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CARACTERIZAÇÃO DO PROCESSO DE CULTIVO E OBTENÇÃO DO ÓLEO ESSENCIAL DE *Plectranthus amboinicus* (Lour.) Spreng. COM ATIVIDADE ANTIMICROBIANA

SÃO LUÍS
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Tese apresentada ao Programa de Pós-Graduação em Biotecnologia da Rede Nordeste de Biotecnologia (RENORBIO) como como requisito final à obtenção do título de Doutora em Biotecnologia.

Orientadora: Profa. Dra. Denise Fernandes Coutinho.
Co-Orientadora: Profa. Dra. Patrícia de Maria Silva Figueiredo.
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Defesa de Tese em 17 de agosto de 2021, pela banca examinadora constituída dos seguintes membros:

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Prof. Dra. Joicy Cortez de Sá Sousa
Examinadora - Universidade CEUMA

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“*Tente (tente)*
E não diga que a vitória está perdida
Se é de batalhas que se vive a vida
Tente outra vez”

Raul Seixas

RESUMO

Plectranthus amboinicus (Lour.) Spreng., da família Lamiaceae, é uma espécie vegetal conhecida popularmente como malva-do-reino ou hortelã-da-folha-grossa, com potencial antimicrobiano. Considerando a necessidade de padronizar fitoterápicos dos aspectos agronômicos até sua produção, esta pesquisa teve por objetivo determinar o período de cultivo e a adubação que fornecessem o óleo essencial (OE) das folhas dessa espécie com melhor resposta antimicrobiana. O cultivo ocorreu na casa de vegetação do Horto de Plantas Medicinais da Universidade Federal do Maranhão, São Luís-MA, entre 2018 e 2019, envolvendo dois tipos de adubação: mista (bovina e avícola) -A e bovina-B ao longo de quatro períodos climáticos (início das chuvas-T1, chuva intensa-T2, final das chuvas-T3 e seca-T4). Foram analisados a fertilidade do solo (FS) e o estado nutricional das folhas (NF) e os OEs foram extraídos por hidrodestilação com determinação de seus rendimentos e da composição química por cromatografia em fase gasosa acoplada em espectrometria de massa. A atividade antimicrobiana foi realizada por difusão em disco com microrganismos de *Candida albicans*, *C. parapsilosis*, *C. krusei*, *Staphylococcus aureus* e *Escherichia coli* com determinação da concentração inibitória mínima, concentração bactericida mínima e concentração fungicida mínima. A análise estatística dos dados multivariados foi realizada através da determinação da variância (ANOVA) e a comparação dos tratamentos foi através do teste t de Student. A análise dos principais componentes (PCA) investigou a similaridade entre os componentes dos OEs nos tratamentos. Os melhores resultados da FS e NF foram obtidos com as adubações B e A, respectivamente. As estações que mais influenciaram alterações na FS e no NF foram os períodos T1 e T4, nos quais foi percebido que enquanto T4 aumentou FS e reduziu NF, T1 atuou de modo inverso. Os rendimentos dos OEs variaram de 0,4 a 1,65%. O NF interferiu na composição química do OE, influenciado também pela adubação e pelo período climático na formação dos constituintes, como cariofileno e γ-terpineno que tiveram suas concentrações alteradas. Entretanto, carvacrol manteve-se sempre como o majoritário em todos os tratamentos, variando apenas seu teor. A melhor atividade antimicrobiana do OE foi obtida em T1 com adubação A contra microrganismos de *C. albicans*, *C. parapsilosis* e *E. coli* e com adubação B, ainda em T1, contra *S. aureus*; enquanto T4 mostrou melhor resposta contra *C. krusei* nos dois tipos de adubação. Dessa forma, constatou-se que o período seco (T4) e o período de transição, que abrange o fim da seca e o início da estação chuvosa (T1), nos dois tipos de adubações testadas, são os mais indicados para obtenção do OE de *P. amboinicus* para desenvolvimento de produtos biotecnológicos com ação antimicrobiana.

Palavras-Chave: Adubação orgânica; Carvacrol; Hortelã da folha grossa; Padronização; Solo.

ABSTRACT

Plectranthus amboinicus (Lour.) Spreng., of the Lamiaceae family, is a plant species popularly known as thick-leaf mint, with antimicrobial potential. Considering the need to standardize phytotherapeutics from agronomic aspects to their production, this research aimed to determine the cultivation period and fertilization that would provide the essential oil (EO) from the leaves of this species with the best antimicrobial response. The cultivation occurred in the greenhouse of the Horta de Medicinal Plants of the Federal University of Maranhão, São Luís-MA, between 2018 and2019, involving two types of fertilization: mixed (bovine and poultry) -A and bovine-B in four climatic periods (early rainfall-T1, heavy rainfall-T2, late rainfall-T3 and dryness-T4). Soil fertility (FS) and leaf nutritional status (NF) were analyzed and the EOs were extracted by hydrodistillation with determination of their yields and chemical composition by gas chromatography coupled to mass spectrometry. The antimicrobial activity was performed by disc diffusion with the microorganisms of *Candida albicans, C. parapsilosis, C. krusei, Staphylococcus aureus* and *Escherichia coli* with determination of the minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration. The best FS and NF results were obtained with fertilizers B and A, respectively. Statistical analysis of the multivariate data was performed by determination of variance (ANOVA) and comparison of the treatments was by Student's t-test. Principal component analysis investigated the similarity between the components of the EOs in the treatments. The stations that most influenced changes in FS and NF were the periods T1 and T4, in which it was perceived that while T4 increased FS and reduced NF, T1 acted in the opposite way. The yields of the EOs ranged from 0.4-1.65%. The NF interfered in the chemical composition of the EO, also influenced by fertilization and the climatic period in the formation of constituents, such as caryophyllene and γ-terpinene, which had their concentrations altered. However, carvacrol always remained the majority in all treatments, varying only its content. The best antimicrobial activity of the EO was obtained at T1 with fertilization A against *C. albicans, C. parapsilosis*, and *E. coli* microorganisms, and with fertilization B, still at T1, against *S. aureus*; while T4 showed the best response against *C. krusei* in both types of fertilization. Thus, it was found that the dry period (T4) and the transition period, which covers the end of the dry season and the beginning of the rainy season (T1), in both types of fertilization tested, are the most suitable for obtaining essential oil from *P. amboinicus* for development of biotechnology products with antimicrobial action.

Keywords: Carvacrol; Organic fertilization; Soil; Standardization; Thick-leaf mint.

LISTA DE SIGLAS E ABREVIATURAS

ANVISA	Agência Nacional de Vigilância Sanitária
CTC	Capacidade de troca de cátions
CBM	Concentração bactericida mínima
CIM	Concentração inibitória mínima
CFM	Concentração fungicida mínima
CG-EM	Cromatografia em fase gasosa acoplada espectrometria de massa
DMAPP	Dimetilalil pirofosfato
FPP	Pirofosfato de farnesil
GPP	Pirofosfato de geranil
IPP	Isopentenil pirofosfato
MS	Ministério da Saúde
OE	Óleo essencial
OMS	Organização Mundial da Saúde
RENAME	Relação Nacional de Medicamentos Essenciais
SB	Soma das bases
SUS	Sistem Único de Saúde
V%	Soma das bases trocáveis

SUMÁRIO

1 INTRODUÇÃO

Desde o aparecimento do homem na Terra, há registros do uso de vegetais como recursos na alimentação e na terapêutica. Atualmente, as plantas medicinais representam um importante recurso terapêutico, além de serem essenciais para o desenvolvimento econômico e social em diversas localidades (ASL ROOSTA et al., 2017; SHEN et al., 2021). Em países asiáticos o cultivo destas plantas serve como fonte de renda da população rural e o comércio dos medicamentos fitoterápicos aquece a economia local (HE et al., 2018; SHEN et al., 2021).

Segundo CECHINEL FILHO & ZANCHETT (2020), cerca de 70% dos novos fármacos e medicamentos, no período de 1981-2010, estavam relacionados direta ou indiretamente aos produtos naturais, principalmente vegetais terrestres e animais marinhos. Foram empregados fármacos isolados desses seres ou como modelos para sintetizar substâncias com efeitos terapêuticos desejados. Levantamentos estendidos até 2014, corroboram a participação dos produtos naturais no desenvolvimento de moléculas terapêuticas visando a produção de novos medicamentos (BERLINCK, et al. 2017; CECHINEL FILHO, 2017).

A biodiversidade vegetal é composta por uma variedade estrutural de compostos moleculares que engrandece e encoraja a pesquisa por novas substâncias e/ou compostos moleculares naturais para o isolamento de fármacos, desenvolvimento de fitoterápicos ou como protótipos na síntese de análogos estruturais e/ ou terapêuticos (BERLINCK, et al. 2017). Responsável por aproximadamente um terço dos vegetais existentes no planeta, a biodiversidade brasileira apresenta-se como uma potencial fonte de recursos a ser explorada. Entretanto, apenas 10% de suas espécies estão sendo estudadas cientificamente (CECHINEL FILHO, 2020).

A fitoterapia compreende o uso de plantas medicinais no tratamento de doenças nas suas mais variadas formas farmacêuticas. É uma prática terapêutica com registros de seu uso pelos povos desde 8.500a.C, sendo considerada uma das mais antigas (LEITE et al., 2020). Em países desenvolvidos, a fitoterapia participa como medicina complementar e alternativa, enquanto nos países de baixa renda integram a atenção primária à saúde devido o menor custo e a fácil aquisição (OLIVEIRA, 2018).

A relevância dos conhecimentos sobre as plantas medicinais para aplicação da fitoterapia foi confirmada pela Organização Mundial da Saúde (OMS), sendo implementadas normas no Brasil para a ampliação segura deste uso através da sua integração no Sistema Único de Saúde (SUS) (BRASIL, 2016).

O maior consumo em medicamentos fitoterápicos está na China, enquanto em Nova Zelândia a exportação de produtos fitoterápicos corresponde a 80% (WHO, 2013). Na Alemanha 40% das prescrições correspondem a produtos vegetais, com um consumo de fitoterápicos por 70% dos alemães (JUTTE et al., 2017; LEITE et al., 2020). Já na América Latina, o Brasil e Cuba apresentam legislações que integram a fitoterapia ao sistema público de saúde (MAZIEIRO & TEIXEIRA, 2020; OLIVEIRA, 2018).

A partir da publicação da Política Nacional de Plantas Medicinais e Fitoterápicos pelo governo brasileiro em 2006 (BRASIL, 2006), o Ministério do Saúde intensificou suas ações para garantir a fitoterapia racional e segura no Brasil, destacando-se algumas publicações. O Formulário Fitoterápico da Farmacopeia Brasileira (FFFB), aprovado em 2011 e atualizado em sua 2ª edição em 2021 que disponibiliza orientações sobre a manipulação e utilização de fitoterápicos que podem ser incluídos nos Programas de Fitoterapia no SUS (BRASIL, 2021; LEITE et al., 2020). A Relação Nacional de Medicamentos Essenciais (RENAME) no âmbito do Sistema Único de Saúde (SUS), desde sua edição em 2007 incluiu plantas medicinais e sua edição mais atual, 2020, apresenta 12 plantas, cujos fitoterápicos podem ser utilizados nas unidades de saúde. O Memento Terapêutico (BRASIL, 2016) traz ainda muitas informações sobre plantas medicinais, importantes para o uso racional desses produtos.

A espécie *Plectranthus amboinicus* (Lour.) Spreng, apesar de não constar em nenhuma publicação da ANVISA/MS, tem demonstrado ser uma planta de interesse para aprofundar seus estudos científicos.

Esta pertence a família Lamiaceae, que caracteriza-se por ser rica em óleos essenciais (OEs), compreende 236 gêneros e aproximadamente 7000 espécies (ABDELATY et al., 2021). O gênero *Plectranthus* é um dos mais importantes da família Lamiaceae, com destaques nas áreas: médica, alimentícia e de cosméticos (AL-JUHANI & KHALIK, 2021; ABDELATY et al., 2021). Apresenta uma vasta distribuição geográfica, principalmente nos trópicos e subtrópicos, podendo ser encontradas na África, América, Oceania and Ásia, (RUAN et al., 2019; MESQUITA et al., 2021).

P. amboinicus (Lour.) Spreng., conhecida como hortelã da folha grossa, é a espécie mais estudada de sua família devido a sua variedade terapêutica (BAÑUELOS-HERNÁNDEZet al., 2020; Terto et al., 2020). Depósitos de patentes com esta espécie ocorreram principalmente em países como Japão, Estados Unidos da América (EUA), Coréia do Sul e China, não sendo encontrados registros no Brasil até o ano de 2019 (BORBA et al., 2021). Esta espécie tem valor econômico e potencial para desenvolvimento de produtos para uso na nutrição e saúde humana pela indústria (KUMAR et al., 2014; WADIKAR & PATKI, 2016).

Destacando-se a necessidade de se validar o uso terapêutico das espécies vegetais da flora brasilieira, é importante ressaltar a necessidade de boas práticas de cultivo para assegurar as melhores condições de cultivo para a obtenção de matérias-primas vegetais. Assim o cultivo de plantas medicinais com o propósito comercial requer um melhor planejamento e organização na produção desejada, a fim de garantir a qualidade da matéria-prima obtida. Além disto, possibilita obter um melhor rendimento nas classes dos fitocompostos esperados e reduz o risco de falsificações, que podem ocorrer com o emprego de espécies erradas e com a presença indesejada de contaminantes, como partes de insetos ou resíduos de agroquímicos (CARVALHO, 2015).

As características dos óleos essenciais, assim como de outros metabólitos secundários produzidos pelos vegetais, são passíveis de alterações qualitativas e quantitativas em decorrência de variações ambientais, sazonais, métodos de cultivos, métodos extrativos, o tempo de colheita (GUIMARÃES et al., 2020a; SILVA et al., 2020) e ainda fatores genéticos e tecnológicos (SALAMON et al., 2018). Dessa forma, adequações nos processos de cultivo, colheita, processamento e armazenamento são recursos que garantem a melhor produção e rendimento de OE (SALAMON et al., 2018).

As diversas condições e fatores a que uma espécie está exposta refletem na produção de seus constituintes químicos e consequentemente na intensidade de sua atividade biológica. Buscando entender melhor o desenvolvimento de *P. amboinicus* (Lour.) Spreng., frente à estas situações, este estudo avaliou o período de cultivo e as condições de adubação para obtenção de OE desta espécie com melhores atividades frente a cepas de microorganismos patogênicos.

Esta pesquisa permite, ainda, agregar conhecimentos sobre a espécie *P. amboinicus* analisando os fatores que podem interferir nas características químicas e biológicas dos seus constituintes, apresentando-se como uma importante ferramenta de análise da qualidade da matéria-prima vegetal além de possibilitar a identificação das condições ideais de cultivo para atingir o melhor aproveitamento da atividade antimicrobiana.

2 REVISÃO DE LITERATURA

2.1 *Plectranthus amboinicus* (Lour.) Spreng.

Plectranthus deriva das palavras gregas: "plectron" (esporão) e "Anthos" (flor), devido ao formato de esporão das flores de algumas espécies deste gênero (STEARN, 1992), enquanto *amboinicus* refere-se a origem em Ambon, ilha montanhosa pertencente as Ilhas Maluku, na Indonésia. Acredita-se que foi levada para as Índias Orientais e África, sendo posteriormente naturalizada na América Latina pelos espanhóis, que a chamaram de orégano da folha larga (KUMAR et al., 2020). Orégano cubano, como também é conhecida, é encontrada em florestas tropicais da Malásia e Indonésia, e por ser facilmente adaptável tornou-se uma planta popular encontrada em toda América tropical (RAO et al., 2010; FREITAS et al., 2014). Tem ocorrência natural nas regiões tropicais e quentes da Ásia, Austrália e África (PRASAD et al., 2020).

P. amboinicus (Lamiaceae) é uma planta ornamental com propriedades terapêuticas e nutricionais, pertencente a subfamília Nepetoideae (ARUMUGAM et al., 2016). Cerca de 70% do uso das plantas do gênero *Plectranthus* decorrem dos valores terapêuticos deste gênero seguido por suas propriedades nutricionais e hortícolas atribuídas à sua natureza aromática e capacidade de produção de OE (AL-JUHANI & KHALIK, 2021; ABDELATY et al., 2021).

Esta espécie é uma herbácea perene, carnuda, com até 1 metro de altura, suculenta de caule quebradiço, folhas pubescentes, duras oval-deltoides, crenada de ápice agudo, bordos dentados e pecíolo grosso com ponta afunilada, dispostas em pares opostos, facilmente multiplicadas por estaquia (FREITAS et al., 2014; SILVA, 2016). As folhas são aromáticas, pubescentes (pêlos grossos) em maior número na superfície inferior, o que lhe confere aspecto fosco (ARUMUGAM et al., 2016). As flores com pedicelo curto e verticilo denso, tem cor púrpura opaca, com cálice no formato de sino. A corola tem cor púrpura opaca sendo mais longa e maior que o cálice (ROSHAN et al., 2010). Raramente são vistas suas flores e sementes (ARUMUGAM et al., 2016; SILVA, 2016).

Apresenta como sinônimos botânicos: *Coleus amboinicus* Lour., *Coleus aromaticus* Benth (LIMA, 2013; FREITAS et al., 2014; TROPICOS, 2021), *Plectranthus aromaticus* Roxb. (BAÑUELOS-HERNÁNDEZ et al., 2020; TROPICOS, 2021) e *Plectranthus unguentarius* Codd (TROPICOS, 2021) sendo popularmente conhecida como *Indian borage* (RUAN et al., 2019), Malva do reino (SOUSA et al., 2021), Hortelã da folha grande (FREITAS et al., 2014), *Mexican mint*/Oregano (BAÑUELOS-HERNÁNDEZet al., 2020), *Spanish thyme*, *Soup mint*, *Mexican mint*, *Indian mint*, *French thyme*, *Country borage* (TROPICOS, 2021).

Seus benefícios nutricionais decorrem da presença de tetraterpenos (40 unidades de carbono) ou carotenoides como: zeaxantina, caroteno, neoxantina, violaxantina e da concentração significativa de minerais como cálcio, potássio e ferro, que são importantes no fortalecimento dos ossos e no melhor funcionamento de órgãos como rins, coração, nervos e músculos (LUKHOBA et al., 2006; SWAMY & SINNIAH, 2015). Na culinária esta espécie é usada como aromatizantes de vinhos e cervejas, no realce do sabor e aroma dos alimentos, em substituição ao orégano, no tempero e recheio de carnes (PRASAD et al., 2020), em molhos de tomates e para marinar mariscos e peixes (MOHANTY et al., 2014). *In natura*, ou crua, é usada em pães (PRASAD et al., 2020). Seu uso ornamental em residências, feito em vasos suspensos, decorre do formato em coração de suas folhas e do aroma que desprende. Apresenta ainda uma variedade ornamental: *P. amboinicus variegata* que possui folhas com bordas brancas (PRASAD et al., 2020).

Na prática popular, as folhas dessa espécie são usadas como expectorante, no tratamento de colites, helmintíase, convulsões e cálculos renais (LIMA, 2013; FREITAS et al., 2014). Em Taiwan, seu uso tópico tem indicações para queimaduras, edemas e *tinea*. Enquanto o seu uso interno tem indicações como carminativos e tratamento de asma, sendo esta espécie muito conhecida por suas características antimicrobianas (WEI et al., 2006). Há também registros de patentes referentes a ações antiinflamatórias, cicatrizantes e antitumorais, tratamento para osteoporose, nutracêuticos e agentes de limpeza de cavidade oral, principalmente com novos métodos de extração ou métodos de melhoria com predominância de patentes de processo (BORBA et al., 2021).

Estudos fitoquímicos descrevem nas folhas a presença de 76 compostos voláteis e 30 compostos não voláteis pertencentes a diferentes classes de constituintes como: monoterpenoides, diterpenoides, triterpenoides, sesquiterpenoides, compostos fenólicos, ésteres, álcoois e aldeídos (ARUMUGAM et al., 2016). O seu OE apresenta compostos bioativos como carvacrol, timol, γ-terpineno, α-terpineol, p-cymeno (ASHAARI et al., 2020), β-caryophylleno, α-humuleno, e β-selineno, α-copaeno, germacreno D, d-cadineno e óxido de caryophyleno (EL-AHMADY, 2014).

O extrato etanólico das folhas demonstrou atividade contra bactéria Gram-positiva *Bacillus cereus* (MUTIA et al., 2021) e ação potencial no tratamento da obesidade em ratos por suprimir o estresse oxidativo e de marcadores inflamatórios (ICAM-1, VCAM-1 e CD40) (HAREFA et al., 2021). Ainda com extrato etanólico, também foram testadas e comprovadas propriedades anticarcinogênicas *in vitro* contra linhagens celulares HeLa, MCF7 e T47D. Nesta pesquisa desenvolveram-se nanopartículas a partir destes extratos para melhorar a biodisponilidade e diminuir os efeitos colaterais tornando-se ideal para medicamentos anticarcinogênicos (HASIBUAN & SUMAIYAH, 2019).

Outro estudo com extrato etanólico das folhas inibiu o crescimento das bactérias *Staphylococcus aureus* e *Streptococcus mutans (*NAZLINIWATY & LAILA, 2019), enquanto no extrato hexânico das folhas foi demonstrada atividade citotóxica e apoptótica em linhagens de células cancerígenas da mama: MDA-MB-231 e MCF7 (ALMALKI et al., 2021).

O óleo essencial (OE) obtido do extrato metanólico das folhas mostrou ação antimicrobiana contra a bactéria *Klebsiella pneumoniae* e antifúngica contra *Candida albicans* (SIVARANJANI et al., 2019), enquanto o OE obtido por hidrodestilação das folhas mostrou melhor ação antimicrobiana contra *S. aureus* (HASSANI et al., 2013).

Estudos com *P. amboinicus* comprovam diferentes atividades farmacológicas de seus OEs, tais como: analgésica (CHIU et al., 2012), atividade larvicida (VIJAYAKUMAR et al., 2015), anticarcinogênica (HASIBUAN & SUMAIYAH, 2019), antinflammatória (LEU et al., 2019), antitrombótica (ASHAARI et al., 2020), antioxidante e antinociceptiva (DURAISAMY et al., 2021). Há registros também que comprovam a eficácia do OE de *P. amboinicus* em modelos *in vivo* por meio da modulação imune, melhorando a epitelização e produção de colágeno na contração da ferida (SIVARANJANI et al., 2019).

Outros estudos com amostras de OE dessa espécie demonstraram ação antifúngica contra: *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Aspergillus oryzae, Fusarium sp., Penicillium species* e *Sacharomyces cerevisiae* (KHARE et al., 2011). OLIVEIRA et al. (2007) demonstraram efeito sinérgico de amostras de OE de *P. amboinicus* quando associado ao itraconazol nas espécies: *C. albicans, C. tropicalis, C. krusei and C. stellatoidea*; e em associação ao cetoconazol também foi observado efeito sinérgico nas espécies*: C. albicans, C. guilliermondii* and *C. stellatoidea*. Estudos recentes demonstraram atividade antimicrobiana dos OEs, contra cepas de bactérias Gram-positivas (VASCONCELOS et al., 2017), Gram-negativas e fungos (SIVARANJANI et al., 2019).

2.2 Óleos essenciais

Os vegetais sintetizam uma variedade de substâncias químicas chamadas de metabólitos que se classificam em metabólitos primários e secundários. Os metabólitos primários são os responsáveis pelo fornecimento de substâncias essenciais ao crescimento e ao desenvolvimento das plantas, enquanto os metabólitos secundários geralmente estão envolvidos com a proteção, defesa e atração de animais polinizadores, além de terem aplicação no setor industrial (CHIOCCHIO et al., 2021).

As classes de metabólitos secundários, ao contrário dos metabólitos primários, têm distribuições restritas a determinados grupos taxonômicos, estando divididas principalmente em três grupos majoritários: terpenos, compostos fenólicos e compostos nitrogenados (BORGES & AMORIM, 2020). Diferentes condições ambientais e de estresse podem afetar a expressão dos genes envolvidos nas vias dos metabólitos secundários, influenciando no crescimento e na sobrevivência das espécies envolvidas (LI et al., 2020). As três rotas principais de formação dos metabólitos secundários são: via acetato malonil, acetato mevalonato, metil-eritritol-P e via shiquimato (SIMÕES et al., 2017).

OE compreendem uma complexa mistura de substâncias, odoríferas, voláteis, líquidas, de natureza lipofílica, constituídas predominantemente por terpenos (monoterpenos e sesquiterpenos) e/ou fenilpropanoides (STEPHANE & JULES, 2020). Os estudos químicos de OE das folhas de *P. amboinicus* demonstram a presença predominante de compostos terpênicos, principalmente monoterpenos e sesquiterpenos (ARUMUGAM & SWAMY, 2016; RUAN et al., 2019).

A produção e a concentração de terpenoides, assim como outros metabólitos secundários, sofrem a interferência de diversos fatores de regulação como: cofatores metálicos, estágio desenvolvimento, fatores ambientais, etc (RIVERA-PEREZ et al., 2015; VATTEKKATTE & BOLAND, 2020).

Terpenos são compostos orgânicos voláteis, constituídos por isopreno (bloco de construção de cinco carbonos-C), derivados do isopentenil pirofosfato (IPP) e do dimetilalil pirofosfato (DMAPP) (LOUIE et al., 2020). O alongamento desta cadeia gera pirofosfato de geranil (GPP) precursor de monoterpenos e o encadeamento com mais outro IPP origina o pirofosfato de farnesil (FPP), precursor dos sesquiterpenos. Tanto GPP quanto FPP são substratos para reações na clivagem mediada por metal da porção pirofosfato e posterior ciclização intramolecular que geram diversos compostos com sistemas de anéis complexos (VATTEKKATTE et al., 2018). São classificados baseados no número de unidades isoprênicas, como exemplo: monoterpenos com duas unidades isoprênicas (10C), sesquiterpenos com três unidades (15C) (TAIZ et al., 2021).

Os pirofosfatos: pirofosfato de geranil (GPP), pirofosfato de farnesil (FPP) e pirofosfato de geranil geranil (GGPP) são substratos que as terpeno-sintases usam na produção dos terpenoides do tipo mono, sesqui e diterpenos (VATTEKKATTE & BOLAND, 2020). A especificidade do produto das terpeno-sintases é conhecida por ser influenciada pelo tipo de cofator de metal ligado pela enzima, como demonstrado em estudos (LOPEZ-GALLEGO et al., 2010; VATTEKKATTE et al., 2018). Esses cofatores metálicos agem neutralizando a carga negativa do pirofosfato, além de propiciar a ionização do difosfato alílico, sendo raras as terpenos sintases sem cofatores metálicos (VATTEKKATTE et al., 2018).

A ativação do pirofosfato ocorre com a participação de íon metálico divalente (WANG et al., 2019). Cofatores metálicos são necessários para as terpeno-sintases, sendo Magnésio (Mg) e Manganês (Mn) os mais encontrados nas monoterpenos e sesquiterpenos sintases (VATTEKKATTE & BOLAND, 2020).

Os OE são encontrados em diversos órgãos, sendo as folhas as que mais produzem e armazenam esses produtos. Estes são obtidos através de várias técnicas como prensagem, enflorage, destilação a vapor, hidrodestilação e extração com líquido supercrítico (BURGER et al., 2019; SIMÕES et al., 2017). Os OEs podem servir como atrativo para polinizadores assim como repelir a ação de predadores (LUZ et al., 2020) e a maioria dos OEs obtidos de plantas vasculares demonstrou ser eficaz no tratamento de infecções fúngicas e bacterianas (GHAVAM et al., 2020).

Suas propriedades terapêuticas estimulam o aumento no uso de produtos naturais (SALAMON et al., 2018), com importância nas áreas farmacêutica, alimentícia, cosmética, agricultura, saneantes (STEPHANE & JULE, 2020) e aplicação como flavorizantes e aromatizantes (SHARMEEN et al., 2021). Devido as suas atividades antimicrobiana, antioxidante e anticâncer têm sido foco de estudos relacionados à fitomedicina e à aromaterapia (STEPHANE & JULES, 2020).

Carvacrol é um monoterpeno isômero do timol (SOUZA et al., 2021). Segundo BADI et al. (2017), carvacrol provavelmente deriva do γ-terpineno por oxidação, enquanto γ-terpineno via p-cimeno é o precursor de thimol. Thimol é um monoterpeno citado na literatura como um dos componentes do OE de *P. amboinicus* e considerado um dos seus quimiotipos (MONZOTE et al., 2020), com provável ação sinérgica entre p-cimeno, γ-terpineno e carvacrol como agentes antimicrobianos e antifúngicos (BADI et al., 2017) e agentes fumigantes, no combate a insetos (PAVELA, 2008).

Testes *in vivo* and *in vitro* demonstraram ação antibacteriana contra *K. pneumoniae* (SOUZA et al., 2021). É também capaz de causar a morte celular bacteriana, por esvaziamento do citoplasma em bactérias do tipo *Streptococcus pyogenes* (WIJESUNDARA et al., 2021) e de inibir a produção de exopolissacarídeo e a origem de biofilmes de *Enterobacter cloacae* (LIU et al., 2021).

As quantidades de OE produzidos sob condições de deficiência hídrica podem ser modificadas dependendo da espécie e magnitude do estresse. A restrição hídrica pode comprometer o teor de OE: a) aumentar, como em espécies de *Ocimum* (SANTOS et al., 2016) e *Origanum vulgare* L. subsp. g*racile* (orégano) (MORSHEDLOO et al., 2017); b) diminuir, como em *Lippia sidoides* Cham (alecrim-pimenta) (ALVARENGA et al., 2011); c) ou até mesmo provocar resultados oscilatórios conforme a intensidade e duração da redução hídrica, como nas espécies de *Cymbopogon* (SANGWAN et al., 1993; SINGH-SANGWAN et al., 1994).

Os OE também podem sofrer alterações qualitativas em sua composição química devido à deficiência hídrica, como demonstrado por BETTAIEB et al. (2009) com *Salvia officinalis* L. (sálvia). Porém, em geral, em OE das espécies sob déficit hídrico há somente alterações quantitativas com aumento do rendimento e do teor de seus componentes majoritários (MCKIERNAN et al., 2016; MAATALLAH et al., 2016). Essa alteração na composição do OE varia em função da intensidade da redução da aplicação hídrica e das características de cada espécie, como verificado em alguns estudos (VAZIN, 2013; YADAV et al., 2014).

2.3 O cultivo de plantas medicinais

As plantas medicinais representam um importante recurso terapêutico tanto como fonte de fármacos como matérias-primas para produção de fitoterápicos que são medicamentos que se caracterizam pela presença de um fitocomplexo, sem apresentar substâncias ativas sintéticas ou mesmo isoladas de plantas. Para a utilização segura e racional das plantas medicinais é necessário realizar estudos que possibilitem sua validação como ferramentas terapêuticas. Os estudos científicos de validação de plantas compreendem diversas metodologias, envolvendo estudos etnodirigidos, botânicos, agronômicos, farmacológicos, de toxicidade, desenvolvimento farmacêutico, além dos ensaios clínicos (BRASIL, 2012; SILVA et al., 2017).

Considerando a Política Nacional de Plantas Medicinais e Fitoterápicos que incentiva o uso sustentável da biodiversidade brasileira, o cultivo de plantas medicinais vem ganhando destaque no meio científico, visando assegurar condições ideiais de plantio e colheitas das espécies vegetais com interesse terapêutico (SCHAEFFER et al., 2021). Assegurando condições ideais de plantio, pode-se garantir etapa importante na qualidade das plantas medicinais (ALIPOURKHASHT et. al., 2021; JAFARI & ALIZADEH, 2021).

As modificações dos habitats e as colheitas de plantas medicinais sem um planejamento e controle ambiental pode ameaçar a existência de algumas espécies. A conservação e o uso sustentável de plantas medicinais na produção de medicamentos fitoterápicos é essencial para a manutenção das espécies e de seus constituintes químicos (RASHID et al., 2021). Parte das plantas medicinais e condimentares cultivadas no país são nativas de outros países e ambientes, como por exemplo *P. amboinicus* que é originária da Índia. Tal fato pode alterar as condições ideais de desenvolvimento, interferindo na produção de suas sementes e/ou flores em algumas regiões e até mesmo nas características qualitativas e quantitativas de seus metabólitos (CARVALHO, 2015).

