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Organic management vs. conventional management influence the antimicrobial activity of essential oils of *Origanum vulgare L*

O manejo orgânico versus convencional influencia a atividade antimicrobiana dos óleos essenciais de *Origanum vulgare* L

El manejo orgánico versus el manejo convencional influye en la actividad antimicrobiana de los aceites esenciales de *Origanum vulgare* L

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Abstract

The objective this stud was to evaluate the antimicrobial activity of essential oils (organic vs. conventional) of *Origanum vulgare* L. in the action against Candida albicans, Escherichia coli and Staphylococcus aureus. The inoculation of rhizobacteria (environmental (ME) and Bacillus subtilis) potentiated the antimicrobial action. The essential oils of Traditional (organic) cultivation presented antibacterial action and antifungal, while the essential oil of conventional cultivation (NPK) showed no any antibacterial or antifungal activity. It was concluded that the formulation of vermicomposting and the use of rhizobacteria are potential technologies and tools for family farmers and traditional community in the cultivation of orégano.

Keywords: Vermicomposting; *S. aureus; E. coli; C. albicans;* Agroecology; Natural antimicrobial.

Resumo

O objetivo deste trabalho foi avaliar a atividade antimicrobiana de óleos essenciais (orgânicos vs. convencionais) de *Origanum vulgare* L. em ação contra *Candida albicans, Escherichia coli* e *Staphylococcus aureus*. A inoculação de rizobactérias (ambientais (ME) e *Bacillus subtilis*) potencializou a ação antimicrobiana. Os óleos essenciais de cultivo tradicional (orgânico) apresentaram ação antibacteriana e antifúngica, enquanto o óleo essencial de cultivo convencional (NPK) não apresentou atividade antibacteriana ou antifúngica.

Concluiu-se que a formulação de vermicompostagem e o uso de rizobactérias são tecnologias e ferramentas potenciais para os agricultores familiares e de tradição no cultivo de orégano.

Palavras-chave: Vermicompostagem; *S. aureus; E. coli; C. albicans*; Agroecologia; Antimicrobiano natural.

Resumen

El objetivo de este trabajo fue evaluar la actividad antimicrobiana de los aceites esenciales (orgánicos vs. convencionales) de Origanum vulgare L. en acción contra Candida albicans, Escherichia coli y Staphylococcus aureus. La inoculación de rizobacterias (ambientales (ME) y Bacillus subtilis) potenció la acción antimicrobiana. Los aceites esenciales de cultivo tradicional (orgánico) mostraron acción antibacteriana y antifúngica, mientras que el aceite esencial convencional (NPK) no mostró actividad antibacteriana o antifúngica. Se concluyó que la formulación de vermicompostaje y el uso de rizobacterias son tecnologías y herramientas potenciales para los agricultores familiares y tradicionales en el cultivo de orégano.

Palabras clave: Vermicompostaje; *S. aureus; E. coli; C. albicans*; Agroecología; Antimicrobiano natural.

1. Introduction

Origanum vulgare L., also known as oregano or marjoram is a medicinal, spicy, generalized-flavored plant belonging to the Family Lamiaceae (Lukas et al., 2015). Because it originates from North Africa, the Mediterranean, the Euro-Siberian and other countries, it is an extremely variable species, presenting five subspecies according to the database (www.theplantlist.org), and a diversity of essential oil chemotypes (EOs) the most commercially important product (Fick et al., 2019; Sarrou et al., 2017; Sarikurkcu et al., 2015; Suzuki et al., 2015).

The genus *Origanum* is known for its expectorant, antispasmodic, carminative, antioxidant properties for gastrointestinal disorders (Kosakowska et al., 2019), antiseptic, anti-inflammatory and analgesic (Dhifi et al., 2016). In addition, it has currently been highly employed in the food and pharmaceutical sector, due to its antimicrobial action in the control of *Candida albicans* (Khan et al., 2019), *Escherichia coli* (Bhat et al., 2018; Nowotarska et al., 2017), *Staphylococcus aureus* (Cui et al., 2019) *Bordetella bronchiseptica*, *Saccharomyces cerevisiae* (Fikry et al., 2019). These activities are attributed to the major constituents present in the EOs, such as carvacrol, terpinen -4 –ol, thimol, geraniol, among others.

