JANDEILSON PEREIRA DOS SANTOS

COLORED SHADE NETS AND GREEN MANURE INFLUENCE ON THE GROWTH, PHYTOCHEMISTRY AND ANTIOXIDANT METABOLISM OF *Origanum majorana* L.

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Aprovada em 16 de fevereiro de 2024.

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LAVRAS - MG
2024

À Deus,

À minha família, em especial aos meus pais, Ana Lucia Pereira dos Santos e José Gomes dos Santos Sobrinho, pelo apoio, dedicação e amor incondicional.

Dedico

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“Tudo o que um sonho precisa para ser realizado é alguém que acredite que ele possa ser realizado.” (Roberto Shinyashiki)

RESUMO

A procura por métodos sustentáveis e eficientes para o crescimento de plantas e produção de compostos bioativos tem impactado o setor agrícola. Neste contexto, fatores como qualidade da luz e adubação verde pode impulsionar práticas agrícolas sustentáveis e maximizar o potencial de espécies como *Origanum majorana* L., conhecida por suas propriedades medicinais e aromáticas. Objetivou-se (I) avaliar o crescimento vegetativo, o teor e a composição química do óleo essencial, o doseamento de compostos fenóis e flavonas/flavonóis totais e a capacidade antioxidante de *O. majorana*. cultivada sob diferentes malhas de sombreamento coloridas. Objetivou-se (II) avaliar o potencial de *Cajanus cajan* como adubo verde no crescimento vegetativo e no metabolismo do óleo essencial, dos pigmentos fotossintetizantes e antioxidante de *O. majorana*. No experimento I, plantas de *O. majorana* foram cultivadas sob três tipos malha de sombreamento 50% (azul, preto, vermelho) e a pleno sol. O acúmulo de matéria seca total e de raiz, taxa assimilatória liquidada, teor e rendimento do óleo essencial e o teor de α-terpineol foram aumentados em 93%, 154%, 43%, 75%, 27% e 62%, respectivamente, nas plantas sob pleno sol em relação a malha azul. Além disso, as plantas sob pleno sol e malha vermelha aumentaram significativamente a matéria seca das folhas, caule e raiz, e a taxa de crescimento relativo. Já a malha vermelha e preta aumentou significativamente a área foliar total e específica, e o teor de γ-terpineno em comparação a malha azul e a pleno sol. Já a malha vermelha proporcionou aumento do teor de ρ-cimeno em 39% em relação a pleno sol. A capacidade antioxidante total e fenóis totais foram significativamente aumentados em pleno sol e malha preta. No experimento II, foram utilizados seis tratamentos sendo cinco doses de adubo verde (0, 150, 300, 450 e 600 g plant^{-1} de *C. cajan*) e um controle (adubação inorgânica) em quatro repetições. As plantas tratadas com 600 g plant^{-1} de *C. cajan* aumentaram a matéria seca das folhas, caule e parte aérea em 338%, 555% e 414% respectivamente, em relação a ausência de adubação. A dose de 450 g plant^{-1} aumentou o teor de clorofila total em 14% e 18% respectivamente em relação a dose 150 g plant^{-1} e ao controle. A menor atividade da superóxido dismutase foi observada para adubação inorgânica. As doses 0 e 150 g plant^{-1} aumentaram a atividade da catalase. No entanto, a atividade da ascorbato peroxidase foi reduzida na ausência de adubação. O rendimento de óleo essencial foi maior com a aplicação de 600 g plant^{-1} de *C. cajan* e adubação inorgânica, atingindo rendimentos de OE de 0,050 e 0,056 mg planta^{-1}, representando um aumento de 406% e 471% respectivamente, em comparação a ausência de adubação. Foi demonstrado que adubação verde influencia positivamente a produção de óleo essencial, alcançando níveis e rendimentos comparáveis a adubação inorgânica. Condições de cultivo a pleno sol ou o uso de malha vermelha e a aplicação de 600 g plant^{-1} de *C. cajan*, podem melhorar o crescimento, o rendimento e a composição química do óleo essencial de *O. majorana*.

Palavras-chave: Manjerona; feijão-guandu; malhas coloridas; metabolitos secundários.

ABSTRACT

The search for sustainable and efficient methods for plant growth and bioactive compound production has impacted the agricultural sector. In this context, factors such as light quality and green manure can boost sustainable agricultural practices and maximize the potential of species such as *Origanum majorana* L., known for its medicinal and aromatic properties. The objectives were (I) to evaluate the vegetative growth, essential oil content and chemical composition, total phenolic and flavones/flavonols content, and antioxidant capacity of *O. majorana* cultivated under different colored shade meshes. (II) to evaluate the potential of *Cajanus cajan* as a green manure on the vegetative growth, essential oil metabolism, photosynthetic pigments, and antioxidant activity of *O. majorana*. In experiment I, O. majorana plants were grown under three types of 50% shade mesh (blue, black, red), and full sun. The accumulation of total dry matter and root dry matter, net photosynthetic rate, essential oil content and yield, and α-terpineol content were increased by 93%, 154%, 43%, 75%, 27%, and 62%, respectively, in plants under full sun compared to the blue mesh. In addition, plants under full sun and red mesh significantly increased leaf, stem, and root dry matter, and relative growth rate. The red and black meshes significantly increased total and specific leaf area, and γ-terpinene content compared to the blue mesh and full sun. The red mesh provided an increase in ρ-cymene content by 39% compared to full sun. Total antioxidant capacity and total phenolics were significantly increased under full sun and black mesh. In experiment II, six treatments were used: five doses of green manure (0, 150, 300, 450 e 600 g plant^{-1} of *C. cajan*) and a control (inorganic fertilization) in four replicates. Plants treated with 600 g plant^{-1} of *C. cajan* increased leaf, stem, and aerial part dry matter by 338%, 555%, and 414%, respectively, compared to no fertilization. The 450 g plant^{-1} dose increased total chlorophyll content by 14% and 18% compared to the 150 g plant^{-1} dose and the control, respectively. The lowest superoxide dismutase activity was observed for inorganic fertilization. Doses of 0 and 150 g plant^{-1} m^{-2} increased catalase activity. However, ascorbate peroxidase activity decreased in the absence of fertilization. Essential oil yield was higher with the application of 600 g plant^{-1} of *C. cajan* and inorganic fertilization, reaching EO yields of 0.050 and 0.056 mg plant^{-1}, representing an increase of 406% and 471%, respectively, compared to no fertilization. Green manure was shown to positively influence essential oil production, achieving levels and yields comparable to inorganic fertilization. Cultivation conditions under full sun or the use of red mesh and the application of 600 g plant^{-1} of *C. cajan* can improve the growth, yield, and chemical composition of the essential oil of *O. majorana*.

Keywords: Marjoram; pigeon pea; colored shade nets; secondary metabolites.

INDICADORES DE IMPACTO

A tese intitulada “Colored shade nets and green manure influence on the growth, phytochemistry and antioxidant metabolism of *Origanum majorana* L.” corrobora com impactos sociais, tecnológicos, econômicos e culturais. Socialmente, promoveu práticas agrícolas sustentáveis que podem ser adotadas por agricultores familiares e pequenos produtores, contribuindo para a inclusão social e melhoria da qualidade de vida no meio rural. No campo da educação, o caráter extensionista do estudo inclui a capacitação de técnicos, docentes e discentes, atuando diretamente para fornecer informações que podem beneficiar comunidades agrícola, fomentando a disseminação de conhecimento científico e prático. No âmbito tecnológico, a adoção de adubação orgânica e o uso de telas de sombreamento coloridas demonstraram melhorar a composição química do solo e a otimização da produção de metabólitos secundários, contribuindo para a sustentabilidade ambiental. Tecnologicamente, a pesquisa introduziu inovações no manejo fitotécnico, que otimizam a produção de óleos essenciais. Economicamente, as técnicas estudadas indicaram maior produtividade e a qualidade do óleo essencial, impulsionando a competitividade dos produtores locais e gerando novas oportunidades de emprego e renda, refletindo-se positivamente no trabalho. Culturalmente, o estudo valoriza o conhecimento tradicional do cultivo de plantas medicinais, integrando-o com práticas modernas de cultivo sustentável. Além disso, a pesquisa tem implicações significativas para o setor industrial, que pode se beneficiar com a maior produção do óleo essencial, ampliando a produção de cosméticos e fármacos de alta qualidade, agregando valor econômico e industrial ao produto. Desse modo, os impactos alinham-se a Agenda 2030 da ONU, promovendo práticas agrícolas sustentáveis, conservando recursos naturais melhorando a qualidade do solo, bem como seu potencial de exploração econômica que impacta na geração de emprego e renda. Assim, a pesquisa demonstra que a integração de adubação orgânica e técnicas avançadas de manejo de luz pode transformar significativamente a produção de plantas medicinais, com amplos benefícios sociais, econômicos, tecnológicos e culturais.

IMPACT INDICATORS

The thesis entitled “Colored shade nets and green manure influence on the growth, phytochemistry and antioxidant metabolism of *Origanum majorana* L.” corroborates social, technological, economic, and cultural impacts. Socially, it promoted sustainable agricultural practices that can be adopted by family farmers and small producers, contributing to social inclusion and improving the quality of life in rural areas. In the field of education, the extensionist nature of the study includes the training of technicians, teachers, and students, acting directly to provide information that can benefit agricultural communities, fostering the dissemination of scientific and practical knowledge. In the technological sphere, the adoption of organic fertilization and the use of colored shade screens have shown to improve the chemical composition of the soil and optimize the production of secondary metabolites, contributing to environmental sustainability. Technologically, the research introduced innovations in phytosanitary management, which optimize the production of essential oils. Economically, the techniques studied indicated higher productivity and quality of the essential oil, boosting the competitiveness of local producers and generating new employment and income opportunities, positively impacting the work. Culturally, the study values traditional knowledge of medicinal plant cultivation, integrating it with modern sustainable cultivation practices. In addition, the research has significant implications for the industrial sector, which can benefit from the increased production of essential oil, expanding the production of high-quality cosmetics and pharmaceuticals, adding economic and industrial value to the product. Thus, the impacts are aligned with the UN's 2030 Agenda, promoting sustainable agricultural practices, conserving natural resources, improving soil quality, as well as its potential for economic exploration that impacts job creation and income generation. Thus, the research demonstrates that the integration of organic fertilization and advanced light management techniques can significantly transform the production of medicinal plants, with broad social, economic, technological, and cultural benefits.

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SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

Origanum majorana L. conhecida popularmente como manjerona, é uma planta aromática versátil conhecida por seu uso na culinária e na perfumaria, bem como por seus benefícios medicinais. Esta planta perene, pertencente à família Lamiaceae, é amplamente distribuída no mundo. É comercialmente cultivada em várias partes do mundo e os principais produtores comerciais do seu óleo essencial são Marrocos, Egito, Tunísia, Bulgária, Espanha, África do Sul, Hungria e Itália (Muqaddas *et al.*, 2016; Khadhri *et al.*, 2019).

A *O. majorana* tem recebido atenção considerável devido às suas propriedades benéficas e a versatilidade de seu óleo essencial (Prerna e Vasudeva, 2015; Tripathy *et al.*, 2017; Bouyahya *et al.*, 2021). Devido ao seu aroma característico a *O. majorana* é utilizada em diversos fins, na culinária para dar sabor a carnes e saladas, como aromatizantes de bebidas e como conservante natural principalmente de carnes (Krishnakumar e Potty, 2012).

O óleo essencial de *O. majorana* é utilizado na perfumaria, na indústria farmacêutica e cosmética (Khadhri *et al.*, 2019). Seu valor terapêutico é atribuído ao seu potencial antibacteriano e antifúngico (Deans e Svoboda, 1990; Ezzeddine *et al.*, 2001), antioxidante e citotóxico (Hussain *et al.*, 2011; Ouedrhiri *et al.*, 2021), antiespasmódico (Sahranavard *et al.*, 2014), hepatoprotetor (Pasavei *et al.*, 2020), anti-inflamatório (Gheitasi *et al.*, 2021) e antidepressivo (Amaghnouje *et al.*, 2020; Sales *et al.*, 2020). Além disso, devido suas propriedades inseticidas e repelentes é utilizado na agricultura como inseticidas e repelentes naturais (Kakouri *et al.*, 2022), e no controle de fitopatógenos devido a sua atividade antimicrobiana (Ghazal *et al.*, 2022).

A biossíntese e o acúmulo de metabólitos secundários são influenciados por alteração no ambiente. Durante o crescimento e desenvolvimento, as plantas interagem com diferentes fatores abióticos, como água, luz, temperatura, solo e nutrição. Esses fatores podem afetar o crescimento, desenvolvimento e expressão de genes envolvidos no metabolismo secundário, que por sua vez afeta a produção de compostos ativos e a produtividade das plantas (Verma e Shukla, 2015; Pant *et al.*, 2021). No cultivo de plantas medicinais, a qualidade e concentração dos princípios ativos desempenham um papel de extrema importância. A implementação de boas práticas agrícolas e o manejo nutricional adequado são estratégias essenciais empregadas para aprimorar a produção agrícola dessas plantas (Singh *et al.*, 2022).

O manejo agronômico desempenha um papel de extrema importância no cultivo de plantas medicinais e aromáticas, impactando diretamente o crescimento, o rendimento e a qualidade dos metabólitos secundários dessas plantas (Chen *et al.*, 2016). Para maximizar a produção e a qualidade dos óleos essenciais, é essencial o uso de técnicas de manejo adequado abrangendo fatores como manejo nutricional e qualidade da luz, entre outros. O uso de fertilizantes orgânicos e/ou minerais no manejo nutricional tem demonstrado melhorar o crescimento das plantas e a qualidade dos óleos essenciais produzidos (Honorato *et al.*, 2022).

A qualidade e intensidade da luz também desempenham um papel significativo no cultivo dessas plantas. Elas podem influenciar diretamente o crescimento da biomassa, a composição química dos óleos essenciais (Costa *et al.*, 2010). Pesquisas recentes têm destacado que a intensidade e a qualidade da luz afetam o tamanho das folhas, o crescimento dos caules, o vigor das plantas, a relação raiz/parte aérea e a síntese de metabólitos secundários em plantas medicinais e aromáticas (Ribeiro *et al.*, 2018; Ribeiro *et al.*, 2022; Honorato *et al.*, 2023).

Dentre as técnicas de manejo, o uso de malhas de sombreamento coloridas para manipular o espectro de luz é uma técnica que pode ser empregada para melhorar a produção de metabólitos secundários e a produção geral da planta. As malhas de sombreamento coloridas alteram os espectros de luz incidente e o microclima dentro do ambiente de cultivo (Thakur e Kumar, 2021). Pesquisas mostram que essas malhas têm um efeito significativo na filtragem da luz incidente de acordo com a cor da tela, alterando os níveis de fotossíntese e levando a um aumento na produção de biomassa e compostos secundários (Costa *et al.*, 2012; Ribeiro *et al.*, 2022; Vij *et al.*, 2022; Honorato *et al.*, 2023).

No que se refere às condições nutricionais de cultivo, o uso da adubação verde é uma técnica importante que pode trazer diversos benefícios para o cultivo de plantas medicinais e aromáticas (Javanmard *et al.*, 2022). Essa técnica é uma alternativa sustentável e eficiente que, além de fornecer nutrientes, promove a melhoria das propriedades físicas e químicas do solo, proporcionando maiores condições para o desenvolvimento das plantas (Zhang *et al.*, 2023).

A adubação verde é relativamente simples e pode ser facilmente adaptada a diferentes culturas, regiões e sistemas de cultivo. De acordo com (Honorato *et al.*, 2022), a adubação verde melhora a produção e a qualidade do cultivo do tomilho, e quando combinado com esterco bovino melhora a produção de óleo essencial e aumenta a produção de alguns metabolitos secundário como timol.

Embora existam estudos que demonstrem a eficácia do uso de malhas de sombreamento e adubação verde na produção de plantas medicinais, aromáticas e condimentares, é importante ressaltar que cada espécie responde de forma específica ao método de manejo adotado. Neste contexto, objetivou-se avaliar o desempenho da planta de *Origanum majorana* L. cultivada sob malhas de sombreamento coloridas e diferentes doses de adubo verde utilizando *Cajanus cajan* (L.) Millsp.

2 REFERENCIAL TEÓRICO

2.1 Aspectos gerais da espécie

A *Origanum majorana* L., tem como sinônimo *Majorana hortensis* Moench. também conhecida como manjerona, manjerona-doce, manjerona verdadeira, é uma espécie perene pertencente à família Lamiaceae, originária do sul da Europa e que ocorre de forma espontânea nas regiões mediterrâneas. É uma espécie de grande importância econômica, sendo amplamente reconhecida como uma erva culinária utilizada em diversos pratos (Krishnakumar e Potty, 2012).

A *O. majorana* é uma planta amplamente cultivada e empregada na culinária como especiaria, contribuindo para realçar o sabor e aroma de uma ampla variedade de pratos (Thanh *et al.*, 2019; Chaudhari *et al.*, 2020). Suas folhas e flores são altamente aromáticas, podendo ser usadas tanto frescas quanto secas em diversos pratos, como sopas, pizzas, carnes, saladas, peixes, omeletes, batatas fritas, molhos e recheios (Van Wyk, 2013).

O. majorana é um arbusto perene espesso e resistente que pode comportar-se como planta anual em algumas condições. Suas hastes são cilíndricas e lenhosas, com uma cor marrom avermelhada, com galhos descendentes e multi-ramificados que se espalham em forma de touceiras, atingindo alturas que variam entre 15 e 80 cm. As folhas são simples e opostas umas às outras, apresentando tricomas. Além disso, os pecíolos possuem uma forma oval alongada e uma coloração cinza distintiva (Faleiro *et al.*, 2005; Pimple *et al.*, 2012).

As plantas de *O. majorana* se caracteriza por suas flores hermafroditas, pequenas e de tonalidade rosa pálido ou branco, com lábios duplos, que florescem em espigas, geralmente no final do verão. As sementes são pequenas, ovais e apresentam uma coloração marrom escura. Suas raízes possuem uma forma subcilíndrica, exibindo rugosidades longitudinais e fissuras transversais. A superfície externa da raiz é marrom escura, enquanto a superfície interna é marrom claro, emitindo odor aromático característico e sabor não amargo (Al-Harbi, 2011; Muqaddas *et al.*, 2016).

É uma espécie de clima tropical, quente e úmido, que não tolera temperaturas abaixo de 10ºC, devendo ser protegida de ventos fortes. A planta desenvolve-se melhor em terrenos arenosos, ricos em matéria orgânica e com boa drenagem (Krishnakumar e Potty, 2012). No entanto, pode também prosperar em uma ampla faixa de pH, em solos ácidos a neutro básico. Em seus habitats naturais de origem e subespontâneos, geralmente crescem em áreas secas e rochosas, de 100 a 1500 m de altitude em relação ao nível de mar (Ietswaart, 1980). A manjerona é facilmente propagada a partir de estacas, demonstrando alta capacidade de enraizamento em diferentes estações do ano (Ziech *et al.*, 2020). Além disso, tem crescimento favorável em áreas sombreadas ou em pleno sol e apresenta boa tolerância a seca (Castro, 2003; Pandey *et al.*, 2019).

As hastes e flores da manjerona possuem propriedades medicinais, podendo conter de 1% a 2% de um óleo essencial composto por terpenóides, taninos, compostos fenólicos, carotenos e vitamina C. A *O. majorana* apresenta várias propriedades farmacológicas relatadas na literatura, tais como função hepatoprotetora (Hassanen, 2012; Pasavei *et al.*, 2020), cardioprotetora e anticoagulante (Ramadan *et al.*, 2013), antiulcerosa (Al-Howiriny *et al.*, 2009), antiproliferativa (Ramazan *et al.*, 2016), antifúngica (Amor *et al.*, 2019), ansiolítica (Amaghnouje *et al.*, 2020; Sales *et al.*, 2020), antidiabética (Tahraoui *et al.*, 2007), anticonvulsivante (Sahranavard *et al.*, 2014), antimutagênica (Qari, 2008), antiprotozoária (Prerna e Vasudeva, 2015), anti-inflamatória (Arranz *et al.*, 2019; Gheitasi *et al.*, 2021), atividades antioxidante (Ouedrhiri *et al.*, 2021) e antibacteriana (Bouyahya *et al.*, 2021).

Além das propriedades medicinais mencionadas, a manjerona é tradicionalmente utilizada no tratamento de distúrbios gastrointestinais, como dores de estômago e cãibras. Além disso, é eficaz contra cefaleias, resfriados e febres. Sua aplicação se estende a problemas digestivos, sendo considerada antitussígena, antiespasmódica e útil no combate à diarreia aguda. Ela também, demonstra efeitos tônicos, expectorantes, estimulantes, carminativos e antissépticos, proporcionando alívio para uma ampla gama de condições (Rauf *et al.*, 2021). A *O. majorana* também pode funcionar como um agente curativo para dor de dente, reumatismo, indigestão e asma. As hastes e folhas da planta são usadas para fazer infusões, tinturas e pós. Pessoas que sofrem de flatulência, náusea, inchaço abdominal e pequenos problemas neurais podem se beneficiar do consumo de uma infusão feita com esta erva (Muqaddas *et al.*, 2016).

2.2 Constituintes fitoquímicos de *Origanum majorana* L.

Os fitoquímicos são compostos químicos naturais sintetizados pelas plantas, que desempenham um papel vital na sua defesa contra estresses bióticos e abióticos (Koç e Karayiğit, 2023). Esta classe heterogênea de compostos, embora não participe em sua maioria de funções metabólicas essenciais (Gutiérrez-Grijalva *et al.*, 2017), desempenha um papel vital como mecanismo de defesa fisiológica contra patógenos, pragas, herbívoros, luz ultravioleta e estresse oxidativo (Rajčević *et al.*, 2023). O conteúdo de fitoquímicos nas plantas é influenciado por vários fatores, incluindo a cultivar, localização geográfica, clima, luz do dia, temperatura, condições do solo, estresse hídrico e época de colheita entre outros (Koç e Karayiğit, 2023). Esta complexidade química também se manifesta nos óleos essenciais.

Os óleos essenciais são misturas complexas de substâncias voláteis, tipicamente lipofílicas, geralmente odoríferas e líquidas. Sua principal característica é a volatilidade, que os diferencia dos óleos fixos (Simões *et al.*, 2010). Esses óleos são produzidos como parte do metabolismo secundário das plantas, e sua composição e intensidade podem variar dependendo da espécie, condições ambientais e estágio de desenvolvimento da planta. Em algumas famílias botânicas, os óleos essenciais são armazenados em estruturas secretoras especializadas, tais como pêlos glandulares, células parenquimáticas diferenciadas, canais oleíferos, bolsas lisígenas ou esquizolisígenas (Dhifi *et al.*, 2016).