A mudança do habitat natural e ainda as alterações climáticas a que o vegetal está exposto podem alterar as características químicas de uma espécie, seja de modo qualitativo ou quantitativo, que refletirá no medicamento fitoterápico a ser produzido (KFOURY et al., 2019; YANG et al., 2019). A geo fitoterapia avalia a adaptação das plantas medicinais em outros ambientes (SHEN et al, 2021). Para minimizar estas interferências aplicam-se métodos de melhoramento no cultivo, como barreiras de ventos, adubação com fontes orgânicas, controle da luminosidade, entre outros (CARVALHO, 2015).

O cultivo com propósito comercial é uma alternativa de manutenção das plantas medicinais e um estímulo econômico ao produtor rural, como na agricultura familiar, garantindo assim a conservação e a sustentabilidade das plantas medicinais (RANA et al., 2020). A importância do cultivo de plantas medicinais decorre do interesse global e do crescente mercado de fitoterápicos, visando aumentar a produção e garantir a sua qualidade (NITHIYA et al., 2015). No BRASIL, devem ser aplicadas as Boas Práticas Agrícolas (BPA) na produção de plantas medicinais para a produção regulamentada de fitoterápicos pela autoridade sanitária. As BPA orientam sobre o cultivo, coleta/colheita, beneficiamento, secagem e armazenamento da espécie vegetal (BRASIL, 2014).

É importante destacar a necessária capacitação dos agricultores que cultivam plantas medicinais como um dos fatores para a garantia da qualidade dos constituintes vegetais que serão usados na produção de fitoterápicos (SILVA & MORAES, 2008; GERAIS, 2016). Pesquisas sobre o cultivo de plantas medicinais registram a importância no manejo de nutrientes para garantir a sustentabilidade do cultivo e qualidade da produção (LEMA & ABEWOY, 2021). Entender como cada espécie reage as alterações climáticas são etapas importantes no cultivo comercial e na garantia de qualidade na matéria prima vegetal produzida (LI et al., 2020; SHEN et al. 2021).

A petição de registro de fitoterápicos no órgão regulador da ANVISA requer a apresentação de algumas informações, como: detalhes da coleta/colheita no laudo de análise da droga vegetal, período e local da coleta; condições do tempo no momento da coleta; estágio de desenvolvimento da planta; se cultivada ou espontânea. Além disso, no caso de plantas originadas de cultivo, deve-se informar as condições de cultivo aplicadas: substrato utilizado, tipo de adubação, modo de irrigação, luminosidade, procedimento de coleta, uso de agrotóxicos. Estas exigências decorrem da influência que as técnicas de cultivo (colheita) ou técnicas extrativistas (coleta) exercem sobre a ação terapêutica da droga vegetal, do derivado vegetal e do produto acabado (BRASIL, 2014).

2.4 Fatores interferentes no desenvolvimento vegetal

A absorção de nutrientes pelos vegetais é influenciada por fatores externos e internos (GUIMARÃES et al., 2020b), que modificam a velocidade de absorção destes nutrientes, como os fatores ambientais e as interações entre estes nutrientes (SANTANA et. al., 2008).

Nas folhas, representantes do principal órgão de fotossíntese, pode haver variações dos metabólitos secundários em decorrência do seu período evolutivo e dos fatores ambientais como: estação do ano, temperatura e umidade do solo (GOMES et al., 2019). O diagnóstico nutricional da planta conduz ao melhor aproveitamento e rendimento deste vegetal, sugestionando as condições mais favoráveis de cultivo. Pode ser feito através da associação e interpretação de dois estudos: análise de solo e análise de tecidos vegetais, o que possibilita a avaliação de diferentes adubações e das causas de deficiências nutricionais (ROZANE et al., 2016).

Os principais fatores extrínsecos que influenciam o crescimento e o desenvolvimento das espécies vegetais são água, elementos minerais na solução do solo, temperatura e luz. Fatores climáticos (como precipitação) e solo (relacionados às condições do solo) afetam o desempenho de todas as plantas, incluindo crescimento, desenvolvimento, reprodução e sobrevivência (TAIZ et al., 2021).

Há estudos que comprovam os efeitos negativos da redução hídrica no cultivo de espécies vegetais (ELSHIBLI et al., 2016; GUIMARÃES et al., 2016), mas apesar da limitação hídrica ocasionar efeitos danosos nos vegetais de modo geral, o excesso de água também não é benéfico para o vegetal. A dinâmica da física do solo é alterada de maneira significativa pela saturação hídrica (GAZOLA et al., 2014). O excesso de água (por irrigação ou pluviosidade) também pode provocar estresse e alterar crescimento e desenvolvimento de espécies vegetais, com redução da produtividade (AZEVEDO et al., 2011; DUTRA et al., 2012).

Os elementos minerais (principalmente nitrogênio, fósforo e nutrientes do solo) são necessários para a construção do corpo vegetal, assim como para as reações bioquímicas por serem constituintes importantes de muitas enzimas e cofatores. A falta desses elementos minerais leva ao comprometimento do crescimento e pode resultar em morte do vegetal (BRUKHIN & MOROZOVA, 2011). Os nutrientes são basicamente transportados para as raízes por fluxo de massa e difusão, portanto a disponibilidade de água no solo influencia a movimentação de nutrientes por esses dois mecanismos para a aquisição de macronutrientes (N, P, K, Ca, Mg e S) e micronutrientes catiônicos (Fe, Mn, Zn e Cu) (OLIVEIRA et al., 2010).

O estado nutricional do solo reflete as suas características químicas (MELO et al., 2015) e interfere na produtividade e no rendimento da espécie cultivada (PARIS et al., 2020). Parte dos solos brasileiros apresentam elevada acidez com baixo teor nutricional e de matéria orgânica (PARIS et al., 2020). É necessário avaliar a fertilidade do solo através de análises químicas para garantir uma adequada nutrição no planejamento dos cultivos. Desequilíbrios nutricionais do solo podem acarretar alterações fisiológicas mais perceptíveis nas folhas dos vegetais e a avaliação deste estado nutricional é feita mediante análise química (COELHO et al., 2008; PREZOTTI & GUARÇONI, 2013).

O estudo foliar pode ser usado como ferramenta de monitoramento do estado nutricional das plantas para o cultivo comercial, por determinar as necessidades e quantidades dos nutrientes a serem utilizados para cada espécie (CAVALCANTI et al., 2021). Desta forma, a análise do solo associada a diagnose foliar direciona sobre o tipo de adubação mais adequada e ao mesmo tempo econômica visando fornecer os nutrientes necessários a uma adequada produção (TECCHIO et al., 2006).

A aplicação de fertilizantes orgânicos e inorgânicos supre as carências nutricionais dos solos usados em cultivos continuados. O uso de fertilizantes orgânicos aumenta a formação de substâncias antioxidantes e bioativas, enquanto os inorgânicos aumentam o rendimento em um menor intervalo de tempo (LEMA & ABEWOY, 2021). Portanto, a adubação orgânica é uma fonte de nutrientes essenciais, de baixo custo e fácil acesso ao produtor (BENEDETTI et al., 2019).

Como as áreas de alimentos, cosméticos, saneantes e produtos farmacêuticos requerem cada vez mais uma produção de óleos essenciais de maior qualidade, oriundos de plantas aromáticas, um novo artifício aplicado está sendo o cultivo destas espécies em áreas de seca (SABRA et al., 2018).

Adequações nos processos de cultivos, colheitas, processamento e armazenamento são recursos que garantem a melhor produção e rendimento dos metabólitos (SALAMON et al., 2018). Entretanto, apesar de existirem pesquisas visando o estabelecimento de técnicas apropriadas de cultivo para obtenção de espécies vegetais de melhor qualidade e rendimento (GAMA et al., 2012; VILANOVA et al., 2018), não há estudos sobre o impacto da variação das condições de cultivo de *P. amboinicus* no teor nutricional de suas folhas e consequentemente na atividade antimicrobiana do seu OE.

3 OBJETIVOS

3.1 Objetivo Geral

Avaliar os processos de cultivo e obtenção de óleo essencial de *Plectranthus amboinicus* (Lour.) Sprengel com melhor atividade antimicrobiana.

3.2 Objetivos Específicos

Caracterizar os tratamentos de solos aplicado nos cultivos de *P. amboinicus*;

Determinar o estado nutricional das folhas originárias dos cultivos;

Determinar o rendimento e os índices físico-químicos dos óleos essenciais obtidos nas extrações das folhas dos diversos cultivos;

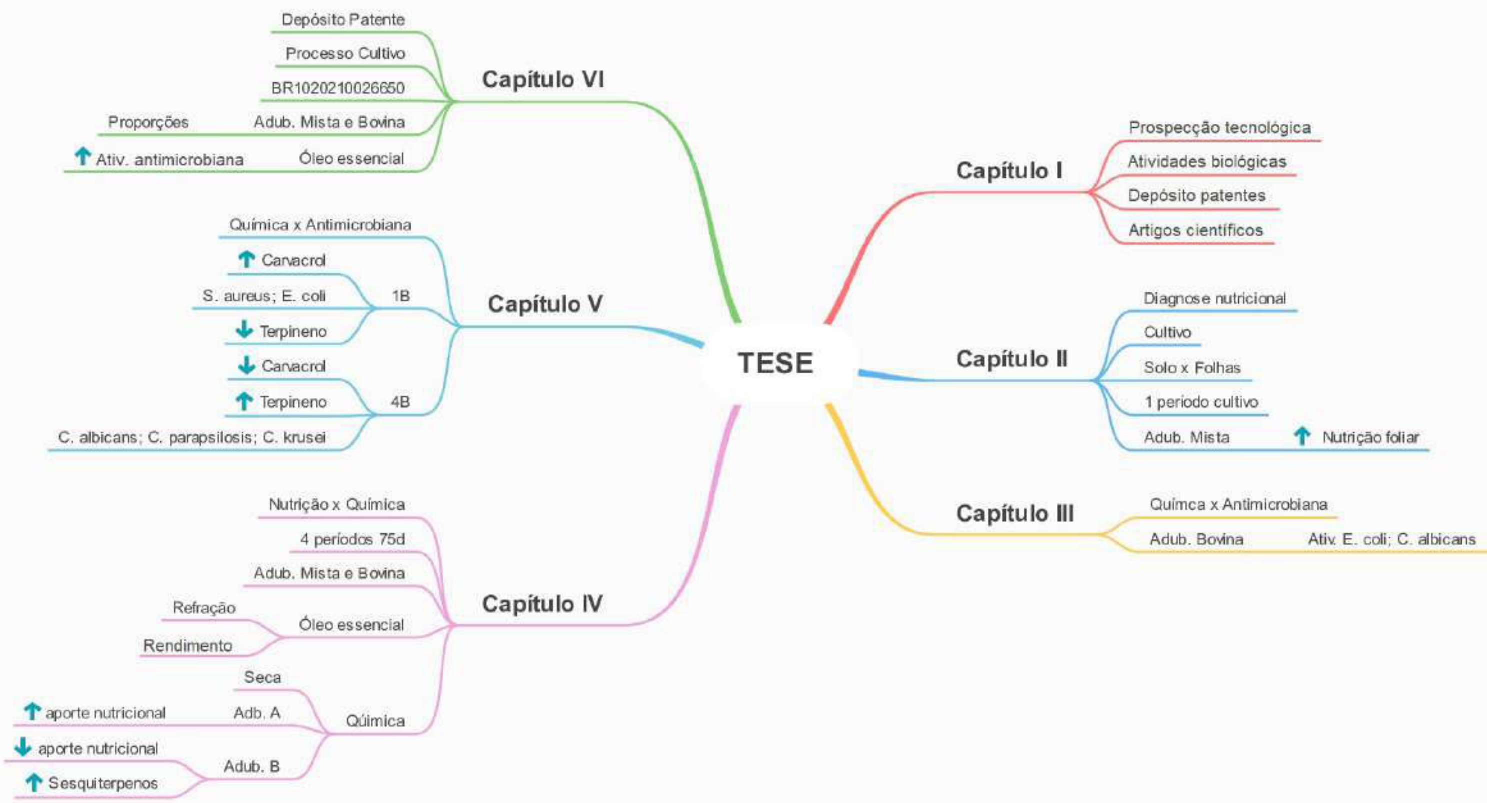
Identificar a composição química dos óleos essenciais em estudo;

Correlacionar o estado nutricional do solo à absorção de nutrientes da folha com a composição química dos óleos essenciais obtidos;

Determinar a atividade antimicrobiana das amostras estudadas de óleos essenciais;

Determinar as condições ideais de cultivo para obter óleo essencial com a melhor atividade antimicrobiana.

4 CAPÍTULOS


TESE
Capítulo I
Prospecção tecnológica
Atividades biológicas
Depósito patentes
Artigos científicos
Capítulo II
Diagnose nutricional
Cultivo
Solo x Folhas
1 período cultivo
Adub. Mista
Nutrição foliar
Capítulo III
Quimca x Antimicrobiana
Adub. Bovina
Ativ. E. coli; C. albicans
Capítulo IV
Nutrição x Química
4 períodos 75d
Adub. Mista e Bovina
Óleo essencial
Refração
Rendimento
Qúimica
Seca
Adb. A
aporte nutricional
Adub. B
aporte nutricional
Sesquiterpenos
Capítulo V
Química x Antimicrobiana
1B
Carvacrol
S. aureus; E. coli
Terpineno
4B
Carvacrol
Terpineno
C. albicans; C. parapsilosis; C. krusei
Capítulo VI
Depósito Patente
Processo Cultivo
BR1020210026650
Adub. Mista e Bovina
Proporções
Óleo essencial
Ativ. antimicrobiana

CAPÍTULO 1

Technological prospection of the biological activities of *Plectranthus amboinicus* (Loureiro) Sprengel: an integrative review

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Technological prospection of the biological activities of *Plectranthus amboinicus* (Loureiro) Sprengel: an integrative review

Prospecção tecnológica das atividades biológicas de *Plectranthus amboinicus* (Loureiro) Sprengel: uma revisão integrativa

Prospección tecnológica de las actividades biológicas de *Plectranthus amboinicus* (Loureiro) Sprengel: una revisión integradora

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Abstract

Plectranthus amboinicus (Loureiro) Sprengel is popularly known as malvariço or indian borage. This paper carries out the technological prospecting of this species with an emphasis on its biological activities. The research consisted of an integrative literature review based on information collected from patent and electronic databases. Initially, 3019 results were obtained, which, after the screening, selection and analysis process, resulted in 55 articles and 48 patent documents. It was found that 77.08% (n=37) of patent deposits refer to obtaining or improving the process and 22.92% (n=11) to the finished product related to the following indications: wound healing, mainly in diabetics,

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followed by treatment of rheumatoid arthritis, hepatocellular carcinomas, breast cancer and melanoma, treatment for acne caused by *P. acnes*, *S. aureus* and *P. aeruginosa*, oral cavity cleansing agent, treatment for osteoporosis, nutraceutical composition for lactation and insect repellent. In the databases of scientific articles, 55 documents were selected, proving the following actions: antimicrobial, antioxidant, anticarcinogenic, anti-inflammatory, diuretic, nephroprotective, larvicide, analgesic, veterinary food additive, anti-leishmania, antipsoriasis, antidiarrheal, antidiabetic, hepatoprotective and anthelmintic. The scientific studies analyzed reaffirm other actions reported in popular use, but which are not described in the patent processes. Therefore, there are many proven biological properties of *P. amboinicus* that should be further explored and exploited in the pharmaceutical, cosmetic and food sectors in order to generate new products and patents.
Keywords: *Plectranthus amboinicus* (Loureiro) Sprengel**;** Prospecting; Malvariço; Indian Borage; Patents.

Resumo
Plectranthus amboinicus (Loureiro) Sprengel é popularmente conhecida como malvariço ou *indian borage.* Esta pesquisa realiza a prospecção tecnológica desta espécie com ênfase em suas atividades biológicas. A pesquisa consistiu em uma revisão integrativa de literatura baseada em informações coletadas em bancos de dados de patentes e bases de dados eletrônicas. Obteve-se inicialmente 3019 arquivos, que após processo de triagem, seleção e análise, resultaram em 55 artigos e 48 documentos de patentes. Constatou-se que 77.08% (n=37) dos depósitos de patentes referem-se a obtenção ou melhoramento de processo e 22.92% (n=11) à produto acabado relacionados as seguintes indicações: cicatrização de ferimentos principalmente em pacientes diabéticos, seguido de tratamento de artrite reumatoide, carcinomas hepatocelular, câncer de mama e melanoma, tratamento para acne causada por *P. acnes*, *S. aureus* e *P. aeruginosa*, agente de limpeza da cavidade oral, tratamento para osteoporose, composição nutracêutica para lactação e repelente de insetos. Nas bases de dados de artigos científicos selecionaram-se 55 documentos, comprovando-se as seguintes ações: antimicrobiana, antioxidante, anticarcinogênica, antiinflamatória, diurética, nefroprotetora, larvicida, analgésica, aditivo alimentar de uso veterinário, anti-leishmania, antipsoríase, antidiarreica, antidiabética, hepatoprotetora e antihelmíntica. Os estudos científicos analisados reafirmam outras ações relatadas no uso popular, mas que não estão descritas nos processos de patentes. Portanto, existem muitas propriedades biológicas comprovadas de *P. amboinicus* que devem ser mais exploradas e aproveitadas nas áreas farmacêutica, de cosméticos e de alimentos visando a geração de novos produtos e patentes.
Palavras-chave: *Plectranthus amboinicus* (Loureiro) Sprengel; Prospecção; Malvariço; Indian Borage; Patentes.

Resumen
Plectranthus amboinicus (Loureiro) Sprengel se conoce popularmente como malvariço o borraja india. Esta investigación realiza la prospección tecnológica de esta especie con énfasis en sus actividades biológicas. La investigación consistió en una revisión integradora de la literatura basada en información recopilada de bases de datos de patentes y bases de datos eletrónicas. Inicialmente se obtuvieron 3019 expedientes que, luego del proceso de cribado, selección y análisis dieron como resultado 55 artículos y 48 documentos de patente. Se encontró que el 77.08% (n=37) de los depósitos de patentes se refieren a la obtención o mejora del proceso y el 22.92% (n=11) al producto terminado con las siguientes indicaciones: cicatrización de heridas principalmente en pacientes diabéticos, seguida de tratamiento de artritis reumatoide, carcinomas hepatocelulares, cáncer de mama y melanoma, tratamiento del acne por *P. acnes*, *S. aureus* y *P. aeruginosa,* agente limpiador de la cavidad oral, tratamiento para la osteoporosis, composición nutracéutica para la lactancia y repelente de insectos. En las bases de datos de artículos científicos se seleccionaron 55 documentos, comprobando las siguientes acciones: antimicrobiano, antioxidante, anticancerígeno, antiinflamatório, diurético, nefroprotector, larvicida, analgésico, aditivo alimentario veterinario, antileishmanial, antipsoriasis, antidiarreico, antidiabético, hepatoprotector y antihelmíntico. Los estudios científicos analizados reafirman otras acciones reportadas de uso popular que no están descritas en los procesos de patente. Por lo tanto, existen muchas propiedades biológicas comprobadas de *P. amboinicus* que deberían explorarse y explotarse más a fondo en los sectores farmacéutico, cosmético y alimentrio para generar nuevos productos y patentes.
Palabras clave: *Plectranthus amboinicus* (Loureiro) Sprengel**;** Prospección; Malvariço; Indian Borage; Patentes.

1. Introduction

Biotechnology research has evolved quickly and safely in the production of information, which can be seen by the growing development of new processes and products. Technological prospecting studies have shown a way of schematizing and informing technoscientific advances, which has narrowed the distance between invention and commercialization of products (Amparo et al., 2012; Caruso & Tigre, 2004; Oliveira & Quental, 2012).

The possibility of identifying and obtaining compounds with varied pharmacological actions has stimulated research on medicinal plants (Souto-Maior et al., 2011). Species of the genus *Plectranthus* (Lamiaceae) are found on four continents: Africa, America, Oceania, and Asia (Arumugam et al., 2016; Ruan et al., 2019).

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Plectranthus amboinicus (Loureiro) Sprengel, popularly known as malvariço or indian borage, is a herbaceous plant, up to one meter long, succulent with a brittle stem, oval leaves with thick apex, jagged edges and a thick petiole with a sharp tip. Phytochemical studies have described the existence of 76 volatile and 30 non-volatile compounds that belong to different classes of constituents, such as monoterpenes, diterpenes, triterpenes, sesquiterpenes, phenolics, flavonoids, esters, alcohols and aldehydes, with diterpenoids, triterpenoids and essential oils (OEs) being the most common metabolites. Some noticeable pharmacological actions of this plant are: antimicrobial, anti-inflammatory, antitumor, healing, antiepileptic, and analgesic activities (Arumugam et al., 2016; Ruan et al., 2019).

The low cost and therapeutic benefits presented have led part of the population to use natural products. New products made from medicinal plants originated in popular knowledge and were later scientifically proven. The systematization of records of the use of this species aims to stimulate access to innovative research and the generation of patents through a selection of bioassays that exhibited biological effects in a simple, sensitive and safe way.

This research sought to identify scientific and technological advances in processes and/or products associated with biological properties, in order to create an updated information source that disseminates the evidence of the pharmacological action. The idea of conducting a logical and rational analysis of the existing data in patent databases and academic research reveals the current panorama of the world market and leads to a compendium of relevant information on the main lines of research developed with *P. amboinicus*.

2. Methodology

The research consisted of an integrative literature review that seeks the synthesis of knowledge and the incorporation of the applicability of the results of significant studies in practice (Silveira, 2005; Souza et al., 2010). It was based on information collected from databases of patents and scientific articles. The question that guided the study was: Are there scientifically proven therapeutic actions by *Plectranthus amboinicus* that have not yet been patented?

2.1 Data sources

Scientific research was carried out in four electronic databases: SCIELO (Scientific Electronic Library Online), Google Scholar, PUBMED (National Library of Medicine of the United States, National Institutes of Health) and CAPES Portal (Coordination for the Improvement of Higher Education Personnel). The technological prospection was also carried out on four patent databases: USPTO (United States Patent and Trademark Office), WIPO (World Intellectual Property Organization), ESPACENET, and INPI (National Institute of Industrial Property). The search was performed by combining two criteria: i) descriptors and ii) search for international patent classification – IPC – codes (prospecting). Scientific names were used for titles and abstracts, with or without the Boolean operator "AND". The descriptors used in the study are described in Figure 1.

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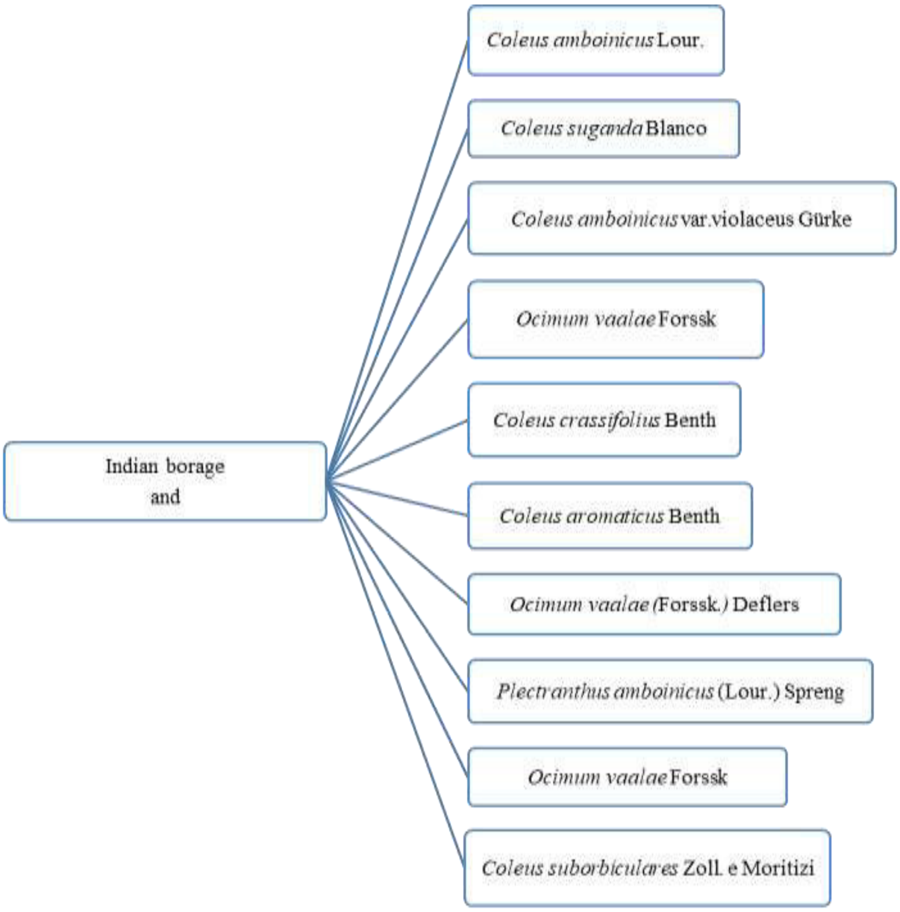
Figure 1. Heteronyms isolated or associated with Boolean operators.



Source: Authors (2021).

2.2 Selection criteria

The selection criteria were based on the inclusion and non-inclusion criteria established by the researchers. Among the 3019 files found, 154 documents (83 articles and 71 patents) were selected for reading in full. The inclusion criterion covered materials that addressed: technological applications, therapeutic uses, formulations developed and the parts used of this species. The non-inclusion criteria were: year of publication of the articles, duplication in the patent databases and academic research consulted, articles and/or patents whose focus was not directed to biological uses and properties, development of formulation or technologies involving this species.

During the research with heteronyms of the species *P. amboinicus* (taken from the site: reflora.jbrj.gov.br) and the Boolean operator "AND" plus "Indian Borage", the files were selected and quantified, excluding those that did not correspond to the criteria. Review articles, conference abstracts and articles that studied their effects associated with other plants or other properties of the same were excluded. The selection and quantification of the data obtained in each analysis process can be seen in Figure 2.

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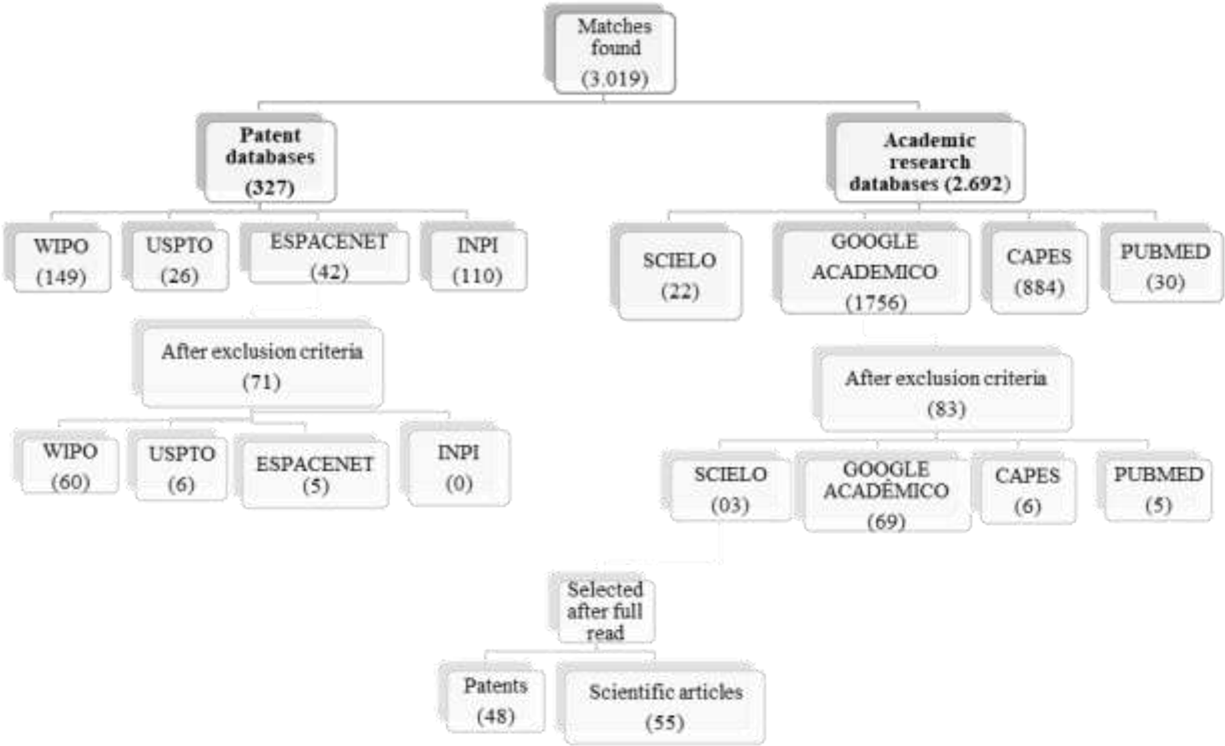
Figure 2. Process of sorting and selecting data in patent and academic research databases.



( ): represents the number of selected files. Souce: Authors (2021).

2.3 Data analysis

Data collection and analysis was carried out in January 2020, through a selection of scientific articles published in the last 10 years (from January 2010 to December 2019) and all published patents found in the patent databases, without temporal (1976 to 2019) or language delimitations, to discuss and cross-check the data found. The list of relevant article references to the research was verified and, then, they were subjected to an exploratory and selective reading to identify data related to the proposed theme according to the objective of the study.

The first step was to analyze the titles and read the abstracts. The files that met the inclusion criteria were read in full and then selected according to the objective of this work. Some aspects that guided the analysis of the archives were the year of publication, research articles and essential observations on the proposed theme. Regarding the analysis of the patents, the criteria used to determine compliance with the research topic were: title, abstract, documentation and patent number. The files that had the scientific or popular name of the plant in the title and/or abstract were selected for further analysis of prospection by classification, year and country.

The extracted data were organized in a Microsoft® Excel spreadsheet. After corrections, the information was exported to the Statistical Package for the Social Sciences (SPSS) software, version 26.0, where statistical processing was performed. To characterize the sample, descriptive statistics were performed using simple frequency.

3. Results and Discussion

The data selection process (scientific articles and data from patent databases) initially generated 3019 files matching the chosen descriptors and study period, including files duplicated by the research sources. Of the 3019 files retrieved, 2692

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results corresponded to scientific articles and 327 corresponded to results in patent databases. The quantification of these data can be observed in Table 1.

After selection and analysis (titles and abstracts), 83 scientific articles and 71 patents issued (154 documents) were chosen to be read in full. For a better selection of data, a search for therapeutic indications was carried out in patent database and academic research, with a subsequent reading in full of the selected results. After a critical review of the articles, 48 patent documents and 55 scientific publications were selected for further analysis and discussion.

The search for scientific articles in the chosen electronic databases provided 2692 initial results, most of them in Google Scholar (n = 1756), followed by the Portal of CAPES (n = 884), PUBMED (n = 30) and SCIELO (n = 22). The search in the patent databases returned 327 results, most of them found in WIPO (n = 149), followed by INPI (n = 110), ESPACENET (n = 42) and USPTO (n = 26).

After the initial results obtained from the patent databases (n = 327) were filtered by the inclusion / exclusion criteria, 57 issued patents were returned. It was observed, however, that 05 of the USPTO results and 03 of ESPACENET results were also filed at WIPO and 1 result was filed. After the final selection, 48 issued patents were chosen for final analysis, all extracted from WIPO.

The databases were searched and the collected date were crossed. Some differences were observed in the results obtained with the use of popular synonyms and their applications in the formulations developed in the selected works (Table 1).

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Table 1. Initial quantification of data obtained with the selected descriptors.

Descriptors	WIPO	USPTO	INPI	CAPES	ESPACENET	GOOGLE SCHOLAR	PUBMED	SCIELO
C.amboinicus Lour	43	7	0	205	12	973	02	2
C. amboinicus Lour And Indian Borage	26	0	0	277	13	424	0	0
Ocimum vaalae Forssk	0	0	04	01	0	0	02	0
Ocimum vaalae Forssk.AND Indian Borage	0	0	0	0	0	0	02	0
Coleus amboinicus var.violaceus Gürke	0	0	36	0	0	3	0	0
Coleus amboinicus var. Violaceus *Gürke* AND Indian Borage	0	0	36	0	0	0	0	0
Coleus amboinicus var.violaceus Gürke	0	0	0	0	0	2	0	0
Coleus amboinicus var.violaceus Gürke AND Indian Borage	0	0	0	0	0	0	0	0
Coleus suganda Blanco	03	0	0	03	01	269	0	0
Coleus suganda Blanco AND Indian Borage	02	0	0	02	0	0	0	0
Plectranthus amboinicus (Lour.) Spreng	50	2	02	205	0	12	22	20
Plectranthus amboinicus (Lour.) Spreng *AND Indian Borage*	11	3	0	31	0	9	02	0
Coleus carnosus Hassk.	02	0	0	02	0	59	0	0
Coleus carnosus Hassk. AND Indian Borage	02	0	0	01	0	6	0	0
Coleus subfrutectosus Summerh.	0	0	0	01	0	2	0	0
Coleus subfrutectosus Summerh. AND Indian Borage	0	0	0	0	0	1	0	0
Coleus aromaticus Benth	05	0	16	100	0	1	0	0
Coleus aromaticus Benth. AND Indian Borage	02	0	0	23	0	0	0	0
Coleus crassifolius Benth	02	0	0	01	16	0	0	0
Coleus crassifolius Benth AND Indian Borage	01	0	0	20	0	0	0	0
Coleus vaalae (Forssk.) Deflers	0	0	0	11	0	0	0	0
Coleus vaalae (Forssk.) Deflers *AND Indian Borage*	0	4	0	01	0	0	0	0
Coleus suborbicularis Zoll. & Moritzi	0	0	0	01	0	0	0	0
Coleus suborbicularis Zoll. & Moritzi AND Indian Borage	0	10	0	0	0	0	0	0
TOTAL	149	26	110	884	42	1756	30	22

Source: Authors (2021).

