Medicinal Plants
Vol. 13 (3), September 2021, 442-451

doi : 10.5958/0975-6892.2021.00050.2

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Research Article

Interactive effects of light intensity and pH on growth parameters of a bulbous species (*Tulbaghia violacea* L.) in hydroponic cultivation and its antifungal activities

Wanga Ncise, Chris W. Daniels, Ninon G.E.R. Etsassala and Felix Nchu*

Department of Horticultural Sciences, Faculty of Applied Sciences, Cape Peninsula University of Technology, Symphony Way, Bellville, 7535, Cape Town, South Africa

Received: March 01, 2021; Accepted: July 14, 2021

ABSTRACT

Tulbaghia violacea L. (Amaryllidaceae) is one of the most harvested bulbous plants of high medicinal value of South Africa in the wild. So far there is no recommended practice of commercial cultivation and knowledge about antimicrobial activity medicinal of this plant species. In this study, we assessed the interactive effects of light intensity and pH on plant growth, nutrient uptake and antifungal activity of extracts of *T. violacea*, grown under hydroponic environments. One-month-old *T. violacea* plantlets were grown under low (40% shade) and high (0% shade) light intensities in an environmentally-controlled greenhouse with different pH exposures. The effects of light intensity and pH on plant height, plant fresh and dry weights, tissue macro-and micro-nutrient contents as well as antifungal activity (minimum inhibitory concentration [MIC]) were evaluated. The highest dry weight was achieved under the high light intensity treatment (0% shade) combined with the pH 4 treatment (8.29 ± 0.80 g plant^{-1}). Light intensity and pH had significant interactive (DF = 2; $P < 0.001$) effects on fresh and dry weights. Among the tissue nutrients assessed, only tissue N content was significantly influenced by the interaction between pH and light intensity. Acetone extracts of plants simultaneously grown under the high light intensity and pH 4 produced the best antifungal activity with a mean MIC values range from 0.97 ± 0.18 to 1.50 ± 0.00 mg mL^{-1} at 18 hr. In conclusion, high light intensity and acidic conditions significantly increased plant biomass and antifungal activity of the plants in hydroponic solution.

Keywords: *Tulbaghia violacea,* hydroponic cultivation, growth parameters, antifungal activity

INTRODUCTION

The *Tulbaghia violacea* is a very important traditional medicinal plant in South Africa. Because of its high demand and no standard practice of cultivation available yet, the supply target cannot be achieved, thereby warranting studies on high-yielding cultivation technologies (Van Wyk *et al.,* 1997). Environmental conditions are among the key factors that affect plant metabolism and medicinal properties (Lin *et al.,* 2006). Environmental stress factors, such as variation in soil and water pH, water content, temperature, and light intensity may cause the accumulation of reactive oxygen species in plants and secondary metabolite contents, affecting plant growth and health as well as plant medicinal value (Daniels *et al.,* 2011).

Potential hydrogen (pH) plays a significant role in plant growth by influencing the availability of various nutrients

*Corresponding author e-mail: felixnchu@gmail.com

for plant uptake in the substrate (Kuhn *et al.,* 1995; Marschner, 1995). As the pH approaches 7.5 and above, phosphorus, iron, manganese, boron, and zinc are less available for uptake in *Artemisia afra* L. (Koehorst *et al.,* 2010). Elements, such as calcium, magnesium, zinc, and copper are less available at pH 5 and below (Brady and Weil, 2008). pH may influence enzyme actions and subsequently, some metabolic processes and plant growth parameters (Stern, 2006). It has also been scientifically proven that the availability of nutrients to plants affect secondary metabolites production and consequently the medicinal properties of extracts derived from these plants (Economakis, 1993; Maggini *et al.,* 2002; Sugumaran *et al.,* 2013). According to previous reports, the optimum pH levels for the cultivation of *T. violacea*, *Allium cepa* and *Allium sativum* are 6.5, 8, and 5.3-6.5, respectively (Minard, 1978; Nock and Mazelis, 1998; Raji *et al.,* 2015). However, studies that have specifically examined the effects of pH on the tissue nutrient accumulation and medicinal properties of these Amaryllidaceae species are not available.

