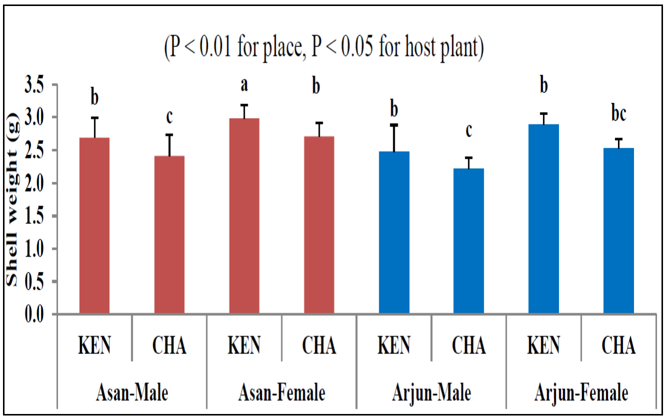

Fig. 3: Shell weight (g) in male and female cocoons reared on two different plants in Kendujuani (KEN) and Chandua (CHA)

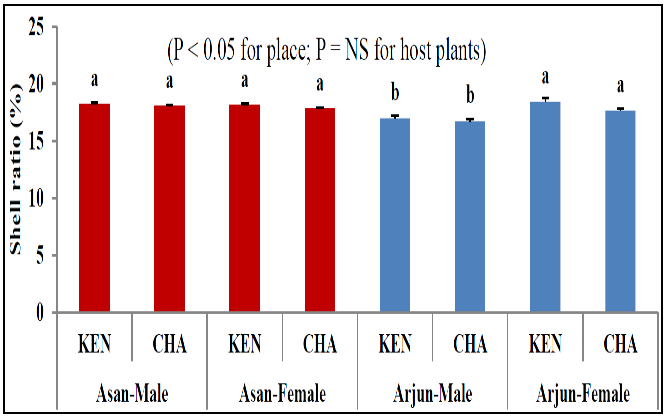

Fig. 4: Shell ratio in male and female cocoons reared on two different plants in Kendujuani (KEN) and Chandua (CHA)

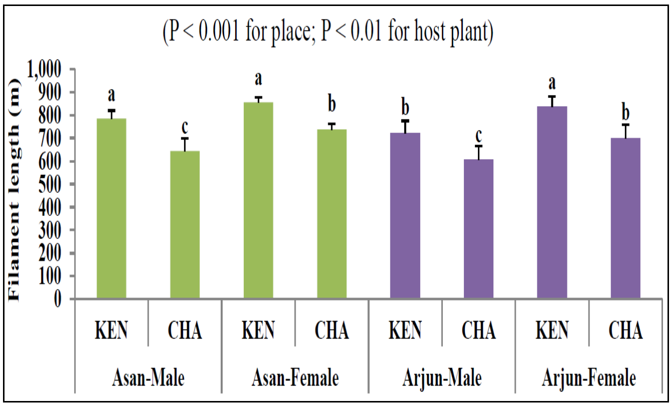

Fig. 5: Filament length (m) in male and female cocoons reared on two different plants in Kendujuani (KEN) and Chandua (CHA)

Discussion

The soil nutrients are essential for plant growth and perform very specific and essential roles such as energy transfer process, enzymes and hormones. There is difference in mechanism by which the primary nutrients are available to the plant and it is a complex process. The timing of uptake of nutrients by the plants and the form in which it is stored in the soil and the mechanism by which it is available to the plants are different in different geographical regions as well as the constituent of the soil.

It has been reported earlier that the quality of leaf is a very important factor for the production of tasar silk (Sahay and Kapila, 1993; Sinha *et al.*, 2002). Physical and chemical properties of leaves directly influence the amount of leaf consumption and digestion in mulberry silkworm and thereby larval growth, larval weight, yield, cocoon and shell weights (Das and Vijayaraghvan, 1990; Ghosh *et al.*, 2000; Zannoon *et al.*, 2012; Zannoon *et al.*, 2012; Kumar *et al.*, 2013).