However, due to chemotypes, the genus Origanum presents a range of major constituents present in the EOs, depending on the origin and cultivation. For studies conducted in Argentina, the compounds o-cymene (14.3%), terpinen-4-ol (12.5%), (E) -b-terpineol (10.4%), thymol (10.1%), y- terpinene (9.1%) and carvacrol (5.6%) (Camiletti et al., 2016). In Brazil, Suzuki et al., (2015), identified the following constituents: y-terpinene (30.5%), carvacrol (15.7%), terpinen- 4-ol (13.0%), geraniol (7.1%) and cis - ocimene (7.0%), while in Túrquia, had a predominance of thymol (58.3%), carvacrol (16.1%), p-cymene (13.5%) and y-terpinene (4.5%) (Sarikurkeu et al., 2015).

This variation in chemical composition occurs because medicinal species may undergo changes due to edaphoclimatic characteristics, such as seasonality, climate, soil type, altitude, nutritional management, harvest time, biotic, abiotic and genetic factors (Luz et al., 2014; Paulus et al., 2013). In addition to these factors, currently a wide variety of elicitor agents, rhizobacteria (PGPR), humic substances, algae extracts, among others, (Bhattacharyya et al., 2012, Olivares et al., 2017; Canellas et al., 2015), has been applied in the cultivation of medicinal species, because they influence ontogeny, secondary metabolite production, biosynthesis, efficiency and absorption of nutrients by species (Pereira et al., 2019).

Thus, the difference in relation to field cultivation, either by the conventional system through the application of synthetic fertilizers or agroecological management, using organic vermicompost or compound, can directly influence the yield, content and phytochemical composition of essential oils. Studies by Esmaielpour et al., (2017) for *Ocimum basilicum L.*, by Ganjali et al., (2017) in the cultivation of *Rosmarinus officinalis L.*, Ayyobi et al., (2014) in the cultivation of *Mentha piperita L.* and Shirin Nikou et al., (2019) for *Origanum vulgare L.*, observed greater efficiency in the application of vermicompost in relation to synthetic fertilizer (NPK) or control (soil).

In addition to the factors mentioned above, worldwide there is a concern about the environment, the production of herbal medicines, among other products used by the food and agricultural industry, which are free of pesticides and synthetic fertilizers (Basak et al., 2020; Tripathy et al. 2015). Thus, the objective of this study was to evaluate the antimicrobial and microbicidal activity of essential oils (organic vs. conventional) of *Origanum vulgare L.* in the action against *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* as a function of the major constituents of phytocomplex.

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2. Material and Methods

2.1 Field conduction and inoculum preparation

The assay was conducted in the field, in the experimental area of the Plant Tissue Culture Laboratory of the Federal University of Lavras (UFLA). The oregano exsicata is deposited in the herbarium ESAL/UFLA, under registration no. 22,156. The seedlings of *Origanum vulgare L.* were obtained from apical cuttings from matrix plants of the Medicinal Garden of UFLA in the Department of Medicinal, Aromatic and Condiment plants, and rooted in expanded polypropylene trays of 128 cells.

After 30 days, the seedlings were transplanted to the field, in 1.8m² beds with 20cm height, for each experimental plot (block), according to the experimental design in randomized blocks, with three replications and eighteen plants per plot. The different treatments used in the experiment are described in Table 1.

Table 1. Treatments used in the experiment.

Treatment	Code	Method
Soil (Testimony)	T1	Testimony without fertilization
Pure vermicompost	T2	Organic
Environmental vermicomposto (EM)	T3	Organic
Commercial vermicompost	T4	Organic
Pure vermicompost + immobilized *Bacillus subtilis*	T3	Organic
Pure vermicompost + suspended *Bacillus subtilis*	T6	Organic
Synthetic fertilizer (NPK)	T7	Conventional

Source: Author, (2020).

