Evaluation of Castor Ecotypes of Selected Regions of the Western Ghats of Karnataka, India through Bio-Chemical Assay

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Abstract: ***An investigation has been conducted to estimate the nutritional value (leaf moisture, chlorophyll, total protein, total sugars, total phenols and mineral constituents) in leaves of identified castor (Ricinus communis L.) ecotypes found in the natural habitat of the selected regions [Heggada Devana Kote, Madikeri, Sakaleshpur, Shimoga, Sirsi and Mysore (control for comparison)] of the Western Ghats of Karnataka. The essence of the study is to select a best castor ecotype(s) for rearing of the domesticated vanya silkworm, Samia cynthia ricini Boisduval, as castor has been adjudged as the better food plant for production of valuable eri cocoons. The castor ecotypes existed in the Western Ghats of Karnataka were identified based on molecular characterization and were authenticated through National Centre for Biotechnology Information (NCBI) by awarding accession numbers viz., KJ000402, KJ000403, KJ000404, KJ000405, KJ000406, KJ000407, KJ130043, KJ130044, KJ130045, KJ130046, KJ130047 and KJ130048. The leaves collected at different heights of the plant in each of the identified castor ecotypes were pooled and processed for estimation of bio-molecules adopting standard protocols. The results of the investigation revealed that, the castor ecotype bearing accession number KJ000406 showed superiority in respect of leaf moisture, chlorophyll, total protein, total sugars, total phenols and major (nitrogen, phosphorus and potassium), secondary (calcium, magnesium and sulphur) and micro (manganese, zinc, iron and copper) nutrients. However, ecotypes bearing accession numbers KJ000404, KJ130043 and KJ130045 stood next best in their superiority for all the biochemical components. Interestingly, KJ130048 ecotype found least in respect of all the bio-molecules analyzed in the leaf samples.***

Keywords: Castor, *Ricinus communis*, Bio-chemical assay, *Samia cynthia ricini*, Western Ghats.

1. Introduction

Castor (*Ricinus communis* L.) belongs to the family Euphorbiaceae, is mainly cultivated as an intercrop with agricultural and horticultural crops. In India, castor is cultivated in an area of 10,96,000 ha with a production and productivity of 16,44,000 tonnes and 1500 kg/ha, respectively (http://faostat3.fao.org). The major castor bean producing states of India are Andhra Pradesh, Orissa, Gujarat, Karnataka, Kerala, Tamil Nadu, Maharashtra, Madhya Pradesh, Rajasthan, Bihar, Uttar Pradesh, Punjab and West Bengal. Apart from oilseed production, the leaves of castor are also used for the rearing of eri silkworm (*Samia cynthia ricini* Boisduval) in north-eastern parts of India as castor being the primary food plant of the eri silkworm.

The food quality relevant to growth, development and reproductive potential depends mainly on nutritional composition, which includes both the absolute and relative amount of water, carbohydrates, proteins, amino acids, lipids, minerals, *etc*. (Slanky and Scriber, 1985). Similarly, Ravikumar (1988) too opined that, rich harvest of cocoons is ensured only when superior quality of leaves is fed to the silkworms. It is known that 25 to 30 per cent of foliage from castor plantations can be utilized for eri silkworm rearing without affecting the seed production and as such, ericulture can be a supportive economy for the poor, dry land cultivators and provide gainful employment to the women (Rao *et al*., 2004, Raghavaiah, 2003).

The Western Ghats covers an area of approximately 160,000 km^2 with an elevation of 300-2700 m and latitudinal extent of 12^0 (8^0 N - 20^0 N). The mean temperature of the coldest month ranges from 25^{0}C at mean sea level to 10^0 C at 2400 m (Daniel, 1997). The entire Western Ghats is known for its biodiversity, richness and endemism of different species and it represents a whole range of gradient, both altitudinal as well as latitudinal in climatic factors, such as total annual rainfall, maximum temperatures, etc.

Castor is rich in varietal composition and many local and high yielding varieties / hybrids are widely grown in Assam and rest of India mainly for oilseed production. Biochemical components of castor leaves like moisture, chlorophyll, protein, amino acids, minerals, *etc*. are known to govern the growth, development and survival of eri silkworms. It has been well documented that nutritive value of the leaves of castor differs considerably with castor varieties/hybrids, seasons and growth phase of the crop. Pandey (1995) also opined that, nutritional status of leaves has been implicated as major factor in the survival of non mulberry silkworms. Apart from the cultivated varieties / hybrids, castor flora available in wild form (perennial) in the Western Ghats region of Karnataka were explored for rearing of the domesticated multivoltine (polyvoltine) eri silkworm as the leaves are available throughout the year in perennial type for eri cocoon production. In this context, an attempt has been made to know the biochemical constituents of leaves in identified castor ecotypes in selected regions of the Western Ghats of Karnataka to choose the best ecotype(s) for rearing of eri silkworm.

