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Seasonal variation in mineral and secondary metabolites of *Sterculia foetida* leaf fodder across different landscapes of Southern Gujarat, India

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ABSTRACT

The three major sources of fodder supply in India are crop residues, cultivated fodder and fodder from common property resources like forests and pastures which are low in productivity. It is essential to explore high yielding perennial multipurpose tree species. Therefore, present study intended to estimate Sterculia foetida leaf fodder macro and micro mineral and secondary metabolite composition to establish landscape and seasonal variation in three districts namely Navsari, Valsad (coastal parts of Arabian Sea) and the Dangs of south Gujarat, India. The study revealed significant variation ($P \leq 0.05$) in macro (nitrogen, phosphorus, calcium and magnesium and micro minerals (iron, copper, zinc and manganese) and secondary metabolites [total tannin content (TTC), total phenolic content (TPC), total flavonoids content (TFC), total alkaloid content (TAC), total terpenoids content (TTeC) and total saponin content (TSC)] in S. foetida leaf fodder among landscapes and seasons. N, P, Ca and Mg ranged from 0.86-1.40, 0.08-0.15, 4.10-7.25 and 0.58-1.88 per cent, respectively among eight landscapes. Out of the 8 macro and micro minerals N, Cu, Fe and Mn was higher Waghai Forest II (L8) landscape whereas P, Ca, Mg and Zn was maximum in Valsad Urban II (L6), and NAU Agricultural I (L1) and NAU Agricultural II (L2). Amongst macro minerals, N and Ca were significantly higher during summer and Mg and P during winter. Similarly, micro minerals namely Cu, Zn, Fe and Mn ranged from 0.88-2.23, 2.23-5.58, 23.60-67.98 and 2.45-7.00 ppm, respectively among landscapes. Leaf fodder TTC, TPC, TFC, TAC, TTeC and TSC ranged from 1.11-1.56, 1.16-2.21, 1.51-2.26, 2.92-3.08, 1.03-1.59 and 6.00-8.16 per cent, respectively. Fe and Mn content was higher during the summer season, and Cu and Zn content in winter season. Out of the eight macro and micro minerals, availability of one or the other recorded higher in winter and summer, indicating good fodder quality in both the seasons. However, out of six secondary metabolites, TTC, TPC, TFC, TAC, and TSC was significantly higher during the winter season and only TTeC was higher in the summer season leaf fodder. Thus, lean period S. foetida leaf fodder could provide many beneficial biological activities due to presence of most of alkaloids and flavonoids at safe limits. Further, the provenances with higher mineral matter and secondary metabolite content could be taken up for improvement programme for quality leaf fodder production.

INTRODUCTION

Livestock play a crucial role in agricultural systems worldwide and are particularly important in India for supporting the livelihoods of smallholder farmers. India, an agrarian country, possesses the second-largest livestock population globally (FAO, 2021), with a total population of 535.08 million animals dominated by cattle, buffaloes, sheep and goats (Anonymous, 2021a). Since 2012, the livestock population has increased by 4.6%, contributing 5.1% to the national gross value added (GVA) and 17.1% to the GVA of agriculture and allied sectors (Anonymous, 2021b; Yadav et al. 2023). This rapid expansion has intensified pressure on available fodder resources required to sustain livestock productivity. Cultivated fodders occupy merely about 4.0% of the cultivable land, leading to substantial deficits of green fodder, dry fodder and concentrate feeds (IGFRI, 2015). Declining grazing lands, overgrazing and limited scope for expanding fodder acreage further aggravate feed scarcity, particularly during lean periods, thereby constraining animal productivity and contributing to low per-animal milk yield in the country (Thakur et al. 2015).

Under such conditions, fodder trees have emerged as an important alternative feed resource in fodder-based land-use systems. Tree leaves and pods/fruits, commonly referred to as "top feeds," provide nutritionally rich fodder at low cost during periods when conventional forages are scarce (Meena et al. 2012; Sukhadiya et al. 2021). Tree leaf fodder is often comparable to leguminous fodder crops in nutritive value (Singh, 1982) and is generally rich in digestible protein, fibre fractions, vitamins and minerals, which enhance rumen microbial activity and improve the utilization of low-quality roughages (Gaikwad et al. 2017; Idan et al. 2020; Sarkwa et al. 2020). Consequently, several fodder tree species have been evaluated for their potential to partially replace concentrate feeds in ruminant diets (Malek et al. 2024a).

The nutritive value of tree fodder is further influenced by species genetics, site characteristics, soil and climatic conditions, management practices and seasonal variations (Navale et al. 2017; Enri et al. 2020, Malek et al. 2024a, 2024b). Seasonal and phenological changes can markedly alter chemical composition and digestibility, emphasizing the importance of identifying optimal harvest periods to ensure consistent fodder quality (Singh and Todaria, 2012; Siulapwa et al. 2016; Malek et al. 2024a).

In addition to proximate and fibre fractions, fodder tree species assessment must also consider their mineral and secondary metabolite composition, as minerals play a vital role in maintaining optimal immuno-physiological functions, feed intake, nutrient utilization and metabolic processes in livestock (Arangasamy et al. 2018). Mineral requirements of small ruminants are dynamic and vary with age, physiological status and production level, while deficiencies particularly of trace minerals are often difficult to diagnose due to subtle clinical symptoms (Manimaran et al. 2024). Although trees and shrubs contribute substantially to mineral nutrition, forage resources alone rarely meet the complete mineral requirements of grazing animals (Sumathi et al. 2017). On the other hand, secondary metabolites, including antioxidant peptides, phenols, flavonoids, phytic acid, trypsin inhibitors, and lectins (Dey *et al.,* 2019; Wong *et al.,* 2020; Sukhadiya *et al.,* 2021) are rich in antioxidant, anti-inflammatory and antibacterial activity (Kumar and Goel 2019; Malik *et al.*, 2025). These phyto groups have been advocated to exhibit positive antioxidant benefits in livestock in terms of favored growth performance, production quality and enhanced endogenous antioxidant systems, directly affecting specific molecular targets or/and indirectly as alleviated conjugates affecting the metabolic pathways (Hamaker and Tuncil 2014; Wong *et al.,* 2020). Consequently, dichotomizing the antioxidant effects and the underlying mechanism of dietary secondary metabolites is a significant subject matter.

