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Chemical and thermal profile of *Plectranthus amboinicus* essential oil for its application as a bioherbicide

Perfil químico e térmico do óleo essencial de *Plectranthus amboinicus* visando sua aplicação como bioherbicida

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The species *Plectranthus amboinicus* is an aromatic herb with great application in popular medicine due to the diversity of biological properties. Chemically, its essential oil (EO) is characterized by two chemotypes, thymol and carvacrol, which vary depending on factors such as seasonality. Despite being an extensively exploited species, studies of the bioherbicidal potential of this species are insufficient. In this context, the EO of *P. amboinicus* leaves, extracted in two different seasonal periods, were characterized regarding chemical profile (by gas chromatography mass spectrometry - GC-MS) and thermal profile (DTG) and was subjected to bioherbicide tests (germination test and seedling development) against *Eragrostis plana*, commonly known as capim annoni, an invader of pastures in the Pampas region. *P. amboinicus* EO was a potent inhibitor of *E. plana* germination, reducing accumulated germination by over 70% when exposed to 0.1% EO, and a complete inhibition of germination was observed when exposed to 0.5%. Following the effects observed in germination, the initial growth of *E. plana* was significantly affected by concentrations above 0.05%. The major constituent identified via GC-MS was carvacrol, representing 87.5% of the volatile composition of *P. amboinicus* leaves. In addition, *P. amboinicus* EO presented high thermal stability up to 100 °C, which is an interesting result regarding its use as a bioproduct.
Keywords: essential oil, bioherbicide, derivative thermogravimetric.

Plectranthus amboinicus é uma espécie herbácea aromática com ampla aplicação na medicina popular devido a suas variadas propriedades biológicas. Quimicamente seu óleo essencial (OE) é caracterizado por dois quimiotipos, timol e carvacrol, que podem variar dependendo de diferentes fatores incluindo sazonalidade. Apesar de ser uma espécie amplamente explorada, os estudos do potencial bioherbicida desta espécie são insuficientes. Nesse contexto, OE das folhas de *P. amboinicus*, extraídos em dois períodos sazonais distintos, foram caracterizados quanto ao perfil químico (por cromatografia gasosa e espectrometria de massa - CG-EM), térmico (DTG) e submetido a testes bioherbicida (teste de germinação e desenvolvimento inicial de mudas) contra *Eragrostis plana*, vulgarmente conhecido como capim annoni, invasor de pastagens na região dos Pampas. O OE de *P. amboinicus* foi um potente inibidor da germinação de *E. plana*, reduzindo a germinação acumulada em mais de 70% quando exposto a 0,1% de OE, e uma inibição completa da germinação foi observada quando exposto a 0,5%. Seguindo os efeitos observados na germinação, o crescimento inicial de *E. plana* foi significativamente afetado por concentrações acima de 0,05%. O principal constituinte identificado por CG-EM foi o carvacrol, representando 87,5% da composição volátil das folhas de *P. amboinicus*. Além disso, o OE de *P. amboinicus* apresentou alta estabilidade térmica até 100 °C, o que é um resultado muito interessante quando se visa seu uso como bioproduto.
Palavras-chave: óleo essencial, bioherbicida, termogravimetria derivada.

1. INTRODUCTION

Plectranthus amboinicus, known as country borage, is a dicotyledonous plant belonging to the family *Lamiaceae* [1]. It is a large succulent aromatic perennial herb [2]. The species, known as "thick-mint", has been used for long as a medicinal plant for many diseases such as respiratory diseases, hepatopathy, renal and vesical calculi, to treat malarial fever, cough, chronic asthma,

hiccough, bronchitis, helminthiasis, colic, convulsions, and epilepsy [3-5]. Some studies provide evidence for its folkloric medicinal uses, such as its analgesic and anti-inflammatory activities [6]. Patel et al. (2010) [7], found significant antioxidant activity. Moreover, antimicrobial [5, 8] and insecticidal potential [9] were investigated.

