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Influences of Soil, Community Composition and Species Traits on Steroidal Sapogenin, Zinc and Iron Concentration in Fruits of *Tribulus terrestris*

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ABSTRACT *Tribulus terrestris* L. (Herbal Viagra) is an annual herb, popularized for its pharmacological properties, especially related with its efficiency for correcting male infertility disorders. In present study, impacts of environmental variables on quantitative characters of steroidal sapogenin, iron and zinc in fruit of *T. terrestris* were assessed. Five different sites within the arid area of the Indian Thar desert were selected and sampled during the pulse event (rainy season). Principal Component Analysis ordinate metabolites along-with soil, community factors and plant variables. Suitability of this factor analysis was assessed through Bartlett's test of sphericity and Kaiser-Meyer-Olkin tests. ANOVA revealed the high level of spatial variability in zinc, iron, steroidal sapogenin and for plant cover ($P<0.01$). Among soil variables, silt and clay fractions are very crucial for enhancing iron and steroidal sapogenin concentration; however, sand fraction and soil pH suppressed iron and zinc concentrations, respectively. Negative relationships were established between soil moisture and steroidal sapogenin. Result revealed saturation growth rate relationships between fruit output and steroidal sapogenin. Thus, present findings can be use as protocol through which different environmental factors can be identified and cognition of these would facilitate harvest of these bio-components in larger quantity.

INTRODUCTION

Tribulus terrestris a cosmopolitan annual herb popularly known as "Herbal Viagra" that indicates its potentiality for correcting various problems associated with male infertility. Many researchers have conducted detailed autecology, phytochemistry and clinical studies of this species. Cohort dependent seedling recruitment, survival and reproductive capacity revealed that seedling survivorships (%) and reproductive capacity associated with emergence timing; earlier emergence supports higher survivorship (64%) as well as higher reproductive output as compared to stand with late emergence (Verdu and Mas 2006). Presence and strength of microalage showed positive relationships with seed germination (Petkov 2010), pollen morphology and seed viability correlated with its reproductive biology (Tsevetkova et al. 2011). Pot experiment with 100mgN/kg and 90mgP/kg showed significant increased in dry matter and sapogenin content in the shoot (Georgiev et al. 2010). Inorganic profile (Na^+, K^+, Mg^{2+}, Cl^-, $CaCO_3^{2-}$, SO_4^{2-}), total hardness and total alkalinity in different compartments were also quantified (Hussain et al. 2011). Effect of spatial factors on deviation from the optimum percentage of plant mineral from its reference value in *T. terrestris* revaled larger nutrient imbalance as compared to their optimum values (Mathur and Sundaramoorthy 2013). Based on the relationships between various micro and micronutrients with edaphic factors, researchers recommended the collection of this species from specific habitat that contents higher amount of clay and silt and lesser amount of sand (Mathur and Sundaramoorthy 2013). Influence of geographical origins (Bulgaria, Greece, Serbia, Macedonia, Turkey, Georgia, Iran, Vietnam and India) on prototribestin, pseuoprotodioscin, dioscin, tribestin and tribulosin concentration in diffrent parts of *T. terrestris* revaled two types of chemotypes (Dincheve et al. 2008). Chemotype one (East South Europe and West Asia) characterized by high concentration of protodioscin and the presence of the sulphur containing sapogenins prottribestin and tribestin, while chemotype two collected from Indian and Vietnam had negligible amount of protodioscin and lack of protoribestin and tribestin. Dincheve et al. (2008) have concluded a product - to product variations in the sapogenin composition and recommended a quality evaluation of different herbal products before their pharmacological consumptions. Phytochemistry and Medicinal properties of steroidal sapogenin had been evaluated by Kang et al. (2014), Folwarczna et al. (2016) and Jayachandran et al. (2016). However, identification of causal factors (soil factors and community dynamics) that affect the quantitative varaitation in such importat plant products are still not explored.

