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Evaluation of the efficiency of allspice, thyme and rosemary essential oils on two foodborne pathogens in *in-vitro* and on alfalfa seeds, and their effect on sensory characteristics of the sprouts

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ABSTRACT

Seeds are usual source of contamination and their sprouts are commonly associated foodborne illness. Therefore, the aim of this study was to evaluate the antibacterial vapor phase efficiency of allspice, thyme and rosemary essential oils on two foodborne pathogens in *in vitro* and on alfalfa seeds, including the chemical profile of the tested EOs and their effect on the sensory characteristics of the sprouts. Antibacterial activity was determined through the minimal inhibitory concentration (MIC) of EOs in vapor phase to inhibit the growth of *Listeria monocytogenes* and *Salmonella* Typhimurium in culture media and on alfalfa seeds. Also, the germination and the effect on sensory characteristics of the sprouts were determined. Thyme EO was the most effective of the tested EOs on culture media and on alfalfa seeds, against both bacteria. When rosemary EO was tested against *L. monocytogenes* in alfalfa seeds, the MIC (4.0 mL/L_{air}) was higher, compared to the one obtained in culture media (2.7 mL/L_{air}). But when this EO was tested against *S.* Typhimurium, the MIC in alfalfa seeds was lower than in culture media (11.7 *vs* 13.3 mL/L_{air}). Allspice EO resulted more effective against both bacteria in alfalfa seeds (6.0 mL/L_{air} for *L. monocytogenes* and 6.7 mL/L_{air} for *S.* Typhimurium), compared to culture media (12.0 mL/L_{air} for *L. monocytogenes* and 13.3 mL/L_{air} for *S.* Typhimurium). Vapor phase EOs MICs resulted in significant ($p \leq 0.05$) decreases of *L. monocytogenes* and *S.* Typhimurium counts compared to the control. There also was a significant ($p \leq 0.05$) difference between systems (*in vitro* or on alfalfa seeds) despite the microorganism or the evaluated EO. Treatment alfalfa seed with vapor phase EOs, did not affect the seed germination. Sensory acceptability of the sprouts, obtained of treated seeds, did not were significant ($p \geq 0.05$) different of the sprouts obtained from the non-treated seeds.

1. Introduction

Bacteria are challenging microorganisms to control in the food industry and are the main cause of foodborne illnesses (USDA, 2012). Sprouts are grown from a great variety of seeds, including alfalfa, which are usually consumed raw in sandwiches or salads, and are known to be a common vehicle for bacterial foodborne illnesses (FDA, 2017). Generally, seeds could contain a microbial load of 6.0 log CFU/g, while sprouts could have counts > 3 logs. In addition, seeds are the most common source of contamination for sprout-associated foodborne illness. Other indirect contamination sources for sprouts could be irrigation water, pre-harvest contamination through fertilizers/manure, or soil quality (Yang et al., 2013). Some contamination sources for seeds, could be irrigation water, pre-harvest contamination through fertilizers/manure, and soil quality (Yang et al., 2013).

The Food and Drug Administration (FDA) mentions that there are 46 reported outbreaks of foodborne illnesses related to sprouts in the United States over 20 years (between 1996 and 2016); detecting microorganisms such as *Salmonella*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7. The prevalence of *Salmonella* in seeds is reported to be higher than in the final product (sprouts) while the prevalence of *L. monocytogenes* in seeds and in sprouts was similar (FDA, 2017).

There has been a worldwide increase in consumer demand for freshly produced fruit and vegetables mainly because they are associated with health benefits. Similarly, the demand for more “natural” products has increased over the years; thus, there is a need to find natural sources of food preservatives, such as herbs and spices, and their extracts and/or essential oils (Hyldgaard et al., 2012; Kim et al., 2012) that may reduce the risk of foodborne illnesses.

