RAFAEL MARLON ALVES DE ASSIS

GREEN MANURE AND GROWTH REGULATORS IN THE PRODUCTION OF BIOMASS, ESSENTIAL OIL AND ANTIOXIDANT CAPACITY OF *Origanum vulgare* L. (LAMIACEAE)

LAVRAS-MG
2023

RAFAEL MARLON ALVES DE ASSIS

GREEN MANURE AND GROWTH REGULATORS IN THE PRODUCTION OF BIOMASS, ESSENTIAL OIL AND ANTIOXIDANT CAPACITY OF *Origanum vulgare* L. (LAMIACEAE)

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Plantas Medicinais, Aromáticas e Condimentares, área de concentração cultivo e manejo sustentável de plantas medicinais, para obtenção do título de Doutor.

Prof. Ph.D. José Eduardo Brasil Pereira Pinto
Orientador

Prof (a). Dr (a). Suzan Kelly Vilela Bertolucci
Co-orientadora

Prof. Dr. Alexandre Alves de Carvalho
Co-orientador

LAVRAS-MG
2023

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Assis, Rafael Marlon Alves de.
Green manure and growth regulators in the production of biomass, essential oil and antioxidant capacity of *Origanum vulgare* L. (Lamiaceae) / Rafael Marlon Alves de Assis. - 2023.
104 p. : il.

Orientador(a): José Eduardo Brasil Pereira Pinto.
Coorientador(a): Suzan Kelly Vilela Bertolucci, Alexandre Alves de Carvalho.
Tese (doutorado) - Universidade Federal de Lavras, 2023.
Bibliografia.

1. Crotalária. 2. Orégano. 3. Metabolito secundário. I. Pinto, José Eduardo Brasil Pereira. II. Bertolucci, Suzan Kelly Vilela. III. Carvalho, Alexandre Alves de. IV. Título.

RAFAEL MARLON ALVES DE ASSIS

GREEN MANURE AND GROWTH REGULATORS IN THE PRODUCTION OF BIOMASS, ESSENTIAL OIL AND ANTIOXIDANT CAPACITY OF *Origanum vulgare* L. (LAMIACEAE)

ADUBO VERDE E REGULADORES DE CRESCIMENTO NA PRODUÇÃO DE BIOMASSA, ÓLEO ESSENCIAL E CAPACIDADE ANTIOXIDANTE DE *Origanum vulgare* L. (LAMIACEAE)

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Plantas Medicinais, Aromáticas e Condimentares, área de concentração cultivo e manejo sustentável de plantas medicinais, aromáticas e condimentares, para obtenção do título de Doutor.

APROVADA em 22 de Fevereiro de 2023.

Dr. Osmar Alves Lameira	Embrapa Amazônia Oriental
Dr. Felipe Campos Figueiredo	IFSULDEMINAS
Dra. Ana Paula Ribeiro Medeiros	SEMAS
Dra. Suzan Kelly Vilela Bertolucci	UFLA

Prof. Ph.D. José Eduardo Brasil Pereira Pinto
Orientador

LAVRAS-MG
2023

Dedico esse trabalho a minha família, em especial aos meus pais, Manoel Gilvan de Assis e Heliana Ferreira Alves, como forma de gratidão por toda a educação e ensinamentos repassados a mim e por serem meu exemplo e inspiração de vida.

AGRADECIMENTOS

Primeiramente, agradeço a Deus pela dádiva e dom da vida, por ter permitido eu concluir mais um importante ciclo da minha vida. Durante essa trajetória sempre levei comigo a seguinte frase do Galilei Galileu: “A ciência humana de maneira nenhuma nega a existência de Deus. Quando considero quantas e quão maravilhosas coisas o homem compreende, pesquisa e consegue realizar, então reconheço claramente que o espírito humano é obra de Deus, e a mais notável”.

À minha família, em especial aos meus pais Manoel e Heliana, ao meu irmão Marcelo Fagner Alves de Assis e ao meu sobrinho Heitor, que me concederam durante essa caminhada todo suporte necessário de união, companheirismo e amor para realização desse sonho.

Ao meu orientador e amigo José Eduardo Brasil Pereira Pinto, que desde o início me deu todo suporte e apoio necessário. Desde o planejamento até a execução dos experimentos esteve sempre presente com disposição para ajudar. Meu muito obrigado por toda amizade e companheirismo durante essa etapa importante de minha vida.

Aos meus coorientadores, Suzan Kelly Vilela Bertolucci e Alexandre Alves de Carvalho que sempre estiveram disponíveis para dar instruções e repassar conhecimentos nos momentos necessários. Muito obrigado por toda dedicação.

Ao meu primeiro orientador da vida acadêmica, Dr. Osmar Alves Lameira que antes da graduação esteve ao meu lado me auxiliando, me orientando e sendo meu exemplo como pessoa e como profissional. Meu muito obrigado por fazer parte da realização e concretização deste sonho.

Aos meus amigos de Pós-Graduação Jandeilson Pereira dos Santos, Jeremias José Ferreira Leite, Lissa Izabel Ferreira de Andrade, Rafaela Melheiros Salomon, Giulia Duarte, João Pedro Miranda Rocha, Júlia Assunção de Castro Oliveira, Adriane Duarte Coelho que contribuiram significativamente para realização deste trabalho. Meus sinceros agradecimentos a vocês!

Aos amigos conterrâneos e da UFLA que desde o mestrado e durante parte do doutorado estiveram presentes me ajudando, Ana Paula, Carol Germano, Diene Xavier, Fernanda Ribeiro, Lucinda Hoffman, Nelma Oliveira, Raíssa Couteiro, Ruanny Vidal, Tainá Rocha, Thainá Oliveira, Marcus Vinicius Prado Alves, Alan da Cunha Honorato, Abel Bastos, Érica Marquez.

Aos meus amigos da Igreja Adventista do Sétimo Dia (IASD) - Central de Lavras, em especial ao Dany Flávio Tonelli e Leticia Klein Tonelli que desde a minha chegada em Lavras

me acolheram como membro da família! Meu muito obrigado pela amizade, confiança e pelos momentos de alegria proporcionados que fizeram a diferença durante essa jornada.

A dona Cidinha (Maria das Graças Oliveira), por toda estadia e cuidado durante esses seis anos. Muito obrigado por tudo!

Aos núcleos de estudos NEMAC (Núcleo de Estudos em Palntas Medicinais Aromática e Condimentares) e NEPRON (Núcleo de Estudos em Produtos Naturais) por toda experiencia proporcionada durante a organização de eventos e nos valiosos momentos de extensão onde repassávamos todos os conhecimentos aprendidos.

Aos Técnicos do Horto de Plantas Medicinais do Departamento de Agricultura (DAG-UFLA), Paulo (Pulhinho, em memória), Leandro (Dico) e Giulia Duarte por toda ajuda e apoio prestado durante a execução dos experimentos.

Ao Laboratório de Fitoquímica DAG-UFLA, por toda estrutura e pela a ajuda da Anette Boari que foi de grande importância.

Ao Laboratório de Cultura de Tecidos em Plantas Medicinais do DAG-UFLA, principalmente pelo técnico Evaldo Arantes de Souza que foi sempre prestativo e colaborativo com as exigências dos trabalhos.

Aos órgãos de fomento, como a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES - Código financeiro 001) pela concessão da bolsa de doutorado que possibilitou meu sustento e recursos para os estudos realizados. Bem como ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e a Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) que em parceria com outros setores também colaboraram de forma significativa.

Á Universidade Federal de Lavras - UFLA por todo suporte estrutural que permitiu a concretização desse trabalho de dissertação.

Ao programa de Pós-Graduação em Plantas Medicinais Aromáticas e Condimentares, pela oportunidade e aos professores da comunidade acadêmica que contribuíram com os conhecimentos e ensinamentos compartilhados.

Enfim, a todos que de alguma forma ajudaram e contribuíram para realização deste sonho. Eu agradeço. MUITO OBRIGADO!!!

RESUMO GERAL

O orégano (*Origanum vulgare* L.) é uma das espécies mais importantes comercialmente da família Lamiaceae, pois tem sido largamente utilizada nas indústrias agrícolas, farmacêuticas e de cosméticos. Dentre as práticas usadas para aumentar a biomassa e produção de óleo essencial nas plantas medicinais encontram-se: adubação verde (AV) e aplicação foliar de reguladores vegetais. No presente estudo objetivou-se: (1) avaliar o efeito da adubação verde (*Crotalaria juncea* L.) na produção de biomassa, composição química do óleo essencial, acúmulo de compostos fenólicos e atividade antioxidante de *O. vulgare* e (2) observar a influência da aplicação foliar de reguladores de crescimento de plantas (RCPs) na produção de biomassa, metabólitos secundários e na atividade antioxidante enzimática *de O. vulgare*. O experimento de adubação verde foi constituído por oito tratamentos: controle (solo); 200 g $vaso^{-1}$ (raiz de crotalária); 150, 300, 450 e 600 g $vaso^{-1}$ parte aérea de crotalaria (PA=folha+caule) + 200 g $vaso^{-1}$ (raiz de crotalária); 600 g $vaso^{-1}$ parte aérea de crotalaria (PA); e controle positivo (esterco bovino 300 g $vaso^{-1}$). Para os RCPs foi utilizado 3 tipos (Giberelina-GA_3, Ácido indolbutírico-AIB, Cinetina-KIN) com 3 concentrações (25, 50, 100 mg L^{-1}) + controle. Foram avaliados o peso seco, pigmentos fotossintéticos, teor e rendimento de óleo essencial e atividade antioxidante. O manejo da AV proporcionou as maiores médias de peso seco nas doses 300, 450 e 600 g (PA+R) e 600 g $vaso^{-1}$ (PA), assim como o esterco bovino (300 g). O tratamento sem AV (controle) resultou em uma produção de óleo essencial 75% menor em comparação ao tratamento 450 g $vaso^{-1}$ AV (PA+R) e de aproximadamente 71% em relação aos tratamentos 300, 600 g AV (PA + R) e 600 g $vaso^{-1}$ AV (PA). No que tange ao experimento com RCPs, a aplicação de GA_3 na dose de 25 mg L^{-1} aumentou o peso seco da folha, caule, raiz, parte aérea e peso seco total em relação ao controle. O maior teor de clorofila *a* foi observado para a dose de KIN25. Os ganhos de teor de óleo com as doses de KIN25 e IBA25 foram de 30,9 e 29,6%, respectivamente, em relação à testemunha e as maiores médias de rendimento foram observadas nas doses mais baixas. Os menores níveis médios de peróxido de hidrogênio (H_2O_2) e malondialdeído (MDA) foram observados na presença dos RCPs. Os RCPs estimularam a atividade antioxidante enzimática, sinalizando proteção ao estresse oxidativo. Em conclusão, o uso de *C. juncea* como AV é um recurso de baixo custo que tem efeito positivo no cultivo de *O. vulgare*, e sua utilização pode reduzir a aplicação de fertilizantes químicos, tornando-se uma estratégia ecologicamente adequada para o cultivo de plantas medicinais. A aplicação foliar de RCPs em baixas doses pode contribuir para aumentar a matéria seca e os constituintes químicos em *O. vulgare*.

Palavras chave: Orégano. Crotalária. Aplicação foliar. Planta medicinal. Metabolito secundário.

ABSTRACT

Oregano (*Origanum vulgare* L.) is one of the most commercially important species of the Lamiaceae family, as it has been widely used in the agricultural, pharmaceutical and cosmetic industries. Among the practices used to increase biomass and essential oil production in medicinal plants are: green manuring (GM) and foliar application of plant regulators. The present study aimed to: (1) evaluate the effect of green manuring (*Crotalaria juncea* L.) on biomass production, essential oil chemical composition, accumulation of phenolic compounds and antioxidant activity of *O. vulgare* and (2) observe the influence of foliar application of plant growth regulators (PGRs) on biomass production, secondary metabolites and enzymatic antioxidant activity of *O. vulgare*. The green manure experiment consisted of eight treatments: control (soil); 200 g pot^{-1} (crotalaria root); 150, 300, 450 and 600 g pot^{-1} sunn hemp aerial part (Sh=leaf+stem) + 200 g pot^{-1} (crotalaria root); 600 g pot^{-1} aerial part of crotalaria (Sh); and positive control (cattle manure 300 g pot^{-1}). For PGRs, 3 types were used (Giberellin-GA_3, Indolebutyric acid-IBA, Kinetin-KIN) with 3 concentrations (25, 50, 100 mg L^{-1}) + control. Dry weight, photosynthetic pigments, essential oil content and yield and antioxidant activity were evaluated. The GM management provided the highest dry weight averages at doses 300, 450 and 600 g (Sh+R) and 600 g pot^{-1} (Sh), as well as cattle manure (300 g). Treatment without GM (control) resulted in a 75% lower essential oil production compared to treatment 450 g pot^{-1} GM (Sh+R) and approximately 71% compared to treatments 300, 600 g GM (Sh + R) and 600 g pot^{-1} GM (Sh). Regarding the experiment with PGRs, the application of GA_3 at a dose of 25 mg L^{-1} increased the dry weight of the leaf, stem, root, shoot and total dry weight in relation to the control. The highest chlorophyll a content was observed for the KIN25 dose. The gains in oil content with the KIN25 and IBA25 doses were 30.9 and 29.6%, respectively, in relation to the control and the highest average yields were observed at the lowest doses. The lowest average levels of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) were observed in the presence of PGRs. PGRs stimulated enzymatic antioxidant activity, signaling protection against oxidative stress. In conclusion, the use of *C. juncea* as GM is a low-cost resource that has a positive effect on the cultivation of *O. vulgare*, and its use can reduce the application of chemical fertilizers, making it an ecologically appropriate strategy for the cultivation of medicinal plants. The foliar application of PGRs at low doses may contribute to increase dry matter and chemical constituents in *O. vulgare*.

Keywords: Oregano. Sunn hemp. Foliar application. Medicinal plant. Secondary metabolite.

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE SIGLAS

APx	Ascorbate Peroxidase
CAT	Catalase
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
2,4-D	2,4-dichlorophenoxyacetic acid
EDTA	Ethylenediaminetetraacetic acid
EO	Essential oil
ERN	Espécies Reativas de Nitrogênio
ERO	Espécies Reativas de Oxigênio
ET	Ethylene
GA_3	Gibberellin
GM	Green manure
GPx	Glutationa Peroxidase
IAA	3-indoleacetic acid
IBA	Indole-3-butyric acid
ISO	"International Organization for Standardization"
ISR	Induced systemic resistance
KIN	Kinetin
LAR	Leaf area ratio
LDW	Leaf dry weight
MDA	Malondialdehyde
MO	Matéria Orgânica
NAA	1-naphthalene-acetic acid
NBT	Nitrotetrazolium blue
ORAC	"Oxygen-radical absorbancy capacity"
PCA	Principal component analysis
PGR	Plant growth regulator
RDW	Root dry weight
ShDW	Shoots dry weight
SAR	Systemic acquired resistance
R/Sh Ratio	Roots-Shoots ratio
SDW	Stem dry weight
SLA	Specific leaf area
SOD	Superoxide dismutase
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TDW	Total dry weight
TLA	Total leaf area
TRAP	"Trapping antioxidant parameter"

SUMÁRIO

PRIMEIRA PARTE

1. INTRODUÇÃO GERAL

O orégano (*Origanum vulgare* L.) é uma espécie vegetal comercialmente importante da família Lamiaceae (MORSHEDLOO et al., 2018), pois tem sido largamente utilizada nas indústrias agrícolas, farmacêuticas e de cosméticos. Essa espécie é bastante conhecida na culinária, sendo usada em molho de tomate, pizzas, salada e até em carnes. Além disso, seus constituintes são utilizados como substância aromatizante em produtos alimentares, bebidas alcoólicas e em perfumaria, por possuir fragrância picante (COQUEIRO et al., 2012; PEREIRA; DOS SANTOS, 2013).

Conforme Pezzani, Vitalini e Iriti (2017) o orégano representa uma fonte promissora de produtos naturais bioativos (óleo essencial, extratos ou compostos puros), pois apresenta potencial como agente protetor em doenças crônico-degenerativas e infecciosas devido às atividades anticâncer, anti-inflamatória, antioxidante e antimicrobiana relacionadas aos seus fitoquímicos bioativos.

Apesar do Brasil possuir extensa área e uma das floras mais ricas do mundo, é um país com grandes gastos em importações de plantas para fins medicinais e ou condimentares (MOSELE; CECCHIN; DEL FRARI, 2010). De acordo com Rodrigues (2016), o Brasil gastou mais de 20.000 milhões U$ com importações de planta medicinais “in natura”. Em adição, Moghaddam e Mehdizadeh (2017) afirmam que vários fatores podem influenciar a composição dos óleos essenciais (OE). Esses fatores podem ser intrínsecos e extrínsecos, incluindo condições de crescimento, clima, altitude, tipo de solo, métodos e práticas agrícolas, estádio de desenvolvimento, parte da planta extraída, tempo de colheita, dentre outros fatores.

Outro ponto importante está relacionado com as práticas de cultivos dentro dessa espécie, pois segundo Skoufogianni, Solomou e Danalatos (2019), apesar da extensa literatura disponível sobre o uso do óleo essencial e da matéria seca foliar do orégano, existem poucos relatos referente ao cultivo desta planta, os quais dependem das condições edafoclimáticas.

A partir disso, a inovação no manejo do cultivo para essa espécie é de grande importância para melhorar as suas práticas agrícolas e, consequentemente, sua produtividade. Dentre as práticas usadas para aumentar a matéria seca e a produção de óleo essencial nas plantas medicinais encontram-se a adubação verde e aplicação foliar de reguladores de crescimento (NAZMY, 2020; MARQUES et al.,2018).

Na dubação verde a *Crotalaria juncea* L. (Leguminosae) é uma das espécies mais utilizadas, pois é adaptada às diferentes condições edafoclimáticas e do solo no Brasil e destaca-se por sua grande capacidade de produção de biomassa, acúmulo de nutrientes e altas qualidades

de seus resíduos (XAVIER; OLIVEIRA; SILVA, 2017). Duarte Junior (2006) avaliando plantas de cobertura para sistema de plantio direto verificou que a *C. juncea* apresentou maior acúmulo de K, Mg, S, Zn e Fe quando comparada com outras espécies de adubo verde. Conforme Lima-Filho et al. (2014) a crotalária pode apresentar médias de 40,5 t/ha de matéria fresca, 17,5 t/ha de matéria seca, relação C/N de 18 e 400 kg ha^{-1} de nitrogênio.

Apesar de serem escassas, algumas pesquisas demonstraram que o uso da adubação verde pode melhorar os rendimentos de biomassa e a produção de óleo essencial. Youn et al. (2017) constataram que o uso da adubação verde com a espécie *Hordeum vulgare* L. (Poeaceae) influenciou no rendimento radicular, no conteúdo total de polifenóis e na atividade antioxidante de *Cynanchum wilfordii* Hemsley (Apocynaceae). Singh et al. (2010) também obsevaram que a adubação verde com *Vigna unguiculata* L. Walp. (Leguminosae) promoveu o aumento da matéria fresca e rendimento de óleo essencial da espécie *Cymbopogon martini* (Roxb.) W. Watson (Poaceae), além de melhorar a eficiência do uso do nitrogênio, promovendo economia de 30 kg/ha.

Outro manejo disponível para aumentar a produção de metabólitos secundários é a aplicação foliar de reguladores de crescimento de plantas (RCPs) como ácido indole-3-butírico (IBA), cinetina (KIN) e giberelina (GA_3) (NAZMY, 2020; BAHMAN; MEHRAFARIN; NAGHDI BADI, 2017; POURHADI et al., 2018; SINGH et al., 2019). O RCP pode influenciar no acúmulo de bioativos de interesse econômico, aumento de biomassa e das atividades antioxidantes.

No caso das plantas medicinais, empregar um manejo adequado durante o cultivo é essencial para garantir a qualidade físico-química da matéria prima vegetal. Portanto, o objetivo deste trabalho foi avaliar o efeito da adubação verde e reguladores de crescimento no crescimento, na produção e análise química do óleo essencial, no acúmulo de compostos fenólicos, na atividade e produção de enzimas antioxidante de *Origanum vulgare*.

2. REFERENCIAL TEÓRICO

2.1 *Origanum vulgare* L.: aspectos botânicos, agronômicos e medicinais

A família Lamiaceae abrange cerca de 200 gêneros e, aproximadamente, 3.200 espécies, distribuídas em todo o mundo (LORENZI; MATOS, 2002). Diversos autores afirmam que a maioria das espécies dessa família já são conhecidas pelo seu uso condimentar, e muitas delas possuem atividade biológica já relatada na literatura. Dentre algumas espécies estudadas as que mais se destacam são: a *Hyptis suaveolens* L. (Cheirosa. Salva limão, bamburral), *H. mutabilis*

(Rich.) Briq., *H. atrorubens* Poit.; *Lavandula angustifolia* Mill (alfazema); *Melissa officinalis* L. (cidreira), *Mentha arvensis* L. (hortelã-do-Brasil), *M. piperita* L. (hortelã), *M. pulegium* L. (poejo), *Ocimum basilicum* L. (majericão), *Origanum vulgare* L. (orégano), *Rosmarinus officinalis* L. (alecrim) e *Salvia officinalis* L. (sálvia) (LORENZI; MATOS, 2008).

O gênero *Origanum* L. apresenta mais de 70 espécies, 49 táxons (espécies e subespécies) e híbridos naturais que são encontrados em quase todos os países do mediterrâneo e também nas zonas temperadas da Ásia e América (KINTZIOS, 2002).

O nome orégano (*Origanum vulgare*) é derivado das palavras "*oros*" = montanha e "*ganos*" = esplendor, ou seja, é uma planta nativa de regiões montanhosas e pedregosas do Sul da Europa e cultivada no Brasil, possuindo caráter heliófila. (CUNHA; ROQUE; GASPAR, 2011; DELAVEUA et al., 1983; LORENZI; MATOS, 2008).

O *Origanum vulgare* é conhecido popularmente como orégano, orégão, ouregão, manjerona-baiana, manjerona-selvagem, manjerona, dentre outros. Essa espécie apresenta características herbácea, perene, ereta, aromática e de hastes algumas vezes arroxeadas. A altura dessa espécie pode variar de 30-50 cm, com folhas de 1-2 cm de comprimento, as flores podem apresentar colorações esbranquiçadas, róseas ou violáceas, reunidas em inflorescências paniculadas terminais (LORENZI; MATOS, 2008).

De acordo com Skoufogianni, Solomou e Danalatos (2019), o orégano pode crescer e se desenvolver de forma ideal em temperaturas entre 18ºC-22ºC. Porém, temperaturas abaixo de 4ºC e acima de 33ºC podem limitar o crescimento da espécie. Além disso, essa espécie pode ser cultivada em uma grande variedade de solo e climas. Um excelente valor de pH do solo é de cerca de 6,8, mas essa planta pode ser encontrada em solos calcários com valores de pH muito mais altos. Geralmente, é necessário um longo período de luz (mais de 12 horas) para um alto teor de óleo essencial e carvacrol (SKOUFOGIANNI; SOLOMOU; DANALATOS, 2019).

A reprodução da espécie pode ser assexuada, por propagação vegetativa, ou sexuada via sementes (PÓVOA, FARINHA e CLARÉ, 2017). Sendo que a propagação por via vegetativa pode ser feita por divisão de rebentos ou através do enraizamento de estacas. Goleniowski, Flamarique e Bima (2003) também afirmaram que a divisão de plantas é usada tradicionalmente para propagar orégano e a propagação via sementes ocorrem altos níveis de heterogeneidade entre as plantas.

No que diz respeito ao uso e efeito medicinal do *Origanum vulgare* pesquisas realizadas em células *in vitro* verificaram que o chá por infusão (3g / 250 mL de água) teve potencial para combater células cancerígenas da próstata (PC3) e do cólon (HT29), além de apresentar atividade antioxidante relacionada aos fenóis totais. Estudos pré-clínicos evidenciaram que o

pó das folhas de orégano é capaz de aumentar a imunidade. Sendo também já comprovado que o óleo essencial dessa espécie possui atividade antimicrobiana (BELTRÁN et al., 2020; FIKRY; KHALIL; SALAMA et al., 2019; KOGIANNOU et al., 2013).

Em relação ao mercado econômico, o orégão (*Origanum vulgare*) é um dos condimentos mais valorizados no mundo, principalmente nos países do mediterrâneo. O mercado total de orégano é de aproximadamente 350-500 toneladas na França, 600 toneladas na Alemanha, 500 toneladas em Inglaterra e 150 toneladas nos Países Baixos. Nos Estados unidos são consumidas mais de 300. 000 tonelada de orégano por ano (PÓVOA; FARINHA; CLARÉ, 2017).

2.2 Óleos essenciais

Conforme a *International Organization for Standardization* - ISO 9235 (2013), o óleo essencial (OE) é o produto obtido a partir de uma matéria-prima natural de origem vegetal, por técnicas que envolvem hidrodestilação, arraste com vapor de água e também pela prensagem do pericarpo de frutos cítricos.

Os OEs podem ser obtidos de diferentes partes das plantas, incluindo flores (*Rosa grandiflora* Wallr. – Rosaceae), folhas (*Menha x piperita* L. – Lamiaceae), frutas (*Citrus limon* (L.) Osbeck – Rutaceae), sementes (*Pimpinella anisum* L. – Apiaceae), ervas (*Cymbopogon citratus* (DC.) Stapf – Poaceae), raízes (*Chrysopogon zizanioides* (L.) Roberty – Poaceae), rizomas (*Zingiber officinale* Roscoe – Zinginberaceae), madeira (*Cedrela fissilis* Vell. – Meliaceae), casca (*Cinnamomum verum* J.Presl – Lauraceae), goma (*Achellia seyal* (Delile) P.J.H. Hurter – Leguminosae), flores de árvores (*Cananga odorata* (Lam.) Hook.f. & Thomson – Annonaceae), bulbos (*Allium sativum* L. – Amaryllidaceae) e botões de flores secos (*Syzygium aromaticum* (L.) Merr. & L.M.Perry – Myrtaceae) (TISSERAND; YOUNG 2013).

Além disso, podem apresentam diversos compostos químicos, sendo que os constituintes presentes nessa categoria de metabólitos das plantas se enquadram em duas principais classes: terpenos e fenilprapanóides. Apesar dos terpenos e seus derivados oxigenados (terpenóides) serem mais abundantes e frequentes nos OE, algumas espécies contêm elevados teores de derivados do chiquimatos (fenilpropanóides) que quando estão presentes proporcionam características de odor e sabor específicos das plantas (MOGHADDAM; MEHDIZADEH, 2017).

De acordo com os mesmos autores, os terpenos e terpenóides resultam da condensação de isopreno (2-metil-1,3-butadieno), uma unidade de pentacarbonato com duas ligações

insaturadas e, portanto, muitas vezes são chamadas isoprenoides. Os terpenos são classificados em diferentes classes estruturais e funcionais, ou seja, de acordo com o número de unidades de isopreno em sua estrutura, por exemplo, hemiterpenos (uma unidade), monoterpenos (duas unidades), sesquiterpenos (três unidades), diterpenos (quatro unidades) e assim por diante.

Já os fenilpropanóides possuem uma ou mais unidades C6-C3, sendo C6 um anel benzeno. Normalmente apresentam um grupo funcional éter metílico ligado ao anel e uma cauda de propenil (cadeia de três carbonos com um C ═ C ligado ao anel por uma extremidade). Muitos dos fenilpropanóides presentes nos OE são fenóis ou éteres fenólicos. Seus principais representantes nos OEs incluem os hidrocarbonetos oxigenados anetol, eugenol e safrol (MOGHADDAM; MEHDIZADEH, 2017).

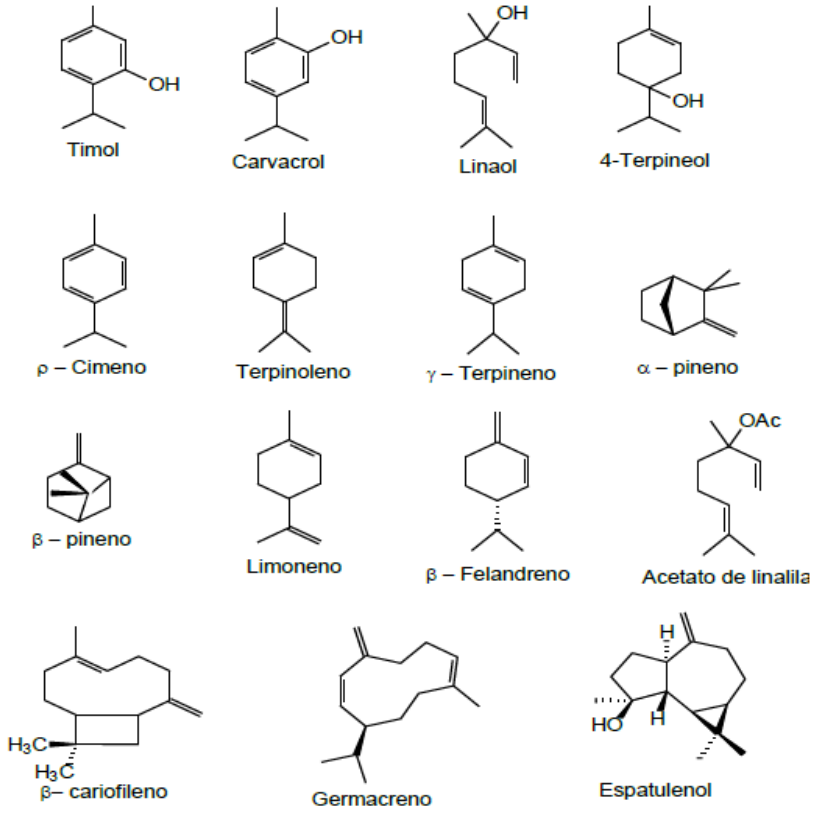
Os principais constituintes presentes nos OEs podem ser originados de três vias biossintéticas, a via do mevalonato que leva aos sesquiterpenos, a via do metileritritolfosfato que leva aos monos e diterpenos e a via do ácido chiquímico que leva aos fenilpropenos (BASER; BUCHBAUER, 2010).

Diversos fatores podem interferir na produção dos óleos essenciais, tanto bióticos quanto abióticos. Os fatores bióticos podem ser os relacionados à planta como, a idade do vegetal (PINTO, 2017), o órgão de armazenamento (MASETTO et al., 2011), o material genético (BOTREL et al., 2009), dentre outros. Já os fatores abióticos como o ambiente, podem ser, a qualidade da luz (CHAVES, 2020), o horário da coleta (QUEIROZ et al., 2016), o período de secagem, (DA SILVA, et al., 2016), a adubação (DE ASSIS, 2020), irrigação (DOS SANTOS et al., 2020) e outros.

Segundo Bakkali et al. (2008), os óleos essenciais exercem um grande e importante papel na proteção das plantas como agentes antibacterianos, antivirais, antifúngicos, inseticidas e também contra herbívoros, reduzindo o apetite. Além disso, o aroma desses óleos atrai polinizadores, auxiliando a dispersão de pólen e sementes das plantas (SIMÕES; SPITZER, 2010). Outro ponto importante está relacionado com a composição do óleo volátil de uma planta, pois é utilizada como fonte potencial de compostos farmacologicamente ativos como analgésicos, anti-inflamatórios, antitumorais, antibióticos e digestivos (GILLIJ; GLEISER; ZYGADLO, 2008).

A composição química do óleo essencial do orégano é caracterizada, principalmente pelas presença de carvacrol, sabineno, p-cimeno, terpineno-4-ol, α-terpineno, γ-terpineno, linalol, 4-terpineol e timol (BLANK et al., 2016; SKOUFOGIANNI; SOLOMOU; DANALATOS, 2019; JAN et al., 2020). Figueredo (2019) representou em forma de estruturas os principais constituintes do óleo essencial do *Origanum vulgare*. conforme demonstrado na Figura 1.

Figura 1 — Estrutura química dos principais constituintes do óleo essencial de *Origanum vulgare*.



Fonte: Figueredo, 2019.

Além disso, diversos estudos comprovaram os efeitos do óleo essencial do orégano, dentre os principais se encontram, efeitos larvacida (CHAVES, 2019), antibacteriano (GOMES et al., 2019), antifúngico (VINCIGUERRA et al., 2019), herbicida (GRUĽOVÁ et al., 2020) e antioxidante (FERREIRA et al., 2019). Sendo também muito utilizado na indústria alimentícia em molhos para salada (RIBES; FUENTES; BARAT, 2019), suplementação alimentar de animais (SALAMON; PORACOVA; HRYTSYNA, 2019) e conservantes alimentícios (JEDDI et al., 2019).

2.3 Adubação verde: histórico e aplicação nas plantas medicinais

Há milhares de anos diversos povos têm praticado a agricultura baseada no manejo dos materiais disponíveis nas propriedades rurais. Nos quais, destacam-se aqueles de origem orgânica (esterco, restos de cultura, composto, etc.) que podem melhorar a qualidade do solo e promover aumento da produtividade vegetal (ESPÍNDOLA; GUERRA; DE ALMEIDA, 1997).

De acordo com Rocha, Rosa e Cardoso (2009), o termo adubação foi usado em 1840, quando o químico alemão Justus von Liebig observou a relação entre o crescimento de plantas

e a utilização de fezes de animais como adubo. Essa prática além de modificar quimicamente a composição do solo, de modo a fornecer à planta os elementos necessários, tem também a capacidade de melhor condicioná-lo fisicamente. Os mesmos autores afirmaram que dentre as diversas formas de adubação orgânica com custo/benefício atrativos e amplos resultados técnicos para solos e plantas, a adubação verde é a de mais fácil aplicação e menor custo.

O adubo verde trata-se da planta cultivada ou não, com a finalidade precípua de elevar a produtividade do solo com a sua massa vegetal, quer produzida no local ou importada (KIEHL, 1960). Desta forma, adubação verde caracteriza-se pela prática de incorporar a massa vegetal não decomposta ao solo, com a finalidade de preservar e/ou restaurar a produtividades das terras agricultáveis (KIEHL, 1960). Ou seja, incialmente o conceito de adubação verde abrangia somente as partes de fertilidade, nutrição e química do solo.

