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Water Stress Effect on Total Antioxiant Activity and Total Phenolic Content of *Solanum scabrum* Mill and *Solanum scabrum* in Kiambu, Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Author OPO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MPN and WM managed the analyses of the study. Author JPO managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: The aims of this study were to investigate water stress effect on total phenolics and total antioxidants of selected African nightshades and determine any possible variations in the amount of total phenolics and total antioxidants among the accessions grown.
Study Design: Study was conducted on the basis of randomized complete block design.
Place and Duration of Study: The study was carried out in Kenyatta University Agricultural farm in Kiambu County of Kenya. Greenhouse experiments were carried out in the same farm.
Methodology: A greenhouse and field experiment was conducted to investigate effects of water stress on total phenolic and total antioxidant contents of *Solanum scabrum* and *Solanum villosum*. The two African nightshades were subjected to different soil water tensions of 15cbars, 50cbars and 85cbars. After a month of transplanting, samples from the different blocks were collected fortnightly and prepared for total phenolic and total antioxidant determination.

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Results: Obtained data showed that *Solanum villosum* had a higher concentration of both the total phenolics and antioxidant activity in the shoots (46.41 g/Kg total phenolic content and 52.68% total antioxidant activity) while *Solanum scabrum* had higher concentration in the roots (25.06 g/Kg total phenolic content and 27.18% total antioxidant activity).
Conclusion: Total phenolics and total antioxidant accumulation not only depend on irrigation variation but also on the accessions grown.

Keywords: Phytochemical concentration; phenolic content; antioxidant activity.

1. INTRODUCTION

Free oxygen radicals, produced as the usual secondary consequence of environmental stresses, are very dangerous for cell components and must be precisely regulated [1]. All plants have developed several antioxidant systems, both enzymatic and non-enzymatic, to scavenge these toxic compounds. The degree of activities of antioxidant systems under drought stress is extremely variable [2]. The defining factors include variation in plant species, in the cultivars of the same species, development and the metabolic state of the plant, and the duration and intensity of the stress [3]. With the ever increasing demand for African nightshade among consumers, there is need to quantify the levels of phytochemicals within the plants as affected by water stress. The present study was aimed at evaluating the total phenolic content and total antioxidant activity of *Solanum scabrum* Mill and *Solanum villosum*.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Materials

The shoots and roots of *Solanum scabum* and *Solanum villosum* were obtained from the experimental plots, weighed and dried in the oven for 24 hours, then reweighed to obtain the biomass and then crushed differently using electric grinder. 5 g of each sample was added to 100 ml labeled measuring cylinder. 50 ml of methanol was added to each sample as an extracting solvent, and the cylinders covered with aluminum foil then left for 60 hours. The contents were then filtered using Whatman filter paper No. 1 and the filtrate kept in disposable sampling tubes for further analysis.

2.2 Analysis for Total Phenolic Content

The total phenol content of the extracts was determined using the Folin-Ciocalteu method [4]. The total phenol content was subsequently calculated using Gallic acid as standard. 0.5 g Gallic acid was dissolved in 10 ml of methanol then diluted using distilled water to 1000 ml equivalent to 500 ppm. This was then diluted to 250 ppm, 125 ppm, 62.5 ppm, 31.15 ppm and 15.625 ppm using distilled water. These provided the calibration solution. Each 1 ml of the different Gallic acid solutions was pipetted into separate test tubes and to each 4 ml of distilled water was added followed by 0.2 ml Folin reagent and mixed well. After 10 minutes 0.4 ml freshly prepared sodium carbonate (prepared by adding 40 g of Na_2CO_3 in 200 ml of distilled water) was added and the solution incubated for 1 hour at 25ºC. To determine phenolic content in the obtained plant extracts, 1 ml of plant extract was pipetted in separate test tubes, and same procedure for preparation of calibration solution employed. Absorbance of each solution was determined at 765 nm against the blank.

2.2.1 Calculation of total phenolic content

$$\text{Total phenolic content} = \left[\frac{\{\text{GAE (mg/L)} \times \text{volume of methanol extract} \times \text{sample weight (Kg/g)}\}}{\text{Dilution factor (L/ml)}}\right]$$

GAE= Gallic acid equivalent.