Another important environmental factor that affects plant growth and physiology is light intensity. When the light intensity changes, it affects plant morphology, physiology, and microstructure (Dia *et al.,* 2009). Each plant requires a certain light intensity to achieve optimal growth. If the intensity of light is too high or too low, the photosynthetic rate is negatively affected (Zhao *et al.,* 2012). Carbohydrate accumulation is influenced by factors such as temperature, relative humidity, and light intensity (Gomes-Laranjo *et al.,* 2006). Under shade conditions, the light becomes limited and consequently, changing temperature, air movement, and carbon dioxide (CO_2); all of which are essential factors for plant growth (Vialet-Chabrand *et al.,* 2017). Plants tend to allocate their photosynthetic end-products to growth processes (Cronin and Hay, 1996). Light is also an essential factor for accumulation of secondary metabolites in plants (Thoma *et al.,* 2020). The quantity and quality of secondary metabolites influence plants' bioactivities (Hou *et al.,* 2010; Ghasemzadeh and Ghasemzadeh, 2011). Currently, however, there is limited scientific literature on the effects of pH treatments under varying light intensities on nutrient uptake and antifungal activity of medicinal plants in hydroponics.

T. violacea is widespread throughout Africa, with the highest population in Southern Africa (Raji *et al.,* 2015). It is one of the most important medicinal plant species for the treatment of cardiovascular diseases in South Africa (Raji *et al.,* 2015). The other common names of this plant is wild garlic, society garlic, sweet garlic (English), wildeknoffel (Afrikaans), isihaqa (Zulu) and itswelelomlambo (Xhosa). *T. violacea* belongs to the family of Amaryllidaceae (Kubec *et al.,* 2002; Harris, 2004). It grows well in soils that are nutrient-poor and have low pH. In South Africa, *T. violacea* bulbs and leaves are traditionally used for the treatment of gastrointestinal ailments, asthma, fever, colds, rheumatism, tuberculosis, paralysis, and stomach problems, while the leaves are used to treat cancer of the oesophagus (Van Wyk *et al.,* 1997; Kulkarni *et al.,* 2005). Furthermore, *T. violacea* is one of the most utilized plants by traditional healers in KwaZulu-Natal. It is difficult to find *T. violacea* outside protected areas because it is excessively harvested by the traditional healers (Aremu and Van Staden, 2013). Published reports suggest *T. violacea* has good antimicrobial, antioxidant, antifungal, and anti-cancer activities (Motsei *et al.,* 2003; Malungane, 2014; Krstin *et al.,* 2018; Takaidza *et al.,* 2015; Ncise *et al.,* 2020). Various classes of compounds with a wide spectrum of pharmaceutical applications were isolated from this species, including thiosulfinate marasmicin, (R(S)R(C))– S-(methylthiomethyl) cysteine-4-oxide and methyl alpha-D-glucopyranoside (Ranglová *et al.,* 2015). The compound allicin occurs in *T. violacea* and is active against microbial infections caused by fungi, viruses, and bacteria in both plants and humans (Malungane *et al.,* 2014). The sulphur-based compounds of *T. vioalcea* have also been found to be active on the cardiovascular system by modulating the renin-angiotensin aldosterone system, the autonomic nervous system, oxidative stress and haemostasis (Raji *et al.,* 2015).

This study aimed to determine the optimum environmental conditions, specifically light intensity and pH levels for cultivating the well-known medicinal plant *T. violacea*, in hydroponic environments with the view of enhancing the yield and quality of chemical constituents for the pharmaceutical companies, traditional healers and horticulture industries. The objective of this study was to assess the interactive effects of light intensity and pH on plant growth, nutrient uptake, and antifungal activity of extracts of *T. violacea* plants, grown hydroponically.