Os óleos essenciais exibem uma composição química complexa, contendo uma ampla gama de compostos orgânicos. Essa mistura inclui concentrações variáveis de hidrocarbonetos terpênicos, álcoois simples e terpênicos, aldeídos, cetonas, fenóis, ésteres, éteres, óxidos, peróxidos, furanos, ácidos orgânicos, lactonas, cumarinas e compostos de enxofre (Dhifi *et al.*, 2016). Geralmente, os compostos terpenóides derivados da rota metabólica do ácido mevalônico, e os fenilpropanóides, derivados da rota do ácido chiquímico, são as classes mais encontradas. Os compostos terpênicos mais frequente nos óleos voláteis são os monoterpenos e os sesqueterpenos (Dewick, 2002).

O óleo essencial extraído da *Origanum majorana* L. é altamente valorizado tanto pelo seu potencial alimentício quanto pelas suas propriedades medicinais e aromáticas, sendo amplamente utilizado na indústria alimentícia, farmacêutica e cosmética (Paudel *et al.*, 2022). A sua composição é rica em compostos voláteis e aromáticos, os quais contribuem para o seu aroma singular, que pode variar de quente e picante a canforado, com toques amadeirados e herbáceos. A cor do óleo essencial pode variar de amarelo pálido a amarelo incolor ou esverdeado, enquanto o seu odor característico é frequentemente descrito como uma mistura de noz-moscada e menta (Pimple *et al.*, 2012).

A composição e a concentração dos componentes do óleo essencial de *Origanum majorana* L. podem variar de acordo com o clima e a região geográfica onde a planta é cultivada. Isso pode levar à presença de diferentes quimiotipos (Fathy *et al.*, 2009). Na literatura, é encontrado dois quimiotipos mais comuns para os óleos essenciais de *Origanum majorana* L., um rico em timol e/ou carvacrol (Sarer *et al.*, 1982; Baser *et al.*, 1993) e o outro contendo terpinen-4-ol e hidrato de sabineno como componentes principais (Vera e Chane-Ming, 1999; Ezzeddine *et al.*, 2001; Banchio *et al.*, 2008). O quimiotipo terpinen-4-ol e hidrato de sabineno é mais comum na Europa, enquanto o quimiotipo rico em timol e/ou carvacrol é mais comum no Oriente Médio e norte da África (Bağci *et al.*, 2017; Ghazal *et al.*, 2022; Kordali *et al.*, 2022).

Conforme relatado na literatura, existe uma considerável variação na composição química do quimiotipo terpinen-4-ol e hidrato de sabineno. De acordo com o estudo de Farsi *et al.* (2019), os principais constituintes são os monoterpenos oxigenados hidrato de *trans*-sabineno (30,39%), terpinen-4-ol (23,73%), hidrato de *cis*-sabineno (6,40%), α-terpineol (5,59 %) e, os hidrocarbonetos monoterpênicos γ-terpineno (7,58%), sabineno (5,66%) e α-terpineno (4,07%). Omidbaigi e Bastan (2005) também relataram como principais compostos o terpinen-4-ol (21,3%), o hidrato de *trans*-sabineno (14,8%), o acetato de hidrato de *cis*-sabineno (10,7%), γ-terpineno (10,7%), α-terpineno (7,3%), sabineno (5,7%), α-terpineol (4,7%), hidrato de *cis*-sabineno (4,5%), limoneno (3,0%) acetato de linalil (3,0%) e terpinoleno (2,6%), os quais representaram 99,2% da composição química total do óleo essencial.

Elansary e Mahmoud (2015) ao avaliar a composição do óleo essencial de 3 acessos de *Origanum majorana* L., observaram como constituintes principais para o acesso OM550 o hidrato de *cis*-sabineno (34,3%), o β-terpineno (18%), o 4-terpineol (15,20%), o terpinoleno (11,8%) e o sabineno (7,40%). Para OM555 foram identificados o α-terpineno (29,20%), hidrato de *cis*-sabineno (15,40%), terpinoleno (19,50%), e 4-terpineol (14,40%). Para o acesso OM560 foram relatados o 4-terpineol (35%), hidrato de *cis*-sabineno (20%), terpinoleno (10,33%), α-terpineno (8,96%) e sabineno (8,40%).

Novak *et al.* (2008), identificaram três quimiotipos diferentes em uma população natural *Origanum majorana* L. no Chipre. Além do quimiotipo mais comum para esta região (quimiotipo sabinil, por conter principalmente sabineno, hidrato de *trans* e *cis*-sabineno e acetato de hidrato de *cis*-sabineno), foram encontrados mais dois quimiotipos: um contendo α-terpineol (73%) e outro caracterizado pela presença de sabinil (41%) e α-terpineol (40%).

Dentre os quimiotipos do óleo essencial de *Origanum majorana* L. descritos na literatura, além dos mencionados anteriormente, há relatos de um rico em terpinen-4-ol (31,6%) e α-Terpineol (11,4%) (Fadel *et al.* (2020). Outros autores, como (Gharib e Silva, 2013; Cunha *et al.*, 2018), também apontaram nos seus estudos os quimiotipos terpinen-4-ol e/ou α-terpineol. Portanto, pode-se observar que há uma variedade de quimiotipos do óleo essencial de *Origanum majorana* L., com diferentes composições químicas e propriedades terapêuticas.

2.3 Estresse oxidativo e metabolismo antioxidante

Radicais livres são gerados continuamente nas plantas e desempenham papéis vitais quando produzidos em quantidades apropriadas, como na transferência de elétrons. Todavia, a superprodução desses radicais nos processos metabólicos pode resultar em danos oxidativos, levando ao desenvolvimento de mecanismos antioxidantes destinados a restringir os níveis intracelulares dessas espécies reativas e controlar a ocorrência de danos associados (Jones, 2013).

A exemplo, as plantas aeróbicas metabolizam em condições fisiológicas uma grande parte do oxigênio (O_2) nas mitocôndrias, principalmente através da cadeia transportadora de elétrons. No entanto, uma pequena porção desse oxigênio é desviada para outras vias metabólicas, onde sofre oxidação univalente, resultando na formação de radicais livres (Sies *et al.*, 2017).

Essa produção contínua de radicais livres durante os processos metabólicos nas plantas leva ao desenvolvimento de mecanismos de defesa antioxidante, cujo objetivo é limitar os níveis intracelulares dessas espécies reativas, controlando assim os danos potencialmente prejudiciais resultantes do estresse oxidativo (Hussain *et al.*, 2019).

As espécies reativas de oxigênio têm a capacidade de remover elétrons de moléculas orgânicas, convertendo-as em radicais, que têm a capacidade de iniciar reações em cadeia. Dentre estes, o superóxido ($O_2^{-\bullet}$) é o primeiro produto gerado durante a redução do oxigênio de seu estado fundamental e pode passar por reações de oxidação e redução. O superóxido pode reagir com algumas moléculas para gerar outras espécies reativas ou pode ser convertido em peróxido de hidrogênio (H_2O_2), seja espontaneamente ou por ação enzimática. Embora o peróxido de hidrogênio não seja um radical livre, ele atua como um agente oxidante ou redutor em diversas reações celulares (Sies, 2017).

Ao contrário do superóxido, o peróxido de hidrogênio é altamente permeável através das membranas e pode inativar diretamente algumas enzimas sensíveis, mesmo em concentrações muito baixas. Devido à sua estabilidade, o peróxido de hidrogênio é menos tóxico em comparação com outras espécies reativas de oxigênio (Sies, 2014). Para regular a produção de ERO em níveis fisiológicos normais e para combater o aumento de ERO em condições de estresse, as plantas desenvolveram uma ampla gama de componentes químicos e estratégias, conhecidas como antioxidantes (Huang *et al.*, 2019; Mansoor *et al.*, 2022).

Esse sistema de defesa antioxidante, tanto enzimático quanto não enzimático, neutraliza a formação de Espécies Reativas de Oxigênio (ERO). Esse mecanismo engloba uma variedade de antioxidantes enzimáticos, como Superóxido Dismutase (SOD), Ascorbato Peroxidase (APX), Catalase (CAT), dentre outras enzimas, de mecanismos não-antioxidantes que atuam de forma coordenada para manter a homeostase redox subcelular, eliminando diversas produções de ERO (Xie *et al.*, 2019).

Os antioxidantes não enzimáticos geralmente são moléculas de baixo peso molecular que complementam a ação das enzimas antioxidantes. A exemplo, os fenólicos, como os flavonoides e os carotenoides (Ahmad *et al.*, 2010).

Os fenólicos, que incluem metabólitos secundários com diversas funções, como os flavonoides, são abundantes em folhas e órgãos reprodutivos e são principalmente localizados no vacúolo e no apoplasto. Portanto, plantas ricas em flavonoides, como as antocianinas, exibem uma maior capacidade antioxidante. Além de agirem na extinção do peróxido de hidrogênio, essas substâncias podem também atuar como quelantes de metais, pois podem doar prótons ou elétrons (Farida *et al.*, 2020). Além disso, observa-se ainda que fatores de estresse abióticos, como por exemplo a radiação UV-B, estimulam a síntese de flavonoides nas plantas, reforçando, assim, o papel protetor desses compostos fenólicos (Pehlivan Karakas *et al.*, 2022).

Outro antioxidante não enzimático são os carotenoides, compostos lipossolúveis derivados de isoprenoides, entre os quais se destacam o β-caroteno, o licopeno e a zeaxantina. Eles são principalmente encontrados em folhas, flores e frutos, desempenhando um papel essencial na absorção de luz durante o processo de fotossíntese, além de desempenharem um papel crucial na proteção das clorofilas contra danos causados pela luz. Os carotenoides são amplamente reconhecidos por suas propriedades antioxidantes, pois têm a capacidade de neutralizar o oxigênio singleto no interior dos cloroplastos e combater radicais que surgem como resultado da peroxidação lipídica (Tuna *et al.*, 2013; Ashraf *et al.*, 2019).

Para manter um controle eficaz sobre a quantidade de ERO no organismo vegetal, uma gama de enzimas antioxidantes, como a SOD e a APX, estão amplamente distribuídas na célula vegetal, ocupando diversos compartimentos, incluindo os cloroplastos, o citosol, as mitocôndrias, os peroxissomos e o apoplasto. Outras enzimas, como a CAT, têm uma localização mais específica, sendo principalmente peroxissomais (Vasconcelos *et al.*, 2009; You e Chan, 2015).

A SOD é uma enzima crucial, desempenha um papel fundamental na conversão do superóxido (O_{2-}) em peróxido de hidrogênio (H_2O_2). Isso ocorre porque o peróxido de hidrogênio é menos reativo e menos prejudicial em comparação com o superóxido, embora, na presença de metais de transição, possa reagir com o superóxido, gerando radicais hidroxila (OH^-) altamente reativos. As SODs são metaloproteínas que podem usar Cu, Zn, Mn ou Fe como cofatores, e estão distribuídas em vários compartimentos celulares, incluindo os cloroplastos, as mitocôndrias, o citosol, os peroxissomos e o apoplasto (Koyro *et al.*, 2012).

Já a CAT é uma enzima que desempenha um papel importante na degradação do H_2O_2, que, embora seja menos reativo que o superóxido, é lipossolúvel e, portanto, capaz de reagir com o superóxido ou metais de transição. Enzimas como as peroxidases, encontradas no citosol e associadas à parede celular, assim como a catalase peroxissomal ou glioxissomal, estão envolvidas nesse processo de eliminação do peróxido de hidrogênio (Vasconcelos *et al.*, 2009).

A APX também desempenha um papel essencial na remoção do H_2O_2 e, assim como seu substrato, o ácido ascórbico, está amplamente distribuída em praticamente todas as organelas celulares. A APX está envolvida nos ciclos da água nos cloroplastos e do glutationa-ascorbato, apresentando uma afinidade maior pelo peróxido de hidrogênio em comparação com a catalase, o que a torna uma peça-chave no metabolismo antioxidante das plantas (Caverzan *et al.*, 2012; Maruta e Ishikawa, 2017; Rajput *et al.*, 2021).

2.4 Importância da luz no crescimento e metabolismo vegetal

A luz é indispensável ao curso de vida das plantas. Os principais fatores relacionados à radiação luminosa incluem fotoperíodo (duração), intensidade (quantidade), direção e qualidade (frequência ou comprimento de onda) (Zoratti *et al.*, 2014). A princípio, a luz desempenha um papel insubstituível na promoção do crescimento e na indução ou regulação do metabolismo das plantas. Em resposta à radiação luminosa, as plantas são capazes de se adaptar às mudanças das circunstâncias pela liberação e acúmulo de vários metabólitos secundários (Yang *et al.*, 2018).

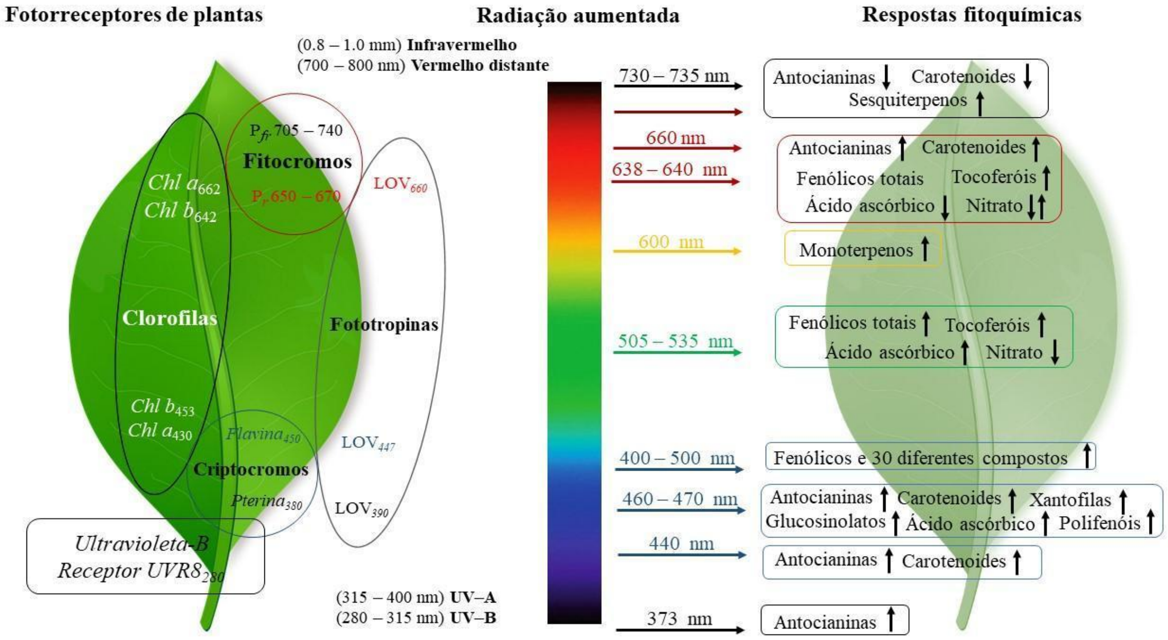
A qualidade da luz é determinada pelo espectro de luz, representado pelos fótons de diferentes comprimentos de ondas, cuja capacidade energética varia de acordo com a faixa eletromagnética visível. A disponibilidade de luz visível nas radiações fotossinteticamente ativas nas regiões azul (420 a 490 nm) e vermelha (620 a 660 nm) é a principal responsável pelas respostas de crescimento das plantas (Holopainen *et al.*, 2018).

Na natureza, a transferência de energia entre as plantas e o meio ambiente ocorre principalmente por meio da radiação solar. A radiação solar é a principal fonte de energia para as plantas, sendo que uma parte significativa dessa energia é convertida em calor e afeta processos como transpiração e fotossíntese (Taiz *et al.*, 2017). Além disso, a radiação solar desempenha um papel importante na determinação da temperatura dos tecidos vegetais, o que por sua vez influencia as taxas de processos metabólicos e o equilíbrio entre eles (Jones, 2013).

As plantas comportam-se de maneira diferente quanto à qualidade a luz. Essas particularidades tornam-se importantes objetos de estudos na busca por produtos de melhor qualidade(Costa *et al.*, 2019). Portanto, a luz é um dos fatores ambientais mais importantes, que influenciam o crescimento e o metabolismo secundário das plantas.

De acordo com (Holopainen *et al*., 2018), os diferentes comprimentos de onda na faixa da luz visível, bem como as faixas de luz UV (<400 *nm*) e vermelho distante (>700 *nm*) desempenham papéis cruciais na modulação das defesas químicas das plantas contra estresses bióticos e abióticos, além de ativar vias metabólicas específicas. Os pigmentos fotorreceptores das plantas respondem a diferentes picos de absorbância, e comprimentos de onda específicos exercem influência sobre os principais grupos fitoquímicos presentes nas culturas, conforme ilustrado na Figura 1.

Figura 1 - Visão geral da influência do comprimento de onda no acúmulo de fitoquímicos de plantas cultivadas em ambientes protegidos.



Adaptado de (Holopainen *et al*., 2018).

As luzes vermelha, azul e UV aumentam a concentração de óleos essenciais em várias espécies em comparação com a luz branca ou a luz solar. No entanto, o nível de aprimoramento varia entre as diferentes espécies, seus constituintes químicos e os tratamentos luminosos aplicados. Por exemplo, o teor total de óleo essencial de *Mentha piperita* L., *Mentha spicata* L. e *Mentha longifolia* L. foi mais elevado sob luz vermelha a 500 µmol m^2 s^{-1} PPF e fotoperíodo de 16 horas por 60 dias, 39% maior do que sob a luz azul e 86% maior do que sob a branca, conforme observado por (Sabzalian *et al*., 2014).

Os compostos fenólicos, flavonóides e antocianinas presentes nas plantas podem ser enriquecidos com luz vermelha, azul ou UV, para fornecer produtos naturais benéficos à saúde dos seres humanos (Dou *et al*., 2017). Os tratamentos monocromáticos de luz vermelha ou azul aumentaram significativamente as concentrações de compostos fenólicos totais, flavonóides e antocianinas nas folhas e raiz de *Rehmannia glutinosa* Libosch. em comparação com a luz branca, e a luz azul foi mais eficiente que a luz vermelha (Manivannan *et al.*, 2015).

A luz vermelha, azul e UV podem ser usadas como processos eficazes para melhorar a biossíntese de metabólitos secundários das plantas. Contudo, além de aumentar as concentrações de óleos essenciais, a qualidade da luz também pode alterar as composições de óleos essenciais das plantas. A exemplo, Noguchi e Amaki (2016) verificaram que *Plectranthus amboinicus* Lour. cultivada sob luz azul exibiu maiores quantidades de compostos do grupo sesquiterpeno, enquanto plantas sob luz vermelha apresentaram maiores quantidades de compostos do grupo monoterpeno. Portanto, a qualidade da luz pode ser manipulada para aumentar as concentrações de compostos direcionadas para diversos fins (Noguchi e Amaki, 2016).

Na agricultura moderna, a manipulação ativa do ambiente de cultivo tem sido amplamente adotada para otimizar a produção e a qualidade das plantas (Dueck *et al.*, 2016). Com a finalidade de melhorar o rendimento e a qualidade das plantas, o conhecimento sobre como as elas respondem à luz, permitiu o desenvolvimento de técnicas para manipulação do espectro de luz solar incidente nos cultivos incluindo o do uso de malhas coloridas. Essas malhas são consideradas uma alternativa eficaz para aprimorar a qualidade da luz (Coles *et al.*, 2021).

Portanto, as malhas de sombreamento coloridas, podem afeta algumas plantas, modificando o espectro de luz e, consequentemente, influenciando o crescimento, desenvolvimento e acúmulo de metabolitos secundários (Ilić e Fallik, 2017). Entretanto, as respostas das plantas a luz variam de acordo com a espécie e a cor da tela utilizada (Costa *et al.*, 2012; Ribeiro *et al.*, 2018; Ribeiro *et al.*, 2022; Vij *et al.*, 2022; Honorato *et al.*, 2023).

Neste sentido, Corrêa *et al.* (2012) estudando o uso de malhas coloridas no cultivo de *Origanum vulgare* L. verificou um crescimento significativo nas plantas, no teor, rendimento e na qualidade do óleo essencial de orégano. Também foi observado que o uso de malhas coloridas somado ao uso de adubação orgânica favorece o crescimento do orégano, aumentando a eficiência do seu uso, conforme relatado por (Oliveira *et al.*, 2017).

Além disso, as malhas coloridas constituem um elemento que combina proteção física (contra aves, granizo, insetos, radiação excessiva), modificações ambientais (umidade, sombra, temperatura), aumenta a proporção relativa de luz difusa (dispersa), juntamente com a filtragem diferenciada da radiação solar alterando o espectro de luz visível, para promover respostas fisiológicas desejáveis, reguladas pela luz (Pérez *et al.*, 2006).

2.5 Adubação verde

A alta demanda por uma agricultura sustentável tem levado à exploração de novos sistemas de produção, que envolvem a substituição de insumos sintéticos por orgânicos. Essa abordagem traz um impacto positivo nas características físico-químicas e biológicas do solo, e consequentemente, resultando em aumento no rendimento das culturas (Pinto *et al.*, 2017). Dentre as alternativas de adubação, destaca-se a adubação verde como uma ferramenta importante em qualquer sistema de produção de plantas, sobretudo em espécies com fins medicinais (Honorato *et al.*, 2022).

O adubo verde é uma prática que consiste em incorporar matéria orgânica ao solo para melhorar as propriedades físico-químicas do mesmo (Rodríguez-Ortiz *et al.*, 2020). Através da incorporação de resíduos de colheita, gramíneas, leguminosas ou outros materiais vegetais no solo, a adubação verde ajuda a aumentar o teor de matéria orgânica, melhorar a estrutura do solo e fornecer uma fonte de nutrientes essenciais para as culturas (Marques *et al.*, 2018). Também contribui para promover a atividade microbiana benéfica no solo, que pode melhorar o crescimento e a produção das plantas. Além disso, o adubo verde pode ajudar a reduzir a erosão e melhorar a infiltração de água, além de fornecer uma fonte natural de supressão de plantas daninhas (Ma *et al.*, 2021).

A adubação verde é especialmente importante para plantas medicinais, aromáticas e condimentares porque essas culturas têm necessidades específicas de nutrientes para produzir os compostos medicinais desejados (Ostadi *et al.*, 2020). Ao usar adubo verde para culturas de plantas medicinais, é importante escolher uma cultura de cobertura que não irá competir com as plantas medicinais por nutrientes ou água. Leguminosas, como *C. cajan*, costumam ser escolhas assertivas para esse fim, pois fixam o nitrogênio no solo, reduzindo a necessidade de adubos sintéticos (Ragozo *et al.*, 2014).

O *C. cajan*, conhecido como feijão guandu, é uma leguminosa nativa da Índia cultivada principalmente por sua capacidade de fixar nitrogênio no solo, tornando-se uma valiosa fonte de adubo verde (Mendonça *et al.*, 2017). Esta planta de crescimento rápido tem a notável habilidade de cobrir o solo rapidamente, desempenhando um papel fundamental na fertilização (Lima Filho *et al.*, 2023).

Além de sua rápida cobertura do solo, o *C. cajan* estabelece relações simbióticas benéficas com fungos e bactérias do solo. Essa simbiose é crucial para converter o nitrogênio atmosférico em uma forma utilizável pelas plantas, reduzindo a necessidade de insumos sintéticos potencialmente danosos ao meio ambiente (Fonseca *et al.*, 2023). A capacidade do

C. cajan de associar-se simbioticamente com bactérias do gênero *Rhizobium* confere-lhe a habilidade de fixar nitrogênio atmosférico (Sarmento *et al.*, 2019).