Objectives

Following the above information and to patch the existing gaps, present study commenced with two objectives (a) quantify spatial variation in Zn, Fe and steroidal sapogenin contents in fruit of *T. terrestris* (b) to assess the potential impacts of soil (organic carbon, nitrogen, phosphorus, moisture, electric conductivity, and proportion of sand, clay, silt and gravel), community (Richness, Relative Importance value of *T. Terrestris* and diversity parameters (Shannon and Weaver index, Simpson index and evenness) and plant variables (fruit output, fruit weight and plant percent cover) on Zn, Fe and steroidal sapogenin. These micronutrients and steroidal sapogenin were selected based on their potential pharmacological roles includes spermatogenesis, sperm dynamics, correcting erectile dysfunction and nitric oxide accumulation (Mathur 2013).

MATERIAL AND METHODS

Coordinates, habitat types and other attributes of selected sites are presented in Table 1. At each site, ten quadrates of 5x5m abutting to each other in a row were laid across the field (Kent and Cokker 1992). Visits were conducted after the rainy season (August) and fifteen plants randomly uprooted at fruiting stage. Total steroidal sapogenin was estimated by spectro-photometric method (Baccou et al. 1977). Standard prepared with diosgenin and ethyl acetate, acetic anhydrite and sulfuric acid were the major reagent. Absorbance of the supernatant fluid was measured at 430 nm. For Zn and Fe, fruits were grounded and digested with tri acid mixture (HNO_3:H_2SO_4: $HCLO_4$ in 9:1:4 ratio) and quantified through Atomic Absorption Spectrophotometer method (Lindsay and Norvell 1978). Standards of Zn and Fe were prepared with $ZnSO_4.7H_2O$ and Fe $(SO_4)_2.6H_2O$, respectively. Analysis of variance (ANOVA) carried out for micronutrient, steroidal sapogenin and fruit parameters in a strip plot design (Gmoez and Gomez 1984). Community parameters like species richness, Simpson's index, Shannon and Weaver's index and evenness or equitability were calculated (Ludwig and Reynods 1999). Soil samples were collected up-to 30 cm depth. Soil textures were estimated using different types of sieves. Soil moisture quantifies immediately after the sampling, while prior to other parameters, samples were air-dried and sieved through 2mm sieve (Jackson 1973). Water- soil suspension (5:1) utilized to measure electric conductivity (EC) and pH with the respective Digital Meters at room temperature. Organic carbon (modified Walkley and Black's method) and total nitrogen (micro-kjheldahl method) were estimated as described by Jackson (1973), the molybdenum blue color method used for quantification of available phosphorus (Allen et al. 2008).

Exploratory Factor Analysis (Principal Component Analysis) was conducted as a data reduction technique. PCA was performed with Pearson's correlation coefficient with the help

Table 1: GPS locations, habitat types and other attributes of sampling sites

Site No.	*Coordinates*		*Habitat types*	*Land use*	*Status*
	Latitude	*Longitude*			
I	26^0 12' 33.7''	73^0 4' 8.4'	Sandy hummocky plain	Grazing land	Degraded
II	26^0 14' 31.6''	73^0 01' 21.1'	Old alluvium plain	Fallow land	Protected with plantation of *Anogeissus penducla*
III	26^0 18' 47.0''	72^0 60' 35.1'	Piedmonts	Forest	Protected with plantation of *Acacia Senegal*
IV	26^0 17' 2.5''	72^0 56' 5.9'	Younger alluvium plain	Hill slope	Grazing and Cutting allowed
V	26^0 20' 58.9''	73^0 3' 57.2'	Hummocky undulating terrains	Croplands	Double cropping system

of Xlstat software. PCA ordinate the steroidal sapogenin, Zn and Fe along-with soil factors (organic carbon, nitrogen, phosphorus, moisture, pH, electric conductivity and fractional distributions of clay, sand, gravel and clay), community factors (Relative Importance Value of *T. terrestris,* Shannon and Weaver's index, Simpson's index, Richness and Evenness) and plant variables (fruit output, fruit weight and plant cover). Suitability of this factor analysis assessed through Bartlett's test of sphericity and Kaiser-Meyer-Olkin (KMO) tests. Appropriate regression equations were selected based on probability level significance and higher R^2 value. This path analysis carried out by Curve Expert software (2001).

