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Research article

Effects of light intensities and varying watering intervals on growth, tissue nutrient content and antifungal activity of hydroponic cultivated *Tulbaghia violacea* L. under greenhouse conditions

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ABSTRACT

Optimization of the quality and quantity of medicinal materials during cultivation could improve the value of medicinal plants. Light intensity and water availability affect physiological processes and growth of plants. *Tulbaghia violacea* L. (Alliaceae) bulbs and leaves are widely used traditionally in southern Africa for treatments of many ailments. The interactive effects of light intensity and watering regime on plant growth, nutrient uptake and antifungal activity of *T. violacea* were evaluated in the current study. Seedlings of *T. violacea* were grown hydroponically under two shading levels (0% and 40%) while being exposed simultaneously to one of three watering intervals: 5-day, 14-day and 21-day. Different growth parameters (plant height, plant fresh and dry weights) and tissue nutrient contents were evaluated at the end of the experiment. The antifungal activity of acetone extracts on *Fusarium oxysporum* were evaluated in a microdilution bioassay. Generally, significantly higher concentrations of macronutrients were recorded in the tissue of plants exposed to shorter watering interval. The results showed that different watering frequencies and light intensities significantly ($p < 0.05$) influenced plant growth parameters (height, and dry and fresh weights). Moreover, there were strong interactive effects of watering frequency and light intensity on most of the plant growth parameters. Remarkably, plants that were simultaneously exposed to the extended watering interval (21-day) and low light intensity showed the best anti-*F. oxysporum* activity. Key findings of this study revealed that shading alleviated the negative effects of water-deficit stress on plant growth and improved antifungal activity.

1. Introduction

The accumulation of biosynthesised secondary metabolites in plants could enhance the value of plant-based medicinal materials (Lubbe and Verpoorte, 2011). However, the physiological and morphological processes in plants are affected by ambient environmental factors. Water, for example, is an essential resource for plant growth and survival, and its availability influences plant physiological processes including biosynthesis of secondary metabolites and enzyme activities. Water has many essential roles in plants: it is used for translocation and distribution of nutrients and metabolites, it maintains rigidity of plant organs, it is a medium for chemical reactions, and it is an essential component of the photosynthetic process (Mengel et al., 2001). Water deficit is the most important limiting factor of plant growth (Marchese et al., 2010). Water deficits can affect photosynthesis through stomata closure and decreased CO_2 diffusion to the chloroplast (Pinheiro and Chaves, 2011). Drought stress is also associated with the enhancement of accumulation of many classes of natural products in plants, such as terpenes, phenols, alkaloids and glucosinolates (Selmar and Kleinwächter, 2013). Leaves of water-stressed *Ctenanthe setosa* (Rosc.) tended to accumulate more carbohydrates of low molecular weight and phenolic acids (Ayaz et al., 2000). A significant increase in both leaf and plant artemisinin was observed among plants that were maintained under water deficit compared to well-watered plants (Marchese et al., 2010). However, Selmar and Kleinwächter (2013) argued that since drought stress also reduces growth and biomass production in most plants, drought stress-related increase in natural product concentrations does not mean that the rate of biosynthesis of natural products in the plants has increased.

Another important and well-studied factor that influences the morphological and physiological processes in plants is light (Humbert et al., 2007). When plants are exposed to low light intensity, they tend to

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have elongated leaves, and increased leaf surface area and plant height (Guo et al., 2013; Setiawati et al., 2018; Rezai et al., 2018). Low irradiance has also been associated with secondary metabolite production (Rezai et al., 2018). High light intensity can induce plants to produce high starch and carbohydrate contents, which contribute positively to their biomass (Köse, 2014). Baligar et al. (2006) reported that in legumes, growth, nutrient uptake and use-efficiency ratios were higher at higher Photosynthetic Photon Flux Density (PPFD) than at lower PPFD. However, when plants are simultaneously exposed to more than one stress factor, the responses are more complex. For instance, it was observed that shading alleviated the negative impact of drought on leaf traits and biomass characteristics of *Acer buergerianum* Miq (Guo et al., 2013). On the other hand, there was no interactive effect between light and water treatments on biomass accumulation in *Quercus suber* L. seedlings (Puértolas et al., 2008). Holmgren (2000) argued that shading could reduce the impact of drought by limiting loss of water in soil during evaporation. Puértolas et al. (2008) and Quero et al. (2006) described three hypotheses to predict the possible responses of plants to the interactive effects of water stress and light availability. These are trade-off hypothesis — plants that are adapted to deep shade may adapt relatively poorly to drought than other plants growing under higher light levels, facilitation hypothesis — shade enhances survival and physiological status of plants by decreasing evaporative demands and radiation loads, and orthogonal hypothesis — the combined effects of shade and water-shortage are independent, and their impacts are orthogonal. Puértolas et al. (2008) further argued that interactive responses are influenced by plant species, the intensity of water stress, the range of light intensities, the traits considered and seedling age or environmental conditions. Currently, however, reports that address the interaction of light and water stresses on bioactivity of medicinal plant extracts are scarce.

