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Evaluating the effect of 40% and 0% shading levels on the secondary metabolites, antifungal and anti-insect activities of extracts of *Allium porrum* cultivated hydroponically under greenhouse conditions

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Background: *Allium* species are generally reputed for their anti-pest properties; however, few studies have focused on optimising the quality and yield of anti-pest bioactive materials from these medicinal plants.

Setting: Laboratory and greenhouse experiments were carried out on the Bellville campus of the Cape Peninsula University of Technology, Cape Town.

Aims: This research study aimed to evaluate the effect of light intensity on the volatile constituents, antifungal and anti-insect activities of extracts obtained from *Allium porrum* L. cultivated hydroponically under greenhouse conditions.

Methods: Seedlings of *A. porrum* were hydroponically grown under 40% shading and 0% shading conditions for 12 weeks. The phytochemical constituents of the aerial parts (leaf and bulb) of *A. porrum* were analysed. The antifungal activity against *Fusarium oxysporum* and the anti-insect activity on the grapevine mealybug (*Planococcus ficus*) was evaluated by microdilution and repellency bioassays, respectively.

Results: Remarkably, the total polyphenol content was statistically higher (DF = 1, 6; $F = 9.17$; $p < 0.05$) in plants exposed to 40% shade treatment. The gas chromatography-mass spectrometry (GC-MS) analysis revealed that the volatile compounds varied significantly (DF = 1; $\chi^2 = 3.435$; $p > 0.05$) between the two treatments. However, although a higher number of compounds (73) occurred in plants exposed to 40% shade than in those exposed to 0% shade (58), the shading effect on the number of compounds was not significant (DF = 1; $\chi^2 = 69.551$; $p > 0.05$). The acetone extracts of *A. porrum* that were cultivated under lower light irradiance showed a higher fungistatic activity against *F. oxysporum* in the antifungal bioassay.

Conclusion: Broadly, this study revealed that lowering light intensity from 313 µmol m^{-2} s^{-1} to 153 µmol m^{-2} s^{-1} favoured a higher phenolic content, volatile constituents and higher anti-*F. oxysporum* activities in leeks.

Keywords: secondary metabolites; anti-*Fusarium oxysporum*; *Allium porrum*; *Planococcus ficus*; insect repellency.

Introduction

Medicinal plants are rich sources of secondary metabolites, including carotenoids, terpenes, alkaloids, phenolic and sulphur compounds (Atanasov et al. 2015; Ramírez-Gómez et al. 2019). They are used as medicinal constituents, flavouring agents and insecticides (Bourgaud et al. 2001; Gandhi, Mahajan & Bedi 2015; Yang et al. 2018). Since the 1850s, pharmaceutical organic chemists have been investigating the phytochemical properties of medicinal plants (Bourgaud et al. 2001; Yang et al. 2018).

Plants can biosynthesise different secondary metabolites (Pagare et al. 2015; Wink 2018). However, the influence of various environmental factors, such as temperature, humidity, light intensity, water supply minerals and CO_2, on the synthesis of secondary metabolites has been demonstrated

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(Akula & Ravishankar 2011; Mohiuddin 2019). Furthermore, the type and concentrations of secondary metabolites synthesised by plants are determined by the physiology, species, genotype and growth stage of the plant (Isa 2019). Over the past few years, many approaches have been established to increase the production of compounds of interest and plant biomass (Bourgaud et al. 2001; Murthy et al. 2014). These strategies include manipulating the cultivation methods, such as amendment of growing substrates with fungi under varying light intensities, water stress and nutrient solutions; for example, light can significantly alter the metabolite concentrations (Akula & Ravishankar 2011; Ma et al. 2010). Shading can induce biochemical changes in plant leaves (Gottschalk 1994). In a study conducted by Hou et al. (2010), low-light intensity significantly increased the accumulation of glycyrrhizic acid in the roots of *Glycyrrhiza uralensis*. Under reduced light intensity, the methylxanthine content was increased in *Ilex paraguariensis* leaves (Coelho et al. 2007).

Secondary metabolites play a vital ecological role in the defence, protection and signalling mechanisms of plants (Griesser et al. 2015). They are being exploited for the control of common plant pests. Extracts rich in bioactive secondary metabolites or plant-based pesticides have been successfully used to control plant pathogens and insect pests under greenhouse and field conditions (Khater 2012; Koul & Walia 2009). Many plant-based agents are readily available commercially as microbicides, insecticides and repellents (Niroumand et al. 2016). Moreover, the demand and market trend are shifting in favour of biorational control methods, including plant-based agents, for they are believed to be environmentally friendly.

The spread of pest in plants is the foremost factor that hinders optimum production of crops. Two of the major pests include grapevine mealybugs (*Planococcus ficus* [Signoret]) and Fusarium wilt (*Fusarium oxysporum*), which are widespread in nature and of economic importance, thus causing significant crop losses. The challenge to control these pests is influenced by several factors, including environment, host response, pest response to pesticide and pesticide resistance (Agrios 1998; Franco, Zada & Mendel 2009; Summerell et al. 2010; Walton et al. 2004). *Allium porrum* L. (leek) and other *Allium* species are used in folk medicine for their antimicrobial properties (Tamokou, Mbaveng & Kuete 2017). The extracts of *Allium* species contain several bioactive constituents, including phenolic compounds, organosulphur compounds, non-structural and soluble carbohydrates, organic acids, and various amino acids (Slimestad, Fossen & Vågen 2007). Leek is closely related to garlic (*Allium sativum*), a species that is renowned for its anti-microbial and anti-arthropod repellent activities (Adenubi et al. 2016; Adetumbi & Lau 1983). It is, therefore, reasonable to assume that *A. porrum* will also have prominent bioactivities against phytopathogens and insects.

