Australian Journal of Crop Science AJCS

AJCS 15(03):416-421 (2021) ISSN:1835-2707
doi: 10.21475/ajcs.21.15.03.p2926

Interaction between mycorrhizal fungi and *Meloidogyne javanica* on the growth and essential oil composition of basil (*Ocimum basilicum*)

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Abstract

Plant-parasitic nematodes and arbuscular mycorrhizal fungi (AMF) have been reported to alter the yield and chemical composition of basil (*Ocimum basilicum*) essential oil. The aim of this study was to evaluate the effectiveness of AMF to control the root-knot nematode *Meloidogyne javanica* in basil and to investigate the effects of nematode-AMF interactions on plant growth, phosphorus (P) absorption, and essential oil composition. The experiment was conducted under greenhouse conditions following a completely randomized 3 × 2 factorial (two fungal species and an uninoculated control × inoculated and uninoculated seedlings) arrangement with 10 replicates. Substrates were inoculated with *Claroideoglomus etunicatum*, *Rhizophagus clarus*, or no fungi (control) and sown with basil seeds. After 20 days, half of the seedlings were inoculated with 4,000 *M. javanica* eggs. After 60 days, the vegetative parameters, P absorption, essential oil composition, nematode population density, AMF root-colonization efficiency, and AMF spore density were determined. The presence of AMF increased the basil's fresh weight and ability to absorb P, while reducing the *M. javanica* reproduction. In total, 21 compounds were identified in basil essential oil, the concentrations of which varied according to the treatments. The major components were eucalyptol, linalool, eugenol, β-elemene, *trans*-α-bergamotene, and τ-cadinol. Inoculation with AMF decreased the linalool levels but increased the amount of eucalyptol. Mycorrhizal plants showed increased shoot height, P uptake, and essential oil yield and a decreased nematode population density in their roots.

Keyword*s*: root-knot nematode; *Ocimum basilicum*; biological control; *Rhizophagus clarus*; *Claroideoglomus etunicatum.*

Introduction

Basil (*Ocimum basilicum* L.) is a culinary and medicinal herb that is rich in essential oils and widely produced by small- and large-scale farmers (Teixeira et al., 2002). Basil essential oil is composed mainly of linalool (up to 82.64%) (Blank et al., 2007), a terpene alcohol with anti-inflammatory and insecticidal properties used in the production of perfumes, cosmetics, and insect repellents (Teixeira et al., 2002; Rabelo et al., 2003).

The growth and development of basil and other aromatic plants can be limited by biotic and abiotic factors. Basil is susceptible to root-knot nematodes, particularly *Meloidogyne javanica* (Treub) Chitwood and *Meloidogyne incognita* (Kofoid & White) Chitwood (Dias-Arieira et al., 2012).

Root-knot nematodes establish a complex parasitic system in the root environment. They induce feeding sites near or in the xylem, which result in the irregular growth of cells adjacent to the feeding sites and cause damage to the vascular cylinder, thus hindering the absorption of water and nutrients and limiting plant development (Lin et al., 2013). Swelling can occur in external root tissues, leading to the formation of root knots or galls. The parasitism of *Meloidogyne* spp. negatively affects the amount of fresh mass in the aerial parts of basil plants, which consequently reduces the yield of essential oil, since the oil is extracted mainly from the aerial parts (Tiwati et al., 2017).

Integrated management strategies are necessary to control nematodes, as there are no nematicides currently available for aromatic plants. Biological agents have shown promise in the control of plant parasites and may contribute to minimizing the risks of environmental contamination (Berry et al., 2009).

