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Research Paper

Variations in phytosterol composition in *Corchorus depressus* and their relation with bottom-up, top-down and plant metabolites

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ABSTRACT

Phytosterol (cholesterol lowering agent) represent a diverse group of natural products and knowledge about their occurrence in various plants and their chemical compositions have largely been studied. In the present study modular, spatial and temporal variations in phytosterol composition in *Corchorus depressus* with in semi arid part of the Indian Thar desert were quantified. The study revealed that among different modules fruit possessed higher phytosterol during pulse events (rain) while during semi-pulse (winter) and non-pulse events (summer) leaves possessed higher phytosterol compare to other modules. In whole plant semi-pulse (winter) conditions favors phytosterol concentration, while non-pulse (summer) conditions inhibit it. Principal component analysis revealed the significant (P<0.05) relationship between phytosterols composition and various bottom up, top down and plant primary and secondary metabolites. Soil pH, evenness and carbohydrate content showed synergistic relation with phytosterols concentration, while plant protein and phenol had antagonistic relation with it.

Keywords: Phytosterols; *Corchorus depressus;* Principal Component Analysis; Bottom Up and Top Down Factors; Soil pH.

INTRODUCTION

Phytosterols (plant sterols) are triterpenes that are important structural components of plant membrane, and free phytosterols serve to stabilize phospholipid bilayers in plant cell membranes just as cholesterol does in animal cell membrane (Moreau, et al., 2002). They are endogenous to all plants (Phillips, et al., 1999). Common known sources are vegetables, wood, nuts and vegetable oils (Milovanovie, et al., 2009). The function of phytosterol in plants is primarily associated with their ability to affect membrane fluidity and water permeability. They also act as plant hormones and hormonal precursors. Phytosteorls have always been a fascinating subject of study because of their diversified physiological and pharmacological effects on animals.

Piironen, et al., (2000) and Mehtiev and Misharin, (2008) have been reviewed the biological activities of phytosterol in mammals and in mammalian cells. In general it has been demonstrated that diet supplemented with phytosterol (>3g/day) lowered blood plasma cholesterol level (7-10%) and LDL cholesterol level (10-15%). Other than cholesterol lowering properties phytosterols have also useful as an anti-inflammatory, anticancer and immune regulatory substance (Carr and Borwn, 2010).

A detailed study of sterol biosynthesis in *Zea Mays* has demonstrated that their composition varies as a function of both plant organ and period of development (Guo, et al., 1995). Sterol inter-conversion is controlled by phyto-hormones level and environmental conditions like light, temperature, water, response to ozone stress, ions etc. (Moreau, et al., 2002; Pavlik, et al., 2010). Okpuzor, et al., (2009) have reported spatial and temporal variation in phytosterol concentration in different brands of vegetable oils. However, Hartman and Benveniste, (1974) have reported that in potato tuber burst of respiration and metabolic activity following slicing of tissue is accompanied by a sharp increase in de nova phytosterols synthesis. Whatever the sources, phytosterol composition varies with species, variety, growing location, season and processing types (Philips, et al., 2002; Vikstrom, et al., 2005).

Phytosterols are not synthesized by animals or human body and can only enter in the body through diet, large number of plants including many angiosperm and gymnosperms have been screened for this natural substance. Phytosterol production in *Digitalis* purpurea has been reported by Evans, (1973) while, Shirley, et al., (1984) have screened 67 different medicinal plants for this natural product. Sarin and Bansal, (2011) quantified phytosterols from *Adhatoda vasica* and *Ageratum conyzoides* from semi arid environment of Rajasthan, India. Very information review on phytosterols concentration in tomato, apple, potato, *Cucumis melo*, carrot, cabbage, canola, *Asparagus*, *Discorea spp.*, *Solanum melongena*, *Trigonella foenum-graceum* have been made by Moreau et al., (2002).

With above information's a study was conducted to quantify the impact of various spatial and temporal events on composition of phytosterol in various modules of *Corchorus depresus.* The objectives is (a) to quantify the impact of various spatial and temporal events on composition of phytosterol in various modules of *Corchorus depresus* under natural conditions and (b) to find out relationship between phtyosterol compositions with bottom up (community dynamics like, Relative Importance Value {RIV} of *C. depressus*, Shannon and Weaver Index {H'}, evenness and richness), top down factors like Soil parameters (organic carbon, nitrogen, moisture, phosphorus, soil pH, electric conductivity) and primary and secondary plant metabolites.