2.3 Antioxidant Determination

The free-radical-scavenging ability of the extracts against DPPH (2, 2-diphenyl-1picrylhydrazyl) free radical was evaluated as described by Akter et al. [5]. This provides information on the reactivity of the test compounds with a stable free radical and gives a strong absorption band at 517nm in the visible region. The following concentrations of the extracts were prepared, 0.05, 0.1, 0.5, 1.0, 2.0 and 5 mg/ml in methanol in cuvette placed in the spectrophotometer (Analar grade). Vitamin C was used as the antioxidant standard at the same concentrations as the extract. One ml of the extract was placed in a test tube, and 3 ml of methanol added followed by 0.5 ml of 1 mM DPPH in methanol. The mixture was shaken vigorously and left to

stand for 5 min. A blank solution was prepared containing the same amount of methanol and DPPH. The absorbance of the resulting solution was measured at 517 nm with a UV-vis spectrophotometer (model Cecil CE: 2041; 2000 series, Shimadzu Corp., Kyoto, Japan). All tests were run in triplicate and the radical scavenging activity was then calculated using the following formula:

$$\% \text{ Antioxidant activity} = \frac{\{\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}\}}{\text{Absorbance}_{\text{Control}}} \times 100$$

2.4 Spectrophotometric Measurement

The absorption at different wavelengths for total phenolic content and antioxidant activity was done using the following spectrophotometer, Cecil CE: 2041; 2000 Series.

2.5 Data Analysis

Obtained experimental data were analyzed using SAS software version 9.00 TS Level 00M0 XP-PRO platform, and SPSS software version 21 was used for the analyses of data gathered from survey. All data were analyzed with an analysis of variance (ANOVA) using the general linear model procedure in SAS (SAS Institute, 2002). The assumptions of variance analysis were tested by ensuring that the residuals were random and homogenous, with a normal distribution about a mean of zero. Means were separated using Fisher's protected least significance difference (LSD) test at the 95% level of probability. Correlation analyses using PROC CORR in SAS were conducted to determine the relationship between measured parameters and phytochemical contents.

3. RESULTS AND DISCUSSION

3.1 Total Leaf and Root Phenolic Content

There was significance water stress effect at P≤ 0.05 on the total leaf phenolic content as in Fig. 1.

Total leaf and root phenolic content were significantly affected by the different irrigation intervals. Data showed that the highest concentration of total phenolic compounds was in the leaves of *Solanum villosum* at 46.41g/Kg and roots of *Solanum scabrum* at 25.06g/Kg. The phenolic contents of both the roots and leaves increased with increasing water stress levels; hence the highest results were obtained from plants irrigated at 85 cbars. Despite variation in the phenolic contents in different seasons, the trend, however, remained the same as shown in Fig. 1.

In response to stress, activates the synthesis of phenolic compounds (especially flavonoids), carotenoids and ascorbic acid [6]. Thus, phenolic compounds provide important physiological and ecological duties, being mainly involved in protection against different types of stress [3]. Besides numerous enzymes (superoxide dismuthase, peroxidase etc.), phenolic compounds are strong antioxidants that help plants to survive stress conditions [7]. Antioxidant compounds such as phenolic compounds are able to prevent oxidative burst of plant cells and thus protect plants from damage of proteins and lipids, DNA and RNA structures [8]. In the

Table 1. Interactions between irrigation intervals and the leaf and root total phenolic content

Variety	Tensiometer readings (cbars)	Green house		Long rainy season		Short rainy season	
		Leaf total phenolic content	Root total phenolic content	Leaf total phenolic content	Root total antioxidant activity	Leaf total phenolic content	Root total phenolic content
Solanum scabrum	15	23.55^{f}	9.49^{e}	11.26^{f}	8.56^{e}	13.78^{f}	6.92^{e}
	50	29.86^{d}	16.45^{c}	19.71^{d}	13.96^{c}	24.65^{d}	13.87^{b}
	85	38.91^{b}	21.81^{a}	24.53^{b}	20.17^{a}	30.66^{b}	16.67^{a}
Solanum villosum	15	27.86^{de}	7.59^{f}	14.24^{e}	7.83^{f}	22.15^{e}	5.57^{f}
	50	36.75^{c}	15.28^{cd}	21.37^{c}	10.65^{d}	29.87^{c}	10.40^{d}
	85	46.41^{a}	18.39^{b}	32.81^{a}	16.62^{b}	$36.26f^{a}$	13.29^{bc}
LSD		**2**	**1.17**	**1.21**	**0.51**	**0.15**	**1.23**
T X V		*	*	*	*	*	*

Means followed by the same letter in a column are not significantly different at P≤ 0.05

present study, the nightshade accessions grown (*S. scabrum* and *S. villosum*) revealed higher contents of total phenols under water-deficit conditions. The promotion of the synthesis of phenolic compounds due to drought was already documented in numerous studies [9]. Increase in phenolic content of both the roots and leaves were directly proportional to the increase in irrigation interval. This result was consistent with similar work done on lettuce by Myong-Min Oh. [10].