Leeks are cultivated in well-drained, nutrient-rich soils under full sunlight. However, because of loss of agricultural land, increasing water scarcity, and the demand for increased production and reduced contamination of legumes, including leeks, many farmers are turning to hydroponic cultivation, which is adaptable and efficient (Fitton et al. 2019). The system can be manipulated to optimise the secondary metabolite contents and bioactivities of plants (Vu et al. 2006). Interestingly, because some studies suggest that shading can influence the production of secondary metabolites in plants, and leek plants can tolerate shading (Chen et al. 2017; Rijck, Schrevens & De Proft 1994; Yang et al. 2018); light intensity could be used to increase secondary metabolite contents as well as the bioactivities of leek plant extracts cultivated hydroponically. Currently, there is a scarcity of information on the effects of shading on the secondary metabolite contents, anti-insect and antifungal activities of *A. porrum*. Previously, Van Der Werf et al. (1996) studied the effect of light intensity on the growth parameters of leek and found that although the plants could grow at low-light intensity, 100 µmol m^{-2} s^{-1}, plant growth was significantly reduced. We have been cultivating leek plants hydroponically in the greenhouse conditions under natural sunlight, all year round to increase the medicinal value of the leek products. This study aimed to ascertain the effect of light intensity on the volatile constituents, antifungal and anti-insect activities of *A. porrum* extracts cultivated hydroponically under greenhouse conditions.

Materials and methods

Plant material

Seedlings of *A. porrum* L. (cultivar: Porbella) were purchased from Stodels Garden Centre, situated at Eversdal Rd, Bellville, 7535, in the Western Cape, South Africa. Roots were gently washed to remove soil particles and were separated. The baseline data were obtained by measuring all plants (height, root length and number of leaves) before transplanting in the greenhouse conditions. After that, 10 plants were used to obtain baseline data of fresh and dry weight.

Greenhouse experimental design

A completely randomised design with a single factor was used to investigate the effect of shading with two shading levels on the secondary metabolites and anti-pest activities of extracts obtained from *A. porrum*. Experimental plants were grown under two levels of shading, that is, 0% shade and 40% shade, under greenhouse conditions. The 40% shade was achieved by covering the metal table (230 cm × 90 cm × 85 cm) with a 40% green polyethylene shade net; the shade net was supplied by the gardening retailer. The 0% shade was achieved by subjecting plants to sunlight entering through the greenhouse's polycarbonate roof cover. It is worth mentioning that we intended to test three shading levels; however, most leek plants had died under 80% shading within 4 weeks post-treatment in a preliminary study. Light irradiance was measured at plant height using a digital lux meter (0.1–40 000 LUX [Unit of illumination]) MT942 Major Tech. Light intensity was converted from Lux to photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}) with a standard conversion factor of 0.0185 for the sunlight light

source (Apogee Instruments 2020). Conversion formula: lux × conversion factor (0.0185) = PPFD (µmol m^{-2} s^{-1}). The average 0% and 40% shade light intensity (PPFD) measured at noon were 313 µmol m^{-2} s^{-1} and 153 µmol m^{-2} s^{-1}, respectively. The experiment was conducted in a greenhouse with the following internal environmental conditions: temperatures ranged from 19 °C to 27 °C, with the average relative humidity ranging from 29% to 67%. One hundred (50 plants per treatment) seedlings were individually transferred into 25-cm pots filled with the following substrate mix: silica sand 25% + perlite 25%+ coco peat 25% + vermiculite 25%. Plants were supplied with a hydroponic fertilizer, Nutrifeed®, purchased from Starke Ayres Pty Ltd, Cape Town. The fertilizer contained the following ingredients: 65 mg kg^{-1} N, 75 mg kg^{-1} S, 130 mg kg^{-1} K, 70 mg kg^{-1} Ca, 22 mg kg^{-1} Mg, 1500 mg kg^{-1} Fe, 10 mg kg^{-1} Mo, 27 mg kg^{-1}, P 20 mg kg^{-1} Cu, 240 mg kg^{-1} Mn, 240 mg kg^{-1} B and 240 mg kg^{-1} Zn. The nutrient solution was applied to each plant as a drench, with each plant receiving 100 mL of the nutrient solution once a week, followed by deionised water (100 mL) once every 3 days. This experiment was conducted in autumn (February-April) under greenhouse conditions in the Department of Horticultural Sciences, Cape Peninsula University of Technology, Bellville Campus.

Phytochemical screening

Total alkaloid assay

This assay followed the method described by Fadhil and Reza (2007). The grounded plant materials (100 mg) of the aerial parts (leaf and the bulb) of *A. porrum* were extracted in 25 mL of 60% aqueous ethanol for 24 h. The mixture was centrifuged (4000 *g*) for 10 min, and the supernatant was used in the assay. Standard atropine solutions (10 mg/L, 20 mg/L, 50 mg/L and 100 mg/L) or extracted supernatant (0.5 mL) were mixed with 5 mL of sodium phosphate buffer (pH 4.7) and 12 mL of bromocresol green solution. After that, 12 mL of chloroform was added to the reaction mixture and then shaken vigorously. The absorbance values of test and standard solutions were ascertained against the reagent blank (chloroform) at 417 nm with a UV-visible spectrophotometer. The total alkaloid content was expressed as milligram of AE/g of the plant extract. Four plants were randomly selected and tested for each light intensity treatment.