RESULTS

Study revealed higher concentrations of zinc (2.781 mg g^{-1}), steroidal sapogenin (1000.41 μg^{-1}) and plant cover (3.23 %) from the plant material collected from site II; however, the lowest values of these parameters (zinc 0.14 mg^{-1}, steroidal sapogenin 548.57 μg^{-1} and plant cover 0.23%) were recorded from material belonging to site III. Fruit output revealed the reverse value as compared to the above-mentioned parameters, being recorded maximum (1673) at site III, while the lowest (622) from site II. These findings are depicted in Table 2 and revealed the high impact of spatial variablities on these plant products Maximum iron (5.26 mg g^{-1}) and fruit weight (3.99 gm) were recorded from site II and V, respectively. Analysis of variance revealed the high level of spatial variability in Zn, Fe and steroidal sapogenin contents. For these parameters, variations were brought by spatial factor ($P<0.01$). Similar result was also recorded for plant cover ($P<0.01$) however, for fruit output and fruit weight significant variations were brought by both spatial factors as well as by replicates itself. ANOVA analysis (Table 3) further confirmed the significant spatial variations in micronutrients, for steroidal saponins and fruit parameters. Such results indicate that for these parameters, collection site should be considered as an important parameters as this factor brought significant quantitative variations.

Range of various soil, community and plant parameters utilized for multivariate analysis (PCA) is presented in Table 4. Among the edaphic factors sand proportion is dominant (30.37-61.04%) followed by clay (27.61-33.35%). While other factors like electric conductivity, soil pH, organic carbon, nitrogen and phosphorus indi-

Table 2: Micronutrient, steroidal sapogenin, fruit output, weight and plant cover at various sites

Site No.	*Fe (mg g^{-1})*	*Zn (mg g^{-1})*	*Steroidal sapogenins (μg g^{-1})*	*Fruit output*	*Fruit Weight (gm)*	*Plant cover (%)*
I	5.265	1.573	634.85	731	3.37	0.8
II	4.73	2.781	1000.41	622	3.39	3.23
III	3.59	0.16	548.57	1673	3.4	0.33
IV	3.526	0.297	582.81	702	3.18	0.3
V	4.414	0.1432	788.99	500	3.99	0.69

Table 3: Analysis of variance of micronutrients, steroidal sapogenin and fruit parameters in *T. terrestris*

Micronutrients	*Variables*	*Total sum of square*	*Mean sum of square*	*Computed F value*
Iron	Replicates	0.55	0.27	0.59^{NS}
	Sites	5.65	1.41	30.70^{**}
Zinc	Replicates	4.66E-04	2.33E-04	$6.18E\text{-}02^{NS}$
	Sites	16.63	4.15	1103.31^{**}
Steroidal Sapogenin	Replicates	121.5	60.75	2.51^{NS}
	Sites	417193.5	104298.4	4323.24^{**}
Fruit Output	Replicates	709	354.5	166.82^{**}
	Sites	26669404	667351	314047.5^{**}
Fruit Weight	Replicates	0.309	0.154	6.31^{*}
	Sites	1.13	0.28	11.64^{**}
Plant Cover	Replicates	0.1029	0.0051	2.02^{NS}
	Sites	23.95	5.987	235.091^{**}

** = $P<0.01$; * = $P<0.05$; NS =Non Significant cating the moderate soil types. Diversity parameters like the Shannon and Weaver index showed moderate diversity (0.23-2.06) with even distribution patters of different species (evenness E5 0.91-1.2). Relative Importance Value of *T. terrestris* indicated that during rainy season this speices become the important component of plant community. Among the plant variables, fruit out put showed greater spatial variability as it ranges from 500 per plant to 1673 per plant. Similar trend was also recorded for percent plant cover (Table 4).