3.2 Leaf and Root Total Antioxidant Activity

There was significance at P≤ 0.05 interaction between watering regimes and the total leaf and root antioxidant activity in greenhouse, season one (long rains) and season two (short rains) as in Fig. 3 and Table 2. Total leaf and root antioxidant activity was significantly affected by the different irrigation intervals. Data showed that *Solanum villosum* had the highest total antioxidant activity in the leaves at 52.68, while *Solanum scabrum* had the highest concentration of the same in the roots at 27.18. The data was obtained from plants irrigated at 85 cbars. Changes in the antioxidant capacity of water-stressed plants paralleled those in the total phenolic compounds. The changes in antioxidant capacity of nightshade plants were roughly reflective of the changes in the total phenolic content.

In moderate water deficit, the total antioxidant activity in the roots among the two varieties had an increasing order of *Solanum scabrum* › *Solanum villosum*, while in the leaves the order was *Solanum villosum* › *Solanum scabrum.* The lowest levels of total antioxidant activity were recorded in the plants irrigated at 15 cbars. This shows that increase in water stress led to increase in phenolic content in the accessions grown. Drought affects not only water relations, but also induces stomatal closure and decreases the photosynthetic rate and growth. Closure of stomata decreases CO_2 concentration in leaf mesophyll tissue and results in an accumulation of NADPH. Under such conditions, where NADP is a limiting factor, oxygen acts as an alternate

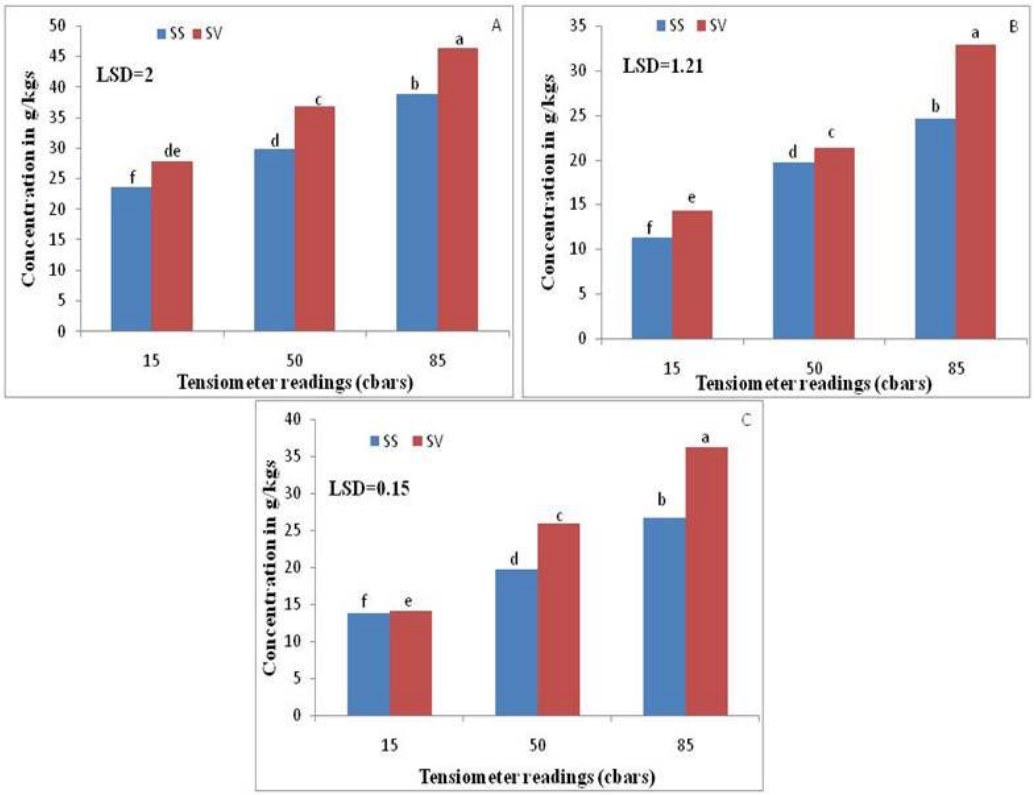

Fig. 1. Water stress effect on total leaf phenolic content in greenhouse (A), long rains (B) and short rains (C) respectively. SS- *Solanum scabrum*, SV- *Solanum villosum*

