The influence of photoselective shade netting on vegetative growth and bioactivity of *Myrsine africana* L. for cosmeceutical production

1. List of abbreviations

MA (*Myrsine africana* L.)
PPE (Porcine pancreatic elastase)
PAR (Photosynthetic active radiation)
R:FR (Red:Far-red)
B:R (Blue:Red)

2. Introduction

The increasing interest in anthropogenically useful plant secondary metabolites necessitates efficient regeneration and commercialisation of desired species (Van Wyk, 2008; 2015). Moreover, biodiversity conservation can be achieved by ensuring sustainable utilisation, regeneration (propagation) and domestication of desired or threatened plant species. This effectively reduces the pressure on this valuable genetic and biochemical resource (Phondani et al., 2016).

Myrsine africana L., the African boxwood, belonging to the Myrsinaceae family, is used in traditional medicine and possesses commercially significant cosmetic properties. *M. africana* is traditionally used for blood purification, justified by the significant anthelmintic and antimicrobial activity (Ahmad et al., 2011a; Ahmad et al., 2011b; Chappuis et al., 2007; Muthee, 2018). Significant cosmetic properties of shoot, leaf and twig extracts include anti-tyrosinase, anti-melanogenesis and elastase inhibition activity, owing to its skin toning, lightening and anti-wrinkle activity (Lall et al., 2017; Momtaz et al., 2008; Stapelberg et al., 2019).

M. africana is a slow growing, evergreen, dioecious, perennial shrub with multiple stems and dark leathery leaves. Lall et al. (2017) elucidated that purification of myrsinoside B, the compound responsible for anti-wrinkle activity of *M. africana* shoot material, is needed to achieve acceptable activity for commercial application due to the slightly insufficient activity of the crude shoot extract. However, since purification is costly, crude extracts are often utilized in formulations. This necessitates large scale production of large quantities of biomass with high bioactivity to meet industry requirements. Therefore, in order to successfully commercialize this cosmeceutical species with significant commercial

potential, the aim of this study was to enhance growth, bioactivity and primary and secondary metabolite content.

Light quantity and quality have been manipulated in horticultural research and industry for numerous decades to augment plant growth and development (Corré, 1983; Stuefer and Huber, 1998; Taiz and Zeiger, 2010). This is because light properties, such as quality (spectral composition), have a prominent influence on the primary metabolism of plants, influencing morphology, cell elongation and resource allocation (Bastías and Corelli-Grappadelli, 2012; Reed et al., 1993). In addition, light properties have a direct effect on secondary metabolite production, as was demonstrated by Ilić and Fallik (2017) and Sivakumar et al. (2017).

Photoselective shade netting is an effective technique for large scale manipulation of light spectral composition. Multiple studies have displayed the enhanced antioxidant and radical scavenging activity of plants cultivated under photoselective shade net (Ilić and Fallik, 2017; Selahle et al., 2014). Furthermore, the manipulation of light properties may be used to improve overall crop health and growth, facilitating desirable resource partitioning, while improving secondary metabolite production and reducing susceptibility to pests.

This study aimed to improve cultivation of *M. africana* for cosmeceutical production using photoselective shade netting. An attempt was made to investigate the influence of modified light properties on plant growth and bioactivity (anti-wrinkle properties) of *M. africana* through selected photoselective shade nets. A further objective was to identify light properties responsible for stimulating desired secondary metabolite production.

Conclusions were made on the influence of photoselective shade net on light properties and the subsequent effect on *M. africana* growth and biochemistry.

3. Materials and methods

2.1. Cultivation, growth and harvesting of samples

A total of 36 sexually mature *M. africana* shrubs, both male and female, were cultivated on the Hillcrest campus of the University of Pretoria, each in 5 L polyethylene plant bags. The plant population constituted 18 male clonal plants and 18 female clonal plants attained from Grassland nursery, Krugersdorp, South Africa. These plants, although young, were sexually mature as flowers were observed. Unfortunately, due to the limited availability of this species at the time, a comparison of male and female individuals could not be done.

The growth medium consisted of a mixture of sandy soil and compost (1:1 ratio). Six individual plants as replicates were grown under each photoselective shade net (Knittex, Randburg, SA) of separate colours, namely, green, black, white and blue at 50% density and red at 80% density. The control involved the cultivation of plants under full-sun exposure. Red photoselective shade net at 80% density was used, as 50% red was not commercially available in South Africa.

Shade net structures with dimensions of 2x2x2 m were constructed from timber and covered in respective shade net. All treatments were irrigated through drip irrigation (2 L/h drippers), receiving 8 L/day over four applications of an hour each. In addition, soluble fertilizers, Hygroponic (Reg. No. K5709) and Solu-Cal (Reg. No. K7583) from Hygrotech, were applied through fertigation at the recommended dosages (25 kg Hygroponic and 25 kg Solu-Cal per hectare per week in 50 000 L of water).

The trial was initiated in December 2017 and initial growth measurements recorded. Shoot material for primary and secondary metabolite analysis was harvested from each replicate in two seasons, namely autumn (April) and spring (November). Roughly 10 g of shoot material was taken from each individual plant for metabolite analysis, weighed and frozen in liquid nitrogen before freeze drying for four days and grinding it to a fine powder using a mechanical grinder.