Arbuscular mycorrhizal fungi (AMF) are symbiotic microorganisms that improve nutrient absorption (Schouteden et al., 2015) and increase pathogen resistance in plants through biochemical, physiological, and molecular mechanisms (Elsen et al., 2008; Vos et al., 2013; Schouteden et al., 2015). Rhizosphere colonization by AMF has been reported to alter the composition of root exudates (Vos et al., 2013) and promote lignification of root cells, conferring resistance to pathogen penetration (Chen et al., 2019). Both mycorrhizae and parasitism can alter the production and

composition of secondary metabolites in herbs (Kapoor et al., 2002; Hassiotis et al., 2014). The use of AMF in conjunction with other biological control microorganisms efficiently controls root-knot nematodes; in addition, these fungi increase the yield of essential oil produced by basil plants parasitized by *Meloidogyne* spp. (Tiwari et al., 2017).
However, little is known about the effects of the interaction between AMF and nematodes on growth and essential oil production in basil. This study aimed to evaluate the effectiveness of AMF in controlling *M. javanica* and investigate the effects of AMF-nematode interactions on basil growth, vegetative development, and essential oil production.

Results and discussion

Plant height, shoot fresh weight, shoot dry weight, root fresh weight, chlorophyll index, and phosphorus concentration

The shoot height, shoot dry weight, and chlorophyll index were not altered by the presence of nematodes or AMF (Table 1). There was an interaction between nematode infection and AMF inoculation for fresh shoot weight. Plants inoculated with *C. etunicatum* had a higher fresh shoot weight than plants not inoculated with AMF, regardless of the nematode infection. Nematode-free plants inoculated with *R. clarus* had a higher fresh shoot weight than the AMF- and nematode-free controls (Table 1). The stimulatory effects of AMF on host plants are well known. For instance, the shoot fresh weight of *Mentha arvensis* L. was shown to increase by up to 207% following inoculation with mycorrhiza (Freitas et al., 2004). However, the beneficial effect of AMF on shoot fresh weight may be affected by the presence of nematodes, because these two microorganisms have similar requirements and ecological niches, thereby competing for colonization space and nutrients (Vos et al., 2013). This fact may explain the lack of effect of *R. clarus* on the shoot dry weight of plants infected by *M. javanica*.
AMF also influenced the root fresh weight, which was highest in plants inoculated with *C. etunicatum* (Table 1). These results further confirmed the beneficial effects of mycorrhizae on root development (Brandon et al., 2004; Sharma and Sharma, 2017). Enhanced root development increases phosphorus (P) uptake (Bressan et al., 2001; Nunes et al., 2009), as evidenced by the high P concentration in plants inoculated with either AMF species. P has low mobility in soil, and plants have developed mechanisms to increase its uptake. One of the main strategies adopted by plants is mycorrhizal symbiosis (Smith et al., 2011). The external hyphae and mycelia of AMF increase the root surface area (Calvet et al., 2003), and AMF arbuscules stimulate the activation of phosphate transporter genes in the host, leading to higher P absorption (Pumplin et al., 2012).

Nematode reproduction, root colonization, and soil mycorrhizal spore density

Inoculation with *R. clarus* reduced the nematode count by 25% compared with the control (from 1865 to 1405 eggs and second-stage juveniles) (Table 2). Inoculation with *C. etunicatum*, however, did not lead to significant differences in the nematode count compared with the controls. This result may have been due to the higher root weight of the AMF-inoculated plants. Both AMF species decreased the density of the nematode population by 36% (Table 2). These results showed that inoculation with the AMF helped to protect the plants against the parasitic pathogens (Talavera et al., 2001; Anjos et al., 2010; Schouteden et al., 2015).
AMF display a range of mechanisms of action against nematodes, including the competition for space and nutrients, changes in radicular exudate composition, production of hormones, and suberization of root tissues. These effects increase the host's resistance to pathogen penetration and reduce the attraction of nematodes to the plant (Pozo et al., 2002; Wipps, 2004; Schouteden et al., 2015). In addition, AMF promote the production of nematode-antagonistic compounds, such as phenolics and phytoalexins, as well as lignin and amino acids, such as phenylalanine and serine (Siddiqui and Mahood, 1996; Vos et al., 2013; Nair et al., 2015).
We highlight that care must be taken when using biological control agents. It is important to inoculate plants with AMF before they are exposed to pathogens, because symbiotic fungi temporarily impair the defense mechanisms of plants during rhizosphere colonization (Schouteden et al., 2015; Brito et al., 2018). This nematode control strategy would be feasible for plants sown in pots and later transplanted to the field, as is the case for most vegetables and herbs.
The effects of the combined AMF inoculation and nematode infection on the percentage of roots that were colonized were not significant. In contrast, AMF independently affected colonization. Whereas the uninoculated control displayed root colonization of 9.36%, plants inoculated with *C. etunicatum* and *R. clarus* exhibited root colonization of 90% or higher, regardless of the nematode infection (Table 2). These results corroborated those of a previous study, which showed that the presence of nematodes reduced AMF colonization by only 16% (Borowicz, 2001). The relationship between the host plant, nematodes, and AMF is complex and may be affected further by the soil and climate conditions. In *Cucumis sativus* L., the presence of *M. incognita* reduced the colonization efficiency of the AMF *Glomus mosseae* Schwarzott, Walker & Schüßler but not of *G. intraradices* Schenck & Smith (Zhang et al. 2008). Likewise, in white clover (*Trifolium repens* L.), *M. incognita* increased the *G. intraradices* colonization efficiency but did not alter that of *G. aggregatum* Schenck & Smithand or *G. mosseae* (Habte et al., 1999).

