EFFECT OF IRRIGATION AND HARVESTING AGE ON THE GROWTH, YIELD AND CHEMICAL COMPOSITION OF *PELARGONIUM SIDOIDES* DC.

Report to the
Water Research Commission (WRC)

by

Nike Lewu[1], Reckson Mulidzi[1], Francis Lewu[2], Takalani Mulaudzi[3], Samuel Oluwafemi[4], Oluwafemi Adebo[5], Ngwatshipane Mashabela[1], Yandiswa Mtimkulu[1,2], Phila-Sande Ntoyi[1,3] and Kundani Khameli[1,4,5]

[1] The ARC-Institute for Fruit, Vine and Wine (ARC Infruitec-Nietvoorbij), Stellenbosch
[2] Department of Agriculture, Faculty of Applied Sciences, Cape Peninsula University of Technology
[3] Department of Biotechnology, Faculty of Natural Sciences, University of the Western Cape
[4] Department of Chemical Sciences, University of Johannesburg
[5] Department of Biotechnology and Food Technology, University of Johannesburg

WRC report no. 3201/1/25
ISBN 978-0-6392-0707-0

MAY 2025

Obtainable from
Water Research Commission
Bloukrans Building, Lynnwood Bridge Office Park
4 Daventry Street
Lynnwood Manor
PRETORIA

This is the final report of WRC project no. C2021/2022-00829.

EXECUTIVE SUMMARY

BACKGROUND

Pelargonium sidoides is a perennial medicinal plant commonly known as geranium, African geranium, Umckaloabo, Uvendle (IsiXhosa), Kalwerbossie (Afrikaans), i-Yeza lezikali (isiXhosa), Icwayiba (IsiXhosa), ikhubalo (IsiXhosa), Rabassam or Rabas (Dutch or Afrikaans) and Khoaara e nyenyane (Sesotho). It belongs to the family Geraniaceae, and it is one of the species of the genus *Pelargonium. P. sidoides* is indigenous to South Africa and Lesotho. In South Africa, its habitat extends along the extreme Eastern edge of the Western Cape, throughout the Eastern Cape, and in various regions across the Gauteng, North-West, Free State, KwaZulu-Natal, and Mpumalanga provinces. It is one of the estimated 3000 medicinal plant species that have been documented as traditional medicine used across South Africa. The medicinal use of *P. sidoides* dates back centuries where it is commonly used by several ethnic groups of the country to treat both human and livestock ailments. It is renowned for its pharmacological properties in treating tuberculosis, bronchitis, cough, fever and other respiratory-related ailments. Traditionally, it is used in the treatment of different health complications such as gonorrhoea, diarrhoea, dysentery, coughs, liver disorders, colic, prolapsed rectum, instila (stomach ailment in infants). Numerous studies have been carried out on the antibacterial and antifungal properties of the species. The medicinally active ingredients are found in the bitter-tasting roots of the plants.

Various phytochemical products have been produced making use of this plant. EPs® 7630 (Umckaloabo®) is the aqueous ethanolic extract of *P. sidoides* that has been evaluated for clinical treatment of ailments such as acute bronchitis and acute tonsillopharyngitis. *P. sidoides* has been effectively transformed into international phytopharmaceuticals and traded under several brand names, such as Linctagon and Umckaloabo. In Mexico and North America, there are a range of solid and liquid herbal supplements containing *P. sidoides*. Various products have since emerged in South Africa and other countries. These products are made solely with *P. sidoides* extract while others are having *P. sidoides* in combination with other medicinal plant extracts. *P. sidoides* is also available as processed materials in modern packaging and in various dosage forms such as teas, tinctures, tablets, capsules or ointments. It also forms part of the multi-million-rand informal markets of medicinal plants

There has been a significant growth in demand for *P. sidoides* from both domestic and foreign pharmaceutical manufacturers. The rapid growth in demand for the species has led to significant over-harvesting of the wild population, threatening population stability. The diminishing supply and increasing demand present an opportunity to cultivate this plant. The World Health Organisation (WHO, 1999) recommended that medicinal plants be cultivated, wherever possible, as the source of supply for the market. However, successful commercial cultivation of *P. sidoides* is hindered by lack of scientific data on its water use and absence of irrigation guidelines. Another challenge is the lack of reliable information on when maturity is attained by this plant. There are conflicting reports about the harvesting age at which maximum medicinal benefits in the roots is attained. Thus, understanding the water needs and maturity stage of crops like *P. sidoides* are important factors in designing appropriate agronomic practices like irrigation management and harvesting at the right age that will ensure optimum productivity, especially for commercial farmers. This way, pressure on the wild populations can be reduced.

Cultivation of *P. sidoides* has several distinct advantages: 1) to ensure supply of roots of the correct species and avoid adulteration, 2) as a conservation strategy, 3) to provide health nourishment for the rural communities when used as supplement to boost the immune system, 4) to ensure a reliable supply of the plant resource to buyers, 5) consumers would be assured that the material was obtained in a legitimate manner, and; 6) it provides an opportunity for sustainable production and increased income generation for the rural poor communities providing plant material for the informal as well as commercial markets. Hence, the need for this research.

AIMS (OBJECTIVES)

In light of the above background, the specific objectives of the project were:

1. To assess the growth of *Pelargonium sidoides* in response to three different irrigation levels
2. To evaluate the effect of different levels of irrigation and harvesting age on the root yield of *P. sidoides*
3. To investigate the effect of water stress on soil microbial enzyme activity in relation to soil fertility/quality
4. To determine the effect of different levels of irrigation and harvesting age on the chemical composition of the roots of *P. sidoides*

METHODOLOGY

The research was a pot study conducted inside a tunnel at the Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Stellenbosch, South Africa (Latitude 33°54’52.38”S Longitude 18°51’40.27”E). There were three (3) irrigation levels at different plant available water (PAW) namely, 75% PAW, 50% PAW and 25% PAW; and three harvest times (6, 12 and 18 months after initiation of water deficit stress). *P. sidoides* planting materials (sprouted tuber cuttings) were obtained from the Agricultural Research Council Vegetable Industrial and Medicinal Plants Institute (ARC VIMP). The plants were grown on loamy soil. Planting of sprouted tuber cuttings of *P. sidoides* took place in November 2022. Data collection commenced in June 2023, once the different water stress levels were introduced as treatments. The first harvest (H1) was done in December 2023 while the second harvest (H2) was carried out in June 2024.

Soil moisture content was monitored using the DFM soil moisture probes linked to a software. The software displays information on when and how much to irrigate in line with the predetermined soil moisture levels set for the different irrigation treatments (75% PAW, 50% PAW and 25% PAW). Data were collected monthly on the morpho-physiological responses and biochemical processes of *P. sidoides* growing under different PAW conditions. The study also monitored the chemical and biological (enzyme activity) changes in the soil due to water stress. Yields were determined at every harvest. This research further assessed the chemical profile and the secondary metabolites in the roots of *P. sidoides* subjected to different levels of water deficit stress. The effects of harvesting age on these metabolites were assessed as well as the nutritional composition of the leaves and roots of the species under different irrigation levels and harvest age.

The final project report covers data collected over about two years of plant growth, with two harvests completed.

The report includes capacity building and knowledge transfer activities which included scientific training of students for postgraduate qualifications, published scientific paper and presentations of research finding at conferences.

RESULTS AND DISCUSSION

After the three different levels of water stress was implemented on the *P. sidoides* plants at seven months after planting, data on growth parameters showed that water stress had no immediate effect on plant height and number of leaves in the first three months of water stress initiation. However, from 4 to 12 months of treatment application, I1 (75% PAW) consistently had higher plant height and number of leaves while I3 (25% PAW) plants had the least during this period of observation. These results could be attributed to better growth in the well-watered plants (75% PAW). For leaf area, the severely stressed plants (25% PAW) consistently had reduced leaf area compared to the other two treatments However, plants grown under the 75% and 50% PAW were more or less comparable with each other in the first 6 months after water stress introduction. Another observation was that there were no differences in leaf area among all treatments from the 7th month onwards, indicating that water stress had no effect on leaf area during this period. *Pelargonium sidoides* is considered a semi-evergreen to evergreen species, depending on environmental conditions. During prolonged drought or cold conditions, it may shed some or all its leaves to conserve energy and water. In this case, it behaves more like a semi-evergreen species. The underground tuberous root system allows it to survive adverse conditions and regrow when the environment becomes favourable again.

Relative water content (RWC) is a physiological trait that gives an indication of the water status of the plant and can also be used to assess a plant's drought tolerance. In this study, as expected the well-watered plants (75% PAW) consistently had higher RWC than the moderately stressed (50% PAW) and the severely stressed (25% PAW) plants, with the most stressed plants having the least RWC. In general, RWC declined during the summer months compared to winter, and this can be attributed to the role played by seasonal conditions. Warmer temperatures typically increase water loss through transpiration, leading to a reduction in RWC. Stomatal conductance is another physiological trait measured in the trial. It is a measure of the water loss from plant leaves via the stomatal aperture. The data collected over 12-month period, starting from July 2023 to June 2024 showed pronounced differences among the treatments with effect from the month of September with I3 (25% PAW) experiencing the most decline in stomatal conductance compared to the other two treatments till the end of the reporting period. The well- watered (75% PAW) plants consistently had the highest stomatal conductance from September 2023 to June 2024. The leaf chlorophyll content is also a significant physiological trait of plants. It represents the photosynthetic capacity of the plant. This physiological parameter shares a similar pattern with the RWC. For the 12-month duration of measurement, the effect of water stress on the leaf chlorophyll content became noticeable from the month of September 2023. Overall, the well-watered plants exhibited the highest level of chlorophyll contents, followed by the moderately stressed plants, while the severely stressed plants had the lowest chlorophyll contents.

For biochemical traits, reactive oxygen species (ROS) response to water depletion levels was determines on the leaves of *P. sidoides* from July to December 2023. Hydrogen peroxide (H_2O_2) level was consistently higher in the severely stressed plants (75% water depletion) throughout the observation period, with 25% water depletion treatment (75% PAW) being the least. ROS level increased with increase in water stress. Severe soil water depletion led to overproduction of H_2O_2, and thus, exacerbating oxidative stress and ultimately damaging the cells of *P. sidoides*. Malondialdehyde (MDA) levels followed the same trend as ROS. Since MDA is a suitable indicator of oxidative stress, the results of this study showed that *P. sidoides* was experiencing oxidative stress under severe water depletion. Proline levels in response to water depletion was also in line with ROS and MDA. Proline contents increased water stress. Indicating that under severe water depletion, *P. sidoides* synthesizes proline as an osmoprotectant counter-act oxidative stress, caused by hydrogen peroxide.

Two harvests (H1 and H2) were carried out and the fresh and dry shoot and root yields documented. The first harvest of *P. sidoides* took place on 05 December 2023, while the second harvest occurred on 04 June 2024. Deficit irrigation did not affect fresh shoot biomass in both harvests. However, shoot dry matter yield were significantly higher in H2 when compared to H1, irrespective of the treatment. Indicating that the longer *P. sidoides* stays before harvest, the higher the shoot dry matter yield. For the root yield, highest fresh root yield came from the well-watered plants while the severely stressed plants had the lowest fresh root yield at H1. On the contrary, the moderately stressed plants gave better fresh root yield compared to the well-watered and the severely stressed plants at H2. The dry root yield in H1 followed the same trend as fresh root yield. However, at H2, the highest dry root yield came from the moderately stressed plants while there were no significant differences in dry root yield between the well-watered and the severely stressed plants.

For soil enzyme activity, there were no significant differences in β-glucosidase activity across all irrigation levels (75% PAW, 50% PAW & 25% PAW) at both harvests. However, a comparison of the two harvests shows that β-glucosidase activity in H1 was significantly higher than of H2. Acid phosphatase activity was not significantly different among treatments in H1 and H2. Urease activity was lower for 25% PAW at H1 while activity at 75% PAW, 50% PAW were not different. However, urease activity remained the same at all irrigation levels during H2. Meanwhile, Urease activity dropped slightly at H2 for I1 and I2 while that of I3 increased when compared with H1. The reduction in enzyme activity in H2 can be attributed to decreased microbial activity and biomass, along with alterations in soil pH and organic matter availability. No significant difference was observed in AI3 across both harvests, although the values in H1 were higher than those in H2. At H1, the AI3 index scores became less negative as the water stress increased while it dropped to the pre-planting level and even lower (at 50% water depletion level) at H2. Overall, soil enzyme activities showed a drastic decline in H2.

Soil micronutrients Cu, Zn, Mn, B and Fe levels before planting, at H1 and H2 were also investigated. Cu level dropped for all treatments at H2, Zn level increased for 50% and 75% soil water depletion levels while that of 25% water depletion decreased at H2, Mn levels increased among all irrigation levels at H2 while B and Fe levels in the soil remained more or less the same at H1 and H2. The observed increase in some micronutrients suggests that *P. sidoides* was not able to assimilate these micronutrients present in the soil and even facilitated increases across treatments. Another reason may be due to organic carbon (C) percentage which increased with every harvest. Soil C is normally responsible for the binding of trace minerals.

For the chemical properties of *P. sidoides* leaves, the levels of N, P, Ca, Mg, Na & Fe in both harvests (H1 and H2) were not different significantly, irrespective of the treatments. One possible explanation for the lack of changes is that *P. sidoides* is recognized for its resilience and stress tolerance and can effectively regulate its metabolism under stress, allowing it to maintain a consistent chemical composition across harvests. There was a reduction in K level at H2 while Mn, Cu and Zn mostly increased at H2. These inconsistencies in nutrient composition of *P. sidoides* leaves may be due to several environmental, and soil related factors.

P. sidoides roots were only analysed for nutritive value during the second harvest (H2). Root nutrient analysis revealed that there were no differences in the nutritional composition of *P. sidoides* despite being subjected to different levels of irrigation. Indicating that water stress did not change the nutrient contents of the species.

There has never been any report on the colour analysis of *P. sidoides* root in both wild and cultivated populations. In this study, the colour analysis at H1 and H2 in response to different irrigation levels shows that water availability and harvesting age affected the colour attributes of *P. sidoides* roots. The lightness (L*) values for the plants harvested at 6-month (H1) after water stress initiation were significantly higher (59.85–60.53) than that of the plants harvested after 12-months of water stress (H2), which had lower lightness values (48.32–49.11). H2 plants are, therefore, lighter in colour compared to H1 plants. The different irrigation levels did not significantly affect lightness, showing that lightness was mainly influenced by plant age rather than irrigation treatment. The redness (a*) results show a clear distinction between the 6-month and 12-month harvests, with significant differences across the irrigation levels. Harvest age strongly influences redness, with H1 harvest showing higher redness values compared to H2 plants. Irrigation level did not influence yellowness at H1. In the 12-month harvest (H2), yellowness (b*) significantly decreases compared to H1. The severely stressed plants (25% PAW) had significantly lower yellowness at H2. This suggests that reduced irrigation at negatively impacts yellowness. Likewise, irrigation level had no effect on chroma among treatments in H1 or H2. However, age at harvest played a significant role. Chroma decreased significantly at H2 when compared to H1. Indicating that water stress reduces the intensity of colour as plants age. Hue values also followed the trend of the other colour indicator. The H1 *P. sidoides* roots had hue values ranging from 52.82° to 55.22°, indicating a yellowish-orange colour. The highest hue value was found in the moderately stressed (50% PAW) plants (55.22°), which suggests that these roots had the most yellowish colour. In contrast, the H2 plants had much lower hue values (38.39°–40.38°), reflecting a shift toward red-orange hues. The moderately stressed (50% PAW) H2 roots had the lowest hue value (38.39°), indicating a greater shift to the red tone. This shift in hue suggests that as the plants age, there might be a change in the types or concentrations of pigments present. The colour variations among *P. sidoides* roots in this study may serve as a direct indicator of quality and clinical effectiveness.

The presence of functional groups in *P. sidoides* roots were analyzed using Perkin Elmer Fourier-transform infrared spectroscopy (FTIR) spectrum spectrometer (Perkin Elmer, Midrand, South Africa). It is a quick, cost-effective technique that requires only a small sample to identify the chemical groups in herbal samples. In this study, the FTIR spectra for *P. sidoides* roots at H1 and H2 depict a variety of peaks suggesting the presence of hydroxyl (OH) groups stretching; N-H stretching vibrations (possibly indicating the presence of amines or amides); C-H bonds; C-H stretching; carbon triple bond (C≡C); C=C (carbonyl amide) stretching vibrations; CH2 bending vibrations (aromantic ring stretches); and C-O stretching vibrations in esters, phenols, or alcohols at different wavelengths. The functional groups such as hydroxyl, amides, alkynes, alkenes, and carbonyl groups that were found in the *P. sidoides* roots in this study correlate with the reported presence of phytochemicals such as oxygenated coumarins, phenolics, flavonoids and proanthocyanidins in *P. sidoides*.

In this study, PCA was used to qualitatively clarify the relationship between spectra obtained from *P. sidoides* root samples in response to different harvest age and irrigation levels. The PCA scores plot show variation at 95.6% for PC1 while PC2 has variation of 3.06%. The PCA scores plot show clear separation between the first harvest (at 6 months after treatment initiation) and second harvest (at 12 months after treatment initiation). It further showed variations between the different irrigation levels at H1 and H2. The use of chemometrics method (PCA) proved to be a valuable aspect in the investigation of the relationship between the different treatments. The PCA score observations correlate with the spectra.

The phytochemical analysis of *P. sidoides* extracts revealed the presence of saponins, flavonoids, terpenoids, phenols, tannins, and coumarins across all irrigation levels and harvest age. However, the steroids and alkaloids tests were negative, suggesting their absence or an indication that they are in levels below the detection limit for the assay adopted in this study.

The TLC results for all the treatments showed the presence of esculin band as compared to the esculin reference on track 6. When viewed under UV light, the bands suggest the different phytochemicals that are separated by the chromatography. The appearance of these bands suggests that the *P. sidoides* roots analyzed in this study contain different phytochemicals. Umckalin and scopoletin bands appeared near the end point. At all irrigation levels, tracks 1- 4 show luminescence at the start point and the end point, which suggests that there were still phytochemicals not separated on the samples. In the High-performance thin layer chromatography (HPTLC) analysis, *P. sidoides* roots separated into seven distinct bands when viewed under UV light at 366 nm with one of them being the marker compound umckalin. The TLC results showing presence of esculin aligns with preliminary phytochemical screening done on the samples, showing the coumarins.

The total phenolic content (TPC) and total flavonoid content (TFC) of *P. sidoides* across two harvests (H1 and H2) under three different irrigation levels (I1, I2 and I3) were investigated. Despite the differences in age at harvest and water stress, none of the variations in TPC are statistically different, indicating that phenolic contents remain relatively stable across the different irrigation levels. For TFC, although there were observable differences at H1 in response to the different irrigation levels, they were not statistically different. Likewise, the TFC for H2 followed the same trend as H1. Although there were observed increases for I1 (59.55 mg QE/g) and I2 (52.01 mg QE/g) when compared to TFC in H1, TFC in I3 tends to decrease (42.62 mg QE/g) compared to H1. This suggests a potential trend where flavonoid content increases over time under moderate irrigation levels (75% and 50% PAW). The phenomenon observed in this current study may be due to excessive water stress at 25% PAW.

The 2,2-Azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of *P. sidoides* across two harvests under different irrigation levels were also studied. For ABTS radical scavenging activity, the results are consistent across both harvests, which shows that the different irrigation levels did not have any significant difference on the ABTS radical scavenging activity of *P. sidoides* roots. The ABTS assay trend observed on harvest age aligns with the trend observed in TPC and TFC results. However, the lack of significant variation suggests that the ABTS radical scavenging activity by *P. sidoides* is unaffected by changes in irrigation levels and harvest age. Unlike ABTS, The DPPH radical scavenging activity shows more variation across treatments and harvests. In Harvest 1, there was no significant difference among the irrigation treatments. For the second harvest however, the DPPH activity was significantly higher in the unstressed plants (I1) than that of the water stressed plants (I3). DPPH radical scavenging activity was higher as the plants grew older.

Extent to which contract objectives have been met

All project objectives have been met. Comprehensive data were collected on two harvests (H1 and H2). Investigation on the morpho-physiological responses, biochemical processes and gene expression of *P. sidoides* growing under different plant available water (PAW) conditions was addressed (Aim 1). The shoot and root yields were determined at every harvest (Aim 2). The study also monitored the chemical and biological (enzyme activity) changes in the soil due to water stress (Aim 3). Determination of the chemical composition of the roots of *P. sidoides* was carried out. The phytochemical analysis, colour profiling, physicochemical properties using FTIR spectroscopy and chemometrics method (PCA), qualitative phytochemical analysis, radicals scavenging assays were all completed; together with the chemical profiling and the determination of the quality of secondary metabolites in the roots of *P. sidoides*, using advanced analytical methods [High Performance Liquid Chromatography (HPLC) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS)]. The study also determined the nutritional composition of the leaves and roots of the species under different irrigation levels and harvest age. The postgraduate students will complete their academic studies outside the contract period of this project.

Other outputs from the project include:

- One review article was published by the Journal of Medicinal Plants for Economic Development
- Two other papers are under preparation by a PhD and an MSc students
- Two oral presentations at local conferences

Pathway to future adoption of project findings by potential beneficiaries

This project aligns well with Goal 1, 2, 3 and 12 of the national Sustainable Development Goals (SDGs) which are:

- SDG 1: End poverty in all its forms everywhere
- SDG 2: End hunger, achieve food security and improved nutrition and promote sustainable agriculture
- SDG 3: Ensure healthy lives and promote well-being for all at all ages
- SDG 12: Ensure sustainable consumption and production patterns

This project aligns with SDG 1 and 2 since the cultivation of *P. sidoides* with limited amount of irrigation will provide an opportunity for sustainable production and increased income generation for the poor rural communities that are providing plant material for the informal as well as commercial markets. Likewise, the study promotes healthy living for everyone, especially among the rural communities who use *P. sidoides* as health supplement for boosting their immune systems (SDG 3). This research is also about sustainable use of water in agriculture, especially for the cultivation of *P. sidoides* (SDG 12). This fact aligns well with the objectives of the WRC in terms of sustainable use of water resources.

Moreover, for the successful cultivation of *Pelargonium sidoides* and the adoption of the irrigation and cultivation guidelines from this project by all stakeholders (established farmers, community members, upcoming and aspiring farmers, researchers and academics), the following should be considered:

- There must be clear understanding of where *Pelargonium sidoides* can potentially be grown, looking at the distribution map for *P. sidoides* in South Africa
- Trainings and workshops must be organised for community members and other stakeholders who are interested in the cultivation of *P. sidoides*
- Popular publication and pamphlets indicating the irrigation and cultivation guidelines for *P. sidoides* must be readily available for all stakeholders

CONCLUSIONS

P. sidoides adopted various strategies in response to water deficit stress, with different irrigation levels and harvest age having diverse impacts on the measured parameters. Overall, the well-watered plants (75% PAW) exhibited superior performance across morphological, physiological, and yield parameters of the plant. However, the fresh root yield of the moderately stressed (50% PAW) was better than that of the well-watered plants during the second harvest (H2). The dry matter yield at H2 also recorded the highest dry yield for plants subjected to 50% PAW. In general, as water deficit became more intense (25% PAW), most of the measured plant parameters experienced a decline. Most crops are typically water dependent. However, looking at the yield, this study demonstrates that *P. sidoides* can be successfully cultivated commercially without the need for complex irrigation programs. The plants are stress tolerant and the fresh and dry root yield of the most stressed plants competed well and, in some cases, even yielded better than the well-watered *P. sidoides* plants. Minimal water use can contribute to the production of more marketable root tubers, which is the most important part of the plant. Another observation from the study is that higher watering frequency increased fresh root mass but did not affect dry root mass. H2 had improved fresh and dry root yield compared to H1, especially for the moderately (50% PAW) and severely (25% PAW) stressed plants, with the well-watered plants (75% PAW) suffering a decline in yield at both harvests. Farmers can reduce production cost vis-à-vis irrigation cost by decreasing watering frequency, as frequent irrigation showed no significant impact on dry root yield. Too much soil moisture also resulted in root rot in some of the well-watered plants. Therefore, it is essential to establish proper irrigation guidelines for the cultivation of *P. sidoides.* The results also show that 75% water depletion (25% PAW) level increases lipid peroxidation in *P. sidoides*. Conversely, this severely stressed level lowers CO_2 assimilation and reduce leaf water potential. Furthermore, 75% water depletion level lowers chlorophyll content and cell growth of *P. sidoides*.

Phytochemical analysis of *P. sidoides* roots was able to show that irrigation and harvest age can affect the chemical composition of the plant. The colour profile analysis revealed that water stress can be a factor that can influence the colour of *P. sidoides* roots. Despite the complexity of FTIR data, the use of chemometrics method (PCA) proved to be a valuable aspect in the investigation of the relationship between the different treatments. Qualitative phytochemical methods were able to show the presence of different phytochemicals, thus, rendering the methods as good starting point for determining the phytochemicals present in the roots of *P. sidoides*. Radicals scavenging assays suggested that both irrigation and harvest age can influence antioxidant activity.

For root nutrient composition, there were no differences in the nutritional composition of *P. sidoides* despite being subjected to different levels of irrigation. Indicating that water stress did not change the nutrient contents of the species.

RECOMMENDATIONS

Based on the above findings, 50% water depletion level (50% PAW) is highly recommended for the cultivation of *P. sidoides* during the vegetative stage to enhance growth and its viability. Growing the species at 50% PAW favours tuber production since highest yield (fresh and dry) was obtained at this irrigation level. Root rot can also be prevented at 50% water depletion level. Age at harvest is also a crucial factor in the cultivation of *P. sidoides*. It is also recommended that harvesting be delayed until the plants are advanced in age before harvesting, since this study recorded highest fresh and dry root yield at the second harvest (H2).

There is a need for more research on the agronomy of *P. sidoides*. The lack of agronomic information may restrict the promotion of cultivation of *P. sidoides*, particularly within commercial farming systems. There are no cultivation and irrigation guidelines for *P. sidoides*. There are also conflicting reports about the harvesting age at which maximum medicinal benefits in the roots is attained. Cultivation might be limited in scope probably due to the long growth cycle, estimated to be 7-9 years before commercial maturity is reached. This Final report only covered two and half years of the research and two years of growth of *P. sidoides,* hence, further research is needed to provide quality findings and irrigation guideline for *P. sidoides* cultivation. Therefore, the extension of this study is recommended as it will address the knowledge gap.

ACKNOWLEDGEMENTS

The project team wishes to thank the following people for their contributions to the project. This project was funded and managed by the Water Research Commission (WRC) of South Africa, and the support is sincerely appreciated. We also thank the Agricultural Research Council for co-funding the research and for providing the facilities to conduct the research. The contribution of the project team members is acknowledged gratefully. Special thanks go to Mr Ulrich Feiter: CEO PARCEVAL (PTY) LTD, Wellington, South Africa for collaboration and support for this project and for donating some *Pelargonium sidoides* plants to the ARC research team for free. Lastly, we gratefully acknowledge the guidance and advice from the following people from the WRC and members of the project reference group:

Dr SN Hlophe-Ginindza	Water Research Commission (Chairperson)
Prof NS Mpandeli	Water Research Commission
Ms M Kapari	Water Research Commission
Prof FR Kutu	University of Mpumalanga
Prof SP Songca	University of KwaZulu-Natal
Prof OA Fawole	University of Johannesburg
Prof OTA Ashafa	University of the Free State
Dr S Nelana	Vaal University of Technology
Dr S Figlan	University of South Africa
Dr R Johnson	Stellenbosch University

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS & ABBREVIATIONS

ABTS	2,2-Azinobis-(3-ethylbenzthiazolin-6-sulfonic acid)
AI3	Alteration Index Three
ARC	Agricultural Research Council
CIE	Commission Internationale de l'Éclairage
CPUT	Cape Peninsula University of Technology
DPPH	2,2-diphenyl-1-picrylhydrazyl
FTIR	Fourier-transform infrared
HPLC	High-performance liquid chromatography
HPTLC	High-performance thin layer chromatography
LC-QTOF-MS	Liquid chromatography quadrupole time-of-flight mass spectrometry
MDA	Malondialdehyde
PAW	Plant Available Water
PCA	Principal component analysis
ROS	Reactive oxygen species
RWC	Relative water content
TEAC	Trolox equivalent antioxidant capacity
TLC	Thin layer chromatography
TFC	Total flavonoid content
TPC	Total phenolic content
UJ	University of Johannesburg
UWC	University of the Western Cape
WHO	World Health Organisation
WRC	Water Research Commission

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

Pelargonium sidoides DC. Is one of the species of the genus *Pelargonium* that is commonly used by several ethnic groups in South Africa. The species is endemic to South Africa and Lesotho. It is widely distributed in South Africa but very prominent in the Eastern Cape, the extreme eastern boundary of the Western Cape, parts of Free State, North West, Mpumalanga and Gauteng Provinces. It is a popular medicinal plant used in traditional medicine mainly for the treatment of respiratory tract infections, such as bronchitis and asthma. The medicinally important part of the species is the perennial tuberous root or rhizome. The tubers are mostly harvested from the wild, while commercial cultivation is rare. *P. sidoides* has been formulated into a highly successful, evidence-based phytopharmaceuticals Eps® 7630 “Umckaloabo®” in Germany and Linctagon® in South Africa. Like many other medicinal plants, *P. sidoides* has experienced extensive wild harvesting of the wild-growing species due to the increasing international demand. The increased rate of wild harvesting diminishes the natural population, thus, making the exploitation of *P. sidoides* unsustainable. In 2009, the medicinal plant was classified as ‘Declining’ on the Red Data list of South African plants (Raimondo et al., 2009), which was later re-classified to ‘Least Concern’ in 2011. To ensure sustainable harvest and prevent depletion of the wild population, cultivation of *P. sidoides* is seen as one of the ways out of these challenges. The World Health Organisation (WHO, 1999) recommended that medicinal plants be cultivated, wherever possible, as the source of supply for the market. At present, cultivation of *P. sidoides* in South Africa is not widespread and the agronomic requirements are unknown. Commercial cultivation of the species will not only ensure sustainability and conservation of the species but will also improve the livelihood of rural harvester communities. Also, it is believed that *P. sidoides* is not easy to cultivate because of the long growth cycle, coupled with the slow recovery rate after wild harvesting, which makes the medicinal plant not economically feasible for most growers. However, the high economic benefits from the ethanolic formulation of *P. sidoides* extract (Eps® 7630) which was about 80 million Euros per annum (Van Wyk, 2011) is an encouragement to promote the cultivation of this plant (Tanga et al., 2018; Yousefian et al., 2023).

However, water is needed for successful cultivation to take place. Information on the water use of *P. sidoides* in a water-challenged country like South Africa is still very scanty. Thus, understanding the water needs of crops like *P. sidoides* is an important factor in designing appropriate irrigation management that will ensure optimum productivity. This way, pressure on the wild populations can be reduced.