Therefore, fodder and other edible tree species intended for inclusion in fodder-based land-use systems need to be systematically evaluated for their mineral matter and other secondary metabolites composition (Sukhadiya et al. 2019, 2021). One such multipurpose tree species is *Sterculia foetida* L. (Jangli Badam or Pinari) which is a large deciduous tree widely distributed across tropical regions, including several parts of India. The species is valued for its multiple uses, and its leaves are reported to be rich in protein and essential minerals, particularly calcium and phosphorus, indicating good potential as ruminant fodder (Orwa et al. 2009). Accordingly, the present study was undertaken to investigate landscape and season-wise variation in *S. foetida* leaf fodder in three districts namely Navsari, Valsad (coastal parts of Arabian Sea) and Dangs of south Gujarat, India. It was hypothesized that variations associated with landscape and season could be effectively exploited for the selection of superior genotypes aimed at improving fodder quality, as well as for identifying the optimal season of harvest to obtain higher mineral content.

MATERIALS AND METHODS

Provenances and geo-climatic conditions

Distantly located eight different landscapes (agricultural, urban and forest landuse) falling in three districts namely Navsari, Valsad (coastal parts of Arabian Sea) and The Dangs of south Gujarat, India, were selected (Table 1 & Fig 1). *NAU agricultural I*, NAU agricultural II, Navsari Urban I and Navsari Urban II landscapes fall under the Navsari district situated near Arabian Sea coast.

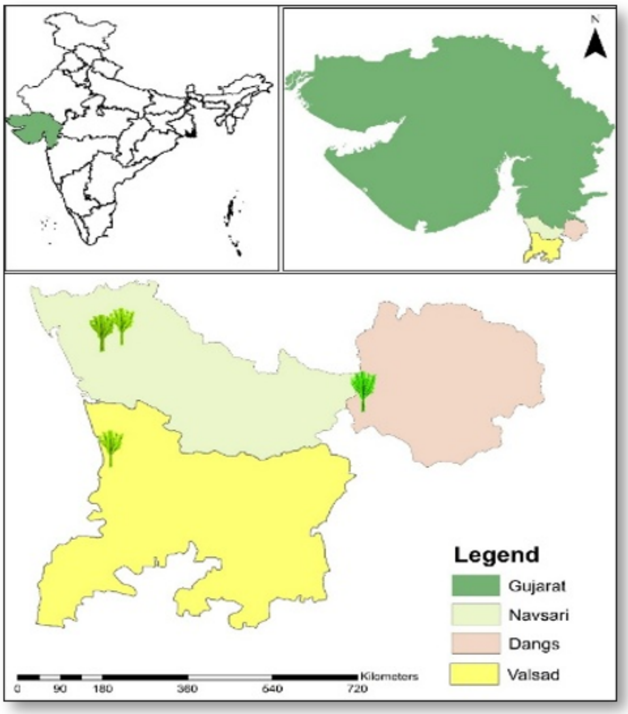

Figure 1: Geographic location of S. foetida landscapes selected in the study

Soils of this region are black rock outcrops, shallow black, brown and alluvial soils of recent origin. The average annual rainfall in the district ranges from 1500 to 3000 mm. The average annual temperature is about 26°C and the maximum temperature may shoot up to 46°C and the winter temperatures 22°C. Valsad Urban I and Valsad Urban II landscapes fall under Valsad district. The selected landscapes are in Valsad city, situated near Arabian Sea coast. Geologically it is

Table 1: Geographical location of *S. foetida* trees in different landscapes of south Gujarat region selected in the present study.

Landscape (L)	Geographical attributes			Average biometric attributes		
	Altitude (m)	Longitude (E)	Latitude (N)	Tree height (m)	DBH (cm)	Canopy area (m^2)
NAU Agricultural I (L_1)	30.4	72°89'49"	20°91'83"	13.7	23.80	51.0
NAU Agricultural II (L_2)	32.6	72°89'16"	20°91'84"	21.2	68.30	99.2
Navsari Urban I (L_3)	30.4	72°94'06"	20°93'54"	22.7	42.20	111.2
Navsari Urban II (L_4)	31.9	72°94'01"	20°93'69"	25.1	40.38	82.9
Valsad Urban I (L_5)	31.6	72°91'38"	20°60'43"	10.1	29.50	245.3
Valsad Urban II (L_6)	39.1	72°92'93"	20°60'80"	16.3	24.50	127.2
Waghai Forest I (L_7)	175.1	73°30'01"	20°45'15"	39.1	54.70	122.9
Waghai Forest II (L_8)	148.5	73°29'52"	20°45'55"	35.0	41.90	141.7

a northern extension of Deccan Plateau of Central India, belonging to late cretaceous-early Eocene age and here, it is followed by Quaternary sediments. The region has black cotton soils. The maximum daily temperature during the year ranges from 32.2°C in August to 41.2°C in April while the minimum temperature ranges from 9.9°C in January to 23.3°C in May.

Waghai Forest I and Waghai Forest II landscapes fall under south Dangs region. Soils of this region are black rock-outcrops, shallow black, brown and alluvial soils of recent origin. The average annual rainfall in the district ranges from 1500 to 3000 mm. Average annual temperature is about 26℃ and maximum temperature may shoot up to 46°C and the winter temperatures 22℃

Sample collection and processing

Five trees were selected randomly from each landscape for leaf fodder sample collection. Four sub-sample (replications) from a composite sample of five trees were drawn for analysis. Tree growth parameters *viz.* average tree height and diameter at breast height (DBH) and average canopy area (m^2) were recorded (Table 1) in all landscapes. Leaf samples were collected from all directions (north-south and east-west) at lower, middle and upper tree canopy. The samples were washed with distilled water in laboratory and air-dried followed by drying in hot air oven at 60℃±5℃ till constant weight was achieved. The dried samples were then finely grounded (particle size<1 mm) using grinder and laboratory grade pulverizer. Leaf fodder macro and micro mineral content was estimated separately for summer (Last week of May) and winter (Third week of December) seasons for two years and average of seasons of 2023 and 2024 and averaged to ascertain the provenance and seasonal variations in mineral matter and secondary metabolites.

Macro and micro mineral estimation

Macro (nitrogen, calcium, phosphorus and magnesium) minerals were estimated by standard procedures (AOAC 2016). Standard curve was prepared (Fig. 2) using different concentrations of potassium dihydrogen phosphate for estimation of phosphorus content. The concentrations of micro minerals (iron, zinc, copper and manganese) were measured using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). The detail procedure followed for each mineral is as described as under.

