

The Variations in the Phytochemical Attributes of *Nerium Oleander*, *Ricinus Communis*, *Calotropis Procera* and *Withania Somnifera* From Three Ecological Zones of Pakistan

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Research Article

Keywords: Ecological zonation, flavonoids, phenolic acids, phytoclimatic diversity, tannins

Posted Date: March 25th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-3308843/v2>

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Additional Declarations: The authors declare no competing interests.

Abstract

The phyto-climatic diversity in a region presents the life form of vegetation. Plants produce vast and diverse variety of phytochemicals to communicate and minimize the climatic threats. In the current study, the variations in the phytochemical attributes of four plant species (*Nerium oleander*, *Ricinus communis*, *Calotropis procera* and *Withania somnifera*) from three ecological zones of Pakistan (sandy desert, irrigated & rain-fed plains) were investigated. The phenolic acids, flavonoids and tannins were quantitatively analyzed by methanolic extraction and standardized methods. The results revealed that zone rainfed lands and zone sandy desert showed the maximum amount of phytochemicals in all species and also exhibited the extreme environmental conditions. The irrigated plains had less stressful environmental condition and maximum amount of soil organic matter that resulted in minimum amount of phytochemicals in plant species. Previous research also supports the evidence that environmental stresses like temperature stresses, water stresses, high light intensities increase, and organic matter hinders the accumulation of phytochemicals. This study proved that same species in different ecological zones show different amounts of phytochemicals due to environmental variations, although environmental stresses exert the effects on plants to produce more phytochemicals.

Introduction

The life form of vegetation in a region can be measured by the plant-environment analogy (Khan et al., 2013). Different ecological factors influence differently the vegetation, plant community, structure, and morphology of plants to adapt to their environment for their better survival of life. These influences could lead to long term adaptive strategies (Salick et al., 2009; Khan et al., 2013). Long term existence of species needs to be differentiated as they have back-and-forth in their abilities to respond constrains of their environment (Tilman, 1988). Ecological attributes that can affect biota of a region are, slope, altitude, topography, soil, habitat, vegetation type, plant community (Ahmad et al., 2008), solar radiation, temperature (Javaid et al., 2010), moisture stress, salinity, water, and precipitation (Shukat & Burhan, 2000).

The phyto-climatic gradient of a region influences the plants to grow healthy i.e., to adapt to their environment (Khan et al., 2013). The effect of climatic diversity on the synthesis of phytochemicals has widely perceived a considerable amount of attention (Krasteva et al., 2013). Plants produce a vast and diverse variety of organic compounds that help them to communicate and overcome the environmental threats (Hussain & Reigosa, 2014; Pavarinia et al., 2012). The main function of plant organic compounds is thought to produce phyto-climatic adaptive mechanisms (Kliebenstein, 2004; Hussain et al., 2012). Different biotic and abiotic stresses influence the accumulation of plant organic metabolites (Naghdibadi et al., 2004; Pavarinia et al., 2012). The diversity in accumulation of phytochemicals is strongly susceptible to temperature, light intensity, altitude, and water stresses (Nikolic and Zlatkovic 2010; Radusiene et al., 2012). For the current investigation four plant species were selected from different zones. The *Nerium oleander* belongs to *Apocynaceae* family. It is a poisonous evergreen shrub that grows to about 6 to 7 meters in height. It loves warmth and tolerates all types of soils. (Huxley et al., 1992).

Ricinus communis belongs to family *Euphorbiaceae*, it is an evergreen glabrous, soft-woody shrub. It grows throughout the warm temperate and tropical regions. (Salihu et al., 2012). *Caloropsis procera* belongs to *Asclepiadaceae* family, it is erect, tall, large, branched and with milky latex shrub (Begum et al., 1987). *Withania somnifera* belongs to family Solanaceae, an erect, evergreen, tormentor shrub, 30-150 cm in height and with stout and fleshy taproots. (Welman, 2011).

Materials and methods

Study area: Pakistan has a great climatic diversity due to variation in topography, altitude, and season throughout the region. PARC (1980) have subjected the country into ten agro-ecological zones, based on physiographic characteristics (Muhammad, 1986; PARC, 1980) (Fig. 1). Only three zones were selected for the present studies. The site of sampling from investigated zones is presented in table 1.

