Cape Peninsula
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The effect of light intensity on the growth and anti-pest activities of leek (*Allium porrum*) against the grapevine mealybug (*Planococcus ficus*) and a phytopathogenic fungus (*Fusarium oxysporum*)

by

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DECLARATION

I, Bulelwa Ntobela, declare that the contents of this dissertation/thesis represent my unaided work and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed Date

DEDICATION

I dedicate this thesis:

- Firstly, to my grandmother Nomalinge Bebeza, uVundle, uBhayi, uKhetshe, Inyok' emnyama eyacanda iziziba, uMevelamhlophe aseCamsholo, uGcwaca, Umsuthu, ndiyabulela ngokundithanda nangeemfundiso zakho.
- Secondly, to mother Marry Ntombizini Liwani, uMangxobe, uMahlathini, ndiyabulela ngemithandazo yakho nangeenkuthazo zakho, kwakufika ubunzima endleleni ubundomeleza maxesha onke.
- Lastly, to my late brother Andile Ntobela, uMqwatho, uDikela, uNoni, uNtswayibane uSidindi, uNomatyala, continue resting in peace.

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LIST OF ACRONYMS

AE Atropine Equivalent

ANOVA Analysis of Variance

B Boron

C Carbon

Ca Calcium

CAF Central Analysis Facilities

Cu Copper

DW Dry Weight

Fe Iron

GAE Gallic Equivalent

GC-MS Gas Chromatography – Mass Spectrometry

K Potassium

Mg Magnesium

MIC Minimum Inhibitory Concentration

Mn Manganese

N Nitrogen

Na Sodium

^{o}C Degrees Celsius

P Phosphorus

P Phosphorus

PDA Potato Dextrose Agar

PPFD Photosynthetic Photon Flux Density

QE Quercitin Equivalent

RH Relative Humidity

S Sulphur

Zn Zinc

ABSTRACT

Pest infestation is a disturbing factor in the agricultural industry. Grapevine mealybug (*Planococcus ficus*) and fusarium wilt, (*Fusarium oxysporum*) are the major pests of economic importance. Control of these pests mainly relies on chemical-based pesticides. However, synthetic pesticides pose severe threats to human health and the environment. Hence, the search for safer alternative pest control measures is intensifying. *Allium porrum* (Alliaceae) is a medicinal plant of the Allium genus extensively recognized to contain many important bioactive agents with remarkable biological activities. Research focusing on optimizing yield and modification of bioactive constituents is intensifying.

This study was designed to determine the effects of varying light intensities on plant growth, bio-screen insecticidal, and antifungal activity of *A. porrum* and to establish the optimum light intensity level for enhancing yield and medicinal properties. The study was divided into two parts to address these objectives. The first part focused on the physiology of of the plant and is presented in chapter two. The second part, covered in chapters 3 and 4, focused on the evaluation of the effect of light intensities on the volatile constituents, antifungal and anti-insect activities of *A. porrum* extract cultivated under greenhouse conditions. The results of the study demonstrated varied effects of light intensities on plant growth parameters. Seasonal changes significantly influenced plants subjected to low light and high light intensities. Aerial-part height was significantly ($P < 0.05$) increased with low light intensity, whereas number of leaves, fresh and dry weights decreased under low light intensity. The association between light intensity and tissue nutrients were examined closely; shading elicited a significant ($DF = 6$; $P < 0.05$) positive response in N, P, and Ca accumulation in the plant tissue. This finding suggests that decreased light intensity favoured the growth of *A. porrum* in height by enhancing macronutrient uptake.

In the second part of the study, presented in chapter three, the seedlings of *A. porrum* were hydroponically grown under low light (40% shade) and high light intensity (0% shade) in 3 different seasons, each running for 12 weeks. The phytochemical analyses of constituents in dry aerial parts of *A. porrum* showed that total polyphenol content was statistically higher ($DF = 1, 6$; $F = 9.17$; $P < 0.05$) in plants exposed to reduced light intensity than in those subjected to high light intensity at 12 weeks post-treatment. Following the gas chromatography mass spectrometry (GC-MS) analysis, the number of known antifungal and anti-insect volatile

compounds plant constituents did not vary significantly, however, higher number of compounds occurred in plants subjected to low light intensity (DF=1; χ^2 =0.44; P > 0.05)**.** The acetone extracts *A. porrum* subjected to lower irradiance exhibited fungistatic effects against *F. oxysporum*. In the grapevine mealybug repellency bioassay, there was no significant difference in different solvent extracts of *A. porrum* subjected to low light and high light intensity at diffent concentrations. The insect repellency tended to be higher at higher concentrations irrespective of the solvent. This study's key findings are: low light intensity has positive effects on volatile constituents, total polyphenol content, fungistatic activities of extracts of *A. porrum*.

Broadly, the results obtained in this study will contribute to the literature of growth, physiological responses, and nutrient content of *A. porrum* subjected to varying light intensities. This study also contributes to the present knowledge on the effect of light irradiance on plant growth and physiology and the biosynthesis of bioactive compounds. This study establishes new protocol for optimised cultivation of leek plants with increased medicinal values. Furthrmore, it opens up new market opportunities to improve profits for farmers.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Introduction

Medicinal plants are a source of many natural and synthetic insecticides and fungicides. Their extracts contain extractable bioactive compounds. These compounds vary according to the plant species, cultivar, and location, as well as ambient environmental conditions (Zlatić and Stanković, 2017). Flavonoids, phenols, phenolic glycosides, sulphur compounds, saponins cyanogenic glycosides, and glucosinolates are recognized medicinal compounds (Bennett and Wallsgrove, 1994; Grayer and Harborne, 1994).

Significant progress has been made to identify and extract pure and crude extracts that are bioactive against many pathogens and pests. Approximately 30-50% of medicine and nutrient rich food are plant-based (Anand et al., 2019). However, the bulk of the medicinal plant studies have focused on their efficacies on pathogens or pests affecting human and animal health. Relatively few studies have concentrated on the development of plant-based agents that are valuable to plant health.

Optimising the yield and quality of secondary metabolites produced by plants is an important aspect of pharmaceutical plant research. Manipulating environmental conditions has been used successfully to improve bioactive compounds in many medicinal plant species (Vu, 2006; Ghasemzadeh and Ghasemzadeh, 2011; Labrooy et al., 2016). Several earlier studies have shown that manipulating light and other environmental factors enhance secondary metabolite contents in plants (Ma et al., 2010; Xu et al., 2020). Light and other environmental growth parameters can be easily controlled. Plants adapt to different light conditions, however, their response depends on the plant species cultivation practices and season (Zhang et al., 2003; Pan and Guo, 2016; Baya, 2016). Photosynthesis is influenced by light intensity (Pan and Guo, 2016; Bayat et al., 2018).

Leek plant is commonly consumed as a vegetable worldwide (Soininen et al., 2014). They are easily cultivated globally under greenhouse and field conditions. Comparable to other *Allium* species, leek extracts contain copious bioactive compounds (Slimestad et al., 2007; Radovanović et al., 2015). These bioactive agents can be used as medicines for their antimicrobial, lipid-lowering, cardiovascular, hypocholesterolemic, antithrombotic,

hypoglycemic, antioxidant (Griffiths et al., 2002; Radovanović et al., 2015). Because this species is easily cultivated in a greenhouse, it is possible to control the yield of plant constituents, including those active against plant pests, by manipulating the abiotic factors.

The spread of plant pests is the main factor that hinders optimum crop production, and two of the major pests are grapevine mealybug (*Planococcus ficus*) and fusarium wilt (*Fusarium oxysoprum*). Both are widespread and are pests of economic importance, causing significant crop loss. Many factors influence the control of pests, and these include host response, pest response to pesticide, and pesticide resistance (Agrios, 1998; Franco et al., 2009; Summerell et al., 2010). Grapevine mealybugs secrete honeydew that attracts ants and inhibits natural enemies' activity (Mgocheki and Addison, 2009). Currently, these pests are mainly controlled using synthetic pesticides. Alternative control measures include biological and cultural control techniques (Walton et al., 2004; Walton and Pringle, 2004; Holm, 2008).

In this study, seedlings of leeks were were subjected to varying light intensities to determine if such variation in light intensity has differential effects on plant growth and leaf extract anti-pest properties. This study's findings will contribute to the existing literature relating to the use of plant cultivation approaches for optimizing bio-efficacy of medicinal plants that will be safer, affordable, readily available, and environmentally friendly. This research study is probably the first that investigates the effects of light intensities on growth and bioactivities of extracts of leek against *P. ficus* and *F. oxysporum*.

1.2 Statement of the research problem

Pest infestations are among the most limiting factor to crop production. Two of some of the most destructive pests of agriculture are greapevine mealybug (*Planococcus ficus* [Signoret]) (Hemiptera: Pseudococcidae) and the phytopathogenic fungus *Fusarium oxysporum* (Nectriaceae). Both have a worldwide distribution, cause huge losses, and are expensive to control. Farmers rely on synthetic pesticides for effectively controlling both pests. These chemicals are unsafe; they are toxic to humans and the environment and have been associated with pesticide resistance development. These risk factors have incentivised the shift to more, sustainable, and environmentally friendly pest control agents (Kulkarni, 2015 in Awasthi 2015). Plant is an essential source of bioactive compounds with repellent and insecticidal properties. Insecticidal and repellent plant species are in high demand. It is causing the overharvesting of wild plants. Cultivation of medicinal plants is regarded as a viable strategy to solve high demand and exploitation of wild species. During cultivation, it is possible to

enhance plant extracts' bioactivity for both pharmaceutical and pest management purposes. The key research question of this study is: Does light intensity influence growth, secondary metabolite production and anti-pests activities of leek plants?. In this greenhouse study, *Allium porrum* (Alliaceae) were cultivated in varying light intensities to assess light intensity effects on plant growth. The light intensity effects on volatile compounds and bioactivities of *A. porrum* extracts against grapevine mealybug *P. ficus* and *F. oxysporum* were also assessed in insect and minimum inhibitory concentration bioassays.

1.3 The Main objectives of the research

This study aimed to determine the effects of varying light intensities on growth, bio-screen insecticidal, antifungal activity of *A. porrum* (Alliaceae), and establish the optimum light intensity level for enhancing yield and medicinal properties.

1.3.1 Specific objectives of the study were:

- To determine the vegetative growth rate of aerial-part length, leaf counts, and biomass yield of *A. porrum* following exposure of the plants to different light intensities.
- To determine the chemical profile of secondary metabolites of extracts of *A. porrum* upon exposure to different light intensities.
- To evaluate the repellency of *A. porrum* extracts of plants grown under different light intensities against grapevine mealybug (*P. ficus*).
- To evaluate the antifungal activity of *A. porrum* extracts of plants grown under different light intensities against *F. oxysporum*.

1.4 The hypotheses of the research

- Different light intensities will have varying effects on the growth rate of aerial part height, number of leaves, and biomass yield of *A. porrum*.
- Different light intensities will have varying effects on the chemical profile of secondary metabolites of extracts of *A. porrum*.
- Different light intensities will have varying effects on contact toxicity and repellency of *A. porrum* extracts on grapevine mealybug (*P. ficus).*
- Different light intensities will have varying effects on the fungistatic and fungicidal activities of *A. porrum* extracts against phytopathogenic fungus *F. oxysporum*.

1.5 Structure of the thesis

This study is a compilation of four succinctly written chapters.

Chapter One: This chapter comprises an introduction, background to the research problem, and literature review.

Chapter Two: This chapter focuses on the effects of varying light intensities on the growth, physiological responses, and nutrient contents of *A. porrum* cultivated hydroponically under greenhouse conditions.