Atmospheric conditions were measured under each treatment, including spectral composition, measured as relative intensity (µmol/m^2/s) of each wavelength (nm), using a spectral radiometer. In addition, Photosynthetic Active Radiation (PAR in µmol/m^2/s) level was measured using a ceptometer, and air temperature and relative humidity were measured using iButton dataloggers.

Growth parameters, namely plant height and canopy size, of each replicate were measured throughout the duration of the experiment. Due to the shrub growth form of this species, plant height was determined by measuring five of the longest shoots from the soil surface, recorded and averaged for each replicate. Canopy size was measured using a mobile application, named Canopeo which measures canopy cover as described by Patrignani and Ochsner (2015), displaying canopy size as a percentage of ground cover, based on the detection of chlorophyll.

2.2. Carbohydrate proportions – starch and sucrose / d-fructose / d-glucose

Sample preparation for each treatment replicate constituted extraction of 20 mg of dry, powdered plant material in 1 mL of ethanol within a polyethylene test tube in a water

bath at 80 °C for 10 minutes. This was followed by centrifuging at 4000 rpm for 10 minutes and collecting the supernatant in a clean test tube. This procedure was repeated at room temperature an additional two times to yield 3 mL of extract for soluble carbohydrate analysis, while the remaining solid pellet was reserved for the total starch quantification.

The extract was further clarified for carbohydrate quantification according to the sample clarification method described in the MEG/K-SUFRG assay handbook (Megazyme, 2020a). Clarification involved the addition of Carrez 1 (8.52 M of potassium hexacyanoferrate (II) ($K_4[Fe(CN)_6].3H_2O$) (Sigma cat. no. P9387)), followed by 0.5 mL of Carrez 2 (25.03 M of zinc sulphate ($ZnSO_4.7H_2O$) (Sigma cat. no. Z4750)) and 1 mL of sodium hydroxide (NaOH) [100 mM] to the extract and filtering it through Whatman No. 2 filter paper, after which the clear solution collected proceeded for carbohydrate analysis.

Carbohydrate analysis was conducted using sucrose, D-fructose and D-glucose assay kits (MEG/K-SUFRG) and Total Starch assay kits (MEG/K-TSTA) (Megazyme) and measured using a spectrophotometer at the appropriate wavelengths. Methodology was followed according to the procedures provided with each assay kit, namely, the AOAC Method 996.11 (Megazyme, 2020b), and K-SUFRG 04/17 method (Megazyme, 2020a), for starch and Sucrose / D-fructose / D-glucose, respectively.

Starch percentage and sucrose, D-fructose and D-glucose content was calculated using the Megazyme Mega-Calc^{TM} total starch and Sucrose / D-fructose / D-glucose calculation spreadsheets provided on the Megazyme website, inserting absorbency values attained during each protocol.

2.3. Bioactivity - elastase inhibition

Ethanolic extracts of shoot material from each treatment replicate were prepared and analysed for elastase inhibition (anti-wrinkle activity), according to the methodology described by Lall et al. (2017).

Elastase inhibition is determined by measuring the extract's ability to inhibit porcine pancreatic elastase (PPE) (Type IV from porcine pancreas – E0248, Sigma USA) according to the methodology of Bieth et al. (1989) and modified by Lall et al. (2017). The release of the colour compound, p-nitroaniline, from the substrate, N-succinyl-ala-ala-ala-p-nitroanilide (S4760, Sigma, USA), was measured spectrophotometrically at a wavelength of 405 nm (Lall et al., 2017).

Briefly, ethanol extracts were prepared by adding 30 ml of ethanol to two grams of the dry, powdered plant material from each sample in a glass container, sealed and placed on

a shaking table for 48 hours. The ethanol fraction was then collected after filtration through a Buchner funnel and the remaining solid was extracted again as above. The two extract fractions from each sample were then combined and concentrated by evaporation using a rotary evaporator to yield the ethanolic extract.

The assay was performed in a 96 well Elisa plate which constituted reaction mixtures containing sample extracts that were serially diluted to yield a concentration range from 250 µg/mL to 7.8 µg/mL. To each well 100 mM Tris buffer (pH 8.0) and 30 µl PPE (5 mM) was added and then incubated at 37 °C for 15 minutes. The reaction was then initiated by addition of 20 µl N-succinyl-ala-ala-ala-p-nitroanilide (4 mM) to each well. The rate of the reaction was obtained by measuring the change in absorbance of the reaction mixture for 15 minutes at 37 °C using an Elisa plate reader at a wavelength of 405 nm.

The release of 1 µM of p-nitroaniline/min is equivalent to one unit of elastastolytic activity. The concentration at which 50 % of the enzyme activity is inhibited, the IC_{50}, was calculated using GraphPad Prism 4 software with appropriate transformations.

2.4. Data analysis

All data was analysed using GraphPad Prism statistical analysis software and the means were separated and compared through the appropriate post-test. Tukey's honestly significant difference (HSD) post hoc test was used for one-way comparisons of the influence of shade net (Table 1), and the Bonferroni post-test for two-way comparisons and analysis of interaction between the influence of shade net and season (Table 2 and Table 3).