Table 1 Ecological conditions of selected sites with their respective zones

No.	Name of zones	Sites	Altitude	Ecological conditions of sites
1	Sandy Desert	Hasilpur (cholistan)	140 m or 459 feet	Arid climate, annual rain fall 100-200 mm, temperature max. 50 °C and mini. 13 °C and sandy soil (Noureen et al., 2008)
2	Northern Irrigated plain	Sahiwal	175 m or 574 feet	Semi-arid climate, annual rain fall 200 mm, temperature max. 45 °C and mini. 7 °C and sandy, loam-clay, loam soil (Arshad et al., 2007).
3	Barani lands (rainfall)	Kalar kahar	644 m or 2114 feet	Hot semi-arid climate, annual rain fall 485 mm, temperature max. 38 °C and mini. 4 °C and sandy, loam-clay, loam soil (Ahmed & Zaman, 2012).

Soil Sampling and Determination of various parameters

Collection of soil sample: All the soil samples were collected from the barren soil as well as under the canopies of selected plants. Soil samples were taken from the depth of 10-15 of the topsoil using a soil digger.

Organic matter determination: Organic matter of soil was determined by the standard method, referred to as the Walkley-Black method explained in Estefan et al. (2013), 10 ml of 1 *N* potassium dichromate solution and 20 ml of concentrated H₂SO₄ were added in 1gm of air-dried soil sample and swirl the beaker to mix the suspension. After 30 minutes, about 200 ml DI water and 10 ml concentrated H₃PO₄ were added using a dispenser and allowed the mixture to cool. Then 10-15 drops of diphenylamine indicator added, and titrated with 0.5 *M* ferrous ammonium sulfate solution, until the color

changed from violet-blue to green. Whole the process was repeated thrice, a blank solution containing all reagents but no soil, also treated in the same way as the soil suspensions.

Quantitative Analysis

Preparation of extract: Powder of each plant leaf sample (10 g) was soaked separately in 75% methanol for 24 hours. The soaked mixture was filtered and obtained residue was re-extracted with the same volume of solvent and filtered again and volume was made up to 100 ml in measuring flask.

Determination of phenolic acids: Total phenolic acid content was estimated by the method described by Taga et al. (1984). Each extract (1ml) was mixed with 10% Folin-ciocalteu reagent (1ml) followed by the addition of saturated solution of Na_2CO_3 (2 ml). The mixture was kept for 30 minutes, and absorbance was measured $\lambda=760$ nm by spectrophotometer. The total phenolic acid contents were calculated as gram gallic acid equivalent 1g/100g dry weight by using the regression equation from the standard curve of Gallic acid.

Determination of flavonoids: The total flavonoid contents were estimated according to the method of Jia and Tang in 1999 with a slight modification. An aliquot (1ml) of each extract (0.1 mg/ml) was added to 4ml ethanol and 0.3 ml NaNO_2 . After ten minutes 10% AlCl_3 (0.3 ml) was added at 25°C. Then 4% NaOH (4 ml) and 30% ethanol (0.4 ml) were thoroughly mixed in it. It was kept standing for 12 minutes and absorbance was measured at $\lambda=510$ nm against blank. The contents of total flavonoids were calculated as 1g/100 g dry weight by the calibration curve of catechin anhydrate.

Determination of tannins: The total tannins content in ground samples were determined by the following method of Makkar as modified by Fagbemi et al., in 2005. To the methanolic extract of each sample (1ml), Folin –Ciocalteu's reagent (0.5ml), saturated Na_2CO_3 solution (1ml) and distilled water (8 ml) were added, mixed well, and allowed to stand for 30 minutes at room temperature. The contents were centrifuged, and absorbance was recorded at $\lambda=725$ nm by using UV-Visible spectrophotometer. The tannins contents in each sample were calculated as g/100 g dry weight by the calibration curve of tannic acid.