3.1 Prospecting of *P. amboinicus* in patent databases

In this study, 48 patents issued until 2019 were identified, 11 patents (22.92%) referring to finished product and 37 (77.08%) to processes. It was observed that the majority of patents are from Japan (n = 14/29.17%), followed by the USA (n = 08/6.67%), South Korea (n = 07/14.58%), China (n = 06/12.50%), Europe (n = 05/10.42%), Canada (n = 04/8.33%), Malaysia (n = 02/4.17%) and, finally, India (n = 01/2.08%) and Russia (n = 01/2.08%).

The analyzed patents on this species aimed at obtaining or improving processes or products with the following indications: wound healing, mainly in diabetic (n = 16/33.3%), treatment of rheumatoid arthritis (n = 14/29.1%), hepatocellular carcinoma, treatment of breast cancer and melanoma (n = 08/16.7%), treatment for acne by *P. acnes*, *S. aureus* and *P.*

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aeruginosa (n = 03/6.3%), oral cavity cleaning agent (n = 02/4.2%), treatment for osteoporosis (n = 02/4.2%), nutraceutical composition for lactation (n = 02/4.2%) and insect repellent (n = 01/2%).

It was also detected in the prospecting research of this species a greater number of patents for isolated use classified in the A61K group (97.3%) and one in the A61P group (2.7%). Associated use patents are classified into the groups listed in Table 2.

Table 2. Patents of the species P. amboinicus (Loureiro) Sprengel classified by International Patent Classification (IPC)

***Patents on isolated use**	**N**	**%**
A61K	36	97.30
A61P	1	2.70
**** Patents on associated use**	**N**	**%**
A61K+A61P	5	45.45
A61K+C11D	2	18.18
A61K+A61P+A61F	2	18.18
A61F+A61P	1	9.09
A61K+A61Q+C11D+B01D	1	9.09

*A61K= preparations for medical, dental, or toilet purposes; A61P= specific therapeutic activity of chemical compounds
or medicinal preparations
**A61F= Bandages, dressings or absorbent pads; A61Q - specific use of cosmetics or similar toilet preparations; B01D - separation processes; C11D= detergent compositions
Source: Authors (2021).

From the data presented above, it can be seen that this species has been more studied in relation to the area of the A61K group (preparations for medical, dental or hygienic use), totaling 36 patents for isolated use (97.3%). The A61K group appears in all 48 patents consulted when associated with other purposes (classifications), directing all studies to applications in humans, mainly for medical, dental or toilet purposes. The pharma industry's R&D (Research and Development) sector in recent decades has shown only slight growth in innovation, while inventions and innovations in medical technologies have advanced more rapidly (Dutta et al., 2019).

According to the data obtained, the patents are mainly process patents, for human health purposes. These patents refer mainly to anti-inflammatory, healing and anti-tumor actions, especially with new extraction methods or improvement methods with a predominance of process patents.

3.2 Prospecting in scientific article databases

The search in academic research databases returned 83 documents, of which 55 were selected after reading in full. The following actions were identified: antimicrobial (n = 22), antioxidant (n = 12), anticarcinogenic (n = 8), anti-inflammatory (n = 7), diuretic and nephroprotective (n = 5), analgesic (n = 3), larvicide (n = 3), veterinary food additive (n = 02), antileishmania (n = 2), antipsoriasis (n = 1), antidiabetic (n = 1), antidiarrheal (n = 1), hepatoprotective (n = 1) and anthelmintic (n = 1). It is noteworthy that of the 55 articles selected, 11 analyzed more than one pharmacological action. Nine of these files simultaneously investigated two pharmacological activities, one file made a study with three pharmacological actions (Bhatt et al., 2013) and the other document (El-hawary et al., 2012) analyzed five pharmacological actions.

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3.2.1 Antimicrobial and antidiarrheal activities

In twenty-two selected articles on antimicrobial activity, studies with extracts and OE extracted from the leaves were found. These studies confirmed its activity against gram negative bacteria: *Enterobacter sp*, *E. coli*, diarrheogenic *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella sp*, *Shigella flexneri*, *Shigella sp*. (*Shigella bayedir*), *Vibrio* sp., *Yersinia enterocolitica* and also against gram positive bacteria: *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Lactobacillus acidophilus*, *Methicillin resistant Staphylococcus aureus*, *Streptococcus mutans* and *Streptococcus pyogenes*.

The hydroalcoholic extract showed satisfactory results against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *S. aureus*, *Shigella bayedir* (Majee et al., 2013; Oliveira et al., 2013). Nanoparticles developed from the hydroalcoholic extract of had satisfactory results against: *C. albicans, Enterococcus faecalis* (Manojkanna et al., 2017), *Lactobacillus* and *Streptococcus mutans* (Manojkanna et al., 2019). In 2017, Sireesha et al. added information on the antibacterial activity of the ethanolic extract proving its action also against *Mycobacterium tuberculosis*.

Analyses with hydroalcoholic extract, carried out in other studies, also demonstrated activity against *Klebsiella pneumoniae* bacteria (Ismayil & Nimila, 2019) with action attributed to the major carvacrol (Goncalves et al., 2012) and against *Aspergillus niger* and *C. albicans* type fungi (El-hawary et al., 2012; Majee et al., 2013; Sivaranjani, 2019).

The hydroalcoholic extract of dry leaves at concentrations of 50mg/ml and 100mg/ml was also able to inhibit the growth of *Pseudomonas aeruginosa* (Sreelakshmy & Thangapandiyan, 2019), *Shigella bayedir* (Ismayil & Nimila, 2019), *Aspergillus niger, Bacillus subtilis* and *Staphylococcus aureus*, and its effect was compared to that of the standard drugs tested: Ampicilline trihydrate and Amphoterecine B (Majee et al., 2013). In the study by Bhatt & Negi (2012), the acetonic extract of the leaves showed superior activity to the ethyl acetate extract against the pathogens of food origin: *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Yersinia enterocolitica*.

In the study by Bhatt et al. (2013) with methanolic extract, the action of this species was proven against pathogens present in food infections such as: *Y. enterocolitica*, *E. coli*, *S. aureus* and *B. cereus*. Years later, Shubha & Bhatt (2015) published research on the prebiotic effect of the aqueous extract proving the ability of this extract to stimulate the growth of *Lactobacillus plantarum*. This probiotic bacterium consumed the constituents of the extract to produce metabolic enzymes, thus stimulating its growth. Still in this study, the extract inhibited the growth of pathogens (*E. coli* and *Salmonella typhimurium*), proving the popular use of this species as an antidiarrheal in foodborne infections.

Extracts and OE of this species were tested to analyse its viability as an anti-biofilm. There are studies that describe the property of nanoparticles, synthesized from the aqueous extract of its leaves treated with zinc nitrate solution, being able to inhibit biofilm formation of methicillin-resistant *Staphylococcus aureus* (Vijayakumar et al., 2015). In the study of efficacy against *Streptococcus pyogenes* biofilms, the methanolic extract of the leaves also showed inhibitory effect functioning as anti-biofilm (Manimekalai et al., 2016; Zhang et al., 2017). The OE tested on oxacillin- and vancomycin-resistant *S. aureus* species also worked as a viable alternative against these biofilms (Vasconcelos et al., 2017).

The OE extracted also showed activity against enteric pathogens: diarrhogenic *Escherichia coli*, *Salmonella sp*., *Shigella sp*. and *Vibrio sp.* in concentrations of 10 µg/ml (Hassani et al., 2012; Sivaranjani et al., 2019).

The leaves of indian borage can be used to treat opportunistic infections in HIV-infected patients, as its OE has an effect against *C. albicans* and *Cryptococcus neoformans* (Asiimwe, 2015). There are also reports of OE activity against fungi of the genus *Candida: C.albicans*, *C.parapsilosis* and *C.tropicalis* (EL-Zefzafy et al., 2016; Manjamalai et al., 2012; Santos et al., 2016; Vijayakumar et al., 2015).

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3.2.2 Antioxidant, anti-inflammatory and analgesic activities

In twelve articles, results showed antioxidant action, *in vitro*, of the leaves, roots and stems of this species. In most of these studies, the use of hydroalcoholic leaves extract showed better activity when compared to other types of solvents (Amarasiri et al., 2018; Bezerra et al., 2017; Bhatt & Negi, 2012; Bhatt et al., 2013; Bole & Kumudini, 2014; El-hawary et al., 2012; Haryani et al., 2018; Hasibuan et al., 2013; Patel et al., 2010a; Prasenjit et al., 2011). In the comparative study carried out by Bhatt & Negi (2012) with different types of solvents: hexane, acetone, ethyl acetate metanol and hydroalcohol, a greater antioxidant activity was detected in the ethyl acetate extract of the leaves, followed by the acetonic extract.

Seven articles reported anti-inflammatory activity, with aqueous, ethanolic extracts and/or OE. The anti-inflammatory activity of the ethanolic extract of the leaves proved to be excellent when compared to indomethacin and hydrocortisone, while the essential oil showed anti-inflammatory activity similar to diclofenac sodium (standard drugs tested) (Devi & Periyanayagam, 2010; El-hawary et al., 2012; Manjamalai et al., 2012).

There are records of the use of aqueous extract to inhibit pain induced by acetic acid and formalin and inflammation induced by carrageenan. It was found that this effect was related to the amount of carvacrol in the aqueous extract, resulting in the modulation of antioxidant enzymes in the liver, leading to a decrease in malondialdehyde, tumor necrosis factor alpha (TNF-α) and cyclooxygenase 2 (COX-2) production of paw edema in rats (Akinbo et al., 2018; Chiu et al., 2012; Leu et al., 2019). Thymoquinone was detected in the hexanic fraction of leaves as a compound that inhibits the expression of TNF-α (Chen et al., 2014).

Recent studies report that the constituents rosmarinic acid, cirsimaritin, salvigenin and carvacrol are correlated with the inhibition of inflammasome and activation of inflammatory responses, with an inhibitory activity observed in NLRP3 inflammasome blockade (Leu et al., 2019).

Three articles were found that prove analgesic activity: Chiu et al., 2012; El-Hawary et al., 2012 and Majee et al., 2013. In two studies, indomethacin was the standard drug used, and for El-Hawary et al. (2012), the best response was in alcoholic extracts and ethyl acetate fractions of the leaves while for Chiu et al. (2012) the best response was in the aqueous extract. In the study by Majee et al., 2013, using pentazocine as the standard drug, the analgesic effect was confirmed in the hydroalcoholic extract of the leaves. These records scientifically prove its popular use in pain relief.

3.2.3 Anticarcinogenic activity

Eight research papers identified anticarcinogenic activity (Bhatt et al., 2013; Hasibuan & Rosidah, 2015; Hasibuan & Sumaiyah, 2019; Hasibuan et al., 2013; Hasibuan et al., 2015; Rosidah & Hasibuan, 2014; Thirugnanasampandan et al., 2015; Yulianto et al., 2017).

In Bhatt et al. (2013) the sample extracted in methanol presented as constituents: total phenols, flavonoids and condensed tannins in greater proportions when compared to the others. Cytotoxic activity against cervical adenocarcinoma cells (HeLa) was detected in the hexane and alcoholic leaves extracts (Hasibuan et al., 2013; Hasibuan & Rosidah, 2015). In the study by Hasibuan et al. (2013) the ethyl acetate extract showed the highest content of phenolic compound and the best antioxidant and anticancer response.The ethanolic extract was transformed into nanoparticles by gelation, to test its cytotoxicity against HeLa, MCF-7 and T47D cells *in vitro*. There are also records of studies showing synergism with doxorubicin in ethyl acetate extract to inhibit metastasis in T47D breast cancer cells (Hasibuan et al., 2015; Rosidah & Hasibuan, 2014).

There are also reports of the anticarcinogenic potential, with application of nanoparticles of the ethanolic extract to improve bioavailability in the investigation of antiproliferative and pro-apoptosis effects in T47D breast cancer cell lines, as well as studies with essential oil in colorectal cancer cell lines (HT-29). It was found that OE can be a protector of DNA

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damage in 3T3-L1 fibroblast cells, depending on the applied concentration (Hasibuan & Sumaiyah, 2019; Thirugnanasampandan et al., 2015).

3.2.4 Diuretic e nephroprotective activities

The diuretic and nephroprotective activities of the ethanolic extract of the leaves were tested using furosemide as a standard drug in nephropathies induced acetaminophen. The research showed an increase in the total production of urine and urinary electrolytes, proving the nephroprotective and antioxidant effects (Palani et al., 2010).

The studies by Amarasiri et al. (2018), El-hawary et al. (2012) and Patel et al. (2010b) evaluating the nephroprotective and diuretic effects of aqueous and ethanolic leaves extracts also showed a significant increase in urinary volume and urinary electrolytes. Analyses with alcoholic leaves extract showed promising results in cisplatin-induced nephropathies, suggesting that the extract increases the expression of TGF-1β (transforming growth factor-1β) and, consequently, inhibits renal necrosis and cellular infiltration (Sahrial & Solfaine, 2019).

3.2.5 Larvicidal, antileishmania e anthelmintic activities

Three articles demonstrated the larvicidal activity of the OE extracted from leaves against *Anopheles stephensi* (mosquito vector of malaria), *Culex quinquefasciatus* and larvae of *Aedes aegypti*. The main phytochemical constituents identified in these studies were carvacrol (28.65%), thymol (21.66%), undecanal (8.29%), γ-terpinene (7.76%), ρ-cymene (6.46%), caryophyllene oxide (5.85%), α-terpineol (3.28%) and β-selinene (2.01%) (Arjunan et al., 2012; Senthilkumar & Venkatesalu, 2010; Vijayakumar et al., 2015).

The larvicidal test determined the LC_{50} (Lethal Concentration - LC) of the OE at 33.54 ppm (after 12 h) and 28.37 ppm (after 24 h), while for LC_{90} the observed values were 70.27 ppm (after 12 h) and 59.38 ppm (after 24 h). The results showed that the OE obtained from leaves is an inexpensive and ecological source of natural larvicidal agent that can be used to control and reduce the population of malaria vector mosquitoes, since there are records of drug resistance among these parasites (Arjunan et al., 2012; Senthilkumar & Venkatesalu, 2010).

The extract was mixed with a solution of zinc nitrate and submitted to the drying process until obtaining the nanoparticles, which showed safe and satisfactory results against *A. stephensi*, *C. quinquefasciatus* and *C. tritaeniorhynchus*. In this proposal, a formulation was developed to achieve better selectivity and less side effects (Vijayakumar et al., 2015).

Regarding antileishmania activity, two articles described this action in the essential oil, *in vitro* and *in vivo*, against *Leishmania (Viannia) braziliensis*. The OE presented antileishmania activity in tests *in vitro* and *in vivo*. The oil inhibited the growth *in vitro* of 107 promastigotes of *Leishmania braziliensis* after 48 hours, by intralesional administration for 14 days. The ethyl acetate extract from leaves also caused an *in vitro* and *in vivo* reduction in the number of promastigotes, in a shorter treatment time, and can be an additive to the existing therapy (Gonçalves, 2017; Lima et al., 2014).

Alcoholic extracts from leaves, roots and stems showed good anthelmintic activity against *Pheritima posthuma* (intestinal parasite). The response was close to the control drugs used in the study, piperazine citrate and albendazole, with the most potent action attributed to the leaves extract and the least potent to the root extract (Prasenjit et al., 2011).

3.2.6 Other identified actions

The improvement of animal feed with the addition of indian borage leaves to obtain weight gain in domestic chickens, are applications for veterinary use. The lowest amount of total coliforms and CFU (colony-forming units) detected in the feces of these birds, was observed in the group with antibiotic-free diet, which were replaced by the leaves extract. Therefore, this

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study proves the bacteriostatic efficiency of the leaves extract, reducing the bacterial count in the feces and indicating a safe alternative to replace antibiotics in poultry feed (Chuchuca et al., 2016).

Another reported veterinary use is of leaves used *in vitro* as a modulator of rumen fermentation. The addition of leaves to the diet can reduce methane generation by up to 30% and increase the digestion of dry matter, ammonia and other fermentation substances, such as volatile fatty acids, whose isomers are beneficial for human health (Yanza et al., 2018).

The study of psoriasis reports an ointment formulation made from ethanolic extract of roots. The antipsoriasis effect was proven to induce psoriasis with topical application of a mixture containing formaldehyde for seven days on the dorsal skin of mice. The effect of ointments containing indian borage extract at concentrations of 0.5% and 1%, was evaluated by the severity index of psoriasis, its phenotypic characteristics such as redness, scales and erythema and the aspects of the epidermal layer of the skin *in vivo*. There was a progressive reduction in the severity of psoriasis lesions, showing good response to its treatment and confirming its traditional use in skin diseases (Vijayalakshmi et al., 2019).

The hepatoprotective effects of the ethanolic and aqueous extract from leaf were tested to induce hepatotoxicity in rats with carbon tetrachloride (CCl_4). Animals with altered liver function in this research were monitored using biochemical markers (aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma-glutamyl transpeptidase and total and direct bilirubin), and those treated with ethanol extract showed more effective recovery of these markers, in compared to those treated with the aqueous extract. And once the hepatocytes have regenerated and the fat changes have normalized, validating the popular use of this species due to its hepatoprotective effect (Patel, 2011).

The antidiabetic activity, in ethanol and acetone extracts of leaves, by alpha-amylase inhibition was confirmed *in vitro* (Bole & Kumudini, 2014). Therapeutic applications of this species can expand its use as a functional nutraceutical food (Bhatt et al., 2013).

This study synthesized the existing knowledge in scientific publications about *P. amboinicus* and its therapeutic properties, as well as identified the existing processes and products involving this species and its applications in order to stimulate innovations in biotechnology.

This prospecting revealed that the benefits of this species can be seen in both humans and animals, with different purposes. The scientific publications analyzed showed that some therapeutic actions of popular use, although tested and validated, are not yet described in patent documents. The good therapeutic performance of this plant contributes to future studies where the information provided here can be used for the developing of innovative products.

4. Conclusions

There was an interdependence between the information found in patent databases and academic research about *P. amboinicus* and its therapeutic actions. And the association of technology with scientific research is essential to validate the traditional use of this species by the population.

These data can leverage research for the generation of pharmaceutical patents, be they process or product patent and the development of products with cosmetic or food applications. Therefore, studies are promising due to the many potentialities of this plant that have not yet been fully elucidated and explored.

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CAPÍTULO 2

Características nutricionais do solo e das folhas no cultivo de *Plectranthus amboinicus* (Lour.) Spreng

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Características nutricionais do solo e das folhas no cultivo de *Plectranthus amboinicus* (Lour.) Spreng

Nutritional characteristics of soil and leaves in the cultivation of *Plectranthus amboinicus* (Lour.) Spreng

Características Nutricionales del suelo y las hojas en el cultivo de *Plectranthus amboinicus* (Lour.) Spreng

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Resumo
Plectranthus amboinicus (Lour.) Spreng., da família Lamiaceae, tem propriedades terapêuticas e nutricionais atribuídas aos seus compostos fitoquímicos naturais, que são altamente valorizados na indústria farmacêutica. O objetivo do estudo foi avaliar as condições nutricionais ofertadas por dois tipos de adubações orgânicas no cultivo dessa espécie e verificar o aproveitamento desses nutrientes pelas folhas do vegetal. A pesquisa foi realizada com adubação de estercos avícola e bovino associados (Tratamento A) e adubação apenas com esterco bovino (Tratamento

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B). Foram mensuradas as propriedades do solo em relação ao: pH, soma das bases (SB), complexo sortivo, teor disponível de fósforo, enxofre e de micronutrientes, saturação do complexo sortivo por bases e capacidade de troca de cátions (CTC). Na diagnose foliar foi realizada a determinação dos teores de macronutrientes e de micronutrientes. O Tratamento B aumentou os teores de Ca e Mg, SB e CTC no solo. O Tratamento A elevou os teores de P, Fe, Mn e Cu no solo e de N (30,8 g/kg), P (4,9 g/kg), B (59 mg/dm^3), Cu (19,1 mg/dm^3), Fe (615 mg/dm^3) e Zn (57 mg/dm^3) nas folhas de *Plectranthus amboinicus*. Houve melhora do teor nutricional das folhas da espécie em estudo pela utilização de esterco avícola associado ao esterco bovino na adubação do solo.
Palavras-chave: Adubação orgânica; Fertilidade do solo; Análise foliar.

Abastract
Plectranthus amboinicus (Lour.) Spreng., from the Lamiaceae family, has therapeutic and nutritional properties attributed to its natural phytochemicals, which are highly valued in the pharmaceutical industry. The objective of the study was to evaluate the nutritional conditions offered by two types of organic fertilizers in the cultivation of this species and to verify the use of these nutrients by the leaves of the vegetable. The research was carried out with fertilization of associated poultry and bovine manure (Treatment A) and fertilization only with bovine manure (Treatment B). Soil properties were measured in relation to: pH, sum of bases (SB), assortment complex, available content of phosphorus, sulfur and micronutrients, saturation of the assortment complex by bases and cation exchange capacity (CTC). In leaf diagnosis, the determination of macronutrient and micronutrient contents was performed. Treatment B increased the levels of Ca and Mg, SB and CTC in the soil. Treatment A increased the levels of P, Fe, Mn and Cu in the soil and N (30.8 g/kg), P (4.9 g/kg), B (59 mg/dm^3), Cu (19,1 mg/dm^3), Fe (615 mg/dm^3) and Zn (57 mg/dm^3) in the leaves of *Plectranthus amboinicus*. There was an improvement in the nutritional content of the leaves of the species under study by the use of poultry manure associated with bovine manure in soil fertilization.
Keywords: Organic fertilization; Soil fertility; Leaf analysis.

Resumen
Plectranthus amboinicus (Lour.) Spreng., de la familia Lamiaceae, posee propiedades terapéuticas y nutricionales atribuidas a sus fitoquímicos naturales, muy valorados en la industria farmacéutica. El objetivo del estudio fue evaluar las condiciones nutricionales que ofrecen dos tipos de fertilizantes orgánicos en el cultivo de esta especie y verificar el aprovechamiento de estos nutrientes por parte de las hojas del vegetal. La investigación se llevó a cabo con fertilización de estiércol de aves y bovino asociado (Tratamiento A) y fertilización solo con estiércol de bovino (Tratamiento B). Las propiedades del suelo se midieron en relación a: pH, suma de bases (SB), complejo de surtido, contenido disponible de fósforo, azufre y micronutrientes, saturación del complejo de surtido por bases y capacidad de intercambio catiónico (CTC). En el diagnóstico foliar se realizó la determinación del contenido de macronutrientes y micronutrientes. El tratamiento B incrementó los niveles de Ca y Mg, SB y CTC en el suelo. El tratamiento A aumentó los niveles de P, Fe, Mn y Cu en el suelo y N (30.8 g/kg), P (4.9 g/kg), B (59 mg/dm^3), Cu (19, 1 mg/dm^3), Fe (615 mg/dm^3) y Zn (57 mg/dm^3) en las hojas de *Plectranthus amboinicus*. Hubo una mejora en el contenido nutricional de las hojas de la especie en estudio por el uso de estiércol de aves asociado a estiércol bovino en la fertilización del suelo.
Palabras clave: Fertilización orgánica; Fertilidad del suelo; Análisis de hojas.

1. Introdução

O planejamento no cultivo de plantas medicinais para comercialização é um requisito necessário na garantia de uma produção adequada e de qualidade (Azevedo & Moura, 2010). Portanto, é importante identificar as condições ambientais de sua origem a fim de proporcionar as características ideais para o seu crescimento e maior produção dos metabólitos responsáveis pela ação terapêutica. Os fatores climáticos exercem influência sobre o desenvolvimento dos vegetais e a produção de seus metabólitos. As plantas medicinais são reconhecidas como aquelas que apresentam em um ou mais de seus órgãos fitoconstituintes ou classes de metabólitos com ação terapêutica (Carvalho, 2015).

Na produção de plantas medicinais inicialmente deve-se selecionar a melhor forma de propagação da espécie, para em seguida proceder ao cultivo da espécie. Na etapa do cultivo, a análise do local e do solo são importantes para estimar o melhor rendimento da produção (Rodrigues, 2004). As três análises de solo mais utilizadas são: análise química do solo, análise física do solo e análise química da planta. Enquanto a análise química avalia a fertilidade, a análise física (granulométrica) caracteriza a textura do solo. A análise foliar surge como um complemento às análises de solo e permite avaliar a interação solo-planta-clima (Chinelato, 2019). Desta forma, a análise química do solo possibilita identificar as carências nutricionais existentes permitindo correções no mesmo (Arruda, et al., 2014), enquanto que a avaliação do estado nutricional do vegetal

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pela análise foliar aliada as análises de solo permite avaliar a adubação mais adequada e econômica no planejamento de cultivos (Tecchio, et al., 2006). Ressalta-se que os resultados obtidos na diagnose foliar não estão exclusivamente ligados as características do solo, pois existem outros fatores interferentes na absorção dos nutrientes pelos vegetais (Santana, et. al., 2007).

No Brasil, o uso de plantas medicinais tem heranças culturais indígenas, africanas e europeias (Azevedo & Moura, 2010). Espécies de Plectranthus (Lamiaceae) possuem uso milenar na medicina popular. Este gênero ocorre em quatro continentes: África, América, Oceania e Ásia (Ruan, 2019).

Plectranthus amboinicus (Lour.) Spreng. pertence à família Lamiaceae e ocorre naturalmente em todos os trópicos e regiões quentes da África, Ásia e Austrália. É nativa da Índia, sendo encontrada em toda América tropical. Esta erva tem propriedades terapêuticas e nutricionais atribuídas aos seus compostos fitoquímicos naturais, que são altamente valorizados na indústria farmacêutica. Possui também propriedades hortícolas devido à sua natureza aromática e capacidade de produção de óleo essencial (Arumugan, et al. 2016).

Apresenta-se como uma erva perene, tortuosa e suculenta com folhas duras oval-deltoides, crenadas de ápice agudo e quebradiço ao ser dobrado. São folhas aromáticas, carnudas, dispostas em pares opostos (duas a duas), facilmente multiplicadas por estaquia mas raramente são encontradas suas sementes e flores (Freitas, 2014).

Popularmente essa planta é conhecida como malvariço, malva do reino, hortelã da folha grande e tem como sinônimos botânicos: *Coleus amboinicus* Lour., *Coleus aromaticus* Benth. (Freitas, 2014). Tem um melhor crescimento em locais semi-sombreado, com solo úmido e pH neutro, desde que não haja excesso de água no solo pois apodrecem suas raízes. Há poucas informações sobre os métodos de cultivos aplicados para esta espécie (Khan, 2013; Arumugan, et al., 2016).

Esta pesquisa busca avaliar as condições nutricionais ofertadas de tratamentos com dois tipos de adubações orgânicas no cultivo da espécie e verificar o aproveitamento destes nutrientes pelas folhas deste vegetal, uma vez que estes nutrientes podem influenciar diretamente nas características químicas e biológicas dos seus metabólitos.

2. Metodologia

O presente estudo é de caráter experimental e possui abordagem quantitativa, como preconizado por Pereira, et al. (2018).

2.1 Características do ambiente do cultivo

Os cultivos foram realizados em São Luís do Maranhão (Brasil) na casa de vegetação do Horto de Plantas Medicinais Berta Lange de Morretes, da Universidade Federal do Maranhão-UFMA (2° 32'S e 44°16 W), com 50% de sombreamento. A região do estudo tem temperatura média é de 27°C e pluviosidade anual média de 1896 mm com variações de 1.700 a 2.300 mm. O clima do tipo Aw', segundo a classificação de Koppen e Geiger, é quente e úmido, com dois momentos de precipitação: um período chuvoso, compreendendo os meses de janeiro a junho e outro período de estiagem, nos meses de julho a dezembro (NUGEO/UEMA, 2002; Climate-Date.org., s.d.).

Os experimentos foram conduzidos em vasos plásticos com capacidade de 10dm^3, preenchidos uniformemente com os dois tipos de tratamento do solo: Substrato A (esterco bovino + esterco avícola + solo) e Substrato B (esterco bovino + solo).

As mudas da espécie *Plectranthus amboinicus* (Lour.) Spreng. foram transplantadas nestes dois sistemas de tratamentos e cultivadas por um período de 75 dias de 25/04/19 a 10/07/19, com irrigação manual alternada de 500mL de água e afastadas espacialmente de modo a reduzir competição por luz solar entre as plantas. Uma exsicata da espécie foi depositada no Herbário "Ático Seabra", no Departamento de Farmácia da UFMA, Brasil (SLS nº.1.477).

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2.2 Análise dos tratamentos com adubação orgânica

Na amostragem dos tratamentos com adubações orgânicas, as amostras simples de cada tratamento, coletadas a uma profundidade de 20cm, foram misturadas para então retirar 50g de amostra composta de cada tratamento. As amostras foram então embaladas em recipientes limpos e enviadas ao laboratório agronômico para serem submetidas as análises de: pH em $CaCl_2$, Complexo Sortivo (teores trocáveis de K, Ca, Mg, Al e H+Al), Teor disponível de Fósforo (P) e de Enxofre (S), teor disponível de micronutrientes (Fe, Cu, Mn, Zn); Saturação do Complexo Sortivo por bases (V), e capacidade de troca de cátions (CTC) conforme a metodologia de Silva (2009).

2.3 Análise foliar

As amostras das folhas cultivadas nos dois tipos de substratos (A e B) foram coletadas no último dia do cultivo no intervalo de 8-9h da manhã. As folhas foram dessecadas, em estufa com circulação de ar a 40ºC até massa constante e acondicionadas em recipientes plásticos. As amostras foram enviadas ao laboratório agronômico para determinação dos teores de: Nitrogênio (N), Potássio (K), Cálcio (Ca), Magnésio (Mg), Enxofre (S), Sódio (Na), Zinco (Zn), Manganês (Mn), Ferro (Fe) e Cobre (Cu) seguindo a metodologia de Carmo, et al. (2000).

3. Resultados e Discussão

As características físico-químicas e nutricionais do solo na utilização dos dois tipos de adubo podem ser observados na Tabela 1. O incremento nos teores de Ca e Mg no Tratamento B (somente esterco bovino) elevou a soma das bases trocáveis (SB) também nesse tratamento, em comparação com o Tratamento A (estercos bovino e avícola associados). Assim, consequentemente, a capacidade de troca iônica (CTC) do solo apresentou comportamento semelhante, com aumento dos valores no Tratamento B e diminuição no Tratamento A (Tabela 1).

Tabela 1 - Valores médios dos atributos químicos do solo com dois diferentes tipos de adubo no cultivo de *Plectranthus amboinicus* (Lour.) Spreng. na profundidade 0-20cm.

TRATAMENTO*	ATRIBUTOS**											
	K	Ca	Mg	Al	H+Al	SB	CTC	V	m	Ca	Mg	K
	..............................cmol/dm³.......................							........................%......................				
A	0,6	3,8	1,9	0,0	0,5	6,4	6,9	92,6	0,0	55,8	28,2	8,6
B	0,3	9,4	3,4	0,0	0,6	13	13,6	95,9	0,0	68,6	25,0	2,3

	pH $CaCl_2$	P	S	Fe	Mn	Cu	Zn
		....mg/dm³..					
A	6,1	132	1,1	165,2	55,1	3,8	16,4
B	6,0	76,4	3,7	28,9	50,0	1,1	23,9

*A: solo + esterco bovino + esterco avícola; B: solo + esterco bovino
** K: Potássio; Ca: Cálcio; Mg: Magnésio; H+Al: Hidrogênio + Alumínio; SB: soma de bases (K+Ca+Mg); CTC: capacidade de troca catiônica; V: saturação por base; $CaCl_2$: Cloreto de cálcio; P: Fósforo; S: Enxofre; Fe: Ferro; Mn: Manganês; Cu: Cobre; Zn:Zinco
Fonte: Autores (2021).