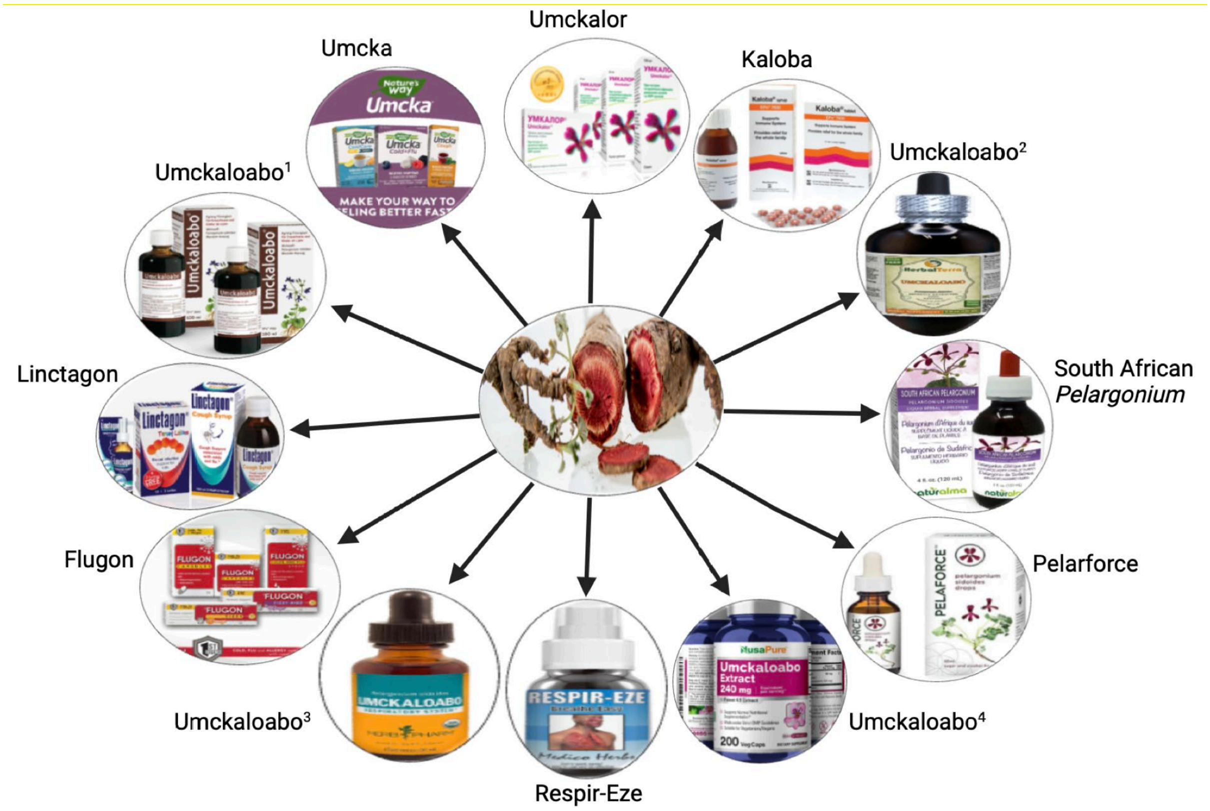


Figure 1.1 Summary of products in the market developed from *P. sidoides* root extract

1.2 PROJECT AIMS

The following were the aims of the project:

1. To assess the growth of *Pelargonium sidoides* in response to three different irrigation levels
2. To evaluate the effect of different levels of irrigation and harvesting age on the root yield of *P. sidoides*
3. To investigate the effect of water stress on soil microbial enzyme activity in relation to soil fertility/quality
4. To determine the effect of different levels of irrigation and harvesting age on the chemical composition of the roots of *P. sidoides*

1.3 APPROACH

This three-year project was executed by a multi-disciplinary team of researchers with expertise in soil and water science, agronomy, biotechnology, chemistry and food technology. The research team which cut across different disciplines were based in three South African universities and Science council, together with two (2) PhD and one (1) MSc students [Ms Yandiswa Mtimkulu (PhD), Ms Kundani Khameli (PhD) and Mr Phila-Sande Ntoyi (MSc)]. The project started with a detailed knowledge review (inception report) which was conducted and submitted as Deliverable 1 to WRC in May 2022. The review captured all aspects of the agronomy and water requirements of *P. sidoides*. The production, wild harvesting, distribution and nutritional requirements, as well as breeding / screening of *P. sidoides* for water use were covered. It further revealed that there are no cultivation and irrigation guidelines for *P. sidoides*. Likewise, there is no information in public domain on the breeding of *P. sidoides* for water use efficiency. Optimum harvesting age for the species is also controversial.

According to the project terms of reference, Deliverable 2 was submitted to WRC in February 2023. The amended version was submitted in June 2023. It covered capacity building and project implementation from the beginning of the project, including the procurement done, installation of tensiometers and irrigation system, pre-planting soil analysis, planting and crop maintenance. Later, tensiometers were replaced with soil moisture probes, for better accuracy. Deliverable 3, which was submitted to WRC in October 2023 reported on the growth performance (morphophysiological parameters) of *P. sidoides* in response to the different irrigation regimes, results of the pre-planting soil physicochemical analysis and enzyme activity (Ms Yandiswa Mtimkulu and Mr Phila-Sande's research work). The 4th Deliverable reported on the effects of the different irrigation levels [75% PAW (I1), 50% PAW (I2) and 25% PAW (I3)] and harvesting age on the yield (shoot and root) of *P. sidoides* after the first harvest (H1) in December 2023. The report also gave insight into the effects of these treatments on soil chemistry as well as the soil enzyme activity after H1. Deliverable 5 described the effect of three different levels of irrigation and harvest age on the chemical composition of the roots of cultivated *P. sidoides*. The report covered Specific Aim 4, which was mainly Ms Kundani Khameli's PhD research work. Ms Mtimkulu also carried out some experiments on the chemical composition of the roots and leaves while Mr Ntoyi conducted some assays on the biochemical traits of the leaves of *P. sidoides*.

P. sidoides is a slow growing plant. After planting, the plants were allowed to establish for about seven months before different levels of water stress levels were introduced as treatments in July 2023. Data were collected on monthly basis on growth, physiological and biochemical parameters in-situ as well as further analysis that were carried out in the laboratory. Due to time constraints, only two harvests were carried out at six months intervals (December 2023 and June 2024). The current Final report is reporting on these two harvests (H1 and H2). The findings of this research highlight the impact of water deficit stress and the age at harvest on the growth, yield and the chemical composition of *P. sidoides*.

1.4 SCOPE OF THE PROJECT

This three-year project (C2021/2022-00829), funded by the Water Research Commission (WRC) of South Africa from 01 April 2022 – 31 March 2025 was awarded to Dr MN Lewu of the Agricultural Research Council (ARC) Infruitec Nietvoorbij, Stellenbosch. The project is titled "Effect of irrigation and harvesting age on the growth, yield and chemical composition of *Pelargonium sidoides* DC", with funding that totalled R1 050 000.00 (VAT inclusive). As noted in the original project proposal as well as the contract, this project was conducted in specific phases and each phase was linked to a specific aim, with the progress reports submitted as Deliverables.

The study aimed to investigate the water use, growth, yield, chemical composition and the optimum harvesting age of *P. sidoides* cultivated inside a tunnel under deficit irrigation. The study provided necessary information on the morpho-physiological responses, biochemical processes, gene expression of *P. sidoides* growing under different plant available water (PAW) conditions. The study also monitored the chemical and biological (enzyme activity) changes in the soil due to water stress. This research further provided information on the chemical profile and the secondary metabolites in the roots of *P. sidoides* subjected to different levels of water deficit stress. Information on the effects of harvesting age on these metabolites were also be provided. The projects submitted a total of 6 Deliverables, including this Final report, which is the 6th Deliverable.

CHAPTER 2: KNOWLEDGE REVIEW

2.1 GENERAL KNOWLEDGE ABOUT *PELARGONIUM SIDOIDES*

Pelargonium sidoides DC. Commonly called geranium, Umckaloabo, Uvendle (isiXhosa), Kalwerbossie (Afrikaans), i-Yeza lezikali (isiXhosa) Icwayiba (isiXhosa), ikhubalo (isiXhosa), Rabassam or Rabas (Dutch/Afrikaans) and Khoaara e nyenyane (Sesotho) is a plant native to South Africa and Lesotho (Brendler and Van Wyk, 2008; Van Niekerk, 2009; Motjotji, 2011). It belongs to the family Geraniaceae, and it is one of the species of the genus *Pelargonium*. *Pelargonium* species are commonly used by several ethnic groups in South Africa because of their medicinal properties. They are evergreen perennials indigenous to temperate and tropical regions of the world, with many species (about 80%) confined to southern Africa, while others occur in Australia, New Zealand and the Far East (Lalli et al., 2006; Mativandlela et al., 2006; Röschenbleck et al., 2014). The genus includes about 280 species of perennials, succulents and shrubs. They usually grow in short grasslands and sometimes with shrubs and trees on stony soil varying from sand to clay-loam, shale or basalt. Though evergreen, they die back in nature during droughts and winter. It survives harsh environmental conditions due to its well-developed tubers or rhizomes (Van der Walt et al., 1988; Mativandlela et al., 2006). The genus is a fundamental part of the Cape flora, and the centre of diversity is in the south-western part of South Africa (Lalli et al., 2006; Röschenbleck et al., 2014). Most of the *Pelargonium* plants cultivated in Europe and North America originated from South Africa (Sayre, 2003). The importance of plants in the genus *Pelargonium* in the traditional herbal medicine in southern Africa is well known and documented. Numerous studies have been carried out on the antibacterial and antifungal properties of the species (Mativandlela et al., 2006; Adewusi and Afolayan, 2009; Saraswathi et al., 2011; Carmen and Hancu, 2014; Džamić et al., 2014; Hamidpour et al., 2017; Gucwa et al., 2018). The medicinally active ingredients are found in the bitter-tasting roots of the plants (Mativandlela et al., 2006).

P. sidoides is indigenous to South Africa and Lesotho (Lewu et al., 2007a; Brendler and Van Wyk, 2008; Van Niekerk, 2009). It is commonly used by several ethnic groups (Khoi/San, Xhosa and Zulu) of the country. The species is highly valued and well known to generations of traditional healers of these regions for its curative properties (Kolodziej et al., 2003; Mativandlela et al., 2006) and thus, used to treat both human and livestock ailments (Van Wyk, 2008). *P. sidoides* is predominantly found in the Eastern Cape Province of South Africa. It is renowned for its pharmacological properties in treating tuberculosis, bronchitis, cough, fever and other respiratory-related ailments (Lewu et al., 2007a; Brendler and Van Wyk, 2008; White et al., 2008; Moyo et al., 2013). The species also has a long tradition of use in the treatment of diarrhoea and other gastrointestinal disorders (Kolodziej et al., 2003; Mativandlela et al., 2006). The health benefits of this medicinal plant continue to stimulate more interest and demand, especially in developed countries. For example, *P. sidoides* has been formulated into phytopharmaceuticals, namely Eps® 7630 (Umckaloabo®, Dr. Willmar Schwabe GmbH & Co. KG Pharmaceuticals, Germany) and Linctagon® (Nativa, South Africa), which originate from the roots of the species (Moyo and Van Staden, 2014). This herbal medicine is extensively used in modern phytotherapy in Europe to cure infectious diseases of the respiratory tract (Mativandlela et al., 2006).

Nowadays, *P. sidoides* is available as processed materials in modern packaging and in various dosage forms such as teas, tinctures, tablets, capsules or ointments. It also forms part of the multi-million-rand informal markets of medicinal plants (Cunningham, 1988; Mander, 1998).

2.1.1 Wild harvesting of *P. sidoides*

Harvesting of *P. sidoides* from the wild is the main source of raw materials, hence, making the wild stock susceptible to over-exploitation (White et al., 2008). Over the years, the species has experienced extensive wild harvesting by plant gatherers for personal use and income generation, which has resulted in localized population declines. Most of the gatherers are from rural communities, with no other source of income and thus, rely heavily on natural resources for sustenance (Mofokeng et al., 2020). The medicinal plant was classified as 'Declining' on the Red Data list of South African plants (Raimondo et al., 2009), although re-classified to 'Least Concern' in 2011 (Moyo and Van Staden, 2014). It is fast becoming endangered due to the unsustainable harvesting of its tubers from the wild populations to produce phytopharmaceuticals for the local and expanding international export markets (Lewu et al., 2006a, 2007b; Colling et al., 2010; Motjotji, 2011; Moyo et al., 2013). This is further exacerbated by the harvesting methods currently used which involve the entire removal of whole plant. To circumvent this challenge and ensure sustainable harvest of *P. sidoides*, collection and bioprospecting permits were introduced to minimize illegal harvest. However, it appears that harvesters are not adhering to the harvest methods provided for gatherers and legislation seems to be lacking in regulating the use of the species. Apart from this, other challenges facing *P. sidoides* population in the wild include habitat transformation and degradation due to urbanisation and agriculture. These factors are now leading to loss of populations at a much faster rate than harvesting (De Castro et al., 2010). Cultivation of *P. sidoides* now seems to be the way forward to achieve sustainable use of the medicinal plant. It is believed that large scale cultivation will ensure constant supply of the species to the pharmaceutical industry as well as for local consumption. Thus, cultivation will not only ensure conservation and sustainable use of the species, but it will also improve the livelihood of the rural harvester communities (Mofokeng et al., 2020). Several large-scale commercial cultivations used to exist in different parts of South Africa which are now negligible due to a range of factors.

2.1.2 Description and Distribution

P. sidoides has wider geographical distribution than its close taxonomic ally *P. reniforme*, which is an indication of its high tolerance of a broad range of environmental conditions. *P. sidoides* can be found growing in short grasslands and on a variety of soils (Van Niekerk, 2009). Although, it may seem difficult to distinguish between the two species, they can only be distinguished by the shape of the leaves and the colour of flowers. *P. reniforme* has grey-green velvety kidney-shaped leaves with pink to magenta flowers, while *P. sidoides* has crowded velvety, cordate, long-stalked leaves with short glandular hairs and dark reddish-purple to almost black flowers. It is a perennial, slightly aromatic plant. Flowers of *P. sidoides* plant are mostly present from

October to January (Motjotji, 2011). Mature tubers have deep red colouration after years of growth. In cross section, young tubers are white, followed by the pink stage and gradually change to the deep red colour at maturity (Van Niekerk, 2009). *P. sidoides* is endemic to South Africa and Lesotho. It is widely distributed in South Africa but very prominent in the Eastern Cape Province. It is also found in the extreme eastern boundary of the Western Cape, parts of Free State, North West, Mpumalanga and Gauteng Provinces (Lewu et al., 2007b; Brendler and Van Wyk, 2008). The plant grows well on average soil that is well drained (for example, stony soil like sand to clay-loam, shale or basalt). The species like alkaline soil, with a pH of 7.6-7.8. Species identification may become problematic in South Africa due to the morphological similarities between *P. reniforme* and *P. sidoides* since both species grow side by side in their wild habitat, especially when harvesting is done in the non-flowering season. However, in Lesotho, there is no species confusion because *P. sidoides* only occurs on its own (White, 2006; Newton, 2009; Motjotji, 2011). Since it is extremely difficult to morphologically detect adulteration from *P. reniforme*, the most reliable method is through chemical analysis.

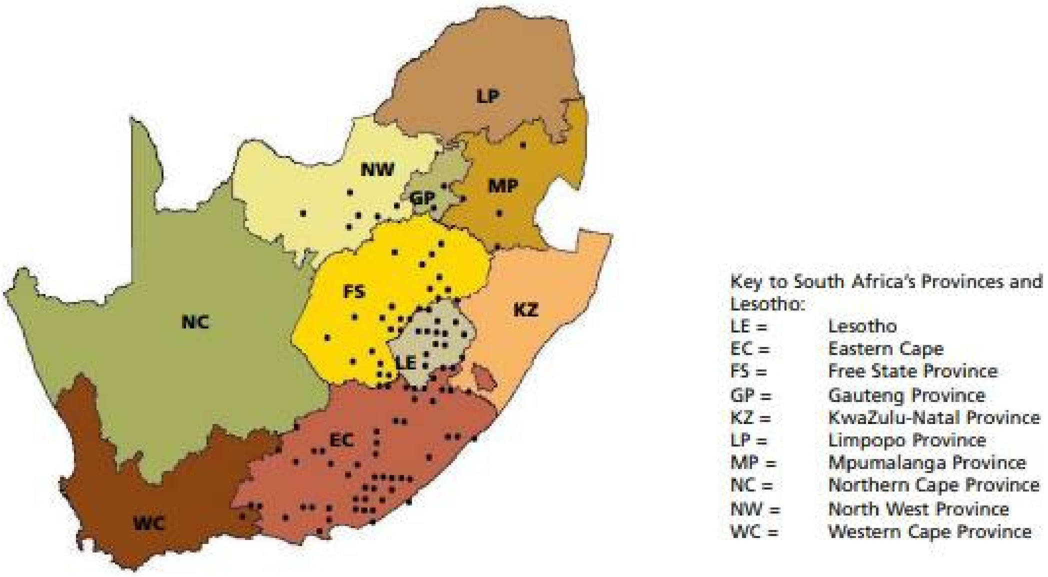


Figure 2.1 Distribution map for *P. sidoides* in South Africa and Lesotho (https://cites.org/sites/default/files/ndf_material/WG2-CS2.pdf)

Phytochemical investigations revealed that the main constituents of *P. sidoides* are phenolic acids (e.g. gallic acid and its methyl ester), proanthocyanidins and several coumarins. Among these coumarins, Umckalin (7-hydroxy-5,6-dimethoxycoumarin) is believed to be the active compound that is responsible for efficacy in *P. sidoides* which is either low or absent in *P. reniforme*. Therefore, Umckalin is a useful marker compounds for *P. sidoides*, since they appear to be absent in *P. reniforme* (Brendler and Van Wyk, 2008; Franco and de Oliveira, 2010). However, while *P. reniforme* has a diterpene, called reniformin in its roots, this compound is absent in *P. sidoides*.

2.1.3 Production of *P. sidoides*

2.1.3.1 Growth of P. sidoides

P. sidoides can undergo both sexual (by seed) and asexual (using tuber or shoot cuttings) reproduction. Although, seed propagation of the species may be limited by their inherent low seed viability and germination potential (Lewu et al., 2006b; Colling et al., 2010), the plant has high resprouting capacity when cuttings are used, thus, making it easy to propagate and produces leafy regrowth within weeks or months. In the wild, the species flourishes well in partially disturbed sites, but seemingly absent from transformed sites such as cultivated fields. *P. sidoides* appears to prefer periodic fire or grazing which remove competition from neighbouring plants. However, the medicinal plant tends to suffer displacement by *Acacia karoo* and die back when the trees become large (Vlok, 2003; Motjotji, 2011). Perennial plants like *P. sidoides* are able to resprout after a disturbance or winter because of the build-up of carbohydrate reserves in the underground tubers or rhizomes, thereby allowing them to persist in many habitats. It is estimated that it takes a period of about seven years for *P. sidoides* tubers to recover to a commercially valuable size and dark-red tuber phase after wild harvesting (Motjotji, 2011).

2.1.3.2 Cultivation of P. sidoides

The World Health Organisation (WHO, 1999) recommended that medicinal plants be cultivated, wherever possible, as the source of supply for the market. Commercial *Pelargonium* cultivation has several distinct advantages, most especially, ensuring supply of roots of the correct species that have documented medicinal efficacy (White et al., 2008). Cultivation of *P. sidoides* as a conservation strategy can increase yield without affecting its bioactivity, while providing nourishment for the rural communities (Mofokeng et al., 2020). Commercial growing would ensure a reliable supply of the resource to buyers (Schippmann et al., 2002). Consumers would be assured that the material was obtained in a legitimate manner and thus, provide an opportunity for sustainable production and increased income generation for the rural poor communities providing plant material for the informal as well as commercial markets (Mofokeng et al., 2020). At present, cultivation of Pelargonium in southern Africa is not widespread (Van Niekerk and Wynberg, 2012).

Cultivation of *P. sidoides* involves planting in established agricultural lands either from seeds or cuttings. Lawrence (2001) indicated that autumn is the most preferable season to propagate *P. sidoides*. These plants receive intensive care until they are harvested. Some of the advantages of cultivation is that it helps to ensure that roots of the correct species are supplied, irrigation can be applied to increase biomass yield and also helps to decrease chemical variability of plants (Mofokeng, 2015). Field cultivation of the species is negligible; therefore, the tubers are mostly wild harvested. As a result of this, there is no recent information on the commercial cultivation of the plant in South Africa (Colling et al., 2010; Mofokeng, 2015). Scientific knowledge on the cultivation of the plant is also lacking, except for a few research trials. Cultivation has been limited in scope probably due to the long growth cycle of the tubers which is estimated to be 8-9 years before commercial maturity is reached. There are also concerns about the potency of active compounds in cultivated plants (Motjotji, 2011; Wynberg et al., 2015).

Studies on the cultivation trials further established that it takes several years for *P. sidoides* tubers to reach commercially desirable maturity (tubers with deep red internal flesh colour). Further research also shows that there are reports of less active substance (Umckalin) being present in the cultivated plants when compared to the ones harvested from the wild (White, 2006; Motjotji, 2011). White (2006) reported that wild-harvested *P. sidoides* tubers had 10 times higher concentrations of Umckalin compared to the propagated plants growing in the same area. Another drawback is that the propagated plants have lighted tuber coloration than the wild-harvested *P. sidoides*. However, the light tuber colouration and low Umckalin levels may be due to the short duration of the study. Longer-term studies may give different results. Schwabe-owned South African company, Parceval (based in Wellington, Western Cape), helps to export the roots of *P. sidoides* from South Africa to Schwabe Pharmaceuticals based in Germany. Schwabe, through Parceval financed a *Pelargonium* cultivation project in the Free State and Eastern Cape Provinces of South Africa as well as in Lesotho. Cultivation of *P. sidoides* in these two countries is currently not widespread. According to Van Niekerk and Wynberg (2012), a 45 ha field on a Parceval-owned farm was abandoned in 2009 due to unsuitable growing conditions. The reasons for non-extensive cultivation of this species are because of a variety of reasons which includes economies of scale and the slow regeneration rate of the plant. Schwabe also established a large-scale cultivation of *P. sidoides* in Kenya and Mexico (ACB, 2008) in order to ensure regular supply of the tubers as permitting arrangements in southern Africa were becoming stricter. This act of cultivating and trading the resource outside the area where it naturally occurs raises important questions about whether the genetic material was exported in compliance with relevant regulations in South Africa and Lesotho (Van Niekerk and Wynberg, 2012). While cultivating *P. sidoides* is being promoted in order to save the wild population, there are concerns of marginalization of small producers, or even excluded entirely if it requires significant capital, land and infrastructures (Wynberg and van Niekerk, 2014).

The tissue culture technique is another way of boosting the production of this species. Tissue culture plantlets of medicinal *Pelargonium* would establish tubers within 6 months and could offer a continuous year-round production in a shorter possible time with mass production in automated systems (Makunga, 2015; Ingarfield, 2018). Moreover, no record has been found on successful cultivation of *P. sidoides* through hydroponics technique. However, growing *P. sidoides* under shade net diminished the root umckalin concentration of the species. Indicating that shade nets were not beneficial to the accumulation of umckalin in the roots of *P. sidoides* (Ntshabele et al., 2013).

In all, White (2006) concluded that greenhouse cultivated plants showed approximately six times greater growth rates, thus, supporting the cultivation of roots to supply future market demand.

2.1.4 Nutritional requirements of *P. sidoides*

Since there was no information on the nutritional requirement or fertilizer guidelines for *P. sidoides*, a field trial was conducted by Mofokeng (2015) to determine the performance of *P. sidoides* in response to nitrogen (N) and soil water management. Application of 100 kg N ha-1 resulted in a significantly higher number of leaves when compared with the 0, 50 and 150 kg N ha-1. N levels of 50 and 100 kg N ha-1 gave a significantly higher biomass compared to the well-watered treatment. In addition, N had a small effect on the tubers irrespective of the N levels applied.

In a study carried out on cultivated *P. sidoides* in Kenya where roots of the medicinal plant which are grown solely for export to Germany; the study determined the correlation of leaf glucose, chlorophyll contents with mineral nutrient uptake of South African geranium (*P. sidoides*). Inorganic and organic fertilizers (worm leachate) of known nutrient levels were applied to the soil. Though the effects of soil nutrients applications on the active ingredients of *P. sidoides* was not carried out, the results showed that uptake of calcium fertilizer resulted in highest increase of glucose while that of worm leachate had the least effect. Also, nutrients uptake increased chlorophyll content. Mineral nutrients have a strong effect on photosynthates and their utilization in the roots which act as the sink organs or storage sites (Kinyua, 2014). The results further showed a correlation between soil mineral nutrients uptakes and leaf glucose level in the *P. sidoides*. Correlations were also observed between leaf chlorophyll and nitrogen fertilizers application. Chlorophyll increased significantly three months after application of CAN and KNO3 fertilizers. Another study reported that nutrient uptake increased in *P. sidoides* in all the different watering levels tested with the exception of Mg (Ingarfield, 2018).

2.1.5 Water requirement of *P. sidoides*

Cultivation of *P. sidoides* requires water. Water is one of the important factors that affect growth and yield, amongst other plant parameters (Mofokeng et al., 2015). However, water shortage is a serious concern for a water-scarce country like South Africa. Pelargonium plants are drought tolerant and do not need to be watered too frequently, with soil being sufficiently dry before watering again, but it is important that roots do not dry out completely (Hall, 2021). However, there are no cultivation guidelines *for P. sidoides*. Hence, the water requirement of P. sidoides is not known. Sustainable use of water has become a priority in agriculture and thus, innovative irrigation management practices are critical. Therefore, due to the aforementioned gaps in research on the cultivation of *P. sidoides*, the current study has become a necessity.

In the absence of relevant information on the agronomic practices required for the cultivation of the species, Mofokeng (2015) investigated a field trial on soil water management of *P. sidoides*. The findings of the study showed that the well-watered plants had significant growth, physiological and root yield advantage over the water stressed plants. The study recommended that *P. sidoides* should be grown under well-watered conditions for a period of about 1-2 years to improve growth and tuber yield. After this stage, water deficit stress may be introduced, which is expected to increase the concentration of important secondary metabolites.

Another study by Mofokeng et al. (2017) showed that *P. sidoides* plants can employ several survival strategies when exposed to water stress. During a prolonged exposure to water stress, the plants reduced plant growth and stomatal conductance and were therefore not affected by terminal water stress. Higher watering frequency only promoted fresh root mass and not dry root mass. This is an important finding since the harvested roots are always supplied in a dry state. Thus, *P. sidoides* farmers can save on irrigation costs by reducing watering frequency, as there was no significant difference in dry root yield.

On the other hand, earlier study found that geographical origin and genetics of plants rather than environmental variation may have an influenced in umckalin production (White, 2006). Research on the effect of water stress reported that water deficit did not result in a significant increase in umckalin concentration in *P. sidoides* cultivated under greenhouse condition. Rather, the control plants (well-watered) yielded umckalin concentrations similar to that of wild harvested plants from high rainfall area, with an advantage of higher growth rates (White, 2006; Brendler and Van Wyk, 2008; White et al., 2008). The regrowth of replanted shoots from which a standard proportion of the root was harvested showed that water availability affected shoot survival but not root regrowth rate. However, the regrowth rates were found to be low, questioning the viability of wild harvest (White, 2006). However, Ingarfield (2018) reported that a certain level of water deprivation (plants watered every 24 days) gave highest values of polyphenols. However, medium to low watering resulted in higher leaf content in *P. sidoides*.

2.1.6 Breeding / screening of *P. sidoides* for water use efficiency

To the best of our knowledge, there is no information in public domain on the breeding of *P. sidoides* for water use efficiency.

2.2 CONCLUSIONS

In this chapter, we provide a review of *Pelargonium sidoides*, although, information was very scanty on the nutritional and water requirements of the species. In addition, no studies have so far investigated the breeding of *P. sidoides* for water use efficiency. Likewise, the age at which *P. sidoides* reaches maturity is also controversial. This study therefore seeks to close the information gap and provide insights on the water use and maturity age of *P. sidoides*, given the limited water resources in South Africa.

CHAPTER 3: EXPERIMENTAL SET UP

3.1 INTRODUCTION

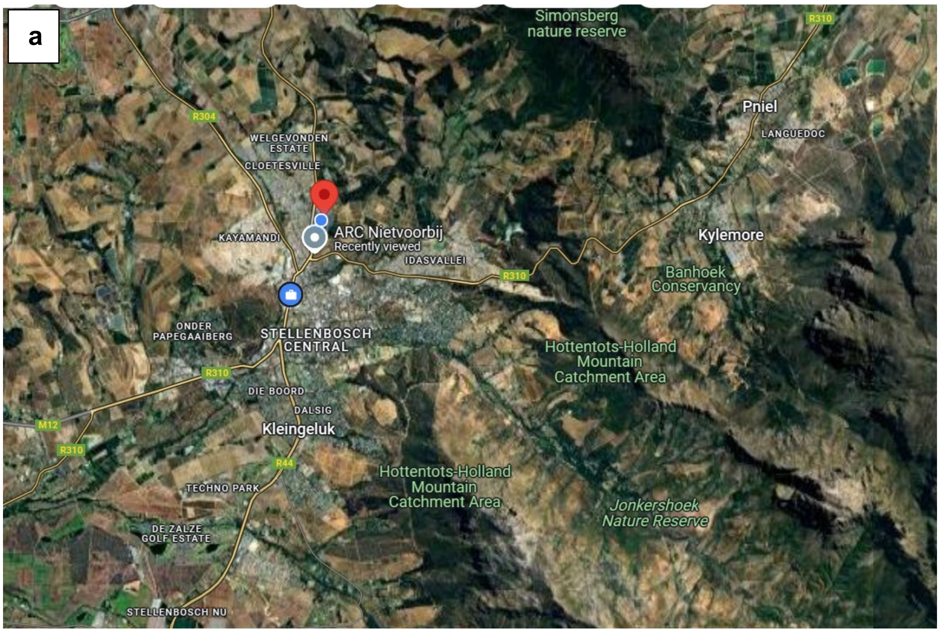
P. sidoides has wider geographical distribution than its close taxonomic ally *P. reniforme*, which is an indication of its high tolerance of a broad range of environmental conditions. *P. sidoides* can be found growing in short grasslands and on different kinds of soil (Van Niekerk, 2009). It is widely distributed in South Africa but very prominent in the Eastern Cape Province. It is also found in the extreme eastern boundary of the Western Cape, parts of Free State, North West, Mpumalanga and Gauteng Provinces (Lewu et al., 2007b; Brendler and Van Wyk, 2008). The plant grows well on average soil that is well drained (for example, stony soil like sand to clay-loam, shale or basalt). The species like alkaline soil, with a pH of 7.6-7.8. Although, seed propagation of the species may be limited by their inherent low seed viability and germination potential (Lewu et al., 2006b; Colling et al., 2010), the plant has high resprouting capacity when cuttings are used, thus, making it easy to propagate and produces leafy regrowth within weeks or months. In the wild, the species flourishes well in partially disturbed sites, but seemingly absent from transformed sites such as cultivated fields. Perennial plants like *P. sidoides* are able to resprout after a disturbance or winter because of the build-up of carbohydrate reserves in the underground tubers or rhizomes, thereby allowing them to persist in many habitats.

The World Health Organisation (WHO, 1999) recommended that medicinal plants be cultivated, wherever possible, as the source of supply for the market. Field cultivation of the species is negligible; therefore, the tubers are mostly wild harvested. As a result of this, there is no recent information on the commercial cultivation of the plant in South Africa (Colling et al., 2010; Mofokeng, 2015). Scientific knowledge on the cultivation of the plant is also lacking, except for a few research trials.