Medicinal plants are an important source and inspiration for discovery of new products for drug development (Xego et al., 2016). Consequently, many research activities have focused on the manipulation of these secondary metabolites in plants and yields of medicinal materials in order to meet the demands of the pharmaceutical industry, traditional healers and the cosmetics industry (Bourgaud et al., 2001). In South Africa, *T. violacea* bulbs and leaves are traditionally used for treatments of gastrointestinal ailments, asthma, fever and tuberculosis; the leaves are used to treat cancer of the oesophagus (Kulkarni et al., 2005; Van Wyk et al., 2009). Previously, crude extracts from *T. violacea* showed good antimicrobial activities against bacterial strains (Ncube et al., 2011). *T. violacea* has been shown to have similar antibacterial and antifungal activities as *Allium sativum* (garlic) (Motsei et al., 2003; Krstin et al., 2018). *T. violacea* is rich in sulphur-containing compounds including thiosulfinate marasmicin (2,4,5,7-tetrathiaoctan-4-oxide) which exhibit significant antimicrobial activities (Ranglová et al., 2015; Takaidza et al., 2015). Kubec et al. (2002) isolated (R(S)R(C))–S-(methylthiomethyl) cysteine-4-oxide from the rhizomes of *T. violacea*. The sulphur compounds in *T. violacea* are unstable and Jäger and Stafford (2012) reported that grinding the rhizome material in liquid nitrogen and extraction with ethanol yielded the best results and the sulphur compounds in the rhizomes decreased rapidly upon storage, after harvest. Methyl alpha-D-glucopyranoside, a bioactive compound that can selectively kill cancer cells was successfully isolated from *T. violacea* using apoptosis-guided purification (Lyantagaye, 2013).

Tulbaghia violacea is regularly harvested from the wild by traditional healers, a practice that may cause decline of the species' populations in the wild (Van Wyk et al., 2009; Mander and Mckenzie, 2005). The persistent high demand might eventually place *T. violacea* at risk of extinction (Mander, 1998; Raimondo et al., 2009). Hence, there is a need to develop optimum cultivation protocols that will ensure improved crop yield and quality of medicinal materials. Plant growth parameters, such as dry and fresh weights, plant height, and antifungal activity of plant extracts are useful indicators of yield and quality of medicinal materials. Since ambient environmental conditions during cultivation can influence plant physiology, plant health and crop yield, a greenhouse is a perfect facility for manipulating most exogenous factors like humidity, light, temperature and water. Furthermore, growing plants in greenhouses could help circumvent many challenges, such as land availability, water availability, season, climate, pests and diseases, which are major concerns with conventional cultivation of indigenous plant species (Pierik, 1987; Arikat et al., 2004).

The objective of this study was to assess the individual and interactive effects of light intensity and watering regime on plant growth, nutrient uptake and antifungal activity of extracts of *T. violacea* plants, grown hydroponically.

2. Methods and materials

2.1. Plant materials

One month old *T. violacea* plantlets of the same cultivar obtained from Best Western Seedlings Nursery (Varkens Vlei Road, Phillipi, Western Cape, 7785, South Africa) in six-pack trays were used in this study. The plantlets were propagated using the division method; the root clumps were separated and gently washed with tap water. Thereafter, the plantlets were transplanted into 15 cm black plastic pots (Plastics for Africa, Somerset West, Cape Town, South Africa) filled with river sand supplied by Builders Warehouse Pty Ltd, Cape Town, South Africa. The potted plants were then spaced 30 cm apart on the concrete floor surface of a climate-controlled greenhouse.

2.2. Experimental design and treatments

A factorial experiment design was used to test the main effects of two factors (shading and watering interval) with two shade levels and three watering intervals on *T. violacea* plants. Five potted plants were randomly allocated and simultaneously treated to one of two shade levels and one of three watering intervals in four replicates. The effects of the interactions of the two factors on plant growth, tissue nutrient contents and antifungal activity of root extracts of *T. violacea* were assessed.