The microorganisms used in this study were *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922) and Candida albicans (ATCC 10231). The inoculums were prepared in suspension form and standardized at 1.5x108 CFU/mL by reading absorbance in spectrophotometer (660 nm) and 75% transmittance.

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2.2 Formulation of vermicompost, Inoculation of rhizobacteria and Chemical characteristics of the planting substrate

Vermicomposting was produced in the Biodiesel Sector - UFLA, through the pre-composting of organic waste from the university restaurant - UR and plant residue from the landscaping of the campus, without prior treatment, packed in masonry blocks with a volume of 1m³ (each cell).

In the pre-composting phase, three cells were used, one was conducted without inoculation, and the other two were inoculated with environmental microorganism (EM) captured in the native forest of the UFLA campus at six points, according to the methodology proposed by Coutinho (2011) and the other cell inoculated with commercial microorganisms – (Korin®), prepared according to the manufacturer's recommendations. After 90 days, with the material still in the pre-maturation phase, cattle manure and californian red earthworm (*Eisenia fetida*) were added in each cell, remaining for another 60 days for biotransformation and full maturation of vermicompost.

After biotransformation of the materials in vermicompost, they were packed in microfiber bags, respecting the inoculations (pre-composting) and sent to the planting area. In the field, pure vermicompost (T1) was separated for fractionation and inoculation of rhizobacteria. While the others, environmental vermicompost (T3) and commercial vermicompost (T4), were applied to the flowerbeds.

The previously reserved pure vermicompost was divided into three parts, being applied in the field respecting the treatments according to the experimental design. The first part was applied pure (without inoculation) and in the other two parts the *Bacillus subtilis* population of 10^9 x plant was inoculated, in the immobilized form (inert in alginate capsules) and suspended, applying 10ml/ plant (liquid in agar solution). The treatments were then made: vermicompost (pure) (T2), vermicompost + *Bacillus subtilis* immobilized (T5) and vermicompost +*Bacillus subtilis* suspended (T6).

2.3 Extraction of essential oil

The oregano stems were cut at 5 cm from the soil, at 95 days of field cultivation, when the first plants started the flowering phase. The material was fractionated, allocated in kraft paper bags and taken to a forced air circulation oven at 30ºC to constant weight.

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For the extractions of essential oils (EOs) the dry aerial part was erased, weighed and packed in a 5L volumetric balloon, submerged in deionized water, where they were hydrodistilled for 3 hours, using a Clevenger type device. After extraction, the EO was purified and fractionated for chemical composition, antimicrobial activity and microbicide analyses.

2.4 Gas chromatography with mass spectrometry detector

The chemical composition was obtained through the gas chromatograph coupled to mass spectrometer (GC-MS) to identify the compounds present in the essential oil, using the QP2010 (Shimadzu) equipment, equipped with fused silica capillary column (30 m long and 0.25mm internal diameter), with RTX stationary phase®-5MS (0.25 μm film thickness) and helium, with drag gas with flow of 1.0 mL/minute.

The mass spectra were obtained by impact of electrons at 70 eV, with a scan of 29 to 400 (m/z), and 1 μL of the prepared oil solution was injected at a concentration of 10 mg. L^{-1} with split ratio of 1:20. and the comparison of mass spectra to the NIST database (spectroteca) was performed.

2.5 Determination of retention indexes with arithmetic indexes (AI)

To verify the arithmetic indexes (AI), a mixture of linear alkanes (C9 -C17) was injected into the chromatograph. Then, the spectra of spectroteca and retention indexes were compared with arithmetic indexes (AI) calculated according to equation 1. The relative percentage of each compound was calculated by the ratio between the area of each peak and the total area of all sample constituents. To assist in the identification and characterization of volatile compounds, the values of the calculated retention indexes were compared with values found in the literature (Adans, 2017).

$$AI_{(x)} = 100_{Pz}+100\ [\ \underline{\{RT_{(x)} - RT_{(Pz)}\}}$$

Where: X: compound of interest; Pz: number of hydrocarbon carbon atoms with retention time prior to the retention time of compound X; $RT_{(x)}$: retention time of compound X; $RT_{(Pz)}$:

is the retention time of the Pz compound; $RT_{(Pz+1)}$: hydrocarbon retention time with retention time after the retention time of compound X.

