

Vermicompost leachates enhance morpho-physiological properties in *Pelargonium sidoides* DC



Authors:

McMaster Vambe¹ 
 Karishma Singh¹ 
 Roger M. Cooposamy¹ 
 Kuben Naidoo¹ 
 Rebecca Zengeni² 

Affiliations:

¹Department of Nature Conservation, Faculty of Natural Sciences, Mangosuthu University of Technology, Umlazi, Durban, South Africa

²Department of Soil Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa

Corresponding author:

McMaster Vambe,
 Mcmastervambe@gmail.com

Dates:

Received: 20 Mar. 2024

Accepted: 13 Apr. 2024

Published: 28 June 2024

How to cite this article:

Vambe, M., Singh, K., Cooposamy, R.M., Naidoo, K. & Zengeni, R., 2024, 'Vermicompost leachates enhance morpho-physiological properties in *Pelargonium sidoides* DC', *Journal of Medicinal Plants for Economic Development* 8(1), a256. <https://doi.org/10.4102/jomped.v8i1.256>

Copyright:

© 2024. The Authors.
 Licensee: AOSIS. This work is licensed under the Creative Commons Attribution License.

Read online:



Scan this QR code with your smart phone or mobile device to read online.

Background: *Pelargonium sidoides* (Geraniaceae family) is extensively used in African folk medicine to manage several diseases including gonorrhoea, tuberculosis, gastro-intestinal, hepatic and menstrual disorders. The plant's parts and efficacious extract-based pharmaceutical products are sold in several local and international markets. However, the growing demand for the herb has resulted in overexploitation of its wild populations, prompting an urgent need to develop sustainable production and conservation strategies.

Aim: The present study investigated the effects of vermicompost leachate (VCL) on morpho-physiological properties of greenhouse-grown *P. sidoides*.

Setting: *Pelargonium sidoides* seeds were supplied by the Mountain Herb Estate (Pretoria, South Africa), and all experiments were conducted at the University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Methods: Six-week-old seedlings of the plant were watered regularly and subjected to 50 mL (soil drench) of VCL (1:5, 1:10 or 1:20; VCL: water, v/v dilution) once a week. After 8 weeks, the seedlings were harvested, and morphological parameters, antioxidant activity, phytochemical and photosynthetic pigment content determined.

Results: The VCL (1:5, 1:10 and 1:20; VCL: water) induced a significant ($p \leq 0.05$) increase in the concentration of phenolic compounds, photosynthetic pigments, canopy size, leaf area, number of tubers, fresh and dry weights in treatments compared to the control. The antioxidant activity also increased by 7% – 27 % in treatments compared to the control.

Conclusions: The study showed that VCL improves the yield and quality of *P. sidoides*.

Contribution: The use of VLC could, therefore, potentially help in the conservation and large-scale production of the herb.

Keywords: bio-stimulant; morpho-physiology; traditional medicine; *Pelargonium sidoides*; phenolics; vermicompost leachates.

Introduction

Pelargonium sidoides DC [Synonym: *Pelargonium sidaefolium* (Thunb)] is a geophyte belonging to the Geraniaceae family. This perennial herb is native to Lesotho and South Africa (Eastern Cape, Free State and Gauteng provinces) (Newton et al. 2008), where it is commonly referred to as the black pelargonium (English), *kalwerbossie*, *rabassam* (Afrikaans), *ikubalo*, *iyeza lesikhali* (isiXhosa), *khoara-nyenyane* (Southern Sotho), *Uvendle* (IsiZulu) and *umckaloabo* (German remedy) (Moyo & Van Staden 2014). Traditional healers in southern Africa use the plant to manage a wide range of medical conditions including abdominal pains, gonorrhoea, hepatic and menstrual disorders (Brendler & Van Wyk 2008). One of the most common folkloric uses of *P. sidoides* is the treatment of tuberculosis, which resulted in the herb being introduced in Europe in the late 1800s (Bladt & Wagner 2007). A rich ethnobotanical history, substantiated with compelling empirical evidence allowed the development of several *P. sidoides* extract-based pharmaceutical products including Umckaloabo, Umkalor and EPs® 7630 (Brendler & Van Wyk 2008). The principal bioactive compounds in these products include oxygenated coumarins (7-hydroxy-5, 6-di-methoxycoumarin; 6,8-dihydroxy-5,7-dimethoxycoumarin), gallic acid-derivatives, flavonoids, phenolic and hydroxycinnamic acid derivatives (Kayser & Kolodziej 1995). However, the ever increasing local and international demand for the herb, coupled with widespread habitat destruction especially in its native areas in sub-Saharan Africa has resulted in significant declines in its wild populations (Lewu, Grierson & Afolayan 2006). Conservation strategies include micro-propagation, genetic engineering and bioreactor technologies (Moyo & Van Staden 2014). Large-scale cultivation of the herb has also been considered a viable option of easing the pressure on wild *P. sidoides* populations (Mofokeng 2015;

Mofokeng et al. 2015). Cultivating the herb using simple and eco-friendly technologies such as the use of bio-stimulants could ensure sustainable production and conservation of this economically valuable plant.

Plant bio-stimulants are substances that, in minute quantities, enhance the yield and quality of plants (Kauffman, Kneivel & Watschke 2007). Vermicompost leachate (VCL) is a plant-based bio-stimulant that stimulates several biochemical, organoleptic and physical properties in plants (Alabi et al. 2012, Aremu, Masondo & Van Staden 2014; Arthur et al. 2012). The bio-stimulant consists of water extracts of earthworm-decomposed organic matter laden with beneficial bacteria and trace amounts of amino acids, humic acids, plant growth regulators (PGR) and their by-products (Aremu et al. 2015; Mackowiak, Grossl & Bugbee 2001; Nardi et al. 2021; Siddiqui et al. 2020). These components work individually or synergistically to stimulate various processes in plants resulting in improved crop quality, yields and resistance to abiotic and biotic stresses (Arthur et al. 2012; Chinsamy, Kulkarni & Van Staden 2013; Kandari, Kulkarni & Van Staden 2011; Vambe et al. 2023).