Quantification and characterization of essential oils

The AMF spore density was influenced by mycorrhizal inoculation. *C. etunicatum* spores were found at 2.47 spore/g soil and *R. clarus* spores at 2.91 spores/g soil (Table 3). Both of these concentrations were significantly higher than that found in the control (0.79 spores/g soil). In plants inoculated with nematodes, the mean AMF spore density was 1.89 spores/g soil, which was lower than that observed in plants that were not inoculated with *M. javanica* (2.22 spore/g soil) (data not shown). The low spore density in plants inoculated with the symbiotic and parasitic organisms was probably due to competition for space and nutrients, as evidenced by the reduction in the nematode population density. For AMF to compete with nematodes, they must be well established in the rhizosphere. The presence of arbuscules is an indication of successful root colonization (Pozo and Azcón-Aguilar, 2007). The results underscored that plants must establish a symbiotic relationship with AMF before being exposed to pathogens.
The yield of essential oil differed significantly between the control (0.20%), *R. clarus*-inoculated plants (0.25%), and *C.*

Table 1. Plant height, shoot fresh weight, shoot dry weight and root fresh weight, chlorophyll index (CI) and phosphorus concentration (P) in basil plants undergoing treatments with mycorrhizae, inoculated or not with *Meloidogyne javanica* (Mj).

Treatments	Height (cm)	Shoot fresh weight (g)		Shoot dry weight (g)	Root fresh weight (g)	CI	P (mg/g)
		With Mj	Without Mj				
Control	55.48^{ns}	39.73 bA	41.38 bA	2.55^{ns}	32.77 b	33.87^{ns}	3.63 b
C. etunicatum	58.88	52.39 aA	58.95 aA	3.96	46.48 a	35.59	4.48 a
R. clarus	59.03	40.06 bB	60.93 aA	3.54	42.40 ab	33.62	4.68 a
CV (%)	9.43	20.35		38.25	29.98	9.89	10.47

Means followed by the same lower-case letter in the column and upper-case in the row do not differ from each other by the Tukey test at 5% probability. Ns = not significant. CV = coefficient of variation.

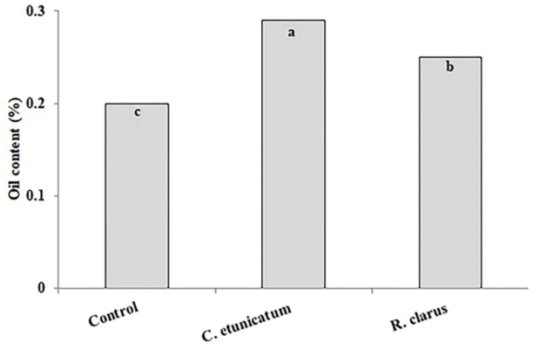

Fig 1. Essential oil content (%) of basil uninoculated and inoculated with *Claroideoglomus etunicatum* or *Rhizophagus clarus*. Columns followed by the same letter are not statistically different (Tukey, $p \leq 0.05$). Coefficient of variation = 6.67%.