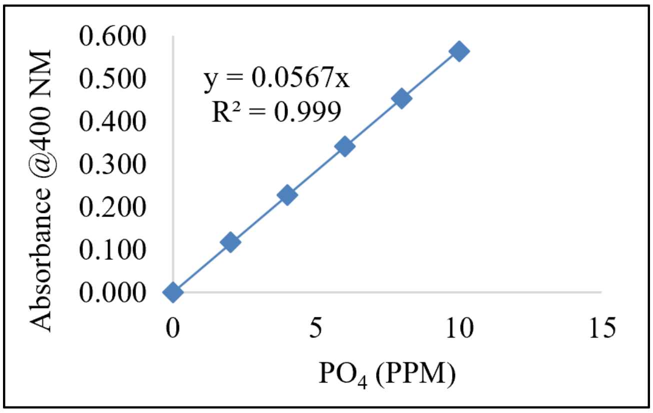

Figure 2: Standard curve of phosphate for estimation of phosphorus in S. foetida leaf sample

Macro minerals

After ashing 25 ml diluted HCl (1:1) was added in crucible containing ash through pipette from side of crucible. The crucible was covered with the watch glass and placed on hot plate for digestion up to 20 min. After cooling the content was filtered through Whatman filter paper no. 40 in to 250 ml volumetric flask by stem funnels. The crucible and filter paper were rinsed with 5% HCl solution and then with distilled water until the content became free from acid. 250 ml aliquot was prepared with distilled water. The aliquot was preserved for the determination of individual minerals.

Nitrogen (N %)

One g oven dried powdered leaf sample was transferred in to a Kjeldahl flask with 3 g of digestion mixture and 10 ml concentrated sulphuric acid (H_2SO_4). Then flask was placed in digestion chamber for 2-3 hr till the solution became clear. After cooling, content was transferred into volumetric flask and final volume was made up to 100 ml adding distilled water. 25 ml boric acid solution containing Tashiro's indicator (methyl red + bromocresol green) was taken in conical flask and placed in such a way that tip of condenser should dip in the content. 25 ml of aliquot was transfered into the distillation unit with 100 ml 40% NaOH solution and apparatus was set up. Care was taken to add NaOH in last and the valve was closed immediately. Water circulation and heater was turned on till 50-70 ml of distillate was collected. The distillate then titrated against standard N/10 H_2SO_4 solution up to faint red to pink color of Tashiro's indicator was reappeared.

Nitrogen (N%) = (V × 0.0014 × D) / (W × A) × 100

Where, **V** =Vol. used in titration

D =Total vol. made by digested sample

W = Weight of sample

Calcium (Ca%)

Twenty-five ml of aliquot was transferred in 250 ml of beaker and 10 ml of 1:2 HCl, 10 ml of saturated ammonium oxalate and 2-3 drops of methyl red indicator was added to the solution. Diluted ammonia solution (1:4) was added in the solution to neutralize the contents, through side walls till the colour of solution became faint pink to colourless. This content was left overnight to settle the precipitate. Solution was filtered through Whatman filter paper no. 40 and then filter paper was transferred along with precipitate in the same beaker and 10 ml of H_2SO_4 was added drop by drop. The solution was titrated against 0.1N $KMnO_4$ up to light pink colour. The formula mentioned below was used to calculate the Ca per cent.

Calcium (%) = [mL of N/10 $KMnO_4$ used × 0.002 × dilution factor / Weight of sample taken for ashing] × 100

Phosphorus (P) (%)

Four ml of Molybdo-Vande reagent, 2 ml aliquot and 4 ml distilled water was taken in test tubes and absorbance was measured at 400 nm against reagent blank in spectrophotometer. The standard curve (Fig. 2a) was prepared by using different concentrations of potassium dihydrogen phosphate. Concentration of phosphorus in the test sample was calculated from standard curve

Magnesium (Mg) (%)

Five ml of aliquot was taken in 150 ml beaker and diluted with 10 ml of distilled water. 5 ml of ammonium buffer, 0.25 g Sodium Diethyldithio Carbamate, 2-3 drops EBT (Eriochrome Black T) was added to the solution, respectively. The content was titrated against 0.02N EDTA (Ehylene diamine tetra-acetic acid) up to sky blue colour. Magnesium content was estimated using the following equation.

Magnesium (%) = {[(mL of EDTA used × 0.01 × 10 × 2000) − (Ca%)] × 12.15} / 1000

Micro minerals

A 0.5 g powdered leaf sample of *S. foetida* was accurately weighed and transferred into a clean digestion tube. To this, 5 ml of concentrated nitric acid (HNO_3) was added. The sample was then left undisturbed overnight to allow for predigestion. On the following day, 10 ml of a diacid mixture (HNO_3:$HClO_4$, 10:4) was added to the tube. The contents were then digested on a hot plate until the solution became clear, indicating complete digestion of the organic matter. After cooling, the digested sample was filtered through Whatman filter paper No. 42 and the filtrate was quantitatively transferred to a 50 ml volumetric flask. The final volume was made up to 50 ml with distilled water.

The concentrations of micronutrients [Iron (Fe ppm), Copper (Cu ppm), Zinc (Zn ppm) and Manganese (Mn ppm)] **of** *S. foetida* leaf fodder samples were then measured using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

Secondary metabolite estimation

For secondary metabolite estimation, 3 g of sample was extracted in 30 ml of 80% methanol, re-extracted 2-3 times using 5 ml of methanol and the supernatants were pooled. The 80% methanolic extract was used to determine total phenol, total flavonoids and total tannin. For estimation of total flavonoids and total phenolic content, quercetin and gallic acid standard curves were used. Alkaloid content was estimated as suggested by Harborne (1998) followed by titrimetric methods (Debnath *et al.*, 2015), flavonoids content by modified aluminum chloride colorimeter method (Kim *et al.*, 2003), phenolic content by folin-ciocalteu colorimetric method (Malick and Singh 1980), saponin content by double extraction gravimetric method (Harborne 1998), tannin content following Folin-Ciocalteu method (Ferguson 1956).

Statistical analysis

Data pertaining to leaf fodder mineral and secondary metabolite composition were statistically analyzed following factorial completely randomized design (CRD) and F-test was done and ANOVA was constructed following model $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$, where Y_{ijk} = the response form the kth experimental unit receiving the i^{th} level of factor A and the j^{th} level of factor B, μ = overall mean, α_i = an effect due to the i^{th} level of factor A, β_j = an effect due to the j^{th} level of factor B, $(\alpha\beta)_{ij}$ = an interaction effect of the i^{th} level of factor A with j^{th} level of factor B, ε_{ijk} = a random error have a normal distribution $(0, \sigma^2)$. Here, landscapes and seasons are considered as factor-I and factor-II, respectively with four replications. Further, treatment means were compared at $P<0.05$. Further, Duncan's multiple range test (DMRT) was adopted to compare the sets of means of each factor (Sheoran et al. 1998).

RESULTS AND DISCUSSION

Macro mineral composition

The results pinned out that percentage of macro minerals namely nitrogen (N), phosphorus (P), calcium (Ca) and magnesium (Mg) in S. foetida *leaf fodder varied* significantly ($P\leq0.05$) among landscapes (Fig. 3a-d) and seasons (Table 2). Results deduced that nitrogen in Waghai Forest I (L_7) landscape fodder was significantly higher (1.25±0.02%) and lowest (1.00%) was in of Waghai Forest II (L_8) landscape (Fig. 3a).