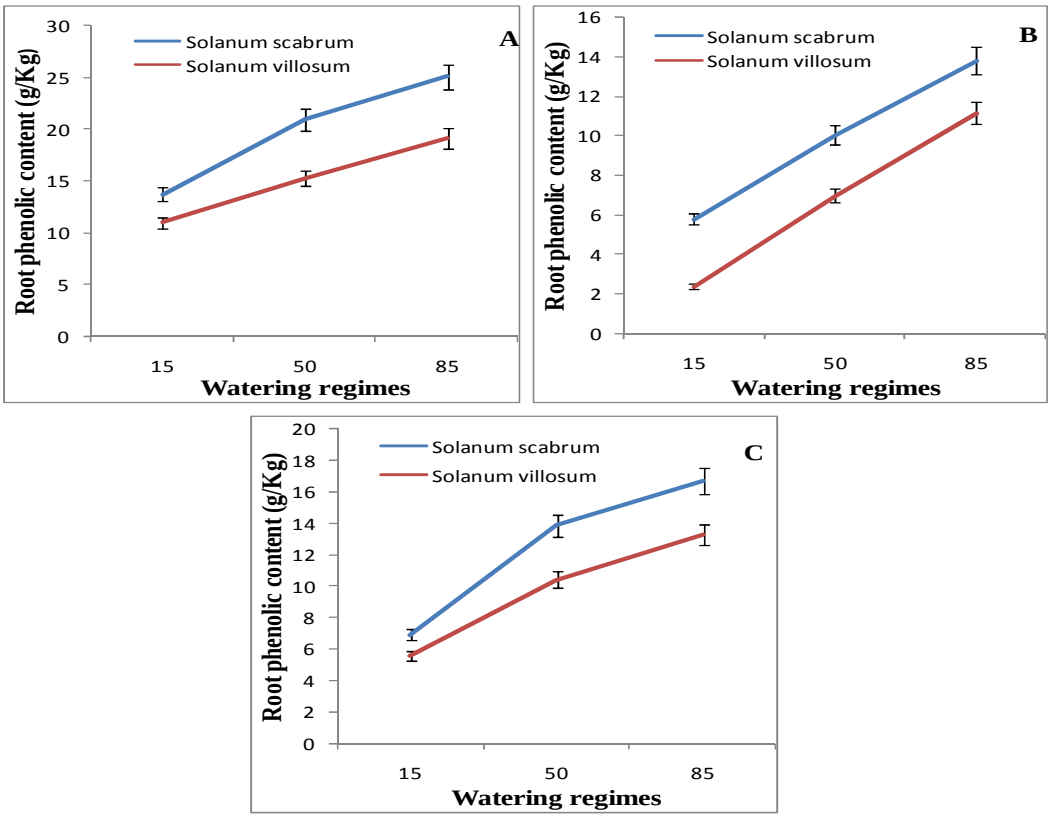

Fig. 2. Water stress effect on total root phenolic content in greenhouse (A), long rains (B) and short rains (C) respectively

acceptor of electrons from the thylakoid electron transport chain, resulting in the formation of superoxide radical (O_2^-) [11]. Superoxide radical and its reduction product H_2O_2 are potentially toxic compounds, and can also combine by the Haber-Weiss reaction to form the highly toxic hydroxyl radical (OH^-) [12]. Under optimal conditions leaves are rich in antioxidant enzymes and metabolites and can cope with reactive oxygen species (ROS), thus minimizing oxidative damage. A large number of reports deal with the deleterious effects of ROS, which production is stimulated under water stress conditions [13].