Results

The results show that in zone 1 *N. oleander* contains the highest amount of phenolic acids (3.251 g) and in zone 2 it contains the minimum (0.45 g) amount, while in zone 3 it has 1.966 g phenolic acids, as arranged in the order zone 1>zone 3>zone 2. It contains the maximum flavonoids (0.132 g) in zone 1 and the minimum (0.048 g) in zone 2, while in zone 3 it has 0.091 g, as arranged in the order zone 1>zone 3>zone 2. It contains maximum tannins (1.369 g) in zone 1 and minimum (0.984 g) in zone 2, while in

zone 3 it has 1.021 g, as arranged in the order zone 1>zone 3>zone 2. These phytochemicals are significantly varied in each zone at the level of 0.05 (Fig. 2).

The results show that *R. communis* has phenolic acids in the decreasing order, zone 3>zone 2>zone 1 as maximum (2.166 g) in zone 3 and minimum (1.897 g) in zone 1. It is found to contain the maximum (0.083 g) and the minimum amounts (0.063 g) of flavonoids in zone 1 and zone 2 respectively while in zone 3 it has 0.081 g, as arranged in the decreasing order zone 1>zone 3>zone 2. It has maximum (1.168 g) and minimum amounts (0.854 g) of tannins in zone 3 and zone 2 respectively while in zone 1 it has tannins 0.944 gm, as arranged in the decreasing order zone 3>zone 1>zone 2. All these phytochemicals are significantly varied at the significance level of 0.05 in all samples (Fig. 3)

The results show that *C. procera* contains phenolic acids in the order zone 2>zone 1>zone 3, as the lowest amount (1.04 g) is present in zone 3 and the highest amount (2.102 g) in zone 1. It contains flavonoids in the order zone 2>zone 3>zone 1, as the lowest amount (0.061 g) is found in zone 1 and the maximum amount (0.074 g) in zone 2. It contains tannins in the order zone 2>zone 1>zone 3, as the least amount (0.836 g) is found in zone 3 and maximum (1.225 g) in zone 2. These phytochemicals are significantly varied at the significance level of 0.05 in all zones (Fig. 4).

The results show that *W. somnifera* contains the highest amount (3.39 g) of phenolic acids in zone 3 and the lowest amount (1.136 g) in zone 1, as in the decreasing order zone 3>zone 2>zone 1. It contains the highest amount (0.011 g) in zone 2 and the minimum (0.088 g) in zone 3 as in the decreasing order zone 2>zone 1>zone 3. It has the highest amount (1.351 g) in zone 3 and minimum amount (0.912 g) in zone 1, as in the decreasing order zone 3>zone 2>zone 1. The two phytochemicals phenolic acids and tannins are significantly varied at the significance level of 0.05 and flavonoids show significance at the level of 0.91 so; it is not significantly varied in all samples of *W. somnifera* (Fig. 5).

Discussion

The phytochemical analysis of all plant samples suggests that the quantities of phytochemicals of each species are significantly varied in studied zones. Previous research revealed that environmental stresses cause the major effect on synthesis of phytochemicals. In the current study plant species selected from three different ecological zones, which are divided on the base of different ecological conditions by PARC, these different ecological conditions can be responsible for varied phytochemical contents of same species in different zones.

The results revealed that maximum phytochemical contents were found in zone 1 and zone 3 and minimum amounts in zone 2. Other researchers reported that both these zones exhibit extreme climatic conditions, as zone 3 is present at the 644 m altitude, zone 2 is at 172 m and zone 1 is at the 140 m. So, zone 3 because of highest altitude faces low temperature, high UVB light, high precipitation, and low atmospheric pressure (Ahmed & Zaman, 2012), while zone 1 has the lowest altitude and faces droughts, low precipitation, and high temperature (Noureen et al., 2008). Environmental stresses i.e., temperature, light intensity, altitude, and water stresses strongly influence the accumulation of phytochemicals (Nikolic

and Zlatkovic 2010; Radusiene et al., 2012). UV-B-treated plants produce significant amounts of flavonoids and phenolics (Gould, 2004). UVB radiation increase phenylalanine ammonia lyase (PAL) activities which can facilitate phenolic accumulation and induce stresses tolerance (Tekelmariam & Black, 2004). It is also reported that flavonoids increase with light intensity forms (Erhard & Gross, 2005). Phenolic and flavonoid concentrations are also influenced by irrigation or precipitation in berries (Cuadra and Harborne, 1996). High temperature also induces increased phytochemical contents in several plant species (Sayre et al., 1953). Bray (2002) reported that chalcone synthase (CHS) an enzyme that is involved in the biosynthesis of flavonoids and phenylalanine that (PAL) is the key enzyme in the phenolic biosynthesis that were up regulated by drought stress in *Arabidopsis* (Bray, 2002). High temperatures, water stresses, extreme light intensities and poor soil quality increase the tannin content of plants (Rhoades, 1979). High temperatures and UV light have been shown to influence flavonoid gene expression (Shamir and Levi-Nissim, 1997).