Chapter Three: This chapter emphases on the effect of light intensities on the volatile constituents, antifungal, and anti-insect activities of *A. porrum* extracts cultivated under greenhouse conditions.

Chapter Four: This chapter comprises a general discussion of the results, implications of these results in this area of study, and recommendations from the author.

1.6 Literature Review

1.6.1 Cultivation of medicinal plants

Medicinal plants have been utilized as traditional medicine for centuries. Overexploitation of those plants has been alarming for posing threat to global biodiversity. South Africa has indegnous plants of more than 30,000 species of higher plants, and at least 3000 species that are used as medicine (Van Wyk and Gericke, 2007). Approximately 80% of the South African people depends on the use traditional medicines for primary health care needs (Street and Prinsloo, 2013). In South Africa, herbal medicine trade is worth an estimation of R2.9 billion annually (Mander et al., 2007). There is increasing pressure for large-scale cultivation techniques due to high consumption and trade in medicinal plants and phytomedicines. Medicinal plants are a source of many natural and synthetic pesticides, and their extracts contain bioactive principles, which are extractable (Loundou, 2018). These compounds may vary according to the plant species (Godwin et al., 1991). The production of secondary metabolites by plants is part of the chemical defense response to increased stress (Isah, 2019). It is also paramount to understand the type of stress environment that influences the production of the targeted agents for the reduction of biomass or plant health loss (De Matos Nunes et al., 2014).

Cultivating some medicinal plants can be complex, geographical, and ecological affect plants' physiological responses (Vines, 2004; Cardoso et al., 2019). Fortunately, plant growth and yield can be manipulated by optimizing cultivation practices (Canter et al., 2005; Amoo, 2014; Jelodar et al., 2014), such as the use of hydroponic systems (Vu et al., 2006; Ncise et al., 2020) and micropropagation (Debnath et al., 2006; Reed et al., 2011; Swain et al., 2016) under environmentally controlled conditions.

1.6.1.2 Hydroponics

The hydroponic system is an attractive alternative method compared to conventional cultivation. The hydroponic system is a soilless cultivation method used to deliver plants with essential macronutrients and micronutrient plants (Hayden, 2006). The hydroponics term was derived from the Greek words '*hydro*' (which means water), and '*ponos*' (which means labour), and precisely means water work. Commercial hydroponics farms were developed in the 1960s and 70s (Resh, 2013; Sharma et al., 2018). This cultivation method makes it easier to control water availability, climate, pests, and diseases (Sharma et al., 2018). Medicinal plants that are

difficult to grow can be cultivated commercially using hydroponic systems (Canter et al., 2005). A protected system offers a solution to medicinal plants' sustainable and uniform growth (Giurgiu et al., 2015). Compared to conventional cultivation methods, hydroponic systems offer high yield and quality of plants (Maboko et al., 2009; Buchanan and Omaye, 2013).

The hydroponic system is an interesting alternative for the cultivation of pharmacological plants (Gontier et al., 2002; Moon et al., 2020). Hydroponics can be used as a sustainable agronomic technology for the optimization of natural molecules manufacturing for pharmaceuticals and cosmetics (Nchu et al., 2018). Plants respond against modifications of the environment by synthesising secondary metabolites (Vu et al., 2006). Further, plants can be grown all year round, and factors such as temperature, humidity, light, and nutrients can be easily manipulated to optimise the targeted plant constituents (Kiferle, 2011). The accumulation of bioactive compounds in medicinal plants using conventional cultivation methods are time-consuming and requires more space (Abeysinghe et al., 2014). Literature reports approaches for enhancing the metabolites profile. The study by Abeysinghe et al. (2020) showed that hydroponically grown *Acmella oleraceae* had higher total flavonoids and phenolic contents when compared to callus culture-growing method. Also, in the study by Vu et al. (2006), it was reported that the hydroponic system improved alkaloid production.

1.6.2 Light

Light influences growth and plant development (Anasori and Asgari, 2009) and the biogenesis of organic compounds (Zavala and Ravetta, 2001; Coelho et al., 2007). Plants respond differently to light contigent upon the stage of plant development, species genotype, light type, and light availability (Ghosh et al., 2018; Isah and Umar, 2020). Low light intensity has an inhibitory effect on plants' productivity and growth by influencing gas exchange (Zavala and Ravetta, 2001; Gregoriou et al., 2007). Shading can significantly reduce the thickness of the leaf, leaf weight and plant material (Gregoriou et al., 2002, 2001; Hou et al., 2010).

The effect of light intensity on the content of various secondary metabolites is either enhanced under low light environments (Ralphs et al., 1998; Hou et al., 2010) or decreased depending on the plant species (Zavala and Ravetta, 2001) (Table 1.a). Light intensity, for example, in the study by Coelho et al. (2007), the content of methylxanthine in leaves of *Ilex paraguariensis* increased with low light intesity, but had an inhibitory effect on resin content in *Grindelia chiloensis* leaves (Zavala and Ravetta, 2001). In *Zingiber officinale*, total flavonoid biosynthesis was highest in the leaves and rhizomes of plants submitted to 310 $\mu mol^{-2}s^{-1}$ (low

light), while total phenolics were higher under 790 µmol^{-2}s^{-1} (high light) (Ghasemzadeh et al., 2010). Exposure of *Camptheca acuminata* to 27% full sunlight increased the concentration of camptothecin (Liu et al., 1997). Hou et al. (2010) observed that low light intensity significantly enhanced the accumulation of glycyrrhizix acid and liquirtin in the roots of *Glycyrrhiza uralensis*. In a study by Devkota et al. (2009), high amounts of asiatic acid were observed in *Centella asiatica* upon exposure to 70% shade.

Table 1.a: Light intensity effects on the content of various plant secondary metabolites**.**

Environmental factor	Plant species	Secondary metabolite	Effect	Author
Low light	*Ilex paraguariensis*	methylxanthine-	increase	Coelho et al., 2007
Low light	*Grindelia chiloensis*	resin	decrease	Coelho et al., 2007
Low light	*Zingiber officinale*	flavonoid	increase	Ghasemzadeh et al., 2010
Low light	*Camptheca acuminata*	camptothecin	increase	Liu et al., 1997
Low light	*Mahonia bodinieri*	Alkaloids	increase	Kong et al., 2016
Low light	*Glycyrrhiza uralensis*	glycyrrhizic acid and liquiritin	increase	Hou et al., 2010

1.6.3 Grapevine mealybug *Planococcus ficus* (Signoret)

Planococcus ficus belongs to the family Pseudococcidae and in the order Hemiptera. It is a small-bodied insect with a body length of about 4 mm, oval-shaped, segmented, and pink. The body is covered by a thin with a layer of wax, fringe of wax, capillary extensions around the body, and thin dark stripes devoid of wax along the back (Picker et al., 2002). According to

Myburgh et al. (1986), adult females are wingless, ovate shaped, about 4 mm long with a fringe of finger-like wax protrusions, and covered with fine, white powdery wax. They are light-slate to flesh-coloured, with a darker line lengthwise down the middle of the back, where the waxy covering is noticeably thinner.

Planococcus ficus is the most serious pest of grapes and also occurs on figs (Myburgh et al., 1986). It feeds on wide range of plants (Picker et al., 2002). *P. ficus* occurs in the Western Cape in spring and summer on grape shoots. Its copious honeydew secretions cause black mould. Mealybugs in warmer regions produce up to six generations per season. They can feed on any plant part, causing yellow leaves and stems, white cottony deposits, sticky residue, and sooty mould (Heyler et al., 2003). According to Tsai et al. (2008), grapevine mealybug is a potential vector of grapevine leafroll-associated viruses (GLRaVs) that causes grapevine leafroll disease. The leafroll disease affects both vine health and quality and signs befall in the autumn when reddening between the leaf veins occurs in dark fruit varieties, leaf chlorosis in white varieties, and downward leaf margin rolling. Chiotta et al. (2010) suggested that *P. ficus* can be regarded as a vector of the pathogen that causes grape skin damage. The damage caused by *P. ficus* leaves table grapes with blemishes and unmarketable fruits (De Villiers, 2006). Severe mealybug infestation causes inhibition of normal ripening processes, and the grapes lack taste and color and result in bunch withering (De Villiers, 2006; De Villiers and Pringle, 2007; Blumberg et al., 1995). Leaves turn yellow and drop prematurely (Walton and Pringle, 2004; Holm, 2008).

In South Africa the method of mealybug control relies on has been the use of chemical insecticides. The active ingredients of the registered pesticides for the control of grapevine mealybug are borax/orange oil, carbaryl, chlorpyrifos, dichlorvos, dimethoate, imidacloprid methidation, mevinphos, profenfos, and prothiofos (South Africa Department of Agriculture, 2007). Chlorpyrifos-methyl insecticide is commonly used for the control of *P. ficus* and is harmful towards *Anagyrus* sp. near *pseudococci*. It was found that the spirotetramat and Prev-Am® can be applied for the control of *P. ficus* (Mansour et al., 2011).

1.6.3.1 Natural enemies of mealybug

The grapevine mealybug in the Cape wineries has three groups of natural enemies. These enemies are: predatory ladybird beetles (Coccinellidae) including *Hyperaspis senegalensis hottentota*, *Scymnus quadrivittatus*, and *Sceloporus angustus* (Picker et al., 2004; Heyler et al.,

2003; Greenwood and Halstead, 2007); the parasitic wasps (Hymenoptera), which includes the encyrtrids, *Leptomastix dactylopii;* and, the lacewings (Chrysopidae) group, includes *Chrysopa pudica* and *C. burgeonina*. Studies suggest that ladybird beetles are more important natural enemies to vine mealybugs in the Cape (Annecke and Moran, 1982). In a recent survey by Walton and Pringle (2004), it was found that predatory beetles, *Nephus angustus* (Casey), *N. quadrivittatus* (Mulsant), *N. binaevatus* (Mulsant), *Nephus* sp., *Hyperaspis felixi* (Mulsant), *Cryptolaemus montrouzieri* (Mulsant), *Scymnus nubilis* (Mulsant), *Cydonia lunata* F., a *Rhizobiellus* sp., and *Hippodamia* sp. are the natural enemies of *P. ficus*. The Nephus genus was found to be the most abundant predatory beetle genus. According to Le Vieux and Malan (2013), *S. yirgalemense* and *H. zealandica* have a potential as biological agents for the control of *P. ficus*.

1.6.3.1.1 Pheromone traps

Use of pheromone traps has shown success in catching *P. ficus* males, especially under low infestation levels of pest populations. Therefore, these traps can be used as a preventative measure in vine propagation units with intolaerance for this vector. In areas with established *P. ficus*, these traps are quick detectors for early preventions of destructive populations of *P. ficus* (Walton et al., 2004).

Karamaouna et al. (2013) claims that their study provided the first investigation on the insecticidal activity of the essential oils derived from two citrus species such as *Citrus limon* and *Citrus sinensis*, and four aromatic plant species such as *Satureja thymbra, Mentha piperita, Lavandula angustifolia,* and *Ocimum basilicum* on *P. ficus*. The findings also showed that limonene in citrus peel was the most toxic against *P. ficus* and did not cause phytotoxicity on grapevine leaves than other essential oils.

1.6.3.1.2 Insecticidal and repellent plants

Medicinal plants have shown potential for the managing many destructive insect pests in the agricultural industry. Also, the use of botanicals contributes to the conservation of natural enemies of pests by reducing the use of synthetic pesticide and their negative impact in non-targeted organisms (Baidoo and Mochiah, 2016). The extracted natural chemicals from plants are used as a substitute for chemical-based pesticides (Suthisut et al., 2011; Hikal et al., 2017). The secondary metabolites are extracted from all plant parts: seeds, leaves, roots, stems, bark,

flowers, and fruits (Cragg and Newman, 2001; Hussein and El-Anssary, 2018). The secondary compounds are grouped into nitrogen containing and non-nitrogen- containing compounds with economically important constituents for control of insect pests (Rossi, 2008). Plant secondary metabolites can mediate signalling pathways that produce plant toxins and be directly toxic to insect pests (War et al., 2019).