4. Results

3.1. Treatment growing conditions

Photoselective shade nets manipulated spectral conditions successfully with regard to spectral distribution and PAR in comparison to the control, as can be observed in Fig. 1 and 2. Spectral composition differed appropriately according to the respective spectral treatment implemented. In addition, all shade nets effectively reduced ultraviolet (UV) radiation, while black shade net reduced the intensity of all wavelengths. White shade net enhanced the intensity of wavelengths beyond 400 nm, while red shade net enhanced wavelengths beyond 580 nm. Blue and green shade net enhanced respective wavelengths while reducing others. Note approximate wavelength ranges for respective colours are: 100-400 nm (UV); 420-500 nm (Blue); 480-560 nm (Green); 660-670 nm (Red); 740-750 nm (Far-red). Furthemore,

higher shade net density reduced PAR most. However, of the 50% density shade nets investigated, black shade net reduced PAR most and blue the least (Fig. 2).

Table 1 displays the ratio of respective spectral composition under each treatment. The red:far-red (R:FR) light ratio is highest under the blue treatment and lowest under the red treatment. The ratio of total photosynthetic active radiation to UVA is higher under white and red shade net treatments, while the blue:red (B:R) light ratio is highest under blue shade net and lowest under red shade net.

Table 1
Comparison of the ratios of relative spectral composition within each shade net structure.

	Control	Black	White	Red	Green	Blue
R:FR	1.66	1.43	1.20	1.09	1.20	2.06
PAR:UVA	2.43	2.92	9.92	39.51	4.32	3.93
B:R	38.71	27.59	10.83	0.72	40.19	63.44

Where, R = Red; FR = Far-red; PAR = Photosynthetic active radiation; UVA = Ultra-violet A; B = Blue.

Temperature and humidity measured hourly under each treatment differed only slightly, thus were not significantly different (results not shown).

3.2. Growth

Photoselective shade net effectively influenced growth of *M. africana* with regard to plant height and canopy size. From the growth parameters observed over the duration of the study period, it was apparent that red, green and black photoselective shade net, increased plant height significantly ($p < 0.001$) in comparison to that of the control. In contrast, blue photoselective shade net inhibited plant height significantly ($p < 0.001$) (Fig. 3). Note that significant differences were only evident from September onwards.

Plants under green, red and black shade net treatments, in the order of reducing canopy size, also had a significantly greater canopy size than those of the other treatments, especially later during the study period (Fig. 4). The Canopeo app displays vegetative cover based on green pigments, which is influenced by plant health and season. Therefore, it appeared as though the canopies became smaller towards winter (May - August) as leaves changed colour, then expanded immensely in spring as the canopies greened up with new shoots. Note that significant differences were only evident from September onwards.

Furthermore, it should be noted that shoots were harvested in April and November, resulting in reduced plant height and canopy size in these months, as can be observed in Fig. 3 and 4.

3.3. Carbohydrate content

3.3.1. Starch content

Starch content did not differ significantly from that of the control. However, in autumn the white shade net treatment resulted in significantly higher starch content than that of the red ($p < 0.01$) and blue shade net treatments ($p < 0.05$), as can be observed in Fig. 5. In spring, starch content of the white shade net treatment was significantly higher ($p < 0.05$) than that of the red, black and blue treatments. The two-way ANOVA of seasonal starch content displayed no significant differences between season and no significant interaction effects.

3.3.2. Soluble carbohydrate content – sucrose / d-glucose / d-fructose

Sucrose content in autumn did not differ significantly between treatments. Furthermore, there were no significant differences in sucrose content of *M. africana* between the two seasons investigated. In addition, both D-glucose and D-fructose content did not differ significantly between treatments, and there were also no significant interaction effects (Table 2).

Table 2

ANOVA of respective carbohydrate content (g/100g dry mass) of *M. africana* L. shoot material attributed to season, shade net treatment and their interaction.

Carbohydrate	Source of Variation	Df	Sum-of-squares	Mean square	F	P-value
Starch	Interaction	5	2.6780	0.5355	0.443	0.8169
	Season	1	3.4320	3.4320	2.837	0.0973
	Treatment	5	50.1000	10.020	8.283	<0.0001
	Residual	60	72.5900	1.2100		
Sucrose	Interaction	5	0.7000	0.1400	1.215	0.3131
	Season	1	0.0047	0.0047	0.041	0.8401
	Treatment	5	2.3650	0.4730	4.106	0.0028
	Residual	60	6.9120	0.1152		
D-glucose	Interaction	5	0.0591	0.0118	0.335	0.8898

	Season	1	0.5049	0.5049	14.320	0.0004
	Treatment	5	0.2382	0.04763	1.351	0.2557
	Residual	60	2.1160	0.03526		
D-fructose	Interaction	5	0.0689	0.01377	0.354	0.8777
	Season	1	0.0833	0.08331	2.141	0.1486
	Treatment	5	0.2321	0.04643	1.193	0.3234
	Residual	60	2.3340	0.03891		

3.4. Bioactivity – elastase inhibition

The elastase inhibition assay revealed some variability between individual plants within single treatments, displaying differences in bioactivity and possibly biochemistry between plant individuals of the same species and ecotype. Elastase inhibition remained relatively consistent in both seasons under black, white, and blue photoselective shade nets (Table 3). Elastase inhibition under black photoselective shade net was the lowest in autumn and significantly lower than the control in autumn but not in spring.

Although there was a significant interaction between season and shade net colour ($p < 0.001$), there was no significant difference between seasons under black, white, and blue treatments (Table 3). However, under red, green and control treatments, elastase inhibition was significantly higher in autumn than in spring.