Experimental plants were cultivated under one of two shade levels (0% and 40% shade) and were simultaneously treated to one of varying watering intervals including 5-day, 14-day and 21-day. The 0% shade treatment was achieved by exposing plants to natural sunlight that entered through the polycarbonate roof cover of the greenhouse, and the average unshaded light intensity (Photosynthetic Photon Flux Density [PPFD]) measured at noon was 807.5 μmol m^{-2} s^{-1}. In the 40% shade treatment, the light intensity was reduced with a 40% black shading screen cloth (Alnet, Epping, Western Cape, South Africa). The black shading screen cloth was suspended horizontally at a height of two meters above the floor surface of the greenhouse using ropes tied to metal poles, and it covered an area of 5 m^2. The potted plants were arranged in randomized complete blocks under the shade net and in the unshaded part of the greenhouse. Twenty plants were randomly allocated to each block of replicates. Plants were drip irrigated with Nutrifeed fertilizer (supplied by Starke Ayres Pty Ltd, Cape Town). The fertilizer contained the following ingredients: N (65000 mg kg^{-1}), P (27000 mg kg^{-1}), K (130000 mg kg^{-1}), Ca (70 mg kg^{-1}), Cu (20 mg kg^{-1}), Fe (1500 mg kg^{-1}), Mo (10 mg kg^{-1}), Mg (22 mg kg^{-1}), Mn (240 mg kg^{-1}), S (75 mg kg^{-1}), B (240 mg kg^{-1}) and Zn (240 mg kg^{-1}). The nutrient solution was prepared by dissolving 60 g of the fertilizer into a 60 L black reservoir filled with municipal tap water. An airstone was placed in each reservoir to add oxygen to the nutrient solution. Two hundred and fifty millilitres of the nutrient solution was applied to each plant daily. The experiment was conducted in a climate controlled greenhouse located at the nursery of the Cape Peninsula University of Technology, Bellville, Western Cape, South Africa from 1 February to 30 April, 2017 (3 months). The temperatures in the climate controlled greenhouse ranged from 24-26 °C during day and 15–20 °C during night and the average relative humidity was 74% RH.

2.3. Data collection (plant growth parameters)

Data of the different plant growth parameters were recorded at the end of the experiment. The height of the plant (from the river sand media level to the tip of the tallest shoot) was recorded at two months post-treatment using a measuring tape. The number of leaves was enumerated at two months post-treatment. At the end of the experiment, plants were harvested and fresh weight was immediately measured. In order to determine the dry weight, harvested plants were placed separately in paper bags and dried in a thermo-oven at 70 °C, and the dried plant samples were weighed.

2.4. Tissue analyses

Leaf samples were analyzed for macronutrients and micronutrients by a commercial laboratory, Bemlab Pty Ltd, Somerset West, South Africa. Leaves were washed with a teepol solution, rinsed with de-ionized water and dried at 70 °C overnight in the thermo-oven. The dried leaves were then milled and ashed at 480 °C and shaken up in a 50:50 Hydrochloric (HCl) (50%) solution for extraction through filter paper (Campell and Plank, 1998; Miller, 1998). Potassium (K), Phosphorus (P), Calcium (Ca), Magnesium (Mg), Sodium (Na), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Z) and Boron (B) contents of the extracts were analysed using the Ash method. Total Nitrogen (N) content of the leaves was determined through total combustion in a Leco N-analyser. The amount of N, P, K, Ca and Mg were converted from percentage (%) to mg gDW^{-1}, while Mn, Fe, Cu, Z and B were converted from percentage (%) to μg gDW^{-1}

2.5. Evaluation of antifungal activity

2.5.1. Plant extract

Plant material (bulbous roots) from the different treatments were excised into smaller pieces and air-dried at 35 °C for 7–14 days. The dried materials were then ground separately to a fine powder using a Jankell and Kunkel model A 10 mill. Acetone is a useful extractant because it dissolves a wide range of hydrophilic and lipophilic compounds and is less toxic (Eloff, 1998). The powdered root material (3 g) was extracted with 60 ml of acetone in a 500 ml glass bottle. The acetone extraction was left overnight, and then filtered with a Whatman No.1 filter paper. The filtrate was left to dry overnight at room temperature (25 °C) and the dried acetone extract was weighed to obtain extract yield.

2.5.2. Microdilution assay

The antifungal activity was evaluated using the minimum inhibitory concentration (MIC) value obtained in a microdilution assay. *Fusarium oxysporum* f. sp. glycines strain (UPFC no. 21) obtained by courtesy of the Phytomedicine Programme, University of Pretoria, South Africa was used as the pathogenic agent in the bioassay. The *F. oxysporum* strain was sub-cultured from stock agar plates and grown into nutrient broth (Merck, South Africa) for 4 h. The concentration of fungal spores in the nutrient broth was determined using a haemocytometer. One hundred microlitres of solution containing crude acetone extracts of plant roots was serially diluted with sterile distilled water in 96-well microplates (two-fold serial dilution). The fungal suspension (100 ml) was added to each well of a 96-well microplate (10^5 spores ml^{-1}). Forty micro litre of 0.2 mg ml^{-1} of *p*-iodonitrotetrazolium chloride (INT) (Sigma) dissolved in sterile distilled water was added to each microplate well, sealed in a plastic bag and incubated at 37 °C and 100% RH. Acetone was used as a negative control. The MIC values were recorded after 6, 12 and 18 h. There were three replicates per treatment and per watering interval. The MIC value (mg ml^{-1}) and the weight of the extract obtained following acetone extraction were used to determine the Total Activity (TA). The unit of TA is ml g^{-1} and it indicates the degree to which the active compounds in one (1) g of plant materials can be diluted and still inhibit the growth of the tested microorganisms (Eloff, 2000, 2004).