Plants from the *Allium* genus are among studied plants due to their high content of bioactive constituents (Elisovetcaia et al., 2018). Many trials have reported *Allium* spp. extracts against a large number of pests. Plant volatiles of *Allium* spp. have repellent and pesticidal properties on some arthropod pests (Denloye et al., 2003; Mann et al., 2011). For example, the essential oils extracted from *Allium tuberosum,* Chinese chives have contact toxicity and repellent activity against *Plurtella xylostella* larvae (Gao et al., 2019). In the study by Nchu et al. (2016), *Allium sativum* exhibited repellent effects on ticks at lower concentrations. Furthermore, Meriga et al. (2012) reported that methanol and aqueous extracts of *Allium sativum* bulbs were significantly insecticidal with a high mortality rate against *Spodoptera litura*. Allicin is a sulfur compound commonly found in *Allium* plants and is effectively used to control agricultural crop-damaging pests, i.e., repellent against fruit flies (Miron et al., 2006). The sulfur volatiles such as disulfides and trisulfides produced in *Allium tuberosum* repelled *Diophorina citri* (Mann et al., 2011). Intercropping with *Allium cepa* and *Allium sativum* has also been proven effective for controlling insect pests by excreting odours responsible for repellent activity (Debra and Misheck, 2014). In addition, pyrethrins derived from pyrethrum are also one of the critical groups of organic insecticidal compounds; it is normally extracted from *Chrysathemum cinerariifolium* (Yang et al., 2012; Bekele, 2018). Seeds of *Cinnamomum camphora* and *Artemisia princeps* Pamp were tested and exhibited high insecticidal and repellent activities against *Sitophillus oryzae* and *Bruchus rugimanus* (Liu et al., 2006). Roots powder of *Chromolaena odorata* were reported to be highly repellent and insecticidal activities and can be used as an effective measure for the control and management of of *Callosobruchus maculatus* (Fab.) (Coleoptera Chrysomelidae) (Osarivekemwen and Benedicta, 2017).

1.6.4 *Fusarium oxysporum* (Hypocreales)

Fusarium oxysporum is a soil-borne pathogenic fungus. It asexually produces different types of spores, such as microconidia, macroconidia, and chlamydospores. These spores can remain dormant in soil for over 30 years, spreading through running water, on-farm implements, and machinery. It consists of over 120 known strains or "special forms" (formae speciales; f. sp.),

with each strain ihabitating a specific host plant in which it causes disease. Altogether, these *F. oxysporum* strains infect and kill an extensive variety of host including commercially harvested crops. Symptoms only appear when transmitted to other individuals, resulting in this fungus being a significant and damaging pest in agriculture (Gonsalves and Ferreira 1993; Miller et al., 1996). The fungus can either penetrate a plant with mycelium or sporangial germinal tube, attacking the roots of the plant. Various *Fusarium* species' spreading is caused by environmental factors, such as temperature, rainfall, soil type, and vegetation (Summerell et al., 2010).

The soil-borne fungus is among the important factors limiting agro-ecosystems' productivity and is challenging to control with conventional strategies using host cultivars with resistance and chemical based fungicides (Bailey and Lazarovits, 2003; Bananomi et al., 2007). However, according to Alabouvette et al. (1996), Fusarium wilt diseases can be controlled by the selection of resistant cultivars. Moreover, there are commercially available resistant cultivars in crops such as tomato and radish. *Bacillus amyloliquefaciens* has long been extensively used as a biological control agent of pathogens living withing the soil (Mari et al., 1996; Yu et al., 2002). A recent study by Sotoyama et al. (2016) revealed that *B. amyloliquefaciens* IUMC7 isolated from mushroom compost has can be used as biocontrol agent due to the growth inhibition of *F. oxysporum* f. sp. *lycopersici* (FOL), and germ tube elongation of FOL. The reduction of soil-borne diseases can be achieved by applying organic amendments, manures, and composts rich in nitrogen (Bailey and Lazarovits, 2003). Application of antagonistic fungi and bacteria isolated from soils have been used as biological control agents against fusarium wilts of numerous crops (Larkin et al., 1996; Leeman et al., 1996; Lemanceau et al., 1992).

Numerous commercial preparations of botanical extracts and essential oils are investigated as potential substitutes to soil disinfection to control fusarium wilt diseases (Bowers and Locke, 2000). A study by Shimoni et al. (1993) has also demonstrated that essential oils extracted from *Majorana syriaca*, can reduce *F. oxysporum* by 85% and it has been concluded that *M. syriaca* can be used as an antifungal agent for use against *F. oxysporum*. There is a high possibility of controlling fusarium wilt disease using crude extracts of medicinal plants.

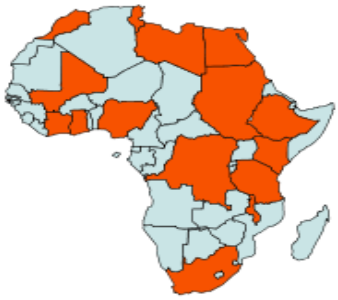

Figure: 1.a: The distribution of Fusarium wilt disease, *Fusarium oxysporum* in Africa. Source: http://oer2go.org/mods/en-infonet/export/printct87$pests.html

1.6.2 Leek (*Allium porrum* L.)

Leeks belong to the family Alliaceae that shows morphological differences from onions. It is larger than onions, and with flattened leaf blades. Leeks have a more delicate taste than onion, though a coarser texture. They are close to the green onion, but their size is larger. Leek is a cultivated form of *Allium ampeloprasum*, a tetraploid (2n=32). (Warade and Shinde, 1998).

Leeks are predominantly grown in northern European countries, such as Belgium, Denmark, and the Netherlands (Warade and Shinde, 1998). In South Africa, leeks are commercially grown as vegetables. They thrive in a sunny sheltered site in well-drained, neutral to slightly acidic soil, and at minimum soil or compost temperature of at least 7 °C (45 °F) to germinate (Biggs et al., 2012). Rapid germination can be encouraged by sowing varieties indoors during late winter at 13-16 °C (55-60 °F) in trays, pots, or seed compost modules. Sowing can be done in glasshouses in the absence of heat and cold frames or under cloches. Seeds are sown late winter to early spring, pot on, hardened off, and transplanted in the late spring (Biggs et al., 2012).

Allium genus is represented by a large variety of chemical compounds. Aside from spirostane- and furostane-type compounds, an exceptional group of open-chain saponins is known to many species. This genus is also a source of unique steroidal sapogenins, regardless of steroidal glycosides low content in *Allium* species, they contribute in sulphur compounds, and to the general bioactivities of these plants. The sulphur compounds of *Allium* plants are mostly represented by *S*-allyl cysteine, alk(en)yl cysteine sulfoxides, thiosulfates, and diallyl mono-di-, and trisulfides, vinyldithiins (Ramiez et al., 2017; Poojary 2017). According to Lamberth et al. (2015), about 30% of agricultural chemicals contains sulphur atoms. Organosulphur compounds contributes plant defence by constituting insecticidal, fungicidal properties

(Hartzell and Lathrop, 1925; Nwachukwu et al., 2012, Sagdic et al., 2012; Sobolewska et al., 2016). Also, *Allium* spp. particularly *Allium sativum* synthesize allicin sulfur compound generated for defence (Slusarenko et al., 2008; Borlinghaus et al., 2014).

1.6.5 References

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CHAPTER TWO

The effects of varying light intensity on the growth, physiological responses, and tissue nutrient contents of *Allium porrum* cultivated hydroponically under greenhouse conditions.

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Abstract

Manipulating cultivation protocols to improve yield and quality of medicinal materials is a crucial aspect of medicinal plant research. Evidences from literature suggest controlling light intensity can influence photosynthetic process, secondary metabolite production, and growth and development of medicinal plants. In this study we investigated the effect of varying light intensity on the growth, chlorophyll contents, and tissue nutrient contents of *Allium porrum*, which like other members of the Allium genus, is recognized as a source of medicinal materials. Allium porrum seedlings were grown hydroponically under low light (40% shade) and high light intensity (0% shade) in three different seasons. Plant growth in response to different light intensities were recorded on the following parameters: number of leaves, plant height, plant fresh and dry weights, and nutrient content. The number of leaves and plant height were recorded on a weekly basis. In all three experiments, plants were harvested at 12 weeks post-treatment and recorded fresh and dry weights. Tissue nutrient content was analysed on dried aerial parts. Light intensities had varied effects on the plant growth parameters of *A. porrum*. Aerial-part height was significantly increased with reduced light intensity (shading), whereas fresh and dry weights, and number of leaves significantly decreased under low light intensity. The interaction between light and different growing seasons significantly ($P < 0.05$) affected plant height, number of leaves, fresh root and aerial-part weight, and aerial-part dry weight. We also found that shading elicited significantly positive effects on N, P, and Ca ($DF = 6$; $P < 0.05$) concentrations in the plant tissue. These findings suggest that decreased light intensity favours the growth of *A. porrum* in height and plant tissue Zn content, while high light intensity favours higher biomass accumulation. Seasonal light intensity and day length variations may have modulated the observed effects on the plant growth parameters.

Key words: Medicinal plants; Light intensity; Biotechnology; *Allium porrum*; Plant cultivation

2.1 Introduction

Leek plants (*Allium porrum* L.) belong to the family Alliaceae, which comprises approximately 600 species. Leek plants (*Allium porrum* L.) belong to the family Alliaceae that comprises approximately 600 species. It is among species of the *Allium* genus that are extensively cultivated for food and pharmaceutical uses (Fattorusso et al., 2001; Sharifi-Rad et al., 2016 Leek has been recognized for centuries as a rich source of medicinal materials. It is rich in pharmacologically active sulphur-based compounds with antibacterial, antifungal and antioxidants (Harris et al., 2001; Griffiths et al., 2002; Sharifi-Rad et al., 2016). Like other *Allium* species, *A. porrum* possesses insecticidal, fungicidal, and pharmaceutical properties(Irkin and Korukluoglu, 2007; Tamokou et al., 2017).

Studies to find enhanced cultivation protocols with the view of improving the yield of this species are on the increase. Leeks can be successfully grown in hydroponic systems (De Rijck et al., 1993). Hydroponics is a sustainable method of cultivating plants (Kumari et al., 2008). The literature provides evidence on how hydroponic system and light can be used to enhance the production of secondary metabolites and biomass production in plants (Vu et al., 2006; Labrooy et al., 2016).

Light is a key factor of the environment that plays a most important role in plants by regulating photosynthesis process, growth, and development (Fukuda et al., 2008; Ruban, 2009; Zervoudakis et al., 2012). Plants can adjust and adapt to varying light intensities. But their response depends on plant species, cultivation practices, season, and light intensity (Zhang et al., 2003; Kozai, 2016). Light being the trigger of photosynthesis can also function as a stress factor in plants when it is too high or too low (Pan and Guo, 2016; Bayat et al., 2018). An increase in light intensity correlates with a net photosynthesis rate increase (Fan et al., 2013). However, exposure of plants to excessively high light conditions can damage the photosynthetic apparatus (Li et al., 2014).