Given that over 400 tonnes of *P. sidoides* tubers are harvested in South Africa every year (Van Niekerk & Wynberg 2012), coupled with the fact that the plant regenerates slowly after harvest (Lewu et al. 2006), there is an urgent need to develop and optimise cultivation techniques for the herb. Large-scale production of the herb will not only provide means to meet both international and local demands but will also work a long way in preventing the inevitable degradation of *P. sidoides* biodiversity. Although efforts have been previously made to standardise cultivation methods for the plant (Mofokeng 2015; Mofokeng et al. 2015), not much has been done to optimise its production using eco-friendly technologies such as bio-stimulants. The present study investigated the bio-stimulating effects of VCL on the yield and quality of *P. sidoides*.

Research methods and design

Plant collection and preparation

Pelargonium sidoides seeds were supplied by the Mountain Herb Estate (Pretoria, South Africa). On the 4th of August 2021, the seeds were sown in nursery trays containing Gromor™ potting mix (30 dm³) and the seedlings raised in a greenhouse at the University of KwaZulu-Natal, Pietermaritzburg campus, South Africa (29°37'S 30°23'E). The relative humidity in the greenhouse ranged between 50% and 60%, while day and night temperatures averaged 25°C and 15°C, respectively. The photosynthetic photon flux density of day hours in the greenhouse was approximately 450 ± 5 µmol m⁻² s⁻¹. After 6 weeks, the seedlings were transplanted into individual pots (1 seedling per pot) containing 1 kg of Gromor™ potting mix. Each treatment had 15 pots randomly arranged on greenhouse benches. Once a week the seedlings were watered and each pot received 50 mL (soil drench) of VCL (1:5, 1:10 or 1:20; VCL: water, v/v

dilution) for 8 weeks (from 12 September to 10 November 2021). Furthermore, 50% Hoagland's solution (HS) was applied as a liquid fertiliser to each seedling based on the properties of Gromor™ potting mix. On weeks 0, 4 and 8, half-strength HS was applied at a rate of 110 mL per pot (100 kg N ha⁻¹). The plants were harvested on the 17th of November 2021, and morphological parameters (area, number and weight of leaves and tubers), antioxidant activity, phenolic compound and photosynthetic pigment content determined immediately.

Leaf area determination

Average leaf area and canopy size for each treatment were estimated using the Easy leaf Area app as previously outlined by Easlon and Bloom (2014). Aerial images of individual leaves and plants were taken using a Samsung (Galaxy A20s) camera phone at an estimated distance of 20 cm directly above the leaf or plant. Leaf area and canopy size estimations were done using the Easy leaf area software (Easlon & Bloom 2014). Ten leaves and 10 plants randomly selected from each treatment were used to determine the leaf area and plant canopy size.

Phenolic composition of selected plants

Source of chemicals

Catechin, Folin C reagents and Gallic acid were all supplied by Sigma-Aldrich Co. (Steinheim, Germany). The other chemicals used included sodium carbonate and sodium nitrate (BDH Chemicals Ltd, England, United Kingdom), ferric ammonium sulphate (Hopkins and Williams Ltd, England, United Kingdom), cyanidin chloride (Carl Roth, GmbH + Co., Karlsruhe, Germany), aluminium chloride and sodium hydroxide (Merck KDA, Darmstadt, Germany).

Extraction for phenolic compounds

Dried-powdered material from each sample were extracted using 50% aqueous methanol at 5 mL/g by sonification in ice water for 30 min. The resultant extracts were then filtered through Whatman No. 1 filter papers and used immediately.

Determination of total phenolic content

The total phenolic content in each sample was determined using the Folin C assay as previously described by Makkar (1999). The assay was done in triplicate and varying quantities of Gallic acid (0.1 mg/mL) were used as standards for generating a calibration curve. After incubating the reaction mixture for 40 min at room temperature, absorbance readings for each sample were taken at 725 nm using a Cary 50 UV-visible spectrophotometer (Varian, Australia). The quantity of total phenolics in each sample was expressed as Gallic acid equivalents (GAE), as determined from the calibration curve.

Assay for condensed tannins

The butanol-hydrochloric acid assay was used to determine the concentration of proanthocyanidins (condensed

tannins) in each sample as previously reported by Makkar (1999). The assay was done in triplicate and cyanidin chloride was used as a standard for plotting the calibration curve. A Cary 50 UV-visible spectrophotometer (Varian, Australia) was used to take absorbance readings of each sample at 550 nm. The condensed tannins content (CTC) in each sample was expressed as cyanidin chloride equivalents (CCE).

Assay for flavonoids

The aluminium chloride assay (Makkar 1999) was used to determine the flavonoid content in each sample. The assay was done in triplicates, and catechin was used to generate the calibration curve. Absorbance readings of the samples were read at 510 nm using a Cary 50 UV-visible spectrophotometer (Varian, Australia), immediately after the mixture was made. The flavonoid content (FC) in each sample was expressed as catechin equivalents (CAE), as estimated from the calibration curve.

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

The free radical scavenging properties of root samples was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as previously reported by Karioti et al. (2004), and Moyo and Van Staden (2014). Dried plant samples were suspended in aqueous methanol (50%) to known concentrations with 50 mg/mL as the highest concentration. A DPPH concentration of 50 μ M in the final reaction was used. Ascorbic acid (AA) and butylated hydroxytoluene (BHT) served as the positive controls, while the negative control consisted of a reaction mixture in which methanol was used instead of a plant extract. The final concentration of the extracts and standards in the bioassay was 0.5 mg/mL. The assay was done in triplicate. The rate of DPPH decolouration in the reaction mixtures over a 30 min time interval in the dark was used to determine the per cent free radical scavenging activity (% RSA).

The % RSA was calculated using the following formulae:

$$\text{RSA (\%)} = [1 - (A_{\text{sample}} - A_{\text{background}}) / A_{\text{control}}] \times 100,$$

where A_{sample} , $A_{\text{background}}$ and A_{control} were the absorbance values of the sample, background solution and negative control, respectively.

β -Carotene-linoleic acid model system

The β -carotene inhibitory activity was determined using the β -carotene-linoleic assay (Amarowicz et al. 2004) using BHT as a positive control. Dried root samples were re-suspended in 50% aqueous methanol to a concentration of 6.25 mg/mL. The initial absorbance was taken at 470 nm ($t = 0$) soon after BHT or plant extracts were added. The rate of β -carotene bleaching (antioxidant activity) was determined from absorbance readings taken at 30 min interval during a 2 h incubation at 50°C in the dark. Four replicates were used per sample.