These properties are often attributed to the essential oils (EOs) of the species. Studies have revealed the presence of 76 volatile constituents in the *P. amboinicus* EO [10]. The prevalent presence of the phenolic compounds carvacrol and thymol has already been observed by several authors [9-11]. The quantitative participation of those chemotypes in the composition of the EO is variable and influenced by several factors.

Many external factors can influence the EOs biosynthesis, such as environmental and agronomic conditions [12-14], stress factors [15, 16], time of harvest and phenology [17-19], different parts of the plants used for the extraction [20, 21] and even epigenetic changes resulting from concrete growing conditions [22, 23]. All those possible variations interfere with the action potential of EOs. Thus, this work aims to compare the chemical profile of the EO of *P. amboinicus* in two well-defined seasons, Amazonian winter/rainy season and Amazonian summer/dry season.

Although *P. amboinicus* EO is well studied, its bioherbicide potential was investigated for the first time in this study, where we tested its effect on germination and initial growth of *Eragrostis plana* Nees (Poaceae), the most abundant invasive plant in the pastures of southern Brazil. Rapid dispersion, facilitated by abundant seed production and allelopathic effects on other plants, made the species a difficult pest to control [24]. The impacts caused by this invasive species mainly refer to the replacement of native vegetation in many regions, which significantly impairs the feeding quality of the herd, causing considerable damage to livestock production [25].

With the intention of proposing a bioproduct for the control of *E. plana*, the *P. amboinicus* EO was also subjected to thermogravimetric analysis to evaluate the thermal behavior of the bioproduct, as well as its stability.

2. MATERIAL AND METHODS

2.1 Plant material

Plectranthus amboinicus leaves were harvested at a private vegetable garden in the city of Marabá, PA (5º 15’26.2’’S - 49º 04’06.1’’W) in two different periods of the year (Amazonian winter and summer). The botanical material was properly identified by Dr. Bernardo Tomchinsky from Universidade Federal do Sul e Sudeste do Pará (Unifesspa), with exsiccate in the herbarium of Casa da Cultura de Marabá, under registration number 6417.

Eragrostis plana seeds, originating from a batch of seeds collected in different cities in areas of the Pampa biome, central-southern region of Rio Grande do Sul (Hulha Negra, Dom Pedrito, São Gabriel and Santa Maria), were used in this study. Prior to the experiments, the seeds were stored in a refrigerator (± 4°C).

2.2 Essential oil extraction

Plectranthus amboinicus air-dried leaves were ground through a Wiley mill equipped with either a l-mm or a 2-mm screen. The following process, hydrodistillation, was performed using Clevenger-type apparatus for three hours to obtain the EO. The oils obtained were dried over anhydrous sodium sulfate and total oil yields were calculated as the rate of the mass of the base vegetable and the oil mass obtained and expressed in percentage. Prior to chemical characterization and bioassays, the oil samples were stored in Eppendorf-type microtubes at -4 °C.

2.3 Chromatographic analysis

The EO composition was analyzed in triplicate by gas chromatography coupled to the mass spectrometer using the Shimadzu GCMS-QP2010 Plus (Shimadzu Corporation, Kyoto, Japan) device equipped with autosampler (AOC – 5000 Plus from Shimadzu). A fused silica capillary column, Rtx - 5MS (30 m x 0.25 mm x 0.25 μm) was used with helium as carrier gas at a constant flow of 1.23 mL/min. The injector and detector temperatures were adjusted to 250 °C and 280 °C, respectively, and the injection volume was 1 μL in split ratio 1:10. The oven temperature started at 40 °C and was kept for 3 min, then increased to 240 °C at 4 °C/min and kept for 5 min, presenting a final time of 58.00 minutes of analysis. The mass spectrum was acquired in the range of 40 m/z to 500 m/z. 70 eV electron impact ionization energy was used.

In order to identify the individual components of the EO, a mixture of hydrocarbons ($C_{10}H_{22}$–$C_{40}H_{82}$) was injected under the same conditions and identification of constituents was then performed by comparing the spectra obtained with those of the database and, considering the relative retention index (RRI), calculated for each constituent as previously described [26].