2.6 Antimicrobial activity and microbicide

The determination of the minimum inhibitory and microbicidal concentrations of oregano essential oils on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* were based on CLSI standards (2008, 2015) for yeasts and bacteria, with some modifications. The test was performed in Muller Hinton (HIMEDIA, India) for activity against *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 25922) and in RPMI -1640 medium (Sigma Aldrich) for activity against *Candida albicans* (ATCC 10231).

The essential oil samples were dissolved in 1mL of dimethylsulfoxide (DMSO) and placed in the plates of 96 wells under serial dilutions (1000 - 10.9375 µg/mL). Aliquots of 20 µL of the inoculum in suspension were added to the corresponding wells in the microplate, after serial dilution of the standard essential oil and antimicrobial.

The standard antimicrobials used were amoxicillin and streptomycin (10 – 0.078µg/mL) for *Staphylococcus aureus* and *Escherichia coli* respectively and fluconazole (40-0.312 µg/mL) for *Candida albicans*. Then the microplates were incubated at 37°C for 24 h. An aqueous solution of resazurin at 0.2%, added at 22h, was used as a developer to determine the viability of the inoculum, indicated by the change of color from blue to pink (SARKER et al., 2007).

The value of the minimum inhibitory concentration (MIC) was determined as the lowest concentration without variation in the color of the developer. To determine the minimum microbicidal concentration (MMC), a 15µL aliquot was removed from the wells that showed no color change and applied to the surface of a Petri dish containing Muller Hinton agar (HIMEDIA, India) for bacteria and Sabouraud agar (HIMEDIA, India) for yeast and the plates were incubated at 37°C for 24 h.

Microbial growth in the plates indicates that the essential oil did not exert a microbicidal action. The experiment was carried out in triplicate. The degree of antimicrobial activity was determined based on the criteria of Holetz et al., (2002), which establishes antimicrobial action as strong (≤ 100 µg/ml), 100 µg/ml), moderate (100 –500 µg/ml), weak (500 -1000 µg/ml) and inactive (≥ 1000 µg/ml).

3 Results and Discussion

3.1 Major constituents of oregano essential oils

Due to the complexity of the composition of the essential oil found in plants, several studies demonstrate its potential against various microorganisms, fungi, bacteria, nematodes, etc., either by the activity of intact essential oil or by its isolated constituents. Thus, for the present study with organic and conventional essential oils, there are differences in the concentration of the major constituents identified by GC-MS (Table 1).

The major constituents identified with their variations in area percentage (Figure 1 and Table 2) were sabinene (3.02 – 3.37%), terpinene (2.52 – 4.49%), o-cymene (5.10 – 10.54%), gamma-terpinene (8.02 -13.42%), 4-thujanol (1.59 – 2.55%), terpinen-4-ol (13.21 - 16.77%) and carvacrol (10.65 - 17.29%). Evaluations of the populations of *Origanum vulgare L.* around the world also identified these and other compounds, such as the majority (Laothaweerungsawat et al., 2020; Morshedloo et al., 2017; Mastro et al., 2015).

The chemical constituent marker of this variety of *Origanum vulgare L* is carvacrol, as identified in this research and reaffirmed by Shiwakoti et al., (2016); Lukas et al., (2015). In a study conducted in Brazil, Pradebon Brondani et al. (2018) and Suzuki et al., (2015), identified a similar phytochemical profile, whose main constituents were c-Terpinene (30.5%), carvacrol (15.7%), terpinen- 4-ol (13.0%), geraniol (7.1%) and schomimene (7.0%).