No decorrer do contexto histórico do século XX essa prática (adubação verde) foi sendo esquecida. Conforme Espíndola, Guerra e De Almeida (1997), nas décadas de 1950 e 1960, os países do terceiro mundo conheceram a "Revolução Verde". A partir de então, os governos locais criaram linhas de crédito atreladas à compra de insumos agropecuários, enquanto as principais escolas de agronomia destes países reformularam seus currículos, valorizando as técnicas associadas ao novo modelo agrícola (ESPÍNDOLA; GUERRA; DE ALMEIDA, 1997).

O uso de maquinário pesado, as sementes de alto rendimento, a adubação de alta solubilidade e o uso de agrotóxicos formam a base tecnológica da Revolução Verde. Sendo que um dos seus principais objetivos era solucionar o problema da fome mundial, onde obtiveram aumentos substânciais de produtividade (entre 1950 e 1984 a produção alimentar dobrou e a disponibilidade de alimento por habitante aumentou em 40%) (DE ASSIS; DE JESUS, 2002; MEIRELLES, 2002).

Porém, com o uso intensivo dos pacotes tecnológicos relacionados à "Revolução Verde" começaram a surgir sérios problemas por volta de 1970. Ocorrendo erosão e degradação dos solos, contaminação das águas, êxodo rural, dependência da agricultura do complexo agroindustrial que a comprimiu, menor qualidades dos alimentos, mortes por intoxicação causadas por agrotóxicos e descapitalização do setor rural (ESPÍNDOLA; GUERRA; DE ALMEIDA, 1997; MEIRELLES, 2002).

A partir disso, buscou-se por uma agricultura mais sustentável, ou seja, aquela ecologicamente correta, economicamente viável, socialmente justa, culturalmente adaptada, que se desenvolve como um processo, numa condição democrática e participativa (De ASSIS; De JESUS, 2002).

Todo esse contexto proporcionou uma visão mais holística do conceito de adubação verde, conceito este que vai além da fertilidade e da química do solo. Portanto, a adubação verde passa a consistir de acordo com Alcântara (2016) e Espíndola, Guerra e De Almeida (1997), na utilização de plantas em sucessão, rotação ou consórcio com as culturas de interesse econômico. Tais plantas podem ser incorporadas ao solo ou mantidas na superfície, proporcionando, em geral, melhoria das características físicas, químicas e biológicas do solo.

Conforme Alcântara (2016), o adubo verde pode ser utilizado das seguintes formas:

1. Rotação: o adubo verde é plantado nos talhões e rotacionado com as culturas. Assim, pelo menos um talhão da propriedade recebe adubação verde uma vez por ano, enquanto os outros recebem as culturas.
2. Sucessão: o adubo verde é semeado no mesmo talhão, antes do plantio da cultura principal, que será semeada depois que o adubo verde for manejado.
3. Consórcio: o adubo verde é semeado nas entrelinhas ou nas próprias linhas da cultura principal. Pode ser também plantado em faixas, intercalada com a cultura principal. Porém, são necessários alguns cuidados com a água, nutriente e a luz para evitar competições entre as culturas.

Além disso, o manejo dessa prática pode variar de acordo com o objetivo desejado. Se o material vegetal for deixado sobre a superfície do solo o objetivo principal visa proteger a cobertura contra erosão e plantas espontâneas, podendo também ajudar a manter a umidade e atenuar as temperaturas do solo. Sendo que neste caso o adubo verde pode ser cortado no florescimento ou após a retirada dos grãos, que servirão de sementes para o próximo ano. Por outro lado, se o adubo verde for incorporado no solo o principal objetivo está relacionado com a melhoria da fertilidade do solo, ou seja, o fornecimento de nutrientes. Neste caso o adubo deve ser incorporado em pleno florescimento, pois apresenta maiores teores de nutrientes nas folhas e caules (ALCÂNTARA, 2016).

Conforme Eiras e Coelho (2011), as plantas da família das Leguminosas são as mais utilizadas como adubo verde. Uma das principais razões está relacionada com o fato das espécies desta família possuir a capacidade de realizar o processo de simbiose com as bactérias fixadoras do N_2 atmosférico e também devido a rusticidade, à elevada produção de matéria seca e ao sistema radicular profundo (SILVA; MENEZES, 2007).

Outros aspectos são que essas espécies trazem algumas vantagens importantes para o solo e para as plantas, como: cobertura do solo evitando o seu aquecimento; controle de erosão; equilíbrio biológico; conservação da umidade no solo; incorporação de nitrogênio (N) ao sistema, através da fixação biológica do N atmosférico; ciclagem de nutrientes das camadas

mais profundas do solo para a superfície, colocando-os na zona onde as plantas cultivadas conseguem retirar (FORMENTINI et al., 2019).

As principais espécies utilizadas na adubação verde são: crotalárias (*Crotalaria juncea* L e *C. spectabilis* L), feijão-de-porco (*Canavalia ensiformis* DC), mucuna anã (*Mucuna deeringiana* (Bort) Merr.), mucuna cinza (*Stizolobium cinereum* Piper & Tracy), mucuna preta (*Styzolobium aterrimum* Piper & Tracy), guandu (*Cajanus cajan* (L.) Millsp.), labe-labe *(Dolichos lablab* L.), leucena (*Leucaena leucocephala* (Lam.) de Wit), gliricídia (*Gliricidia sepium* (Jacq.) Walp.*)*, sabiá (*Mimosa caesalpiniifolia* Benth), canafístula (*Peltophorum dubium* (Spreng.) Taub.) (EIRAS; COELHO, 2011; TEODORO et al., 2011). Sendo que algumas dessas espécies são bastante valorizadas por apresentarem efeitos alelopáticos como no caso da *Canavalia ensiformes* (PEREIRA et al., 2018) e controle de nematoides *Crotalaria* sp. (COLTRO-RONCATO et al., 2015).

Dentre as espécies usadas na adubação verde a *Crotalaria juncea* L. é uma das mais utilizadas, pois é adaptada às diferentes condições edafoclimáticas e do solo no Brasil e destaca-se por sua grande capacidade de produção de biomassa, acúmulo de nutrientes e altas qualidades de seus resíduos (XAVIER; OLIVEIRA; SILVA, 2017). Duarte Junior (2006) avaliando plantas de cobertura para sistema de plantio direto verificou que a *C. juncea* apresentou maior acúmulo de K, Mg, S, Zn e Fe quando comparada com outras espécies de adubo verde. Conforme Lima-Filho et al. (2014) a crotalária pode apresentar médias de 40,5 t/ha de massa fresca, 17,5 t/ha de massa seca, relação C/N de 18 e 400 kg ha^{-1} de nitrogênio. Além disso, essa espécie pode ser usada para recuperar áreas degradas (RIBEIRO, 2018), para o controle de nematoide (PINHEIRO; MELO; RAGASSI, 2019) e plantas daninhas (FERRACO et al., 2019).

Além das fabaceas (leguminosas), as espécies da família das Poaceae (gramíneas) também são utilizadas na adubação verde (ROMAGNA; JUNGES; MICHELON, 2019). Principalmente se o objetivo é a manutenção da cobertura do solo, e não a incorporação de resíduos no solo, pois a relação C/N mais elevada dessa família implica em maior permanência dos resíduos no solo, favorecendo o estabelecimento da cobertura (MENEZES; LEANDRO, 2004). Dentre as espécies utilizadas está o milheto (*Pennisetum glaucum* (L.) R.Br.), milho (*Zea mays* L.), aveia branca (*Avena sativa* L.) e aveia preta (*Avena strigosa* Schreb.), azevém (*Lolium multiflorum* Lam.) e centeio (*Secale cereale* L.) (DE SOUZA et al., 2016; INOUE et al., 2019; TIVELLI; PURQUERIO; KANO, 2010.)

Atualmente existem poucos trabalhos relacionados aos efeitos da adubação verde no cultivo de plantas medicinais. Porém, pesquisas demonstram que esse manejo pode aumentar o teor de óleo essenciais (CORRÊA; CASTELLANE; JORGE, 1997), elevar a produção de

biomassa e de óleo essencial (SINGH et al., 2010), aumentar a biomassa de folhas, caules e frutos (LINHARES et al., 2012; MOREIRA et al., 2012), bem como, maior acúmulo de raízes, rizomas e teor de fenóis totais melhorando a eficiência do sistema de defesa antioxidante (FERDOUS et al., 2018; YOUN et al., 2017).

2.4 Reguladores de crescimentos

Os biorreguladores ou reguladores vegetais são compostos orgânicos, naturais ou sintéticos, que em pequenas quantidades, inibem ou modificam de alguma forma processos morfológicos e fisiológicos do vegetal, podendo ser aplicadas diretamente em folhas, frutos e sementes, provocando alterações nos processos vitais e estruturais, com a finalidade de incrementar a produção, reduzir o tamanho das plantas e modular o crescimento vegetal (BELTRÃO et al., 2010).

Por definição, o hormônio vegetal ou fitohormônio é uma substância química biologicamente ativa, produzida por uma planta que, em baixas concentrações (10^{-15} a 10^{-9} M) regula determinados processos fisiológicos. Em geral, é produzida em uma determinada parte da planta e translocada para promover a ação em outro local do vegetal (BIASI, 2002). No que diz respeito ao desenvolvimento vegetal, o mesmo é regulado por nove hormônios principais: auxinas, giberelinas, citocininas, etileno, ácido abscísico, brassinosteroides, jasmonatos, ácido salicílico e estrigolactonas (TAIZ et al., 2017).

Os fitormônios são substâncias naturais produzidas pelo próprio vegetal, enquanto os termos, regulador de crescimento de plantas (PGRs) ou regulador vegetal são empregados para todas as substâncias naturais (produzidas por fungos, por exemplo) ou artificiais, que possuem efeito no crescimento e desenvolvimento das plantas (DE MELO et al., 2002).

De acordo com Povh e Ono (2006), o uso de reguladores vegetais está sendo muito utilizado na agricultura devido às suas influências positivas na quantidade e qualidade de produção. No entanto, pouco se conhece sobre os efeitos destas substâncias em plantas medicinais e aromáticas (POVH; ONO, 2006).

Os reguladores de crescimento têm sido utilizados em plantas medicinais, podendo influenciar a produção de óleo essencial por meio de alterações no metabolismo secundário, efeitos no crescimento das plantas (biomassa de folhas ou flores), biossíntese de óleo essencial e número de estruturas de armazenamento de óleo, produzindo alterações quantitativas e qualitativas. Por outro lado, o tempo de aplicação exógena e o tipo de reguladores de

crescimento podem afetar o conteúdo de óleo essencial (SHARAFZADEH; ZARE, 2011; PRINS; VIEIRA; FREITAS, 2010).

A influência de auxinas, citocininas e giberelinas no crescimento e na produção de metabolitos secundários de algumas plantas medicinais da família Lamiaceae, promoveram maiores acúmulo de matéria fresca e seca, maior produção de óleo essencial, mudanças na composição química, maior teor do composto majoritário, conforme reportado por Sharafzadeh e Zare (2011).

O termo auxina é de origem grega (*auxein*) que significa crescer ou aumentar, e possui importante papel na regulação do crescimento e desenvolvimento do vegetal. O ácido 3-indolacético (AIA) é a forma mais abundante e fisiologicamente mais importante dentre as auxinas, cuja fórmula molecular é $C_{10}H_9NO_2$ (TAIZ et al., 2017). Dependendo da espécie, idade da planta, estação do ano e condições sob as quais a mesma se desenvolve, outras auxinas naturais podem ser encontradas, como o ácido-4-cloroindolil-3- acético (4-cloro AIA), o ácido fenilacético (PLA) e ácido indol-3-butírico (AIB). Além dessas moléculas os ácidos 1-naftaleno-acético (ANA), 2,4-diclorofenoxiacético (2,4-D) e 2-metóxi-3,6-diclorobenzoico (dicamba) são usados amplamente como reguladores de crescimento e herbicidas na agricultura. (MERCIER, 2004; TAIZ et al., 2017).

Estudos demonstraram que AIB e ANA são usualmente empregados no enraizamento de espécies (CAVALLI 2017; KAMILA et al., 2020; OLIVEIRA et al., 2011). No entanto, a aplicação exógena de diferentes reguladores de crescimento pode aumentar o crescimento e a produção de biomassa (JUN et al., 2018). Segundo Petri et al. (2016), o AIB é utilizado para induzir a formação de raízes em estacas herbáceas e lenhosas e em cultura de tecidos. Ludwig-Müller (2000) afirma que quando aplicado exogenamente, o AIB tem uma variedade de efeitos diferentes no crescimento e desenvolvimento das plantas. Como demonstrado por Xu et al. (2012), pois a aplicação foliar de AIB promoveu maior acúmulo de biomassa de raiz, caule e folha na espécie *Pinus yunnanensis* Franch. (Pinaceae).

Ja às giberelinas são sintetizadas em locais onde a divisão celular é mais intensa, como ápice dos ramos em crescimento, ápice das raízes novas, folhas jovens e sementes, pois os teores são mais elevados (PETRI et al., 2016). As giberelinas podem se movimentar em dois sentidos nas plantas. Portanto, transloca tanto no xilema (das raízes para as folhas) como no floema. Entre as principais funções fisiológicas estão a divisão celular, crescimento, inibição da indução floral, partenocarpia e retardo do processo de senescência, sendo que as principais moléculas ativas são, GA_3, GA_4 e GA_7 (PETRI et al. 2016).

Conforme Iftikhar et al. (2019), a aplicação foliar de ácido giberélico (GA) em altas concentrações (200 mg L^{-1}) aumentou a biomassa, fotossíntese, nutrientes e produtividade da espécie *Triticum aestivum* L. (Poaceae) sob estresse com nanopartículas de zinco. Em adição, Kaplan et al. (2019) observaram que a aplicação de GA_3 nas concentrações 100 e 200 mg L^{-1} demonstraram influenciar significativamente o teor de ácido fenólico na espécie *Vitis* sp.

Já as citocininas estimulam a divisão de células vegetais em combinação com auxinas. A molécula indutora da citocinese foi denominada cinetina. A cinetina é uma citocinina sintética, mas sua estrutura é similar à das citocininas de ocorrência natural. Esse fitohôrmonio têm efeitos em muitos processos fisiológicos e de desenvolvimento, incluindo senescência foliar, dominância apical, formação e atividade dos meristemas apicais (TAIZ et al., 2017). Outro papel importante que as citocininas desempenham é na interação das plantas com fatores bióticos e abióticos, abrangendo os estresses salino e hídrico, os macronutrientes (incluindo nitrogênio, fósforo e enxofre), as relações simbióticas com bactérias fixadoras de nitrogênio e fungos micorrízicos arbusculares, bem como bactérias patogênicas, fungos, nematódeos e vírus (TAIZ et al., 2017).

Em condições *in vitro* o uso do 6-benzilaminopurina (BAP), ácido a-naftalenacético (ANA) e ácido indole-3-butírico (AIB) proporcionaram teores significativamente maiores de antocianina total e fenólico na espécie *Origanum vulgare* (PANDEY et al., 2019). Pal, Mahajan e Agnihotri (2016) comprovaram que aplicação foliar de cinetina na espécie *Rosa* × *damascena* Herrm. (Rosaceae) melhorou a produção de flores e teor de óleo essencial. Em adição, a aplicação foliar exógena da cinetina estimulou o metabolismo da cafeína, trigonelina, ácido 5-cafeoilquinóico, mangiferina, antocianinas e conteúdo fenólico total. Além de aumentar a atividade fotossintética e a capacidade total de eliminação de radicais das plantas de café (ACIDRI et al., 2020).

2.5 Radicais livres e sistemas antioxidantes

Os radicais livres podem ser classificados como as moléculas orgânicas e inorgânicas e os átomos que contêm um ou mais elétrons não pareados, com existência independente. Essa característica faz dos radicais livres moléculas altamente instáveis, com meia-vida curtíssima e muito reativas quimicamente. Sendo que a presença dos radicais é crítica para a manutenção de muitas funções fisiológicas normais (HALLIWELL, 1994; POMPELLA, 1997).

A oxidação faz parte de um dos processos fundamentais na vida dos seres aeróbicos e do metabolismo. Portanto, os radicais livres são originados naturalmente ou por alguma

disfunção biológica e estão envolvidos na produção de energia, fagocitose, regulação do crescimento celular, sinalização intercelular e síntese de substâncias biológicas importantes. Porém, o excesso de radicais provoca efeitos maléficos ao organismo, como a peroxidação dos lipídios de membrana, agressão às proteínas dos tecidos e membranas, às enzimas, carboidratos e ao ácido desoxirribonucleico (DNA) (MIRANDA, 2010). Conforme Xie et al. (2019) o estresse oxidativo prejudicial ocorre quando a um estado de desequilíbrio entre a produção de ERO e a neutralização de radicais livres por antioxidantes, resultando em danos aos componentes celulares, incluindo lipídios, ácidos nucléicos, metabólitos e proteínas, que finalmente leva à morte de células nas plantas.

Esses radicais podem ser encontrados como derivados do oxigênio (EROs) ou como derivado nitrogênio (ERN). As principais EROs são $O_2^{\bullet-}$ (ânion-radical superóxido), $HO^{\bullet}$ (radical hidroxilo), HO_2 (radical hidroperoxil), $ROO^{\bullet}$ (radical peroxilo), $RO^{\bullet}$ (radical alcoxilo), H_2O_2 (peróxido de hidrogênio), HClO (ácido hipocloroso), e $^1O_2$ (oxigênio singleto). Já as ERN são $NO^{\bullet}$ (óxido nítrico), NO_2 (dióxido de nitrogênio), N_2O_3 (trióxido de dinitrogênio) e $ONOO^-$ (peroxinitrito) (ALI et al., 2008; EVANS; HALLIWELL, 1999).

Os componentes antioxidantes são compostos de substâncias lipossolúveis (hidrofóbicas) e solúveis em água (hidrofílicas). Os antioxidantes à base de plantas são principalmente hidrofílicos, nos quais alguns casos são fenólicos, flavonóides, superóxido dismutase (SOD), catalase (CAT), glutationa peroxidase (GPx), além de ácidos úrico, lipóico, benzóico e ascórbico. Por outro lado, os antioxidantes hidrofóbicos estão ligados à membrana biológica funcional, encontrada em carotenóides, tocoferóis, vitamina K, ubiquinona e fosfolipídios (HAIDA; HAKIMAN, 2019). Além disso, de acordo com Jacob (1995), os antioxidantes doam seus elétrons para neutralizar os radicais livres exógenos e endógenos.

Conforme Haida e Hakiman (2019), os antioxidantes são categorizados de acordo com as linhas de defesa: antioxidante preventivo como a linha de defesa inicial, antioxidante de eliminação de radicais na segunda linha de defesa e, por último, reparo e enzimas de *novo* como terceira linha de defesa, conforme a seguir:

A primeira linha de defesa dificulta a produção de radicais livres (que levam ao estresse oxidativo) por meio de enzimas, como SOD, CAT, GPx, glutationa redutase e por meios não enzimático, como selênio (Se), manganês (Mn), cobre (Cu), ferro (Fe) e Zinco (Zn).

A segunda linha de defesa inibe a produção de espécies danificadas, além de tornar os radicais livres menos prejudiciais, reduzindo ainda mais os danos causados pela reação oxidativa. Alguns excelentes eliminadores de radicais livres são as vitaminas E e C, flavonóides e ácido úrico.

A terceira linha de defesa serve para reparar DNAs danificados, proteínas, peróxidos e lipídios oxidados, além de inibir a propagação da reação em cadeia no radical peroxil lipídico. Como por exemplo as polimerases, glicosilases, nucleases e proteases (IGHODARO; AKINLOYE, 2018; LOBO et al., 2010).

Além dessa classificação, Cotinguiba et al. (2015) afirmam que os antioxidantes podem apresentar algumas divisões quanto a sua classe (enzimática e não enzimática). A atividade enzimática são os compostos capazes de bloquear a iniciação da oxidação, ou seja, as enzimas que removem as espécies reativas ao oxigênio. Já a atividade não enzimática são moléculas que interagem com as espécies radicalares e são consumidas durante a reação. Nesta classificação, incluem-se os antioxidantes naturais e sintéticos.

Os sistemas antioxidantes complexos são muito importantes para proteger membranas celulares e organelas de efeitos prejudiciais de espécies ativas de oxigênio. Estes incluem sistemas antioxidantes enzimáticos e não enzimáticos (PADMAJA; SRAVANTHI; HEMALATHA, 2011). Em relação aos enzimáticos, as principais enzimas atuantes são a catalase (CAT), superóxido dismutase (SOD), ascorbato peroxidase (APx) e glutationa-peroxidase (GSH-PX) (BARBOSA et al., 2014).

Em relação à enzima SOD, conforme Fridovch (1986) a mesma desempenha papel chave no sistema de defesa antioxidante através da dismutação do ânion radical superóxido ($O_2^{\bullet-}$) a peróxido de hidrogênio (H_2O_2) e oxigênio (O_2). Desta forma a atividade da enzima interfere na concentração das duas espécies reativas de oxigênio (EROs) envolvidas na reação de Haber-Weiss, fazendo parte do mecanismo central de defesa dos organismos, evitando a formação do radical •OH (LEÓN et al., 2002).

As SODs são metaloenzimas que ocorrem em três diferentes formas moleculares, contendo os metais Mn, Fe ou Cu/Zn como grupos prostéticos. A Cu/Zn-SOD é encontrada no estroma dos cloroplastos, a Mn-SOD e a Fe-SOD são encontradas tantos em células eucarióticas quanto em procarióticas, na matriz mitocondrial (FRIDOVCH, 1986; MALLICK; MOHN, 2000).

A catalase é encontrada em todos os organismos vivos, sendo descrita em 1901 por Loew (FRUGOLI et al., 1996), é uma enzima tetramérica que contém grupos heme. Por possuir ampla distribuição e capacidade de degradar rapidamente o peróxido de hidrogênio em água (H2O) e oxigênio (O2), a CAT desempenha um papel fundamental nos sistemas que capacitam os organismos a viverem em ambientes aeróbicos (MITSUDA, 1956; McDONALD, 1999; MALLICK; MOHN, 2000).

A APX é uma heme-proteína, classificada com classe I da superfamília das peroxidases, com várias formas isoenzimáticas, diversamente reguladas. Normalmente, as isoformas podem ser encontradas em citosol, mitocôndrias, peroxissomos, cloroplastos e parede celular. Como agente redutor a APX exige o ácido ascórbico. Tem alta afinidade com o H_2O_2, permitindo a eliminação (H_2O_2) mesmo em baixas concentrações (BARBOSA et al., 2014).

A quantificação de enzimas antioxidantes nas plantas medicinais é uma ferramenta importante para mensurar o potencial antioxidante das mesmas. Pesquisa realizada Padmaja, Sravanthi e Hemalatha (2011), demonstrou que a espécie *Adhatoda vasica* Nees (Acanthaceae) apresentou maior atividade antioxidante enzimática e não enzimática em comparação com *Sesbania grandiflora* (L.) Pers. (Leguminosae) devido os maiores teores das enzimas CAT, SOD, peroxidases e oxidase do ácido ascórbico. Ou seja, as atividades antioxidantes das espécies vegetais estão diretamente relacionadas com as atividades dessas enzimas. Ferrari et al., (2020) também observaram a presença das enzimas CAT, SOD e ascorbato peroxidase-APx na espécie *Curcuma longa* L. (Zingiberaceae) cultivada com fungos micorrízicos, onde essas enzimas atuam na eliminação de radicais livres favorecendo a atividade antioxidante.

Em relação aos métodos analíticos da capacidade antioxidante, Vasconcelos et al. (2007) afirmaram que os diversos testes propostos na literatura variam quanto ao tipo de radicais gerados, ao indicador de oxidação escolhido e ao método usado para sua detecção e quantificação. São chamados ensaios de captação ("trap assays"). Em todos esses ensaios, um radical é gerado e reage com moléculas-alvo, para produzir cor, fluorescência, quimioluminescência, perda ou ganho de sinais de ESR ("Electron Spin Resonance" ou Ressonância do Spin Eletrônico) ou outra mudança mensurável. A presença de antioxidantes altera esses sinais, o que permite sua análise quantitativa.

Dentre os testes mais utilizados, citam-se TRAP (Total Radical – "Trapping Antioxidant Parameter"), ORAC ("Oxygen-Radical Absorbancy Capacity"), teor de polifenóis, ácidos fenólicos totais, flavonoides totais, ácido ascórbico total, teor de antociania, atividade de eliminação de DPPH, ensaio férrico de redução de poder antioxidante (FRAP), atividade de eliminação de peróxido de hidrogênio (H_2O_2), atividade de eliminação de óxido nítrico, atividade de eliminação de radicais superóxido, atividade de eliminação de radicais hidroxila e ensaio de fosfomolibdato (capacidade antioxidante total) (HAIDA; HAKIMAN, 2019; VASCONCELOS et al., 2007).

REFERÊNCIAS

ACIDRI, R. *et al.* Exogenous kinetin promotes the nonenzymatic antioxidant system and photosynthetic activity of coffee (*Coffea arabica* L.) plants under cold stress conditions. **Plantas** , v. 9, n. 2, p. 281, 2020. https://doi.org/10.3390/plants9020281

ALCÂNTARA, F. **Saber e fazer agroecologia: O que é e como fazer adubação verde.** Embrapa Arroz e Feijão. Folders, 1. Ed. n. 5, 2016.

ALI, S. S. *et al*. Indian medicinal herbs as sources of antioxidants. **Food Research International**, *41*, 1–15. (2008). https://doi.org/10.1016/j.foodres.2007.10.001

BAHMAN, S.; MEHRAFARIN, A.; NAGHDI BADI, H. Influence of gibberellic acid, indole butyric acid, and methanol on morpho-physiological and phytochemical traits in *Thymus vulgaris* L. **فصلنامه علمی پژوهشی گیاهان دارویی**, v. 1, n. 61, p. 33-44, 2017. Disponível em: http://jmp.ir/article-1-1641-en.html. Acesso: 17 jun. 2020.

BAKKALI, F. *et al*. Biological effects of essential oils - A review. **Food and Chemical Toxicology**, 46, pp.446-475, 2008. https://doi.org/10.1016/j.fct.2007.09.106

BARBOSA, M. R. *et al*. Geração e desintoxicação enzimática de espécies reativas de oxigênio em plantas. **Ciência Rural**, v. 44, p. 453-460, 2014. https://doi.org/10.1590/S0103-84782014000300011

BASER, K.; BUCHBAUER, G. **Handbook of Essential Oils: Science, Technology, and Applications.** CRC Press, Boca Raton, FL. 2010.

BELTRÁN, J. M. G. *et al*. Effects of dietary *Origanum vulgare* on gilthead seabream (*Sparus aurata* L.) immune and antioxidant status. **Fish & Shellfish Immunology**, v. 99, p. 452-461, 2020. https://doi.org/10.1016/j.fsi.2020.02.040

BELTRÃO, N. E. de M. *et al*. Resposta da mamoneira a aplicação de doses de regulador de crescimento. In: **Embrapa Algodão-Artigo em anais de congresso (ALICE)**. In: Congresso Brasileiro De Mamona, 4.; Simpósio Internacional de Oleaginosas Energéticas, 1., 2010, João Pessoa. Inclusão social e energia: anais. Campina Grande: Embrapa Algodão, 2010.

BIASI, L. A. Reguladores de crescimento vegetal. In: Wachowicz, C.M.; Carvalho, R. I.N. (eds.). **Fisiologia Vegetal: Produção e Pós-colheita.** Curitiba: Editora Champagnat, 2002. 63-94 p.

BLANK, D. E. *et al*. Composição química e citotoxicidade de *Origanum vulgare* L. e *Rosmarinus officinalis* L. **Science And Animal Health**, v. 4, n. 2, p. 117-130, 2016. https://doi.org/10.15210/sah.v4i2.6569

BOTREL, P. P. *et al*. Teor e composição química do óleo essencial de *Hyptis marrubioides* Epling (Lamiaceae) em diferentes genótipos. **Revista Brasileira de Plantas Medicinais**, v. 11, n. 2, p. 164-169, 2009. https://doi.org/10.1590/S1516-05722009000200009

CAVALLI, J. **Influência do ácido indolbutírico e comprimento de estacas na propagação vegetativa de lavanda (*Lavandula dentata*)**. 2017. 32 p. Trabalho de Conclusão de Curso - Universidade Tecnológica Federal do Paraná, PR. 2017. Disponível em: http://repositorio.utfpr.edu.br/jspui/handle/1/10745. Acesso: 17 jun. 2020.

CHAVES, M. C. **Leds no cultivo in vitro e na produção de compostos voláteis de *Lippia filifolia* Mart. & Shauer.** 2020. 45 p. Dissertação (Mestrado em Agronomia/Fisiologia Vegetal)-Universidade Federal de Lavras, Lavras, 2020. Disponílvel em: https://locus.ufv.br//handle/123456789/11567. Acesso: 17 jun. 2020.

CHAVES, R. do S. B. **Avaliação da atividade larvicida do extrato bruto etanólico e óleos essenciais das folhas de *Origanum majorana* L. e *Origanum vulgare* L. frente ao *Aedes aeggypti* (Linnaeus, 1762).** 2019. 111 p. Dissertação (Mestrado em Ciências Farmacêuticas) – Departamento de Pós-Graduação, Universidade Federal do Amapá, Macapá, 2019. http://repositorio.unifap.br:80/jspui/handle/123456789/358

COLTRO-RONCATO, S. *et al*. Fitoquímicos como controle alternativo de nematoides. In: KUHN, O. J; NUNES, R. V; STANGARLIN, J. R; RAMPIM, L. **Ciências Agrárias Tecnologias e Perspectivas.** 1. ed. cap. 10. Universidade Estadual do Oeste do Paraná, 2015. 190 p.

COQUEIRO, D. P. *et al*. Efeitos do chá de orégano (*Origanum vulgare*) no perfil bioquímico de ratos Wistar. **Sci Med**, v. 22, n. 4, p. 191-196, 2012. Disponível em: https://www.researchgate.net/publication/286104331. Acesso: 17 jun. 2020.

CORRÊA, J. C.; CASTELLANE, P. D.; JORGE, N. J. Influence of organic and chemical fertilization on the yield of flowers, contents and composition of essential oil of (*Chamomilla recutita* (L.) Rauschert). In: **II WOCMAP Congress Medicinal and Aromatic Plants, Part 3: Agricultural Production, Post Harvest Techniques, Biotechnology 502**. 195-202 p. 1997. https://doi.org/10.17660/ActaHortic.1999.502.31

COTINGUIBA, G. G. *et al*. Método de avaliação da defesa antioxidante: uma revisão de literatura. **Journal of Health Sciences**, v. 15, n. 3, 2015. https://doi.org/10.17921/2447-8938.2013v15n3p%25p

CUNHA, A. P.; ROQUE, O. R.; GASPAR, N. **Cultura e utilização das plantas medicinais e aromática**, Fundação Calouste Gulbenkian, Lisboa, 2011. Dispovível em: http://hdl.handle.net/10400.15/591. Acesso: 19 jun. 2020.

DA SILVA, T. I. *et al*. Influência do período de secagem e horário de colheita no teor de óleo essencial de *Lippia alba* (MILL.). **Cadernos de Cultura e Ciência**, v. 15, n. 1, p. 29-38, 2016.

DE ASSIS, R. L.; DE JESUS, E. L. Histórico, Conceitos e Princípios da Agroecologia. In: PADOVAN, M. P; URCHEI, M. A; MERCANTE, F. M; CARDOSO, S. **Agroecologia em Mato Grosso do Sul: Princípios, Fundamentos e Experiências**. 1. ed., cap. 2. Ed. eletrônica: Eliete do Nascimento Ferreira, 2002. Embrapa Agropecuária Oeste; Campo Grande: IDATERRA, 2002. 41- 45 p. Disponível em: http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/247552. Acesso: 29 mai. 2020.

DE ASSIS, R. M. A. *et al*. Arbuscular mycorrhizal fungi and organic manure enhance growth and accumulation of citral, total phenols, and flavonoids in *Melissa officinalis* L. **Industrial Crops and Products**, v. 158, p. 112981, 2020. https://doi.org/10.1016/j.indcrop.2020.112981

DE MELO, N, F. *et al*. Introdução aos hormônios e reguladores de crescimento vegetal. In: **Embrapa Semiárido-Artigo em anais de congresso (ALICE)**. In: Seminário Coda de Nutrição Vegetal, 1. Anais... Petrolina: CODA, 2002.

DE SOUZA, J. L. *et al*. Utilização de espécies vegetais como cobertura de solo no sistema plantio direto e como adubação verde na Região Serrana do ES. **Revista Científica Intelletto**. v.1, n.2, 87-102 p. 2016. Disponível em: http://biblioteca.incaper.es.gov.br/digital/handle/123456789/2987. Acesso: 04 jun. 2020

DELAVEAU, P. *et al*. **Segredos e virtudes das plantas medicinais.** Selecções do Reader's Digest, Lisboa, 1983.

DOS SANTOS, H. T. *et al*. Effects of irrigation management and seasonal stages on essential oil content and biomass of *Origanum vulgare* L. **Notulae Scientia Biologicae**, v. 12, n. 1, p. 42-56, 2020. https://doi.org/10.15835/nsb12110588

DUARTE JUNIOR, J. B. **Avaliação agronômica da cana-de-açúcar, milho e feijão em sistema de plantio direto em comparação ao convencional em Campos dos Goytacazes-RJ**. 2006. 284 p. Tese. Doutorado em Produção Vegetal – Campos dos Goytacazes – RJ, Universidade Estadual do Norte Fluminense – UENF, 2006.

EIRAS, P. P.; COELHO, F. C. Utilização de leguminosas na adubação verde para a cultura de milho. **InterSciencePlace**, v. 1, n. 17, 2011.

ESPÍNDOLA, J. A. A; GUERRA, J. G; DE ALMEIDA, D. L. **Adubação verde: Estratégia para uma agricultura sustentável.** Embrapa Agrobiologia-Documentos (INFOTECA-E), 1997.