O *C. cajan*, apresenta baixa relação C:N e alta fixação de N, podendo ser uma escolha adequada para ser utilizada como adubo verde. A relação C/N da biomassa da cultura de cobertura é um fator muito importante a ser considerado ao selecionar uma planta para esse fim. O *C. cajan* é uma que pode ser utilizada como adubo verde e pode apresentar rendimento de biomassa seca entre 8 e 12 t ha^{-1}, Relação C:N 15-22 e potencial de fixação de nitrogênio de 37 a 280 kg ano (Lima Filho *et al.*, 2023). a adição de resíduos de leguminosas com baixa composição C:N pode resultar na mineralização do N (Rodríguez *et al.*, 2022). A rápida decomposição da biomassa do *C. cajan* libera nutrientes de maneira eficiente, tornando-os prontamente disponíveis para as plantas, acelerando a ciclagem de nutrientes no solo.

A eficácia na utilização de adubo verde em cultivos de plantas medicinais tem sido demonstrada. Marques *et al.* (2018) verificou que *Mucuna aterrima* (Pipper & Tracy.) aumentou a produção de biomassa e o rendimento de óleo de *Lippia alba* (Mill.). A adubação verde melhora a estrutura do solo, o que pode aumentar a disponibilidade de nutrientes para as plantas medicinais. Além disso, o uso de adubação orgânica pode contribuir nas relações simbióticas com microrganismos, favorecendo no aumento de biomassa, e consequentemente, no rendimento de óleo essencial e constituintes químicos (Assis *et al.*, 2020).

No geral, a incorporação de adubo verde nas práticas de cultivo de plantas medicinais pode levar a plantas mais saudáveis, rendimentos mais altos e uma abordagem mais sustentável da agricultura (Lei *et al.*, 2022). Pesquisas que comprovem a eficácia do uso de adubos verdes como *C. cajan* no cultivo de plantas medicinais, incluindo *Origanum majorana* L., são necessárias para reduzir o uso de insumos químicos e maximizar a relação custo/benefício, considerando fatores como produtividade, qualidade da planta, e perfil fitoquímico.

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SEGUNDA PARTE - ARTIGOS

ARTIGO 1 – COLORED NETS AFFECT *Origanum majorana* L. GROWTH, VOLATILE AND PHENOLIC PHYTOCHEMICALS, AND ANTIOXIDANT CAPACITY

MALHAS COLORIDAS AFETAM O CRESCIMENTO, OS FITOQUÍMICOS VOLÁTEIS E FENÓLICOS E A CAPACIDADE ANTIOXIDANTE DE *Origanum majorana* L.

ABSTRACT

The increased search for sustainable and efficient methods to optimize plant growth and bioactive compounds' production has boosted the agricultural sector. Quality of light plays key role in plants' development, mainly in species like *Origanum majorana* L., which is known for its medicinal and aromatic properties. The aim of the current study is to assess vegetative growth, essential oil content and chemical composition, phenolic compounds and total flavones/flavonols' concentration, and antioxidant capacity of *O. majorana* plants grown under different colored shading nets. In order to do so, *O. majorana* plants were grown in four different environments, three of them were under shading nets (blue, black, red) and in full sun. The following parameters were assessed 120 days after seedling transplantation: stem, leaf, root and shoot dry matter; root:shoot ratio; total leaf and specific leaf area; leaf area and leaf weight ratios; relative growth and net assimilation rates; chlorophyll *a* and *b*; total chlorophyll; carotenoids; total antioxidant capacity; total phenol and flavones/flavonols' concentrations; as well as essential oil content, yield and chemical composition. Growing *O. majorana* under different colored shading meshes and in full sunlight has significantly affected the analyzed response variables. The accumulation of total dry matter and root dry matter, as well as the liquid assimilation rate, the content and yield of essential oil, and the α-terpineol content were significantly increased by 93%, 154%, 43%, 75%, 27%, and 62%, respectively, in plants cultivated under full sun compared to those cultivated under the blue net. Additionally, plants grown under full sun and the red net showed a statistically significant increase in leaf, stem, and root dry matter, as well as in relative growth rate. On the other hand, the red and black nets significantly increased total and specific leaf area, as well as the γ-terpinene content compared to the blue net and full sun. The red net increased the ρ-cymene content by 39% compared to full sun. Total antioxidant capacity and total phenolics were significantly increased in full sun and under the black net. Finally, manipulating the light spectrum over *O. majorana* grown either in full sunlight or under red shading net can be a strategy used to get essential oil with differentiated aromatic features, without plant production losses.

Keywords: Marjoram; colored shade nets; biomass; essential oil.

RESUMO

O aumento da busca por métodos sustentáveis e eficientes, visando otimizar o crescimento de plantas e a produção de compostos bioativos, tem impulsionado os setores agrícolas. Nesse contexto, a qualidade da luz desempenha um papel fundamental no desenvolvimento de plantas, especialmente em espécies como *Origanum majorana* L., conhecida por suas propriedades medicinais e aromáticas. Objetivou-se avaliar o crescimento vegetativo, o teor e a composição química do óleo essencial, o doseamento de compostos fenóis e flavonas/flavonóis totais e a capacidade antioxidante de *O. majorana* cultivada sob diferentes malhas de sombreamento coloridas. Plantas de *O. majorana* foram cultivadas em quatro ambientes, sendo três sob malhas de sombreamento (azul, preto, vermelho) e um em pleno sol. Após 120 dias do transplantio, foram avaliados os seguintes parâmetros: matéria seca de caule, folhas, raízes, parte aérea e total, razão raiz/parte aérea, área foliar total e específica, razão área foliar, razão peso foliar, taxa de crescimento relativo e taxa assimilatória liquida, clorofila *a*, *b*, *a/b* e total, carotenoides, capacidade antioxidante total, doseamento de fenóis e flavonas/flavonóis totais, assim como o teor, o rendimento e a composição química do óleo essencial. O cultivo de *O. majorana* sob diferentes malhas de sombreamento coloridas e a exposição à pleno sol teve um impacto significativo nas variáveis analisadas. O acúmulo de matéria seca total e de raiz, bem como a taxa assimilatória liquidada, o teor e rendimento do óleo essencial e o teor de α-terpineol foram significativamente aumentados em 93%, 154%, 43%, 75%, 27% e 62%, respectivamente nas plantas cultivadas sob pleno sol em comparação com aquelas cultivadas sob a malha azul. Além disso, as plantas cultivadas sob pleno sol e malha vermelha apresentaram um aumento estatisticamente significativo na matéria seca das folhas, caule e raiz, assim como na taxa de crescimento relativo. Por outro lado, as malhas vermelha e preta aumentaram significativamente a área foliar total e específica, bem como o teor de γ-terpineno em comparação a malha azul e a pleno sol. A malhas vermelha aumentou o teor de ρ-cimeno em 39% em comparação com pleno sol. A capacidade antioxidante total e fenóis totais foram significativamente aumentadas em pleno sol e na malha preta. Conclui-se que a manipulação do espectro luminoso no cultivo de *O. majorana* sob luz solar plena ou sob malha vermelha podem ser uma estratégia para obtenção de óleo essencial com características aromáticas diferenciadas, sem perdas de produção vegetal.

Palavras-chave: Manjerona; malhas de sombreamento colorida; biomassa; óleo essencial.

1 INTRODUCTION

Marjoram (*Origanum majorana* L.), which is synonymous with *Majorana hortensis* Moench, belongs to family Lamiaceae, which, in its turn, plays great economic and gastronomic importance role. This plant is widely acknowledged for its culinary applications, since its leaves and flowers, either fresh or dried, are used to enhance the flavor of a wide variety of dishes, such as soups, pizzas, meats, salads, fish, omelets, French fries, sauces and fillings (Thanh *et al.*, 2019; Chaudhari *et al.*, 2020).

In addition to its culinary application, marjoram is also used in Ethnomedicine to treat several diseases. The literature reports the use of its leaves to treat gastrointestinal disorders and infections (Vogl *et al.*, 2013), respiratory tract disorders (Charles, 2013a) and hypertension (Tahraoui *et al.*, 2007). On the other hand, *O. majorana* essential oil is used to treat asthma, indigestion and headaches, as well as rheumatoid pains (Baâtour *et al.*, 2012; Erenler *et al.*, 2016). Furthermore, the *O. majorana* has several proven pharmacological properties, such as antioxidant (Erenler *et al.*, 2016; Paudel *et al.*, 2022), antibacterial (Amor *et al.*, 2019), hepatoprotective (Mossa *et al.*, 2013), cardioprotective (Ramadan *et al.*, 2013), antiulcer (Al-Howiriny *et al.*, 2009), anti-inflammatory (Arranz *et al.*, 2015) and antifungal (Paudel *et al.*, 2022) activities.

However, the growth, development, and accumulation of secondary metabolites in medicinal and aromatic plants can be significantly influenced by environmental factors (Milenković *et al.*, 2021). Light intensity and quality exert direct influence on various aspects of plants, such as leaf size, stem growth, vigor, stem-to-root ratio, photoperiod control, metabolite synthesis, among others (Costa *et al.*, 2010).

According to Stagnari *et al.* (2018), the light spectrum transmitted through shading nets impacts the accumulation of secondary metabolites in medicinal plants. In this context, it is possible to change plant metabolism through exposure to a specific light spectrum, as demonstrated in plants of the Lamiaceae family by (Hosseini *et al.*, 2018; Milenković *et al.*, 2019; Milenković *et al.*, 2021). In order to improve the yield and quality of essential oil in plants, various management techniques have been employed. One of these techniques is the use of colored shading nets to manipulate the light spectrum transmitted to the plants (Ribeiro *et al.*, 2018; Coles *et al.*, 2021; Ribeiro *et al.*, 2022; Honorato *et al.*, 2023; Viana *et al.*, 2023).

Photoselective shading nets, in addition to manipulating incident-light quality and microclimate, can be used as alternative to protect plants from adverse environmental conditions, such as excessive exposure to solar radiation, high temperatures, wind, hail, birds and pests (Buthelezi *et al.*, 2016; Ilić and Fallik, 2017; Milenković *et al.*, 2019).

Positive results have been observed for phenolic compound levels and antioxidant capacity in other crops subjected to colored nets, such as lettuce (*Lactuca sativa* L.) (Ilić and Fallik, 2017), chilli pepper (*Capsicum annum* L.) (Díaz-Pérez *et al.*, 2020), thyme (*Thymus vulgaris L.*) (Honorato *et al.*, 2023), as well as for the content, yield and quality of oregano essential oil (*Origanum vulgare* L.) (Corrêa *et al.*, 2012), *Pogostemon cablin* (Blanco) Bentha (Ribeiro *et al.*, 2018) and rosemary (*Lippia gracilis* Schauer) (Viana *et al.*, 2023). However, it is important having in mind that these responses are species-specific, as observed for lemon balm (*Melissa officinalis* L.) (Russo and Honermeier, 2017) and thyme (*Thymus vulgaris* L.) (Honorato *et al.*, 2023), as well as that light spectrum manipulation did not increase leaf dry mass production and essential oil content.

Accordingly, the search for information about *Origanum majorana* L. behavior under the straight influence of light quality can help identifying patterns and correlations between light quality and key aspects, such as growth rate, secondary metabolites' synthesis and physiological processes. These discoveries have the potential to not only improve the understanding about this specific species, but also enable relevant practical applications, such as optimizing agricultural cultivation and production. In light of the foregoing, the aims of the current study were to assess vegetative growth, essential oil content and chemical composition, as well as measuring total phenols, flavones/flavonols, and the antioxidant capacity of *O. majorana* plants grown under different colored nets.

2 MATERIALS AND METHODS

2.1 Plant materials and experimental procedures

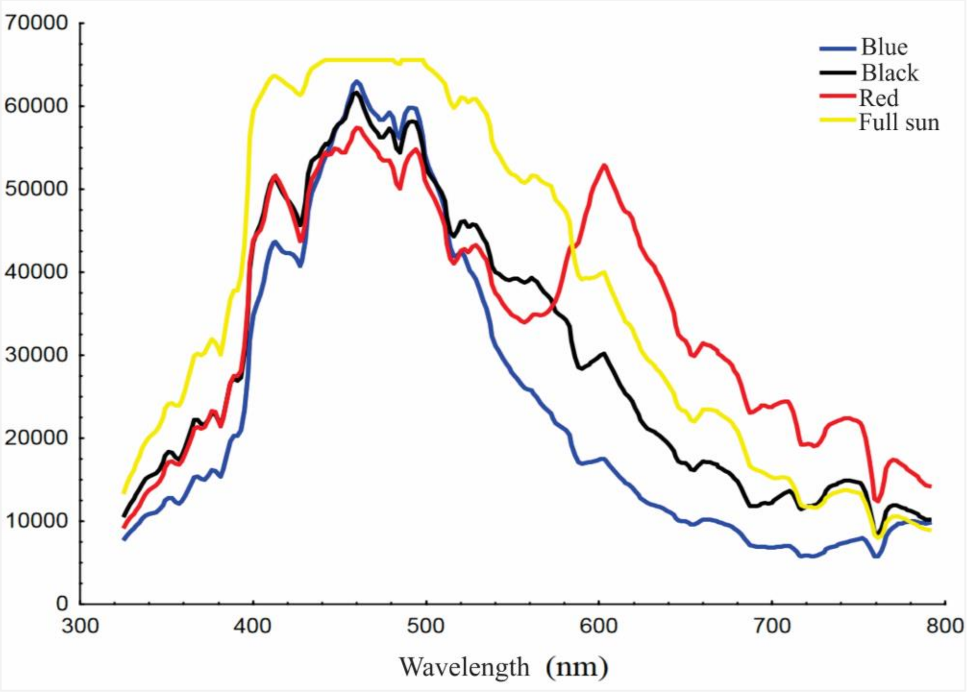
The experiment was conducted in the experimental area of the Medicinal Plants' sector at Federal University of Lavras (UFLA), which is located at the following geographic coordinates: latitude 21º14'43" S and longitude 44º59'59" W, altitude of 918 m. According to Köppen's classification (Alvares *et al.*, 2013), the prevalent climate in the region is Cwa, with two well-defined seasons: dry winter, with mild temperatures, and hot and rainy summer (Martins *et al.*, 2018). The species exsiccate of *Origanum majorana* L. (mixed chemotype α-terpineol, γ-terpinene, and ρ-cymene) was deposited in the herbarium of the Agricultural Research Company of Minas Gerais (also known as EPAMIG), under n. 58898.

Origanum majorana L. seedlings were obtained through apical cuttings (±5 cm), placed in polyethylene tray covered with commercial substrate, and kept in greenhouse under micro sprinkler irrigation. Seedlings measuring approximately 10 cm were transplanted to 10 dm^3 pots (29 x 24.5 x 19 cm, respectively height, top and bottom diameters), based on using soil and sand (2:1) + 150 g of chicken manure as substrate.

The substrate soil comprised a dystrophic red-yellow Oxisol with the following chemical features: pH in water = 6.4; K (mg/dm^3) = 97.8; P-Rem (mg/L) = 1.7; Ca^{2+}, Mg^{2+}, Al^{3+}, H+Al (cmolc/ dm^3) = 1.4; 0.02; 0.00; and 1.7; base saturation index (V%) = 52.38; organic matter (dag/kg) = 1.0; Zn, Fe, Mn, Cu, B and S (mg/dm^3) = 0.3; 27.6; 7.0; 2.5; 0.06; and 60.2, respectively.

The experiment has followed a completely randomized design with four treatments and five repetitions (five plants per repetition) - 100 plants, in total. The experimental unit consisted of a pot containing a single plant, and the spacing between pots was maintained at 50 x 40 cm. Treatments were represented by culture environments under ChromatiNet® shading nets (50%) in the following colors: blue (854 µmol m^{-2} s^{-1}), black (1,131 µmol m^{-2} s^{-1}), red (834 µmol m^{-2} s^{-1}) and full sunlight (2,361 µmol m^{-2} s^{-1}). Light intensity was measured with QSO-S Procheck + Sensor-PAR Photon Flux (Decagon Devices-Pullman-Washington-USA). Absolute light spectrum composition of the colored shading nets is shown in Figure 1.

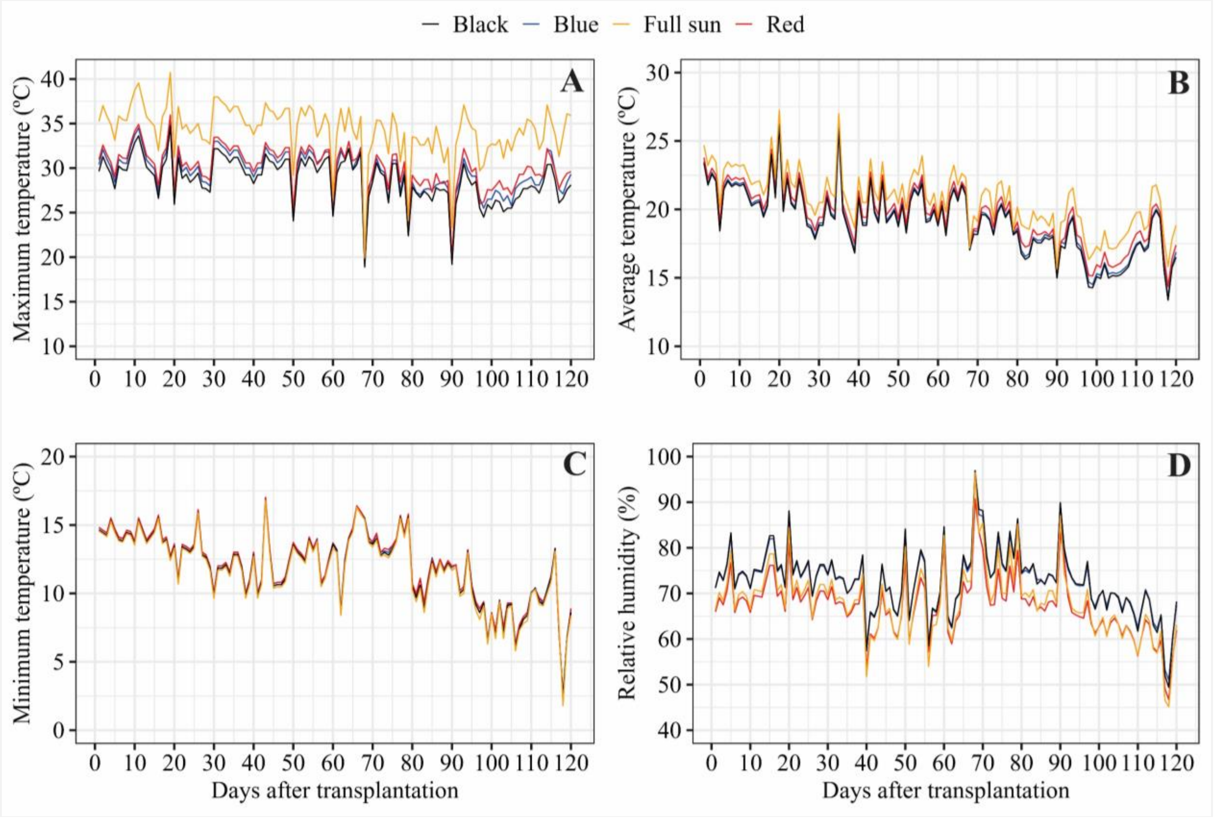
Figure 1 - Composition of the absolute light spectrum of the colored shading nets (blue, black, red) and full sun in the wavelength range of 300-800 nm.



Source: Author (2024).

Elitech Brasil® Datalogger (RC-51H model) was positioned in each treatment to monitor temperature (°C) and relative humidity (%) conditions. The data loggers were programmed to take readings every hour for 120 days. Maximum air temperatures were recorded in full sunlight conditions - values reached approximately 36°C, and their peak reached 40°C at 20 days after transplantation (DAT) (Figure 2C).

Figure 2 - Temperature and humidity during the experiment. Relative humidity (A), average temperature (B), maximum temperature (C), and minimum temperature (D).



Source: Author (2024).

Irrigation was performed manually based on 48-hour watering shift and on applying a varying irrigation blade depending on culture evapotranspiration, calculated by the pot weighing method. It was done to raise the substrate moisture to field capacity and maintain it at approximately 70%, a soil moisture level that allows plants to withdraw water and nutrients adequately.

2.2 Biomass production and growth analysis

At 120 days after transplantation, the plants were harvested by cutting the entire plant. Then, they were segmented into root, stem, and leaf, and dehydrated in a forced-air oven at 37°C until reaching a constant weight. Subsequently, the following growth parameters were assessed: stem (StDM), leaf (LDM), root (RDM), shoot (SDM) and total (TDM) dry matter – results were expressed in g plant^{-1}. The root:shoot (R:S) ratio was calculated based on dividing root dry matter by shoot (leaves + stem) dry matter (Benincasa, 2003).

In total, 20 random fully expanded leaves were collected from each repetition and scanned for leaf area (LA) calculation purposes. LA was measured in previously calibrated Motic® Plus 2.0 software. Subsequently, leaves with known LA were placed in greenhouse equipped with forced ventilation oven (at 37°C), until they reached constant weight. With the area of the 20 leaves in cm² (A20L), the dry matter of the 20 leaves in g^{-1} (DM20L), and the total dry matter of leaves in g per plant^{-1} (TDML), the total leaf area was determined in dm² (TLA), using the formula:

$$\mathrm{TLA} = \left(\frac{\mathrm{TDML}\ x\ \mathrm{A20L}}{\mathrm{DM20L}}\right)/100$$

From the TLA values, the following indices were calculated: Specific leaf area (SLA = LA / LDM), leaf area ratio (LAR = LA / TDM), leaf weight ratio (LWR = LDM / TDM) according to the equations from (Benincasa, 2003).

The Relative Growth Rate (RGR in g g^{-1} week^{-1}) was determined using the formula RGR = $(\ln W_2 - \ln W_1) / (t_2 - t_1)$, where W_1 and W_2 refer to the dry weight of two samplings at t_1 (transplanting) and t_2 (120 DAT). The Net Assimilation Rate (NAR in g dm^{-2} week^{-1}) was calculated based on the dry matter produced per leaf area per unit of time using the formula NAR = $[(W_2 - W_1) / (A_2 - A_1)] \times [(\ln W_2 - \ln W_1) / (t_2 - t_1)]$, where W_1 and W_2 represent the initial and final dry weights, and A_1 and A_2 refer to the LA at t_1 (Transplanting) and t_2 (120 DAT), respectively (Alvarenga *et al.*, 2015).