MATERIALS AND METHODS

Plant materials were collected from five natural sites during three distinct seasonal events, i.e. rain (Pulse events), winter (semi-pulse events) and summer (non-pulse events). These events were considered as resource gradient with in semi arid system. *C. depressus* is a woody perennial plant inhabiting many parts of the word particularly on compact soil. Whole plant have been traditionally use as aphrodisiac. Mathur (2005) established the clinical validation of its traditional medicinal property. During the study period mean annual precipitation ranges from 0.004 to 260 mm, average winter (January) temperature ranges from 10.7°C to 23°C while, mean summer (June)

temperature ranges from 28.7 to 42.2°C. Relative humidity ranges from 31 to 91% (Morning) and 08% to 68% (Evening).

The coordinates (Table 1) and other attributes like edhaphic, plant metabolites and community dynamics have been mentioned in (Table 2)**.** The phytosterols concentration in the ethanolic plant extract was done by sperctro-photometrically through Liebermin - Burchard method (Bloor, 1916). In this method the standard was prepared with 20 mg. cholesterol and Acetic-anhydride-H_2SO_4 was the main reagent. The mechanism of this test includes that acetic anhydride break down the sterol into chleostra-3,5 diene and H_2SO_4 converted it into Bioscholestra -3,5 diene monosulphonate, this produces green colour which measured spectro-photometrically at 680 nm (Okpuzor, et al., 2009). This method was chosen for its simplicity and rapid analysis and it generally gave an approximate level of phytosterol. Further, HPLC protocol available only for some particular phytosterol composition of vegetable oil (Okpuzor, 2009).

Statistical analysis: Two way Analysis of Variance in strip plot design were carried out, where vertical factor was site and different seasons were considered as horizontal factor (Gomez and Gomez, 1984).

Multivariate analysis**:** Principal Component Analysis (PCA) was performed to know the relation among phytosterol concentration and with various bottom up (community dynamics like, Relative Importance Value {RIV} of *C. depressus*, Shannon and Weaver Index {H'}, evenness and richness), top down factors like Soil parameters (organic carbon, nitrogen, moisture, phosphorus, soil pH, electric conductivity) and primary and secondary plant metabolites like (protein, phenol, carbohydrate, steroidal saponins, alkaloids, phosphorus, calcium, sodium, potassium, and iron). Ranges of these parameters are presented in Table 2. The interpretation of the correlation circle was carried out under following criteria, when two variables are far from the center, then if they are: close to each other, they are significantly positively correlated (r close to 1), if they are orthogonal, they are not correlated (r close to 0), if they are on opposite side of the center, then they are significantly negatively correlated (r close to -1). Squared cosines were used to link the variable with the corresponding axis and the greater the squared cosine, the greater the link with the corresponding axis. However, in order to determine basic soil, diversity (vegetation) and plant metabolites variables sustaining these interrelationships, the concept of component defining variables (CDV) which stipulates the selection and subsequent naming of variables with the highest component loading (correlation coefficient) as variables that provide the best relationships (Iwara, et al., 2011) was employed.

RESULTS AND DISCUSSION

Among different modules fruit possessed higher phytosterol during pulse events, while during semi-pulse and non-pulse events leaves possessed higher phytosterol compare to other modules (Table 3). Since the whole plant is useful for its medicinal properties, total phytosterol concentration during various spatial and temporal events were also quantified (Table 4). Results revealed that C. *depressus* possessed higher phytosterol during semi-pulse events, followed by pulse and non pulse events. Thus, semi-pulse (winter) conditions favors phytosterols concentration, while non-pulse (summer) conditions inhibit it. It is evident that in plants the sterol pathway consists of a sequence of more than 30 enzymes-catalyzed reactions, all of which are found in

the plant membranes and temperature sensitive (Benveniste, 1986; Piironen, et al., 2000). Sterols are controlled by phyto-hormones level and environmental conditions and they are involved in the regulation of membrane properties in response to changing growth conditions (Lindsey, et al., 2003). Present results can be supported by the finding of Whitaker (1993; 1999) that stated the storage of mature green tomato fruit at (2°C) (chilling) resulted in about a two fold increase in the level of free sterol. Analysis of variance revealed that variance in phytosterol brought by sites, seasons, plant parts as well as by their interaction also, $P<0.01$ (Table 5).