Cultivation of *P. sidoides* requires water. Water is one of the important factors that affect growth and yield, amongst other plant parameters (Mofokeng et al., 2015). However, water shortage is a serious concern for a water-scarce country like South Africa. Pelargonium plants are drought tolerant and do not need to be watered too frequently, with soil being sufficiently dry before watering again, but it is important that roots do not dry out completely (Hall, 2021). However, there are no cultivation guidelines for *P. sidoides*. Hence, the water requirement of *P. sidoides* is not known. Sustainable use of water has become a priority in agriculture and thus, innovative irrigation management practices are critical. Therefore, due to the aforementioned gaps in research on the cultivation of *P. sidoides*, the current study has become a necessity.

3.2 TRIAL SITE

The research was a pot study conducted inside a tunnel at the Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Stellenbosch, South Africa (Latitude 33°54’52.38”S Longitude 18°51’40.27”E). The experiment was laid out in a randomized block design with four replications that are drip irrigated. Treatment consisted of three (3) irrigation levels (75% PAW, 50% PAW and 25% PAW) and three harvest times (four, eight and 12 months after initiation of water deficit stress). However, for this Final report, two harvests (H1 and H2) were focussed on. Plants were maintained for about seven (7) months to ensure good establishment of the *P. Sidoides* plants, since they grow slowly. The plants were given booster NPK fertilizer at planting to get the plants established. Thereafter, insect pests were controlled using Makhro Cyper® (active ingredient: cypermethrin, 200 g L-1) whenever there were pest problems. Initiation of treatments started in the first week of July 2023 while data collection commenced immediately. Data were collected monthly. For weed control, weeds were manually hand-pulled after irrigation, when the soil was wet.



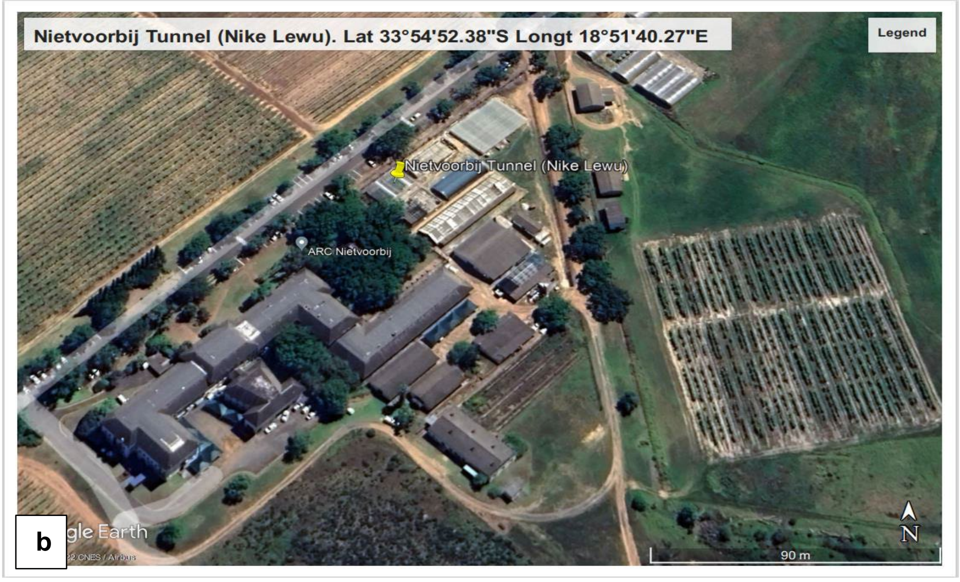


Figure 3.1a) Map indicating the trial location at ARC Nietvoorbij, b) Location of the tunnel used for the trial at ARC Nietvoorbij, Stellenbosch (Source: Google Earth)

Figure 3.2 Trial layout in the tunnel

Soil water content was monitored with the aid of DFM soil moisture probes, linked to a DFM software. Representative pots were installed with one probe per pot. The software was monitored daily from a computer to know which treatment was due for irrigation, in line with the pre-set levels of soil water depletion, which are 75% PAW, 50% PAW and 25% PAW.

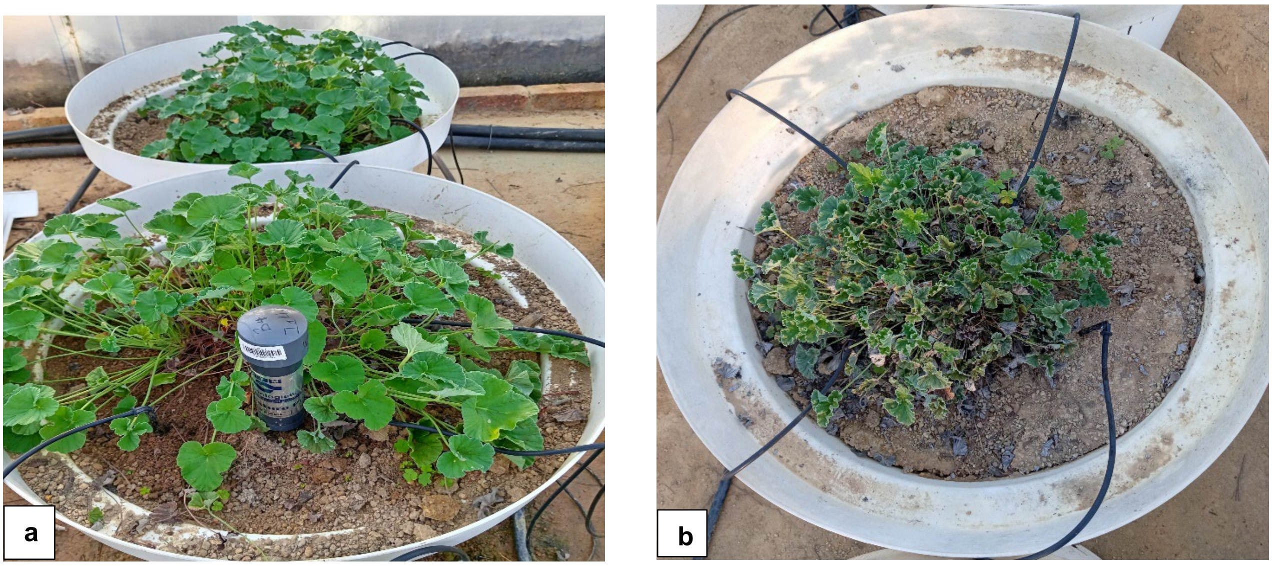
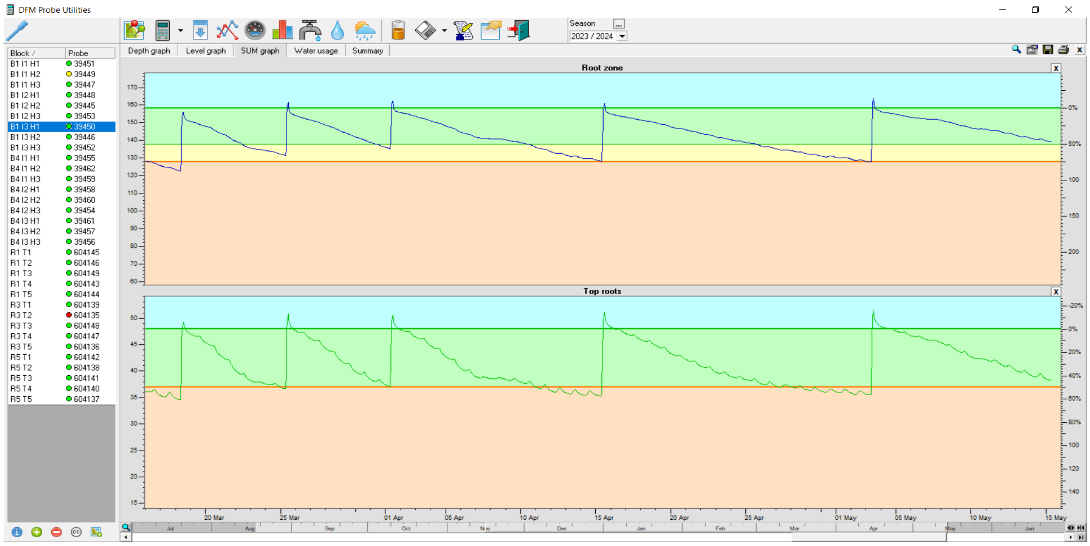


Figure 3.3 a) DFM soil moisture probe installed in a well-watered (75% PAW) *Pelargonium sidoides* pot, b) severely stressed (25% PAW) *P. sidoides* pot



Block	Probe
B1 I1 H1	● 39451
B1 I1 H2	● 39449
B1 I1 H3	● 39447
B1 I2 H1	● 39448
B1 I2 H2	● 39445
B1 I2 H3	● 39453
B1 I3 H1	● 39450
B1 I3 H2	● 39446
B1 I3 H3	● 39452
B4 I1 H1	● 39455
B4 I1 H2	● 39462
B4 I1 H3	● 39459
B4 I2 H1	● 39458
B4 I2 H2	● 39460
B4 I2 H3	● 39454
B4 I3 H1	● 39461
B4 I3 H2	● 39457
B4 I3 H3	● 39456
R1 T1	● 604145
R1 T2	● 604146
R1 T3	● 604149
R1 T4	● 604143
R1 T5	● 604144
R3 T1	● 604139
R3 T2	● 604135
R3 T3	● 604148
R3 T4	● 604147
R3 T5	● 604136
R5 T1	● 604142
R5 T2	● 604138
R5 T3	● 604141
R5 T4	● 604140
R5 T5	● 604137

Season: 2023 / 2024

Figure 3.4 Graph showing irrigation frequency of one of the probes at 75% soil water depletion (25% PAW) level

3.3 CONCLUSIONS

The Final report focussed on two harvests (H1 and H2). *P. Sidoides* is a slow growing crop, therefore, to ensure good establishment of the plants, they were grown for about seven (7) months before the initiation of treatments, which started in the first week of July 2023. Data collection commenced immediately on a monthly basis. Soil water content was monitored with the aid of DFM soil moisture probes, linked to a DFM software. Representative pots were installed with one probe per pot.

CHAPTER 4: GROWTH OF *PELARGONIUM SIDOIDES* IN RESPONSE TO THREE DIFFERENT IRRIGATION LEVELS

4.1 INTRODUCTION

Pelargonium sidoides DC. is one of the estimated 3000 medicinal plant species that have been found to be used in traditional medicine across South Africa (Van Wyk et al., 1997). About 350 species of medicinal plants are the most used and traded (South African National Biodiversity Institute, 2006). However, Van Wyk (2008) claims that about 38 indigenous species have been commercialized to some extent. *P. sidoides* has popular usage for the treatment of respiratory related ailments, such as bronchitis and asthma (Lewu et al., 2007a; Brendler and Van Wyk, 2008; Van Niekerk, 2009). Hence, the traditional knowledge associated with this plant and its uses is widely spread across cultural groups and geographical areas (Watt and Breyer-Brandwijk, 1962; Hutchings et al, 1996; Brendler and Van Wyk, 2008). *P. sidoides* is available as processed materials in modern packaging and in various dosage forms such as teas, tinctures, tablets, capsules or ointments. Several others are also produced for multi-million rand informal markets (Cunningham, 1988; Mander, 1998; Williams et al., 2000).

Like many other medicinal plants, *P. sidoides* has experienced extensive wild harvesting due to the increasing international demand (Lewu et al., 2006b). Thus, leading to localised overexploitation of its wild populations in Southern Africa. *Pelargonium sidoides,* listed as 'declining' in 2009 (Raimondo et al., 2009), faces endangerment due to overharvesting for phytopharmaceuticals, driven by local and international market growth (Colling et al., 2010). According to the World Health Organization (WHO, 1999), cultivation is the preferred method of sourcing medicinal plants for commercial purposes. Additionally, cultivating *P. sidoides* is considered a key solution to addressing the sustainability challenges facing its wild harvest, thus, mitigating the risk of population depletion and promoting long-term availability.

However, water availability is a critical factor in successful *P. sidoides* cultivation. South Africa's water-scarce context underscores the need for better understanding of *P. sidoides* water usage, and yet relevant information remains scarce. Therefore, determining the water requirements of crops such as *P. sidoides* is crucial for developing effective irrigation strategies that maximize productivity. As a result, the strain on wild populations can be alleviated. According to Ayana (2011), the integration of deficit irrigation into water management practices is essential for mitigating water scarcity in arid and semi-arid environments. Research shows that deficit irrigation, when applied judiciously during certain developmental phases, can potentially boost crop yields (Bilibio et al., 2011).

Currently, there are no guidelines on the water requirements of young *P. sidoides*. However, it is thought that substantial water wastage may occur during cultivation if there is no clear understanding of the water requirement of this species, due to a lack of information to guide irrigation scheduling. Therefore, the aim of this Chapter was to determine the morphophysiological responses of *P. sidoides* subjected to different levels of irrigation over time. These data are useful in developing irrigation guidelines for *P. sidoides*.

4.2 MATERIALS AND METHODS

This experiment was set up in a tunnel at the ARC Nietvoorbij campus, Stellenbosch, South Africa (Latitude 33°54'52.38"S Longitude 18°51'40.27"E). *Pelargonium sidoides* plant materials (sprouted root tubers) were acquired from the ARC-Vegetable, Industrial, and Medicinal Plants (ARC-VIMP) gene bank, with accession number M2009/38. Planting of sprouted root tubers was done in November 2022, utilizing, accession number M2009/38. The experiment was laid out in a randomized block design with four replications that were drip irrigated (Figure 4.1). There were 3 irrigation levels (I1 = 75% PAW, I2 = 50% PAW & I3 = 25% PAW) and three harvest time (6, 12 and 18 months after water stress initiation). The maximum soil water content was at 100% pot capacity, from this, a predetermined depletion level of 25%, 50% and 75% of moisture was permitted for each level of irrigation before replenishing back to 100% pot capacity. I1 treatment was referred to as the well-watered or control treatment, while 12 and I3 were the moderately and severely water stress treatments respectively. An experimental unit consisted of five pots, each containing a single *P. sidoides* plant, giving a total of 180 pots.

Each experimental treatment was fitted with a DFM soil moisture probe to monitor soil moisture at the root zone. Drip irrigation system was installed for irrigation. After planting, crops were maintained and well-watered with the same quantity of water for excellent establishment until the end of June 2023. In July 2023, different irrigation (I1, I2 and I3) levels were introduced as treatments. These irrigation levels are synonymous with 25% (75% PAW or I1), 50% (50% PAW or I2) and 75% (25% PAW or I3) soil water depletion levels. Data collection began in July 2023 and continued monthly for a period of 12 months for this Final report. However, for the MSc student (Mr Ntoyi) working on the morphology, physiology, biochemical and molecular traits of *P. sidoides*, the data were collected over 6 months (July to December 2023). This Chapter presents findings on the effect of the different irrigation levels [75% PAW (I1), 50% PAW (I2) and 25% PAW (I3)] on the growth, physiological, biochemical and molecular traits of *P. sidoides* during the period of growth.

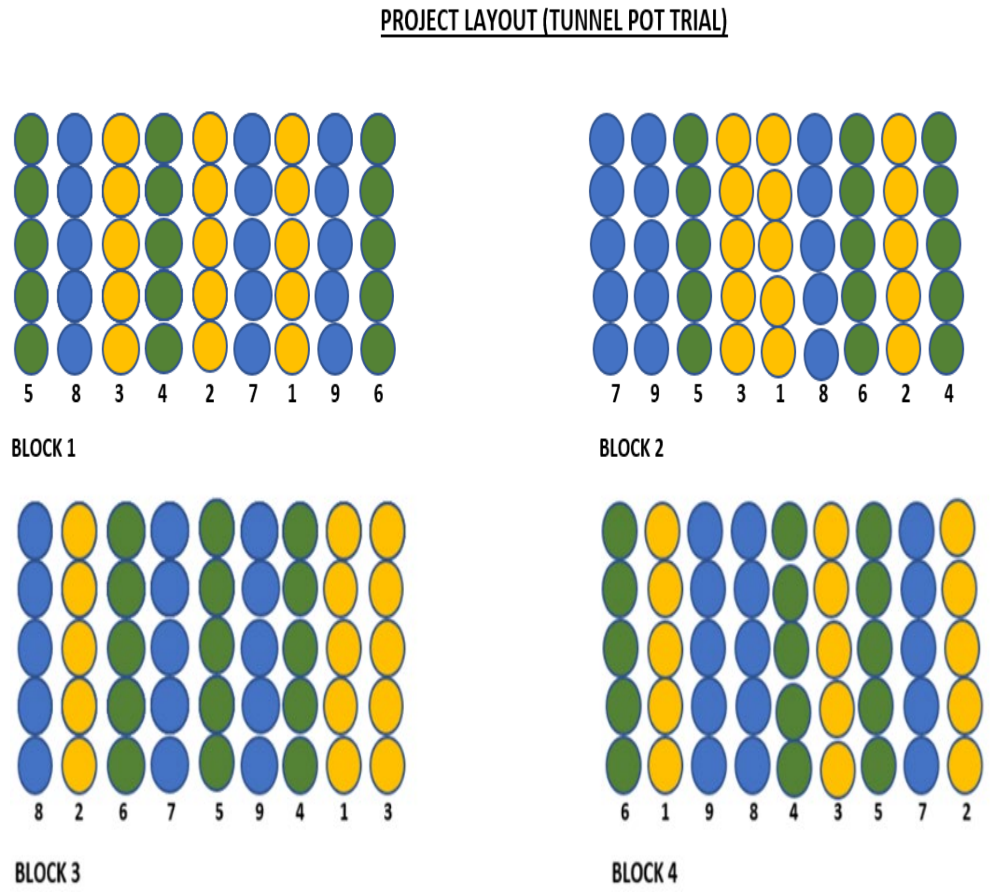


Figure 4.1 Trial layout.

Yellow = Irrigation 1 (H1), Green = Irrigation 2 (H2) Blue = Irrigation 3 (H3). Each irrigation level appearing three times per block because of the three different harvest times.

4.2.1 Morphological and physiological traits

For morphological parameters, plant height of selected plants was taken from the base of the stem to the growing tip of the last leaf using a 30 cm ruler. The number of leaves were counted manually.

The area of a heart-shaped *Pelargonium sidoides* leaf was used to estimate the leaf area per leaf, employing a specialized formula:

$$\text{Leaf area} = \frac{(a \times b \times \pi)}{2 \times \sqrt{2}}$$

a = length (cm)
b = width (cm)
π = 3.1 m
π = 3.14 the ratio of a circle's circumference to its diameter

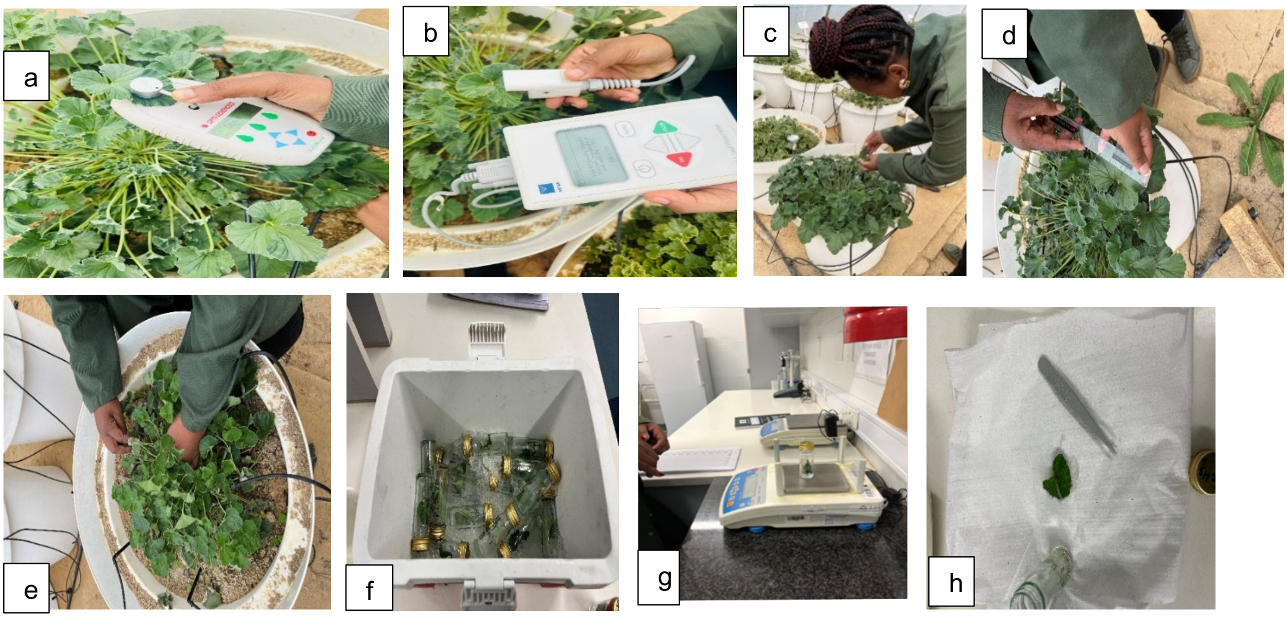


Figure 4.2 Determination of a) chlorophyll content, b) stomatal conductance, c) plant height, d) leaf length & width, e) number of leaves, f-h) relative water content.

However, for physiological traits, leaf chlorophyll content was measured on the top-most leaf using chlorophyll content meter (CCM-200 PLUS). Stomatal conductance was measured with SC-1 leaf porometer (Decagon Devices, Pullman, USA). Measurements were taken on the abaxial (bottom) side of the leaf at midday which corresponds with the peak period of environmental factors. Relative water content (RWC) was measured using an improved method of Sade et al. (2015). Determination of chlorophyll a and b was carried out using the method of Mulaudzi et al. (2023) while total chlorophyll content was assayed in accordance with Arnon (1949) method.

4.2.2 Biochemical traits

Malondialdehyde (MDA) and reactive oxygen species (ROS) were assayed in accordance with the method of Azeem et al. (2023) while proline content was assayed in accordance with the method of Carillo and Gibon (2011).

Figure 4.3 Leaf sampling for analysis of biochemical traits

4.3 RESULTS AND DISCUSSION

4.3.1 Growth parameters

Table 4.1 presents the effects of different irrigation levels over 12 sampling months on leaf area, number of leaves, and plant height. Data on growth parameters showed that water stress had no immediate effect on plant height and number of leaves in the first three months of water stress initiation. However, from 4 to 12 months of deficit irrigation, I1 (75% PAW) consistently had higher plant height and number of leaves while I3 (25% PAW) plants had the least during this period of observation. These results could be attributed to better growth in the well-watered plants (75% PAW). For leaf area, the severely stressed plants (25% PAW) consistently had reduced leaf area compared to the other two treatments However, plants grown under the 75% and 50% PAW were more or less comparable with each other in the first 6 months after water stress introduction. Another interesting observation was that there were no differences in leaf area among all treatments from the 7th month onwards, indicating that water stress had no effect on leaf area during this period. *Pelargonium sidoides* is considered a semi-evergreen to evergreen species, depending on environmental conditions. During prolonged drought or cold conditions, it may shed some or all its leaves to conserve energy and water. In this case, it behaves more like a semi-evergreen species. The underground tuberous root system allows it to survive adverse conditions and regrow when the environment becomes favourable again.

Table 4.1 Growth parameters of *P. sidoides* as influenced by different irrigation levels and age

Month	Irrigation level	Plant height (cm)	No. leaves	Leaf area (cm^2)
1	I1	24.1875 a	410.50a	3.8921a
1	I2	24.1250 a	405.50a	3.2786b
1	I3	23.9125 a	403.50a	2.9135b
2	I1	23.7125 a	407.88a	6.6662a
2	I2	23.0625a	403.13a	6.0839a
2	I3	22.8875a	400.63a	5.1980b
3	I1	20.3650a	401.23 a	9.5616a
3	I2	20.1000a	376.38a	8.0629ab
3	I3	19.9000a	352.13 a	6.6489b
4	I1	18.3875a	354.00 a	9.0345a
4	I2	16.0375b	306.38ab	7.6809ab
4	I3	12.6250c	261.75b	6.7157b
5	I1	18.0875a	297.250a	8.5540a
5	I2	15.6750b	251.875b	7.2369b
5	I3	12.2625c	211.375 c	6.2025c
6	I1	18.4875a	336.63a	8.9109a
6	I2	15.5500b	252.88b	7.7938ab
6	I3	12.2750c	203.13c	6.7833b
7	I1	19.3125a	218.875a	8.553a
7	I2	15.7000b	183.625b	8.334a
7	I3	12.6500c	142.125c	8.156a
8	I1	18.6625a	176.000a	8.727a
8	I2	15.9375b	138.750b	8.722 a
8	I3	12.8500c	83.625c	7.702a
9	I1	18.3750a	166.750a	9.002a
9	I2	16.0000b	132.250b	7.509a
9	I3	12.8500c	106.375c	6.947a
10	I1	20.1125a	181.875a	9.316a
10	I2	16.8625b	152.000b	7.715a
10	I3	14.1625 c	127.250c	7.456a
11	I1	20.0250a	152.500a	10.219a
11	I2	16.5375b	124.625b	8.343a
11	I3	13.6750c	101.375c	7.494a
12	I1	17.4625a	144.750a	9.377a
12	I2	15.0125b	106.500b	9.021a
12	I3	12.4375 c	89.375c	7.920a

Values with the same letters in the same column are not significantly different ($P \leq 0.05$).

4.3.2 Physiological parameters

4.3.2.1 Relative water content (RWC)

Leaf relative water content (RWC) is an important physiological trait that gives an indication of the water status of the plant and can also be used to assess a plant's drought tolerance. It reflects the balance between water supply to the leaf tissue and transpiration rate (Lugojan and Ciulca, 2011). In this study, as expected, the well-watered plants (75% PAW) consistently had higher RWC than the moderately stressed (50% PAW) and the severely stressed (25% PAW) plants, with the most stressed plants having the least RWC (Table 4.2). RWC in I1 was initially high in July (86.16145%) but decreased to 72.9935% by November this decline can be attributed to the seasonal conditions during summer. I2 followed a similar pattern as I1, starting at 86.1502% in July, declining to 62.618% in December. I3 (severely stressed) was always significantly lower compared with I1 and 12 from September till the end of the sampling period June 2024. In general, RWC declined during the summer months compared to winter, and this can be attributed to the role played by seasonal conditions. Warmer temperatures typically increase water loss through transpiration, leading to a reduction in RWC.

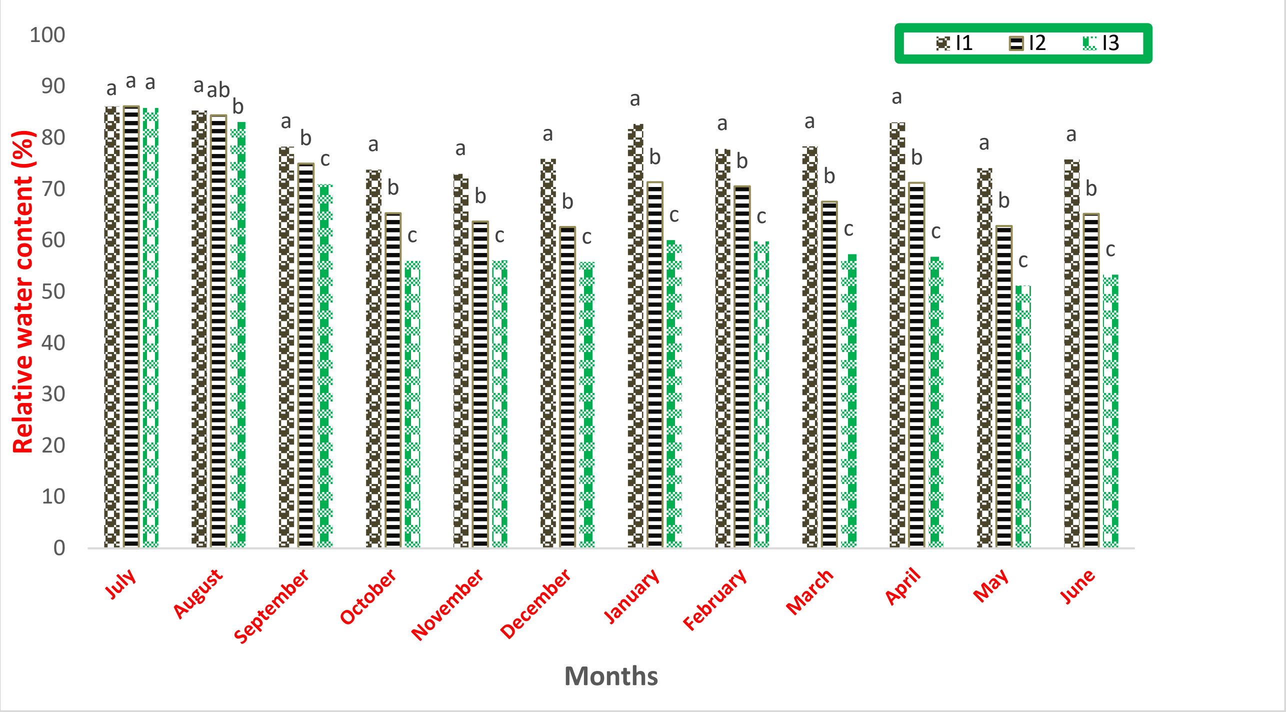


Figure 4.4 Effect of different irrigation levels on relative water content of *Pelargonium sidoides* over a 12-month sampling periods. Means with the same letter in the same month are not significantly different (P≤0.05).

4.3.2.2 Stomatal conductance

Stomatal conductance is another physiological trait measured in this trial. It is a measure of the water loss from plant leaves via the stomatal aperture and driven by the gradient between internal and external water vapor pressures (Horton and Hart, 1998). No significant differences were observed between treatments during the first month (July) of observation (Figure 4.5). This could be because the plants had not yet reached a threshold where stomatal closure is triggered as leaf water potential approaches a critical stress level caused by water deficit irrigation (Mofokeng et al. 2015). The data collected over 12-month period, starting from July 2023 to June 2024 showed pronounced differences among the treatments with effect from the month of September with I3 (25% PAW) experiencing the most decline in stomatal conductance compared to the other two treatments till the end of the reporting period (Figure 4.5). The well- watered (75% PAW) plants consistently had the highest stomatal conductance from September 2023 to June 2024. A decline was observed in October across all treatments, with I3 experiencing a sharp drop. This could be attributed to the seasonal change from winter to spring, thereby causing an abrupt change in weather pattern. The results show that CO_2 assimilation and leaf water potential were reduced by severe water depletion level.