Extraction and quantification of photosynthetic pigments

Three technical replicates (7 mg DW) per plant sample were extracted with acetone using a bead mill, and absorbance readings of the extracts were taken at 470, 653 and 666 nm using a Varian Cary 50 UV-VIS spectrophotometer (Varian, Australia). The concentration of total photosynthetic pigments, chlorophyll a (*Chl a*) and chlorophyll b (*Chl b*) were determined using a formula developed by Németh (1998). The quantity of photosynthetic pigments in each sample were expressed in mg g⁻¹FW.

Statistical analysis

All results were presented as mean $\pm$ standard error. One way analysis of variance (ANOVA) was used to determine differences among treatments, while the Duncan's multiple range test was used to separate statistically significance ($p \leq 0.05$) means. Graphs were constructed using Graph Pad Prism version 5.0 for Windows (Graph Pad Software Inc., San Diego, CA), while data analysis was done using the Statistical Package for the Social Sciences (SPSS) version 24.0 for Windows (IBM SPSS Inc., Chicago, IL).

Results and discussion

Although VCL treatments did not induce significant increases in the average number of leaves per plant, notable improvements in plant canopy cover (3- to 5-fold increase) and leaf area (3- to 6-fold increase) were observed among treatments (Table 1, Figure 1 and Figure 2). Furthermore, there was a 3-fold increase in the average number of tubers per plant in all treatments, compared to the control (Figure 2 and Figure 3a). Data from the present study also show that there was a 2-fold increase in fresh root weight (Figure 3b), as well as a 2.6-fold increase in dry root weight (Figure 3b) in all treatments compared to the control. Furthermore, fresh shoot weight doubled, while dry shoot weight tripled in nearly all treatments compared to the control (Figures 3c).

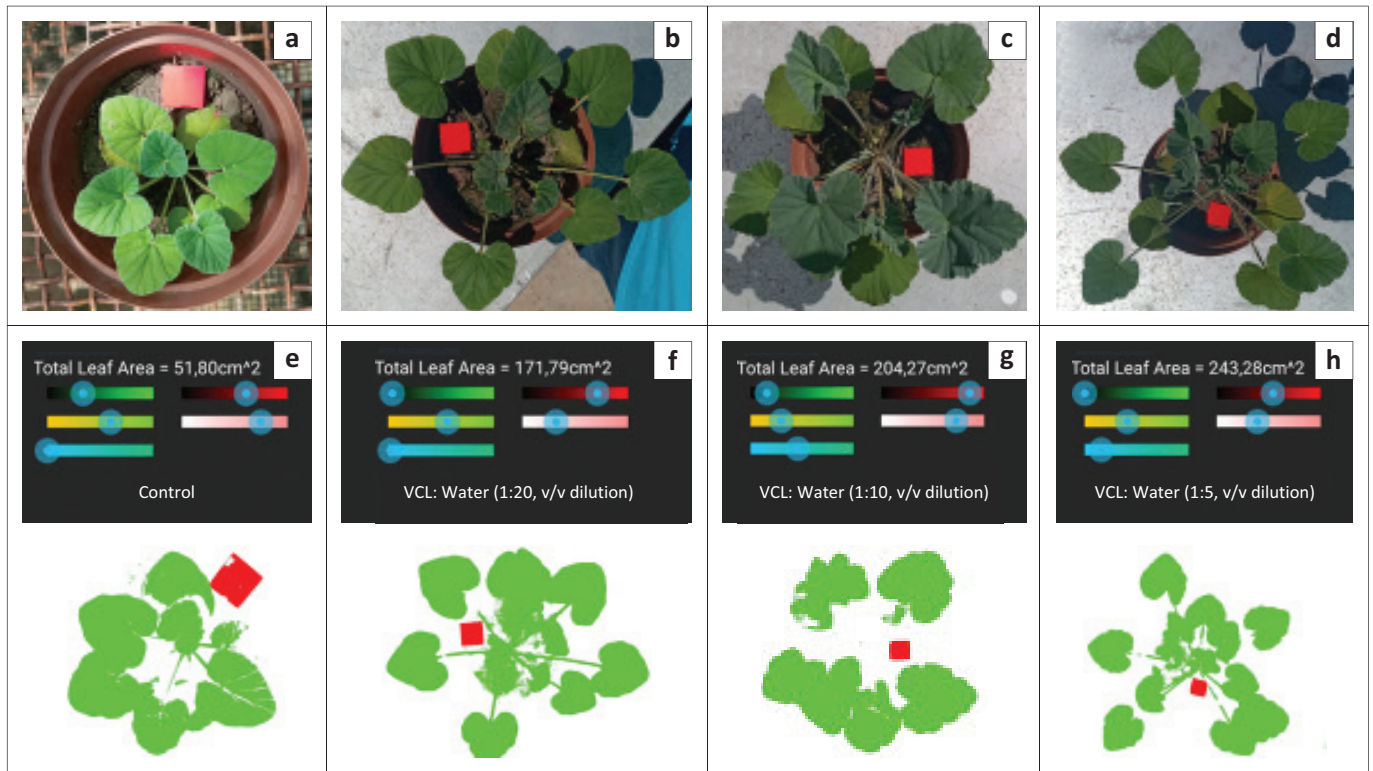
Vermicompost leachate also significantly ($p \leq 0.05$) increased the concentration of photosynthetic pigments in *P. sidoides* seedlings (Figure 4). All treatments stimulated > 1.5-fold increase in the concentration of total chlorophyll content in the seedlings, compared to the control. Vermicompost leachate also doubled the concentration of chlorophyll a in nearly all treatments (Figure 4). However, none of the

TABLE 1: Effects of vermicompost leachate (VCL) on leaf area and canopy size of green-house grown *Pelargonium sidoides* seedlings.