A number of studies have found that lack of light lowers photosynthesis, resulting in decreased plant growth (Pierson et al., 1990; Chang, 2005; Zavala and Ravetta, 2001; Putri et al., 2018). For example, *P. lactiflora* plants grown under shade had decreased photosynthetic capacity (Zhao et al., 2010). Meanwhile, other studies showed that low light intensity increases plant height, leaf size, and chlorophyll content (Zervoudakis et al., 2012; Rezai et al., 2018). Under low light intensity, plant productivity is inhibited by affecting gas exchange (Gregoriou et al.,

2007; Hou et al., 2010). Light is used during photosynthesis to generate ATP and NADPH, which are needed for converting carbon to carbohydrate (Hou et al., 2010; Gregoriou et al., 2007). Under low irradiation conditions, insufficient ATP is produced to enable carbon fixation and carbohydrate synthesis, which inhibits plant growth (Shao et al., 2014; Feng et al., 2019). Photosynthetic rate is influenced by light, temperature, and carbon dioxide externally and by chlorophyll internally (Emerson, 1929; Rathore and Jasrai, 2013). Lower light conditions significantly reduce the plant biomass with changes in the biomass allocation to different plant organs (Poorter and Nagel, 200). The exposure of plants to high light intensity favoured the yield and nutrient composition of the above-ground portion of *H. cordata* (Li et al., 2015).

The chlorophyll content is an essential pigment for transforming solar radiation energy into chemical energy stored in leaves (Steele et al., 2008; Ma et al., 2018). Chlorophyll is the basic unit of plant photosynthesis; the concentration and composition directly influence the photosynthetic rate (Fan et al., 2013; Ahmad et al., 2018). Chlorophyll synthesis and photo-oxidation depends on light availability (Srichaikul et al., 2011; Zervoudakis et al., 2012), but the pattern is rather unclear. According to Park and Masaru (2018), low light decreases plants' chlorophyll content, resulting in decreased leaf thickness and leaf mass. However, other studies reported an increase of chlorophyll with decreasing light intensity, for example, in tomato seedlings (Wang et al., 2010), sage (Rezai et al., 2018), *Anglaonema commutatum* (Dibenedetto, 1991).

Thus far, research on the cultivation of *A. porrum* with the intent of optimizing yield and chlorophyll, through manipulation of light intensity, is rare. This study's objective was to determine the effects of varying light intensities on the growth, chlorophyll content, and nutrient uptake of *Allium porrum* in the greenhouse conditions.

2.2 Materials and Methods

2.2.1 Plant material

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

2.2.2 Greenhouse experimental design

A single factorial experiment design was used to investigate the effect of one factor (shading) with two shading levels on *A. porrum*. The experimental trials were replicated three times, each running for 12 weeks in different seasons. Trial 1 ran from June to August (winter), trial 2 from September to December (spring-summer), and experiment 3 from February to April (autumn). The experiment was conducted in the greenhouse of the Department of Horticultural Sciences, Cape Peninsula University of Technology, Bellville Campus. Experimental plants (160 seedlings) were individually transplanted into 25 cm pots filled with the following substrate mix: silica sand 25% + perlite 25%+ coco peat 25% + vermiculite 25%. Data such as the number of leaves and above-ground plant length (cm) at the beginning and end of the experiment using a measuring tape to determine plant growth in response to different light intensities. The relative growth of plant height and number of leaves at the end of the experiment was estimated as follows: final measurement – initial measurement. Fresh weights of plants were recorded immediately after harvesting plants at 12 weeks post-treatment. The plant materials were dried in an oven at 25 ºC for 14 days and weighing to obtain the dry weights of the aerial-part and root (g). Treatments were based on two levels of irradiance i.e., high light intensity (0% shade) and low light intensity (40% shade) in the greenhouse. Low light intensity (40% shade) was obtained by covering a metal table (230x90x85 cm) with 40% green polyethylene shade net obtained from Stodels Pty Ltd, Garden Centre, Cape Town (Figure 2.a). High light intensity (0% shade) was obtained by subjecting plants to sunlight entering through the greenhouse's polycarbonate roof cover (Figure 2.a). Light irradiance was measured at plant height using digital lux meter (0.1- 400, 00 LUX) MT942 Major Tech. Light intensity was converted from Lux to PPFD (µmol m^{-2} s^{-1}) using a standard conversion factor of 0.0185 for sunlight light source (Apogeeinstruments, 2020). The conversion formula was

lux × conversion factor (0.0185) = PPFD (µmol m^{-2} s^{-1}). The average of 0% and 40% shade light intensities (Photosynthetic Photon Flux Density [PPFD]) measured at noon were 313 and 153 µmol m^{-2} s^{-1}, respectively. The temperatures ranged from 19 °C to 27 °C, and the average relative humidity ranged from 29 to 67%. Because the trial was repeated in different seasons with different light intensities and day lengths, the interactive effects of season and light intensity was also evaluated using a Two-Way Analysis of Variance (anova). Plants were supplied with a hydroponic fertilizer, Nutrifeed®, bought from Starke Ayres Pty Ltd, Cape Town. The fertilizer contained the following ingredients: 65 mg kg^{-1} N, 27 mg kg^{-1} P, 130 mg kg^{-1} K, 70 mg kg^{-1} Ca, 20 mg kg^{-1} Cu, 1500 mg kg^{-1} Fe, 22 mg kg^{-1} Mg, 75 mg kg^{-1} S, 240 mg kg^{-1} Mn, 240 mg kg^{-1} B, 10 mg kg^{-1} Mo, and 240 mg kg^{-1} Zn. The nutrient solution was prepared according to the recommended dosage with deionized water and applied as a drench to each plant. Each plant received 100 ml of the nutrient solution once a week, followed by deionized water once every three days.

Figure 2.a: High light intensity: 0% shade (A), Low light intensity: 40% Shade (B).

2.2.3 Chlorophyll content

Chlorophyll extraction was based on the method described by Arnon (1949). At twelve weeks post-treatment, *porrum* aerial parts from the trial 3 (autumn) were used for the chlorophyll analyses. Finely cut fresh leaves (1 g) was grinded with 20 ml of 80% acetone using a pestle and mortar. The paste was separated by centrifuge at 5000 rpm for 5 minutes. The supernatant was transferred, and this procedure was repeated up until the residues became colourless. The extract solutions absorbance was read at 645 nm and 663 nm against the solvent (80% acetone) blank. To estimate the chlorophyll contents, the concentrations of chlorophyll a, chlorophyll b, and total chlorophyll were calculated using the following equations: Total Chlorophyll: 20.2(A645) + 8.02(A663); Chlorophyll a: 12.7(A663) – 2.69(A645); Chlorophyll b: 22.9(A645) – 4.68(A663).

2.2.4 Tissue analysis

Leaf samples of plants grown in trial 3 (autumn) were by a commercial laboratory, Bemlab (Pty) Ltd **(**Gant's Sentrum, 16 Van Der Berg Cres**,** Strand, South Africa), for macro- and micro-elements analysis using inductively coupled mass spectrometry (ICP-MS) and a LECO-nitrogen analyser for total-nitrogen (N) with suitable standards as previously described [66-68], Teepol solution was used to wash leaves, rinsed with deionized water, and dried in an oven at 65 ℃ for 48 hours. Five grams of milled dried leaves were transferred to porcelain crucible and ashed using an oven for heating at a 480 ^{o}C. Afterwards, the ash was cooled at a room temperature, then shaken up in a 50:50 HCl (50%) solution, and the extract was filtered through a Whatman No. 1 filter paper. The Carbon (C), phosphorus (P) and boron (B) were assessed directly in final digests. The Calcium (Ca), magnesium (Mg), potassium (K), copper (Cu), Sodium (Na), iron (Fe), manganese (Mn) and zinc (Zn) were analysed using the Ash method.

2.2.5 Statistical Analysis

The experimental data collected for plant growth parameters, chlorophyll content, tissue nutrient content were collected and analysed following one-way analysis of variance (ANOVA). Tukey HSD test was used to separate the means at a level of significance, $P< 0.05$. Two-way analysis of variance (ANOVA) to analyse the interactive effects of light intensity and season. Statistical analyses were performed using Statistica 13.3.1 software (TIBCO software Inc., Palo Alto, USA).

2.3 Results

2.3.1 Plant height

Higher mean heights were obtained in plants subjected to low light intensity compared with those under high light intensity in all trials (Table 2.a). Plant height of *A. porrum* varied significantly (DF = 2,177; P < 0.005) among different trials in both low light (40% shade) and high light (0% shade) intensities following one-way ANOVA analysis. Plants grown under low light intensity was significantly taller in trial 3 (autumn), and followed by trail 1 (winter), and the shortest height was observed in plants grown in trail 2 (spring-summer) (DF = 2,177; F = 28.99; P < 0.005) (Table 2.a). A similar trend was observed in plants grown under the higher light intensity. A significantly taller mean height was observed in plants grown in trial 3, followed by trial 1 and trial 2, which recorded the lowest height (DF = 2,177; F = 73.74; P < 0.05) (Table 2.a). Based on a two-way ANOVA analysis, the interaction between light and different trials (seasons) showed a significant (DF =2, 354; F= 8.75; P < 0.05) influence on plant height of *A. porrum*.

2.3.2 Number of leaves

Plants subjected to the higher light intensity significantly (P < 0.005) produced more leaves when compared to those subjected to low light intensity (40% shade) in all three trials at 12 weeks post-treatment. There was a significant difference (DF = 2,177; F = 8.03; P < 0.05) in the mean number of leaves among trials under low light intensity (40% shade). The highest mean number of leaves of *A. porrum* was obtained in trial 3 (autumn) and trail 2 (spring-summer), respectively, and trial 1 (winter) produced the lowest number of leaves (DF = 2,177; F = 8.19; P < 0.05) (Table 2.a). There were no significant differences (DF = 2,177; F = 2.66; P > 0.05) in the mean number of *A. porrum* leaves among the growing seasons under high light intensity. Although no significant difference was obtained under high light intensity, trial 1 (winter) showed a slightly higher number of leaves, followed by plants grown in trial 2 (spring-summer). The lowest number was observed in season 3, respectively (Table 2.a). The interaction between light intensity and season significantly influenced the number of leaves of *A. porrum* (DF = 2,354; F = 8.75; P < 0.05).

2.3.3 Fresh aerial-part weight

When aerial-part fresh weight were compared between varying light intensities, plants subjected to high light intensity had significantly ($P < 0.005$) more weight when compared to those subjected to low light intensity in all three seasons at 12 weeks post-treatment. The fresh aerial weight (g) of *A. porrum* was influenced by the significant interaction between light and trial (season) ($DF = 2, 174$; $F = 3.41$; $P < 0.05$) (Table 2.a). Generally, plants grown under high light intensity were significantly heavier than those exposed to low light intensity in all the growing seasons.

2.3.4 Fresh root weight

The root fresh weight (g) of *A. porrum* among trails under both low light (40% shade) ($DF = 2, 87$; $F = 30.04$; $P < 0.05$) and high light intensity (0% shade) ($DF = 2, 87$; $F = 3.28$; $P < 0.05$) differed significantly at 12 weeks post-treatment. The fresh root weight of plants in trial 3 (autumn) was significantly heavier, followed by trial 2 (spring-summer), and the lowest was observed in trial 1 (winter), under low light intensity (Table 2.a). Fresh root weight of *A. porrum* maintained in high light intensity produced heavier roots in trial 1 (winter) and trial 3 (autumn), while trial 2 (spring-summer) showed the lowest fresh root weight (Table 2.a). Generally, the interactive effect of light and trial (season) on the fresh root weight of *A. porrum* was significant ($DF = 2, 174$; $F = 25.72$; $P < 0.05$).