In autumn both green and red shade net treatments resulted in significantly higher bioactivity than all other treatments, 18.59 ± 0.39 µg/mL and 19.28 ± 0.36 µg/mL, respectively (Fig. 6). In spring, red, white, and blue shade net treatments resulted in significantly higher elastase inhibition, between 36.41 ± 7.7 and 41.34 ± 2.4 µg/mL, than remaining treatments (Fig. 6 and Table 3). These findings suggest that photoselective shade net may influence secondary metabolite production of *M africana.*

Table 3

Mean seasonal elastase inhibition (Concentration required to inhibit 50% of the elastase enzyme, IC_{50} expressed as µg/mL) of *M. africana* L. shoot material following cultivation under respective spectral treatments. Different letters represent significant differences ($p < 0.001$) between treatments within each season. P-values < 0.001 display significant differences between seasons.

Treatment	Autumn (µg/mL)	Spring (µg/mL)	P-value
Control	37.93 ± 3.61 a	57.79 ± 5.67 a	< 0.001
Red	19.28 ± 0.63 b	41.34 ± 2.40 b	< 0.001
Green	18.59 ± 0.39 b	54.49 ± 3.45 a	< 0.001
Black	57.79 ± 6.66 c	56.97 ± 2.23 a	ns
White	38.89 ± 3.15 a	41.48 ± 0.78 b	ns
Blue	37.56 ± 3.79 a	36.51 ± 7.73 b	ns

Where, ns = Not significant.

5. Discussion

The spectral compositions observed in this study appeared similar to those in similar studies with regard to wavelength peaks and relative intensities (Arthurs et al., 2013; Shahak, 2006; Shahak et al., 2008). Differences in spectral quality were observed under clear sky conditions, as cloud cover negated the influence of photoselective shade net on spectral composition (data not shown). Therefore, the influence of photoselective shade net may be more prominent in areas experiencing less cloud cover per annum.

PAR levels were logically reduced most by higher shade net density (80%), namely, red and black shade net at 80% density. However, black shade net at 50% density reduced PAR most in comparison to other shade net treatments at an equal shade net density of 50%. This is due to the fact that black shade net reduces transmittance and does not scatter light, while white shade net reduces transmittance but scatters light well (Selahle et al., 2014).

Minor differences regarding temperature and humidity under respective treatments were consistent with the findings of Gaurav et al. (2016). The influence of red shade net on temperature (reduced in all seasons) and humidity (highest in winter) is most likely due to the shade net density. It should be noted that these conditions may have a direct influence on plant growth and phytochemistry, especially since reduced temperatures would reduce stress, photoinhibition and may enhance resource use efficiency (Arthurs et al., 2013; Stamps, 2009). Moreover, higher relative humidity reduces the vapour pressure deficit, which enhances stomatal conductivity and the rate of photosynthesis (Mortensen and Gislerød, 1999).

With regard to growth, *M. africana* grew most under red, green and black shade net treatments and least under blue shade net over the study period (expressed as plant height and canopy size). Although temperature was highest under red shade net, this was not the case

under green and black treatments, as black most often resulted in the lowest temperatures. In addition, although relative humidity was most often highest under black shade net, this was opposite for green and red treatments. Therefore, in this case, the effect on growth was probably not attributed to temperature and/or humidity. This may be confirmed by the growth inhibition observed under the blue shade net treatment, which displayed no extraordinary influence on temperature nor humidity, attributing the observed effects on growth to the manipulation of light properties.

In the current study, PAR level was highest under blue shade net in comparison to other shade net treatments, yet growth was significantly reduced in comparison to the control and other shade net treatments. The observed effects on elongation under red and blue treatments are consistent with findings of Warrington and Mitchell (1976), displaying elongation stimulated by red-biased light and inhibited by blue-biased light. Therefore, it is evident that light quality (spectral composition) is probably responsible for the observed growth inhibition rather than light quantity.

Enhanced plant height was expected under red photoselective shade net due to the shade avoidance response induced by lower R:FR light ratio (Table 1), as explained by Smith (1982). However, the elongation observed under red shade net may also be attributed to the excessive reduction in incident radiation and PAR as a result of the shade net density. This is confirmed by the findings of Retamales et al. (2006), which displayed no significant effect of red shade net, both at 35% and 50% shade density, on the elongation of blueberry shoots. However, Retamales et al. (2006) observed that 50% red shade net reduced PAR similar to black shade net at 35% and 50% density, although black shade net stimulated elongation significantly at both densities. Therefore, it is apparent that a wider range of factors may be responsible for the enhanced elongation observed in both studies. Furthermore, one should consider that certain effects may be species dependent (Boardman, 1977).

Growth stimulation experienced under black photoselective shade net may be a result of reduced temperatures and PAR. The study by Stamps and Chandler (2008) also revealed enhanced plant height and leaf yield of *Pittosporum tobira* and *Aspidistra elatior* by black shade net, in comparison to those under grey, red and blue shade net at equal densities. The review by Vandenbussche et al. (2005) elaborated on factors inducing the shade avoidance response in plants, stating that reduced light intensity may also induce the shade avoidance response, stimulating elongation and expansion. Therefore, it is evident that the observed stimulation of plant height and canopy size of *M. africana* under red and black shade net may be a result of reduced PAR levels.