Table 4: Range of soil, community and plant variables utilized for multivariate analysis

Parameters	*Range*
Edaphic Factors	
Clay (%)	27.61- 33.35
Silt (%)	3.63- 26.87
Sand (%)	30.37- 61.04
Gravel (%)	2.765- 23.25
Electric Conductivity (mSm)	0.143- 0.46
Organic Carbon (mg/100 gm)	145- 225
Nitrogen (mg/100 gm)	15.76- 37.05
Phosphorus (mg/100 gm)	21.38- 54.06
Moisture (%)	4.19- 7.98
pH	8.47- 9.11
Community Parameters	
Richness (Menhinick)	0.562- 0.8
Shannon Weaver Index (H')	0.23- 2.06
Simpson index (λ)	0.15- 0.46
Evenness (E5)	0.91- 1.2
Relative Importance Value of *T. terrestris*	20.61- 35.72
Plant Variables	
Fruit out put	500-1673
Fruit weight	3.18- 3.99
Percent plant cover	0.3- 3.23

The Kaiser-Meyer-Olkin (KMO) is an index used to examine the appropriateness of factor analysis. A high value (between 0.5 and 1.0) indicates the suitability of factor analysis. In the present study, KMO was 0.536, which indicates appropriate use of factor analysis (Table 5). For the Bartlett's test of sphericity, there are two levels to interpret this test (a) H_0: There is no correlation significantly different from 0 between the variables and Ha: at least, one of the correlations between the variables is significantly different from 0. As the computed p-value is higher than the significance level = 0.05, one should reject the null hypothesis H0 and accept the alternate. In other words, we can conclude that there are significant relationships between variables (Table 5). Literature suggested that 80 percent cumulative percentage of variance in PCA indicates perfect variables copious on four principal axes that, ultimately nullify to horse shoe or arch effect as well as usefulness of this multivariate technique (Wei-Gang et al. 2008). In the present study, cumulative percentages indicates that first four axes together accounted 100 percent variation in the data set (Fig. 1 and Table 6) with their individual contribution being 40.86, 30.30, 18.7, and 10.0 percent respectively. For each component, variables with loading ≥0. 70 were identified as significant variable and used for path analysis and for discussion (Iwara et al. 2011). With the help of these 21 variables, five selected sites oriented that showed sites I and II and sites III and IV grouped together while site V was oriented far away from other sites showed its dissimilarity with other sites (Fig. 2).

Table 5: Bartlett's sphericity and Kaiser-Meyer-Olkin sampling adequacy tests

Chi-square (Critical value)	*244.808*
DF	210
p-value	0.251
Alpha	0.05
KMO	0.536

Relationships with Soil Factors

Regression analysis revealed synergistic relationships between silt content and iron concentration in fruit of *T. terrestris* that follows a linear path (Iron concentration = 3.22+0.0918 Silt content R^2 = 0.984**, P<0.01). However, sand content inhibit it in quadratic fashion (Iron concentration = 4.42+0.062 Sand Content+-0.0012 Sand Content^2, R^2 =0.965**, P<0.01). Soil pH linearly and negatively affects the zinc concentration (Zinc Concentration = 40.018 +-4.397 Soil pH, R^2 = 0.91*, P<0.05). Soil electric conductivity exhibited the similar relationships with steroidal sapogenin (Steroidal Sapogenin = 1220.62+-1478.52 Electric Conductivity, R^2 = 0.961**, P>0.01). Soil moisture inhibits steroidal sapogenin content in a quadratic fashion (Steroidal Sapogenin = -2190.31+1123.6 Soil Moisture +-98.01 Soil Moisture^2, R^2 =0. 993**, P<0.01) and from the present study, it was emerged that 5.07 percent soil moisture seems to be most appropriate for its high concentration and after that thresh-

Table 6: Eigen value analysis and other attributes obtained from principal component analysis