Essential oils (EOs) are complex mixtures of volatile and aromatic

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compounds, extracted from different parts of plants (Burt, 2004), including EOs extracted from allspice (*Pimenta dioica*), thyme (*Thymus vulgaris*), and rosemary (*Rosmarinus officinalis*). These EOs have shown antimicrobial activity (in liquid or vapor phase) against different microbial strains, such as *L. monocytogenes*, *Staphylococcus aureus*, *St. epidermidis*, *Micrococcus luteus*, *Bacillus cereus*, *E. coli*, *Pseudomonas aeruginosa*, *Enterococcus feacalis*, *Vibrio alginolyticus*, *Salmonella* Typhimurium, *Rhizoctonia solani*, *Macrophomia phaseolina*, *Salmonella* Senftenberg, *S.* Give, *Aspergillus ochraceus*, *A. parasiticus*, and *A. niger* (Boskovic et al., 2016; Du et al., 2009; Han et al., 2014; Khaledi et al., 2015; Kim et al., 2016; Mattos De Oliveira et al., 2013; Miladi et al., 2013). However, when EOs are applied in liquid phase (directly), they produce a significant impact on the sensory attributes of the food because of their strong aroma and flavor. Unlike liquid phase, vapor phase application (indirectly) of EOs, requires lower concentrations for their use as antimicrobials. Therefore, vapor phase application could be a solution to the adverse effects of the intense aroma and flavor of EOs in foods (Lee et al., 2018).

There are few studies that mention the use of allspice, thyme, and rosemary EOs as antimicrobials against *Salmonella* Typhimurium and *L. monocytogenes* in vapor phase, and even fewer assessing their antibacterial activity in alfalfa seeds. Thus, the aim of this research was to evaluate the antibacterial vapor phase efficiency of allspice, thyme and rosemary essential oils on two foodborne pathogens *in vitro* and on alfalfa seeds, including a chemical profile of the tested EOs and their effect on the sensory characteristics of the sprouts.

2. Materials and methods

2.1. Bacterial strains

Bacterial strains (*Salmonella enterica* serovar Typhimurium ATCC 14028 and *Listeria monocytogenes* Scott A) were obtained from the Food Microbiology Laboratory strain collection of Universidad de las Americas Puebla (UDLAP, Mexico, Puebla), and were maintained on Tryptic Soy Agar (TSA; Difco, BD, Sparks, MD) slants at 5 °C.

Cultures were prepared by inoculating the bacteria (*S.* Typhimurium and *L. monocytogenes*) into 10 mL of Tryptic Soy Broth (TSB; Difco, BD, Sparks, MD) and incubated at 35 °C for 24 h. Inoculum cell concentration was adjusted to 10^8 or 10^5 CFU/mL for subsequent use in the culture mediums and seeds respectively (Reyes-Jurado et al., 2016).

2.2. Essential oils, components and plant materials

Allspice (*Pimenta dioica*) EO was obtained from Liquid Gold® (Evansville, IN) while thyme and rosemary EOs were purchased from Hersol® laboratories (San Mateo Atenco, Estado de México, Mexico). Alfalfa seeds were purchased from Hortaflor® (Rancho Los Molinos, Tepoztlán, Morelos, Mexico).

2.3. Gas chromatography/mass spectrometry (GC/MS) analysis

The studied EOs were analyzed by a gas chromatographer with a 6850 Series Network (Agilent Technologies, Santa Clara, CA), equipped with a mass selective detector (5975C VL) and with a triple-axis detector (Agilent Technologies). Separation of the components was achieved by an HP-5MS (5% phenyl – 95% polydimethylsiloxane) capillary column (30 m by 0.35 mm, 0.25 μm film thickness). The carrier gas was helium at a constant flow mode of 1.5 mL/min. The temperature of the column was initially around 60 °C for 10 min, increasing every 5 min until reaching 240 °C, and maintained at 240 °C for 50 min. The injector temperature was 240 °C. Retention indices were calculated by a homologous series of n-alkanes C_8 to C_{18} (Sigma, St. Louis, MO). Compounds were identified by comparing mass spectra obtained with the reported in the US NIST (National Institute of Standard Technology) Library, and Shimadzu retention index (RI) isothermal equation (Reyes-Jurado et al., 2016).