The enhanced growth observed under green photoselective shade net may be attributed to spectral composition due to similarities of PAR, temperature and RH with that of white and control treatments, respectively. The R:FR light ratio under green photoselective shade net (Table 1) was low, possibly responsible for the observed growth stimulation or shade avoidance response. Photoreceptors which perceive red and blue light, such as certain phytochromes and cryptochromes, have been shown to respond to green light, inducing similar shade-avoidance responses. The review by Wang and Folta (2013) emphasised the involvement of green light in the shade-avoidance response, elaborating on green light stimulated elongation. Therefore, the enhanced growth of *M. africana* observed under green photoselective shade net is most likely due to the shade avoidance response.

The observed growth inhibition under blue shade net is consistent with the findings of Rajapakse and Shahak (2007), which illuminated the inhibitory effect of copper sulphate ($CuSO_4$) light filters on elongation. This is due to the perception of reduced R:FR light ratio and enhanced blue light by the phytochrome and cryptochrome photoreceptors, respectively (Ahmad et al., 2002; Folta et al., 2003; Rajapakse and Shahak, 2007). Studies by Lin (2000); Neff and Chory (1998) and Poppe et al. (1998) elucidated that blue-light inhibited elongation is a result of the perception of blue light by cryptochrome 1 and phytochrome A photoreceptors, which inhibit cell elongation/ expansion. Furthermore, the study by Lee et al. (2010) displayed a clear influence of B:R light ratios on plant the primary and secondary metabolism of lettuce (*Lactuca sativa* L.).

Photoselective shade net had a significant influence on the starch content of *M. africana* shoots in both seasons and on sucrose content in spring. In addition, starch content of *M. africana* shoots, influenced by photoselective shade net, exhibited similar trends in both seasons, with the white treatment resulting in significantly higher starch content than that of the red, black and blue treatments. The study by Shin et al. (2008) elucidated higher starch content in leaves resulting from blue light alone, in comparison to the influence of red light alone, however, the combination of blue and red light enhanced starch content most. In addition, fluorescent light resulted in less starch than both blue and B:R combination, which may display the influence of B:R light ratio.

The reduced PAR levels under red and black shade net treatments may be responsible for reduced starch content. However, the enhanced blue light under the blue treatment may be responsible for the reduced starch content in this case. Higher R:FR and B:R light ratios were observed in the current study, which appear to correlate with significantly reduced starch and sucrose content of *M. africana* shoot material. Moreover, the study by Runkle and Heins

(2001) revealed the independent influence of blue light and R:FR light ratio on stem elongation. The study by Rajapakse and Kelly (1995) found that cultivation of chrysanthemums under a $CuSO_4$ filter, which significantly increased the R:FR- and B:R-light ratios (Rajapakse and Shahak, 2007), resulted in dwarfed growth and reduced carbohydrate concentrations (including starch). Therefore, dwarfed growth of *M. africana* under blue shade net may be attributed to the influence of spectral filters on blue, red and far-red light ratios and the subsequent effect on carbohydrate metabolism.

Green shade net reduced R:FR and increased B:R light ratios, which may have been responsible for the enhanced growth response. However, carbohydrate content was not significantly different from that of the control, although starch content appeared to be higher in both seasons. The study by Muneer et al. (2014) investigated the influence of monochromatic green, red and blue light on lettuce growth, photosynthesis and protein synthesis, and found that green light alone failed to enhance either. Furthermore, Xiaoying et al. (2012) highlighted the importance of red, green and blue light separately and in combination for net photosynthesis and revealed the poor performance of green light alone. This illustrates the importance of wavelength combinations, and proportions thereof, for plant growth. Therefore, spectral composition under green photoselective shade net may be ideal for *M. africana* growth.

The current study revealed a significant influence of season, shade net density and photoselective shade net colour on elastase inhibition. Anti-wrinkle activity of *M. africana* shoot material, specifically elastase inhibition, was investigated by Lall et al. (2017), revealing significant elastase inhibition with a 50% inhibitory concentration (IC_{50}) of 28.04 µg/ mL.

Low elastase inhibition (high IC_{50}) resulted from black shade net treatment in both seasons. Black photoselective shade net significantly reduced total irradiance level, along with PAR, which has also been shown to reduce secondary metabolite content, consistent with the findings of Mosaleeyanon et al. (2005), Ghasemzadeh, et al. (2010) and Murthy et al. (2014). Due to the effect of black photoselective shade net on all light wavelengths, absorbing all wavelengths and scattering none (Fig. 1), this may reduce metabolic activity, metabolite synthesis, and consequently reduce bioactivity (Selahle et al., 2014).

Significantly higher elastase inhibition that was experienced in autumn under red and green photoselective shade net may have been influenced by the phytochrome system due to changes in R:FR light ratios, as was explained by Goren et al. (2011). This is supported by the study of Ahmad et al. (2011b), which displayed enhanced antioxidant potential of *Stevia*

under green and red light. However, enhanced quantities of green light, known to stimulate the shade-avoidance response (Wang and Folta, 2013), did not enhance elastase inhibition under green shade net in spring. Therefore, enhanced bioactivity resulting from the red treatment may be a result of the excessively reduced irradiance, a potential stress response due to insufficient radiation, which may have stimulated the shikimic acid pathway (Ibrahim et al., 2010).