2.6. Statistical analysis

The experimental data for plant growth parameters, tissue nutrient content, and minimum inhibitory concentration and total activity were collected and analysed using one-way and two-way analyses of variance (ANOVA). The means were compared using Tukey HSD at $p < 0.05$ level of significance. These computations were performed using PAST (Hammer et al., 2001) and graphs were plotted on MS Excel 2018.

3. Results

3.1. Number of leaves

At higher light intensity, there was a significant difference ($DF = 2, 9$; $F = 0.8$; $p < 0.05$) in the mean number of leaves produced by plants among the different watering interval treatments. Significantly higher mean numbers of leaves were recorded in plants subjected to 5-day (10.3 ± 0.4) and 14-day watering intervals (10.3 ± 0.4) compared to those in the 21-day watering interval (8 ± 0.4) under low light intensity (Figure 1). When plants exposed to 5-day and 14-day watering intervals were compared, no significant difference ($p > 0.05$) was observed under 0% shading and 40% shading. However, plants maintained under the 21-day watering interval and low light intensity had significantly ($DF, 2, 6$; $F = 0.2$; $p < 0.05$) more leaves than the 21-day counterparts under higher irradiance. No significant interaction between light intensity and watering interval ($DF = 2$; $F = 4.2$; $p > 0.05$) on number of leaves of this species was observed.

3.2. Plant height

There was a significant difference ($DF = 2, 9$; $F = 0.6$; $p < 0.05$) in plant heights among the different watering interval treatments under the higher light intensity (0% shading). Plants subjected to the 5-day watering interval and high irradiance had a significantly higher mean height (25.2 ± 0.7 cm) compared to those under the 14-day watering interval (19.5 ± 0.6 cm) (Figure 2). The lowest mean height was obtained with plants exposed to the 21-day watering interval at 0% shading (Figure 2). Under low light intensity, plant heights also varied significantly ($p < 0.001$) among the different watering regimes. The tallest plants were observed in the 5-day watering regime (34.2 ± 0.9 cm) followed by the 14-day watering interval (28.8 ± 1.0 cm), and the shortest was obtained in the 21-day watering interval (23.5 ± 1.0 cm) under low light intensity (Figure 2). However, when plants exposed to 40% shading and 0% shading were compared to each other for the varying watering intervals, higher mean heights were obtained in plants grown under the lower light condition for all three watering intervals. The interactive effect between light and watering interval was significant ($DF = 2$; $F = 4.2$; $p < 0.01$) in influencing the height of *T. violacea*.

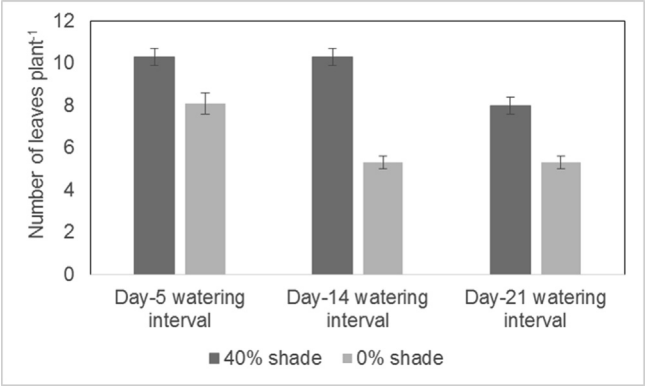

Figure 1. Mean ± SE number of leaves per plant of *T. violacea* grown under 40% shade (low light) and 0% shade (high light) conditions while exposed to different watering regimes at two months post treatment.