EVANS, P.; HALLIWELL, B. Free radicals and hearing: Cause, consequence, and criteria. **Annals of the New York Academy of Sciences**, 884,19–40. 1999. https://doi.org/10.1111/j.1749-6632.1999.tb08633.x

FERDOUS, M. *et al*. Effect of green manure along with nitrogenous fertilizer on the growth and yield of turmeric (*curcuma longa* L.) of Bangladesh. **International Journal of Advanced Geosciences.** 2018. https://doi.org/10.14419/ijag.v6i1.11713

FERRACO, M. *et al*. Phytoremediation of contaminated soil with sulfentrazone by different density of *Crotalaria juncea*. **Planta Daninha**, v. 37, 2019. https://doi.org/10.1590/S0100-83582019370100008

FERRARI, M. P. De S. *et al*. Substrate-associated mycorrhizal fungi promote changes in terpene composition, antioxidant activity, and enzymes in *Curcuma longa* L. acclimatized plants. **Rhizosphere**, v. 13, p. 100191, 2020. https://doi.org/10.1016/j.rhisph.2020.100191

FERREIRA, V. R. F. *et al*. Colorimetric, electroanalytical and theoretical evaluation of the antioxidant activity of Syzygium aromaticum L., *Origanum vulgare* L., *Mentha spicata* L. and

Eremanthus erythropappus M. essential oils, and their major constituents. **New Journal of Chemistry**, v. 43, n. 20, p. 7653-7662, 2019. https://doi.org/10.1039/C8NJ05893H

FIGUERÊDO, J. de S. Estudo fitoquímico e potencial biológico de *Origanum vulgare* (orégano). 2019. 75 p. Dissertação (Mestrado em Química) - Universidade Federal do Piauí, Piauí, 2019.

FIKRY, S; KHALIL, N; SALAMA, O. Chemical profiling, biostatic and biocidal dynamics of *Origanum vulgare* L. essential oil. **AMB Express**, v. 9, n. 1, p. 41, 2019. https://doi.org/10.1186/s13568-019-0764-y

FORMENTINI, E A. *et al*. **Cartilha sobre adubação verde e compostagem.** 2019.

FRIDOVICH, I. Superoxide dismutases. In: **Advances in enzymology and related areas of molecular biology**. v.58, 61-97 p. 1986. https://doi.org/10.1002/9780470122860

FRUGOLI, J.A. Catalase is encoded by a multigene family in *Arabidopsis thaliana* (L.) Heynh. **Plant Physiology**, v.112, n.1, p.327-336, 1996. https://doi.org/10.1104/pp.112.1.327

GILLIJ, Y. G.; GLEISER, R. M.; ZYGADLO, J. A. Mosquito repellent activity of essencial oils of aromatic plants growing in Argentina. Bioresou. **Technol.**, v. 99, p. 2507-25-15, 2008. https://doi.org/10.1016/j.biortech.2007.04.066

GOLENIOWSKI, M. E.; FLAMARIQUE, C.; BIMA, P. Micropropagation of oregano (*Origanum vulgare*× applii) from meristem tips. **In Vitro Cellular & Developmental Biology-Plant**, v. 39, n. 2, p. 125-128, 2003. https://doi.org/10.1079/IVP2002361

GOMES, P. R. B. *et al*. Estudo da composição química e aplicação do óleo essencial *Origanum vulgare* L como agente antibacteriano em sururu (*Mytella charruana*) in natura. **Revista Virtual de Química**, v. 11, n. 6, 2019. http://dx.doi.org/10.21577/1984-6835.20190119

GRUĽOVÁ, D. *et al*. Thymol Chemotype *Origanum vulgare* L. Essential Oil as a Potential Selective Bio-Based Herbicide on Monocot Plant Species. **Molecules**, v. 25, n. 3, p. 595, 2020. https://doi.org/10.3390/molecules25030595

HAIDA, Z.; HAKIMAN, M. A comprehensive review on the determination of enzymatic assay and nonenzymatic antioxidant activities. **Food science & nutrition**, v. 7, n. 5, p. 1555-1563, 2019. https://doi.org/10.1002/fsn3.1012

HALLIWELL, B. Free radicals and antioxidants: a personal view. **Nutrition Reviews**, New York, v.52, n.8, p.253-265, 1994. https://doi.org/10.1111/j.1753-4887.1994.tb01453.x

IFTIKHAR, A. *et al*. Effect of gibberellic acid on growth, photosynthesis and antioxidant defense system of wheat under zinc oxide nanoparticle stress. **Environmental Pollution**, v. 254, p. 113109, 2019. https://doi.org/10.1016/j.envpol.2019.113109

IGHODARO, O. M.; AKINLOYE, O. A. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role

in the entire antioxidant defence grid. **Alexandria Journal of Medicine**, v. 54, n. 4, p. 287–293, 1 dez. 2018. https://doi.org/10.1016/j.ajme.2017.09.001

INOUE, M. H. *et al*. Seletividade de herbicidas aplicados em pré-emergência em cultivares de girassol. **Revista de Ciências Agrárias Amazonian Journal of Agricultural and Environmental Sciences**, v. 62, 2019. http://dx.doi.org/10.22491/rca.2019.3000

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION-ISO. **Aromatic natural raw materials – Vocabulary**. ISO 9235:2013. Genebra, 2013.

JACOB, R. A. The integrated antioxidant system. **Nutrition Research**, 15, 755–766. 1995. https://doi.org/10.1016/0271-5317(95)00041-G

JAN, S. et al. Biological efficacy of essential oils and plant extracts of cultivated and wild ecotypes of *Origanum vulgare* L. **BioMed Research International**, v. 2020, 2020. https://doi.org/10.1155/2020/8751718

JEDDI, S. *et al*. Effect of chitosan edible preservative coatings on *Origanum vulgare* L. essential oil on rainbow trout (*Oncorhynchus mykiss*) in refrigerator. **Journal of Innovation in Food Science and Technology**, v. 11, n. 1, 2019.

JUN, L. V. *et al*. Aplicação de reguladores de crescimento em plantas de *Stipa krylovii* em áreas de pastagem de xilin gol. **Planta Daninha**, v. 36, 2018. https://doi.org/10.1590/S0100-83582018360100049

KAMILA, P. K. *et al*. Effect of auxins on rooting of stem cuttings in *Hypericum gaitii*. **Journal of Herbs, Spices & Medicinal Plants**, p. 1-12, 2020. https://doi.org/10.1080/10496475.2020.1749207

KAPLAN, M. *et al*. Effect of gibberellic acid (GA3) inflorescence application on content of bioactive compounds and antioxidant potential of grape (*Vitis* L.)'Einset Seedless' berries. **South African Journal of Enology and Viticulture**, v. 40, n. 1, p. 1-10, 2019. http://dx.doi.org/10.21548/40-1-3004

KIEHL, E. J. **Contribuição para o estudo da poda e da decomposição de adubos verdes.** 1960. 113 p. Tese (Livre-Docência) - Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, 1960. Disponível em: https://repositorio.usp.br/item/000725838. Acesso: 29 mai. 2020.

KINTZIOS, S. E. **Oregano - The genera Origanum and Lippia.** Agricultural University of Athens, Athens, Greece. 2002.

KOGIANNOU, D. A. A. *et al*. Herbal infusions; their phenolic profile, antioxidant and anti-inflammatory effects in HT29 and PC3 cells. **Food and chemical toxicology**, v. 61, p. 152-159, 2013. https://doi.org/10.1016/j.fct.2013.05.027

LEÓN A. M. *et al*. Antioxidative enzimes in cultivars of peppers plants with different sensitivity to cadmium. **Plant Physiology and Biochemistry**, 40:813-820, 2002. https://doi.org/10.1016/S0981-9428(02)01444-4

LIMA-FILHO, DE L. O. F. *et al*. **Adubação verde e plantas de cobertura no Brasil: fundamentos e prática**. Embrapa, 2014.

LINHARES, P. C. F. *et al*. Rendimento de coentro (*Coriandrum sativum* L.) em sistema de adubação verde com a planta jitirana (*Merremia aegyptia* L.). **Revista brasileira de plantas medicinais**, v. 14, n. SPE, p. 143-148, 2012. https://doi.org/10.1590/S1516-05722012000500003

LOBO, V. *et al*. Free radicals, antioxidants and functional foods: Impact on human health. **Pharmacognosy Reviews**, v. 4, n. 8, p. 118–126, 2010. https://doi.org/10.4103/0973-7847.70902

LORENZI, H; MATOS, F. J. A. **Plantas Medicinais no Brasil**: Nativas e Exóticas. Instituto Plantarum de Estudos da Flora Ltda. Nova Odessa, SP, 2002, 512 p.

LORENZI, H; MATOS, F. J. A. **Plantas Medicinais no Brasil**: Nativas e Exóticas. Instituto Plantarum de Estudos da Flora Ltda. 2ª Ed. Nova Odessa, SP, 2008, 324 p.

LUDWIG-MÜLLER, J. Indole-3-butyric acid in plant growth and development. **Plant Growth Regulation**, v. 32, n. 2-3, p. 219-230, 2000. https://doi.org/10.1023/A:1010746806891

MALLICK N; MOHN F. H. Reactive oxygen species: response of alga cells. **Journal of Plant Physiology**, 157:183-193, 2000. https://doi.org/10.1016/S0176-1617(00)80189-3

MARQUES, C. T. S. *et al*. Improvement of biomass and essential oil production of *Lippia alba* (Mill) NE Brown with green manures in succession. **Industrial Crops and Products**, v. 112, p. 113-118, 2018. https://doi.org/10.1016/j.indcrop.2017.10.065

MASETTO, M. A. M. *et al*. Teor e composição do óleo essencial de inflorescências e folhas de *Lavandula dentata* L. em diferentes estádios de desenvolvimento floral e épocas de colheita. **Revista Brasileira de Plantas Medicinais**, v. 13, n. 4, p. 413-421, 2011. https://doi.org/10.1590/S1516-05722011000400007

McDONALD, M. B. Seed deterioratio: physiology, repair and assessement. **Seed Science and Technology**, Zurich, v. 27, n. 1, p. 177-237, 1999.

MEIRELLES, L. Agricultura Ecológica e Agricultura Familiar. In: PADOVAN, M. P; URCHEI, M. A; MERCANTE, F. M; CARDOSO, S. **Agroecologia em Mato Grosso do Sul: Princípios, Fundamentos e Experiências**. 1. ed., cap. 2. Ed. eletrônica: Eliete do Nascimento Ferreira, 2002. Embrapa Agropecuária Oeste; Campo Grande: IDATERRA, 2002. 51 p. Disponível em: http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/247552. Acesso: 29 mai. 2020.

MENEZES, L. A. S; LEANDRO, W. M. Avaliação de espécies de coberturas do solo com potencial de uso em sistema de plantio direto. **Pesquisa Agropecuária Tropical**, v. 34, n. 3, p. 173-180, 2004. Disponível em: https://revistas.ufg.br/pat/article/view/2311. Acesso: 29 mai. 2020.

MERCIER, H. Auxinas. In: KERBAUY, G.B. **Fisiologia Vegetal**. Rio de Janeiro: Guanabara Koogan, 2004. 452 p.

MIRANDA, C. A. S. F. de. **Atividade antioxidante de óleos essenciais de folhas de diversas plantas.** 2010. 151 p. Dissertação (Mestrado em Agroquímica), Programa de PósGraduação em Agroquímica, Universidade Federal de Lavras, Lavras, 2010.

MITSUDA, H. **Studies on Catalase**. Bulletin of the Institute for Chemical Research, Kyoto University, 34(4): 165-192. 1956. https://doi.org/10.1080/03758397.1955.10857290

MOGHADDAM, M; MEHDIZADEH, L. Chemistry of essential oils and factors influencing their constituents. In: **Soft chemistry and food fermentation.** Academic Press, 2017. 379-419 p. https://doi.org/10.1016/B978-0-12-811412-4.00013-8

MOREIRA, D. G. *et al*. 005-Adubação verde no crescimento e na produção de *Schinus terebinthifolius*, *Hibiscus sabdariffa* e *Jacaranda decurrens*. **Cadernos de Agroecologia**, v. 7, n. 2, 2012.

MORSHEDLOO, M. R. *et al*. Essential oil profile of oregano (*Origanum vulgare* L.) populations grown under similar soil and climate conditions. **Industrial Crops and Products**, v. 119, p. 183-190, 2018. https://doi.org/10.1016/j.indcrop.2018.03.049

MOSELE, S. H.; CECCHIN, D.; DEL FRARI, R. V. Estudo em inteligência competitiva para a cadeia produtiva de plantas medicinais e condimentares. **Perspectiva Erechim**. v.34, n.127, p. 73-83, 2010.

NAZMY, A. Effect of benzyl adenine, indole acetic acid and gibberellic acid on vegetative growth, chemical constituents and volatile oil attributes of sweet basil plants. **Egyptian Journal of Horticulture**, v. 47, n. 1, p. 41-56, 2020. https://doi.org/10.21608/ejoh.2020.23401.1126

OLIVEIRA, L. M. *et al*. Propagação vegetativa de *Hyptis leucocephala* Mart. ex Benth. e *Hyptis platanifolia* Mart. ex Benth. (Lamiaceae). **Revista Brasileira de Plantas Medicinais**, v. 13, n. 1, p. 73-78, 2011. https://doi.org/10.1590/S1516-05722011000100011

PADMAJA, M.; SRAVANTHI, M.; HEMALATHA, K. P. J. Evaluation of antioxidant activity of two Indian medicinal plants. **Journal of Phytology**, 2011.

PAL, P. K.; MAHAJAN, M.; AGNIHOTRI, V. K. Foliar application of plant nutrients and kinetin modifies growth and essential oil profile in *Rosa damascena* under acidic conditions. **Acta Physiologiae Plantarum**, v. 38, n. 7, p. 176, 2016.

PANDEY, A. *et al*. Phenolic compounds, antioxidant capacity and antimutagenic activity in different growth stages of in vitro raised plants of *Origanum vulgare* L. **Molecular biology reports**, v. 46, n. 2, p. 2231-2241, 2019. https://doi.org/10.1007/s11738-016-2187-6

PEREIRA, J. C. *et al*. Potencial alelopático e identificação dos metabólitos secundários em extratos de *Canavalia ensiformis* L. **Revista Ceres**, v. 65, n. 3, p. 243-252, 2018. https://doi.org/10.1590/0034-737X201865030004

PEREIRA, R.; DOS SANTOS, O. G. Plantas condimentares: cultivo e utilização. **Embrapa Agroindústria Tropical-Documentos (INFOTECA-E)**, 2013. Disponível em: http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/981610. Acesso: 04 jul. 2020.

PETRI, J. L. *et al*. Reguladores de crescimento para frutíferas de clima temperado. **Embrapa Uva e Vinho-Livro científico (ALICE)**, 2016. Disponível em: http://www.alice.cnptia.embrapa.br/alice/handle/doc/1067694. Acesso: 19 jun. 2020.

PEZZANI, R.; VITALINI, S.; IRITI, M. Bioactivities of *Origanum vulgare* L.: an update. **Phytochemistry reviews**, v. 16, n. 6, p. 1253-1268, 2017. https://doi.org/10.1007/s11101-017-9535-z

PINHEIRO, J. B.; MELO, R. A de C.; RAGASSI, C. F. Manejo de nematoides em hortaliças sob plantio direto. **Embrapa Hortaliças-Circular Técnica (INFOTECA-E)**, 2019. Disponível em: http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/1120353. Acesso: 02 jul. 2020.

PINTO, V. dos S. **Influência da idade da planta na composição química do óleo essencial de *Lippia alba* e de um ciclo de seleção recorrente na atividade formicida.** 2017. 66 f. Dissertação (Mestrado em Agricultura e Biodiversidade) - Universidade Federal de Sergipe, São Cristóvão, SE, 2017. Disponível em: http://ri.ufs.br/jspui/handle/riufs/6766. Acesso: 26 jun. 2020.

POMPELLA, A. Biochemistry and histochemistry of oxidant stress and lipid peroxidation. **International Journal of Vitamin and Nutrition Research**, Bern, v.67, n.5, p.289-297, 1997.

POURHADI, M. *et al*. Allometric ratio of menthol and menthone to pulegone in the essential oil of peppermint (*Mentha piperita* L.) affected by bioregulators. **Research Journal of Pharmacognosy**, v. 5, n. 3, p. 21-29, 2018. https://doi.org/10.22127/rjp.2018.64861

POVH, J. A; ONO, E. O. Rendimento de óleo essencial de *Salvia officinalis* L. sob ação de reguladores vegetais. **Acta Scientiarum. Biological Sciences**, v. 28, n. 3, p. 189-193, 2006.

PÓVOA, O; FARINHA, N.; CLARÉ, C. Adaptação ao cultivo de oregão (*Origanum vulgare* L.) na região de Elvas. **Revista de Ciências Agrárias**, v. 40, n. SPE, p. 61-70, 2017. https://doi.org/10.19084/RCA16182

PRINS, C. L.; VIEIRA, I. J.C.; FREITAS, S. P. Growth regulators and essential oil production. **Brazilian Journal of Plant Physiology**, v. 22, p. 91-102, 2010. https://doi.org/10.1590/S1677-04202010000200003

QUEIROZ, T. B. *et al*. Teor e composição química do óleo essencial de erva-baleeira (*Varronia curassavica* Jacq) em função dos horários de coleta. **Revista Brasileira de Plantas Medicinais**, v. 18, n. 1, p. 356-362, 2016. http://dx.doi.org/10.1590/1983-084X/15_116

RIBEIRO, M. C. **Crescimento Vegetativo de *Crotalaria juncea* em misturas solo/agregado reciclável visando recuperação de áreas degradadas degradadas.** 2018. 109p. Dissertação

(Programa de Pós-Graduação em Sistemas de Infraestrutura Urbana) - Pontifícia Universidade Católica de Campinas, Campinas-SP. 2018.

RIBES, S; FUENTES, A; BARAT, J. M. Effect of oregano (*Origanum vulgare* L. ssp. hirtum) and clove (*Eugenia* spp.) nanoemulsions on *Zygosaccharomyces* bailii survival in salad dressings. **Food chemistry**, v. 295, p. 630-636, 2019. https://doi.org/10.1016/j.foodchem.2019.05.173

ROCHA, J. C; ROSA, A. H; CARDOSO, A. A. **Introdução à química ambiental**. Artmed Editora, 2009, 180 p.

RODRIGUES, W. Competitividade e mudança institucional na cadeia produtiva de plantas medicinais no Brasil. **Interações (Campo Grande)**, v. 17, p. 267-277, 2016.

ROMAGNA, I. S.; JUNGES, E.; MICHELON, C. J. Incidência de fungos em sementes de leguminosas e gramíneas utilizadas como adubação verde. **Anais do Salão Internacional de Ensino, Pesquisa e Extensão**, v. 10, n. 2, 2019.

SALAMON, I; PORACOVA, J; HRYTSYNA, M. Oregano essential oil (*Origanum vulgare* L.), as a food-supplement in a rearing of piglets. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series**: Veterinary Sciences**, v. 21, n. 95, p. 55-61, 2019. https://doi.org/10.32718/nvlvet9510

SHARAFZADEH, S.; ZARE, M. Influence of growth regulators on growth and secondary metabolites of some medicinal plants from Lamiaceae family. **Adv. Environ. Biol**, v. 5, p. 2296-2302, 2011.

SILVA, T. O; MENEZES, R. S. C. Adubação orgânica da batata com esterco e, ou, *Crotalaria juncea*: II-disponibilidade de N, P e K no solo ao longo do ciclo de cultivo. **Revista Brasileira de Ciência do Solo**, v. 31, n. 1, p. 51-61, 2007. https://doi.org/10.1590/S0100-06832007000100006

SIMÕES, C. M. O; SPITZER, V. **Óleos Voláteis**. In: Farmacognosia: da planta ao medicamento. SIMÕES, C.M.O. (Org.) 6 ed. 1 reimp. Porto Alegre: Editora: UFRGS, Florinópois, Editora UFSC, 2010.

SINGH, M. *et al*. Cowpea (*Vigna unguiculata* L. Walp.) as a green manure to improve the productivity of a menthol mint (*Mentha arvensis* L.) intercropping system. **Industrial Crops and Products**, v. 31, n. 2, p. 289-293, 2010. https://doi.org/10.1016/j.indcrop.2009.11.004

SINGH, M. *et al*. Kinetin Alleviates UV-B-Induced Damage in *Solanum lycopersicum*: Implications of Phenolics and Antioxidants. **Journal of Plant Growth Regulation**, v. 38, n. 3, p. 831-841, 2019. https://doi.org/10.1007/s00344-018-9894-8

SKOUFOGIANNI, E.; SOLOMOU, A. D.; DANALATOS, N. G. Ecology, Cultivation and Utilization of the Aromatic Greek Oregano (*Origanum vulgare* L.): A Review. **Notulae Botanicae Horti Agrobotanici Cluj-Napoca**, v. 47, n. 3, p. 545-552, 2019. https://doi.org/10.15835/nbha47311296

TAIZ, L. *et al*. **Fisiologia e desenvolvimento vegetal**. Artmed Editora, 719 p, 2017.

TEODORO, R. B. *et al*. Aspectos agronômicos de leguminosas para adubação verde no Cerrado do Alto Vale do Jequitinhonha. **Revista Brasileira de Ciência do Solo**, v. 35, n. 2, p. 635-640, 2011. https://doi.org/10.1590/S0100-06832011000200032

TISSERAND, R; YOUNG, R. **Essential oil safety: A guide for health care professionals**. Elsevier Health Sciences, 2013.

TIVELLI, S. W; PURQUERIO, L. F. V.; KANO, C. Adubação verde e plantio direto em hortaliças. **Pesquisa & Tecnologia**, v. 7, n. 1, p. 1-7, 2010.

VASCONCELOS, S. M. L. *et al*. Espécies reativas de oxigênio e de nitrogênio, antioxidantes e marcadores de dano oxidativo em sangue humano: principais métodos analíticos para sua determinação. **Quím Nova**, v. 30, No. 5, 2007. https://doi.org/10.1590/S0100-40422007000500046

VINCIGUERRA, V. *et al*. Chemical characterization and antifungal activity of *Origanum vulgare*, *Thymus vulgaris* essential oils and carvacrol against Malassezia furfur. **Natural product research**, v. 33, n. 22, p. 3273-3277, 2019. https://doi.org/10.1080/14786419.2018.1468325

XAVIER, F. A. da S.; OLIVEIRA, J. I. A.; SILVA, M. R. Decomposition and nutrient release dynamics of shoot phytomass of cover crops in the Recôncavo Baiano. **Revista Brasileira de Ciência do Solo**, v. 41, 2017.

XIE, Xiulan *et al*. The roles of environmental factors in regulation of oxidative stress in plant. **BioMed research international**, v. 2019, p. 11, 2019. https://doi.org/10.1155/2019/9732325

XU, Y. *et al*. Growth promotion of Yunnan pine early seedlings in response to foliar application of IAA and IBA. **International journal of molecular sciences**, v. 13, n. 5, p. 6507-6520, 2012.

YOUN, C. K. *et al*. Effects of incorporation of green manure crops on growth and quality in Cynanchum wilfordii Hemsley. **Korean Journal of Medicinal Crop Science**, v. 25, n. 2, p. 115-120, 2017. https://doi.org/10.7783/KJMCS.2017.25.2.115

SEGUNDA PARTE – ARTIGOS

ARTIGO I

Green manure (*Crotalaria juncea* L.) increases biomass production, essential oil yield, and accumulation of bioactive compounds in *Origanum vulgare* L.

Elaborado de acordo com as normas do periódico Journal of Applied Research on Medicinal and Aromatic Plants

ABSTRACT

Oregano is a plant that is widely used across the world for medicinal and seasoning purposes. Organic fertilizers are important in terms of environmental protection and improve the soil structure, fertility and biological activity. The present study was undertaken to avaluate the effect of green manure (GM) (*Crotalaria juncea* L.) on biomass accumulation, volatile and phenolic compounds production and antioxidant activity in *Origanum vulgare* L. The treatments consisted of GM doses of 0, 150, 300, 450, and 600 g pot^{-1} with shoots (Sh = leaf+stem) and 200 g of sunn hemp roots (R), with 600 g of only shoots (Sh), with 200 g of only roots of GM, and with soil and 300 g of tanned manure per pot. At 90 days, the following were evaluated: leaf dry weight (LDW), stem dry weight (SDW), root dry weight (RDW), shoot dry weight (ShDW = LDW+SDW), total dry weight (TDW = LDW+SDW+RDW), root-to-shoot ratio (R/Sh), total leaf area, content and yield of essential oil, total phenols, and total flavonoids. There were significant effects of the GM doses on the dry weight of oregano. The highest means of the LDW, SDW, RDW, ShDW, and TDW were observed in the presence of both GM (300, 450, and 600 g pot^{-1} GM (Sh+R) and 600 g pot^{-1} GM (Sh) and cattle manure (300 g). For the content and yield of essential oil, GM manure resulted in the highest means, as well as the highest accumulation of flavonols/flavones. For essential oil production, the treatment without GM (control soil) showed a 75% lower production compared to the 450 g pot^{-1} GM (Sh+R) treatment and in a decrease of approximately 71% in comparison to that in the treatments with doses of 300, 600 g GM (Sh+R), and 600 g pot^{-1} GM (Sh). Based on the principal component analysis (PCA), both the organic GM (300, 450, 600 g pot^{-1} GM (Sh+R), and 600 g pot^{-1} GM (Sh) and manure fertilization (cattle manure) positively influenced the dry weight and content, yield, and chemical constituents of the essential oil, as well as the accumulation of total flavonoids in oregano. According to these findings, organic management through the use of the GM *C. juncea* L. may be beneficial for cultivating *O. vulgare* L. because it can increase the production of biomass and the active components present in this species. The use of *C. juncea* L. as GM is an inexpensive resource that has a positive effect on the cultivation of *O. vulgare* L. plants, and its utilization can reduce the use of chemical fertilizers, making it an ecologically appropriate strategy for the cultivation of medicinal plants.

keywords: organic cultivation, secondary metabolite, sabinene hydrate-trans, thymol, terpinen-4-ol, sunn hemp.

1. Introduction

Plants used for medicinal, aromatic, and seasoning purposes are widely used and consumed worldwide; among the most consumed species is oregano, which is known mainly for its use in cooking, often in tomato sauce, pizzas, salads, and meat (De Oliveira et al., 2017). In addition, the constituents of this spice are used in the agricultural, pharmaceutical, and cosmetic industries as a flavoring substance in food products, alcoholic beverages, and perfumery due to its spicy fragrance (Coqueiro et al., 2012; Pereira and Dos Santos, 2013).

Of the various essential oils produced worldwide, oregano oil is valued and important to several industrial segments. Its essential oil is used for the production of biodegradable films (Medeiros, 2017); as an antimicrobial to fight bacteria, fungi, and yeasts (Jan et al., 2020); as an antioxidant (Tapiero et al., 2019); in food (Migliorini et al., 2019); in medicine as a therapy for chronic rhinosinusitis (Alagawany et al., 2020; Kamaneh et al., 2020); and in the food industry (Lee et al., 2020). In addition, an important aspect related to the cultivation practices of this species related to the lack of research on it because despite the extensive literature available on the use of essential oil and oregano biomass, there are few reports on the cultivation of this plant (Skoufogianni et al., 2019).

According to Marques et al. (2018), techniques such as organic fertilization, minimum cultivation, and all organic agricultural practices are recommended for the cultivation and management of medicinal plants. Within these management recommendations, the preservation of active ingredients is encouraged by developing plants that are more resistant to and free of chemical pesticides that can compromise the chemical composition of a plant, changing it or even making it unusable as a medicinal plant (Marques et al., 2018).

Several studies show that over the long term, in comparison with using mineral fertilizers, using organic fertilizers (animal manure, plant residues, among others) is less expensive; improves soil structure, texture, and aeration; and increases the water holding capacity and organic matter (OM) content of soil, but mineral fertilizers in the short term bring benefits (rapid availability of nutrients) but in the long term causes serious side effects such as soil compaction, erosion, soil toxicity, and decline in total fertility (Assefa and Tadesse, 2019; Li et al., 2018; Ma et al., 2021).

Among the various forms of organic fertilization with good costs/benefits and broad technical results for soils and plants, green fertilization is the easiest to apply and least costly (Rocha et al., 2009). A green fertilization system is characterized by the use of plants in succession, rotation, or intercropping with crops of economic interest. Such plants can be incorporated into the soil or removed and maintained on the surface, providing a general

improvement in the physical, chemical, and biological characteristics of soil (Alcântara, 2016; Espíndola et al., 1997; Meena et al., 2020).

Eiras and Coelho (2011) reported that plants in the legume family (Fabaceae) are the most commonly used as green manure (GM). One of the main reasons for their use is related to the fact that the species in this family have the ability to perform the symbiosis process with atmospheric N2-fixing bacteria, and that they are hardy, high dry matter production, and deep root systems (Meena et al., 2020; Silva and Menezes, 2007). Among the species used in GM, *Crotalaria juncea* L. is one of the most used because it adapts to different climatic conditions and soil types in Brazil and stands out for its large biomass production capacity (21-60 t ha^{-1}), nutrient accumulation (150-450 kg of N ha^{-1}), and high residue quality (Araújo et al., 2010; Xavier et al., 2017).

The management of GM was tested and applied to *Ocimum selloi* Benth. (Lamiaceae) and *Echinacea purpurea* (L.) Moench (Compositae), and no increases in dry matter and chemical compounds were observed (Morais and Barbosa, 2012; Susanti et al., 2017). However, other studies have shown that this type of management can provide higher dry matter yields, essential oil production, bioactive constituents, and enzymatic activity (Adeniji and Kumoye, 2020; He et al., 2018; Honorato et al., 2022; Massey et al., 2021), showing that the production of medicinal plants is influenced by the management of GM as well as by the quality of the organic waste used.

To the authors knowledge, to date, there is a lack of studies relating the use of GM in the cultivation of the species *Origanum vulgare*. Thus, proposing new management systems to improve its biomass and essential oil production, as well as its constituent assets, is of great importance for providing a quality material to the market, as it is widely used. Given the above information, the present study was undertaken to avaluate the effect of green manure (GM) (*Crotalaria juncea* L.) on biomass accumulation, volatile and phenolic compounds production and antioxidant activity in *Origanum vulgare* L.

2. Materials and methods

2.1. Vegetal material and Experimental conditions

Preliminary experiments were carried out in two consecutive years in the same location with several species to test the effect of green manure on plants. Experiments were conducted in pots of 10 L. The experiment was carried out in the Medicinal Plant Garden of the Federal University of Lavras, Brazil, located at the geographic coordinates 21º 14’S and 45º 00W, at 918 m altitude. The mean climatic conditions during the growing seasons were 18,57◦C

(minimum) and 28,95◦C (maximum) as average daily temperature and 71.83% as mean relative humidity.

The exsiccate of the species is deposited in the ESAL Herbarium of the Department of Biology (number 22.156). The oregano seedlings were produced using apical cuttings (approximately 4 to 5 cm in length). The plants were kept in a greenhouse with automated irrigation for 30 days and then the plants were transplanted into 10 L pots.

Six sunn hemp seeds were sown in 10 L pots containing soil and sand (2:1). At the beginning of flowering, the shoots (Sh = leaf+stem) was removed and ground, and the soil present in the pots was broken up so that the roots (R) were incorporated together with the aerial part according to each treatment.

Sunn hemp was chopped in a forage chipper, and then, the biomass was incorporated into the soil. The cultivation was performed 30 days after the incorporation of GM into the pots. The experiment was in a randomized block design (RBD) with eight treatments: control (soil); 200 g pot^{-1} (sunn hemp root); 150, 300, 450, and 600 g pot^{-1} (sunn hemp shoot) + 200 g pot^{-1} (sunn hemp root); 600 g pot^{-1} (sunn hemp shoot); and positive control (cattle manure 300 g pot^{-1}). Each treatment consisted of 4 replicates (five plants per replicate), totaling 160 experimental units. Irrigation was performed two/three times a week according to the needs of the plants. A substrate sample was collected for a soil analysis of each treatment. The chemical characteristics of the substrate are described in Table 1. Table 2 shows the nutritional characterization of sunn hemp and the amount of nutrients added to the soil according to each dose.

Table 1
Soil chemical characterization after 90 days according to the doses of green manure (*Crotalaria juncea*) used in the cultivation of *Origanum vulgare*.

Soil parameters	Control soil	Cattle manure	200 g GM (R)	150	300	450	600	600 g GM (Sh)
				..... g GM (Sh+R)				
pH (H_2O)	6.30	5.60	5.50	5.50	5.70	5.80	6.00	6.10
K (mg/dm^3)	47.84	96.50	103.63	100.80	78.21	88.80	96.90	72.34
P (mg/dm^3)	0.84	12.52	0.00	0.00	0.00	0.00	0.00	0.00
Ca (cmolc/dm^3)	1.76	2.18	1.37	2.55	2.85	2.47	1.56	1.93
Mg (cmolc/dm^3)	0.39	0.62	0.30	0.50	0.51	0.52	0.38	0.50
Al (cmolc/dm^3)	0.10	0.00	0.00	0.10	0.10	0.10	0.00	0.00
H+Al (cmolc/dm^3)	1.40	1.90	1.60	1.50	1.50	1.60	1.60	1.60
EBS (cmolc/dm^3)	2.27	3.05	1.94	3.31	3.56	3.22	2.19	2.62
t (cmolc/dm^3)	2.37	3.05	1.94	3.41	3.66	3.32	2.19	2.62
T (cmolc/dm^3)	3.67	4.95	3.54	4.81	5.06	4.82	3.79	4.22
V (%)	61.92	61.56	54.8	68.80	70.40	66.80	57.74	61.98
m (%)	4.22	0.00	0.00	2.93	2.73	3.01	0.00	0.00
OM (dag/kg)	1.18	2.03	1.37	1.66	1.94	1.69	1.71	1.81
P-Rem (mg/L)	12.50	21.80	15.70	18.20	19.80	18.40	16.30	15.50
Zn (mg/dm^3)	1.00	2.60	0.90	2.30	2.40	2.40	1.00	1.50
Fe (mg/dm^3)	41.70	34.30	24.70	25.70	25.20	25.00	24.90	25.90
Mn (mg/dm^3)	15.60	14.90	9.60	14.00	14.00	13.80	11.50	12.20
Cu (mg/dm^3)	1.48	1.27	1.14	1.20	1.10	1.18	1.15	1.21
B (mg/dm^3)	0.01	0.13	0.05	0.04	0.11	0.13	0.03	0.04
S (mg/dm^3)	2.20	7.60	7.90	6.40	6.80	7.10	8.00	6.40

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.