Improved bioactivity that resulted from blue and white shade net treatments in spring may be a result of enhanced blue light level. The study by Lee et al. (2010) demonstrated the influence of blue light on anthocyanin production, while the study by Johkan et al. (2010) supported it. In addition, Ahmad et al. (2016) reported enhanced antioxidant activity due to blue light stimulated accumulation of phenolic and flavonoid compounds. The study by Ohashi-Kaneko et al. (2007) elucidated the involvement of blue light and glucose during phenylpropanoid biosynthesis for conversion of aglycones to their glucosides, of which, myrsinoside B is a derivative. Therefore, it is understandable that enhanced blue light under respective photoselective shade net may enhance elastase inhibition. This may also be the reason why elastase inhibition is low under black shade net.

Seasonal variation in bioactivity was only observed for the control, red and green shade net treatments, which displayed significantly ($p < 0.001$) higher elastase inhibition in autumn. Furthermore, the current study displayed little seasonal fluctuation under blue, black and white photoselective shade net treatments. This may be a result of neutralizing effects of these photoselective shade nets with regard to natural seasonal fluctuations in spectral composition (Uddin et al., 2001). This is supported by the findings of Arthurs et al. (2013), illuminating the influence of seasonal variation in light quality as relative wavelength intensities shifted depending on season, although wavelength ratios remained consistent under the respective shade nets.

The results attained in this study with regard to elastase inhibition has industry application value, revealing the most appropriate season to harvest *M. africana* shoot material and which shade net would be preferable. Green photoselective shade net would be ideal for seasonal harvesting in autumn due to enhanced bioactivity and growth, whereas white shade net would be ideal for year-round harvesting due to consistency regarding bioactivity, without compromising on vegetative growth.

6. Conclusions

Photoselective shade net is effective for the manipulation of spectral composition and intensity. Black shade net reduces PAR most as it reduces light intensity across all wavelengths and scatters none. In addition, all shade nets may be used to reduce UV stress and enhance resource use efficiency, although the influence on the medicinal species and compound/s of interest should be considered. Red, green and black shade net may be used to enhance plant height and canopy size of *M. africana*, while blue shade net may be used to inhibit plant elongation. These effects may be attributed to the R:FR light ratio and blue light content experienced under these shade nets. Furthermore, red, black and blue shade net treatments resulted in the lowest starch and sucrose contents, which may be a result of low PAR level and light intensity and a high proportion of blue light.

Elastase inhibition was highest under red and green shade net in autumn. Therefore, it may be desirable to cultivate *M. africana* under green photoselective shade net to enhance biomass production with no significant compromise on bioactivity. For cosmeceutical production, *M. africana* may be cultivated under white shade net with multiple harvests per year of consistent bioactivity, alternatively under green or red shade net with harvesting in autumn only to obtain material with enhanced bioactivity. However, due to the relatively slow growth of this species, it may be desirable to practice seasonal harvesting.

Acknowledgements

The authors would like to thank all involved in this research project, from those who conceptualized, conducted, and analysed the studies, to those who kindly provided insight and guidance, and those who provided moral support throughout the project. In addition, the authors gratefully appreciate the University of Pretoria, its affiliates and supporting staff for all their assistance.

Funding

This work was financial supported by the National Research Foundation (SARCHI) [grant number: 98334].

Declaration

The authors declare that there were no competing interests.

Author contributions

ZSC: Conceptualization; Investigation; Methodology; Data curation; Formal analysis; Writing – original draft. **ESDT**: Supervision; Writing – review & editing. **JMS**: Supervision; Funding acquisition; Writing – review & editing. **NL**: Supervision; Funding acquisition; Writing – review & editing. **BP**: Mentorship; Software; Data curation; Writing – review & editing.

References

Ahmad, B., Azam, S., Bashir, S., Hussain, F., Chaudhary, M.I., 2011a. Insecticidal, brine shrimp cytotoxicity, antifungal and nitric oxide free radical scavenging activities of the aerial parts of *Myrsine africana* L. African Journal of Biotechnology 10 (8), 1448-1453.

Ahmad, B., Azam, S., Bashir, S., Khan, I., Ali, N., Chaudhary, M.I., 2011b. Phytotoxic, Antibacterial and Haemagglutination activities of the aerial parts of *Myrsine africana* L. African Journal of Biotechnology 10 (1), 97-102.

Ahmad, M., Grancher, N., Heil, M., Black, R.C., Giovani, B., Galland, P., Lardemer, D., 2002. Action spectrum for cryptochrome-dependent hypocotyl growth inhibition in *Arabidopsis*. Plant Physiology 129 (2), 774-785.

Ahmad, N., Rab, A., Ahmad, N., 2016. Light-induced biochemical variations in secondary metabolite production and antioxidant activity in callus cultures of *Stevia rebaudiana* (Bert). Journal of Photochemistry and Photobiology B: Biology 154, 51-56.

Arthurs, S.P., Stamps, R.H., Giglia, F.F., 2013. Environmental modification inside photoselective shadehouses. HortScience 48 (8), 975-979.

Bastías, R.M., Corelli-Grappadelli, L., 2012. Light quality management in fruit orchards: physiological and technological aspects. Chilean Journal of Agricultural Research 72 (4), 574.

Bieth, J.G., Dirrig, S., Jung, M.-L., Boudier, C., Papamichael, E., Sakarellos, C., Dimicoli, J.-L., 1989. Investigation of the active center of rat pancreatic elastase. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology 994 (1), 64-74.

Boardman, N.K., 1977. Comparative photosynthesis of sun and shade plants. Annual Review of Plant Physiology 28 (1), 355-377.