2.4. Vapor phase antibacterial activity in vitro

2.4.1. Inverted Petri dish method

Plates prepared with TSA were platted with 50 μL of inoculum of each bacteria, using a spiral plater Autoplate 4000 (Spiral Biotech, Norwood, MA).

Minimum inhibitory concentration (MIC) refers to the minimum concentration necessary to inhibit the visible growth of the studied microorganism (López-Malo et al., 2005.). This value was considered to evaluate the antibacterial activity using the inverted Petri dish technique. This method consists in placing a sterile paper disc (Whatman No. 1, diameter 55 mm) on the lid of the Petri dish and impregnating it with a known volume of EOs. The volumes tested varied from 5 to 1900 μL, depending on the tested EO and the studied bacteria. The previously inoculated culture medium with the paper disc on the lid was immediately inverted, sealed with Parafilm® and incubated at 35 °C for 24 h (Miladi et al., 2013). Quantification of colony forming units (CFU/mL) was performed when growth was observed, using a Q-Count counter and corresponding software (Spiral Biotech, Norwood, MA). The obtained MICs were expressed as mL of EO per L of air. Tests were performed by triplicate.

2.5. Vapor phase antibacterial activity in vivo

2.5.1. Seed disinfection

Alfalfa seeds were disinfected by submersing them in a 70% ethanol solution for 1 min, followed by dipping in 0.75% NaClO solution for 3 min, and then rinsed four times with sterilized distilled water. Seeds were dried overnight using Whatman filter paper sheets at 35 °C in a laminar flow hood (Kotana et al., 2013).

2.5.2. Seed inoculation

For seed inoculation, 1 g of disinfected seeds (approximately 420 seeds) and 1 mL of the adjusted inoculum were transferred to a tube with 8 mL of TSB, and incubated with shaking (BT25, Yamato Scientific Co., Japan) at 35 °C for 1 h. Inoculated seeds were then drained and dried overnight using Whatman No, 1 filter paper sheets at 35 °C in a laminar flow hood (Kotana et al., 2013).

2.5.3. Seed treatment with essential oils

The antibacterial effect of the essential oils against the studied bacteria was quantified by minimum inhibitory concentration (MIC) using an airtight container (*ca.* 1.5 L, 21 cm long X 11.5 cm wide X 10.5 cm high). One gram of inoculated seeds with *L. monocytogenes* or *S.* Typhimurium was placed inside of an airtight container over a plastic mesh attached to a circular glass container (7 cm diameter, 3 cm high), and exposed to different concentrations of EO vapors (Lee et al., 2018) at room temperature for 24 h.

2.5.4. Antibacterial activities of essential oils in alfalfa seeds

After the treatment with EO vapors, the seeds were crushed in a sterile mortar and pestle with 9 mL of sterile peptone water (1.0 g/L) until a seed slurry was obtained. One milliliter of the slurry was serially diluted in 9 mL of sterile peptone water and poured plated (1 mL) in TSA. Petri dishes were incubated at 35 °C for 24 h and colony forming units (CFU/mL) were quantified (Lee et al., 2018; Singh et al., 2003). Tests were performed by triplicate.

2.5.5. Seed germination on Petri dish

Seed germination percentage was determined by incubating (at room temperature) 10 dry, inoculated seeds treated with EOs in a Petri dish (60 × 15 mm) over sterile Whatman No. 1 filter paper and wetted with 4 mL sterilized distilled water. Germinated seeds were considered

germinated if a 2-mm radicle had emerged; they were observed under a microscope magnified 20× (Forty, American Optical Corporation, USA) and counted after 24 h of incubation. Germination was calculated by percentage (Kotana et al., 2013). Tests were performed by triplicate.

