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Volatile organic compounds of jack bean leaves (*Canavalia ensiformis* (L.) DC) and their potential use in the ecological management of pests

Compostos orgânicos voláteis de folhas de feijão-de-porco (*Canavalia ensiformis* (L.) DC) e seu potencial uso no manejo ecológico de pragas

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ABSTRACT

Jack bean (*Canavalia ensiformis* (L.) DC) is widely used as green manure and for the phytochemical control of agricultural pests. However, information on the volatile organic compounds (VOCs) emitted by its leaves is still lacking, which could help to develop sustainable pest management strategies based on chemical ecology. This study aimed to determine the VOC profile of jack bean leaves and assess its potential for pest management applications. Leaf samples from greenhouse-grown plants were used for VOC extraction using solid-phase microextraction (SPME). VOCs were analyzed by gas chromatography coupled with mass spectrometry (GC-MS). The linear retention index was calculated using retention time values from both compounds extracted from samples and a homologous series of *n*-alkanes (C_7–C_{30}), with additional confirmation with analytical standards when available. We identified a total of 46 compounds, with green leaf volatiles (GLVs) exhibiting the highest relative abundance. The detected GLVs included (*Z*)-3-hexenal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*E*,*E*)-2,4-hexadienal, (*E*,*E*)-2,4-heptadienal, 1-hexanol, 2-ethyl-1-hexanol, (*E*)-2-octenal, nonanal, (*E*,*E*)-2,6-nonadienal, (*Z*)-3-nonenol and 1-nonanol. We additionally identified terpenes such as isothujone, β-cyclocitral, γ-isogeraniol, cis-geraniol, pulegone, β-citral, β-cyclohomocitral, geranial, α-ionone, β-ionone, dihydroactinolide, α-bisabolol, and pythan. Several of these compounds have been previously associated with plant defense mechanisms against key agricultural pests. We discuss the potential application of the identified VOCs in pest management, particularly in agroecosystems where jack bean is used as green manure.

Index terms: Cover crop; sustainable management; volatiles; HP-SPME.

RESUMO

O feijão-de-porco (*Canavalia ensiformis* (L.) DC) é uma planta utilizada como adubo verde em diferentes culturas e no manejo fitoquímico de pragas. Contudo faltam dados sobre os compostos orgânicos voláteis (COVs) liberados pelas folhas para apoiar seu uso no manejo sustentável de pragas recorrendo a ecologia química. O objetivo desse trabalho foi determinar o perfil de COVs das folhas do feijão-de-porco com potencial de aplicação no manejo de pragas. Amostras foliares de feijão-de-porco cultivadas em casa de vegetação foram utilizadas para extração dos compostos utilizando a técnica de micro-extração em fase sólida (SPME). A análise foi feita através da cromatografia gasosa acoplada à espectrometria de massas (GC-MS). Para a identificação dos COVs, foi feito o cálculo do índice de retenção linear, utilizando os valores de tempo de retenção dos compostos na amostra e de uma série homóloga de n-alcanos (C_7-C_{30}), além da injeção de padrões analíticos disponíveis. Foram confirmados 46 compostos, sendo os voláteis de folhas verdes (VFV´s) os de maior abundância relativa. Os VFV's encontrados foram (*Z*)-3-hexenal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*E*,*E*)-2,4-hexadienal, (*E*,*E*)-2,4-heptadienal, 1-hexanol, 2-ethyl-1-hexanol, (*E*)-2-octenal, nonanal, (*E*,*E*)-2,6-nonadienal, (*Z*)-3-nonenol and 1-nonanol. Outro grupo presente também foi o dos terpenos: isothujone, β-cyclocitral, *γ*-isogeraniol, cis-geraniol, pulegone, β-citral, β-cyclohomocitral, geranial, α-ionone, β-ionone, dihydroactinidiolide, α-bisabolol, phytan. Alguns dos compostos identificados já foram associados à defesa de plantas contra pragas e seu uso potencial para o manejo de pragas em agroecossistemas foi discutido, particularmente onde essa espécie é utilizada como adubo verde.

Termos para indexação: Cultura de cobertura; manejo sustentável; voláteis; HS-SPME.

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Introduction

Jack bean (*Canavalia ensiformis* (L.) DC) is a biennial shrubby and creeping species of the Fabaceae family, reaching up to 1.2 m in height (Silva-López, 2012). It is widely used as green manure due to its symbiotic association with nitrogen-fixing microorganisms, which convert atmospheric nitrogen into plant-assimilable forms (Ngwenya et al., 2022). Additionally, jack bean has allelopathic properties, inhibiting the growth of invasive plant species (Mendes & Rezende, 2014).