Table 2
Nutritional characterization of green manure (*Crotalaria juncea*) and the amount of macro- and micronutrients incorporated in the soil according to each dose used in the cultivation of *Origanum vulgare*.

Treatments		Macronutrients						Micronutrients				
		N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
Shoots (leaf+stem) g pot^{-1}	Roots g pot^{-1}	..... g kg^{-1}						..... mg kg^{-1}				
Green manure		34.5	1.9	9.9	11.2	3.2	2.1	24.9	6.3	655.1	50.5	34.3
Root GM		9.7	0.6	13.4	3.1	0.9	0.7	12.7	8.8	881	18.8	12.7
		..... g pot^{-1}						..... g pot^{-1}				
150	200	7.1	0.4	4.2	2.3	0.7	0.4	6.2	2.7	274.6	11.4	7.6
300	200	12.3	0.7	5.7	4.0	1.2	0.8	10.0	3.7	372.8	18.9	12.8
450	200	17.4	1.0	7.2	5.6	1.6	1.1	13.7	4.6	471.1	26.5	17.9
600	200	22.6	1.2	8.6	7.3	2.1	1.4	17.4	5.5	569.3	34.0	23.1
600	-	20.7	1.1	5.9	6.7	1.9	1.26	14.9	3.8	393.0	30.3	20.6
-	200	1.9	0.1	2.7	0.6	0.2	0.1	2.5	1.8	176.3	3.8	2.5
Control soil (-)		-	0.0	0.03	0.14	0.006	0.002	0.02	1.4	43.0	15.3	0.46
Cattle manure (+)		11.0	5.0	12.0	7.0	4.0	2.0	140.0	57.0	3,3811	920.0	72.0

2.2. Plant growth

At 90 days after transplanting, the following characteristics were evaluated: leaf dry weight (LDW- g plant^{-1}); stem dry weight (SDW- g plant^{-1}); root dry weight (RDW- g plant^{-1}); shoot dry weight (ShDW- g plant^{-1} = LDW+SDW); total dry weight (TDW- g plant^{-1} = LDW+SDW+RDW); and the root-to-shoot ratio (R/Sh). The total leaf area (TLA) was measured using *ImageJ* ® *software*. The following relationships were also observed: leaf area ratio (LAR = TLA/TDW) and specific leaf area (SLA= TLA/LDW).

2.3. Photosynthetic pigments and nutritional analysis

The photosynthetic content was extracted and analyzed according to De Assis et al. (2020). The samples were prepared in quintuplicate, and three aliquots (3 mL) of each replicate were transferred to a quartz cuvette; the optical density values were read in a spectrophotometer Tecan Infinity M200 PRO, a 480, 649 e 665 nm, operated in the I -Control® data processing system (version 3.37), against blank DMSO. The wavelengths and equations adopted for these calculations were based on the methodology of Wellburn (1994): Chlorophyll *a* 649 = (12.47 x A665) - (3.62 x A649); Chlorophyll *b* 665 = (25,06 x A649) - (6,5 x A665); Carotenoids 480= (1000 x A480 – 1.29 x C*a* – 53.78 x C*b*)/ 220; Total chlorophyll = chlorophyll *a* + chlorophyll *b*.

To determine the accumulation of N, P, K, Ca, Mg, S, B, Cu, Mn, Zn, and Fe in the dried leaves of *Origanum vulgare*, 2 g of each treatment was sampled for analysis. Macronutrients were expressed in g kg^{-1} and micronutrients in mg kg^{-1} of dry leaf weight.

2.4. Extraction of essential oil

The essential oil was extracted from 20 g of dried leaves placed in a 2,000 mL volumetric flask filled with 500 mL of distilled water by hydrodistillation in a modified Clevenger apparatus. The extraction time was fixed at 90 min from the boiling point. The extraction was performed in quadruplicate for each treatment. The essential oil was weighed, stored in an airtight amber bottle, and kept in a refrigerator at 4 °C. The essential oil content was expressed in g 100 g^{-1} of leaf dry weight, and the yield was expressed in g $plant^{1}$.

2.5. Analysis of essential oil

The analysis of essential oil was analyzed according to De Assis et al. (2020). Briefly, the quantitative and qualitative chemical analyses of the essential oil were performed in triplicate in the Agilent® 7890A Gas Chromatography (GC) system operated in an MSD CHEM Station Ver. E.02.02.1431 data processing system equipped with injector/autosampler CombiPAL Autosampler System (CTC Analytic AG, Switzerland) and Flame Ionization Detector (FID). Retention index was calculated using the equation of Van Den Dool and Dec Kratz, (1963); the evaluations of the retention index described in the literature were consulted (Adams, 2007).

2.6. Analysis of phenolic compounds and antioxidant activities

2.6.1 Preparation of extracts

The extracts were prepared according to the methodology of Baranauskaité et al. (2016) with adaptations, namely, 3 g of leaves sprayed and weighed on an analytical balance was added to a 500 mL volumetric flask for extraction in 300 mL of ethanol (92.8°) by thermal reflux. The extraction occurred for 360 min, and then, the extracts were placed in a rotary evaporator under reduced pressure for solvent evaporation to obtain the crude extracts.

Subsequently, 10 mg of the crude extracts was weighed in microtubes (1.5 mL), 1 mL of ethanol (92.8°) was added, and the samples were sonicated for 10 min. Next, the samples were vortexed and centrifuged for 10 min at 10,000 rpm, and the supernatant was collected to obtain the ethanolic extract at a concentration of 10 mg mL^{-1}.

2.6.2 Total Phenols

The total phenol content was determined in the Folin-Ciocalteau reagent based on the method of Slinkard and Singleton (1977). In total, 150 μL of ethanolic leaf extract was placed in microtubes at a concentration of 10 mg mL^{-1} and added to 300 μL of Folin-Ciocalteau reagent (10% v/v) and 375 μL of sodium carbonate (7% w/v). The samples were homogenized by vortexing and then incubated at room temperature in the dark for 120 min. After the incubation period, the reaction was centrifuged for 10 min at 10,000 rpm. Subsequently, the supernatant (275 μL) was added to the microplate and the absorbance was measured at 760 nm. The calibration curve was generated from the standard of gallic acid in the concentration of distilled water ($y= 0.0997 + 13.184x$ $r^2 = 0.9989$) ranging from 0.0078 to 0.25 mg mL^{-1}. The results were expressed in mg Equivalent Gallic Acid (EAG) g dry $leaf^{-1}$.

2.6.3 Total flavonols/flavones

The flavonols/flavones contents were quantified as described by Woisky and Salatino (1998). An aliquot of 100 μL of the ethanolic extract of the leaf was placed on microplates at a concentration of 10 mg mL^{-1}, and then, 100 μL of aluminum chloride solution (10% w/v) was added. The calibration curve was generated from the quercetin standard, with 70% alcohol ($y = 0.0962 + 19.302 x$ $r^2 = 0.9951$) at a concentration ranging from 0.0078 to 0.125 mg mL^{-1}. The tests were performed in quintuplicates and the results were expressed in mg Quercetin Equivalent (EQ) g dry $leaf^{-1}$.

2.6.4 Total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) was measured based on the ammonium molybdate reduction method described by Prieto et al. (1999). Two hundred microliters of the extracts (10 mg mL^{-1}) was mixed with 1500 μL of the reagent solution (0.6 M sulfuric acid, 28 mM monobasic sodium phosphate, and 4 mM ammonium molybdate). After 90 minutes of incubation at 95°C, the samples were cooled to room temperature, and their absorbances were measured at 695 nm. The calibration curve of aqueous ascorbic acid solution was constructed from the absorbances found ($y = 0.053 + 14.261x$ $r^2 = 0.9997$) at a concentration ranging from 0.0078 to 0.25 mg mL^{-1}. The tests were performed in quintuplicate and the results expressed in mg Equivalent in Ascorbic Acid (EAA) g dry $leaf^{-1}$.

2.6.5 Free radical scavenging activity (DPPH)

The scavenging activity of 1,1-diphenyl-1,2-picrylhydrazyl (DPPH) radicals was determined by the method proposed by Brand-Williams et al. (1995). In microplates, 20 μL of

the sample was added to 260 µL of a methanolic solution of DPPH. The mixture was incubated for 60 minutes in the dark at room temperature. After this period, the absorbance was measured at 517 nm. BHT (butylhydroxytoluene) was used as a standard (positive control). The free radical scavenging activity was performed in quintuplicate. Subsequently, the IC_{50} was calculated, corresponding to the concentration of extract capable of inhibiting 50% of DPPH radicals, according to the equation:

$$\% IC_{50} = \frac{Ac - At}{Ac} \times 100$$ where, Ac: control absorbance; At: test absorbance.

The results of the DPPH free radical capture assay were expressed in the Antioxidant Activity Index (AAI) proposed by Scherer and Godoy (2009), where the plant extract is considered to have low antioxidant activity ($AAI \leq 0.5$); moderate antioxidant activity ($0.5 \leq AAI \leq 1.0$); strong antioxidant activity ($1.0 \leq AAI \leq 2.0$); and, very strong antioxidant activity ($AAI \geq 2.0$).

2.6.6 Ability to absorb oxygen radicals (ORAC)

The analysis was based on the method of Ou et al. (2001). Onto 96-well black microplates, 30 µL of the sample and 150 µL of fluorescein (70 mM) prepared in phosphate buffer (75 mM and pH 7.4) were added. The microplate was preincubated for 10 minutes at 37°C. After this period, 30 µL of the 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) radical (12 mM) was added, and the fluorescence was recorded every minute for 150 minutes. As a control, the Trolox standard was used at concentrations from 0.25 to 0.015625 mg mL^{-1}. As a blank, 30 µL of phosphate buffer (70 mM and pH 7.4) was used instead of AAPH. All treatments were evaluated in triplicate.

Results were calculated using a regression equation between Trolox concentration and net AUC (area under the curve) ($AUC_{sample} - AUC_{white}$), with ORAC values expressed as µmol equivalent of Trolox per gram of leaf dry weight (µmol ET g^{-1} of LDW).

2.7 Statistical analysis

The data were subjected to variance analysis. The means were compared using the Scott–Knott test ($p < 0.05$) with the statistical program SISVAR® (Ferreira, 2019). Principal component analysis (PCA) was performed using Statistica® software, version 10.0 (StatSoft - Tulsa, USA).

3 Results and discussion

3.1. Biomass production and essential oil content and yield

The influence of GM doses on leaf dry weight (LDW), stem (SDW), root (RDW), shoot (ShDW), total (TDW), root/shoot ratio (R:Sh) and content and essential oil yield are shown in Table 3. There was a significant difference in the doses of GM for all variables of dry weight and essential oil of oregano. The greatest differences were observed for the doses of 300 g pot^{-1} GM (Sh+R), 450 g pot^{-1} GM (Sh+R), 600 g pot^{-1} GM (Sh+R), 600 g pot^{-1} GM (Sh), and cattle manure, as they resulted in the highest means for LDW, SDW, RDW, ShDW, and TDW compared to those for the control soil, 150 g pot^{-1} GM (Sh+R) and 200 g pot^{-1} GM (R). Thus, the presence of GM and cattle manure were important for oregano dry weight gain, especially in relation to the control soil treatment and the dose containing only sunn hemp roots (200 g pot^{-1} GM) as it obtained a lower mean dry weight.

For LDW, the highest means were 8.8, 8.71, 8.56, and 8.38 g for the doses 300 g pot^{-1} GM (Sh+R), 600 g pot^{-1} GM (Sh), 450 g pot^{-1} GM (Sh+R), and cattle manure (300 g pot^{-1}), respectively. Compared to the control soil, which had the lowest mean LDW (2.82 g) and SDW (1.30 g), these same doses of GM and cattle manure increased the production of dry weight 3-fold for LDW and 4-fold for SDW. This result demonstrates that the management of GM can result in significant gains in the production of oregano leaves and, therefore, can be adopted as an agricultural strategy to meet industrial demands since the leaves concentrate greater amounts of essential oil, which has high market value. Doses of 300 g pot^{-1} GM (Sh+R), 450 g pot^{-1} GM (Sh+R), 600 g pot^{-1} GM (Sh), and cattle manure increased ShDW and TDW more than 3-fold compared to that produced in the treatment without GM (control soil) (Table 3).

Table 3

Doses of green manure (*Crotalaria juncea*) on dry weight production and essential oil of *Origanum vulgare* Leaf (LDW), stem (SDW), root (RDW), shoot (ShDW), total dry weight (TDW) and essential oil (EO).

Treatments		LDW	SDW	RDW	ShDW	TDW	Ratio	EO	Yield
Shoots (leaf+stem) g pot^{-1}	Roots g pot^{-1}	g plant^{-1}					R/ShDW	Content %	mg EO plant^{-1}
150	200	5.58c	3.10c	6.03c	8.69d	14.70c	0.70c	0.74b	40.0d
300	200	8.80a	5.91a	11.00b	14.70a	25.71a	0.75c	0.79b	70.0b
450	200	8.56a	6.24a	10.60b	14.80a	25.40a	0.72c	0.91a	80.0a
600	200	7.84b	5.31b	9.82b	13.15c	22.97b	0.72c	0.91a	70.0b
600	-	8.71a	5.92a	12.10a	14.60a	26.70a	0.83b	0.82a	70.0b
-	200	3.24d	1.51d	4.22d	4.75e	8.97d	1.00a	0.67b	20.0e
Control soil (-)		2.82d	1.30d	4.16d	4.12e	8.28d	0.94a	0.91a	20.0e
Soil + Cattle manure (+)		8.38a	5.98a	10.58b	14.36a	24.94a	0.76c	0.71b	60.0c
CV%		4.98	4.50	8.76	4.76	6.53	6.12	4.98	5.41

Means followed by the same letter in the column are not significantly different according to the Scott-Knott test ($p \leq .05$). CV: Coefficient of variation.

Despite the lack of studies relating to GM and medicinal plants, some studies demonstrate the potential of this management strategy. Diniz et al. (2017) found similar results when testing doses of *Crotalaria juncea* (0, 3, 6, and 9 t/ha) because they observed that increasing doses of GM provided higher mean dry weight in *Brassica oleracea* L. (Brasicaceae). Marques et al. (2018), when testing sources of GM in succession, also observed that compared to the control, sunn hemp provided dry weight gains (11.4%) in *Lippia alba* (Mill.) N.E.Br. ex Britton & P.Wilson. (Verbenaceae). The positive effects of using GM, as well as the combination of other organic fertilizers or low doses of mineral fertilizers have been also reported (Massey et al., 2021; Peralta-Antonio et al., 2021; Rothé et al., 2019; Watthier et al., 2020).

Linhares et al. (2012) showed that the doses of GM (*Merremia aegyptia* (L.) Urb. - Convolvulaceae) also increased agronomic yields (height, number of stems per plant, yield, and shoot dry weight) of the species *Coriandrum sativum* L. (Apiaceae). These results confirm the importance of this management technique in cultivating plants used for aromatic, seasoning, and medicinal purposes, as the significant plant growth and consequently higher plant dry weight that occur GM are notorious.

It is possible to observe that in comparison to the control soil and 200 g pot^{-1} GM (R) treatments, the treatments with GM and cattle manure had higher shoot growth, which consequently influenced the dry weight (Figure 1). The higher mean dry weight of oregano can

be explained by the greater availability of nutrients (K, Ca^{+2}, Mg^{+2}, Zn, B, and S) and organic matter (OM) (Table 1 and Table 2) due to the increase in the doses of GM when compared to the treatment without GM. According to Meena et al. (2020), GM can fix atmospheric nitrogen and provide biomass that stimulates microbial diversity, consequently improving soil fertility and releasing nutrients throughout the crop cycle.

Fig. 1. *Origanum vulgare* plants cultivated under the influence of different doses of green manure (*Crotalaria juncea*). 1) 150 g shoot + 200 g root/pot, 2) 300 g shoot + 200 g root/pot, 3) 450 g shoot + 200 g root/pot, 4) 600 g shoot + 200 g root/pot, 5) 600 g shoot/pot, 6) soil + 200 g root, 7) soil (control -) and 8) soil + cattle manure 300 g/pot (control +).

Rosa et al. (2017) studied the quality of the soil cultivated with cover crops, and among the species studied, *Crotalaria juncea* experienced increases in carbon in the fulvic acid fraction, which contributed to aspects of soil fertility. According to Vakeesan and Nishanthan (2008), this increase in organic carbon and consequently in soil OM may have been related to the plant tissues that partially decomposed or decomposed green fertilizers, which fed the beneficial soil organisms.

The R:Sh indicates the preferential allocation of dry mass to the root system (Table 3 and Figure 1). In comparison to the control soil treatment and 200 g pot^{-1} GM (R), the plants managed with GM and the treatment with cattle manure resulted in a lower R:Sh ratio; i.e., there was a greater allocation of dry biomass to the shoots (Figure 1). The highest averages of R:Sh in the treatments without GM and the 200 g pot^{-1} GM (R) dose may have occurred due to

the lower levels of nutrients in the soil; thus, the lower growth of the shoots may have occurred due to the plants attempting to allocate more mass to the roots to increase absorption efficiency.

The content (%) and yield (mg EO plant^{-1}) of oregano essential oil showed that the doses of GM resulted in a significant difference (Table 3). The doses of 450 and 600 g pot^{-1} GM (Sh+R) and the control soil resulted in the highest mean essential oil content (0.91%), followed by the 600 g pot^{-1} GM (Sh) dose at 0.82%. For essential oil production (mg EO plant^{-1}), the lowest means were observed in the control soil and at the dose of 200 g pot^{-1} GM (R), both resulting in 20 mg EO plant^{-1}. Although the control soil had a high essential oil content (0.91%), the yield of essential oil was 75% lower compared to that resulting from the dose of 450 g pot^{-1} GM (Sh+R) and by approximately 71% compared to that resulting from the doses of 300, 600 g pot^{-1} GM (Sh+R), and 600 g pot^{-1} GM (Sh). In comparison to the control soil, the treatment with cattle manure also showed an increase of 67% more essential oil, demonstrating the importance of organic fertilization for oregano.

The higher production of essential oil influenced by the doses of GM may be related to the better chemical characteristics of the soil and greater availability of nutrients provided by *Crotalaria juncea* (Table 1 and Table 2). Silva et al. (2021) state that the species of the genus *Crotalaria* stand out within the Fabaceae family because they contribute to the accumulation of OM and nutrients, in addition to protecting the soil against erosion. Marques et al. (2018) showed that different sources of GM in succession, including sunn hemp, increased the production of essential oil in *Lippia alba* by approximately 14% compared to the control.

The use of organic fertilization and green fertilization in *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson (Poaceae) significantly increased the content and yield attributes, as well as oil composition, soil nutrient status, and microbial populations (Massey et al., 2021). Bidgoli and Mahdavi (2018) observed that nitrogen doses combined with GMs (*Medicago sativa* L. - Leguminosae and *Secale montanum* Guss. - Poaceae) resulted in higher oil content and yield in *Mentha x piperita* L. Honorato et al. (2022) also demonstrated that sunn hemp doses (0, 3, 6 and 9 kg m^2) increased biomass, essential oil content and yield in *Thymus vulgare* L. (Lamiaceae) when compared to control.

3.2. Photosynthetic pigments and leaf area

The mean values of photosynthetic pigments and oregano leaf area indices grown under different doses of GM are shown in Table 4. There was no significant difference in GM doses for the following variables: carotenoids, specific leaf area (SLA), and leaf area ratio (LAR). However, for the photosynthetic pigments chlorophyll *a* and *b* and total leaf area (TLA), there

was a significant difference between the doses of GM (Table 4). It was observed that the highest means of chlorophyll *a* occurred in the presence of GM (150, 300, 450, and 600 g pot^{-1} GM). However, the cattle manure and the control soil, both with 0.67 mg. g^{-1} FW, and the 200 g pot^{-1} GM (R) treatment with 0.64 mg g^{-1} FW, recorded the lowest means of chlorophyll *a*. This higher accumulation of chlorophyll *a* may be related to the higher concentrations of nitrogen and magnesium (Table 2) in the soil from the doses of GM, as these nutrients are part of the chlorophyll molecule (Li et al., 2021).

In comparison to the other treatments, the cattle manure and the 450 g pot^{-1} GM (Sh+R) dose resulted the lowest chlorophyll *b* means, both with 0.58 mg g^{-1} FW. For total chlorophyll, the highest means were observed in the 150 g pot^{-1} GM (Sh+R) and 200 g pot^{-1} GM (R) treatments, at 146 and 148 mg g^{-1} FW, respectively.

Photosynthetic pigments play a direct role in photosynthesis because they use solar energy to synthesize complex carbon compounds (carbohydrates and the release of oxygen from carbon dioxide and water). The energy stored in these molecules can be used to boost cellular processes in a plant and serve as an energy source (Taiz et al., 2017). In addition, most of the total green pigments found in plants correspond to chlorophyll *a*, and chlorophyll b is a supplementary pigment (Borrmann, 2009). Similar results were also found by Mutisya et al. (2014), who observed that in comparison to the treatment without GM, the treatments with doses of GM (*Sesbania sesban* (L.) Merr. – Leguminosae) increased the photosynthetic pigments of chlorophyll *a* in *Solanum nigrum* L. (Solanaceae) Corrêa et al. (2009) observed the influence of organic fertilization on *Origanum vulgare* and found higher levels of photosynthetic pigments in organically fertilized plants compared to the control.

Table 4
Effect of green manure (*Crotalaria juncea*) doses on photosynthetic pigments and total leaf area (TLA), leaf area ratio (LAR) and specific leaf area (SLA) of *Origanum vulgare*.

Photosynthetic Pigments						Growth analysis.....		
Treatments		Chlorophlly			Carotenoids	TLA	LAR	SLA
Shoots (leaf+stem) g pot^{-1}	Roots g pot^{-1}	a	b	Total				
		..... mg. g^{-1} FW.....				cm^{-2}	..... cm^{-2}. g	
150	200	0.73a	0.73a	1.46a	0.25a	516.8b	59.1a	92.4a
300	200	0.71a	0.67a	1.31b	0.23a	680.9a	49.1a	80.9a
450	200	0.72a	0.58b	1.37b	0.23a	813.1a	53.7a	90.9a
600	200	0.72a	0.70a	1.35b	0.24a	744.9a	54.4a	88.6a
600	-	0.72a	0.70a	1.39b	0.24a	833.0a	56.1a	87.9a
-	200	0.64b	0.71a	1.48a	0.24a	240.8c	60.5a	87.5a
Control soil (-)		0.67b	0.71a	1.34b	0.25a	275.2c	53.8a	80.3a
Soil + Cattle manure (+)		0.67b	0.58b	1.36b	0.24a	793.1a	54.7a	94.3a
CV%		3.7	5.7	4.9	4.1	15.3	13.2	11.46

Means followed by the same letter in the column are not significantly different according to the Scott-Knott test ($p \leq .05$). FW: fresh weight, CV: Coefficient of variation.

The highest mean TLA occurred in the presence of GM (300, 450, 600 g pot^{-1} GM and 600 g pot^{-1} (Sh)), as well as in the presence cattle manure. By relating these data to the dry weight data (Table 3), it can be inferred that the larger leaf area, combined with the higher chlorophyll *a* content (Table 4), contributed to a greater accumulation of LDW, RDW, ShDW, and TDW in relation to the control soil and 200 g pot^{-1} GM (R), which had the lowest mean leaf area, at 275.2 and 240.8 cm^{-2} g, respectively. This smaller leaf area may be related to a possible stress condition caused by the low content of nutrients and OM in the soil in these treatments.

According to Taiz et al. (2017), plants have plasticity and develop the ability to modify leaf morphology to allow them to avoid or mitigate the effects of abiotic extremes. Such mechanisms include changes in leaf area, leaf orientation, leaf folding, trichomes, and waxy cuticles. In addition, large and flat leaves provide optimal surfaces for the production of photosynthesis. However, they can be harmful to the growth and survival of agricultural crops under stressful conditions because they expose a large surface area to water evaporation, which can lead to rapid depletion of soil water (Taiz et al., 2017).

3.3. Leaf nutrient accumulation

Table 5 shows the accumulation of macro- and micronutrients in dry leaves of *O. vulgare* grown under the influence of doses of *Crotalaria juncea*. In general, in relation to macronutrients, the treatment without GM had higher accumulations of Ca, and S at 24.5 and

4.9 g kg^{-1}, respectively. For N, P and K, the highest accumulations (18.6, 2.2, and 24.4 g kg^{-1}, respectively) were observed in the presence of GM at 600, 450 g pot^{-1} GM (Sh+R), and 200 g pot^{-1} GM (R), respectively.

The higher levels of Ca, and S in the control soil in comparison to those in the treatments with GM may be related to plant growth since the plants without GM had less growth (Table 3 and Fig. 1) than the fertilized plants. Chemical and environmental factors that cause changes in growth rates and nutrient absorption will affect the concentrations of the nutrients in plant tissue (Fontes, 2001). If a plant has a low growth rate and nutrients continue to be absorbed, then concentration of nutrients will occur; however, if a plant grows rapidly, then nutrient dilution will occur (Maia et al., 2005).

Table 5
Accumulation of macro and micronutrients in dry leaves of *Origanum Vulgare* cultivated under the influence of doses of green manure (*Crotalaria juncea*).

Treatments		Macronutrients						Micronutrients				
		N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
Shoots (leaf+stem) g pot^{-1}	Roots g pot^{-1}	..... g kg^{-1}						..... mg Kg^{-1}				
150	200	14.7	2.1	22.8	23.3	1.8	3.6	51.8	11.7	2,300	65.8	66.2
300	200	14.7	2.1	19.0	23.4	1.7	3.0	52.2	10.8	1,135	57.2	61.5
450	200	15.2	2.2	19.9	22.9	1.7	3.0	52.2	11.0	2,697	56.1	65.8
600	200	18.6	2.0	21.4	22.9	1.9	3.4	48.8	10.3	1,576	72.8	57.5
600	-	14.1	1.9	19.4	22.1	1.8	3.0	47.9	10.1	3,302	70.8	54.5
-	200	14.6	2.0	24.4	23.1	1.9	4.6	49.1	11.1	2,087	122	68.3
Control soil (-)		15.6	2.1	23.8	24.5	2.0	4.9	47.4	11.0	1,514	97.2	68.9
Soil+Cattle manure (+)		15.1	2.4	20.8	22.0	2.3	3.3	44.7	11.3	2,023	61.6	77.8

Vieira et al. (2019) tested types of fertilization (organic and chemical) in the species *Justicia pectoralis* Jacq. (Acanthaceae) and observed higher accumulation of macronutrients (K, Ca, and Mg) in the treatment that did not receive fertilization than in the fertilization treatments. Studies corroborate the higher accumulation of N, P, and K provided by GM because they showed that both organic management via animal manure and GM resulted in higher accumulation of leaf macronutrients in the species *Acmella oleracea* (L.) R.K.Jansen (Compositae) and *Bactris gasipaes* Kunth. (Arecaceae) (Magalhes et al., 2020; Nordi, 2021).

Regarding micronutrients, it was also observed that doses of GM, 150, 300, 450 g pot^{-1} GM (Sh+R) and 600 g pot^{-1} GM (Sh) influenced a greater accumulation of B, Cu, and Fe. According to Batista et al. (2018), the higher levels of macro- and micronutrients in oregano

leaves may be related to the increase in OM in the soil provided by GM because OM is a controlling component of nutrient availability in the soil. Thus, nutrient availability can occur through mineralization or through the formation of organometallic complexes (soluble or insoluble), which prevent micronutrients from interacting with soil minerals or other dissolved ions that are easily absorbed by the plant (Batista et al., 2018).

3.4. Chemical composition of the essential oil

The chemical constituents of the essential oil of *Origanum vulgare* grown under different doses of *Crotalaria juncea* are shown in Table 6. It was observed that the doses of GM influenced the chemical composition of the essential oil of oregano expressed in relative area rate. The six main classes of compounds found were alkenyl alcohol, monoterpene hydrocarbons, oxygenated monoterpenes, phenolic monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes. The same classes of constituents in the chemical composition of the essential oil of oregano was also reported by De Mastro et al. (2017). Regarding the qualitative aspect of the essential oil of oregano, δ-cadinene was detected only in the 200 g pot^{-1} of GM (R), 150 g pot^{-1} of GM (Sh+R), and cattle manure treatments.

Hydrocarbon monoterpenes were the main class present in the essential oil of oregano, recorded at levels between 55.31 and 60.91%. The control soil treatment, followed by doses of 450, 300 and 600 g pot^{-1} of GM (Sh+R), presented the highest means, at 60.91, 60.71, 60.66, and 60.48%, respectively.

Tabela 6
Chemical composition of *Origanum vulgare* essential oil cultivated under different doses of green manure (*Crotalaria juncea*).

Compound	RI	Control	Cattle manure	200 g GM (R)	150	300	450	600	600 g GM (Sh)	CV%
					..... g GM (Sh+200g R)					
Alkenyl alcohol		**2.6a**	**0.96f**	**2.1b**	**2.1b**	**1.7e**	**1.8d**	**1.9c**	**2.0c**	
1-Octen-3-ol	971	2.6a	0.96f	2.1b	2.1b	1.7e	1.8d	1.9c	2c	2.1
Monoterpenes Hydrocarbons		**60.91a**	**55.31d**	**58.43b**	**59.05b**	**60.66a**	**60.71a**	**60.48a**	**57.18c**	
α-Thujene	925	0.47a	0.11g	0.32b	0.33b	0.23f	0.25e	0.27d	0.29c	2.8
α-Pinene	931	0.27a	nd	0.17c	0.18b	0.12f	0.11f	0.14e	0.16d	3.6
Myrcene	990	1.65a	0.87f	1.44b	1.48b	1.22e	1.29d	1.36c	1.43b	2.1
α-Phellandrene	1005	0.16b	0.12d	0.17b	0.17b	0.15c	0.19a	0.19a	0.17c	3.3
α-Terpinene	1015	1.82a	1.35e	1.53c	1.72b	1.42d	1.68b	1.66b	1.83a	1.8
o-Cymene	1023	5.7a	3.63e	5.6a	5.3b	4.86d	4.98c	5.0c	4.85d	1.6
Sylvestrene	1027	1.75a	1.18d	1.67b	1.62b	1.47c	1.63b	1.65b	1.63b	1.8
1,8-Cineole	1030	0.49b	0.46d	0.49b	0.49b	0.51a	0.52a	0.50a	0.47c	1.7
(Z)-β-Ocimene	1035	4.13a	3.31e	3.92b	4.16a	3.64d	3.78c	3.99b	4.21a	1.7
(E)-β-Ocimene	1045	0.66a	0.53e	0.63b	0.68a	0.58d	0.61c	0.64b	0.67a	1.7
γ- Terpinene	1056	7.52b	6.64d	7.01c	7.74b	7.0c	7.56b	7.55b	8.03a	1.5
cis-Sabinene hydrate	1066	3.56d	3.69b	3.43e	3.40e	3.77a	3.62c	3.58d	3.18f	0.63
Terpinolene	1087	0.59d	0.65b	0.53e	0.62c	0.53e	0.64b	0.62c	0.68a	1.7
trans-Sabinene hydrate	1101	32.14e	32.77d	31.52f	31.16g	35.16a	33.85b	33.33c	29.58h	0.3
Oxygenated monoterpenes		**11.79g**	**16.31a**	**11.78g**	**12.66e**	**12.46f**	**13.40c**	**12.88d**	**13.87b**	
Terpinen-4-ol	1176	6.13f	9.87a	5.44h	6.87d	6.05g	7.2c	6.75e	7.71b	0.3
α-Terpineol	1190	2.88f	3.49a	2.87f	3.02e	3.06d	3.13b	3.02e	3.08c	0.5
Linalool acetate	1257	2.78e	2.95d	3.47a	2.77e	3.35b	3.07c	3.11c	3.08c	0.8
Phenolic monoterpenes		**15.58c**	**17.48a**	**17.48a**	**16.91b**	**16.84b**	**17.01b**	**16.55b**	**17.83a**	
Thymol methyl ether	1234	1.26b	1.17c	1.38a	1.08d	1.0e	1.0e	0.98e	1.1d	1.2
Carvacrol methyl ether	1243	0.95f	1.04b	1.11a	1.0d	0.97e	0.98e	0.95f	1.02c	0.6
Thymol	1294	13.17c	15.06a	14.75b	14.59b	14.64b	14.82b	14.42b	15.49a	2.0
Carvacrol	1302	0.20c	0.21c	0.24a	0.24a	0.23b	0.21c	0.20c	0.22b	2.5
Sesquiterpene hydrocarbons		**5.83c**	**6.38a**	**6.5a**	**5.99b**	**5.39d**	**4.45e**	**5.61d**	**6.05b**	
β-Bourbonene	1382	0.28b	0.29b	0.32a	0.27c	0.25d	0.19g	0.23f	0.24e	2.3
(E)-Caryophyllene	1416	1.99b	2.11a	2.13a	1.94c	1.74d	1.47e	1.75d	1.99b	1.2
α -Humulene	1450	0.22b	0.22b	0.28a	0.21b	0.20c	0.16d	0.19c	0.22b	3.0
Germacrene D	1478	1.75c	1.96a	1.92a	1.83b	1.68d	1.41e	1.70d	1.94a	1.8
Byciclogermacrene	1493	1.59c	1.67b	1.72a	1.62c	1.52d	1.22f	1.74e	1.66b	1.8
δ-Cadinene*	1521	nd	0.13	0.13	0.12	nd	nd	nd	nd	
Oxygenated sesquiterpene		**2.08b**	**2.02b**	**2.45a**	**1.96c**	**1.92c**	**1.66e**	**1.72e**	**1.84d**	
Spathulenol	1574	1.16b	1.13b	1.35a	1.09b	1.09b	0.97c	1.0c	1.08b	3.4
Caryophyllene oxide	1580	0.92b	0.89b	1.10a	0.87b	0.83c	0.69e	0.72e	0.76d	3.2
Total % of compounds		98.79	98.46	98.74	98.67	98.97	99.03	99.14	98.77	
Number of compounds		29	29	30	30	29	29	29	29	

RI: retention index relative to alkane series (C_8 – C_{20}) on HP-5MS column. Means followed by the same letter within the same line belong to the same group according to the Scott-Knott test ($p \leq .05$). GM (Green Manure); R (Root); Sh (Leaf + stem). *Compound was not detected in all treatments, CV: Coefficient of variation.