Treatments	Control	VCL		
		1:5	1:10	1:20
Average canopy size (cm ²)	48.09 $\pm$ 3.21 ^a	246.65 $\pm$ 5.97 ^d	201.97 $\pm$ 6.81 ^c	173.41 $\pm$ 2.21 ^b
Average leaf area (cm ²)	4.91 $\pm$ 1.56 ^a	24.03 $\pm$ 67.00 ^d	16.74 $\pm$ 2.34 ^c	12.45 $\pm$ 3.14 ^b

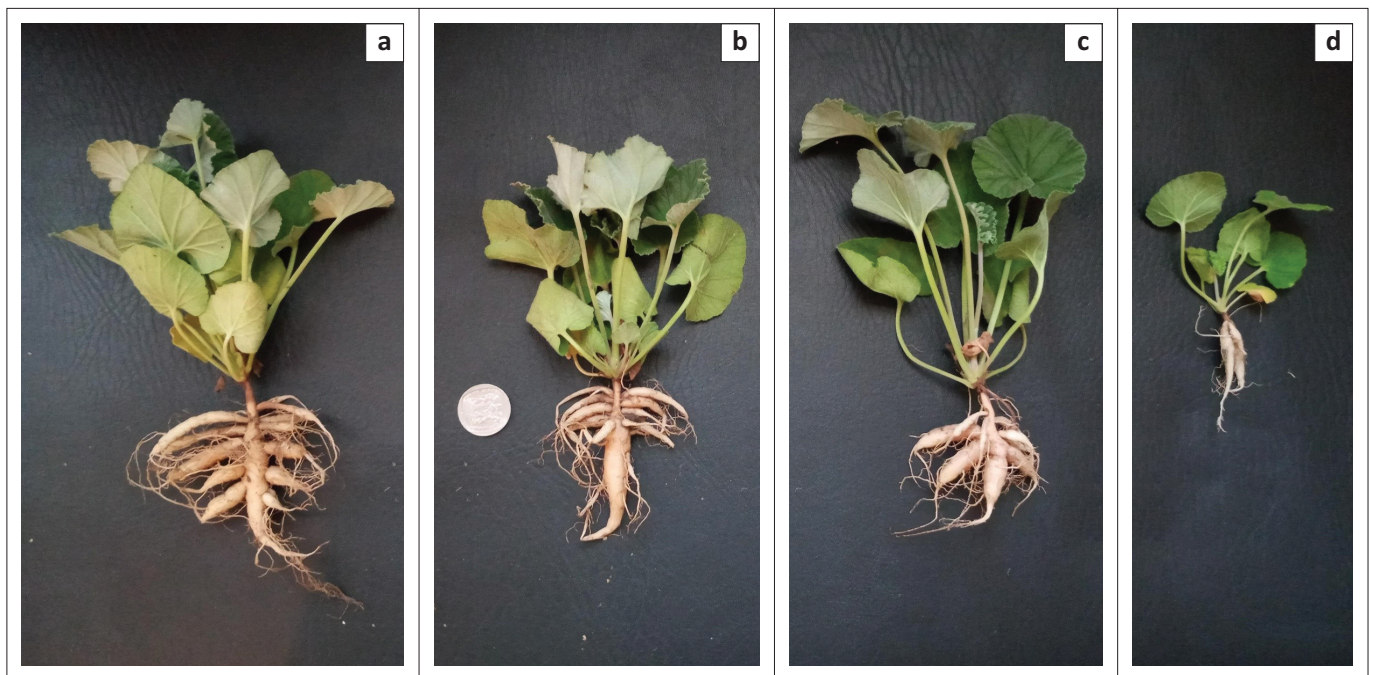
Note: Values represents mean $\pm$ standard error, $n = 10$. Values with different letters are significantly ($p \leq 0.05$) different according to the Duncan's Multiple Range Test. VCL (1:5) = VCL (VCL: Water, v/v dilution); VCL (1:10) = (VCL: Water, v/v dilution); VCL (1:20) = (VCL: Water, v/v dilution).

VCL, vermicompost leachate.



Picture taken by: Easy leaf area software.

FIGURE 1: Estimation of total leaf area using the Easy Leaf Area software (Easlon & Bloom 2014). (a–d) Depicts actual plant (*Pelargonium sidoides*). (e–h) Shows images processed by the Easy Leaf area software.



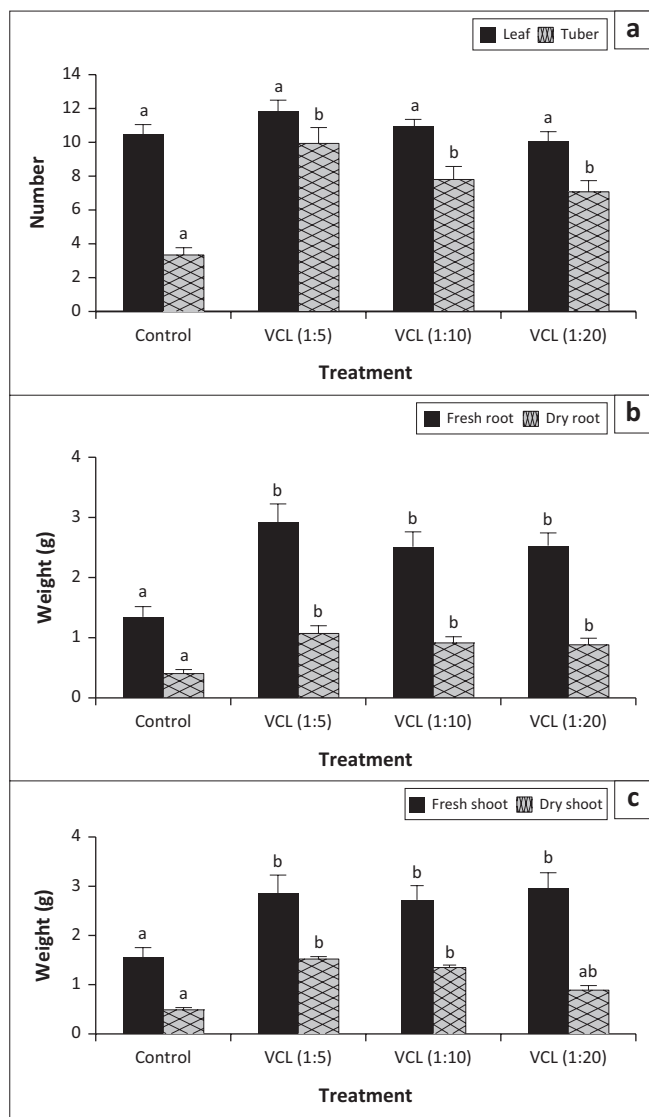
Picture taken by: Easy leaf area software.

FIGURE 2: Effects of vermicompost leachate (VCL) on morphological features of green-house grown *Pelargonium sidoides* seedlings. (a) VCL: Water (1:5, v/v dilution). (b) VCL: Water (1:10; v/v dilution). (c) VCL: Water (1:20, v/v dilution). (d) Control.

treatments stimulated an increase in the concentration of chlorophyll b except VCL: Water (1:5, v/v).