	F1	*F2*	*F3*	*F4*
Eigen-value	8.581	6.364	3.939	2.117
Variability (%)	40.862	30.303	18.756	10.079
Cumulative %	40.862	71.165	89.921	100.000
Iron	**0.796**	0.029	0.553	-0.243
Zinc	**0.927**	-0.360	-0.099	-0.020
Fruit output	**-0.799**	-0.466	-0.296	-0.580
Fruit weight	0.072	**0.924**	0.088	-0.364
Percent plant cover	**0.896**	-0.086	-0.435	-0.022
Clay	0.690	0.235	-0.666	-0.161
Silt	**0.843**	-0.120	0.468	-0.237
Sand	**-0.758**	0.274	-0.573	0.148
Gravel	0.254	-0.470	**0.844**	0.033
Electric conductivity	**-0.918**	-0.097	0.347	-0.162
Soil organic carbon	-0.384	**-0.818**	0.115	-0.413
Soil nitrogen	0.351	0.593	0.011	**-0.725**
Soil phosphorus	0.237	**0.897**	0.364	-0.081
Moisture	-0.557	**-0.794**	0.232	0.069
pH	**-0.773**	0.449	0.411	-0.177
Steroidal sapogenin	**0.870**	0.322	-0.373	0.011
Richness	**-0.709**	0.583	0.105	0.384
Shannon Weiver Index	0.665	0.021	0.176	**0.726**
Simpson index	-0.126	**0.943**	0.300	-0.065
Evenness	0.337	0.021	**0.935**	0.104
RIV of *T. terrestris*	-0.429	**0.888**	0.006	0.168

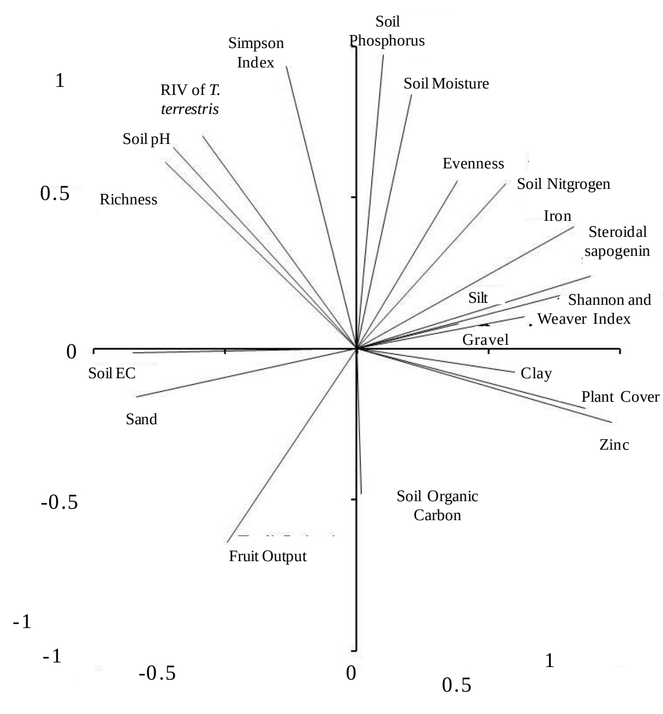

Fig. 1. Principal component analysis with showing plant metabolites and their relationships with various environmental variables