Segundo Ronquim (2020), a CTC dos solos representa a graduação da capacidade de liberação de vários nutrientes, favorecendo a manutenção da fertilidade por um prolongado período e reduzindo ou evitando a ocorrência de efeitos tóxicos da

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aplicação de fertilizantes, se a maior parte da CTC do solo está ocupada por cátions essenciais como Ca^{2+}, Mg^{2+} e K^{+}, pode-se dizer que esse é um solo bom para a nutrição das plantas (Tabela 1).

Percebe-se que o aumento da CTC no Tratamento B se deu pelo aumento de Ca (9,4 $cmol/dm^3$) e Mg (3,4 $cmol/dm^3$) corroborando com os achados de Granjeiro, et al. (2011) que afirma que o acúmulo de Ca praticamente duplica o teor de Mg, como verificado em nosso estudo.

Entretanto, foi possível constatar que o Tratamento A (estercos bovino e avícola associados) promoveu aumento de nutrientes P, Fe, Mn e Cu, quando comparado ao Tratamento B (apenas esterco bovino), como pode ser observado na Tabela 1. Isso demonstra a importância do esterco avícola para o fornecimento desses nutrientes ao solo.

A maior disponibilidade de P no Tratamento A (132 mg/dm^3) provavelmente ocorreu pelo aumento de fornecimento de ácidos orgânicos, característicos dos resíduos do esterco de aves, e que ocupam o mesmo sítio de adsorção do fosfato, o que permite a disponibilidade à planta (Andrade, et al., 2003). Enquanto que o aumento considerável de Fe (165,2 mg/dm^3) Mn (55,1 mg/dm^3) e Cu (3,8 mg/dm^3) no Tratamento A pode ser devido ao emprego de rações ricas em nutrientes, bem como pela presença frequente de menor teor de água, fezes e urina misturadas no esterco avícola, como de galinha, conforme mencionado por Marrocos (2011).

Do ponto de vista fisiológico, os nutrientes podem ser agrupados em quatro grupos, onde: P é um nutriente do segundo grupo, importante no armazenamento de energia da planta; Mn pertence ao terceiro grupo com função de cofator enzimático e regulador de potenciais osmóticos; enquanto Fe e Cu são do quarto grupo, nutrientes envolvidos em reações redox com importantes funções em reações envolvendo transporte de elétrons (Taiz, et al., 2016; Kerbauy, 2019)

A amostragem dos tratamentos dos solos permite identificar as características qualitativas e quantitativas dos nutrientes neles existentes, orientando sobre o uso sustentável e econômico dos solos (Vieira, et. al., 2020).

Os macronutrientes N, P, K, Ca, Mg e S (também chamados de nutrientes principais) são absorvidos pela planta em maior proporção que os micronutrientes B, Zn, Cu, Fe, Mo, Cl e Mn (também chamados de elementos traço). Para torná-los disponíveis, o solo deve ser bem manejado (Ronquim, 2010).

Dentre os macronutrientes, verificou-se em nosso estudo que houve aumento de N (30,8 g/kg) e P (4,9 g/kg) nas folhas de *Plectranthus amboinicus* submetidas ao Tratamento A (estercos bovino e avícola associados), como consta na Tabela 2. De acordo com Weinärtner, et al. (2006) o esterco de aves é muito rico em N, o que torna esse material bastante interessante na composição de um substrato, pois favorece a oferta desse nutriente; enquanto que o aumento do teor de P, deve-se a maior disponibilidade desse nutriente no solo no Tratamento A (Tabela 1) e, consequentemente, à planta (Tabela 2), o que é característico do esterco de ave segundo estudos de Andrade, et al. (2003).

Tabela 2 - Característica nutricional das folhas de *Plectranthus amboinicus* (Lour.) Spreng. com dois tipos de tratamentos com adubações orgânicas.

TRATAMENTO*	**NUTRIENTES****										
	N	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**
	....................g/Kg....................						**....................mg/dm³....................**				
A	30,8	4,9	60,1	20,7	9,3	0,7	59,0	19,1	615	43	57
B	16,8	3,6	61,6	37,8	11,7	0,7	54,1	8,9	295	54	48

*A: Solo + esterco bovino + esterco avícola ; **B: Solo + esterco bovino
**N: Nitrogênio; P: Fósforo; K: Potássio; Ca: Cálcio; Mg: Magnésio; S: Enxofre; B: Boro; Cu: Cobre; Fe: Ferro; Mn: Manganês; Zn: Zinco
Fonte: Autores (2021).

Em relação aos micronutrientes, como mostrado na Tabela 2, ocorreu aumento nos teores de B (59 mg/dm^3), Cu (19,1 mg/dm^3), Fe (615 mg/dm^3) e Zn (57 mg/dm^3) nas folhas de *Plectranthus amboinicus* submetidas ao Tratamento A (estercos

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bovino e avícola associados). A concentração e o acúmulo de metais nos tecidos da planta dependem de sua disponibilidade na solução do solo, pois a concentração desses na raiz e na parte aérea aumenta com o aumento da sua concentração na solução do solo (Marques, et al., 2000). Além disso, as plantas podem ser acumuladoras, indicadoras ou excluidoras de metais, dependendo da capacidade de absorção, translocação e concentração dos metais na raiz e nas folhas (Baker, 1987). Percebe-se que no caso de Fe e Cu o aumento no teor desses compostos nas folhas se deu pelo maior incremento na disponibilidade do solo (Tabela 1), enquanto que B e Zn foram acumulados por características próprias da espécie.

4. Conclusão

O uso da adubação com estercos avícola e bovino associados promoveu aumento no teor nutricional das folhas de *Plectranthus amboinicus*, em comparação à adubação apenas com esterco bovino. São necessários outros estudos comparativos que permitam avaliar a influência das variações sazonais com esta espécies. A análise deste tipo de estudo com a mesma duração de cultivo, em um intervalo de 365 dias, ampliará as informações sobre as prováveis alterações que o vegetal possa apresentar.

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CAPÍTULO 3

Características químicas e atividade antimicrobiana do óleo essencial de *Plectranthus amboinicus* (Lour.) Spreng. cultivada com adubações distintas

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Características químicas e atividade antimicrobiana do óleo essencial de *Plectranthus amboinicus* (lour.) Spreng. cultivada com adubações distintas

Chemical characteristics and antimicrobial activity of the essential oil of *Plectranthus amboinicus* (lour.) Spreng. cultivated with different fertilizers

Características químicas y actividad antimicrobiana del aceite esencial de *Plectranthus amboinicus* (lour.) Spreng. cultivado con diferentes fertilizantes

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Resumo

Objetivo: avaliar se há diferenças no rendimento, na composição química e consequentemente na atividade antimicrobiana dos óleos essenciais de *Plectranthus amboinicus* quando submetida a cultivos com adubações diferentes em um mesmo período do ano. Metodologia: *Plectranthus amboinicus* foi cultivada com dois tipos de adubação diferentes durante 75 dias. A extração do óleo essencial das folhas ocorreu por hidrodestilação com Clevenger e a identificação dos seus constituintes químicos foi realizada por cromatografia gasosa acoplada a espectrometria de massas. A atividade antimicrobiana foi determinada pela técnica de microdiluição em caldo.

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Resultados: Foram constatadas variações no rendimento, na composição química e na atividade antimicrobiana do óleo essencial nas amostras cultivadas com adubações orgânicas diferentes em uma mesma época. Os melhores resultados foram obtidos na amostra de óleo essencial adubada com esterco bovino, tendo um rendimento de 1,65% e como majoritários Carvacrol (58,6%), γ-Terpineno: (15,1%) e p-Cymeno: (8,2%). Conclusão: Os majoritários Carvacrol, γ-Terpineno e p-Cymeno estiveram mais abundantes no cultivo com adubação orgânica do tipo esterco bovino. Da mesma forma, o melhor potencial antimicrobiano detectado do óleo essencial contra *E. coli* e *C. albicans* decorreu do cultivo das plantas com adubação orgânica do tipo esterco bovino. Os dados sugerem que este tipo de adubação seja adequado para produzir o óleo essencial com melhor atividade antimicrobiana e maior qualidade. Portanto, é possível melhorar o rendimento, a concentração dos constituintes e sua resposta antimicrobiana através de uma adubação orgânica acessível e de baixo custo.
Palavras-chave: *Plectranthus amboinicus;* Óleo essencial; Atividade antimicrobiana; Adubação orgânica; Composição química.

Abstract
Objective: To evaluate if there are differences for yield, chemical composition and, consequently, antimicrobial activity of the essential oils of *Plectranthus amboinicus* when submitted to crops with different fertilizations in the same period of the year. Methodology: *Plectranthus amboinicus* was grown with two different types of fertilization for 75 days. The extraction of essential oil from the leaves was through hydrodistillation with Clevenger and the identification of its chemical constituents was performed by gas chromatography coupled with mass spectrometry. The antimicrobial activity was determined by the broth microdilution technique. Results: Variations in yield, chemical composition and antimicrobial activity of essential oil were found in samples grown using different organic fertilizers at the same time. The best results were obtained in the sample of essential oil fertilized with bovine manure, yielding 1.65% and Carvacrol (58.6%), γ-Terpinene: (15.1%) and p-Cymene: (8.2%) as majorities. Conclusion: The majorities Carvacrol, γ-Terpinene and p-Cymene were more abundant in the cultivation with organic fertilization of bovine manure type. Likewise, the best antimicrobial potential detected by the essential oil against *E. coli* and *C. albicans* was due to the cultivation of plants with organic fertilization of bovine manure type. The data suggest that this type of fertilization is adequate to produce the essential oil with better antimicrobial activity and higher quality. Therefore, it is possible to improve the yield, the concentration of the constituents and their antimicrobial response through an affordable organic fertilizer.
Keywords: *Plectranthus amboinicus;* Essential oil; Antimicrobian activity; Organic fertilization; Chemical composition.

Resumen
Objetivo: evaluar si existen diferencias en rendimiento, composición química y, en consecuencia, en la actividad antimicrobiana de los aceites esenciales de *Plectranthus amboinicus* cuando se someten a cultivos con diferentes fertilizaciones en el mismo período del año. Metodología: Se cultivó *Plectranthus amboinicus* con dos tipos diferentes de fertilización durante 75 días. La extracción del aceite esencial de las hojas se realizó mediante hidrodestilación con Clevenger y la identificación de sus componentes químicos se realizó mediante cromatografía de gases acoplada a espectrometría de masas. La actividad antimicrobiana se determinó mediante la técnica de microdilución en caldo. Resultados: Se encontraron variaciones en el rendimiento, la composición química y la actividad antimicrobiana del aceite esencial en muestras cultivadas con diferentes fertilizantes orgánicos al mismo tiempo. Los mejores resultados se obtuvieron en la muestra de aceite esencial fertilizado con estiércol bovino, con un rendimiento del 1,65% y como mayoritaria Carvacrol (58,6%), γ-Terpineno: (15,1%) y p-Cymeno: (8,2%). Conclusión: Las mayorías Carvacrol, γ-Terpineno y p-Cymeno fueron más abundantes en el cultivo con fertilización orgánica de tipo estiércol bovino. Asimismo, el mejor potencial antimicrobiano detectado por el aceite esencial contra *E. coli y C. albicans* se debió al cultivo de plantas con fertilización orgánica del tipo estiércol bovino. Los datos sugieren que este tipo de fertilización es adecuada para producir el aceite esencial con mejor actividad antimicrobiana y mayor calidad. Por lo tanto, es posible mejorar el rendimiento, la concentración de los componentes y su respuesta antimicrobiana a través de un fertilizante orgánico asequible.
Palabras clave: *Plectranthus amboinicus;* Aceite esencial; Actividad antimicrobiana; Fertilización orgánica; Composición química.

1. Introdução

Os óleos essenciais ou óleos voláteis constituem uma mistura líquida de substâncias odoríferas voláteis e lipofílicas produzidas por plantas aromáticas, que podem ter variações decorrentes de fatores ambientais, sazonais e extrativos. Os óleos essenciais podem ser originados pela via acetato ou chiquimato e pertencem em sua maioria à classe dos monoterpenoides, sendo encontrados também nos sesquiterpenos e fenilpropanoides (Benelli & Mehlhorn, 2018; Coutinho, et. al., 2020).

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Nos vegetais suas principais funções são de defesa contra predadores, microrganismos patogênicos e na atração de polinizadores. São encontrados principalmente em estruturas celulares específicas como: células oleíferas, bolsas secretoras, canais secretores e tricomas glandulares das plantas aromáticas. Nas angiospermas dicotiledôneas são encontrados principalmente nas famílias: Lamiaceae, Myrtaceae, Lauraceae, Verbenaceae, Piperaceae e Asteraceae (Baser & Buchbauer, 2015; Simões, et al., 2017).

A família Lamiaceae (Labiatae) é composta por cerca de 200 gêneros e 3.200 espécies com amplo uso na culinária e no tratamento de diversas doenças (Punet Kumar, et. al., 2020). Como integrante desta família, *Plectranthus amboinicus* (Lour.) Spreng, popularmente conhecida como malvariço, hortelã da folha grossa ou Indian borage, também tem suas aplicações na culinária como tempero e na medicina popular. As suas folhas podem ser consumidas cruas, usadas como aromatizantes ou adicionadas como ingredientes na preparação de alimentos tradicionais (Arumugan, et al., 2016).

Plectranthus amboinicus (Lour.) Spreng é uma herbácea perene que atinge em média 1m de altura e pode viver de 3 até 10 anos (Khalid & El-Gohary, 2014). Tem folhas ovais, suculentas, com ápice agudo ou obtuso e superfícies crenadas (Punet Kumar, et. al., 2020). Apresenta pêlos glandulares na superfície inferior das folhas, pecíolo grosso e caule quebradiço. Suas flores têm coloração violácea, sendo difícil observar sementes e flores dessa planta em algumas localidades (Prasad, et al., 2020).

Esta espécie tem como metabólitos secundários 76 constituintes voláteis como: β-cariofileno, carvacrol, timol e 30 compostos não voláteis pertencentes a diferentes classes de fitoquímicos, como: terpenos (monoterpenos, diterpenos, triterpenos e sesquiterpenos), fenólicos, flavonoides, ésteres, álcoois e aldeídos. Estudos citaram diversas propriedades farmacológicas, incluindo atividades antimicrobiana, antiinflamatória, analgésica, diurética, citotóxica, cicatrizante, larvicida e antioxidante (Arumugam & Swamy, 2016; Ruan, 2019; Sany, et.al., 2020).

Estudos reforçam que os metabólitos secundários produzidos pelos vegetais estão susceptíveis à alterações em sua síntese e proporção, decorrentes da fase de desenvolvimento de vegetal, de fatores genéticos e ambientais, como: temperatura, umidade, luminosidade, oferta de nutrientes e disponibilidade hídrica (Coutinho, et al., 2020; Luz, et. al., 2020).

A variedade de condições e fatores a que uma espécie está exposta refletem na produção de seus constituintes químicos, e consequentemente na intensidade de sua atividade biológica. Buscando entender melhor o comportamento de *Plectranthus amboinicus* (Lour.) Spreng, frente à estas situações, a pesquisa teve como objetivo avaliar se há diferenças (qualitativa e quantitativa) na composição química dos óleos essenciais e consequentemente na atividade antimicrobiana desta espécie quando submetida a cultivos com adubações diferenciadas em um mesmo período.

2. Metodologia

O presente estudo é de caráter experimental e possui abordagem quantitativa, como preconizado por Pereira, et al. (2018).

2.1 Cultivo e amostra

No Estado do Maranhão predomina um clima tropical estando a 24m acima do nível do mar. O município de São Luís localizado no Estado do Maranhão tem um clima do tipo Aw' quente e úmido, segundo Koppen e Geiger, e apresenta com duas situações definidas de precipitação: um período chuvoso, que se estende de janeiro a junho e um de estiagem de julho a dezembro. Tem uma temperatura média de 27°C e pluviosidade anual média 1896mm (Martins, et. al., 2020; da Silva, et. al., 2020).

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2.2 Características do cultivo e coleta

O cultivo de 75 dias da espécie compreendeu o período de 25/04/19 até 10/07/19 na casa de vegetação do Horto de Plantas Medicinais Berta Lange de Morretes, da Universidade Federal do Maranhão- UFMA ((2° 32'S e 44°16 W), com 50% de sombreamento. Uma exsicata da espécie foi depositada no Herbário "Ático Seabra", no Departamento de Farmácia da UFMA, Brasil (SLS nº.1.477).

Os cultivos foram realizados aplicando dois tipos de tratamento do solo com adubação orgânica: Tratamento A (esterco bovino + esterco avícola + solo) e Tratamento B (esterco bovino + solo).

2.3 Extração do óleo essencial

Folhas frescas coletadas de cada Tratamento (A e B) foram dessecadas à 40ºC por 72 horas e então submetidas a hidrodestilação por três horas usando aparelho tipo Clevenger (Brasil, 2019). As amostras de óleo essencial (OE) foram centrifugadas (4000 rpm/30 min) e desidratadas com sulfato de sódio anidro (Na_2SO_4). Em seguida, foram acondicionadas em ampolas de vidro âmbar e mantidas sob refrigeração (2−8°C) até o momento das análises de determinação do seu conteúdo e rendimento seguindo a metodologia de Coutinho, et. al., (2007).

2.4 Análise química dos óleos essenciais (OE)

A análise das amostras de OE foi com a técnica de cromatografia gasosa acoplada ao espectrômetro de massas (GC-MS-QP2020-Shimadzu). As condições aplicadas foram: coluna capilar BPX5 (30m x 0,25mm x 0,25µm), com faixa de temperatura de 60ºC-240ºC (3°C/min); gás carreador hélio (3ml min^{-1}) e volume injetado de 1 µ L (em hexano). A temperatura do injetor foi de 280ºC, com pressão na coluna de 111,5 KPa e velocidade linear de 48,9 cm/s e modo *scan* 0.5s. Os componentes foram identificados por comparação com os padrões de fragmentação encontrados nos espectros de massa descritos nas espectrotecas (NIST, 2005) e na literatura (Adams, 2007).

2.5 Determinação do rendimento do óleo essencial

Os rendimentos dos OE foram expressos em porcentagem aplicando-se a equação abaixo (Rodrigues, et al., 2011):

Eq. (1) *% Rendimento óleo essencial = Moe/Mp X 100*

M_{oe}= massa do óleo essencial extraído;

Mp= massa da matéria prima usada na extração

2.6 Atividade antimicrobiana

Quatro cepas de fungos e duas cepas de bactérias foram analisadas na determinação da atividade antimicrobiana. Foram testadas as seguintes cepas testadas: *C. albicans* (ATCC 14053 e ATCC 90028), *C. parapsilosis* (ATCC 22019), *C. Krusei* (ATCC 6528), *S. aureus* (ATCC 25923) *e E. coli* (ATCC 35218). Como controle positivo os padrões usados foram o Cloranfenicol e a Nistatina.

Os microrganismos foram reativados por semeadura em caldo BHI (Ágar de Infusão Cérebro-Coração) à 35 °C por 24h. Na sequência foram semeados em placas com ágar nutriente e incubados à 35°C durante 24h. As colônias isoladas foram ressuspendidas em 3 mL de solução salina 0,9% obedecendo a escala de 0,5 McFarland (1,5 x 10^8 UFC/ml) (CLSI, 2020).

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2.6.1 Determinação da Concentração Inibitória mínima (CIM)

A técnica de microdiluição em caldo com microplaca de 96 poços, contendo 100 µL de Caldo Mueller Hinton em cada um deles foi aplicada para avaliação do potencial antifúngico dos OE. H e no controle positivo foi adicionado 20 µL de antimicrobiano (Cloranfenicol 0,02mg/mL ou Nistatina 100.000 UI). Em seguida, acrescentou-se 5 µL da suspensão microbiana em todos os poços. A microplaca foi incubada por 24-48h à 37°, realizando-se a revelação desta com 30 µL de resazurina 0,015% em todos os poços. As análises foram realizadas em triplicata e a concentração inibitória mínima (CIM) foi determinada com a técnica de microdiluição conforme descrito pelo Clinical and Laboratory Standards Institute (CLSI, 2020).

2.6.2 Determinação da Concentração Bactericida Mínima (CBM) e Concentração Fungicida Mínima (CFM)

A CBM foi avaliada nas concentrações onde não houve crescimento do microrganismo no teste da MIC, através de semeadura em placa de petri com ágar Mueller Hinton. Após a incubação das placas em estufa bacteriológica por 24/48h, houve a leitura do teste para verificar a presença de ação bactericida ou bacteriostática nas amostras do óleo essencial (CLSI, 2020).

3. Resultados e Discussão

3.1 Análise química e rendimento

A amostra que recebeu o tratamento B de adubação orgânica (esterco bovino + solo) apresentou um rendimento do óleo essencial de 1,65% sendo superior ao obtido no cultivo com o tratamento A (esterco bovino + esterco avícola + solo) que pode ser visualizado na Tabela 1. Segundo Oliveira (2020), o rendimento do OE é influenciado pelo estado de nutrição da planta, forma de cultivo, fatores ambientais e estágio de crescimento do vegetal. Outro interferente no rendimento do OE como a variação sazonal foi relatado no estudo de Zouari-Bouassida, et al., (2018) em que eles constataram diferenças no rendimento de OE entre as estações analisadas. Portanto, apesar dos dois tratamentos terem ocorrido na mesma época e com a mesma técnica extrativa é possível que a diferença no tipo de adubação possa ter refletido em um melhor rendimento da espécie *P. amboinicus*.

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Tabela 1 - Composição química e rendimento dos óleos essenciais de *Plectranthus amboinicus* com adubações orgânicas diferentes**.**

	RI	Compostos	Tratamentos	
			A	B
1	902	α-Thujene	1,08	1,03
2	919	(+)-4-Carene	3,17	2,67
3	948	α-Pinene	0,45	0,38
4	958	Myrcene	1,58	1,04
5	962	1-Octen-3-ol	0,15	-
6	964	β-Phellandrene	0,33	-
7	969	α-Phellandrene	0,47	0,39
8	998	γ-Terpinene	14,43	15,09
9	1018	Limonene	0,59	-
10	1041	Sabinene hydrate	0,35	-
11	1042	p-Cymene	7,43	8,21
12	1137	Terpinen-4-ol	1,32	1,08
13	1262	Carvacrol	49,42	58,66
14	1371	Linalyl propionate	-	0,24
15	1430	α-Bergamotene	5,76	2,86
16	1494	Caryophyllene	8,46	5,05
17	1500	β-Bisabolene	0,33	-
18	1507	Caryophyllene oxide	1,18	0,56
19	1579	Humulene	2,94	1,49
		Monoterpenos hidrocarbonetos	29,53	28,81
		Monoterpenos oxigenados	51,09	59,74
		Sesquiterpenos hidrocarbonetos	17,49	9,40
		Sesquiterpenos oxigenados	1,18	0,56
		Outros	0,15	0,24
		Total identificado (%)	99,44	98,75
		Rendimento (%)	1,2	1,65

(-) Não detectado
RI - Indice de retenção linear da biblioteca (Adams, 2007).
Fonte: Autores (2021).

Ainda nesta mesma tabela os dados revelam que a quantidade de compostos químicos identificados do OE da amostra A (n=18) foi superior aos da amostra B (n=14). Cinco compostos foram detectados apenas na Amostra A: *β-Phellandrene, 1-Octen-3-ol, β-Bisabolene, Limonene e Sabinene hydrate*, enquanto o *Linalyl propionate* foi identificado apenas na Amostra B. O componente mais abundante nas duas amostras foi o carvacrol (A=49,4%, B=58,6%), seguido por γ-terpineno (A=14,4%, B=15,1%) e p-cymeno (A=7,4%, B=8,2%), estando presentes em maior proporção na amostra do tratamento B. Somente o cariofileno (A=8,4%, B=5%), dentre os majoritários, foi detectado em maior concentração na amostra A.

O estudo realizado no Brasil por Santos, et al., (2015) apresentou semelhança na composição qualitativa dos componentes majoritários variando, entretanto no número de compostos obtidos no OE e nas concentrações dos mesmos, como por exemplo o carvacrol (37,7%). Quantidades aproximadas de compostos identificados foram detectadas também no estudo de Khalid & El-Gohary (2014), realizado no Egito, apesar de haver diferenças nos constituintes identificados e em suas concentrações como os majoritários carvacrol (15,9%) e γ-terpinene (2,5%). Resultado diferenciado foi observado no estudo comparativo entre salinidade e dose de fertilizante de Mesquita et. al. (2017) que obteve como constituinte principal o Timol (53,2-93,6%) enquanto o carvacrol esteve em menores concentrações (13,1-24,5%). É importante destacar que a concentração de carvacrol (A=49,4%, B=58,6%) produzida nesta pesquisa com cultivos analisando dois tipos adubação orgânica, foi superior à dos estudos acima mencionados.

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3.2 Atividade antimicrobiana

Os dados apresentados na Tabela 2, demonstram que a melhor atividade antifúngica ocorreu na concentração de 3,90mg/mL para as estirpes de *C. albicans* ATCC 14053 e ATCC 90028 na amostra do tratamento B.

Quanto à atividade antibacteriana os melhores resultados encontrados foram para as estirpes de *E. coli* ATCC 35218, tendo no tratamento B a menor CIM (0,97mg/mL). Carvacrol é conhecido por ter ação bactericida e/ou bacteriostática dependendo da concentração usada e ainda pela capacidade de destruição da membrana celular da *E.coli, S. typhimurium* e *Shigella flexneri* (Gonçalves, et.al., 2012), o que reforça os resultados encontrados nesta pesquisa cuja concentração de carvacrol foi de 58,6%.

Tabela 2 – Concentrações inibitória, fungicida e bactericida mínima dos óleos essenciais de *Plectranthus amboinicus* com adubações orgânicas diferentes**.**

CEPAS	TRATAMENTO A		TRATAMENTO B	
	CIM	**CBM/CFM**	**CIM**	**CBM/CFM**
C. albicans ATCC 14053	7,81	7,81	3,90	3,90
C. albicans ATCC 90028	15,62	15,62	7,81	7,81
C. parapsilosis ATCC 22019	7,81	31,25	7,81	15,62
C krusei ATCC 6528	3,90	3,90	3,90	3,90
S. aureus ATCC 25923	3,90	3,90	15,62	15,62
E. coli ATCC 35218	1,95	1,95	0,97	1,95

CIM: Concentração Inibitória Mínima (mg/ml)
CBM/CFM: Concentração Bactericida/Fungicida Mínima (mg/ml)
Fonte: Autores (2021).

Em um estudo realizado por EL-Zefzafy (2016) com óleo essencial de *Plectranthus amboinicus* para avaliar a atividade antimicrobiana, a CIM para *C. albicans* foi de 1,4µg/ml e para *E. coli* foi de 8,2µg/ml. Este mesmo estudo também identificou nos componentes do óleo essencial como majoritário o Limoneno (42%), seguido do β Myrcene β (11,3%). Estes dados diferem dos resultados encontrados nesta pesquisa o que segundo Oliveira (2020) podem ser justificados pela possibilidade de alterações nos componentes dos óleos essenciais, qualitativa e quantitativa, decorrentes de fatores como origem botânica, origem geográfica, sazonalidade, entre outros.

Analisando ainda os dados da Tabela 2 observa-se que na maioria dos testes, exceto para *S. aureus*, a amostra do Tratamento B respondeu com níveis de concentração iguais ou menores do que o Tratamento A. Portanto, a partir desses dados pode-se inferir que a variação nas concentrações dos compostos majoritários e consequentemente na resposta antimicrobiana pode estar associada ao tipo de adubação aplicada. A adubação orgânica do tratamento B (esterco bovino) mostra-se como a mais indicada no cultivo, nestas condições, uma vez que apresentou resultados iguais ou melhores do que o tratamento A. Além disto, é um tipo de adubação mais simples e de menor custo quando comparada a aplicada no tratamento A (esterco bovino + esterco avícola).

4. Considerações Finais

A pesquisa demonstrou que houve diferenças no rendimento, na composição química e na atividade antimicrobiana do óleo essencial ao comparar amostras cultivadas da mesma espécie com adubações diferentes em um mesmo período (chuvoso). Os resultados obtidos comprovaram um potencial antibacteriano contra *E. coli* e antifúngico contra *C. albicans* principalmente no óleo essencial originado das plantas cultivadas com adubação orgânica do tipo esterco bovino. Os dados sugerem que este tipo de adubação seja suficiente para produzir óleo essencial com maior qualidade e melhor atividade antimicrobiana. Portanto,

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é possível melhorar o rendimento, a concentração dos constituintes e a resposta antimicrobiana do óleo essencial através de uma adubação orgânica acessível e de baixo custo.

São necessários outros estudos para avaliar se essas diferenças podem ser mais acentuadas com as variações sazonais. A análise deste tipo de estudo com a mesma duração em um intervalo de 365 dias, poderá refletir melhor as alterações sofridas pelo vegetal e as consequências na produção de seus metabólitos secundários.

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CAPÍTULO 4

Analysis of the nutritional state and the essential oil of leaves of *Plectranthus amboinicus* (Lour.) Spreng. as a function of seasonal variation and organic substrate

Artigo submetido no periódico South African Journal of Botany

South African Journal of Botany

Effect of seasonal climate and organic substrate on nutritional state and essential oil of Plectranthus amboinicus (Lour.) Spreng. in the Brazilian Legal Amazon

--Manuscript Draft--

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<tr><td>Abstract:</td><td>Plectranthus amboinicus (Lour.) Spreng., popularly known as oregano, has antimicrobial, anti-inflammatory, antitumor, healing, and larvicidal properties, among others. We examined how leaf nutritional status and essential oil (EO) of P. amboinicus changes by seasonality associated with organic fertilizer. This research was conducted in a area of Braziliam Amazon and was assessed with two organic fertilizer at the beginning of the rainy season, during the rainy season, end of the rainy season and the dry season. The composition of EO was analised by CG-MS. The EO differed qualitatively and quantitatively between fertilizers and seasonal variations, with the better yield occurring at the beginning of the rainy season. The samples had 26 different constituents, 8 of which are common to all samples: caryophyllene oxide, humulene, caryophyllene, α-bergamotene, carvacrol, terpinen-4-ol, γ-terpinene, and p-cymene. The constituents at higher concentrations in all samples were: caryophyllene, γ-terpinene, p-cymene, and carvacrol, with the latter having the highest concentration. Mixed fertilizer influenced increase in the N, P, S, B and Cu content in leaves. The Fe and B in leaves of P. amboinicus were more sensitive to dry season. The mixed fertilizer (with bovine and poultry manure) in the dry season resulted in the better leaf nutritional status and, consequently, higher carvacrol content. Changes in chemical composition and essential oil yield were influenced by both the type of organic fertilizer and seasonality.</td></tr>
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	Katia Castanho Scortecci kacscort@yahoo.com Research the mechanism of plant response to antibiotic stresses and the prospection of plant biomolecules for biological and pharmacological activities.

Dear Editor of South African Journal of Botany,

The manuscript "Effect of seasonal climate and organic substrate on nutritional state and essential oil of *Plectranthus amboinicus* (Lour.) Spreng in the *Brazilian* Legal Amazon" is an original study conducted in a area of in the *Brazilian* Amazon in the dry season, at the beginning of the rainy season, during the rainy season and end of the rainy season, and the effect of seasonality with two types of organic fertilize were assessed by in the leaf nutritional status and in the chemical composition of the essential oil of *Plectranthus amboinicus* (Lour.) Spreng

Although much is known about how water availability or fertilization changes the of production and crops of plant, there is a surprisingly lack of knowledge on how water availability associated with organic fertilize affects the leaf nutritional status and the chemical composition of the essential oil, especially in species in the Amazon.

The target audience includes all researchers working with plant, as the results of the research allow to guide the cultivation according to the better understanding of the behavior of the species the seasonal change with the use of organic fertilization, as an alternative to the use of chemical fertilizers. This journal was chosen due to its excellent (high) concept, credibility, and wide visibility, which allows to disseminate this acquired knowledge to several countries. I count on your collaboration in allowing the analysis of the manuscript in this journal.

Yours sincerely,
Elizabeth Borba

HIGHLIGHTS

The dry season is the most suitable for the cultivation of *P. amboinicus*.