Findings from the present study suggest that VCL significantly ($p \leq 0.05$) enhanced the concentration of

phenolic compounds in greenhouse-grown *P. sidoides* seedlings. For instance, while the average total phenolic compounds (TPC) in the leaves of the control was 14.01 mg GAE/g, it ranged between 19.6 mg GAE/g and 22.9 mg GAE/g in leaf samples from the treatments (Figure 5a).



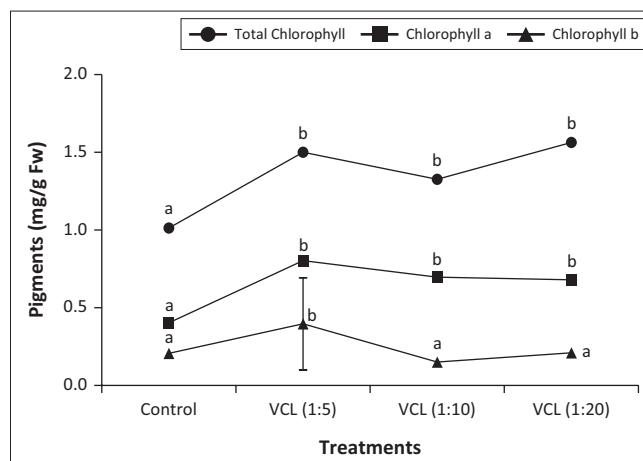
Note: Bars (Mean $\pm$ SE) of each growth parameter (leaf, tuber, root and shoot) with different letter(s) are significantly different according to Duncan's multiple range test at 5% significant level ($p < 0.05$).

VCL, vermicompost leachate.

FIGURE 3: Morphological effects of VCL on greenhouse grown *Pelargonium sidoides* seedlings.

The concentrations of TPC in all three treatments were, however, not statistically different from each other. Furthermore, root TPC in the control was 19.01 mg GAE/g, while it ranged between 26 mg GAE/g and 38 mg GAE/g in treatments (Figure 5a). The highest root TPC was observed in seedlings treated with the highest concentration of VCL (VCL: water [1:5, v/v dilution]), while the lowest concentration of VCL used in the current study (VCL: water [1:20, v/v dilution]) did not cause a notable increase in TPC in the roots, compared to the control.

There was a 2-fold increase in the concentration of tannins (9.5 mg CCE/g – 15.3 mg CCE/g) in the roots of seedlings treated with VCL, compared to the control (6.54 mg CCE/g, Figure 5b). The highest CTC in the roots was obtained from seedlings subjected to VCL: water (1:10, v/v dilution), while results from the VCL: water (1:5, v/v dilution) and VCL: water (1:20, v/v dilution) treatments were



Note: Different letters denote significantly different values according to Duncan's multiple range test ($p < 0.05$).

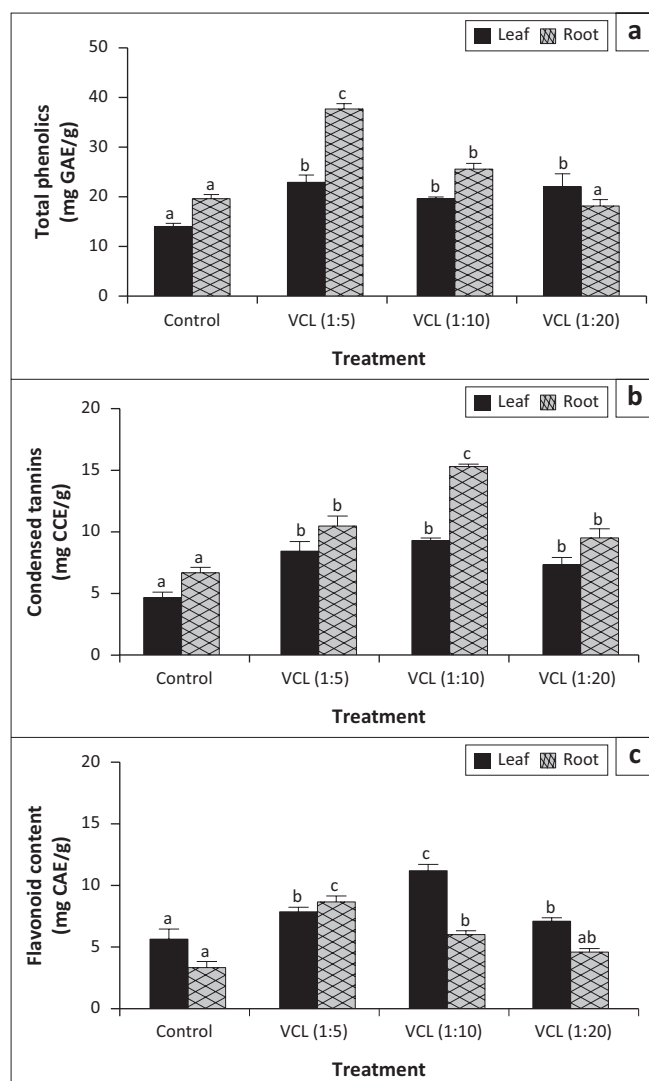
VCL, vermicompost leachate.

FIGURE 4: Effect of VCL on photosynthetic pigments of *Pelargonium sidoides* seedlings grown under greenhouse conditions.

comparable to each other (Figure 5b). The concentration of condensed tannins in leaves were comparable among treatments, though statistically different from the control (Figure 5b).

There was a direct relationship between the concentration of VCL used and the concentration of flavonoids in the roots. Among the root treatments, the highest and lowest FC were 8.66 mg CAE mg/g and 4.6 mg CAE mg/g, respectively (Figure 5c). In leaves, however, the highest FC (11.89 mg CAE/g) was observed in samples treated with VCL: water (1:10, v/v dilution). The other two treatments gave results comparable to each other (7.5 mg CAE/g – 8.1 mg CAE/g, Figure 5c). The optimum bio-stimulant concentration in this case was, therefore, VCL: water (1:10 v/v dilution, Figure 5c). The trend (bell curve) observed in Figure 5c (root FC) was akin to that observed in the case of root CTC (Figure 5b). It was interesting to note that the lowest VCL dilution used in the current study (VCL: water [1:20, v/v dilution]) induced a significant increase in all assessed phenolic compounds in the plant, except for root TPC and root FC (Figure 5a and c, respectively).