It is stated that Asan food plant is superior over all other food plants (Dash *et al.*, 1992). Rearing of tasar silkworm on Asan is known to contribute for better productivity. Leaf protein in Kendujuani showed the highest value which was significantly higher than that Chandua, the work of Deka and Kumari (2013) corroborates with the findings of present study, that leaf proteins have an important role for production of silk as it is also expressed as cocoon traits. Tasar silkworm has tremendous ability to convert leaf protein of food plants into silk. Kendujuani is placed at comparatively high altitude is most suitable for Daba race indicates a better source of protein for the larva of tasar silk moth as dietary proteins provided essential amino acids needed for building of new tissues, enzymes and also protein content of tender leaves is absorbed by silkworm gut transferred to the body matter and cocoon formation (Krishnaswami, 1978). Plant protein and carbohydrate content may vary in addition to environmental factors, including the amount of light it receives, composition of soil and water inputs (Levitt, 1980; Leclerc, 2003; Sala *et al.*, 2012). In our study high concentration of protein in the leaves of Asan as compared to Arjun indicates a better source of protein for the larva of tasar silk moth.

Our results also demonstrate high carbohydrate concentration of food at Kendujuani while lowest at Chandua, indicating high carbohydrate content of food found to be gaining in larval mass as reported earlier (Bernays and

Chapman, 1994). Carbohydrates are utilised by the silkworm as an energy source and synthesizes both lipids and amino acids (Borah and Boro, 2020). Deka and Kumari (2013) stated that higher value of total carbohydrate content of leaf of *T. tomentosa* might be due to the higher photosynthesis rate which might be true for our case in Kendujuani.

Plants produce a large variety of secondary products that contain a phenol group, a hydroxyl functional group or an aromatic ring called phenol (Hattenschwiler and Vitousek, 2000). In our study, the level of total leaf phenol content in Chandua was significantly higher than that of the Kendujuani that supports the findings of Sawa *et al*. (1999) that phenols can act as antioxidants by radical scavenging, in which they break the free radical chain reaction through hydrogen atom donation and many plant phenolic compounds found to be protect leaves from photo damage. In our study, the high concentration of total phenolics in the leaves of Asan suggest that the highest antioxident property is associated with the respective host plant tissue as reported for mulberry (Dimitrova *et al*., 2015; Yu *et al*., 2018).

Chlorophyll is the most important naturally occurring pigment found in plants, which helps in conversion of solar energy to chemical energy through photosynthesis process (Pareek *et al*., 2017). In our present investigation total chlorophyll concentration was highest for both plants at Kendujuani. This indicates that the chlorophyll content of primary food plants play a pivotal role for the successful larval rearing resulting to higher cocoons as well as better quality of silk for commercial purpose as reported by Baskey *et al*. (2019). Chlorophyll has thus a direct relationship with silkworm carbohydrate metabolism (Kar *et al*., 2012).

Availability of higher concentration of N, P, K and OC in both soil and leaves at Kendujuani compared to Chandua is indicative of higher rate of natural nutrient recycling process in that region. The amount of nitrogen present in the soil is manifested in the leaves. The manifestation of nitrogen also increases the water content of the leaf as a result increases the number of herbivores insect population feeding on the plant (Slansky and Scriber, 1985). Situated at the foot hill of Similipal, Kendujuani offers as the best place for tasar silkworm rearing targeted at higher productivity. Further studies are required on soil microbes and associated factors those are helpful in enriching the soil of that habitat as there was no nutrient replenishment.

Conclusion

Comparative study on soil and leaf micronutrients, and biochemical parameters at two diversified places of Mayurbhanj revealed wide range of variation. The higher level of N, P, K, OC as well as leaf proteins, carbohydrates and chlorophyll are clearly reflected in the form for economic traits of cocoons. This suggests application of required nutrients in order to achieve higher tasar silk production. On the other hand, rearing in *Adapahis* in the line of Kendujuani, adjacent to or inside the natural forests may be explored for tasar silkworm rearing.

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