Mixed fertilizer influenced increase in the N, P, S, B and Cu content in leaves.

Fe and B in leaves of *P. amboinicus* were more sensitive to dry season.

The lowest essential oil yields of *P. amboinicus* occurred in intense rain.

Mixed fertilization is recommended as alternative substrate for *P. amboinicus*.

Effect of seasonal climate and organic substrate on nutritional state and essential oil of *Plectranthus amboinicus* (Lour.) Spreng. in the Brazilian Legal Amazon

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ABSTRACT

Plectranthus amboinicus (Lour.) Spreng., popularly known as oregano, has antimicrobial, anti-inflammatory, antitumor, healing, and larvicidal properties, among others. We examined how leaf nutritional status and essential oil (EO) of *P. amboinicus* changes by seasonality associated with organic fertilizer. This research was conducted in a area of Braziliam Amazon and was assessed with two organic fertilizer at the beginning of the rainy season, during the rainy season, end of the rainy season and the dry season. The composition of EO was analised by CG-MS. The EO differed qualitatively and quantitatively between fertilizers and seasonal variations, with the better yield occurring at the beginning of the rainy season. The samples had 26 different constituents, 8 of which are common to all samples: caryophyllene oxide, humulene, caryophyllene, α-bergamotene, carvacrol, terpinen-4-ol, γ-terpinene, and p-cymene. The constituents at higher concentrations in all samples were: caryophyllene, γ-terpinene, p-cymene, and carvacrol, with the latter having the highest concentration. Mixed fertilizer influenced increase in the N, P, S, B and Cu content in leaves. The Fe and B in leaves of *P. amboinicus* were more sensitive to dry season. The mixed fertilizer (with bovine and

poultry manure) in the dry season resulted in the better leaf nutritional status and, consequently, higher carvacrol content. Changes in chemical composition and essential oil yield were influenced by both the type of organic fertilizer and seasonality.

Keywords: Oregano; Organic fertilization; Leaf nutrition; Essential oil; Carvacrol

1. Introduction

The Brazilian Amazon encompasses nine states and for the purposes of planning and management, the region was established as the Legal Amazon. The region represents around 60% of the Brazil's territory (Domingues et al., 2020).

Until a few decades ago, in the Amazon, there were only two seasons, the rainy season and the less rainy season. Today, the Amazon passes from catastrophic floods to droughts so radical that even water is lacking (Marengo and Souza Jr, 2018). The information about the plant vulnerability by the climate change are important tool to can be considerations into species management plans (Wilkening et al., 2019). For this reason, the knowledge of how they behave with climate change becomes essential for the preservation of species, even in seasonal conditions, such as those in the Amazon region.

The main extrinsic factors that influence the growth and development of plant species are water, mineral elements in the soil solution, temperature and light. Climatic factors (such as rainfall) and soil (related to soil conditions) impact all plant performance, including growth, development, reproduction and survival (Taiz et al., 2021).

External and internal factors influence the absorption of nutrient by plants (Guimarães et al., 2020a). In leaves, which are the main photosynthetic organ, the content of secondary metabolites can vary depending on the growth stage and environmental factors such as: season, temperature and soil moisture (Gomes et al., 2019). The nutritional analysis of the plants leads to the better use and yield of the analyzed plant, suggesting the most favorable conditions for its cultivation. This can be done through the association and interpretation of two studies: soil analysis and analysis of plant tissue, enabling the evaluation of different fertilizers and the causes of nutritional deficiencies (Rozane et al., 2016).

Nutrients are basically transported to the roots by mass flow and diffusion, so water availability in the soil influences the nutrient movement by these two mechanisms to the acquisition of macronutrients (N, P, K, Ca, Mg, and S) and cationic micronutrients (Fe, Mn, Zn, and Cu) (Oliveira et al., 2010).

The dry season usually reduces the absorption of nitrogen and other nutrients absorbed through the mass flow process (Vieira and Mochel Filho, 2010). Lack of these mineral elements leads to the impairment of plant growth reducing the vigor and may result in plant death (Brukhin and Morozova, 2011). Several studies confirme the negative effects of water deficit on the cultivation of plant (Niculcea et al., 2013; Elshibli et al., 2016; Guimarães et al., 2016).

Although water limitation causes harmful effects in most species, excess water is also not beneficial for the plant. The dynamics of soil physics are significantly altered by water saturation (Gazola et al., 2014). Excess water (through irrigation or rainfall) can also cause stress and alter the growth and development of plant species, with reduced productivity, as demonstrated by Pravuschi et al. (2010), Azevedo et al. (2011) and Dutra et al. (2012). Drought stress tolerance is seen in all plants but its extent varies from species to species (Akula and Ravishankar, 2011).

Essential oils (EOs) comprise a complex mixture of odorous, volatile, liquid and lipophilic substances, predominantly consisting of terpenes (monoterpenes and sesquiterpenes) and/or phenylpropanoids (Stephane and Jules, 2020). That compounds are also subject to qualitative and quantitative changes due to environmental and seasonal variations, methods of cultivation and harvest time (Guimarães et al., 2020b; Silva et al., 2020).

The yield and the content of EOs under water deficient conditions can be modified depending on the species and magnitude of the stress. Water restriction can compromise the content of essential essentials: a) increase, as in *Ocimum africanum* Lour (Santos et al., 2016); b) decrease, as in *Lippia menosides* Cham (Alvarenga et al., 2011); c) or even cause oscillatory results according to the intensity and duration of the water reduction, as in the species of *Cymbopogon* (Singh-Sangwan et al., 1994).

The EOs can also have qualitative changes in their chemical composition due to water deficiency, as demonstrated by Bettaieb et al. (2009) with *Salvia officinalis* L. However, in general, in EOs of species under water deficit there are only quantitative changes with increased yield and the content of its major components (Meira et al., 2013; Mckiernan et al., 2016; Maatallah et al., 2016). This change in the composition of the EOs varies according to the intensity of the reduction in water application and the characteristics of each species, as seen in the studies by Simon et al. (1992) and Yadav et al. (2014).

Plants of the genus *Plectranthus* (Lamiaceae) have a wide geographical distribution, occurring in Africa, America, Oceania and Asia (Mesquita et al., 2020) and comprises species cultivated as ornamental plants or as sources of essential oils. In over 85% of the literature, documentation of *Plectranthus* is on the therapeutic values of this genus followed by its nutritional and horticultural properties attributed to its aromatic nature and essential oil producing capability (Al-Juhani and Khalik, 2021; Hamed et al., 2021).

Plectranthus amboinicus (Lour.) Spreng., so known as mexican mint /oregano (Bañuelos-Hernández et al., 2020), is a useful edible herb and stands out as the most studied species in the Lamiaceae family (Terto et al., 2020). This herb is widely used by indigenous people of tropical rain forests for culinary and medicinal purposes. The leaves of the plant are often eaten raw or used as flavoring agents, or incorporated as ingredients in the preparation of traditional food (Khare et al., 2011; Mohanty et al., 2014; Prasad et al., 2020). The chopped leaves are also used as a substitute for *Salvia officinalis* in meat stuffing (Khare et al., 2011). *P. amboinicus* is also grown as an ornamental plant for its fresh aroma and attractive heart-shaped foliage or with white-edged (Arumugam et al., 2016).

This species is used also to ornamental (Prasad et al., 2020) and have economic value and potential for development products for use to nutrition and human health by industry (Kumar et al., 2014; Wadikar and Patki, 2016). The benefits of this species can be seen in both humans and animals, with different purposes and there are also patent registrations refer mainly to anti-inflammatory, healing and anti-tumor actions, treatment for osteoporosis, nutraceuticals and oral cavity cleaning agent, especially with new extraction methods or improvement methods with a predominance of process patents (Borba et al., 2021).

However, despite the existence of several studies aimed at establishing appropriate cultivation techniques for obtaining better quality and yielding plant species (Reimberg et al., 2009; Millani et al., 2010; Vilanova et al., 2018), there are no studies on the impact of the variation in the cultivation conditions of *P. amboinicus* on the nutritional content of its leaves and its EOs.

Analyzes on the oriented cultivation of plant species record the importance in the management of nutrients to guarantee the sustainability of this cultivation and the quality of production (Lemma and Abewoy, 2021). The knowledge how each species reacts to climate change are important steps in plant management (Li et al., 2020; Shen et al. 2021).

Thus, this research deal with production of *P. amboinicus* evaluated in the nutritional status of the leaf and chemical composition of the essential oil under tropical conditions in an area of the Legal amazon in the beginning of the rainy season, during the rainy season, end of the rainy season and dry season with two types of organic fertilization.

2. Material and methods

2.1. Experiment location and characterization of the collection zone

The study was conducted in an area of Brazilian Amazon (called Maranhense Amazon), in São Luís, Maranhão, Brazil (2°32'S and 44°16'W). This region has an average temperature of 27 °C and an average annual rainfall of 1896 mm (precipitation ranging from 1700 to 2300 mm). The predominant climate is tropical and the region is 24 m above sea level. According to the Köppen and Geiger classification, the climate is Aw', hot and humid, with a rainy season from January to June and dry season from July to December (NUGEO, 2002).

2.2. Cultivation and collection of plant material

Cultivations took place from November 2018 to October 2019 in a greenhouse at the "Berta Lange de Morretes" Medicinal Plants Garden of the Federal University of Maranhão - UFMA, São Luís, Maranhão, Brazil. The plants were grown with 50% shade.

The experiments included seedlings of the species *P. amboinicus*, which were transplanted in black plastic pots (perforated at the bottom) with a capacity of 10 dm^3. The experimental design was completely randomized with three replications in a factorial scheme (2 x 4). The treatments consisted of two types of substrate and four harvest periods, covering the dry and rainy seasons of the region, as shown in Table 1.

Table 1

Treatments applied to *Plectranthus amboinicus* (Lour.) Spreng.

Treatment (T)		Substrate	Cultivation and collection
1	A	soil with bovine and poultry manure (mixed)	beginning of the rainy season (11/02/18 - 01/15/19)
	B	soil with bovine manure	
2	A	soil with bovine and poultry manure (mixed)	intense rainy season (01/28/19 – 04/12/19)
	B	soil with bovine manure	
3	A	soil with bovine and poultry manure (mixed)	end of the rainy season (04/25/19 – 07/10/19)
	B	soil with bovine manure	
4	A	soil with bovine and poultry manure (mixed)	dry season (07/23/19 - 10/05/19)
	B	soil with bovine manure	

The cultivation lasted 75 days, with manual irrigation of 500 mL of water on alternate days. An exsiccata of the species (SLS nº 1477) was deposited at Herbarium Ático Seabra, in the Department of Pharmacy, UFMA, Brazil.

2.3. Analysis of substrates with organic fertilizers

Samples from treatments with organic fertilizers (A and B) were collected at a depth of 20 cm. The soil sampling occurred 30 days after the beginning of cultivation.

The soil cation exchange capacity (CEC) (calcium-Ca and magnesium-Mg) and the micronutrient content (Ca; Mg; copper – Cu; iron – Fe; zinc – Zn; and manganese - Mn) were determined by spectrophotometry of atomic absorption. The micronutrients potassium (K) and phosphorus (P) were extracted by Mehlich-1, while sulfur (S) was extracted by turbidimetry with spectrophotometric analysis. Potassium (K) was analyzed by flame spectrophotometry, while P was analyzed by ultraviolet-visible spectrophotometry (UV-VIS), following the methodology of Silva (2009) and Embrapa (2011). Nitrogen (N) content was analyzed using the micro-Kjeldahl method (Raij et al., 2001; Silva, 2009; Embrapa, 2011). The available boron (B) was determined by extraction with hot water and the content of organic matter (OM) by colorimetry (Raij et al., 2001).

2.4. Analysis of leaf nutritional status

The collections for leaf analysis were carried out at 8-9 a.m. every 75 days after planting, in four periods, as shown in Table 1. The collected leaves were desiccated in an oven with air circulating at 40 ºC, until constant weight. Chemical analyzes were carried out using techniques and methodologies: nitric-perchloric solubilization for P (colorimetry); flame photometry for K and sodium (Na); atomic absorption for Ca, Mg, Cu, Fe, Mn and Zn; turbidimetry for S; sulfuric solubilization (Kjeldahl) for N and dry solubilization (colorimetry) for B (Carmo et al., 2000).

2.5. Extraction of essential oils

Fresh leaves of *P. amboinicus* were dried at 40 ºC for 72 hours and then subjected to hydrodistillation for three hours in a Clevenger type apparatus (Brasil, 2019). The essential oils (EOs) were centrifuged (4000 rpm / 30 min), dehydrated with anhydrous sodium sulfate (Merck Chemical Co., Germany) and packed in amber glass ampoules. The material was kept refrigerated (2 - 8 ℃) until use. The content and yield of EOs were determined according to the methodology of Coutinho et al. (2007).

2.6. Chemical analysis of essential oils

Essential oil (EO) samples were analyzed using gas chromatography coupled to mass spectrometry (GC-MS - QP2020 - Shimadzu), under the following conditions: BPX5 capillary column (30 m x 0.25 mm x 0.25 µm) with temperature between 60 ºC and 240 ºC (3 ℃/ min); helium carrier gas (3mL min^{-1}); and injected volume of 1 µ L diluted in hexane. The injector temperature was 280 ºC, with column pressure of 111.5 KPa, linear speed of 48.9 cm/sec and scan mode of 0.5 s/scan. The mass spectrometer operated with an electron impact (EI) ionization detector at 70 eV, with automatic scanning in a range of 37–660 Daltons at 0.5 sec. The components were identified by comparison with the fragmentation patterns of the mass spectra described both in mass spectral libraries (NIST, 2005) and in the literature (Adams, 2007). Each constituent was quantified using the area normalization method (%).

2.7. Determination of essential oil yield

Essential oil yields were expressed as a percentage using the equation below (Rodrigues et al., 2011):

Eq. (1): $\%\ oil\ yield = W_{eo}(g)/W_2(g) \times 100$

W_{eo} = weight of the essential oil extracted;

W_2 = weight of the raw material used in the extraction

2.8. Determination of physicochemical indices of the essential oil

The determination of relative density and the refractive index (ABBE refractometer, model Q7678, at 22.4 ºC) followed the methodologies described in the 6th edition of the Brazilian Pharmacopoeia (Brasil, 2019).

2.9. Parameters analyzed

The analysis of substrates considered the following parameters: pH in $CaCl_2$; assortment complex (K, Ca, Mg, Al and H + Al); P, S and micronutrient (Fe, Cu, Mn, Zn) content; soil base saturation (V%); and cation exchange capacity (CEC). The parameters analyzed in the study of leaf nutritional status were the levels of N, K, Ca, Mg, S, Na, Zn, Mn, Fe and Cu.

The analyzes to determine the chemical attributes of the soil were: pH ($CaCl_2$), OM, S, Fe, Mn, Cu, P, K, Ca, Mg and potential acidity (H + Al). The results of these analyzes made it possible to calculate the CEC, base saturation of soil (V%) and sum of bases (SB).

The physicochemical analysis comprised the relative density and refractive index of the EO. For relative density, the mass of EO was analyzed in relation to that of water, while the refractive index considered the relationship between atmospheric air and EO.

The chemical analysis comprised the content and yield of EO, as well as the presence and/or absence of chemical components in the EO of the leaves of each treatment.

2.10. Statistical analyzes

Statistical analyzes were performed using the Statistical Package for the Social Sciences - SPSS Version 26. Multivariate data analysis was performed through analysis of variance (ANOVA) and Tukey test; treatments were compared using the Student's t test.

Principal Component Analysis (PCA; R CORE TEAM, 2019) was used to investigate similarities between EO components in the treatments. All graphics were made using the Sigma Plot 10.0 program (Systat Software, San Jose, CA). All data were analyzed by the programs R (R CORE TEAM, 2019) and Sisvar 5.6 (FERREIRA, 2011).

3. Results

3.1. Soil analysis

The analysis of the soil assortment complex showed no significant change for the K content and, consequently, for the H + Al content with fertilizers A and B in the analyzed periods (Table 2). Fertilizer B significantly influenced the increase of Ca content in the soil in all rainy seasons (T1, T2 and T3). This contributed to the SB and CEC also being influenced by fertilizer B, which increased their values in relation to fertilizer A. The Ca was the nutrient that most contributed to these increases in SB and CEC, resulting in the increase of this compound in all treatments with fertilizer B. The V% was also more influenced by fertilizer B, except in T2 (Table 2).

Table 2

Average values of chemical attributes of the soil as a function of organic fertilizers in each climatic period in the cultivation of *Plectranthus amboinic*us (Lour.) Spreng.

TREATMENT (T)*		**ATTRIBUTE**** **K^+**	**Ca^{2+}**	**Mg^{2+}**	**$H^+ + Al^{3+}$**	**SB**	**CEC**	**V**	**Ca^{2+}**	**Mg^{2+}**	**K^+**
		..........cmol/dm^3..........						**..........%..........**			
1	A	0.2^{ns}	2.0^{b}	1.1^{ns}	0.7^{ns}	3.3^{b}	4.1^{b}	81.9^{b}	49.0^{b}	27.3^{a}	5.6^{a}
	B	0.2^{ns}	7.8^{a}	2.4^{ns}	1.2^{ns}	10.4^{a}	11.7^{a}	89.7^{a}	66.9^{a}	20.7^{b}	2.0^{b}
2	A	0.2^{ns}	2.6^{b}	1.1^{b}	0.5^{ns}	3.9^{b}	4.4^{b}	89.0^{ns}	59.5^{b}	25.8^{ns}	3.7^{ns}
	B	0.4^{ns}	8.7^{a}	2.7^{a}	0.6^{ns}	11.7^{a}	12.5^{a}	95.4^{ns}	70.6^{a}	21.5^{ns}	3.3^{ns}
3	A	0.6^{ns}	3.8^{b}	1.9^{ns}	0.5^{ns}	6.4^{b}	6.9^{b}	92.6^{b}	55.8^{b}	28.2^{ns}	8.6^{a}
	B	0.3^{ns}	9.3^{a}	3.4^{ns}	0.6^{ns}	13^{a}	13.6^{a}	95.9^{a}	68.6^{a}	25^{ns}	2.3^{b}
4	A	0.6^{ns}	5.5^{ns}	2.8^{ns}	1.1^{ns}	8.9^{b}	9.9^{b}	89.3^{b}	54.9^{ns}	28.4^{ns}	6.0^{a}
	B	0.7^{ns}	9.9^{ns}	5.2^{ns}	0.8^{ns}	15.7^{a}	16.6^{a}	95.4^{a}	60.1^{ns}	31.1^{ns}	4.2^{b}
		pH $CaCl_2$	**OM**	**P**	**S**		**Fe**	**Mn**	**Cu**		**Zn**
			..........mg/dm^3..........								
1	A	5.6^{ns}	23.2^{b}	59.1^{b}	0.4^{ns}		252.3^{a}	21.1^{b}	2.5^{ns}		6.5^{b}
	B	5.7^{ns}	50.4^{a}	117.4^{a}	0.7^{ns}		30.7^{b}	100.0^{a}	4.5^{ns}		20.2^{a}
2	A	5.7^{ns}	26.6^{b}	95.7^{b}	0.9^{ns}		106.4^{a}	44.9^{b}	3.3^{ns}		12.3^{b}
	B	6.0^{ns}	43.7^{a}	123.9^{a}	1.7^{ns}		31.7^{b}	110.9^{a}	2.9^{ns}		28.6^{a}
3	A	6.1^{ns}	44.4^{b}	132.0^{a}	1.1^{b}		165.2^{a}	55.1^{a}	3.8^{ns}		16.4^{ns}
	B	6.0^{ns}	57.8^{a}	76.4^{b}	3.7^{a}		28.9^{b}	50.0^{b}	1.1^{ns}		23.9^{ns}
4	A	5.4^{ns}	49.2^{b}	127.7^{a}	4.0^{b}		71.1^{a}	45.0^{b}	4.3^{ns}		15.6^{ns}
	B	5.8^{ns}	61.7^{a}	95.7^{b}	19.3^{a}		37.6^{b}	51.0^{a}	1.3^{ns}		24.3^{ns}

* A: mixed fertilizer; B: bovine fertilizer; 1: beginning of the rainy season; 2: intense rainy season; 3: end of the rainy season; 4: dry season.
** K: Potassium; Ca: Calcium; Mg: Magnesium; H + Al: Hydrogen + Aluminum; SB: sum of bases (K + Ca + Mg); CEC: cation exchange capacity; V: base saturation; $CaCl_2$: Calcium chloride; P: Phosphorus; S: Sulfur; Fe: Iron; Mn: Manganese; Cu: Copper; Zn: Zinc; OM: organic matter.
Note: means with different lower case letters in the columns differ from each other by the LSD test at 5% for the fertilizers used within each climatic period; ns = not significant by Student's t test.

Fertilizer B significantly increased the values of OM in all treatments in relation to fertilizer A. In addition, it positively influenced the Mn content in all treatments, except in T3. However, fertilizer A influenced the Fe content; this micronutrient increased in all treatments with mixed fertilizer in relation to fertilizer B. The fertilizers used did not significantly influence Cu. Furthermore, the P content increased with the use of fertilizer B in T1 and T2 and with the use of fertilizer A in T3 and T4 treatments (Table 2).

The analysis of the chemical attributes of the soil under climatic variation (Table 3) showed that this variation did not significantly influence the Ca and K content for fertilizer A. However, T1 and T2 treatments were responsible for the lowest SB, CEC and

V% values due to the most significant reduction of these compounds in these seasons (48.97% Ca and 3.70% K).

Table 3

Average values of soil analysis as a function of climatic periods in each organic fertilization in the cultivation of *Plectranthus amboinicus* (Lour.) Spreng.

TREATMENT (T)*	ATTRIBUTE**									
	K^{+}	Ca^{2+}	Mg^{2+}	$H^{+} + Al^{3+}$	SB	CEC	V	Ca^{2+}	Mg^{2+}	K^{+}
	..................................cmol/dm^{3}.............................						...................%..................			
A										
1	0.23^{a}	2.33^{a}	1.10^{b}	0.73^{a}	3.30^{b}	4.07^{c}	81.90^{b}	48.97^{b}	27.33^{a}	5.63^{b}
2	0.20^{a}	2.63^{a}	1.07^{b}	0.47^{a}	3.87^{b}	4.43^{c}	89.00^{a}	59.53^{a}	25.83^{a}	3.70^{c}
3	0.63^{a}	3.83^{a}	1.90^{ab}	0.47^{a}	6.37^{ab}	6.93^{b}	92.60^{a}	55.80^{a}	28.20^{a}	8.60^{a}
4	0.57^{a}	5.47^{a}	2.83^{a}	1.13^{a}	8.87^{a}	9.93^{a}	89.33^{a}	54.93^{ab}	28.43^{a}	6.00^{b}
B										
1	0.17^{a}	7.77^{a}	2.40^{a}	1.20^{a}	10.43^{c}	11.67^{c}	89.73^{b}	66.90^{b}	20.70^{c}	1.97^{b}
2	0.40^{a}	8.73^{a}	2.73^{a}	0.60^{a}	11.70^{bc}	12.50^{bc}	95.37^{a}	70.63^{a}	21.47^{bc}	3.27^{ab}
3	0.33^{a}	9.33^{a}	3.40^{a}	0.63^{a}	13.00^{b}	13.60^{b}	95.90^{a}	68.63^{ab}	25.00^{b}	2.33^{b}
4	0.67^{a}	9.93^{a}	5.23^{a}	0.80^{a}	15.70^{a}	16.63^{a}	95.37^{a}	60.10^{ab}	31.07^{a}	4.20^{a}
	pH $CaCl_2$	OM	P	S		Fe	Mn	Cu		Zn
		....mg/dm^{3}...								
A										
1	5.57^{a}	23.17^{d}	59.10^{d}	0.44c		252.30^{a}	21.10^{c}	2.50^{a}		6.50^{b}
2	5.67^{a}	26.57^{c}	95.67^{c}	0.87^{b}		106.40^{c}	44.90^{b}	3.30^{a}		12.30^{ab}
3	6.07^{a}	44.37^{b}	132.00^{a}	1.10^{ab}		165.20^{b}	55.10^{a}	3.80^{a}		16.40^{a}
4	5.40^{a}	49.20^{a}	127.70^{b}	3.97^{a}		71.10^{c}	45.00^{b}	4.30^{a}		15.60^{a}
B										
1	5.70^{a}	50.43^{c}	117.40^{b}	0.70^{c}		30.67^{a}	100.00^{b}	4.50^{a}		20.20^{b}
2	6.00^{a}	43.70^{d}	123.90^{a}	1.73^{c}		31.70^{a}	110.90^{a}	2.90^{a}		28.60^{a}
3	6.00^{a}	57.83^{b}	76.40^{d}	3.73^{b}		28.93^{a}	50.00^{c}	1.10^{a}		23.90^{ab}
4	5.80^{a}	61.73^{a}	95.73^{c}	19.30^{a}		37.60^{a}	51.00^{c}	1.30^{a}		24.30^{ab}

* A: mixed fertilizer; B: bovine fertilizer; 1: beginning of the rainy season; 2: intense rainy season; 3: end of the rainy season; 4: dry season.
** K: Potassium; Ca: Calcium; Mg: Magnesium; H+Al: Hydrogen + Aluminum; SB: sum of bases (K + Ca + Mg); CEC: cation exchange capacity; V: base saturation; $CaCl_2$: Calcium chloride; OM: organic matter; P: Phosphorus; S: Sulfur; Fe: Iron; Mn: Manganese; Cu: Copper; Zn: Zinc.
Note: means with different lower case letters in the columns differ from each other by the LSD test at 5% for the climatic periods analyzed in each fertilization by ANOVA test, Post Hoc Tukey test.

Under climatic variation, the assortment complex behaved similarly with fertilizer B (Table 3). This indicates that the type of fertilizer influenced the changes in soil attributes more strongly than climatic period (Table 2). However, the values of the parameters were more stable, as in Mg, which did not change with climatic variation when fertilizer B was used (Table 3).

Regarding the nutritional content of the soil under climatic variation, fertilizer A reduced the content of most micronutrients (P, S, Mn and Zn), except Fe (252.3 mg/dm^3) in T1. The Fe content increased by 71.82% in T1 in relation to the lowest value obtained in T4. Noteworthy is the linear increase in OM and S over the analyzed climatic periods, with lower values (23.2 and 0.4 mg/dm^3, respectively) in T1 and the highest values (49.2 and 3.97 mg/dm^3, in that order) in T4. These values correspond to increases of 47.15% and 90%, respectively, in these two parameters in the dry season in relation to the other periods (Table 3).

However, fertilizer B led to a linear increase in S values in the analyzed seasons, with the lowest value (0.7 mg/dm^3) occurring in T1 and the highest value (19.3 mg/dm^3) in T4, similar to what occurred in treatments with fertilizer A. The T4 treatment also stands out as the period of greatest increase in S content, corresponding to an increase of 96.4% in relation to T1 and a 6.4% in relation to the same period (dry season) with fertilizer A.

The T1 and T4 treatments were the ones that most influenced soil fertility. Considering the parameters of the assortment complex, these two periods also had a greater influence on soil nutrient content. In general, this content increased more during the dry season (T4), regardless of fertilizer type. The results of this study showed an improvement in soil fertility at T4 compared to other treatments.

3.2. Leaf diagnosis

The leaf analysis showed a higher influence of fertilization on the K content, followed by the content of N, Ca, Cu and Mg. Fertilizer A influenced the increase in the nutrient content in leaves more than the fertilizer B. In other words, more compounds increased in content with fertilizer A than with fertilizer B. Fertilizer A increased the levels of N, P, S, B and Cu, while fertilizer B increased only the levels of Mn, K and Ca (Table 4).

Table 4

Nutritional analysis of leaves of *Plectranthus amboinicus* (Lour.) Spreng as a function of organic fertilizers in each climatic period.

TREATMENT (T)*		NUTRIENT**										
		N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
		……g/Kg……					……mg/dm³……					
1	A	14.0^{ns}	5.5^{ns}	80.6^{a}	18.6^{ns}	9.9^{ns}	1.1^{ns}	49.2^{ns}	17.0^{ns}	418.0^{ns}	45.0^{ns}	32.0^{ns}
	B	14.0^{ns}	4.9^{ns}	75.2^{b}	19.1^{ns}	9.6^{ns}	1.2^{ns}	47.9^{ns}	16.1^{ns}	410.0^{ns}	54.0^{ns}	33.0^{ns}
2	A	26.6^{a}	6.1^{ns}	54.7^{b}	28.7^{a}	11.0^{ns}	1.5^{ns}	60.3^{a}	17.6^{ns}	241.0^{ns}	36.0^{b}	40.0^{ns}
	B	21.0^{b}	4.8^{ns}	65.2^{a}	19.8^{b}	8.8^{ns}	1.1^{ns}	47.9^{b}	11.2^{ns}	333.0^{ns}	61.0^{a}	34.0^{ns}
3	A	30.8^{a}	4.9^{ns}	60.1^{ns}	20.7^{b}	9.3^{ns}	0.7^{ns}	59.0^{ns}	19.1^{a}	615.0^{ns}	43.0^{ns}	57.0^{ns}
	B	16.8^{b}	3.6^{ns}	61.6^{ns}	37.8^{a}	11.7^{ns}	0.7^{ns}	54.1^{ns}	8.9^{b}	295.0^{ns}	54.0^{ns}	48.0^{ns}
4	A	35.0^{ns}	6.4^{a}	71.6^{a}	18.7^{ns}	9.1^{ns}	1.6^{a}	7.8^{ns}	19.7^{a}	235.0^{ns}	30.0^{b}	36.0^{ns}
	B	25.2^{ns}	3.6^{b}	57.6^{b}	25.6^{ns}	9.3^{ns}	0.6^{b}	36.5^{ns}	8.8^{b}	226.0^{ns}	61.0^{a}	40.0^{ns}

* A: mixed fertilizer; B: bovine fertilizer; 1: beginning of the rainy season; 2: intense rainy season; 3: end of the rainy season; 4: dry season.
** N: Nitrogen; P: Phosphorus; K: Potassium; Ca: Calcium; Mg: Magnesium; S: Sulfur; B: Boron; Cu: Copper; Fe: Iron; Mn: Manganese; Zn: Zinc.
Note: means with different lower case letters in the columns differ from each other by the LSD test at 5% for the fertilizers used within each climatic period; ns = not significant by Student's t test.

Regarding soil nutrition, the fertilizers used showed an inverse correlation. Fertilizer A decreased the content of Fe in the leaves and increased the content of Fe content in the soil, while the opposite occurred with fertilizer B. This higher content of nutrient in the soil indicates less absorption of nutrients by *P. amboinicus*.

In relation to climatic variation, fertilizer A caused oscillations in the content of most compounds of the leaves of *P. amboinicus* throughout the seasons, except for P, Cu and Mn, whose contents were not influenced by climatic variation (Table 5). It is noteworthy that the Fe content decreased significantly (237 mg/dm^3) in T4, with a higher value (615 mg/dm^3) in T3; and B showed a great difference between the highest and lowest value, with the highest value (60.3 mg/dm^3) in T2 and the lowest value (7.8 mg/dm^3) in T4. Thus, the levels of B and Fe decreased by 87.06% and 38.54%, respectively, in the dry period (T4) in relation to the rainy period (T2 and T3) (Table 5).

Regarding the nutritional content of the leaves of *P. amboinicus* with fertilizer B, K and Fe showed linear behavior, with lower values (57.6 g/Kg and 226 mg/dm^3, respectively) in T4 and higher values (75.2 g/Kg and 410 mg/dm^3, respectively) in T1, in contrast to N and Fe with fertilizer A (Table 5).

Table 5

Nutritional analysis of leaves of *Plectranthus amboinicus* (Lour.) Spreng as a function of climatic periods in each organic fertilization.