2. Materials and Methods

2.1 Study Area

Current investigation has an attempt to explore the genetic variability exists in castor under natural habitat in five selected regions [Heggada Devana Kote, Madikeri, Sakaleshpur, Shimoga, Sirsi and Mysore (control for comparison)] of the Western Ghats of Karnataka (Fig. 1) to identify the best ecotype(s) based on bio-chemical composition of leaves for rearing of the eri silkworm. In each of the selected region, based on morphological features (colour) of the castor plant, two types namely pink and green castor were found and were characterized through DNA sequencing and later authenticated from the National Centre for Biotechnology Information (NCBI) by obtaining accession numbers (Table1).

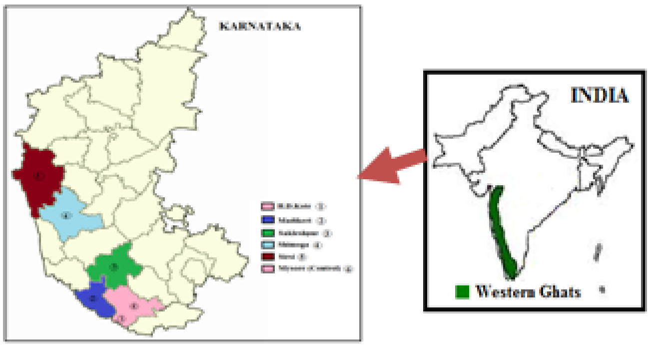

Figure 1: Maps showing selected regions of the Western Ghats of Karnataka

Table 1: Selected regions of the study area with accession numbers

Code	*Region*	*Accession No.*
S-1	Heggada Devana Kote (Pink)	KJ000402
S-2	Heggada Devana Kote (Green)	KJ000403
S-3	Madikeri (Pink)	KJ000404
S-4	Madikeri (Green)	KJ000405
S-5	Sakaleshpur (Pink)	KJ000406
S-6	Sakaleshpur (Green)	KJ000407
S-7	Shimoga (Pink)	KJ130043
S-8	Shimoga (Green)	KJ130044
S-9	Sirsi (Pink)	KJ130045
S-10	Sirsi (Green)	KJ130046
S-11	Mysore (Pink)	KJ130047
S-12	Mysore (Green)	KJ130048

2.2 Collection of Leaf Samples

The castor leaf samples was collected from the identified castor ecotypes in five regions of the Western Ghats of Karnataka at different maturity levels *viz*., tender (from top 3^{rd} to 5^{th}), middle (from 6^{th} to 9^{th}) and mature (from 10^{th} leaf downwards) and composite samples were prepared and dried under shade for three days and later in hot air oven at 70°C until constant weight. The dried leaf samples of each ecotype were ground into fine powder and preserved in butter paper bags for chemical analysis.

2.3 Biochemical Estimation of Leaf Samples

Moisture content of the leaf was estimated by taking the difference between the fresh and dry weights and expressed in percentage on fresh weight basis (A.O.A.C., 1970). Chlorophyll content of leaf was estimated by following the procedure outlined by Hiscox and Israelstam (1979) at wave lengths of 645 and 663 nm using spectrophotometer. The chlorophyll 'a', 'b' and total were computed using the formula suggested by Arnon (1949) and expressed in mg/g of fresh leaf tissue.

Total protein content in leaf samples was estimated as per the procedure described by Lowry *et al.* (1951) at optical density of 660 nm and calculated with the help of standard curve using Bovine Serum Albumin as standard and expressed in mg/g on dry weight basis. Total sugars in leaf samples was estimated as per the procedure of Dubios *et al.* (1956) using glucose as standard with an optical density of 495 nm and expressed in mg/g on dry weight basis. Total phenol content in leaf was estimated using Folin-Ciocalteau reagent (catechol used as standard) and measured at an optical density of 650 nm (Sadasivam and Manickam, 2008) and expressed in mg/g on dry weight basis. Nitrogen content in leaf was analyzed using Micro-Kjeldhal method (A.O.A.C., 1970), while phosphorus, potassium, calcium, magnesium and sulphur contents were estimated as per the procedure outlined by Jackson (1973) and expressed in percentage on dry weight basis. The micronutrients like copper, zinc, iron and manganese were estimated using an atomic absorption spectrophotometer and expressed in ppm on dry weight basis (Jackson, 1973).