Table 2: Seasonal variation of *S. foetida* leaf fodder macro mineral composition in different landscapes of south Gujarat, India

Landscape (L)	**Macro mineral (%)**							
	Nitrogen		Phosphorus		Calcium		Magnesium	
	Season							
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
NAU Agricultural I (L_1)	1.11^{gh}	0.89^{j}	0.08^{i}	0.135^{bc}	6.58^{b}	4.58^{g}	1.31^{d}	1.76^{ab}
NAU Agricultural II (L_2)	1.19^{ef}	1.05^{hi}	0.117^{f}	0.13^{de}	5.48^{e}	4.83^{f}	0.85^{ef}	1.81^{ab}
Navsari Urban I (L_3)	1.24^{de}	1.05^{hi}	0.113^{fg}	0.128^{e}	7.13^{a}	4.93^{f}	1.23^{d}	1.55^{c}
Navsari Urban II (L_4)	1.28^{cd}	1.04^{i}	0.117^{f}	0.14^{a}	6.70^{b}	4.83^{f}	1.20^{d}	1.84^{a}
Valsad Urban I (L_5)	1.38^{a}	1.06^{hi}	0.102^{h}	0.143^{a}	6.20^{c}	5.43^{e}	0.81^{f}	1.35^{d}
Valsad Urban II (L_6)	1.22^{de}	0.87^{j}	0.132^{cde}	0.139^{ab}	6.10^{c}	5.33^{e}	1.00^{e}	1.34^{d}
Waghai Forest I (L_7)	1.31^{bc}	1.2^{ef}	0.13^{de}	0.117^{f}	4.48^{g}	5.4^{e}	0.87^{ef}	1.72^{ab}
Waghai Forest II (L_8)	1.37^{ab}	1.14^{fg}	0.109^{g}	0.134^{cd}	5.83^{d}	5.48^{e}	1.28^{d}	1.67^{bc}
Mean	1.26^{a}	1.04^{b}	0.112^{b}	0.133^{a}	6.06^{a}	5.10^{b}	1.07^{a}	1.63^{b}
SEm(±)S	0.01		0.001		0.02		0.02	
SEm(±) S×L	0.02		0.002		0.06		0.05	

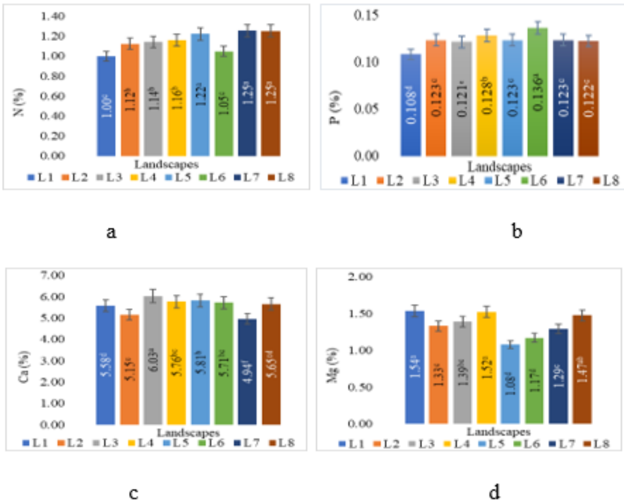

Figure 3: Variation in S. foetida leaf fodder macro minerals (%), a) nitrogen (SEm=±0.02), b) phosphorus (SEm=±0.001), c) calcium (SEm=±0.04) and d) magnesium (SEm=±0.04), among different landscapes (L1 to L8 as given in table 1), (SEm(±)-Standard error of mean), different superscript letters with treatment means in each bar are significantly different according to Duncan's multiple range test (P ≤ 0.05).

Highest P content amounting to 0.136±0.001% was in leaf fodder of Valsad Urban II (L_6) landscape. Lowest P (0.108%) content was in the leaf samples from NAU agricultural I (L_1) landscape (Fig. 3b). Ca content (Fig. 3c) was maximum (6.03±0.04%) in leaf fodder of Navsari Urban I (L_3) landscape. Lowest (4.94%) Ca was in Waghai Forest I (L_7) leaf fodder. Leaf fodder Mg of NAU agricultural I (L_1) landscape was maximum (1.54±0.04%) and lowest (1.08%) was recorded in leaf fodder from Valsad Urban I (L_5) landscape (Fig. 3d). Study expressed that *S. foetida* leaf fodder macro minerals exhibited significant seasonal variation (Table 2). Leaf fodder N (1.26±0.01%) and Ca (6.06±0.02%) was significantly higher in summer season (S_1), whereas Mg (1.63±0.02%) and P (0.133±0.001%) was higher during winter season (S_2).

L-Landscape, S_1-Summer season, S_2-Winter season, SEm(±)-Standard error of mean, different superscript letters with treatment means in same column and row are significantly different, and same letters in column and row are not significantly different according to Duncan's multiple range test ($P \leq 0.05$)

The interaction effect between seasons (S_1=summer, S_2=winter) and landscapes was also statistically ($P \leq 0.05$) significant for all the *S. foetida* leaf fodder macro minerals (Table 2). Highest (1.38±0.02%) N content was due to interaction effect of summer season fodder and Valsad Urban I (L_5) landscape. The interaction between winter season leaf fodder and Valsad Urban I (L_5) landscape resulted in maximum (0.143±0.002%) P content. Significantly maximum (7.13±0.06%) Ca was due to interaction effect of summer season fodder of Navsari Urban I landscape (L_3) however, it was lowest (4.48%) during summer season in Waghai Forest I (L_7) landscape. Interaction due to winter season and Navsari Urban II (L_4) landscape leaf fodder gave significantly higher Mg (1.84±0.05%) however, lowest was because of summer and Valsad Urban I (L_5) landscape fodder interaction effect (Table 2).

Mineral in animal diets required to maintain optimal immuno-physiological function and competence, optimal feed intake and nutrient utilization, food metabolism, inhibition of development of metabolic and oxidative stress, linked to hematopoiesis, metabolism and other vital life functions and significantly impact livestock productivity (Arangasamy et al. 2018). Thus, top feeds, as referred to trees, with higher mineral content in any season if fed to livestock would help improve livestock performance resulting in improvement of life of livestock as well as human by having better milk and other livestock products. This is expected to address United Nations Sustainable Development Goal of responsible consumption and production (SDG 12:) and SDG 15: life on land.