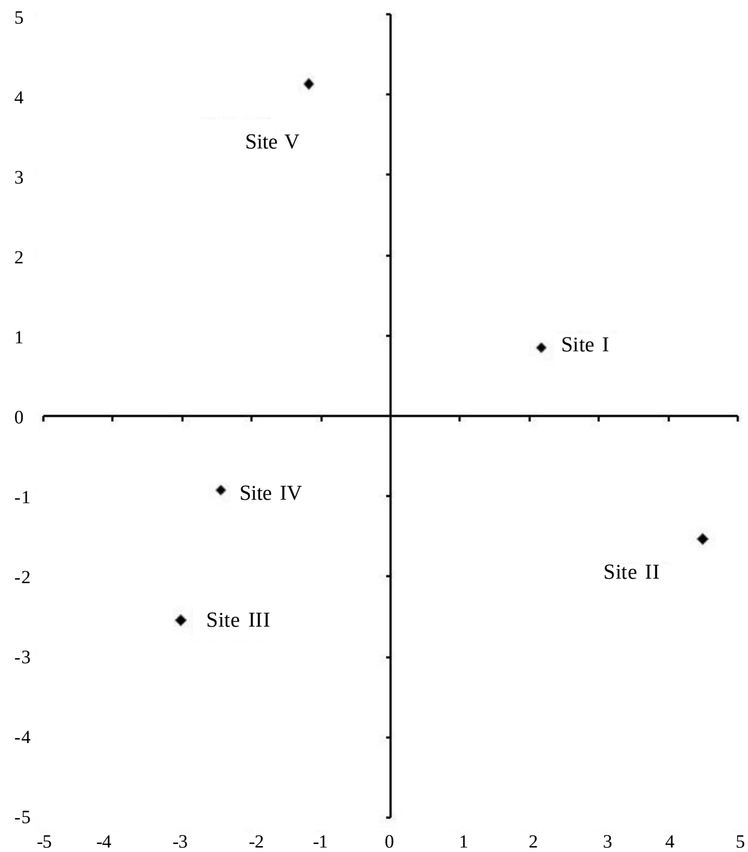

Fig. 2. Principal component analysis showing the positios of various sites in relation to their exploratory and dependent variables

old, the concentration of this secondary metabolite reduces drastically. Clay content supports the steroidal sapogenin in linear fashion (Steroidal sapogenin = -1450.37+ 73.068 x Clay Content, $R^2 = 0.922^*$ $P<0.05$). Besides these important micronutrient and secondary metabolites, soil factors also affect the fruit output as well as fruit weight. Soil organic carbon supported the production of fruits in the quadratic manner (Fruit output = 7386.78+-85.13+ Soil Organic Carbon+0.262 Soil Organic Carbon^2, $R^2 = 0.922^*$, $P<0.05$). For fruit weight, soil Phosphorus (Fruit Weight = 3.903+-0.045 Soil Phosphorus +0.00089 Soil Phosphorus^2, $R^2 = 0.962^{**}$, $P<0.01$) and soil nitrogen (Fruit Weight = 4.288+-0.11 Soil Nitrogen + 0.0029 Soil Nitrogen^2, $R^2 = 0.992^{**}$, $P<0.01$) also show their synergistic relationships in quadratic fashions.

Relationships with Community Diversity

Community richness exhibited negative exponential relationships with zinc concentration (Zinc Concentration = 3876e^ (-12.75 Richness), $R^2 = 0.971^{**}$, $P<0.01$). Among the community diversity parameters, both Shannon and Weaver index (H') and Simpson index showed the quadratic relationships with fruit output; however, Shannon and Weaver's index had a negative relationship with it (Fruit Output = 2059.98+-1857.82 Shannon and Weaver's Index (Diversity) +584.44 Shannon and Weaver's Index (Diversity)^2, $R^2 = 0.975^{**}$, $P<0.01$) while Simpson's index had a positive relation (Fruit Out Put = 4.033+-6.311 Simpson Index +13.51 Simpson Index (Dominance Index)^2, $R^2 = 0.963^{**}$, $P<0.01$).

