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Effect of Phosphorus Deficiency on Phenolics and Antioxidants Content of Two African Nightshade Varieties Grown in Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Author OOJ designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors JPGO, PN and WM reviewed the study design and all drafts of the manuscripts. Authors OOJ and JPGO managed the analyses of the study and performed the statistical analysis. Author OOJ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Indigenous vegetables form an integral part of the Kenyan diets, most commonly consumed being the African nightshade. These vegetables contain important phenolics and antioxidants that have medicinal and good health attributes. Their production has strongly been associated with environmental stresses, and phosphorus as one the limiting nutrients had been suspected to play key role. To investigate the effect of phosphorus stress on Total Phenolic Content (TPC) and Total Antioxidants Activity (TAA) on nightshade, greenhouse and field experiments were conducted. Two commonly grown varieties *(Solanum villosum-SV* and *Solanum scabrum-SS)* were planted in both conditions; done under long (May-July 2014) and short rain seasons (August-October 2014). It was laid as Randomized Complete Block Design with split plot arrangement. The two varieties were the

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main plot and phosphorus levels (0, 20, 40 and 60 kg/ha) constituted the subplot with four replicates. Gallic Acid (standard) Extraction method was used to analysis TPC. Diphenyl picryl hydrazyl method was used to analyze TAA where Vitamin C was standard. Data on TPC and TAA were recorded and later the effects resulting from these treatments analyzed using ANOVA and mean separation using Least Significant Difference (LSD) at p≤0.05. The TPC and TAA were significantly affected by different phosphorus levels (p≤0.05). TPC and TAA decreased with increase in phosphorus. *SV* had higher TPC and TAA (6.09 mg/g and 38.58% respectively) as compared to *SS* that had 5.49 mg/g (TPC) and 35.92% (TAA). *SV* had more phenolics and antioxidants in the shoots than roots, the converse was found for *SS*. Both varieties at 40 kgP/ha offered the best tradeoff between yield and secondary metabolites (phenolics and antioxidants). Study recommends 40 kgP/ha as it had the highest levels of phenolics and antioxidants. Further research needs to be done on other important antioxidants like anthraquinones and how different levels of macronutrients affect their production.

Keywords: African nightshade; phosphorus; phenolics; antioxidant.

1. INTRODUCTION

African nightshades are rich in important phytochemicals that are essential for human health. Medical evidence increasingly suggests that consumption of diets rich in phytochemicals has a protective effect against cardiovascular disease and certain forms of cancer [1]. Although plants contain components which may lead to overall health benefits including proteins, amino acids, vitamins, and fiber, recent research has focused on the role of secondary plant metabolites, particularly phenolics and antioxidants in disease prevention [2].

Although the nutritional benefits derived from eating phytochemical rich plant foods are well known, foods and beverages containing the highest phytochemical levels are often lacking or absent in many diets, particularly in Kenya and other developing countries [3]. Therefore, there has been growing interest in developing simple methodologies to increase both phenolics and antioxidants concentrations in more commonly consumed plant foods [4]. This is more interesting for the case of African nightshade vegetables and the need to enhance their overall nutritional value.

Food insecurity and malnutrition is also an issue of concern in Kenya [5] and other countries in Sub Saharan Africa. Over 60% of the rural populations live below the poverty line, resulting in malnutrition and 1.2 billion of world's population has poor health. The poverty situation has been worsened by the high prevalence of HIV/AIDs where 2.5 million Kenyans are infected with about 200,000 new infections per year [6]. Nevertheless, Kenya is endowed with agricultural biodiversity like African nightshade which could contribute significantly in the management of the HIV/AIDs infected and affected persons. These vegetables have high micronutrients, medicinal properties and other benefits but it has been underutilized for equally along time.

When plants undergo phosphorus deficiency, they are known to release organic acids in the roots rhizosphere to help in the mobilization and acquisition of available phosphorus [6]. Similarly plants are known to release Reactive Oxygen/Nitrogen species during phosphorus deficiencies, these species aid in the scavenging of available stored phosphorus with the help of enzymes [7]. During scavenging, an enzymes activity destroys plant organelles hence for these to be stopped; there is the release of antioxidants that prevent further reaction within the plant. This is among simple mechanisms? Plants employ to increase both phenolics and antioxidants concentrations in more commonly consumed plant foods [7].

Therefore information from this study was intended to provide knowledge on the bioactive components of African nightshade and identify the levels of phosphorus which is optimal for production of phenolics and antioxidants. The objectives of this study was (i) to determine the effect of phosphorus deficiency on total phenolic and antioxidants contents and their distribution between the shoots and roots of the two nightshade varieties.

2. MATERIALS AND METHODS

2.1 Greenhouse and Field Experiments

A greenhouse and field experiments were conducted at Kenyatta University farm Nairobi

County, Kenya. The site lies at an altitude of latitude 110' 0.012" S and longitude 3649'59.880" E. The average amount of rainfall received is 989 mm per year. Temperature ranges between 12.8 degrees Celsius during the cold month and 24.6 degrees Celsius during the hot seasons. The soils are loamy, acidic, well drained and moderately deep (Table 1).