2.3 Photosynthetic pigments

Photosynthetic pigments' (chlorophylls *a* and *b*, total chlorophylls and carotenoids) extraction and quantification processes have followed the methodology by (Hiscox and Israelstam, 1979), with adaptations by (Barnes *et al.*, 1992). In total, 50 mg of photosynthetically active fresh leaves were collected and placed right away in Falcon tubes filled with 10 ml of dimethylsulfoxide (DMSO) saturated with $CaCO_3$, at the proportion of 5 g L^{-1} DMSO, based on the protocol by (Santos *et al.*, 2008). Samples were analyzed in spectrophotometer, at wavelengths of 649, 665 and 480 nm, after 48-h incubation in greenhouse at 65°C. Results were expressed in µg g^{-1} of fresh leaf. The adopted equations were based on wavelength, according to the method by (Wellburn, 1994), wherein:

$$Chlorophyll\ a_{649} = (12.47\ x\ A_{665}) - (3.62\ x\ A_{649});$$
$$Chlorophyll\ b_{665} = (25.06\ x\ A_{649}) - (6.5\ x\ A_{665});$$
$$Carotenoids_{480} = (1000\ x\ A_{480} - 1.29\ x\ C_a - 53.78\ x\ C_b)/220.$$

The sum of chlorophyll equations (a + b) provided the total chlorophyll content - all values were expressed in mg g^{-1} of fresh matter. The chlorophyll a/b ratio was estimated by dividing the content of Chl *a* by Chl *b*.

2.4 Phenolic compounds and antioxidant capacity quantitation

Majorana originum extracts were prepared based on using 3 g of LDM sprayed in 300 ml ethanol (92.8° INPM), and kept in heat reflux for 6 h. This procedure was followed by residue concentration in rotavap under reduced pressure to remove the solvent and to get the crude extract. These extracts were diluted in ethanol (92.8° INPM), at the concentration of 10 mg mL^{-1}, to prepare the samples.

Total phenol levels in the samples were determined by the colorimetric method based on using Folin-Ciocalteau reagent, in compliance with the colorimetric method described by (Slinkard and Singleton, 1977). A 150 µL sample was mixed to 300 µL of Folin-Ciocalteau reagent and 375 µL of NA_2CO_3 at 7%. Absorbance was measured at 760 nm, after 120-min incubation at room temperature. Total phenol levels were expressed in milligram of gallic acid equivalent (mg GAE) per g of LDM^{-1} (mg GAE/g) based on a calibration curve (y = 0.213 + 13.501, R^2 = 0.99).

Flavones/flavonols contents were determined based on the method described by (Woisky and Salatino, 1998), with modifications. The aliquot of 100 µL of ethanolic leaf extract was added to 100 µL of $AlCl_3$ solution at 10%. Absorbance was measured at 420 nm, after 40-min incubation at room temperature. Total flavones/flavonols levels were expressed in quercetin equivalent milligrams (QE) per g LDM^{-1} (mg QE/g), based on a calibration curve (y = 0.0962 + 19.302x R^2 = 0.99).

Total antioxidant capacity (TAC) was measured based on the ammonium molybdate reduction method described by (Prieto *et al.*, 1999). In total, 200 µl of sample was mixed to 1,500 µL of reagent solution (H_2SO_4 0.6 M, NaH_2PO_4 28 mM, $(NH_4)_6Mo_7O_{24}$ 4 mM) - the mix was incubated at 95°C for 90 min. Solution absorbance was measured at 695 nm. The calibration curve (y = 0.053 + 14.261x R^2 = 0.99) was built and results were expressed in milligrams of ascorbic acid equivalent (AAE) per g of LDM^{-1} (mg AAE/g).

2.5 Essential oil distillation and chemical analysis

Essential oil (EO) extracted from *Origanum majorana* L was hydrodestiled in modified Clevenger device based on using 40 g of LDM per 1 L of distilled water added to a 2 L distillation flask. The EO was purified through liquid-liquid partition conducted with CH_2CL_2 (3 x 5 mL). The organic phase was combined, treated with anhydrous $MgSO_4$ and filtered. The EO was collected and transferred to an amber bottle in order to have its essential oil content (mg/100 g^{-1} of leaf dry matter) and essential oil yield (mg/$plant^{-1}$) determined. The EO was stored under refrigeration (at 4°C) until chemical analysis time.

Quantitative EO analyses were performed through gas chromatography coupled to a hydrogen flame (CG-FID) ionization detector in gas chromatography system Agilent®7890a operated with HP GC Chemstation Ver. A.01.14 data processing system and equipped with injector/automatic sampler (Combapal Autosampler System, CTC Analytic AG, Switzerland).

The oil was diluted in ethyl acetate (1%, V/V) and automatically injected in the chromatographer at injection volume of 1.0 µL, at 50:1 split mode ratio. Injector and detector temperature was kept at 220°C and 240°C, respectively. The initial oven temperature was 60°C, with temperature ramp at 3°C min^{-1}, until it reached 240°C. This ramp was followed by another one at 10°C min^{-1} until it reached 280°C. Analyses were performed in triplicate (n = 3) and analyte concentrations were expressed in relative area rate standardized to chromatographic peaks.

Qualitative analyses were performed through gas chromatograph mass spectrometry (GC-MS) carried out in Agilent ® 5975C mass spectrometer operated through 70-eV electron impact ionization, at 1.0 scan/s scanning mode and 40–500 m/z mass acquisition range. The same operation conditions adopted for quantitative analyses were herein used. Retention rates corresponding to the co-injection of *n*-alkane standards, C_8-C_{20} (Sigma Chemical Co., St. Louis, MO, USA), were calculated based on the equation by (Van Den Dool and Dec. Kratz, 1963). Chemical constituents were identified by comparing their calculated retention rates and mass spectra to retention index data available in the literature (Adams, 2007), and in the Mass Spectral Library of the National Institute of Standards and Technology (Nist, 2008).

Statistical analysis

Experimental data were subjected to statistical analysis in R software version 4.1.2. Variance homogeneity (Levene and Bartlett, $p \geq 0.05$) and data normality (Shapiro-Wilk, $p \geq 0.05$) tests were initially performed based on using the “car” package (Fox *et al.*, 2022).

Residues showing normality were subjected to analysis of variance (ANOVA) through F Test ($p \leq 0.05$).

Mean values were compared through Tukey test ($p \leq 0.05$) based on using the “Expde.pt” package (Ferreira *et al.*, 2021). In addition, principal component analysis (PCA) was performed based on using the fviz_pca_biplot function of “factoextra” package to analyze the effect of treatments in a broader manner (Kassambara and Mundt, 2020).

3 RESULTS AND DISCUSSIONS

3.1 Dry matter yield and growth analysis

Results have indicated that the dry matter yield of *O. majorana* plants was significantly influenced by culture under colored nets (Table 1). Plants grown under full sun conditions showed higher accumulation of dry matter in vegetative organs compared to those under the blue and black shading nets. However, there was no significant difference between plants grown in full sun and those cultivated under the red shading net for the variables of stem, leaf, and aboveground dry matter. A significant reduction in biomass accumulation was observed in *O. majorana* plants grown under blue and black shading nets. The negative effect of colored shading nets was also observed by(Costa *et al.*, 2012; Honorato *et al.*, 2023).

Table 1 – Accumulation of leaves (LDM), stems (StDM), shoot (SDM), roots (RDM), and total (TDM) dry matter, and root-to-shoot ratio (R/SR) in *Origanum majorana* grown under different light spectra.

Treatments	LDM	StDM	SDM	RDM	TDM	R/SR
			g^{-1}			g g^{-1}
Blue	11.60±0.4 c	4.47±0.7 c	16.07±1.0 c	10.97±0.5 d	27.04±1.1 d	0.68±0.0 b
Black	13.69±1.5 bc	5.60±0.3 b	19.29±1.6 b	13.25±0.4 c	32.54±1.8 c	0.69±0.1 b
Red	14.43±1.8 ab	6.44±0.6 ab	20.87±2.3 ab	16.31±0.8 b	37.17±1.8 b	0.84±0.2 b
Full sun	16.21±1.3 a	7.24±0.7 a	23.45±1.2 a	27.85±0.7 a	52.23±1.9 a	1.17±0.2 a
VC (%)	9.57	10.19	8.11	3.59	4.56	15.73

* Means followed by the same letter in the column do not differ significantly by Tukey's test ($p \leq 0.05$). Means ± standard deviation (n = 5), CV: coefficient of variation.

Source: Author (2024).

Leaf dry matter is an important indicator of plants’ ability to intercept and absorb light (Sercu *et al.*, 2017; Hosseini *et al.*, 2023). Stress caused by low light intensity can inhibit photosynthesis and affect gas exchange processes, a fact that reduces plant growth rates. Photosynthesis is a light-dependent process that converts solar energy into chemical energy, which is essential for plant growth and development processes. Plants have a hard time carrying out efficient photosynthesis under limited light conditions, and it results in decreased biomass production (Gregoriou *et al.*, 2007; Thakur and Kumar, 2021).

In the present study, *O. majorana* plants grown under full sun conditions exhibited superior accumulation of LDM, StDM, and SDM compared to plants cultivated under blue and black shading nets. However, there was no significant difference between plants grown under the red shading net and those under full sun conditions. Furthermore, it was observed that plants grown under full sun conditions increased their LDM, StDM, and SDM by 62%, 40%, and 46%, respectively, compared to those under the blue shading net (Table 1). This finding has indicated that leaves of *O. majorana* plants grown under high light intensity conditions were capable of intercepting and absorbing more light, as well as of assimilating more CO_2, than leaves of plants grown under shady conditions. Plants grown under high light intensity conditions produced thicker leaves, and it increased their photosynthetic capacity per unit area and improved their ability to use light for carbon fixation purposes (Terashima *et al.*, 2005). According to Thakur and Kumar (2021), there is close correlation between the amount of photons reaching the leaves and increased chlorophyll, ATP and NADPH production.

The blue shading net absorbs spectral bands of ultraviolet, red and far-red light, as well as enriches blue light spectra. Blue light triggers morphological and physiological responses capable of inhibiting cell division and expansion processes. This factor enables the development of thinner leaves with reduced biomass and lower leaf dry weight in comparison to plants grown under red light condition (Izzo *et al.*, 2020). Furthermore, blue light can change the activation of photoreceptors and, consequently, change plants' phototropism. It can cause dwarfism in plants grown under blue shading nets (Ilić and Fallik, 2017). Reduced dry matter under shading conditions was observed for other species belonging to family Lamiaceaey, such as *Ocimum basilicum* L. (Martinez-Gutierrez *et al.*, 2016), *Melissa officinalis* L. (Oliveira *et al.*, 2016) and *Thymus vulgaris* L. (Honorato *et al.*, 2023).

On the other hand, red shading nets absorb spectral bands of ultraviolet, blue and green light, as well as enrich red and far-infrared light spectra (Sivakumar *et al.*, 2018). Red light stimulates shade avoidance traits in plants, such as increased hypocotyl elongation, internodal distance and leaf surface. This stimulus contributes to plants' growth, overall development and vegetative vigor (Zhang, H. *et al.*, 2023).

Radiation plays key role in both directly and indirectly regulating plant growth and development (Taiz *et al.*, 2017). Adaptations experienced by plants in their photosynthetic apparatus in response to ambient light conditions have straight impact on their overall growth.

These morphophysiological responses by plants are not only determined by light intensity, but also by variations in light quality (Silva *et al.*, 2013).

Plants grown in full sunlight recorded higher root dry matter (RDM) accumulation than plants grown under colored shading nets (Figure 3D). The greater allocation of photoassimilates to root growth in plants under full sun conditions may be a strategy to enhance water and nutrient absorption capacity, given that such environments tend to be drier. Similar behavior was observed for other species grown under experimental conditions similar to the herein adopted ones (Costa *et al.*, 2010; Ribeiro *et al.*, 2018).

Shading nets had negative influence on the development of *O. majorana* roots. This outcome can be attributed to light intensity effect on auxin synthesis, which is an important root growth regulator. According to Van Gelderen *et al.* (2018), cortex cells account for regulating auxin synthesis; yet, light is a factor capable of interfering with this process.

O. majorana plants grown in full sun conditions showed an increase in total dry matter (TDM) of 93.16%, 61.51%, and 40.52% compared to blue, black, and red shading nets, respectively (Table 1). These findings demonstrate that shading conditions with colored nets, to which marjoram cultivation was subjected, are detrimental to the growth of this species.

Plants grown under full sunlight condition have allocated higher amounts of assimilates to the roots and, consequently, they recorded higher root:shoot ratio (R:S) than plants grown under shading conditions (Table 1). Just as observed for RDM, the higher R:S ratio observed for plants grown under full sunlight condition has indicated that direct sunlight incidence and high temperatures observed throughout the day exposed plants to higher stress levels. Root:Shoot ratio is a variable directly linked to plants’ water status. Therefore, the higher the R:S ratio, the higher the water stress in plants (Silva *et al.*, 2012).

Differences in leaf morphological parameters were also observed depending on the adopted shading nets (Table 2). Plants grown under red and black shading nets recorded significant increase in total leaf area (TLA) in comparison to plants grown under full sunlight and blue shading nets. Acclimation in shaded environments can favor bigger leaf area due to cells’ expansion under low light intensity in order to get higher light incidence for photosynthesis purposes (Ilić *et al.*, 2015).

Table 2 - Total leaf area (TLA), specific leaf area (SLA), leaf area ratio (LAR), leaf weight ratio (LWR), relative growth rate (RGR), and net assimilation rate (NAR) of *Origanum majorana* grown under different light spectra.

Treatments	TLA	SLA	LAR	LWR	RGR	NAR
	dm^2	$dm^2\ g^{-1}$		$g\ dm^2$	$dm^2\ g^{-1}$	
Blue	20.78 ±0.9 b	1.76 ±0.1 b	0.78 ±0.1 a	0.43 ± 0.0 a	0.60 ±0.0 c	0.42 ±0.0 b
Black	28.06 ±2.9 a	2.07 ±0.2 a	0.84 ±0.1 a	0.42 ±0.0 a	0.62 ±0.0 b	0.39 ±0.0 b
Red	29.86 ±1.8 a	2.09 ±0.0 a	0.79 ±0.1 a	0.38 ±0.0 b	0.63 ±0.0 ab	0.38 ±0.0 b
Full sun	22.21 ±2.4 b	1.38 ±0.1 c	0.47 ±0.1 b	0.32 ±0.0 c	0.65 ±0.0 a	0.59 ±0.1 a
CV (%)	8.38	5.93	13.37	4.44	1.78	13.73

* Means followed by the same letter in the column do not differ significantly according to Tukey's test ($p \leq 0.05$). Means ± standard deviation ($n = 5$), CV: coefficient of variation.

Source: Author (2024).

Leaves of plants grown under open field conditions are exposed to higher light intensity and it can lead to increased water loss due to transpiration. Therefore, leaf area is an important factor for plants' water balance. Plants can reduce the size of their leaves to avoid evapotranspiration, since such reduction decreases the amount of light absorbed by plants and their photosynthesis rate. This factor can lead to reduced plant growth and essential oil yield rates (Harish *et al.*, 2022).

O. majorana plants grown under full sunlight conditions recorded significant average reduction of 43%, 71.92%, and 28.13% respectively in specific leaf area (SLA), leaf area ratio (LAR) and leaf weight ratio (LWR) in comparison to plants grown under shading conditions (Table 2).

This response can be interpreted as plants' adaptation to minimize likely damage caused by excessive solar radiation, while reducing water losses associated with full sunlight environment. The SLA relates a morphological component, leaf surface area, to an anatomical component, leaf dry biomass (Benincasa, 1988), and is considered an important functional trait that can affect light interception and leaf longevity (Wright *et al.*, 2004). Generally, plants grown under shading conditions tend to develop a higher SLA to enhance light capture efficiency and maximize carbon gain in these environments, resulting in a reduction in leaf thickness and mass per unit area (Liu *et al.*, 2016).

TLA directly influences LAR, as the larger the leaf area, the greater the leaf area ratio. This means that as the plant develops more leaves, the proportion of leaf area to the total area occupied by the plant increases. The increase in LAR represents a plant adaptation to low light conditions, indicating a greater proportion of photosynthetically active tissue in the form of leaf area (Patterson, 1980; Souza *et al.*, 2010).

Excessive irradiance can be harmful to photosynthetic tissues, since it forces plants to develop smaller and thicker leaves, and it results in higher leaf mass per unit area, mainly under intense light conditions. These leaf morphology adaptations enable optimizing heat dissipation, and contribute to avoid damage caused by overheating and to reduce transpiration rates (Huang *et al.*, 2021).

Plants grown under shading nets recorded LAR value significantly higher than that observed for plants grown in full sunlight. This finding may indicate that shading nets have influenced plants' morphophysiology, which recorded higher leaf area expansion and, consequently, larger useful photosynthetic area. Low LAR and LWR values (Table 1) observed for plants grown under full sunlight conditions have indicated plants' ability to adapt to different light intensity conditions (Aguilera *et al.*, 2004)

Although plants grown under full sunlight condition presented significant reduction in SLA, LAR and LWR, they recorded higher relative growth rate (RGR) and net assimilation rate (NAR) (Table 2). These plants were more efficient in absorbing light and it has corroborated the high dry matter yield. According to (Benincasa, 2003), SLA, LAR and LWR indicate leaf thickness, relative assimilatory surface and leaf tissue proportion, as well as plants' ability to adapt to low light incidence.

Leaves of *O. majorana* plants grown under full sunlight conditions recorded SLA and LWR values lower than those observed for leaves grown under shading conditions, and it suggested thicker palisade parenchyma and, consequently, higher chlorophyll concentration per leaf area unit. This adaptation can enable higher light absorption per leaf area unit under high light intensity conditions (Moraes *et al.*, 2001).

3.2 Photosynthetic pigments

Changes in the composition of light reaching plants grown under colored shading nets can trigger different specific morphogenetic and photosynthetic responses. These changes result from variations in both the quantity and quality of the radiation falling on plants (Ilić *et al.*, 2017). In addition, they can lead to differences in photosynthetic pigments' content and composition. Chlorophyll levels in *O. majorana* plants were overall affected by light conditions (Table 3).

Table 2 - Chlorophyll a (Chl.*a*), chlorophyll b (Chl.*b*), chlorophyll *a*/*b* ratio (Chl.a/Chl.b), total chlorophyll (Total Chl), and carotenoids (Car) of *Origanum majorana* grown under different light spectra.

Treatments	Chl.*a*	Chl.*b*	Chl.*a*/Chl.*b*	Chl.total	Car
	µg g^{-1} FL^{-1}			µg g^{-1} FL^{-1}	
Blue	724.11±11.0 a	570.51±10.3 a	1.27±0.0 c	1294.61±13.7 a	198.45±19.7 a
Black	711.31±32.4 ab	444.13±20.8 b	1.60±0.1 b	1155.44±49.2 b	204.13±33.5 a
Red	672.58±12.1 b	226.06±19.9 c	2.99±0.3 a	898.63±23.7 c	182.14±16.5 a
Full sun	540.17±32.4 c	539.42±27.8 a	1.0±0.1 d	1079.59±50.9 b	188.29±25.9 a
CV (%)	3.67	4.64	8.23	3.43	12.81

* Means followed by the same letter in the column do not differ significantly according to Tukey's test ($p \leq 0.05$). Means ± standard deviation (*n* = 5), FL: fresh leaves, CV: coefficient of variation.

Source: Author (2024).

Chlorophyll *a* content has significantly decreased in plants grown under full sunlight condition (Table 3). Although chlorophyll molecules absorb blue and red wavelengths, plants grown under red shading nets recorded the lowest chlorophyll *b* and total chlorophyll levels. Carotenoid content did not show significant differences among treatments.

Plants grown under blue shading net recorded total chlorophyll content significantly higher than that observed for other treatments. Oliveira *et al.* (2016) observed similar results for total chlorophyll content in *Melissa officinalis* L. plants grown under blue shading net. According to (Poudel *et al.*, 2008), blue light influences chloroplasts' development and chlorophyll synthesis in plant cells, in a positive and coordinated manner. This factor can explain the increased total chlorophyll concentration in *O. majorana* plants.

Plants grown in environments subjected to reduced light intensity tended to show higher chlorophyll concentrations per leaf weight unit. Although leaves grown under shading net conditions are not directly exposed to sunlight, they tend to produce additional chlorophyll *a* and *b* to capture diffuse radiation (Taiz *et al.*, 2017).

3.3 Phenolic compounds and antioxidant capacity

Plants grown under black shading net and full sunlight conditions recorded higher antioxidant capacity than plants grown under blue and red shading nets (Table 4). This higher antioxidant capacity could be indicative that the plants were under oxidative stress.

Table 3 - Total antioxidant capacity (TAC) in mg ascorbic acid equivalents per gram of fresh leaf (mg AAE g), Total phenols in mg gallic acid equivalent per gram of fresh leaf (mg GAE g), and Total flavones/flavonols in mg quercetin equivalent per gram of fresh leaf (mg QE g) of *Origanum majorana* grown under different light spectra.

Tratamento	TAC (mg AAE g)	Total phenol (mg GAE g)	Total flavones/flavonols (mg QE g)
Blue	25.07±0.2 b	97.43±1.9 c	13.54±0.3 a
Black	26.78±0.3 a	164.23±3.6 a	8.91±0.4 b
Red	19.90±0.2 c	79.43±1.3 d	13.94±0.5 a
Full sun	27.43±0.3 a	145.38±2.3 b	5.58±0.2 c
CV %	1.08	1.99	3.52

* Means followed by the same letter in the column do not differ significantly according to Tukey's test ($p \leq 0.05$). Means ± standard deviation ($n = 5$), CV: coefficient of variation.

Source: Author (2024).

When plants are exposed to high levels of sunlight, as in the case of plants under full sun conditions, it can lead to oxidative imbalance, which in turn triggers increases in the production of reactive oxygen species in plant cells (Rao *et al.*, 2016). Black shading nets exhibit significantly higher blue light transmittance compared to other shading nets. Greater blue light transmittance through black nets enhances the production of antioxidants and antioxidant scavenging activity in vegetables grown under black nets (Ilić and Fallik, 2017). This may explain the higher total antioxidant capacity observed in plants cultivated under black shading nets and full sun conditions.

Plants grown under black shading nets have accumulated significantly higher total phenol levels. This response can be attributed to shading impact on plants’ antioxidant system. Black shading nets can reduce the amount of solar radiation reaching plants by creating environments with lower light intensity. This growing condition type can boost the production of secondary metabolites, such as phenolic compounds, as plants’ adaptive response to environmental stress (Ilić *et al.*, 2017). According to (Buthelezi *et al.*, 2016), black shading nets have also increased total phenol contents in *Coriandrum sativum* L leaves.

Plants dynamically respond to growing conditions by adapting to them through mechanisms, such as phenolic compounds’ synthesis. These compounds play crucial role as defense mechanisms against biotic and abiotic stressors (Cheok *et al.*, 2017; Jiménez-Viveros *et al.*, 2023). Phenolic compounds are produced by plants in order to adapt to biotic and abiotic conditions, such as infections, wounds, water stress, cold, light intensity, among others (Murthy *et al.*, 2014; Naikoo *et al.*, 2019).

The synthesis of flavonoids and other phenolic compounds is influenced by a series of internal and external factors - light is one of the main regulators. The biosynthesis of phenolic compounds is intrinsically linked to both active photosynthetic radiation and spectral quality of light. Manipulating light conditions can induce changes in metabolite content; therefore, it can change photoprotection mechanisms (Qaderi *et al.*, 2023).