TREATMENT (T)*	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	NUTRIENT**										
	..............................g/Kg............................					..............................mg/dm^3..............................					
A											
1	14.0^{c}	5.5^{ns}	80.6^{a}	18.6^{b}	9.9^{ab}	1.1^{ab}	49.2^{b}	17.0^{a}	418.0^{bc}	45.0^{ns}	32.0^{b}
2	26.6^{b}	6.1^{ns}	54.7^{b}	28.7^{a}	11.0^{a}	1.5^{a}	60.3^{a}	17.6^{a}	241.0^{c}	36.0^{ns}	40.0^{ab}
3	30.8b^{a}	4.9^{ns}	60.1^{ab}	20.7^{ab}	9.3^{ab}	0.7^{b}	59.0^{a}	19.1^{a}	615.0^{a}	43.0^{ns}	57.0^{a}
4	35.0^{a}	6.4^{ns}	71.6^{ab}	18.7^{b}	9.1^{b}	1.6^{a}	7.8^{c}	19.7^{a}	235.0^{c}	30.0^{ns}	36.0^{b}
B											
1	14.0^{d}	4.9^{ns}	75.2^{a}	19.1^{b}	9.6^{a}	1.2^{a}	47.9^{a}	16.1^{a}	410.0^{a}	54.0^{b}	33.0^{b}
2	21.0^{b}	4.8^{ns}	65.2^{b}	19.8^{b}	8.8^{a}	1.1^{ab}	47.9^{a}	11.2^{a}	333.0^{ab}	61.0^{a}	34.0^{b}
3	16.8^{c}	3.6^{ns}	61.6^{bc}	37.8^{a}	11.7^{a}	0.7^{ab}	54.1^{a}	8.9^{b}	295.0^{bc}	54.0^{b}	48.0^{a}
4	25.2^{a}	3.6^{ns}	57.6^{c}	25.6^{ab}	9.3^{a}	0.6^{b}	36.5^{b}	8.8^{b}	226.0^{c}	61.0^{a}	40.0^{ab}

* A: mixed fertilizer; B: bovine fertilizer; 1: beginning of the rainy season; 2: intense rainy season; 3: end of the rainy season; 4: dry season.
** N: Nitrogen; P: Phosphorus; K: Potassium; Ca: Calcium; Mg: Magnesium; S: Sulfur; B: Boron; Cu: Copper; Fe: Iron; Mn: Manganese; Zn: Zinc.
Note: means with different lower case letters in the columns differ from each other by the LSD test at 5% for the climatic periods analyzed in each fertilization by ANOVA test, Post Hoc Tukey test. Ns: not significant.

The significant reduction of Fe again stands out, with a decrease of 55.12% of its content in T4 in relation to the higher value obtained with fertilizer B, similar to what occurred with fertilizer A (Table 5).

The climatic periods had less influence on the variation of the nutrient content in leaves with fertilizer B than with fertilizer A. For the latter, the nutrients P, Mg, B, Cu and Mn varied widely in the analyzed seasons. A probable explanation for this is the greater stimulation of the microbiota by fertilizer A, leading to a cascade effect on soil fertility and on the nutritional status of the soil, favoring the absorption of nutrients by the plant. In this way, the plant was able to assimilate more easily the nutrients resulting from the decomposition of poultry manure present in fertilizer A, which contributed to lower values of nutrients in the soil.

As was the case for the complex parameters of soil assortment and nutritional status of soil, T1 and T4 were also the ones that most influenced the leaf nutritional content in the species under study. However, T4 increased the nutrient content in the soil and decreased the nutrient content in the leaves, with the opposite occurring in T1. This demonstrates that the nutrients at higher levels in the soil were the least absorbed by *P.*

amboinicus during the rainy season (T1) and vice versa, justifying the reduction of these nutrients in the soil in T1, the time of greatest absorption by the species under study.

3.3. Chemical analysis and yield

The data obtained show that the compounds isolated from the EO varied quantitatively and qualitatively depending on the cultivation period. In other words, the chemical composition differed between the samples. These differences were only quantitative in some cases and qualitative and quantitative in others. Only in T4 there was a change in the content of constituents, with the same chemical composition in both types of fertilizers.

Twenty-six distinct constituents were identified in EO samples, eight of which are common to all samples analyzed: caryophyllene oxide, humulene, caryophyllene, α-bergamotene, carvacrol, terpinen-4-ol, γ-terpinene and p-cymene. The amounts of the isolated constituents were similar in the rainy seasons (T2 and T3), even with different fertilizers (Table 6).

Fertilization altered the second major component in T2 and in T4, in which fertilizers A and B contributed more significantly to the formation of γ-terpinene and caryophyllene, respectively. The first major component (carvacrol) remained in the same position despite the change in the type of fertilizer in all periods, with a change only in its content.

Table 6

Chemical analysis and yield of essential oils from leaves of *Plectranthus amboinicus* (Lour.) Spreng as a function of organic fertilizers in each climatic period.

	COMPOUND		**TREATMENT (T)****							
			1		**2**		**3**		**4**	
			A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
1	α-Thujene	902*	-	-	0.58^{b}	2.30^{a}	1.08^{ns}	1.03^{ns}	1.60^{ns}	2.20^{ns}
2	(+)-4-Carene	919	-	-	-	-	3.17^{a}	2.67^{b}	-	-
3	(+)-2-Carene	946	0.21^{a}	0.12^{b}	-	-	-	-	-	-
4	α-Pinene	948	-	-	0.24^{a}	-	0.45^{ns}	0.40^{ns}	0.62^{b}	0.89^{a}
5	Myrcene	958	-	-	0.98^{ns}	0.93^{ns}	1.60^{a}	1.04^{b}	2.98^{b}	3.65^{a}
6	1-Octen-3-ol	962	0.00^{b}	0.35^{a}	-	-	0.15^{a}	-	2.92^{ns}	2.86^{ns}
7	β-Phellandrene	964	-	-	0.26^{a}	-	0.32^{a}	-	-	-
8	α-Phellandrene	969	-	-	0.28^{ns}	0.30^{ns}	0.47^{ns}	0.40^{ns}	0.65^{ns}	0.87^{ns}
9	**γ-Terpinene**	998	**2.19^{a}**	**1.46^{b}**	**19.84^{a}**	**8.87^{b}**	**14.43^{b}**	**15.10^{a}**	**14.16^{b}**	**15.04^{a}**
10	Limonene	1018	-	-	0.30^{ns}	0.30^{ns}	0.63^{a}	-	0.82^{b}	1.10^{a}
11	Sabinene hydrate	1041	-	-	-	-	0.35^{a}	-	-	-
12	**p-cymene**	1042	**1.37^{a}**	**1.21^{b}**	**7.43^{a}**	**6.30^{b}**	**7.43^{b}**	**8.21^{a}**	**8.63^{b}**	**10.04^{a}**
13	Terpinolene	1052	-	-	-	-	-	-	4.80^{b}	5.75^{a}
14	Terpinen-4-ol	1137	0.33^{b}	0.97^{a}	0.76^{b}	2.60^{a}	1.32^{a}	1.08$_{b}$	0.77^{b}	1.63^{a}
15	Borneol	1138	-	-	0.06^{a}	-	-	-	-	-
16	**Carvacrol**	1262	**82.25^{a}**	**80.54^{b}**	**55.13^{a}**	**47.60^{b}**	**49.42^{b}**	**58.67^{a}**	**37.52^{a}**	**29.20^{b}**
17	Linalyl propionate	1371	-	-	-	-	0.24^{a}	-	-	-
18	α-Bergamotene	1430	3.89^{a}	3.77^{b}	3.26^{b}	8.43^{a}	5.76^{b}	2.86^{a}	6.47^{a}	5.37^{b}
29	(E)-β-Farnesene	1440	-	0.09^{a}	-	0.67^{a}	-	-	-	-
20	Aromadendrene oxide-(2)	1462	-	0.32^{a}	-	0.67^{a}	-	-	0.69^{ns}	0.40^{ns}
21	**Caryophyllene**	1494	**6.06^{b}**	**6.72^{a}**	**6.73^{b}**	**12.30^{a}**	**8.46^{a}**	**5.05^{b}**	**10.00^{b}**	**16.14^{a}**
22	β-Bisabolene	1500	-	0.21^{a}	-	0.80^{a}	0.33^{a}	-	0.44^{a}	0.32^{b}
23	Caryophyllene oxide	1507	1.30^{a}	1.13^{b}	1.43^{ns}	1.70^{ns}	1.18^{a}	0.57^{b}	1.72^{a}	1.26^{b}
24	Humulene	1579	1.66^{ns}	1.71^{ns}	1.78^{b}	4.40^{a}	2.94^{a}	1.51^{b}	3.96^{a}	2.90^{b}
25	Humulene epoxide II	1592	-	0.21^{a}	0.18^{a}	-	-	-	-	-
26	Geranyl-α-terpinene	1962	-	-	0.52^{a}	-	-	-	-	-
	Hydrocarbon monoterpenes		3.76^{a}	3.14^{b}	29.91^{a}	18.83^{b}	29.53^{a}	28.81^{b}	34.22^{b}	39.53^{a}
	Oxygenated monoterpenes		82.58^{a}	81.51^{b}	55.92^{a}	50.20^{b}	51.13^{b}	59.74^{a}	38.30^{a}	30.83^{b}
	Hydrocarbon sesquiterpenes		11.61^{b}	12.50^{a}	11.85^{b}	26.56^{a}	17.51^{a}	9.40^{b}	20.90^{b}	21.83^{a}
	Oxygenated sesquiterpenes		1.30^{ns}	1.67^{ns}	1.61^{b}	2.35^{a}	1.20^{a}	0.56^{b}	2.41^{b}	4.55^{a}
	Others		-	-	0.52^{a}	-	0.15^{b}	0.24^{a}	2.92^{ns}	2.86^{ns}
	Total identified (%)		99.25^{a}	98.81^{b}	99.81^{a}	97.93^{b}	99.44^{a}	98.75^{b}	98.71^{b}	99.60^{a}
	Yield (%)		1.52^{ns}	1.60^{ns}	0.40^{ns}	0.51^{ns}	1.20^{b}	1.65^{a}	1.24^{a}	0.67^{b}

* Linear retention index of the library (Adams, 2007).

**A: mixed fertilizer; B: bovine fertilizer; 1: beginning of the rainy season; 2: intense rainy season; 3: end of the rainy season; 4: dry season.

Note: data correspond to mean values (n = 3). Means with different lower case letters in the columns differ by the LSD test at 5% for the climatic periods in each fertilization by the ANOVA test, Post Hoc Tukey test; ns: not significant; -: not detected.

The presence of Mn in fertilizer B may have stimulated the production of caryophyllene. This compound was detected in higher concentrations in leaves cultivated with fertilizer B in the same climatic periods in which this change occurred (T2 and T4). In turn, Mn was detected in lower concentrations in leaves cultivated with fertilizer A, while N and P were detected in higher concentrations with this same fertilizer (Table 5).

When considering only the climatic period (T1, T2, T3 and T4), within each type of fertilizer (A and B), the first major component (carvacrol) remained superior in relation to the other compounds in all periods. Nevertheless, fertilizer A led to a linear decrease in its values from period 1 to 4, in which the levels of carvacrol were 82.25%, 55.1%, 49.42% and 37.52% in T1, T2, T3 and T4, respectively. Fertilizer A decreased the content of carvacrol as the rainy season intensified and during the period of intense drought. In other words, the carvacrol content was lower in intense rain (T2 and T3) and drought (T4) conditions.

Fertilizer B led to a similar reduction in the content of carvacrol, which had a higher value in T1 (80.54%) and a lower value (29.2%) in T4. However, the reduction was not linear, as the content fluctuated according to intensity of the rains (T2 and T3). In these rainy periods, the content of carvacrol increased from 47.59% (T2) to 58.66% (T3), showing that the increase in rain can increase the content of carvacrol in samples grown with fertilizer B (Table 7).

The second major compound of EO changed from caryophyllene in T1 to γ-terpinene in T3. During these periods, these compounds were more sensitive to climate change than to fertilization, with the change of the second major compound being due to climatic season and not to the type of fertilizer. It is the opposite of what happened in the period of intense rains (T2) and in the dry season (T4), where there was the change of the second major compound (from caryophyllene to γ-terpinene) due to changes in fertilization, as already mentioned (Table 6).

The production of caryophyllene fluctuated in a cyclical pattern over climatic seasons with fertilizer B. The content of this compound decreased in T1, increased in T2, decreased in T3 and increased again in T4. The lowest value (5.05%) occurred at the end of the rainy season (T3) and the highest value (16.14%) in the dry season (T4). With fertilizer A, however, the caryophyllene content increased linearly from T1 to T4, with values from 6.06% to 10.1%, respectively (Table 7).

Table 7

Chemical analysis and yield of essential oils from leaves of *Plectranthus amboinicus* (Lour.) Spreng as a function of climatic periods in each organic fertilization.

* Linear retention index of the library (Adams, 2007).
**A: mixed fertilizer; B: bovine fertilizer; 1: beginning of the rainy season; 2: intense rainy season; 3: end of the rainy season; 4: dry season.
Note: data correspond to mean values (n = 3). Means with different lower case letters in the columns differ

COMPOUND			TREATMENT**							
			A				B			
			1	2	3	4	1	2	3	4
1	α-Thujene	902*	-	0.58^{c}	1.08^{b}	1.60^{a}	-	2.30^{a}	1.03^{c}	2.20^{b}
2	(+)-4-Carene	919	0.00^{b}	-	3.17^{a}	-	-	-	2.67^{a}	-
3	(+)-2-Carene	946	0.21^{a}	-	-	-	0.12^{a}	-	-	-
4	α-Pinene	948	-	0.24^{c}	0.45^{b}	0.62^{a}	-	-	0.40^{b}	0.89^{a}
5	Myrcene	958	-	0.98^{c}	1.60^{b}	2.98^{a}	-	0.93^{c}	1.04^{b}	3.65^{a}
6	1-Octen-3-ol	962	-	-	0.15^{b}	2.92^{a}	0.35^{b}	0.00^{c}	-	2.86^{a}
7	β-Phellandrene	964	-	0.26^{b}	0.32^{a}	-	-	-	-	-
8	α-Phellandrene	969	-	0.28^{c}	0.47^{b}	0.65^{a}	-	0.30^{c}	0.40^{b}	0.87^{a}
9	**γ-Terpinene**	998	**2.19^{c}**	**19.84^{a}**	**14.43^{b}**	**14.16^{b}**	**1.46^{c}**	**8.87^{b}**	**15.10^{a}**	**15.04^{a}**
10	Limonene	1018	-	0.30^{c}	0.63^{b}	0.82^{a}	-	0.30^{b}	-	1.10^{a}
11	Sabinene hydrate	1041	-	-	0.35^{a}	-	-	-	-	-
12	**p-Cymene**	1042	**1.37^{c}**	**7.43^{b}**	**7.43^{b}**	**8.63^{a}**	**1.21^{d}**	**6.30^{c}**	**8.21^{b}**	**10.04^{a}**
13	Terpinolene	1052	-	-	-	4.80^{a}	-	-	-	5.75^{a}
14	Terpinen-4-ol	1137	0.33^{c}	0.76^{b}	1.32^{a}	0.77^{b}	0.97^{ns}	2.60^{ns}	1.08^{ns}	1.63^{ns}
15	Borneol	1138	-	0.06^{a}	-	-	-	-	-	-
16	**Carvacrol**	1262	**82.25^{a}**	**55.13^{b}**	**49.42^{c}**	**37.52^{d}**	**80.54^{a}**	**47.60^{c}**	**58.67^{b}**	**29.20^{d}**
17	Linalyl propionate	1371	-	-	0.24^{a}	-	-	-	-	-
18	α-Bergamotene	1430	3.89^{c}	3.26^{d}	5.76^{b}	6.47^{a}	3.77^{c}	8.43^{a}	2.86^{d}	5.37^{b}
19	(E)-β-Farnesene	1440	-	0.08^{a}	-	-	0.09^{b}	0.67^{a}	-	-
20	Aromadendrene oxide-(2)	1462	-	-	-	0.69^{a}	0.32^{b}	0.67^{a}	-	0.40^{c}
21	**Caryophyllene**	1494	**6.06^{d}**	**6.73^{c}**	**8.46^{b}**	**10.00^{a}**	**6.72^{c}**	**12.30^{b}**	**5.05^{d}**	**16.14^{a}**
22	β-Bisabolene	1500	-	-	0.33^{b}	0.44^{a}	0.21^{c}	0.80^{a}	-	0.32^{b}
23	Caryophyllene oxide	1507	1.30^{c}	1.43^{b}	1.18^{d}	1.72^{a}	1.13^{c}	1.70^{a}	0.57^{d}	1.26^{b}
24	Humulene	1579	1.66^{d}	1.78^{c}	2.94^{b}	3.96^{a}	1.71^{c}	4.40^{a}	1.51^{d}	2.90^{b}
25	Humulene epoxide II	1592	-	0.18^{a}	-	-	0.21^{a}	-	-	-
26	Geranyl-α-terpinene	1962	-	0.52^{a}	-	-	-	-	-	-
Hydrocarbon monoterpenes			3.76^{d}	29.91^{b}	29.53^{c}	34.22^{a}	3.14^{d}	18.83^{c}	28.81^{b}	39.53^{a}
Oxygenated monoterpenes			82.58^{a}	55.92^{b}	51.13^{c}	38.30^{d}	81.51^{a}	50.20^{c}	59.74^{b}	30.83^{d}
Hydrocarbon sesquiterpenes			11.61^{d}	11.85^{c}	17.51^{b}	20.90^{a}	12.50^{c}	26.56^{a}	9.40^{d}	21.83^{b}
Oxygenated sesquiterpenes			1.30^{c}	1.61^{b}	1.20^{d}	2.41^{a}	1.67^{c}	2.35^{b}	0.56^{d}	4.55^{a}
Others			-	0.52^{c}	0.15^{b}	2.92^{a}	-	-	0.24^{b}	2.86^{a}
Total identified (%)			99.25^{c}	99.81^{a}	99.44^{b}	98.71^{d}	98.81^{b}	97.93^{d}	98.75^{c}	99.60^{a}
Yield (%)			1.52^{a}	0.40^{d}	1.20^{c}	1.24^{b}	1.60^{b}	0.51^{d}	1.65^{a}	0.67^{c}

by the LSD test at 5% for the climatic periods in each fertilization by the ANOVA test, Post Hoc Tukey test; ns: not significant; -: not detected.

With fertilizer B, the constituent γ-terpinene increased linearly from T1 to T4, with its content of 1.46% and 15.04%, respectively. However, fertilizer A led to a slight fluctuation in these values over the seasons. In this treatment, the γ-terpinene content decreased (2.19%) at the beginning of the rainy season (T1), increased significantly (19.84%) in the intense rainy season (T2) and decreased again (14.43%) at the end of the rainy season (T3), remaining stable (14.16%) in the dry season (T4) (Table 7).

Thus, the T4 treatment led to a decrease in the content of the major component carvacrol and an increase in the content of both γ-terpinene, as the second major component with fertilizer A and caryophyllene, as the second major component with fertilizer B. The three chemical components demonstrated greater sensitivity to climate changes (drought and rain) than to type of fertilizer. The T1 and T4 treatments were the most influential periods in the changes in the soil and in the nutrient content in the leaves of the species under study (Table 7).

According to leaf analysis, the greater production of γ-terpinene at the end of the rainy season (T3) in relation to the beginning of the rainy season (T1) may relate to the greater supply of N in the same period (Tables 4 and 5).

The chemical characteristics of the EO samples obtained from the crop varied not only qualitatively but also quantitatively as a function of climatic period and fertilizer type. The treatment with fertilizer A, with the exception of T1, was the one with the highest number of constituents. It is also noteworthy that despite having the same amount of constituents in T4, fertilizers A and B differed for these constituents. There were differences between some of the compounds produced.

For both types of fertilizer (A and B), the lowest EO yields occurred during the period of intense rain (T2). In turn, the highest yields occurred in T1, corresponding to the end of the dry season and the beginning of the rainy season, with rainfall rates still not very high in this region (Tables 6 and 7).

3.4. PCA analysis

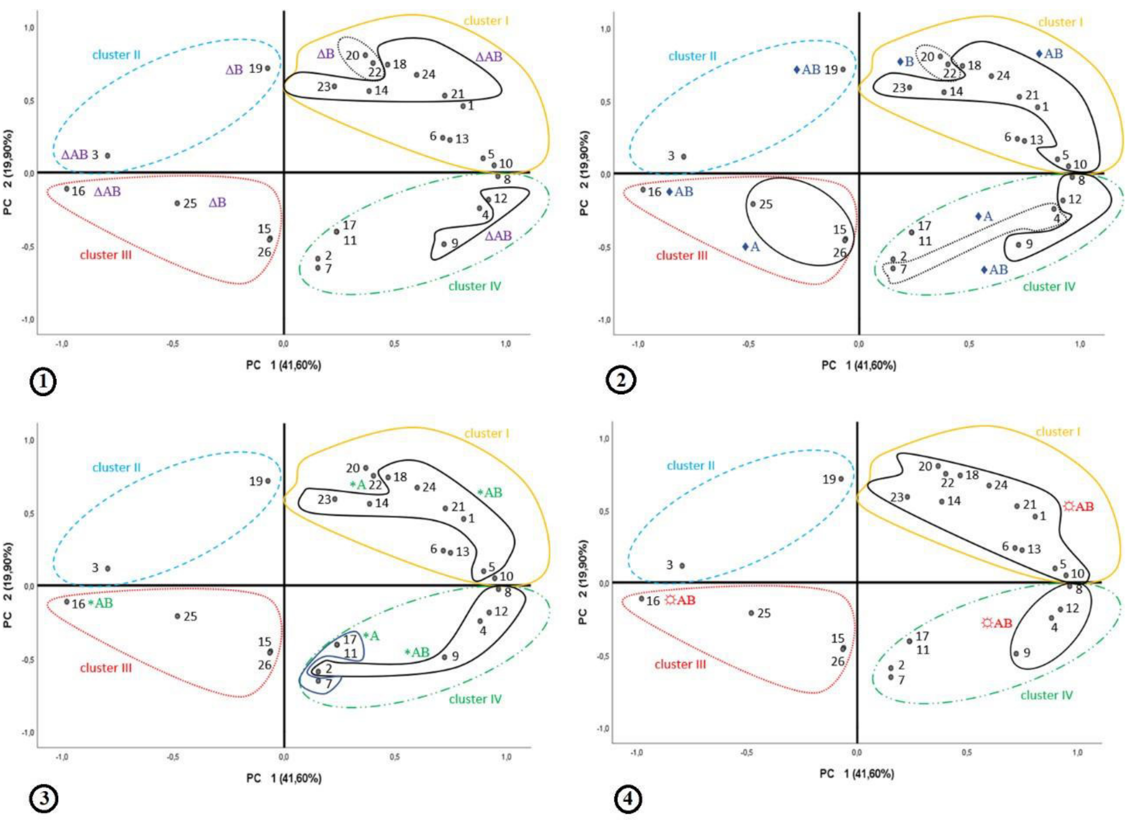
Figure 1 shows PCA as a function of organic fertilizations in each climatic period. The two principal components (PCs), responsible for 41.60% and 19.90% of the data variance, respectively, led to the formation of 4 clusters. Considering fertilizers A and B, these samples were grouped mainly with positive axis values: 12 compounds had a positive correlation with PC 1 and PC 2 (cluster I) and 8 compounds had a positive correlation only with PC 1 (cluster 4). In addition, fertilizers A and B showed greater homogeneity in T4 in cluster I. This treatment, together with T3, did not contribute to the formation of cluster II.

In cluster I, fertilizers A and B contributed to the grouping of 8 sesquiterpenes (α-bergamotene, (E)-β-farnesene, aromadendrene oxide-(2), caryophyllene, β-bisabolene, caryophyllene oxide, humulene and humulene epoxide II) in T4. The T1 and T2 treatments were responsible for the greater influence of fertilizer B in the grouping of these sesquiterpenes in this cluster. In turn, in T3 treatment, fertilizer A was the one that most contributed to the cluster of sesquiterpenes (α-bergamotene, caryophyllene, β-bisabolene, caryophyllene oxide, humulene). This was due to the aromadendrene oxide-(2), which was no longer produced in T3, and β-bisabolene, which in the other climatic periods was produced with fertilizer B. Fertilizers A and B also influenced the distribution of monoterpenes (α-thujene, myrcene, limonene, terpinolene and terpinen-4-ol) in cluster I.

In cluster II, PC 2 separated the sesquiterpene (E)-β-farnesene and the monoterpene (+)-2-carene. The presence of these compounds was independent of type of fertilizer, both in T2 for the sesquiterpene and in T1 for the monoterpene.

In cluster III, the monoterpene carvacrol was separated in all seasons, regardless of the type of fertilizer, and borneol was separated under the influence of fertilizer A only in T2. The sesquiterpene humulene epoxide II also showed a negative correlation with PC 1 and PC 2, with the influence of fertilizers B and A in T1 and T2, respectively. It is noteworthy that in T2, fertilizer A also contributed to the isolation of a rare diterpene, geranyl-α-terpinene, in cluster III.

Cluster IV was responsible for separation only monoterpenes ((+)-4-Carene, α-Pinene, β-phellandrene, α-phellandrene, γ-terpinene, sabinene hydrate, p-cymene and linalyl propionate). Fertilizers A and B equally contributed to the presence of these compounds, except in T2 and T3, in which compounds α-pinene, β-phellandrene, sabinene hydrate and linalyl propionate correlated only with fertilizer A.



A: bovine + livestock; B: bovine; Δ: beginning of the rainy season (1); ♦: intense rainy season (2); *: end of the rainy season (3); ☼: dry season (4).

Fig. 1. PCA analysis of the essential oils from leaves of *Plectranthus amboinicus* (Lour.) Spreng as a function of organic fertilizers in each climatic period. Projection of the average levels of essential oil compounds in the first two principal axes (+ and - indicate positive and negative correlations with the axes, respectively). The numbers represented in the plan correspond to the compounds reported in Table 6.

Figure 2 shows PCA as a function of the climatic periods in each organic fertilization. Principal components 1 and 2, accounting for 50.03% and 32.19% of the data variance, respectively, promoted the formation of 3 clusters. Most of the compounds were grouped in cluster I, correlating positively with PC 1 and PC 2 both in the analysis of climatic seasons with fertilizer A (with 14 compounds) and with fertilizer B (with 10 compounds). However, the opposite occurred for the distribution of compounds to form cluster II.

The samples of EO with fertilizer A, consisting of 9 compounds, showed the second largest distribution and a positive correlation with PC 2, while the samples with fertilizer B, with 7 compounds, showed the second largest distribution and a positive correlation with PC 1. In both in the analyzes, cluster III corresponded negatively with

PC 1 and PC 2, with 2 and 3 compounds in the analysis of samples with fertilizers A and B, respectively (Fig. 2).

In cluster I of the analysis of samples with fertilizer A (Fig. 2-A), the T4 treatment was the one that most contributed to the grouping of compounds (n = 14 elements), while T1 was the climatic period that least influenced the grouping of compounds with a positive correlation with PC 1 and PC 2, with only 6 elements. Monoterpenes (α-thujene, α-pinene, myrcene, α-phellandrene, limonene, p-cymene, terpinolene and terpinen-4-ol) predominated in cluster I, where only p-cymene and terpinen-4-ol were not influenced by climatic seasons with fertilizer A.

The monoterpenes α-thujene, α-pinene, myrcene, α-phellandrene and limonene were not detected in T1 and terpinolene was present only in T4. The change in climatic seasons also did not influence the detection of sesquiterpenes (α-bergamotene, caryophyllene, β-bisabolene and humulene), except for aromadendrene oxide-(2), detected only in T4 (Fig. 2-A).

Monoterpenes ((+)-4-Carene, β-phellandrene, γ-terpinene, sabinene hydrate, borneol and linalyl propionate) also predominated in cluster II of the analysis of samples with fertilizer A (Fig. 2-A), in which only γ-terpinene was not influenced by climatic seasons. The T3 treatment was the climatic period that most contributed to the grouping of monoterpenes in cluster II (n = 5 elements), in which (+)-4-carene, sabinene hydrate and linalyl propionate were detected only in that season. However, the T2 treatment was responsible for the presence of the sesquiterpenes (E)-β-farnesene and humulene epoxide II and the diterpene geranyl-α-terpinene with fertilizer A, in cluster II (Fig. 2-A).

Only two monoterpenes were grouped in cluster III (Fig. 2-A), in which carvacrol was not influenced by climatic seasons with fertilizer A, unlike (+)-2-carene, detected only in T1. The PCA of samples with fertilizer B (Fig. 2-B) allowed a better understanding of the influence of the two fertilizers and the four climatic seasons on the chemical compounds of *P. amboinicus.*

The production of some compounds ceased under the influence of fertilization in certain climatic seasons. This was the case of sesquiterpenes β-bisabolene (more sensitive to fertilizer B in T3) and aromadendrene oxide-(2) (more sensitive to fertilizer A in rainy seasons, T1, T2 and T3) and monoterpenes limonene (sensitive to fertilizer B in T1 and T3) and α-pinene (sensitive to fertilizer B in T2) (Fig. 2).

Other compounds were more sensitive to only one type of fertilizer, without the influence of the climatic seasons. This occurred for the sesquiterpene caryophyllene oxide, which was sensitive to fertilizer A and was detected in all climatic seasons with fertilizer B and for the monoterpenes α-phellandrene, p-cymene and terpinolene, which were sensitive only to fertilizer B. With fertilizer A, these monoterpenes correlated positively with PC 1 and PC 2 (cluster I); with fertilizer B, they started to have a positive correlation only with PC 1 (cluster II). Similarly, γ-terpinene and (+)-4-carene monoterpenes went from a negative correlation with PC 1 and PC 2 with fertilizer A to a positive correlation only with PC 1 with fertilizer B (Fig. 2).

Noteworthy, a similar fact occurred with the monoterpene α-pinene and the sesquiterpene humulene epoxide II, in which fertilizer B led to a change in grouping due to PCs. More specifically, α-pinene left cluster I with fertilizer A and went to cluster II with fertilizer B and humulene epoxide II went from cluster II with fertilizer A to cluster III with fertilizer B. This revealed that monoterpenes were more sensitive to changes with fertilizer B than sesquiterpenes (Fig. 2).

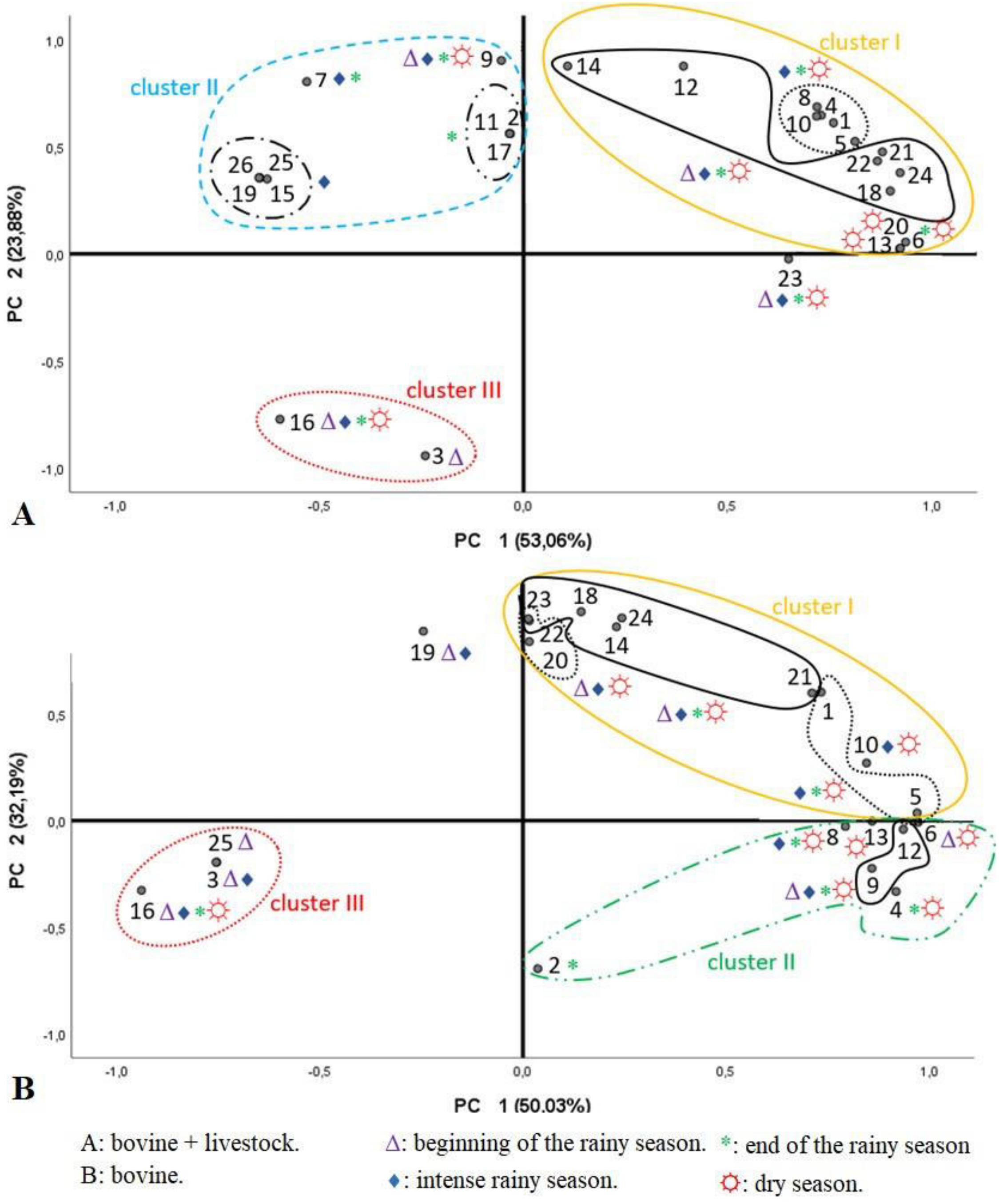


Fig. 2. PCA analysis of the essential oils from leaves of *Plectranthus amboinicus* (Lour.) Spreng as a function of climatic periods in each organic fertilization. Projection of the average levels of essential oil compounds in the first two principal axes (+ and - indicate positive and negative correlations with the axes, respectively). The numbers represented in the plan correspond to the compounds reported in Table 7.