Research on the chemical potential of this species has focused on bioactive compounds such as ureases (Postal et al., 2012), canatoxin (Oliveira et al., 1999), lectins (Sprawka et al., 2014), recombinant peptides (Dos Santos et al., 2019), and some molecules with nematicidal activity (Rocha et al., 2017)

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important for managing both insect pests and plant pathogens. However, information on the volatile organic compounds (VOCs) emitted by jack bean leaves is still lacking, which could further help to develop sustainable pest and disease control strategies for agriculture based on chemical ecology.

VOCs include a wide range of chemical groups, attracting interest across multiple research fields, particularly in the context of sustainable and environmentally friendly agriculture (Makhlouf et al., 2024). Studies on VOCs have demonstrated their importance in pest control and the selection of pest-resistant crops (Foba et al., 2023), as well as in pest management through the attraction of natural predators (Turlings & Wäckers, 2004) and forming a natural barrier (Bybee-Finley & Ryan, 2018). In this context, VOCs play a crucial role in managing vector-borne and other plant diseases, as they can directly inhibit pathogen growth or activate plant defense systems (Razo-Belman & Ozuna, 2023). Additionally, VOCs help in the discovery of bioactive compounds for developing insect-repellent and/or attractant formulations (Braasch, Wim & Kaplan, 2012). Together, these studies contribute to developing strategies such as traps containing plant volatiles for pest monitoring, natural enemy identification, and insect capture (Reddy & Guerreiro, 2004). VOCs can be synthesized for field application or provided through intercropping with cover crops.

To date, VOCs emitted by leaves of *Canavalia* species have not been investigated. However, VOCs from legumes such as faba bean, chickpea, and cowpea, species belonging to the same family as *Canavalia*, have been shown to influence various insect-plant interactions. In addition, Makhlouf et al. (2024) reviewed the role of VOCs in cool-season legumes, highlighting their application in managing plant diseases and pests.

VOCs modulate insect behavior by prolonging landing time on plants, facilitating host location, attracting parasitoid wasps, reducing oviposition, and repelling pest insects. Makhlouf et al. (2024) also highlighted that VOCs can influence the development of fungal diseases and mediate plant-plant interactions. Both pathogen infection and herbivory can trigger VOC release, potentially enhancing the resistance of neighboring plants. Research on the Fabaceae species *Crotalaria nitens* Kunth has demonstrated a significant increase in VOC emission in response to herbivory by *Utetheisa ornatrix* (L.) caterpillars, reinforcing the importance of VOCs in plant-insect interactions (Prada, Stashenko & Martínez, 2021).

Research on VOCs from jack beans is limited to the study by Barros et al. (2014), who identified VOCs with nematicidal activity in macerated seeds. In this study, we aimed to characterize the VOC profile of jack bean leaves, focusing on bioactive compounds with the potential to repel pest insects and/or attract natural enemies. Our findings position jack bean as a promising candidate in integrated pest management strategies, including push-pull systems. We also discuss the ecological significance of the identified VOCs in pest management.

Material and Methods

Chemicals

Standard solutions of *n*-alkanes (C_7–C_{30}), benzaldehyde, 1-octen-3-ol, (E)-2-octenal, 1-octanol, decanal, 1-decanol, and β-ionone were purchased from Sigma-Aldrich (Burlington, MA, USA). All standards used in the GC-MS analysis had a purity of ≥95%.

Biological assay

The experiment was conducted at Embrapa Cassava and Fruit Crops, located in Cruz das Almas, Bahia, Brazil (12°40′29.9″S; 39°06′9.9″W), under hot and humid tropical climate conditions and natural light. Jack bean plants (*C. ensiformis*) were individually grown in plastic pots with a 3 kg capacity, using a 1:1 mixture of soil and pine bark as the growing substrate. The mean daily air temperature in the screen house ranged between 22 and 34 °C and the mean daily humidity was 80%. Plants were watered three times weekly and fertilized every 15 days with a solution containing micronutrients [nitrogen (94.3 mg L^{-1}), potassium (79.7 mg L^{-1}), phosphorus (23.8 mg L^{-1}), magnesium (5.47 mg L^{-1}), calcium (58.07 mg L^{-1}), iron (0.71 mg L^{-1}), copper (0.98 mg L^{-1}), zinc (0.18 mg L^{-1}), manganese (0.26 mg L^{-1}), boron (0.12 mg L^{-1}), and molybdenum (0.04 mg L^{-1})]. When necessary, pest management was conducted manually to prevent interference from chemical products. For the detection and identification of VOCs, three plants were used, with each specimen sampled four times (each extraction from a different leaf) (Mesquita et al., 2017; Silva et al., 2017). A blank control (empty vial following the same conditions as the samples) was included to check for potential contamination during the extraction and analysis process.

Solid-phase microextraction (SPME)

A fresh leaf was sectioned from the plants, cut into small pieces, placed into a 20 mL glass vial until the weight reached 0.3 grams, and then macerated with a glass rod. Each plant was treated as a distinct sample, with a total of three plants included in the study. Each sample underwent four replications, all performed on the same day. The experiment was conducted over three consecutive days, with one plant (i.e., one sample) processed per day. Once prepared, vials were sealed and coated with Teflon® and kept at room temperature for 11 min to allow the volatiles to reach equilibrium within the confined space. Afterward, vials were placed on a hot plate at 57 °C for 47 min to enhance compound volatilization.