High N levels in leaves favor the retention of flowers and fruits, favor vegetative growth and promotes apical branching which is good for any fodder species being used in lean period. It also accentuates the strength of the demand of the shoot, thus affecting the reproductive structures (Gonzalez et al. 2000). Phosphorous is essential for strengthening the skeleton, teeth, improving blood plasma, assimilation of carbohydrates, fats protein synthesis and necessary for enzyme activation. Phosphorous is the most limiting mineral affecting the productivity of grazing animals throughout the world because of low availability from range plants (Soni et al. 2014). Calcium is an important mineral for cattle and small ruminants, as it is involved in blood clotting, muscle contraction, transmission of nerve impulses, regulation of the heart, secretion of hormones, and enzyme activation and stabilization (Soni et al. 2014) and tree leaves are considered as a good source of Ca (Shinde et al. 2009).

Higher Ca of tree fodder than the recommended levels in the diet for ruminant animals could be advantageous for high yielding ruminants during early stage of lactation (Khan et al. 2008). Magnesium is essential in energy metabolism, transmission of the genetic code, membrane transport, and nerve impulse transmission. Ruminants are generally at risk from hypomagnesaemia when the forage contains less than 0.20 per cent Mg. Magnesium is considered to be an important factor in the occurrence tetany in animals (Soni et al. 2014) and it is advocated to fed animals with top feed (Shinde et al. 2009). The Mg levels were comparable to earlier reported ranges (Jeyaleela et al. 2015, Aye and Adegun 2013, Otache and Agbajor 2017). Zinc is not stored in the body and must be supplemented regularly to maintain optimal immuno-physiological function in animals. Zinc and copper are the second and third most essential trace minerals next to iron. Copper (Cu) and zinc (Zn) in animal diets significantly impact livestock productivity. Copper is required for animals as a trace element. It is intimately linked to hematopoiesis, metabolism and other vital life functions (Arangasamy et al. 2018).

Among the macro-minerals, N in *S. foetida* ranged from 0.86–1.40%, aligning well with commonly fed tree fodders including *Melia dubia* (Jeyaleela et al. 2015, Malek et al. 2024a), *Melia azedarach* (Uppal and Sharma, 2004), *Grewia tenax* (Saleem et al. 2012) and *Celtis australis* (Singh et al. 2022). The P concentration (0.08–0.15%) also remained within the reported spectrum for tropical tree species (Gaikwad et al. 2017, Wankhede and Jain, 2011, Niranjan et al. 2007) and comparable values have been noted in *Melia dubia* (Jeyaleela et al. 2015), *Quercus leucotrichophora* (Kumar et al. 2017a) and several multipurpose tree species (Bakshi and Wadhwa 2004, Shah et al. 2019, Navale et al. 2022). The observed P values indicate that *S. foetida* may contribute meaningfully to meeting the dietary requirement of 0.12–0.24% for ruminants (Khan et al. 2008). The Ca concentration was relatively high (4.10–7.25%), demonstrating that *S. foetida* is a Ca-rich fodder comparable to *M. dubia* (Jeyaleela et al. 2015), *Moringa oleifera* (Moyo et al. 2011) and other widely utilized tree fodders (Niranjan et al. 2007, Gaikwad et al. 2017, Navale et al. 2022). Magnesium content (0.58–1.88%) exceeded the deficiency threshold of 0.20% (Grunes et al. 1970, Soni et al. 2014), remaining comparable to values reported for *Grewia villosa* (Saleem et al. 2012), *Moringa oleifera* (Moyo et al. 2011) and *Ficus capensis* (Achi et al. 2017). The macro-mineral profile of *S. foetida* suggests its potential to support skeletal development, metabolism, rumen microbial activity and physiological homeostasis in ruminants (Jacobson et al. 1972, Kumar and Soni 2014a&b, Soni et al. 2014).

Micro mineral composition

Study indicated that composition of micro mineral i.e. copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn) in *S. foetida* leaf fodder varied significantly ($P \le 0.05$) among the landscapes (Fig. 4a-d) and seasons (Table 3).

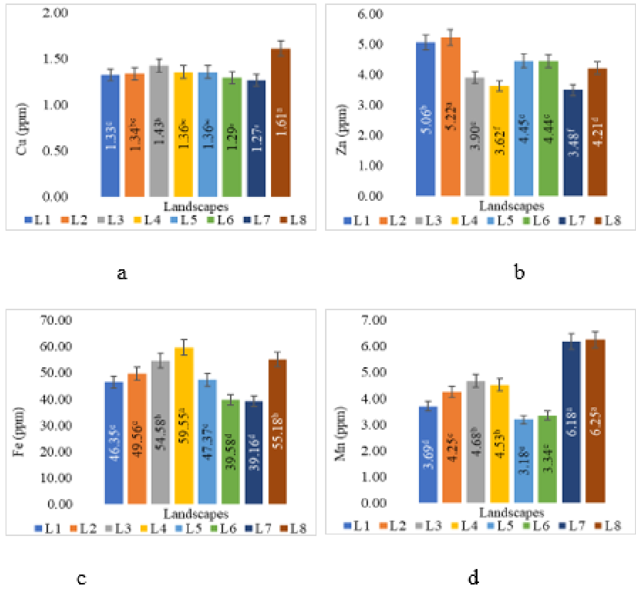

Figure 4: Variation in S. foetida leaf fodder micro minerals (ppm) a) copper (SEm=±0.03), b) zinc (SEm=±0.05), c) iron (SEm=±1.24) and d) manganese (SEm=±0.06), among different landscapes (L1 to L8 as given in table 1), SEm(±)-Standard error of mean, different superscript letters with treatment means in each bar are significantly different according to Duncan's multiple range test (P ≤ 0.05)

The Cu content was significantly higher (1.61±0.03 ppm) in Waghai Forest II (L_8) landscape and lowest (1.27 ppm) in Waghai Forest I (L_7) landscape leaf fodder (Fig. 4b). Significantly maximum (5.22±0.05 ppm) Zn was in NAU agricultural II (L_2) and lowest (3.48 ppm) was in Waghai Forest I (L_7) landscape fodder (Fig. 4c).Fe content in leaf fodder of Navsari Urban II (L_4) landscape was significantly maximum (59.55±1.24 ppm) and lowest (39.16 ppm) was of Waghai Forest I landscape (Fig. 4a). Further, maximum manganese (Mn) (6.25±0.06 ppm) was in leaf fodder of Waghai Forest II (L_8) landscape (Fig. 4d). The lowest (3.18 ppm) Mn content was in leaf fodder of Valsad Urban I (L_5) landscape.

The results deduced that seasonally, leaf fodder Fe and Mn concentration was significantly ($P \le 0.05$) higher (53.02±0.62 and 4.71±0.03 ppm, respectively) during the summer season (S_1), whereas, Cu and Zn content was higher (1.65±0.02 and 4.52±0.02 ppm, respectively) in winter season (Table 3). Study further pinned out that the interaction between landscapes and seasons (S_1=summer, S_2=winter) was statistically significant for all micro mineral components except iron (Table 3). Interaction, winter season and Waghai Forest II (L_8) landscape resulted in maximum copper content of 2.11±0.04 ppm. Maximum Zn content (5.60±0.06 ppm) was due to winter season fodder interaction with Navsari agricultural II (L_2) landscape (Table 3). Significantly maximum (6.75±0.08 ppm) Mn content was as a result of interaction between winter season and Waghai Forest II (L_7) landscape.