All VCL treatments significantly ($p \leq 0.05$) improved the antioxidant activity of the plant as shown in Table 2. The DPPH assay revealed that VCL treatments increased the anti-oxidant activity of the plant by between 10% and 27%. The highest DPPH inhibitory activity was observed in samples subjected to VCL: water (1:10, v/v dilution). The difference between the antioxidant activities of samples treated with VCL: water (1:5, v/v) and VCL: water (1:20, v/v) were not statistically significant. Data from the β -carotene inhibition assay also demonstrated that VCL improved the antioxidant properties of the seedlings by 7% – 17%, with the VCL: water (1:5, v/v dilution) treatment yielding the highest inhibitory activity (50%). Treatments subjected to VCL: water (1:10, v/v dilution) and VCL: water (1:20, v/v dilution) yielded results comparable to each other but statistically different ($p \leq 0.05$) from the



Note: CAE, catechin equivalents; CCE: cyanidin chloride equivalents; DPPH = 2, 2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalents; v/v = volume to volume dilutions with water. Bars ($\pm$ SE) of leaf and root with different letter(s) are significantly different according to Duncan's multiple range test at 5% significant level ($p < 0.05$).

VCL, vermicompost leachate.

FIGURE 5: Effect of VCL on phytochemical content of greenhouse – grown *Pelargonium sidoides*.

TABLE 2: Antioxidant activity of the roots of *Pelargonium sidoides* seedlings treated with vermicompost leachate (VCL).

Treatments	Inhibition activity (%)	
	DPPH	β -Carotene
Control (-ve)	19.62 $\pm$ 4.1 ^a	32.41 $\pm$ 0.15 ^a
VCL (1:5, v/v)	33.54 $\pm$ 1.2 ^b	49.50 $\pm$ 5.55 ^c
VCL (1:10, v/v)	46.91 $\pm$ 3.6 ^c	38.00 $\pm$ 1.30 ^b
VCL (1:20, v/v)	29.73 $\pm$ 5.9 ^b	40.10 $\pm$ 2.80 ^b
Ascorbic acid	96.50 $\pm$ 0.2	-
Butylated hydroxytoluene	94.90 $\pm$ 1.95	98.45 $\pm$ 0.87

Note: Values represents mean $\pm$ standard error, $n = 3$. Values with different letters are significantly ($p \leq 0.05$) different according to the Duncan's Multiple Range Test. VCL (1:5) = VCL (VCL: Water, v/v dilution); VCL (1:10) = (VCL: Water, v/v dilution); VCL (1:20) = (VCL: Water, v/v dilution).

VCL, vermicompost leachate; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

negative control and the treatment subjected to VCL: water (1:5, v/v dilution, Table 2).

The current study revealed for the first time the bio-stimulating effects of VCL on greenhouse grown *P. sidoides*.

Although the bio-stimulant did not cause a significant change in the average number of leaves per plant (Figure 2 and Figure 3a), there were notable improvements in several morpho-physiological parameters of the plant such as antioxidant activity, canopy size, leaf area, average number of tubers per plant, plant weight, photosynthetic pigment and phenolic compound content. Canopy size and leaf area increased in a concentration depended manner (Table 1), suggesting that the efficacy of the principal bioactive constituents in VCL improved as their concentration in VCL increased. Data from Figure 5b (root CTC) and C (leaf FC) also suggested that the principal bioactive compounds in VCL had a threshold concentration beyond which no further increases in VCL concentration resulted in a corresponding increase in the concentration of phenolic compounds in the plant. Evidence from the current study also suggested that the lowest VCL concentration (VCL: water, 1:20, v/v) used induced a statistically significant ($p \leq 0.05$) change in almost all evaluated parameters compared with the control. This observation suggested that the bioactive agent(s) in VCL were able to significantly improve plant metabolic, growth and developmental processes in minute quantities. All these observations, when put together, seem consistent with the effects of several bio-stimulating agents on plants. Empirical evidence from previous studies implicated PGRs (or their derivatives) and humic acids (HA) as the principal plant bio-stimulating agents in VCL (Alabi et al. 2012; Aremu et al. 2015; Arthur et al. 2012).

Aremu et al. (2015) reported the presence of abscisic acid, auxins, brassinosteroids, cytokinins and gibberellins in VCL. These well-known phyto-hormones work individually or synergistically to regulate copious morpho-physiological processes in plants. Notable improvements in chlorophyll production observed among treatments in the present study (Figure 4) could, therefore, probably be attributed to the presence of cytokines and gibberellins in VCL. Previous studies demonstrated that exogenous applications of these two hormones significantly improve the production of chlorophyll pigments and photosynthetic efficiency in *Camellia sinensis* (tea plant), *Cinchona ledgeriana* (red cinchona), *Avena sativa* (oats) and *Solanum lycopersicum* (tomatoes) (Chauhan et al. 2018; Maxiselly et al. 2021; Rosniawaty et al. 2020; Siddiqui et al. 2020). The individual or combined effects of auxins, cytokinins, gibberellins and brassinosteroids probably also contributed to increases in the number and size of tubers, canopy size, leaf area and plant weight among treatments in the current study (Alabi et al. 2012; Chinsamy et al. 2013; Aremu et al. 2015; Nardi et al. 2002; Nardi, Schiavon & Francioso 2021).

The plant bio-stimulatory effects exhibited by VCL in the current study could also partly be because of the effects of HA on *P. sidoides*. Humic acid is known to stimulate cell elongation, cell permeability, photosynthesis and respiration in several plants (Nardi et al. 2002; 2021). Some reports also suggests that HA enhances secondary metabolism in plants (Canellas et al. 2015; Schiavon et al. 2010), thus perhaps explaining why in the present study,

the concentration of phenolic compounds was higher in virtually all treatments than the control (Figure 5). Furthermore, the presence of more phenolic compounds in plants treated with VCL probably justified the treatment's enhanced antioxidant activities compared to the control (Table 2). Humic acid has also been reported to stimulate plant growth indirectly by increasing the biomass of beneficial microorganism in the soil. Some reports also suggests that HA enhances the adsorption and bio-availability of nutrients in the root zone (Li et al. 2019; Mackowiak et al. 2001; Maji et al. 2017; Sun et al. 2020). Vermicompost leachate therefore, probably also enhanced the yield and quality of *P. sidoides* by improving the biological, chemical and physical properties of soils.