Chappuis, F., Sundar, S., Hailu, A., Ghalib, H., Rijal, S., Peeling, R.W., Alvar, J., Boelaert, M., 2007. Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? Nature Reviews Microbiology 5 (1), 873-882.

Corré, W., 1983. Growth and morphogenesis of sun and shade plants I. The influence of light intensity. Plant Biology 32 (1-2), 49-62.

Folta, K.M., Lieg, E.J., Durham, T., Spalding, E.P., 2003. Primary inhibition of hypocotyl growth and phototropism depend differently on phototropin-mediated increases in cytoplasmic calcium induced by blue light. Plant Physiology 133 (4), 1464-1470.

Gaurav, A.K., Raju, D., Janakiram, T., Singh, B., Jain, R., Krishnan, S.G., 2016. Effect of coloured shade net on production of *Dracaena fragrans*. The Horticultural Society of India (Regd.) 73 (1), 94-98.

Ghasemzadeh, A., Jaafar, H.Z., Rahmat, A., Wahab, P.E.M., Halim, M.R.A., 2010. Effect of different light intensities on total phenolics and flavonoids synthesis and anti-oxidant activities in young ginger varieties (*Zingiber officinale* Roscoe). International Journal of Molecular Sciences 11 (10), 3885-3897.

Goren, A., Alkalia-Tuvia, S., Perzelan, Y., Aharon, Z., Fallik, E., 2011. Photoselective shade nets reduce postharvest decay development in pepper fruits. Advances in Horticultural Science 25 (1), 26-31.

Ibrahim, M.H., Jaafar, H.Z., Rahmat, A., Rahman, Z.A., 2010. The relationship between phenolics and flavonoids production with total non structural carbohydrate and photosynthetic rate in *Labisia pumila* Benth. under high CO2 and nitrogen fertilization. Molecules 16 (1), 162-174.

Ilić, Z.S., Fallik, E., 2017. Light quality manipulation improves vegetable quality at harvest and postharvest: A review. Environmental and Experimental Botany 139, 79-90.

Johkan, M., Shoji, K., Goto, F., Hashida, S.-n., Yoshihara, T., 2010. Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. HortScience 45 (12), 1809-1814.

Lall, N., Kishore, N., Fibrich, B., Lambrechts, I.A., 2017. In vitro and In vivo activity of *Myrsine africana* on elastase inhibition and anti-wrinkle activity. Pharmacognosy Magazine 13 (52), 583.

Lee, J.-G., Oh, S.-S., Cha, S.-H., Jang, Y.-A., Kim, S.-Y., Um, Y.-C., Cheong, S.-R., 2010. Effects of red/blue light ratio and short-term light quality conversion on growth and anthocyanin contents of baby leaf lettuce. Protected Horticulture and Plant Factory 19 (4), 351-359.

Lin, C., 2000. Plant blue-light receptors. Trends in Plant Science 5 (8), 337-342.

Megazyme, 2020a. Sucrose/D-Fructose/D-Glucose Assay Kit - K-SUFRG 04/17. Available at https://www.megazyme.com/documents/Booklet/K-SUFRG_DATA.pdf (Accessed May 2020).

Megazyme, 2020b. Total starch assay procedure - K-TSTA-50A / K-TSTA-100A 08/19. Available at https://www.megazyme.com/documents/Booklet/K-TSTA-100A_DATA.pdf (Accessed May 2020).

Momtaz, S., Lall, N., Basson, A., 2008. Inhibitory activities of mushroom tyrosine and DOPA oxidation by plant extracts. South African Journal of Botany 74 (4), 577-582.

Mortensen, L.M., Gislerød, H.R., 1999. Influence of air humidity and lighting period on growth, vase life and water relations of 14 rose cultivars. Scientia Horticulturae 82 (3-4), 289-298.

Mosaleeyanon, K., Zobayed, S., Afreen, F., Kozai, T., 2005. Relationships between net photosynthetic rate and secondary metabolite contents in St. John's wort. Plant Science 169 (3), 523-531.

Muneer, S., Kim, E., Park, J., Lee, J., 2014. Influence of green, red and blue light emitting diodes on multiprotein complex proteins and photosynthetic activity under different light intensities in lettuce leaves (*Lactuca sativa* L.). International Journal of Molecular Sciences 15 (3), 4657-4670.

Murthy, H.N., Lee, E.-J., Paek, K.-Y., 2014. Production of secondary metabolites from cell and organ cultures: strategies and approaches for biomass improvement and metabolite accumulation. Plant Cell, Tissue and Organ Culture (PCTOC) 118 (1), 1-16.

Muthee, J.K., 2018. Anthelmintic efficacy and safety of selected medicinal plants against mixed gastrointestinal nematodes in artificially infected sheep.

Neff, M.M., Chory, J., 1998. Genetic interactions between phytochrome A, phytochrome B, and cryptochrome 1 during *Arabidopsis* development. Plant physiology 118 (1), 27-35.

Ohashi-Kaneko, K., Takase, M., Kon, N., Fujiwara, K., Kurata, K., 2007. Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. Environmental Control in Biology 45 (3), 189-198.

Patrignani, A., Ochsner, T.E., 2015. Canopeo: A powerful new tool for measuring fractional green canopy cover. Agronomy Journal 107 (6), 2312-2320.