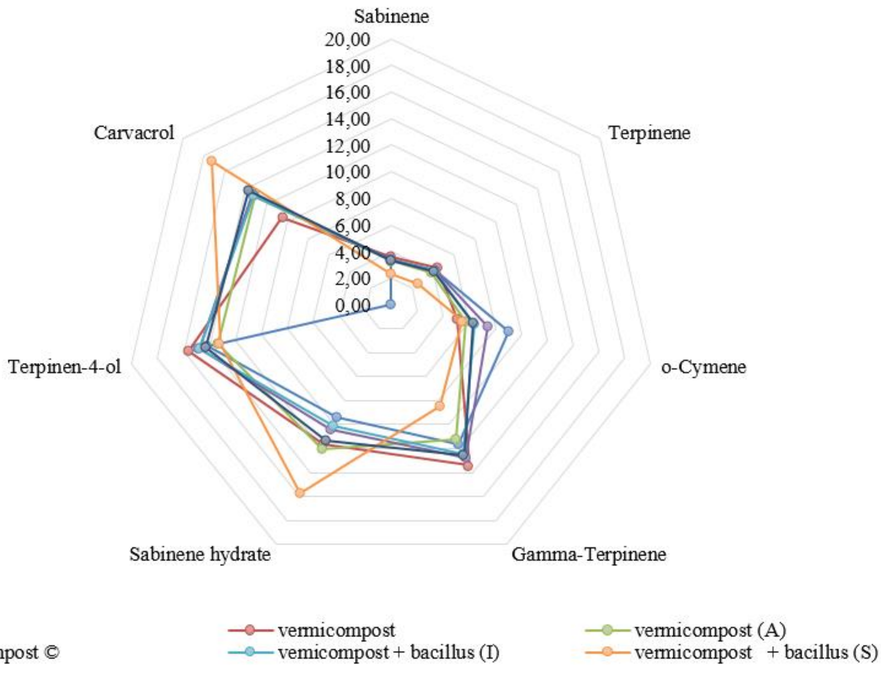
Table 2. Majority constituents (area%) hydrodistilled essential oils of dried herbs of Origanum vulgare L. submitted to different nutritional managements (organic and conventional).

Components	IR *	IR**	CAS	Treatments (area %)						
				T1	T2	T3	T4	T5	T6	T7
Sabinene	971	969	3387-41-5	3,37	3,59	3,31	3,41	3,36	2,33	3,37
Terpinene	1015	1014	99-86-5	4,22	4,49	3,83	4,19	4,12	2,52	4,07
o-Cymene ou p-cymene	1022	1022	527-84-4	9,05	5,10	5,74	7,39	6,37	5,44	6,25
Gamma-Terpinene	1056	1054	99-85-4	11,69	13,42	11,21	12,77	12,44	8,45	12,59

Sabinene hydrate -trans	1098	1065	17699-16-0	9,41	11,69	12,01	10,41	10,06	15,76	11,34
Terpinen-4-ol	1175	1174	562-74-3	14,15	15,66	13,36	14,83	14,81	13,21	14,27
Carvacrol	1295	1298	499-75-2	-	10,50	13,10	13,32	13,24	17,29	13,76

IR* Calculated arithmetic index; IR** Arithmetic index of Adans; CAS: record number of the chemical compound; T1: soil; T2: vermicompost; T3: vermicompost (A); T4: vermicompost ©; T5: vermicompost + bacillus (I); T6: vermicompost + bacillus (S); T7: NPK. Source: Authors, ;(2020).

Figure 1. Major constituents of the essential oils of *Origanum vulgare L.* submitted to different nutritional managements (agroecological and conventional).



Source: Authors, (2020).

When the concentrations of the constituents are evaluated in relation to the characteristics of the treatments (control, conventional and organic) there are specific differences in relation to the major constituents, which are of great importance for the chemical, pharmaceutical and agrochemical industry (Hasenclever, et al., 2017). In this context, when comparing conventional and organic management, higher levels of active constituents were identified for the following treatments: carvacrol (17.29%) and sabinene hydrate (15.76%) for pure vermicompost + immobilized *bacillus subtilis* (T6), terpinen-4-ol

(15.66%) and gamma – terminene (13.42%), for pure vermicompost (T2), o-cymene/p-cymene (9.05%) (control) (T1).