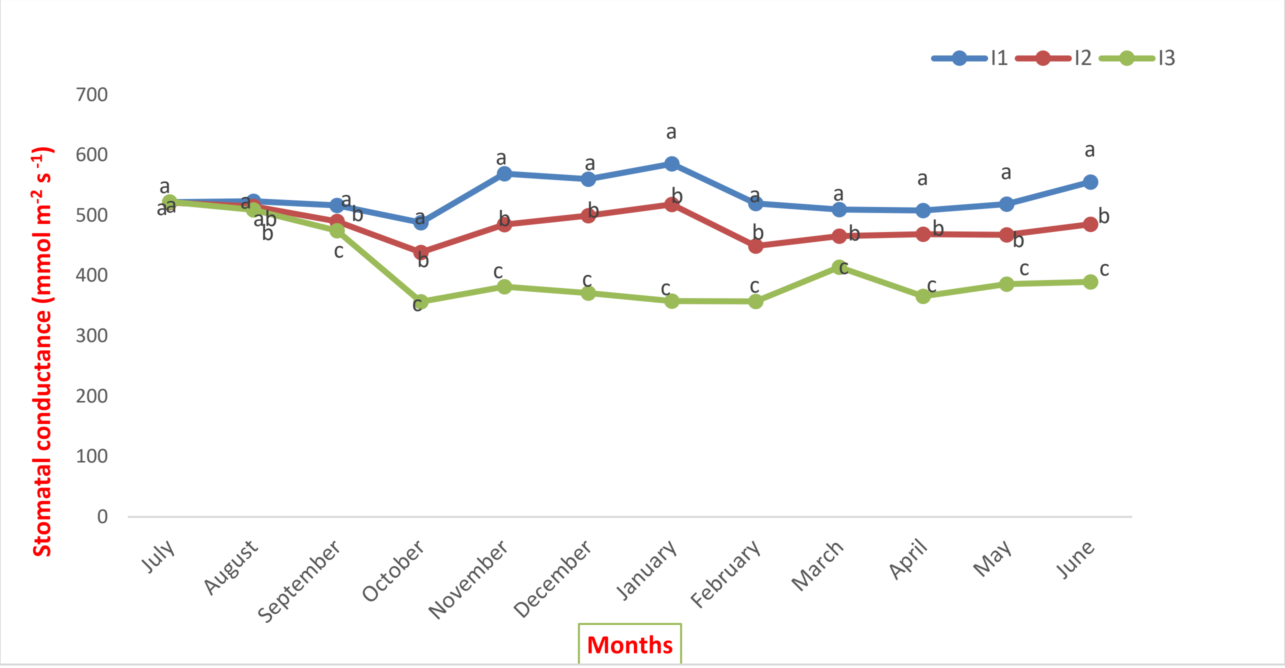


Figure 4.5 Effect of different irrigation levels on the stomatal conductance of *Pelargonium sidoides* over a 12-month sampling period. Means with the same letter in the same month are not significantly different (P≤0.05)

4.3.2.3 Chlorophyll content

Leaf chlorophyll content was determined using a chlorophyll content meter CCM-200 plus. Figure 4.6 shows the effect of different irrigation levels on chlorophyll content of *Pelargonium sidoides* over a period of 12 months. The leaf chlorophyll content is also a significant physiological trait of plants. It represents the photosynthetic capacity of the plant. This physiological parameter shares a similar pattern with the RWC. For the 12-month duration of measurement, the effect of water stress on the leaf chlorophyll content became noticeable from the month of September 2023. Overall, the well-watered plants exhibited the highest level of chlorophyll contents, followed by the moderately stressed plants, while the severely stressed plants had the lowest chlorophyll contents. October shows a sharp decline across all irrigation treatments, followed by a significant recovery in November. This could be attributed to change in season.

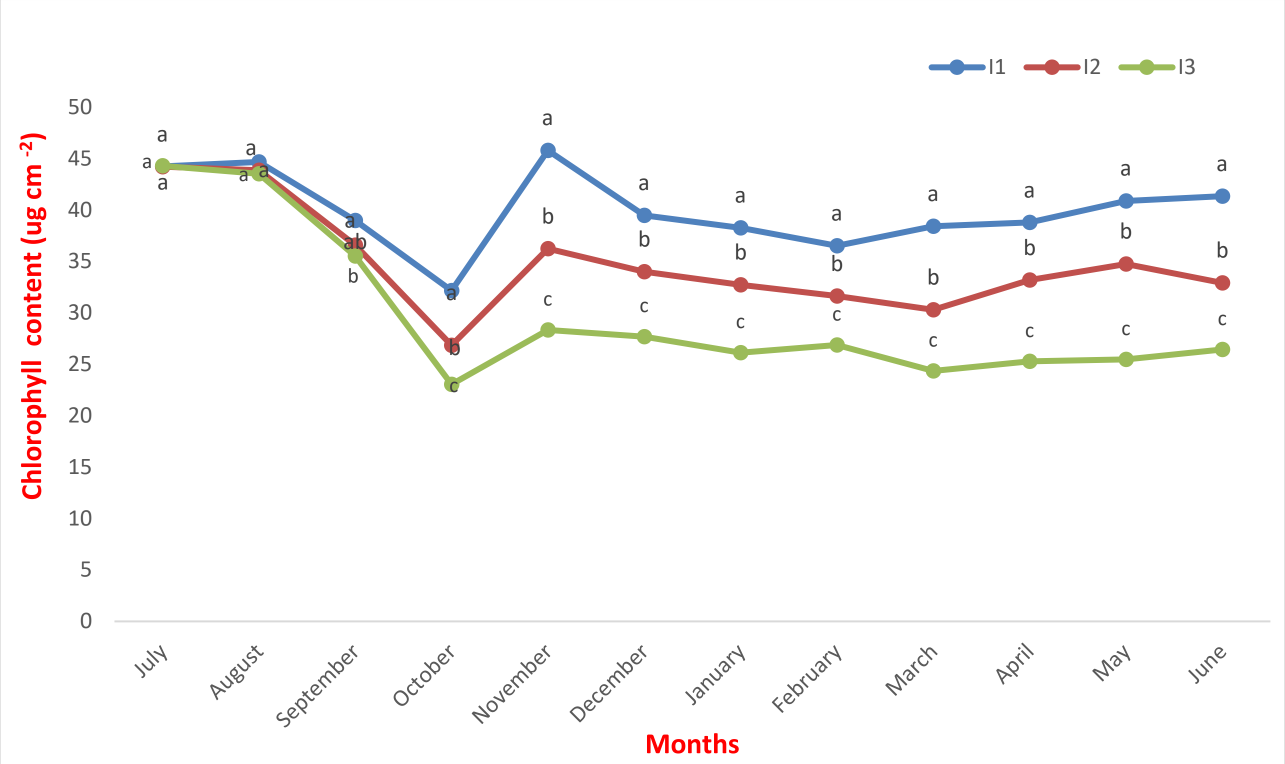


Figure 4.6 Effect of different irrigation levels on chlorophyll content of *Pelargonium sidoides* during the duration of sampling period. Means with the same letter in the same month are not significantly different (P≤0.05)

4.3.3 Biochemical traits

4.3.3.1 ROS, MDA and proline levels in *P. sidoides* after six months of water stress

Data were collected on the biochemical traits of *P. sidoides* using the leaves of the species. Stress markers like reactive oxygen species (ROS), malondialdehyde (MDA) and proline contents were analysed to determine the response of *P. sidoides* to deficit irrigation during growth, from July to December 2023. Hydrogen peroxide (H_2O_2) level was consistently higher in the severely stressed plants (75% water depletion) throughout the observation period, with 25% water depletion treatment (75% PAW) being the least. ROS level increased with increase in water stress. Severe soil water depletion led to overproduction of H_2O_2, and thus, exacerbating oxidative stress and ultimately damaging the cells of *P. sidoides*. Malondialdehyde (MDA) levels followed the same trend as ROS. Since MDA is a suitable indicator of oxidative stress, the results of this study showed that *P. sidoides* was experiencing oxidative stress under severe water depletion. Proline levels in response to water depletion was also in line with ROS and MDA. Proline contents increased water stress. Indicating that under severe water depletion, *P. sidoides* synthesizes proline as an osmoprotectant counter-act oxidative stress, caused by hydrogen peroxide.

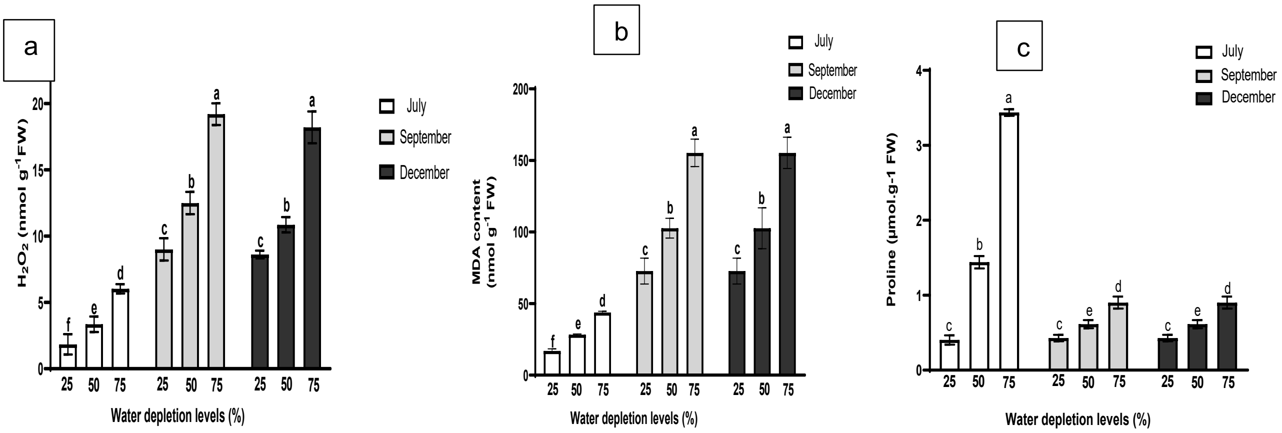


Figure 4.7 a) Reactive oxygen species (ROS), b) Malondialdehyde (MDA), c) proline contents in response to water depletion. Bars with different letters are significantly different ($P < 0.05$)

4.3.4 Chlorophyll a, b and total chlorophyll contents

Chlorophyll content shows that water stress had no effect on chlorophyll b, especially at I1 and I2. However, chlorophyll contents decreased with increase in water stress (Table 4.2). Whereas, over the months, as the plants grew older, chlorophyll a and chlorophyll b contents became more pronounced, and the highest levels were recorded at the third harvest (H3). It should also be noted that lowest concentration of chlorophyll b was recorded at H3. In general, 75% water depletion level lowered chlorophyll contents and cell growth of *Pelargonium sidoides*.

Table 4.2 Chlorophyll a, b and total chlorophyll contents of *P. sidoides* subjected to different water depletion levels

Water depletion level	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total Chlorophyll (mg/g FW)
25% (I1)	10.1658^{a}	2.2158^{a}	12.2750^{a}
50% (I2)	8.3700^{b}	2.4967^{a}	10.4867^{b}
75% (I3)	5.0567^{c}	1.7467^{b}	6.0225^{c}
Pr>F	**0.0001**	**0.00025**	**0.0001**
LSD	**0.2735**	**0.3968**	**0.2715**
Months			
July (H1)	8.2000^{b}	3.2833^{a}	10.1000^{b}
September (H2)	5.3892^{c}	1.7925^{b}	7.2708^{c}
December (H3)	10.0033^{a}	1.3833^{c}	11.4133^{a}
Pr>F	**0.0001**	**0.0001**	**0.0001**
LSD	**0.2735**	**0.3968**	**0.2715**
Irrigation X Month			
I1 X H1	11.3000^{b}	3.3500^{a}	14.3000^{a}
I2 X H1	8.5000^{c}	3.2000^{a}	10.5000^{c}
I3 X H1	4.8000^{f}	3.3000^{a}	5.5000^{f}
I1 X H2	7.3075^{d}	2.0775^{b}	9.4050^{d}
I2 X H2	5.4100^{e}	2.1300^{b}	7.5400e
I3 X H2	3.4500^{g}	1.1700^{c}	4.8675^{g}
I1 X H3	11.8900^{a}	1.2200^{c}	13.1200^{b}
I2 X H3	11.2000^{b}	2.1600^{b}	13.4200^{b}
I3 X H3	6.9200^{d}	0.7700^{c}	7.7000^{e}
Pr>F	**0.0001**	**0.0264**	**0.0001**
LSD	**0.4737**	**0.6872**	**0.4702**

Bars with different letters are significantly different ($P < 0.05$)

4.4 CONCLUSIONS

P. sidoides displayed a variety of responses to water deficit stress during its growth. For most of the morphological parameters like plant height, leaf area and number of leaves, *P. sidoides* performed better at the 75% PAW while the least performer was the 25% PAW. Relative water content, stomatal conductance and chlorophyll content were all higher at 75% PAW while the least was observed at 25% PAW. While water stress did not have any effect on chlorophyll b at I1 and I2, the lowest level was recorded in I3. In general, the levels of chlorophyll a, chlorophyll b and total chlorophyll decreased with increase in water stress, with plants at 75% water depletion level (severely stressed plants) having the lowest chlorophyll contents. However, for the stress markers ROS, MDA and proline, these parameters were all the highest levels at the 25% PAW, thus, indicating high level of stress when there was very limited water in the soil.

CHAPTER 5: YIELD OF *PELARGONIUM SIDOIDES* IN RESPONSE TO DIFFERENT LEVELS OF IRRIGATION AND HARVESTING AGE

5.1 INTRODUCTION

Global warming brings about climate changes, limited water resources and water restrictions for agricultural activities which compelled producers to invest in crops and different agricultural management practices that are more adapted to the potential unfriendly future climate (Botai et al., 2017; Galindo et al., 2018; Otto et al., 2018; Burls et al., 2019). An efficient adaptation to the changing climate on farms at the sector and policy levels is a prerequisite for reducing negative impacts and obtaining possible benefits (Olesen and Bindi, 2002) from land use and land management as well as changes in inputs of water, nutrients, and pesticides (Olesen, 2006). In Western Cape agriculture, water demands are likely to increase in future with the expected increase in temperature and evaporation due to climate change effects (Harris, 2018; Otto et al., 2018). This will in turn result in increased irrigation demand by crops. The increased unstable climate change means there may be a prolonged drought with severe consequences in the future just as it happened in 2015-2017 (Otto et al., 2018; Burls et al., 2019). Irrigation volume for agricultural sector is unlikely to increase since the Department of Water and Sanitation has capped agricultural allocations to the current level. In addition, a significant reduction in stream flow is predicted for the Western region of South Africa, where several Western Cape water management areas are already water stressed. Hence, improved water use and productivity in agriculture is a necessity in order to provide the needed water for the projected increased water demand for human consumption and industrial production by 2030 (Bester, 2011). Some of the most wide-ranging adaptations involve changes in water management and water conservation (Olesen, 2006; Taparauskiene and Miseckaite, 2014).

Cultivation of *P. sidoides* requires water. Water is one of the important factors that affect growth and yield, amongst other plant parameters (Mofokeng et al., 2015). However, water shortage is a serious concern in a water scarce country like South Africa. Although, *Pelargonium* are drought tolerant and do not need to be watered too frequently, it still requires that the soil is not too dry before watering again. It is important that roots do not dry out completely to ensure survival of the plants (Hall, 2021). However, there are no cultivation guidelines for *P. sidoides*. Hence, the water requirement of the species is unknown. Sustainable use of water has become a priority in agriculture. Therefore, innovative irrigation management practices are critical. Hence, the current study has become a necessity. The aim of this Chapter was to provide information on the yield of *P. sidoides* in response to different levels of irrigation and harvesting age.

5.2 MATERIALS AND METHODS

5.2.1 Total biomass (shoot and root yield)

Harvesting of the *P. sidoides* was done at six (6) months intervals. This Chapter only reports on two harvests (H1 and H2) carried out in December 2023 (H1) and June 2024 (H2). Pots were fully irrigated a day prior to harvesting to facilitate easy pull out of plants during harvesting process. The shoots were detached from the roots. The root tubers were rinsed with water to remove soil debris and later blotted with tissue paper. The fresh root tubers and shoots were weighed separately and recorded as fresh weight. Both shoot and root samples were oven-dried at 70°C until constant weight and the dry weight recorded.

Figure 5.1 Harvesting of *P. sidoides* shoot and roots a-c) fresh shoot, d) dry shoot, e-g) fresh root, h-k) dry root

5.3 RESULTS AND DISCUSSION

5.3.1 Shoot yield

Figure 5.2 illustrates the fresh shoot yield of *P. sidoides* under different irrigation levels, across two harvests (H1 and H2). The two harvests were carried out and the fresh and dry shoot and root yields documented. The first harvest of *P. sidoides* took place on 05 December 2023, while the second harvest occurred on 04 June 2024. Deficit irrigation did not affect fresh shoot biomass in both harvests (Figure 5.2). However, shoot dry matter yield were significantly higher in H2 when compared to H1, irrespective of the treatment (Figure 5.2). Indicating that the longer *P. sidoides* stays before harvest, the higher the shoot dry matter yield.

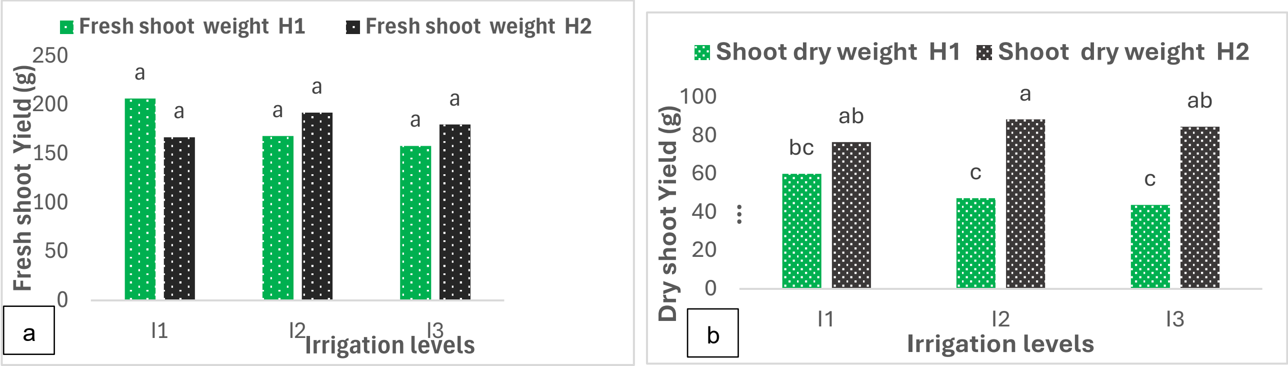


Figure 5.2 a) fresh shoot yield, b) dry shoot yield of *P. sidoides* at Harvest 1 and Harvest 2. Bars with the same letter are not significantly different (P≤0.05)

5.3.2 Root yield

Figure 5.3 illustrates the fresh root yield for both H1 and H2 across different irrigation levels. For the root yield, highest fresh root yield came from the well-watered plants while the severely stressed plants had the lowest fresh root yield at H1. On the contrary, the moderately stressed plants gave better fresh root yield compared to the well-watered and the severely stressed plants at H2. The dry root yield in H1 followed the same trend as fresh root yield. However, at H2, the highest dry root yield came from the moderately stressed plants while there were no significant differences in dry root yield between the well-watered and the severely stressed plants.

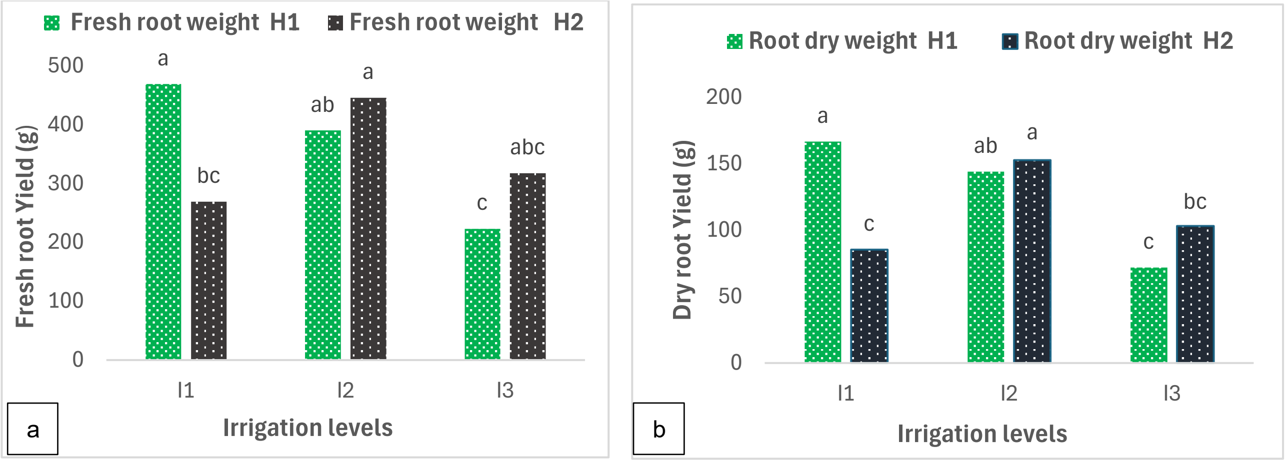


Figure 5.3 a) Fresh root yield, b) Dry root yield of *P. sidoides* over two harvests (H1 and H2). Bars with the same letter are not significantly different (P≤0.05).

5.4 CONCLUSIONS

The fresh and dry yield patterns of *P. sidoides* revealed that water stress did not affect fresh shoot mass at either of the two harvests. Dry shoot yield was generally higher at harvest 2. The severely stressed plants gave the least fresh and dry root shoot yield at H1. The highest dry root yield came from the moderately stressed plants (50% PAW).

CHAPTER 6: EFFECT OF WATER STRESS ON SOIL MICROBIAL ENZYME ACTIVITY IN RELATION TO SOIL FERTILITY/QUALITY UNDER *PELARGONIUM SIDOIDES* CULTIVATION

6.1 INTRODUCTION

Information is lacking on soil biological activities, including enzyme activity and their relationship in the soil environment in which *Pelargonium sidoides* is growing. Soil enzyme activity is one of the key soil fertility parameters as enzymes assist in mineralizing organic nutrients, thereby making them available for plants and soil microorganisms (Al et al., 2015). Enzymes indicate soil quality, as many soil enzymes respond almost immediately to changes in soil fertility status (Tejada et al., 2008; Guangming et al., 2017), and soil water content. According to Kaurin et al. (2018), soil texture, pH, and nutrient availability contribute to soil enzyme and microbial activities.

Soil enzyme activity is sensitive to environmental and soil changes (Alkorta et al., 2003). Some of the most sensitive enzymes are urease, phosphates and β-glucosidase (Guangming et al., 2017; Asadishad et al., 2018). These are responsible for promoting the hydrolysis of nitrogen (N) containing organic matter, the cycling of phosphorus (P), and the hydrolysis of glycosides, which play a crucial role in soil carbon (C) cycling, respectively (Guangming et al., 2017; Asadishad et al., 2018; Kaurin et al., 2018). The activity of soil enzymes has been used in soil health research (Alkorta et al., 2003; Meena and Rao, 2021; Kanté et al., 2021). Therefore, the aim of this Chapter was to assess the effect water stress on soil microbial enzyme activity in relation to soil fertility under *P. sidoides* cultivation.

6.2 MATERIALS AND METHODS

Immediately after each harvest (H1 and H2) of *P. sidoides*, soil samples were taken per treatment to assess the deficit irrigation effect on soil enzyme activity. The soil enzymes analyzed were β-glucosidase, acid phosphatase, and urease, using methods of Eivazi and Tabatabai (1988), Icoz and Stotzky (2008), and Kandeler and Gerber (1988), respectively. The enzyme activity data were then converted to Alteration Index Three (AI3) scores using the methods described in the work of Huyssteen et al. (2020). The substrates used for the analysis of the enzymes β-glucosidase, acid phosphatase, and urease were 4-MUB-β-D-glucoside, 4-MUB-phosphate, and a urea solution, respectively.

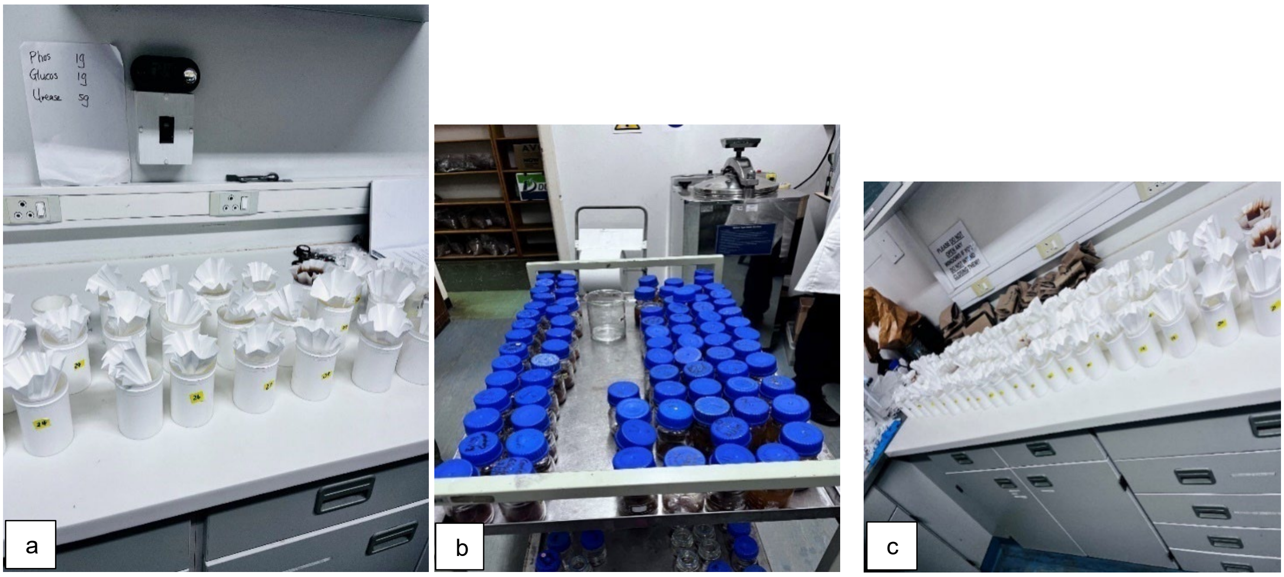


Figure 6.1 a-c) Different stages of β-glucosidase, acid phosphatase, and urease analysis

6.3 RESULTS AND DISCUSSION

Preplanting values for soil enzyme activity were recorded at the beginning of the study to assess the change over time brought about by water deficit stress treatments (I1, I2, and I3). There were no significant differences in β-glucosidase activity across all irrigation levels (75% PAW, 50% PAW & 25% PAW) at both harvests (Table 6.1). However, a comparison of the two harvests shows that β-glucosidase activity in H1 was significantly higher than of H2. Acid phosphatase activity was not significantly different among treatments in H1 and H2. Urease activity was lower for 25% PAW at H1 while activity at 75% PAW, 50% PAW were not different. However, urease activity remained the same at all irrigation levels during H2. Meanwhile, Urease activity dropped slightly at H2 for I1 and I2 while that of I3 increased when compared with H1. The reduction in enzyme activity in H2 can be attributed to decreased microbial activity and biomass, along with alterations in soil pH, reduced soil fertility and organic matter availability (since there was no fertilizer application to supply nutrients). No significant difference was observed in AI3 across both harvests, although the values in H1 were higher (though not significant) than those in H2. At H1, the AI3 index scores became less negative as the water stress increased while it dropped to the pre-planting level and even lower (at 50% water depletion level) at H2. All three activities did show a tendency to decrease as water stress increased in the order I1 > I2 > I3 (Table 6.1), especially at H1. Overall, soil enzyme activities showed a drastic decline in H2. The enzyme activity results are similar to that of stomatal conductance, RWC, chlorophyll, MDA and ROS contents in that they all declined with increasing water stress.

Table 6.1 Soil enzyme activity after the first and second harvest

Irrigation	β-glucosidase	Acid-phosphatase	Urease	AI3
Harvest 1				
I1	79.73^{a}	340.96^{a}	20.055^{a}	-20.568^{a}
I2	78.65^{a}	321.45^{a}	19.350^{a}	-19.287^{a}
I3	76.23^{a}	254.55^{a}	13.991^{b}	-14.157^{a}
Harvest 2				
I1	18.997^{b}	197.36^{ab}	14.657^{b}	-14.179^{a}
I2	18.438^{b}	162.18^{b}	17.263^{ab}	-12.770^{a}
I3	16.473^{b}	203.32^{ab}	15.598^{ab}	-14.905^{a}
Preplanting (Baseline)	59.657	208.851	23.515	-14.73

Values with the same letters in the same column are not significantly different ($P \leq 0.05$).

6.4 CONCLUSIONS

Overall, soil enzyme activities showed a drastic decline in H2. Deficit irrigation did not affect β-glucosidase activity within treatments, although, activity decreased at H2. The same trend was observed for Acid-phosphatase and urease. No significant difference was observed in AI3 across both harvests. At H1, the AI3 index scores became less negative as the water stress increased while it dropped to the pre-planting level and even lower (at 50% water depletion level) at H2.

CHAPTER 7: EFFECT OF DIFFERENT LEVELS OF IRRIGATION AND HARVESTING AGE ON THE CHEMICAL COMPOSITION OF THE ROOTS OF *P. SIDOIDES*

7.1 INTRODUCTION

Pelargonium sidoides is commonly known as black *Pelargonium* or ‘Umckaloabo’. It is a perennial medicinal plant that is native to South Africa and Lesotho. In South Africa, its habitat extends along the extreme Eastern edge of the Western Cape, throughout the Eastern Cape, and in various regions across the Gauteng, North-West, Free State, KwaZulu-Natal, and Mpumalanga (Viljoen et al., 2022). This plant belongs to the Geraniaceae family, which has about 270 species of different perennial small shrubs (Kolodziej, 2007). *P. sidoides* is characterized by its dark red to purplish flowers, heart shaped green leaves, and tuberous roots (Viljoen et al., 2022).

The medicinal use of *P. sidoides* dates back centuries. Traditionally, it is used in the treatment of different health complications such as gonorrhoea, diarrhoea, dysentery, coughs, liver disorders, colic, prolapsed rectum, and “instila” which is stomach ailment in infants (Alossaimi et al., 2022; Moyo and van Staden, 2014). Its introduction to Europe in the late 19th century, particularly as a remedy for tuberculosis by Charles Henry Stevens, marked the beginning of its international commercialisation (Moyo and van Staden, 2014). The plant's roots are the primary source of its medicinal properties, leading to the development of standardized extract known as EPs 7630, which is approved for treating acute bronchitis (Witte et al., 2020).

A range of ill-health conditions for which *P. sidoides* is utilized against has expanded from its original traditional uses. Numerous pharmacological studies encompassing in vitro studies accessing the antiviral, antibacterial, antimycobacterial, antifungal, and antiparasitic activities have been published (Moyo and van Staden, 2014). In vivo studies on antiviral, anti-coagulant and central nervous system activity of this plant have been conducted as well. Additionally, clinical trials have been carried out to investigate the plant’s effect on ailments such as acute bronchitis, acute rhinosinutis and common cold, with minimal side effects (Schapowal et al., 2019; Kamin et al., 2012; Bachert et al., 2009). The plant has exhibited high therapeutic properties with evident cytoprotective, antimicrobial, and immune-enhancing effects (Kolodziej, 2011).

The pharmacological effectiveness of *P. sidoides* is attributed to highly oxygenated coumarins such as umckalin, scopoletin, and esculin, along with the presence of other compounds such as gallic acid and flavonoids (Alossaimi et al., 2022). Several studies have reported these metabolites and out of the pool of secondary metabolites produced by *P. sidoides*, umckalin is considered a marker (Brendler and Van Wyk, 2008; Hauer et al., 2009; Masondo and Makunga, 2019; Van Wyngaard et al., 2023). The chemical composition of the roots of cultivated *P. sidoides* at three irrigation conditions and three harvest ages is being explored in this part of the study. This is done using various laboratory methods and analysis. The aim of this Chapter was to determine the effect of age and water deficit stress on the chemical composition of *P. sidoides* root.