Evaluation of micro-minerals indicated that Cu (0.88–2.23 ppm), Zn (2.23–5.58 ppm), Fe (23.60–67.98 ppm) and Mn (2.45–7.00 ppm) fell within safe nutritional limits (Anon. 2012) and were comparable to values reported for various fodder tree species such as *Psidium guajava*, *Bambusa bambos*, *Albizia lebbeck*, *Aegle marmelos* and *Olea glandulifera*

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Table 3: Seasonal variation of *S. foetida* leaf fodder micro mineral composition in different Landscapes of Gujarat, India

Landscape (L)	Micro minerals (ppm)							
	Copper		Zinc		Iron		Manganese	
	Season							
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
NAU Agricultural I (L_1)	1.10^{f}	1.55^{cde}	5.49^{a}	4.63^{c}	51.71^{cd}	40.99^{f}	3.59^{i}	3.8^{ghi}
NAU Agricultural II (L_2)	1.10^{f}	1.58^{cd}	4.84^{b}	5.60^{a}	54.36^{cd}	44.75^{ef}	4.01^{fg}	4.49^{e}
Navsari Urban I (L_3)	1.05^{f}	1.80^{b}	3.90^{f}	3.90^{f}	60.05^{ab}	49.1^{de}	5.53^{d}	3.83^{gh}
Navsari Urban II (L_4)	1.11^{f}	1.60^{cd}	3.51^{g}	3.73^{f}	63.13^{a}	55.98^{bc}	5.40^{d}	3.65^{hi}
Valsad Urban I (L_5)	1.05^{f}	1.66^{c}	4.75^{bc}	4.15^{e}	51.28^{cd}	43.46^{f}	3.33^{j}	3.04^{k}
Valsad Urban II (L_6)	1.11^{f}	1.48^{de}	4.71^{bc}	4.18^{e}	43.89^{ef}	35.26^{g}	4.15^{f}	2.54^{l}
Waghai Forest I (L_7)	1.10^{f}	1.44^{e}	2.54^{i}	4.43^{d}	44.58^{ef}	33.74^{g}	5.61^{d}	6.75^{a}
Waghai Forest II (L_8)	1.11^{f}	2.11^{a}	2.85^{h}	5.58^{a}	55.18^{bc}	55.18^{bc}	6.09^{c}	6.41^{b}
Mean	1.09^{b}	1.65^{a}	4.07^{b}	4.52^{a}	53.02^{a}	44.81^{b}	4.71^{a}	4.31^{b}
SEm(±)S	0.02		0.02		0.62		0.03	
SEm(±)S×L	0.04		0.06		1.75		0.08	

Note: L-Landscape, S_1-Summer season, S_2-Winter season, SEm(±)-Standard error of mean, different superscript letters with treatment means in same column and row are significantly different, and same letters in column and row are not significantly different according to Duncan's multiple range test (P ≤ 0.05)

(Gaikwad et al. 2017, Navale et al. 2022). The Cu concentrations recorded in the present study were comparable to values reported for *M. dubia* (Jeyaleela et al. 2015) and several fodder tree species evaluated by Bakshi and Wadhwa (2004). The Zn content aligned closely with earlier reports in tree fodder (Navale et al. 2022, Gaikwad et al. 2017), while Fe content confirmed earlier conclusions that tree leaves generally exhibit higher iron concentrations than herbaceous forages (Shinde et al. 2009). Leaf age, phenology, season, location and altitude significantly affect fodder mineral matter composition and variation in the present findings may be ascribed to such influences (Gonzalez et al. 2000; Mir et al. 2018).

Seasonal analysis revealed distinct mineral distribution patterns in *S. foetida.* N, Ca, Fe and Mn were higher in summer, whereas P, Mg, Cu, and Zn concentrations were higher during winter. Similar seasonal mineral dynamics have been reported in *M. dubia*, *C. australis*, *G. tenax*, and *Prunus armeniaca* (Saleem et al. 2012, Singh et al. 2022, Kadir et al. 2019, Malek et al. 2023, Navale et al. 2022). These variations may be attributed to leaf maturity, nutrient remobilization, environmental stress, altitude and phenological stage (Gonzalez et al. 2000, Mir et al. 2018). It is comprehended that Mg content range in *S. foetida* fodder, reported in present study, could be considered to overcome occurrence of hypomagnesaemia and tetany (Soni et al. 2014) issues in animals as it contains good percentage of it. Thus, the macro- and micro-mineral concentrations observed in *S. foetida* leaf fodder were found to be within acceptable ranges to support growth, development and other essential physiological functions in livestock.

Secondary metabolite composition

In general, total tannin content (TTC), total phenolic content (TPC), total flavonoids content (TFC), total alkaloid content (TAC), total terpenoids content (TTeC) and total saponin content (TSC) exhibited significantly variation (*P≤0.05*) among the selected 8 landscapes (Fig. 5a-f) and seasons (Table 4). The results indicated that the significantly highest (1.56±0.02%) leaf TTC was in Valsad Urban II (L_6) and lowest (0.84±0.02%) was in Waghai forest I (L_7) landscape (Fig. 5a). Maximum TPC (2.21±0.01%) was recorded in Valsad Urban II (L_6) landscape (Fig. 5b) whereas, the lowest (1.16±0.01%) was in NAU agricultural I (L_1) leaf sample. Similarly, TFC was highest (2.62±0.02%) in Valsad Urban II (L_6) landscape, whereas lowest (1.16±0.02%) was in NAU agricultural I (L_1) landscape (Fig. 5c). TAC (3.08±0.01%) was in leaf samples from NAU agricultural II (L_2), with the lowest (2.91±0.01%) in L_7 landscape (Fig. 5d). Similarly, significantly maximum (1.59±0.07%) TTeC was recorded in L_4 landscape and lowest (1.03±0.07%) was in L_6 landscape leaf sample (Fig. 5e). *S. foetida* leaf TSC of Valsad Urban II (L_6) landscape was highest (8.16±0.06%) and lowest (6.00±0.06%) TSC was in Valsad Urban I (L_5) landscape (Fig. 5f).