For headspace adsorption (HS-SPME) of VOCs, a carboxen/polydimethylsiloxane (CAR/PDMS, 75 µm) solid-phase microextraction fiber (Supelco, Bellefonte, PA, USA) was used. After extraction, the fiber was retracted and inserted into the gas chromatography injector for 3 min at 250°C (adapted from Mesquita et al., 2017).

Gas chromatography coupled to a mass spectrometer (GC-MS) analysis

GC-MS analysis was performed on a Shimadzu instrument, model GCMS-QP2010 Plus (Kyoto, Japan) using splitless injection mode. VOCs were separated on an HP-5MS capillary column (5%-phenyl-methylpolysiloxane; 30 m × 0.25 mm ID × 0.25 µm, Restek, Bellefonte, USA), using helium (99.999%) as carrier gas at a flow rate of 0.61 mL min^{-1}. The oven temperature was adjusted as follows: the column temperature started at 30 °C (15 min); it was heated at 1 °C min^{-1} to 40 °C (holding for 2 min); heated at 1 °C min^{-1} to 60 °C (holding for 2 min); heated at 5 °C min^{-1} to 110 °C; heated at 10 °C min^{-1} to 200 °C, and then heated at 25 °C min^{-1} to 280 °C. Each run lasted 71 min (Figure 1). The mass detector conditions were: the transfer line temperature was set to 220 °C, the ion source temperature to 220 °C, and the electron impact ionization mode set at 70 eV.

The chromatograms were analyzed using GCMS Solution software (version 4.20) and the identification of VOCs was carried out (i) comparing the GC retention times and mass spectra with those of pure analytical standards from Sigma-Aldrich, when available; (ii) cross-referencing all mass spectra with the NIST 147 2008 mass spectral library; and (iii) determining the linear retention index (LRI) values using a homologous series of n-alkanes (C_7–C_{30}) and comparing them to those reported in the literature for similar chromatographic columns.

Statistical analysis

The relative composition of VOCs was expressed as means ± standard deviation, considering four replicates per plant and three plants. Analysis of variance (ANOVA), followed by Tukey's test, was performed to determine statistically significant differences among means ($p < 0.05$) using XLStat software (version 7.8).

Results and Discussion

We identified a total of 46 VOCs (Table 1) in the leaves of the evaluated jack bean plants, of which 32 had higher relative abundance. Twelve of these compounds are green leaf volatiles (GLVs) that have 6 to 9 carbons, including aldehydes, alcohols, and esters (Figure 2): (*Z*)-3-hexenal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*E*,*E*)-2,4-hexadienal, (*E*,*E*)-2,4-heptadienal, 1-hexanol, 2-ethyl-1-hexanol, (*E*)-2-octenal, nonanal, (*E*,*E*)-2,6-nonadienal, (*Z*)-3-nonen-1-ol, and 1-nonanol. Among these GLVs, (E)-2-hexenal had the highest mean relative abundance, followed by (Z)-3-hexenal (Table 1)

GLVs are released in response to various stimuli and serve multiple functions, including defense against pathogens and herbivores (Ul-Hassan, Zainal, & Ismail, 2015). Turlings et al. (1991) identified the GLVs (*Z*)-3-hexenal and (*E*)-2-hexenal in the natural blend emitted by corn seedlings infested with *Spodoptera exigua* (Hübner) larvae. Furthermore, they demonstrated that this blend attracted the female parasitoid wasp *Cotesia marginiventris* (Cresson). The presence of these GLVs in jack bean leaves suggests that this species could be a good candidate for testing its attractiveness to natural enemies of pests.

(*E*)-2-hexen-1-ol, another GLV identified in this study, is also emitted by healthy leaves from susceptible lines of *Brassica chinensis* L. and *Brassica napus* L. (Yan et al., 2023). In both behavioral assays and under laboratory and field conditions, (E)-2-hexen-1-ol has attracted the pest *Plutella xylostella* (L.), also known as 'diamondback moth'. The authors suggested that (E)-2-hexen-1-ol could serve as an attractant in a 'push and pull' cultivation strategy. Additionally, (Z)-3-hexenal, when present in a ternary blend, showed attractiveness to both sexes of *Ectropis obliqua* (Prout, 1915), a major pest of tea plants (Sun et al., 2016). 1-nonanol, also present in the leaves of tomato and