Marculescu et al. (2002) stated that the use of organic fertilizers plays an essential role in the development of plants and in the biosynthesis of organic substances, because when using organic management (manure), they noted that the amount of active ingredient was high in the species *Chrysanthemum balsamita* L. (Compositae). Pereira et al. (2020) showed that organic management provides better bioactivity of the essential oil of oregano because it resulted in antibacterial and antifungal activity, whereas conventional cultivation (NPK) showed no activity.

The three main chemical constituents found were *trans*-sabinene hydrate, thymol and terpinen-4-ol (Fig. 2). The major compound was *trans*-sabinene hydrate. It reached the highest content at 300 and 450 g pot^{-1} of GM (Sh+R) accounting for 35.16 and 33.85%, respectively, that is, a production of 8.6 and 5.0% higher compared to the treatment without green fertilization (control soil), which presented 32.14% relative area.

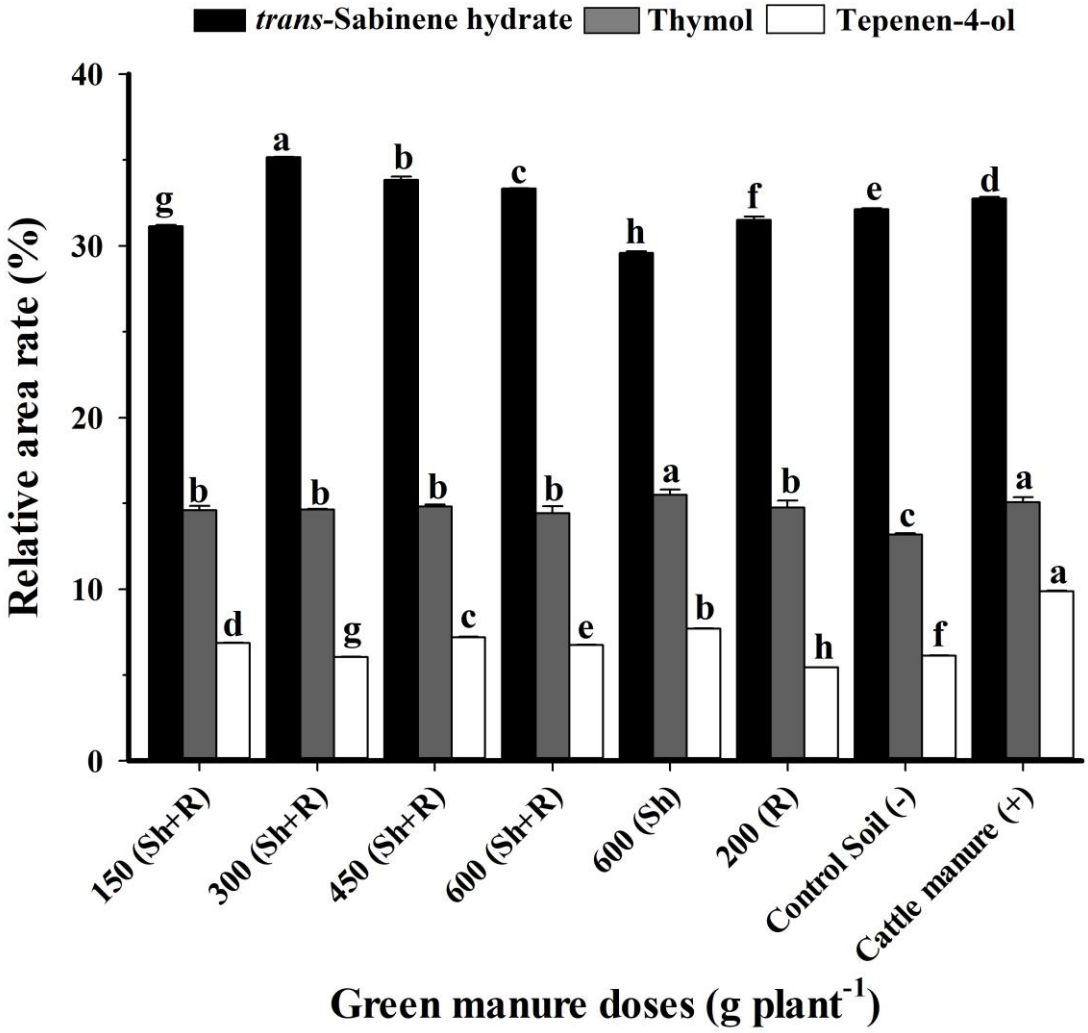


Fig. 2. Major chemical constituents present in the essential oil of *Origanum vulgare* cultivated under the influence of doses of green manure (*Crotalaria juncea*).GM (Green Manure); R (Root); Sh (Shoot = leaf+stem). Means followed by the same letter in each chemical constituent are not significantly different by the ScottKnott test ($p < 0.05$).

Similar results were found by Quiroga et al. (2011), who observed the same major compounds in the chemical composition of the essential oil of several oregano species. Corrêa et al. (2010) also observed higher content of *trans*-sabinene hydrate, thymol, and terpinen-4-ol from the oil of *Origanum vulgare* in the presence of organic fertilizer doses (manure) than in the presence of the control (zero dose).

Regarding thymol (Fig. 2), it was observed that the highest means occurred in the presence of GM rather than in the control soil treatment, with emphasis on the dose of 600 g pot^{-1} of GM (Sh) and cattle manure, which provided the highest mean of this constituent, at 15.49 and 15.06%, respectively. These doses produced approximately 15 and 12.5% more thymol than the control soil, which produced 13.17%.

According to Ezzat Abd El-Hack et al. (2016), thymol has several applications, acting in several industries, as an antispasmodic agent, antioxidant, antimicrobial, immunomodulatory, anticancer agent and anti-inflammatory agent, suppressing harmful compounds/free radicals, with the ability to change the intestinal microbiota and increase digestion, absorption, and metabolism of nutrients.

The same authors stated that this constituent is also used in dietary supplementation as a food additive in the production of animals, fish, and birds because it improves productive and reproductive performance, nutrient bioavailability, and immunity. In addition, recent studies have shown that thymol may be a potential natural selective herbicide in monocotyledonous plant species (Gruľová et al., 2020).

Terpinen-4-ol (Fig. 2) had the highest content (9.87, 7.71, and 7.2%) in the cattle manure, 600 g pot^{-1} GM (Sh), and 450 g pot^{-1} GM (Sh+R) treatments, respectively. This constituent also has several applications because it is used to increase resistance of fruits against pathogens through its antibacterial and anticancer properties and used for the production of antimicrobial nanofibers (Cordeiro et al., 2020; Li et al., 2020; Nakayama et al., 2017; Nepomuceno et al., 2018; Zhang et al., 2018; Zhao et al., 2021).

3.5. Analysis of phenolic compounds and antioxidant activities

The amount of total phenol and flavonols/flavones constituits, as well as the antioxidant activity of the extracts of dried leaves of *Origanum vulgare* grown under different doses of *Crotalaria juncea*, are shown in Table 7. It was observed that for all variables (total phenols, total flavonoids, TAC, ORAC and DPPH), there were significant differences due to the doses of GM.

For the total phenol content, the treatment without fertilization (control soil) had the highest mean at 482 mg of EGA g^{-1} dry leaf. The lowest means occurred with doses of 300, 450, 600 g pot^{-1} GM (Sh+R), and 600 g pot^{-1} GM (Sh). However, the highest levels of total flavonols/flavones occurred with 450 and 600 g pot^{-1} GM (Sh+R) at 12.2 and 12.1 mg of EQ g^{-1} dry leaf, respectively. The lowest mean occurred in the control soil treatment at 6.9 mg of EQ g^{-1} dry leaf.

Tabela 7
Total phenols, total flavonols/flavones and antioxidant activity of extracts from dry leaves of *Origanum vulgare* cultivated under the influence of doses of green manure (*Crotalaria juncea*).

Treatments		Total phenols	Total flavonoids	TAC	ORAC	
Shoots (leaf+stem) g pot^{-1}	Roots g pot^{-1}	mg of EGA g^{-1}	mg of EQ g^{-1} (dry leaf)	mg of EAA g^{-1}	µmol ET g^{-1}	DPPH (AAI)
150	200	214b	9.20c	27.7d	531a	3.2a
300	200	181c	8.20d	31.9a	525a	2.8b
450	200	191c	12.2a	27.5d	498b	3.1a
600	200	192c	12.1a	32.4a	501b	2.9b
600	-	179c	10.6b	27.8d	451c	2.9b
-	200	216b	7.90e	29.6c	419d	3.0b
Control soil (-)		482a	6.90e	30.9b	522a	2.7c
Soil + Cattle manure (+)		209b	7.60e	27.6d	534a	2.5d
CV%		8.6	6.6	1.6	3.6	3.6

Means followed by the same letter in the column do not differ from each other by the ScottKnott test ($p < 0.05$), CV: Coefficient of variation. GM (Green Manure); R (Root); Sh (Shoot = leaf+stem). Total antioxidant capacity (TAC), ability to absorb oxygen radicals (ORAC), free radical scavenging activity (DPPH). Equivalent gallic acid (EAG); quercetin equivalent (EQ); equivalent in ascorbic acid (EAA); equivalent of Trolox (ET).

The influence of GM doses on the accumulation of flavonols/flavones compounds and antioxidant activity may be related to the nutrition of oregano plants. According to Gobbo-Neto and Lopes (2007), the availability of nutrients should be taken into account in the cultivation of medicinal plants because to influencing primary metabolism, nutrient availability can alter the production of secondary metabolites. For *Origanum vulgare*, studies have already shown that the concentrations of bioactive compounds in plants are significantly affected by some chemical properties of the soil (pH, N, Ca, P, and Mg) (Klimienė et al., 2021).

According to Choi et al. (2002), due to the complexity of the chemical substances present in the extracts, it is necessary to evaluate the antioxidant capacity of the plant with at least two methods. Thus, it was observed that for the three types of antioxidant analyses performed (TAC, ORAC, and DPPH), in comparison to the other treatments, the doses of GM provided greater activity.

For the TAC, which was performed based on the reduction of Mo^{+6} to Mo^{+5}, the highest means were observed with doses of 300 and 600 g pot^{-1} GM (R +Sh) at 31.9 and 32.4 mg of EAA g^{-1} dry leaf, respectively. For the ORAC, which consisted of the decrease in fluorescence caused by the AAPH radical, the cattle manure and the GM doses of 150 and 300 g pot^{-1} GM (Sh+R) resulted in the highest means at 534, 531 and 525 µmol of ET g^{-1} dry leaf, respectively, followed by the that in the control soil at 522 µmol of ET g^{-1} dry leaf.

For the free radical scavenging activity (DPPH), all extracts showed very strong antioxidant activity because they had an AAI above 2. The highest means were recorded with doses of 150 and 450 g pot^{-1} of GM (Sh+R) at 3.2 and 3.1, respectively.

Kawthar et al. (2017) obtained similar results because the antioxidant activity and phenol content were higher in the species *Matricaria chamomilla* L. (Compositae) when the management of GM (*Anabaena azollae* Strasb. – Euphorbiaceae) combined with organic compost was used.

Another factor that may have contributed to the higher content of chemical constituents and antioxidant activity was the use of organic fertilizer because Matlok et al. (2020) observed that organic fertilizers result in the production of dried oregano with a much higher bioactive potential than that treated with mineral fertilizer. Kazimierczak et al. (2015) also observed that in comparison to a conventional system, an organic system provided higher production of phenolic acids and total flavonoids in the species (Lamiaceae) *Mentha x piperita* L., *Salvia officinalis* L, *Melissa officinalis* L, and *Rosmarinus officinalis* L. The results of this study confirm the importance of organic management for the production of secondary metabolites in medicinal plants, especially in the species *Origanum vulgare*.

3.6. Principal Components Analysis (PCA)

The PCA results showed an overview of the doses of GM applied to oregano, as they explained 82.08% of the total variance (Fig. 3). There was 66.87% variation associated with principal component 1, while principal component 2 explained 15.21% of the variation.

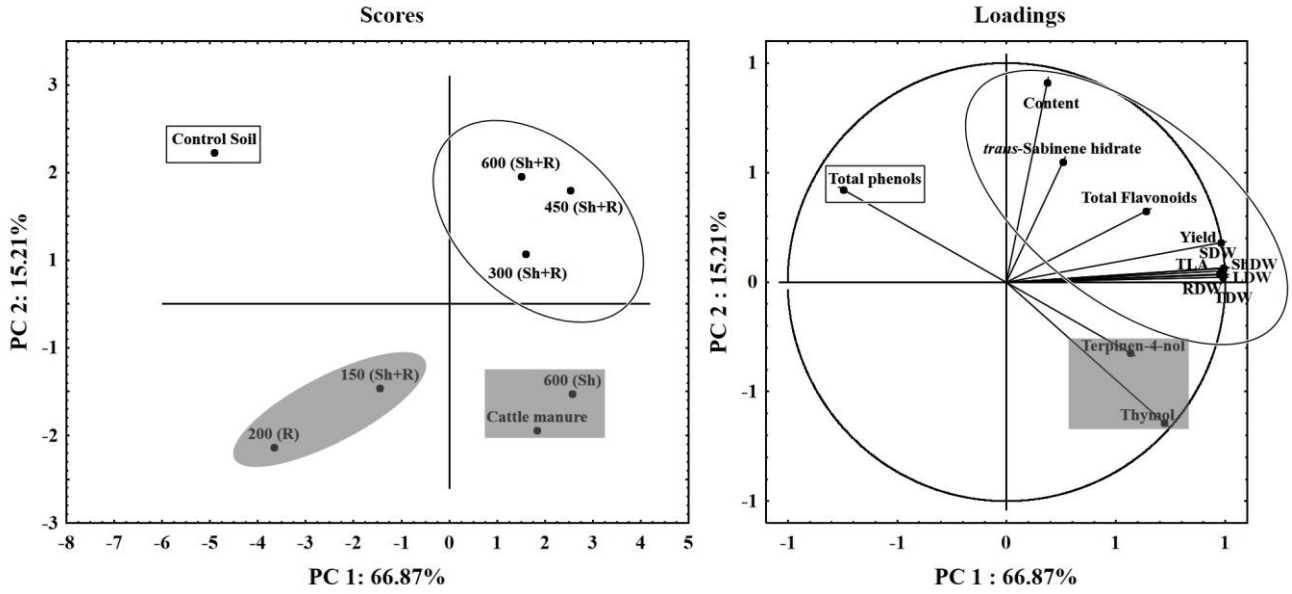


Fig. 3. Principal component analysis (CPA) on composite matrix correlation using data for LDW, SDW, RDW, TDW, TLA, content, yield, essential oil major compounds, phenols and total flavonoids.

Four distinct groups were observed: first, control soil; second, 200 g pot^{-1} GM (R) and 150 g pot^{-1} GM (Sh+R); third, 300, 450, and 600 g pot^{-1} GM (Sh+R); and fourth, 600 g pot^{-1} GM (Sh) and cattle manure. The load analysis showed that the doses of GM (300, 450, and 600 g pot^{-1} GM – Sh+R) positively influenced the dry weight (LDW, SDW, RDW, ShDW, TDW), TLA, and oil content and yield, as well as the accumulation of total flavonoids in oregano. Conversely, the cattle manure and the 600 g pot^{-1} GM (Sh) dose contributed to the production of thymol and terpinen-4-ol. It was also found that the 200 g pot^{-1} (R) and 150 g pot^{-1} GM (Sh+R) dose treatments did not influence the observed variables of oregano production.

The present study showed that the management of GM and cattle manure significantly influenced the production and quality of essential oil of *Origanum vulgare*, in addition to resulting in a greater accumulation of bioactive constituents.

4. Conclusion

The doses of *Crotalaria juncea* provided significant dry weight gains in *Origanum vulgare*, contributing to the higher essential oil yield and significant accumulation of the main chemical constituents, specifically *trans*-sabinene hydrate, thymol, and terpinen-4-ol, which have great value to various industrial sectors. In addition, the presence of GM increased the total flavonoid content, causing greater antioxidant activity. These findings suggest that organic management through the use of the GM *C. juncea* may be beneficial for cultivating *O. vulgare* because it can increase the production of biomass and the active principles of this species.

Acknowledgements

The authors would like to thank the Universidade Federal de Lavras (UFLA) for all its support in terms of structure and resources, as well as the funding agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES – Finance Code 001).

Credit author statement

All authors contributed substantially to the work reported. RMAA, SKVB, and JEBPP conceived the study, designed the experiments; RMAA, JPS, ACH, JPMR, and AAC, performed experiments, analyzed data; RMAA, JPMR and SKVB did the chemical analyses. RMAA, SKVB, and JEBPP wrote the manuscript. All authors read and approved the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERÊNCIAS

Adams, R.P., 2007. Identification of essential oil components by gas chromatography/mass spectrometry. Allured publishing corporation, Carol Stream IL, USA (2007).

Adeniji, A.A., Kumoye, D.E., 2020. Effects of incorporated green manure and inorganic fertilizer on amaranth (*Amaranthus caudatus* L) vegetable. Int. J. Trend. Sci. Res. Dev. (IJTSRD) 4, 1414-1419.

Alagawany, M., Abd El-Hack, M.E., Farag, M.R., Shaheen, H.M., Abdel-Latif, M.A., Noreldin, A.E., Khafaga, A.F., 2020. The applications of *Origanum vulgare* and its derivatives in human, ruminant and fish nutrition–a review. . Ann. Anim. Sci. 20, 389-407. https://doi.org/10.2478/aoas-2020-0004.

Alcântara, F., 2016. Knowing and doing agroecology: what it is and how to make green manure. Embr. Arroz. Feijão, 2.

Araújo, E.d.S., Guerra, J.G.M., Espindola, J.A.A., da Cruz, L.C.J., Alves, B.J.R., Urquiaga, S., 2010. Nitrogen balance in sunn hemp seed production area under the conditions of the baixada Fluminense. Embr. Agrobiol-Bol. Pes. Desenv. 66 (INFOTECA-E), 20.

Assefa, S., Tadesse, S., 2019. The principal role of organic fertilizer on soil properties and agricultural productivity-a review. Agric. Res. Tech: Open Access J 22, 556192. https://doi.org/10.19080/ARTOAJ.2019.22.556192.

Baranauskaitė, J., Jakštas, V., Ivanauskas, L., Kopustinskienė, D.M., Drakšienė, G., Masteikova, R., Bernatonienė, J., 2016. Optimization of carvacrol, rosmarinic, oleanolic and ursolic acid extraction from oregano herbs (*Origanum onites* L., *Origanum vulgare* spp. hirtum and *Origanum vulgare* L.). Nat. Prod. Res. 30, 672-674. https://doi.org/10.1080/14786419.2015.1038998.

Batista, M.A., Inoue, T.T., Esper Neto, M., Muniz, A.S., 2018. Principles of soil fertility, fertilization and mineral nutrition. Hort. frut. Maringá: EDUEM, 113-162. https://doi.org/10.7476/9786586383010.0006.

Bidgoli, R.D., Mahdavi, M.J., 2018. Effect of nitrogen and two types of green manure on the changes in percentage and yield of peppermint (*Mentha piperita*) essential oil. Not. Sci. Biol. 10, 245-250. https://doi.org/10.15835/nsb10210275.

Borrmann, D., 2009. Influence of drought stress on chemical and biochemical characteristics of soybean and on chlorophyll degradation, with focus on the formation of non-coloured chlorophyll catabolites, Food Science. University of São Paulo, São Paulo, SP, p. 125.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 28, 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5.

Choi, C.W., Kim, S.C., Hwang, S.S., Choi, B.K., Ahn, H.J., Lee, M.Y., Park, S.H., Kim, S.K., 2002. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Sci. 163, 1161-1168. https://doi.org/10.1016/S0168-9452(02)00332-1.

Coqueiro, D.P., dos Santos, B.P.C., Guiguer, E.L., Barbalho, S.M., Souza, M.d.S.S., Araújo, A.C., Da Silveira, T.C., Scacco, G., Tiveron, A.M., Costa, J.M., 2012. Effects of oregano (*Origanum vulgare*) tea on the biochemical profile of Wistar rats Sci. Med. 22, 191-196.

Cordeiro, L., Figueiredo, P., Souza, H., Sousa, A., Andrade-Júnior, F., Medeiros, D., Nóbrega, J., Silva, D., Martins, E., Barbosa-Filho, J., Lima, E., 2020. Terpinen-4-ol as an antibacterial and antibiofilm agent against *Staphylococcus aureus*. Int. J. Mol. Sci. 21, 4531. https://doi.org/10.3390/ijms21124531.

Corrêa, R.M., Pinto, J.E.B.P., Reis, E.S., Costa, L., Alves, P.B., Niculan, E.S., Brant, R.S., 2010. Organic fertilization on phytomass production and essential oil content and quality of oregano (*Origanum vulgare* L.) under protected cultivation. Rev. Bras. Pl. Med. 12, 80-89. https://doi.org/10.1590/S1516-05722010000100012.

Corrêa, R.M., Pinto, J.E.B.P., Reis, É.S., Oliveira, C.d., Castro, E.M.d., Brant, R.d.S., 2009. Leaf anatomical traits of oregano (*Origanum vulgare* L.) plants, subjected to different sources and levels of organic fertilization. Acta Sci-Agron. 31, 439-444. https://doi.org/10.4025/actasciagron.v31i3.690.

De Assis, R.M.A., Carneiro, J.J., Medeiros, A.P.R., de Carvalho, A.A., da Cunha Honorato, A., Carneiro, M.A.C., Bertolucci, S.K.V., Pinto, J.E.B.P., 2020. Arbuscular mycorrhizal fungi and organic manure enhance growth and accumulation of citral, total phenols, and flavonoids in *Melissa officinalis* L. Ind. Crops Prod. 158, 112981. https://doi.org/10.1016/j.indcrop.2020.112981.

De Mastro, G., Tarraf, W., Verdini, L., Brunetti, G., Ruta, C., 2017. Essential oil diversity of *Origanum vulgare* L. populations from southern Italy. Food Chem. 235, 1-6. https://doi.org/10.1016/j.foodchem.2017.05.019.

De Oliveira, V.C., Dos Santos, A.R., De Souza, G.S., Dos Santos, R.d.S., 2017. Physiological responses of oregano plants (*Origanum vulgare* L.) cultivated under colored meshes and with organic fertilizers. Rev. Colomb. Cienc. Hortic. 11, 400-407. https://doi.org/10.17584/rcch.2017v11i2.7591.

Diniz, E.R., Vargas, T.O., Pereira, W.D., Santos, R.H., Urquiaga, S., Modolo, A.J., 2017. Levels of *Crotalaria juncea* on growth, production, recovery and efficiency of the use of N in broccoli. Hort. bras. 35, 395-401. https://doi.org/10.1590/S0102-053620170313.

Eiras, P.P., Coelho, F.C., 2011. Use of legumes in green manure for corn crop. Inter. Sci. Place 4, 96-124.

Espíndola, J.A.A., Guerra, J.G., De Almeida, D., 1997. Green manure: strategy for sustainable agriculture. Embr. Agrobiol-Bol. Pes. Desenv. Doc. (INFOTECA-E), 21.

Ezzat Abd El-Hack, M., Alagawany, M., Ragab Farag, M., Tiwari, R., Karthik, K., Dhama, K., Zorriehzahra, J., Adel, M., 2016. Beneficial impacts of thymol essential oil on health and production of animals, fish and poultry: a review. J. Essent. Oil Res. 28, 365-382. https://doi.org/10.1080/10412905.2016.1153002.

Ferreira, D.F., 2019. Sisvar: a computer analysis system to fixed effects split plot type designs. Braz. J. Biom. 37, 529-535. https://doi.org/10.28951/rbb.v37i4.450.

Fontes, P.C.R., 2001. Diagnosis of the nutritional status of plants. UFV, Viçosa, MG. 122.

Gobbo-Neto, L., Lopes, N.P., 2007. Medicinal plants: factors of influence on the content of secondary metabolites. Quím. Nova 30, 374-381. https://doi.org/10.1590/S0100-40422007000200026.

Gruľová, D., Caputo, L., Elshafie, H.S., Baranová, B., De Martino, L., Sedlák, V., Gogaľová, Z., Poráčová, J., Camele, I., De Feo, V., 2020. Thymol chemotype *Origanum vulgare* L. essential oil as a potential selective bio-based herbicide on monocot plant species. Molecules 25, 595. https://doi.org/10.3390/molecules25030595.

He, C., Zhong, S., Yan, J., Wang, F., Li, Y., Zhang, H., Huang, Y., 2018. Effect of Chinese milk vetch (*Astragalus sinicus* L.) as a green manure on grape productivity and quality, nutrient contents, and microbiologic properties of vineyard soils. Fujian J. Agric. Sci. 33, 1151-1157.

Honorato, A.d.C., Maciel, J.F.A., de Assis, R.M.A., Nohara, G.A., de Carvalho, A.A., Pinto, J.E.B.P., Bertolucci, S.K.V., 2022. Combining green manure and cattle manure to improve biomass, essential oil, and thymol production in *Thymus vulgaris* L. Ind. Crops Prod. 187, 115469. https://doi.org/10.1016/j.indcrop.2022.115469.

Jan, S., Rashid, M., Abd_Allah, E.F., Ahmad, P., 2020. Biological efficacy of essential oils and plant extracts of cultivated and wild ecotypes of *Origanum vulgare* L. Biomed Res. Int. 2020, 8751718. https://doi.org/10.1155/2020/8751718.

Kamaneh, M.D.S.-R., Qaraaty, M., Tabarrai, M., Mazidi, M., Mojahedi, M., Mazandarani, M., Behnampour, N., 2020. Effect of oregano oil (*Origanum vulgare* L.) on chronic rhinosinusitis: A randomized, double-blind, clinical trial. Indian J. Tradit. Knowl. 19, 341-349.

Kawthar, A., Ashour, H., Fatma, S., 2017. Growth characters and some chemical constituents of *Matricaria chamomilla* L. plants in relation to green manure and compost fertilizer in sandy soil. Middle East J. 6, 76-86.

Kazimierczak, R., Hallmann, E., Rembiałkowska, E., 2015. Effects of organic and conventional production systems on the content of bioactive substances in four species of medicinal plants. Biol. Agric. Hortic. 31, 118-127. https://doi.org/10.1080/01448765.2014.977948.

Klimienė, A., Klimas, R., Shutava, H., Razmuvienė, L., 2021. Dependence of the concentration of bioactive compounds in *Origanum vulgare* on chemical properties of the soil. Plants 10, 750. https://doi.org/10.3390/plants10040750.

Lee, S., Kim, H., Beuchat, L.R., Kim, Y., Ryu, J.-H., 2020. Synergistic antimicrobial activity of oregano and thyme thymol essential oils against *Leuconostoc citreum* in a laboratory medium and tomato juice. Food Microbiol. 90, 103489. https://doi.org/10.1016/j.fm.2020.103489.

Li, J., Wen, Y., Li, X., Li, Y., Yang, X., Lin, Z., Song, Z., Cooper, J.M., Zhao, B., 2018. Soil labile organic carbon fractions and soil organic carbon stocks as affected by long-term organic and mineral fertilization regimes in the North China plain. Soil Tillage Res. 175, 281-290. https://doi.org/10.1016/j.still.2017.08.008.

Li, Y., Xu, X., Lei, B., Zhuang, J., Zhang, X., Hu, C., ... & Liu, Y. 2021. Magnesium-nitrogen co-doped carbon dots enhance plant growth through multifunctional regulation in photosynthesis. Chemical Engineering Journal, 422, 130114. https://doi.org/10.1016/j.cej.2021.130114

Li, Z., Wang, N., Wei, Y., Zou, X., Jiang, S., Xu, F., Wang, H., Shao, X., 2020. Terpinen-4-ol enhances disease resistance of postharvest strawberry fruit more effectively than tea tree oil by activating the phenylpropanoid metabolism pathway. J. Agric. Food Chem. 68, 6739-6747. https://doi.org/10.1021/acs.jafc.0c01840.

Linhares, P., Pereira, M., Dias, M., Holanda, A., Moreira, J., 2012. Yield of coriander (*Coriandrum sativum* L.) in a system of green manure with the plant scarlet starglory

(*Merremia aegyptia* L.). Rev. Bras. Pl. Med. 14, 143-148. https://doi.org/10.1590/S1516-05722012000500003.

Ma, D., Yin, L., Ju, W., Li, X., Liu, X., Deng, X., Wang, S., 2021. Meta-analysis of green manure effects on soil properties and crop yield in northern China. Field Crops Res. 266, 108146. https://doi.org/10.1016/j.fcr.2021.108146.

Magalhes, W.L.E., Sá, F.P.d., Vieira, L.M., Maggioni, R.d.A., Lima, E.A.d., 2020. Green manure in commercial peach palm plantation: biomass and nutritional content. Embr. Flores. Comun. Téc. (INFOTECA-E).

Maia, C.E., de Morais, E.R.C., Filho, F.d.Q.P., Gueyi, H.R., de Medeiros, J.F., 2005. Nutrients content in leaves of muskmelon irrigated with waters of different salinities. Rev. Bras. Eng. Agríc. Ambient. v9, 292-295. https://doi.org/10.1590/1807-1929/agriambi.v9nsupp292-295.

Marculescu, A., Sand, C., Barbu, C., Bobit, D., Hanganu, D., 2002. Possibilities of influencing the biosynthesis and accumulation of the active principles in *Chrysanthemum balsamita* L species. Rom. Biotechnol. Lett. 7, 577-584.

Marques, C.T.d.S., Gama, E.V.S., da Silva, F., Teles, S., Caiafa, A.N., Lucchese, A.M., 2018. Improvement of biomass and essential oil production of *Lippia alba* (Mill) N.E. Brown with green manures in succession. Ind. Crops Prod. 112, 113-118. https://doi.org/10.1016/j.indcrop.2017.10.065.

Massey, A., Meena, R.N., Meena, A.K., 2021. Effects of organic manures and green manuring on growth, yield, economics and quality of lemongrass (*Cymbopogon Flexuosus* L.) in custard apple (*Annona Squamosa* L.) based agri-horti system. Bangladesh J. Bot. 50, 499-506. https://doi.org/10.3329/bjb.v50i3.55828.

Matłok, N., Stępień, A.E., Gorzelany, J., Wojnarowska-Nowak, R., Balawejder, M., 2020. Effects of organic and mineral fertilization on yield and selected quality parameters for dried herbs of two varieties of oregano (*Origanum vulgare* L.). Appl. Sci. 10, 5503. https://doi.org/10.3390/app10165503.

Medeiros, J.A.S., 2017. Embedded biodegradable film of encapsulated oregano essential oil, Materials Engineering. Federal Technological University of Paraná, Londrina, Paraná., p. 62.

Meena, A.L., Karwal, M., Raghavendra, K., Kumar, S., 2020. Green manure: a complete nutrient source for sustainable soil health in modern agriculture. Food Sci. Rep. 1, 67.

Migliorini, M.J., Boiago, M.M., Roza, L.F., Barreta, M., Arno, A., Robazza, W.S., Galvão, A.C., Galli, G.M., Machado, G., Baldissera, M.D., Wagner, R., Stefani, L.C.M., Silva, A.S.D.A., 2019. Oregano essential oil (*Origanum vulgare*) to feed laying hens and its effects on animal health, An. Acad. Bras. Cienc., p. 20170901.

Morais, L., Barbosa, A., 2012. Influence of green manure and different organic manure on production of aereal phytomass of *Ocimum selloi*. Rev. Bras. Pl. Med. 14, 246-249. https://doi.org/10.1590/S1516-05722012000500020.

Mutisya, M.D., Okello, V.S., Anyango, S.P., Masila, M.J., 2014. Effects of fresh leaf materials of *Sesbania sesban* (L.) Merrill on the growth and photosynthetic pigments of nightshade (*Solanum nigrum* L. var. popolo). Int. J. Agron. Agric. Res. v4., 10-21.

Nakayama, K., Murata, S., Ito, H., Iwasaki, K., Villareal, M.O., Zheng, Y.W., Matsui, H., Isoda, H., Ohkohchi, N., 2017. Terpinen-4-ol inhibits colorectal cancer growth via reactive oxygen species. Oncol. Lett. 14, 2015-2024. https://doi.org/10.3892/ol.2017.6370.

Nepomuceno, N.C., Barbosa, M.A., Bonan, R.F., Oliveira, J.E., Sampaio, F.C., Medeiros, E.S., 2018. Antimicrobial activity of PLA/PEG nanofibers containing terpinen-4-ol against Aggregatibacter actinomycetemcomitans. J. Appl. Polym. Sci. 135, 45782. https://doi.org/10.1002/app.45782.

Nordi, N.T., 2021. Organic fertilization in the production and accumulation of nutrients in jambu plants, Faculty of Agronomic Sciences. Paulista State University, Botucatu, São Paulo., p. 73.

Ou, B., Hampsch-Woodill, M., Prior, R.L., 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. Journal of Agricultural and Food Chemistry 49, 4619-4626. https://doi.org/10.1021/jf010586o.

Peralta-Antonio, N., Watthier, M., Silva Santos, R.H., 2021. Green manure and mineral fertilizer in sequential cropping: effect on dry matter, yield, accumulation and recovery efficiency of nutrients. Commun. Soil Sci.Plant Anal. 52, 322-337. https://doi.org/10.1080/00103624.2020.1845354.

Pereira, M.M.A., Morais, L.C., Zeneratto, N.J., Reis, W.d.S.M., Gómez, O.C., Luiz, J.H.H., Moreira, D.M.B., Pasqual, M., Dória, J., 2020. Organic management vs. conventional management influence the antimicrobial activity of essential oils of *Origanum vulgare* L. Res. Soc. Dev. 9, 4239118504. https://doi.org/10.33448/rsd-v9i11.8504.