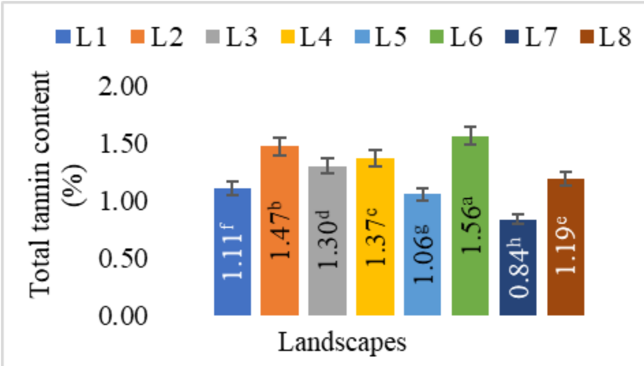

Figure 5: a

S_1- summer season, S_2-winter season; TTC-total tannin content, TPC-total phenolic content, TFC-total flavonoids content, TAC-total alkaloid content, TTeC-total terpenoids content, TSC-total saponin content, SEm-Standard error means, different superscript letters with treatment means in same column and row are significantly different according to Duncan's multiple range test (*P* ≤ 0.05); n=48

Further, results pointed out that there was significant seasonal variation (*P≤0.05*) in *S. foetida* leaf fodder secondary metabolites (Table 4). Leaf TTC, TPC, TFC, TAC, and TSC was significantly higher (1.75±0.01%, 2.279±0.004%, 2.50±0.01%, 3.01±0.004%, and 7.94±0.03%, respectively) during the winter season (S_2). In contrast, TTeC was notably higher (1.43±0.01%) in the summer season (S_1) leaf fodder. The interaction between season and landscape for various secondary metabolites was statistically significant (Table 4). The interaction effect of winter season × Valsad Urban II landscape (L_6) lead to highest TTC, TPC and TFC (2.11±0.03, 2.860±0.012 and 3.26±0.02% respectively) and it was lowest

Table 4: Seasonal variation in secondary metabolites of *S. foetida* leaf fodder in different landscapes of south Gujarat, India

Landscape (L)	Secondary metabolites											
	TTC		TPC		TFC		TAC		TTeC		TSC	
	Season											
	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2
NAU Agricultural I (L1)	0.77^{ij}	1.45^{f}	0.762^{m}	1.551^{h}	1.42^{j}	1.6^{i}	2.92^{e}	3.05^{b}	1.6^{bc}	0.79^{gh}	5.49^{i}	7.61^{e}
NAU Agricultural II (L2)	0.97^{h}	1.98^{b}	1.236^{i}	2.454^{c}	1.78^{h}	2.74^{c}	2.99^{c}	3.16^{a}	1.53^{c}	1.03^{f}	6.09^{h}	8.39^{c}
Navsari Urban I (L3)	0.73^{jk}	1.88^{c}	1.100^{j}	2.150^{f}	1.29^{k}	2.32^{e}	2.83^{h}	3.03^{b}	1.63^{b}	1.21^{e}	5.5^{i}	7.75^{de}
Navsari Urban II (L4)	0.81^{i}	1.93^{bc}	1.021^{k}	2.199^{e}	1.39^{j}	2.38^{e}	2.91^{ef}	3.04^{b}	1.75^{a}	1.43^{d}	6.31^{h}	8.66^{b}
Valsad Urban I (L5)	0.54^{l}	1.58^{e}	0.860^{l}	2.032^{g}	1.09^{l}	2.07^{f}	2.86^{gh}	2.97^{cd}	1.41^{d}	0.74^{h}	5.41^{i}	6.59^{g}
Valsad Urban II (L6)	1.02^{h}	2.11^{a}	1.561^{h}	2.860^{a}	1.97^{g}	3.26^{a}	2.94^{de}	2.99^{c}	1.18^{e}	0.88^{g}	6.8^{g}	9.53^{a}
Waghai Forest I (L7)	0.30^{m}	1.37^{g}	0.663^{n}	2.659^{b}	0.93^{m}	3.09^{b}	2.88^{fg}	2.94^{de}	1.14^{e}	1.01^{f}	5.57^{i}	7.07^{f}
Waghai Forest II (L8)	0.67^{k}	1.71^{d}	1.023^{k}	2.326^{d}	1.43^{j}	2.55^{d}	2.92^{e}	2.93^{e}	1.19^{e}	1.18^{e}	5.59^{i}	7.91^{d}
Mean	0.73^{b}	1.75^{a}	1.028^{b}	2.279^{a}	1.41^{b}	2.50^{a}	2.91^{b}	3.01^{a}	1.43^{a}	1.03^{b}	5.85^{b}	7.94^{a}
SEm(±)S	0.01		0.004		0.01		0.004		0.01		0.03	
SEm(±) S×L	0.03		0.012		0.02		0.012		0.03		0.08	

(0.30±0.03, 0.663±0.012 and 0.93±0.02% respectively) due to Waghai Forest I (L_7) landscape and summer season fodder. Highest (3.16±0.012%) TAC was during winter season in leaf fodder of NAU agricultural II (L_2) landscape whereas, summer season fodder of Navsari Urban II (L_4) recorded lowest (2.91±0.012%). The interaction, summer fodder harvesting and Navsari Urban II landscape (L_4) resulted in highest (1.75±0.03%) TTeC, and it was lowest (0.74±0.03%) due to interaction Valsad Urban I (L_5) and winter fodder samples. Highest (9.53±0.08%) TSC was recorded due to interaction effect of winter season fodder and Valsad Urban II landscape (L_6) whereas, it was lowest (5.41±0.08%) as a result of Valsad Urban I (L_5) landscape fodder harvested in summer (Table 4).

In the present study, the range of TTC (0.21-2.34%), TPC (0.40-2.91%)**,** TFC (0.86-3.59%), TAC (2.83 to 3.17), TTeC (0.70-1.80%) and TSC (5.04 to 9.63) in *S. foetida* are in agreement with that reported in tree species used as top feed (Moyo *et al.,* 2011; Achi *et al.,* 2017; Malek *et al.,* 2025). Apparently, in our study all *S. foetida* leaf non-volatile compounds (TTC, TPC, TFC, TAC, and TSC) were higher during the winter season as compared to summer. These findings have congruousness with those of Malek *et al.* (2025) reported in *M. dubia*, Navale *et al.* (2017) in *Pittosporum floribundum* and Hawu *et al.* (2025) in *Searsia lancea* and *Searsia pyroides.* In contrast, TTeC was higher in the summer season leaf fodder. Riddhi *et al.* (2024) reported higher terpenoid in *Mesosphaerum suaveolens* and Mahmoud *et al.* (2024) in *Polyalthia suberosa* during summer season as compared to winter. Seasonal variation in secondary metabolites can be attributed to a variety of factors, including species, organ, climate pattern, region, habitat, altitude and climate variability. It can also be responsive to ecological stresses (Sala-Carvalho *et al.,* 2019; Riddhi *et al.,* 2024).