Seeds of two African nightshade varieties (*Solanum scabrum* and *Solanum villosum*) were pre-germinated in a nursery. After 4 weeks, six seedlings were transplanted in each 20 Kgs plastic pots (34 cms diameter and 30 cms depth) filled with 20 Kgs of sterilized sand. Treatments included four phosphorus levels with two varieties of African nightshade replicated four times. Treatments included four phosphorus levels (0, 6, 12 and 18 g/pot) with two varieties of African nightshade replicated four times (given a total plots of 32). All pots were administered with recommended basal plant nutrients namely; N, K and other micronutrients through Hoagland solution.

2.2 Experimental Layout and Management

Field experiments were conducted over two cropping seasons, the long rains of May to July 2014 and the short rains season of August to October 2014. Planting was done at Kenyatta University farm as split plot arrangements with two varieties being the main plot measuring (3x3)m and varying phosphorus levels (0, 20, 40 and 60 kg/ha) constituting the subplot in Randomized Complete Block Design.

Primary tillage was done to a moderate tilth after which 6-week-old seedlings were transplanted. Appropriate rates of Triple Super Phosphate fertilizer were administered into 15 cm deep drilled holes on the respective plots at the time of transplanting. Calcium ammonium nitrate (26% N) at 60 $KgNha^{-1}$ and Muriate of potash (60% K_2O) at 30 Kg K ha^{-1} were uniformly administered and incorporated into the soil in both seasons. The aim was to supply sufficient amounts of N and K to ensure the two nutrients were not limiting factors on plant growth when studying the effects of phosphorus.

Seedlings of African nightshade varieties were transplanted at a spacing of (30x30) cm in 32 plots on 1st May, 2014 (Long rains) in the first season and 5th August, 2014 (Short rains) in the 1745 meters above sea level and is within second season. The fields were kept weed-free by manually weeding. Insect pests and diseases were controlled using abamectin and copper sulfate respectively. Destructive sampling was done at random starting at 3 weeks after transplanting and thereafter each subsequent week up to the fourth harvest. For every harvest, samples were oven dried at 60℃ for 72 hours and samples stored for analysis of total phenolic content and total antioxidant activity.

2.3 Extraction and Analysis of Plant Materials

Methanol extraction was applied to powdered oven dried plant samples (shoot and root). Five grams of the powdered plant material in a flask was soaked with 50ml methanol and allowed to stand for 48-72 hrs. It was then filtered through Whatman filter paper No. 1 and distilled using rotary evaporator (Bibby Sterilin Ltd, RE 100B, UK) at 60℃ until methanol-free solid powder was obtained. The resulting extracts were labeled as methanol extracts and preserved at 5℃ in airtight bottles awaiting phenolics and antioxidant analysis.

2.4 Total Phenolic Content Analysis

To determine TPC, Gallic was used as a standard. An amount of 0.500 g of Gallic was weighed and dissolved in 10 ml of methanol and diluted to 100 ml using distilled H_2O and 200 g of sodium carbonate was weighed and added in 800 ml of distilled water [8]. The solution was brought to automatic boiling after cooling, few crystals of hydrous sodium carbonate was added and after 24 hours. It was later filtered and topped up to 1L using distilled water.

To prepare a calibrated curve 0, 1, 2, 3, 5 and 10 ml of the above Gallic solution were added into 100 ml volumetric flasks, diluted to volume with water to give the following concentrations; 0, 50, 100, 150, 250 and 500 mg/L gallic acid. From each calibration solution sample or blank, 1 ml was pipetted into separate test tubes and to each, 4 ml of distilled water and 0.2 ml of Folin reagent was added and mixed well. The solutions were left at ambient temperature for 2 hours and the absorbance of each solution was determined at 765 nm against blank. Absorbance verse concentration graph was plotted to determine the equation of regression (R^2) [8].

Table 1. Initial soil properties for the field experimental site

Parameter	pH	% N	% Olsen P	% Org. P	% Clay	% Silt	% Sand	% Zn	% Cu
Value	5.45	0.12	1.49	1.53	33.7	30.3	36	1.11	1.15

2.5 Determination of Antioxidant Activity

The radical-scavenging activity was determined using diphenyl picryl hydrazyl (DPPH) radical according to [8]. This provided information on the reactivity of the test compounds with a stable free radical and gave 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) to come up with a calibration curve.

Vitamin C was used as the antioxidant standard at the same concentrations as the extract. One ml of the extract was poured in a test tube, and 3 ml of methanol added, followed by 0.5 ml of 1 mM DPPH in methanol. The mixture was then 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-visible spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan). All tests were run in triplicate and the radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = \{[Ab-Aa]/Ab\} \times 100$$

Where:

Ab = absorption of the blank sample and
Aa = absorption of the extract.