Pereira, R., Dos Santos, O., 2013. Spice plants: cultivation and use. Embr. Agroind. Trop. Doc. (INFOTECA-E) 1, 55.

Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269, 337-341. https://doi.org/10.1006/abio.1999.4019.

Quiroga, P.R., Riveros, C.G., Zygadlo, J.A., Grosso, N.R., Nepote, V., 2011. Antioxidant activity of essential oil of oregano species from Argentina in relation to their chemical composition. Int. J. Food Sci. Technol. 46, 2648-2655. https://doi.org/10.1111/j.1365-2621.2011.02796.x.

Rocha, J.C., Rosa, A.H., Cardoso, A.A., 2009. Introduction to environmental chemistry. Artmed Editora. 256

Rosa, D.M., Nóbrega, L., Mauli, M.M., De Lima, G.P., Pacheco, F.P., 2017. Humic substances in soil cultivated with cover crops rotated with maize and soybean. Rev. Ciênc. Agron. 48, 221-230. https://doi.org/10.5935/1806-6690.20170026.

Rothé, M., Darnaudery, M., Thuriès, L., 2019. Organic fertilizers, green manures and mixtures of the two revealed their potential as substitutes for inorganic fertilizers used in pineapple cropping. Sci. Hort. 257, 108691. https://doi.org/10.1016/j.scienta.2019.108691.

Scherer, R., Godoy, H.T., 2009. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. Food Chem. 112, 654-658. https://doi.org/10.1016/j.foodchem.2008.06.026.

Silva, M.A., Nascente, A.S., Frasca, L.L.d.M., Rezende, C.C., Ferreira, E.A.S., Filippi, M.C.C.d., Lanna, A.C., Ferreira, E.P.d.B., Lacerda, M.C., 2021. Isolated and mixed cover crops to improve soil quality and commercial crops in the Cerrado. Res. Soc. Dev. 10, 11101220008. https://doi.org/10.33448/rsd-v10i12.20008.

Silva, T.O.d., Menezes, R.S.C., 2007. Organic fertilization of potato with manure and, or, *Crotalaria juncea*: II-soil N, P, and K availability throughout the growing season. Rev. Bras. Ciênc. Solo 31, 51-61. https://doi.org/10.1590/S0100-06832007000100006.

Skoufogianni, E., Solomou, A.D., Danalatos, N.G., 2019. Ecology, cultivation and utilization of the aromatic greek oregano (*Origanum vulgare* L.): a review. Not. Bot. Horti Agrobot. Cluj-Napoca 47, 545-552. https://doi.org/10.15835/nbha47311296.

Slinkard, K., Singleton, V.L., 1977. Total phenol analysis: automation and comparison with manual methods. Am. J. Enol. Vitic. 28, 49.

Susanti, D., Widodo, H., Hartanto, E., 2017. The effect green manure of *Tithonia diversifolia* and dung manure on yield of *Echinacea purpurea*. Bul. Penelit. Tanam. Rempah dan Obat 28, 127-136. http://dx.doi.org/10.21082/bullittro.v28n2.2017.127-136.

Taiz, L., Zeiger, E., Møller, I.M., Murphy, A., 2017. Plant physiology and development. Artmed Editora. 700.

Tapiero, J., Salamanca, G., Marín, C., 2019. Analysis of volatile compounds and antioxidant activity of the essential oil of oregano (*Origanum vulgare* L.). Adv. Med. Plant Res. 7., 54-60. https://doi.org/10.30918/AMPR.72.19.022.

Vakeesan, A., Nishanthan, T., 2008. Green manures: nature's gift to improve soil fertility. 24., 2.

Van Den Dool, H., Dec. Kratz, P., 1963. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. J. Chromatogr. A 11, 463-471. https://doi.org/10.1016/S0021-9673(01)80947-X.

Vieira, R., Vieira, A., Rezende, C., Peixoto, J.d.C., 2019. Growth, nutrient accumulation and phytochemical prospection of *Justicia pectoralis* Jacq as a function of fertilization type. Rev. Bras. Agropec. Sust. 9, 27-33. https://doi.org/10.21206/rbas.v9i04.8601.

Watthier, M., Peralta Antonio, N., Gomes, J.A., Rocha, S.B.F., Santos, R.H.S., 2020. Decomposition of green manure with different grass: legume ratios. Arch. Agron. Soil Sci. 66, 913-924. https://doi.org/10.1080/03650340.2019.1644622.

Wellburn, A.R., 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144, 307-313. https://doi.org/10.1016/S0176-1617(11)81192-2.

Woisky, R.G., Salatino, A., 1998. Analysis of propolis: some parameters and procedures for chemical quality control. J. Apic. Res. 37, 99-105. https://doi.org/10.1080/00218839.1998.11100961.

Xavier, F.A.d.S., Oliveira, J.I.A., Silva, M.R.d., 2017. Decomposition and nutrient release dynamics of shoot phytomass of cover crops in the recôncavo baiano. Rev. Bras. Ciênc. Solo 41. https://doi.org/10.1590/18069657rbcs20160103.

Zhang, Y., Feng, R., Li, L., Zhou, X., Li, Z., Jia, R., Song, X., Zou, Y., Yin, L., He, C., Liang, X., Zhou, W., Wei, Q., Du, Y., Yan, K., Wu, Z., Yin, Z., 2018. The antibacterial mechanism of terpinen-4-ol against *Streptococcus agalactiae*. Curr. Microbiol. 75, 1214-1220. https://doi.org/10.1007/s00284-018-1512-2.

Zhao, L., Duan, F., Gong, M., Tian, X., Guo, Y., Jia, L., Deng, S., 2021. (+)-Terpinen-4-ol Inhibits *Bacillus cereus* biofilm formation by upregulating the interspecies quorum sensing signals diketopiperazines and diffusing signaling factors. J. Agric. Food Chem. 69, 3496-3510. https://doi.org/10.1021/acs.jafc.0c07826.

ARTIGO II

Foliar application of plant growth regulators increases the production of biomass, content, yield and chemical constituents of *Origanum vulgare* essential oil

Elaborado de acordo com as normas do periódico plant physiology reports

Abstract

Origanum vulgare L. is used in the prevention of neurodegenerative diseases; for feeding poultry, fish, and cattle; and in the food industry. The aim of this study was to evaluate the influence of the foliar application of plant growth regulators (PGRs) on the biomass, secondary metabolites and antioxidant enzymatic activity. The experiment was in a double factorial design with a control, 3 types of regulators and 3 concentrations. After 90 days, the dry weight, essential oil analysis and photosynthetic pigments were measured. The effects of PGRs on antioxidative system and some of the antioxidant enzyme activities; such as superoxide dismutase, catalase and ascorbate peroxidase were investigated. The use of PGRs, branch length gain was greater than that in the control. The application of GA_3 at concentration of 25 mg L^{-1} increased the leaf, stem, root, shoot and total dry weight when compared to the control. The highest chlorophyll a content was observed for the KIN25 concentration. The oil content gains from the KIN25 and IBA25 concentrations were 30.9 and 29.6%, respectively, compared to the control and yields in the lower concentrations. The highest thymol content was observed in plants that received the $GA_3$50 concentration (24.80%), which was 12.14% higher compared to the control. The lowest mean hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels were observed in the presence of the PGRs. The PGRs stimulated enzymatic antioxidant activity, decreasing oxidative stress signaling. These findings suggest that foliar application of PGRs at low concentrations may contribute to increase biomass and active ingredients.

Keywords: Medicinal plants; Secondary metabolite; Gibberellic acid; Indolebutyric acid; kinetin.

Introduction

Commonly known as oregano, *Origanum vulgare* L. (Lamiaceae) originates and is widely distributed in the Mediterranean, Euro-Siberian and Iranian-Turanian regions (Lukas et al., 2015). This species has nutritional importance because it is a widely appreciated natural condiment. At the industrial level, it is used in perfumery and personal hygiene, while in medicine, it is used in the preparation of anesthetics, sedatives, antispasmodics, antirheumatics and expectorants (Tintaya & Hurtado, 2019).

The global production of oregano generates an approximate value of US $22.5 million per year (Henry et al., 2020). Oregano essential oil has great value because it has antimicrobial, antiradical, and acaricidal activities and plays a role in the prevention of phytopathogens (Gonçalves et al., 2021; Kačániová et al., 2012; Qiao et al., 2021). In addition, oregano can be used as an antidiabetic and food supplement (Yu et al., 2021); in the prevention of neurodegenerative diseases (Ligaj et al., 2021); in the feeding of poultry, fish, and cattle (Guato et al., 2020; Henry et al., 2020; Santos et al., 2020); and in the food industry (Coutinho et al., 2020; de Marins et al., 2021; Galindo et al., 2019; Prete et al., 2020).

According to Pinto and Bertolucci (2002), oregano does not behave in the same way year-round. The plant undergoes changes, and therefore, the concentration of active ingredients may vary seasonally. Among the various abiotic and biotic factors, such as fertilization (Odivi et al., 2021), drying (Alqarni et al., 2022), irrigation (Souza et al., 2021), ontogeny (Yeşil & Özcan, 2021) and genetics (Lal et al., 2021), that interfere with the production of essential oil, the use of growth regulators has been highlighted in recent years as a widely applicable tool for the improvement of the production of secondary metabolites in medicinal plants (Jamwal et al., 2018).

Plant growth regulators (PGRs), also known as hormones, are natural or synthetic products that affect development and metabolic processes in plants (Fishel, 2006; Rademacher, 2015). Among the nine main hormones that regulate plant development are auxins, gibberellins and cytokinins (Taiz et al., 2017).

Sharafzadeh and Zare (2011) state that the use of growth regulators can influence the production of essential oil through effects on plant growth (biomass of leaves or flowers), biosynthesis of essential oil and number of oil storage structures. In addition, the timing of exogenous application and the type of growth regulator applied can also affect the essential oil content.

According to the same authors, among the main growth regulators used to influence the production of secondary metabolites in species of the family Lamiaceae, auxins, cytokinins and gibberellins stand out.

Studies have shown that the application of indole-3-butyric acid (IBA) and gibberellic acid (GA_3) improved the phytochemical characteristics of the species *Thymus vulgare* L. (Lamiaceae) (Bahman et al., 2017). The use of IBA improved vegetative growth, the yield of chalices and the phytochemical parameters of *Hibiscus sabdariffa* L. (Malvaceae) (El-Kinany et al., 2020). Hasan-Beigi, Saidi and Mohammadi (2021) found that the foliar action of gibberellic acid (GA_3) along with salicylic acid increased the dry biomass and essential oil content in *Echinacea purpurea* (L.) Moench. (Compositae). Regarding kinetin (KIN), Khan et al. (2022) observed that foliar application provided a higher yield of essential oil and active constituents in the species *Mentha arvensis* L. (Lamiaceae).

In this context, the objective of this study was to observe the influence of the foliar application of plant growth regulators on the production of biomass, phtosynthetic pigments, volatile compunds and the antioxidant enzymatic activity of *Origanum vulgare*.

Materials and Methods

Study Area and Seedling Production

The experiment was carried out in the greenhouse of the Tissue Culture Laboratory of the Department of Agriculture (DAG) of the Federal University of Lavras, Brazil, located at the geographic coordinates 21º 14'S and 45º 00W, at 918 m altitude. A specimen material of the species is already deposited in the ESAL Herbarium, of the Department of Biology at UFLA and corresponds to nº 22.156.

Seedlings were obtained by vegetative propagation of apical cuttings of parent plants (5-7 cm). The cuttings were rooted in 128-cell styrofoam trays with the commercial substrate Basaplant®. After the cuttings rooted (30 days), the plants were transplanted to pots (10 L) containing a mixture of dystrophic red Latosol and sand (2:1) and 300 g cattle manure per pot. The chemical characteristics of the substrate (2:1 soil + 300 g of cattle manure) are shown: pH in water = 5.4; K, Na (mg/dm^3) = 217.25; 72; P-Rem ($mg\ L^{-1}$) = 17; Ca^{2+}, Mg^{2+}, Al^{3+}, H+Al ($cmolc/dm^3$) = 2.53; 0.72; 0.10; 1.30; base saturation index (V%) = 74.5; organic matter ($dag\ kg^{-1}$) = 2.28; Zn, Fe, Mn, Cu, B and S (mg/dm^3) = 2; 34; 13.50; 1.47; 0.22; 11.20 respectively.

Experime ntal Design

The experimental design was completely randomized in a double factorial scheme (3 types of regulators x 3 concentrations + 1 control) and four replicates; each replicate contained four plants, totaling 160 experimental units distributed in 10 treatments, as shown in Table 1.

Table 1 Description and abbreviations of each treatment used in the experimente.

No.	Treatment description	Abbreviation
T1	Gibberellic acid 25 mg L^{-1}	$GA_3$25
T2	Gibberellic acid 50 mg L^{-1}	$GA_3$50
T3	Gibberellic acid 100 mg L^{-1}	$GA_3$100
T4	Indole-3-butyric acid 25 mg L^{-1}	IBA25
T5	Indole-3-butyric acid 50 mg L^{-1}	IBA50
T6	Indole-3-butyric acid 100 mg L^{-1}	IBA100
T7	Kinetin 25 mg L^{-1}	KIN25
T8	Kinetin 50 mg L^{-1}	KIN50
T9	Kinetin 100 mg L^{-1}	KIN100
T10	Control - H_2O + Tween 80 (0.05%)	Control

GA3 (Gibberellic acid); IBA (Indole-3-butyric acid); KIN (Kinetin)

Freshly, 500 mL of 100 mgL^{-1} stock solution was prepared for each PGR (GA_3, IBA and KIN). The other concentrations (50 and 25 mg L^{-1}) were prepared by stock solution diluition. After, Tween 20 (0.05%) was added to each solution and the control. An amount of 200 mL was sprayed to the point of dripping three times.

The first and second applications were done 30 and 60 days after transplanting, respectively. The third application was performed 72 hours before harvest (87 days after transplanting) during the 90-day cycle of cultivation of the species. Plant irrigation was carried out two to three times a week, maintaining the field capacity between 80 and 90%. The temperature and humidity (Fig. 1) were measured with a Datalogger Elitech® during the 90 days of oregano cultivation (spring-summer).

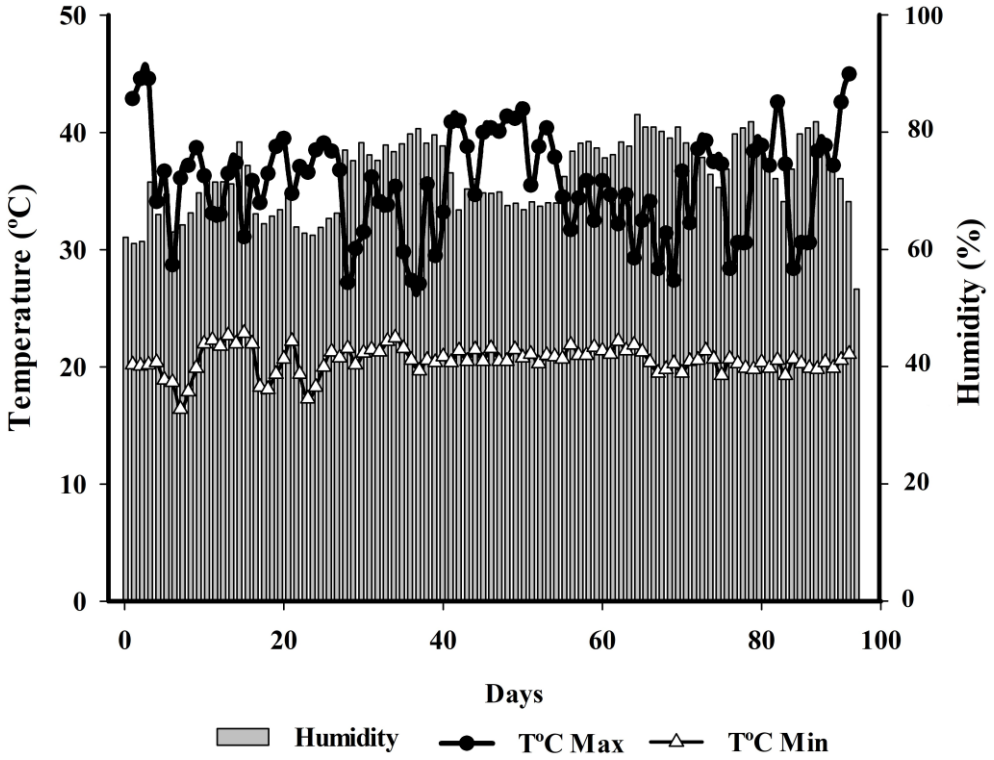


Fig. 1 Daily averages of temperature and humidity during the 90 days of oregano cultivation in a greenhouse using Datalogger Elitech®. Spring-summer

Growth Analysis

At 90 days after transplanting, the length of the longest branch was measured using a tape measure, and the results were expressed in cm. To obtain dry matter, the leaves, stems and roots were placed in Kraft paper and kept in a forced air oven at 40 °C until reaching a constant weight. Then, each part of the plant was weighed, and the result was expressed in grams (g). The parameters evaluated were the leaf (LDW), stem (SDW), root (RDW), shoot (ShDW) and total (TDW) dry weight and root-shoot ratio (R/Sh).

Photosynthetic Pigments

The photosynthetic content was extracted and analyzed based on the methods developed by Hiscox and Israelstam (1979) and Barnes et al. (1992). Fresh leaves of oregano (50 mg) were weighed and incubated in 10 mL of dimethyl sulfoxide (DMSO) saturated with calcium carbonate ($CaCO_3$) according to methods described by Santos et al. (2015). Then, the samples were kept in an oven at 65 °C for 48 h.

The samples were prepared in quintuplicate, and three aliquots (3 mL) of each replicate were transferred to a quartz cuvette; the optical density values were read in a TECAN INFINITY M200 PRO spectrophotometer at 480, 649 and 665 nm. The spectrophotometer was

operated in the I -Control® data processing system (version 3.37) against a blank sample containing DMSO. The specific optical density value recorded for each sample was calculated based on the average of the readings applied to the aliquots.

The wavelengths and equations adopted for these calculations were based on the methodology of Wellburn (1994):

Chlorophyll *a* 649 = (12.47 x A665) - (3.62 x A649);

Chlorophyll *b* 665 = (25.06 x A649) - (6.5 x A665);

Carotenoids 480 = (1000 x A480 - 1.29 x Ca - 53.78 x Cb)/220.

Total Chlorophyll = Chlorophyll *a* + Chlorophyll *b*

Extraction of Essential Oil

The essential oil was extracted using the hydrodistillation technique in a modified Clevenger apparatus. Forty grams of dry leaves was placed in a 2,000 mL volumetric flask and filled with 1,000 mL of distilled water. From the boiling point, the extraction time was fixed at 90 min. Four extractions were performed for each treatment. The essential oil was weighed, stored in an airtight amber bottle and kept in a refrigerator at 4 °C. The essential oil content was expressed in g 100 g^{-1} of leaf dry mass, and the yield was expressed in g $plant^{-1}$.

Chemical Analysis of Essential Oil

The qualitative and quantitative analyses of the essential oil were performed according to de Assis et al. (2020). Briefly, measurements were performed in triplicate, on the Agilent® 7890A Gas Chromatography (GC) system, operated on MSD CHEM Station Ver. E.02.02.1431 data processing system equipped with CombiPAL autosampler/injector Autosampler System (CTC Analytic AG, Switzerland) and Flame Ionization Detector (FID). Qualitative analyzes were performed on an Agilent® MSD 5975C selective mass detector (Agilent Technologies, California, USA) operated by electronic impact ionization at 70 eV, in scan mode, 1.0 s^{-1} scan speed and mass acquisition interval of 40–400 mz^{-1}. Chemical constituents were identified by comparing their retention rates relative to the co-injection of a standard *n*-alkane solution (C_8-C_{20}; Sigma-Aldrich®, St. Louis, USA), using the Van Den Dool and Kratz (1963) equation. Oregano EO constituents were identified by comparing mass spectra from the NIST/EPA/NHI library database (NIST, 2003) and based on retention rates described in the literature (Adams, 2007).

Extraction and Quantification of Hydrogen Peroxide (H_2O_2) and Malondialdehyde (MDA)

The extraction of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) (lipid peroxidation product) was performed according to Biemelt et al. (1998). A total of 0.2 g of fresh leaves was macerated in liquid N_2 with polyvinylpyrrolidone (PVPP). Then, the material was placed in an Eppendorf tube, homogenized in 1,500 µL of trichloroacetic acid (TCA) at 0.1% (w/v) and centrifuged at 12,000 × g for 15 minutes. The supernatant was separated to obtain the sample solution, which was stored at -20 °C until quantitative analyses were performed in quadruplicate.

Hydrogen Peroxide (H_2O_2)

Incubation was according to Velikova et al. (2000). In each microplate well it has been added 45 µL of 10 mmol L^{-1} potassium phosphate buffer solution (pH 7.0), 90 µL of potassium iodide and 45 µL of the sample solution. Readings were taken at 390 nm. The calculation of the H_2O_2 concentration of the samples was performed based on the straight-line equation given by the curve obtained from the densities, and the results were expressed in mmol H_2O_2 g^{-1} FW.

Malondialdehyde (MDA)

Incubation was according to Buege and Aust (1978). Na amount of 250 µL of solution with 0.5% thiobarbituric acid (TBA) and 10% trichloroacetic acid (TCA) was added in microtube together with 125 µL of the sample solution. The solution was homogenized and placed in a water bath at 95°C for 30 min. After cooling, 200 µL was taken for reading, performed at 535 and 600 nm. MDA contents were calculated using an extinction coefficient of 1.56 x $^{10-5}$ and the results expressed in ηmol MDA g^{-1} FW.

Extraction of Antioxidant Enzymes and Enzymatic activity

The extraction of the enzymes (SOD, CAT and APx) was prepared according to Biemelt et al. (1998). Fresh leaves (0.2 g) were macerated in liquid nitrogen with polyvinylpyrrolidone (PVPP). Subsequently, the material was placed in Eppendorf tubes and homogenized with 1,500 µL of extraction buffer solution composed of 375 µL of 400 mM potassium phosphate (pH 7.8), 15 µL of 10 mM EDTA, 75 µL of 200 mM ascorbic acid and 1,035 µL of distilled water. The reagent solution was centrifuged for 10 min at 13,000 g in a Nl 1801 benchtop microcentrifuge, and the supernatant (sample solution) was collected and stored in Eppendorf tubes at -20 °C until quadruplicate analysis.

Enzymatic Activity of Superoxide Dismutase (SOD), Catalase (CAT) and Ascorbate Peroxidase (APX)

Superoxide dismutase **(**SOD) activity was based on the ability of the enzyme to inhibit the photoreduction of nitrotetrazolium blue (NBT). The methodology was performed according to Giannopolitis and Ries (1977). Briefly, the incubation buffer solution was used, containing 100 µL of 100 mM potassium phosphate (pH 7.8), 40 µL of 70 mM methionine, 3 µL of 10 µM EDTA, 15 µL of 1 mM NBT and 2 µL of 0.2 mM riboflavin and distilled water, pipetted into a microplate along with 10 µL of the sample solution. Then the plate was illuminated for 7 min and subsequently read at 560 nm. The results were expressed as U SOD activity min^{-1} g^{-1} FW.

Catalase (CAT) incubation was performed according to Havir and Mchale (1987). Briefly, 90 µL of buffer solution containing 200 mM potassium phosphate (pH 7.0), previously incubated in a water bath at 30ºC, and 72 µL of distilled water were pipetted into a microplate along with 9 µL of the sample solution. The 9 µL hydrogen peroxide (250 mM) was added only at the time of reading, performed at 240 nm every 15 seconds for 3 min. The activity was calculated using an extinction coefficient of 36 M^{-1} cm^{-1}.

Ascorbate peroxidase (APX) incubation was performed according to Nakano and Asada (1981). Briefly, 90 µL of 200 mM potassium phosphate buffer solution (pH 7.0), 9 µL of 10 mM ascorbic acid and 63 µL of distilled water were added to the microplate along with 9 µL of the sample solution. And, 9 µL of hydrogen peroxide (250 mM) was added only at the time of reading, performed at 290 nm every 15 seconds for 3 min. The activity was calculated using an extinction coefficient of 2.8 M^{-1} cm^{-1}.

Statistical Analysis

The data were subjected to analysis of variance, and the means were compared using the Scott–Knott test ($p < 0.05$) with R software. Principal component analysis (PCA) was performed using Statistica® software, version 10.0 (StatSoft - Tulsa, USA).

Results and Discussion

Plant Growth Analysis

There was a significant interaction between the types of PGRs (GA_3, IBA, KIN) and concentrations (25, 50, 100 mg L^{-1}) used in oregano cultivation. Gibberellin provided longer branch length than the other regulators (IBA and KIN) and the control (Fig. 2) The 100 mg L^{-1} concentration of GA_3 yielded a length of 107.9 cm, significantly different from the highest

concentrations of IBA (86.7 cm) and KIN (90.8 cm) and the control, in which a length of 82.8 cm was obtained (Figure 2). The 100 mg L^{-1} concentration of GA_3 provided branch length gains of 19.6, 15.8 and 23.3% compared to the IBA100 and KIN100 concentrations and the control, respectively.

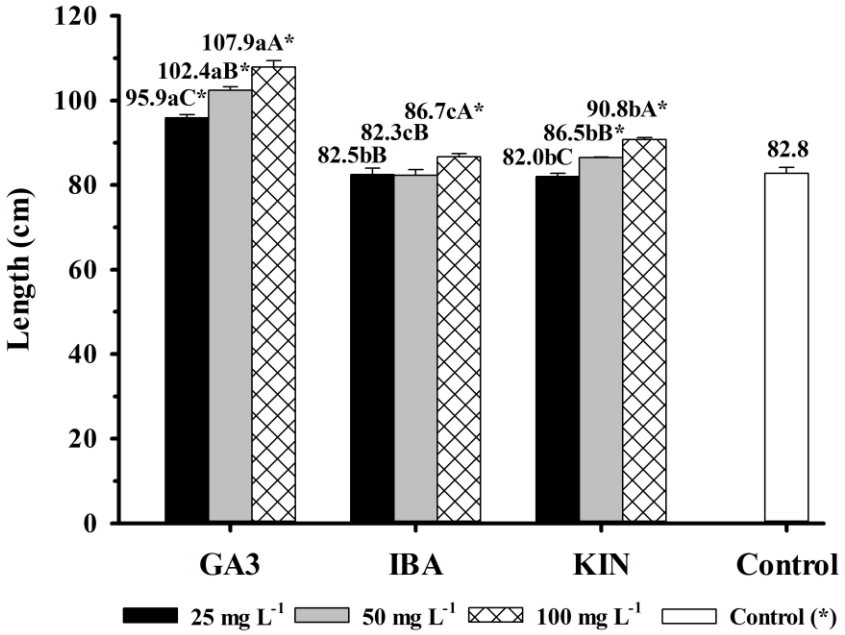


Fig. 2 Influence of foliar application of plant growth regulators-PGRs with different concentrations on the length of the largest branch of *Origanum vulgare* grown in a greenhouse. The means followed by the same lowercase letters between the PGR types with the same concentration and capital letters for the concentration factor within the PGRs do not differ from each other by the Scott-Knott test ($p < 0.05$). Means followed by (*) differ from the control treatment by Dunnett's test ($p < 0.05$). GA_3 (Gibberellic acid); IBA (Indole-3-butyric acid); KIN (Kinetin).

The greater mean length of the oregano branches in the gibberellin treatment than in the control, IBA and KIN treatments can be explained by the effects of gibberellin on plant growth and development. According to Taiz et al. (2017), one of the main roles of biologically active gibberellins is the promotion of cell elongation and induction of internode elongation in plants.

Dadkhah et al. (2016) found similar results; these authors also observed that the growth of the species *Satureja hortensis* L. (Lamiaceae) was higher following the exogenous application of GA_3 at the highest concentrations (100, 200 and 300 ppm). The results of this study are also in agreement with those from the study by Sajid et al. (2015), who found that foliar application of PGRs (GA and BAP) promoted greater height with increasing concentrations (0, 25, 50, 100 mg L^{-1}) among the species *Gladiolus grandiflorus* Andrews (Iridaceae). In addition to these studies, other studies have shown that compared to a control, the exogenous application of PGRs affects plant growth (Arunadevi et al., 2019; Shah et al., 2021; Tadayon & Hosseini, 2022).

Figure 3 shows the influence of the foliar application of different concentrations of PGRs on the leaf LDW, stem SDW, root RDW, shoot ShDW, total TDW and root/shoot ratio R:Sh of oregano. There was a significant interaction for all observed dry weight variables (LDW, SDW, RDW, ShDW, TDW, and R:Sh). Foliar application of the PGRs GA_3, IBA and KIN provided significant increases in oregano dry weight compared to the control. The application of 25 mg L^{-1} of GA_3 increased the LDW, SDW, RDW, ShDW and TDW by approximately 3.5, 21.8, 14.0, 14.1 and 12.9%, respectively, compared to the control treatment (without application of PGRs).

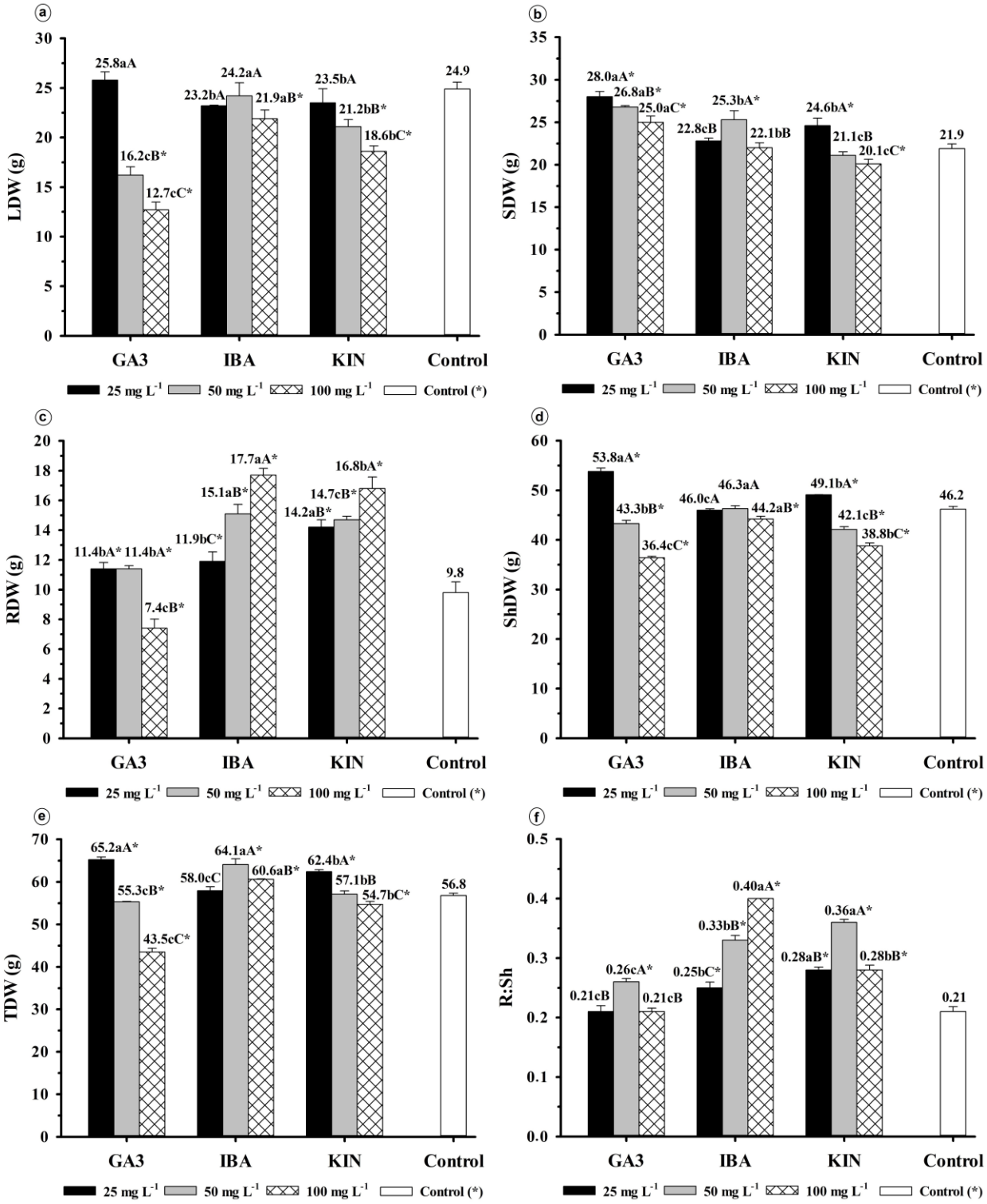


Fig. 3 Dried matter accumulation in *Origanum vulgare* grown in a greenhouse with diferente concentrations of plant growth regulators-PGRs. The means followed by the same lowercase letters between the PGR types with the same concentration and capital letters for the concentration factor within the PGRs do not differ from each other by the Scott-Knott test ($p < 0.05$). Means followed by (*) differ from the control treatment by Dunnett's test ($p < 0.05$). GA_3 (Gibberellic acid); IBA (Indole-3-butyric acid); KIN (Kinetin). Leaf dry weight (LDW), stem dry weigth (SDW), root dry weigth (RDW), shoot dry weight (ShDW), total dry weight (TDW), root-shoot ratio (R:Sh).

The lowest LDW, SDW, ShDW and TDW accumulations of oregano was observed at the highest concentration of PGRs, especially with the application of $GA_3$100. and KIN100. It

is important to note that the highest mean branch length (Fig. 2), observed at the $GA_3$50 and $GA_3$100 concentrations, did not reflect the LDW gain (Fig. 3A) but rather significant increases in SDW (Fig. 3B), with values of 26.8 and 25.0 g, respectively.

There was an increase in the RDW as the IBA and KIN concentrations increased, and the highest mean RDW (17.7 g) was observed following the IBA100 concentration. For GA_3, the opposite pattern was observed, since at the highest concentration ($GA_3$100), there was a lower RDW (7.4 g) demonstrated in Figure 3C.