METHODS AND MATERIALS

Plant materials

One-month old *T. violacea* plantlets of the cultivar Silver lace were obtained from Best Western Seedlings Nursery (Varkens Vlei Road, Phillipi, Western Cape, 7785, South Africa). The plantlets were then propagated using the propagation method. The root clumps were divided and gently washed with tap water. The plantlets were then transplanted into 15 cm black plastic pots (Plastics for Africa, Somerset West, Cape Town, 7130) filled with river sand obtained from Builders Warehouse (Pty) Ltd, Cape Town. Plants were then placed on the concrete floor surface of the greenhouse and spaced 30 cm apart.

Greenhouse experiment

Experimental layout

The potted plants were arranged in a completely randomized design with 2 x 3 factorial combinations under the shaded and unshaded part of the greenhouse. Forty individually potted plants were randomly allocated to each of the six treatment combinations. Twenty plants were randomly selected in each treatment group for plant growth assessments and antifungal bioassays and the remaining 20 plants were used for analysis of tissue nutrient contents. Experimental plants were cultivated under one of two shade levels (0 and 40% shade corresponding to high and low light intensities, respectively) and were simultaneously treated to one of varying pH levels: pH 4, 6 and 8. The 0% shade treatment was achieved by exposing plants to natural sunlight that entered through the greenhouse's polycarbonate roof cover and the average unshaded light intensity (Photosynthetic Photon Flux Density [PPFD]) measured at noon was 807.5 ìmol 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.

Plants were drip-irrigated with Nutrifeed fertilizer (supplied by Starke Ayres Pty Ltd, Cape Town). The fertilizer contained the following elements: 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 mgkg^{-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 air stone 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: S33° 54' 0, E18° 38' 0 from 1 February to 30 April (Autumn), 2017 (3 months). The greenhouse temperature ranged from 24-26°C during day and 15–20°C during night and the average relative humidity was 74% RH.

Growth parameters

The different plant growth parameters were recorded at the end of the experiment, at two months post-treatment. The data for each of the growth parameters (plant height, number of leaves, fresh and dry weights) were obtained from 20 plants, representing 20 replicates per experimental unit. The height of the potted plant (from the surface of the river sand (medium) to the tip of the tallest shoot) was recorded at two months post-treatment using a measuring tape. The number of leaves per plant was also 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 35°C for 7–14 days, after which the dried plant samples were weighed.

Tissue analyses

Fresh leaf samples from 20 plants, representing 4 replicates, per experimental unit were analyzed for tissue macro-nutrients and micro-nutrients with help of Bemlab Pvt Ltd in Somerset West, South Africa. Five plants were grouped together to achieve at least 100 g for the tissue analyses. 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 acid (HCl) (50%)

solution for extraction through filter paper. 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 DW^{-1}, while Mn, Fe, Cu, Z and B were converted from percentage (%) to µg DW^{-1}

Evaluation of antifungal activity

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. The powdered root material (3 g) was extracted with 60 mL of acetone in a 500 mL glass bottle. Acetone is a useful extractant because it extracts a wide range of hydrophilic and lipophilic compounds and is less toxic (Eloff, 1998). 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.

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 hr. The concentration of fungal spores in the nutrient broth was determined using a haemocytometer. One hundred microliters 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 iodonitrotetrazolium chloride (INT) (Sigma) dissolved in sterile distilled water was added to each 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 18 hr. 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, 1998)

Statistical analyses

The experimental data for plant growth parameters, tissue nutrient content, and minimum inhibitory concentration and total activity were collected and analysed using 2 x 3 factorial one-way ANOVA to assess effects of pH or light intensity on height, leaf number, fresh and dry weights and antifungal activity and two-way ANOVA to check for the interactive effects between the independent factors pH and light intensity on height, leaf number, fresh and dry weights, tissue nutrient contents, and antifungal activity) analyses of variance. 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.

RESULTS

Height and number of leaves

The plant height ranged from 28.38±0.93-37.35±0.99 cm $plant^{-1}$ and did not vary significantly (df = 2, 57; $P> 0.05$) among pH treatments in both low and high light intensities for all the pH levels. However, plants grown under low light intensity produced the highest mean height values at pH 6 (37.35±0.99 cm $plant^{-1}$), closely followed by pH 4, and the lowest was obtained at pH 8 respectively (Table 1). On the other hand, for high light intensity, the pH 8 treatment produced the highest mean height (29.35±0.72 cm $plant^{-1}$) followed by pH 6 and 4 (Table 1). There was a significant difference in the heights of *T. violacea* between low and high light intensities for all the three-pH levels (Table 1). Based on a two-way ANOVA, the interaction between light and pH was significant (df, 2; F=0.009; $P< 0.001$) in influencing the studied species' height.