The application of vermicompost and rhizobacteria in the cultivation and management of *Origanum vulgare L.* (oregano) was efficient in the production of the major constituents of the essential oil (EO). The highest concentrations of the compounds were identified in the organic treatments when the application of conventional synthetic fertilizer (NPK) was compared. The results obtained in this study correspond to those obtained by Esmaielpour et al.,(2017); Ganjali et al., (2017); Ayyobi et al.,(2014); Shirin Nikou et al., (2019), in which the application of vermicompost associated or not with rhizobacteria in the cultivation of medicinal plants were more efficient than the application of synthetic fertilizer NPK.

It is known that EO is influenced by edaphoclimatic characteristics, such as climate, soil type, altitude, nutritional management, seasonality, biotic and abiotic factors (Luz et al., 2014; Paulus et al., 2013). Thus, the differences in the concentration of the major compounds identified in this study are related to the characteristics of the EOs obtained through the different crops and field managements.

According to Prabha et al., (2008), vermicomposting is rich in macro and micronutrients, humic substances, phytohormones and growth-promoting microorganisms in plants. The activity of the earthworm in biodegradation increases the population of beneficial microorganisms in the soil (Pathma and Sakthivel, 2012, 2013), producing plant hormones in their secretions, whose nutrients are molded the forms readily available to plants, which represents beneficial factors for plant production (Ayyobi et al. 2014; Chatterjee et al.2014).

And when associated with the use of elicitor agents, such as growth-promoting rhizobacteria in plants, such as (*Bacillus subtilis, environmental and commercial microorganisms*) applied in this field study (planting and management), they can influence ontogeny, production of secondary metabolites, biosynthesis, efficiency and absorption of nutrients by plants, resulting in changes in the concentrations of the phytochemical profile of the EOs (Pereira et al., 2019; Chavarria et al., 2018; Trinh et al., 2018; Silva et al., 2017; Leite et al., 2016). While studies conducted from the application of synthetic fertilizers (NPK) report that fertilizer results in rapid growth of shoot biomass and may influence the reduction of the concentration of bioactive constituents (dilution). This factor can affect not only the content and yield of the EO itself, but also modify the phytochemical profile of the species (Kazimierczak et al., 2015; Luz et al., 2014; Ayyobi et al., 2014).

In this sense, from the results obtained in this research, it is possible to infer not only that organic managements have great potential in the production of the essential oil of rich

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oregano, in major constituents of pharmacological, industrial and agrochemical interest; but also that the application of synthetic fertilizer (NPK) can be replaced by organic (agroecological) formulations, corroborating the research conducted by Basak et al., (2020); Tripathy et al., (2015); Hossaini et al., (2016).

3.2 Antimicrobial activity and microbicide

The antimicrobial activity of the essential oils of *Origanum vulgare L.* (oregano) against the bacteria *Staphylococcus aureus* and *Escherichia coli*, and the fungus *Candida albicans* were evaluated by the minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) (Table 3).

Candida albicans was the microorganism most susceptible to antimicrobial action of oregano essential oils, regardless of treatment, presenting moderate antifungal action according to the criteria of Holetz et al (2002). For bacteria, the oil obtained through the application of vermicompost + *Bacillus subtilis* (S) – (T6) was the best result, with moderate antimicrobial action (250-500 µg/mL) for both species. This action may be due to the higher concentration of carvacrol (17.29%), usually the majority constituent of the studied species, as found in studies conducted by Nowotarska et al., (2017).

These results are in accordance with the work of Khan et al., (2019) in which the antimicrobial action of EO of oregano collected in Jordan and Saudi Arabia against *Escherichia coli* was tested, where total inhibition occurred at a concentration of 200 and 300 µg/mL, respectively. However, the EOs for soil (control) (T1) and organic EOs, such as the application of environmental vermicompost (A) (T3), commercial vermicompost (C) (T4) and vermicompost + *Bacillus subtilis* (I) (T5), showed action while the EO obtained through the application of pure vermicompost (T2) had no antibacterial activity.

Table 3. Antimicrobial activity of *Origanum vulgare L.* essential oils on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* in µg/mL.