The quantitative analysis was performed with a gas chromatograph (Shimadzu GC 2010) equipped with a DB-5 column (30 m × 0.25 mm × 0.25 μm, J &W Scientific), an autosampler (AOC 20i) and a flame ionization detector (FID). The method conditions were the following: the initial temperature of the column oven was 40 °C and was kept for 3 min, then increased to 240 °C at 4 °C/min and kept for 5 min. The carrier gas was hydrogen at 1.23 mL/min, and the injector and detector temperatures were 250 and 280 °C, respectively. The total time of analysis was 58.00 minutes and the injection volume was 1 μL with split ratio 1:10.

2.4 Thermogravimetric analysis

Plectranthus amboinicus EO sample (19.037 mg) was subjected to thermogravimetric analysis (TGA) using a Shimadzu equipment (DTG-60H model). The heating rate was 10 °C/min from room temperature (25 °C) to 700 °C. Air was employed as carrier gas in a flow rate of 50 mL/min.

2.5 Bioherbicidal assays

The EO from the dry season was diluted in absolute ethyl alcohol (1:1 – v/v) prior to the treatment composition to allow homogenization in aqueous solution. The experiments consisted of nine treatments: control (distilled water); alcohol control; and 0.005; 0.01; 0.05; 0.1; 0.5; 1.0 and 1.5% (v/v) of the EO diluted in alcohol. The alcohol control was established with the highest concentration of ethyl alcohol used in the dilution to eliminate any effects of alcohol in the treatments.

After mild disinfestation with distilled water, hypochlorite (2.5%), commercial detergent and 70% alcohol, the *E. plana* seeds were arranged on blotter-type germination paper in germination boxes (Gerbox, capacity 250 mL, 11 x 11 x 3.5 cm). The germination paper was moistened with the respective solution of each treatment in a volume of 5 mL per replicate. Every 4 days, 2 mL of this solution were applied to avoid dehydration. Four replicates were used per treatment, each one consisting of a germination box with 20 *E. plana* seeds. The tests were performed in a climate-controlled chamber at a temperature of 25 ± 2 ºC and a photoperiod of 12 hours. For standardization purposes, a germinated seed was considered a seed that emitted a radicle of at least 2 mm in length. The following variables were evaluated:

Accumulated Germination - total of germinated seeds at 10 days after incubation. The results were expressed as percentages of germination.

Dead - were considered dead all non-germinated seeds and those that did not evolve to seedling after root protrusion.

Daily count of germinated seeds up to 10 days after incubation yielded the following indexes:

Germination Speed Index (GSI) - calculated by the following formula:

GSI = G1/N1 + G2/N2 + ... + Gn/Nn.

Germination Speed Coefficient (GSC) - calculated by the following formula:
GSC = G1 + G2 + G3 + … + Gn/(G1.N1 + G2.N2 + G3.N3 + … + Gn.Nn),

Average Germination Time (AGT) - calculated by the following formula:
AGT = (G1.N1 + G2.N2 + G2.N3… + Gn.Nn)/(G1 + G2 + G3 +… + Gn),

where: G1, G2, ... Gn = number of normal seedlings computed in the first count, the second count and the last count; N1, N2, ... Nn = number of days of sowing at the first, second and last count [27].

The initial growth of the *E. plana* was evaluated at 10 days after sowing, when the height of the seedlings was measured with a ruler. The number of tillers was counted, and damage was estimated by assigning a rating scale (0 to 3 where 0 stands for "without damage") and the seedlings were dried in a drying oven at 60 ºC until reaching a constant mass. Then, the dry mass was measured with an analytical scale.

2.6 Statistical analysis

The bioherbicidal experiments were repeated three times with four replicates per treatment, each consisting of a germination box with 20 *E. plana* seeds. The percentage data were previously submitted to arc sine transformation to meet the assumptions of the mathematical model. Subsequently, all variables were submitted to analysis of variance, and the mean values were compared by the Tukey test ($P < 0.05$) using the statistical software SISVAR 5.6 [28].