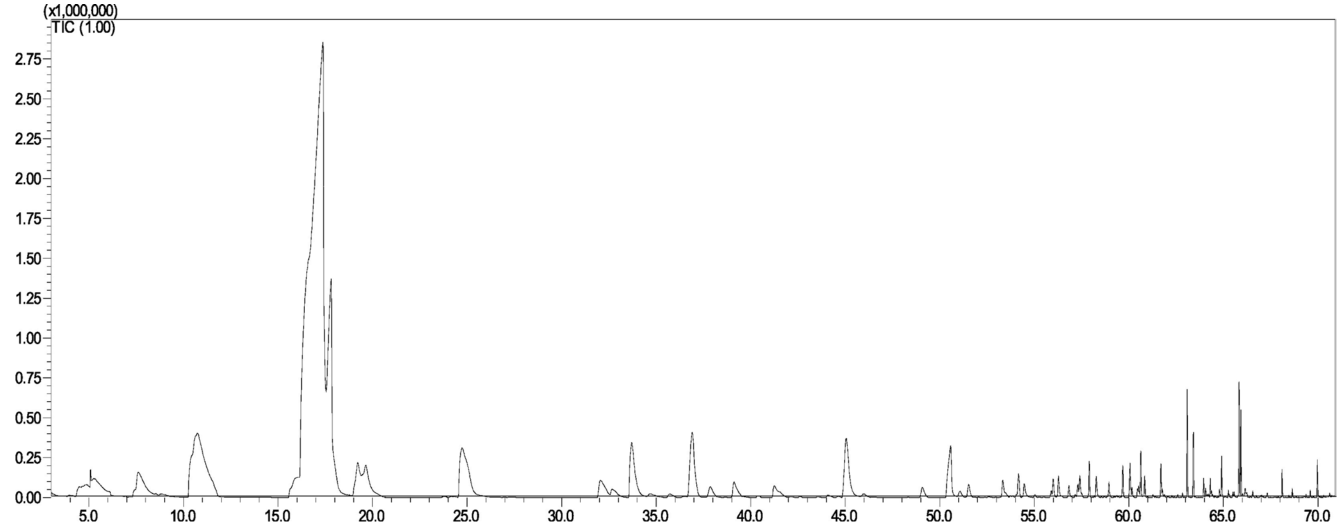

Figure 1: Example of a VOC chromatogram of jack bean leaves obtained through HS-SPME/GC-MS. The total analysis time (71 min) is on the horizontal axis, and the signal intensity in arbitrary units is on the vertical axis.

eggplant, has been associated with the attraction behavior of *Tuta absoluta* (Meyrick) (Chen et al., 2023). (*E*)-2-hexenal and (*Z*)-3-hexenal, the most abundant GLVs in jack bean leaves, were increased in corn plants of the 'Prosna' variety when infected by fungi from different *Fusarium* species (Piesik et al., 2011). The amounts of induced volatiles were higher 7 days after infection compared to 3 days, especially in plants infected through the leaves rather than via soil. Additionally, neighboring uninfected plants also exhibited increased production of these volatiles. The authors suggested that GLVs are among the volatiles that can trigger defense responses in neighboring uninfected plants. Olfactometry tests showed that infected plants were able to attract the larvae of the beetle *Oulema melanopus* (L.), an economically significant pest of wheat. These findings suggest that certain herbivores may be attracted to these GLVs, which signal the presence of vulnerable plants (Piesik et al., 2011).

Table 1: VOCs identified in jack bean leaves using SPME and GC-MS analysis.