Relationships with Species Variables

Both zinc (Zinc concentration = 0.070+0.860 percent cover of *T. terrestris* $R^2 = 0.905^*$, $P<0.05$) and steroidal sapogenin contents (Steroidal sapogenin = 562.80+138.61 percent cover of *T. terrestris* $R^2 = 0.914^*$, $P<0.05$) showed linear synergistic relationships with the percent cover of *T. terrestris.*

Relationship of Fruit Output with Steroidal Sapogenin

In the present study saturation growth rate model was established between fruit output and steroidal sapogenin, and this relationship explained as y=ax/(b+x), where y was fruit output and x was steroidal sapogenin content in fruit. Thus the equation can be elaborate as:

$$\text{Fruit Output} = \frac{156.96 \text{ x sterioidal sapogenin}}{(-493.33+\text{steroidal sapogenin})}$$

DISCUSSION

Cardio-protective and other beneficial role of steroidal sapognin in biological system have been reviewed by Folwarczna et al. (2016) and Jayachandran et al. (2016) while, Mathur (2016) provided the biological significance of Zn and Fe on sperm mobility and for correcting male infertility. Present study suggested that among soil factors silt and clay fractions are very crucial for enhancing the iron and steroidal sapogenin concentration for this species, respectively, however; sand fraction and soil pH negatively affect the iron and zinc concentrations, respectively. Many physical and chemical properties of soil may greatly affect Fe availability and Fe use efficiency in plants. These factors include soil moisture, pH, texture, irrigation frequency and synergistic and antagonistic relationships of silt and sand with iron, respectively (Chahabra et al. 1996; Sharma et al. 1996; Nazif et al. 2006; Karaman 2012; Rengel 2015). Further previous results indicates that zinc availability decreases 100-fold for each unit increase in pH (Gangloff et al. 2002; Camberato and Maloney 2012). Typically, soil pH level greater than 7 is associated with Zn deficiency. Eroded areas in soils derived from calcareous parent materials are also associated with Zn deficiency because erosion exposes the high calcium carbonate subsoil which has low Zn availability. Total Zn supply can be low in sandy soils and organic soils, and in conjunction with a high pH can exacerbate the low levels of Zn and cause more severe deficiencies. Soil moisture also negatively affects the steroidal sapogenin. This finding can be supported by the results of El-Sayed et al. (2008) and Odjegba and Alokolaro (2013) where they reported the increase in steroidal sapogenin contents during water stress conditions in *Acalypha wilkesiana* and *Tribulus* species, respectively. This increase could be related to its protective role against oxidative stress (Lin et al. 2009). Among the plant community, present study suggested that habitats with lower diversity (richness and H') and higher dominance of *T. terrestris* indicates higher zinc concentration and fruit output. Similarly linear synergistic relationsips between Zn and percent cover of *T. terrestris* indicated that vegetative growth supports the concentrations of Zn and steroidal sapogenin contents in fruit of this species.

Further negative saturation growth rate relationship between steroidal sapogenin and fruit output suggests that higher content of steroidal sapogenin in fruit inhibited its production and in present study, highest fruit output (1673) was found at the lowest value of steroidal sapogenin content (548.57 µg g^{-1}). The synergistic and antagonistic relationships of steroidal sapogenin with percent plant cover and fruit output, respectively, can be explained through sucrose metabolism and phosphorus uptake and utilization efficiency of species. Since steroidal sapogenin is made up of two types of sugar, predominantly sucrose, after the establishment of the seedling, this sucrose interrupts the phosphorus transportation in fruit and through this mechanism ultimately suppresses the reproductive output but switch on the vegetative growth. These results are in agreement with the studies made by Brilz and Biondini (2002), Gaucher et al. (2004) and White and Veneklaas (2012).

CONCLUSION

In conclusion, silt and clay fractions, low community diversity and high percent cover of *T. terrestris* were the most crucial factors for harvesting higher amount of zinc, iron and steroidal sapogenin contents. From present study, moderate water stress condition is recommended for optimizing yield of steroidal sapogenin, while salinity (higher pH and electric conductivity) and sand fraction were identified as major inhibitory factors for this important secondary metabolite. Saturation growth rate model between fruit output and steroidal sapogenin suggested that plant with higher vegetative growth favoured higher steroidal sapogenin content. Thus, studied parameters can be use as an indicating factor for harvesting higher concentration of the bioactive component and modification in such micro-climatic conditions can enhance the pharmacological values of a product prepared with this species.

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