Results indicate that zone 1 has lowest and zone 2 has maximum amount of soil organic matter from open soils as well as under plant canopies (Fig. 6 & 7). Other research also support the evidence of less soil organic matter in zone 1 than in zone 2 (Ahmed & Zaman, 2012; Noreen et al., 2008). The zone 1 is a deserted area and consists of less vegetation, as vegetation plays very important role in enrichment of nutrient poor sandy soil (Noreen et al., 2008). Soils generally show a rapid decrease in organic matter from the surface downwards and away from the vegetation (karim et al., 2009).

On the other hand, zone 2 is cultivated area, as plant residues help to increase the soil organic matter of a region (karim et al., 2009). In zone 3 soil organic matter is present as more than in zone 1 and less than in zone 2, other research also supports this evidence (Ahmed & Zaman, 2012). It shows that soil organic matter in zone 2 is more than other zones; it can be due to its highly cultivated soil.

The results indicate that phenolic acids of *R. communis*, *C. procera* and *W. somnifera* are weak positively and flavonoid and tannin of *C. procera* are strong positively correlated with the soil organic matter. But all other phytochemicals of four plants are negatively correlated with the soil organic matter (Table 2). Zone 2 has different environmental conditions than zone 1 and zone 3, for example it doesn't faces environmental stresses and has high soil organic matter that is less in other zones. This zone has less stress able conditions of temperature, precipitation and light intensity that less influence the accumulation of phytochemicals by plants. The result indicates that plants produce lowest amounts of phytochemicals in zone 2 than in other zones. The results also reveal that soil organic matter is negatively correlated with the phytochemicals. So, it predicts that high organic matter has negative impact on the phytochemicals in zone 2. It is reported that higher soil fertility actually hinders the accumulation of phytochemicals (Oh, 2008). Fernandez-Esobar et al., (2006) suggested that application of organic matter to soil increases microbial activity that leads to the fixation of plant nutrients including N, while excessive nitrogen produce negative impacts on phenolic contents (Fernandez-Esobar et al.,

2006). So, it reveals that in zone 2 no environmental stresses and high soil organic matter lead to the less accumulation of phytochemicals by plants.

Conclusion

All these results lead to the conclusion that different plant species and even same specie produces different amounts of phytochemicals in response of different ecological conditions. Plants produce high amounts of phytochemicals in response of environmental stresses like temperature stresses, water stresses, high light intensities and low soil organic matter. But the most favorable conditions for the phytochemicals accumulation are low temperature, moderate organic matter high UVB light and droughts, in other words at high altitudes plants contain more phytochemicals.

Declarations

Contributing Author Details; S.A.K. and K.T. perform the experiments and wrote the main manuscript. Z.A.S. supervise the work. KFA. Proofread the Manuscript ZAS & KFA reviewed/finalize the manuscript.

Competing Interest: It is declared that there is no competing interest.

Ethical Approval: NA

Approval for Reproducing Image: NA

Funding: Authors declare that funding was not received from any source for this research work.

Availability of data and materials: All the data to support this study are included in the manuscript.

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Table 2

Variables		Soil organic matter			
		<i>N. oleander</i>	<i>R. communis</i>	<i>C. procera</i>	<i>W. somnifera</i>
<i>N. oleander</i>	Soil organic matter	1			
	Phenolic acids	-.989*			
	Flavonoids	-.983			
	Tannins	-.805			
<i>R. communis</i>	Soil organic matter	.999*	1		
	Phenolic acids	.154	.113		
	Flavonoids	-.983	-.990*		
	Tannins	-.462	-.498		
<i>C. procera</i>	Soil organic matter	1.000**	.997*	1	
	Phenolic acid	.499	.534	.473	
	Flavonoids	.971	.960	.977	
	Tannins	.846	.867	.830	
<i>W. somnifera</i>	Soil organic matter	-.047	-.088	-.018	1
	Phenolic acids	.471	.435	.498	.859
	Flavonoids	-.998*	-1.000**	-.995*	.118
	Tannins	-.177	-.217	-.148	.991*

Table. 2 Correlation between phytochemicals of different plants and soil organic matter

Soil samples were collected from the depth of 10-15 of the topsoil using a soil digger.