3. RESULTS AND DISCUSSION

3.1 Chemical profile

The comparison between the oil yields obtained in winter (0.2582 ± 0.0781) and summer (0.2680 ± 0.0126), as well as the chemical profile of the EO samples, showed that the environmental conditions in these two periods of the year did not significantly affect the metabolism of *P. amboinicus*. Yields between 0.12% [11] and 0.43% [29] of EO from leaves have been reported for this species. From the analysis of the chemical profile, the predominance of terpene class was observed with the majority production of carvacrol chemotype, representing an average of 87.5% of the volatile composition of *P. amboinicus* leaves (Table 1). These results were in accordance with [30].

Table 1. Chemical profile of essential oil of Plectranthus amboinicus *leaves.*

Peaks	RT[a] (min)	Compounds	IR[b]	IR[c]	Summer ± SD[d]	Winter ± SD[d]
1	4.13	β-pinene	995	979	0.14 ± 0.06	0.19 ± 0.09
2	4.62	α-terpinene	1025	1017	0.01 ± 0.01	0.01 ± 0.00
3	4.75	*p*-cimene	1032	1024	1.01 ± 0.46	1.41 ± 0.70
4	5.26	γ-terpinene	1064	1059	0.40 ± 0.17	0.60 ± 0.29
5	5.51	*cis*-Sabinene hydrate	1080	1070	0.07 ± 0.04	0.09 ± 0.05
6	6.00	Linalool	1108	1096	0.02 ± 0.01	0.03 ± 0.01
7	6.09	*trans*-Sabinene hydrate	1113	1098	0.22 ± 0.10	0.29 ± 0.14
8	7.31	NI[e]	1175	NI	1.45 ± 0.81	1.51 ± 0.38
9	7.52	Borneol	1185	1169	0.95 ± 0.67	1.15 ± 0.48
10	7.68	Terpinen-4-ol	1193	1177	0.59 ± 0.4	0.30 ± 0.19
11	8.04	NI	1211	NI	0.25 ± 0.11	0.12 ± 0.02
12	8.67	NI	1240	NI	0.48 ± 0.28	0.63 ± 0.27
13	9.36	NI	1273	NI	0.08 ± 0.02	0.05 ± 0.01
14	9.75	NI	1291	NI	0.09 ± 0.01	0.07 ± 0.03
15	9.91	Thymol	1299	1290	0.28 ± 0.01	0.26 ± 0.03