Conclusion

The current study revealed that VCL, a plant-based bio-stimulant, enhances the yield and quality of green-house grown *P. sidoides* by > 3-fold. Evidence from the study strongly suggests that VCL-induced increases in leaf area, plant canopy size and photosynthetic pigments result in significant improvements in the photosynthetic capacity of the plant, which in turn results in increased yields (more tubers per plant and higher tuber weights than the control, Figure 3). Apart from increasing yields, VCL also stimulated increases in the antioxidant potential and concentration of phenolic compounds in *P. sidoides* tubers, which are the main part of the plant predominantly used in both traditional and conventional medicine. Given that antioxidants and phenolic compounds suppress the onset and progression of several medical disorders, enhancing their concentration in *P. sidoides* could potentially improve the plant's efficacy. Integrating VCL in *P. sidoides* cultivating practices could therefore bear numerous socio-economic benefits, as it could enhance both the quality and yields of this economically important medicinal plant.

Although findings from the current research are generally encouraging, more work still needs to be done for us to fully comprehend the manifold benefits and potential risks (if any) associated with integrating VCL in *P. sidoides* cultivation practices. It is therefore imperative that the effects of VCL on the pharmacological, morpho-physiological and toxicological properties of the plant, as well as the environment be thoroughly investigated. Although a dilution of VCL: water (1:5, v/v) generally yielded the best overall results in the current study, field trials should also be conducted to standardise the use of VCL on a large-scale. It could also be interesting to know if using different VCL application techniques has any beneficial effects on the plant. In the present study the mode of VCL application used was soil drenching, and as such foliar application and a combination of the two methods could be tried in future studies. It is also worth exploring the potential benefits of applying VCL in combination with other organic bio-stimulants (e.g., eckol, smoke water, karrikinolide, Kalpak, etc.) with organic fertilisers on the yield and quality of the plant. The potential use of VCL to alleviate abiotic and biotic stresses associated with the plant should also be investigated.

Acknowledgements

The authors wish to thank the Mangosuthu University Research Directorate for their financial support in conducting this research. The authors would also like to thank Dr M.G. Kulkarni for assisting with data analysis.

Competing interests

The author(s) declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

M.V. conceived the original idea, conducted the trial and wrote the first draft, K.S., R.Z., K.N. and R.M.C. edited the manuscript, provided resources and supervised the project.

Ethical considerations

This article followed all ethical standards for research without direct contact with human or animal subjects.

Funding information

Mangosuthu University Research Directorate is thanked for their financial support.

Data availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

References

- Alabi, O., Arthur, G., Cooposamy, R., Mbanjwa, K.N.S. & Tshapha, L., 2012, 'Vermicompost leachate a viable bio-stimulant for tomato growth and yield (*Solanum lycopersicum*)', *Indian Journal of Agricultural Research* 1, 1–6.
- Amarowicz, R., Pegg, R., Rahimi-Moghaddam, P., Barl, B. & Weil, J., 2004, 'Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies', *Food chemistry* 84(4), 551–562. [https://doi.org/10.1016/S0308-8146\(03\)00278-4](https://doi.org/10.1016/S0308-8146(03)00278-4)
- Aremu, A.O., Masondo, N.A. & Van Staden, J., 2014, 'Physiological and phytochemical responses of three nutrient-stressed bulbous plants subjected to vermicompost leachate treatment', *Acta Physiologiae Plantarum* 36, 721–731. <https://doi.org/10.1007/s11738-013-1450-3>
- Aremu, A.O., Stirk, W.A., Kulkarni, M.G., Tarkowská, D., Turečková, V., Gruz, J. et al., 2015, 'Evidence of phytohormones and phenolic acids variability in garden-waste-derived vermicompost leachate, a well-known plant growth stimulant', *Plant Growth Regulation* 75, 483–492. <https://doi.org/10.1007/s10725-014-0011-0>
- Arthur, G.D., Aremu, A.O., Kulkarni, M.G. & Van Staden, J., 2012, 'Vermicompost leachate alleviates deficiency of phosphorus and potassium in tomato seedlings', *HortScience* 47(9), 1304–1307. <https://doi.org/10.21273/HORTSCI.47.9.1304>
- Bladt, S. & Wagner, H., 2007, 'From the Zulu medicine to the European phytomedicine Umckaloabo®', *Phytomedicine* 14(Suppl. 1), 2–4. <https://doi.org/10.1016/j.phymed.2006.11.030>
- Brendler, T. & Van Wyk, B.-E., 2008, 'A historical, scientific and commercial perspective on the medicinal use of *Pelargonium sidoides* (Geraniaceae)', *Journal of Ethnopharmacology* 119(3), 420–433. <https://doi.org/10.1016/j.jep.2008.07.037>
- Canellas, L.P., Da Silva, S.F., Olk, D.C. & Olivares, F.L., 2015, 'Foliar application of plant growth-promoting bacteria and humic acid increase maize yields', *Journal of Food, Agriculture and Environment* 13, 131–138.