3 soils samples were collected and mixed from each data.

Figures

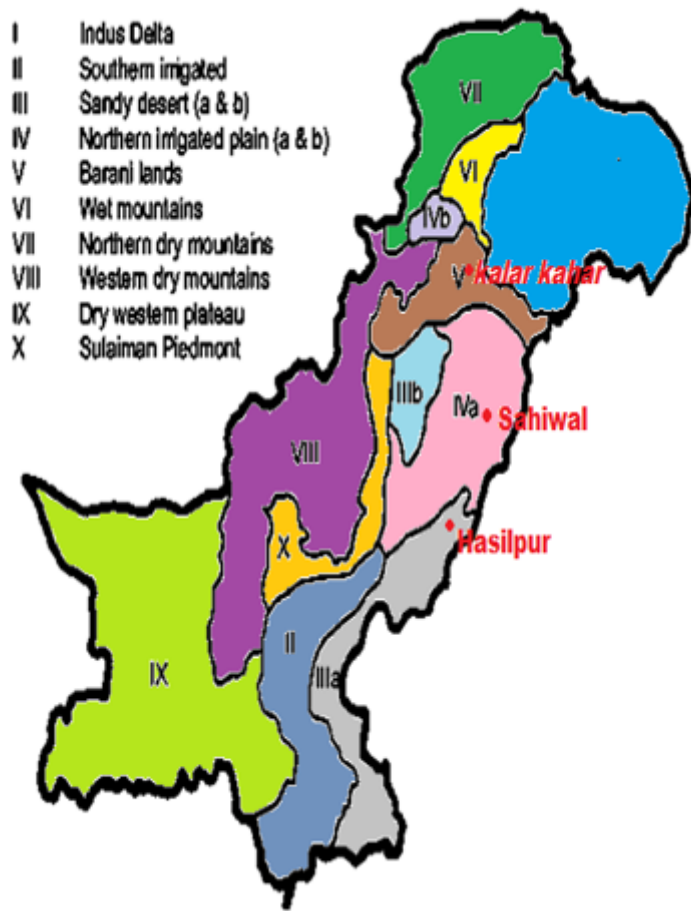


Figure 1

Map of Pakistan with agro-ecological zones (PARC1980)