2.6. Sensory evaluation

Triangle tests were utilized to compare treated alfalfa sprouts (with each of the three tested EOs, allspice, thyme and rosemary) with non-treated alfalfa sprouts. The amount of EO used on treated seeds for this evaluation was the MIC obtained from the seed treatment with EOs previously explained. The sensory panel group consisted of 51 untrained panelists, and regular consumers of alfalfa sprouts; for each test, three samples were simultaneously presented, one different from the other two, all were randomly selected from treated or untreated alfalfa sprouts.

For sample preparation, the sprouting method reported by Landry et al. (2014) was utilized with some modifications. Treated and untreated alfalfa seeds were placed in a 750 mL flask and soaked with 112.5 mL (15%) of drinking water at 25 °C for 24 h. The water was then removed, sprouting was continued for 3 days at the same temperature and then alfalfa sprouts were stored at 5 °C until used, but for no longer than 1 day.

With the purpose of having samples at room temperature, they were taken out of the fridge 1 h before the test; then placed in a white plate and presented to panelists. Samples were accompanied by a neutral flavored cookie and a glass of water to clean panelists' palates. Tests were performed at UDLAP's Sensory Evaluation Laboratory (Stone et al., 2012).

Complementary to the triangle test, panelists were asked to mark the degree of difference that they detected (slight, moderate, strong, or extreme); and their preference among the samples (equal samples or the different one). Finally, panelists were encouraged to write additional comments.

2.7. Statistical analyses

MICs obtained data were analyzed using a general linear model with Minitab statistical package (ver. 17, Minitab Inc., State College, PA). Statistical analysis of the germination data was performed by ANOVA and Tukey's mean comparison tests ($p \leq 0.05$), using Minitab and the results of the triangle tests were analyzed with minimum number of correct judgments to establish significance ($p \leq 0.05$) for triangle tests presented by Stone et al. (2012).

3. Results and discussion

3.1. Chemical composition of the essential oils

Allspice, thyme, and rosemary EOs main components were identified by GC–MS, and their calculated retention indices are reported in Table 1. The main component of allspice EO was eugenol (89.55%); M-cymene (36.77%) and thymol (16.98%) of thyme EO; α-pinene (27.39%), camphor (20.64%) and 1,8 – cineole (20.89%) of rosemary EO. Burt (2004) mentioned that rosemary EO may contain 6–14% of 1,8–cineole and 2–10% of β–pinene; thyme EO, 10–64% of thymol, 2–10% of carvacrol, and 2–31% γ–terpinene; this chemical composition is similar to that obtained in our analysis. Miladi et al. (2013) found in rosemary EO, α–pinene, 1,8–cineole, β–pinene, and camphene as the main components; again similar to the reported results in this study except for α–pinene. For thyme EO, these authors reported that thymol, p-cymene, and γ-terpinene were its main components. On the other hand, Attokaran (2011) stated that allspice EO contained 80–87% of eugenol, 4–8% β-caryophyllene, and 0.2–0.5% of β-phyllandrine; which coincide with our findings, especially in the case of eugenol.

Table 1
Main components of allspice, thyme, or rosemary essential oils (EOs) determined by gas chromatography–mass spectrometry.

Compound	Percentage in allspice EO	Percentage in thyme EO	Percentage in rosemary EO	Retention Index
Eugenol	89.55	–	–	1356
α-Terpineol	2.04	–	4.25	1457
Caryophyllene oxide	1.48	3.52	–	1582
1,8 – cineole	1.06	–	20.89	1026
α-Cadinol	0.86	–	–	1652
M-Cymene	–	36.77	–	1082
Thymol	–	16.98	–	1288
Caryophyllene	–	9.20	–	1417
γ-Terpinene	–	7.83	–	1055
α-Pinene	–	5.38	27.39	0930
Camphor	–	–	20.64	1141
Camphene	–	–	7.16	0946
Borneol	–	–	3.69	1165

3.2. Vapor phase antibacterial activity

The antibacterial activity of allspice, thyme, or rosemary EOs against *L. monocytogenes* and *S.* Typhimurium was tested by the inverted Petri dish method and the obtained results are shown in Fig. 1; where it can be observed that the three tested EOs exhibited antibacterial effects (at different concentrations) against the two studied bacteria.