Total flavonol assay

The total flavonol content was evaluated using quercetin as a standard at 0 mg/L, 5 mg/L, 10 mg/L, 20 mg/L, 40 mg/L, and 80 mg/L in 95% ethanol (Sigma-Aldrich, South Africa) (Daniels et al. 2011). Crude extract solution (12.5 µL) of the aerial parts was prepared by mixing 12.5 µL of 0.1% hydrochloric acid (HCl) (Merck, South Africa) in 95% ethanol in the sample wells followed by 30-min incubation at room temperature. The total flavonoid concentration in ethanol extracts was expressed as milligram quercetin equivalent per gram dry weight (mg QE/g DW). Four plants were randomly selected and tested for each light intensity treatment.

Total phenolic assay

The total phenolic content of dry material (leaf and the bulb) of leeks was assessed with the Folin-Ciocalteau assay, following methods by Singleton, Orthofer and Lamuela-Raventós (1999). Using a 96-well microplate, 25 µL of the sample was mixed with 125 µL of Folin-Ciocalteau reagent (1:10 dilution with distilled water; Merck, South Africa). After 5 min, 100 µL of 7.5% Na_2CO_3 solution was added to the mixture in each well. The total phenolic content of dry leaf material of leek was expressed as milligrams of gallic acid equivalents (GAEs) per gram dry mass (mg GAE/ g dw). All samples were analysed in duplicates. Four randomly selected plants were tested for each light intensity treatment.

Headspace gas chromatography-mass spectrometry analysis

Sample preparation

The volatile compound profiles of 12-week-old *A. porrum* (Leek) potted samples were analysed from eight randomly selected potted plants, that is, four from each treatment; 40% shade and 0% shade. Plant aerial parts (leaf and bulb) were cut into small sections and freeze dried at -80 °C overnight. Liquid nitrogen was used for crushing plant material, and 1 g was weighed into a solid-phase microextraction (SPME) vial. Thereafter, 2 mL of 12% soaking alcohol solution (v/v) at pH 3.5 was added to each vial, followed by 3 mL of 20% saturated NaCl solution. Sample vials were vortexed, and the headspace of the sample was analysed using divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre (grey).

Chromatographic separation

The separation of volatile compounds was carried out using a gas chromatograph (6890N, Agilent Technologies Network) combined to an inert XL EI/CI mass selective detector (model 5975B, Agilent Technologies Inc., Palo Alto, CA), which was operated in a full scan mode. The GC-MS system was combined to a CTC Analytics PAL autosampler. The separation of volatiles from the samples was performed on a polar ZB-WAX (30 m, 0.25 mm ID, 0.25-µm film thickness) Zebron 7HGG007-11 capillary column. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The injector temperature was maintained at 250 °C, with the split ratio set at 5:1. The initial temperature was set at 35 °C for 6 min, at a rate of 3 °C/min to 70 °C for 5 min, and then at 4 °C/min to 120 °C for 1 min. Finally, the temperature was increased to 240 °C at a rate of 20 °C/min and held for 2.9 min. The transfer line temperature was set at 250 °C, whilst the ion source and quadrupole temperatures were maintained at 230 °C and 150 °C, respectively. The mass spectrometer was operated under electron impact mode at an ionisation energy of 70 eV, scanning from 35 m/z to 500 m/z. Retention time and mass spectrum with 50% matching with the internal standard and reference library enabled identification of the volatile compounds.

Preparation of plant extracts: Antifungal bioassay

A preliminary experiment conducted in our laboratory had revealed that the extracts of the roots did not have had

weaker antifungal and repellent activities than the aerial parts, and acetone extracts were more active than ethanol and dichloromethane extracts. Hence, only the aerial parts (leaf and bulb) of *A. porrum* and acetone extracts were used in this bioassay. Fresh aerial parts (leaf and the bulb) of plants obtained from the 40% and 0% shade treatments were crushed with a porcelain pestle and mortar for 5 min. Acetone is a regularly used solvent for extraction because it is less toxic, volatile, and dissolves a wide range of lipophilic and hydrophilic elements (Eloff 1998). The crushed plant material (5 g) was extracted with 25 mL of acetone (99.9%) (Merck Pty Ltd, South Africa) and kept at room temperature for 18 h, followed by filtration with Whatman No. 1 filter paper. Four randomly selected plants from each light intensity treatment were evaluated. Filtrates were placed under a fume hood for 48 h to allow complete evaporation of acetone. The obtained residues were weighed to obtain yield and reconstituted in 50% aqueous acetone to obtain a starting concentration of 6 mg/mL.