R:Sh parameter is one of the important variables for evaluating plant growth and development because it expresses the influence of root growth in relation to that of shoots and the influence of shoot growth relative to roots (Benincasa, 2003; do Nascimento et al., 2020). All the treatments showed lower values of R:Sh than one (1.0), demonstrating higher dry weight production among the aerial plant parts at the expense of root production.

Studies have shown that foliar application of PGRs directly influences plant growth and development. GA_3, for example, is responsible for controlling various aspects of plant growth and physiology, such as stem elongation, shoot growth, and juvenile leaf stage for adults (Gupta & Chakrabarty, 2013; Shah et al., 2021). Sajid et al. (2015) observed that the highest GA_3 concentration (25, 50 and 100 mg L^{-1}) resulted in a greater weight of the species *Gladiolus grandiflorus* Andrews. Hasan and Ismail (2018) observed that spraying 150 mg L^{-1} of GA_3 on peanuts significantly increased plant height, total dry weight and number of twigs.

These last results are in contrast to those observed for oregano, for which higher weights were observed for the lowest concentration ($GA_3$25), proving that each species is influenced differently by PGR concentration. In the present study, oregano performed better with lower concentrations of PGRs ($GA_3$25, IBA50, KIN25) than in the control.

Regarding IBA, Kulkarni et al. (2013) observed that the foliar application of IBA (5 µM) to mustard increased the dry weights of the aerial parts and roots of the plant, corroborating the results of the present study, the foliar application of IBA stimulated the growth of both the aerial parts and roots of oregano in relation to the control. Arora et al. (2020) also observed that the exogenous application of IBA increased the fresh and dry root weights, plant height, number of branches, shoot diameter and shoot fresh weight in the medicinal species *Punica granatum* L. (Lythraceae) relative to the control. These results confirm the role of auxin (IBA) in the process of cell growth and division as well as in plant rooting (Inoue et al., 2022; Taiz et al., 2017).

For KIN, which is related to the activity of the apical meristem and breaking the dormancy of buds and shoots (Ramy et al., 2019; Shah et al., 2021), studies have shown that

spraying KIN (0.5, 1.0 or 5 µM) increased shoot length and the fresh and dry biomasses of soybean plants (Hamayun et al., 2015). El et al. (2018) also found that the exogenous application of kinetin (75 mg L^{-1}) increased the fresh and dry weights of *Dendranthema grandiflorium* (Kitam.) Kitam. (Compositae).

Analysis of Photosynthetic Pigments

The influence of different concentrations of PGRs on the photosynthetic pigments of *Origanum vulgare* is shown in Figure 4. There was a significant response for chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoids. For the PGRs GA_3 and KIN, there were differences between the concentrations, with the highest averages observed for KIN25 (0.69 mg g^{-1} FW) and for $GA_3$50 and $GA_3$100 (both with 0.68 mg g^{-1} FW) (Fig. 4A). However, there were no significant differences in chlorophyll *a* between the IBA concentrations, and the values ranged from 0.63 to 0.66 mg g^{-1} FW. Regarding the control, the mean chlorophyll *a* content (0.50 mg g^{-1} FW) was significantly lower than that in the PGRs treatments. In the case of the KIN25 concentration (0.69 mg g^{-1} FW), the production of chlorophyll *a* was 27.54% higher than that in the control. In addition, it is worth noting that the higher means of chlorophyll *a* present in the KIN25 concentration (0.69 mg g^{-1} FW) may have contributed to the increases in the LDW, SDW, ShDW and TDW, as shown in Figure 3.

For chlorophyll *b* (Fig. 4B), the highest mean was observed in the control (0.64 mg g^{-1} FW). Among the PGRs, GA_3 showed an increase in chlorophyll *b* production according to the increase in concentrations, with 0.51 mg g^{-1} FW at the maximum concentration ($GA_3$100). The highest mean of total chlorophyll (Fig. 4C) was also found at the highest concentration ($GA_3$100), with 1.24 mg g^{-1} FW, which was 8.1% more than that in the control (1.14 mg g^{-1} FW), which may have contributed to the increase in SDW (Fig. 3B). In addition, the maximum concentrations, IBA100 and KIN100, caused the lowest total chlorophyll production, both with 0.96 mg g^{-1} FW, which were significantly lower than the values observed in the control. This may have triggered the lower growth of oregano in the IBA100 and KIN100 concentrations, resulting in low LDW, SDW, ShDW and TDW (Fig. 3).

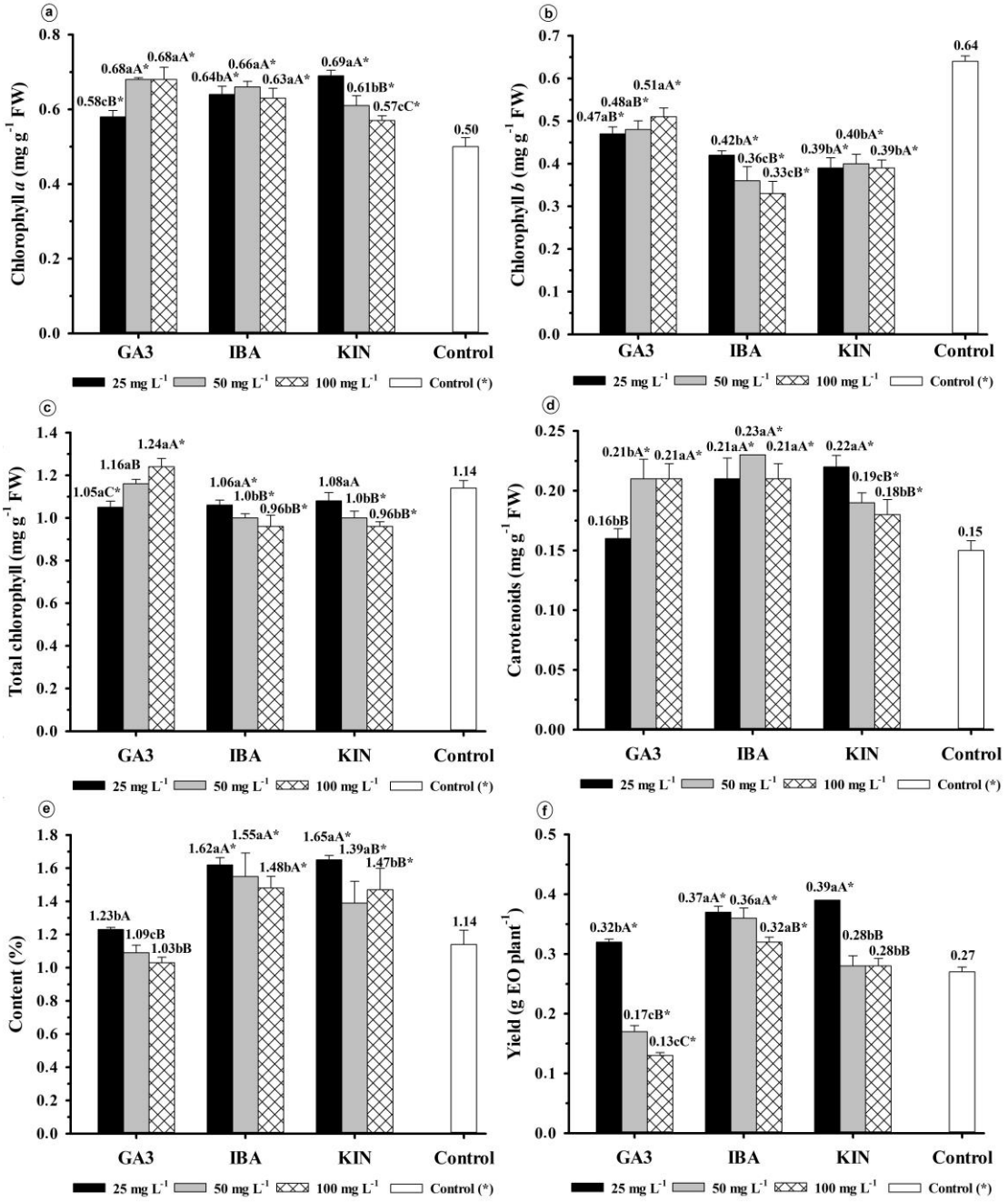


Fig. 4 Photosynthetic pigments and essential oil content and yield of *Origanum vulgare* grown in greenhouse with different concentrations of plant growth regulators-PGRs. The means followed by the same lowercase letters between the PGR types with the same concentration and capital letters for the concentration factor within the PGRs do not differ from each other by the Scott-Knott test ($p < 0.05$). Means followed by (*) differ from the control treatment by Dunnett's test ($p < 0.05$). GA_3 (Gibberellic acid); IBA (Indole-3-butyric acid); KIN (Kinetin)

Compared to the control, the application of PGRs caused higher production of carotenoids at all concentrations (Fig. 4D). The highest average was recorded at the IBA50 concentration with a value of 0.23 mg g^{-1} FW, which was 34.8% higher than that in the control

(0.15 mg g^{-1} FW). There was no significant difference between the IBA concentrations, with the variation in the values ranging from 0.21 to 0.23 mg g^{-1} FW.

According to Cheng and Liu (2010), among the photosynthetic pigments, chlorophyll is one of the most important because it is related to the absorption, transmission and transformation of light energy in the photosynthetic process. Regarding the higher chlorophyll content provided by the application of PGRs, similar results were found for GA_3, which resulted in an increase in chlorophyll with increasing concentrations. The higher chlorophyll content at higher concentrations may be due to the inhibition of pigment degradation mediated by GA_3 (Xiao et al., 2022). Saleem et al. (2020) observed the same behavior in *Corchorus capsularis* L. (Malvaceae) under copper stress, applying concentrations of 10, 50 and 100 mg L^{-1}. However, the highest levels of chlorophyll in *C. capsularis* resulted in a higher dry weight per plant, unlike oregano, in which a lower dry weight per plant was observed as the GA_3 concentrations increased. This result shows that high concentrations of GA_3 can cause different effects depending on the species.

In the case of the lowest concentration (25 mg L^{-1}) of the PGRs IBA and KIN, for which higher pigments and consequently higher LDW were observed (Fig. 3a), studies showed that both PGRs stimulated the production of chlorophyll. Mansouri and Talebizadeh (2017) observed similar results because low concentrations of IBA (0.01, 0.1 and 1 μM) had a stimulatory effect on chlorophyll *a* and the accumulation of carotenoids in the species *Nostoc linckia* [Roth] Bornet et Flahault (Noscotaceae). The authors Bashri et al. (2021) found that the lowest concentrations of KIN (10, 50 μM) significantly improved growth and photosynthetic activity in *Trigonella foenum-graecum* L. (Leguminosae).

The higher chlorophyll *a* content in the presence of PGRs can be explained by the fact that auxins (IBAs) influence the promotion of pigment synthesis and/or delay its degradation (El Karamany et al., 2019). Exogenous application of cytokinins (KIN) can increase the activity of the enzyme ribulose 1,5-diphosphate carboxylase (RuBisCO), promoting greater efficiency of carboxylation and photosynthesis (Amaro et al., 2020). The lower levels of total chlorophyll in the IBA100 treatment may have occurred due to the high concentration of auxin (IBA), which induces the synthesis of 1-aminocyclopropane-1-carboxylic acid, the key regulatory enzyme in ethylene biosynthesis because at high levels, this enzyme may lead to the degradation of photosynthetic pigments (Hunt et al., 2010).

The greater production of carotenoids in oregano treated with PGRs than in the control may have contributed to a greater photosynthetic activity and consequently to the TDW gain, mainly with the application of lower concentrations. since carotenoids are a group of isoprenoid

compounds that comprise a wide range of structures and can act as accessory pigments in photosynthesis, increasing light uptake and conferring photoprotective properties to plants (Havaux, 2014). In addition, because it is one of the most appreciated condiments in the world, the high content of carotenoids in oregano may be of interest and add market value. Studies have shown that among plant pigments, carotenoids have bioactive properties and can be beneficial for human health (Amengual, 2019). According Nabi et al. (2020), this pigment is also widely appreciated for its industrial applications and can be used as an additive for food, feed, supplements and natural dyes.

Essential Oil Content, Yield and Constituents

There was a significant interaction between the PGRs and the concentrations, both for the content (Fig. 4E) and the yield (Fig. 4F) of essential oil. The highest means of essential oil content occurred in plants treated with the lowest concentrations of PGRs (KIN25: 1.65%; IBA25: 1.62%; and $GA_3$25: 1.23%). The KIN25 and IBA25 concentrations stood out; these concentrations resulted in increases of 30.9 and 29.6%, respectively, relative to the mean essential oil content in the control (1.14%).

The highest concentrations of the PGRs ($GA_3$100, IBA100 and KIN100), resulted in the lowest average oregano essential oil content; these results demonstrated the greater sensitivity of the plants to the $GA_3$100 concentration, which resulted in the lowest essential oil content (1.03%). These results show that the oil content of the species *Origanum vulgare* L. can vary both by the type of PGR and by the foliar concentration applied.

The highest yields of oregano oil also occurred at the lowest concentrations, KIN25, IBA25 and $GA_3$25, with 0.39, 0.37 and 0.32 g EO plant^{-1}, respectively, while the control yielded 0.27 g EO plant^{-1}. The essential oil content in the control decreased by 30.8, 27 and 15.6% relative to that in plants that received the KIN25, IBA25 and $GA_3$25 concentrations, respectively.

Plant hormones, in addition to stimulating plant growth and development, also influence the biosynthesis of terpenes in a large number of aromatic plant species, affecting the content and quality of essential oils (Bano et al., 2016; Prins et al., 2010). This fact was observed in the results of this study because the KIN25, IBA25 and $GA_3$25 concentrations resulted in higher contents and yields than the control (without the application of PGRs).

Singh et al. (2016) observed that the application of auxins (IAA and NAA) and GA_3 increased the essential oil content at concentrations of 25, 50, 100 ppm in *Matricaria chamomilla* L. (Compositae). Mahmoudi et al. (2022) observed that the application of GA_3

(125, 250, 375 µM) increased the essential oil content in *Ocimum basilicum* L. (Lamiaceae) when compared to the control. The species *Mentha arvensis* L. (Lamiaceae), when treated with GA_3 (1, 10, 100 µM), showed higher oil yield than untreated plants, and the increase due to the 1 µM concentration was 44.60% (Bose et al., 2013). In this study, increased oil yield in oregano was observed in plants treated with $GA_3$25, with contents 15.6% greater than that in the control.

Abbas and EL-Saeid (2012) observed that for lemongrass, plants in the control had a lower oil content than plants that received IBA concentrations (25, 50, 100 ppm). However, regarding the oil yield per lemongrass plant, the control stood out relative to the IBA concentrations. This is different from the results presented in this study, which showed that the IBA25 concentration increased the oil yield by 27% in oregano when compared to the control.

Conversely, KIN application increased the oil content and yield in *Mentha arvensis* L. by 46.6% and 50.80%, respectively, compared to the control plants (Khan et al., 2022), corroborating the data reported in this study, since the KIN25 concentration also increased the content (30.9%) and the yield (30.8%) of oil in oregano compared to that in the control.

The chemical constituents of the essential oil of *Origanum vulgare* are shown in Table 2. The application of PGRs influenced the synthesis of chemical compounds of the essential oil of oregano. The main constituents found were o-cymene, γ-terpinene, *trans*-sabinene hydrate, terpinen-4-ol and thymol, totaling more than 67.23% of the oil compounds. The highest averages of *trans*-sabinene hydrate were observed in the presence of PGRs, where the KIN100 (31.56%) and $GA_3$100 (31.22%) concentrations increased the contents by approximately 25.2 and 24.4%, respectively, compared to that in the control, for which the lowest mean (23.61%) value was observed. The same pattern occurred for thymol, since the highest mean content was observed for the $GA_3$50 concentration (24.80%), which was 12.14% higher than that for the control (21.79%). Higher levels of terpinen-4-ol and γ-terpinene were also observed in the presence of PGRs than in the control.

Five classes of constituents were observed: monoterpene hydrocarbons, oxygenated monoterpenes, phenolic monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes. The highest averages of monoterpene hydrocarbons were observed at the KIN25 concentration, with 57.6%. However, higher concentrations of gibberellin ($GA_3$100 and $GA_3$50) caused a decrease in the production of monoterpene hydrocarbons, with values of 51.3 and 49.2%, respectively, which may be associated with the absence of the constituents α-pinene and β-pinene in both treatments. For the levels of oxygenated monoterpenes, phenolic monoterpenes and oxygenated sesquiterpene, the highest means were observed at the $GA_3$50 concentration.

The variation in the chemical composition of oregano essential oil may be related to the foliar application of PGRs, which can affect the enzymatic pathways of terpenoid biosynthesis (Sangwan et al., 2001). According to Assaf et al. (2022), at optimal concentrations, PGRs may favor the accumulation of secondary metabolites in species of the family Lamiaceae. Elsayed et al.(2021) found that the application of GA_3 (25 mg L^{-1}) in chamomile increased the main component of the oil. El-Kinany et al. (2020) demonstrated that the application of IBA (100 ppm) improved the chemical compounds in *Hibiscus sabdariffa*. Thakur and Kumar (2020) observed a higher β-myrcene content in *Rosa damascena* treated with KIN (35 mg L^{-1}). These findings corroborate the data presented in this study because in the case of oregano, there was greater production of major constituents (*trans*-sabinene hydrate, thymol, terpinen-4-ol and γ-terpinene) in the presence of PGRs.

Table 2 Influence of foliar application of plant growth regulators-PGRs with different concentrations on the chemical constituents of the essential oil of *Origanum vulgare* grown in a greenhouse

Compound	IR	% Área										CV%
		Control	GA3 (mg L^{-1})			IBA (mg L^{-1})			KIN (mg L^{-1})			
			25	50	100	25	50	100	25	50	100	
Alkenyl alcohol		**2.04d**	**2.03d**	**0.63i**	**0.76h**	**1.32g**	**1.47e**	**2.60b**	**2.28c**	**2.68a**	**1.41f**	1.8
1-Octen-3-ol	972	2.04d	2.03d	0.63i	0.76h	1.32g	1.47e	2.60b	2.28c	2.68a	1.41f	1.8
Monoterpenes Hydrocarbons		**50.70d**	**55.34a**	**48.60e**	**50.51d**	**51.03d**	**53.47c**	**54.62b**	**55.62a**	**54.57b**	**54.74b**	1.0
α-Thujene	925	0.33e	0.35d	0.08i	0.10h	0.17g	0.19f	0.45b	0.38c	0.48a	0.19f	2.7
α-Pinene*	932	0.18	0.19	nd	nd	0.07	0.09	0.25	0.20	0.27	0.08	
β-Pinene*	976	0.12	0.12	nd	nd	0.07	0.08	0.15	0.13	0.15	0.07	
Myrcene	990	1.50c	1.52c	0.67g	0.76f	1.13e	1.19d	1.76a	1.60b	1.74a	1.11e	1.5
α-Phellandrene	1005	0.27c	0.30c	0.24d	0.26d	0.30c	0.30c	0.39a	0.37a	0.33b	0.29c	9.5
α-Terpinene	1016	2.62c	2.48c	1.33f	1.43f	2.01d	1.71e	2.0d	2.79b	2.98a	2.05d	3.9
o-Cymene	**1023**	**4.52d**	**3.23f**	**6.25b**	**5.55c**	**6.20b**	**7.8a**	**3.69e**	**3.27f**	**3.20f**	**2.69g**	1.5
Sylvestrene	1027	1.76c	1.75c	1.18f	1.28e	1.60d	1.66d	2.10a	2.03a	1.95b	1.56d	3.5
1,8-Cineole	1030	0.20b	0.20b	0.18c	0.15d	0.22a	0.19c	0.22a	0.18c	0.20b	0.18c	2.3
(Z)-β-Ocimene	1036	3.41c	3.61b	2.22h	2.44g	2.58f	2.69e	3.90a	3.56b	3.87a	3.11d	1.3
(E)-β-Ocimene	1046	0.40f	0.45d	0.26j	0.29i	0.33g	0.31h	0.52a	0.46c	0.49b	0.41e	1.4
γ- terpinene	**1057**	**8.51c**	**9.65a**	**2.88g**	**3.52f**	**5.09e**	**3.49f**	**9.60a**	**9.33b**	**9.75a**	**7.65d**	1.6
cis-Sabinene hydrate	1065	2.35e	2.77c	3.03a	2.91b	2.90b	2.91b	2.63d	2.66d	2.65d	2.99a	1.3
Terpinolene	1087	0.92a	0.81c	0.59d	0.60d	0.78c	0.64d	0.87b	0.93a	0.98a	0.80c	4.0
***Trans*-Sabinene hydrate**	**1101**	**23.61e**	**27.91c**	**29.69b**	**31.22a**	**27.58c**	**30.22b**	**26.09d**	**27.73c**	**25.53d**	**31.56a**	1.4
Oxygenated monoterpenes		**15.21b**	**12.77f**	**17.06a**	**14.40c**	**15.20b**	**14.55c**	**13.33d**	**12.47g**	**13.34d**	**13.02e**	1.0
Terpinen-4-ol	**1177**	**10.38b**	**7.42f**	**10.83a**	**8.17d**	**9.42c**	**8.29d**	**7.74e**	**7.11g**	**8.39d**	**7.27f**	1.2
α -Terpineol	1190	3.13c	2.95e	3.47a	3.16c	3.39b	3.356b	3.01d	2.94e	2.95e	3.04d	1.1
Carvone*	1237	nd	nd	nd	nd	nd	nd	0.26	nd	nd	nd	
Linalyl acetate*	1253	nd	nd	0.13	nd	nd	0.13	nd	nd	nd	nd	
Linalool acetate	1257	1.70h	2.40e	2.63d	3.07a	2.39e	2.77c	2.32f	2.42e	2.0g	2.71b	1.1
Phenolic monoterpenes		**23.82c**	**23.34d**	**26.74a**	**25.57b**	**25.79b**	**25.17b**	**22.91e**	**22.88e**	**22.18f**	**23.21d**	1.3
Thymol methyl ether	1234	0.61a	0.22g	0.31d	0.34d	0.26f	0.32d	0.31d	0.31d	0.49b	0.39c	3.3
Carvacrol methyl ether	1243	1.08a	0.91e	1.08a	1.02c	1.0c	1.06b	0.93e	0.93e	0.96d	1.05b	1.5
Thymol	**1295**	**21.79d**	**21.75d**	**24.80a**	**23.70b**	**23.94b**	**23.29c**	**21.28e**	**21.20e**	**20.36f**	**21.42e**	1.3
Carvacrol	1302	0.34e	0.46c	0.55a	0.51b	0.59a	0.50b	0.39d	0.44c	0.37d	0.35e	4.5
Sesquiterpene hydrocarbons		**5.83a**	**5.50b**	**3.99g**	**5.05c**	**3.50h**	**2.63i**	**4.18f**	**4.68e**	**4.93d**	**5.44b**	1.0
Elixene*	1366	nd	nd	0.11	0.10	nd	0.10	nd	nd	nd	nd	
β-Bourbonene*	1382	nd	nd	nd	0.07	nd	nd	nd	nd	nd	nd	
(E)-Cariophyllene	1417	2.04b	1.78d	1.93c	2.17a	1.54g	1.46i	1.45i	1.51h	1.58f	1.73e	1.0
α -Humulene	1450	0.21b	0.19c	0.20b	0.23a	0.17d	0.15e	0.15e	0.17d	0.17d	0.18c	3.7
Germacrene D	1479	2.30a	2.10b	1.31h	1.79e	1.26i	0.79j	1.53g	1.74f	1.92d	2.04c	1.4
Byciclogermacrene*	1494	1.10	1.28	0.26	0.51	0.40	nd	0.92	1.13	1.13	1.36	
δ-Cadinene	**1522**	0.18a	0.15b	0.18a	0.18a	0.13c	0.13c	0.13c	0.13c	0.13c	0.13c	3.5
Oxygenated sesquiterpene		**0.98e**	**0.65f**	**1.93a**	**1.81b**	**1.31d**	**1.72c**	**0.56g**	**0.55g**	**0.51h**	**0.62f**	2.3
Spathulenol	1575	0.78e	0.52f	1.52a	1.45b	1.04d	1.30c	0.44g	0.42g	0.37h	0.44g	2.1
Caryophyllene oxide	1580	0.20d	0.13f	0.41a	0.36b	0.27c	0.42a	0.12f	0.13f	0.14f	0.18e	4.1
Total % of compounds		98.58	99.63	98.95	98.10	98.15	99.01	98.20	98.48	98.21	98.44	
Number of compounds		30	30	30	30	30	31	31	30	30	30	

RI: retention index reltive to alkane series ($C_8 - C_{20}$) on HP-5MS column. Means followed by the same letter within the same line belong to the same group according to the Scott-Knott test ($p \leq .05$). R (Root); Sh (Leaf + stem). *Compound whose statistical analysis was not performed because it was not detected in all treatments, CV: Coefficient of variation.

The lower means of monoterpene hydrocarbons present at the GA_3100 (51.3%) and GA_350 (49.2%) concentrations may have occurred due to the influence of gibberellin on the metabolic pathway of terpenoids. Mansouri et al. (2011) found that GA_3 caused a decrease in the activity of one of the key enzymes for the synthesis of terpenes (DXS: 1-deoxy-D-xylulose-5-phosphate synthase) compared to that in control plants. In addition, the GA_3 treatment decreased the number and percentage of monoterpenes in the treated plants (Mansouri et al., 2011). Hazzoumi et al. (2014) noted in a study that the use of GA_3 (70 mg L^{-1}) suppressed β-cedrene and azulene in *Ocimum gratissimum* L. (Lamiaceae) Similar to Ghassemi-Golezani et al. (2022), these authors found that GA_3 (1 mM) reduced the contents of most of the constituents in *Anethum graveolens* L. (Apiaceae) Similarly, the essential oil constituents α-pinene and β-pinene were not found in oregano when treated with the GA_3100 and GA_350 concentrations.

Oxidative Stress and Enzymatic Antioxidant Activity

For oxidative stress and enzymatic activity there was a significant response for hydrogen peroxide (H_2O_2), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) (Fig. 5). The highest mean H_2O_2 content was observed for the GA_3100 concentration, with 7.9 µmol H_2O_2 g^{-1} FW (Figure 5A). However, the lowest means of H_2O_2 also occurred in the presence of PGRs, namely, at the GA_325, GA_350 and IBA100 concentrations, with 3.2, 3.3 and 3.4 µmol H_2O_2 g^{-1} FW, respectively, differing significantly from the control, which had an average of 5.5 µmol H_2O_2 g^{-1} FW.

In the absence of PGR, an increase in lipid peroxidation measured as malondialdehyde (MDA) was observed. The control treatment had the highest content of MDA, with 6.2 ηmol MDA g^{-1} FW, which was significantly higher than that of the oregano plants treated with PGRs. The MDA production in the control was 95.8% and 95.3% higher than that in the IBA50 (2.6 ηmol MDA g^{-1} FW) and KIN100 (2.9 ηmol MDA g^{-1} FW) treatments, respectively (Figure 5B).

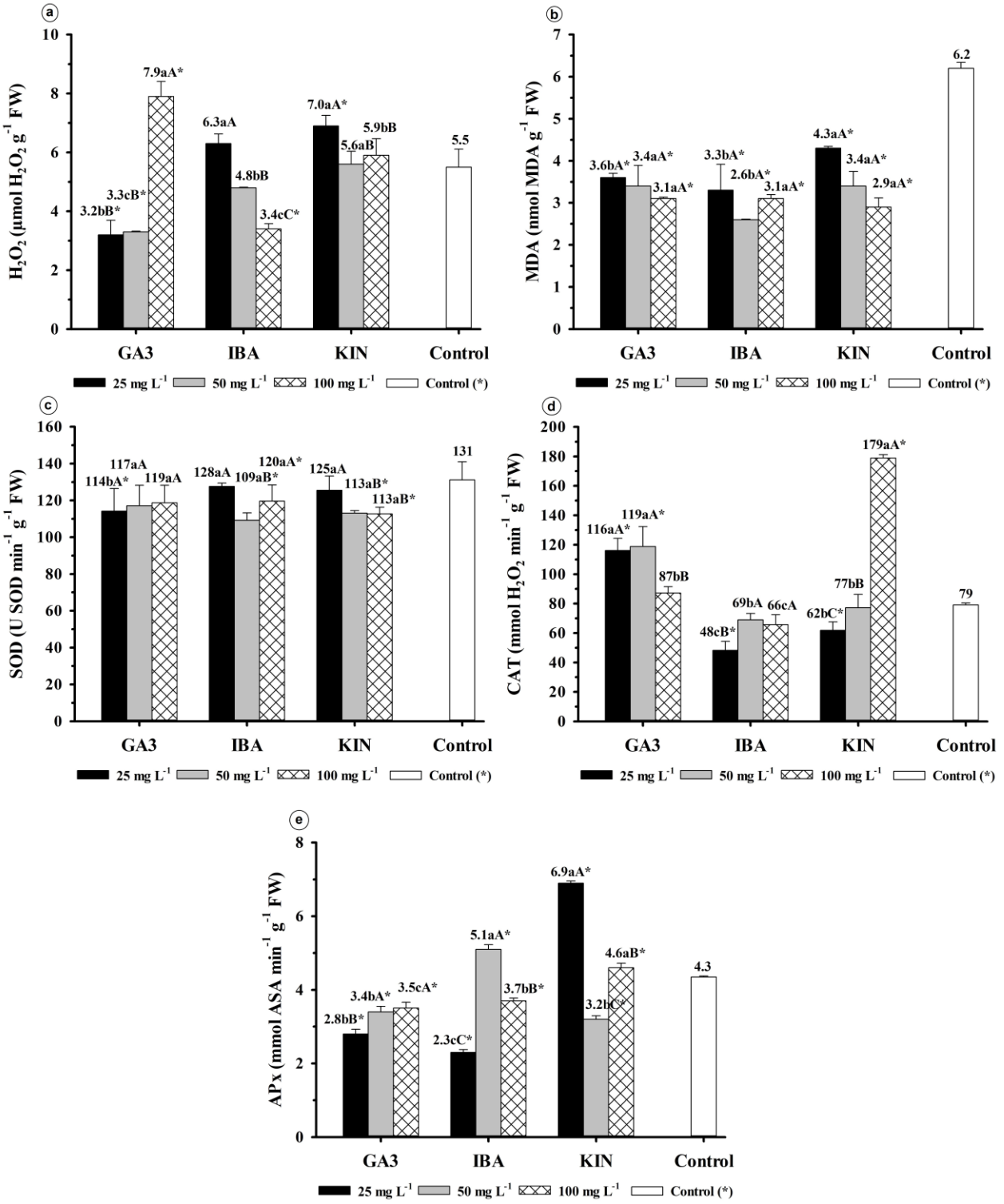


Fig. 5 Influence of foliar application of plant growth regulators-PGRs with different concentrations on the oxidative stress of *Origanum vulgare* grown in a greenhouse. The means followed by the same lowercase letters between the PGR types with the same concentration and capital letters for the concentration factor within the PGRs do not differ from each other by the Scott-Knott test ($p < 0.05$). Means followed by (*) differ from the control treatment by Dunnett's test ($p < 0.05$). GA_3 (Gibberellic acid); IBA (Indole-3-butyric acid); KIN (Kinetin). Hydrogen peroxide (H_2O_2), malondialdehyde (MDA), Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX)

Regarding the activity of antioxidant enzymes, it was generally observed that the presence of PGRs stimulated the antioxidant defense system of oregano. For SOD, the highest

activity occurred in the control, with 131 U SOD min^{-1} g^{-1} FW, but this value did not differ statistically from the SOD values observed for the IBA25 and KIN25 concentrations, which also showed high activity, with 128 and 125 U SOD min^{-1} g^{-1} FW, respectively (Figure 5C). Conversely, for CAT, there was a higher activity at concentrations of KIN100, $GA_3$50 and $GA_3$25, with 179, 119 and 116 mmol H_2O_2 min^{-1} g^{-1} FW, respectively; these values were significantly higher than that observed in the control (79 mmol H_2O_2 min^{-1} g^{-1} FW) (Figure 5D).

For the antioxidant enzyme APX, the highest activity was observed at the KIN25 and IBA50 concentrations, with values of 6.9 and 5.1 mmol ASA min^{-1} g^{-1} FW, respectively, and activity levels 37.7 and 15.7% higher than that in the control (4.3 mmol ASA min^{-1} g^{-1} FW) (Figure 5E).

The finding that the SOD activity level was higher than the CAT and APX activity levels can be explained by the fact that the SOD enzyme is the first to act in the detoxification process, i.e., the first barrier against oxidative damage; the main function of SOD is the conversion or dismutation of toxic radicals (superoxide, $O_2^{\bullet-}$) in H_2O_2 and molecular oxygen (O_2). The enzymes CAT and APX act in the dismutation of H_2O_2 in water and oxygen, minimizing oxidative stress (García-Caparrós et al., 2021; Ozden et al., 2009).

The foliar application of the concentrations of PGRs stimulated the activation of the enzymatic antioxidant system; this is because there was a lower signaling of oxidative stress in the oregano plants treated with PGRs, which had lower MDA contents than those in the control. Studies have shown that PGRs play an important role in the adaptation and minimization of oxidative stress in plants by activating the enzymatic antioxidant system (Nazir et al., 2020; Olteanu et al., 2009). Jan et al. (2021) observed that foliar application of GA_3 and auxin (indole acetic acid-IAA) combined with EDTA significantly increased antioxidant activities (CAT and SOD) and reduced MDA levels. Ahanger et al. (2018) and Kaya et al. (2018) also demonstrated that foliar application of KIN activated the antioxidant defense system and reduced oxidative stress in *Zea mays* L. (Poaceae).

Although the control showed high antioxidant activity (SOD), there were still higher levels of H_2O_2 and MDA. The higher enzymatic antioxidant activity of SOD in the control may have occurred in an attempt to mitigate cellular oxidative stress due to the high levels of H_2O_2 and MDA, as studies have shown that high levels of H_2O_2 and MDA are one of the main responses to stress conditions. To stabilize this process, it is necessary to activate the enzymatic antioxidant system (García-Caparrós et al., 2021).