Plants grown under blue and red shading nets recorded significantly higher total flavones/flavonols contents. Spectral light composition can cause “compensation” between plants’ optimal growth and defense systems. Enhanced blue light (400–500 nm), for example, can significantly increase the biosynthesis of epidermal flavonoid compounds (Hoffmann *et al.*, 2015).

These results highlight the important role played by quality of light in regulating secondary metabolites’ production in plants. Different shading net colors and spectral light features can play crucial role in modulating plants’ secondary metabolism by affecting phenolic compounds and flavonoids’ synthesis in response to the surrounding environment. This complex regulation of secondary metabolites may be an adaptive strategy adopted to optimize plant growth and protection from environmental stress (Katerova *et al.*, 2017).

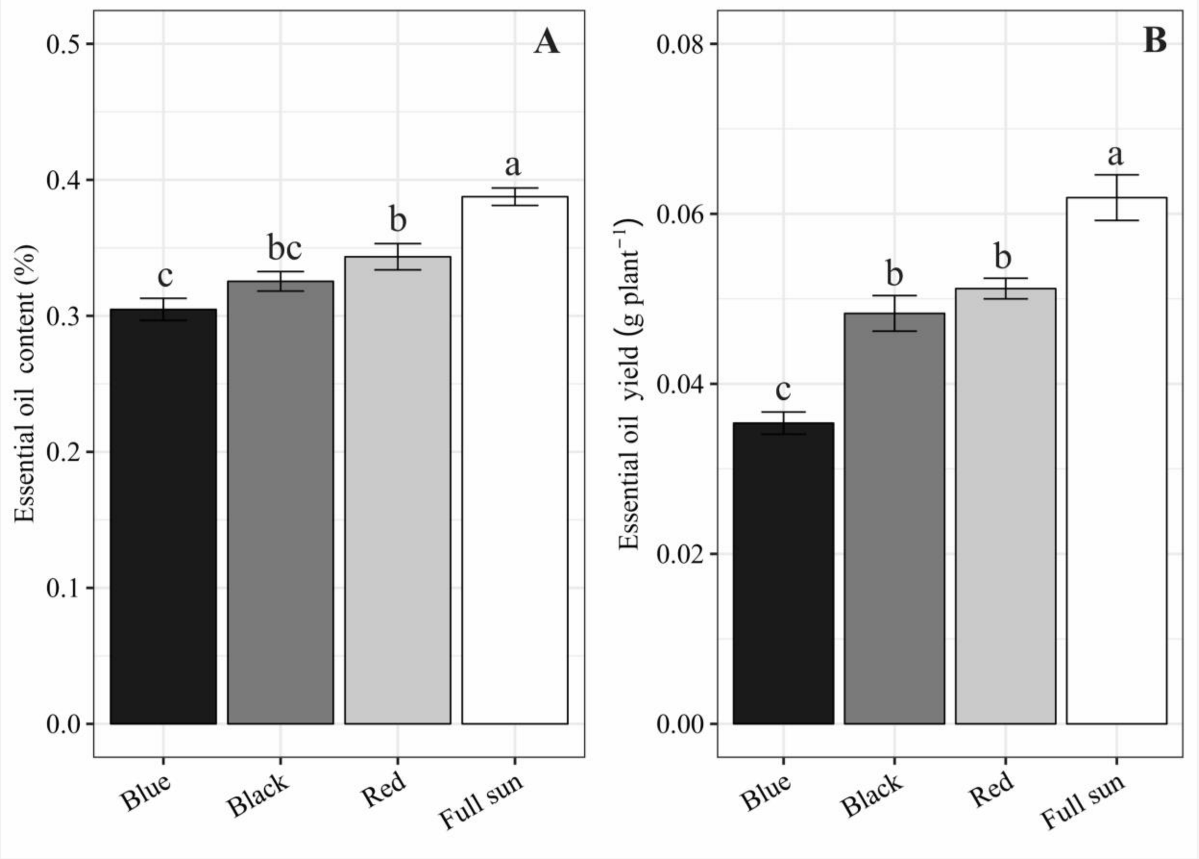
Some secondary metabolism constituents, such as phenols and flavonoids, are directly linked to plants’ interaction with the environment and to abiotic factors they are exposed to (Borges *et al.*, 2017). Thus, the fact that each of the analyzed compounds was expressed in a different manner in each treatment has indicated that the way solar irradiation fell on plants may have generated different responses in the biosynthesis of these compounds.

Furthermore, temperature may have directly influenced total phenol and flavonoid levels. This factor can be explained by the amplitude in the maximum temperature range between shading net types and full sunlight (Figure 2C). The highest temperature observed under full sunlight and red shading net conditions may have led to compounds’ degradation. Thus, there was reduction in these compound levels; consequently, they did not accumulate in plants’ leaves.

3.4 Essential oil distillation and chemical analysis

Shading nets had significant impact on *Origanum majorana* L essential oil (EO) content and yield. Plants grown in full sunlight recorded statistically higher EO content and yield than plants grown under shading conditions, as shown in Figure 3.

Figure 3 - Content (A) and yield (B) of essential oil from *Origanum majorana* grown under different light spectra.



*Bars followed by the same letter do not differ significantly according to Tukey's test ($p \leq 0.05$).
Source: Author (2024).

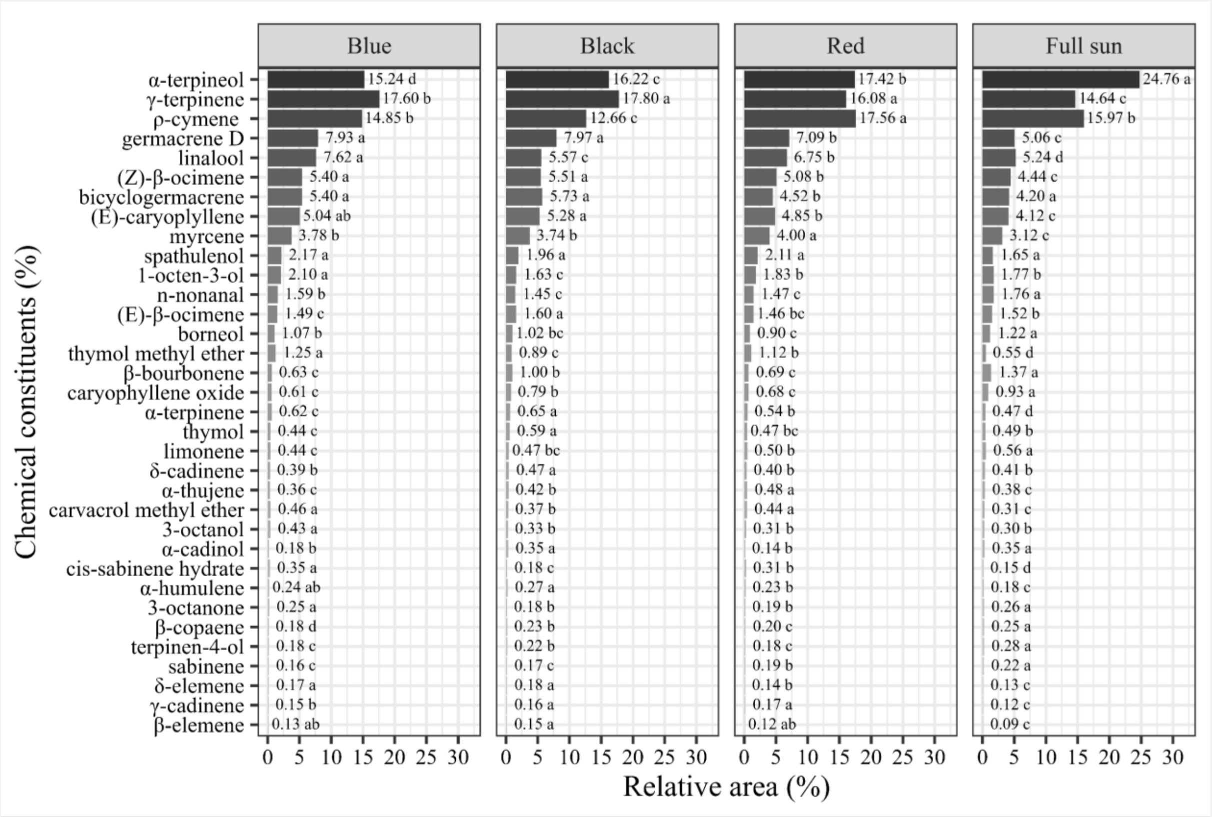
Plants grown in full sunlight recorded EO content equal to 0.39% and yield equal to 0.062 g plant^{-1}. They also recorded increase by 27.19% in EO content and by 74.96% in yield in comparison to plants grown under blue shading net.

These results have suggested that direct sunlight incidence played important role in *O. majorana* EO production. Intense and unfiltered sunlight provided the ideal conditions for bioactive compounds' growth and accumulation in this plant species. However, it is important emphasizing that shading nets can be useful in certain contexts, such as protecting plants from high temperatures, reducing disease incidence or controlling the quality of light and changing bioactive compounds' concentration (Buthelezi *et al.*, 2016; Milenković *et al.*, 2021). However, in the specific case of *O. majorana*, results have indicated that plants grown under full sunlight condition recorded significant increase in EO levels and yield in comparison to plants grown under shading conditions.

Qualitative and quantitative differences were observed in the chemical composition of essential oil extracted from *O. majorana* L. leaves (Figure 6). In total, 34 chemical constituents were identified – they accounted for 95.65%-98.29% of EO total chemical composition. Nine

(9) of these compounds recorded relative peak area rate higher than 3% (α-terpineol, γ-terpinene, ρ-cymene, germacrene D, linalool, biciclogermacrene, β-(Z)-ocymene, (E)-caryophyllene, myrcene), and it represented 82.06% of EO total chemical composition, on average.

Figure 4 - Chemical composition of the essential oil of *Origanum majorana* grown under different light spectra.



*Means followed by the same letter on the y-axis do not differ significantly according to Tukey's test ($p \leq 0.05$).

Source: Author (2024).

The essential oil extracted from *O. majorana* plants comprised a complex mix of monoterpenes and sesquiterpenes. Monoterpenes, such as α-terpineol (from 15.24% to 24.76%), γ-terpinene (from 14.64% to 17.80%), and ρ-cymene (from 12.66% to 17.56%), were the main compounds in it. Plants exposed to red light recorded significant increase in γ-terpinene and ρ-cymene concentrations - γ-terpinene is one of the precursor constituents of *ρ*-cymene (Kamitsou *et al.*, 2014). It has a wide spectrum of applications in the pharmaceutical, cosmetic, food and fine chemical industries, as well as likely uses in agriculture. Its antioxidant, antimicrobial and insecticidal properties turn it into a component of interest in several research and development fields (Schwab *et al.*, 2013; Gong and Ren, 2020; Mollica *et al.*, 2022; Mandal *et al.*, 2023). Moreover, *ρ*-Cimene is a valuable aromatic monoterpene produced from terpenes.

It is used as important intermediate in the industrial synthesis of p-cresol and in the production of high-quality chemicals, such as flavorings, fragrances, perfumes, fungicides and pesticides (Kamitsou *et al.*, 2014).

The type of exposure to light (full sunlight or shading net) had significant effect on the chemical composition of the investigated essential oil. Plants exposed to full sunlight recorded higher α-terpineol (24.76%) concentration than plants grown under shading conditions, regardless of net color. This outcome has suggested that direct sunlight had positive influence on the production of this non-phenolic monoterpene.

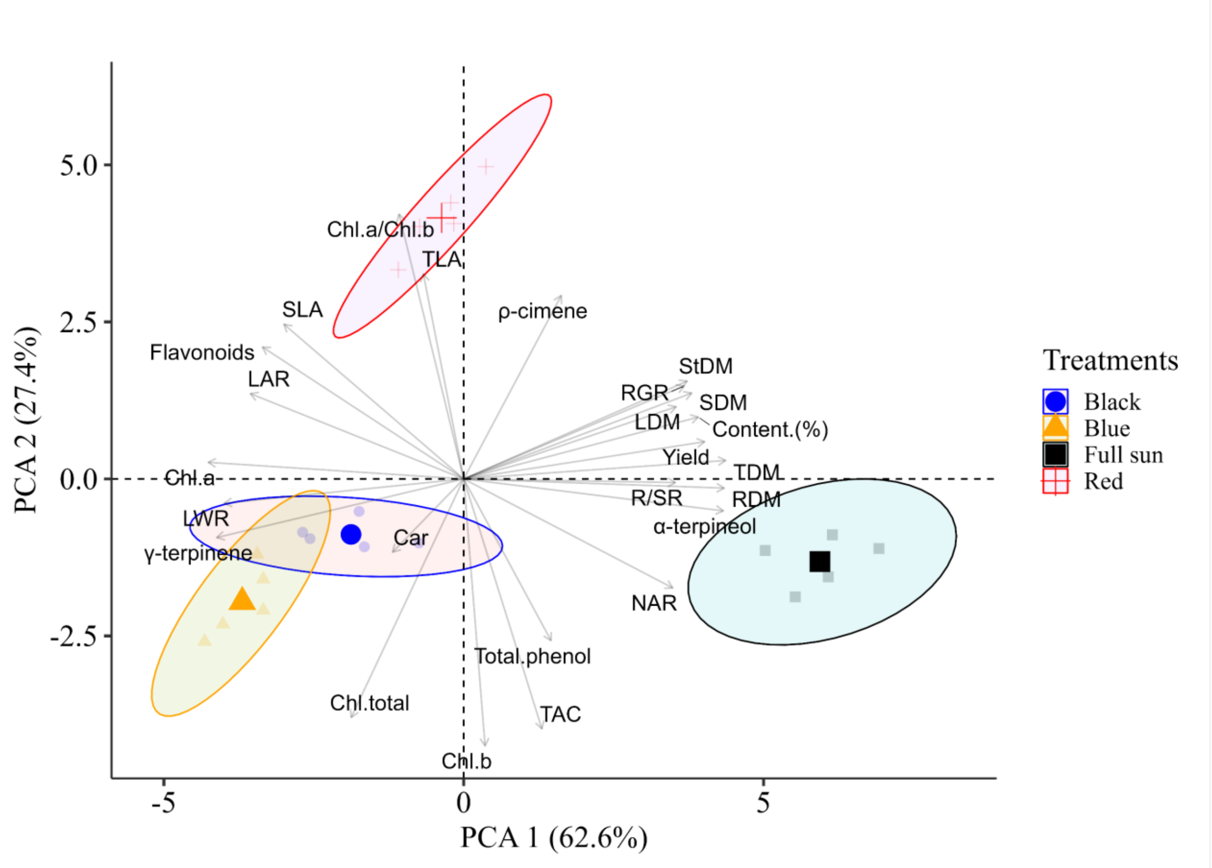
Several studies have evidenced that quantitative and qualitative variations in *Origanum* EO depend on environmental factors influenced by geographic location type, harvest time and EO extraction conditions (Baranauskaitė *et al.*, 2016). Results observed in these studies were in compliance with data previously recorded in the literature, according to which, *O. majorana* is featured by the prevalence of γ-terpinene, α-terpineol and ρ-cymene (Gharib and Silva, 2013; Elansary and Mahmoud, 2015; Cunha *et al.*, 2018; Fadel *et al.*, 2020). The essential oil extracted from *Origanum majorana* L. analyzed by (Mossa and Nawwar, 2011) presented 4-terpineol (29.97%), γ-terpinene (15.40%), trans-sabinene hydrate (10.93%) and α-terpinene (6.86%) as its major constituents.

3.5 Principal Component Analysis

Principal Component Analysis (PCA) was applied to investigate association among different culture conditions, and growth variables and features measured in *O. majorana* plants. PCA is a dimensionality reduction technique aimed at transforming a set of correlated variables into a smaller set of uncorrelated components called principal components (Younes *et al.*, 2023).

PCA was used to investigate association between plant-growth environments and the investigated response variables. The graphical representation of the first two principal components (PC1 + PC2) accounted for 89.9% of the accumulated variance (Figure 5). The first two components accumulated high total data variance rate and it suggested that these components were capable of capturing most of the relevant information in the investigated variables. According to (Mardia *et al.*, 1979), total data variation higher than 80% satisfactorily explains the variability recommended by the analyzed variables.

Figure 5 - Principal component analysis (PCA) relating to *Origanum majorana* grown under different light spectra with the analyzed variables.



Source: Author (2024).

Based on PCA (Figure 5), the loads (correlations) of the main components have indicated that PC1 was strongly associated with growth variables (StDM, LDM, SDM, RDM and NAR). PC1 has explained 62.4% of analyzed data - most growth variables recorded positive correlation higher than 0.80 (StDM, LDM, SDM, RDM, TDM, AFT, RGR, essential oil yield and α-Terpineol) and full sunlight conditions prevailed over these variables. PCA results corroborated what was previously discussed, when it was pointed out that plants grown in full sunlight recorded the best results for dry matter accumulation variables. Furthermore, these plants recorded higher RGR and NAR values, and it indicated their higher light absorption and biomass accumulation efficiency. Therefore, it is recommended preferably growing *O. majorana* in areas fully exposed to sunlight.

It is worth emphasizing that other variables, such as LWR, LAR, Chlorophyll *a* content and γ-terpinene content, were also influenced by different shading conditions. The herein conducted analysis has evidenced significant negative correlations, since PC1 values lower than -0.80 have indicated strong association between these variables and the use of blue and black shading nets. These correlations have indicated that blue and black shading nets had positive effect on these variables. These results both corroborated and completed previous analyses and interpretations.

Sarfaraz *et al.* (2023) assessed the influence of different light spectra on *Origanum majorana* L. and *Origanum vulgare* L. growth. They observed the highest amount of chlorophyll *a,* chlorophyll *b* and carotenoids in plants belonging to both species grown under blue light condition. These findings are consistent with the results obtained in the present study.

On the other hand, PC2 has explained 27.5% of total data variance and mainly represented TAC, chlorophyll *b* and total chlorophyll, which presented significant correlation (Figure 5). This finding has suggested that blue and black shading nets and full sunlight have influenced these specific features. Variables recording negative correlation in both components (PC1 and PC2) comprised LWR, total Chlorophyll, carotenoids, and γ-terpinene, with blue and black shading nets being the dominant conditions. Based on these results, red shading nets have positively influenced these variables. Thus, blue and black shading nets had negative effect on growth variables, α-terpineol concentration, and essential oil content and yield, whereas the red shading net had positive impact on total flavones/flavonols content, Chl*a*/Chl*b* ratio, SLA, TLA, and ρ-cymene concentration. Therefore, it is recommended using red shading nets when sunlight availability is very intense and when plant growth in full sunlight is not feasible.

4 CONCLUSION

The current results highlight the importance of not only taking into consideration light intensity, but also its spectral quality at the time to plan plant growth to optimize specific features. Each variable can be influenced by shading conditions and spectral light composition, in different manners. Therefore, it is essential understanding light-plant interactions to help maximizing the desired results in agricultural production.

Finally, *Origanum majorana* L. plants have shown better response to culture carried out under full sunlight conditions, as well as increased essential oil yield and α-terpineol accumulation. However, red shading nets can be used as alternative to increase γ-terpinene and ρ-cymene accumulation in environments lacking shading structure.

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ARTIGO 2 - GREEN MANURE INCREASES THE BIOMASS PRODUCTION, CONTENT, YIELD AND CHEMICAL CONSTITUENTS OF *Origanum majorana* L. ESSENTIAL OIL

ADUBAÇÃO VERDE AUMENTA A PRODUÇÃO DE BIOMASSA, TEOR, RENDIMENTO E CONSTITUINTES QUÍMICOS DO ÓLEO ESSENCIAL DE *Origanum majorana* L.

ABSTRACT

Environmental protection is one of the seventeen goals of UN's 2030 Agenda for Sustainable Development. Thus, implementing sustainable agricultural practices, such as green manure, contributes to UN sustainability goals, enables understanding their effects on medicinal plants' development and quality, as well as maximizes its potential for economic exploitation. The aim of the current study is to assess the potential of *Cajanus cajan* to be used as green manure to enable *O. majorana* vegetative growth and essential oil metabolism, photosynthetic pigments and antioxidant activity. The experiment comprised 6 treatments and 4 repetitions (5 plants per repetition), and it totaled 120 plants. Treatments comprised 5 doses of green manure (0, 150, 300, 450 e 600 g plant^{-1} of *C. cajan*) and one chemical control (inorganic fertilizer). Leaf, stem and root dry matter, absolute growth rate, net assimilation rate, root/shoot ratio, leaf area, leaf area ratio, specific leaf area, leaf weight ratio, total photosynthetic pigment content, essential oil content and yield, essential oil chemical composition, nutrient accumulation and antioxidant enzyme activity were assessed after 120-day cultivation. Results have shown that using 600 g plant^{-1} of *C. cajan* resulted in significant increases in the accumulation of dry matter in leaves, stem, and shoot parts of 338%, 555%, and 414%, respectively, compared to the 0 g plant^{-1} dose. The dose of 450 g plant^{-1} of *C. cajan* resulted in the highest total chlorophyll content by 14% and 18%, compared to the 150 g plant^{-1} dose and the control, respectively. However, it did not statistically differ from the 300 and 600 g plant^{-1} doses. The inorganic fertilizer recorded the lowest SOD activity, whereas the lowest *C. cajan* doses (0 and 150 g plant^{-1}) increased CAT activity. APX activity decreased in the absence of fertilization. Increasing *C. cajan* doses did not influence MDA and H_2O_2 levels. Furthermore, the essential oil yield was significantly higher with 600 g plant^{-1} of *C. cajan* and inorganic fertilizer, reaching 0.050 and 0.056 mg plant^{-1}, an increase of 406% and 471%, respectively, compared to the absence of fertilization. In addition, α-Terpineol stood out as the major compound in the essential oil, since it reached higher concentrations in plants treated with 600 g plant^{-1} of *C. cajan*. These findings suggest that this specific dose can be especially relevant for companies that use α-Terpineol in their products. The application of 600 g plant^{-1} of *C. cajan* not only resulted in the best performances in growth, nutrient accumulation and antioxidant activity, but also consolidated *C. cajan* effectiveness as fundamental green manure strategy for the sustainable production of aromatic plants, such as *O. majorana*. This factor reinforced its potential benefit to sustainable agriculture and to the essential oil industry.

Keywords: Marjoram; pigeon pea; chemical composition, α-terpineol.