Antifungal bioassay: Minimum inhibitory concentration

The microdilution method described by Nchu et al. (2010) was used to determine the minimum inhibitory concentration (MIC) of plant extracts. The *A. porrum* extract solution of the aerial parts (leaf and the bulb) were serially diluted two folds to obtain the following concentrations in each row of a 96-well microplate: 6.000 mg/mL, 3.000 mg/mL, 1.500 mg/mL, 0.750 mg/mL, 0.375 mg/mL, 0.188 mg/mL, 0.094 mg/mL and 0.047 mg/mL. We used a fungal culture of *F. oxysporum* f. sp. glycines strain (UPFC No. 21) maintained in the Horticulture Research Laboratory at the Cape Peninsula University of Technology, Bellville campus. The *F. oxysporum* was sub-cultured from stock agar plates and then grown in a nutrient broth (Merck, South Africa) for 4 h. The fungal suspension (100 µL) was added to each well of a 96-well microplate (10^5 cells/mL) containing the serially diluted extracts. Searls Mancozeb fungicide ® (Stodels Pty Ltd, Bellville) was prepared and served as a positive control, and acetone served as a negative control. Forty microlitre of 0.2 mg/mL of p-iodonitrotetrazolium chloride (INT) (Sigma) was dissolved in sterile distilled water and added to each microplate well, sealed in a plastic bag and incubated at 37 °C under 100% RH. The antifungal bioassay (MIC) comprised three replicates per treatment. The MIC values were recorded after 6 h, 12 h and 18 h.

Preparation of extracts for the insect bioassay

Fresh plant materials from aerial parts of *A. porrum* (leaf and the bulb) under 40% and 0% shade treatments were crushed with a porcelain pestle and mortar for 5 min. The crushed material was extracted separately with ethanol, acetone and dichloromethane. Six grams of the crushed samples were mixed separately with acetone, ethanol and dichloromethane to obtain a concentration of 20 w/v%. The extraction process lasted for 5 h and then filtration, using Whatman No. 1 filter paper, into a centrifuge tube. A two-fold dilution was carried out to obtain 10 w/v%, 5 w/v% and 2.5 w/v% concentrations for all the solvents. The aerial parts of five plants from each light intensity treatment were used to prepare each solvent extract.

Insect rearing

Grapevine mealybug (*Planococcus ficus*) was obtained from the Agricultural Research Council (Stellenbosh, South Africa) courtesy of Dr. Achiano Kwaku. Insects were reared on butternut squash in the darkroom at 25 °C in 60% RH at Research Laboratory, Department of Horticultural Sciences, Cape Peninsula University of Technology. Adult female mealybugs were tested on four treatments, including control, and replicated five times per treatment.

Repellency bioassay

The repellency bioassay followed methods described by Koschier et al. (2000) with slight modifications. In this bioassay, the Y-tube olfactometer made of a transparent plexi glass tube was used. One arm of the olfactometer was used as a treatment arm, and the other arm was used as a control arm. The aerial parts of extracts were evaluated at four rates (20 w/v%, 10 w/v%, 5 w/v% and 2.5 w/v%), including a commercial synthetic insecticide, Kemprin (Cypermethrin pyrethroid 200 g/L), as the positive control. One hundred microlitres of ethanol, acetone and dichloromethane extracts of leeks were applied separately on a 4-cm diameter filter paper (Whatman no. 1) for the test arm. The pure solvents were treated as a negative control and applied to another filter paper piece (4 cm diameter). ProTek Kemprin® (Stodels Pty Ltd) solution (100 µL) was applied on the same size and type of filter paper. The ends of the Y tube were covered with test filter paper and control filter papers. By employing a membrane pump at the base of the Y tube, the air was sucked out, producing an airflow of 10 cm/sec in the Y-tube olfactometer. Ten adult female grapevine mealybugs were released in the base tube of the Y-tube olfactometer using a camel-hair brush. The experimental time was recorded from the time the air suction tube was connected to the glass Y. Once the grapevine mealybugs reached the Y junction in the glass tube, and they had to choose between the airflow loaded with the solvent and airflow loaded with the extracts. The choice was recorded once the mealybugs reached the far end of one arm. If all 10 mealybugs did not respond after 10 min, no score was recorded, and the bioassay was repeated. The bioassay was replicated five times, with each completed after 10 min. The mean percentage repellency was calculated using the following formula: Repellence (%) = C-T/C×100, where C = number of insects in the control arm and T = number of insects in the extract-treated arm. After each replication, the olfactometer was cleaned with the solvent.

Statistical analysis

The experimental data (MIC, total polyphenols, alkaloids and flavonols, number of volatile compounds and percentage repellency) are presented as mean ± SE in tables. The data on polyphenol, alkaloid, flavonol and MIC were analysed using one-way analysis of variance. The post hoc Tukey HSD test

was used to separate the means at a level of significance, $p < 0.05$. Statistical analyses were performed using Statistica 13.3.1 software (TIBCO Software Inc., Palo Alto, USA). The non-parametric Kruskal-Wallis test was used to compare the number of insects repelled by the shade-treated and unshaded plant extracts for the different solvents and extract concentrations, as well as the volatile constituents in plants. The post hoc Mann-Whitney test was used to separate the medians at a level of significance, $p < 0.05$. These computations were performed using PAST version 3.21 (Hammer, Harper & Ryan 2001).

Ethical considerations

Ethical clearance to conduct this study was obtained from the Ethics Committee of the Faculty of Applied Sciences, Cape Peninsula University of Technology (No. 213011514/04/2019).

Results

Quantification of plant secondary metabolites

The results of the phytochemical constituents revealed that the polyphenol content in the aerial parts (leaf and bulb) was significantly higher (DF = 1, 6; F = 9.17; $p < 0.05$) in plants under 40% shading than those subjected to 0% shade treatment. There were no significant differences between treatments in total alkaloid (DF = 1, 6; F = 2.73; $p > 0.05$) and flavonol (DF = 1, 6; F = 1.31; $p > 0.05$) contents; however, higher yields of these constituents were observed in plants subjected to 40% shading (Table 1).