2.3.5 Aerial-part dry weight

When aerial-part dry weights were compared between low and high light intensities, plants subjected to the higher light intensity produced higher aerial-part dry weight in all growing trials (seasons). There was a significant difference in the aerial-part dry weights (g) of *A. porrum* among growing seasons in both low light ($DF = 2, 87$; $F = 22.69$; $P < 0.05$) and high light intensity at 12 weeks post-treatment ($DF = 2, 87$; $F = 8.27$; $P < 0.005$). Under low light intensity, plants grown in trial 2 (spring-summer) had significantly heavier dry weight (g), followed by trial 3 (autumn) when compared to those grown in trial 1 (winter) (Table 2.a). Under high light intensity, trials 3 (autumn) and 2 (spring-summer) produced heavier aerial-part dry material, while trial 1 (winter) showed the lowest aerial part dry weight (Table 2.a). The interactive effect between trial (season) and light intensity on dry weight of *A. porrum* was significant ($DF = 2, 174$; $F = 6.44$; $P < 0.05$).

2.3.6 Root dry weight

There was a significant difference in root dry weight (g) of *A. porrum* among varying seasons under both low light (DF = 2,87; F = 82.36; P < 0.05) and high light intensities (DF = 2,87; F = 20.39; P < 0.005) at 12 weeks post-treatment following one-way Anova. Trial 2 (spring-summer) produced a significantly higher root dry weight (g) under low light intensity compared to trial 1 (winter) and s trial 2 (spring-summer) (Table 2.a). When trials were compared within high light intensity, dry root weight was higher in both trial 2 (spring-summer) and trial 3 (autumn), and lowest in the trial 1 (winter) (Table 2.a). The high light intensity produced heavier root dry weight than those under low light intensity in all the growing seasons. There was no significant interactive influence between light and season (DF = 2,174; F = 2.11; P > 0.05) based on two-way ANOVA analysis.

Table 2.a: Effects of varying light intensities (40% and 0% shade) on the growth parameters of *A. porrum*.

Light irradiance level	Season	Light intensity (PPFD: µmol m-2s-1)	Plant height (cm)	Number of leaves	Fresh weight (g)		Dry weight (g)	
					Aerial-part	Root	Aerial-part	Root
40% shade	Trial 1 (Winter)	106.5	50.2±0.9bB	2.8±0.1aB	25.6±1.4aA	4.5±0.4aA	2.1±0.1aA	0.3±0.03aA
	Trial 2 (Spring-Summer)	217.5	45.2±0.8bA	3.4±0.1aA	32.6±0.7aB	10.3±0.7aC	3.5±0.2bC	1.8±0.1aB
	Trial 3 (Autumn)	134.9	53.5.±0.6bC	3.3±0.1aA	22±1.2aA	7.2±0.8aB	2.8±0.2aB	0.5±0.1aA
0% Shade	Trial 1 (Winter)	224.2	46.3±1.1aB	4.1±0.2bA	33.8±1.2bA	16.4±1.8bA	3.3±0.2bB	1.6±0.1bA
	Trial 2 (Spring-Summer)	440.9	35.8±0.8aA	3.8±0.1bA	35.3±0.7bA	13.2±0.7bA	3.9±0.1aA	2.6±0.2bB
	Trial 3 (Autumn)	273.6	50.6±0.7aC	3.7±0.1bA	29.2±1.2bB	15.9±0.9bA	4.1±0.2bA	1.5±0.1bA

*The same lowercases between light irradiance levels for each season in the same column indicates no significant difference following Tukey's test ($P > 0.05$). The same uppercase letter among seasons for each light irradiance level in the same column indicates no significance difference following Tukey's test ($P > 0.05$).

2.3.7 Tissue Analysis

2.3.7.1 Macronutrients

Leaf samples from the third trial (autumn) were analysed for tissue macro-and micro-nutrient contents. No significant differences (DF = 1, 6; P > 0.05) in the levels of C, K, Mg, and Na contents in aerial plant tissue were observed between treatments. But plant nutrient contents of N, P, and Ca were significantly higher in aerial parts of plants grown under 40% shade (low light) than in 0% shade (high light intensity) (Table 2.b). When plant nutrient contents were compared between treatments, N, K, and C contents differed significantly (DF = 1, 6; P < 0.05) between 0% and 40% shading treatments (Table 2.b). However, plant nutrients were higher in plants exposed to low light intensity than in high light intensity.

Table: 2.b: Tissue macronutrient contents (Mean ± SE mg/kg) for *A. porrum* grown under low light (40% shade) and high light (0% shade) intensity at 12 weeks post-treatment.

Nutrients (mg/kg)	**Light irradiance**	
	High light intensity (0% shade)	**Low light intensity (40% shade)**
C	385975±2346.4dA	383400±2387.8eA
N	29625±404.9bA	31200±488.2cB
P	3975±103.1aA	5525±193.1abB
K	42750±1436.1cA	46500±1707.8dA
Ca	5300±57.7aA	6850±572.3bB
Mg	5475±125aA	6125±363.7abA
Na	852.3±73aA	1021.5±226aA

*Means followed by the same uppercase letters in the same row are not significantly different following Tukey's test (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).

2.3.7.2 Micronutrients

Statistically, there were no significant differences ($P > 0.05$) in the tissue nutrient contents of Mn, Fe, Cu, and B among aerial-parts in varied light intensities (Table 2.c). Nonetheless, Zn's tissue content was significantly higher in unshaded plants than unshaded plants ($DF= 1, 6$; $F = 6.01$; $P < 0.05$) (Table 2.c).

Table 2.c: Tissue micronutrient contents (Mean ± SE mg/kg) for *A. porrum* subjected low light (40% shade) or high light (0% shade) intensity at 12 weeks post treatment.

Nutrients	**Light irradiance**	
	High light intensity (0% shade)	**Low light intensity 40% shade**
Mn	38.8±2.7aA	34.5±2.0cA
Fe	75.9±12.0cA	77.2±11.2dA
Cu	3.8±0.3bA	4.0±0.7aA
Zn	15.7±1.6abB	11.8±0.4abA
B	32.5±0.6aA	29.4±1.5bcA

*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 are not significantly different following Tukey's test ($P > 0.05$).

2.3.8 Chlorophyll contents

Leaf samples from the trial 3 (autumn) were analysed for tissue chlorophyll contents. No significant differences ($P > 0.05$) in chlorophyll a, chlorophyll b, and total chlorophyll contents between low and high light treatments was detected (Figure 2.b). However, through observations, the highest chlorophyll concentration was found in plants exposed to low light intensity (40% shade). Generally, plants subjected to low light intensity showed an increased amount of chlorophyll a, chlorophyll b, total chlorophyll with mean concentrations of 0.6±0.03 µg/ml, 0.2±0.03 µg/ml, and 0.7±0.06 µg/ml, respectively (Figure 2.b). Plants maintained under high light intensity (0% shade) resulted in low chlorophyll content compared with those grown under low light intensity (40 % shade) at 12 weeks post-treatment.

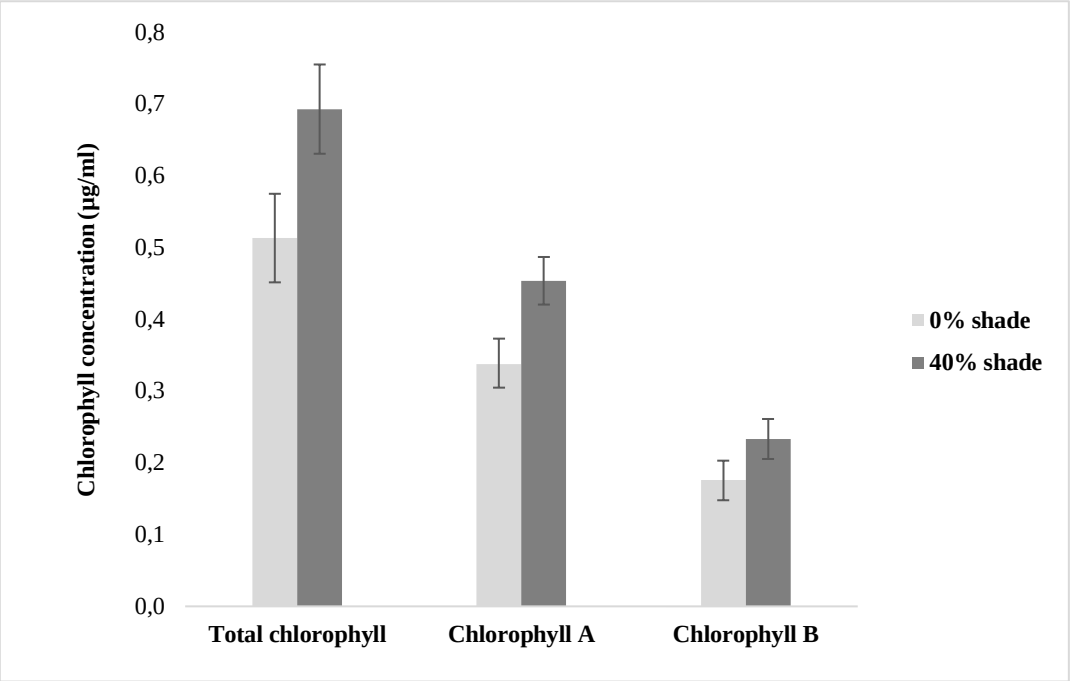

Figure 2.b: The chlorophyll content (Chlorophyll a, Chlorophyll b, Total chlorophyll a+b) of *A. porrum* plants grown under varying light intensities (40% and 0% shade).

2.4 Discussion

Generally, the effects of light intensity on growth parameters were influenced by seasonal changes except in root dry weight. According to the results of the study, generally, the low intensity was positively associated with higher plant height, total chlorophyll content, tissue Zn content. The height and tissue nutrient contents increased with reduced light intensity, while number of leaves, fresh and dry weights decreased under low light with intensity. The interaction between light and different growing seasons significantly ($P < 0.05$) affected the growth response in parameters such as plant height, number of leaves, fresh root and aerial-part weight, aerial-part dry weight. Specifically, this study results demonstrated that plants subjected to low light intensity in trials 3 (autumn) and 1 (winter) obtained higher mean height of *A. porrum*. These results corroborate with previous results; a related species, *Tulbaghia violacea*, grown under low light intensity (40 % shade) had significantly higher mean height and lower fresh and dry aerial parts weights when compared with those subjected to 0% shading treatment (Ncise et al., 2020). Similar findings on low light intensity inducing reduced plant growth have been reported on *Salvia officinalis* (Zervoudakis et al., 2010), *Afzelia xylocarpa* (Phonguodume et al., 2012), and *Lilium auratum* (Zhang et al. (2015).

The significant interactive effects of season and light, possibly mediated by varying day lengths, may have contributed to the varied growth responses observed in this study. Although the day length parameter was not recorded in this study, summer months in the Western Cape Province have the most extended day length (Table S1). It is worth mentioning that the period of exposure to light was probably longer during spring and summer compared with winter, irrespective of shading treatments.

Various studies reported that plants grown under low light intensity had increased chlorophyll content and reduced fresh weight (Khan et al., 2000; Caruso et al., 2004; Kubatsch et al., 2007). The results of this study is inconsistent with those of Perrin and Mitchell (2013) in terms of increased plant height and chlorophyll content under high light intensity. Under shade conditions, the distribution of photosynthates in plant tissues favors elongation of shoots and leaf area to enhance light capture capability (Khan et al., 2000; Caruso et al., 2004; Kubatsch et al., 2007). Thakur et al. (2009) suggested that these effects might be a result of etiolation due to shading. The response of plants grown in different light intensities may vary depending on plant type, species, and season (Phonguodume et al., 2012; Bayat et al., 2018). In addition, the leaves of plants exposed to low light tend to be thinner than plants subjected to high light intensity (Wu et al., 2017). Enhanced shading decreases leaf thickness and leaf dry matter content (Yang et al., 2019). Low light conditions deter plant development and productivity by affecting the gas exchange (Zavala and Ravetta, 2001; Fan et al., 2013). The reduction in light intensity affects carbon balance in plants due to the increase of carbohydrate demand while decreasing its production (Feng et al., 2019).