ROS cause lipid peroxidation and consequently membrane injuries, protein degradation, enzyme inactivation [12], thus induce oxidative stress. Tolerant genotypes, therefore, should not only be able to retain sufficient water under drought, but should also have a highly active system to protect against oxidative injury, and *Solanum villosum* exhibits more of this than *Solanum scabrum*. Plants possess several tissue antioxidant enzymes for protection against ROS, as superoxide dismutase (SOD), ascorbate peroxidase (APOX), guajakol peroxidase (GPOX), reductase (GR) and catalase (CAT). These enzymes either quench toxic compounds or regenerate antioxidants with the help or reducing power provided by the photosynthesis [14]. During drought conditions high activities of antioxidant enzymes are associated with lower levels of lipid peroxidation, being connected to drought tolerance [15]. In fact, an increased metabolic capacity of these enzymes may be part of a general antioxidative system in plants involving regulation of protein synthesis or gene expression [16]. Low-molecular weight antioxidants are presented by carotenoids, tocopherols, glutathione and ascorbic acid.

Apart from their obvious role as enzyme substrates, they can react chemically with almost all forms of ROS .Among substances able to protect plant cell from oxidative attack, a specific role of polyamines in preventing photooxidative damages is reported [17]. Genotypes of the same species respond differentially to environmental stresses and oxidative injury, as a result of genetic based differences in their

antioxidant systems as in *Solanum villosum* and *Solanum scabrum.* That provides an important tool to have an insight into the physiological mechanisms operative in stress tolerant genotypes [12]. According to Foyer et al. [18] much of the injuries caused by exposure to biotic and abiotic stresses are associated with oxidative damage at a cellular level, the chloroplasts being an important site of H_2O_2 generation. Blokhina et al. [19] established that at the end of drought period, an increased H_2O_2, and OH^- production was observed in young bean plants, therefore revealing a state of oxidative stress in cells. H_2O_2 is a strong oxidant produced mainly as a result of scavenging of superoxide radical, and its higher concentration is injurious to cells, resulting in a localized oxidative damage, lipid peroxidation, and disruption of metabolic function and losses of cellular integrity at sites where it accumulates [20]. It is well known that H_2O_2, similar to glutathione, has multi-functional interactive roles in the early stages of plant stress response. H_2O_2 can diffuse to relatively long distances, causing changes in the redox status to surrounding cells and tissues where, at relatively low concentrations, may trigger an antioxidative response [18]. Rather than just the scavenging capacity, a fine-tuning of H_2O_2 levels is essential for an efficient control. The rationale of this assumption is that H_2O_2, whilst deleterious to some cellular components, is essential to plants in various biosynthetic reactions and, as suggested by some authors, possibly also in signal transduction pathways, which could contribute to plant defense [21]. In fact, the drought induced production of H_2O_2 in the mesophyll cells may be associated with changes in the cell wall structure [17]. Furthermore, H_2O_2 is necessary for the peroxidase-mediated oxidative polymerization of cynnamil alcohols to form lignin, and several enzymatic systems have been proposed as responsible for hydrogen peroxide production, on the surface of plant cells [22]. It may be therefore suggested that the increased level of H_2O_2 observed by many authors in the drought treated plants is due to oxidative damages, but eventually may also have a signal function. H_2O_2, OH^- and other ROS can be expected to be responsible for the lipid peroxidation [23].