3.5. Determination of physicochemical indices of the essential oil (EO)

The range of variation in the relative density of EO samples was d_{20}^{20}: 0.8594 - 0.9119 for fertilizer A and d_{20}^{20}:0.7281 - 0.92 for fertilizer B. The T2 treatment was the one with the highest relative density, while T1 presented the lowest density of EO, regardless of type of fertilizer (Table 8).

Table 8

Average values of the physicochemical indices of the essential oil from leaves of *Plectranthus amboinicus* (Lour.) Spreng as a function of organic fertilizers in each climatic period.

TREATMENT (T)*		REFRACTIVE INDEX (n^{20}_D)	RELATIVE DENSITY (g/mL d_{20}^{20})
1	A	1.510	0.8594
	B	1.512	0.7281
2	A	1.504	0.9048
	B	1.502	0.9200
3	A	1.502	0.9119
	B	1.505	0.8493
4	A	1.502	0.8735
	B	1.503	0.8573

* A: mixed fertilizer; B: bovine fertilizer; 1: beginning of the rainy season; 2: intense rainy season; 3: end of the rainy season; 4: dry season.

The refractive indices (n^{20}_D) varied between 1.502 - 1.512. The highest values for the refractive index were obtained with the T1 treatment. In each period between fertilizations, the densest samples of EO had a lower refractive index (e.g. sample 1A was more denser and had a lower refractive index than sample 1B).

4. Discussion

4.1. Soil chemical analysis

Some Brazilian soils have high acidity with low fertility and low organic content. This causes nutritional deficiencies (Paris et al., 2020) that can compromise the cultivation and production of plant species (Vieira and Weber, 2017). The elements considered essential to the plant, such as micronutrients, concentrate mainly in organic matter (Oliveira et al., 2016).

The cation exchange capacity (CEC), the sum of bases (SB) and the base saturation of soil (V%) indicate the general soil fertility conditions. The CEC indicates the total value of cations that the soil can adsorb, corresponding to the negative charges present in the soil; it is calculated by the sum of Ca, Mg, K, Na and Al + H. A soil is suitable for plant nutrition if cations such as Ca, Mg and K comprise most of the CEC (Ronquim, 2010; Silva, 2018). The SB, on the other hand, represents the sum of the contents of exchangeable cations, except H and Al (SB = Ca + Mg + K), referring to the negative colloid loads filled by the bases (Silva, 2018). The V% informs the percentage of anions occupied by available bases in the soil, indicating the percentage of CEC in the soil that is filled by exchangeable bases (Teixeira et al., 2017; Silva, 2018).

The higher content of soil nutrients obtained with fertilizer B correlated with the fertility data obtained by the parameters CEC, SB and V%. Initially, comparing the data obtained for fertilizers A and B with the specialized literature, the present study expected to find greater soil fertility with fertilizer A (mixed). Such information stems from the fact that the literature (Kiehl, 1985; Raij et al., 2001) indicates that the average chemical composition of poultry manure is higher than that of bovine manure in terms of nutrient content.

Duarte et al. (2010) showed that the reduction in the supply of P increases the production of different secondary metabolites, which correlate with the accumulation of terpenoid, high content of P in the soil and the enrichment of the cultivation media. This corroborates the results of the present study, in which the soil with fertilizer B was richer in P in periods T1 and T2, while the soil with fertilizer A showed higher contents of P in T3 and T4.

The high Fe content in fertilizer A may be due to the use of rations rich in this nutrient (Marrocos et al., 2012), since several metals are added to the birds' diet to facilitate weight gain and disease prevention (Han et al., 2000). Supplements of unabsorbed residual metals end up in chicken manure and different feed formulations can alter the levels of metals in poultry fertilizers. This can increase the concentrations of these metals in the soil, even to toxic levels (Foust et al., 2018).

The analytical results showed that the soil with bovine fertilizer had more nutrients than the soil with mixed fertilizer. It was expected that the treatment with mixed fertilizer, constituted by the mixture of bovine and poultry manure, would increase the content of these nutrients and, consequently, the soil fertility in relation to the use of fertilizer B (bovine manure) only; however, this did not occur. This was probably due to the balance of nutrients in the soil-plant relationship, in which the absorption of these nutrients by the plant favored the use of fertilizer A (mixed manure). Noteworthy, soil sampling occurred 30 days after the beginning of cultivation.

The nutrient balance corresponds to the difference between the input of nutrients into an agricultural system (mainly animal manure and fertilizers) and the output of nutrients from the system (the absorption of nutrients for the production of crops and pastures) (OECD, 2021).

The first factor to be considered for this imbalance of soil nutrients between treatments A and B is the relationship between the availability of soil nutrients and their absorption by the species under study. This corroborates Silva et al. (2014), who cites the faster decomposition of poultry and mixed (poultry and bovine) fertilizers and, consequently, a greater supply of nutrients to the plant in relation to bovine fertilizer.

Before plants can absorb nutrients from plant and animal waste present in organic fertilizers, organic compounds must be degraded normally by the action of soil microorganisms. This involves a process called mineralization, which depends on many factors, including temperature, availability of water and oxygen and type and number of microorganisms present in the soil. As a consequence, the rate of mineralization is highly variable and nutrients become available to plants for periods ranging from days to months or years (Taiz et al., 2021).

Sousa et al. (2020) cultivated *Plectranthus ornatus* with different organic substrates and obtained the best results with poultry fertilizer. This reinforces the hypothesis of the influence of nutrient balance for the results obtained in the present study. Thus, it is likely that *P. amboinicus* absorbed lower amounts of nutrients Zn, S and Mn from fertilizer B and less Fe from fertilizer A, increasing the content of these elements in soils with fertilizers B and A, respectively. The same may have happened with the elements Cu and P, in which the levels obtained in the soils with fertilizers A and B correspond to the inverse of the results obtained in leaf analysis.

The levels of organic matter (OM) were high in treatment B in all periods analyzed, showing their interaction with CEC and SB, which were also high in this treatment in all periods. According to Silva et al. (1999) and Silva (2018), CEC correlates positively with the quantity and quality of organic matter. Thus, soils with high values of this attribute have a greater capacity to retain water and nutrients, favoring plant productivity.

In addition, rainfall decreased soil fertility in both T1 (initial phase) and T2 (most intense phase), probably due to leaching and the consequent loss of nutrients by surface transport, which reduces the amount of elements available in the soil for the plants. According to Dubeux and Sollenberger (2020), leaching occurs when nutrients move with water beyond the root zone, decreasing the capacity of plants to absorb nutrients.

Another factor to be consider is the influence of seasonality on the absorption of macroelements by the species under study. Thus, the rains may have contributed to a greater absorption of Mg by the plant, decreasing the parameters of the assortment complex and, consequently, the Mg content in the soil in this season in relation to the dry season (T4). Some plant species, such as *Ouratea spectabilis* (Mart.) Engl. (Leitão and Silva, 2004) and *Pennisetum pedicellatum* Trin. (Ziblim et al., 2012), present well-defined seasonal patterns of water and nutrient use.

Water is also an important factor that influences soil nutrient availability and microbial activity in terrestrial ecosystems (Clark et al., 2009). The rate of absorption of nutrients is independent of the rate of absorption of water, but the content of nutrients on the root surfaces depends a lot on the content of water in the soil. The water content in the soil is important because it affects the growth of the root and the transport of nutrients to the root surface, both in the flow of water created by transpiration (mass flow) and in the diffusive flow towards or away from the root (Smethurst, 2004).

The fact that the possible relationship between the availability of nutrients in the soil and the absorption of nutrients by the species under study depends on seasonal factors may also have contributed to the imbalance of nutrients in the soil observed in the data obtained. This is the case of the species *Pennisetum pedicellatum*, which presents a recommendation for harvesting in the rainy season, as this season is responsible for higher levels of K, Mg and P in relation to the dry season (Ziblim et al., 2012).

With respect to Ca and Mg, it is important to monitor the relationships between soil nutrients, rather than looking for levels of Ca, Mg or other suitable elements in the soil. The availability of these elements depends on these relationships and the interaction of these macronutrients in the soil-plant system must be measured by the absorption by the plant (Salvador et al., 2011).

An appropriate proportion of organic substrate not only provides sufficient nutrients, but also improves the soil environment and leads to higher yields (Geng et al., 2019). Sousa et al. (2020) compared the types of fertilizers in the cultivation of

Plectranthus ornatus and obtained better results for poultry manure in relation to bovine manure and goat manure.

Although the nutrient contents in the soil are lower in treatment A than in treatment B, they indirectly reflect the nutrient contents in the leaves. This suggests that these values are lower in the soil due to the greater use of nutrients by *P. amboinicus*, detected by means of higher levels of nutrients in the analyzed leaves. This is evident in the analysis of nutritional status of the leaf for both types of treatment.

4.2. Leaf analysis

In the nutritional analysis of leaves of *P. amboinicus*, the N content varied from 14 g to 35 g / Kg with organic fertilization, in which treatment 4A had the highest value. When evaluating the effect of organic fertilization with bovine and poultry manure on the cultivation of lettuce for a period of thirty-three days, studies with other species of medicinal plants found a decrease in the N of the manure due to leaching and volatilization (Pinto et al., 2016).

Fertilization of poultry manure must take into account the cultivation requirements, the speed of decomposition of the manure for nutrient release and the nutrient content. This is because most of these nutrients are organic compounds and need to be mineralized to become available to plants (Schmidt and Knoblauch, 2020).

Geng et al. (2019) showed that the average absorption of N in response to the treatments had the following order: poultry manure > bovine manure > chemical fertilizer. Poultry manure usually favors all microbial groups, especially ammonium producers. This is due to the amounts of soluble carbohydrates, low C/N ratio and low lignin content, resulting in high microbial activity and, therefore, faster decomposition of applied poultry manure (Griffiths et al., 1994), increasing the supply of N by the plant.

This greater microbial activity in soils fertilized with poultry manure also helps in the release of P in the soil and its consequent absorption by the plant (Zai et al., 2010). It is worth mentioning that microorganisms are the main agents of nutrient mineralization. About 90% of nutrients are mineralized by microorganisms, making them available in the soil solution and, consequently, in plants (Lavelle, 2000).

In the leaves of *P. amboinicus*, Fe and B were more sensitive to the dry season (T4) than the other nutrients. Thus, this period was responsible for the lower values of these nutrients. For Fe, these variations may be due to its property to receive or donate hydrogen according to the characteristics of the environment (Cardoso and Andreote, 2016). In addition, Fe can act as an electron acceptor in redox processes, especially those related to soil microbiota. In these processes, the decrease in the redox potential leads to the transition from the predominance of aerobic to facultative and then anaerobic microorganisms (Cardoso and Andreote, 2016; Moreira and Moreira, 2006).

According to Lima Filho and Malavolta (1997), plants deficient in B have less complexation with phenols, which increases the phenolic alcohol content. The B available in the soil is present mainly in organic matter (OM) and there is a lack of this nutrient in sandy soils with little organic matter, high precipitation rates and low CEC (Tomicioli et al., 2021). However, the results of the present study differ from those of Tomicioli et al. (2021) since the lowest contents of B in the leaves of *P. amboinicus* occurred in the dry period (T4), with both types of organic fertilizer.

This demonstrates that even when the analysis indicates adequate levels of nutrients in the soil, there is no guarantee that the plants will be adequately supplied. Factors such as limited moisture, compacted soils, among others, compromise the transport of soil nutrients and their absorption by plants (Lima Neto et al., 2020).

The use of poultry manure (fertilizer A) made the chemical composition of *P. amboinicus* in general more stable and less influenced by climate change, mainly in relation to monoterpenes: p-cymene, γ-terpinene and carvacrol. Probabily, these three compounds did not suffer climatic influence with the use of poultry manure due to the greater supply of Fe by this fertilizer. Thus, the greater nutritional supply provided by fertilization of poultry manure contributed to making the stressful environment (rainfall stress in T1, T2 and T3) more comfortable for the plant. This increase in supply reduced the influence of this abiotic factor by stimulation the synthesis of geranyl pyrophosphate (GPP), which is a precursor molecule for the synthesis of monoterpenes and terpene synthases (Hilgers et al., 2021).

With the use only bovine manure (fertilizer B), the plants directed the synthesis of terpenes for the production of sesquiterpenes, such as caryophyllene, to reduce stress and improve comfort. This is because this fertilizer had lower Fe content, which was insufficient for plant demand. The studies by Duarte et al. (2010) demonstrated a positive correlation between caryophyllene and metal ions such as Mn, Fe, Cu and Zn. The production and concentration of terpenoids and other secondary metabolites depend on several regulatory factors such as: metal cofactors, stage of developmental and environmental factors (Rivera-Perez et al., 2015; Vattekkatte et al., 2020).

It is noteworthy that the greater production of caryophyllene from the use of isolated bovine manure, especially in the dry season (T4), may also have occurred due to the greater stimulus of caryophyllene synthase from farnesyl pyrophosphate (FPP, precursor to sesquiterpenes) for increase caryophyllene content. This compound has strong repellent activity against predators and parasites (Hilgers et al., 2021), which are more active and diversified in the dry season in tropical regions (Silva et al., 2017).

The data obtained in this study corroborate the premises established by Lima Filho and Malavolta (1997) who cite a direct relationship between fertilizer (its dose and nutritional contribution), leaf content and production. A study carried out with *Pennisetum pedicellatum* described that the levels of P, K and Mg were higher in the rainy season than in the dry season. The only exception was Ca, whose content was higher in the dry season (Ziblim et al., 2012).

4.3. Chemical analysis and yield

Terpenes are volatile organic compounds that consist of isoprene (a five-carbon building block) and derivatives from isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) (Louie et al., 2020). The elongation of this chain generates geranyl pyrophosphate (GPP), a precursor to monoterpenes; and chaining with another IPP gives rise to farnesyl pyrophosphate (FPP), a precursor to sesquiterpenes. Both GPP and FPP are substrates for reactions in metal-mediated cleavage of pyrophosphate moiety and further intramolecular cyclization, which generate several compounds with complex ring systems (Vattekkatte et al., 2018). Terpenes are classified based on the number of isoprene units, for instance: monoterpenes with two isoprene units (10C), sesquiterpenes with three units (15C) (Taiz et al., 2021).

It is worth mentioning that the total monoterpene content gradually decreased from T1 to T4 (86.34% - 72.52%) in fertilization A. In addition, the Mn content in leaves cultivated with fertilizer A followed a similar behavior. However, the total levels of monoterpenes fluctuated between T1 and T4 in fertilization B, being higher in T1 and T3 (84.65 - 88.55%) and lower in T2 and T4 (69.03 - 70.36%). In this fertilization, the leaf contents of Mn were inversely proportional, that is, lower in T1 and T3 and higher in T2 and T4. Frick et al. (2013) suggested a correlation between the levels of Co or Mn and the production of monoterpene and between the levels of Mg and the production of sesquiterpene. The authors claim that the specificity of these metal ions in reactions with enzymes produced by the species of beetle *P. cochleariae* correlates with the production of monoterpenes, sesquiterpenes and diterpenes.

Pyrophosphates geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP) and geranyl-geranyl pyrophosphate (GGPP) are substrates that terpene synthases use in the production of monoterpenes, sesquiterpenes and diterpenoids (Vattekkatte and Boland, 2020). The specificity of the terpene synthase product depends on the type of metal cofactor bound to the enzyme, as shown in the literature (Lopez-Gallego et al., 2010; Vattekkatte et al., 2018). These metal cofactors neutralize the negative charge of pyrophosphate and enable the ionization of allyl diphosphate. Terpene synthases without metal cofactors are rare (Vattekkatte et al., 2018).

The activation of pyrophosphate occurs with the participation of divalent metal ions (Wang et al., 2019). Metal cofactors are necessary for terpene synthases, with prevalence of Mg and Mn in monoterpene and sesquiterpene synthases (Vattekkatte and Boland, 2020).

The metal ion in greater concentration in the leaves of *P. amboinicus* in both types of fertilization (A and B) and in all climatic periods was Fe. The lowest concentration of this nutrient occurred in T4, with both fertilizers. The monoterpene carvacrol showed a similar behavior, remaining the main constituent in both types of fertilization and in all climatic periods. Its lowest concentration also occurred in T4, with both fertilizers. In addition, two other constituents (γ-terpinene and p-cymene) present in all climatic periods and in the two types of fertilization may also explain the high levels of Fe in the species *P. amboinicus* in this region of Brazil. According to Taiz et al. (2021), Fe plays an essential role in the formation of cytochrome-type enzymes.

Cytochrome P450 monooxygenases (P450s) are classes of enzymes present in various organisms. The largest group corresponds to the P450s detected in plants (4,266 enzymes), responsible for a structural variety of several compounds. These enzymes are heme-dependent, that is, they need the participation of Fe to catalyze a variety of reactions in the plant, being important for the synthesis and structural diversity of monoterpenes (Crocoll, 2011).

Carvacrol probably derives from γ-terpinene by oxidation (Badi et al., 2017). Our study shows this correlation since the period with the lowest γ-terpinene content was the one with the highest production of carvacrol (T1). In turn, γ-terpinene via p-cymene is the precursor of thymol (Badi et al., 2017), a monoterpene not detected in our study, but which is mentioned in the literature as one of the components of the EO of *P.*

amboinicus, being one of its chemotypes (Monzote et al., 2020). There is a probable synergistic action between p-cymene, γ-terpinene and carvacrol as antimicrobial and antifungal agents (Badi et al., 2017) as well as fumigants in the fight against insects (Pavela, 2008).

Crocoll (2011) mentions cytochrome P450 monooxygenases as catalysts for the reactions of formation of thymol and carvacrol through the conversion of γ-terpinene via p-cymene. It is important to highlight that in our study with *P. amboinicus*, the constituents: carvacrol, γ-terpinene, p-cymene and caryophyllene were present in higher concentrations in all periods and fertilizers analyzed in relation to with the other constituents identified in the analyzes. Thus, our study with *P. amboinicus* corroborates with Crocoll (2011) who cites the correlation between carvacrol, γ-terpinene and p-cymene, which are mediated by the same enzyme.

Baranauskienė et al. (2003) found that the use of nitrogen fertilizers increases the yield of EO in crops. In that study, the authors cultivated *Thymus vulgaris* and obtained the compounds thymol (44.4 - 58.1%), p-cymene (9.1 - 18.5%), γ-terpinene (6.9 - 18.9%) and carvacrol (2.4 - 4.2%).

The four main constituents identified in our study were: carvacrol, γ-terpinene, caryophyllene and p-cymene, which were also present in the study by Rodrigues et al. (2013), but in different proportions. Other studies had different results, such as that of Merlin et al. (2020) who identified 18 constituents in the EO of *P. amboinicus*, the main ones being: carvacrol (38.48 - 51.07%), trans-caryophyllene (19.80 - 26.65%), α-bergamotene (14.20 - 18.38%) and α- humulene (6.26 - 8.37%).

Bandeira et al. (2011) identified 14 constituents, the major ones being: trans-caryophyllene (25.53%), caryophyllene oxide (9.76%), β-bourbonene (6.30%) and α-cupene (3.13%). In turn, when studying oils extracted from leaves in different periods, El-Hawary et al. (2012b) identified 74 constituents that varied in different seasons, namely: α-humulene (11.14% in winter and 12.70% in summer), thymol (13.02% in autumn) and β-copaene-4-α-ol (9.37% in spring). These results reveal variations affecting the plant due to several factors, such as the growth stage and the nutrition provided (Lermen et al., 2015). The results for the major carvacrol compound varied from 29.2 to 82.25% between fertilizations and climatic periods. These results were close to those of Pinheiro et al. (2015) and distinct from those of Khalid et al. (2014) in Egypt, in which the contents ranged from 13.2 to 15.9% between seasons.

The yields obtained in our study, with the exception of the T2 treatment (0.4 - 0.51%), were higher than those presented by Khalid et al. (2014), Pinheiro et al. (2015) and Bandeira et al. (2011), with 0.8%, 0.41% and 0.43%, respectively.

Despite the chemical analysis pointing to the dry season (T4) and the beginning of the rainy season (T1) as the most prominent in the production of chemical compounds of *P. amboinicus*, PCA confirmed that this species presents better results for chemical composition in the dry season, both quantitatively and qualitatively. This corroborates with Arumugam et al. (2016), who indicate the dry season as the best growing season for this species. This is likely to have occurred because rain is a stressful factor for the species under study. This species occurs naturally in the tropics and hot regions of Australia, Africa and Asia, thus being adapted to dry seasons (Prasad et al., 2020). Therefore, it is suggested that changes in the chemical composition and yield of EO in *P. amboinicus* correlate both with organic fertilization and climatic period.

4.4. Physicochemical indices

The refractive index is important in the identification of substances and in the detection of impurities and can be used to determine the purity of volatile oils (Brasil, 2019). The values of physicochemical indices were close to those of Sathyan et al. (2018), who obtained a density of 0.9207 g/mL and a refractive index of 1.34. However, they differed from those of Lopes et al. (2017), who obtained a density of 1.5 g/mL and a refractive index of 0.9167.

5. Conclusions

The type of fertilizer applied and the climatic periods influenced the nutritional status of the leaves and, consequently, the chemical composition of the EO of *P. amboinicus*. The oriented cultivation of *Plectranthus amboinicus* allows for a better yield and chemical composition of EO.

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Elizabeth Regina de Castro Borba: Conceptualization, Methodology, Data curation, Writing - original draft, Validation, Formal analysis, Writing - review & editing. **Tássio Rômulo Silva Araújo Luz:** Methodology, Writing - review & editing. **Yan Michel Lopes Fernandes:** Methodology, Writing - review & editing. **Thiago Castro Mubárack**: Methodology, Writing - review & editing. **Daniella Patrícia Brandão Silveira**: Methodology, Writing - review & editing. **Ana Zélia Silva:** Methodology, Writing - review & editing. **Patrícia de Maria Silva Figueiredo:** Methodology, Writing - review & editing. **Odair dos Santos Monteiro:** Methodology, Writing - review & editing. **Crisálida Machado Vilanova:** Supervision, Writing - review & editing, Project administration. **Denise Fernandes Coutinho**: Supervision, Writing - review & editing, Project administration, Funding acquisition

Declaration of Competing Interest

All authors declare that there are no conflicts of interest in this work.

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CAPÍTULO 5

Chemical composition and antimicrobial activity of *Plectranthus amboinicus* (Lour.) Spreng in different climate seasons and fertilizations

Artigo submetido no periódico Industrial Crops and Products

Industrial Crops & Products

Chemical composition and antimicrobial activity of Plectranthus amboinicus (Lour.) Spreng in different climate seasons and fertilizations

--Manuscript Draft--

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Abstract:	Plectranthus amboinicus (Lour.) Sprengel is a plant species known for its diversity of biological activities, including antimicrobial activity. This study evaluated the antimicrobial properties of this species in crops with different organic fertilizers in different climatic periods. The species was cultivated with mixed organic fertilization (bovine and poultry - A) and bovine fertilization (B) at the beginning of the rainy season (T1), in the intense rainy season (T2), at the end of the rainy season (T3) and in the dry season (T4). The samples of essential oil (EO) from the leaves were extracted by hydrodistillation followed by chemical identification by gas chromatography coupled to the mass spectrometer (GC-MS). Antimicrobial activity was performed by disc diffusion with the strains of C. albicans (ATCC 14053 and ATCC 90028), C. parapsilosis (ATCC 22019), C. krusei (ATCC 6528), Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 35218), by means of microdilution techniques (to determine the minimum inhibitory concentration - MIC) and sowing in plates (to determine the minimum bactericidal concentration - MBC and minimum fungicidal concentration - MFC). We observed variation in the essential oils' antimicrobial potential, with T1 and T4 treatments and fertilization A standing out with better results in relation to the strains used. Carvacrol, caryophyllene and γ-terpinene are probably responsible for antimicrobial activity. The climatic period and the applied fertilization influenced the composition of the essential oil and, consequently, its antimicrobial characteristics, being determining factors in the quality of the vegetable raw material to obtain the expected biological action.
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Dear Editor,

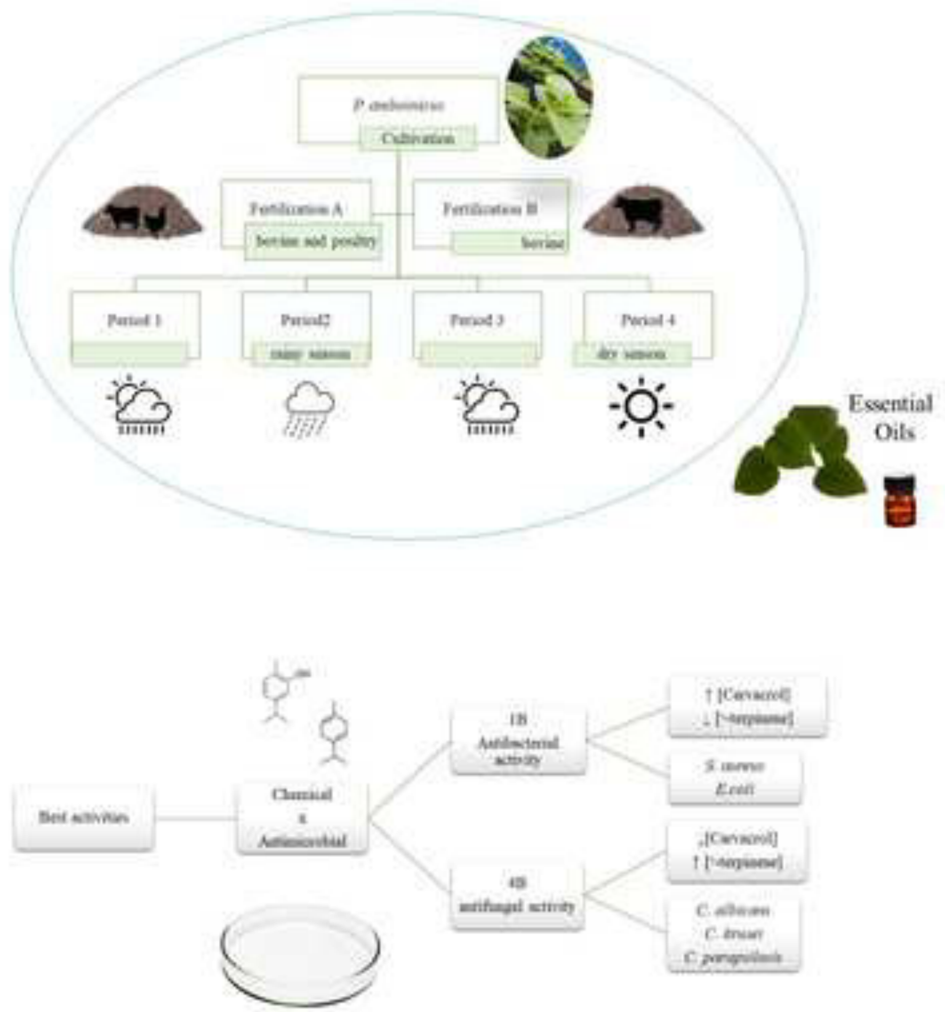
The manuscript “Chemical composition and antimicrobial activity of *Plectranthus amboinicus* (Lour.) Spreng in different climatic seasons and fertilizations” is an original study carried out in an area of the Brazilian Amazon in the dry season, at the beginning of the rainy season, during the season rainy season, and at the end of the rainy season in order to evaluate the effect of seasonality with two types of organic fertilizers on the chemical composition of the essential oil and on the antimicrobial specificity of the essential oils of *P. amboinicus* (Lour.) Spreng.

Although much is known about how availability of water or fertilization alters plant production and crops, there is a surprising lack of knowledge about how the availability of water associated with organic fertilization affects the chemical composition of essential oil and consequently its response in antimicrobial activity, especially in species in the Amazon.

This journal was chosen due to its excellent concept, credibility and wide visibility, which allows us to disseminate our findings to several countries. We hope you consider our research and findings worthy of publication.

Best Regards,

Elizabeth Borba


Cultivation
Fertilization A
bovine and poultry
Fertilization B
bovine
Period 1
Period2
rainy season
Period 3
Period 4
dry season
Essential
Oils
Best activities
Chemical
x
Antimicrobial
Antibacterial
activity
antifungal activity
S. aureus
E.coli

Chemical composition and antimicrobial activity of *Plectranthus amboinicus* (Lour.) Spreng in different climate seasons and fertilizations

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Abstract

Plectranthus amboinicus (Lour.) Sprengel is a plant species known for its diversity of biological activities, including antimicrobial activity. This study evaluated the antimicrobial properties of this species in crops with different organic fertilizers in different climatic periods. The species was cultivated with mixed organic fertilization (bovine and poultry - A) and bovine fertilization (B) at the beginning of the rainy season (T1), in the intense rainy season (T2), at the end of the rainy season (T3) and in the dry season (T4). The samples of essential oil (EO) from the leaves were extracted by hydrodistillation followed by chemical identification by gas chromatography coupled to the mass spectrometer (GC-MS). Antimicrobial activity was performed by disc diffusion with the strains of *C. albicans* (ATCC 14053 and ATCC 90028), *C. parapsilosis* (ATCC 22019), *C. krusei* (ATCC 6528), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 35218), by means of microdilution techniques (to determine the minimum inhibitory concentration - MIC) and sowing in plates (to determine the minimum bactericidal concentration - MBC and minimum fungicidal concentration - MFC). We observed variation in the essential oils' antimicrobial potential, with T1 and T4 treatments and fertilization A standing out with better results in relation to the strains used. Carvacrol, caryophyllene and γ-terpinene are probably responsible for antimicrobial activity. The climatic period and the applied fertilization influenced the composition of the essential oil and, consequently, its antimicrobial characteristics, being determining factors in the quality of the vegetable raw material to obtain the expected biological action.

Keywords Antimicrobial profile Carvacrol Climatic variation Essential oil mint Mexican Organic fertilization

1. Introduction

Essential oils (EOs) are volatile oils consisting mainly of monoterpenes and sesquiterpenes (Stephane and Jule, 2020). The majority of EOs obtained from vascular plants have been demonstrated to be effective in treating fungal and bacterial infections (Ghavam et al., 2020).

Every year, the pharmaceutical and food industries produce over 1,000 tons of EOs exploitable in different fields to develop eco-friendly and safe antimicrobial inhibitors (Scotti et al., 2021). The EOs are subject to qualitative and quantitative changes due to environmental, genetic and technological factors, as well adjustments in cultivating, harvesting, processing and storage processes that ensure the best production and yield of EOs (Salamon et al., 2021).

The use of organic fertilizers increases the formation of antioxidant and bioactive substances, while inorganic ones increase the yield in a shorter period of time (Lema and Abewoy, 2021). However, despite several studies aimed at establishing appropriate cultivation techniques for better quality and yield of plant species (Vilanova et al., 2018), there are no studies on the impact of the variation in *Plectranthus amboinicus* cultivation conditions.

Plectranthus amboinicus (Lour.) Spreng. (Lamiaceae), known as Mexican mint/oregano (Bañuelos-Hernández et al., 2020) is widely used for culinary and medicinal purposes (Arumugam et al., 2016). This plant is grown as an ornamental for its fresh aroma and attractive heart-shaped or white-edged foliage (Prasad et al., 2020) and also has economic value and potential to be developed into products for use by the nutrition and human health industry (Wadikar and Patki, 2016).