Treatment	*Staphylococcus aureus*		*Escherichia coli*		*Candida albicans*	
	MIC	MMC	MIC	MMC	MIC	MMC
T1	500-1000	>1000	500-1000	>1000	175-250	500-1000
T2	>1000	>1000	>1000	>1000	175-250	>1000

T3	500-1000	>1000	500-1000	>1000	175-250	>1000
T4	500-1000	>1000	500-1000	>1000	175-250	>1000
T5	500-1000	>1000	500-1000	>1000	175-250	>1000
T6	250-500	>1000	250-500	>1000	175-250	>1000
T7	>1000	>1000	>1000	>1000	>1000	>1000
Amoxicillin	0.078-0.156	5-10	--	--	--	--
Streptomycin	--	--	0.156 -0.312	5-10	--	--
Fluconazole	--	--	--	--	3.125-6.25	20-40

MIC: minimum inhibitory concentration; MMC: minimum microbicidal concentration; T1: soil; T2: vermicompost; T3: vermicompost (A); T4: vermicompost ©; T5: vermicompost + Bacillus (I); T6: vermicompost + Bacillus (S); T7: NPK. Source: Authors, (2020).

When the conventional EO obtained from the application of synthetic fertilizer NPK (T7) was evaluated, it was verified that it did not present antimicrobial activity for any of the microorganisms used in the experiment. While the microbicidal action occurred only for *C. albicans* in the tests using the EO obtained through the control treatment (soil) (T1).

The results of this study for *C. albicans* are corroborated by other studies (Bhat et al., 2018; Cleff et al., 2010; Manohar et al., 2001). At different concentrations oregano EO was able to significantly reduce the production of the phospholipase enzyme produced by *C. albicans* strains isolated from the oral mucosa of patients with stomatitis (Brondani et al., 2018). Other C*andida* species were also susceptible to antifungal activity of the essential oil of oregano (Cleff et al., 2010; Chami et al., 2004), as well as in the present work.

Essential oils, a product of the secondary metabolism of many plants, contain distinct components. Thus, the antimicrobial effect depends on the variety and concentration of the compounds, either by the activity of the intact essential oil or individually by the substances present in its composition. Bakkali et al. (2008) state that the antimicrobial action of EO is mainly attributed to their major compounds.

Chromatographic analyses of oregano EO revealed the presence of terpinen-4-ol, carvacrol, γ-terpinene and sabinene hydrate among its main constituents (Table 1), varying its concentration as a function of cultivation (control, conventional and agroecological). The differences in the minimum inhibitory concentrations in each treatment in this study are due to the different chemical compositions of the EO obtained in the different nutritional managements performed in the field (in planting and management until harvest).

In this sense, one of the most studied major compounds of oregano (EO) is carvacrol, a substance well known for its antimicrobial action. An experiment with *Escherichia coli* O157: H7 demonstrated that carvacrol and thymol were able to decrease the intracellular ATP pool and increase extracellular ATP, indicating a disruptive action on the bacterial cytoplasmic membrane (Helander et al., 1998; Li et al., 2017; Nowotarska et al., 2017).

Monoterpene, p-cymeno and γ-terpinene hydrocarbons, often found in oregano EO, including those used in this study, did not present an inhibitory effect against *Escherichia coli* O157: H7 when tested in isolation (Burt et al., 2005). However, when combined with carvacrol, synergism occurred against the bacterium *Bacillus cereus* in *in vitro* test and also in rice (Ultee et al., 2000). Both oregano EO and carvacrol, present among the major compounds of all EOs used in this study, have already had their antimicrobial action proven in several previous studies (Ebani et al., 2020; Lu et al., 2018; Mayai et al., 2019; Dos Santos Rodrigues et al., 2018; dos Santos Rodrigues et al., 2017; da Silva Santos et al., 2017; Souza et al., 2016; Burt et al., 2005).