2.6 Statistical Analysis

Data were analyzed using SAS version 9 software, where analysis of variance (ANOVA) and correlation among the variable were performed. Where appropriate, means were separated using least significant difference (LSD) test at 5% significant level. Relationship between treatments and variables were established using linear regression, stepwise selection model.

3. RESULTS AND DISCUSSION

3.1 Total Phenolic Content in Shoot and Root

Shoot and root TPC were significantly affected ($P \leq 0.05$) by different levels of Phosphorous in greenhouse, long and short rains. Mean values of the data showed maximum shoot TPC in plants with no P application (6.09 mg/g for *S. villosum* and 5.49 mg/g for *S. scabrum*) whereas root TPC was 2.18 mg/g for *S. villosum* and 5.09 mg/g for *S. scabrum*. Shoot TPC was 4.93 mg/g for *S. villosum* and 3.88 mg/g for *S. scabrum* at 20 kgP/ha whereas root TPC had 1.77 mg/g for *S. villosum* and 4.78 mg/g for *S. scabrum*. Phosphorus application at 40 kg/ha resulted in shoot TPC of 3.31 mg/g for *S. villosum* and 2.54 mg/g for *S. scabrum* whereas root TPC resulted in 1.11 mg/g for *S. villosum* and 2.22 mg/g for *S. scabrum* for (Figs. 1 and 2). The lowest shoot and root TPC (1.62 mg/g for *S. villosum* and 1.20 mg/g for *S. scabrum*) and (0.41 mg/g for *S. villosum* and 1.42 mg/g for *S. scabrum*) respectively were produced by plants treated with 60 kgP/ha (Figs. 1 and 2).

Plant species have different ways of mobilizing phosphorus from the soil [8]. During phosphorus deficiency, plants secrete organic compounds such as mucilage, organic acids, phosphatases, and some specific signaling substances, which are key drivers of various rhizosphere activities [9]. The chemical and biological processes in the rhizosphere not only determine mobilization and acquisition of soil nutrients as well as microbial dynamics, but also control nutrient use efficiency of crops, thus profoundly influencing crop productivity [9-12].

Phosphorus at 0 kg/ha recorded the highest TPC. High amounts of phosphorus required by the plants triggers the release of plant phenolics in accordance with the plant phosphorus requirement thus the more severe the deficiency, the higher the release of phenolics. Different plant species have different reservoirs for phenolics [13]. For instance, in the case of this study, *S. scabrum* reserves more phenolics in the roots compared to *S. villosum* that stores more in shoots hence when P deficiency was experienced in *S. scabrum*, it probably releases phenolics from the roots that aid in mobilization of available P from the rhizosphere making it available to the plant.

The release of phenolics in form of organic acids in soil probably led to the lowering of root rhizosphere pH (Fig. 3). Similar results were

obtained by [14] who indicated that genotypes exhibited greater variation for root morphological traits and phenolic exudation under varied P sources. They further reported that genotypes varied widely in P uptake under P deficiency.