Results of PCA analysis presented in Table 6 and Figure 1. PCA were considered useful if their cumulative percentage of variance approached 80% (Wei et al., 2008). In present investigation cumulative percentage indicates that first four axes together accounted 84.38% variability in the data set (Table 6) with their individual contribution being 36.66%, 20.79%, 18.69%, and 8.28%, respectively. On each component, variable with loading ≥0.70 were identified as significant variable and used for path analysis and for discussion (Iwara, et al., 2011). Results revealed that among 21 different variables 13 variables were significant. Among the primary and secondary metabolites phytosterol, steroidal saponin, protein, phenol, and potassium are significantly correlated with F1 component; however, carbohydrate and plant phosphorus are well linked with F2 component. Among community dynamics and soil parameters Relative Importance Value, H', evenness, soil phosphorus and soil pH related with component F1. While soil organic carbon are well related with F3 component. Correlation circle (Figure 1 and Table 7) revealed that plant protein [r (regression significance) = -0.59; $P<0.05$], phenol (r = -0.62, $P< 0.050$), carbohydrate (r = 0.97, $P<0.01$) soil pH (r = 0.76, $P<0.01$) and evenness (r = 0.57, $P< 0.05$) were significantly related with phytosterols. Thus among plant metabolites protein and phenol showed negative relation with phytosterols however, carbohydrate showed synergistic relation with it. Among soil parameters soil pH seems to be controlling factor for phytosterols composition in *C. depressus*. Significant positive relation between evenness and phytosterols revealed that uniform habitat Phyto-diversity supports the phytosterols compositions in this plant. Negative relation between phytosterols and phenol (Tocopherol) were demonstrated by Vlahakis and Hazerbroek, (2000). In the present paper it can be hypothesized that temperature is the main factor association with this negative relation between them, phytosterols synthesis generally triggered by cool (winter) temperature (Whitaker, 1993 and 1994) whereas the phenolic compound increases during stress or elevated temperature (Mathur, 2005). Synergistic relation between phytosterols and soil pH and antagonistic relation between phytosterols and plant protein have been explained by Bhardwaj et al (2010) in Lupin plant. Ozsahin and Yilmaz (2010) have been reported positive relation between phytosterols and carbohydrate in Apricot fruit.

CONCLUSION

The present study provides useful information about variation in phytosterols compositions during various spatial and temporal events as well as factors affecting it. Such studies are useful for formulation of phytosterols based product. Study revealed that winter season and soil pH is the most crucial factors for harvesting phytosterols in larger quantities.

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Table-1: Geographical locations of various study sites.

Site	North	East
Site 1	26^0 12' 48.4"	73^0 4' 7.8"
Site 2	26^0 11' 33.4"	73^0 3' 6.1"
Site 3	26^0 14' 47.01"	73^0 0.0' 58.9"
Site 4	26^0 14' 12.4"	73^0 01' 24.2"
Site 5	26^0 21' 54.5"	73^0 03' 48.9"

Table-2: Metabolites, edhaphic factors and community parameters of the studied locations.

Plant Metabolites and Minerals	
Steroidal saponins (mg/ g)	12.64-211.64
Protein (mg/ g)	6.24-30.67
Phenol (mg/ g)	1.85-251.55
Alkaloids (%)	0.07-3.38
Carbohydrate (mg/ g)	52.64-375.07
Phosphorus (mg/ g^-)	21.18-73.61
Calcium (mg/ g)	21.53-44.9
Sodium (mg/ g)	1.37-16.13
Potassium (mg g^{-1})	1.92-82.45
Iron (mg g^{-1})	1.84-36.56
Soil Parameters	
Clay (%)	17.09-29.18
Silt (%)	17.89-25.43
Sand (%)	23.53-43.3
Gravel (%)	10.23-33.5
Electric conductivity ($mScm^{-1}$)	0.06-0.93
Organic Carbon (mg100 g^{-1})	24.97-785
Moisture (%)	0.18-13.98
pH	7-8.81
Nitrogen (mg100 g^{-1})	10.83-118.7
Phosphorus (mg100 g^{-1})	5.54-62.47
Community Parameters	
Relative Importance Value (RIV) of *C. depressus*	6.25-49.11
Species richness	0.505-0.93
Shannon and Weaver Diversity Index (H')	1.04-2.29
Evenness	1.54-2.2

Table-3: Range of Phytocholesterol (mg/g) in various modules during various temporal events.