This species has about 30 nonvolatile compounds, belonging to different phytochemical classes, such as flavonoids, esters, alcohols and aldehydes, and 76 volatile constituents, mainly from the terpene class (Sobrinho et al., 2020; Nakasugi et al., 2021)

P. amboinicus is a prominent plant used in traditional homeopathic medicine because it has anti-inflammatory (Leu et al., 2019), antioxidant, and antinociceptive (Duraisamy et al., 2021) pharmacological activities. In a previous studies, the EO of *P. amboinicus* has shown antimicrobial activity against gram-positive (Vasconcelos et al., 2017) and gram-negative bacteria strains and fungi (Sivaranjani et al., 2019).

However, although several studies indicate that this species has antimicrobial activity (Sreelakshmy and Thangapandiyan, 2019), no studies have been done on how varying cultivation conditions of *P. amboinicus* effects its antimicrobial activity.

Therefore, considering that plants are susceptible to changes in their chemical composition, and, consequently, in their biological activity, this study sought to characterize the antimicrobial activity of the EO of *P. amboinicus* leaves and how its chemical composition, is affected by type of fertilization used during cultivation and collection in different climatic periods.

2. Material and methods

2.1. Samples

The seedlings were grown in 50% shade in the greenhouse of the Horta de Medicinal Plants Berta Lange de Morretes, of the Federal University of Maranhão-UFMA, located in São Luís-MA Brazil (2°32' S, 44°16 W). The crops evaluated two types of organic substrates in four different periods, covering the dry and rainy seasons of the region, as shown in Table 1.

Table 1

Conditions for the crops of *Plectranthus amboinicus* (Lour.) Spreng.

Treatment (T)		Substrate	Cultivation and collection
1	A	soil with bovine and poultry manure (mixed)	beginning of rainy season (11/02/18 – 01/15/19)
	B	soil with bovine manure	
2	A	soil with bovine and poultry manure (mixed)	intense rainy season (01/28/19 – 04/12/19)
	B	soil with bovine manure	
3	A	soil with bovine and poultry manure (mixed)	end of rainy season (04/25/19 – 07/10/19)
	B	soil with bovine manure	
4	A	soil with bovine and poultry manure (mixed)	dry season (07/23/19 – 10/05/19)
	B	soil with bovine manure	

The cultivation lasted 75 days, with manual irrigation of 500 mL of water on alternate days. An exsiccata of the species (SLS nº 1477) was deposited at Herbarium Ático Seabra, in the Pharmacy Department of UFMA, Brazil.

2.2. Extraction of EOs

The fresh leaves were dried at 40 ºC for 72 hours and then subjected to hydrodistillation for three hours in a Clevenger type apparatus (Brasil, 2019). The EOs were centrifuged (4,000 rpm/30 min), dehydrated with anhydrous sodium sulfate (Merck Chemical Co., Germany), packed in amber glass ampoules and kept refrigerated (2 to 8 °C) until use. The content and yield of EOs were determined according to the methodology of Coutinho et al. (2007).

2.3. Chemical analysis of EOs

The EOs were analyzed using gas chromatography coupled to mass spectrometry (GC-MS - QP2020 - Shimadzu), under the following conditions: BPX5 capillary column (30 m x 0.25 mm x 0.25 µm) with temperature between 60 ºC and 240 ºC (3 ℃/min); helium carrier gas (3 mL/min); and injected volume of 1 µL diluted in hexane. The injector temperature was 280 ºC, with column pressure of 111.5 KPa, linear speed of 48.9 cm/s and scan mode of 0.5 s/scan. The mass spectrometer operated with an electron impact (EI) ionization detector at 70 eV, with automatic scanning in a range of 37 to 660 Daltons at 0.5 s. The components were identified by comparing with the fragmentation patterns of the mass spectra described both in mass spectral libraries (NIST, 2005) and in the literature (Adams, 2007). Each constituent was quantified using the area normalization method (%).

2.4. Antimicrobial activity

In determining the antimicrobial activity, four strains of fungi and two strains of bacteria were selected: *Candia albicans* (ATCC 14053 and ATCC 90028), *C. parapsilosis* (ATCC 22019) and *C. Krusei* (ATCC 6528); *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 35218), gram-positive and gram-negative, respectively.

The microbial strains were seeded by sowing in BHI broth (Brain Heart Infusion) at 35 °C for 24 h and incubated at 35 ºC until they reached exponential growth phase (4 to6 h). After this period, the culture cell density was adjusted in 0.9% sterile saline solution, until obtaining a microbial suspension with turbidity comparable to that of the McFarland 0.5 standard solution (1.5 x 108 CFU/mL), according to the Clinical and Laboratory Standards Institute (CLSI, 2020).

2.5. Determination of the minimum inhibitory concentration (MIC)

The antifungal potential of EOs was evaluated, according to the standards of the CLSI, using the broth microdilution technique using a 96-well microplate, containing 100µL of Mueller Hinton broth in each well. A 100-µL aliquot of EO was added in serial dilutions. In the positive control, 20 µL of antimicrobial (Chloramphenicol 0.02 mg/mL or Nystatin 100,000 IU) was added. Then, 5 µL of the microbial suspension was added to all wells. The microplate was incubated for 24 to 48 h at 37 °C. The development was carried out by adding 30 µL of 0.015% resazurin in all wells, with subsequent reading to assess the MIC of the EO samples (CLSI, 2020).

2.6. Determining minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

In the well where there was no growth of the microorganism in the MIC test, MBC/MFC was evaluated by sowing in a petri dish with Mueller Hinton agar. These plates were incubated and, after 24 h, MFC/MBC was defined as the lowest concentration of EO capable of causing the death of the inoculum (CLSI, 2020).

2.7. Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences - SPSS Version 26. Multivariate data analysis was performed through analysis of variance (ANOVA) followed by Tukey test. The results were expressed as mean.

Differences between data sets were considered as significant when the *P* value was less than 0.01 by Tukey test.

3. Results

In the chemical composition of the EO samples, 26 constituents were identified, varying the substances according to the period of cultivation and the type of fertilization, with 08 constituents common to all analyzed samples:carvacrol, caryophyllene, γ-terpinene, p-cymene, terpinen-4-ol, caryophyllene oxide, α-bergamotene and humulene.

There was a decrease in carvacrol in the analyzed periods (T1 to T4) in fertilization A, from 82.25% in T1 to 37.52% in T4 (driest period in the region). In fertilization B, there was a similar result, going from 80.54% in T1 to 29.2% in T4, demonstrating the effect of the climatic period in the production of this constituent regardless of the type of fertilization (Table 2 and 3).

Table 2

Caracterização química e rendimento dos óleos essenciais de *Plectranthus amboinicus* (Lour.) Spreng. resultantes de adubações orgânicas e períodos climáticos distintos.

COMPOUND			TREATMENT (T)**							
			1		2		3		4	
			A	B	A	B	A	B	A	B
1	α-Thujene	902*	-	-	0.58^{b}	2.30^{a}	1.08^{ns}	1.03^{ns}	1.60^{ns}	2.20^{ns}
2	(+)-4-Carene	919	-	-	-	-	3.17^{a}	2.67^{b}	-	-
3	(+)-2-Carene	946	0.21^{a}	0.12^{b}	-	-	-	-	-	-
4	α-Pinene	948	-	-	0.24^{a}	-	0.45^{ns}	0.40^{ns}	0.62^{b}	0.89^{a}
5	Myrcene	958	-	-	0.98^{ns}	0.93^{ns}	1.60^{a}	1.04^{b}	2.98^{b}	3.65^{a}
6	1-Octen-3-ol	962	0.00^{b}	0.35^{a}	-	-	0.15^{a}	-	2.92^{ns}	2.86^{ns}
7	β-Phellandrene	964	-	-	0.26^{a}	-	0.32^{a}	-	-	-
8	α-Phellandrene	969	-	-	0.28^{ns}	0.30^{ns}	0.47^{ns}	0.40^{ns}	0.65^{ns}	0.87^{ns}
9	**γ-Terpinene**	998	**2.19^{a}**	**1.46^{b}**	**19.84^{a}**	**8.87^{b}**	**14.43^{b}**	**15.10^{a}**	**14.16^{b}**	**15.04^{a}**

10	Limonene	1018	-	-	0.30^{ns}	0.30^{ns}	0.63^{a}	-	0.82^{b}	1.10^{a}
11	Sabinene hydrate	1041	-	-	-	-	0.35^{a}	-	-	-
12	**p-cymene**	1042	**1.37^{a}**	**1.21^{b}**	**7.43^{a}**	**6.30^{b}**	**7.43^{b}**	**8.21^{a}**	**8.63^{b}**	**10.04^{a}**
13	Terpinolene	1052	-	-	-	-	-	-	4.80^{b}	5.75^{a}
14	Terpinen-4-ol	1137	0.33^{b}	0.97^{a}	0.76^{b}	2.60^{a}	1.32^{a}	1.08_{b}	0.77^{b}	1.63^{a}
15	Borneol	1138	-	-	0.06^{a}	-	-	-	-	-
16	**Carvacrol**	1262	**82.25^{a}**	**80.54^{b}**	**55.13^{a}**	**47.60^{b}**	**49.42^{b}**	**58.67^{a}**	**37.52^{a}**	**29.20^{b}**
17	Linalyl propionate	1371	-	-	-	-	0.24^{a}	-	-	-
18	α-Bergamotene	1430	3.89^{a}	3.77^{b}	3.26^{b}	8.43^{a}	5.76^{b}	2.86^{a}	6.47^{a}	5.37^{b}
29	(E)-β-Farnesene	1440	-	0.09^{a}	-	0.67^{a}	-	-	-	-
20	Aromadendrene oxide-(2)	1462	-	0.32^{a}	-	0.67^{a}	-	-	0.69^{ns}	0.40^{ns}
21	**Caryophyllene**	1494	**6.06^{b}**	**6.72^{a}**	**6.73^{b}**	**12.30^{a}**	**8.46^{a}**	**5.05^{b}**	**10.00^{b}**	**16.14^{a}**
22	β-Bisabolene	1500	-	0.21^{a}	-	0.80^{a}	0.33^{a}	-	0.44^{a}	0.32^{b}
23	Caryophyllene oxide	1507	1.30^{a}	1.13^{b}	1.43^{ns}	1.70^{ns}	1.18^{a}	0.57^{b}	1.72^{a}	1.26^{b}
24	Humulene	1579	1.66^{ns}	1.71^{ns}	1.78^{b}	4.40^{a}	2.94^{a}	1.51^{b}	3.96^{a}	2.90^{b}
25	Humulene epoxide II	1592	-	0.21^{a}	0.18^{a}	-	-	-	-	-
26	Geranyl-α-terpinene	1962	-	-	0.52^{a}	-	-	-	-	-
	Hydrocarbon monoterpenes		3.76^{a}	3.14^{b}	29.91^{a}	18.83^{b}	29.53^{a}	28.81^{b}	34.22^{b}	39.53^{a}
	Oxygenated monoterpenes		82.58^{a}	81.51^{b}	55.92^{a}	50.20^{b}	51.13^{b}	59.74^{a}	38.30^{a}	30.83^{b}
	Hydrocarbon sesquiterpenes		11.61^{b}	12.50^{a}	11.85^{b}	26.56^{a}	17.51^{a}	9.40^{b}	20.90^{b}	21.83^{a}
	Oxygenated sesquiterpenes		1.30^{ns}	1.67^{ns}	1.61^{b}	2.35^{a}	1.20^{a}	0.56^{b}	2.41^{b}	4.55^{a}
	Others		-	-	0.52^{a}	-	0.15^{b}	0.24^{a}	2.92^{ns}	2.86^{ns}
	Total identified (%)		99.25^{a}	98.81^{b}	99.81^{a}	97.93^{b}	99.44^{a}	98.75^{b}	98.71^{b}	99.60^{a}
	Yield (%)		1.52^{ns}	1.60^{ns}	0.40^{ns}	0.51^{ns}	1.20^{b}	1.65^{a}	1.24^{a}	0.67^{b}

* Linear retention index of the library [21].
**A: mixed fertilizer; B: bovine fertilizer; 1: beginning of the rainy season; 2: intense rainy season; 3: end of the rainy season; 4: dry season.
Note: data correspond to mean values (n = 3). Means with different lower case letters in the columns differ by the LSD test at 5% for the climatic periods in each fertilization by the ANOVA test, Post Hoc Tukey test; ns: not significant; -: not detected.

The compounds caryophyllene and γ-terpinene were also more sensitive to the climatic period than fertilization. Caryophyllene had greater synthesis in the most adverse climatic periods, such as in the intense rainy season (T2) and in the dry season (T4), with reduced concentration in the milder seasons, beginning and end of the rainy season. The variations in carvacrol, γ-terpinene and caryophyllene were due to the climate changes in the region at T4 (Table 3).

Table 3

Caracterização química dos óleos essenciais de *Plectranthus amboinicus* (Lour.) Spreng. por diferentes estações climáticas nas adubações orgânicas mista e bovina.

COMPOUND			TREATMENT**							
			A				B			
			1	2	3	4	1	2	3	4
1	α-Thujene	902*	-	0.58^{c}	1.08^{b}	1.60^{a}	-	2.30^{a}	1.03^{c}	2.20^{b}
2	(+)-4-Carene	919	0.00^{b}	-	3.17^{a}	-	-	-	2.67^{a}	-
3	(+)-2-Carene	946	0.21^{a}	-	-	-	0.12^{a}	-	-	-
4	α-Pinene	948	-	0.24^{c}	0.45^{b}	0.62^{a}	-	-	0.40^{b}	0.89^{a}
5	Myrcene	958	-	0.98^{c}	1.60^{b}	2.98^{a}	-	0.93^{c}	1.04^{b}	3.65^{a}
6	1-Octen-3-ol	962	-	-	0.15^{b}	2.92^{a}	0.35^{b}	0.00^{c}	-	2.86^{a}
7	β-Phellandrene	964	-	0.26^{b}	0.32^{a}	-	-	-	-	-
8	α-Phellandrene	969	-	0.28^{c}	0.47^{b}	0.65^{a}	-	0.30^{c}	0.40^{b}	0.87^{a}
9	**γ-Terpinene**	998	**2.19^{a}**	**19.84^{b}**	**14.43^{c}**	**14.16^{d}**	**1.46^{a}**	**8.87^{b}**	**15.10^{c}**	**15.04^{c}**
10	Limonene	1018	-	0.30^{c}	0.63^{b}	0.82^{a}	-	0.30^{b}	-	1.10^{a}
11	Sabinene hydrate	1041	-	-	0.35^{a}	-	-	-	-	-
12	**p-Cymene**	1042	**1.37^{a}**	**7.43^{b}**	**7.43^{b}**	**8.63^{c}**	**1.21^{a}**	**6.30^{b}**	**8.21^{c}**	**10.04^{d}**
13	Terpinolene	1052	-	-	-	4.80^{a}	-	-	-	5.75^{a}
14	Terpinen-4-ol	1137	0.33^{c}	0.76^{b}	1.32^{a}	0.77^{b}	0.97^{ns}	2.60^{ns}	1.08^{ns}	1.63^{ns}
15	Borneol	1138	-	0.06^{a}	-	-	-	-	-	-
16	**Carvacrol**	1262	**82.25^{a}**	**55.13^{b}**	**49.42^{c}**	**37.52^{d}**	**80.54^{a}**	**47.60^{b}**	**58.67^{c}**	**29.20^{d}**
17	Linalyl propionate	1371	-	-	0.24^{a}	-	-	-	-	-
18	α-Bergamotene	1430	3.89^{c}	3.26^{d}	5.76b	6.47^{a}	3.77^{c}	8.43^{a}	2.86^{d}	5.37^{b}
19	(E)-β-Fanesene	1440	-	0.08^{a}	-	-	0.09^{b}	0.67^{a}	-	-
20	Aromadendrene oxide-(2)	1462	-	-	-	0.69^{a}	0.32^{b}	0.67^{a}	-	0.40^{c}
21	**Caryophyllene**	1494	**6.06^{a}**	**6.73^{b}**	**8.46^{c}**	**10.00^{d}**	**6.72^{a}**	**12.30^{b}**	**5.05^{c}**	**16.14^{d}**
22	β-Bisabolene	1500	-	-	0.33^{b}	0.44^{a}	0.21^{c}	0.80^{a}	-	0.32^{b}
23	Caryophyllene oxide	1507	1.30^{c}	1.43^{b}	1.18^{d}	1.72^{a}	1.13^{c}	1.70^{a}	0.57^{d}	1.26^{b}
24	Humulene	1579	1.66^{d}	1.78^{c}	2.94^{b}	3.96^{a}	1.71^{c}	4.40^{a}	1.51^{d}	2.90^{b}
25	Humulene epoxide II	1592	-	0.18^{a}	-	-	0.21^{a}	-	-	-
26	Geranyl-α-terpinene	1962	-	0.52^{a}	-	-	-	-	-	-
Hydrocarbon monoterpenes			3.76^{d}	29.91^{b}	29.53^{c}	34.22^{a}	3.14^{d}	18.83^{c}	28.81^{b}	39.53^{a}
Oxygenated monoterpenes			82.58^{a}	55.92^{b}	51.13^{c}	38.30^{d}	81.51^{a}	50.20^{c}	59.74^{b}	30.83^{d}
Hydrocarbon sesquiterpenes			11.61^{d}	11.85^{c}	17.51^{b}	20.90^{a}	12.50^{c}	26.56^{a}	9.40^{d}	21.83^{b}
Oxygenated sesquiterpenes			1.30^{c}	1.61^{b}	1.20^{d}	2.41^{a}	1.67^{c}	2.35^{b}	0.56^{d}	4.55^{a}
Others			-	0.52^{c}	0.15^{b}	2.92^{a}	-	-	0.24^{b}	2.86^{a}
Total identified (%)			99.25^{c}	99.81^{a}	99.44^{b}	98.71^{d}	98.81^{c}	97.93^{b}	98.75^{d}	99.60^{a}
Yield (%)			1.52^{a}	0.40^{d}	1.20^{c}	1.24^{b}	1.60^{b}	0.51^{d}	1.65^{a}	0.67^{c}

* Linear retention index of the library [21].
**A: mixed fertilizer; B: bovine fertilizer; 1: beginning of the rainy season; 2: intense rainy season; 3: end of the rainy season; 4: dry season.
Note: data correspond to mean values (n = 3). Means with different lower case letters in the columns differ by the LSD test at 5% for the climatic periods in each fertilization by the ANOVA test, Post Hoc Tukey test; ns: not significant; -: not detected.

The EOs had the same chemical composition regardless of fertilizers in the dry season (T4), without changing the main component: carvacrol. However, in the intense rainy season (T2), fertilizations A and B led to the formation of γ-terpinene and caryophyllene, respectively, as the second major component (Table 2).

The chemical composition of EO were significantly influenced in periods T1 and T4. T1 had the highest yield of EO in the two types of fertilization (A = 1.52 and B = 1.6%), while T2 had the lowest yields of EO (A = 0.4 and B = 0.51%) in the fertilizations. In fertilization B, γ-terpinene gradually increased over the periods, from 1.46% in T1 to 15.04% in T4, with an inverse production to carvacrol in these same conditions (Table 3).

3.1 Fertilizers, climate change and antimicrobial activity

Fertilization A provided the highest number of constituents in the EO, except in T1, and also the best antifungal response in T1 against the strains of *C. albicans* (Table 4). With fertilization A, the highest value of carvacrol (82.25%) and the lowest concentrations of caryophyllene (6.06%), γ-terpinene (2.19%) and p-cymene (1.37%) were obtained (Table 2 and 3).

Table 4

Antibacterial activity of leaf essential oils of *Plectranthus amboinicus* against pathogens.

Treatment (T)	*C albicans* ATCC 14053		*C albicans* ATCC 90028		*C parapsilosis* ATCC 22019		*C krusei* ATCC 6528		*S aureus* *ATCC 25923*		*E coli* ATCC 35218	
	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC/ MFC
A												
1	0.1220^{d}	0.2441^{c}	0.1220^{c}	0.2441^{d}	0.1220^{d}	0.1220^{d}	0.2441^{b}	0.2441^{c}	0.2441^{c}	0.4882c*	0.1220^{d}	0.2441b*
2	1.9531^{b}	7.8125^{a}	1.9531^{b}	7.8125^{b}	3.9062^{b}	3.9062^{b}	3.9062^{a}	7.8125^{a}	1.9531^{b}	3.9062^{a}	0.9765^{c}	1.9531^{c}
3	7.8100^{a}	7.8100^{a}	15.620^{a}	15.620^{a}	7.8100^{a}*	31.250^{a}	3.9000^{a*}	3.9000^{b*}	3.9000^{a}	3.9000^{a}	1.9500^{b}	1.9500^{c}*
4	0.9765^{c}	1.9531^{b}	1.9531^{b}	1.9531^{c}	0.4882^{c}	0.9765^{c}	0.1220^{c*}	0.1220^{d*}	1.9531^{b*}	1.9531^{b}*	3.9062a*	7.8125^{a*}
B												
1	0.2441^{b}	0.4882^{b}	0.2441^{c}	0.4882^{b}	0.9765^{b}	0.9765^{b}	0.1220^{c}	0.4882^{c}	0.1220^{d}	0.4882b*	0.2441^{c}	0.2441^{d*}
2	0.2441^{b}	0.2441^{b}	0.4882^{b}	0.4882^{b}	0.2441^{c}	0.9765^{b}	0.4882^{b}	0.9765^{b}	0.2441^{c}	0.2441^{c}	0.2441^{c}	0.9765^{c}
3	3.9000^{a}	3.9000^{a}	7.8100^{a}	7.8100^{a}	7.8100a*	15.620^{a}	3.9000^{a}*	3.9000a*	15.620^{a}	15.620^{a}	0.9700^{b}	1.9500^{b*}
4	0.2441^{b}	0.4882^{b}	0.2441^{c}	0.2441^{c}	0.0610^{d}	0.1220^{c}	0.1220^{c*}	0.1220^{d*}	1.9531^{b}*	1.9531^{d}*	3.9062^{a*}	7.8125^{a*}

Treatments: A: mixed fertilizer; B: bovine fertilizer; 1: beginning of the rainy season; 2: intense rainy season; 3: end of the rainy season; 4: dry season.
MIC, MBC, MFC = mg/mL
Note: means with * in columns do not differ significantly ($P > 0.01$) with fertilizations considering the same climatic period by Tukey test and means with different lower case letters in the columns differ from each other considering the climatic seasons analyzed within each fertilization by the ANOVA test, Post Hoc Tukey test.

When comparing fertilization A, it can be seen that fertilization B potentiated antifungal inhibition against *C. albicans* in three climatic periods, except in T1. The fungi *C. albicans*, *C. parapsilosis* and *C. krusei* in fertilization B were more sensitive in T4 (dry period), having the best activity against *C. parapsilosis* (MIC = 61 µg/mL and MFC = 122 µg/mL) in T4. The best results against *S. aureus* (MIC = 122 µg/mL and MBC = 244.1 µg/mL) were also with fertilization B in T1 (Table 4).

The best antimicrobial activity against *C. krusei* was in T4 regardless of fertilization, with values of 122 µg/mL for MIC and MFC (Table 4). Both treatments showed similar chemical characteristics, with slightly differing concentrations of the compounds. Only in these treatments were the presence of terpinolene and the lowest content of carvacrol detected, with an increase in the content of γ-terpinene in fertilization A and caryophyllene in fertilization B. Although carvacrol (29.2% to 37.52%) is still the main component in T4, the levels of caryophyllene (10% to16.14%) and γ-terpinene (14.16% to15.04%) increased, suggesting the importance of these two constituents in the activity against *C. krusei* (Table 2 and 3).

Analyzing the climate parameter, better antimicrobial activity was obtained in period 1, except for *C. krusei*, with fertilization A (Table 4). However, we found a greater climatic influence on antimicrobial activity with B fertilization, with better responses in period 1 for *E. coli*, in period 2 for *C. albicans* (ATCC 14053) and *S. aureus* and in period 4 for *C. krusei*, *C. parapsilosis* and *C. albicans* (ATCC 90028).

4. Discussion

Fertilization A showed better activity at the beginning of the rainy season (T1), producing metabolites with better antifungal response to *C. albicans* and *C. parapsilosis* and antibacterial against *E. coli* and *S. aureus* (Table 4). EL-Zefzafy et al. (2016) and Oliveira et al. (2007) identified inhibitory activity with MIC of 1.4 µg/mL and 40 µL/mL, respectively, against *C. albicans*. In a study by Manjamalai et al. (2012), evaluating activity against *E. coli*, the MIC of 50 µg/mL obtained with the EOs was associated with the presence of carvacrol (14%), p-cymene (10%) and thymol (18%) identified in greater concentrations. In the assays by Erny Sabrina et al. (2014) with *P. amboinicus* EO, the activity against *E. coli* was obtained with MIC of 780 µg/mL, with carvacrol (19.29%), camphor (17.96%) and з- carene (20.78%) being identified as main constituents.

Fertilization B provided the best EO yields, mainly in T1 and T3, without positively or negatively affecting the antimicrobial activity. The yields obtained in this study were higher than those of Bandeira et al. (2011), Khalid et al. (2014) and Pinheiro et al. (2015) with 0.8%, 0.41% and 0.43%, respectively.

Regardless of the type of fertilization, T1 provided the best antibacterial response against *E. coli* and *S. aureus* with the best MIC (A = 244 µg/mL and B = 122 µg/mL). During this period, the main constituent, carvacrol, was at its highest concentration (A = 80.54 and B = 82.25%), followed by caryophyllene (6.06-6.72%) and γ-terpinene (2.19-1.46%). The cultivation occurred in the transition from the dry period to the beginning of the rainy season, with 40 mm rainfall at the beginning of cultivation (November) reaching values of 300 mm at the time of collection, with an average of 210 mm of rainfall. Similar results were identified by Vasconcelos et al. (2017), having 88.17% carvacrol in the EOs of this species and action against *S. aureus* with MIC of 250 µg/mL, including oxacillin and vancomycin-resistant strains, while Manjamalai et al. (2012) achieved MIC of 25 µg/mL with the EOs.

The conditions and culture characteristics of the EO obtained from 1A showed a better response against the gram negative bacteria *E. coli* (MIC = 122 µg/mL and MBC = 244 µg/mL) (Table 4). These findings were superior to those found in studies of EOs by Aguiar et al. (2014) and Rodrigues et al. (2013) against *E. coli*, with MIC of 128 µg/mL and 256 µg/mL, respectively. However, the study by EL-Zefzafy et al. (2016) with 10 µL of EO extracted from the aerial parts demonstrated excellent antimicrobial activity with MIC of 8.2 µg/mL and 6 µg/mL against *E. coli* strains and MIC of 1.4 µg/mL against *C. albicans*.

Sample 4B had the best antifungal response against *C. parapsilosis* (MIC = 61 µg/mL and MBC = 122 µg/mL). This treatment covered the dry season in the region, with reduced precipitation levels from 130 mm (July) to 10 mm (October). Greater activity was detected in the study by Oliveira et al. (2007) with EOs from *P. amboinicus* leaves that resulted in a MIC of 40 µl/mL compared to *C. parapsilosis*. Therefore, the dry period (T4) favored the best conditions in the production of metabolites with antifungal activity against *C. parapsilosis* and *C. krusei* (Table 4).

It is important to note that the best antimicrobial responses were obtained in the T4 and T1 periods, during the transition from a dry climatic season (T4), with minimum rainfall and a higher temperature, to the beginning of the rainy season (T1), with less precipitation compared to the other rainy periods (T2 and T3). These conditions favored the production of certain metabolites, driven by organic fertilization. Our results were similar to those of Arumugam et al. (2016) who evaluated the dry period as ideal for cultivating *P. amboinicus* and, therefore, adapted to the dry season, according to Prasad et al. (2020).

5. Conclusion

It is inferred that changes in chemical composition, EO yield, and consequent antimicrobial activity are affected by both the type of organic fertilization and the climatic period of cultivation. Identifying the conditions of greatest antimicrobial activity optimizes the cultivation and production of vegetable raw material for the desired purpose.

The results of this research confirm the antimicrobial action of the species. Cultivation conditions (period and type of fertilization) influenced the specificity, type and intensity of antibacterial activity of EOs produced by *P. amboinicus*. Our study reinforces the importance of standardizing the cultivation of species of economic, industrial, and medicinal interest.

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Elizabeth Regina de Castro Borba: Conceptualization, Methodology, Data curation, Writing - original draft, Validation, Formal analysis, Writing - review & editing. **Tássio Rômulo Silva Araújo Luz:** Methodology, Writing - review & editing. **Yan Michel Lopes Fernandes:** Methodology, Writing - review & editing. **Thiago Castro Mubárack**: Methodology, Writing - review & editing. **Daniella Patrícia Brandão Silveira**: Methodology, Writing - review & editing. **Ana Zélia Silva:** Methodology, Writing - review & editing. **Patrícia de Maria Silva Figueiredo:** Methodology, Writing - review & editing. **Odair dos Santos Monteiro:** Methodology, Writing - review & editing. **Crisálida Machado Vilanova:** Supervision, Writing - review & editing, Project administration. **Denise Fernandes Coutinho**: Supervision, Writing - review & editing, Project administration, Funding acquisition

Declaration of competing interest

All authors declare that there are no conflicts of interest in this work.

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Conflict of interest

No conflict of interest declared.

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DECLARATION OF INTEREST STATEMENT

Declaration of competing interest

All authors declare that there are no conflicts of interest in this work.

CAPÍTULO 6

PROCESSO DO CULTIVO DA PLANTA *Plectranthus amboinicus* (Lour.) Spreng

Patente de Invenção - Pedido de depósito de patente

INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

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Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

Número do Processo: BR 10 2021 002665 0

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Título da Invenção ou Modelo de Utilidade (54): PROCESSO DO CULTIVO DA PLANTA PLECTRANTHUS AMBOINICUS (LOUR.) SPRENG

Resumo: A presente invenção refere-se a um processo do cultivo da planta Plectranthus amboinicus (Lour.) Spreng que visa otimizar a obtenção de constituintes químicos do seu óleo essencial que apresentem a melhor ação antimicrobiana decorrente da variação sazonal e do tipo de adubação orgânica proposta. O processo do cultivo da planta Plectranthus amboinicus (Lour.) Spreng analisa o tipo de adubação, o sombreamento e o período do ano determinando o período de cultivo de Plectranthus amboinicus (Lour.) Spreng em que a atividade antimicrobiana do óleo essencial é mais eficaz definindo a qualidade do material obtido para aplicação nas indústrias: farmacêutica, a de cosmético e a alimentícia.

Inventor 3 de 4

5 CONCLUSÃO

- Os períodos que mais influenciaram a fertilidade do solo e o teor nutricional foliar de *P. amboinicus* foram T1 (início da estação chuvosa) e T4 (estação seca);
- Percebeu-se que enquanto T4 propiciou aumento de teor de nutrientes no solo e reduziu na folha, T1 atuou de modo inverso.
- O cultivo mais favorável para as folhas foi com adubação mista (A) na estação chuvosa por acarretar em melhor aproveitamento dos nutrientes do solo;
- O estado nutricional das folhas interferiu nas composição química do óleo essencial que também demonstrou a interferência do tipo de adubação orgânica e da estação climática;
- O majoritário carvacrol manteve-se sempre nessa posição em todos os períodos climáticos e nos dois tipos de adubação, variando apenas o seu teor;
- O segundo componente majoritário sofreu alteração entre duas substâncias: cariofileno e γ-terpineno;
- A estação seca (T4) diminuiu o teor do carvacrol e aumentou o teor de outros dois constituintes: γ-terpineno e cariofileno. Estes três componentes químicos demonstraram maior sensibilidade às alterações climáticas (seca e chuvosa) do que as adubações testadas;
- O início da estação chuvosa (T1) e a estação seca (T4) foram os períodos que mais influenciaram na produção e atividade antimicrobiana do óleo essencial, semelhante ao ocorrido com o estado nutricional solo e da folha.
- Observou-se que o período climático interferiu na composição química e consequentemente na ação antimicrobiana do vegetal, independente do tipo de adubação. O que significa que os dois tipos de adubação testada proporcionaram efeitos semelhantes ou próximos quando nos períodos climáticos em que identificaram-se os melhores resultados. Sendo assim pode-se optar por um cultivo comercial com adubação B, por estar relacionada a um melhor rendimento do óleo essencial e ser economicamente mais viável.

O cultivo orientado de *Plectranthus amboinicus* permite obter o teor de óleo essencial com maior rendimento, que influencia diretamente na especificidade e na intensidade da atividade antimicrobiana, garantindo uma produção com maior qualidade e resposta terapêutica. Definir as condições ideais de cultivo e caracterizar a química, a físico-química e o potencial microbiológico do óleo essencial originam as especificações para o controle de qualidade da matéria-prima vegetal, particularmente de *P. amboinicus* (Lour.) Spreng.

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