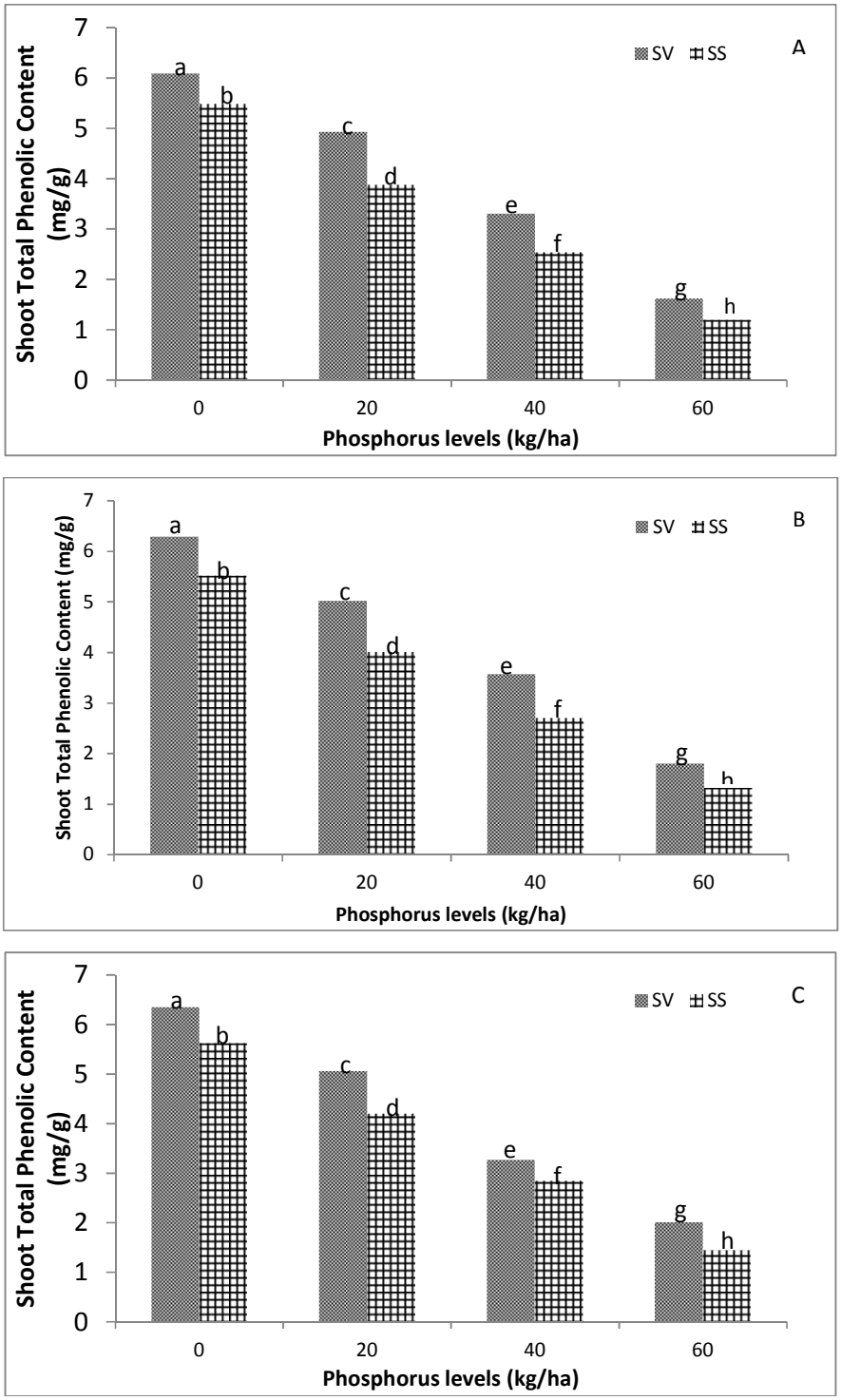

Fig. 1. Effects of phosphorus levels on shoot total phenolic content of two African nightshade varieties for (A) greenhouse, (B) long rains and (C) short rains respectively

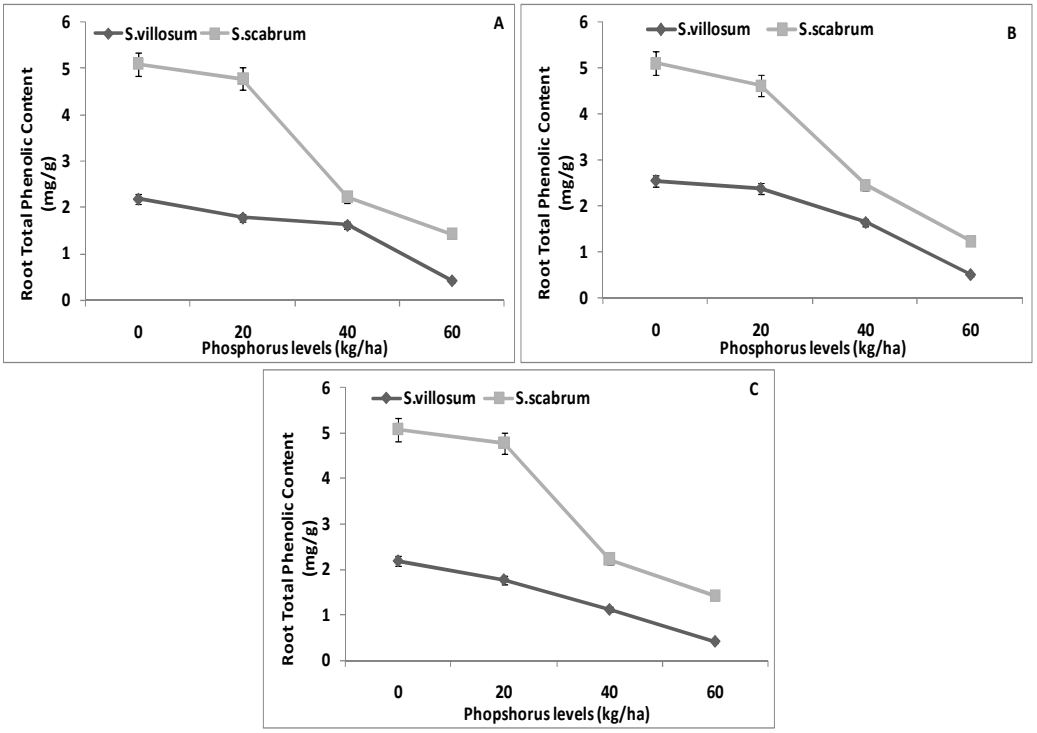

Fig. 2. Effects of phosphorus levels on root total phenolic content of two African nightshade varieties for (A) greenhouse, (B) long rains and (C) short rains respectively