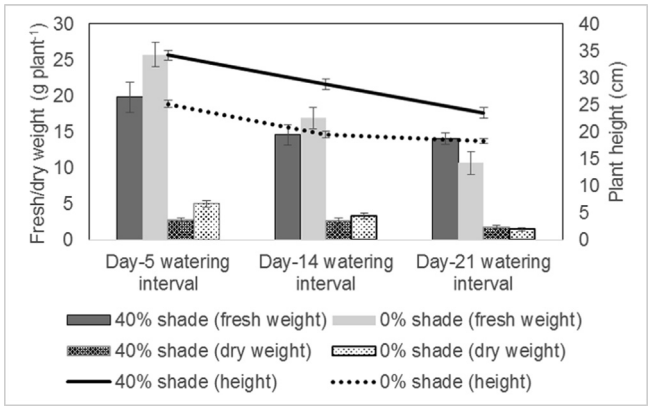

Figure 2. Mean ± SE fresh weight, dry weight and height of *T. violacea* grown under low light (40% shade) and high light (0% shade) conditions while exposed to different watering regimes at two months post treatment.

3.3. Fresh weight

There were significant differences (DF = 2, 9; F = 1.2: $p < 0.05$) among watering intervals in the mean fresh weights after two months of cultivation under high light intensity. Unshaded plants that were watered at 5-day intervals had significantly higher ($p < 0.05$) mean fresh weights (25.7 ± 2.1 g plant^{-1}) when compared to both 14-day (16.9 ± 1.4 g plant^{-1}) and 21-day (10.7 ± 0.8 g plant^{-1}) watering intervals (Figure 2). The highest mean value for total fresh weight in plants grown under higher light intensity was recorded in the 5-day watering interval (25.7 ± 2.1 g plant^{-1}) followed by the 14-day watering (16.9 ± 1.4 g plant^{-1}) and lowest was obtained in the 21-day watering interval (10.7 ± 0.8 g plant^{-1}) (Figure 2). In plants grown under low light intensity, there was a significant difference ($p < 0.05$) among the different watering regimes. For low light, the highest mean value for total fresh weight was recorded in the 5-day watering interval, while the 14-day watering interval (14.6 ± 1.5 g plant^{-1}) and 21-day day watering intervals (14.1 ± 1.2 g plant^{-1}) followed (Figure 2). However, despite the drop in the fresh weight with increasing watering intervals in the shaded plants, the shaded plants had significantly ($p < 0.05$) higher fresh weight compared to the unshaded plants at under 21-day watering intervals. In this study, the highest mean value for total fresh weight was observed in plants grown under high light intensity and watered at the 5-day interval. The interaction between light intensity and watering interval on fresh weight of *T. violacea* was significant (DF = 2; F = 4.3; $p < 0.05$).

3.4. Dry weight

Under 0% shade condition, the total dry weights significantly differed (DF = 2, 9; F = 1.5; $p < 0.05$) among the different watering regimes. The 5-day watering interval (5.0 ± 0.5 g plant^{-1}) treatment had the heaviest dry weight for leaves compared to the 14-day watering interval (3.3 ± 0.4 g plant^{-1}) and 21-day watering interval (1.5 ± 0.2 g plant^{-1}) for plants grown under high light intensity (Figure 2). For plants grown under low light intensity, there was no significant difference in dry weights when day watering intervals (5, 14, and 21) were compared. Interestingly, plants that were concurrently exposed to 40% shading and 21-day watering interval had significantly ($p < 0.05$) higher dry weight than plants that were simultaneously exposed to the higher irradiance and 21-day watering interval. Under high watering frequencies, plants exposed to higher light intensities had higher mean dry weights; however, the reverse was observed at the lowest watering frequency. A statistically significant interaction was detected between shading and watering interval on the dry weight of *T. violacea* (DF = 2; F = 7.3; $p < 0.05$).

3.5. Tissue analysis

3.5.1. Macronutrients

Broadly, tissue macronutrients contents correlated with watering frequency; plants took up more macronutrients in the higher frequency watering intervals (Table 1). Plant tissue nutrient contents of N, P, K, Mg observed under shade were significantly higher than the unshaded plants under the 5-day watering interval (Table 1). Significant interactive effects (DF = 2; $p < 0.05$) of light intensity and watering regime on N, Mg and Ca were observed.

3.5.2. Micronutrients

The nutrient contents of Cu and Fe under low and high light intensities did not show significant differences among the watering regimes (Table 2). However, there were significant differences ($p < 0.05$) on the tissue nutrient contents of Mn and B among plants in the different watering intervals. The nutrient contents for B did not vary in the tissue of plants grown under 0% shading and 40% shading for all the watering interval treatments. Generally, there was no clear pattern in the values for tissue micronutrients in plants grown under the low light intensity and those in the high light intensity.

3.6. Antifungal activity

There was no significant difference ($p > 0.05$) in the MIC values among the different watering interval treatments. Based on the MIC

Table 1. Tissue macronutrient contents (Mean ± SE mg gDW^{-1}) for *T. violacea* grown under low light (40% shade) or high light (0% shade) intensity and simultaneously exposed to one of varied watering regimes for two months.