Thyme EO displayed the strongest antibacterial activity against both bacteria (0.4 mL/L_{air} for *L. monocytogenes* and 1.8 mL/L_{air} for *S.* Typhimurium) followed by rosemary EO (2.7 mL/L_{air} for *L. monocytogenes* and 13.3 mL/L_{air} for *S.* Typhimurium), and allspice EO (12.0 mL/L_{air} for *L. monocytogenes* and 13.3 mL/L_{air} for *S.* Typhimurium). Obtained MICs for thyme EO were higher than those found in the literature, but MICs for allspice and rosemary were similar to those reported. Nodorostova et al. (2009) evaluated the antibacterial effect of thyme EO against *L. monocytogenes* by the disc volatilization method, and determined a MIC of 0.26 $\mu L/cm^3$, lower than that reported in this study. Using the same method, Mattos De Oliveira et al. (2013) tested the vapor phase antimicrobial activity of rosemary EO against *L. monocytogenes* and reported that with 0.72 $\mu L/cm^3$ there was an insignificant reduction of bacterial growth. Likewise, Du et al. (2009) studied the vapor-phase diffusion of allspice EO from tomato films against *S. enterica* and *L. monocytogenes*, where 3% (w/w) of the EO, slightly reduced bacterial growth.

Furthermore, in this study we observed that *L. monocytogenes* was more susceptible to EOs than *S.* Typhimurium, as reported by various authors (Han et al., 2014; Soković et al., 2010; Techathuvanana et al., 2014). Resistance of Gram-negative bacteria, could be related to their hydrophilic cell wall, which helps them against penetration of hydrophobic compounds found in EOs (Rivera Calo et al., 2015). We observed that different factors affect MICs, such as tested EO, microorganism, medium and method of study, which makes it difficult to compare results from different authors.

MICs of allspice, thyme, or rosemary EOs against *L. monocytogenes* or *S.* Typhimurium in inoculated alfalfa seeds are displayed in Fig. 1. As in the culture media, the most effective tested EO against studied bacteria was thyme (0.8 mL/L_{air} for *L. monocytogenes* and 3.4 mL/L_{air} for *S.* Typhimurium); both MICs were higher than those in culture media. When rosemary EO was tested against *L. monocytogenes* in alfalfa seeds, the MIC (4.0 mL/L_{air}) was higher compared to the one obtained in culture media (2.7 mL/L_{air}). But when this EO was probed against *S.* Typhimurium, the MIC in alfalfa seeds was lower (11.7 mL/L_{air} *vs* 13.3 mL/L_{air}). Allspice EO was more effective against both studied bacteria in alfalfa seeds (6.0 mL/L_{air} for *L. monocytogenes* and 6.7 mL/L_{air} for *S.* Typhimurium) compared to the values obtained in culture