Phondani, P.C., Bhatt, I.D., Negi, V.S., Kothyari, B.P., Bhatt, A., Maikhuri, R.K., 2016. Promoting medicinal plants cultivation as a tool for biodiversity conservation and livelihood enhancement in Indian Himalaya. Journal of Asia-Pacific Biodiversity 9 (1), 39-46.

517 Poppe, C., Sweere, U., Drumm-Herrel, H., Schäfer, E., 1998. The blue light receptor
518 cryptochrome 1 can act independently of phytochrome A and B in *Arabidopsis thaliana*.
519 The Plant Journal 16 (4).
520 Rajapakse, N.C., Kelly, J.W., 1995. Spectral filters and growing season influence growth and
521 carbohydrate status of *Chrysanthemum*. Journal of the American Society for
522 Horticultural Science 120 (1), 78-83.
523 Rajapakse, N.C., Shahak, Y., 2007. Light-Quality Manipulation by Horticulture Industry.
524 Annual Plant Reviews: Light and Plant Development 30, 290-312.
525 Reed, J.W., Nagpal, P., Poole, D.S., Furuya, M., Chory, J., 1993. Mutations in the gene for
526 the red/far-red light receptor phytochrome B alter cell elongation and physiological
527 responses throughout *Arabidopsis* development. The Plant Cell 5 (2), 147-157.
528 Retamales, J., Montecino, J., Lobos, G., Rojas, L., 2008. Colored shading nets increase yields
529 and profitability of highbush blueberries. In: XXVII International Horticultural
530 Congress, Seoul, Korea, 2006, pp. 193-197.
531 Runkle, E.S., Heins, R.D., 2001. Specific functions of red, far red, and blue light in flowering
532 and stem extension of long-day plants. Journal of the American Society for Horticultural
533 Science 126 (3), 275-282.
534 Selahle, M.K., Sivakumar, D., Soundy, P., 2014. Effect of photo-selective nettings on
535 post-harvest quality and bioactive compounds in selected tomato cultivars. Journal of the
536 Science of Food and Agriculture 94 (11), 2187-2195.
537 Shahak, Y., 2008. Photo-selective netting for improved performance of horticultural crops. A
538 review of ornamental and vegetable studies carried out in Israel. In: XXVII International
539 Horticultural Congress, Seoul, Korea, 2006, pp. 161-168.
540 Shahak, Y., Gal, E., Offir, Y., Ben-Yakir, D., 2008. Photoselective shade netting integrated
541 with greenhouse technologies for improved performance of vegetable and ornamental
542 crops. In: International Workshop on Greenhouse Environmental Control and Crop
543 Production in Semi-Arid Regions, Tucson, USA, 2008, pp. 75-80.
544 Shin, K.S., Murthy, H.N., Heo, J.W., Hahn, E.J., Paek, K.Y., 2008. The effect of light quality
545 on the growth and development of in vitro cultured *Doritaenopsis* plants. Acta
546 Physiologiae Plantarum 30 (3), 339-343.
547 Sivakumar, D., Jifon, J., Soundy, P., 2017. Spectral quality of photo-selective shade nettings
548 improves antioxidants and overall quality in selected fresh produce after postharvest
549 storage. Food Reviews International, 1-18.

Smith, H., 1982. Light quality, photoperception, and plant strategy. Annual Review of Plant Physiology 33 (1), 481-518.

Stamps, R., Chandler, A., 2008. Differential effects of colored shade nets on three cut foliage crops. In: XXVII International Horticultural Congress, Seoul, Korea, 2006, pp. 169-176.

Stamps, R.H., 2009. Use of colored shade netting in horticulture. HortScience 44 (2), 239-241.

Stapelberg, J., Nqephe, M., Lambrechts, I., Crampton, B., Lall, N., 2019. Selected South African plants with tyrosinase enzyme inhibition and their effect on gene expression. South African Journal of Botany 120, 280-285.

Stuefer, J.F., Huber, H., 1998. Differential effects of light quantity and spectral light quality on growth, morphology and development of two stoloniferous *Potentilla* species. Oecologia 117 (1-2), 1-8.

Taiz, L., Zeiger, E., 2010. Plant Physiology, 5th ed. Sunderland, Massachusetts, Sinauer Associates Inc.

Uddin, A.J., Hashimoto, F., Kaketani, M., Shimizu, K., Sakata, Y., 2001. Analysis of light and sucrose potencies on petal coloration and pigmentation of *Lisianthus* cultivars (in vitro). Scientia Horticulturae 89 (1), 75-84.

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

Van Wyk, B.-E., 2015. A review of commercially important African medicinal plants. Journal of Ethnopharmacology 176, 118-134.

Vandenbussche, F., Pierik, R., Millenaar, F.F., Voesenek, L.A., Van Der Straeten, D., 2005. Reaching out of the shade. Current Opinion in Pant Biology 8 (5), 462-468.

Wang, Y., Folta, K.M., 2013. Contributions of green light to plant growth and development. American Journal of Botany 100 (1), 70-78.

Warrington, I., Mitchell, K., 1976. The influence of blue-and red-biased light spectra on the growth and development of plants. Agricultural Meteorology 16 (2), 247-262.

Xiaoying, L., Shirong, G., Taotao, C., Zhigang, X., Tezuka, T., 2012. Regulation of the growth and photosynthesis of cherry tomato seedlings by different light irradiations of light emitting diodes (LED). African Journal of Biotechnology 11 (22), 6169-6177.