RESUMO

Um dos dezessete objetivos da Agenda 2030 para o desenvolvimento sustentável da ONU é a proteção ao meio ambiente. Assim a condução de práticas agrícolas mais sustentáveis, como a adubação verde, além de contribuir para as metas de sustentabilidade da ONU, trazem a compreensão dos seus efeitos no desenvolvimento e qualidade das plantas medicinais, bem como contribui para a maximização do potencial de exploração econômica. Objetivou-se avaliar o potencial de *Cajanus cajan* como adubo verde no crescimento vegetativo e no metabolismo do óleo essencial, dos pigmentos fotossintetizantes e antioxidante de *O. majorana*. Foram utilizadas 6 tratamentos e 4 repetições, contendo cinco plantas por repetição, totalizando 120 plantas. Os tratamentos consistiram em cinco doses de adubo verde (0, 150, 300, 450 e 600 g planta^{-1} de *C. cajan*) e um controle (fertilizante químico). Após 120 dias de cultivo foram analisados pesos secos das folhas, caule e raízes, taxa de crescimento absoluto, taxa assimilatória líquida, relação raiz/parte aérea, área foliar, razão de área foliar, área foliar específica, razão de peso foliar, teores de pigmentos fotossintetizantes totais, teor e rendimento de óleo essencial, composição química do óleo essencial, acúmulo de nutrientes e atividades de enzimas antioxidantes. Os resultados indicaram que a dose de 600 g planta^{-1} de *C. cajan* resultou em aumentos significativos no acúmulo de matéria seca das folhas, caule e parte aérea de 338%, 555% e 414% respectivamente, em comparação com a dose 0 g planta^{-1}. A dose de 450 g planta^{-1} de *C. cajan* aumentou o teor de clorofila total 14% e 18%, em relação a dose 150 g planta^{-1} e ao controle, respectivamente. No entanto, não diferiu estatisticamente das doses 300 e 600 g planta^{-1}. A menor atividade da enzima superóxido dismutase foi observada para o controle, enquanto as menores doses (0 e 150 g planta^{-1}) aumentaram a atividade da enzima catalase. No entanto, a atividade da enzima ascorbato peroxidase foi reduzida na ausência de adubação. O aumento das doses de *C. cajan* não afetaram os níveis de MDA e H_2O_2. Além disso, o rendimento de óleo essencial foi significativamente maior com 600 g planta^{-1} de *C. cajan* e fertilizante químico, atingindo 0,050 e 0,056 mg planta^{-1}, representando um aumento de 406% e 471% respectivamente, em comparação com a ausência de adubação. O α-terpineol destacou-se como o composto majoritário no óleo essencial, atingindo concentrações mais elevadas nas plantas tratadas com 600 g planta^{-1} de *C. cajan*. Esses resultados sugerem que esta dosagem específica pode ser especialmente relevante para indústrias que utilizam o α-terpineol em seus produtos. Conclui-se que a aplicação da dose de 600 g planta^{-1} de *C. cajan* não apenas resultou nos melhores desempenhos em termos de crescimento, acúmulo de nutrientes e atividade antioxidante, mas também consolidou a eficácia do *C. cajan* como uma estratégia de adubo verde fundamental para a produção sustentável de plantas aromáticas, como *O. majorana*, reforçando seu potencial benéfico na agricultura sustentável e na indústria de óleos essenciais.

Palavras-chave: Manjerona, feijão-guandu, composição química, α-terpineol.

1 INTRODUCTION

According to the 2030 Agenda for Sustainable Development, environmental degradation is one of the most urgent issues to be solved by nowadays' society (United Nations, 2015). Most environmental impacts are caused by the agricultural sector, as reported by Tan *et al.* (2022). According to the aforementioned authors, the organic agriculture practice is often seen as solution for this issue, since its food production process causes less damage to the ecosystem, to plant and animal species, and to humans, in general.

Accordingly, green manure is a strategy comprising the cultivation of specific plants, mainly leguminous plants locally produced or not, which can be used as cover crops or incorporated to the soil to help improving its physical, chemical and biological properties, and to preserve and/or restore soil organic matter and nutrient content (Maitra *et al.*, 2018). Legumes stand out among green manure sources due to their ability to biologically fix atmospheric nitrogen, which helps improving soil mineral composition and, consequently, enables plants' healthy growth (Meena *et al.*, 2018). Pigeon pea (*Cajanus cajan* (L.) Millsp.) is a legume species widely used as green manure. Its dry biomass yield ranges from 8 to 12 t ha^{-1}, its C:N ratio is 15:22 and its nitrogen fixation potential ranges from 37 to 280 kg year (Lima Filho *et al.*, 2023). This fast-growing shrubby plant is capable of covering the ground quite fast and it plays key role in soil fertilization. Furthermore, its symbiotic association with bacteria belonging to genus *Rhizobium* enables it to fix atmospheric nitrogen and to enrich the soil with this essential nutrient (Sarmento *et al.*, 2019).

In addition to environmental factors, agronomic practices, such as soil phytomanagement, can also influence medicinal and aromatic plants' growth and chemical composition (Murillo-Amador *et al.*, 2015; Virga *et al.*, 2020; Malaka *et al.*, 2023). Marjoram (*Origanum majorana* L. syn. *Majorana hortensis* Moench), which belongs to family *Lamiaceae*, is a perennial herbaceous plant that stands out for its culinary applications and medicinal properties. It is widely used as aromatic herb and condiment in different cultures, given its mild and pleasant flavor, as well as to prepare a wide variety of dishes (Thanh *et al.*, 2019; Chaudhari *et al.*, 2020). Furthermore, *O. majorana* leaves are used to treat different diseases, such as gastrointestinal disorders and infections (Vogl *et al.*, 2013), respiratory disorders (Charles, 2013b) and hypertension (Tahraoui *et al.*, 2007). Its essential oil is appreciated for its antioxidant (Ouedrhiri *et al.*, 2021), antimicrobial (Della Pepa *et al.*, 2019; Paudel *et al.*, 2022), anti-inflammatory (Arranz *et al.*, 2015), anticancer (Athamneh *et al.*, 2020) and antidepressant activity (Abbasi-Maleki *et al.*, 2020). In addition to its pharmacological activity, the essential oil extracted from *O. majorana* plants has repellent and insecticidal properties (Giatropoulos *et al.*, 2018; Prabu *et al.*, 2020a; Aboelhadid *et al.*, 2022), and it arouse increasing interest in using *O. majorana* essential oil in medicinal and industrial applications.

However, increasing medicinal and aromatic plants' yield and maintaining their quality are challenging tasks, mainly due to indiscriminate use of chemical inputs and pesticides in agricultural systems, a practice that has negative impact on soil, environmental and human health (Baiyeri and Olajide, 2023). Consequently, sustainable agricultural practices based on medicinal and aromatic plants have been explored, and green manure emerged as promising alternative to increase plant yield, to improve soil fertility and to preserve the ecosystem (Assis *et al.*, 2020; Honorato *et al.*, 2022; Lima Filho *et al.*, 2023).

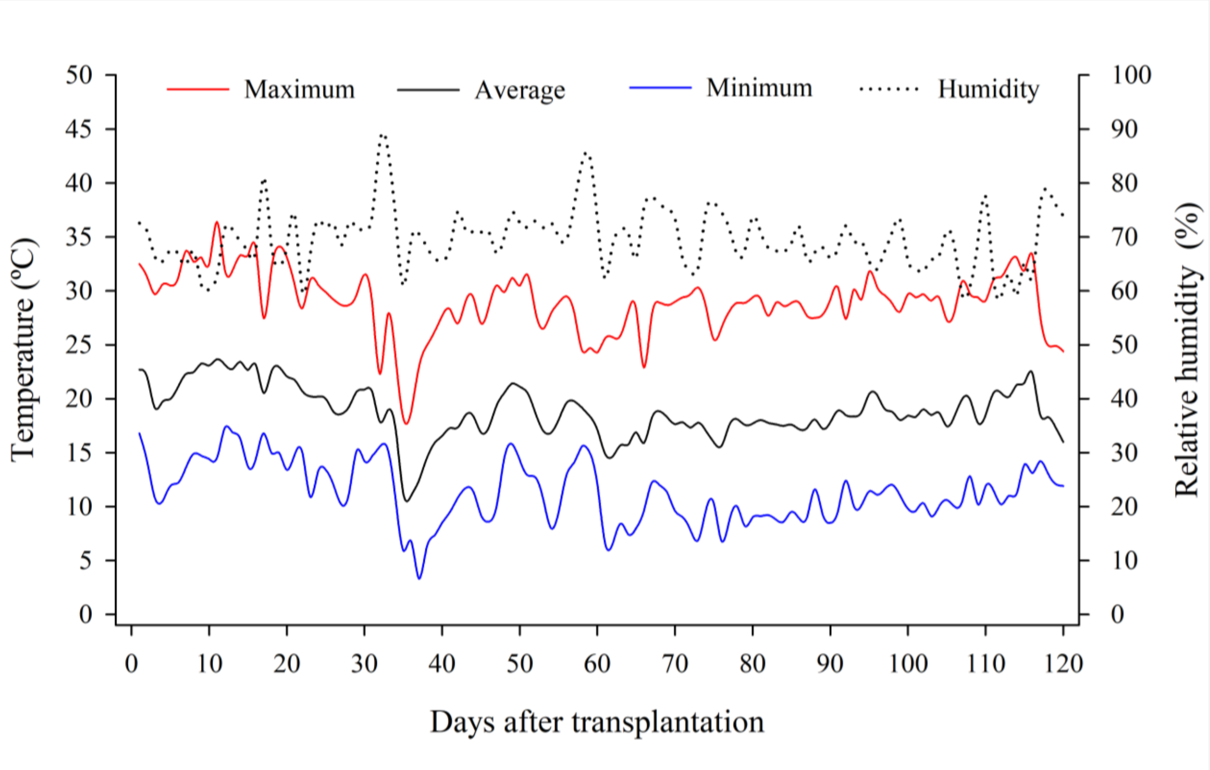
The hypothesis to be herein investigated is that *C. cajan* application as green manure can have significant influence on *O. majorana* features, since it changes plant's properties and positively affects its growth, as well as the composition and quality of its essential oil. In addition, this application enables proposing management procedures based on sustainability principles. Therefore, the aim of the current study was to investigate the potential of *C. cajan* to be used as green manure to improve *O. majorana* vegetative growth and essential oil metabolism, as well as its photosynthetic pigments and antioxidant activity.

2 MATERIALS AND METHODS

2.1 Plant materials and experimental procedures

The experiment was conducted in the experimental area of the Medicinal Plants sector of the Agriculture Department, Federal University of Lavras, Brazil (geographic coordinates 21°14'43" S and 44°59'59" W, and altitude of 918 m). According to Köppen's classification (Alvares et al., 2013), climate in this region is of the Cwa type, with two well-defined seasons: dry winter with mild temperatures, and hot and rainy summer (Martins *et al.*, 2018). Environmental conditions, such as temperature and relative humidity, were monitored during the experiment in Elitech® RC-51H digital datalogger, scheduled to take readings every hour for 120 days, as shown in Figure 1. The investigated species (*Origanum majorana* L., mixed chemotype α-terpineol, γ-terpinene, and ρ-cymene) was herborized and deposited in the herbarium of the Agricultural Research Company of Minas Gerais State (EPAMIG - Empresa de Pesquisa Agropecuária de Minas Gerais) under registration number 58898.

Figure 1 - Recording temperature and humidity data during the experiment.



Source: Author (2024).

O. majorana seedlings were obtained through apical cuttings (approximately 5 cm long) deriving from mother plants grown in the Medicinal Plant Garden. These cuttings were placed in polyethylene trays covered with commercial substrate and kept in greenhouse under controlled irrigation condition to promote seedling rooting and growth.

Pigeon pea (*Cajanus cajan* (L.) Millsp.) plants were grown under no-till system from November to March. Plants were collected at early flowering stage and crushed in chopper and forage harvester equipment (chopped uniformity 5 mm and 13 mm). Subsequently, fresh *C. cajan* matter doses were incorporated to the substrate in the pots, for 30 days.

After the green manure incorporation period was over, seedlings (approximately 10 cm) were transplanted to 10 dm^3 pots (29 x 24.5 x 19 cm, respectively height, top and bottom diameters) filled with substrate comprising dystrophic red-yellow Latosol and sand, at the ratio of 2:1 + treatment. Chemical features of the soil used in the substrate were set as follows: pH in water = 6.4; K (mg/dm^3) = 97.8; P-Rem (mg/L) = 1.7; Ca^{2+}, Mg^{2+}, Al^{3+} and H+Al ($cmolc/dm^3$) = 1.4, 0.02, 0.00 and 1.7; base saturation index (V%) = 52.38; organic matter (dag/kg) = 1.0; Zn, Fe, Mn, Cu, B and S (mg/dm^3) = 0.3, 27.6, 7.0, 2.5, 0.06 and 60.2, respectively.

Crushed material samples were collected, dehydrated in forced-air oven at 65°C until reaching constant weight and sent to the laboratory for green manure nutritional analysis purposes. Nutritional report has indicated N, P, K, Ca, Mg and S (g kg^{-1}) concentrations = 26.7, 1.9, 14.3, 6.3, 1.6 and 1.4, respectively; as well as B, Cu, Fe, Mn and Zn (mg kg^{-1}) concentrations = 22.2, 11.7, 476.6, 51.2 and 15.5, respectively.

The experiment has followed a randomized block design, with 6 treatments and 4 repetitions (5 plants per repetition) – 120 plants, in total. The experimental unit consisted of a pot containing one plant, with spacing between pots maintained at 50 x 50 cm. Treatments consisted in 5 green manure doses (0, 150, 300, 450 and 600 g $plant^{-1}$ of *C. cajan*) and one inorganic fertilizer control – NPK, 2.78 g $plant^{-1}$ of urea (45% N), 14.29 g $plant^{-1}$ of single superphosphate (21% P_2O_5), and 3.77 g $plant^{-1}$ of potassium chloride (60% K_2O), based on guidelines set in the recommendations' manual (Blanco, 2018). Irrigation was manually performed, based on one 48-hour irrigation shift, when a 46-mm irrigation layer (on average) was applied to keep the substrate close to field capacity.

Soil samples of each treatment were collected at 120 days after *O. majorana* seedling transplantation. Soil analysis results are shown in Table 1.

Table 1 - Soil analysis according *Cajanus cajan* dose.

Sample	pH	N	K	P	Na	Ca	Mg	Al	H+Al	EBS	t	T	V	m	OM	P.Rem
g $plant^{-1}$		g kg^{-1}	mg dm^{-2}		cmolc dm^{-3}								%		dag kg^{-1}	mg L^{-1}
0	5.80	0.76	37.07	0.01	9.00	1.18	0.25	0.10	0.90	1.53	1.63	2.24	62.76	6.13	0.87	9.60
150	6.40	0.95	67.46	0.02	9.00	1.34	0.26	0.10	1.40	1.77	1.87	3.17	55.93	5.35	0.98	11.10
300	6.40	1.01	84.08	0.01	10.00	1.49	0.34	0.00	1.30	2.05	2.05	3.35	61.06	0.00	1.04	13.30
450	6.60	1.05	98.17	0.03	9.00	1.56	0.35	0.10	1.30	2.15	2.26	3.45	62.48	4.42	1.29	14.00
600	6.60	1.26	108.32	0.20	11.00	1.60	0.36	0.10	1.40	2.24	2.34	3.64	61.48	4.27	1.44	15.60
Control	6.00	0.76	100.13	11.44	10.00	2.26	0.25	0.10	1.60	2.77	2.87	4.37	63.31	3.48	0.70	11.70

pH in water; EBS – exchangeable base sums; CEC (t) – effective cation exchange capacity; CEC (T) – cation exchange capacity pH 7.0; V – base saturation index; m – aluminum saturation index; OM – Organic matter; P-Rem – remaining phosphorus.

Source: Author (2024).

2.2 Plant growth analysis

Harvest was carried out at 120 days after seedling transplantation by cutting entire plants. Plants were split into root, stem and leaves, and subjected to dehydration in forced air oven at 37°C, until reaching constant weight. Subsequently, the following growth parameters were assessed: leaf (LDM), stem (StDM), root (RDM), shoot (SDM) and total (TDM) dry matter – results were expressed as g $plant^{-1}$. Root:Shoot ratio (R:S) was calculated by dividing root dry weight by shoot (leaves + stem) dry weight (Benincasa, 2003).

Twenty (20) leaves were randomly collected from each repetition and scanned to determine leaf area (LA). LA was measured in previously calibrated Motic® plus software 2.0. Subsequently, leaves with known leaf area were dried in forced air oven at 37°C, until reaching constant weight. With the area of the 20 fully expanded leaves in cm^2 (A20L), the dry matter of the 20 leaves in g^{-1} (DM20L), and the total dry matter of leaves in g $plant^{-1}$ (TDML), the total leaf area (TLA) was determined in dm^2, using the formula:

$$\mathrm{TLA} = \left(\frac{\mathrm{TDML}\ x\ \mathrm{A20L}}{\mathrm{DM20L}}\right)/100$$

TLA values were used to calculate the following indices: Specific leaf area (SLA = LA / LDM), leaf area ratio (LAR = LA / TDM), leaf weight ratio (LWR = LDM / TDM), based on equations proposed by (Benincasa, 2003).

The Relative Growth Rate (RGR in g g^{-1} $week^{-1}$) was determined using the formula RGR = $(\ln W_2 - \ln W_1) / (t_2 - t_1)$, where W_1 and W_2 refer to the dry weight of two samplings at t_1 (transplanting) and t_2 (120 DAT). The Net Assimilation Rate (NAR in g dm^{-2} $week^{-1}$) was calculated based on the dry matter produced per leaf area per unit of time using the formula NAR = $[(W_2 - W_1) / (A_2 - A_1)]$ x $[(\ln W_2 - \ln W_1) / (t_2 - t_1)]$, where W_1 and W_2 represent the initial and final dry weights, and A_1 and A_2 refer to the LA at t_1 (Transplanting) and t_2 (120 DAT), respectively (Alvarenga *et al.*, 2015).

2.3 Leaf nutrient analysis

After all treatments were over, dry leaf samples were collected from each treatment and subjected to analysis at the Agricultural Analysis Laboratory 3rlab, Lavras City, Minas Gerais State, Brazil. It was done to determine the levels of essential nutrients, such as N, P, K, Ca, Mg, S, B, Cu, Mn, Zn and Fe, in *Origanum majorana* L. leaves.

Macronutrient and micronutrient accumulation was calculated by taking into consideration the analysis results, as well as the mean leaf weight per plant in each treatment. These values were expressed in milligrams per plant (mg $plant^{-1}$) and provided a detailed view of the amount of nutrients accumulated by each plant under different experimental conditions.

2.4 Photosynthetic pigments

Photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids) extraction and quantification were carried out based on the methodology by Hiscox and Israelstam (1979) adapted by (Barnes *et al.*, 1992). In order to do so, 50 mg of fresh photosynthetically active leaves were collected and placed in Falcon tubes filled with 10 mL of dimethyl sulfoxide (DMSO) saturated with $CaCO_3$ at the proportion of 5 g L^{-1} of DMSO, based on the protocol by (Santos *et al.*, 2008).

The samples were incubated in oven at 65°C, for 48 hours. Then, they were analyzed in spectrophotometer at wavelengths of 649 nm, 665 nm and 480 nm. Results were expressed as mg g^{-1} of fresh leaf. The herein used equations were based on wavelength, according to the method by Wellburn (1994), wherein:

$$Chlorophyll\ a_{649} = (12.47\ x\ A_{665}) - (3.62\ x\ A_{649})$$

$$Chlorophyll\ b_{665} = (25.06\ x\ A_{649}) - (6.5\ x\ A_{665})$$

$$Carotenoids_{480} = (1000\ x\ A_{480} - 1.29\ x\ chl_a - 53.78\ x\ chl_b)/220$$

The sum of chlorophyll (a + b) equations provided the total chlorophyll content - all values were expressed as mg g^{-1} of fresh matter.

2.5 Antioxidant Metabolism

The activity of enzymes, such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), was determined after enzymatic extraction to assess plants' antioxidant responses. SOD activity was expressed as SOD units per minute per milligram of fresh leaf (U SOD $min^{-1}.mg^{-1}$.FL). It was measured based on the method proposed by Giannopolitis and Ries (1977), by using Nitroblue Tetrazolium (NBT) photoreduction inhibition at 560nm.

CAT activity was expressed as nanomoles of hydrogen peroxide consumed per minute per milligram of fresh leaf (ηmol H_2O_2 $min^{-1}.mg^{-1}$.FL). It was determined based on H_2O_2 consumption at 240nm for 3 minutes, based on the method by Havir and Mchale (1987). Finally, APX activity was expressed as nanomoles of ascorbate (ASA) oxidized per minute per milligram of fresh leaf (ηmol ASA $min^{-1}.mg^{-1}$.FL). It was assessed based on monitoring ascorbate oxidation at 290nm for 3 minutes, as described by Nakano and Asada (1981) and (Kumar, 2022).

Hydrogen peroxide (H_2O_2) and lipid peroxidation production were quantified based on the malondialdehyde (MDA) quantification method to assess reactive oxygen species. Hydrogen peroxide was measured in micromoles per milligram of fresh leaf (µmol $H_2O_2.mg^{-1}$.FL) based on standard curve comprising known concentrations of H_2O_2 (Velikova *et al.*, 2000). Lipid peroxidation was assessed through malondialdehyde (MDA) test, which was used to quantify thiobarbituric acid-reactive oxygen species, as described by (Buege and Aust, 1978).

Results of this assay were expressed as nanomoles per milligram of fresh leaf (ηmol MDA.mg^{-1}.FL).

2.6 Essential oil content and chemical composition

Essential oil (EO) extracted from *Origanum majorana* L. plants was obtained through hydrodistillation process conducted in modified Clevenger apparatus, based on using 40 g of LDM per 1 L of distilled water. Subsequently, the EO underwent purification process based on using liquid-liquid partition with CH_2Cl_2 (3 x 5 mL). The resulting organic phase was combined each other, treated with anhydrous $MgSO_4$ to remove impurities and, then, filtered. The oil was collected and stored in amber bottle. Essential oil content (mg/100 g^{-1} of leaf dry matter) and yield (mg/plant^{-1}) were determined. Subsequently, EOs were stored under refrigeration at 4ºC, until chemical analysis time.

Oil quantitative analysis was carried out in gas chromatographer coupled to hydrogen flame ionization detector (GC-FID), in Agilent®7890A gas chromatography system operated in HP GC ChemStation software, version A.01.14 and equipped with CombiPAL auto injector/sampler (CTC Analytic AG, Switzerland). The oil was diluted in ethyl acetate (1%, v/v) and automatically injected into the chromatograph at injection volume of 1.0 µL, in split mode, at the ratio of 50:1. Both the injector and detector temperatures were maintained at 220°C and 240°C, respectively. The initial oven temperature was 60°C. Temperature ramp of 3°C min^{-1} up to 240°C was adopted, and it was followed by another ramp of 10°C min^{-1} up to 280°C. Analyses were performed in triplicate (n = 3); analyte concentrations were expressed as percentage of the normalized relative area of chromatographic peaks.

Qualitative analyses were carried out through gas chromatography mass spectrometry (GC-MS) in Agilent ® 5975C mass spectrometer operated by electron impact ionization at 70 eV, in scan mode of 1.0 scan/s, with mass acquisition range of 40–400 m/z. The same operating conditions used in quantitative analyses were herein adopted. Retention indices corresponding to co-injection of n-alkane standards, C_8–C_{20} (Sigma Chemical Co., St. Louis, MO, USA) were calculated based on the equation by Van Den Dool and Dec. Kratz (1963). EO chemical constituents were identified by comparing their calculated retention indices to those reported in the literature (Adams, 2007) and by comparing mass spectra from the National Institute of Standards and Technology library database (Nist and Technology, 2008).