2.4 Statistical Analysis

The data obtained in the current investigation was subjected to one way analysis of variance at $P \leq 0.05$ through SPSS for Windows version 20.0. The probability values showing < 0.05 are designated as significant and those values showing > 0.05 as non-significant.

3. Results and Discussion

3.1 Leaf moisture

Moisture content when scored on fresh weight basis in leaves of castor ecotypes which were found in the selected regions of the Western Ghats of Karnataka differed significantly with higher content being in KJ000406 accession (75.42%) followed by KJ000404 (74.23%), KJ130043 (70.56%), KJ130045 (69.81%), KJ000402 (69.28%) and KJ000407 accessions (68.33%). However, lower leaf moisture content was found with KJ130048 accession (62.60%) (Fig. 1a). The increase in leaf moisture content in fewer ecotypes might be enhancement of hydrogen ion concentration in plant sap due to accumulation of chlorides and less moisture loss by evapo-transpiration in the leaves (Eaton, 1942). According to Sastry (1988), leaf moisture and its retention for longer duration increases the feeding efficiency resulting in enhancement of growth rate of silkworm larvae. These results are in conformity with the observations of Kaleemurrahman and Gowri (1982), Sannappa and Jayaramaiah (2002) and Chandrappa *et al.* (2005) who observed variations in moisture content of leaves among the castor genotypes.

3.2 Chlorophyll 'a'

Chlorophyll 'a' content on fresh weight basis in castor ecotypes which were found in the selected regions of the Western Ghats of Karnataka varied considerably with highest being in KJ000404 accession (2.254 mg/g). The ecotypes bearing accession numbers KJ000406 (2.248

mg/g), KJ130043 (2.246 mg/g), KJ130045 (2.139 mg/g) and KJ000402 (2.106 mg/g) stood next best. The lowest Chlorophyll 'a' content was found with KJ130048 accession (1.892 mg/g) (Fig. 1b).

3.3 Chlorophyll 'b'

Chlorophyll 'b' content when measured on fresh weight basis too varied markedly among the castor ecotypes with higher value being in KJ000406 accession (0.871 mg/g), next in the order were KJ000404 (0.838 mg/g), KJ130045 (0.837 mg/g), KJ130043 (0.824 mg/g), KJ000402 (0.747 mg/g), KJ000407 (0.626 mg/g) and KJ000405 accessions (0.588 mg/g). However, KJ130048 recorded least Chlorophyll 'b' content of 0.462 mg/g (Fig. 1b).

3.4 Total Chlorophyll

Total chlorophyll content on fresh weight basis also noticed marked variations in different castor ecotypes that were collected from different regions of the Western Ghats of Karnataka with greater amount being in KJ000406 accession (3.128 mg/g) followed by KJ000404 (3.099 mg/g), KJ130043 (3.078 mg/g), KJ130045 (2.984 mg/g), KJ000402 (2.860 mg/g), KJ000407 (2.713 mg/g) and KJ000405 accessions (2.655 mg/g). However, less amount of total chlorophyll was found with KJ130048 accession (2.359 mg/g) (Fig. 1b).

Silkworm synthesizes red fluorescent protein with the help of chlorophyll in the presence of light, which has anti-viral property, thus reduces the incidence of viral diseases in silkworms. Kaleemurrahman and Gowri (1982), Sannappa and Jayaramaiah (2002) and Chandrappa *et al*. (2005) who also observed variations in chlorophyll content among the castor genotypes. The results of Sengupta *et al*. (2008) also confirmed that the variations were evident in chlorophyll content among the castor genotypes in the Gangetic alluvial soil of West Bengal.