16	9.97	NI	1301	NI	0.13 ± 0.03	0.09 ± 0.02
17	10.07	Carvacrol	1306	1299	87.93 ± 1.55	87.62 ± 1.35
18	12.69	*E*-caryophyllene	1430	1419	1.12 ± 0.36	1.25 ± 0.54
19	12.88	α-*trans*-bergamotene	1439	1434	0.45 ± 0.16	0.52 ± 0.2
20	13.45	α-humulene	1466	1454	0.30 ± 0.09	0.36 ± 0.15
21	13.98	NI	1492	NI	0.02 ± 0.02	0.04 ± 0.01
22	14.29	α-muurolene	1507	1500	0.01 ± 0.01	0.04 ± 0.03
23	14.55	β-bisabolene	1520	1505	0.01 ± 0.01	0.02 ± 0.01
24	14.57	NI	1521	NI	0.02 ± 0.01	0.02 ± 0.01
25	14.85	NI	1535	NI	0.02 ± 0.01	0.03 ± 0.03
26	15.22	*cis*-Sesquisabinene hydrate	1554	1544	0.02 ± 0.01	0.07 ± 0.1
27	15.45	NI	1565	NI	0.02 ± 0.01	0.05 ± 0.03
28	16.04	Caryophyllene oxide	1595	1583	2.59 ± 0.47	1.92 ± 0.35
29	16.39	NI	1613	NI	0.03 ± 0.01	0.02 ± 0.01
30	16.52	NI	1620	NI	0.03 ± 0.03	0.03 ± 0.03
31	16.60	Humulene epoxide II	1624	1608	0.44 ± 0.13	0.35 ± 0.05
32	17.04	NI	1647	NI	0.02 ± 0.01	0.03 ± 0.01
33	17.18	Caryophylla-4(12),8(13)-dien-5-ol	1655	1640	0.04 ± 0.02	0.04 ± 0.01
34	17.56	NI	1675	NI	0.01 ± 0.01	0.01 ± 0.01
35	17.75	14-hydroxy-(Z)-Caryophyllene	1685	1667	0.19 ± 0.09	0.29 ± 0.07
36	17.86	14-hydroxy-9-*epi*-(E)-Caryophyllene	1691	1669	0.14 ± 0.06	0.08 ± 0.01
37	18.52	NI	1725	NI	0.03 ± 0.02	0.04 ± 0.04
38	18.72	14-hydroxy-α-Humulene	1736	1714	0.02 ± 0.02	0.05 ± 0.03
39	20.21	NI	1816	NI	0.02 ± 0.02	0.00 ± 0.01
40	20.82	NI	1854	NI	0.03 ± 0.03	0.02 ± 0.03
41	21.00	NI	1865	NI	0.02 ± 0.01	0.03 ± 0.03
42	21.67	NI	1906	NI	0.02 ± 0.02	0.02 ± 0.02
43	22.07	NI	1931	NI	0.02 ± 0.02	0.04 ± 0.05
44	23.68	NI	2030	NI	0.03 ± 0.02	0.02 ± 0.02
45	23.86	NI	2041	NI	0.02 ± 0.01	0.01 ± 0.01
46	24.10	NI	2056	NI	0.04 ± 0.03	-
47	24.19	NI	2062	NI	0.01 ± 0.01	0.01 ± 0.01
48	24.28	NI	2068	NI	0.01 ± 0.01	0.02 ± 0.03
49	24.64	NI	2091	NI	0.02 ± 0.02	0.01 ± 0.01
50	25.10	NI	2121	NI	0.02 ± 0.01	0.01 ± 0.01
51	25.23	NI	2129	NI	0.03 ± 0.03	0.03 ± 0.02
52	25.42	NI	2142	NI	0.02 ± 0.02	0.03 ± 0.01
53	25.67	NI	2159	NI	0.03 ± 0.02	0.04 ± 0.03
54	26.29	NI	2200	NI	0.02 ± 0.01	0.01 ± 0.01
55	31.58	NI	2588	NI	0.02 ± 0.01	0.01 ± 0.00
		Total identified			**96.95**	**96,96**

[a] Retention Time; [b] Experimental Retention Index; [c] Literature Retention Index; [d] Standard Deviation [e] Not Identified.

3.2 Thermal profile

By using the TGA tests, it was possible to build graphs presenting the weight loss curve and the derivative thermogravimetric (DTG) curve, as can be seen in Figure 1.

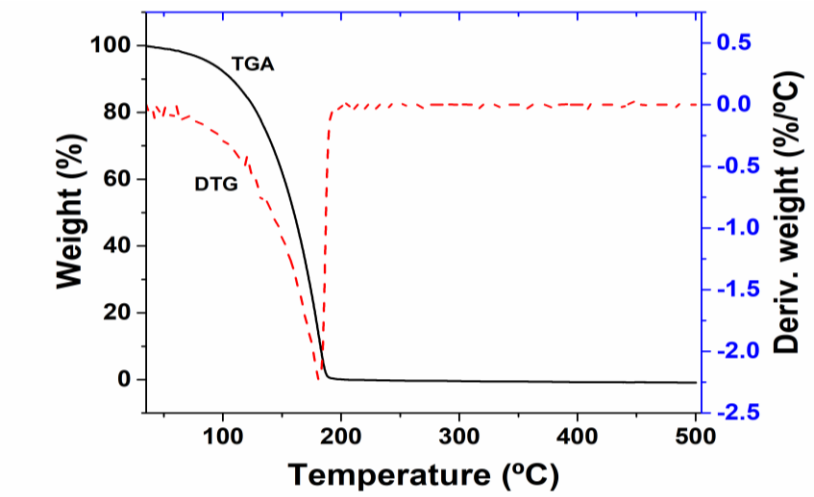

Figure 1. Representation of TGA (solid black line) and DTG (dashed red line) curves for Plectranthus amboinicus *essential oil.*