Watering interval	N		P		K	
	High light	Low light	High light	Low light	High light	Low light
5-day	56.0 ± 1.0aB	61.0 ± .0.4aA	5.0 ± 0.2aB	6.0 ± 0.1aA	69 ± 0.5aB	80.2 ± 0.3aA
14-day	49.0 ± 3.7baA	39.1 ± 0.4cB	3.0 ± 0.5bA	2.5 ± 0.2cA	45.1 ± 8.3bB	70.3 ± 2.4bA
21-day	48.0 ± 1.1aA	46.3 ± 1.3bA	4.0 ± 0.2abA	3.6 ± 0.1bA	56.6 ± 0.1abB	65.3 ± 2.0bA
Interactive effect (Two-way anova)	DF = 2; F = 9.25; $p < 0.05$		DF = 2; F = 3.2; $p > 0.05$		DF = 2; F = 2.91; $p > 0.05$	
Watering interval	Ca		Mg		Na	
	High light	Low light	High light	Low light	High light	Low light
5-day	14.7 ± 1.3aA	13.6 ± 0.3cA	5.6 ± 0.4aA	4.0 ± 0.1abB	2.9 ± 0.1aA	27 ± 0.1cA
14-day	14.4 ± 3.6aA	21.0.±1.0aA	3.4 ± 0.8bA	4.5 ± 0.2aA	2.9 ± 0.6aB	6.0 ± 0.2aA
21-day	15.2 ± 1.1aA	16.8 ± 0.6bA	3.9 ± 0.2abA	3.5 ± 0.1bA	3.0 ± 0.2aB	4.8 ± 0.1bA
Interactive effect (Two-way anova)	DF = 2; F = 4.67; $p < 0.05$		DF = 2; F = 5.72; $p < 0.05$		DF = 2; F = 17.67; $p < 0.001$	

Means followed by the same lowercase letters in the same column are not significantly different following Tukey's test ($p > 0.05$). Means followed by the same uppercase letters in the same row, for each nutrient and each watering interval, are not significantly different following Tukey's test ($p > 0.05$).

Table 2. Tissue micronutrient contents (Mean ± SE μg gDW^{-1}) for *T. violacea* grown under low light (40% shade) or high light (0% shade) intensity and simultaneously exposed to one of varied watering regimes for two months.

Watering interval	Mn		Fe		Cu		Zn		B	
	High light	Low light	High light	Low light	High light	Low light	High light	Low light	High light	Low light
5-day	59.0 ± 2.5aA	61.5 ± 2.3aA	196.3 ± 21.7aA	113.8 ± 7.7aB	2.8 ± 0.3aA	2.8 ± 0.5aA	29.8 ± 1.4aA	31.0 ± 2.1bA	91.5 ± 4.6aA	85.8 ± 1.1aA
14-day	26.5 ± 5.8bA	30.0 ± 2.7bA	129.8 ± 33.7aB	176.5 ± 14.1aA	2.5 ± 0.6aA	2.0 ± 0.0aA	21.3 ± 3.7aB	40.8 ± 3.3aA	39.5 ± 9.2bA	61.3 ± 3.0bA
21-day	27.3 ± 2.0bA	15.5 ± 0.9bB	121.8 ± 10.3aA	115.0 ± 12.8aB	2.0 ± 0.0aA	2.5 ± 0.6aA	23.5 ± 0.6aB	31.5 ± 1.3bA	46.8 ± 1.7bA	42.3 ± 1.3cA
Interactive effect (Two-way anova)	DF = 2; F = 3.83; p < 0.05		DF = 2; F = 3.83; p < 0.05		DF = 2; F = 0.6; p > 0.05		DF = 2; F = 7.60; p < 0.001		DF = 2; F = 4.15; p < 0.05	

Means followed by the same lowercase letters in the same column are not significantly different following Tukey's test (p > 0.05). Means followed by the same uppercase letters in the same row, for each nutrient and each watering interval, are not significantly different following Tukey's test (p > 0.05).

results, the strongest inhibition of *F. oxysporum* by the bulbous root extracts was observed in plants that were simultaneously subjected to the 5-day watering interval and under the higher light intensity with the value of 0.1 ± 0.0 mg ml^{-1} after 6 h followed by plants exposed to the 14-day watering interval. The interaction between light intensity and watering interval in influencing the antifungal activity of bulbous root extracts of *T. violacea* was not significant (DF = 2; F = 1.1; p > 0.5) (Table 3). The total activity value for plants that were exposed to the higher irradiance was significantly higher under the higher watering interval (5-day) compared to plants that were exposed to low irradiance and the 5-day watering interval, which was 85.1 ± 5.2 ml g^{-1} at 6 and 12 h (Table 4). However, bulbous root extracts of plants grown under low light intensity showed significantly higher (p < 0.05) activity on *F. oxysporum* under extended watering interval, up to 118.4 ± 3.8 ml g^{-1} (total activity) at 6 h was recorded at the 21-day watering interval and low light intensity (Table 4). There was a significant interactive effect (DF = 2; F = 56.1; p < 0.01) between light intensity and watering interval on the total activity of the bulbous root extracts of this species.