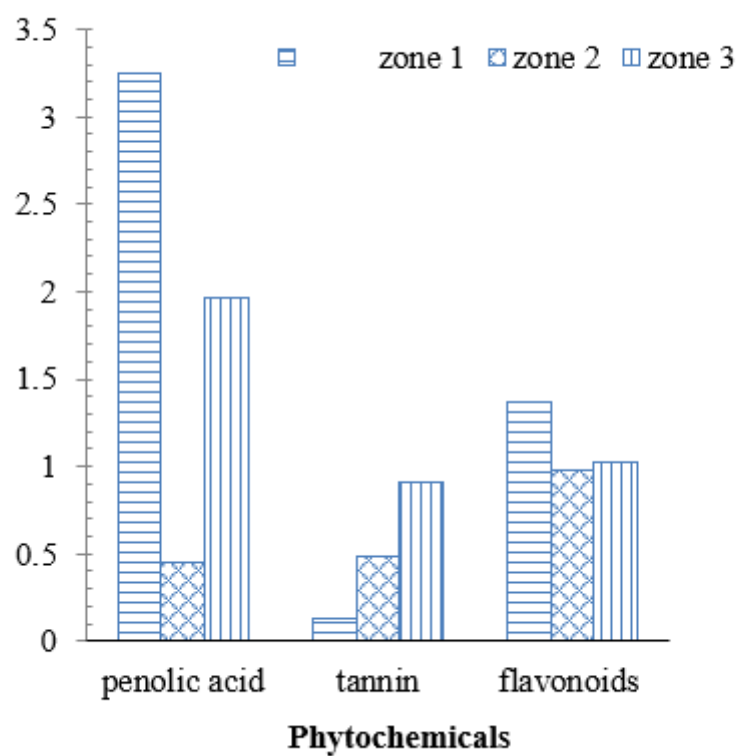


Figure 2

phytochemical variation of *N. oleander* in 3 ecological zones

zone 1-sandy desert; zone 2-northern irrigated plane; and zone 3-barani lands

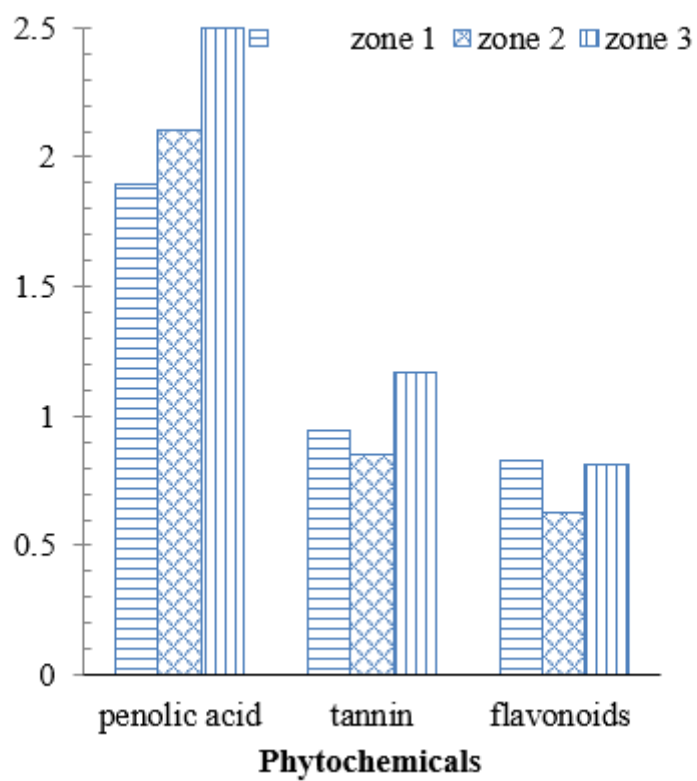


Figure 3