Table 1: The effects of different pH treatments (pH 4, 6 and 8) on the height, number of leaves, and dry and fresh weights of *T. violacea* grown under high and low light intensities

Growth Parameters	Treatments	High light (0%) *Mean ± SE	Low light (40%) *Mean ± SE
Height (cm plant^{-1})	pH 4	28.38±0.93aB	37.09±0.86aA
	pH 6	28.40±1.18aB	37.35±0.99aA
	pH 8	29.35±0.71aB	35.72± 0.87aA
Number of leaves per plant	pH 4	11.65±0.48aA	10.60±0.71aA
	pH 6	11.40±0.31aA	9.55±0.27aB
	pH 8	10.55±0.65aA	9.10±0.54aA
Fresh weight (g plant^{-1})	pH 4	39.40±3.77aA	24.00±2.10aB
	pH 6	34.30±2.92aA	21.24±1.44aB
	pH 8	33.75±2.32aA	19.35±2.36aB
Dry weight (g plant^{-1})	pH 4	8.28±0.80aA	4.39±0.39aB
	pH 6	5.56±0.58bA	4.11±0.35aA
	pH 8	3.99±0.37bA	4.04±0.65aA

*Means of each nutrient with the same lowercase letters in the same column are not significantly different (P > 0.05) among plants exposed to different pH levels and simultaneously to high or low intensity; means of each nutrient with the same uppercase letters in the same row are not significantly different (P > 0.05) between plants exposed to high and low intensities and simultaneously to pH level 4, 6 or 8. Tukey test was used to separate means.

There was no significant difference in the number of leaves for all treatments under high light intensity (df = 2, 57; F=1.21; *P* = 0.3362) and low light intensity (df, 2, 57; F=1.21; *P* = 0.405) following one-way ANOVA analysis (Table 1). However, higher mean value of number of leaves was observed at pH 4 (10.9 ± 0.49 plant^{-1}) and pH 6 (10.90±0.331 plant^{-1}) compared to pH 8 (10.55±0.66 plant^{-1}) under high light, respectively. Based on a two-way ANOVA, the interaction between light and pH was not significant (df = 2; F=1.09; *P*< 0.5) in influencing the number of leaves.

Fresh weight and dry weight

There was no significant difference in fresh weights of *T. violacea* among pH treatments in both high light intensity (df = 2, 57; F=1.03; *P* > 0.05) and low light intensity (df = 2; 57; F=1.39; *P* >0.05), respectively (Table 1). However, plants grown under high light intensity produced the highest mean value of total fresh weight in pH 4 (39.41 ± 3.77 g plant^{-1}) followed by pH 6 and 8 compared with the corresponding pH levels under low light intensity. Similarly, the plants grown under low light intensity had higher total fresh plant weight at pH 4 (24.06 ± 2.105 g plant^{-1}) when compared with pH 6 and 8, respectively. Significantly (df = 2, 57; P < 0.05) higher fresh weights were obtained among plants exposed to high light than low light intensities at all pH levels. Overall, the interaction between light and pH was significant (df = 2; F = 0.001; *P*< 0.001) in influencing the fresh weight of this species.

The results revealed a significant difference (df = 2, 57; F=12.63; *P*< 0.05) in total dry weights; plants exposed simultaneously to high light intensity and pH 4 produced the highest dry weight (8.28 ± 0.80 g plant^{-1}) than those under high light at pH 6 and pH 8 (Table 1). On the other hand, under low light intensity, plant weights did not show significant variations (*P* > 0.05) among the different pH treatments. Generally, high light yielded a better mean dry weight than low light at lower pH levels. There was a significant interactive effect (df = 2; F=6.4; *P*< 0.05) between pH and light intensity on the dry weight of this species.