Table 2. *Meloidogyne javanica* total and per gram of root, root colonization and soil mycorrhizal spore density in basil submitted to treatments with *Claroideoglomus etunicatun* and *Rhizophagus clarus*.

Treatments	Eggs+J2 total	Eggs+J2/g of root	% root colonization	Spore density (spores/gdry soil)
Control	1865 a	66 a	9.36 b	0.79 b
C. etunicatum	1859 a	42 b	90.28 a	2.47 a
R. clarus	1405 b	42 b	91.64 a	2.91 a
CV (%)	18.20	33.94	8.07	32.43

Means followed by the same letter in column are not different by Tukey test at 5% probability. CV = coefficient of variation.

Table 3. Percentage of basil essential oil as a function of mycorrhizal inoculation in the presence and absence of *Meloidogyne javanica*.

N°	Compound	[ab]RI	-F/-N	-F/+N	Ce/-N	Ce/+N	Rc/-N	Rc/+N
1	Eucalyptol	11.988	2.77	7.66	25.54	25.44	8.06	18.21
2	Linalool	14.431	43.72	38.47	t	t	t	t
3	Isoborneol	16.227	0.73	t	2.34	2.34	5.70	t
4	α Terpineol	16.948	1.67	1.28	4.48	4.48	6.09	0.73
5	Bornylacetate	19.577	1.72	1.00	4.29	4.29	2.73	2.20
6	Eugenol	21.804	20.60	25.25	t	t	t	31.05
7	β Elenene	22.478	2.01	4.42	5.17	5.17	10.52	11.66
8	trans α Bergamotene	23.609	9.58	7.85	14.87	14.87	17.60	t
9	α Guaiene	23.685	0.32	0.43	1.14	1.14	1.37	4.19
10	(E) β Famesene	24.003	1.04	t	t	t	1.72	0.95
11	Humulene	24.099	0.78	t	1.66	1.66	1.09	0.98
12	Germacrene D	24.793	3.28	2.64	6.94	6.94	7.39	5.08
13	Azulene	25.354	1.18	1.09	2.95	2.95	2.32	1.88
14	Naphthalene	25.597	2.82	2.35	5.96	5.95	5.15	4.38
15	Epicubenol	28.000	0.87	0.67	1.99	2.00	1.14	2.05
16	τ-Cadinol	28.653	5.90	4.83	13.34	14.34	6.57	12.88
17	β Myrcene	10.844	t	0.61	1.63	1.63	t	t
18	Bornanone	15.693	t	0.53	t	t	9.20	t
19	Benzofuran	25.382	t	t	t	t	4.13	t
20	Epicurzerenone	28.015	t	t	t	t	5.99	t
21	(Z) 1,3,6-Octatriene, 3,7-dimethyl	12.653	t	t	3.49	3.49	t	t
	Total		98.99	99.08	95.79	96.69	96.77	96.24

[a]Base identification of retention index using *n*-alcanos C_8 - C_{25}in column DB-5 (fenilmetilsiloxane 5%); [b]Base identification of comparison of spectral mass with *GC-MS Postrun Analysis* software; t: trace. -F/-N: without AMF and without nematode; -F/+N: without AMF and with nematode; Ce/-N: AMF *C. etunicatum* without nematode; Ce/+N: AMF *C. etunicatum* + nematode; Rc/-N: AMF *R. Clarus* without nematode; Rc/+N: AMF *R. clarus*+nematode.