2.7 Statistical analysis

Collected experimental data were subjected to statistical analysis in R software version 4.1.2. Homogeneity of variance (Levene and Bartlett, $p \geq 0.05$) and normality (Shapiro-Wilk, $p \geq 0.05$) tests were initially applied with “car” package (Fox *et al.*, 2022). Then, residues meeting the normality criterion were subjected to analysis of variance (ANOVA) through F test ($p \leq 0.05$).

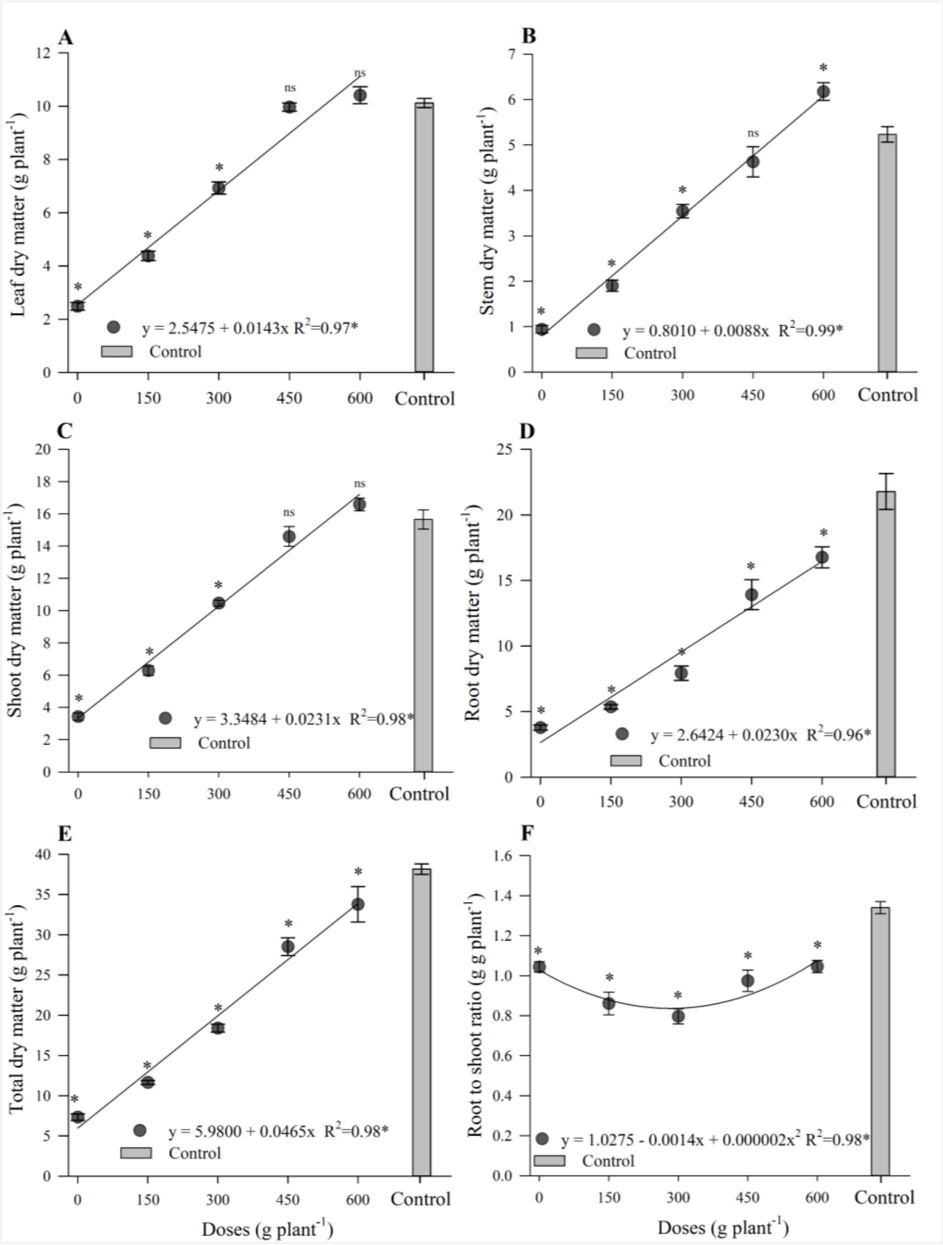
Means were compared through Tukey test ($p \leq 0.05$) and regression analysis for quantitative factor based on using the “ExpDes.pt” package (Ferreira *et al.*, 2021). A Dunnett test was performed to compare the means of the green manure doses against the control (inorganic fertilizer) using the dbc.ad function from the "Tratamentos.ad" package (Azevedo, 2022). Moreover, principal component analysis (PCA) was conducted based on using the fviz_pca_biplot function from the “factoextra” package to perform a broader analysis of treatments’ effects (Kassambara and Mundt, 2020).

3 RESULTS AND DISCUSSION

3.1 Plant growth analysis

Green manure incorporation had significant impact on *O. majorana* plants' growth parameters. Increased *C. cajan* dose led to linear increase in LDM, StDM and RDM accumulation (Figure 2).

Figure 2 –Leaf dry matter [LDM (A)], stem dry matter [StDM (B)], shoot dry matter [SDM (C)], root dry matter [RDM (D)], total dry matter [TDM (E)], and Root to shoot ratio [R/S ratio (F)] of *Origanum majorana* as a function of different doses of *Cajanus cajan*



(^{ns}) no difference, (*) differs from control by Dunnett test ($p \leq 0.05$).
Source: Author (2024).

Increase in *C. cajan* doses was closely related to the increase recorded for dry matter accumulation in all vegetative organs of the plant (Figures 2 and 3). This finding highlights the effectiveness of using *C. cajan* as green manure to enhance *O. majorana* biomass yield. According to Ma *et al.* (2021), green manure application has significantly improved soil quality, biological activity, as well as organic matter, nitrogen, phosphorus, potassium contents, among other nutrients, and it resulted in increased plant growth and dry matter accumulation.

Figure 3 - *Origanum majorana* L. grown with different doses of *Cajanus cajan* L.

Source: Author (2024).

C. cajan application as green manure had significant impact on *O. majorana* shoot growth. The highest LDM (11.12 g plant^{-1}) was achieved with the application of 600 g plant^{-1} of *C. cajan*, resulting in a 338% increase compared to the 0 g plant^{-1} dose. However, the dose of 600 g plant^{-1} of *C. cajan* did not differ from the control according to the Dunnett test (Figure 2A). *O. majorana* leaves are the plant organ of greatest interest for the investigated species. This finding indicates that green manure with *C. cajan* can be used as a sustainable alternative to conventional fertilization for the cultivation of *O. majorana*.

Furthermore, the application of the aforementioned *C. cajan* dose also resulted in a 555% higher increase in StDM in comparison to the other assessed conditions (Figure 2B). According to Meena *et al.* (2018), using *C. cajan* as green manure can help providing continuous nutrient supply to plants, boosting beneficial biological activity in the soil and improving overall plant growth conditions. Therefore, it explains the positive results observed for *O. majorana* shoot growth.

The accumulation of SDM was proportional to the increase in the green manure dose, with the dose of 600 g plant^{-1} resulting in an increase of 414% compared to the control. However, there was no significant difference between this dose and the control (Figure 2C). The increased biomass production observed in *O. majorana* plants due to increased green manure doses may be associated with the gradual release and mineralization of macro and micronutrients during the decomposition process. This factor may have contributed to improve soil structure, as well as to increase its porosity, and water and nutrient retention, which resulted in better crop development (Meena *et al.*, 2018; Meena and Lal, 2018).

The increase in the dose of the green manure *C. cajan* resulted in a linear increase in RDM. The maximum accumulation of RDM was observed in plants fertilized with 600 g plant^{-1} *C. cajan*, 523% higher than plants that did not receive green manure. However, inorganic fertilization resulted in an even greater increase in RDM, with an increment of 724% compared to plants without green manure. This increase corresponded to a 32% increase in RDM compared to plants receiving 600 g plant^{-1} of *C. cajan* (Figure 2D). This result indicates that inorganic fertilization had a more pronounced effect on RDM accumulation compared to the highest dose of green manure.

This response can be attributed to higher phosphorus (P) and lower nitrogen (N) availability in the soil, as shown in Table 1. This phenomenon happens because root growth is more sensitive to phosphorus (P) limitation, which forces plants exposed to lower N:P supply ratios to allocate more resources for root system growth purposes. On the other hand, plants exposed to higher N:P supply ratios tend to allocate more resources to the shoot (Luo *et al.*, 2016).

The accumulation of dry matter in plants is an important indicator of crop growth and development. The use of green manure and inorganic fertilizers can have a significant impact on this accumulation of dry matter. The maximum estimated accumulation of TDM was observed in plants fertilized with 600 g plant^{-1} of *C. cajan*, 361% higher than those that did not receive green manure. However, inorganic fertilization resulted in an even greater increase in TDM, 420% higher compared to plants without green manure. This increase corresponds to a 13% increase in TDM compared to plants receiving 600 g plant^{-1} of *C. cajan* (Figure 2E).

Chemical fertilization led to the highest R:S ratio (1.34) in comparison to green manure treatments (Figure 2D). This finding suggests that chemical fertilization led to disproportionately higher resource allocation to the roots than to the shoot. Plants can adjust the allocation of resources, such as photoassimilates, to the roots in response to nutritional conditions. Plants grown under high nutrient availability conditions may prefer to allocate more resources to the shoot. However, plants grown under nutritional stress conditions can allocate higher resource proportion to the roots in order to optimize nutrient uptake from the soil (Peng *et al.,* 2019).

The R:S ratio is closely related to plants' nutritional conditions. In addition, it is highly plastic in response to changes in nutritional conditions (Luo *et al.*, 2016). Plants grown under some stress conditions can allocate disproportionately more photoassimilates to the roots in order to increase nutrient acquisition (Lambers *et al.*, 2008).

3.2 Plant growth indices

Fertilization with *C. cajan* has significantly affected all vegetative parameters of *O. majorana* plants (Total leaf area (TLA), specific leaf area (SLA), leaf area ratio (LAR), leaf weight ratio (LWR), relative growth rate (RGR) and net assimilation rate (NAR), as shown in Table 2. Results in the current study have indicated that fertilization with *C. cajan* has significantly increased total leaf area (TLA) in *O. majorana* plants. The highest TLA value was observed for plants subjected to the highest *C. cajan* dose (600 g plant^{-1}).

Table 2 – Total leaf area (TLA), specific leaf area (SLA), leaf area ratio (LAR), leaf weight ratio (LWR), relative growth rate (RGR), and net assimilation rate (NAR) of *Origanum majorana* cultivated as a function of *Cajanus cajan* dose.

Doses	TLA	SLA	LAR	LWR	RGR	NAR
g plant^{-1}	dm^2	dm^2 g^{-1}		g dm^2	dm^2 g^{-1}	
0	4.64 e	1.86 c	0.63 b	0.34 ab	0.41 d	0.25 c
150	8.07 d	1.84 d	0.69 ab	0.38 a	0.48 c	0.31 b
300	14.07 c	2.03 a	0.77 a	0.38 a	0.55 b	0.35 b
450	16.98 b	1.76 e	0.60 b	0.34 ab	0.59 a	0.42 a
600	19.61 a	1.88 b	0.58 b	0.31 bc	0.61 a	0.43 a
Control	16.80 b	1.66 f	0.44 c	0.27 c	0.60 a	0.46 a

* Means followed by the same letter in the column do not differ significantly according to the Tukey test ($p \leq 0.05$).

Source: Author (2024).

TLA is an important physiological parameter used to estimate plants' response to different stimuli. It is closely related to light interception, photosynthetic capacity, dry matter accumulation, as well as to plant metabolism, growth and yield (Jain and Sandhu, 2019; Banerjee *et al.*, 2022). Results in the current study suggest that fertilization with *C. cajan* can be an effective strategy to help increasing TLA in *O. majorana* plants. This increase, in its turn, can help improving *O. majorana* plant growth, yield and quality.

SLA refers to the leaf surface area:plant mass ratio. The higher the SLA, the higher the leaf surface area:plant mass ratio. This is an important factor because it is closely linked to plants' ability to capture solar energy to perform photosynthesis. Plants presenting high SLA often have an advantage over others, given their greater ability to capture solar radiation. This ability can lead to higher photosynthesis rate, which, in its turn, increases plant growth (Huang *et al.*, 2021).

However, it is essential emphasizing that the amount of applied green manure mass plays key role in changing plants' SLA. Green manure decomposition and the subsequent mineralization of plant material nutrients are closely linked to the applied mass amount. This association, in its turn, can influence plant-specific leaf area conversion (Diniz *et al.*, 2014).

Variable "LWR" plays crucial role in the process to understand plants' growth and resource allocation strategies. On the other hand, LAR refers to the association between the leaf area accounting for intercepting light energy and CO_2, and the total dry mass resulting from photosynthesis. As plants grow, there is increased interference from upper leaves, and it tends to reduce the useful area (Benincasa, 2003).

Results have indicated that *O. majorana* plants subjected to chemical fertilization recorded the lowest LAR and LWR values. These results were in compliance with the R:S ratio, and it pointed out that *O. majorana* plants subjected to chemical control reallocated greater amount of photoassimilates to other plant parts, since they prioritized root growth and reduced SLA, as shown in Table 1.

Plants presenting lower LAR and LWR values may stress lower investment in leaf biomass in comparison to their total mass, and it suggests the adoption of an adaptive strategy to prioritize the development of other organs, such as roots. It is done to explore a larger soil area for nutrients' capture. Assimilates' allocation to other plant parts can lead to leaf weight lower than that of other structures (Chang and Zhu, 2017; Durand *et al.*, 2018).

C. cajan doses of 450 and 600 g plant^{-1} recorded RGR and NAR results similar to those observed for chemical fertilizer – results recorded for these doses were significantly higher than those observed for other *C. cajan* doses. RGR enables assessing plants' relative growth, overtime; therefore, high RGR means that plants are growing fast, whereas high NAR indicates that plants are producing more organic matter than consuming it (Lima *et al.*, 2021). These findings can highlight that these doses were more effective in promoting organic matter growth and assimilation by *O. majorana* plants, besides corroborating previously presented results observed for dry matter gain.

3.3 Leaf nutrient analysis

The herein adopted treatments had significant effect on nutrients’ content and accumulation in *O. majorana* leaf dry matter. The highest N accumulation values were observed in plants fertilized with doses of 450 and 600 g plant^{-1} of *C. cajan*. Nitrogen (N) concentration in leaves of *O. majorana* plants subjected to the highest green manure doses assessed in the current study was approximately 11.6% higher than that observed in the absence, or at the lowest dose, of this fertilizer, and 26.7% higher than that observed for plants subjected to chemical fertilizer application. Plants fertilized with 600 g plant^{-1} of green manure accumulated the highest K concentrations (24.0 g kg^{-1}), which were 14.16% higher than those observed for plants subjected to chemical fertilizer application, and 21.66% higher than those observed for non-fertilized plants (Table 3).

Table 3 – Nutrients accumulation in dried leaves of *Origanum majorana* grown with different doses of *Cajanus cajan* and controls (no fertilizer and inorganic fertilization).

Treatment	**N**	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**
g plant^{-1}	**macronutrients (g kg^{-1})**						**micronutrients (mg kg^{-1})**				
0	16.1	1.6	18.8	9.4	1.10	1.10	24.9	9.00	412.7	79.9	48.1
150	16.00	1.50	20.70	8.00	1.20	1.30	29.80	10.80	552.30	48.40	57.80
300	16.90	1.30	22.30	7.60	1.20	1.20	31.50	11.10	1445.10	47.00	57.10
450	18.10	1.20	21.30	7.10	1.20	1.40	34.90	11.30	1782.90	42.20	44.10
600	18.20	1.20	24.00	6.90	1.20	1.30	34.00	11.00	792.00	37.00	50.70
Control	13.30	1.30	20.60	9.30	0.80	1.00	25.10	5.20	948.30	61.20	34.90

Source: Author (2024).

Nitrogen (N) levels observed in the soil corroborated biomass results, according to which, *C. cajan* fertilization provided higher N supply as green manure doses increased. This factor led to higher leaf expansion and, consequently, to higher biomass production. Organic fertilizer increased microbial activity and nutrient availability in the soil, and it increased plants’ essential nutrients-uptake efficiency and increased essential oil yield (Meena *et al.*, 2018).

Surprisingly, 450 g plant^{-1} of green manure led to high Fe accumulation in *O. majorana* leaves. Iron is an essential micronutrient for most living organisms given to its critical role in metabolic processes. Iron plays significant role in several plant physiological and biochemical pathways, besides being a fundamental component of vital enzymes. Moreover, it is required for broad biological functions, such as chlorophyll synthesis, as well as chloroplast structure and function maintenance (Rout and Sahoo, 2015).

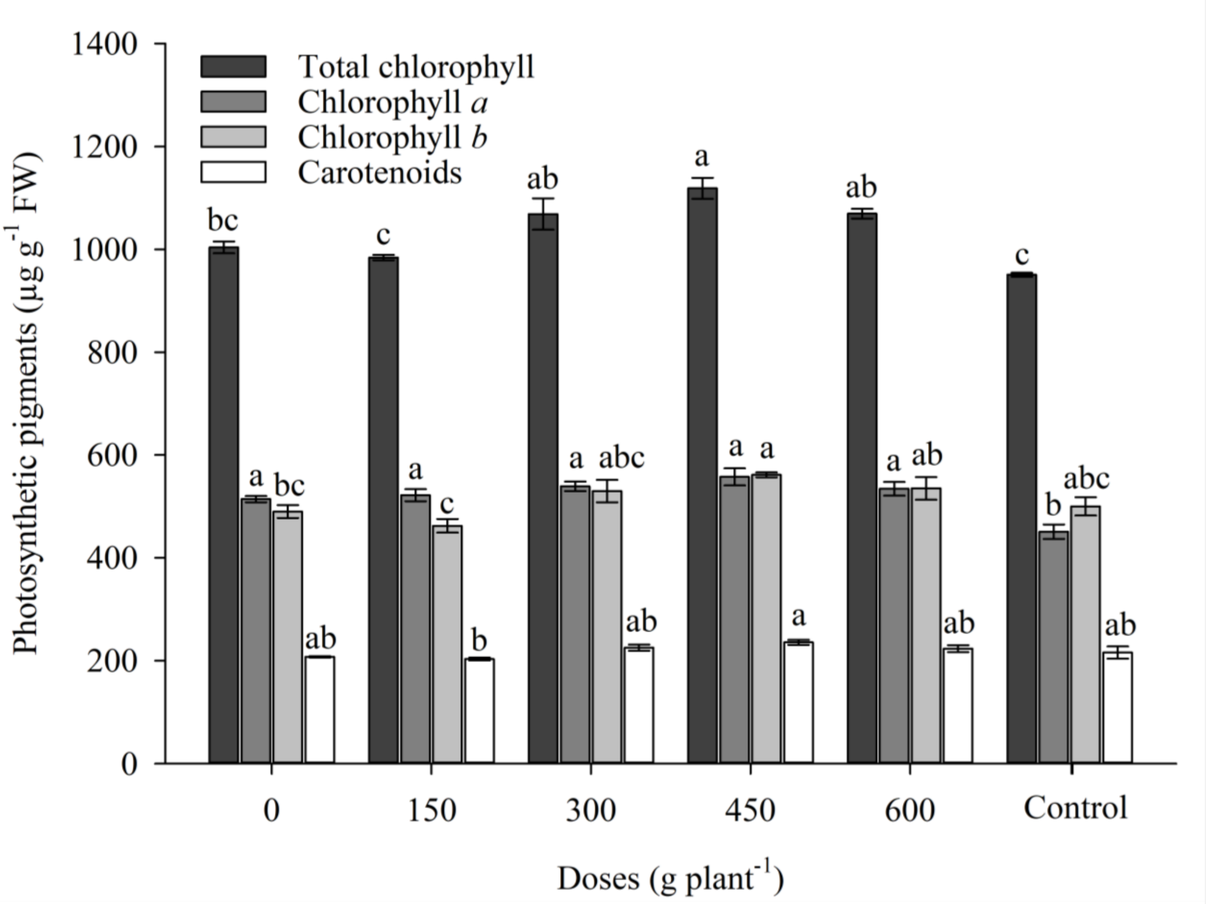
Furthermore, iron is an essential element for several functions in the human body, such as the synthesis of oxygen-carrying proteins like hemoglobin and myoglobin, and the formation of heme enzymes involved in electron transfer and oxidation reduction processes. It plays key role in metabolic processes, such as oxygen transport, DNA synthesis and electron transport (Abbaspour *et al.*, 2014).

Iron deficiency is the sixth most severe human health issue. It is called ‘hidden hunger’ and can lead to health issues, such as anemia, fatigue and impaired cognitive function (Masuda *et al.*, 2020). The high iron content found in species *C. cajan* (476.6 mg kg^{-1}) may have contributed to increase iron content in *O. majorana* plants. Plants’ biofortification with iron and supplementation with iron salts are important strategies to fight iron deficiency and to improve human nutrition (Abbaspour *et al.*, 2014). Therefore, results in the current study have suggested that iron accumulation in *O. majorana* plants can be an interesting biofortification strategy. In addition, it can be beneficial for both medicinal and culinary purposes, since it can increase marjoram’s nutritional value.

3.4 Photosynthetic pigments

Green manure has significantly influenced photosynthetic pigments’ levels in *O. majorana* plants (Figure 4). The dose of 450 g $plant^{-1}$ of green manure promoted the highest total chlorophyll accumulation (1,118.69 µg g^{-1} FW), although it was not significantly different from that observed for doses of 300 and 600 g $plant^{-1}$ of green manure. Chlorophyll is the photosynthetic pigment accounting for light absorption during photosynthesis (Lima *et al.*, 2017; Gotoh *et al.*, 2018). Increased chlorophyll observed in plants treated with green manure has suggested that these plants presented higher light capturing capacity, which may have contributed to increase the photosynthesis rate and, consequently, *O. majorana* plants’ growth and biomass production.

Figure 4 - Concentrations of chlorophyll *a* (µg g^{-1} FW), chlorophyll *b* (µg g^{-1} FW), Total chlorophyll (µg g^{-1} FW), and carotenoids (µg g^{-1} FW) of *Origanum majorana* grown with different doses of *Cajanus cajan*.



*Means followed by the same letter on the x-axis (within doses) do not differ significantly according to the Tukey test ($p \leq 0.05$).

Source: Author (2024).

Another possible explanation for total chlorophyll accumulation increase may be associated with high iron (Fe) levels found in *Origanum majorana* leaves (1,782.90 mg kg^{-1}) and in the green manure "*C. cajan*" (476.6 mg kg^{-1}). Iron (Fe) plays fundamental and limiting role in chlorophyll biosynthesis (Hänsch and Mendel, 2009). Oftentimes, 80% of iron in plants is found in photosynthetic cells, which are used to synthesize heme molecules, mainly chlorophyll, cytochromes, electron transport chain and core proteins in the stroma. Therefore, micronutrient deficiency has negative impact on photosynthetic machinery structure and function (Sági-Kazár *et al.*, 2022; Mahawar *et al.*, 2023).