Nº	Compound	Group compound	Retention time (min)	LRI_{exp}	LRI_{lit}	Mean value (%)	Standard deviation
1.	(*E*)-2-pentenal	aldehyde	7.584	745	746	1.354[e]	± 0.538
2.	(*Z*)-3-hexenal[a]	aldehyde	10.736	794	795	9.316[b]	± 3.908
3.	(*E*)-2-hexenal[a]	aldehyde	17.232	856	856	33.723[a]	± 5.007
4.	(*E*)-2-hexen-1-ol[a]	alcohol	19.254	871	868	0.938[f]	± 0.562
5.	1-hexanol[a]	alcohol	19.602	873	870	1.747[e]	± 0.766
6.	(*E*,*E*)-2,4-hexadienal[a]	aldehyde	24.701	905	906	5.751[c]	± 6.044
7.	benzaldehyde[b]	aldehyde	32.008	955	955	1.590[f]	± 0.953
8.	5-methyl-3-heptanone	ketone	32.642	958	939	0.435[g]	± 0.142
9.	5,5-dimethyl-2(5H)-furanone	lactone	34.661	970	945	0.158[i]	± 0.083
10.	1-octen-3-ol[b]	alcohol	36.938	982	955	4.404[d]	± 2.806
11.	2,3-octanedione	ketone	37.81	987	987	0.595[g]	± 0.239
12.	(*E*,*E*)-2,4-heptadienal[a]	aldehyde	41.216	1006	1006	0.609[eg]	± 0.176
13.	dihydro-quinone	ketone	44.048	1029	1031	0.078[j]	± 0.034
14.	2-ethyl-1-hexanol[a]	alcohol	44.993	1036	1038	4.087[d]	± 1.389
15.	(*E*)-2-octenal[a, b]	aldehyde	48.999	1065	1065	0.655[eg]	± 0.490
16.	isothujone	terpene	51.024	1079	1081	0.306[g]	± 0.221
17.	1-octanol[b]	alcohol	51.483	1082	1082	0.427[g]	± 0.171
18.	1-undecyne	alkyne	53.295	1094	1095	0.399[g]	± 0.093
19.	nonanal[a]	aldehyde	54.066	1099	1100	0.379[g]	± 0.187
20.	phenylethyl alcohol	alcohol	54.445	1105	1105	0.309[g]	± 0.099
21.	cis-3-hexenyl isobutyrate	ester	56.811	1149	1145.3	0.233[h]	± 0.102
22.	(*E*,*E*)-2,6-nonadienal[a]	aldehyde	57.145	1155	1153.1	0.051[j]	± 0.017
23.	(*Z*)-3-nonen-1-ol[a]	alcohol	57.281	1158	1156	0.159[h]	± 0.033
24.	4-ethyl-benzaldehyde	aldehyde	57.384	1160	1163	0.474[g]	± 0.162
25.	1-nonanol[a,b]	alcohol	58.263	1175	1175	0.264[h]	± 0.115
26.	cis-3-hexenyl butyrate	ester	58.931	1187	1187	0.254[h]	± 0.102
27.	decanal[b]	aldehyde	59.667	1201	1201	0.373[g]	± 0.435
28.	*β*-cyclocitral	terpene	60.023	1213	1214	0.521[g]	± 0.096
29.	*γ*-isogeraniol	terpene	60.152	1218	1221.8	0.135[i]	± 0.045
30.	cis-geraniol	terpene	60.444	1228	1228	0.147[i]	± 0.053
31.	pulegone	terpene	60.512	1230	1233	0.084[i]	± 0.027
32.	n-valeric acid cis-3-hexenyl ester	ester	60.621	1234	1235.8	0.704[e]	± 0.445

Continue...

Table 1: Continuation.

Nº	Compound	Group compound	Retention time (min)	LRI$_{exp}$	LRI$_{lit}$	Mean value (%)	Standard deviation
33.	*β*-citral	terpene	60.826	1241	1241	0.268^{h}	± 0.072
34.	*β*-cyclohomocitral	terpene	61.262	1256	1254	0.065^{j}	± 0.029
35.	geranial	terpene	61.687	1271	1271	0.389^{g}	± 0.149
36.	1-decanol^{b}	alcohol	61.762	1274	1274	0.063^{j}	± 0.061
37.	cis-3-hexenyl hexanoate	ester	64.057	1378	1376	0.103^{i}	± 0.032
38.	*α*-ionone	terpene	64.88	1426	1426	0.584^{g}	± 0.149
39.	paeonol	phenol	65.263	1451	1451	0.066^{j}	± 0.041
40.	*β*-ionone^{b}	terpene	65.827	1488	1488.4	1.110^{e}	± 0.237
41.	dihydroactinidiolide	terpene	66.544	1539	1538	0.069^{j}	± 0.025
42.	butanoic acid, decyl ester	ester	67.317	1596	1597	0.149^{i}	± 0.101
43.	*α*-bisabolol	terpene	68.276	1677	1678	0.049^{j}	± 0.040
44.	pentadecanal	aldehyde	68.64	1710	1710	0.044^{j}	± 0.017
45.	phytan	terpene	69.37	1791	1795	0.021^{k}	± 0.011
46.	methyl margarate	ester	70.297	2018	2021	0.013^{k}	± 0.007

^{a}Green leaf volatiles, ^{b}Identification confirmed by comparison with mass spectra and retention times of analytical standards; LRI$_{exp}$ = relative retention index of the n-alkane standard (C_7 to C_{30}) obtained on an HP–5MS capillary column. LRI$_{lit}$ = retention index published in the literature. Significant differences (ANOVA, Tukey test; p<0.05) among the mean relative composition (*n*=3) are indicated by letters.