TGA graphic presented just a single decomposition step, representing a weight loss of 99.04% on the temperature range from 100 °C to 190 °C, and this result agrees with what was previously reported in the literature [30] considering the related plant is of the carvacrol chemotype. The TGA analysis showed that the EO used in this study has a high thermal stability until 100 °C, which is an important result taking into account the proposal to use the referred EO as bioherbicide against a species of *E. plana*, which is invasive of pasture, and thus encourages researchers in order to demonstrate the proof-of-concept of this present study.

3.3 Bioherbicidal assays

The bioherbicidal potential of the EO of *P. amboinicus* leaves did not statistically differ in the different collection seasons, Amazonian winter/rainy season and Amazonian summer/dry season (data not shown). Thus, the presented results refer to the oil from the dry season.

The *P. amboinicus* EO proved to be a potent inhibitor of *E. plana* germination. Accumulated germination was reduced by over 70% on exposure to 0.1% EO of *P. amboinicus* and a complete inhibition of germination was observed from 0.5%. Consequently, mortality followed this response, reaching 100% from 0.5% EO. Regarding germination speed data (GSI, GSC and AGT), it was possible to observe a delay in germination from the concentration of 0.05% EO (Table 2). Although in this study an evident reduction in the germination rate was soon perceived, in some cases the allelopathic effect in plants is most often expressed not by the amount of germinated seeds, but rather by the retardation of their germination [31]. The retardation can give the necessary time to harvest the crop without loss in its production.

Table 2. Plectranthus amboinicus *essential oil on* Eragrostis plana *germination.*

Treatments	Accumulated germination (%)	Dead (%)	GSI	GSC	AGT
Control	81.67 a*	18.33 d	5.47 a	0.27 a	3.82 c
Alcohol Control	75.00 a	25.00 d	4.78 a	0.24 a	4.13 c
0.005 %	21.67 bc	78.33 bc	2.04 bc	0.20 abc	4.94 bc
0.01 %	31.67 b	68.33 c	2.54 b	0.23 ab	4.44 c
0.05 %	15.00 cd	85.00 ab	0.69 cd	0.15 bc	6.64 ab
0.1 %	8.33 cd	91.67 ab	0.28 cd	0.14 c	6.83 a
0.5 %	0 d	100 a	ng**	ng	ng

* Means followed by the same letter in the column do not differ by Tukey test at 5% probability. GSI: germination speed index; GSC: germination speed coefficient; AGT: average germination time; CV: Coefficient of Variation. ** ng: non-germinated.

Following the effects observed in germination, the initial growth of *E. plana* was significantly affected by concentrations above 0.05% of the *P. amboinicus* EO (Figure 2).

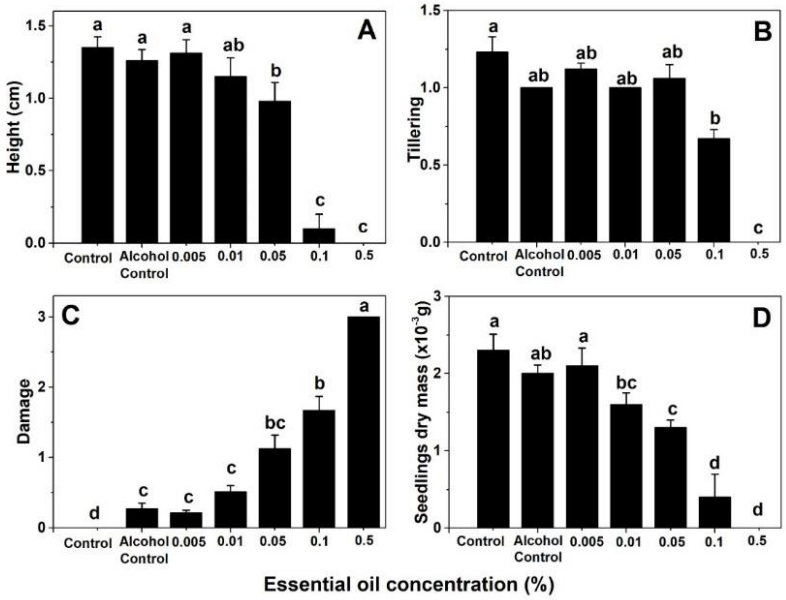

Figure 2. Concentrations of Plectranthus amboinicus *essential oil in the initial growth of* Eragrostis plana. *A: Seedlings height (cm); B: Tillering; C: Damage; D: Seedlings dry mass (g).*