7.2 MATERIALS AND METHODS

7.2.1 Experimental design and sampling

The trial was set up at the Agricultural Research Council, Nietvoorbij campus, Stellenbosch. Irrigation treatments and different harvesting age are the two factors considered in the trial. *P. sidoides* plants were allowed to grow for seven months prior to introduction of water stress conditions, which are three different irrigation levels: 25, 50, and 75% of plant available water (PAW). The plants were then scheduled for harvest every six months after the introduction of water deficit stress, while maintaining the three pre-determined irrigation levels (25, 50 and 75% PAW). The experimental set up was in four replicates. The harvested root samples of *P. sidoides* were oven-dried, milled into powder and subjected to laboratory analysis.

The leaves and roots of *P. sidoides* were also analysed for chemical (nutrient) composition using the method described by the Association of Official Analytical Chemists (AOAC, 1984).

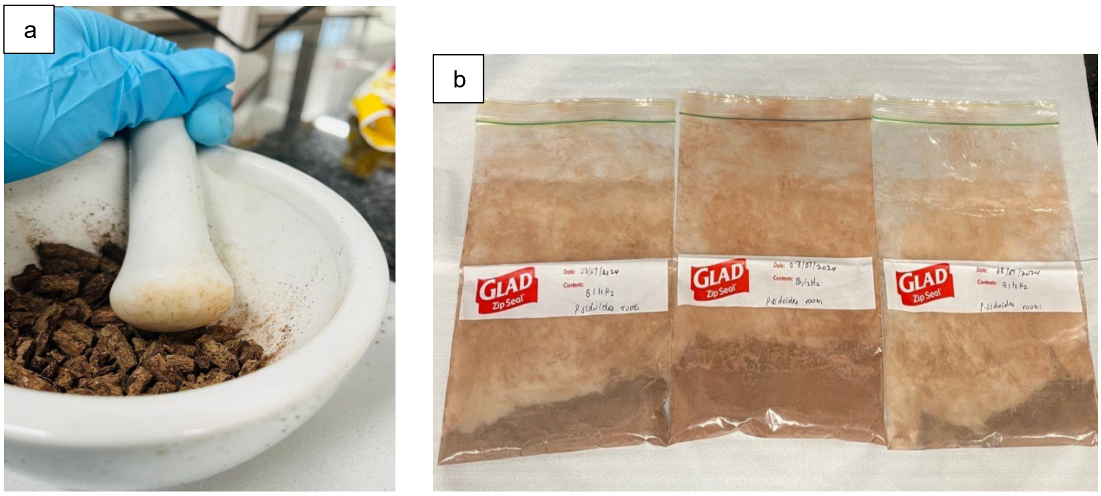


Figure 7.1 a) Crushing of *P. sidoides* dried root, b) Powdered *P. sidoides* root samples

7.2.2 Colour profiling of *P. sidoides* samples

The colour analysis of *P. sidoides* samples were evaluated using chroma meter-410 connected with a Data Procesor-400 (Ver. 1.20) (Konica Minolta, Inc., Tokyo, Japan). The colour profiles: *L** (lightness), *a** (redness), *b** (yellowness) were recorded and chroma and Hue angle calculated using the following formula: Chroma = Sqrt (a *) 2 + (b *) 2, Hue = arctan (b * / a *) (Mudau et al., 2022).

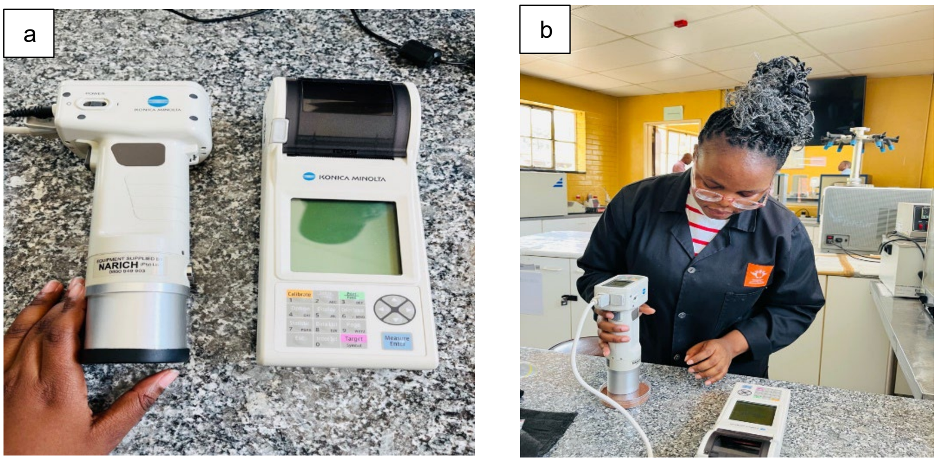


Figure 7.2 a) Colorimeter machine for colour analysis, b) Colour analysis of powdered *P. sidoides* root

7.2.3 Physicochemical properties of P. sidoides

The presence of functional groups in *P. sidoides* samples were analyzed using Perkin Elmer Fourier-transform infrared spectroscopy (FTIR) spectrum spectrometer (Perkin Elmer, Midrand, South Africa). The analysis was done using a direct method where the blank is scanned in the absence of any material on the spectrometer. The samples were analyzed from a wavenumber of 500 to 4000 cm-1. To clean the spectrometer between analysis, 70% ethanol was used. The Origin software (Version 9.1 OriginLab, Massachusetts, USA) was used to draw the FTIR spectra obtained. The results were then further subjected to principal component analysis (PCA) scores using SIMCA software (Version 17 Sartorius, Gottingen, Germany) (Adebo et al., 2018).

7.2.4 Extraction for phytochemical screening

Powdered *P. sidoides* samples were extracted using 11% ethanol. The samples were dissolved in 11% ethanol, sonicated at room temperature in ultrasonic bath (Eins Sci laboratory equipment, Johannesburg, South Africa) for 1 hour, centrifuged and filtered using 0.22 µm syringe filters before analysis.

7.2.5 Qualitative preliminary phytochemical screening

There are different methods used for phytochemical screening of medicinal plants. In this study the following methods were used to qualitatively determine the presence of different phytochemicals:

- The Froth test (Nortjie et al., 2022) was utilized to detect saponins.
- Flavonoids were identified using the sulfuric acid test (Nortjie et al., 2022) and the alkaline reagent test (Sudha et al., 2020).
- Steroids were determined through the acetic anhydride test (Nortjie et al., 2022).
- Terpenoids were assessed with the Salkowski test (Dahanayake et al., 2019).
- Tannins were tested using the Ferric chloride test, Alkaline reagent test (Kausar et al., 2021), and Gelatin test (Abubakar and Haque, 2020).
- Phenols were detected with the Ellagic acid test (Kausar et al., 2021), Ferric chloride test (Nortjie et al., 2022), and Lead acetate test (Banerjee et al., 2022).
- Hager's reagent test (Ali et al., 2018) was used to identify alkaloids.
- Coumarins were tested using a 10% sodium hydroxide solution (Ali et al., 2018).

7.2.6 Phytochemical profiling

P. sidoides extracts were subjected to further qualitative phytochemical profiling by thin layer chromatography. In this analysis the reference solutions of umckalin, scopoletin, and esculin were separately dissolved in 1 mL of methanol. Aluminium- baked TLC254 plates were used for stationary phase. To make the mobile phase water, methanol and ethyl acetate were used at a ratio of 10:14:76, v/v/v. Ten (10) µL of test sample and reference solutions were spotted on the TLC plate. The plates were then allowed to develop to a path of 10 cm and then air-dried. After this, the plates were viewed under ultraviolet light at a wavelength of 365 nm (European Pharmacopoeia, 2023).

7.2.7 Quantitative phytochemical analysis

7.2.7.1 Total phenolic content

The determination of total phenolic content was carried out using the Folin-ciocalteu (F-C reagent method). The F-C reagent working solution was made by mixing F-C reagent with distilled water at a ratio of 1:15. To make gallic acid standard curve, concentrations of 0, 0.01, 0.02, 0.05, 0.1, and 0.25 mg/mL were used. Ten (10) µL of the extracts were dispersed in 96 well plate, then followed by the addition of 50 µL of F-C phenol reagent and 50 µL of 7.5% Na2CO3. The plate was then incubated in the dark at ambient temperature for 30 mins. After the incubation period, the plate was analysed using ultraviolet spectroscopy (Accuris smart reader 96, Edison, United States) at a wavelength of 750 nm. The total phenolic content was then expressed in gallic acid equivalent per mg (Ahmed et al., 2019).

7.2.7.2 Total Flavonoid content

Total flavonoid contents of the samples were determined by aluminium chloride method. An amount of 10 µL extracts were distributed into each well of a 96-well microplate. Subsequently, 30 µL of 2.5 % NaNO2 and 30 µL of 1.25% AlCl3 were sequentially dispensed into each well. Then 100 µL of 2% NaOH was dispensed in each well, resulting in a total volume of the mixture being 170 µL. A standard curve for Quercetin (QE) was established using concentration ranging from 0 to 0.25 mg/mL. The 96 well plate was incubated in the dark for 30 mins. The absorbance of the samples was subsequently assessed at 450 nm using a spectrophotometer (Accuris smart reader 96, Edison, United States). The total flavonoid content was then expressed in quercetin equivalent per mg (Moyo et al., 2020).

7.2.8 Antioxidants capacity

7.2.8.1 2,2-Azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) (ABTS) radical scavenging assay

The radical scavenging activity of 2,2-Azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) (ABTS) was assessed in this study. A mixture of ABTS and potassium persulphate was prepared and incubated in the dark for 16 hours to generate ABTS radicals. After incubation, a working solution was created by diluting the reaction mixture with distilled water. Trolox, ranging from 0 to 0.5 mg/ml, was used to make a standard curve. In a 96-well plate, 2 µL of the extracts were dispersed followed by 200 µL of ABTS working solution, then incubated for 1 minute at room temperature in the dark. The absorbance was measured at 734 nm, and the Trolox equivalent antioxidant capacity (TEAC) was calculated based on the reduction in absorbance (Van Wyngaard et al., 2023).

7.2.8.2 Inhibition % of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

To evaluate the radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH), a working solution of 0.1 mM concentration was prepared. Trolox standard curve was prepared by making use of concentrations ranging from 0 to 0.5 mg/ml. In a 96-well plate, 2 µL of the samples was mixed with 200 µL of the prepared DPPH solution. The mixture was then incubated in darkness for 30 mins at room temperature. Following incubation, the absorbance was measured at 517 nm. The Trolox equivalent antioxidant capacity (TEAC) /mg was calculated (Mareček et al., 2017).

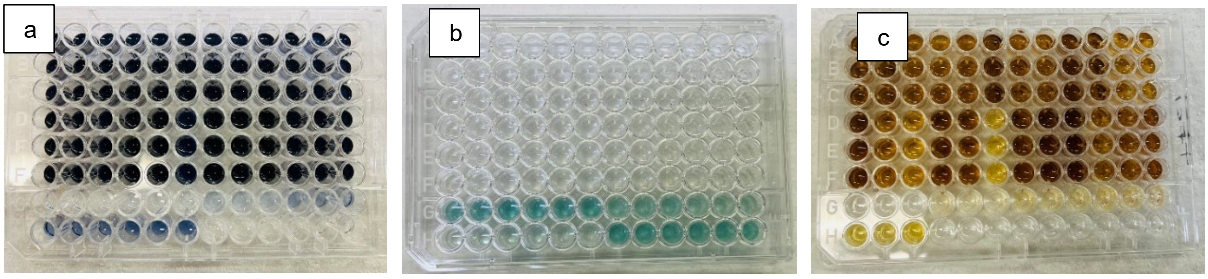


Figure 7.3 Determination of a) Analysis of total phenolic content in 96 well plate, b) ABTs radical scavenging assay in 96 well plate, c) Analysis of total flavonoid content in 96 well plate.

7.2.9 Nutrient composition of *Pelargonium sidoides* shoot and root

After harvest, the leaf and root samples of *P. sidoides* were oven-dried at 70°C until constant weight. Milled into powder and stored in the refrigerator until analysis. The nutritional composition was determined using the method described by the Association of Official Analytical Chemists (AOAC, 1984).

7.3 RESULTS AND DISCUSSION

7.3.1 Colour profiling

The science of colour measurements is called colorimetry. Colorimetry is of importance in medicinal plant authentication, quality assessment and differentiation (Ideh and Ogunkunle, 2019). The colour parameters are recorded in the *L*a*b** colour space as established by 'CIE' (Commission Internationale de l'Éclairage) (Gómez-Polo et al., 2016). The colour space *L*a*b** values represent *L** (black to white component), and the chromaticity coordinates, *a** (+ red to green component, redness) and *b** (+ yellow to blue component, yellowness) (Jiménez-Zamora et al., 2016). Chroma is a measurement of the saturation/vividness of a colour. Completely vivid colours such as pure red and pure blue are 100% saturated (Kasajima, 2019). Hue gives a colour its name such as red, blue, and green. Hue angle ranges from 0 to 360, where 0 (or 360) represents maximum redness, 90 maximum yellowness, 180 maximum greenness and 270 maximum blueness (Scalisi et al., 2022).

There has not been any report on the colour analysis of *P. sidoides* root samples in both wild and cultivated populations. Table 7.1 shows the colour profile results for harvests one and two under different irrigation levels. The colour analysis of *P. sidoides* roots at 6- and 12-months harvest age, under high, medium, and low (75, 50, and 25 plant available water) irrigation levels shows that water availability and harvesting age affected the plant's colour attributes. The lightness (*L**) values for the 6-month samples were significantly higher (59.85–60.53), indicating that these plants were significantly lighter in colour compared to the 12-month samples, which had lower lightness values (48.32–49.11). In the 6- and 12-months root samples, irrigation levels did not significantly affect lightness, showing that lightness was mainly influenced by plant age rather than irrigation treatment.

The redness (a*) results show a clear distinction between the 6-month and 12-month harvests, with significant differences across the irrigation levels. The 6-month harvest samples consistently exhibit higher redness values, ranging from 7.58 ± 0.31 (high irrigation) to 7.44 ± 0.33 (low irrigation). These values are significantly higher compared to the 12-month harvest, where redness values range from 6.05 ± 0.51 (medium irrigation) to 5.14 ± 0.52 (low irrigation). The 6-months samples, regardless of irrigation levels, belong to distinct groups (^{c} and ^{bc}) compared to the 12-month samples (^{a} and ^{ab}), with some overlap observed between medium irrigation at 12 months (6.05 ± 0.51^{ab}) and the medium irrigation of the 6-month group (6.91 ± 0.59^{bc}). This suggests that harvest age strongly influences redness, with samples at six months of harvest showing higher redness values. Yellowness (b*) values in 6 months harvest show no significant differences among the irrigation treatments, with high (9.99 ± 0.13), medium (9.93 ± 0.39), and low irrigation (10.33 ± 0.21) values. This indicates that irrigation level did not influence yellowness at this harvest age. In the 12-month harvest, yellowness (*b**) significantly decreases compared to the 6-month harvest, with values of 4.85 ± 0.25, 4.79 ± 0.43, and 4.10 ± 0.34 for high, medium, and low irrigation levels, respectively. Low irrigation shows a significantly lower yellowness value within this harvest group than medium and high irrigation treatments. This suggests that reduced irrigation at 12 months negatively impacts yellowness. However, the high and medium irrigation levels do not show a significant difference.

The chroma results at the 6-month harvest show no significant differences among the irrigation levels, with values of 12.55 ± 0.18, 12.11 ± 0.51, and 12.73 ± 0.21 for high, medium, and low irrigation, respectively. This indicates that at 6 months harvest stage, irrigation level did not significantly influence the saturation or vividness of colour. In contrast, for the 12-month harvest, chroma decreases significantly, with values of 7.50 ± 0.51, 7.73 ± 0.56, and 6.58 ± 0.54 for high, medium, and low irrigation, respectively. The low irrigation treatment shows the lowest chroma value, significantly different from the medium irrigation treatment, indicating that water stress reduces the intensity of colour as plants age. High irrigation, however, does not significantly differ from medium or low.

The 6-month samples had hue values ranging from 52.82° to 55.22°, indicating a yellowish-orange colour. The highest hue value was found in the 6-Month-Medium sample (55.22°), which suggests that this sample had the most yellowish colour. In contrast, the 12-month samples had much lower hue values (38.39°–40.38°), reflecting a shift toward red-orange hues. The 12-month-medium sample had the lowest hue value (38.39°), indicating a greater shift to the red tone. This shift in hue suggests that as the plants age, there might be a change in the types or concentrations of pigments present. Saganuwan (2018) detailed that colours may suggest the presence of chemical compounds that may have pharmacological activity within plant samples. Plants and plant-parts that are black in colour may possess anticancer activity whereas plants parts that are white in colour may have activities such as antidiabetic, antiasthmatic, antihypertensive and antioxidant. Plants that are yellow, red, purple, pink, and cyan in colour may have antioxidant and antimicrobial activities. The colour variations amongst can serve as a direct indicator of quality and clinical effectiveness (Liu et al., 2023).

Table 7.1 CIELAB colour analysis results of *P. sidoides* roots under different irrigation and harvest age

Sample	Lightness (*L**)	Redness (a*)	Yellowness (*b**)	Chroma	Hue °
6 Months-High	59.85 ± 0.67^{b}	7.58 ± 0.31^{c}	9.99 ± 0.13^{c}	12.55 ± 0.18^{c}	52.82 ± 1.27^{b}
6 Months-Medium	60.44 ± 0.87^{b}	6.91 ± 0.59^{bc}	9.93 ± 0.39^{c}	12.11 ± 0.51^{c}	55.22 ± 2.30^{b}
6 Months-Low	60.53 ± 0.64^{b}	7.44 ± 0.33^{c}	10.33 ± 0.21^{c}	12.73 ± 0.21^{c}	54.24 ± 1.49^{b}
12 Months-High	49.11 ± 0.57^{a}	5.71 ± 0.52^{a}	4.85 ± 0.25^{b}	7.50 ± 0.51^{ab}	40.38 ± 2.10^{a}
12 Months-Medium	48.89 ± 0.77^{a}	6.05 ± 0.51^{ab}	4.79 ± 0.43^{b}	7.73 ± 0.56^{b}	38.39 ± 2.73^{a}

12 Months-Low	48.32 ± 0.38^{a}	5.14 ± 0.52^{a}	4.10 ± 0.34^{a}	6.58 ± 0.54^{a}	38.66 ± 2.65^{a}

Data presented as mean value ± standard deviation. In the same column, different superscript letters in different treatments indicate that they are significantly different at a significance level of 0.05.

7.3.2 Physicochemical properties

Fourier transform infrared (FTIR) spectroscopy has proven to be a significant analytical instrument within the field of phytomedicine over recent decades. It is a quick, cost-effective technique that requires only a small sample to identify the chemical groups in herbal samples (Martins et al., 2023). FTIR spectra allow for the detection of specific wavenumber peaks that indicate the presence of chemical bonds in the phytocompounds (Mulaudzi et al., 2024). The mid-infrared spectrum results are divided into four distinct regions. The first is the single bond region, spanning 2500-4000 cm^{-1}. The second is the triple bond region, ranging from 2000-2500 cm^{-1}. The third region, known as the double bond region, lies between 1500-2000 cm^{-1}. Lastly, the fingerprint region, covering 600-1500 cm^{-1}, contains a complex array of absorption bands, making it highly specific to individual molecules (Nandiyanto et al., 2019).

The FTIR spectra for *P. sidoides* roots are presented in Figure 7.4A (harvest 1) and 7.1B (harvest 2). The spectra depict peaks observed at 3848 cm-1 and 3671 cm-1, which suggest the presence of hydroxyl (OH) groups stretching. The bands at 3224 and 3302 cm-1 suggest the presence of N-H stretching vibrations, possibly indicating the presence of amines or amides. The peaks at 2974 and 2976 cm-1 signify C-H bonds. The bands at 2918 and 2918 cm-1 also signify the C-H stretching. Bands at 2275 and 2319 cm-1 signify carbon triple bond (C≡C). Peaks observed at 1928, 1922, 1620 and 1617 cm-1 correspond to C=C (carbonyl amide) stretching vibrations. Peaks at 1408 and 1417 cm-1 indicate CH2 bending vibrations (aromantic ring stretches). Peaks at 1328, 1235,1227, 1056, and 885 cm-1 are attributed to C-O stretching vibrations in esters, phenols, or alcohols. Similar peak at 1040 cm-1 assigned to C-O was reported by Badeggi et al. (2020). The bands at 765 and 753 cm-1 signify bending vibrations of C-H out of plane bending that is typically found in aromantic rings. The peak at 547 and 531cm-1 skeletal vibrations of aromatic structures. The functional groups such as

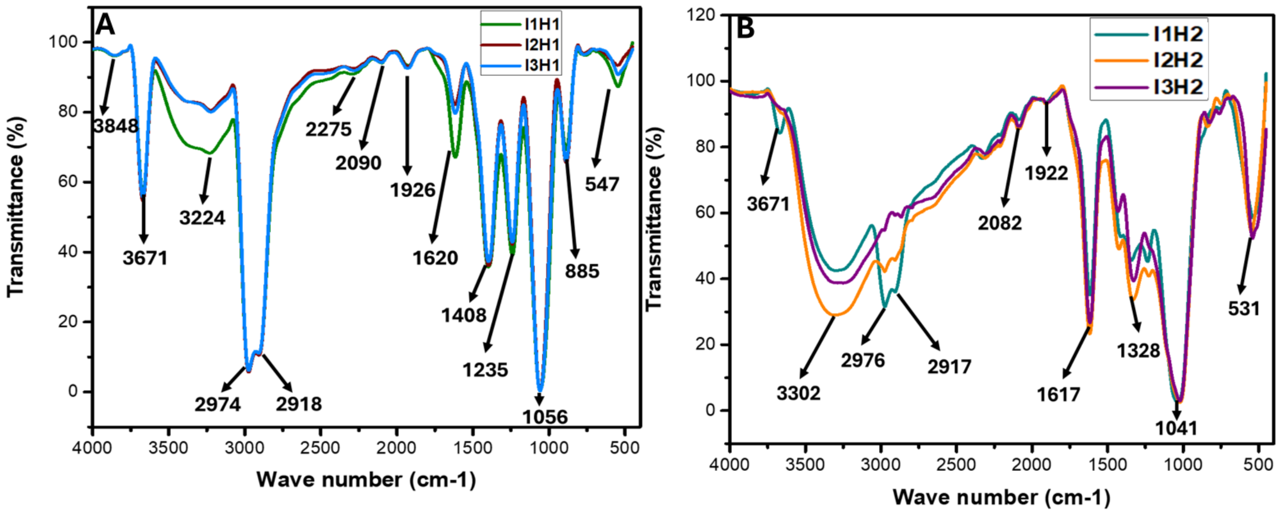


Figure 7.4 FTIR spectra of the first harvest (A) and second harvest (B) of *P. sidoides* roots showing the effect of three different irrigation levels (I1=75% PAW, I2=50% PAW, I3=25% PAW)

hydroxyl, amides, alkynes, alkenes, and carbonyl groups that were found in the *P. sidoides* roots correlate with the reported presence of phytochemicals such as oxygenated coumarins, phenolics, flavonoids and proanthocyanidins in *P. sidoides* samples (Van Wyngaard et al., 2023; Jekabsone et al., 2019; Kolodziej, 2007). In particular, the phenolic and flavonoids are discussed in subsequent sections of this Chapter.

Chemometrics is the application of mathematical and statistical methods to analyze chemical data. This focuses on extracting meaningful information from complex chemical systems, often utilizing techniques from multivariate statistics, applied mathematics, and computer science (Huang, 2022). Principal Component Analysis (PCA) is a fundamental unsupervised technique in chemometrics, particularly useful for analyzing complex chemical data derived from various measurements, such as spectroscopic data (Kharbach et al., 2023). In qualitative analysis, PCA is the most widely used method for unsupervised analysis (Mulaudzi et al., 2024; Umar et al., 2023).

In this study, PCA was used to qualitatively clarify the relationship between spectra obtained from *P. sidoides* root samples in response to different harvest age and irrigation levels. The PCA scores plot show variation at 95.6% for PC1 while PC2 has variation of 3.06% (Figure 7.5A and 7.5B). The PCA scores plot show clear separation between the first harvest (at 6 months after treatment initiation) and second harvest (at 12 months after treatment initiation) (Figure 7.5A), with first harvest (6 Months) located on the first and fourth quadrants while the second harvest (12 Months) is located on the second and third quadrants. In figure 7.5B the plot shows variations between the first harvest irrigation levels, with the 75% PAW level on the first quadrant while irrigation level at 50% and 25% are closely packed on the fourth quadrant. This observation correlates with the spectra as the transmittance of functional groups at 75% PAW varies from the 50% and 25% PAW. The proximity of irrigation at 50% and 25% PAW suggests that the physicochemical properties of the samples are similar. On the second harvest, the roots from the severe stressed (25% PAW) *P. sidoides* plants show more variance from irrigation at 75% and 50% PAW that are showing close properties. The PCA score relationship in the second harvest correlates with quantitative flavonoids results (Table 7.3) where irrigation at 75% and 50% PAW were 59.55 and 52.01 mg QE/g respectively, on PCA these scores are both located on the second quadrant. At 25% PAW, the total flavonoid content was 42.62 mg QE/g and this sample is located on the third quadrant.

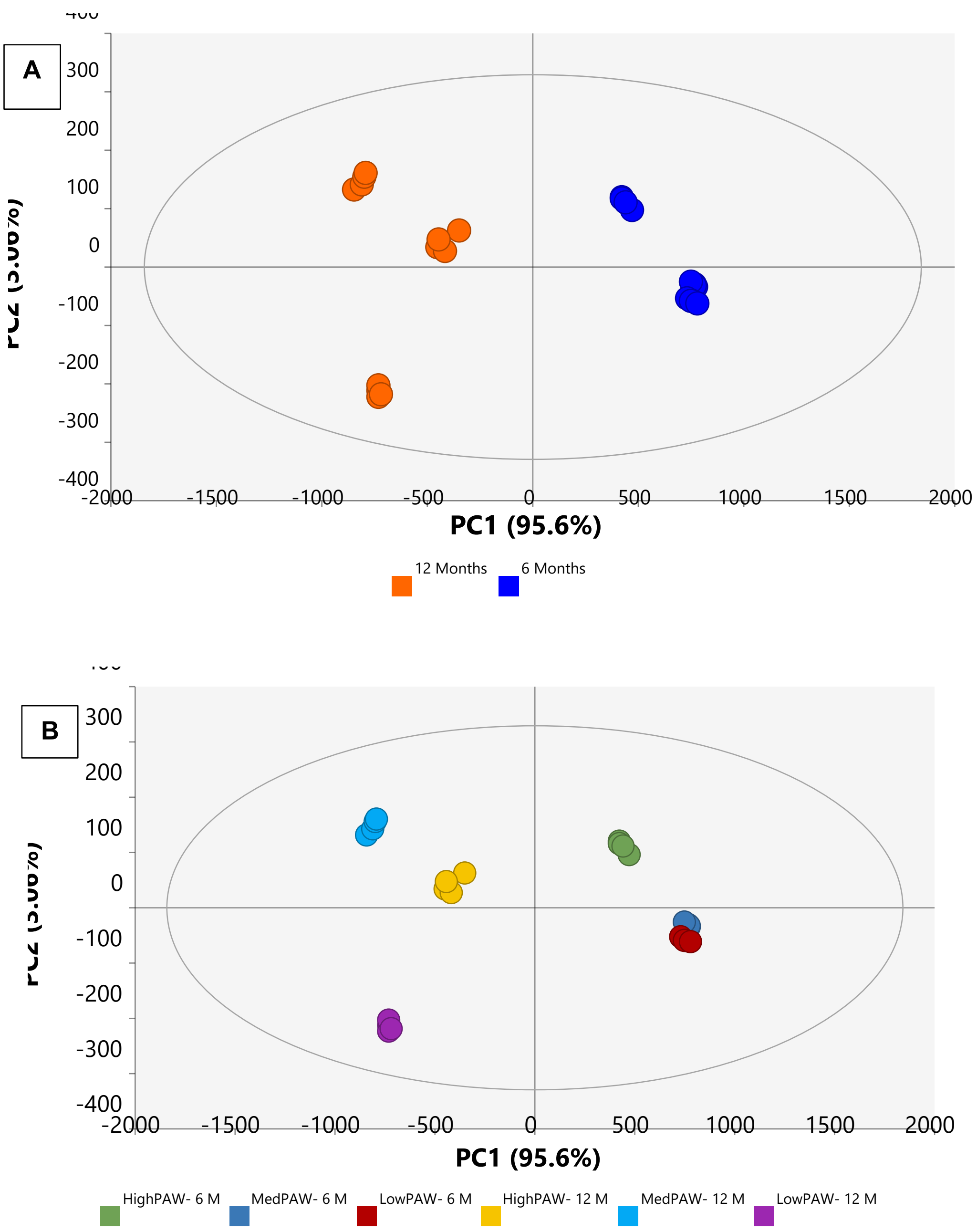


Figure 7.5 Principal component assay scores of *P. sidoides* FTIR data (A) Two harvests (6 and 12 Months after treatment application); (B) different levels of irrigation, high (75% PAW), med (50% PAW), low (25% PAW) and harvest at 6 and 12 Months

7.3.3 Preliminary phytochemical screening

Preliminary phytochemical screening is one of the first tests done in medicinal plants for qualitative determination of phytochemical compounds. These tests are relatively cost effective, easy to perform and time efficient (Maheshwaran et al., 2024). They are used to determine the presence or absence of phytochemical compounds such as saponins, flavonoids, terpenoids, phenols and others (Nortjie et al., 2022). The phytochemical analysis of *P. sidoides* extracts (Table 7.2) revealed the presence of saponins, flavonoids, terpenoids, phenols, tannins, and coumarins across all irrigation levels and harvest ages. However, the steroids and alkaloids tests were negative, suggesting their absence or an indication that they are in levels below the detection limit for the assay adopted in this study. Steroids are usually found in vast plant samples. However, they are usually in small quantities. Their biological activity may be because of synergistic interaction with compounds such as polyphenols (Gadauche et al., 2023). In a study done by Kgatshe et al. (2019), the authors also reported the absence of alkaloids in *P. sidoides*. Saponins are known for their antimicrobial, antitumor, and antioxidant properties (Wang et al., 2022; Sharma et al., 2021). Flavonoids exhibit a range of

Table 7.2 Preliminary phytochemical screening of *P. sidoides* roots at different harvesting age in response to different levels of irrigation.

Phytochemical	**Test**	**Harvest 1**			**Harvest 2**		
		75% PAW	50% PAW	25% PAW	75% PAW	50% PAW	25% PAW
Saponins	Froth test	+	+	+	+	+	+
Flavonoids	Sulfuric acid test	+	+	+	+	+	+
	Alkaline reagent test	+	+	+	+	+	+
Steroids	Acetic anhydride test	-	-	-	-	-	-
Terpenoids	Salkowski test	+	+	+	+	+	+
Tannins	Ferric chloride test	+	+	+	+	+	+
	Alkaline reagent test	+	+	+	+	+	+
	Gelatin’s test	+	+	+	+	+	+
Phenols	Ellagic acid test	+	+	+	+	+	+
	Ferric chloride test	+	+	+	+	+	+
	Lead acetate test	+	+	+	+	+	+
Alkaloids	Hager’s reagent test	-	-	-	-	-	-
Coumarins		+	+	+	+	+	+

+ = Presence; - = Absence, at different levels of irrigation; PAW = Plant Available Water pharmacological activities, including antimicrobial, anticancer, and neuroprotective effects, as well as applications in natural dyes, cosmetics, and skincare products (Ullah et al., 2020). Terpenoids are noted for their antibacterial, antiviral, and anti-inflammatory properties (Kamran et al., 2022; Yang et al., 2020). Phenols have been reported to have cardioprotective, antimicrobial, and antioxidant effects (Cosme et al., 2020). Tannins possess bactericidal, antioxidant, and anti-inflammatory properties (Maugeri et al., 2022; Pizzi, 2021).