4. Discussion and conclusion

The watering regime significantly affected the growth parameters as leaf number, fresh and dry weights, and plant height reduced with increasing watering intervals. Generally, the shorter watering interval (5-day) had higher fresh and dry mean weights compared to plants exposed to the longer watering intervals. These results are consistent with the findings of Xego et al. (2016), which showed that more abundant growth in *Siphonochilus aethiopicus* correlated with shorter watering intervals. In another study, water deficit due to long watering intervals had significant negative effects on plant height, leaf number, and induced a higher biomass of adventitious and tap roots of mango (*Mangifera indica*) (Luvaha et al., 2008).

Interestingly, the positive effects of a shortened watering interval on growth and biomass observed in this study correlated well with the increased tissue macronutrients (N, P and K) contents. This observation provides a plausible explanation of the mechanism through which watering interval can influence growth of plants. Higher tissue nitrogen content increases plant growth rates and shifts plant biomass partitioning to aboveground structures (Sims et al., 2012). On the other hand, a decrease in water availability can reduce nutrient uptake, transportation and availability (Menzel et al., 1986; Jimenez et al., 2009; Bistal et al., 2018). Nitrogen availability and the internal N status of plants correlate positively with shoot:root ratios (Ingestad and Ågren, 1991). Potassium and phosphorus are other essential macronutrients that affect physiological processes and influence plant growth and metabolism (Abdolzadeh et al., 2019; Wang et al., 2013).

Variable effects of shading on growth parameters, such as plant height, leaf number and dry and fresh weights were recorded in this study. The plants grown under the 40% shade produced significantly higher mean height and lower fresh and dry weights of aerial parts than

Table 3. Minimum inhibitory concentration (Mean ± SE) on *F. oxysporum* by acetone extracts of *T. violacea* grown under low light (40% shade) or high light (0% shade) conditions and simultaneously exposed to one of varied watering regimes for two months.

Watering interval	MIC (mg ml^{-1}) at 6H		MIC (mg ml^{-1}) at 12H		MIC (mg ml^{-1}) at 18H	
	High light	Low light	High light	Low light	High light	Low light
5-day	0.1 ± 0.0abA	0.1 ± 0.0abA	0.4 ± 0.0aA	0.4 ± 0.0bA	0.8 ± 0.0bA	0.8 ± 0.0bA
14-day	0.3 ± 0.1bA	0.3 ± 0.1aA	0.4 ± 0.0bA	0.4 ± 0.0bA	0.8 ± 0.0bA	0.8 ± 0.0bA
21-day	0.4 ± 0.0aA	0.4 ± 0.0aA	0.8 ± 0.0aA	0.8 ± 0.0aA	1.5 ± 0.0aA	1.5 ± 0.0aA
ns						

Means followed by the same lowercase letters in the same column are not significantly different following Tukey's test (p > 0.05). Means followed by the same uppercase letters in the same row for 6H, 12H or 18H and each watering interval, are not significantly different following Tukey's test (p > 0.05).
ns. denotes interactive effect between watering interval and light intensity at 6H, 12H and 18H was not significantly (DF = 2; p > 0.05) following two-way Anova.

Table 4. Total activity (Mean ± SE) of acetone root extracts of *T. violacea* grown under low light (40% shade) or high light (0% shade) intensity and simultaneously exposed to one of varied watering regimes for two months.

Watering interval	Total activity (ml g^{-1}) at 6H		Total activity (ml g^{-1}) at 12H		Total activity (ml g^{-1}) at 18H	
	High light	Low light	High light	Low light	High light	Low light
5-day	85.1 ± 5.2aA	37.1 ± 1.2cB	85.1 ± 5.2aA	18.6 ± 0.6cB	11.76 ± 1.3aA	9.3 ± 0.3bA
14-day	41.7 ± 7.6bB	78.8 ± 12.7bA	10.4 ± 1.9bB	4.0 ± 7.6aA	8.44 ± 0.5bB	13.6 ± 1.0bA
21-day	20.9 ± 4.96cB	118.4 ± 3.8aA	7.3 ± 3.2cB	29.3 ± 0.6bA	5.22 ± 1.2cB	14.7 ± 0.3bA
Interactive effect (Two-way anova)	DF = 2; F = 56.2; p < 0.001		DF = 2; F = 12.5; p < 0.001		DF = 2; F = 23.8; p < 0.001	