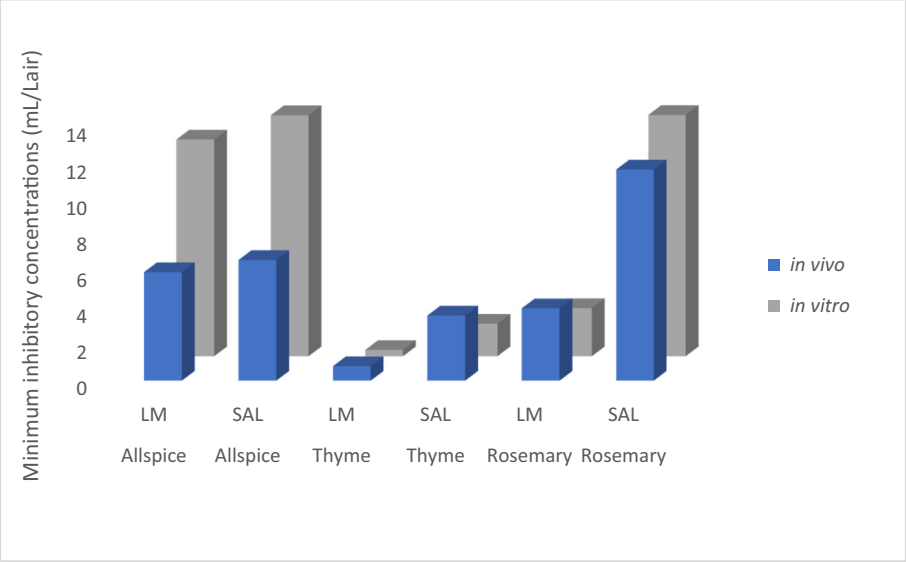

Fig. 1. Minimum inhibitory concentrations (MICs, mL/Lair) of allspice, thyme, or rosemary essential oils against *L. monocytogenes* (LM) and *S.* Typhimurium (SAL).

Table 2
Percentage of germinated alfalfa seeds treated with the vapor phase essential oil (EO).

Bacteria inoculated in seeds	EO utilized for the antibacterial treatment	Concentration of the EO (mL/L_{air})	Germinated seeds (%)
L. monocytogenes	Allspice	6.0	100[a]
	Thyme	0.8	100[a]
	Rosemary	4.0	100[a]
S. Typhimurium	Allspice	6.7	100[a]
	Thyme	3.6	100[a]
	Rosemary	11.7	100[a]

[a] Indicates that there were no significant differences ($p > 0.05$) between samples.

media (12.0 mL/L_{air} for *L. monocytogenes* and 13.3 mL/L_{air} for *S.* Typhimurium) as can be seen in Fig. 1. Vapor phase EOs MICs resulted in significant ($p \leq 0.05$) decreases of *L. monocytogenes* and *S.* Typhimurium viable cells compared to the control. Between treated alfalfa seeds and the control seeds, there was a decrease of 5 log CFU/g. between treated culture medium and the control medium, the decrease was of 7 log CFU/g. There also was a significant ($p \leq 0.05$) difference between systems (*in vitro* or on alfalfa seeds) regardless of the tested microorganism or the EO.

Our findings demonstrate an effective vapor phase antibacterial activity of allspice, thyme and rosemary EOs in alfalfa seeds, against *S.* Typhimurium and *L. monocytogenes*. The antibacterial effect of thyme EO (against both bacteria) and rosemary EO against *L. monocytogenes* tended to be lower when they were utilized in alfalfa seeds than on laboratory media, while the vapors of allspice EO were more effective to inhibit bacterial growth bacteria in alfalfa seeds. An explanation for these results would be that it is harder to remove or inactivate pathogens when they penetrate vegetable surfaces (Seo and Frank, 1999). Nuñez and D'aquino (2012) reported that the presence of organic matter reduced the antibacterial effect of clove EO and that this decrease varied from one microorganism to another. Consequently, it is important to consider the influence of surface structures of alfalfa seeds. The antibacterial efficacy of vapors of the EOs, could also be affected by different factors (Lee et al., 2018) such as relative humidity, pH, water activity, among others, between the studied systems.

Lee et al. (2018) evaluated the antimicrobial activities of gaseous thyme EO against *L. monocytogenes* on laboratory media and on radish sprouts through the MIC. These authors found that there was a significant ($p \leq 0.05$) decrease of *L. monocytogenes* counts, which agree with our findings. There are a few studies related to alfalfa seeds and sprouts treated with EOs, but the comparison was difficult since different methods and EOs were used against diverse microorganisms.