Tissue analysis

Macro-nutrient

Under high light intensity, the levels of tissue macro-nutrients (P, K, Ca, Mg and Na) in plants did not vary

significantly ($P > 0.05$) among pH treatments (Table 2). For plants grown under low light intensity, the level of macro-nutrients (N, P, Ca, Mg, and Na) were significantly different ($P < 0.05$) at different pH levels. The highest N uptake was recorded in plants exposed to pH 4 level under low light intensity (598.50 ± 5.69 mg DW^{-1}), P (59.50 ± 0.50 mg DW^{-1}) and at pH 8 level–plants macro-nutrients uptake for Ca (2025 ± 2.90 mg/kg), Mg (46.75 ± 3.79 mg DW^{-1}) and Na (7475.25±306.05 mg DW^{-1}). Among the tissue macronutrient contents assessed, only tissue N content was significantly (df = 2; F =35.45; P <0.01) influenced by the interaction between pH and light intensity.

Micro-nutrient

For plants grown under high light intensity, tissue micro-nutrients (Mn, Zn and B) were significantly influenced ($P < 0.05$) by pH treatments (Table 3). The highest mean value of tissue micro-nutrients were obtained in plants grown under high light and at pH 4 for Zn (80±9.714 µg DW^{-1}), Mn (100 ± 2.67 µg DW^{-1}), Fe (232.25 ± 32.11 µg DW^{-1}) and, pH 6 for B (74.25 ± 1.931 mg/kg), respectively. Under low light intensity, the highest mean tissue micro-nutrient contents Cu (4.75 ± 0.25 µg DW^{-1}), Zn (60 ± 2.27 µg DW^{-1}) and B (77.25 ± 2.17 µg DW^{-1}). Generally, many of the micro-nutrients were more concentrated in plants grown under low pH. The tissue micro-nutrient contents among pH treatments varied significantly in the low light and high light intensities (Table 3). The pH treatments significantly ($P < 0.05$) affect the uptake of iron under low and high light conditions. The interaction between light and pH did not significantly (df = 2; F = 0.001; $P < 0.01$) influenced tissue contents of the micro-nutrients.

Table 2: Macro-nutrient contents following exposure of *T. violacea* to different pH treatments and low and high light intensities in the leaf for 2 months post-treatment

Nutrient	Treatments	High light (0% shade) *Mean±SE µg DW^{-1}	Low light (40% shade) *Mean±SE µg DW^{-1}
N	pH 4	546.00±10.90aA	598.50±5.69aA***
	pH 6	577.00±6.13aA	447.00±12.70bB
	pH 8	571.75±4.11aA	561.50±30.05a A
P	pH 4	67.50±4.40aB	59.50±0.5bA
	pH 6	55.75±0.47aA	38.50±1.44bB
	pH 8	52.00±1.224aA	50.25±2.21aA
K	pH 4	722.25±9.31aA	758.75±10.30aA
	pH 6	662.25±8.61bB	766.00±11.45aA
	pH 8	658.5±5.79bB	788.00±8.41aA
Ca	pH 4	127.75±3.52aA	138.25±2.95aA
	pH 6	138.75±6.20aA	202.50±2.90bB
	pH 8	106±3.34bB	130.5±7.27aA
Mg	pH 4	38.25±0.75aA	30.75±0.85bB
	pH 6	39.75±1.88aB	39.25±2.86aB
	pH 8	44.75±1.18aA	46.75±3.79aA
Na	pH 4	2090.5±181.17aB	1567.25±99.75bB
	pH 6	1247.5±63.21cB	2532.75±247.88bB
	pH 8	7917±241.10aA	7475.25±306.05aA

*Means of each nutrient with the same lowercase letters in the same column are not significantly different (P > 0.05) among plants exposed to different pH levels and simultaneously to high or low intensity; means of each nutrient with the same uppercase letters in the same row are not significantly different (P > 0.05) between plants exposed to high and low intensities and simultaneously to pH level 4, 6 or 8. Tukey test was used to separate means.*** Significant (P < 0.01) interactive effect of pH and light intensity on a tissue nutrient content following two-way ANOVA.