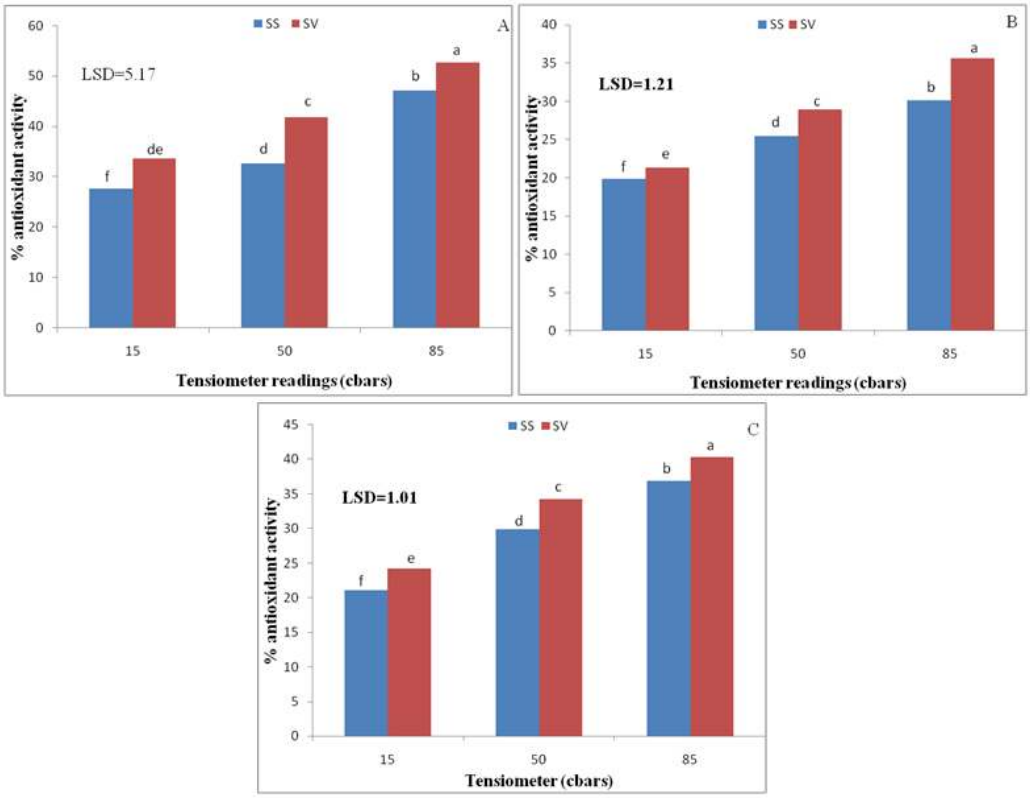

Fig. 3. Water stress effect on the total leaf antioxidant activity in greenhouse (A), long rains (B) and short rains (C) respectively. SS- *Solanum scabrum*, SV- *Solanum villosum*.

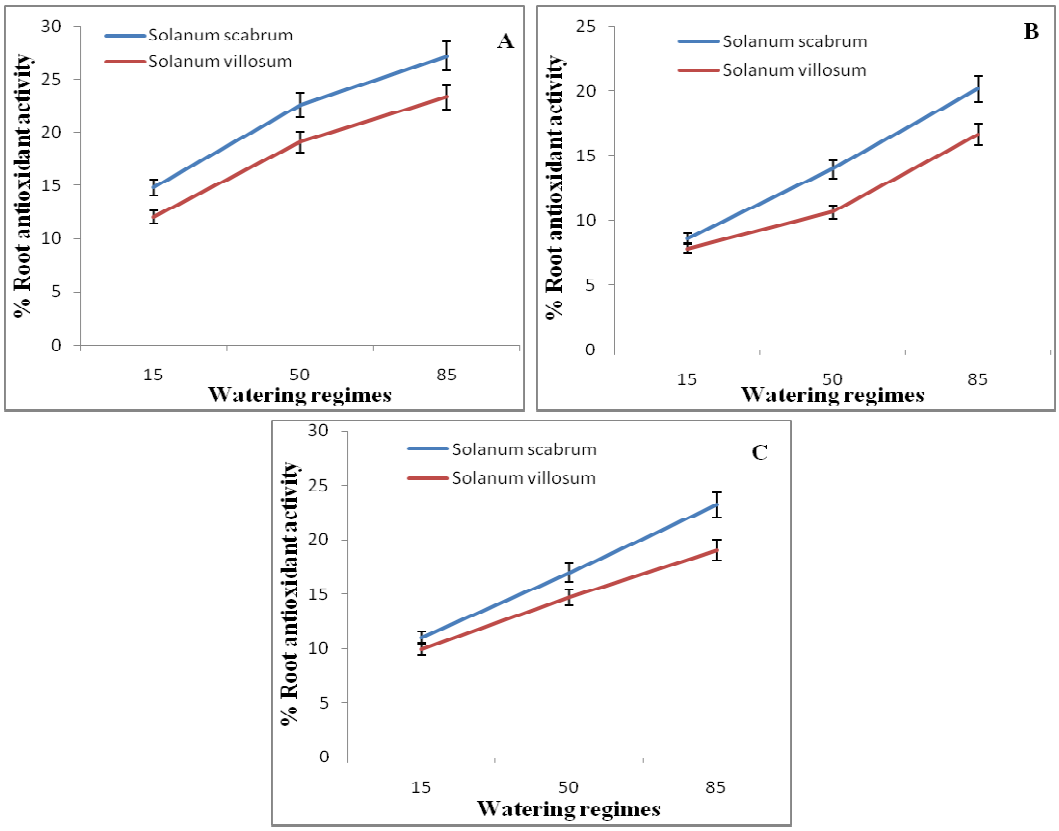

Fig. 4. Water stress effect on the total root antioxidant activity in greenhouse (A), long rains (B) and short rains (C) respectively