Coumarins are known for their diverse pharmacological activities, including anti-inflammatory, anticoagulant, and neuroprotective effects (Kgatshe et al., 2019). These findings align with previous studies confirming the presence of saponins (Kgatshe et al., 2019), phenols (Yousafean et al., 2023), tannins (Schötz and Nöldner, 2007), flavonoids (Mativandlela et al., 2007), terpenoids (Panara et al., 2021) and coumarins (Mativandlela et al., 2007) in extracts of *P. sidoides*.

7.3.4 Qualitative phytochemical profiling

Thin layer chromatography (TLC) is used for phytochemical profiling. It is a crucial method in herbal medicine, widely used for the characterization and quality control of natural remedies. It serves as a straightforward and traditional technique for analyzing quality markers in natural products (Chen et al., 2021). The TLC results for all the treatments in this study showed the presence of esculin band as compared to the esculin reference on track 6 (Figure 7.6). When viewed under UV light, the bands suggest the different phytochemicals that are separated by the chromatography. The appearance of these bands suggests that the *P. sidoides* roots analyzed in this study contain different phytochemicals. Umckalin and scopoletin bands appeared near the end point. At all irrigation levels, tracks 1- 4 show luminescence at the start point and the end point, which suggests that there were still phytochemicals not separated on the samples. In the High-performance thin layer chromatography (HPTLC) analysis, *P. sidoides* roots separated into seven distinct bands when viewed under UV light at 366 nm with one of them being the marker compound umckalin (Maree and Viljoen, 2012). Another study by Mofokeng (2015), used TLC to analyze *P. sidoides* root samples. The results showed the presence of scopoletin and esculin in 12 treatments. However, the TLC plate did not indicate any differences in compound concentrations between samples, since all treatment showed similar UV light absorbance. The TLC results showing the presence of esculin aligns with preliminary phytochemical screening done on the samples, showing the coumarins.

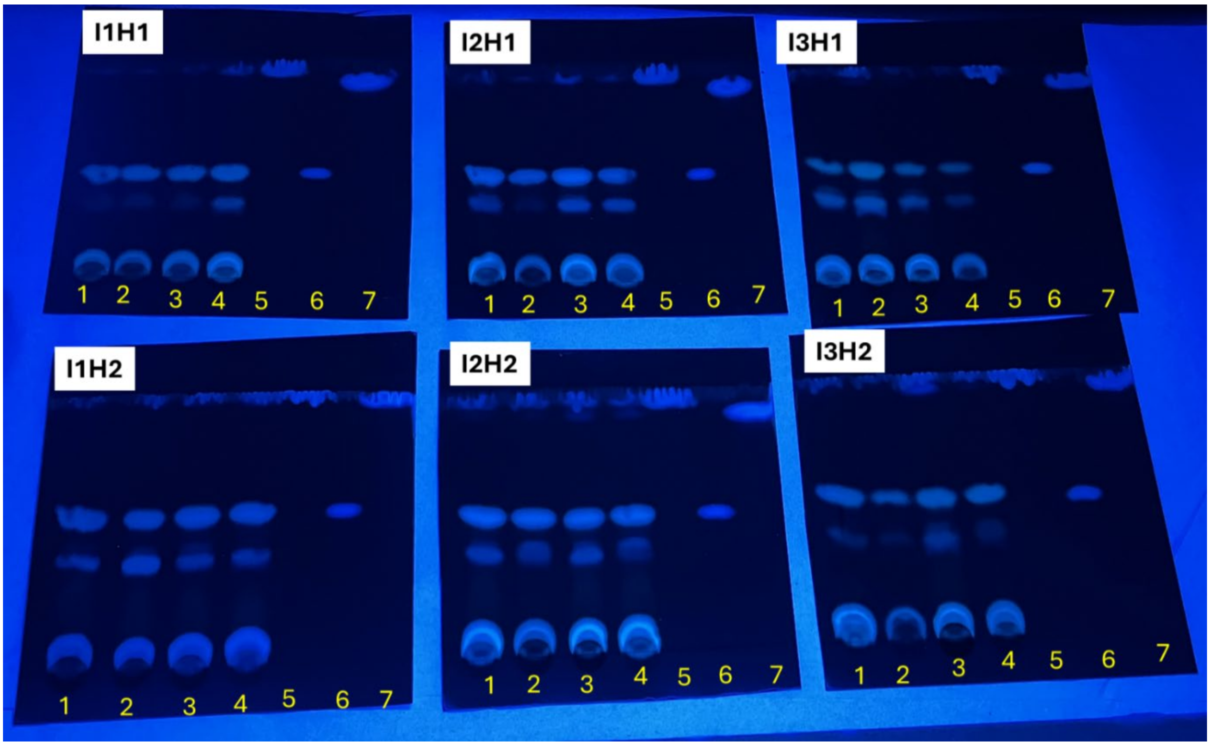


Figure 7.6 Thin layer chromatography (TLC) of the first and second harvests (H1 and H2) of *P. sidoides* roots at different irrigation levels (I1=75% PAW, I2=50% PAW & I3=25% PAW).
1 – 4 = repetition of the same treatment, compared with reference compounds (5 = Umckalin, 6 = Esculin and 7 = Scopoletin).

7.3.5 Quantitative phytochemical analysis

7.3.5.1 Total phenolic content (TPC) and Total flavonoid content (TFC)

Phenolic compounds are one of the most important metabolites, the determination of their content is an important parameter to evaluate the pharmacological potential of plant extracts (Chiavaroli et al., 2023). Flavonoids are known for their important roles in preventing diseases that are associated with oxidative stress (Nwozo et al., 2023). The results for total phenolic content (TPC) and total flavonoid content (TFC) of *P. sidoides* across two harvests (H1 and H2) under three different irrigation levels (I1, I2 and I3) are presented in Table 7.3. For both TPC and TFC, different irrigation levels and harvest age did not have any effect on these parameters. In this study, despite the differences in age at harvest and water stress, none of the variations in TPC and TFC are significantly different from one another, indicating that phenolic and flavonoid contents remain relatively stable across the different irrigation levels irrespective of the harvest age.

Moreover, the quantitative results of TPC and TFC align with the results obtained for qualitative primary phytochemical analysis and TLC. However, the lack of statistically significant difference in TPC and TFC assay results suggests that irrigation and age at harvest do not have any effect on TPC and TFC of *P. sidoides* roots.

The indifference regardless of irrigation and harvest age may be due to the ability of plants to mitigate stress conditions and still be able to produce secondary metabolites. The production of metabolites by plants is seen as an adaptive mechanism to handle stressful conditions in a changing and challenging environment (Isah, 2019). The production of signalling molecules such as abscisic acid and jasmonic acid mitigate water stress in plants, they upregulate the production of metabolites such as phenols, flavonoids, terpenoids and tannins (Mundim and Pringle, 2018).

Table 7.3 Total phenolic and total flavonoid content of *P. sidoides* aqueous ethanolic extracts of two different harvests and at three irrigation levels

Irrigation (PAW) %	Total phenolic content (mg GAE/g)		Total flavonoid content (mg QE/g)	
	Harvest 1	Harvest 2	Harvest 1	Harvest 2
75	6.02 ± 0.97^a	6.84 ± 1.045^a	39.42 ± 4.92^a	59.55 ± 18.39^a
50	5.39 ± 1.34^a	6.14 ± 1.29^a	38.20 ± 10.34^a	52.01 ± 16.83^a
25	5.91 ± 1.49^a	5.89 ± 2.75^a	45.3 3± 12.85^a	42.62 ± 31.52^a

PAW = Plant available water. Data presented as mean value ± standard deviation. On same test (TPC or TFC), same superscript letters in different treatments indicate that they are not significantly different ($p \leq 0.05$).

7.3.6 Antioxidants capacity

7.3.6.1 ABTS and DPPH radical scavenging activity

Oxidative stress is linked with the progression of several diseases that are chronic and degenerative. Increasing the production of free radicals leads to oxidative stress (Teleanu et al., 2022). Antioxidants are the key players in controlling free radical levels. Phytochemicals are believed to be among the most powerful antioxidants which makes them an interest (Chiavaroli et al., 2023). The type and prevalence of antioxidant classes present in a plant sample can influence the measured antioxidant capacity (Pisoschi et al., 2016). Table 7.4 presents the ABTS and DPPH radical scavenging activities of *P. sidoides* across two harvests under different irrigation levels. For ABTS radical scavenging activity, the results are consistent across both harvests, which shows that the different irrigation levels did not have any significant difference on the ABTS radical scavenging activity of *P. sidoides* roots. The ABTS assay trend observed on harvest age aligns with the trend observed in TPC and TFC results. However, the lack of significant variation suggests that the ABTS radical scavenging activity by *P. sidoides* is unaffected by changes in irrigation levels and harvest age.

The DPPH radical scavenging activity shows more variation across treatments and harvests (Table 7.4). In Harvest 1, there is no significant difference in DPPH across the irrigation treatments. For the second harvest however, the DPPH activity was significantly higher in the well-watered plants (75% PAW) than that of the severely stressed plants (25% PAW). Moreover, compared to Harvest 1 with DPPH values ranging from 4.13 – 4.46 mg TEAC/g, Harvest 2 had DPPH values ranging from 4.82 – 5.41 mg TEAC/g. This indicates that DPPH radical scavenging activity was higher as the plants grew older. In addition, the DPPH radical scavenging assay of *P. sidoides* trends shown at different harvest age are similar to the observed trend for TPC and TFC. Although, there were no significant differences in TPC and TFC, however, there were observable increases in the values obtained for the older plants. This suggests that compared with H1, the second harvest at 12 months post treatment had higher levels of antioxidants that can scavenge DPPH. The correlation between quantitative phytochemical analysis and DPPH radical scavenging assay implies that phenols and flavonoids may be the prevalent molecules responsible for scavenging DPPH radicals.

Table 7.4 ABTS and DPPH radical scavenging activity of *P. sidoides* root extract presented in trolox equivalent antioxidant capacity of three irrigation levels and two harvest age (at 6- and 12-months post treatments).

Irrigation (PAW) %	**ABTS radical scavenging activity (mg TEAC/g)**		**DPPH radical scavenging activity (mg TEAC/g)**	
	Harvest 1	**Harvest 2**	**Harvest 1**	**Harvest 2**
75	3.42 ± 0.02^{a}	3.39 ± 0.05^{a}	4.31 ± 0.19^{cd}	5.41 ± 0.23^{a}
50	3.43 ± 0.03^{a}	3.40 ± 0.03^{a}	4.46 ± 0.12^{cd}	5.27 ± 0.23^{ab}
25	3.41 ± 0.01^{a}	3.38 ± 0.05^{a}	4.13 ± 0.39^{d}	4.82 ± 0.81^{bc}

PAW = plant available water. Data presented as mean value ± standard deviation. On same test (ABTS or DPPH), same superscript letters in different treatments indicate that they are not significantly different ($p \leq 0.05$).

7.3.7 Nutrient composition of *Pelargonium sidoides* leaf

The chemical properties of *P. sidoides* leaves showed that both harvest age and water deficit stress had no influence on N, P, Ca, Mg, Na and Fe contents of the species (Table 7.5). One possible explanation for the lack of changes in N, P, Ca, Mg, Na and Fe contents in the leaves of *P. sidoides* across both harvests may be due to the resilience and stress tolerance of this medicinal plant (Manganyi et al., 2018). The plant may effectively regulate its metabolism under stress, allowing it to maintain a consistent chemical composition across harvests. Potassium (K) levels in I1 were significantly high at H1 but showed a sharp decline in H2. However, Mn, Cu and Zn mostly increased at H2. These inconsistencies in nutrient composition of *P. sidoides* eaves may be due to several environmental, and soil related factors such as, reduced soil moisture.

7.3.8 Nutrient composition of *Pelargonium sidoides* root

The roots of *Pelargonium sidoides* were only analysed for nutritive value during the second harvest (H2). Root nutrient analysis revealed that there were no differences in the nutritional composition of *P. sidoides* despite being subjected to different levels of irrigation (Table 7.6). Indicating that water stress would not change the nutrients contents in the roots of the species. Nutritional composition of *P. sidoides* remains the same, whether the plants are growing under harsh condition of drought or have adequate access to soil water.

Table 7.5 Leaf chemical composition of *P. sidoides* during the first (H1) and second (H2) harvests

Treatment		Parameters										
Harvest 1		**N**	**P**	**K**	**Ca**	**Mg**	**Na**	**Mn**	**Fe**	**Cu**	**Zn**	**B**
	I1	1.2725^{a}	0.24000^{a}	1.4075^{a}	1.7150^{a}	0.26250^{a}	109.33^{a}	209.50^{ab}	368.8^{a}	5.550^{b}	368.8^{a}	48.425^{ab}
	12	1.2250^{a}	0.29000^{a}	1.4075^{a}	1.8150^{a}	0.30500^{a}	153.58^{a}	161.50^{b}	437.5^{a}	6.525^{ab}	437.5a	38.450^{b}
	13	1.2400^{a}	0.28750^{a}	1.3175^{ab}	1.9575^{a}	0.34000^{a}	186.95^{a}	177.50^{ab}	423.8^{a}	5.800^{ab}	423.8^{a}	53.025^{a}
Harvest 2												
	I1	1.1375^{a}	0.31250^{a}	0.9950^{c}	2.0825^{a}	0.33250^{a}	160.25^{a}	244.50^{a}	715.0^{a}	102.60^{a}	64.38^{b}	38.500^{b}
	12	1.0925^{a}	0.30750^{a}	1.1375^{bc}	2.1400^{a}	0.27500^{a}	109.35^{a}	252.75^{a}	646.3^{a}	57.15^{b}	65.00^{b}	48.700^{ab}
	13	1.2400^{a}	0.28750^{a}	1.2000^{abc}	2.0675^{a}	0.33500^{a}	124.25^{a}	218.50^{ab}	711.3^{a}	68.30^{b}	46.75^{b}	50.025^{a}
	LSD	0.3017	0.0782	0.2589	0.5168	0.0845	94.328	76.769	557.22	3.238	33.445	10.387

Values with the same letters in the same column are not significantly different (P≤0.05).

Table 7.6 Root nutritional composition of *P. sidoides*

Treatment	Parameters										
	N	**P**	**K**	**Ca**	**Mg**	**Na**	**Mn**	**Fe**	**Cu**	**Zn**	**B**
I1	0.7600^{a}	0.22500b	0.6650a	0.9300a	0.26000a	488.0a	78.43a	451.5a	7.850a	60.30a	9.5250a
12	0.8325^{a}	0.30250ab	0.7350a	0.6375a	0.29500a	335.9a	61.58a	387.5a	5.775a	49.80a	8.4000a
13	1.2350^{a}	0.35500a	0.6550a	0.8025a	0.33250a	544.5a	59.73^{a}	161.0a	9.150a	55.78a	9.4000a
LSD	0.5517	0.091	0.395	0.385	0.1361	545.99	65.992	804.83	5.4403	31.426	1.6633

Values with the same letters in the same column are not significantly different ($P \leq 0.05$).

7.3.9 Soil chemical composition

7.3.9.1 Soil micronutrient

The levels of micronutrients in the soil after the first and second harvests (H1 and H2) of *P. sidoides* are presented in Table 7.7. Preplanting values for soil chemical properties were recorded at the beginning of the study to assess the change brought about by water deficit stress treatments over time. Soil micronutrients Cu, Zn, Mn, B and Fe were investigated. Cu level dropped for all treatments at H2, Zn level increased in the 50% and 75% soil water depletion levels while that of 25% water depletion decreased at H2, Mn levels increased among all irrigation levels at H2 while B and Fe levels in the soil remained more or less the same at H1 and H2. The observed increase in some micronutrients suggests that *P. sidoides* was not able to assimilate these micronutrients although, present in the soil and even facilitated increases across treatments. Another reason may be due to soil carbon (C) percentage which increased with every harvest. Soil C is normally responsible for the binding of trace minerals. This outcome was also corroborated by the results of the root nutritional composition in this study. Despite the varying levels of these microminerals in the soil with some increases, the micronutrient contents in the roots of *P. sidoides* still remained the same. This indicates that the observed increase in the levels of these minerals at H1 and H2 compared to the preplanting levels did not reflect in the root composition, since the micronutrients levels remained the same.

Table 7.7 Effect of diverse levels of irrigation on soil micronutrients at the first (H1) and second (H2) harvest of *P. sidoides*

	Treatment	Cu (mg/kg)	Zn (mg/kg)	Mn(mg/kg)	B(mg/kg)	Fe(mg/kg)
Harvest 1						
	I1	0.86750^{a}	3.7250^{a}	14.275^{b}	0.47000^{a}	145.75^{a}
	I2	0.76250^{ab}	2.8000^{ab}	10.550^{b}	0.45500^{a}	142.75^{a}
	I3	0.74750^{b}	2.2000^{b}	9.525^{b}	0.45750^{a}	137.75^{a}
Harvest 2						
	I1	0.66250^{bc}	2.5500^{ab}	43.425^{a}	0.43000^{a}	239.25^{a}
	I2	0.53750^{c}	3.4250^{ab}	27.250^{ab}	0.42250^{a}	184.50^{a}
	I3	0.56000^{c}	2.9250^{ab}	27.925^{ab}	0.42750^{a}	215.00^{a}
Baseline		0.49	0.92	1.90	<0.10	65.30
LSD		**0.1359**	**1.3136**	**20.446**	**0.0875**	**108.75**

Values with the same letters in the same column are not significantly different (P≤0.05).

7.3.9.2 Soil chemical properties

The soil chemical properties after the first (H1) and second (H2) harvest show that the pH, NO_3-N, P and the cation exchange capacity (CEC) pH remained unchanged and did not differ significantly from the initial levels in the soil prior to the commencement of the study (Table 7.8), the soil remained acidic. Water stress and age at harvest did not affect these soil parameters. The level of Ca, NH_4-N and K decreased at H2 compared to H1. Within the same harvest age, water stress did not affect Ca, NH_4-N and K concentrations either at H1 or H2. However, the level of these nutrients at H1 were significantly higher than that of H2. This is an indication that the soil on which the *P. sidoides* plants were growing diminished in Ca, NH_4-N and K, with time. On the contrary, carbon (C) and Mg increased significantly at H2 compared to H1. However, among irrigation levels within the same harvest age, C and Mg concentrations remained unchanged. This higher levels of C and Mg at H2 suggested that soil elements increased with time. This was observed from the preplanting to the second harvest stage which increased in the order initial < H1 < H2. Sodium (Na) and potassium (K) levels in the soil were higher when the soil was well-watered while the soil Na and K slightly decreased at harvest 2 (H2).

Table 7.8 Effect of diverse levels of irrigation on soil chemical properties at the first (H1) and second (H2) harvest of *P. sidoides*

	Treatments	pH (KCl)	Ca (mg/kg)	NH$_4$-N (mg/kg)	NO$_3$-N (mg/kg)	CEC (cmol/kg)	C (%)	Na (mg/kg)	K (mg/kg)	Mg (mg/kg)	P (Bray2) (mg/kg)
Harvest 1	**I1**	4.9800a	661.70a	19.970a	2.530a	4.0625a	0.8075b	31.650bc	187.25a	145.70b	97.23a
		4.6775a	637.23a	18.630a	4.900a	3.6400a	0.7175b	27.576c	128.00ab	145.90b	61.75a
		4.6875a	611.18a	18.950a	2.630a	3.6200a	0.7100b	28.850c	139.00ab	152.88b	64.25a
Harvest 2	**I1**	4.8250a	492.75b	4.350b	5.475a	3.7650a	1.1125a	41.700ab	98.58b	187.93a	82.10a
	I2	4.7750a	482.80b	5.000b	6.400a	3.6625a	1.1150a	46.875a	92.40b	175.18a	44.38a
	I3	4.7750a	509.95b	5.650b	6.400a	3.5650a	1.2425a	49.225a	91.32b	187.48a	60.33a
Baseline		**4.53**	**318.00**	**11.48**	**5.52**	**2.50**	**0.43**	**45.00**	**77.00**	**67.00**	
LSD		**0.37**	**7.8297**	**2.5477**	**4.4461**	**0.8738**	**0.2397**	**0.0681**	**71.97**	**0.2903**	**67.016**

Values with the same letters in the same column are not significantly different (P≤0.05).

7.4 CONCLUSIONS

Phytochemical analysis of *P. sidoides* roots was able to show that irrigation and harvest age can affect the chemical composition of the plant. The colour profile analysis revealed that water stress can be a factor that can influence the colour of *P. sidoides* roots. Despite the complexity of FTIR data, the use of chemometrics method (PCA) proved to be a valuable aspect in the investigation of the relationship between the different treatments. Qualitative phytochemical methods were able to show the presence of different phytochemicals, thus, rendering the methods as good starting point on determining the phytochemicals present in the roots of *P. sidoides*. Radicals scavenging assays suggested that both irrigation and harvest age can influence antioxidant activity.

Both harvest age and water deficit stress had no impact on N, P, Ca, Mg, Na and Fe contents of *P. sidoides* shoot. Potassium (K) levels in I1 were significantly high at H1 but showed a sharp decline in H2. However, Mn, Cu and Zn mostly increased at H2. These inconsistencies in nutrient composition of *P. sidoides* eaves may be due to several environmental, and soil related factors such as, reduced soil moisture. Water stress did not change the nutrients contents in the roots of the species. Nutritional composition of *P. sidoides* remains the same, whether the plants are growing under harsh condition of drought or have adequate access to soil water.

Soil chemical analysis showed an increase in some micronutrients which suggests that *P. sidoides* was not able to assimilate these micronutrients although, present in the soil and even facilitated increases across treatments. This may be due to soil C which increased with every harvest. Soil C is normally responsible for the binding of trace minerals. Soil loses its NH_4-N concentration with time if there is no additional supply of nitrogen to the soil. Moreover, water deficit stress did not affect the level of NH_4-N. Sodium (Na) and potassium (K) levels in the soil were higher when the soil was well-watered while the soil Na and K slightly decreased at harvest 2 (H2).

CHAPTER 8: CONCLUSIONS AND RECOMMENDATIONS

8.1 GENERAL CONCLUSIONS

In this study we investigated the effect of different irrigation levels and harvest age on the growth, yield and chemical composition of *Pelargonium sidoides*. The study started with a detailed knowledge review (inception report) which was conducted and submitted as Deliverable 1 to WRC in May 2022. The review focussed on all aspects of the agronomy and water requirements of *P. sidoides*. The production, wild harvesting, distribution and nutritional requirements, as well as breeding / screening of *P. sidoides* for water use were covered. It further revealed that there are no cultivation and irrigation guidelines for *P. sidoides*. We were unable to extract any information from the public domain on the breeding of *P. sidoides* for water use efficiency. Optimum harvesting age for the species is also controversial.

The trial was a pot study conducted inside a tunnel at the Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Stellenbosch, South Africa (Latitude 33°54’52.38”S Longitude 18°51’40.27”E). The project had four Objectives, and all the four Objectives were successfully executed. There were three irrigation levels (75% PAW, 50% PAW and 25% PAW). Harvesting was done twice, at 6 months intervals. Plants were grown for 7 months before deficit irrigation treatments were applied. Data were collected monthly on morphophysiological and biochemical traits, soil chemical properties, soil enzyme activity, root and shoot yield, chemical composition of the shoot and root of *P. sidoides*.

P. sidoides adopted various strategies in response to water deficit stress, with different irrigation levels and harvest age having diverse impacts on the measured parameters. Overall, the well-watered plants (75% PAW) exhibited superior performance across morphological, physiological, and yield parameters of the plant. However, the fresh root yield of the moderately stressed (50% PAW) was better than that of the well-watered plants during the second harvest (H2). The dry matter yield at H2 also recorded the highest dry yield for plants subjected to 50% PAW. In general, as water deficit became more intense (25% PAW), most of the measured plant parameters experienced a decline. Most crops are typically water dependent. However, looking at the yield, this study demonstrates that *P. sidoides* can be successfully cultivated commercially without the need for complex irrigation programs. The plants are stress tolerant and the fresh and dry root yield of the most stressed plants competed well and, in some cases, even yielded better than the well-watered *P. sidoides* plants. Minimal water use can contribute to the production of more marketable root tubers, which is the most important part of the plant. Another observation from the study is that higher watering frequency increased fresh root mass but did not affect dry root mass. H2 had improved fresh and dry root yield compared to H1, especially for the moderately (50% PAW) and severely (25% PAW) stressed plants, with the well-watered plants (75% PAW) suffering a decline in yield at both harvests. Farmers can reduce production cost vis-à-vis irrigation cost by decreasing watering frequency, as frequent irrigation showed no significant impact on dry root yield. Too much soil moisture also resulted in root rot in some of the well-watered plants. Therefore, it is essential to establish proper irrigation guidelines for the cultivation of *P. sidoides.* The results show that 75% water depletion (25% PAW) level increases lipid peroxidation in *P. sidoides*. Conversely, this severely stressed level lowers CO_2 assimilation and reduce leaf water potential. Furthermore, 75% water depletion level lowers chlorophyll content and cell growth of *P. sidoides*.

Phytochemical analysis of *P. sidoides* roots show that irrigation and harvest age can affect the chemical composition of the plant. The colour profile analysis revealed that water stress can be a factor that can influence the colour of *P. sidoides* roots. Despite the complexity of FTIR data, the use of chemometrics method (PCA) proved to be a valuable aspect in the investigation of the relationship between the different treatments. Qualitative phytochemical methods were able to show the presence of different phytochemicals, thus, rendering the methods as good starting point on determining the phytochemicals present in the roots of *P. sidoides*. Radicals scavenging assays suggested that both irrigation and harvest age can influence antioxidant activity.

For root nutrient composition, there were no differences in the nutritional composition of *P. sidoides* despite being subjected to different levels of irrigation. Indicating that water stress would not change the nutrients contents of the species. Both harvest age and water deficit stress had no impact on N, P, Ca, Mg, Na and Fe contents of *P. sidoides* shoot (leaves). K levels in I1 were significantly high at H1 but showed a sharp decline in H2. However, Mn, Cu and Zn mostly increased at H2. These inconsistencies in nutrient composition of *P. sidoides* leaves may be due to several environmental, and soil related factors such as, reduced soil moisture.

Soil chemical analysis showed an increase in some micronutrients which suggests that *P. sidoides* was not able to assimilate these micronutrients although, present in the soil and even facilitated increases across treatments.

Overall, soil enzyme activity showed a drastic decline in H2. Deficit irrigation did not affect β-glucosidase activity within treatments, although, activity decreased at H2. The same trend was observed for Acid-phosphatase and urease. No significant difference was observed in AI3 across both harvests. At H1, the AI3 index scores became less negative as the water stress increased while it dropped to the pre-planting level and even lower (at 50% water depletion level) at H2. The decline in soil enzyme activity and AI3 index scores as the plants progressed in age indicated that soil fertility status had declined, and this could be attributed to diminishing nutrients in the soil which in turn had a negative impact on the microbial activity and microbial community structure.

8.2 RECOMMENDATIONS AND FUTURE RESEARCH WORK

From the findings from this study, 50% water depletion level (50% PAW) is highly recommended for the cultivation of *P. sidoides*. Growing the species at 50% PAW favours tuber production since highest yield (fresh and dry) was obtained at this irrigation level. Root rot can also be prevented at 50% water depletion level. Age at harvest is another crucial factor in the cultivation of *P. sidoides*. It is also recommended that harvesting be delayed until the plants are advanced in age, since this study recorded highest fresh and dry root yield at the second harvest (H2).

However, there is a need for more research on the agronomy of *P. sidoides*. The lack of agronomic information may restrict the promotion of cultivation of *P. sidoides*, particularly within commercial farming systems. There are no cultivation and irrigation guidelines for *P. sidoides*. There are also conflicting reports about the harvesting age at which maximum medicinal benefits in the roots is attained. Cultivation might be limited in scope probably due to the long growth cycle, estimated to be 7-9 years before commercial maturity is reached. This Final report only covered two and half years of the research and just two years of growth of *P. sidoides,* therefore, the extension of this study is recommended as it will address the knowledge gap. Therefore, further research is needed in order to provide quality findings and irrigation guideline for *P. sidoides* cultivation.

REFERENCES

ABUBAKAR, A.R., HAQUE, M., 2020. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences, 12(1), pp.1-10.

ACB, 2008. Knowledge not for sale: Umckaloabo and the Pelargonium patent challenges. Briefing paper, ACB, Johannesburg.

ADEBO, O.A., NJOBEH, P.B., KAYITESI, E., 2018. Fermentation by *Lactobacillus fermentum* strains (singly and in combination) enhances the properties of ting from two whole grain sorghum types. Journal of Cereal Science, 82, pp.49-56.

ADEWUSI, E.A., AFOLAYAN, A.J., 2009. Antibacterial, antifungal and antioxidant activity of the roots and leaves of *Pelargonium reniforme* Curtis (Geraniaceae). African Journal of Biotechnology, 8(22), 6425-6433.

AHMED, M., JI, M., SIKANDAR, A., IRAM, A., QIN, P., ZHU, H., JAVEED, A., SHAFI, J., IQBAL, Z., FARID IQBAL, M., SUN, Z., 2019. Phytochemical analysis, biochemical and mineral composition and GC-MS profiling of methanolic extract of Chinese arrowhead *Sagittaria trifolia* L. from Northeast China. Molecules, 24(17), 3025.

AL, C., SUN, J.W., WANG, X.B., LIAND, G.Q., HE, P., ZHOU, W., 2015. Advances in the study of the relationship between plant rhizodeposition and soil microorganism. Journal of Plant Nutrition and Fertilizers, 21(5): 1343-1351. [in Chinese].

ALI, S., KHAN, M.R., IRFANULLAH, SAJID, M., ZAHRA, Z., 2018. Phytochemical investigation and antimicrobial appraisal of *Parrotiopsis jacquemontiana* (Decne) Rehder. BMC Complementary and Alternative Medicine, 18, pp.1-15.

ALKORTA, I., AIZPURUA, A., RIGA, P., ALBIZU, I., AMÉZAGA, I., GARBISU, C., 2003. Soil enzyme activities as biological indicators of soil health. Reviews on Environmental Health, 18(1): 65-73.

ALOSSAIMI, M.A., ALZEER, M.A., ABDEL BAR, F.M., ELNAGGAR, M.H., 2022. *Pelargonium sidoides* root extract: Simultaneous HPLC separation, determination, and validation of selected biomolecules and evaluation of SARS-CoV-2 inhibitory activity. Pharmaceuticals, *15*(10), 1184.

AOAC 1984. Official methods of analysis of the Association of Official Analytical Chemists. 14th ed.

Washington, DC.

ARNON, D.I., 1949. Copper enzymes in isolated chloroplasts. polyphenoloxidase in *Beta vulgaris*. Plant Physiology, 24(1), 1–15.