- Chauhan, A., Rajput, N., Kumar, A., Verma, J. & Chaudhry, A., 2018, 'Interactive effects of gibberellic acid and salt stress on growth parameters and chlorophyll content in oat cultivars', *Journal of Environmental Biology* 39, 639–646. <https://doi.org/10.22438/jeb/39/5/MRN-615>
- Chinsamy, M., Kulkarni, M.G. & Van Staden, J., 2013, 'Garden-waste-vermicompost leachate alleviates salinity stress in tomato seedlings by mobilizing salt tolerance mechanisms', *Plant Growth Regulation* 71, 41–47. <https://doi.org/10.1007/s10275-013-9807-6>
- Easlon, H.M. & Bloom, A.J., 2014, 'Easy Leaf Area: Automated digital image analysis for rapid and accurate measurement of leaf area', *Applications in Plant Sciences* 2(7), 1400033. <https://doi.org/10.3732/apps.1400033>
- Kandari, L., Kulkarni, M. & Van Staden, J., 2011, 'Vermicompost leachate improves seedling emergence and vigour of aged seeds of commercially grown *Eucalyptus species*', *Southern Forests: A Journal of Forest Science* 73(2), 117–122. <https://doi.org/10.2989/20702620.2011.610923>
- Karioti, A., Hadjipavlou-Litina, D., Mensah, M.L., Fleischer, T.C. & Skaltsa, H., 2004, 'Composition and antioxidant activity of the essential oils of *Xylopia aethiopica* (Dun) A. Rich.(Annonaceae) leaves, stem bark, root bark, and fresh and dried fruits, growing in Ghana', *Journal of Agricultural and Food Chemistry* 52(26), 8094–8098. <https://doi.org/10.1021/jf040150j>
- Kauffman, G.L., Kneivel, D.P. & Watschke, T.L., 2007, 'Effects of a biostimulant on the heat tolerance associated with photosynthetic capacity, membrane thermostability, and polyphenol production of *Perennial Ryegrass*', *Crop Science* 47(1), 261–267. <https://doi.org/10.2135/cropsci2006.03.0171>
- Kayser, O. & Kolodziej, H., 1995, 'Highly oxygenated coumarins from *Pelargonium sidoides*', *Phytochemistry* 39(5), 1181–1185. [https://doi.org/10.1016/0031-9422\(95\)00166-5](https://doi.org/10.1016/0031-9422(95)00166-5)
- Lewu, F., Grierson, D. & Afolayan, A., 2006, 'The leaves of *Pelargonium sidoides* may substitute for its roots in the treatment of bacterial infections', *Biological Conservation* 128(4), 582–584. <https://doi.org/10.1016/j.biocon.2005.10.018>
- Li, Y., Fang, F., Wei, J., Wu, X., Cui, R., Li, G. et al., 2019, 'Humic acid fertilizer improved soil properties and soil microbial diversity of continuous cropping peanut: A three-year experiment', *Scientific Reports* 9, 12014. <https://doi.org/10.1038/s41598-019-48620-4>
- Mackowiak, C., Grossl, P. & Bugbee, B., 2001, 'Beneficial effects of humic acid on micronutrient availability to wheat', *Soil Science Society of America Journal* 65(6), 1744–1750. <https://doi.org/10.2136/sssaj2001.1744>
- Maji, D., Misra, P., Singh, S. & Kalra, A., 2017, 'Humic acid rich vermicompost promotes plant growth by improving microbial community structure of soil as well as root nodulation and mycorrhizal colonization in the roots of *Pisum sativum*', *Applied Soil Ecology* 110, 97–108. <https://doi.org/10.1016/j.apsoil.2016.10.008>
- Makkar, H., 1999, Quantification of tannins in tree foliage. A laboratory manual for the FAO/IAEA coordinated research project on 'Use of nuclear and related techniques to develop simple tannin assay for predicting and improving the safety and efficiency of feeding ruminants on the tanniniferous tree foliage', pp. 1–29, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna.
- Maxiselly, Y., Anjasari, I., Sutari, W., Ariyanti, M., Soleh, M., Sari, R. et al., 2021, Stimulation effect of synthetic plant growth regulator (GA3 and BAP) on young cinchona plant (*Cinchona ledgeriana*) grown in lowland, p. 012016, IOP Conference Series: Earth and Environmental Science, IOP Publishing, East Java, ID.
- Mofokeng, M., Steyn, J., Du Plooy, C., Prinsloo, G. & Araya, H., 2015, 'Growth of *Pelargonium sidoides* DC. in response to water and nitrogen level', *South African Journal of Botany* 100, 183–189. <https://doi.org/10.1016/j.sajb.2015.05.020>
- Mofokeng, M.M., 2015, Growth, yield and chemical composition of *Pelargonium sidoides* DC. in response to nitrogen and soil water management, University of Pretoria, Pretoria.
- Moyo, M. & Van Staden, J., 2014, 'Medicinal properties and conservation of *Pelargonium sidoides* DC', *Journal of Ethnopharmacology* 152(2), 243–255. <https://doi.org/10.1016/j.jep.2014.01.009>
- Nardi, S., Pizzeghello, D., Muscolo, A. & Vianello, A., 2002, 'Physiological effects of humic substances on higher plants', *Soil Biology and Biochemistry* 34(11), 1527–1536. [https://doi.org/10.1016/S0038-0717\(02\)00174-8](https://doi.org/10.1016/S0038-0717(02)00174-8)
- Nardi, S., Schiavon, M. & Francioso, O., 2021, 'Chemical structure and biological activity of humic substances define their role as plant growth promoters', *Molecules* 26(8), 2256. <https://doi.org/10.3390/molecules26082256>
- Németh, J., 1998, *A biológiai vízminősítés módszerei. Környezetgazdálkodási Intézet, TOI Környezetvédelmi Tájékoztató Szolgálat, Budapest.*
- Newton, D., Letsela, T., Lijane, T., Mafatle, N., Manyama, P., Naha, S. et al., 2008, 'Development of a non-detriment finding process for *Pelargonium sidoides* in Lesotho', CITES NDF Workshop Case Studies, pp. WG2–CS2, Cancun.
- Rosniawaty, S., Anjasari, I., Sudirja, R., Harjanti, S. & Mubarak, S., 2020, 'Application of coconut water and benzyl amino purine on the plant growth at second centering of tea (*Camellia sinensis*) in lowlands area of Indonesia', *Research on Crops* 21(4), 817–822. <https://doi.org/10.31830/2348-7542.2020.125>
- Schiavon, M., Pizzeghello, D., Muscolo, A., Vaccaro, S., Francioso, O. & Nardi, S., 2010, 'High molecular size humic substances enhance phenylpropanoid metabolism in maize (*Zea mays* L.)', *Journal of Chemical Ecology* 36, 662–669. <https://doi.org/10.1007/s10886-010-9790-6>
- Siddiqui, M.H., Alamri, S., Alsubaie, Q.D. & Ali, H.M., 2020, 'Melatonin and gibberellic acid promote growth and chlorophyll biosynthesis by regulating antioxidant and methylglyoxal detoxification system in tomato seedlings under salinity', *Journal of Plant Growth Regulation* 39, 1488–1502. <https://doi.org/10.1007/s00344-020-10122-3>
- Sun, Q., Liu, J., Huo, L., Li, Y. C., Li, X., Xia, L. et al., 2020, 'Humic acids derived from Leonardite to improve enzymatic activities and bioavailability of nutrients in a calcareous soil', *International Journal of Agricultural and Biological Engineering* 13(3), 200–205. <https://doi.org/10.25165/j.ijabe.20201303.5660>
- Vambe, M., Coopsoosamy, R.M., Arthur, G. & Naidoo, K., 2023, 'Potential role of vermicompost and its extracts in alleviating climatic impacts on crop production', *Journal of Agriculture and Food Research* 12, 100585. <https://doi.org/10.1016/j.jafr.2023.100585>
- Van Niekerk, J. & Wynberg, R., 2012, 'The trade in *Pelargonium sidoides*: Rural livelihood relief or bounty for the "bio-buccaneers"?', *Development Southern Africa* 29(4), 530–547. <https://doi.org/10.1080/0376835X.2012.715440>