The chlorophyll content is an imperative factor in determining photosynthetic rate and production of dry matter (Li et al., 2018; Feng et al., 2019). In low light conditions, the chlorophyll content increases due to reduce photo-oxidatio (Okunlola and Adelusi, 2014). Several studies have reported that chlorophyll contents increase with decreasing light intensity (Resurreccion et al., 2002; Rezai et al., 2018; Setiawati et al., 2018). Also Zhang et al. (2015), found that shade treatments 80% and 75% increased Chl a content when compared with 0% shade in Oriental lily (*Lilium auratum* L.) cv. Sorbonne. In this study, although no significant effect on Chl a, Chl b, Chl a + b contents of *A. porrum* occurred, we observed that plants exposed to low light intensity showed a slight increase amount of chlorophyll a, chlorophyll b, chlorophyll a+b.

When the association between light intensity, tissue nutrient contents were examined, it was observed that shading elicited a significantly positive response in N, P, and Ca accumulation in the plant tissue. N, P, Ca are essential nutrients required by plants for plant growth, development, productivity, and metabolism (Razaq et al., 2017; Malhotra et al., 2018; Nchu et al., 2018). According to Zhou et al. (2019), an increase in nitrogen correlates with the increase in chlorophyll content and electron capacity. However, it is crucial to establish whether N, P, and Ca are linked to secondary metabolites, structural compounds, or unused inorganic ions. Therefore, based on the results obtained in this study, it is reasonable to argue that the interaction of shade and season influenced nutrient, potassium, and calcium uptake, which influenced plants' growth. This study further demonstrated that seasonal light effect is a significant factor for the response of plant growth parameters.

2.5 Conclusion

In conclusion, the growth *of A. porrum* in height was favoured by decreased light intensity. The total plant biomass and the number of leaves produced decreased with reduced light intensity. The level of the studied tissue nutrient contents (N, P, and Ca) was higher in plants exposed to low light intensity. The present study contributes to the literature on plant growth, physiological responses, and tissue nutrient contents of *Allium porrum* subjected to varying light intensities in different seasons. Our results will serve as a fundamental basis for the standardized cultivation of *A. porrum* for medicinal or nutraceutical purposes. These results may open possible market opportunities to improve economy and profits for farmers and avoid the use of synthetic pesticides.

2.6 Recommendation

Based on the findings of this study, it is recommended that there is a need to evaluate the daylength, photosynthetic rate, and growing substrate moisture content in order to better understand the effect of different light intensities in plant growth, development, and secondary metabolite yield and quality.

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Supplementary Table

Table S1. Estimated day lengths in the city of Cape Town from January – December 2020.

Months	Average Daylight
January	14
February	13
March	12
April	11
May	10
June	10
July	10
August	10
September	11
October	13
November	13
December	14

Source:Timeanddate.com(https://www.timeanddate.com/sun/south-africa/cape-town?month=4&year=2019)

CHAPTER THREE

The effect of light intensities on the volatile constituents, antifungal and anti-insect activities of *Allium porrum* extracts cultivated under greenhouse conditions.

Abstract

Enhancing secondary metabolism in plants by manipulating the abiotic such as light intensity during cultivation of medicinal plants could improve the quality and yield of bioactive materials, with anti-pest properties derived from medicinal plants. *Allium porrum* is a commonly cultivated vegetable in Africa and is amongst *Allium* genus plants that are widely studied for their insecticidal, fungicidal, and pharmaceutical properties. Plant pest spread is the foremost factor that hinders optimum crop production, and two of the major pests are grapevine mealybugs, *Planococcus ficus* and Fusarium wilt, *Fusarium oxysporum*. Therefore, this study aimed to ascertain the effect of light intensities on the volatile constituents, antifungal and anti-insect activities of *Allium porrum* extracts cultivated under greenhouse conditions. Seedlings of *A. porrum* were hydroponically grown under low light (40% shade) and high light intensity (0% shade) for 12 weeks. The phytochemical constituents were analysed from dry aerial parts of *A. porrum*. The antifungal activities against *Fusarium oxysporum* and the anti-insect activities on the grapevine mealybug (*P. ficus*) were evaluated in a Minimum Inhibitory Concentration (MIC) and repellency bioassays, respectively. Remarkably, the total polyphenol content was statistically higher ($DF = 1, 6$; $F = 9.17$; $P < 0.05$) in plants exposed to low light intensity compared high light; however, the plants used in this study were equally rich in alkaloids and flavonol. Following the gas chromatography mass spectrometry (GC-MS) analysis, the number of known antifungal and anti-insect volatile compounds plant constituents did not vary significantly; however, higher number of compounds occurred in plants subjected to low light intensity ($DF=1$; $\chi^2 =0.44$; $P > 0.05$). No clear trend in relative area ratios for the individual volatiles between low light intensity and high light intensity was detected. The acetone extracts *A. porrum* subjected to lower irradiance showed better fungistatic activities against *F. oxysporum*. In the repellency bioassay, no significant effects were found among extracts of plants from low light and high light intensity at all concentrations for all three tested solvents. The insect repellency tended to be higher at increased concentrations for all extracts. The key finding of this study is the positive influence of low light intensity effects on volatile constituents and fungistatic activities.

Keywords: Secondary metabolites; Antifungal; *Allium porrum*; *Planococcus ficus*; Repellency

3.1 Introduction

Historically, medicinal plants have established their importance as a source of novel molecules (Atanasov et al., 2005). Secondary metabolites include carotenoids, terpenes, alkaloids, phenolic and sulphur compounds (Ramírez-Gómez et al., 2019). Since the 1850s, pharmaceutical organic chemists gained interest in the novel phytochemicals and have investigated their chemical properties extensively (Bourgaud et al., 2001; Yang et al., 2018). Due to their significant biological activities, they are used as medicinal constituents, flavouring agents, and insecticides (Bourgaud et al., 2001; Gandhi et al., 2015; Yang et al., 2018).

Plants are able to biosynthesize different secondary metabolites (Pagare et al., 2015; Wink, 2018). However, the influence of various environmental factors, such as temperature, humidity, light intensity, the supply of water, minerals, and CO_2, in the synthesis of secondary metabolites have been demonstrated (Akula and Ravishankar, 2011; Mohiuddin, 2019). Also, the type and concentrations of secondary molecules synthesized by a plant are determined by the plant physiology, species, genotype, and growth stage of the plant (Isa 2004). Many approaches have been established over the past years for biomass increase of the production of compounds of interest (Bourgaud et al., 2001; Murthy et al., 2014). These strategies include manipulating the cultivation methods, such as amendment of growing substrates with entomopathogenic fungi, light, water stress, and use of different nutrient mixes**.** Cultivation of plants under controlled environments using hydroponic systems is an attractive strategy to enhance secondary metabolism (Gontier et al., 2002; Dayani and Sabzalian, 2016). In several investigations, light significantly altered the metabolite concentrations (Ma et al., 2010; Akula and Ravishankar, 2011). The shade has an inducing effect on the biochemical changes in plant leaves (Gottschalk, 1994). In a study by Hou et al. (2010), low light intensity significantly increased the accumulation of glycyrrhizic acid and liquirting in the roots of *Glycyrrhiza uralensis*. Under reduced light intensity, methaylxanthine content was increased in *Ilex paraguariensis* leaves (Coelho et al., 2007).

Secondary metabolites play a vital ecological role within the defence, protection, and signalling mechanisms of plants (Griesser et al., 2015). Plant secondary metabolites are exploited for control of common plant pests. Extracts rich in bioactive metabolites or plant-based pesticides have been successfully used to control plant pathogens and insect pests under field greenhouse and field conditions (Koul and Walia, 2009; Khater, 2012). Many plant-based pest control 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, which include plant-based agents, for they are believed to environmentally friendly.

Plant pest spread is the foremost factor that hinders optimum crop production, and two of the major pests are grapevine mealybugs, *Planococcus ficus* and Fusarium wilt, *Fusarium oxysporum*. Both are widespread, and are pests of economic importance, 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, 19998, Walton et al., 2004; Franco et al., 2009, Summerell et al., 2010). *Allium porrum* is among Allium species used in folk medicine for their antimicrobial, antifungal, pharmaceutical purpose (Tamokou et al., 2017). *Allium* species extracts contain a number of bioactive agents, including phenolic compounds, organosulphur compounds, non-structural and soluble carbohydrates, organic acids and various amino acids (Slimestad et al., 2007). Although this is a commonly cultivated plant, the anti-insect and antifungal activities against plant pests have not been studied. Hence, this study aimed to ascertain the effect of light intensities on the volatile constituents, antifungal and anti-insect activities of *Allium porrum* extracts cultivated under greenhouse conditions.

3.2 Materials and Methods

3.2.1 Plant Material

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

3.2.2 Greenhouse experimental design

The third trial (Autumn) was used for this part of the study. Experimental plants were obtained from plants grown under two levels of irradiance, i.e., high light intensity (0% shade) and low light intensity (40% shade) in the greenhouse. Low light intensity (40% shade) was obtained by covering metal table (230x90x85 cm) with 40% green polyethylene shade net obtained from Stodels Pty Ltd, Garden Centre, Cape Town. High light intensity (0% shade) was obtained by subjecting plants to sunlight entering through the greenhouse's polycarbonate roof cover. Light irradiance was measured at plant height using digital lux meter (0.1- 400, 00 LUX) MT942 Major Tech. Light intensity was converted from Lux to PPFD (µmol m-2 s-1) using a standard

conversion factor of 0.0185 for sunlight light source (Apogeeinstruments, 2020). Conversion formula: lux × conversion factor (0.0185) = PPFD (µmol m-2 s-1). The average 0% and 40% shade light intensities (Photosynthetic Photon Flux Density [PPFD]) measured at noon were 313 and 153 µmol m-2 s -1, respectively. The experiment was conducted in a greenhouse with controlled internal environmental conditions. The temperatures ranged from 19 °C to 27 °C, and the average relative humidity ranged from 29 to 67%. One hundred 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®, bought from Starke Ayres Pty Ltd, Cape Town. The fertilizer contained ingredients as follow: 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 deionized water once every three days. This experiment was conducted in summer at Glasshouse Nursery (Horticulture Research 1), Cape Peninsula University of Technology, Bellville Campus.

3.2.3 Phytochemical screening

3.2.3.1 Total alkaloids assay

This assay followed a method as reported Fadhil and Reza (2007). Grinded plant material of *A. porrum* were extracted with 10 ml of 60% ethanol for 2 hours. The mixture was centrifuged (4000×g for 10 min) and the supernatant was used in the essay. Standard atropine solutions with 2 ml of the extract supernatant were mixed with 5 ml sodium phosphate buffer and 12 ml of bromocresol green solution. Thereafter, the mixture was added with 12 ml of chloroform and shaken vigorously. The test and standard solution absorbance were ascertained against the reagent blank at 417 nm with a UV/Visible spectrophotometer. The expression of total alkaloid content was mg of AE/g of extract.

3.2.3.2 Total flavonoids assay

The total flavonoid content was evaluated using quercetin following methods described by Daniels et al. (2011), adopted as a standard for 0, 5, 10, 20, 40, and 80 mg/L in 95% ethanol (Sigma-Aldrich, South Africa). Crude extracts solution (12.5 µl) was prepared by mixing 12.5 µl of 0.1% Hydrochloric acid (HCl) (Merck, South Africa) in 95% ethanol in the sample wells and followed by 30 minutes incubation at a room temperature. The total flavonoid concentration in ethanol extracts were expressed as mg quercetin equivalent per g dry weight (mg QE/gDW).