3.5 Total Protein

The identified castor ecotypes of the selected regions of the Western Ghats of Karnataka exhibit marked differences in total protein content in leaves when scored on dry weight basis with highest being in KJ000406 accession (28.74 mg/g) followed by KJ000404 (26.89 mg/g), KJ130043 (25.44 mg/g), KJ130045 (24.03 mg/g), KJ000402 (23.35 mg/g) and KJ000407 accessions (22.49 mg/g). The lowest total protein content was present in KJ130048 accession (18.23 mg/g) (Fig. 1c). Protein content in leaf is a major source for silkworm to synthesize the silk which consists of two proteins namely fibroin and sericin (Rangaswami *et al*., 1976). Similarly, Bongale and Chaluvachari (1995) also opined that, protein content of mulberry leaves has a profound impact on larval growth particularly in silk gland development and cocoon characters of silkworm.

3.6 Total Sugars

Total sugar content when measured on dry weight basis varied significantly among the leaves of castor ecotypes identified from the selected regions of the Western Ghats of Karnataka. Considerably higher sugar content was found with KJ000406 accession (20.46 mg/g), the ecotypes bearing accession numbers KJ000404 (20.02 mg/g), KJ130043 (20.01 mg/g), KJ130045 (18.35 mg/g), KJ000402 (17.33 mg/g), KJ000407 (16.99 mg/g) and KJ000405 (16.27 mg/g) stood next best with respect to sugar content. On the other hand, KJ130048 accession recorded least sugar content of 12.54 mg/g (Fig. 1c). Sugar plays an important role in determining the quality of leaf that in turn influences growth, development and health of silkworm. As per Horie (1978), more carbohydrate content in leaves is helpful in producing fat body glycogen which in turn increases the trehalose content in haemolymph.

3.7 Total Phenols

The variations were evident in total phenol content (on dry weight basis) among the castor ecotypes identified from the selected regions of the Western Ghats of Karnataka. Total phenol content was lower in KJ000406 accession (20.76 mg/g), the accessions KJ000404 (22.00 mg/g), KJ130043 (23.46 mg/g), KJ130045 (25.41 mg/g), KJ000402 (26.52 mg/g), KJ000407 (28.42 mg/g) and KJ000405 (30.51 mg/g) ranked next in the order with respect to total phenol content. However, higher total phenol content was registered in KJ130048 accession (37.36 mg/g) (Fig. 1d). The current results corroborate with the observations of Sarmah *et al*. (2011), who too recorded variations in phenol content among different castor accessions. Rao *et al*. (2009) opined that high phenol content in castor leaves affects feeding of silkworm. Thus it can be inferred that, lesser the phenol content in leaves better the growth, development and survival of silkworms as these were found anti-nutritional.

3.8 Major Nutrients

Nitrogen content in leaves when scored on dry weight basis in the identified castor ecotypes from the selected regions of the Western Ghats of Karnataka showed significant variations with more being in KJ000406 accession (5.973 %) followed by KJ000404 (5.276 %), KJ130043 (5.235 %), KJ130045 (5.134 %), KJ000402 (4.924 %), KJ000407 (4.914 %), KJ000405 (4.841 %) and KJ130044 accessions (4.626 %). However, nitrogen content was less in KJ130048 accession (4.028 %) (Fig. 2a). Nitrogen content in leaf is known to influence the quality of leaf especially its protein content, in addition, it also controls the plant reproduction cycle (Shankar, 1997).

In the current investigation, the castor ecotypes did not exert significant influence on phosphorus content in leaves when measured on dry weight basis, however, marginally higher phosphorus content was recorded in KJ000406 accession (0.461 %), while phosphorus content was lower with KJ130048 accession (0.251 %) (Fig. 2a). The presence of higher phosphorus content in leaves enhances the levels of total sugars, besides responsible for improving the growth and development of silkworm larvae (Ray *et al*., 1973).

Potassium content when scored on dry weight basis in leaves of castor ecotypes identified from the selected regions of the Western Ghats of Karnataka too showed non-significant variations with more content being in KJ000406 accession

(1.141 %) and the same was less in KJ130048 accession (0.771 %) (Fig. 2a). Shankar *et al*. (1999) opined that the silkworms fed on leaves with higher content of potassium are known to increase the body weight of silkworm consequently enhances the cocoon production.

Further, EL-Shaarawy *et al.* (1975) recorded higher nitrogen, phosphorus and potassium contents with bloomy red variety of castor than the bloomy green variety confirming that these contents varied with the varieties of castor. Sannappa and Jayaramaiah (1999) and Chandrashekhar *et al*. (2013) also recorded variations in major nutrients among the castor genotypes cultivated under rainfed condition. Thus it is inferred that, the nutritive values in leaves differs due to genotypes / ecotypes, seasons, regions, *etc*.