Gas chromatography-mass spectrometry analysis

Gas chromatography-mass spectrometry analysis was performed on extracts of shoots of *A. porrum*. As presented in Table 2, compounds with more than 50% match with the mass spectral library were selected, and a wide range of volatile compounds were detected. Although the number of volatile compounds (73) detected in plants exposed to 40% shade was higher than those (58) in 0% shade plants, it was not statistically significant (DF = 1; $\chi^2 = 3.435$; $p > 0.05$). However, the compounds present in the plants from both treatments varied significantly (DF = 1; $\chi^2 = 65.065$; $p < 0.01$) – 79% of the compounds were different. Only 24 of the 107 compounds identified occurred in plants exposed to both treatments. The compounds included well-known antifungal and anti-insect constituents, such as b-ionone, dimethyl trisulfide, methyl palmitate and 1,3-dithiane (Table 2).

TABLE 1: Content of polyphenols (mg GAE/g DW), flavonols (mg QE/g DW) and alkaloids (mg AE/DW) in aerial part samples of leeks cultivated under different light intensities in the greenhouse conditions.

Treatments	Alkaloids (mg AE/g DW)	Polyphenols (mg GAE/g DW)	Flavonols (mg QE/g DW)
40% shade	3.4 ± 0.2a	2.1 ± 0.2b	1.1 ± 0.1a
0% shade	3.0 ± 0.2a	1.4 ± 0.1a	0.8 ± 0.2a

GAE, gallic acid equivalents; QE, quercetin equivalent; DW, dry weight; AE, atropine equivalent.

Means with the same lower case letters in the same column are not significantly different when treatments are compared using the Tukey test at $p < 0.05$.

Minimum inhibitory concentration

There was a significant difference ($p < 0.05$) in the MIC values between the two light intensities. The inhibition of *F. oxysporum* was significantly higher in acetone extracts obtained from aerial parts of *A. porrum* that were subjected to lower light irradiance (40% shading) at 6 h (DF = 3, 28; F = 1195.55; $p < 0.05$), 12 h (DF = 3, 28; F = 409.06; $p < 0.05$) and 18 h (DF = 3, 28; F = 294.77; $p < 0.05$) post-treatment (Table 3). Generally, the acetone extracts of *A. porrum* that were exposed to 40% shade or 0% shade had the lowest MIC values when compared with the tested positive control (Mancozeb) (DF = 3, 28; $p < 0.05$).

Repellence bioassay

Generally, the Y-olfactometer assay showed that the insect repellency induced by the different plant concentrations (20 w/v%, 10 w/v%, 5 w/v%, 2.5 w/v%) increased significantly (DF = 3; $p < 0.05$) with increasing extract concentration in the 40% and 0% shade treatments (Table 4). Shading and the solvent used for extraction did not significantly ($p > 0.05$) affect insect repellency. At the highest concentration (20 w/v%), there were no significant differences (DF = 6; $p > 0.05$) in insect repellency by the different extracts from plants exposed to both low-light and high-light intensities and Kemprin (positive control) (Table 4); however, acetone and dichloromethane extracts induced the highest mean insect repellency.

Discussion

In this study, phytochemical screening revealed the presence of phenolic compounds, flavonols and alkaloids in the leek plants. Also, subjecting *A. porrum* to 40% shading was significantly associated with a higher total phenolic content in the aerial parts of the plant extracts. Total alkaloid and total flavanol contents were also higher in the shaded plants than the unshaded plants; however, the differences were not significant.

Cultivation of plants under low-light conditions induces biochemical changes in plant leaves (Gottschalk 1994) and an increase of secondary metabolites (Coelho et al. 2007), which could be caused by a trade-off between growth and defence under low-light irradiance (Hou et al. 2010). The production of secondary metabolites is considered as an adaptation of plants to withstand unfavourable environmental changes; the process involves the synthesis of complex chemical types and interactions in the structural and functional stabilisation through signalling processes and pathways (Edreva et al. 2008; Isah 2019).

Based on the gas chromatography-mass spectrometry (GC-MS) analysis, up to 107 compounds, 50% matching with the mass spectra library, were detected in extracts of the leek plants grown under different light intensities, with compounds such as methyl nonanoate, methyl myristate and ethyl pelargonate being present exclusively in plants subjected to low-light intensity. Like other *Allium* species, leek plants

TABLE 2: Volatile compounds, with a mass spectral library match of at least 50%, present in aerial parts (leaf and bulb) of leek plants (*Allium porrum*) exposed to 40% shade and 0% shade for 12 weeks.