It has been known that the major components of allspice, thyme, or rosemary EOs (Table 1) can be related with the observed antibacterial effect; the main component of tested allspice EO was eugenol (representing 89.55% of the total EO), which has been used to protect foods from different microorganisms during storage, and it has been reported as an effective antibacterial against *B. cereus*, *B. subtilis*, *St. aureus*, *E. coli*, *P. aeruginosa* and *Salmonella typhi* trough the inhibition of amino acid decarboxylase, the interruption of amylase and protease production, and by the deterioration of the cell wall (Lee et al., 2018). The tested thyme EO major component was thymol, which also has demonstrated antibacterial effects against *B. subtilis*, *E. coli*, *Klebsiella pneumoniae* and *St. aureus;* this compound causes lipid perturbation in the microbial plasma membrane and penetrates the microorganism's cell in order to exert antimicrobial effects (Kumar Trivedi et al., 2015; Lee et al., 2018). The tested rosemary EO main component was 1,8 – cineole (or eucalyptol), which according to Kumar Sahoo et al. (2011) is an effective antimicrobial against *M. luteus*, *S. epidermis*, *B. subtilis*, *E.*

Table 3
Number of panelist responses that identify the odd sample during the triangle test among samples of sprouted seeds treated or untreated with tested essential oils (EOs).

Comparison	Number of correct answers/total tests
Sprouts from seeds treated with allspice EO *vs* untreated seeds sprouts	15/51[a]
Sprouts from seeds treated with thyme EO *vs* untreated seeds sprouts	17/51[a]
Sprouts from seeds treated with rosemary EO *vs* untreated seeds sprouts	20/51[a]

[a] Indicates that there were no significant differences ($p > 0.05$) between samples.

coli, *S. aureus* and *P. aeruginosa*. The antimicrobial effect of eucalyptol has been attributed to its lipophilic character enabling it to enter the membrane structures, resulting in membrane expansion, enhanced permeability and fluidity; furthermore, it makes iron transport processes difficult and inhibits respiration (Zengin and Baysal, 2014).

The antibacterial activity of EOs can be associated to the interactions among all their components and not only due to their major ones (Lobritz et al., 2014). Beside their main components, the tested allspice, thyme, or rosemary EOs have other components with antimicrobial properties, such as α-terpineol, α-cadinol, caryophyllene, camphor, and borneol (Table 1).

Finally, according to the obtained results of the vapor phase antibacterial activity *in vivo* and on alfalfa seeds, it could be said that allspice, thyme and rosemary EOs antibacterial activity are more effective against Gram-positive bacteria, making their performances as antimicrobial agents, dependent on bacteria type. Despite this, we determined that the studied EOs are effective antimicrobial agents for alfalfa seeds disinfection.

3.3. Seed germination

Table 2 presents the germination percentage of alfalfa seeds treated with tested EOs; the results show that none of the treatments applied to the seeds affected their germination, despite the different concentrations of EOs used. In all cases, 100% of seeds germinated. Evidently, no significant ($p > 0.05$) difference was found between the applied treatments.

3.4. Sensory analysis

The triangle test was performed with the objective of determining whether sprouted seeds treated with EOs were different from the non-treated ones. Results are shown in Table 3; treatment of alfalfa seeds with vapor phase EOs did not significantly ($p \geq 0.05$) affect the sensory acceptability of the sprouts (Stone et al., 2012).

For most judges who identified the different sample; the degree of difference among the samples was moderate for those samples treated with allspice or thyme EO, and slightly for those sprouted seeds treated with rosemary EO. Furthermore; most of the panelists preferred sprouts from seeds treated with allspice or rosemary EOs over untreated samples. In the case of the sprouts treated with thyme EO, panelists preferred untreated samples. Finally, additional comments from a few panelists suggested that sprouts treated with rosemary EO had acid and bitter flavors, while sprouts treated with thyme EO had bitter flavors. Gabrovská et al. (2005) mentions acid flavor as a descriptor of alfalfa sprouts, while Troszyńska et al. (2007) describe bitter as a basic taste in germinated seeds.

Thus, our findings indicate that tested EOs exhibited vapor phase antibacterial activity against *L. monocytogenes* or *S.* Typhimurium in culture media and in alfalfa seeds without significantly ($p > 0.05$) impacting the sensory acceptability of the sprouts; this is useful for further investigation regarding other sprouts, it is necessary to evaluate these EOS at semi-commercial level, as well as for studying inactivation of different foodborne pathogens with EOs in vapor phase in diverse food products.

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Conflict of interest

No conflict of interest declared.

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