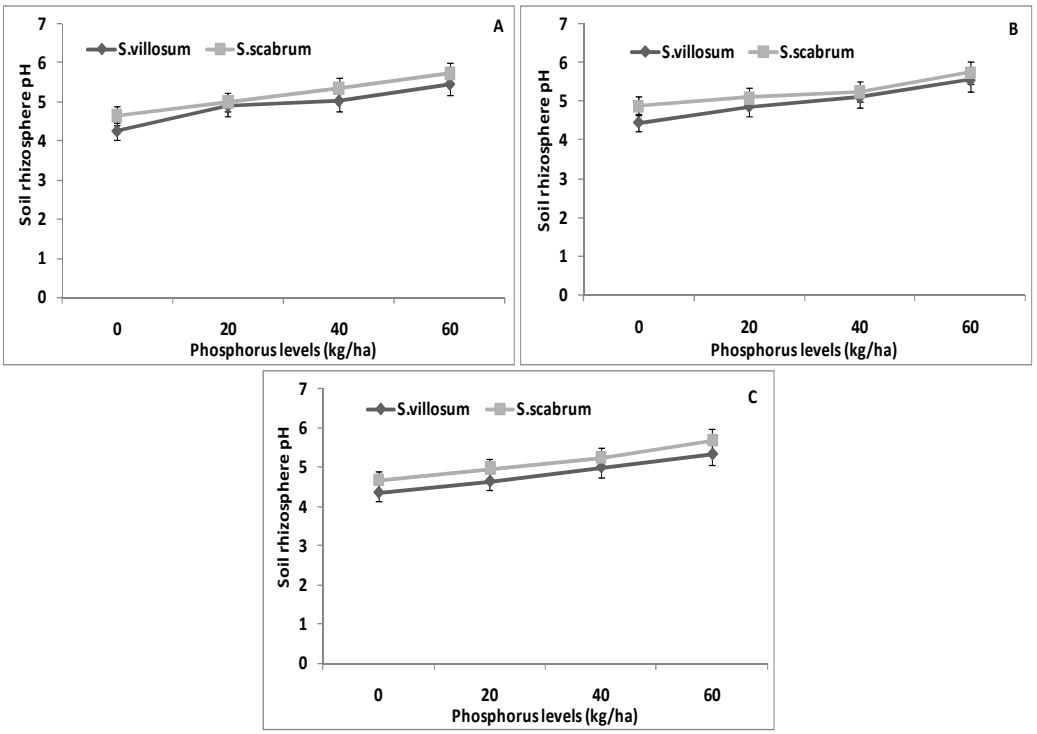

Fig. 3. Effect of phosphorus levels on soil rhizosphere pH of (A) greenhouse, (B) long rains and (C) short rains respectively 40 days after transplanting

In a previous experiment during P deficiency, P was mobilized from the older leaves to the younger leaves; the resulting leaves were abscissed on the surface of the soil above the rhizosphere [15]. The decomposition process of the leaves releases phenolics that were stored in the leaf vacuole to the rhizosphere that would consequently leach to the soil to facilitate mobilization of available P [16]. The release of organic acids in form of phenolic content causes acidification of the rhizosphere.

Leaf total phenolic content for greenhouse, long and short rains are presented in Table 2 showed significant interaction (P≤ 0.05) between variety and phosphorus rate on leaf TPC indicating that varieties responded differently to P application in terms of Leaf TPC.

Given that varieties responded similarly to P application, the differences in their shoot TPC at similar P rates was attributed to their inherent secondary metabolite composition; *S. villosum* had higher secondary metabolite in leaves than in roots that enabled it repel herbivores during stressful situations and also to acquire more P from the soil than *S. scabrum* [17]. The converse is true for *S. scabrum.*

3.2 Total Antioxidant Activity in Root and Shoot

Shoot and root TAA were significantly (P≤ 0.05) affected by different levels of Phosphorous in the greenhouse, long and short rains. Mean values of the data showed that maximum shoot TAA was recorded on plants without P application kg/ha (38.58% for *S. villosum* and 30.92% for *S. scabrum*) whereas root TAA was 37.25% for *S. villosum* and 40.11% for *S. scabrum*. Phosphorus applied at 20 kg/ha produced shoots TAA (34.49% for *S. villosum* and 25.38% for *S. scabrum* whereas root TAA was 33.36% for *S. villosum* and 50.11% for *S. scabrum*). Phosphorus applied at 40 kg/ha resulted in shoot TAA of 30.75% for *S. villosum* and 17.5% for *S. scabrum* while root TAA was 30.44% for *S. villosum* and 39.33 for *S. scabrum* (Figs. 4 and 5). The lowest Shoot TAA (23.71% for *S. villosum* and 10.38% for *S. scabrum*) and root TAA (20.92% for *S. villosum* and 22.7% for *S. scabrum*) were produced by plants treated with 60 kgP/ha (Figs. 4 and 5).