3.2.3.3 Total phenolic assay

The total phenolic content of dry material of leeks was assesssed with Folin- Ciocalteau assay, following methods by Singleton et al. (1999). Using a 96-well microplate, 25µl of the sample 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. Total phenolic content of dry leaf material of leeks was expressed as milligrams of gallic acid equivalents (GAE) per 100 grammes dry mass (mg GAE/100 g dw). All samples were analysed in duplicates.

3.2.4 Headspace GC-MS analysis

3.2.4.1 Sample Preparation

The volatile compound profiles of 12 weeks old *A. porrum* (Leek) potted samples were analyzed from 8 potted plants, i.e., four from each treatment; low light (40% shade) and high light (0% shade). Plant aerial parts were cut into small portions and freeze-dried at -80℃ overnight. Liquid nitrogen was used for crushing plant material, and 1 g was weighed into solid phase micro extraction (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 fiber (gray).

3.2.4.2 Chromatographic separation

The volatile compounds separation was carried out using a gas chromatograph (6890N, Agilent Technologies Network) joined to an inert XL EI/CI Mass Selective Detector (model 5975B, Agilent Technologies Inc., Palo Alto, CA). The GC-MS system was joined to a CTC Analytics PAL auto sampler. The present volatiles separation in the samples was done on a polar ZB-WAX (30 m, 0.25 mm ID, 0.25 µm film thickness) Zebron 7HGG007-11 capillary column. Helium gas was used as the carrier at a constant flow rate of 1 ml/min. The injector temperature was set at 250 °C, and with the split ratio set at 5:1. The Mass Selective Detector and mass spectrometer were operated under full scan mode and electron impact mode at ionization energy of 70 eV, scanning from 35 to 500 m/z, respectively. Retention time and mass spectrum with 90% matching with internal standards and reference library enabled the identification of the volatile compounds.

3.2.5 Preparation of material extracts: Antifungal Bioassay

Fresh plant material of *A. porrum* aerial parts subjected to varied treatments were crushed with a porcelain pestle and mortar for five minutes. Acetone is a regularly used solvent for extraction due to less toxicity, 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 hours, followed by filtration with Whatman No. 1 filter paper. Filtrates were placed under a fume hood for 8 hours to allow complete evaporation of acetone. The obtained residues were weighed to obtain yield and reconstituted in acetone to a 6 mg/ml concentration.

3.2.6 Antifungal bioassay: Minimum inhibitory concentration

The microdilution method was followed a described by Nchu et al. (2010) with slight modification in to determine the minimum inhibitory concentration (MIC) of extracts plant extracts. *A. porrum* extracts were mixed with acetone to obtain a preliminary concentration of 6 mg/ml, which was serially diluted subsequently. In each successive serial dilution, the starting concentration was diluted in two-fold. Fungal culture, *Fusarium oxysporum* f. sp. glycines strain (UPFC no. 21) was obtained from cultures maintained in the Horticulture Research Laboratory of Cape Peninsula University of Technology, Bellville campus. *F. oxysporum* was sub-cultured from stock agar plates and grown into Nutrient Broth (Merck, South Africa) for four hours. The fungal culture (100 µl) was added to each well of the 96-well microplates (10^5 cells/ ml). Searls Mancozeb fungicide ® (Stodels Pty Ltd, Bellville) was prepared and served as a positive control, and acetone served as a negative control. Forty microliter (40 µl) 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 ºC at 100% RH. The antifungal bioassay (MIC) comprised of three replicates per treatment. The minimum inhibitory concentration values were recorded after 6, 12 and 18 h.

3.2.7 Preparation of material extracts: Insect bioassay

Fresh plant material of *A. porrum* aerial parts subjected to varied treatments were crushed with a porcelain pestle and mortar for five minutes. The extraction method was done with three varying solvents; ethanol, acetone, and dichloromethane. The extraction process lasted for five hours, and then filtration, using Whatman no. 1 filter paper, into a centrifuge tube. Six grams of the crushed samples were mixed separately with acetone, ethanol and dichloromethane to

obtain a concentration of 20 w/v%. Two-fold dilution was carried out to obtain 10 w/v%, 5 w/v%, and 2.5 w/v% concentrations for all the solvents.

3.2.8 Insect culture

Grapevine mealybug *(Planococcus ficus*) were obtained from Agricultural Research Council courtesy of Dr. Achiano Kwaku. Insects were reared on butternut squash in the darkroom at 25 ºC and 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.

3.2.9 Repellency bioassay

The repellency bioassay followed methods described by Koschier et al. (2000) but modified. 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, 10, 5, 2.5 w/v %), including a commercial synthetic insecticide, Kemprin (Cypermethrin pyrethroid 200 g/l) as the positive control. One hundred microlitres of each of ethanol, acetone, and dichloromethane extracts of leeks were applied 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 (one hundred microlitres) was applied on the same size and type of the filter paper; clean filter papers were treated as a control. The ends of the Y tube were covered with test filter paper and control filter papers. By means of membrane pump, at the base of the Y tube the air is sucked off, producing an airflow of 10 cm/sec in the Y tubes and the base tube. Ten adult female grapevine mealybugs were released within the first centimetre of the base tube of Y olfactometer using a camel-hair brush. 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, they had to choose between the control airflow and airflow loaded with odour of the extracts. Once the mealybugs reached the far end of one arm, the choice was recorded. If all ten mealybugs did not respond after 10 minutes, no score was recorded, and the experiment was repeated. Experiments were replicated five times, each completed after 10 minutes. The mean percentage repellency was calculated using the following formula: Repellence (%) = C-T/C×100, where C= number of insects in the control arm, T= number of insects in the extract-treated arm. After each replication, all parts of the set-up were cleaned with the solvent.

3.2.10 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 or graphs. The data on polyphenol, alkaloid, flavonol, and MIC were analysed using one-way analysis of variance (ANOVA) and 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 between the shade treated and unshaded plant extracts for the different solvents and extracts concentrations, and it was followed by the post-hoc Mann-Whitney test to separate the means at a level of significance $P< 0.05$. These computations were performed using PAST version 3.21 (Hammer et al. 2001).

3.3 Results

3.3.1 Quantification of plant secondary metabolites

The results of the phytochemical constituents showed that the polyphenols content was significantly higher ($DF = 1, 6$; $F = 9.17$; $P < 0.05$) in plants under low light intensity than those subjected to high light intensity. There were no significant differences between treatments in alkaloids ($DF=1, 6$; $F=2.73$; $P > 0.05$) and flavonol ($DF = 1, 6$; $F = 1.31$; $P > 0.05$) contents; however, higher yields of these constituents were observed in plants subjected to low light intensity (Table 3.a).

Table 3.a Content of polyphenols (mg GAE/g DW), Flavonols (mg QE/g DW), alkaloids (mg AE/ DW) in aerial part samples of leeks cultivated under different light intensities in the greenhouse conditions.

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

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

3.3.2 GC-MS Analysis

GC-MS analysis was carried out on extracts of shoots of *A. porrum*. Compounds with more than 90% matching percentage with GC-MS library data were selected and presented in Table 3.b. A wide range of volatile compounds was detected. The compounds included popular antifungal and anti-insect constituents such as Beta ionone, Dimethyl trisulfide, Ethyl palmitate, Methyl palmitate, and 1,3 Dithiane. The number of plant constituents did not vary significantly, however, a higher number of compounds occurred in plants subjected to low light intensity (DF=1; χ^2 =0.44; P > 0.05). No clear trend in relative area ratios for the individual volatiles between low light intensity and high light intensity was detected (Table 3.b).

Table 3.b: Volatile compounds with a match quality of at least 90% present in shade (40% Shade) and control treatment (0% Shade) of aerial parts of *A. porrum*.

	Low light intensity (40% Shade)	**Highlight intensity (0% Shade)**
1	Dodecane	Dodecane
2	Ethyl_hexanoate	Ethyl_hexanoate
3	2,4-dimethyl_thiophene	2,4-dimethyl_thiophene
4	Methyl_propyl_disulfide	Methyl_propyl_disulfide
5	Dimethyl-disulfide	Dimethyl-disulfide
6	1,3- Dithiane	1,3- Dithiane
7	Dimethyl_trisulfide	Dimethyl_trisulfide
8	Hexanal	Hexanal
9	trans-Propenyl_propyl_disulfide	trans-Propenyl_propyl_disulfide
10	Ethyl_octanoate	Ethyl_octanoate
11	Ethyl_palmitate	Ethyl_palmitate
12	3,5,5-trimethyl-2-cyclohexen-1-one	3,5,5-trimethyl-2-cyclohexen-1-one
13	Ethyl_pentadecanoate	Ethyl_pentadecanoate

14	Methyl_laurate	Methyl_laurate
15	Ethyl_laurate	Ethyl_laurate
16	Phenethyl_alcohol	Phenethyl_alcohol
17	beta-Ionone	beta-Ionone
18	Ethyl_myristate	Ethyl_myristate
19	Methyl_palmitate	Methyl_palmitate
20	Methyl_myristate*	
21	Methyl_nonanoate*	
22	Ethyl_pelargonate*	

*Denotes compounds that are only present in only detected in at least control or shade treated plants.

Table 3.c: Commonly known insecticidal and antifungal volatiles that were detected in *Allium porrum* and their relative area ratios were selected following gas chromatography-linked mass spectrometry analysis of control and shade (40%) treated plants.

Compound	Activity	Reference	Area ratio	
			Low light intensity (Shade 40%)	Highlight intensity (Control)
Dimethyl-disulfide	Antifungal	Gerik (2005), Wang et al. (2009); Yan et al. (2019)	0.02±0.01a	0.22±0.09a
Methyl-propyl-disulfide	Antifungal	Pyun & Shin (2006); Prithiviraj et al. (2004)	0.12±0.02b	0.63±0.13a
1,3- Dithiane	Insecticide, Antifungal	Giannini et al. (2004); Sanei-Dehkordi et al. (2019)	0.12±0.01a	0.36±0.10a
Hexanal	Antifungal, Insecticidal	Mohan et al. (2017); Kashima et al. (2011)	0.10±0.01b	0.28±0.03a
Methyl-nonanoate	Antifungal	James-Meyer & Coles (2015)	0.01±0.01	—
Beta-Ionone	Antifungal, Repellent	Weissling et al. (1989); Kunz (1990); Sas & Adams (1999)	0.12±0.02b	0.22±0.02a
Methyl-myristate	Antifungal	Muhammad et al. (2016)	0.01±0.00	—
Methyl-palmitate	Antifungal, Insecticidal	Pinto et al. (2017), Muhammad et al. (2016)	0.02±0.00a	0.02±0.00a

Ethyl-palmitate	Antifungal, Insecticidal	Mamarozikov et al. (2019), Choi et al. (2010)	0.03±0.01a	0.04±0.01a
Dimethyl-trisulfide	Antifungal, Insecticidal	Kocić-Tanackov et al. (2012); Liu et al (2014); Tang et al. (2019)	0.08±0.01a	1.10±0.55a
No. of compounds			10	8

Mean values followed by the same letter in the row do not show significance at P > 0.05 following comparison using the Tukey test. Comparison between the number of compounds present (DF=1; χ^2=0.44; P>0.05) following Past Kruskal-Wallis test.

3.3.3 Minimum inhibitory concentration

There was a significant difference (P < 0.05) in the MIC values between the different light intensities. The inhibition of *F. oxysporum* was significantly stronger in acetone extracts of aerial parts of *A. porrum* subjected to lower irradiance 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.d). Generally, the acetone extracts of *A. porrum* exposed to both low light and high light intensity had the lowest MIC values when compared to the tested positive control (Mancozeb) (DF = 3, 28; P < 0.05).