Table 3: Micro-nutrient contents following exposure of *T. violacea* to different pH treatments and low and high light intensities in the leaf for 2 months post-treatment

Nutrient	Treatments	High light (0% shade) *Mean±SE µg DW^{-1}	Low light (40% shade) *Mean±SE µg DW^{-1}
Mn	pH 4	100±2.67aA	114.5±2.21bA
	pH 6	65.75±5.07bB	83.75±3.42cB
	pH 8	17.75±1.43cAB	561.5±30.05aA
Fe	pH 4	232.25±32.11aA	116.75±4.02bB
	pH 6	168.25±10.37bA	137.5±8.08aA
	pH 8	202.25±17.52aA	165.25±19.26aA
Cu	pH 4	3.25±0.25aA	4.75±0.25aA
	pH 6	1.75±0.25bB	2.00±0.40bA
	pH 8	1.00±0.00bB	2.00±0.00bA
Zn	pH 4	80.00±9.71aA	60.00±2.27aA
	pH 6	23.50±1.32bB	39.00±1.22bB
	pH 8	19.25±1.70cC	36.50±2.50bB
B	pH 4	61.50±4.11bB	77.25±2.17aA
	pH 6	74.25±1.93bA	59.5±1.84bB
	pH 8	56.25±1.65cB	73.75±0.94aA

*Means of each nutrient with the same lowercase letters in the same column are not significantly different (P > 0.05) among plant exposed to different pH levels and simultaneously to high or low intensity; means of each nutrient with the same uppercase letters in the same row are not significantly different (P > 0.05) between plants exposed to high and low intensities and simultaneously to pH level 4, 6 or 8. Tukey test was used to separate the means.

Table 4: The effect of different pH treatments on minimum inhibitory concentration (at 18 hr in MIC bioassay) of acetone extracts obtained from bulbous roots of *T. violacea* when grown under low or high light intensity

Hours MIC	Treatments	High light (80%) *Mean±SE mg mL^{-1}	Low light (40%) *Mean±SE mg mL^{-1}
18 h	pH 4	0.93±0.19aB	1.50±0.00aA
	pH 6	1.31±0.18aA	1.31±0.18aA
	pH 8	1.50±0.00aA	0.93±0.18bB

*Means with the same lowercase letters in the same column are not significantly different and means with the same uppercase letters in the same row are not significantly different (P > 0.05) following the Tukey test**.**

Antifungal activity

The MIC values did not reveal a clear pattern between low light and high light intensities and pH levels. There were significant differences ($P< 0.05$) between plants that were exposed to low and high light intensities at pH 8 and pH 4 (Table 4). Acetone extracts of *T. violacea* exposed to pH 4 and to high light conditions exhibited the best antifungal activity with a MIC value of 0.93 ± 0.19 mg mL^{-1} in the anti-*F. oxysporum* bioassay at 18 h. Based on the two-way ANOVA, the interaction between light and pH did not significantly influence this species' antifungal activity. Total activities obtained at the different pH levels with pH 8 consistently yielded the lowest total activities, which was statistically significant (Table 5). However, the interaction between light and pH on total activity was not significant (df, 2; F = 6.4; $P > 0.5$).

DISCUSSION

This study demonstrated that light intensity and pH affect *T. violacea* growth, tissue nutrient contents and antifungal

Table 5: The effects of different pH treatments on total activities (based on 18hr MIC) of acetone extracts obtained from bulbous roots of *T. violacea* when grown under low and high light intensity.

Hours Total activity	Treatments	High light (0% shade) *Mean±SE mL g^{-1}	Low light (40% shade) *Mean±SE mL g^{-1}
18 h	pH 4	10.33±1.42aB	18.1075±0.91abA
	pH 6	6.99±1.11bB	21.22±5.23aA
	pH 8	3.44±1.00cB	13.55±3.82cA

*Means with same lowercase letters in the same column are not significantly different and means with same uppercase letters in the same row are not significantly different ($P > 0.05$) following the Tukey test

activity. Specifically, this study shows that plant biomass as well as antifungal activity are enhanced under conditions of high light intensity and low pH. Low pH and high light also favoured the accumulation of tissue nutrients (K, Fe, Zn, and Cu) in plants.