Phosphorus plays an important role in nutrients stress condition for plant survival [9]. In shortage of phosphorus, plants sensitivity increases [17]. Plants receiving no P had the highest release of reactive oxygen species (ROS) [17]. The ROS production in plants involves the release of cognate enzymes such as Super Oxide Dismutase (SOD), Ascorbate Peroxidase and Glutation Reductase [17] that catalyze the reaction responsible for mobilizing the available phosphorus stored in plant. The release and reactions of the enzymes cause digestion of other tissues within the plant hence this calls for their regulation [18].

To prevent excessive destruction of tissue by the ROS enzymes, it is thought that there is release of antioxidants that counter-reacts the activities of ROS thus preventing further tissue damage [18]. Therefore, it was observed that plants that were cultivated without P in the current experiment had the highest antioxidant activity (38.58% for *S. villosum* and 35.92% for *S. scabrum*) followed by 20 kgP/ha then 40 kgP/ha and finally 60 kgP/ha. It appears like the more severe the deficiency the higher the production of antioxidant. This may thus explain the reduction of antioxidant with the decrease in phosphorus deficiency severity (Figs. 4 and 5).

Table 2. Effect of phosphorus rate and variety on shoot total phenolic content in greenhouse, long and short rains

Varieties	P levels	Greenhouse	Long rains	Short rains
		Shoot TPC	Shoot TPC	Shoot TPC
S. villosum	0	6.09^{a}	6.29^{a}	6.35^{a}
	20	4.93^{c}	5.02^{c}	5.07^{c}
	40	3.31^{e}	3.57^{e}	3.27^{e}
	60	1.62^{g}	1.80^{g}	2.02^{g}
S. scabrum	0	5.49^{b}	5.52^{b}	5.63^{b}
	20	3.88^{d}	4.01^{d}	4.21^{d}
	40	2.54^{f}	2.71^{f}	2.85^{f}
	60	1.20^{h}	1.32^{h}	1.45^{h}
P X V	*	*	*	*

Means followed by the same letter within the same column are not significantly different (P≤0.05).
** Significant F values at P≤0.05 and NS-Not significant*