ASADISHAD, B., CHAHAL, S., AKBARI, A., CIANCIARELLI, V., AZODI, M., GHOSHAL, S., TUFENKJI, N., 2018. Amendment of agricultural soil with metal nanoparticles: effects on soil enzyme activity and microbial community composition. Environmental Science and Technology, 52(4): 1908-1918.

AYANA, M., 2011. Deficit Irrigation Practices as Alternative Means of Improving Water Use Efficiencies in Irrigated Agriculture: Case Study of Maize Crop at Arba Minch, Ethiopia. African Journal of Agricultural Research, 6, 226-235.

AZEEM, M., PIRJAN, K., QASIM, M., MAHMOOD, A., JAVED, T., MUHAMMAD, H., YANG, S., DONG, R., ALI, B., RAHIMI, M., 2023. Salinity stress improves antioxidant potential by modulating physio-Biochemical responses in *Moringa oleifera* Lam. Scientific Reports, 13(1), p.2895.

BACHERT, C., SCHAPOWAL, A., FUNK, P., KIESER, M., 2009. Treatment of acute rhinosinusitis with the preparation from *Pelargonium sidoides* EPs 7630: a randomized, double-blind, placebo-controlled trial. Rhinology, 47(1), p.51.

BADEGGI, U.M., LAWAL, B.A., AKINFENWA, A.O., AYIPO, Y.O., AZEH, Y., DAGACI, M.Z., 2020. Physicochemical properties and in vitro stability studies of green synthesized gold nanoparticles using *Pelargonium sidoides*. Nigerian Journal of Technology, 39(3), pp.785-791.

BANERJEE, N., BISWAS, S., HOSSAIN, C.M., BASAK, P., 2022. Effectiveness of onion (*Allium cepa* L.) skin in human health. In Contemporary Medical Biotechnology Research for Human Health (pp. 115-125). *Academic Press*.

BESTER, S.E., 2011. Estimating the threat of water scarcity in the Breede River Valley: A forecast-based analysis. Final year project report. Department of Industrial Engineering, University of Stellenbosch.

BILIBIO, C., CARVALHO, J.D.A., HENSEL, O., RICHTER, U., 2011. Effect of different levels of water deficit on rapeseed (*Brassica napus* L.) crop. Ciência e Agrotecnologia, 35, pp.672-684.

BOTAI, C.M., BOTAI, J.O., DE WIT, J.P., NCONGWANE, K.P., ADEOLA, A.M., 2017. Drought characteristics over the Western Cape Province, South Africa. Water, 9: 876.

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), pp.420-433.

BURLS, N.J., BLAMY, R.C., CASH, B.A., SWENSON, E.T., AL FAHAD, A., BOPAPE, M.J.J., STRAUS, D.M., REASON, C.J.C., 2019. The Cape Town "Day Zero" drought and hadley cell expansion. Climate and Atmospheric Science 27.

CARILLO, P., GIBON, Y., 2011. Protocol: extraction and determination of proline. PrometheusWiki.

CARMEN, G., HANCU, G., 2014. Antimicrobial and antifungal activity of *Pelargonium roseum* essential oils. Advanced Pharmaceutical Bulletin, 4(Suppl. 2), 511-514.

CHEN, Y., LI, L., XU, R., LI, F., GU, L., LIU, H., WANG, Z., YANG, L., 2021. Characterization of natural herbal medicines by thin-layer chromatography combined with laser ablation-assisted direct analysis in real-time mass spectrometry. Journal of Chromatography A, 1654, 462461.

CHIAVAROLI, A., LIBERO, M.L., DI SIMONE, S.C., ACQUAVIVA, A., NILOFAR, RECINELLA, L., LEONE, S., BRUNETTI, L., CICIA, D., IZZO, A.A., ORLANDO, G., 2023. Adding new scientific evidences on the pharmaceutical properties of *Pelargonium quercetorum* Agnew extracts by using in vitro and in silico approaches. Plants, 12(5), p.1132.

COLLING, J., GROENEWALD, J.H., MAKUNGA, N.P., 2010. Genetic alterations for increased coumarin production lead to metabolic changes in the medicinally important *Pelargonium sidoides* DC (Geraniaceae). Metabolic Engineering, 12(6), 561-572.

COSME, P., RODRÍGUEZ, A.B., ESPINO, J., GARRIDO, M., 2020. Plant phenolics: Bioavailability as a key determinant of their potential health-promoting applications. Antioxidants, 9(12), p.1263.

CUNNINGHAM, A.B., 1988. An investigation of the herbal medicine trade in Natal/KwaZulu (No. 29). Institute of Natural Resources, University of Natal.

DAHANAYAKE, J.M., PERERA, P.K., GALAPPATTY, P., PERERA, H.D.S.M., ARAWWAWALA, L.D.A.M., 2019. Comparative phytochemical analysis and antioxidant activities of Tamalakyadi decoction with its modified dosage forms. Evidence-based Complementary and Alternative Medicine, 2019.

DE CASTRO, A., VLOK, J., MCLLELAN, W., 2010. Field survey of the distribution of *Pelargonium sidoides* and size of selected sub-populations. *Resource Assessment study conducted for the South African National Biodiversity Institute*.

DŽAMIĆ, A.M., SOKOVIĆ, M.D., RISTIĆ, M.S., GRUJIĆ, S.M., MILESKI, K.S., MARIN, P.D., 2014. Chemical composition, antifungal and antioxidant activity of *Pelargonium graveolens* essential oil. Journal of Applied Pharmaceutical Science, 4(3), 1-5.

EIVAZI, F., TABATABAI, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biology and Biochemistry, 20(5), pp.601-606.

EUROPEAN PHARMACOPOEIA., 2023. European Pharmacopoeia, 11th edn. EDQM Council of Europe, Strasbourg, 1661.

FRANCO, L., DE OLIVEIRA, B.H., 2010. Determination of umckalin in commercial tincture and phytopreparations containing *Pelargonium sidoides* by HPLC: Comparison of sample preparation procedures. Talanta, 81(4-5), 1368-1372.

GADOUCHE, L., ALSOUFI, A.S.M., PACHOLSKA, D., SKOTAREK, A., PĄCZKOWSKI, C., SZAKIEL, A., 2023. Triterpenoid and steroid content of lipophilic extracts of selected medicinal plants of the Mediterranean region. Molecules, 28(2), p.697.

GALINDO, A., COLLADO-GONZALEZ, J., GRINAN, I., CORELL, M., CENTENO, A., MARTIN-PALOMO, M. J., GIRON, I.F., RODRIGUEZ, P., CRUZ, Z.N., MEMMI, H., CARBONELL-BARRACHINA, A.A., HERNANDEZ, F., TORRECILLAS, A., MORIANA, A., LOPEZ-PEREZ, D., 2018. Deficit irrigation and emerging fruit crops as a strategy to save water in Mediterranean Semiarid Agro systems. Agricultural Water Management, 202, 311-324.

GÓMEZ-POLO, C., MUÑOZ, M.P., LUENGO, M.C.L., VICENTE, P., GALINDO, P., CASADO, A.M.M., 2016. Comparison of the CIELab and CIEDE2000 color difference formulas. The Journal of Prosthetic Dentistry, 115(1), pp.65-70.

GUANGMING, L., XUECHEN, Z., XIUPING, W., HONGBO, S., JINGSONG, Y., XIANGPING, W., 2017. Soil enzymes as indicators of saline soil fertility under various soil amendments. Agriculture, Ecosystems & Environment, 237: 274-279.

GUCWA, K., MILEWSKI, S., DYMERSKI, T., SZWEDA, P., 2018. Investigation of the antifungal activity and mode of action of *Thymus vulgaris*, *Citrus limonum*, *Pelargonium graveolens*, *Cinnamomum cassia*, *Ocimum basilicum*, and *Eugenia caryophyllus* essential oils. Molecules, 23(5), p.1116.

HALL, B., 2021. *Pelargonium* care: how to keep them happy. Oct 15, 2021. The indoor nursery.

HAMIDPOUR, R., HAMIDPOUR, S., HAMIDPOUR, M., MARSHALL, V., HAMIDPOUR, R., 2017. *Pelargonium graveolens* (Rose Geranium)-A novel therapeutic agent for antibacterial, antioxidant, antifungal and diabetics. Archives in Cancer Research, 5(1), 1-5.

HARRIS, J., 2018. Western Cape Sustainable Water Management Plan, 2017-2022. Western Cape Government, Environmental Affairs and Development Planning. 2018. Pp. 62.

HAUER, H., GERMER, S., ELSÄßER, J., RITTER, T., 2009. Benzopyranones and their sulfate esters from *Pelargonium sidoides*. Planta Medica, 350-352.

HORTON, J.L., HART, S.C., 1998. Hydraulic lift: a potentially important ecosystem process. Trends in ecology & evolution, 13(6), pp.232-235.

HUANG, Y., 2022. Chemometric methods in analytical spectroscopy technology. In *Chemometric Methods in Analytical Spectroscopy Technology* (pp. 1-29). Singapore: Springer Nature Singapore.

HUTCHINGS, A., SCOTT, A.H., LEWIS, G., CUNNINGHAM, A., 1996. *Zulu medicinal plants. An inventory*, University of Natal Press, Pietermaritzburg.

ICOZ, I., STOTZKY, G., 2008. Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biology and Biochemistry, 40(3): 559-586.

IDEH, J., OGUNKUNLE, A., 2019. Colourimetric analysis of some powdered medicinal herbs from Ogbomoso, Nigeria. Journal of Medicinal Plants for Economic Development, 3(1), pp.1-8.

INGARFIELD, P.J., 2018. Effect of water stress and arbuscular mycorrhiza on the plant growth and antioxidant potential of *Pelargonium reniforme Curtis and Pelargonium sidoides* DC (Masters dissertation, Cape Peninsula University of Technology).

ISAH, T., 2019. Stress and defense responses in plant secondary metabolites production. Biological Research, 52.

JEKABSONE, A., SILE, I., COCHIS, A., MAKRECKA-KUKA, M., LAUCAITYTE, G., MAKAROVA, E., RIMONDINI, L., BERNOTIENE, R., RAUDONE, L., VEDLUGAITE, E., BANIENE, R., 2019. Investigation of antibacterial and antiinflammatory activities of proanthocyanidins from *Pelargonium sidoides* DC root extract. Nutrients, 11(11), p.2829.

JIMÉNEZ-ZAMORA, A., DELGADO-ANDRADE, C., RUFIÁN-HENARES, J.A., 2016. Antioxidant capacity, total phenols and color profile during the storage of selected plants used for infusion. Food Chemistry, 199, pp.339-346.

KAMIN, W., ILYENKO, L.I., MALEK, F.A., KIESER, M., 2012. Treatment of acute bronchitis with EPs 7630: randomized, controlled trial in children and adolescents. Pediatrics International, 54(2), pp.219-226.

KAMRAN, S., SINNIAH, A., ABDULGHANI, M.A., ALSHAWSH, M.A., 2022. Therapeutic potential of certain terpenoids as anticancer agents: a scoping review. Cancers, 14(5), p.1100.

KANDELER, E., GERBER, H., 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biology and Fertility of Soils, 6: 68-72.

KANTÉ, M., RIAH-ANGLET, W., CLIQUET, J.B., TRINSOUTROT-GATTIN, I., 2021. Soil enzyme activity and stoichiometry: Linking soil microorganism resource requirement and legume carbon rhizodeposition. Agronomy, 11(11): 2131.

KASAJIMA, I., 2019. Measuring plant colors. Plant Biotechnology, 36(2), pp.63-75.

KAURIN, A., CERNILOGAR, Z., LESTAN, D., 2018. Revitalisation of metal-contaminated, EDTA-washed soil by addition of unpolluted soil, compost and biochar: Effects on soil enzyme activity, microbial community composition and abundance. Chemosphere, 193: 726-736.

KAUSAR, F., KIM, K.H., FAROOQI, H.M.U., FAROOQI, M.A., KALEEM, M., WAQAR, R., KHALIL, A.A.K., KHUDA, F., ABDUL RAHIM, C.S., HYUN, K., CHOI, K.H., 2021. Evaluation of antimicrobial and anticancer activities of selected medicinal plants of Himalayas, Pakistan. Plants, 11(1), p.48.

KGATSHE, M., AREMU, O.S., KATATA-SERU, L., GOPANE, R., 2019. Characterization and antibacterial activity of biosynthesized silver nanoparticles using the ethanolic extract of *Pelargonium sidoides* DC. Journal of Nanomaterials, (1), p.3501234.

KHARBACH, M., ALAOUI MANSOURI, M., TAABOUZ, M., YU, H., 2023. Current application of advancing spectroscopy techniques in food analysis: data handling with chemometric approaches. Foods, 12(14), p.2753.

KINYUA, J.K., 2014. *Correlation of leaf glucose content with mineral nutrients uptake of South African geranium (Pelargonium sidoides)* (Masters dissertation, Egerton university).

KOLODZIEJ, H., 2007. Fascinating metabolic pools of *Pelargonium sidoides* and *Pelargonium reniforme*, traditional and phytomedicinal sources of the herbal medicine Umckaloabo®. Phytomedicine, 14, pp.9-17.

KOLODZIEJ, H., 2011. Antimicrobial, antiviral and immunomodulatoru activity of *Pelargonium sidoides* (Eps® 7630) in the context of health promotion. Pharmaceuticals, 4(10), 1295-1314.

KOLODZIEJ, H., KAYSER, O., RADTKE, O.A., KIDERLEN, A.F., KOCH, E., 2003. Pharmacological profile of extracts of *Pelargonium sidoides* and their constituents. Phytomedicine, 10, 18-24.

LALLI, J.Y., VILJOEN, A.M., BAŞER, K.H.C., DEMIRCI, B., ÖZEK, T., 2006. The essential oil composition and chemotaxonomical appraisal of South African *Pelargoniums* (Geraniaceae). Journal of Essential Oil Research, 18(sup1), pp.89-105.

LAWRENCE, E. 2001. *Pelargonium sidoides* DC. South African National Diversity Institute website. www.plantzafrica.com/frames/plantsfram.html

LEWU, F.B., GRIERSON, D.S., AFOLAYAN, A.J., 2006a. Clonal propagation of *Pelargonium sidoides*: A threatened medicinal plant of South African Journal of Biotechnology, 5(2), 123-125.

LEWU, F.B., GRIERSON, D.S., AFOLAYAN, A.J., 2006b. The leaves of *Pelargonium sidoides* may substitute for its roots in the treatment of bacterial infections (short communication). Biological Conservation, 128, 582-584.

LEWU, F.B., GRIERSON, D.S., AFOLAYAN, A.J., 2007a. Morphological diversity among accessions of *Pelargonium sidoides* DC. in the Eastern Cape, South Africa. Genetic Resources and Crop Evolution, 54, pp.1-6.

LEWU, F.B., ADEBOLA, P.O. AFOLAYAN, A.J. 2007b. Commercial harvesting of *Pelargonium sidoides* in the Eastern Cape, South Africa: striking a balance between resource conservation and rural livelihoods. Journal of Arid Environment, 70(2), 380-388.

LIU, Z., CHENG, Y., CHAO, Z., 2023. A comprehensive quality analysis of different colors of medicinal and edible honeysuckle. Foods, 12(16), p.3126.

LUGOJAN, C., CIULCA, S., 2011. Evaluation of relative water content in winter wheat. Journal of Horticulture, Forestry and Biotechnology, 15: 173-177.

MAHESHWARAN, L., NADARAJAH, L., SENADEERA, S.P.N.N., RANAWEERA, C.B., CHANDANA, A.K., PATHIRANA, R.N., 2024. Phytochemical Testing Methodologies and Principles for Preliminary Screening/Qualitative Testing. Asian Plant Research Journal, 12(5), pp.11-38.

MAKUNGA, N.P., 2015. Cultivation method for medically valuable *Pelargonium*. Innovus University of Stellenbosch, Technology Transfer, 2015.

MANGANYI, M.C., REGNIER, T., KUMAR, A., BEZUIDENHOUT, C.C., ATEBA, C.N., 2018. Biodiversity and antibacterial screening of endophytic fungi isolated from *Pelargonium sidoides*. South African Journal of Botany, 116, pp.192-199.

MANDER, M., 1998. Marketing of indigenous medicinal plants in South Africa: a case study in KwaZulu-Natal.

MAREČEK, V., MIKYŠKA, A., HAMPEL, D., ČEJKA, P., NEUWIRTHOVÁ, J., MALACHOVÁ, A., CERKAL, R., 2017. ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt. Journal of Cereal Science, 73, pp.40-45.

MAREE, J.E., VILJOEN, A.M., 2012. Phytochemical distinction between *Pelargonium sidoides* and *Pelargonium reniforme*—A quality control perspective. South African Journal of Botany, 82, pp.83-91.

MARTINS, M.D.F., MARCON, M.V., EGG, C.M.S., DIAS, D.T., MANFRON, J., NADAL, J.M., FARAGO, P.V., NOVATSKI, A., 2023. Botanical authentication of “espinheira-santa” [*Monteverdia ilicifolia* (Mart. ex Reissek) Biral] samples by FTIR spectroscopy coupled with PCA and photoacoustic spectroscopy. Brazilian Archives of Biology and Technology, 66(spe), p.e23230615.

MASONDO, N.A., MAKUNGA, N.P., 2019. Advancement of analytical techniques in some South African commercialized medicinal plants: Current and future perspectives. South African Journal of Botany, 126, 40-57.

MATIVANDLELA, S.P.N., LALL, N., MEYER, J.J.M., 2006. Antibacterial, antifungal and antitubercular activity of (the roots of) *Pelargonium reniforme* (CURT) and *Pelargonium sidoides* (DC) (Geraniaceae) root extracts. South African Journal of Botany, 72(2), 232-237.

MATIVANDLELA, S.P.N., MEYER, J.J.M., HUSSEIN, A.A., LALL, N., 2007. Antitubercular Activity of Compounds Isolated from *Pelargonium sidoides*. Pharmaceutical Biology, 45(8), 645-650.

MAUGERI, A., LOMBARDO, G.E., CIRMI, S., SÜNTAR, I., BARRECA, D., LAGANÀ, G., NAVARRA, M., 2022. Pharmacology and toxicology of tannins. Archives of Toxicology, 96(5), pp.1257-1277.

MEENA, A., RAO, K.S., 2021. Assessment of soil microbial and enzyme activity in the rhizosphere zone under different land use/cover of a semiarid region, India. Ecological Processes, 10: 16.

MOFOKENG, M.M., 2015. Growth, yield and chemical composition of *Pelargonium sidoides* DC. in response to nitrogen and soil water management (Doctoral dissertation, University of Pretoria).

MOFOKENG, M.M., STEYN, J.M., DU PLOOY, C.P., PRINSLOO, G., ARAYA, H.T., 2015. Growth of *Pelargonium sidoides* DC. in response to water and nitrogen level. South African Journal of Botany, 100, 183-189.

MOFOKENG, M.M., ARAYA, H.T., MASHELA, P.W., 2017. Stomatal Conductance, Growth and Yield of *Pelargonium sidoides* DC. in Response to Watering Frequency and Terminal Water Stress. Journal of

Agricultural Science and Technology, 7, 513-521.

MOFOKENG, M.M., PRINSLOO, G., ARAYA, H.T., DU PLOOY, C.P., SATHEKGE, N.R., AMOO, S.O., STEYN, J.M., 2020. Yield and metabolite production of Pelargonium sidoides DC. in response to irrigation and nitrogen management. Metabolites, 10(6), p.219.

MOTJOTJI, L., 2011. Towards sustainability of harvesting the medicinal plant *Pelargonium sidoides* DC. (GERANIACEAE) (Masters dissertation).

MOYO, M., AREMU, A.O., GRUZ, J., ŠUBRTOVÁ, M., SZÜČOVÁ, L., DOLEŽAL, K., VAN STADEN, J., 2013. Conservation strategy for *Pelargonium sidoides* DC: phenolic profile and pharmacological activity of acclimatized plants derived from tissue culture. Journal Ethnopharmacology, 149(2), 557-561.

MOYO, S.M., SEREM, J.C., BESTER, M.J., MAVUMENGWANA, V., KAYITESI, E., 2020. The impact of boiling and in vitro human digestion of *Solanum nigrum* complex (Black nightshade) on phenolic compounds bioactivity and bioaccessibility. Food Research International, 137, p.109720.

MOYO, M., VAN STADEN, J., 2014. Medicinal properties and conservation of *Pelargonium sidoides* DC. Journal of Ethnopharmacology, 152(2), 243-255.

MUDAU, M., MASHAU, M.E., RAMASHIA, S.E., 2022. Nutritional quality, antioxidant, microstructural and sensory properties of spontaneously fermented gluten-free finger millet biscuits. Foods, 11(9), p.1265.

MULAUDZI, N., RASEALOKA, L.T., MAANO, G.H., MOHLAPI, T.C., MOSHIDI, P.M., MOHLABE, N.N., 2024. HPTLC Profiling, Quality Control and FTIR Coupled with Chemometrics Analysis for *Securidaca longipenduculata* Fresen. Analytical and Bioanalytical Chemistry Research, 11(2), pp.191-199.

MULAUDZI, T., SIAS, G., NKUNA, M., NDOU, N., HENDRICKS, K., IKEBUDU, V., KOO, A.J., AJAYI, R.F., IWUOHA, E., 2023. Seed priming with MeJA prevents salt-induced growth inhibition and oxidative damage in *Sorghum bicolor* by inducing the expression of jasmonic acid biosynthesis genes. International Journal of Molecular Sciences, 24(12), p.10368.

MUNDIM, F.M., PRINGLE, E.G., 2018. Whole-plant metabolic allocation under water stress. Frontiers in Plant science, 9, p.852.

NANDIYANTO, A.B.D., OKTIANI, R., RAGADHITA, R., 2019. How to read and interpret FTIR spectroscope of organic material. Indonesian Journal of Science and Technology, 4(1), pp.97-118.

NEWTON, D., 2009. A preliminary assessment of South Africa's role in the *Pelargonium sidoides* (DC) and *P. reniforme* (Curtis) medicinal plant trade. Draft document, contribution to Regional BMP (Biodiversity

Management Plan) for *Pelargonium sidoides*, WWF Germany.

NORTJIE, E., BASITERE, M., MOYO, D., NYAMUKAMBA, P., 2022. Extraction methods, quantitative and qualitative phytochemical screening of medicinal plants for antimicrobial textiles: a review. Plants, 11(15), p.2011.

NTSHABELE, P.M., SOUNDY, P., APOSTOLIDES, Z., 2013. Shade net effects on chemical composition of *Pelargonium sidoides* (geraniaceae). In *II All Africa Horticulture Congress*. Acta Horticulturae, 1007, 959-965.

NWOZO, O.S., EFFIONG, E.M., AJA, P.M., AWUCHI, C.G., 2023. Antioxidant, phytochemical, and therapeutic properties of medicinal plants: A review. International Journal of Food Properties, 26(1), pp.359-388.

OLESEN, J.E., 2006. Reconciling adaptation and mitigation to climate change in Agriculture. Journal de Physique. IV (Proceedings) France, 139, 403-411.

OLESEN, J.E., BINDI, M., 2002. Consequences of climate change for European Agricultural Productivity, land use and policy. European Journal of Agronomy, 16(4), 239-262.

OTTO, F.E.L., WOLSKI, P., LEHNER, F., TEBALDI, C., VAN OLDENBORGH, G.J., HOGESTEEGER, S., SINGH, R., HOLDEN, P., FUCKER, N.S., ODOULAMI, R.C. NEW, M., 2018. Anthropogenic influence on drivers of the Western Cape drought 2015-2017. Environmental Research Letters, 13:124010.

PANARA, A., AALIZADEH, R., THOMAIDIS, N.S., 2022. Chemical characterisation of *Pelargonium sidoides* root based on LC-QToF-MS non-target screening strategies. Phytochemical Analysis, 33(1), pp.40-56.

PISOSCHI, A.M., POP, A., CIMPEANU, C., PREDOI, G., 2016. Antioxidant capacity determination in plants and plant-derived products: a review. Oxidative Medicine and Cellular Longevity, (1), p.9130976.

PIZZI, A., 2021. Tannins medical/pharmacological and related applications: A critical review. Sustainable Chemistry and Pharmacy, 22, p.100481.

RAIMONDO, D., VON STADEN, L., FODEN, W., VICTOR, J.E., HELME, N.A., TURNER, R.C., KAMUNDI, D.A., MANYAMA, P.A., 2009. Red list of South African plants 2009. Strelitzia 25. South African National Biodiversity Institute, Pretoria.

RÖSCHENBLECK, J., ALBERS, F., MÜLLER, K., WEINL, S., KUDLA, J., 2014. Phylogenetics, character evolution and a subgeneric revision of the genus *Pelargonium* (Geraniaceae). Phytotaxa, 159, 31-76.

SADE, N., GALKIN, E., MOSHELION, M., 2015. Measuring Arabidopsis, tomato and barley leaf relative content (RWC). Bio-protocol, 5(8), pp.e1451-e1451.

SAGANUWAN, S.A., 2018. Colours are indicators of phytochemical components and medicinal properties of plants: A review of natural spectroscopy. European Journal of Medicinal Plants.

SANBI, 2006. *A South African response to the global strategy for plant conservation*, SANBI biodiversity series 1, South African National Biodiversity Institute, Pretoria.

SARASWATHI, J., VENKATESH, K., BABURAO, N., HILAL, M.H., RANI, A.R., 2011. Phytopharmacological importance of *Pelargonium* species. Journal of Medicinal Plant Research, 5(13), 2587-2598.

SAYRE J.K., 2003. Scented Geraniums or *Pelargoniums*. Bottlebrushpress.com.

SCALISI, A., O'CONNELL, M.G., ISLAM, M.S., GOODWIN, I., 2022. A fruit colour development index (CDI) to support harvest time decisions in peach and nectarine orchards. Horticulturae, 8(5), p.459.

SCHAPOWAL, A., DOBOS, G., CRAMER, H., ONG, K.C., ADLER, M., ZIMMERMANN, A., BRANDES-SCHRAMM, J., LEHMACHER, W., 2019. Treatment of signs and symptoms of the common cold using EPs 7630-results of a meta-analysis. Heliyon, 5(11).

SCHIPPMANN, U., LEAMAN, D.J., CUNNINGHAM, A.B., 2002. Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. Biodiversity and the ecosystem approach in agriculture, forestry and fisheries.

SCHÖTZ, K., NÖLDNER, M., 2007. Mass spectroscopic characterisation of oligomeric proanthocyanidins derived from an extract of *Pelargonium sidoides* roots (EPs® 7630) and pharmacological screening in CNS models. Phytomedicine, 14, pp.32-39.

SHARMA, P., TYAGI, A., BHANSALI, P., PAREEK, S., SINGH, V., ILYAS, A., MISHRA, R., PODDAR, N.K., 2021. Saponins: Extraction, bio-medicinal properties and way forward to anti-viral representatives. Food and Chemical Toxicology, 150, p.112075.

SUDHA, R., PHILIP, X.C., SURIYAKUMARI, K.V.P., 2020. Phytochemical constituents of leaves of *Moringa oleifera* grow in Cuddalore District, Tamil Nadu, India. SBV Journal Basic, Clinical and Applied Health Science, 3(4), pp.164-167.

TANGA, M., LEWU, F.B., OYEDEJI, O.A., OYEDEJI, O.O., 2018. Cultivation of medicinal plants in South Africa: a solution to quality assurance and consistent availability of medicinal plant materials for commercialization. Academia Journal of Medicinal Plants, 6(7), 168-177.

TAPARAUSKIENĖ, L., MISECKAITĖ, O., 2014. Effect of Mulch on Soil Moisture Depletion and Strawberry Yield in Sub-Humid Area. Polish Journal of Environmental Studies, 23(2), 475-482.

TEJADA, M., GONZALEZ, J.L., GARCÍA-MARTÍNEZ, A.M., PARRADO, J., 2008. Effects of different green manures on soil biological properties and maize yield. Bioresource Technology, 99(6): 1758-1767.

TELEANU, D.M., NICULESCU, A.G., LUNGU, I.I., RADU, C.I., VLADÂCENCO, O., ROZA, E., COSTĂCHESCU, B., GRUMEZESCU, A.M., TELEANU, R.I., 2022. An overview of oxidative stress, neuroinflammation, and neurodegenerative diseases. International Journal of Molecular Sciences, 23(11), p.5938.

ULLAH, A., MUNIR, S., BADSHAH, S.L., KHAN, N., GHANI, L., POULSON, B.G., EMWAS, A.H., JAREMKO, M., 2020. Important flavonoids and their role as a therapeutic agent. Molecules, 25(22), p.5243.

UMAR, A.H., SYAHRUNI, R., RANTETA'DUNG, I., RAFI, M., 2023. FTIR-based fingerprinting combined with chemometrics method for rapid discrimination of *Jatropha spp*. (Euphorbiaceae) from different regions in South Sulawesi. Journal of Applied Pharmaceutical Science, 13(1), pp.139-149.

VAN DER WALT, J.J.A., VORSTER, P.J., 1988. *Pelargoniums* of Southern Africa. Vol. 3. Cape Town, National Botanic Gardens Kirstenbosch, South Africa.

VAN NIEKERK, M.J., 2009. The contribution of the trade in *Pelargonium sidoides* to rural livelihoods in South Africa and Lesotho (Master's thesis, University of Cape Town).

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), pp.530-547.

VAN WYK, B.E., 2008. A broad review of commercially important southern African medicinal plants. Journal of Ethnopharmacology, 119(3), pp.342-355.

VAN WYK, B.E., 2011. The potential of South African plants in the development of new food and beverage products. South African Journal of Botany, 77(4), pp.857-868.

VAN WYK, B.E., VAN OUDTSHOORN, B., GERICKE, N., 1997. *Book. Medicinal plants of South Africa*, pp. 304.

VAN WYNGAARD, J., FAMUYIDE, I.M., INVERNIZZI, L., NDIVHUWO, K.K., TORDIFFE, A.S.W., MAHARAJ, V.J., MCGAW, L.J., 2023. Optimizing extraction of *Pelargonium sidoides* roots: Impact of ethanol concentration on biological activity of extracts. South African Journal of Botany, 162, pp.667-679.

VILJOEN, A., SANDASI, M., FOUCHE, G., COMBRINCK, S., VERMAAK, I. (eds)., 2022. The South African Herbal Pharmacopoeia: Monographs of Medicinal and Aromatic Plants. Academic Press.

VLOK, J., 2003. Assessment of veld harvesting of *Pelargonium sidoides* and *Pelargonium. reniforme* in the Eastern Cape. First survey report. Report commissioned by Parceval (PTY) LTD, Wellington, South Africa. 27pp.

WANG, Y., MA, Y., TAO, L., ZHANG, X., HAO, F., ZHAO, S., HAN, L., BAI, C., 2022. Recent advances in separation and analysis of saponins in natural products. Separations, 9(7), p.163.

WATT, J.M., BREYER-BRANDWIJK, M.G., 1962. The medicinal and poisonous plants of southern and eastern Africa being an account of their medicinal and other uses, chemical composition, pharmacological effects and toxicology in man and animal., 2nd ed. E&S Livingstone, Edinburg.

WHITE, A.G., 2006. *The effect of geography, cultivation and harvest technique on the Umckalin concentration and growth of Pelargonium sidoides (Geraniaceae)* (Masters dissertation, Rhodes University).

WHITE, A.G., DAVIES-COLEMAN, M.T., RIPLEY, B.S., 2008. Measuring and optimising umckalin concentration in wild-harvested and cultivated Pelargonium sidoides (Geraniaceae). South African Journal of Botany, 74(2), pp.260-267.