In this context, when observing the compound found in higher concentration in five of the seven oils applied in this study (Figure 1) terpinen-4-ol, it is verified that the antimicrobial action of this substance against a variety of microorganisms, such as *Bordetella bronchiseptica*, *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *S. aureus*, *Escherichia coli* and *Candida albicans* is corroborated through the results found by other authors (Fikry et al. , 2019; Hammer et al., 2012; Mondello et al., 2006). The mechanism of action of terpinen-4-ol may be related to damage to the plasma membrane and loss of cytoplasmic material (Carson et al., 2002). In addition to antimicrobials, terpinen-4-ol and carvacrol have already demonstrated a larvicidal potential against *Anopheles stephensi*, *A. subpictus Culex quinquefasciatus* and *C. tritaeniorhynchus* and nematicida (Govindarajan et al., 2016)

The wide variety of substances present in EO prevents their antimicrobial activity from being attributed to a single mechanism of action (BURT, 2004). Because they are hydrophobic in nature, essential oils have the ability to penetrate microbial cells causing a series of changes in their structure and also functionality (Nazzaro et al., 2013). However, the antimicrobial potential of the EO has been increasingly explored, both for medicinal use in humans and animals and for the food industry with the aim of replacing traditional preservatives (Souza et al., 2018; Busatta et al., 2007) by EOs. Thus, the advance in the production of organic EO is of paramount importance for the advancement of these sectors, considering that the population in general has demanded more and more natural products, exempt from the application of synthetic fertilizers.

According to Basak et al., (2020), historically many researchers claim there is no difference in the chemical composition between organic and conventional foods (Dangour et al., (2010) and Rosen, (2010), however there is controversy regarding this statement. Since the results obtained in this work demonstrate that organic EOs of oregano present antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, and especially antifungal against *Candida albicans*, which can be attributed mainly to the presence of terpinen-4-ol and carvacrol in its chemical composition. While the EO obtained from conventional cultivation (NPK) did not present antimicrobial action for this study.

As previously mentioned, for the characterization of chemical constituents, the positive effects of organic essential oils are related to several biotic and abiotic factors that allowed a differential of EO in relation to conventional management (Basak et al., (2020); Tripathy et al., (2015); Luz et al., 2014; Ayyobi et al. 2014; Chatterjee et al.2014; Paulus et al., 2013). However, although production systems directly influence the composition of essential oil, more research is needed on the antimicrobial and microbicidal action of organic essential oils, in order to prove efficacy and quality, as well as to emphasize the importance in the transition of the cultivation of medicinal plants from the conventional to the organic (agroecological) system.

The use of synthetic fertilizers (NPK) are allowed in the cultivation of medicinal plants, but, environmentally, the continuous use increases the amount to be applied, the producer becomes dependent on the industry, as well as leads to the exhaustion of soils and natural resources. Continuous use is not due to the quality of the final product itself (dry plant or essential oil), but is associated with the model of food and product production, established by hegemonic technological packages (agribusiness/multinationals), from the green revolution, and replicated by small producers. That historically were naturalized to a single possible model of agriculture, either due to the ease of acquisition and/or available resources, imposition of the market (Ayyobi et al., 2014).

In this context, there is currently a growing demand for medicinal plants, essential oils, cosmetics, herbal medicines, among other products used by the food, pharmaceutical and agricultural industry, free of pesticides and synthetic fertilizers. Whether by improving quality of life, product quality, as well as reducing the negative effects caused to the environment (Basak et al., 2020; Kazimierczak et al. 2015; Tripathy et al. 2015). In addition to the fact that

RDC No. 26/2014 approved periodic inspection of chemical residues in the commercialization of medicinal plant products, in force since 2018 in Brazil, being a major concern in relation to the export market of herbal medicines.

4 Conclusion

• The essential oils of organic culture presented antibacterial action for *Staphylococcus aureus* and *Escherichia coli*, and antifungal against *Candida albicans*;

• The essential oil of conventional culture showed neither antibacterial nor antifungal activity;

• Vermicomposting can be widely applied in the cultivation of *Origanum vulgare L.*

• The inoculation of rhizobacteria (environmental (ME) and *Bacillus subtilis*) enhanced the antimicrobial and microbicidal action of organic essential oils;

• The production of organic (agroecological) essential oils, through the formulation of vermicomposting, are potential technologies and tools for family farmers.

• Carvacrol is the major constituent and have contributed to antimicrobial activity;

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