n	40% shade treatment	RT	*n*	0% shade treatment	RT
	Compounds			Compounds	
1			1	Formic acid	7.8086
2	Sulphurous acid	**7.8505**	2	Sulphurous acid	**7.8193**
3	-		3	Methylamine	9.3791
4	Cyclotetrasiloxane	**9.6199**	4	Cyclotetrasiloxane	**9.6303**
5	Decane	**9.3791**	5	Decane	**9.379**
6	Cyclopentasiloxane	**18.8856**	6	Cyclopentasiloxane	**18.8646**
7	Piperidine	17.0638		-	
8	2-Ethyl-trans-2-butenal	17.0639		-	
9	(E)-3-Methyl-2-hexene	17.0638		-	9.6409
	-		7	Dimethyl disulfide	12.063
	-		8	Dimethyl sulfone	12.0698
10	2-Decanol	18.6657		-	
	-		9	Cyclohexanone	17.0639
	-		10	Furan, 2,5-dihydro-2,5-dimethyl-	17.0639
11	Undecane	19.5661		-	
	-		11	Methyl dehydrolithofellate	17.0535
12	Dodecane	19.7337		-	
	-		12	5-Methyl-1-hepten-4-ol	18.6658
13	Hexadecane	19.765		-	
	-		13	2-Pentanol, 4-methyl-	18.7285
	-		14	2-Hexanol	18.6972
14	9-Azabicyclo[6.1.0]non-8-ene	20.8748		-	
15	Hexanoic acid	21.5239		-	
	-		15	Tridecane	19.6186
	-		16	Tricosane	19.6498
	-		17	Nonadecane	19.7022
16	Butanoic acid	21.5449		-	
17	Ethyl hexanoate	21.6077		-	
	-		18	5-Hydroxy-5-dideutero-1,2-pentadiene	19.7442
	-		19	Cyclohexane	20.4979
	-		20	1-Methyl-2-(gamma-hydroxy) propylacetylene	20.4981
18	3-Hexene	**46.2429**	21	3-Hexene	**20.5085**
19	Methyl propyl disulphide	**20.9640**	22	Methyl propyl disulphide	**20.9650**
20	Thiophene	**22.1417**	23	Thiophene	**22.1102**
21	1,3-Dithiane	**24.0053**	24	1,3-Dithiane	**22.6548**
22	Cyclohexasiloxane	**27.8791**	25	Cyclohexasiloxane	**27.8999**
23	1,3,7-Octatrien-5-yne	**22.4034**			
24	3,4-Dimethylthiophene	**22.1521**	26	3,4-Dimethylthiophene	**22.1207**
25	Methyl-*trans*-propenyl-disulphide	**24.0576**	27	Methyl-*trans*-propenyl-disulphide	**22.6651**
26	3-Octanol	**30.1614**	28	3-Octanol	**30.1614**
27	Ethyl amyl carbinol	**30.1615**	29	Ethyl amyl carbinol	**30.1616**
	-	**27.9**	30	Dimethyl trisulfide	**28.5176**
28	Heptanoic acid	29.5542		-	
29	3-Methyl-1-cyclopentene	29.4914		-	
30	Trans-propenyl propyl disulphide	**30.3604**	31	Trans-propenyl propyl disulphide	**30.3289**
31	Octanoic acid	**29.6275**	32	Octanoic acid	**31.8262**
	-		33	Trans-2-hexenol	30.5593
32	2-Ethoxycyclopenta[b]pyridine	30.308		-	
	-		34	2-Hexen-1-ol	30.5489
	-		35	Cyclopentanol	30.5592
33	N-Tetradecane	30.5802		-	
	-		36	Pyrazine	31.6588
34	Dodecane	30.5907		-	
35	Tetradecane	30.6012		-	
	-		37	*Cis*-propenyl propyl disulphide	31.4491

Table 2 continues →

TABLE 2 (Continues...): Volatile compounds, with a mass spectral library match of at least 50%, present in aerial parts (leaf and bulb) of leek plants (*Allium porrum*) exposed to 40% shade and 0% shade for 12 weeks.

n	40% shade treatment	RT	*n*	0% shade treatment	RT
	Compounds			Compounds	
36	Docosane	30.6012		-	
	-		38	Hexadecanoic acid	31.8261
37	Ethyl caprylate	31.8052			
			39	Ethane	32.4647
	-		40	1-Methoxy-4-(1′-methylethyl) cyclohexa-1,4-diene	39.8355
38	Octanoic acid, ethyl ester	31.8366		-	
			41	3H-1,2,4-Triazole-3-thione	51.5303
39	Methyl nonanoate	34.3284		-	
40	Ethyl pelargonate	36.2758		-	
41	2-Cyclohexen-1-one	38.443		-	
	-		42	3-Flavanol	46.6513
	-		43	4-Ethylbenzaldehyde	47.0386
42	Isophorone	38.443		-	
43	Decanoic acid	38.935		-	
44	Pulegone	39.825		-	
45	Benzeneethanol	**49.3315**	44	Benzeneethanol	**49.3315**
46	Benzene	**33.7212**	45	Benzene	**47.0387**
47	Decanoic acid	41.3431		-	49.3314
48	Beta-cyclocitral	**39.8355**	46	Beta-cyclocitral	**39.8356**
49	1,2,4-Trithiolane, 3,5-diethyl	**45.154**	47	1,2,4-Trithiolane, 3,5-diethyl-	**45.1647**
50	Ethyl laurate	**48.138**	48	Ethyl laurate	**48.1274**
51	beta-Ionone	**49.9597**	49	beta-Ionone	**49.9596**
52	Tetradecanoic acid	**51.8338**	50	Tetradecanoic acid	**51.8337**
53	Dodecanoic acid	47.1538		-	
	-		51	5-Methyl-2,4-diisopropylphenol	49.9597
54	5,6,7,8-Tetrahydrochinoxaline	47.0386		-	
	-		52	2-Methoxy-4-aminophenol	51.2893
55	Benzaldehyde, 4-ethyl-	47.0386		-	
56	1H-Inden-5-ol	47.0386		-	
	-		53	1H-Pyrazole	53.3415
57	Dodecanoic acid	47.1433		-	
58	Methyl laurate	47.1538		-	
	-		54	Methyl 11-(2,3-dideuterocyclopentan-1-yl)undecanoate	54.1476
59	Methyl palmitate	**48.1379**	55	Methyl palmitate	**54.6291**
60	Pentadeuteroethylbromide	**53.3414**	56	Pentadeuteroethylbromide	**53.3413**
	-		57	Benzenamine	57.4664
	-		58	Cis-4,9-Dioxadodeca-6-ene-1,11-diyne	57.8853
61	Edulan I	49.9597		-	
62	Indole-2-one	49.9597		-	
63	2-Methoxy-4-aminophenol	51.2893		-	
64	4-Fluoro-6-aminopyrimidine	53.3414		-	
65	1H-Pyrazole, 4-nitro-	53.3414		-	
66	Pyrrolo[1,2-b]isothiazole, 5,6-dihydro-2-methyl-	53.3414		-	
67	Pentadecanoic acid	54.158		-	
68	Pyrrolidine	54.7443		-	
69	4,5-Dimethylthiazole	54.7443		-	
70	Phenol, 2,6-bis(1,1-dimethylethyl)-	55.1422		-	
71	4-Nonanol, 2,6,8-trimethyl-	57.8433		-	
72	2-(5-chloro-2-methoxyphenyl) pyrrolidine	58.325		-	
73	4-Carbomethoxy-6,7-dimethoxy-3(3′,4′-dimethoxyphenyl)-isoquinoline	60.1467			