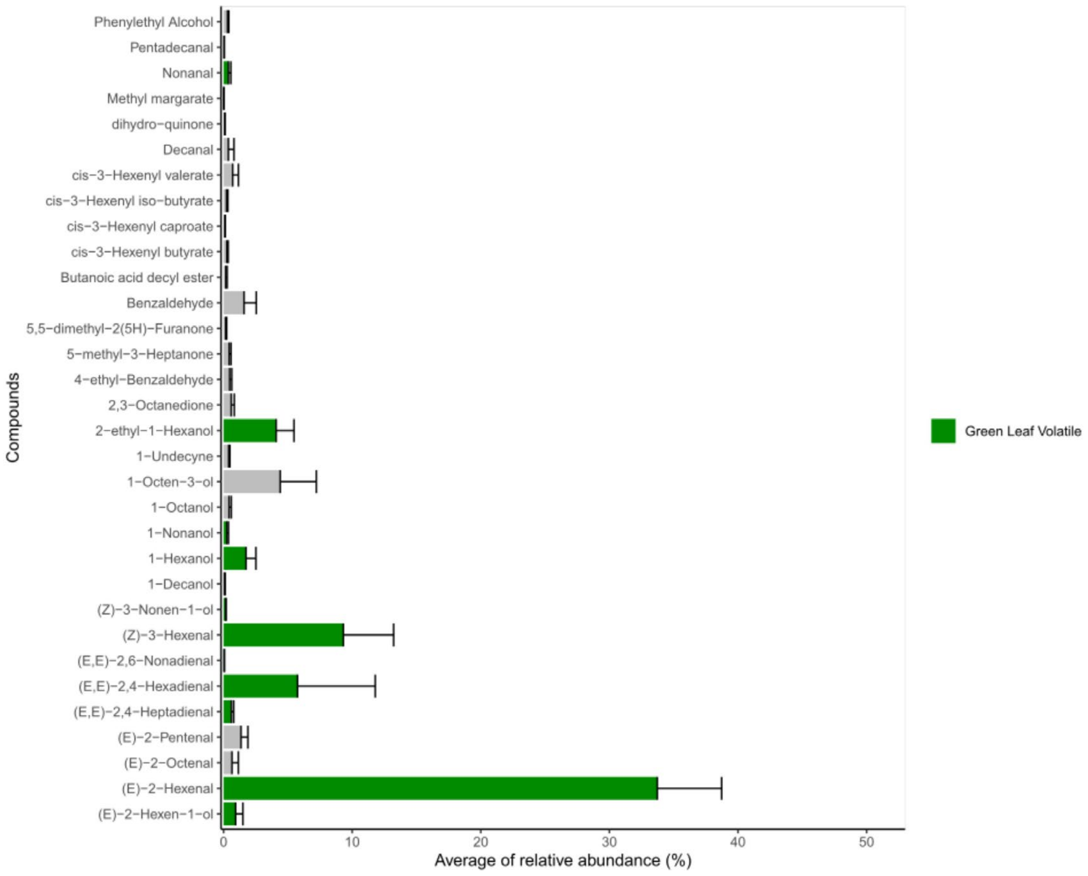

Figure 2: VOCs relative abundance in jack bean leaves. Data are means ± standard deviation (*n*=3). GLVs are highlighted in green.

Yan et al. (2023) further support the idea that GLVs can serve as olfactory cues, signaling the presence of suitable plants for feeding and also playing a role in the location of mating partners (Scala et al., 2013). The detection of different GLVs in the chemical profile of jack bean leaves reinforces the potential of this species not only as green manure but also as a candidate for agricultural pest management.

Pentyl leaf volatiles (PLVs) are another class of compounds closely related to GLVs, as they are produced through the oxygenation of linolenic acid mediated by lipoxygenase enzymes that target membrane fatty acids (Ul-Hassan, Zainal, & Ismail, 2015; Matsui & Koeduka, 2016). PLVs can be 5-carbon alcohols (like (*E*)-2-pentenal), ketones, or aldehydes. These volatiles are released by plants in response to both abiotic stress and defense against pathogens (Gorman et al., 2021). The characterization and synthesis of PLVs are relatively recent in the study of plant metabolites.

The high relative abundance of compounds derived from enzymatic reactions acting on membrane fatty acids can be attributed to the leaf maceration method employed in this study. The mechanical action of maceration breaks down plant cells, potentially releasing fatty acids from the membrane and making them available as substrates for enzymes involved in the GLV biosynthesis pathway. Similarly, the mechanical action of herbivory can trigger the synthesis of GLVs (Ul-Hassan, Zainal, & Ismail, 2015). Therefore, we would expect that in jack bean seedlings subjected to herbivory, GLVs would be among the VOCs with the highest relative abundance.

Other VOCs emitted by jack bean leaves include the phenylpropanoids benzaldehyde and 4-ethyl-benzaldehyde (Table 1). Benzaldehyde, along with the GLVs (E)-2-hexenal, 1-hexanol, and the aldehyde decanal, is part of the volatile blend released by *Vicia faba* L., which attracts the aphids *Aphis fabae* Scopoli (Makhlouf et al., 2024). 4-ethyl-benzaldehyde is one of the volatiles emitted by soybean also detected by both sexes of the bean bug *Riptortus pedestris* (Fabricius) (Makhlouf et al., 2024).

The terpenes isothujone, β-cyclocitral, γ-isogeraniol, cis-geraniol, pulegone, β-citral, β-cyclohomocitral, geranial, α-ionone, β-ionone, dihydroactinolide, α-bisabolol, and phytan were also detected in our work (Table 1). Among these, β-ionone, followed by α-ionone and β-cyclocitral, had the highest relative abundances (Figure 3). These three terpenes are apocarotenoids synthesized by the enzymes carotenoid dioxygenases (Havaux, 2020) and have also been investigated in the context of pest management.