Table 2. Interactions between irrigation intervals and the total leaf and root antioxidant activity

Variety	Tensiometer readings (cbars)	Green house		Long rainy season		Short rainy season	
		Leaf total antioxidant activity	Root total antioxidant activity	Leaf total antioxidant activity	Root total antioxidant activity	Leaf total antioxidant activity	Root total antioxidant activity
Solanum	15	27.7^{f}	14.84^{f}	19.83^{f}	8.56^{e}	21.1^{f}	11.05^{e}
scabrum	50	32.66^{de}	22.59^{bc}	25.41^{d}	13.96^{c}	29.89^{d}	17.01^{c}
	85	47.04^{b}	27.18^{a}	30.14^{b}	20.17^{a}	36.87^{b}	23.22^{a}
Solanum	15	33.64^{d}	12.06^{e}	21.34^{e}	7.83^{f}	24.19^{e}	9.95^{f}
villosum	50	41.87^{bc}	19.11^{d}	28.95^{c}	10.65^{d}	34.22^{c}	14.74^{d}
	85	52.68^{a}	23.35^{b}	35.6^{a}	16.62^{b}	40.35^{a}	19.04^{b}
LSD		**5.17**	**0.76**	**1.21**	**0.51**	**1.01**	**1.11**
T X V		*	*	*	*	*	*

Means followed by the same letter in a column are not significantly different at P≤ 0.05

As reported by Sgherri and Navari-Izzo [24] the increase in the activity of scavenging enzymes could be due either to an adaptive change in catalytic properties or to the transcription of the corresponding silent genes. This could be related to enhanced levels of free radicals or other ROS in plant cells and correlate with a temporal coordination of the production of H_2O_2 via SOD and destruction of this peroxide by APOX and CAT. Such coordinated responses are believed to promote plant tolerance to oxidative stress [25]. It is also possible that increased SOD activity could alter the expression of other metabolic processes associated with water stress. Thus, Gupta et al. [26] have demonstrated that enhanced activity of Cu, Zn SOD in transgenic plants was associated with increased activity of APOX. Some other authors also reported an increase in SOD activity in plants under oxidative stress [20]. It appears that

relative tolerance of plant genotypes, as reflected by its lower lipid peroxidation and higher membrane stability, is related with the levels of its antioxidant enzymes activity. APOX, Cu, Zn-SOD and CAT are involved in overcoming of oxidative stress. The increased activities of antioxidant enzymes act as a damage control system and, thus, provide protection from oxidative stress, resulting in lower LPO and higher membrane stability in tolerant genotypes. The literature analyzed in this review complexity of tolerance of plants to water deficit and supports the statements of many authors that the flexibility of cell metabolism and its acclimation to changes in environmental conditions is a first essential step in stress avoidance [27]. The wider the range of adaptation capacity of plants, the better they are protected against various stresses. The changes in program of plant development are always associated with changes in their physiological and biochemical program and activity.

In spite of intensive investigation of the problem of water deficit tolerance, many of its aspect remain to be explored. Water deficit induces expression of particular genes and this is associated in most cases with adaptive responses of stressed plants. The functions of many of them are still not established. Similar results were obtained by Myung Min Oh [10] on lettuce seedlings subjected to different water stress levels. Furthermore, numerous studies have shown that drought stress can induce a wide range of antioxidants in a number of plant species [28].

4. CONCLUSION

Overall, from the results of this experiment, it can be concluded that water stress significantly increases the total phenolic content and total percentage antioxidant activity. The severe water stress treatment (85 cbars) increased total phenolic content in *S. villosum* to 46.41 g/Kg GAE and to 38.91 g/Kg GAE in *S. scabrum*. The same stress increased total percentage antioxidant activity to 47.04 in *S. scabrum* and 52.68 in *S. villosum*. From the experiment it's eminent that phytochemical concentration not only depend on soil water status but also on the accession grown.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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