Allelopathic effects of this species had already been shown by El-Rokiek et al. (2018) [32], who showed that aqueous extracts from *P. amboinicus* leaves were a potent inhibitor of the growth of weeds (*Phalaris minor* and *Anagalis arvensis*) present in the cultivation of peas (*Pisum sativum*). Furthermore, the authors demonstrated a higher productivity in the cultivation of *P. sativum* using 25% *P. amboinicus* leaf extract.

Several biological activities have previously been attributed to the isomeric chemotypes thymol and carvacrol, such as antifungal activity [33- 36], insecticide, phytotoxic among others [33, 37].

Pinheiro et al. (2015) [11] reported that the EO of *P. amboinicus* and its chemotypes, carvacrol and thymol, inhibited the germination and decreased root and aerial growth of *Lactuca sativa* and *Sorghum bicolor*. Lima et al. (2019) [38] tested thymol in seed treatment; they noted that thymol did not impair the germination and the GSI of soybean seeds. Instead, the use of high concentrations affected the seedling phenotype; doses up to 1 mg/L of thymol showed deleterious effects on roots and seedlings. Moreover, Assis Alves et al. (2018) [35] verified that inhibition of germination brought by thymol in weed species was as intensive as the germination suppression promoted by the synthetic herbicide dichlorophenoxyacetic acid (2,4-D).

Motivated by the research carried out by Bendre et al. (2018) [39], who compiled studies highlighting the extensive effects of carvacrol on various agricultural pests, it is suggested that the allelopathic effects of *P. amboinicus*, found in this study, can be attributed to this main constituent of its EO, the carvacrol.

Previous studies demonstrate the mechanism of action of carvacrol which may also be occurring for the inhibition of germination and initial growth verified in the present study. For example, Gill and Holley (2006) [40] observed that carvacrol at the concentration of 5.0 or 10 mmol/L caused inhibition of the activity of enzyme adenosine triphosphatase (ATPase), which is responsible for catalyzing the process of breaking adenosine triphosphate (ATP) into adenosine diphosphate (ADP) plus inorganic phosphate. The impairment of this metabolic pathway impacts several physiological processes for plant growth and development, both pre and post germination [41].

A more recent study verified the potential of carvacrol, as well as other compounds, in controlling weeds *Amaranthus retroflexus*, *Avena fatua*, *Eleocharis bonariensis*, and *Portulaca oleracea* and could be a good candidate for bioherbicide formulations. This study also confirmed that the exposure time and the applied concentration influenced the degree of toxicity [42].

Thus, the present study corroborates the previously registered research and presents the EOs of *P. amboinicus* leaves as a potential matrix for the development of a bioproduct to be explored as a bioherbicide in the control of *E. plana*, due to the high efficiency in the control of germination and initial growth of this invader (0.1%). In addition, both the plant and its constituents of EO are considered safe for the environment and human health since they are used in flavorings and food infusions in traditional medicine.

4. CONCLUSION

In the perspective of the application of natural products as bioherbicides, in this work the *P. amboinicus* essential oils were analyzed for their chemical and thermal profile and subjected to tests aimed at inhibiting germination and development of *E. plana*. The high efficiency in *E. plana* control presented by essential oils (0.1%), as well as thermal stability up to 100 °C encourages the development of complementary studies necessary for the formulation of a bioproduct with bioherbicidal properties to combat this weed.

This is the first study that searched the bioherbicide potential of *P. amboinicus* essential oil against *E. plana*, an invasive species that is difficult to control.

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