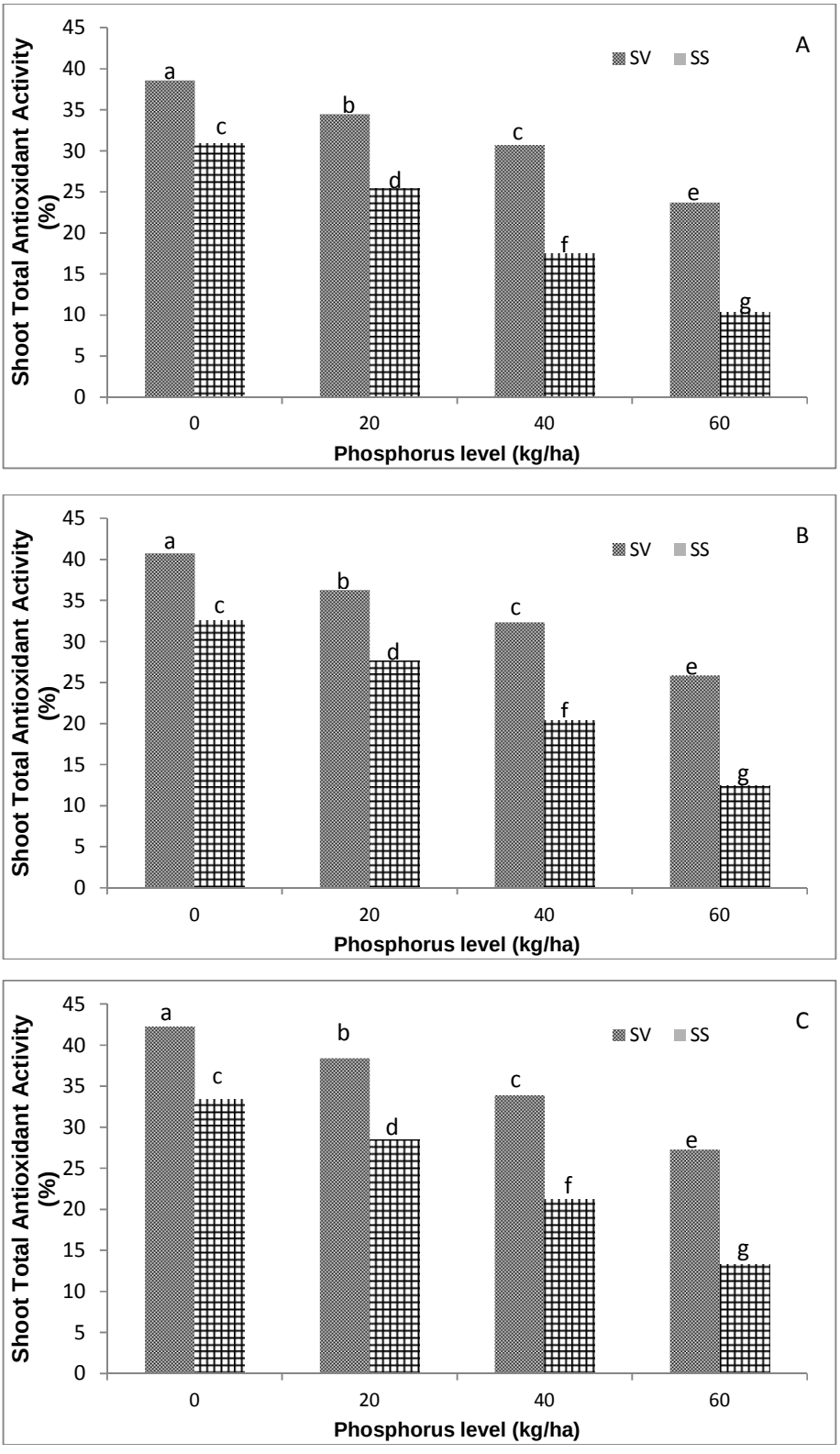

Fig. 4. Effects of phosphorus levels on shoot total antioxidant activities of two African nightshade varieties for (A) greenhouse, (B) long rains and (C) short rains respectively

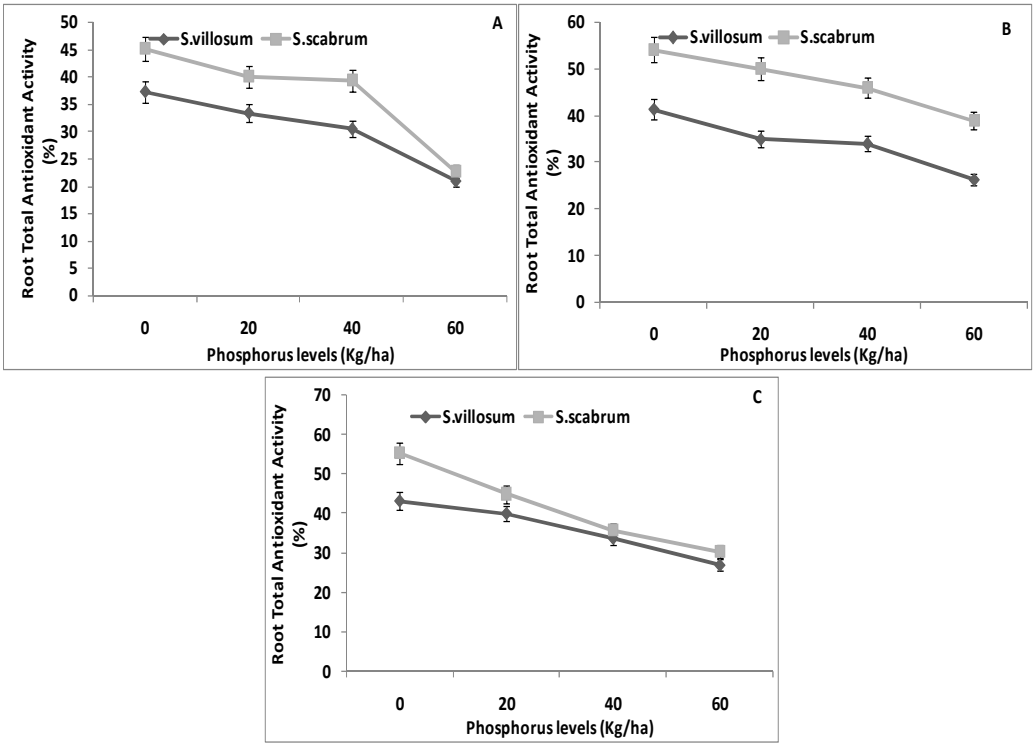

Fig. 5. Effects of phosphorus levels on root total antioxidant activities of two African nightshade varieties for (A) greenhouse, (B) long rains and (C) short rains respectively

The difference in antioxidant activities between shoots and roots were probably as a result of the location of the plant phosphorus reservoir. *S. villosum* reserves most of its secondary metabolites in the leaves whereas *S. scabrum* reserves in the roots. Similar results were obtained by [19] in the study of Sunflower varieties under phosphorus deficiency which showed the increase in antioxidant activity.

Shoot total antioxidant activities for greenhouse, long and short rains are presented in Table 3. There was significant interaction at (P≤ 0.05) between variety and phosphorus rate on shoot TAA indicating that varieties responded differently to P application in terms of shoot TAA.