Phytochemicals variation of *C. procera* in 3 ecological zones

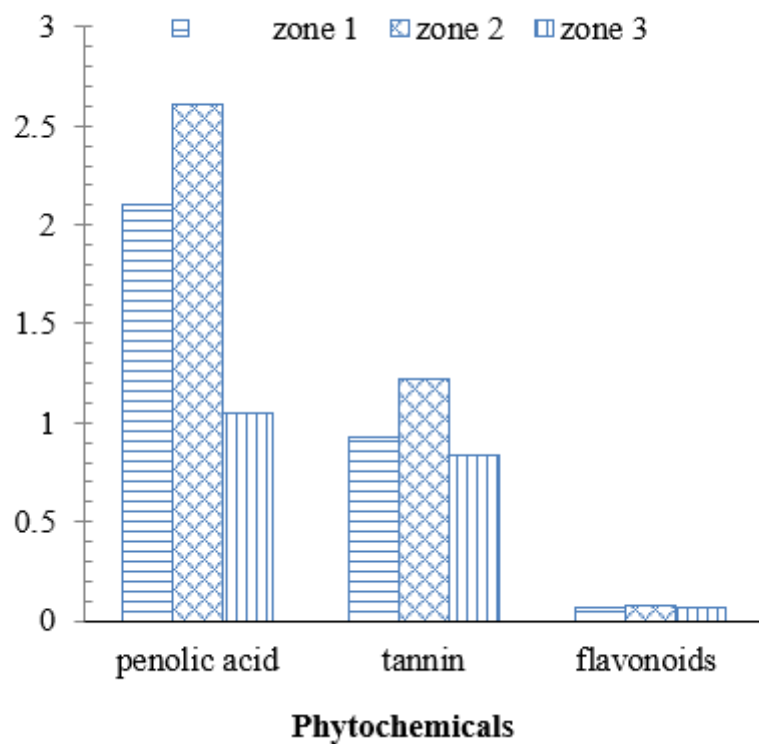


Figure 4

Phytochemical variation of *R. communis* in 3 ecological zones

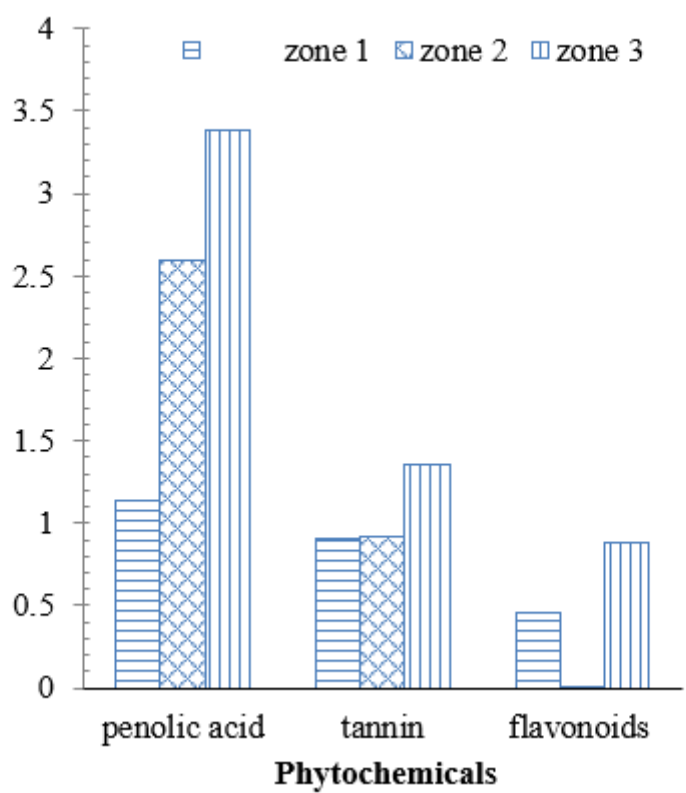


Figure 5

Phytochemical variation of *W. somnifera* in 3 ecological zones