WHO, 1999. Quality control methods for medicinal plant materials. Geneva.

WILLIAMS, V.L., VICTOR, J.E., CROUCH, N.R., 2013, ‘Red listed medicinal plants of South Africa: Status, trends, and assessment challenges’, South African Journal of Botany, 86, 23-35.

WITTE, K., KOCH, E., VOLK, H.D., WOLK, K., SABAT, R., 2020. The herbal extract EPs® 7630 increases the antimicrobial airway defense through monocyte-dependent induction of IL-22 in T cells. Journal of Molecular Medicine, 98, pp.1493-1503.

WYNBERG, R., LAIRD, S., VAN NIEKERK, J., KOZANAYI, W., 2015. Formalization of the natural product trade in southern Africa: unintended consequences and policy blurring in biotrade and bioprospecting. Society & Natural Resources, 28(5), 559-574.

WYNBERG, R., VAN NIEKERK, J., 2014. Governance, equity and sustainability in non-timber forest product value chains. In *Governance for Justice and Environmental Sustainability: Lessons Across Natural Resource Sectors in Sub-Saharan Africa*, eds M. Sowman and R. Wynberg, R (Earthscan/Routledge). 279-304.

YANG, W., CHEN, X., LI, Y., GUO, S., WANG, Z., YU, X., 2020. Advances in pharmacological activities of terpenoids. Natural Product Communications, 15(3), p.1934.

YOUSEFIAN, Z., HAMIDOGHLI, Y., GOLKAR, P., MIRJALILI, M.H., 2023. Growth patterns and biological activities of *Pelargonium sidoides* DC. hairy root cultures: A commercially feasible industrial scale-up to improve yields. Industrial Crops and Products, 194, p.116272.

APPENDIX A: CAPACITY BUILDING

Student recruitment

The involvement of postgraduate students in the project was to increase research capacity. Three postgraduate students were recruited for this project *(Table A1)*.

Ms Yandiswa Mtimkulu is studying towards a PhD degree in Agriculture at Cape Peninsula University of Technology (CPUT) under the tutelage of Prof FB Lewu (supervisor). Drs MN Lewu and AR Mulidzi are the co-supervisors. Her research topic is "The assessment of growth, water requirements and harvesting time of *Pelargonium sidoides* grown in a tunnel". Ms Mtimkulu's research focus was on Objectives 1 to 3 of this project. She looked into the soil parameters (chemical and biological), morpho-physiological responses and the yield of both the above and below ground biomass of *P. sidoides* subjected to different irrigation levels. The effects of harvest time on these parameters were also part of her focus area. Ms Mtimkulu also determined the nutritional composition of the shoot and roots of *P. sidoides*. She already published a review paper on this project; and also presented her findings on the project at the Indigenous Plant Use Forum (IPUF) conference in August 2024.

Mr Phila-Sande Prudent Ntoyi registered at the Department of Biotechnology, University of the Western Cape (UWC). Supervised by Prof Takalani Mulaudzi-Masuku while Drs Lewu and Mulidzi are his co-supervisors. The title of his research is "Investigating the effect of water stress on the growth and chemical composition of *Pelargonium sidoides"*. Mr Ntoyi focussed on Objectives 1 & 2, and also on the biochemical traits, but mainly on the leaves of *Pelargonium sidoides*. He determined the morphological, physiological and biochemical responses of *P. sidoides* growing under different levels of PAW. Mr Ntoyi attended the Combined Congress in January 2024 where he gave an oral presentation on some of his findings on this research project. The title of his presentation is "Investigating the effect of water stress on growth and biochemical response of *Pelargonium sidoides*".

Ms Kundani Khameli is the third student recruited for the project. She registered at the University of Johannesburg (UJ) in July 2023 for a PhD degree in Biotechnology under the supervision of Prof O Adebo. Her co-supervisors are Prof SO Oluwafemi, Prof Takalani Mulaudzi-Masuku and Dr Lewu. Her research topic is "Effect of irrigation and harvesting age on the chemical composition of the roots of *Pelargonium sidoides* DC." Ms Khameli's research focus is on the qualitative and quantitative analysis of the secondary metabolites in the roots of *P. sidoides* and how these parameters are affected by harvesting age. Ms Khameli is currently wrapping up her PhD study. She is working on Specific Aim 4 of this project.

Table A1: Details of Postgraduate students

Name	Gender	Qualification	University
Yandiswa Mtimkulu	Female	PhD	CPUT
Phila-Sande Prudent Ntoyi	Male	Masters	UWC
Kundani Khameli	Female	PhD	UJ

Table A2: Details of the project team

Name	Institution	Role
Dr MN Lewu	ARC Infruitec-Nietvoorbij	Project leader
Dr AR Mulidzi	ARC Infruitec-Nietvoorbij	Team member & Co-supervisor (Mr Ntoyi; Ms Mtimkulu & Ms Khameli)
Prof T Mulaudzi	UWC	Supervisor (Mr Ntoyi); co-supervisor (Ms Khameli)
Prof FB Lewu	CPUT	Team member; Supervisor (Ms Mtimkulu)
Prof SO Oluwafemi	UJ	Team member & co-supervisor (Ms Khameli)
Prof O Adebo	UJ	Supervisor (Ms Khameli)
Ms Y Mtimkulu	ARC Infruitec-Nietvoorbij & CPUT	Student (PhD)
Ms Khameli Kundani	UJ	Student (PhD)
Mr P Ntoyi	ARC Infruitec-Nietvoorbij & UWC	Student (Masters)

Other project outputs:

- One peer reviewed article was published by the Journal of Medicinal Plants for Economic Development (by Ms Yandiswa Mtimkulu)
- Two other papers are under preparation by a PhD (Ms Kundani Khameli) and an MSc (Mr Phila-Sande Prudent) students
- Two oral presentations at local conferences (Ms Yandiswa Mtimkulu; and Mr Phila-Sande Prudent Ntoyi)

Staff development

This project provided empowerment to technical staff of Agricultural Research Council (ARC) to develop capacity in this field. The staff members were trained on how to use modern technology to monitor soil water content, using the DFM soil moisture probes linked to a software. Both technicians and research assistants gained exposure on the installation and maintenance of the irrigation systems and data collection on *P. sidoides*.

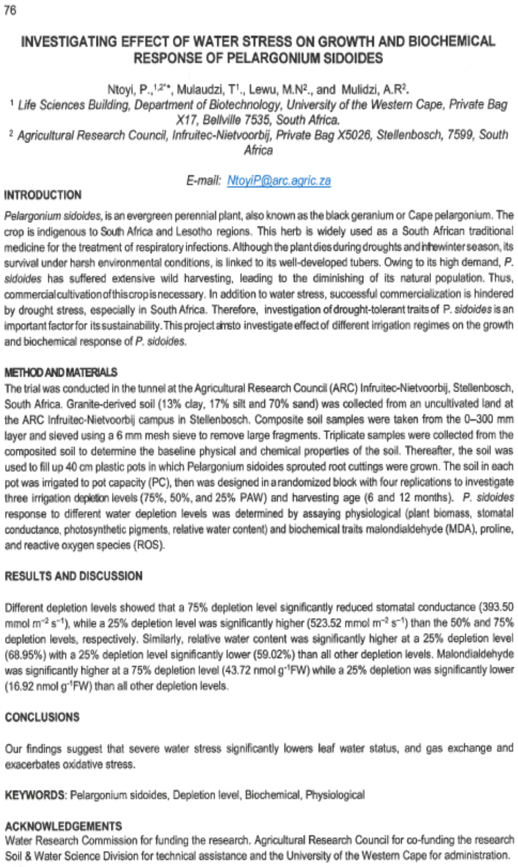
Other collaborations

Apart from academic collaborations, the project team worked with Parceval Pty Ltd, located in Wellington Western Cape, South Africa. This industry collaboration was helpful in different ways right from the beginning of the research to date. Through the founder and CEO, Mr Ulrich Feiter, the project team received unwavering support and collaboration. The company donated planting materials (*Pelargonium sidoides* plants) to the ARC research team for free. The team was also invited for a tour of Parceval production facility where we were able to see the production process. Later, the project team was allowed to ask a variety of questions to which Mr Feiter gave constructive answers to. The question-and-answer section helped to boost the understanding of the research team on *Pelargonium sidoides* further.

Community development

The preliminary irrigation guidelines from this project will assist members of the community who are interested in becoming *P. sidoides* farmers to not irrigate excessively, and not to over or under irrigate. Farmers will become aware of sustainable irrigation management for *P. sidoides*.

APPENDIX B: ORAL PRESENTATION AT COMBINED CONGRESS BY MR P. NTOYI (MASTERS STUDENT)



76

INVESTIGATING EFFECT OF WATER STRESS ON GROWTH AND BIOCHEMICAL RESPONSE OF PELARGONIUM SIDOIDES

Ntoyi, P.,[1,2*], Mulaudzi, T[1]., Lewu, M.N[2]., and Mulidzi, A.R[2].
[1] *Life Sciences Building, Department of Biotechnology, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa.*
[2] *Agricultural Research Council, Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa*

E-mail: NtoyiP@arc.agric.za

INTRODUCTION

Pelargonium sidoides, is an evergreen perennial plant, also known as the black geranium or Cape pelargonium. The crop is indigenous to South Africa and Lesotho regions. This herb is widely used as a South African traditional medicine for the treatment of respiratory infections. Although the plant dies during droughts and in the winter season, its survival under harsh environmental conditions, is linked to its well-developed tubers. Owing to its high demand, *P. sidoides* has suffered extensive wild harvesting, leading to the diminishing of its natural population. Thus, commercial cultivation of this crop is necessary. In addition to water stress, successful commercialization is hindered by drought stress, especially in South Africa. Therefore, investigation of drought-tolerant traits of P. *sidoides* is an important factor for its sustainability. This project aims to investigate effect of different irrigation regimes on the growth and biochemical response of *P. sidoides*.

METHOD AND MATERIALS

The trial was conducted in the tunnel at the Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Stellenbosch, South Africa. Granite-derived soil (13% clay, 17% silt and 70% sand) was collected from an uncultivated land at the ARC Infruitec-Nietvoorbij campus in Stellenbosch. Composite soil samples were taken from the 0–300 mm layer and sieved using a 6 mm mesh sieve to remove large fragments. Triplicate samples were collected from the composited soil to determine the baseline physical and chemical properties of the soil. Thereafter, the soil was used to fill up 40 cm plastic pots in which Pelargonium sidoides sprouted root cuttings were grown. The soil in each pot was irrigated to pot capacity (PC), then was designed in a randomized block with four replications to investigate three irrigation depletion levels (75%, 50%, and 25% PAW) and harvesting age (6 and 12 months). *P. sidoides* response to different water depletion levels was determined by assaying physiological (plant biomass, stomatal conductance, photosynthetic pigments, relative water content) and biochemical traits malondialdehyde (MDA), proline, and reactive oxygen species (ROS).

RESULTS AND DISCUSSION

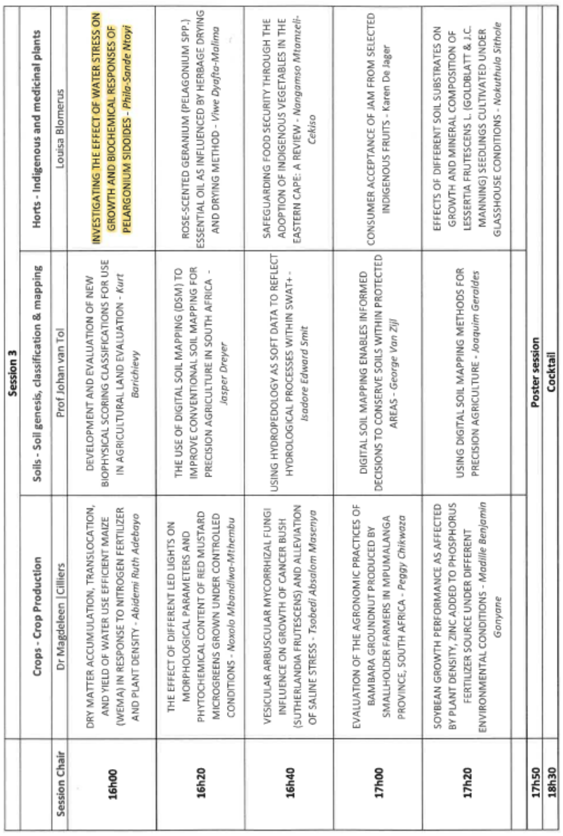
Different depletion levels showed that a 75% depletion level significantly reduced stomatal conductance (393.50 mmol m^{-2} s^{-1}), while a 25% depletion level was significantly higher (523.52 mmol m^{-2} s^{-1}) than the 50% and 75% depletion levels, respectively. Similarly, relative water content was significantly higher at a 25% depletion level (68.95%) with a 25% depletion level significantly lower (59.02%) than all other depletion levels. Malondialdehyde was significantly higher at a 75% depletion level (43.72 nmol g^{-1}FW) while a 25% depletion was significantly lower (16.92 nmol g^{-1}FW) than all other depletion levels.

CONCLUSIONS

Our findings suggest that severe water stress significantly lowers leaf water status, and gas exchange and exacerbates oxidative stress.

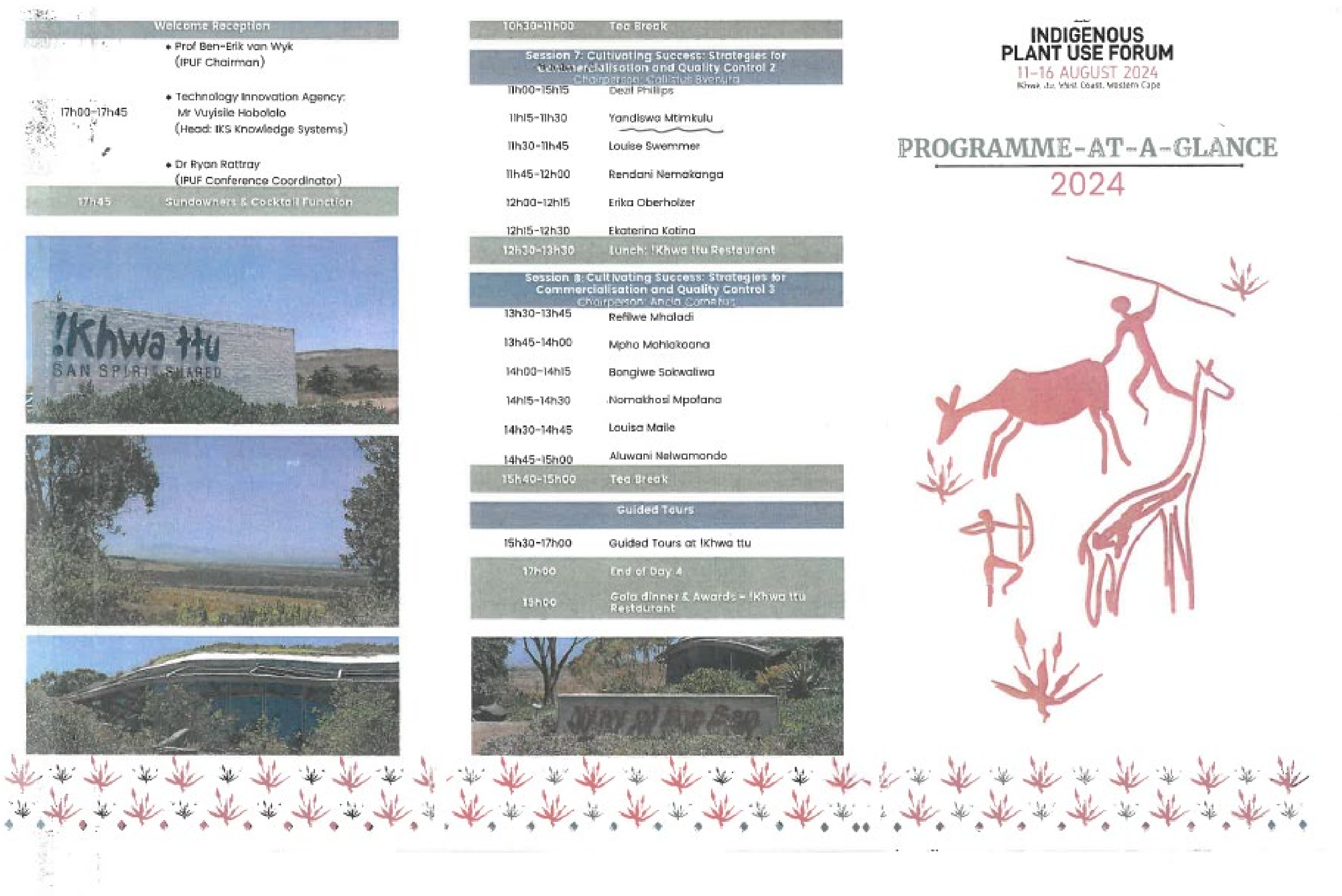
KEYWORDS: Pelargonium sidoides, Depletion level, Biochemical, Physiological

ACKNOWLEDGEMENTS
Water Research Commission for funding the research. Agricultural Research Council for co-funding the research Soil & Water Science Division for technical assistance and the University of the Western Cape for administration.



	Session 3		
	Crops - Crop Production	**Soils - Soil genesis, classification & mapping**	**Horts - Indigenous and medicinal plants**
Session Chair	Dr Magdeleen |Cilliers	Prof Johan van Tol	Louisa Blomerus
16h00	DRY MATTER ACCUMULATION, TRANSLOCATION, AND YIELD OF WATER USE EFFICIENT MAIZE (WEMA) IN RESPONSE TO NITROGEN FERTILIZER AND PLANT DENSITY - *Abidemi Ruth Adebayo*	DEVELOPMENT AND EVALUATION OF NEW BIOPHYSICAL SCORING CLASSIFICATIONS FOR USE IN AGRICULTURAL LAND EVALUATION - *Kurt Barichievy*	INVESTIGATING THE EFFECT OF WATER STRESS ON GROWTH AND BIOCHEMICAL RESPONSES OF PELARGONIUM SIDOIDES - *Phila-Sande Ntoyi*
16h20	THE EFFECT OF DIFFERENT LED LIGHTS ON MORPHOLOGICAL PARAMETERS AND PHYTOCHEMICAL CONTENT OF RED MUSTARD MICROGREENS GROWN UNDER CONTROLLED CONDITIONS - *Noxolo Mbandlwa-Mthembu*	THE USE OF DIGITAL SOIL MAPPING (DSM) TO IMPROVE CONVENTIONAL SOIL MAPPING FOR PRECISION AGRICULTURE IN SOUTH AFRICA - *Jasper Dreyer*	ROSE-SCENTED GERANIUM (PELAGONIUM SPP.) ESSENTIAL OIL AS INFLUENCED BY HERBAGE DRYING AND DRYING METHOD - *Viwe Dyafta-Malima*
16h40	VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI INFLUENCE ON GROWTH OF CANCER BUSH (SUTHERLANDIA FRUTESCENS) AND ALLEVIATION OF SALINE STRESS - *Tsobedi Absalom Masenya*	USING HYDROPEDOLOGY AS SOFT DATA TO REFLECT HYDROLOGICAL PROCESSES WITHIN SWAT+ - *Isadore Edward Smit*	SAFEGUARDING FOOD SECURITY THROUGH THE ADOPTION OF INDIGENOUS VEGETABLES IN THE EASTERN CAPE: A REVIEW - *Nangamso Mtamzeli-Cekiso*
17h00	EVALUATION OF THE AGRONOMIC PRACTICES OF BAMBARA GROUNDNUT PRODUCED BY SMALLHOLDER FARMERS IN MPUMALANGA PROVINCE, SOUTH AFRICA - *Peggy Chikwaza*	DIGITAL SOIL MAPPING ENABLES INFORMED DECISIONS TO CONSERVE SOILS WITHIN PROTECTED AREAS - *George Van Zijl*	CONSUMER ACCEPTANCE OF JAM FROM SELECTED INDIGENOUS FRUITS - Karen De Jager
17h20	SOYBEAN GROWTH PERFORMANCE AS AFFECTED BY PLANT DENSITY, ZINC ADDED TO PHOSPHORUS FERTILIZER SOURCE UNDER DIFFERENT ENVIRONMENTAL CONDITIONS - *Madlile Benjamin Gonyane*	USING DIGITAL SOIL MAPPING METHODS FOR PRECISION AGRICULTURE - *Joaquim Geraldes*	EFFECTS OF DIFFERENT SOIL SUBSTRATES ON GROWTH AND MINERAL COMPOSITION OF LESSERTIA FRUTESCENS L. (GOLDBLATT & J.C. MANNING) SEEDLINGS CULTIVATED UNDER GLASSHOUSE CONDITIONS - *Nokuthula Sithole*
17h50	**Poster session**		
18h30	**Cocktail**		

APPENDIX C: ORAL PRESENTATION AT IPUF BY MS Y. MTIMKULU (PhD STUDENT)



Welcome Reception

17h00–17h45	• Prof Ben-Erik van Wyk (IPUF Chairman) • Technology Innovation Agency: Mr Vuyisile Hobololo (Head: IKS Knowledge Systems) • Dr Ryan Rattray (IPUF Conference Coordinator)
17h45	Sundowners & Cocktail Function

10h30–11h00	Tea Break

Session 7: Cultivating Success: Strategies for Commercialisation and Quality Control 2
Chairperson: Callistus Bvenura

11h00–15h15	Dezil Phillips
11h15–11h30	Yandiswa Mtimkulu
11h30–11h45	Louise Swemmer
11h45–12h00	Rendani Nemakanga
12h00–12h15	Erika Oberholzer
12h15–12h30	Ekaterina Kotina
12h30–13h30	Lunch: !Khwa ttu Restaurant

Session 8: Cultivating Success: Strategies for Commercialisation and Quality Control 3
Chairperson: Ancia Cornelius

13h30–13h45	Refilwe Mhaladi
13h45–14h00	Mpho Mohlakoana
14h00–14h15	Bongiwe Sokwaliwa
14h15–14h30	Nomakhosi Mpofana
14h30–14h45	Louisa Maile
14h45–15h00	Aluwani Nelwamondo
15h40–15h00	Tea Break

Guided Tours

15h30–17h00	Guided Tours at !Khwa ttu
17h00	End of Day 4
19h00	Gala dinner & Awards – !Khwa ttu Restaurant

INDIGENOUS
PLANT USE FORUM
11–16 AUGUST 2024
!Khwa ttu, West Coast, Western Cape

PROGRAMME-AT-A-GLANCE
2024

APPENDIX D: A REVIEW ARTICLE BY MS Y. MTIMKULU (PhD STUDENT)

Journal of Medicinal Plants for Economic Development
ISSN: (Online) 2616-4809, (Print) 2519-559X

Page 1 of 8 Review Article

Cultivation and beneficial uses of *Pelargonium sidoides* DC. – A review

Authors:
Yandiswa Mtimkulu[1,2]
Muinat N. Lewu[2]
Azwimbavhi R. Mulidzi[2]
Francis Lewu[1]

Affiliations:
[1]Department of Agriculture, Faculty of Applied Sciences, Cape Peninsula University of Technology, Cape Town, South Africa

[2]Department of Soil and Water Science, Faculty of Soil Science, Agricultural Research Council, Stellenbosch, South Africa

Corresponding author:
Yandiswa Mtimkulu,
mtimkuluy@arc.agric.za

Dates:
Received: 08 Feb. 2024
Accepted: 26 Mar. 2024
Published: 16 May 2024

How to cite this article:
Mtimkulu, Y., Lewu, M.N., Mulidzi, A.R. & Lewu, F., 2024, 'Cultivation and beneficial uses of *Pelargonium sidoides* DC. – A review', *Journal of Medicinal Plants for Economic Development* 8(1), a246. https://doi.org/10.4102/jomped.v8i1.246

Background: *Pelargonium sidoides* (*P. sidoides*) DC. (Geraniaceae) is one of several geophytic species of the genus that are important traditional medicines in South Africa. *P. sidoides* has been identified as a potential future economic species known to cure various ailments, including respiratory infections.

Aim: This review was aimed at addressing concerns around the overexploitation of *P. sidoides* in the wild.

Setting: This review provides an overview of *P. sidoides* cultivation and usage.

Method: A comprehensive literature search involving mainly electronic and library sources of information was used to collate and synthesise published data.

Results: According to the findings of the study, there has been a huge increase in demand for the plant and it has been overexploited locally as a result of increased domestic and global demand from native consumers and the pharmaceutical industries.

Conclusion: The review emphasises the necessity of cultivation in ensuring the sustainability of *P. sidoides* in the wild. Cultivation is a crucial component of conservation attempts which is under threat because of increasing urbanisation, habitat degradation, and population growth. Furthermore, producing medicinal plants allows new rural farmers to produce them as a new crop option, reducing unsustainable wild collection and competition with established commercial farmers who mostly raise food crops. Lastly, the study reveals the benefits in cultivating medicinal plants namely the strengthening of primary healthcare through traditional medicine, the preservation of indigenous knowledge, local economic growth, and job creation.

Contribution: The benefits of cultivation and using *P. sidoides* medicinally are reviewed in this essay.

Keywords: Geraniaceae; *Pelargonium reniforme*; *Pelargonium sidoides*; Schwabe; Cultivation; Geraniaceae.

Introduction

Pelargonium sidoides (*P. sidoides*) DC. (Geraniaceae) is one of the estimated 3000 medicinal plant species that have been documented as traditional medicine used across South Africa (Van Wyk, Oudtshoorn & Gericke 1997). About 350 species of medicinal plants are the most commonly used and traded (SANBI 2006). However, Van Wyk (2008) claims that about 38 indigenous species have been commercialised to some extent (Table 1). These plants are available as processed materials in modern packaging and in various dosage forms such as teas, tinctures, tablets, capsules or ointments. Several others are also produced for multi-million rand informal markets (Cunningham 1988; Mander 1998).

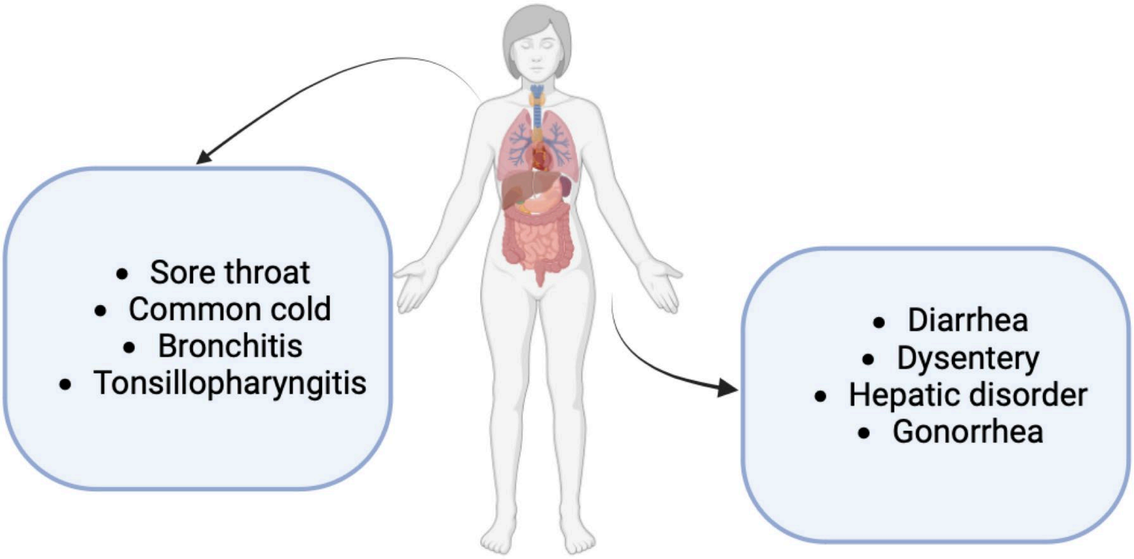
The genus *Pelargonium* is a fundamental part of the Cape flora, and the centre of diversity is the South-Western part of South Africa (Lalli et al. 2006). Most of the *Pelargonium* plants cultivated in Europe and North America originated from South Africa (Sayre 2003). They are adapted to a wide Faltitudinal range, spanning from near sea level in the Eastern Cape of South Africa to 2746 m above sea level in the Lesotho highlands (Newton et al. 2013).The species is commonly called geranium, 'umckaloabo', Uvendle (isiXhosa), Kalwerbossie (Afrikaans), i-Yeza lezikali (isiXhosa), Icwayiba (isiXhosa), ikhubalo (isiXhosa), Rabassam or Rabas (Dutch or Afrikaans), and Khoaara e nyenyane (Sesotho). *P. sidoides* is a native plant to South Africa and Lesotho (Brendler & Van Wyk 2008). The plant has well-developed tubers that enable it to survive harsh environmental conditions, and also circumvent the perennial grass fires that occur across its distribution (Van der Walt & Demarne 1989). Numerous studies have been carried out on the antibacterial and

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

APPENDIX E: PRE-PLANTING SOIL PHYSICAL AND CHEMICAL PROPERTIES

Parameters	Value
Physical properties	
Soil texture	Loam
Sand (%)	72
Silt (%)	12
Clay (%)	16
Chemical properties	
C (%)	0.43
CEC (cmol/kg)	2.50
pH (KCl)	4.53
Electrical Conductivity (mS/cm)	0.282
P (Ambic 1) (mg/kg)	8.10
K (mg/kg)	77.00
NH_4-N (mg/kg)	11.48
NO_3-N (mg/kg)	5.52
Cu (mg/kg)	0.49
Zn (mg/kg)	0.92
Mn (mg/kg)	1.90
B (mg/kg)	<0.10
Fe (mg/kg)	65.30
S (mg/kg)	6.00
Exchangeable cations (cmol/kg)	
Na	0.20
K	0.20
Ca	1.59
Mg	0.56
Base saturation (%)	
Na	5.85
K	5.85
Ca	46.49
Mg	16.37

APPENDIX F: SUMMARY OF AILMENTS THAT CAN BE TREATED WITH *P. SIDOIDES*



APPENDIX G: EARLY STAGES OF THE TRIAL

APPENDIX H: ROOT ROT OF *P. SIDOIDES* AT 75% PLANT AVAILABLE WATER

APPENDIX I: IRRIGATION GUIDELINES OF *P. SIDOIDES*

These are preliminary irrigation guidelines since the project was only funded for three years and maturity of *Pelargonium sidoides* was not yet attained at this age. The study only covered two and half years of the research, therefore further research is needed in order to provide quality findings and irrigation guideline for *P. sidoides* cultivation.

However, based on the work done so far and the project results, these preliminary irrigation guidelines are proposed:

(i) At least 50% plant available water depletion should be allowed between irrigations to allow sufficient aeration since 75% plant available water can result in root rot of *P. sidoides*

(ii) *P. sidoides* grows well on average soil that is well drained, therefore, the internal drainage in the root zone of cultivated *P. sidoides* must not be restricted, to avoid root rot

(iii) Irrigation water must not percolate beyond the root depth to avoid over irrigation and leaching of soil nutrients

(iv) Irrigating at 50% PAW will yield fresh root that is on par with the 75% PAW; whereas for dry root yield, the 50% PAW ensures the highest yield while the lowest root yield will be obtained at 25% PAW due to root rot limiting yield

(v) Farmers can reduce production cost vis-à-vis irrigation cost by decreasing watering frequency, as frequent irrigation showed no significant impact on dry root yield