Given that varieties responded similarly to P application, the differences in their leaf TAA at

Table 3. Effect of phosphorus rate and variety on shoot total antioxidant activity (%) in greenhouse, long and short rains

Varieties	P levels	Greenhouse	Long rains	Short rains
		Shoot TAA	Shoot TAA	Shoot TAA
S. villosum	0	38.58^{a}	40.76^{a}	42.30^{a}
	20	34.49^{b}	36.30^{b}	38.40^{b}
	40	30.75^{c}	32.40^{c}	33.90^{c}
	60	23.71^{e}	25.90^{e}	27.30^{e}
S. scabrum	0	30.92^{c}	32.60^{c}	33.40^{c}
	20	25.38^{d}	27.60^{d}	28.50^{d}
	40	17.50^{f}	20.40^{f}	21.20^{f}
	60	10.38^{g}	12.50^{g}	13.30^{g}
P X V	*	*	*	*

Means followed by the same letter within the same column are not significantly different (P≤0.05).
** Significant F values at P≤0.05 and NS-Not significant*

similar P rates was attributed to their inherent secondary metabolite composition; *S. villosum* has higher secondary metabolite that enables it repel herbivores during stressful situations and also acquire more P from the soil than *S. scabrum.* Similar results were obtained by [20] who worked with production of phytochemicals on medicinal plants.

4. CONCLUSION AND RECOMMENDATION

The two Solanum varieties showed difference in leaf total phenolics and antioxidant, with higher concentration recorded in *Solanum villosum* than *Solanum scabrum*. However *Solanum scabrum* was superior on root total phenolics and antioxidants. Therefore, where modest leave phenolics and antioxidants harvest is of importance, Phosphorus at 40 kg/ha is recommended. There is need to profile a range of specific phenolics, understand partitioning in different organs and check whether there is differential excretion of these phenolics in the rhizosphere in different nightshades and indigenous vegetables in general.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Surch YJ. Cancer chemoprevention with dietary phytochemicals. Nat. Rev. 2003;7.
2. Arts W, Petersen M, Hollman P. Polyphenols and disease risk in epidemiological studies. Clinical Nutrition. 2005;81:46.58.
3. Petersen M, Hollman P, Simmonds J. Molecules of interest: Rosmarinic acid. Phytochemicals; 2003.
4. Beecher GR. Overview of dietary flavonoids: Nomenclature, occurrence and intake. J. Nutrition. 2003;133:109-114.
5. Schreiner M, Shen J, Li H. Vegetable crop management strategies to increase the quantity of phytochemicals. Eur. J. Nutrition. 2006;117-121.
6. Government of Kenya. World Health Organization template on Food and Nutrition. 2002;3:45-78.
7. Wright DM, Jordan GJ, Lee WG, Duncan RP, Forsyth DM, Coomes DA. Do leaves of plants on P-impoverished soils contain high concentrations of phenolic defence compounds? Functional Ecology. 2010;3: 56-82.
8. Ayoola B, Beecher R, Hurdlen P. Overview of dietary flavonoids: Nomenclature, occurrence and intake. J. Nutrition. 2003; 103:91-124.
9. Hinsinger H. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: A review. Plant and Soil. 2009;237(2):173-195.
10. Richardson E, Simmonds J, Mullaney E. Inositol phosphates: Linking agriculture and the environment. CAB International, Wallingford, UK. 2009;304-311.
11. Wissuwa M, Mazzola M, Picard C. Novel approaches in plant breeding for rhizosphere-related traits. Plant Soil. 2009; 321:409–430.
12. Zhang Y, Seeram N, Lee R, Feng L, Heber D. Isolation and identification of strawberry phenolics with antioxidant and human cancer cell anti-proliferative properties. J. Agric. Food Chem. 2010;56:670-675.
13. Ryans D, Robards K, Prenzier P, Antolovich M. Applications of mass spectrometry to plant phenols. Trends Analysis. Chem. 1999;18:362-371.
14. Kajjidoni ST, Salimath PM, Alagawadi AR, Vidyarani PK, Kataraki PG. Responses of advanced breeding lines of black gram to phosphorus Solubilizing bacteria and P sources. Proc. of First Nation. Symposium. On Mineral Phosphate Solubilization. 2009;218-220.
15. Cordell D, Drangert J, White S. The story of phosphorus: Global food security and food for thought. Global Environmental Change. 2009;19:292–305.
16. Cakmak I, Marschner H. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. Plant Physiology. 2005;98:1222-1227.
17. Cakmak I. Activity of ascorbate dependent H_2O_2 scavenging enzymes and leaf chlorosis enhance magnesium and potassium deficient leaves, but not in

phosphorus deficient leaves. J. Exp. Bot. 2003;12(3):56-64.

18. Ren AZ, Gao YB and Zhao F. Response of *Neotyphodieum lolii*-infected perennial ryegrass to phosphorus deficiency. Plant Soil Environment. 2007;53(3):113-119.
19. Mishra NP, Mishra RK, Singhal GS. Changes in the activities of anti-oxidant enzymes during exposure of intact Wheat leaves to strong visual light at different temperatures in the presence of protein synthesis inhibitors. J. Plant Physiology. 2005;102:903-910.
20. Habibi D, Jar M, Mahmoudi A. Antioxidative enzymes in Sunflower subjected to drought stress. 4th International Crop Science Congress. Brisbane, Australia; 2004.

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