3.9 Secondary Nutrients

Calcium content in leaves when measured on dry weight basis showed variation among the identified castor ecotypes in the selected regions of the Western Ghats of Karnataka. Significantly more calcium content was recorded in KJ000406 accession (3.481 %), while KJ000404 (2.914 %), KJ130043 (2.514 %), KJ130045 (2.447 %), KJ000402 (2.214 %), KJ000407 (2.148 %), KJ000405 (2.141 %) and KJ130044 accessions (2.138 %) ranked next best. The castor ecotype bearing accession number KJ130048 recorded less calcium content of 1.264 per cent (Fig. 2b).

Notable differences were evident in foliar magnesium content among the identified castor ecotypes with highest being in KJ000406 accession (2.414 %). The accessions KJ000404 (1.914 %), KJ130043 (1.814 %), KJ130045 (1.814 %), KJ000402 (1.714 %) and KJ000407 (1.714 %) stood next best in respect of magnesium content. Magnesium content was notably lowest in KJ130048 accession (0.514 %) (Fig. 2b). Magnesium is essential for photosynthesis as it involves in the synthesis of chlorophyll and enzymes besides helps in respiration of plants.

Sulphur content (on dry weight basis) showed marked variation in identified castor ecotypes collected from different regions of the Western Ghats of Karnataka with higher (0.904%) being in KJ000406 accession followed by KJ000404 (0.584 %), KJ130043 (0.514 %), KJ130045 (0.494 %), KJ000402 (0.474 %) and KJ000407 accessions (0.444 %). However, KJ130048 accession registered lower sulphur content of 0.124 per cent (Fig. 2b).

Secondary nutrients in leaves did exhibit variations among the castor genotypes when they were raised under rainfed condition (Sannappa and Jayaramaiah, 1999; Chandrashekhar *et al*., 2013). Both major and secondary nutrients are involved in the synthesis of lower molecular weight organic compounds such as amino acids, amides, peptides and urides and directly participate in the synthesis of proteins, nucleic acids, fat and carbohydrates, co-enzymes and energy metabolism. In addition, they are in close relation with respiration, photosynthesis and other metabolic activities in crop plants (Shree *et al*., 2005).

3.10 Micro nutrients

In respect of manganese content (dry weight basis), variations were noticed in leaves of castor ecotypes collected from the selected regions of the Western Ghats of Karnataka. Higher manganese content was recorded in KJ000406 accession (185.0 ppm) when compared to the other ecotypes namely KJ000404 (183.3 ppm), KJ130043 (160.0 ppm), KJ130045 (136.7 ppm), KJ000402 (135.0 ppm), KJ000407 (88.33 ppm), KJ000405 (86.87 ppm), KJ130044 (85.00 ppm), KJ130046 (66.67 ppm), KJ000403 (65.00 ppm) and KJ130047 accessions (60.00 ppm). However, magnesium content was lower in KJ130048 accession (58.33 ppm) (Fig. 2c).

Zinc content in castor leaves of different ecotypes when scored on dry weight basis exhibit significant variations with highest content in KJ000406 accession (107.5 ppm) followed by KJ130043 (89.17 ppm), KJ000404 (85.83 ppm), KJ130045 (84.17 ppm), KJ000402 (82.50 ppm), KJ000407 (79.17 ppm) and KJ000405 accessions (77.17 ppm), while it was lowest in KJ130048 accession (32.50 ppm) (Fig. 2c).

The castor ecotypes identified from different regions of the Western Ghats of Karnataka showed significant variations in iron content of leaves when measured on dry weight basis. The iron content was more in KJ000406 accession (766.0 ppm), next best were KJ000404 (756.0 ppm), KJ130043 (754.0 ppm), KJ130045 (719.0 ppm), KJ000402 (679.0 ppm), KJ000407 (654.0 ppm) and KJ000405 accessions (619.0 ppm). However, KJ130048 accession registered less iron content of 407.0 ppm (Fig. 2d).

Significant variations were recorded in copper content in leaves of identified castor ecotypes of the selected regions of the Western Ghats of Karnataka with more content in KJ000406 accession (362.5 ppm), accessions KJ000404 (276.5 ppm), KJ130043 (275.2 ppm), KJ130045 (263.5 ppm), KJ000402 (260.5 ppm), KJ000407 (258.5 ppm), KJ000405 (249.5 ppm) and KJ130044 (240.5 ppm) stood next best. However, less content of copper was observed in KJ130048 accession (73.00 ppm) (Fig. 2d).