β-ionone is found in the odor of several carotenoid-rich vegetables, making it highly relevant to the food and cosmetics industries. In addition, it serves as a repellent for certain herbivores, a feeding deterrent for others, and an oviposition deterrent (Paparella, Shaltiel-Harpaza, & Ibdah, 2021). Cáceres et al. (2016) demonstrated that the repellent effect of *Arabidopsis thaliana* (L.) Heynh. on *Phyllotreta cruciferae* (Goeze), *Tetranychus urticae* (Kock), and *Bemisia tabaci* (Gennadius) was associated with an increased release of β-ionone. This compound also inhibits herbivory by *P. cruciferae* on *B. napus* (Gruber et al., 2009).

Exposure of *Solanum lycopersicum* L. leaves to α-ionone induced plant defenses, reducing oviposition and survival rates of *Frankliniella occidentalis* (Pergande) females (Murata, Kobayashi & Seo, 2020). Additionally, this volatile attracts male *Bactrocera latifrons* (Hendel) flies (Flath et al., 1994).

β-cyclocitral has also been described as a repellent for *Tetranychus urticae* (Nyalala, Petersen & Grout, 2013). Furthermore, β-cyclocitral was more abundant in grapevine leaves of resistant genotypes inoculated with *Plasmopara viticola* Berl. & de Toni compared to susceptible genotypes (Lazazzara et al., 2018). Based on this finding, these authors also tested the effect of the volatiles indirectly through the volume of air in leaf discs. Paper discs were used on the lid of Petri dishes, soaked in different solutions of volatiles without direct contact with the leaf discs. At concentrations of 0.05 and 0.005 mg/L in the air volume, *β*-cyclocitral reduced symptoms of the disease caused by *P. viticola*. This same study also identified that the PLV (*E*)-2-pentenal, also found in our study, is constitutively produced in greater abundance in a resistant grapevine genotype compared to a susceptible one. When applied to the air volume at concentrations of 0.5, 1.25, and 2.5 mg/L, the infected leaves also showed lower disease severity. Both (E)-2-pentenal and *β*-cyclocitral decreased the length and width of fungal sporangia.

Other terpenes found in this work are also relevant for pest management. For example, geranial repels *Sitophilus zeamais* Mots. (Reis et al., 2016). Geranial is one of the primary components of the essential oil from *Cymbopogon citratus* (DC.) Stapf, which exhibits toxic effects on the beetle species *Ulomoides dermestoides* (Fairmaire, 1893) (Plata-Rueda et al., 2020).

Some compounds identified in this study, such as 1-octen-3-ol and 5-methyl-3-heptanone, do not yet have a well-defined metabolic classification or elucidated biosynthetic pathway, despite being reported in previous research. Leaves of *Arabidopsis thaliana* (L.) Heynh. plants exposed to 10 µL of 1-octen-3-ol diluted in dichloromethane and vaporized showed higher activation of immune defense genes compared to the control dichloromethane alone 24 hours after the treatments (Kishimoto et al., 2007). Additionally, leaves treated with 1-octen-3-ol exhibited smaller lesions caused by the fungus *Botrytis cinerea* Pers. ex Fr. (Kishimoto et al., 2007). Furthermore, 5-methyl-3-heptanone has been shown to attract females of *Ceratitis capitata* (Wiedemann), a fruit fly of economic importance due to the damage it causes to fruit crops, as demonstrated in field tests (Casaña-Giner et al., 2001).

Green manure plants are widely used to improve soil chemical properties and contribute to the overall balance of agroecosystems (Rivera et al., 2020). Understanding the volatiles they emitted can provide valuable insights into their potential for pest control. Consequently, studying these volatiles adds another advantage to using green manure plants in agriculture.