Another important point is the high levels of carotenoids present in plants treated with PGRs (Fig. 4D), which may have contributed to a lower signaling of oxidative stress; these

results indicate that among the various functions of carotenoids in plants, carotenoids can act in the elimination of free radicals and protect against lipid peroxidation of the membrane (Hashim et al., 2020; Johnson et al., 2007; Lim et al., 1992; Mortensen & Skibsted, 1997). The lower carotenoid content in the control (Fig. 4D) may have increased the chances of stress and consequently oxidative damage.

These results indicate that the application of PGRs may have minimized the production of reactive oxygen species (ROS) and consequently decreased the effects of stress conditions, such as temperature and humidity variation, during the growth and development cycle of oregano (Fig. 1).

Principal Component Analysis

Principal component analysis (PCA) was used to evaluate the effects of the interactions between growth regulator application and the evaluated parameters so that more information could be extracted from the results (Fig 6). PCA was performed for a correlation matrix including the variables dry weight, essential oil content and essential oil yield, and the two major components of the essential oil explained 74.38% of the total variation.

The graph of scores in Figure 3 shows the separation of the PCA into three groups: the first group includes GA50 and GA100, the second group includes Kin50 and IBA100 and the third group includes IBA25, IBA50, Kin25 and GA25. Based on these separations, each treatment was in different quadrants of the graph, indicating that there was a difference between the treatments. The group with the GA50 and GA100 treatments (Group 1) positively influenced the content of the two major compounds thymol and *trans*-sabinene hydrate and the SDW. There was a positive correlation between the two major compounds of the essential oil and SDW. In addition, the analysis of the vectors showed that the two major compounds of the essential oil and SDW were negatively correlated with the vegetative growth variables of the

plant (LDW, TDW and ShDW), corroborating the hypothesis of environmental stress caused by the application of growth regulator in the GA50 and GA100 concentrations.

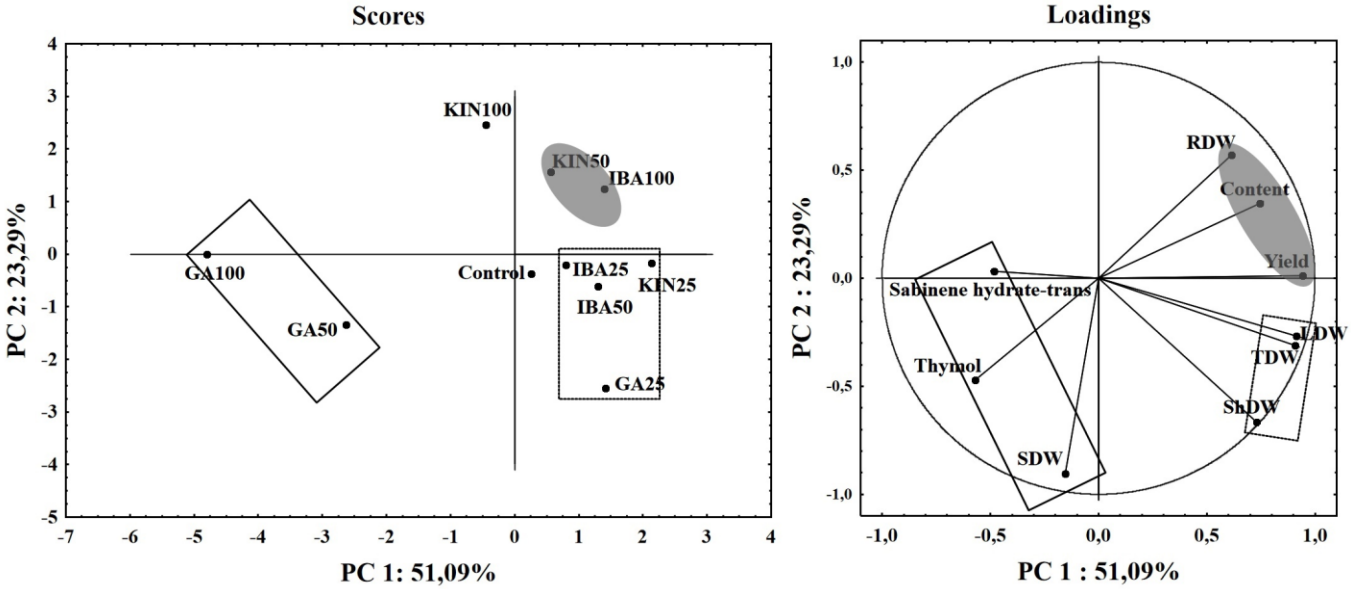


Fig. 6 Principal component analysis (PCA) of the constructed in matrix correlation using data for leaf dry weight (LDW), stem (SDW), shoot (ShDW), root (RDW), total (TDW), essential oil content, essential oil yield, thymol and sabinene hydrate-trans compounds with concentrations of GA_3 (Gibberellic acid); IBA (Indole-3-butyric acid); KIN (Kinetin)

Positive correlations were observed between the KIN50 and IBA100 treatments and the content and yield of essential oil and RDW (Fig. 3). With the application of lower concentrations of KIN, IBA and GA, there was greater weight gain (LDW, TDW and ShDW). These treatments were negatively correlated with the contents of thymol and *trans*-sabinene hydrate.

This analysis provides additional and important information about the results. The PCA showed that there was a difference between the types and concentrations of PGRs and the development of plants and the volatile compounds of *Origanum vulgare*.

Conclusions

Foliar application of PGRs at the lowest concentrations ($GA_3$25, IBA25 and KIN25) resulted in higher dry weight in the species *Origanum vulgare*, contributing to significant gains in essential oil content and yield. The use of PGRs also caused a higher concentration of carotenoids and major constituents of the essential oil, especially trans-sabinene hydrate and thymol. In addition, the presence of PGRs decreased the signaling of oxidative stress in this species, which may have contributed to a better performance of oregano. Therefore, the results of this study suggest that the foliar application of PGRs at low concentrations may contribute to increased production of biomass, carotenoids and volatile compounds in oregano.

Acknowledgements The authors would like to thank the Universidade Federal de Lavras (UFLA) for all its support in terms of structure and resources, as well as the funding agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES – Finance Code 001).

Author Contribution All authors contributed substantially to the work reported. RMAA, SKVB, and JEBPP conceived the study, designed the experiments; RMAA, ACH, JPS, LIFA, JJFL and AAC, performed experiments, analyzed data; RMAA and SKVB did the chemical analyses. RMAA, SKVB, and JEBPP wrote the manuscript. All authors read and approved the manuscript.

Declarations

Conflict of interest Authors declare that they have no conflict of interest.

References

Abbas, S. M., & EL-Saeid, H. M. (2012). Herb, oil yield and hormonal content of lemon grass in relation to some growth regulators. *J Funct Environ Botany 2*(2), 113-117. https://doi.org/10.5958/j.2231-1742.2.2.011

Adams, R. P. (2007). *Identification of essential oil components by gas chromatography/mass spectrometry*. Allured publishing corporation Carol Stream, IL USA.

Ahanger, M. A., Alyemeni, M. N., Leonard, W., Alamri, S. A., Pravej, A., Muhammad, A., & Parvaiz, A. (2018). Potential of exogenously sourced kinetin in protecting *Solanum lycopersicum* from NaCl-induced oxidative stress through up-regulation of the antioxidant system, ascorbate-glutathione cycle and glyoxalase system. *PLoS ONE*, *13*(9), e0202175. https://doi.org/10.1371/journal.pone.0202175

Alqarni, M. H., Salkini, A. A., Abujheisha, K. Y., Daghar, M. F., Al-khuraif, F. A., & Abdel-Kader, M. S. (2022). Qualitative, quantitative and antimicrobial activity variations of the essential oils isolated from *Thymus Vulgaris* and micromeria fruticosa samples subjected to different drying conditions. *Arab J Sci Eng*, *47*(6), 6861-6867. https://doi.org/10.1007/s13369-021-06469-8

Amaro, A. C. E., Souza, E. R., Santos, L. S., Baron, D., Ono, E. O., & Rodrigues, J. D. (2020). Foliar application of plant growth regulators to "Crimson Seedless" grapevine influences leaf age. *Aust J Crop Sci*, *14*(3), 422-430. https://doi.org/10.21475/ajcs.20.14.03.p1874

Amengual, J. (2019). Bioactive properties of carotenoids in human health. *Nutrients*, *11*(10), 2388. https://doi.org/10.3390/nu11102388

Arora, R., Sangwan, A. K., Singh, N. P., & Sharma, S. (2020). Impact of IBA and bio-inoculants on growth and rhizogenesis in pomegranate (*Punica granatum*). *Indian J Agric Sci*, *90*(3), 560-564.

Arunadevi, A., Rajangam, J., & Venkatesan, K. (2019). Effect of plant growth regulators on growth, yield and quality of acid lime (*Citrus aurantifolia Swingle*.) var. PKM 1. *J Pharmacogn Phytochem*, *8*(3), 3438-3441.

Assaf, M., Korkmaz, A., Karaman, Ş., & Kulak, M. (2022). Effect of plant growth regulators and salt stress on secondary metabolite composition in Lamiaceae species. *S Afr J Bot*, *144*, 480-493. https://doi.org/10.1016/j.sajb.2021.10.030

Bahman, S., Mehrafarin, A., & Naghdi Badi, H. (2017). Influence of gibberellic acid, indole butyric acid, and methanol on morpho-physiological and phytochemical traits in *Thymus vulgaris* L [Influence of Gibberellic Acid, Indole Butyric Acid, and Methanol on Morpho-physiological and Phytochemical Traits in Thymus vulgaris L.]. *J Med Plants*, *16*(61), 33-44.

Bano, U., Khan, A. F., Mujeeb, F., Maurya, N., Tabassum, H., Siddiqui, M. H., Haneef, M., Osama, K., & Farooqui, A. (2016). Effect of plant growth regulators on essential oil yield in aromatic plants. *J Chem Pharm Res*, *8*(7), 733-739.

Barnes, J. D., Balaguer, L., Manrique, E., Elvira, S., & Davison, A. W. (1992). A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. *Environ Exp Bot*, *32*(2), 85-100. https://doi.org/10.1016/0098-8472(92)90034-Y

Bashri, G., Singh, S., Prasad, S. M., Ansari, M. J., Usmani, S., Alfarraj, S., Alharbi, S. A., & Brestic, M. (2021). Kinetin mitigates Cd-induced damagesto growth, photosynthesis and PS II photochemistry of *Trigonella* seedlings by up-regulating ascorbate-glutathione cycle. *PLoS ONE*, *16*(6), e0249230. https://doi.org/10.1371/journal.pone.0249230

Benincasa, M. M. P. (2003). *Análise de crescimento de plantas: noções básicas* (Vol. 2). Funep Jaboticabal, pp 41.41.

Biemelt, S., Keetman, U., & Albrecht, G. (1998). Re-Aeration following hypoxia or anoxia leads to activation of the antioxidative defense system in roots of wheat seedlings. *Plant Physiol*, *116*(2), 651-658. https://doi.org/10.1104/pp.116.2.651

Bose, S. K., Yadav, R. K., Mishra, S., Sangwan, R. S., Singh, A. K., Mishra, B., Srivastava, A. K., & Sangwan, N. S. (2013). Effect of gibberellic acid and calliterpenone on plant growth attributes, trichomes, essential oil biosynthesis and pathway gene expression in differential manner in *Mentha arvensis* L. *Plant Physiol Biochem*, *66*, 150-158. https://doi.org/10.1016/j.plaphy.2013.02.011

Buege, J. A., & Aust, S. D. (1978). *[30] Microsomal lipid peroxidation*. Academic Press.https://doi.org/https://doi.org/10.1016/S0076-6879(78)52032-6.

Cheng, Z., & Liu, L. (2010). Detection of vegetation light use efficiency based on chlorophyll fluorescence spectrum. *Trans Chin Soc Agric*, *26*(2), 74-80.

Coutinho, M. G. S., Neves, A. M., Batista, A. S. M., & dos Santos, F. R. O. (2020). Use of essential oils in cheese conservation: Review. *Rev ILCT*, *75*(2), 126-141. https://doi.org/10.14295/2238-6416.v75i2.777.

Dadkhah, A., Ghorbanian, S., & Rassam, G. (2016). Foliar application effect of gibberelic acid on growth, volatile oil and nutrients content of *Satureja hortensis* L. *Z Fur Arznei-Gewurzpflanzen*, *21*(2), 76-79.

de Assis, R. M. A., Carneiro, J. J., Medeiros, A. P. R., de Carvalho, A. A., da Cunha Honorato, A., Carneiro, M. A. C., Bertolucci, S. K. V., & Pinto, J. E. B. P. (2020). Arbuscular mycorrhizal fungi and organic manure enhance growth and accumulation of citral, total phenols, and flavonoids in *Melissa officinalis* L. *Ind Crops Prod*, *158*, 112981. https://doi.org/10.1016/j.indcrop.2020.112981

de Marins, A. R., Sartorelli, A., Silva, L. A. d., Campos, T. A. F. d., Artilha, C. A. F., Silva, N. M. d., Feihrmann, A. C., Gomes, R. G., & Marinho, M. T. (2021). Influence of the addition of rosemary (*Rosmarinus officinalis* L.) and oregano (*Origanum vulgare*) on the lipid stability, texture and sensory characteristics of pork burgers. *Res Soc Dev*, *10*(11), e201101119477. https://doi.org/10.33448/rsd-v10i11.19477

do Nascimento, A. R. L., Freire, C. S., Silva, A. F. A. d., Oliveira, E. C. A. d., Freire, F. J., & Santos, R. L. d. (2020). Management of nitrogen fertilization in alkaline soil cultivated with sorghumsudan. *Rev Geama Sci J Environ ScI Biotechnol*, *6*(2), 72-80.

El-Kinany, R. G., Salama, Y. E., Rozan, M. A., Bayom, H. M., & Nassar, A. M. K. (2020). Impacts of humic acid, Indole butyric acid (IBA) and arbuscular mycorrhizal fungi (*Glomus mosseae*) as growth promoters on yield and phytochemical characteristics of *Hibiscus Sabdariffa* (Roselle). *Alex Sci Exch J*, *41*(JANUARY- MARCH), 29-41. https://doi.org/10.21608/asejaiqjsae.2020.73036

El Karamany, M. F., Sadak, M. S., & Bakry, B. A. (2019). Improving quality and quantity of mungbean plant via foliar application of plant growth regulators in sandy soil conditions. *Bull Natl Res Cent 43*(1), 61. https://doi.org/10.1186/s42269-019-0099-5

El, M., Eman, M., Gomaa, A. O., Youssef, A. S. M., & El-Nemr, A. M. (2018). Effect of kinetin and GA3 treatments on growth and flowering of *Dendranthema grandiflorium* cv. Art Queen plants. *Middle East J Agric*, *7*(3), 801-815.

Elsayed, s. M., E.El-Gohary, A., Hendawy, S., & Abd El-Naby, S. (2021). Mitigation of heat stress effects on chamomile and its essential oil using melatonin or gibberellic acid and some agricultural treatments. *Egypt J Chem*, *64*(10), 6017-6028. https://doi.org/10.21608/ejchem.2021.80586.3993

Fishel, F. M. (2006). *Plant Growth Regulators. EDIS*.

Galindo, M. V., Paglione, I. d. S., Balan, G. C., Sakanaka, L. S., & Shirai, M. A. (2019). Antimicrobial and antioxidant activity of chitosan/gelatin and essential oil edible film. *Seg Aliment Nutr*, *26*(0), e019008. https://doi.org/10.20396/san.v26i0.8653865

García-Caparrós, P., De Filippis, L., Gul, A., Hasanuzzaman, M., Ozturk, M., Altay, V., & Lao, M. T. (2021). Oxidative stress and antioxidant metabolism under adverse environmental conditions: a review. *Bot Rev*, *87*(4), 421-466. https://doi.org/10.1007/s12229-020-09231-1

Ghassemi-Golezani, K., Nikpour-Rashidabad, N., & Samea-Andabjadid, S. (2022). Application of growth promoting hormones alters the composition and antioxidant potential of dill essential oil under salt stress. *Sci Rep*, *12*(1), 14349. https://doi.org/10.1038/s41598-022-18717-4

Giannopolitis, C. N., & Ries, S. K. (1977). Superoxide dismutases: I. occurrence in higher plants. *Plant Physiol*, *59*(2), 309-314. 10.1104/pp.59.2.309

Gonçalves, D. C., Tebaldi de Queiroz, V., Costa, A. V., Lima, W. P., Belan, L. L., Moraes, W. B., Pontes Póvoa Iorio, N. L., & Corrêa Póvoa, H. C. (2021). Reduction of Fusarium wilt symptoms in tomato seedlings following seed treatment with *Origanum vulgare* L. essential oil and carvacrol. *Crop Prot*, *141*, 105487. https://doi.org/10.1016/j.cropro.2020.105487

Guato, A., Gomes, F. A., Freitas, H. J., Santos, F. G. d. A., Zanfangnini, L. G., Sandra, I. d. O., & Ávila, R. L. (2020). Oregano extract (*Origanum vulgare*) in male free-range broilers reared in western amazon. . *S Am J of Basic Educ Techn Technol*, *7*(1), 325-342.

Gupta, R., & Chakrabarty, S. K. (2013). Gibberellic acid in plant. *Plant Signal Behav*, *8*(9), e25504. https://doi.org/10.4161/psb.25504

Hamayun, M., Hussain, A., Khan, S. A., Irshad, M., Khan, A. L., Waqas, M., Shahzad, R., Iqbal, A., Ullah, N., Rehman, G., Kim, H.-Y., & Lee, I.-J. (2015). Kinetin modulates physio-hormonal attributes and isoflavone contents of soybean grown under salinity stress [Original Research]. *Front Plant Sci*, *6*. https://doi.org/10.3389/fpls.2015.00377

Hasan-beigi, H., Saidi, M., & Mohammadi, M. (2021). Effects of gibberellic acid and salicylic acid application on morphophysiological characteristics and essential oil yield of *Echinacea purpurea* (L.) Moench. *Iran J Appl Res Med Aromat Plants 36*(6), 1038-1051. https://doi.org/10.22092/ijmapr.2021.342971.2795

Hasan, M., & Ismail, B. (2018). Effect of gibberellic acid on the growth and yield of groundnut (*Arachis hypogaea* L.). *Sains Malays*, *47*(2), 221-225. https://doi.org/10.17576/jsm-2018-4702-02

Hashim, A. M., Alharbi, B. M., Abdulmajeed, A. M., Elkelish, A., Hozzein, W. N., & Hassan, H. M. (2020). Oxidative stress responses of some endemic plants to high altitudes by intensifying antioxidants and secondary metabolites content. *Plants*, *9*(7). https://doi.org/10.3390/plants9070869

Havaux, M. (2014). Carotenoid oxidation products as stress signals in plants. *Plant J*, *79*(4), 597-606. https://doi.org/10.1111/tpj.12386

Havir, E. A., & McHale, N. A. (1987). Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. *Plant Physiol*, *84*(2), 450-455. https://doi.org/10.1104/pp.84.2.450

Hazzoumi, Z., Moustakime, Y., & Amrani, J. K. (2014). Effect of gibberellic acid (GA), indole acetic acid (IAA) and benzylaminopurine (BAP) on the synthesis of essential oils and the isomerization of methyl chavicol and trans-anethole in *Ocimum gratissimum* L. *SpringerPlus*, *3*(1), 321. https://doi.org/10.1186/2193-1801-3-321

Henry, L.-C., Salgado-Moreno, S., Ávila-Ramos, F., Gutiérrez-Leyva, R., Domínguez-Rebolledo, A., Ayala-Martínez, M., & Escalera-Valente, F. (2020). Review of the use of oregano spp. oil in animal health and production. *Abanico Agrof*, *2*, 1-22. https://doi.org/10.37114/abaagrof/2020.1

Hiscox, J. D., & Israelstam, G. F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canad J Bot*, *57*(12), 1332-1334. https://doi.org/10.1139/b79-163

Hunt, R. W., Chinnasamy, S., Bhatnagar, A., & Das, K. C. (2010). Effect of biochemical stimulants on biomass productivity and metabolite content of the microalga, *Chlorella sorokiniana*. *Appl Biochem Biotechnol*, *162*(8), 2400-2414. https://doi.org/10.1007/s12010-010-9012-2

Inoue, S., Ilogu, C., & Sobze, J.-M. (2022). Effects of indole-3-butyric acid and age of stem cuttings on root morphology, growth, and survival of *Cornus sericea*. *J For Res*, 1-8. https://doi.org/10.1007/s11676-022-01490-5

Jamwal, K., Bhattacharya, S., & Puri, S. (2018). Plant growth regulator mediated consequences of secondary metabolites in medicinal plants. *J Appl Res Med Aromat Plants*, *9*, 26-38. https://doi.org/10.1016/j.jarmap.2017.12.003

Jan, A. U., Hadi, F., Shah, A., Ditta, A., Nawaz, M. A., & Tariq, M. (2021). Plant growth regulators and EDTA improve phytoremediation potential and antioxidant response of *Dysphania ambrosioides* (L.) Mosyakin & Clemants in a Cd-spiked soil. *Environ Sci Pollut Res*, *28*(32), 43417-43430. https://doi.org/10.1007/s11356-021-13772-2

Johnson, M., Havaux, M., Triantaphylidès, C., Ksas, B., Pascal, A., Robert, B., Davison, P., Ruban, A., & Horton, P. (2007). Elevated zeaxanthin bound to oligomeric LHCII enhances the resistance of *Arabidopsis* to photo-oxidative stress by a lipid-protective, anti-oxydant mechanism. *J Biol Chem*, *282*(31), 22605-22618. https://doi.org/10.1074/jbc.M702831200

Kačániová, M., Vukovič, N., Hleba, L., Bobková, A., Pavelková, A., Rovná, K., & Arpášová, H. (2012). Antimicrobial and antiradicals activity of *Origanum vulgare* L. and *Thymus vulgaris* essential oils. *J Microbiol Biotechnol Food Sci*, *2*(1), 263-271.

Kaya, C., Akram, N. A., & Ashraf, M. (2018). Kinetin and indole acetic acid promote antioxidant defense system and reduce oxidative stress in maize (*Zea mays* L.) plants grown at boron toxicity. *J Plant Growth Regul*, *37*(4), 1258-1266. https://doi.org/10.1007/s00344-018-9827-6

Khan, M. M. A., Khanam, N., Uddin, M., Mishra, R. K., & Khan, R. (2022). Nanotized kinetin enhances essential oil yield and active constituents of mint via improvement in physiological attributes. *Chemosphere*, *288*, 132447. https://doi.org/10.1016/j.chemosphere.2021.132447

Kulkarni, M. G., Stirk, W. A., Southway, C., Papenfus, H. B., Swart, P. A., Lux, A., Vaculík, M., Martinka, M., & Staden, J. V. (2013). Plant growth regulators enhance gold uptake in *Brassica juncea*. *Int J Phytoremediation*, *15*(2), 117-126. https://doi.org/10.1080/15226514.2012.683207

Lal, R. K., Gupta, P., Srivastva, S., Chanotiya, C. S., Mishra, A., Yadav, A., & Maurya, R. (2021). Delineating photosynthesis, essential oil yield performance, and their component traits under genotype × traits × environments interactions in *Ocimum genotypes*. *S Afr J Bot*, *141*, 54-65. https://doi.org/10.1016/j.sajb.2021.03.039

Ligaj, M., Kobus-Cisowska, J., Szczepaniak, O., Szulc, P., Kikut-Ligaj, D., Mikołajczak-Ratajczak, A., Bykowski, P., Szymanowska, D., Przeor, M., Polewski, K., & Jarzębski, M. (2021). Electrochemical screening of genoprotective and antioxidative effectiveness of *Origanum vulgare* L. and its functionality in the prevention of neurodegenerative disorders. *Talanta*, *223*, 121749. https://doi.org/10.1016/j.talanta.2020.121749

Lim, B. P., Akihiko, N., Junji, T., Kazunobu, T., Tetsuya, S., & Kozo, T. (1992). Antioxidant activity of xanthophylls on peroxyl radical-mediated phospholipid peroxidation. *Biochim Biophys Acta Bioenerg*, *1126*(2), 178-184. https://doi.org/10.1016/0005-2760(92)90288-7

Lukas, B., Schmiderer, C., & Novak, J. (2015). Essential oil diversity of European *Origanum vulgare* L. (Lamiaceae). *Phytochemistry*, *119*, 32-40. https://doi.org/10.1016/j.phytochem.2015.09.008

Mahmoudi, D., Shekari, F., Afsahi, K., & Maleki, A. (2022). Evaluation of the responses of basil to the application of salicylic acid and gibberellic acid. *Zemdirbyste-Agric*, *109*(2), 131-138. https://doi.org/10.13080/z-a.2022.109.017

Mansouri, H., Asrar, Z., & Amarowicz, R. (2011). The response of terpenoids to exogenous gibberellic acid in *Cannabis sativa* L. at vegetative stage. *Acta Physiol Plant*, *33*(4), 1085-1091. https://doi.org/10.1007/s11738-010-0636-1

Mansouri, H., & Talebizadeh, R. (2017). Effects of indole-3-butyric acid on growth, pigments and UV-screening compounds in *Nostoc linckia*. *Phycol Res*, *65*(3), 212-216. https://doi.org/10.1111/pre.12177

Mortensen, A., & Skibsted, L. H. (1997). Importance of carotenoid structure in radical-scavenging reactions. *J Agric Food Chem*, *45*(8), 2970-2977. https://doi.org/10.1021/jf970010s

Nabi, F., Arain, M. A., Rajput, N., Alagawany, M., Soomro, J., Umer, M., Soomro, F., Wang, Z., Ye, R., & Liu, J. (2020). Health benefits of carotenoids and potential application in poultry industry: A review. *J Anim Physiol Anim Nutr*, *104*(6), 1809-1818. https://doi.org/10.1111/jpn.13375

Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol*, *22*(5), 867-880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Nazir, F., Fariduddin, Q., & Khan, T. A. (2020). Hydrogen peroxide as a signalling molecule in plants and its crosstalk with other plant growth regulators under heavy metal stress. *Chemosphere*, *252*, 126486. https://doi.org/10.1016/j.chemosphere.2020.126486

NIST. (2003). National institute of standards and technology chemistry web book. *National Institute of Standards and Technology, Washington*.

Odivi, g. A., Fallah, S., karimi, M., & lorigooini, Z. (2021). The effect of organic and chemical fertilizers on growth, yield and essential oil of hyssop *Hyssopus officinalis* L. *J Soil Manage Sustain Prod*, *11*(2), 143-158. https://doi.org/10.22069/ejsms.2021.18578.1992

Olteanu, Z., Oprică, L., Truță, E., & Zamfirache, M. M. (2009). Response of barley seedlings to oxidative stress generated by treatments with growth hormones. *J Exp Mol Biol*, *10*(1), 29-36.

Ozden, M., Demirel, U., & Kahraman, A. (2009). Effects of proline on antioxidant system in leaves of grapevine (*Vitis vinifera* L.) exposed to oxidative stress by H_2O_2. *Sci Hortic*, *119*(2), 163-168. https://doi.org/10.1016/j.scienta.2008.07.031

Pinto, J., & Bertolucci, S. (2002). *Cultivation and processing of medicinal plants*. Federal University of Lavras, UFLA. Foundation to Support Teaching and Extension Studies, FAEPE. MG.

Prete, R. d. O., Serafim, R. Â., Souza, D. d. F. M. d., Sakanaka, L. S., & Yamaguchi, M. M. (2020). Characterization and application of oregano oil as a natural antioxidant in fresh sausage. *Braz J Dev*, *6*(7), 44109-44118. https://doi.org/10.34117/bjdv6n7-144

Prins, C. L., Vieira, I. J. C., & Freitas, S. P. (2010). Growth regulators and essential oil production. *Braz J Plant Physiol*, *22*(2), 91-102. https://doi.org/10.1590/S1677-04202010000200003

Qiao, Y., Yu, Z., Bai, L., Li, H., Zhang, S., Liu, J., Gao, Z., & Yang, X. (2021). Chemical composition of essential oils from *Thymus mongolicus*, *Cinnamomum verum*, and *Origanum vulgare* and their acaricidal effects on *Haemaphysalis longicornis* (Acari: Ixodidae). *Ecotoxicol Environ Saf*, *224*, 112672. https://doi.org/10.1016/j.ecoenv.2021.112672

Rademacher, W. (2015). Plant growth regulators: backgrounds and uses in plant production. *J Plant Growth Regul*, *34*(4), 845-872. https://doi.org/10.1007/s00344-015-9541-6

Ramy, G. E. K., Atef, M. K. N., & Ahmed, A. A. E. S. (2019). The role of benzyl amino purine and kinetin in enhancing the growth and flowering of three gaillardia varieties. *Alex J Agric Res*, *64*(5), 277-288. https://doi.org/10.21608/alexja.2019.80484

Sajid, M., Anjum, M. A., & Hussain, S. (2015). Foliar application of plant growth regulators affects growth, flowering, vase life and corm production of *Gladiolus grandiflorus* L. under calcareous soil. *Bulg J Agric Sci*, *21*(5), 982-989.

Saleem, M. H., Fahad, S., Adnan, M., Ali, M., Rana, M. S., Kamran, M., Ali, Q., Hashem, I. A., Bhantana, P., Ali, M., & Hussain, R. M. (2020). Foliar application of gibberellic acid endorsed phytoextraction of copper and alleviates oxidative stress in jute (*Corchorus capsularis* L.) plant grown in highly copper-contaminated soil of China. *Environ Sci Pollut Res*, *27*(29), 37121-37133. https://doi.org/10.1007/s11356-020-09764-3

Sangwan, N. S., Farooqi, A. H. A., Shabih, F., & Sangwan, R. S. (2001). Regulation of essential oil production in plants. *Plant Growth Regul*, *34*(1), 3-21. https://doi.org/10.1023/a:1013386921596

Santos, E. L., Silva, T. d. J., Oliveira, J. M. d., Lima, M. R. d., Machado, S. S., Reis Neto, J., Costa, C. P. A. d. S., Dantas, P. C., & Soares, E. C. (2020). Oregano extract additive in diets for betta splendens. *Res Soc Dev*, *9*(10), e6649109009. https://doi.org/10.33448/rsd-v9i10.9009

Santos, R. P., Da Cruz, A. C. F., Iarema, L., Kuki, K. N., & Otoni, W. C. (2015). Protocolo para extração de pigmentos foliares em porta-enxertos de videira micropropagados. *Ceres*, *55*(4).

Shah, S. H., Islam, S., Parrey, Z. A., & Mohammad, F. (2021). Role of exogenously applied plant growth regulators in growth and development of edible oilseed crops under variable environmental conditions: a Review. *J Soil Sci Plant Nutr*, *21*(4), 3284-3308. https://doi.org/10.1007/s42729-021-00606-w

Sharafzadeh, S., & Zare, M. (2011). Influence of growth regulators on growth and secondary metabolites of some medicinal plants from Lamiaceae family. *Adv Environ Biol*, *5*(8), 2296-2302.

Singh, A., Bhatt, S. S., Pandey, Y., Kumar, N., & Adarsh, A. (2016). Efficacy of auxins and gibberellic acid on floral attributes and essential oil yield on German chamomile (*Matricaria chamomilla* L.). *Med Plants-Int J Phytomed Relat Ind*, *8*(1), 52-55. https://doi.org/10.5958/0975-6892.2016.00007.1

Souza, J. V. R. S., Ming, L. C., Santos, M. A. L., Simon, J. E., Juliani, H. R., & Saad, J. C. C. (2021). Effect of water regime and harvest stage on essential oil accumulation in basil plant growing in sandy soil. *Irrig Sci*, *39*(4), 493-503. https://doi.org/10.1007/s00271-021-00719-1

Tadayon, M. S., & Hosseini, S. M. (2022). Increasing the efficiency of supplemental foliar nutrition on improving reproductive disorders of pistachio by application of plant growth regulators. *J Plant Growth Regul*, *41*(2), 657-671. https://doi.org/10.1007/s00344-021-10330-5

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2017). *Fisiologia e desenvolvimento vegetal*. Artmed Editora.

Thakur, M., & Kumar, R. (2020). Foliar application of plant growth regulators modulates the productivity and chemical profile of damask rose (*Rosa damascena* Mill.) under mid hill conditions of the western Himalaya. *Ind Crops Prod*, *158*, 113024. https://doi.org/10.1016/j.indcrop.2020.113024

Tintaya, F. C., & Hurtado, H. H. (2019). Economic analysis of the production of oregano (*Origanum vulgare* L) in the fstablishments of la yarada in the district of tacna. *Cienc Desarro*(17), 61-66. https://doi.org/10.33326/26176033.2014.17.408

Van Den Dool, H., & Dec. Kratz, P. (1963). A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. *J Chromatogr A*, *11*, 463-471. https://doi.org/10.1016/S0021-9673(01)80947-X

Velikova, V., Yordanov, I., & Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Sci*, *151*(1), 59-66. https://doi.org/10.1016/S0168-9452(99)00197-1

Wellburn, A. R. (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J Plant Physiology*, *144*(3), 307-313. https://doi.org/10.1016/S0176-1617(11)81192-2

Xiao, X., Yang, M., Dong, W., Zhou, C., Shi, L., Chen, W., Cao, S., Yang, Z., & Li, S. (2022). Gibberellic acid inhibited chlorophyll degradation in post-harvest okras. *Postharvest Biol Technol*, *190*, 111951. https://doi.org/10.1016/j.postharvbio.2022.111951

Yeşil, M., & Özcan, M. M. (2021). Effects of harvest stage and diurnal variability on yield and essential oil content in *Mentha* × *piperita* L. *Plant Soil Environ*, *67*(7), 417-423. https://doi.org/10.17221/114/2021-pse

Yu, H., Zhang, P., Liu, H., Sun, X., Liang, J., Sun, L., & Chen, Y. (2021). Hypoglycemic activity of *Origanum vulgare* L. and its main chemical constituents identified with HPLC-ESI-QTOF-MS [10.1039/D0FO03166F]. *Food Funct*, *12*(6), 2580-2590. https://doi.org/10.1039/d0fo03166f