In the current study, pH did not have a significant effect on plant height and number of leaves. Previously, Shetty and Prakash (2010) reported that supplementing pH 4-treated rice plants with phosphorus yielded the highest mean height, which was significantly higher when compared with higher pH treatments. Generally, the plants' fresh and dry weights decreased as the pH increased. Dry weight had a significant ($P< 0.05$) inverse relationship with pH under high light condition. The highest mean dry weight was obtained in plants subjected to pH 4 treatment and grown under high light intensity, while the lowest dry weight was obtained in plants subjected to pH 8. These results corroborate with other reported results, which showed that plants grown at pH 4.5 were heavier than plants grown at pH 5.7 (Hipps *et al.,* 2004; Anugoolprasert *et al.,* 2012). In a previous study, vigorous growth of apple seedlings occurred at pH 3.8 (Li and Utkhede, 1991). Although the fresh weights decreased with increased pH under low and high light conditions, the differences in weights were not significant ($P > 0.05$).

Variations in the tissue micro-and macro-nutrients' contents among plants exposed to pH and light intensity levels were significant for most of the studied nutrients. The availability of these nutrient elements plays a significant contributory role in the action of enzymes that stabilizes the metabolic processes that influence the plants' fresh weight (Brady and Weil, 2008; Stern, 2006; Chatzistathis and Therios, 2013). Interestingly, tissue nitrogen that is essential for plant growth was significantly influenced by the interaction of pH and light intensity. In the tissue analysis, nutrient elements such as K, Fe, Zn, and Cu were significantly enhanced in plants exposed to the pH 4 in the high light intensities. Some of these nutrients also play a significant role in the synthesis phenolic compounds, potentially influencing the medicinal properties of the plant (Bhat *et al.,* 2020). The accumulation of high quantities of micro-nutrients could have favoured the synthesis of secondary metabolites and consequently the higher total activities observed at the lower pH, especially among unshaded plants. However, excess uptake of micro-nutrients can also lead to toxicity in plants (da Silva *et al.,* 2016; Gupta and Gupta, 2017).

Tulbaghia violacea has been identified as a possible source of antifungal active compounds with the potential to be applied as a natural fungicide against plant pathogens in the agricultural industry (Eksteen *et al.,* 2001). The MIC results obtained in this study indicate that the pH treatments have a greater significant influence on *T. violacea* root extracts; more pronounced inhibition of growth of *F. oxysporum* was observed at pH 4 compared with pH 6 and 8 treatments after 18 hr in the MIC bioassay. When plants were exposed to different environmental stresses, they accumulated high levels of bioactive compounds (Ncube *et al.,* 2011a). Kane *et al.* (2006) reported that nutrient solution and pH significantly influenced onion plants' physiological variables. Previous studies on *T. violacea* showed good MIC results when its extract inhibited an *Escherichia coli* bacteria strain commonly found in HIV/AIDs patients (Ncube *et al.,* 2011b). The highest mean value of the total activity was obtained in plants grown under pH 4 and highlight intensity. Plants grown under low light intensity subjected to the pH 6 treatments yielded significantly higher total activities.

In conclusion, this study's results suggest the highest and quality production of dry matter and antifungal activity of extracts of *T. violacea* are enhanced under high light and low pH conditions. Furthermore, pH and light intensity interactively influenced the yield of *T. violacea* during hydroponic cultivation. This study contributes to the current knowledge on the cultivation of this important medicinal species and promotes the cultural and traditional use of *T. violacea*. Farmers could use the current findings to produce high quality medicinal materials of *T. violacea* for personal use and for commercial trade.

ACKNOWLEDGEMENTS

This work was supported by the Cape Peninsula University of Technology through the University Research Fund (URF), grant number R166.

Conflict of interest

The authors declare no conflict of interest.

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