Events	Root	Stem	Leaves	Fruit
Pulse	0.050-0.177 ± 0.049	0.11-0.193 ±0.072	0.116-0.271 ±0.065	0.151-0.355 ±0.080
Semi-pulse	0.203-0.361 ± 0.066	0.284-0.599 ± 0.14	0.416-0.967 ± 0.21	0.082-0.404 ± 0.12
Non-pulse	0.035-0.088 ± 0.021	0.040-0.123 ± 0.031	0.069-0.131 ± 0.025	0.016-0.092 ± 0.026

Table-4: Total phytosterols concentration (mg/g) at various spatial and temporal events.

Events	Site 1	Site 2	Site 3	Site 4	Site 5
Pulse	1.50± 0.30	2.20 ±0.20	2.0±0.10	1.90 ±0.40	1.20 ±0.20
Semi-pulse	5.60 ±0.120	3.30 ±0.40	6.20 ±0.60	5.6 ±0.70	6.0 ±0.95
Non-pulse	0.60 ±0.04	0.80 ±0.10	1.0 ±0.09	1.10 ±0.13	0.50 ±0.12

Table -5: Analysis of Variance for Phytosterol.

Source of Variation	Mean Square	Computed F Value
Sites	53940.75	144.95**
Season	2085162	49920.73**
Site X Season	53146.88	346.645**
Plant Parts	279820.7	159.28**
Site X Plant Parts	28121	151.67**
Season X Plant Parts	139764	753.85**
Site X Season X Plant Part	21798	117.57**

- ** = $P<0.01$ level

Table- 6: Eigenvalues and other attributes of Principal Component Analysis.

	F1	F2	F3	F4
Eigenvalue	7.699	4.366	2.190	1.740
Variability (%)	36.663	20.791	18.697	8.287
Cumulative %	36.663	57.454	76.147	84.380
Phytocholestrol	-0.73	-0.666	0.041	-0.217
Steroidal saponins	0.799	-0.316	-0.014	0.055
Protein	0.718	0.289	0.203	0.160
Phenol	0.900	-0.016	0.149	0.215
Alkaloid	0.302	-0.624	0.450	0.457
Total Sugar	-0.566	-0.712	-0.114	-0.265
Plant P	-0.313	0.817	-0.086	0.211
Plant Ca	-0.622	0.051	0.548	-0.418
Plant Na	-0.024	-0.587	-0.389	-0.076
Plant K	-0.834	-0.393	-0.107	0.139
Fe	-0.316	-0.368	0.414	0.623
Electric conductivity	-0.491	0.186	-0.405	0.164
Organic carbon	-0.127	0.138	0.748	-0.231
Moisture	-0.266	0.655	0.398	0.022
Soil pH	-0.702	-0.279	0.211	0.013
Soil P	-0.792	0.149	0.327	-0.116
Soil nitrogen	0.490	-0.526	0.225	-0.356
RIV *C. depressus*	0.781	-0.517	-0.087	0.100
Richness	-0.540	-0.314	0.199	0.634
H'	-0.779	0.407	-0.261	0.263
Evenness	-0.71	-0.285	-0.262	0.072

- Variables underlines with eigenvectors (coefficients) >±70 are considered.

Table-7: Correlation matrix between phytosterols and other parameters.

	Phytosterols	Protein	Phenol	Carbohydrate	pH
Phytosterol	-				
Protein	**-0.59**	-			
Phenol	**-0.62**	**0.68**	-		
Carbohydrate	**0.97**	**-0.63**	**-0.55**	-	
pH	**0.76**	-0.50	**-0.52**	**0.69**	-
Evenness	**0.57**	-0.41	**-0.57**	0.51	**0.53**

- Bold letters indicates significant values (P<0.05).

Figure-1: Correlation Bi-plot of Principal Component Analysis.