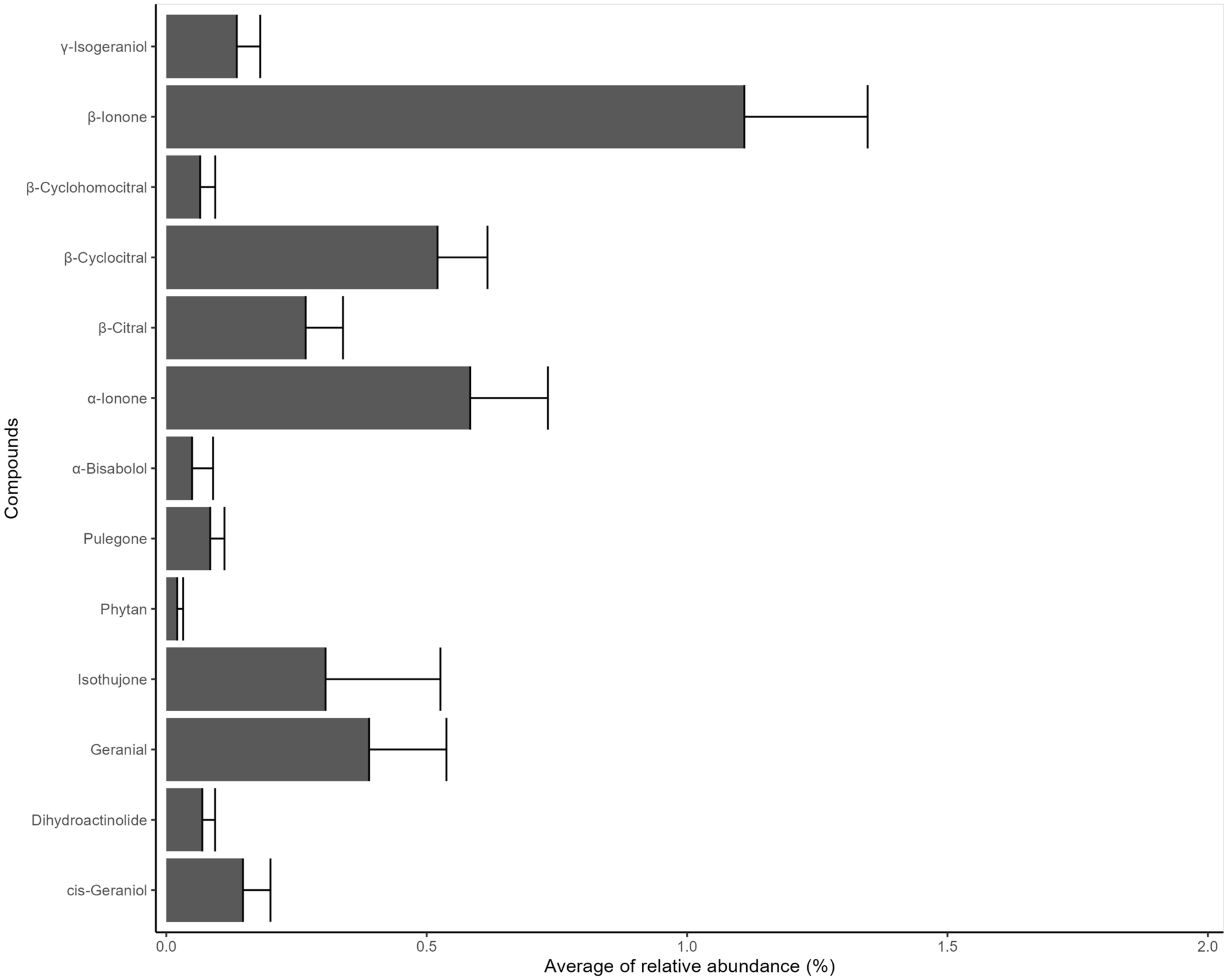

Figure 3: Relative abundances of terpenes identified in the VOC profile of jack bean leaves. Data means ± standard deviations (*n*=3).

Several studies have highlighted the importance of jack beans in intercropping systems. For example, the intercropping of *Zea mays* cultivars Emap11 and Pan5195 with jack beans not only increased grain yield but also reduced root necrosis caused by the nematode *Pratylenchus zeae* Graham in the Emap11 cultivar (Arim et al., 2006). These authors also reported a decrease in the nematode population in the roots of the Emap11 cultivar. Additionally, Agboka, Gounou and Tamo (2006) found that intercropping corn with jack beans significantly reduced the number of eggs and larvae of *Mussidia nigrivenella* Ragonot, a major pest of corn. Based on these findings, the authors recommended using jack beans as an attractant plant in a 'push and pull' strategy for pest management. Species of the genus *Crotalaria* are also used as green manure, and Prada, Stashenko and Martínez (2021). have provided evidence of the importance of their foliar volatiles in pest control. The volatiles emitted by the *Crotalaria* leaves following an attack by *U. ornatrix* modified the behavior of the caterpillars, making them prefer non-attacked leaves for oviposition.

In crops where the 'push and pull' strategy is used, volatiles emitted from the aerial part of the plant play a critical role in pest management (Munawar et al., 2023; Pickett et al., 2014). The VOC profile identified in jack bean plants presents a novel perspective for studies on this species within systems that rely on attractant and/or repellent strategies. Further research utilizing olfactometry and experimental plantings is essential to elucidate the impact of jack bean volatiles on pest management in various crops where it is used as green manure.

Conclusions

This research is the first to examine the leaf VOCs produced by jack bean leaves. The VOCs identified in this study could serve as a foundation for future research on the attractiveness or repellency of pests and phytopathogens, including insect vectors of pathogens, recruitment of biological control agents, as well as the management of agricultural pathogens. Such studies are essential for understanding the complex interactions among plants, herbivores, and other organisms, and for promoting ecologically sustainable crop protection.

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Author contribution

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