Micronutrients are metal or metal containing inorganic compounds. They function as intracellular messengers or enzymatic cofactors. Micronutrients as the name implicates are in small quantities for the proper execution of vital activities in living things (Cohen, 2004, Lehninger, 1993 and Francisco and Rosario, 1996). As in vertebrates, the essentiality and intake of micronutrients (Beck, 1972) is well established in insect nutrition also (Dadd, 1977). Micronutrients have its own importance in insect diet particularly in the preparation of insect artificial diets. They require micronutrients for proper growth and survival (Cohen, 2004). In the case of lepidopteron insects, the absence or presence of micronutrients in the diet can influence on its behaviour and immunocopentance (Popham and Shelby, 2006). Lokanath *et al*. (1986) reported that manganese and iron have the capability to improve the larval development, filament length, cocoon weight and yield.

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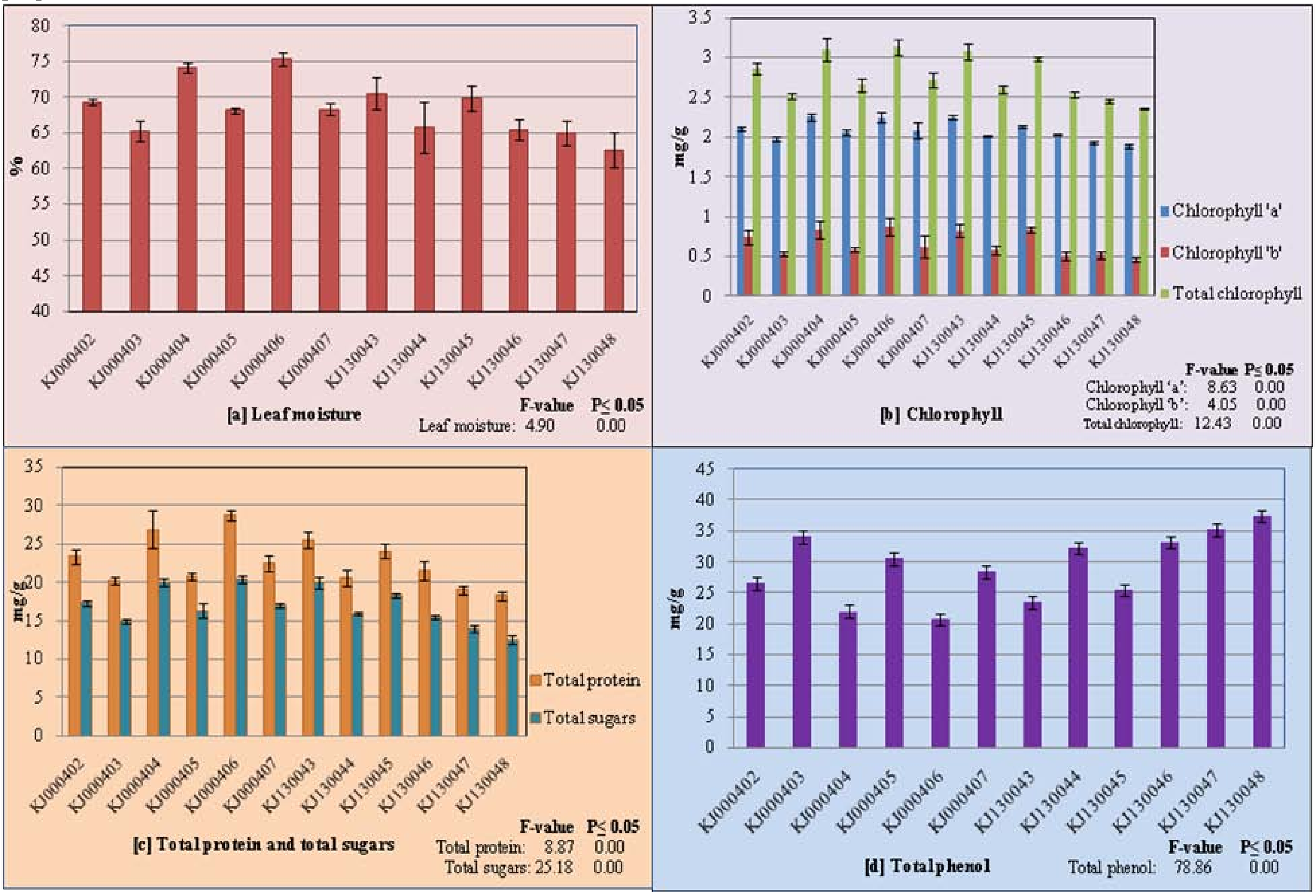