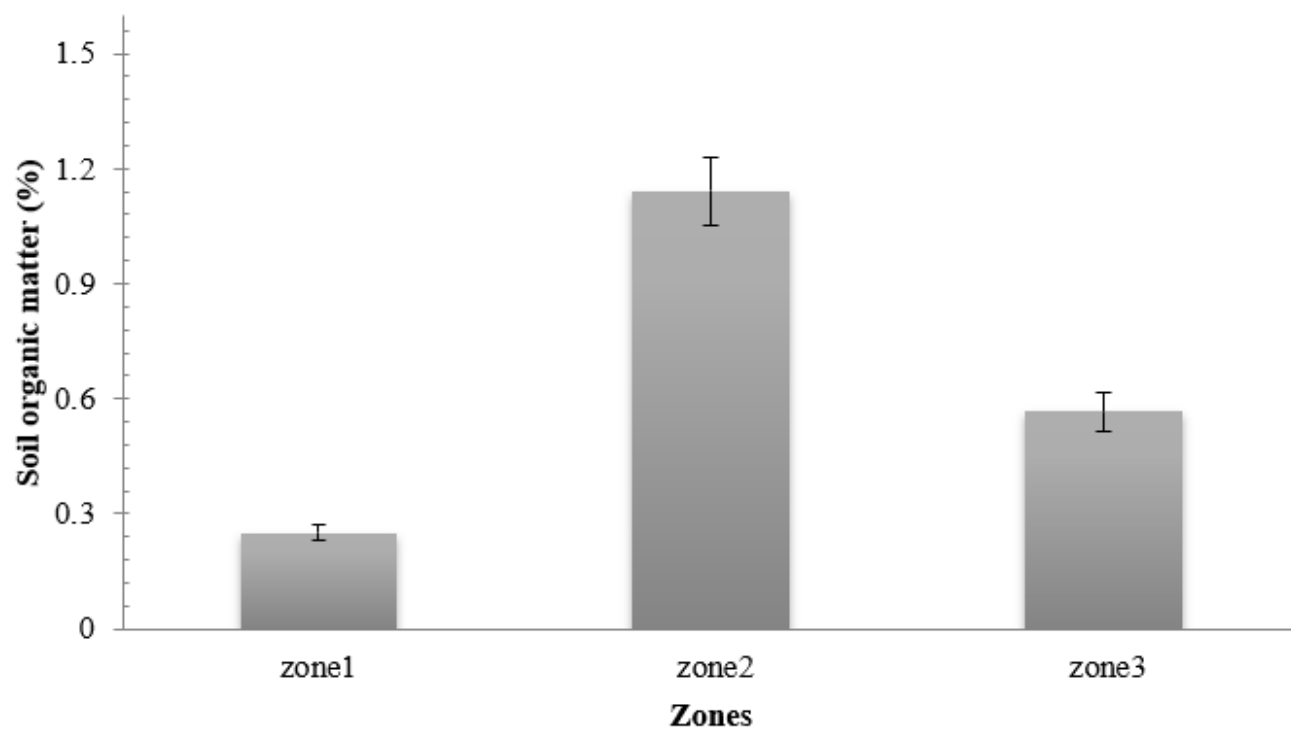


Figure 6

Soil organic matter % in three ecological zones.

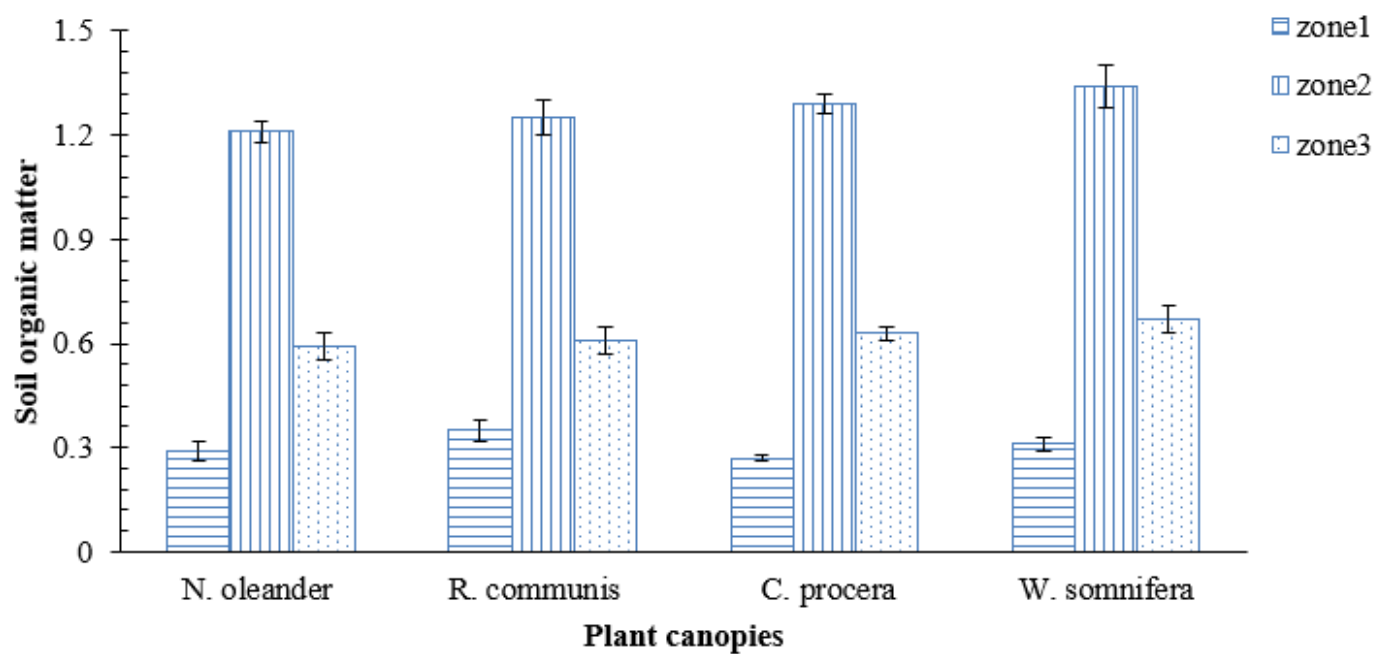


Figure 7

Means and standard deviation of soil organic matter under plant canopies of *N. oleander*, *R. communis*, *C. procera* and *W. somnifera*