Chlorophyll content has significantly decreased in plants subjected to positive control (chemical) in comparison to the other treatments. The highest chlorophyll *b* content (561.30 µg g^{-1} FM) was observed after the application of 450 g $plant^{-1}$ of green manure, but it did not statistically differ from contents observed for plants treated with 300 and 600 g $plant^{-1}$ of it.

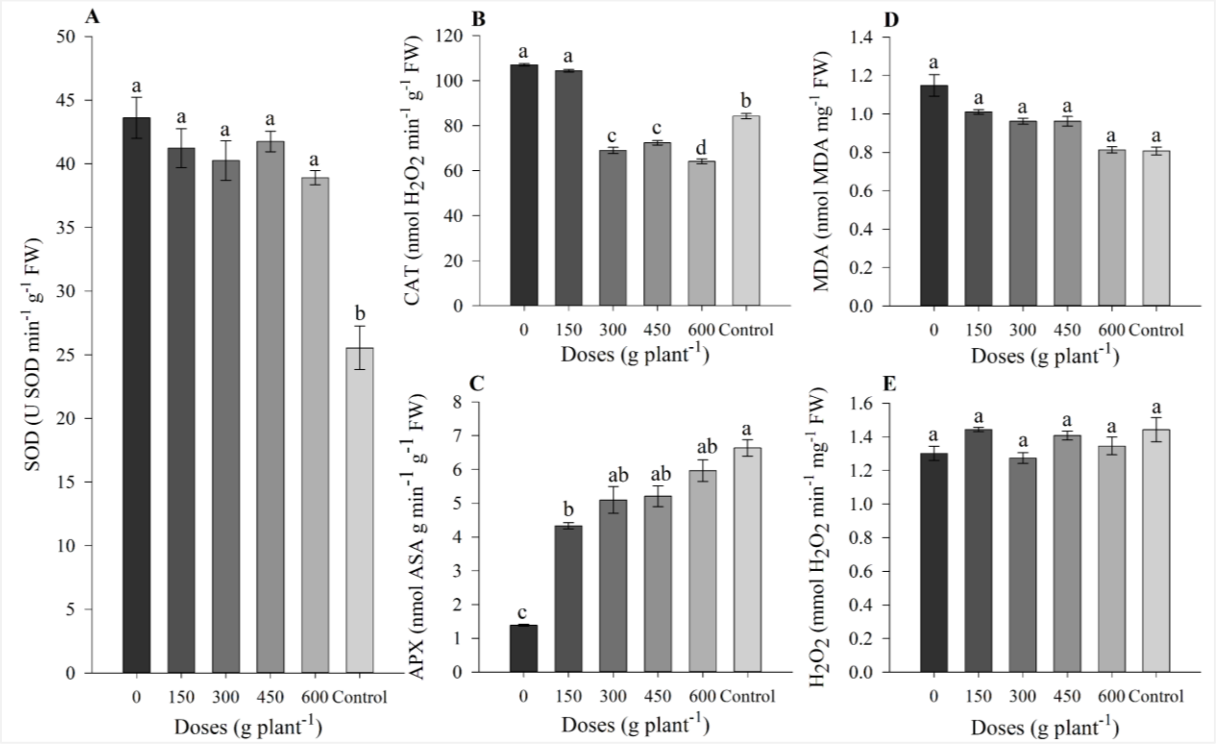
Total carotenoids recorded statistically significant difference between plants treated with 450 and 150 g plant^{-1} of green manure (Figure 4).

Nutrient supply is essential for plant growth and health. Thus, fertilization plays key role in this process. However, limited nutrient supply leads to oxidative stress in plants, which, in its turn, can manifest itself as lower chlorophyll and carotenoid concentrations in leaves (Isah, 2019).

3.5 Antioxidant metabolism

Antioxidant metabolism responses changed depending on the enzyme or on the oxidative stress biological marker among treatments (Figure 5). Plants subjected to chemical fertilization recorded the lowest superoxide dismutase (SOD) activity, which was significantly different from that of other treatments (Figure 5A).

Figure 5 – Contents of superoxide dismutase (U SOD min^{-1} g^{-1} FW), catalase (ηmol H_2O_2 min^{-1} g^{-1} FW), and ascorbate peroxidase (ηmol ASA g min^{-1} g^{-1} FW), lipid peroxidation (ηmol MDA min^{-1} mg^{-1} FW), and hydrogen peroxide (ηmol H_2O_2 min^{-1} mg^{-1} FW) of *Origanum majorana* grown with different doses of *Cajanus cajan*.



*Means followed by the same letter on the x-axis (within doses) do not differ significantly according to the Tukey test ($p \leq 0.05$).

Source: Author (2024).

SOD is the first line of defense against oxidative damage. This enzyme, which is found in all cell types, is a key factor for antioxidant metabolism. It catalyzes the conversion or dismutation of toxic radicals, such as superoxide anion, into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) to protect cells from oxidative stress (Chung, 2017). Results in the current study have suggested that the lower SOD activity observed in plants subjected to chemical control may be associated with low copper availability in the soil. Copper is one constituent of one of SOD isoforms, as well as an essential cofactor for the Cu-SOD isoform function. Therefore, deficiency in this micronutrient, which plays essential role in SOD's active structure formation and maintenance processes, can decrease its activity (Tavanti *et al.*, 2021).

Catalase (CAT) activity, which is regulated by several abiotic conditions leading to stress, was influenced by green manure doses applied to *O. majorana* plants. Plants treated with 0 and 150 g plant^{-1} of green manure recorded higher CAT activity than plants subjected to other treatments (Figure 5B). On the other hand, the lowest activity of this enzyme was observed in plants treated with the highest green manure dose (600 g plant^{-1}), and it pointed out that the high CAT activity observed in plants subjected to negative control and to the lowest *C. cajan* dose may be indicative of oxidative stress caused by nutrient deficiency (Hasanuzzaman *et al.*, 2020). Reactive oxygen species' (ROS) production can increase in case of nutritional deficiency. This process requires higher CAT activity to convert hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2), in order to mitigate damage caused by H_2O_2 accumulation (Ganie *et al.*, 2011).

The lowest ascorbate peroxidase (APX) activity was observed in plants subjected to the control dose (0 g plant^{-1}) and it was significantly different from the APX activity observed for plants subjected to the other green manure doses (Figure 5C). The APX enzyme plays crucial role in protecting plants from oxidative stress, since it is involved in hydrogen peroxide (H_2O_2) decomposition in both cytosol and chloroplasts (Dumanović *et al.*, 2021). Its partitioning can be correlated to the result observed for photosynthetic pigments (Figure 4), since the lowest APX activity took place in plants presenting lower chlorophyll content.

The lowest Fe levels found in leaf tissues (Table 3) may have impaired APX activity in these plants, since APX is a Fe-carrying enzyme. Reduced APX activity is associated with plants sensitive to iron deficiency (Caverzan *et al.*, 2012). Studies have shown that APX activity is lower under Fe deficiency conditions (Santos *et al.*, 2019; Rahman *et al.*, 2021).

On the other hand, the highest APX activity was mainly observed in plants subjected to chemical control. SOD activity compensation by APX suggests plants' adaptive response mechanism to low iron availability conditions, and it highlights the important role played by these enzymes' interaction in plants antioxidant response (Santos *et al.*, 2019).

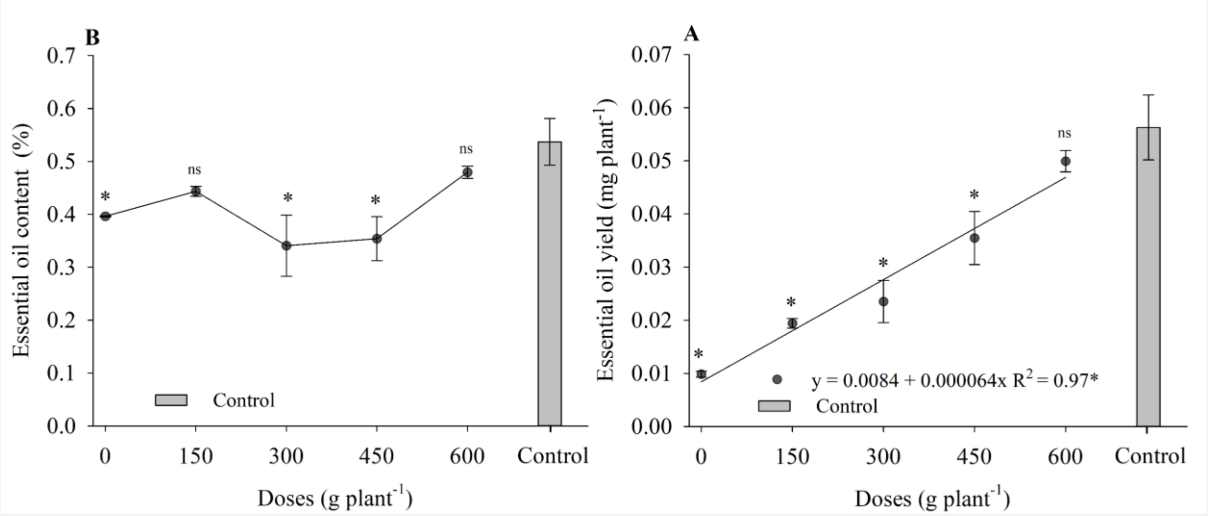
The levels of oxidative stress markers, such as MDA and H_2O_2, did not change among treatments (Figures 5D and E). The role played by reactive oxygen species in plant defense is not fully understood, but it is consensus that their accumulation can lead to oxidative damage. However, differences in enzyme activity among *C. cajan* doses may have suppressed ROS production to a level tolerable by plants.

3.6 Essential oil yield and chemical composition

Green manure has influenced *O. majorana* leaf essential oil (EO) content and yield. The highest EO contents (0.48% and 0.52%) were observed after 600 g plant^{-1} of *C. cajan* and inorganic fertilization, resulting in EO levels of 0.48% and 0.52%, respectively (Figure 6A). Likewise, the highest EO yields (0.055 and 0.056 mg plant^{-1}) were also observed after 600 g plant^{-1} of *C. cajan* and inorganic fertilizer application, representing an increase of 406% and 471%, compared to the absence of fertilization, respectively (Figure 6B).

According to Marques *et al.* (2018), EO production from many medicinal, aromatic and spicy plants is favored by high nutrient availability and accumulation conditions, as well as by soil physical and biological conditions. Therefore, green manure can be a viable alternative to replace chemical fertilization in *O. majorana* plant production focused on extracting its EO.

Figure 6 - Essential oil content (A) and essential oil yield (B) of *Origanum majorana* grown with different doses of *Cajanus cajan* and with inorganic fertilization (control).



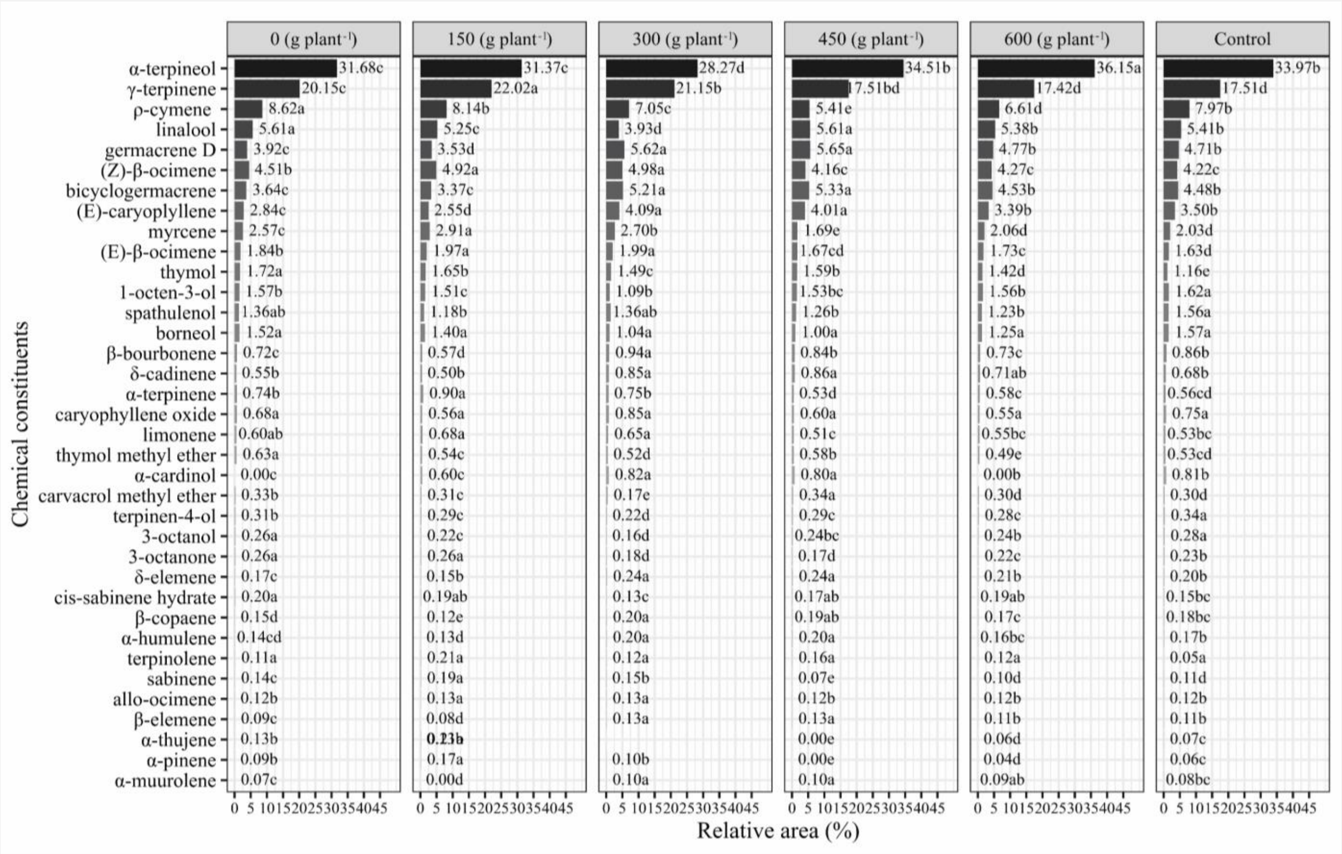
(^{ns}) no difference, (*) differs from control by Dunnett test (p ≤0.05).
Source: Author (2024).

Variations observed in EO levels in response to different fertilizer doses may be associated with a complex interaction between nutritional stress and the biosynthesis of plants' secondary compounds. Plants subjected to lower fertilizer doses can experience nutritional stress and trigger defense responses, such as producing volatile compounds. On the other hand, higher fertilizer doses can promote favorable environment for plant growth and EO yield.

Adequate nutrient availability can stimulate metabolic pathways accounting for producing these secondary compounds and lead to higher EO levels (Yang *et al.*, 2018).

Chemical analysis applied to *O. majorana* essential oil has highlighted incidence of 36 chemical constituents accounting for 97.79%-98.78% of its total chemical composition. Fourteen (14) of these compounds presented relative percentage area greater than 1% and accounted for approximately 90% of the total chemical composition of the investigated EO (Figure 7). EO composition strongly depends on environmental and plant growth conditions, and it is corroborated by differences in volatile constituents' levels in different *C. cajan* and inorganic fertilizer doses.

Figure 7 – Chemical composition of the essential oil of *Origanum majorana* grown with different doses of *Cajanus cajan* and with inorganic fertilization (control).



*Means followed by the same letter on the x-axis (within doses) do not differ significantly according to Tukey's test ($p \leq 0.05$).

Source: Author (2024).

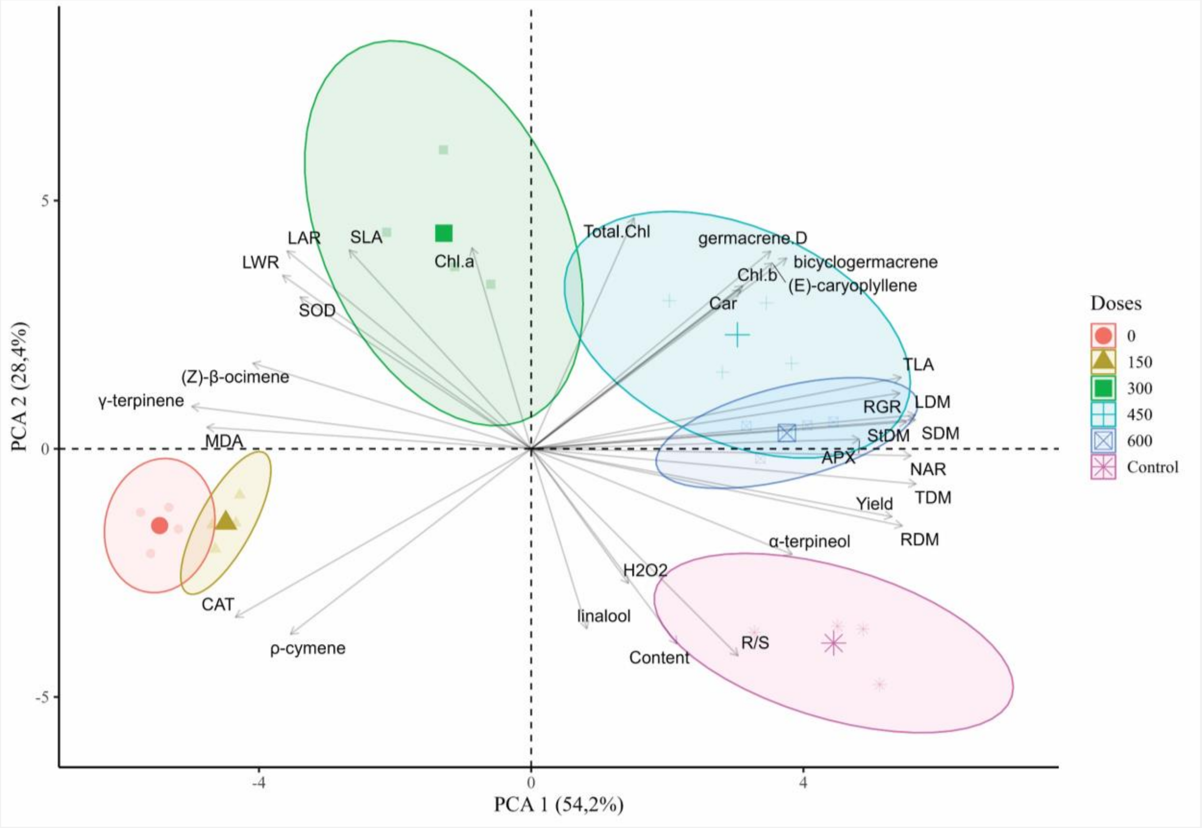
α-Terpineol was the major compound found in *O. majorana* EO. Its concentration was significantly higher at *C. cajan* dose of 600 g plant^{-1} and its percentage value reached 36.15% (Figure 7). α-Terpineol prevalence in *O. majorana* essential oil, mainly at the highest *C. cajan* doses, has relevant implications for the industrial sector (Prabu *et al.*, 2020b).

α-Terpineol has the potential to be used by the cosmetic industry to provide different aromatic features for perfumery and skincare products (Ouedrhiri *et al.*, 2021). Its antimicrobial and refreshing properties also make it essential to personal hygiene products. Moreover, its therapeutic properties provide potential health benefits (Ghazal *et al.*, 2022). These findings highlight the importance of taking into consideration fertilization with *C. cajan* as strategy to increase α-Terpineol concentration in *O. majorana* EO, as well as its usefulness for industries that use this chemical constituent in the composition of their products.

3.7 Principal Component Analysis

Principal component analysis (PCA) is a multivariate statistical technique used to reduce data dimensionality to enable clearer visualization of data patterns and variations (Abbas *et al.*, 2023). Based on Figure 8, data variations were explained by 82.6% of total variance. The first two components, PC1 and PC2, accounted for explaining 54.2% and 28.4% of data variation, respectively.

Figure 1 - Principal Component Analysis (PCA) relating to *Origanum majorana* grown with different doses of *Cajanus cajan* and with inorganic fertilization (control).



Source: Author (2024).

Based on the high variance rate explained by the first two principal components (PC1 and PC2), these components were capable of capturing most of the relevant information provided by the investigated variables. According to (Mardia *et al.*, 1979), total data variation higher than 80% is considered satisfactory to explain the variability recommended by the analyzed variables.

Based on PCA (Figure 8), the correlation among the main PC1 components was strongly associated with several variables, such as total Chl, Chl *b*, carotenoids, (E)-caryophyllene, germacrene D, bicyclogermacrene, linalool, α-terpineol, TLA, RGR, LDM, StDM, APX, NAR, TDM, EO yield and content, RDM and H_2O_2.

According to this association, PC1 captured the joint variations of these variables and it suggested that they may be interrelated or share common factors capable of affecting their values. The presence of prevalent groups of these parameters at doses of 450 and 600 g plant^{-1} of *C. cajan* and chemical fertilizer has pointed out that these green manure and chemical fertilizer doses corroborate previously discussed results.

On the other hand, PC2 has shown positive correlation to LAR, LWR, SLA, Chl *a*, SOD, γ-terpinene, (Z)-β-ocimene, MDA, total Chl, Chl *b*, carotenoids, (E)-caryophyllene, germacrene D, bicyclogermacrene, TLA, RGR, LDM and StDM - the most prevalent groups comprised *C. cajan* doses of 300 and 450 g plant^{-1}.

PCA enabled the comprehensive interpretation of results, as well as identifying specific groups of parameters associated with *C. cajan* doses of 450 and 600 g plant^{-1} and chemical fertilizer. These groups presented better responses than the other *C. Cajan* doses, and it indicated that these doses were more effective in positively influencing *O. Majorana* plant growth, antioxidant metabolism and chemical features. Identifying specific parameter groups enabled the best crop management to achieve sustainability and to increase *O. Majorana* yield.

4 CONCLUSION

Just like inorganic fertilization, green manure based on *C. cajan* application was beneficial to *O. Majorana* plants' growth, antioxidant metabolism and volatile chemical constituents. The dose of 600 g plant^{-1} of *C. cajan* led to the best results, which were equivalent to those observed for inorganic fertilization in terms of plant growth, nutrient accumulation, antioxidant activity, and EO content and yield. It is also worth emphasizing the increased α-terpineol concentration in EO extracted from plants treated with this *C. cajan* dose, since this species can be used as alternative natural source of this chemical constituent in companies that use it in their products' formulation.

Therefore, findings in the current study helped better understanding the effects of green manure based on *C. cajan* application on different aspects of *O. majorana* plants, besides enabling the adoption of an approach focused on optimizing its plant, EO and α-terpineol yield. In addition, using green manure, such as *C. cajan*, is a sustainable and low-cost alternative to grow *O. majorana* plants.

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