Figure 1: Bio-chemical constituents of identified castor ecotypes in the selected regions of the Western Ghats of Karnataka

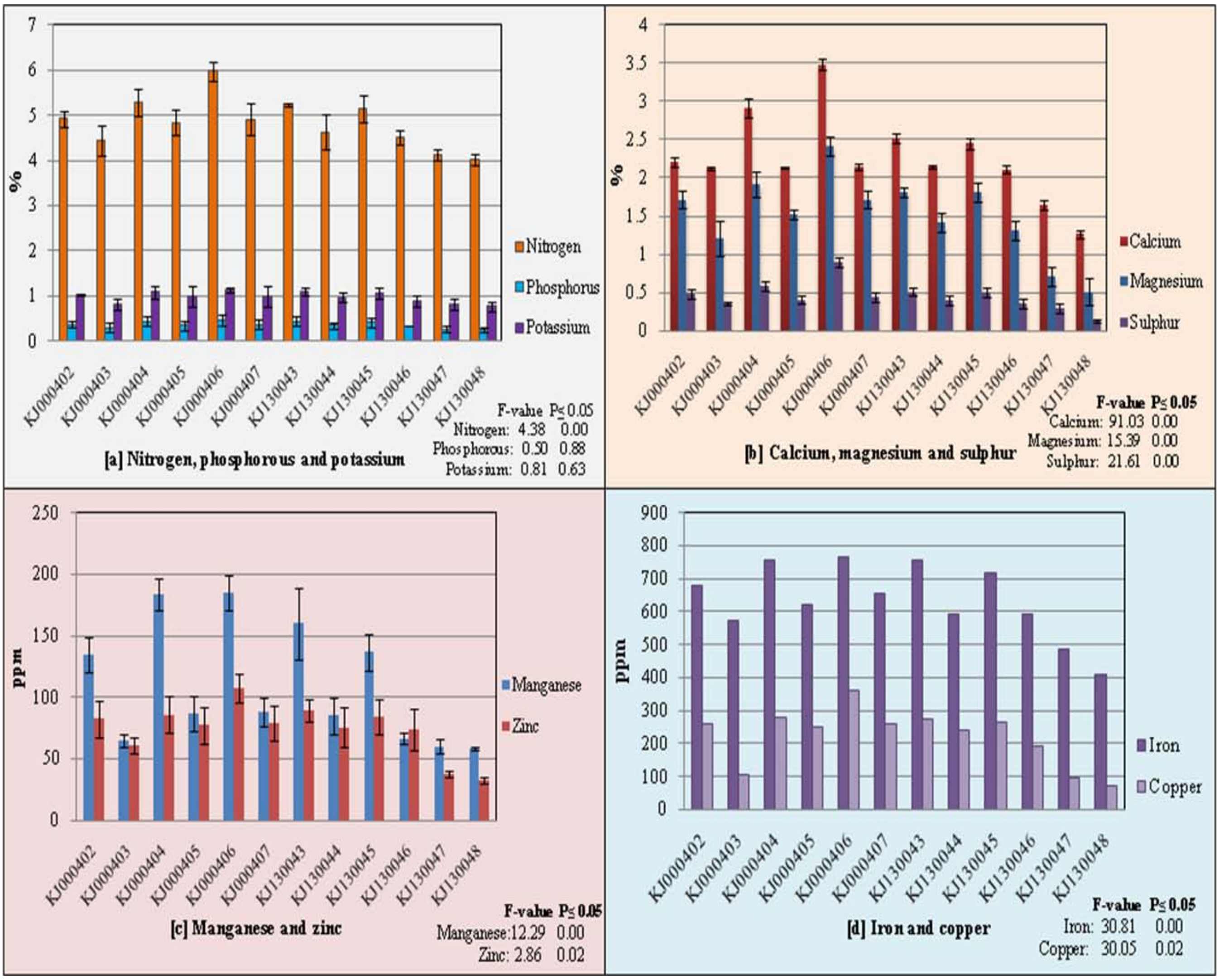

Figure 2: Macro and micro nutrients of identified castor ecotypes in the selected regions of the Western Ghats of Karnataka