RT, retention time.

Bold indicates the occurrence of the same volatile compound in at least one plant in the 40% and 0% shade treatments.

contain many bioactive agents (Radovanović et al. 2015; Slimestad et al. 2007). The identified compounds following the GC-MS analysis are known to exhibit broad-spectrum bioactivity against plant pests (Table 2). For example, dimethyl disulphide (DMDS), which is common in the *Allium* genus, is known for high toxicity against plant pests (Ajwa et al. 2010; Dugravot et al. 2002). Dimethyl disulphide is used as an alternative to methyl bromide (CH_3Br) to control plant fungal pathogens (Wang et al. 2011). Other important compounds detected included b-ionone, which is known to possess antifungal and insect repellent properties (Kunz & Novartis 1990; Sas & Adams 1999; Weissling et al. 1989). Furthermore, 1,3-dithiane and dimethyl trisulfide have antifungal and insecticidal properties (Giannini et al. 2004; Kocić-Tanackov et al. 2012; Liu et al. 2014; Muhammad et al. 2016; Pinto et al. 2017; Sanei-Dehkordi et al. 2019; Tang et al. 2019).

Many studies have characterised the compounds in leek plants, and Schreyen et al. (1976) identified 67 compounds in leek essential oil by comparing the retention times and mass spectra with those of the standards; some of these compounds were also identified in the current study. Noleau, Richard and Peyroux et al. (1991) identified many sulphur compounds in the essential oil from the leaves of *A. porrum* using spectroscopic methods, including two-dimensional nuclear magnetic resonance spectroscopy. Fattorusso et al. (2001) isolated flavonoid glycosides such as kaempferol 3-O-[2-O-(*trans*-3-methoxy-4-hydroxycinnamoyl)-beta-D-galactopyranosyl]-(1-->4)-O-beta-D-glucopyranoside and kaempferol 3-O-[2-O-(*trans*-3-methoxy-4-hydroxycinnamoyl)-beta-D-glucopyranosyl]-(1-->6)-O-beta-D-glucopyranoside from the bulbs of leek. *Allium porrum* can synthesis thiosulfinates and disulfides.

TABLE 3: Minimum inhibitory concentration (Mean ± SE) on *Fusarium oxysporum* by acetone extracts obtained from aerial parts of *Allium porrum* grown under 40% shading and 0% shading conditions at 12 weeks post-treatment.

Treatments	MIC (mg mL^{-1}) at 6 h	MIC (mg mL^{-1}) at 12 h	MIC (mg mL^{-1}) at 18 h
Acetone extracts 40% shade	0.8 ± 0.1c	0.8 ± 0.1c	1.9 ± 0.2c
Acetone extracts 0% shade	1.2 ± 0.1b	1.4 ± 0.3b	3.0 ± 0.0b
Mancozeb	6.0 ± 0.0a	6.0 ± 0.0a	6.0 ± 0.0a
Acetone (-ve control)	> 6.0 ± 0.0a	> 6.0 ± 0.0a	> 6.0 ± 0.0a

MIC, minimum inhibitory concentration.

*Means with the same lowercase in the same column are not significantly different ($p > 0.05$) following the Tukey test. 40% shade; 0% shade.

A few studies have demonstrated that the production of these secondary metabolites by *A. porrum* can be increased by manipulating ambient biotic and abiotic factors. For example, Lundegårdh et al. (2008) reported that exposing leek plants to mineral fertilizer and compost increased the concentration of S-alk(en)yl-L-cysteine sulfoxides (ACSOs) in the plants, whilst green manure induced a high L-ascorbic content. They also reported that sulphur in green manure strongly correlated with an increased level of ACSOs in leek. Exposure of leek (*A. porrum*) to intensive attack by the leek moth *Agrotis ipsilon* induced increased levels of the sulphur precursor, sulphur precursor propyl-cysteine sulfoxide and volatile sulphur compounds in the plants (Dugravot et al. 2005). In this study, we demonstrated that 40% shading can induce increased production of volatile and phenolic contents by *A. porrum*.