Tree leaves showed high digestibility values associated with low TTC levels (Forwood and Owensby, 1985). Mostly in ruminant, the protein tannin complex dissociates and the protein can be digested in the lower gut. Tannins at higher levels become disadvantageous as they reduce the digestibility of fibre in the rumen by inhibiting the activity of bacteria and anaerobic fungi (Akin and Rigsby, 1983). Also, at safe levels tannins are known for their antibacterial, antiviral, and antiparasitic effects (Tong *et al.,* 2022). Phenolic compounds also exhibit beneficial properties; for instance, they act as antioxidants and are capable of preventing diseases such as cancer (Zarfeshany *et al.*, 2014). Alkaloids are known to possess antibacterial properties, which may benefit livestock health (Achi *et al.*, 2017), although certain types may exhibit anti-nutritional effects (Kassa *et al.*, 2015). Terpenoids are bioactive compounds known for their role in enhancing internal resistance to parasites in livestock (Kramer, 2014), and they exhibit a wide range of therapeutic activities, including anti-parasitic, anti-microbial, anti-inflammatory, and anti-viral effects (Dash *et al.*, 2022). Saponins derived from plants are recognized as important dietary supplements due to their antimicrobial properties and their role in protecting plants from microbial pathogens (Achi *et al.*, 2017).(fig 6b)

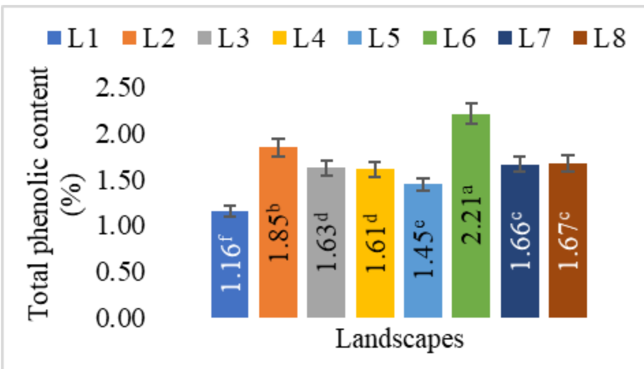

Figure 6: b

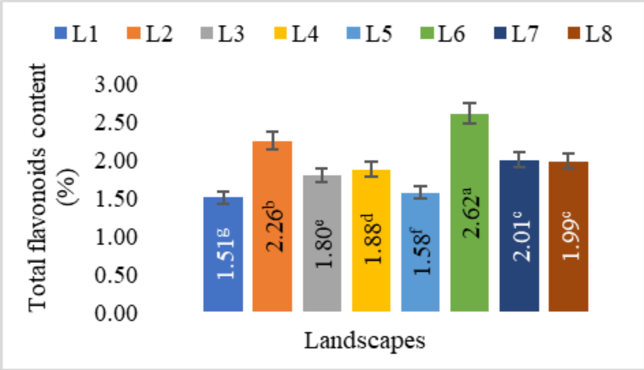

Figure 7: c

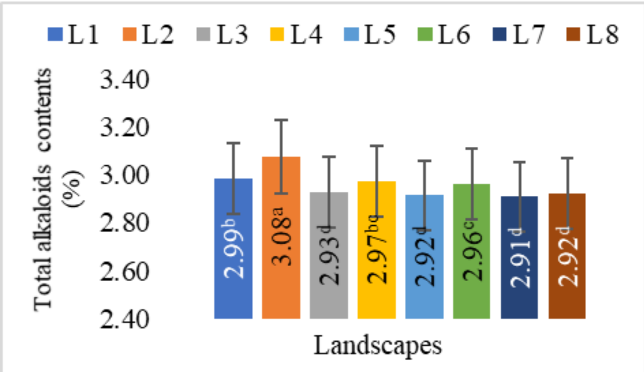

Figure 8: d

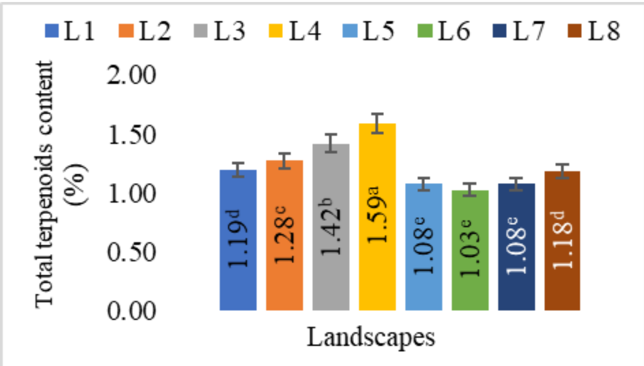

Figure 9: e

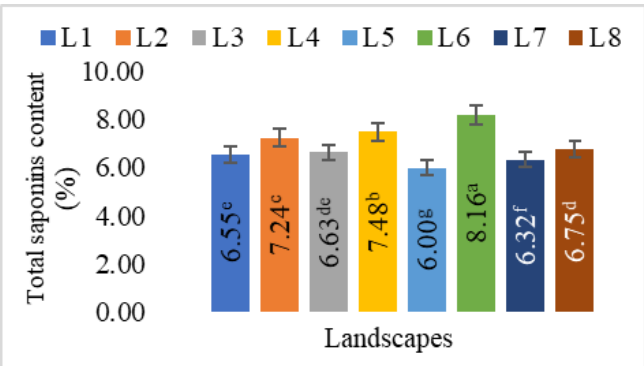

Figure 10: f

CONCLUSION

The study revealed significant variation in macro and micro minerals in *S. foetida* leaf fodder among landscapes and seasons. The interaction between landscape and season was also statistically significant for all the mineral components except iron. Out of the 8 macro and micro minerals N, Cu, Fe and Mn was higher Waghai Forest II (L_8) landscape whereas P, Ca, Mg and Zn was maximum in Valsad Urban II (L_6), and NAU Agricultural I (L_1) and NAU Agricultural II (L_2). N and Ca were significantly higher during summer and Mg and P was higher during winter. Fe and Mn content was significantly higher during the summer season, whereas, Cu and Zn content was higher in winter season. Overall, study divulged that there was fair and square availability of 8 minerals during both the seasons *i.e.* the availability of 4 each was higher in winter and summer season, indicating good fodder quality in both the seasons. As regards secondary metabolites, TTC, TPC, TFC, TAC, and TSC content was higher in lean period *i.e.* winter while TTeC was higher in the summer leaf fodder. Thus, lean period *S. foetida* leaf fodder could provide many beneficial biological activities due to presence of most of alkaloids and flavonoids at safe limits. Thus, *S. foetida* leaf fodder can be categorized as mineral, energy, alkaloid and flavonoid rich fodder. Further, the landscapes with higher mineral matter could be utilized for further improvement programme to improve fodder quality.(fig 7c)(fig 8d)(fig 9e)

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