Table 3.d: Minimum inhibitory concentration (Mean ± SE) on *Fusarium oxysporum* by acetone extracts obtained from aerial parts of *Allium porrum* grown under low light or high light conditions 12 weeks post-treatment.

Treatments	MIC (mg ml^{-1}) at 6 hr	MIC (mg ml^{-1}) at 12hr	MIC (mg ml^{-1}) at 18 hr
Low light intensity (40% shade)	0.8±0.1c	0.8±0.1c	1.9±0.2c
High light intensity (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	6.0±0.0a	6.0±0.0a	6.0±0.0a

*Means with the same lowercase in the same column are not significantly different (P> 0.05) following the Tukey test. Low light intensity (40% shade); High light intensity (0% shade).

3.3.4 Repellence bioassay

Besides ethanol and acetone extracts of plants treated to high light intensity, the Y-olfactometer assay showed that the insect repellency induced by the different concentrations (20 w/v %, 10 w/v %, 5 w/v %, 2.5 w/v %) varied significantly depending on the concentration of the extracts used (Table 3.e). Moreover, the different solvents of extract of plants treated under low and high light intensity did not have an influence in the repellecy percentage, solvents showed similar response in both treatments at all concentrations ($DF=6$ $P > 0.05$ and $DF= 12$; $P > 0.05$). The insect repellency tended to be higher at higher concentrations irrespective of the solvent and light intensity.

Table 3.e: Repellent effects of aerial part extracts of *A. porrum* against grapevine mealybug (*P. ficus*).

Treatments	Mean percentage repellency ±SE					
	20(w/v%)	10(w/v%)	5(w/v%)	2.5(w/v%)	χ^2 (DF=3)	P_{value}
Dichloromethane (40% shade)	93.2±6.8	86±5.79	68.33±4.85	46.53±6.13	12.57	0.00
Dichloromethane (0% shade)	86±9.79	78.33±6.23	63.33±5.65	43.33±4.08	10.84	0.01
Ethanol (40% shade)	78.33±6.24	78.33±12.24	75.33±2.44	44.86±7.26	9.02	0.02
Ethanol (0% shade)	81.66±7.63	83.33±6.97	66.66±11.78	58.33±7.45	4.72	0.17
Acetone (40% shade)	95±5	84.33±6.74	66±6.59	53.33±8.57	10.52	0.01
Acetone (0% shade)	82.66±4.61	61.66±11.66	64.99±8.08	59.33±8.05	5.53	0.12
Kemprin (positive control)	96±4	96.66±3.33	96±5	88.33±7.26	5.53	0.12
Low light intensity χ^2(DF=6)	3.76	2.76	8.44	10.29		
P_{value}	0.15	0.36	0.02	0.01		

High light intensity χ^2(DF=6)	3.02	6.45	7.48	9.95	
P_{value}	0.32	0.07	0.04	0.01	

The repellency data is represented as mean±se. 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 extracts concentrations, and it was followed by the post-hoc Mann-Whitney test to separate the means at a level of significance $P< 0.05$. Chisquare and Pvalue statistics on the right represent comparison among different concentrations in each row running from left to right. Chisquare and Pvalue statistics on the bottom represents comparison between treatments in different extraction solvents running from top to bottom in the same column for each light intensity.

3.4 Discussion and Conclusion

In this study, phytochemical screening revealed the presence of flavonoids, alkaloids, polyphenols in *A. porrum* plants. Also, subjecting *A. porrum* to low light intensity was significantly associated with higher number of bioactive compounds including polyphenols in extracts of aerial parts. Up to 22 compounds were detected in *A. porrum* extracts of plants grown under different light intensities, with compounds such as methyl nonanoate, methyl myristate, ethyl pelargonate present exclusively in plants subjected to low light intensity. Cultivation of plants under low light conditions induces biochemical changes in plant leaves (Gottschalk, 1994). An increase of secondary metabolites under low light intensity is associated with the decrease in the biomass accumulation (Coelho et al., 2007), this could possibly be caused by the trade-off between growth and defense under low light irradiance (Hou et al., 2010). The production of secondary metabolites is considered an adaptive capacity of plants to withstand unfavourable environmental changes that involves synthesis of complex chemical types and interactions in the structural and functional stabilization through signalling processes and pathways (Edreva et al., 2008; Isah, 2019).

Following the gas chromatography mass spectrometry (GC-MS) analysis, the number of known antifungal and anti-insect volatile compounds plant constituents did not vary significantly, however, a higher number of compounds occurred in plants subjected to low light intensity (DF=1; χ^2 =0.44; P > 0.05). Like other *Allium* species, leek contains many bioactive agents (Slimestad et al. 2007; Radovanović et al., 2015). According to previous studies, the identified compounds by GC-MS analysis are important compounds known to exhibit broad-sprectrum bioactivity against plant pests (Table 3.c). For example, dimethyl disulfide (DMDS) is common in the sulfur cycle and known for high toxicity against plant pests (Dugravot et al., 2002; Ajwa et al., 2010). Further, DMDS is used as an alternative to methyl bromide (CH3Br) to control plant fungal pathogens (Wang et al., 2011). The important compounds detected also include beta-ionone known to possess antifungal, repellent activities (Weissling et al., 1989, Kunz, 1990; Sas and Adams, 1999). Also, ethyl-palmitate, 1,3- Dithiane, and Dimethyl-trisulfide have possess 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).

The minimum inhibitory concentration bioassay results showed that the acetone extracts of the aerial part of *A. porrum* subjected under low light intensity had the most growth inhibitory effect against *F. oxysporum* when compared with those exposed to high light intensity. Overall,

acetone extracts of *A. porrum* were found to be bioactive against *F. oxysporum* in both low light intensity and high light intensity. Also, in the study by Radovanović et al. (2015), it was reported that the ethanolic extracts of the *Allium porrum* showed a positive antimicrobial activity on *Bacillus subtilis, Staphylococcus aureus and Aspergillus niger.* Recently, Ncise et al. (2020) reported that extracts of *Tulbaghia violacea* exposed under a decreased light intensity had antifungal activity against *F. oxysporum*. Interestingly, the phytochemical analysis results correlate with the inhibitory effect exhibited against *F. oxysporum* in this study. The presence of the bioactive compounds could influence the antifungal activities of aerial part extracts of *A. porrum*. The findings in the study by Zuo et al. (2015), where a plant of the same species as tested in this study showed similar compounds and were reported to have a significant inhibitory effect against *Fusarium oxysporum* f. sp. cubense tropical race 4. Their findings corroborates with the findings of this study.

For the repellent activity no significant effects were found among extracts of plants subjected to varying light intensities tested in all varying concentrations in all three tested solvents. However, the insect repellency tended to increase with increased concentrations irrespective of the solvent. Broadly, these results suggest that the extracts may contain compounds that deter rather than repel the insect and *A. porrum* extracts can repel grapevine mealybug. The active compounds could be non-volatile in nature. Some of the identified compounds were reported to have repellent activities, for example, dimethyl trisulfide against the mite *Tetranychus urticae* (Hincapié et al. 2008), methyl nonanoate against European corn borer *Ostrinia nubilalis* (Sole et al. 2012), methyl palmitate and methyl myristate against *M. domestica* (Henderson et al. 1991). Also, methyl palmitate had antifeedant properties against *M. persicae* and *Diuraphis noxia* (Santana et al. 2012). Mobki et al. (2014) reported that the garlic extracts which is in the same genus as leeks, strongly repelled *T. castaneum*. Likewise, in Nchu et al. (2016), dichloromethane extract of garlic was demonstrated to be repellent against *H. rufipes*.

Generally, cultivation of *A. porrum* under lower light intensity influenced the biosynthesis of secondary metabolites and antifungal activities of *A. porrum*. Although we demonstrated that *A. porrum* extract can repelled the grapevine mealybug, no clear trend observed in the grapevine mealybug repellence of the aerial part extracts of *A. porrum* in terms of low light intensity effects. The findings of the current study contribute to the literature on the effect of low light intensity for the biosynthesis of the compounds of medical and economic importance. Also, this study opens up possible market opportunities to improve cultivation practices and the profits for farmers.

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CHAPTER FOUR

4.1 General Discussion, Conclusion and Recommendations

4.1.1 General Discussion

The demand for the biologically active natural products and ecologically friendly pest management has increased due to the negative impacts caused by synthetic pesticides. Biological pesticides offer an effective alternative solution in pest control against chemical based pesticides. This study searched for a sustainable solution to pest management of fungal pathogen *F. oxysporum* and *P. ficus*. Biotechnology cultivation systems make it practical to manipulate medicinally important compounds present in plants and obtain high yields (Canter, 2005). Growing medicinal plants under low light intensity have been reported to be effective and that may contribute to the reduction in the use of chemical products in pest control.

The interaction between light and different growing seasons significantly affected the growth response in parameters such as plant height, number of leves, fresh root and shoot weight, shoot dry weight. Light effects on growth parameters were significantly influenced by seasonal changes. In comparison of varying light intensities between growth parameters, reduced light intensity positively influenced plant height in *A. porrum*. However, number of leaves, fresh aerial part and root weight, and dry aerial part and roots reduced significantly under low light intensities. The elongation of internodes could cause an increased plant height in order to increase the capability of light capture during photosynthesis. Moreover, there were significant different differences in tissue nutrient contents of nitrogen, phosphorus, and calcium. These nutrients are essential for plant productivity. Light is a significant factor that can influence nutrient uptake in plants by means of photosynthesis process. Also, water availability stimulates nutrient uptake; however, moisture content in the growing substrate was not evaluated in this study.

Generally, acetone extracts of *A. porrum* grown under different light intensities showed antifungal activity against *F. oxysporum*. Moreover, plants subjected to low to light intensity showed significantly stronger inhibition compared to acetone extracts of plants exposed to high light intensity. These results may be correlated with higher contents of polyphenols found in extracts of *Allium porrum* in this study. Polyphenols are known to possess inhibitory activities against fungi and other microbial pathogens.

Moreover, repellent activity of different solvents and concentrations of aerial part extracts of *A. porrum* grown under different light intensities were assessed against *P. ficus* (grapevine

mealybug). Generally, the results demonstrated that varying light intensities did not influence the repellent activities of extracts *A. porrum* on all tested solvents at the different concentrations. The insect repellency increased with at higher concentrations irrespective of different solvents. Volatiles such as Dimethyl-disulfide, Methyl-propyl-disulfide, 1,3- Dithiane, Hexanal, Methyl-nonanoate, Beta-Ionone, Methyl-myristate, Methyl-palmitate, Ethyl-palmitate, Dimethyl-trisulfide were identified in *A. porrum* extracts using GC-MS analysis, a higher number of compounds was identified in plants subjected to reduced light intensity. The identified compounds are known to have antifungal and anti-insect activities. These results suggest that the compounds observed in this study might have influenced the repellency and antifungal activities of *A. porrum* extracts.

4.1.2 Conclusion

In conclusion, manipulating light intensity during cultivation of plants may be an exciting approach for optimizing the biological constituent, anti-insects and antifungal activities of *A. porrum*. This study's key findings are: low light intensity has positive effects on volatile constituents, total polyphenol content, fungistatic activities of extracts of *A. porrum*. Studies on *A. porrum* against plant pests are rare; this is the first study to report repellent activities of leeks against grapevine mealybug. These findings will contribute to the existing literature on the correlation of light intensity and synthesis of plant volatiles. These results may open possible market opportunities to improve economy and profits for farmers and avoid the use of synthetic pesticides.

4.1.3 Recommendations

According to the outcomes of this study, it is necessary to evaluate the daylength, photosynthetic rate, and growing substrate moisture content in order to better understand the effect of different light intensities in plant growth, development, and secondary metabolite yield and quality.