In the antifungal bioassay, the aerial parts of *A. porrum* exposed to the lower light intensity induced a higher inhibitory effect against *F. oxysporum* than 0% shading, correlating, interestingly, with the higher phenolic content in plants exposed to low-light intensity. Previously, Radovanović et al. (2015) had reported that the ethanolic extracts of *A. porrum* are bioactive against *Bacillus subtilis*, *Staphylococcus aureus and Aspergillus niger.* Recently, Ncise et al. (2020) reported that extracts of *Tulbaghia violacea* exposed to a decreased light intensity showed better antifungal activity against *F. oxysporum* than those from the same species that were cultivated under higher light intensity. The presence of the bioactive compounds, in terms of higher phenolic, alkaloid and flavonol contents, could influence the antifungal activities of extracts obtained from aerial parts of *A. porrum*. The results of this study corroborated with those obtained from the study by Zuo et al. (2015), which demonstrated that *A. porrum* had a significant inhibitory effect against *F. oxysporum* f. sp. cubense tropical race 4.

No significant effects were found between extracts of plants subjected to varying light intensities at all concentrations for the three tested solvents in the repellency bioassay. However, the insect repellency increased with increased concentrations, irrespective of the extracting solvent. A recent study showed mixed effects of leek as a companion plant on aphid (*Myzus persicae*) colonisation of sweet pepper (*Capsicum annuum L.*);

TABLE 4: Repellent effects (mean percentage repellency) of extracts of *Allium porrum* from aerial parts against grapevine mealybug (*Planococcus ficus*).

Treatments	Mean percentage repellency ± SE					
	20 (w/v%)	10 (w/v%)	5 (w/v%)	2.5 (w/v%)	χ^2 (DF = 3)	*p*
Dichloromethane (40% shade)	93.33 ± 6.67aA	86 ± 5.79abA	63.33 ± 5.65bAB	39.99 ± 4.08bB	13.61	0.002
Dichloromethane (0% shade)	86 ± 9.79aA	78.33 ± 6.23abA	63.33 ± 5.65bA	43.33 ± 4.08bB	10.84	0.01
Ethanol (40% shade)	78.33 ± 6.24aA	78.33 ± 12.24abAB	75.33 ± 2.44bAB	44.99 ± 7.26bB	9.02	0.02
Ethanol (0% shade)	81.66 ± 7.63aA	83.33 ± 6.97abA	66.66 ± 11.78abA	58.33 ± 7.45abA	4.72	0.17
Acetone (40% shade)	95 ± 5aA	84.33 ± 6.74abAB	66 ± 6.59bBC	53.33 ± 8.57abC	10.52	0.01
Acetone (0% shade)	82.66 ± 4.61aA	61.66 ± 11.66bAB	64.99 ± 8.08bBC	59.33 ± 8.05abBC	5.53	0.12
Kemprin (+control)	96 ± 4aA	96.66 ± 3.33aA	96 ± 5aA	88.33 ± 7.26aA	5.53	0.12
χ^2(DF = 6)	6.01	7.50	11.2	15.4	-	-
p	0.30	0.22	0.06	0.12	-	-

*Means with the same lowercase in the same column are not significantly different ($p > 0.05$). Means with the same uppercase in the same row are not significantly different ($p > 0.05$). The non-parametric Kruskal-Wallis test was used to compare the number of insects repelled between the shade-treated and unshaded plant extracts for the different solvents and extract concentrations, which was followed by the post hoc Mann-Whitney test to separate the means at a level of significance, $p < 0.05$.

whilst the volatile compounds of leeks had a negative effect on aphids' feeding behaviour, no effect was observed on the orientation of the aphids (Baudry et al. 2021). Some of the compounds identified in leeks in this study have repellent activities; for example, dimethyl trisulfide can repel the red spider mite *Tetranychus urticae* (Hincapié, López & Torres et al. 2008). Methyl nonanoate repels European corn borer *Ostrinia nubilalis* (Sole et al. 2010). Methyl palmitate has been reported as repellents of *M. domestica* (Henderson, Wells & Jeanne 1991). Also, methyl palmitate possesses antifeedant properties against *M. persicae* and *Diuraphis noxia* (Santana et al. 2012). Mobki et al. (2014) reported that the extracts of garlic, which is in the same genus as leeks, strongly repelled *T. castaneum.*

The key finding in this study is lowering light intensity from 313 µmol m^{-2} s^{-1} to 153 µmol m^{-2} s^{-1} favoured higher phenolic content, volatile constituents and higher anti-*F. oxysporum* activities in leeks. However, it did not affect the repellent activity against mealybug. Elucidating the effect of light intensity is difficult because there are many interactive factors, including temperature, relative humidity or cultivation method (Thoma et al. 2020). In the current greenhouse study, the possibility that other environmental factors might have interacted with the shading factor cannot be ruled out. Future studies will focus on identifying the phenolic compounds that may be influencing the anti-*F. oxysporum* activity observed in this study. Nevertheless, this study provides a simple hydroponic cultivation protocol for improving the medicinal value of leek products.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

B.N., F.N. and O.O.O. conceived and designed the greenhouse experiments. B.N. conducted the field experiments. B.N., F.N., O.O.O., E.A.A. and NGERE wrote the manuscript. BN, FN, NGERE, EAA and FR conducted the analytical investigations. FN, EAA and OOO supervised the analytical investigations. F.N., F.R. and O.O.O. provided resources to conduct the greenhouse experiments and analytical investigations. All authors contributed to manuscript revision, reading and approved the submitted version.

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Data availability

The data that support the findings of this study are available on request from the corresponding author, F.N.

Disclaimer

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