Means followed by the same lowercase letters in the same column are not significantly different following Tukey's test (p > 0.05). Means followed by the same uppercase letters in the same row, for 6H, 12H or 18H and each watering interval, are not significantly different following Tukey's test (p > 0.05).

those grown in the 0% shade treatment and short watering intervals (5-day and 14-day). However, under the longest watering interval (21-day) and 40% shading, higher number of leaves ($p < 0.05$) and higher dry weights ($p > 0.05$) were recorded when compared to 0% shading. These results are in agreement with that of Zervoudakis et al. (2012) on *Salvia officinalis* L., which showed that dry mass, number of leaves and physiological parameters had a strong positive correlation with the light intensity, and plant's height and leaf photosynthetic pigments were increased among low light treated plants. Fiorucci and Fankhauser (2017) postulated that low photosynthetically active radiation can induce pronounced phenotypic responses in some species, such as elongation of stem-like structures, elevation of leaves, as well as reduced branching and acceleration of flowering. Under a short-term low irradiance (shade treatments at 5-day), chlorophyll b transiently increased in *Brassica campestris*, but extension of shading time to a 15-day period led to significant decreases in relative chlorophyll a and anthocyanin (Zhu et al., 2017).

There are few studies done on the interactive effects of low light intensity and limited water on plant growth and secondary metabolite synthesis (Kitao et al., 2000; Hazrati et al., 2016). In the present study, plants subjected to both the 21-day watering interval and low light intensity produced more leaves than those grown under the higher light intensity and equivalent watering interval. Research done by Sack et al. (2003) reported that shading could mitigate the negative impact of water stress. Under limited light, plants may accumulate carbohydrates in leaves; these soluble sugars may reduce water loss through turgor maintenance and reduction of stomatal aperture (Morgan, 1984; Abrams, 1986; Augé et al., 1998). Löf et al. (2005) reported an interaction between irradiance and water stress on biomass partitioning in *Fagus sylvatica* seedlings. Yang et al. (2008) argued that plants in shade invest more to produce shoots and leaves than biomass. Broadly, these studies corroborate our finding that watering regime and light intensity have intercative effects on plant growth parameters. Hazrati et al. (2016) reported that about 50% of total solar radiation and irrigation after depleting 40% of soil water content were the most efficient treatments for chlorophyll fluorescence and pigments of *Aloe vera* L. A key finding in this study is that shading alleviated the negative effects of water deficit stress on plant growth. This is in agreement with the report of Guo et al. (2013), in which drought alleviated shading effects on *Acer buergerianum* Miq., and the aboveground facilitation hypothesis (Holmgren, 2000; Guo et al., 2013). Nevertheless, it is worth mentioning that the responses of plants to water and light stress also depend on species. For example, Liu and Su (2016) reported that under low light, *Taxus yunnanensis* produced larger leaves and a higher shoot axis length per unit dry mass under high light, whereas the leaf size and biomass yield of *T. chinensis* were not sensitive to light.

Secondary metabolites play an important role in plant defence. They protect plants against pathogens and herbivory (Compean and Ynalvez, 2014; Pagare et al., 2015). Drought stress can induce plants to produce higher concentrations of secondary metabolites. In this study, although there were no significant differences in the MIC values between plants in low and high light intensities, acetone bulbous root extracts of the plants that were exposed to the longest watering interval (21-day) and 40% shading (low light intensity) yielded the highest (118.4 ± 3.8; $p < 0.05$) total activity. The increase in total activity suggests that there was an interaction between watering interval and light intensity in relation to the yield of acetone extract during extraction. Hazrati et al. (2016) reported that conditions of irradiance of full sunlight and water deficit stress favoured increased anthocyanin production in *Aloe vera*.

In conclusion, broadly, three trends occurred in the results. Firstly, the total weight of *T. violacea* increased with shorter watering intervals under high irradiance. Secondly, shading alleviated the negative effect of water deficit stress on plant growth. Thirdly, the longest watering interval plants (21-Day) had the highest total activity of bulbous root extracts. Furthermore, light intensity and watering interval had significant interactive effects on antifungal activity and plant growth. These results also suggested that nutrient supply and subsequent tissue nutrient levels might be modulating the responses, such as plant growth and biomass, and antifungal activity of plant extracts in relation to light intensity and watering regime. Future studies should investigate the interactive effects of water deficit and shading on production of bioactive compounds in *T. violacea*.

Declarations

Author contribution statement

Wanga Ncise: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Chris W. Daniels: Conceived and designed the experiments; Wrote the paper.

Felix. Nchu: Conceived and designed the experiments; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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