etunicatum-inoculated plants (0.29%) (Fig. 1). In total, 21 compounds were identified in the basil essential oil, and the major components were eucalyptol (2.77–25.54%), linalool (t–43.72%), eugenol (t–31.05%), β-elemene (2.01–11.66%), *trans*-α-bergamotene (t–17.60%), and τ-cadinol (4.83–14.34%). The concentration of the major components varied from 11.66 to 43.72% (Table 3). A significant increase in the yield of essential oil with AMF inoculation was also observed by Urcoviche et al. (2015), who inoculated *Mentha crispa* L. with *C. etunicatum* under low soil P conditions. In the current study, significant increases in the shoot fresh weight and P uptake in AMF-inoculated basil were observed, which probably favored the production of secondary metabolites (Copetta et al., 2007). P is a major constituent of ATP. It is responsible for storing and transporting energy for endergonic processes such as the synthesis of organic compounds. Terpenes, the main components of essential oils, are produced by phosphorylation reactions, in which ATP is the main energy donor. Therefore, plants with low concentrations of P show reduced levels of phosphorylation and, consequently, low terpene concentrations (Rodrigues et al., 2004).

In the absence of AMF, linalool and eugenol were the major essential oil components, as also reported by Ichimura et al. (1995) for plants fertilized with phosphate. The presence of AMF inhibited eugenol production and promoted linalool synthesis (>38.47%), except in plants inoculated with *R. clarus* and root nematodes, in which eugenol was the major essential oil component (31.05%). Eugenol was also present in non-mycorrhizal plants (up to 25.25%). Rizvi et al. (2014) reported that clove basil essential oil, composed of 77.8% eugenol, showed antifungal activity. Morrelli et al. (2017), in studying the antimicrobial activity of the essential oil of basil inoculated with AMF and fertilized with humic substances, found that eugenol (33.90%) was detected only in non-mycorrhizal plants. Altogether, these results show that AMF drastically reduces eugenol production in basil. Further studies are needed to confirm this finding.

Linalool is an acyclic monoterpene tertiary alcohol widely used in the cosmetic and perfume industries because of its fragrance properties. Previous studies have reported its antinociceptive, antileishmanial, antimicrobial, and antifungal properties (Smith and Contractual, 2014; Hanif et al., 2017; Morh et al., 2017). Linalool can cause protein denaturation and cell dehydration in microorganisms, leading to cell death. This mechanism of action is comparable to that of chlorhexidine, an important antimicrobial agent (Camargo and Vasconcelos, 2014). Linalool was the major compound in the essential oil extracted from plants not inoculated with AMF. Nematode infection did not have a significant effect on the linalool concentration.

To date, few reports have focused on the influence of AMF on the essential oil composition of aromatic and medicinal plants, particularly the interaction between AMF and other biotic factors such as nematodes on the synthesis of secondary metabolites. Our results indicated that AMF altered the biosynthetic pathways of secondary metabolites in plants, affecting the composition of essential oils.

Materials and methods

Experimental design

The experiment was conducted in a greenhouse (23°47'25"S 53°15'32"W, 405 m above sea level), following a completely randomized 3 × 2 factorial design with 10 replicates. Treatments consisted of inoculation with AMF (two fungal species and an uninoculated control) and *M. javanica* (inoculated and uninoculated seedlings).

Claroideoglomus etunicatum (Becker & Gerd.) Walker & Schüßler (syn. *Glomus etunicatum*) and *Rhizophagus clarus* (Nicolson & Schenck) Walker & Schüßler (syn. *Glomus clarum*) were obtained from the Glomales Germplasm Bank (UNIPAR, Umuarama, Brazil). Seeds of basil cv. Maria Bonita were sown in polystyrene trays containing a commercial substrate (Bioplant®, Bioplant Agrícola Ltd., Nova Ponte, Brazil). Substrates were inoculated with 250 spores/kg of *C. etunicatum* or *R. clarus*, and uninoculated controls were prepared according to Urcoviche et al. (2015).

At 20 days after germination, seedlings were transplanted into pots containing 3 L of a 2:1 mixture of soil and sand, previously autoclaved (120°C, 2 h), adjusted with 1.82 g of limestone, and fertilized with 0.68 g of NPK (02-16-06). On the same day, half of the seedlings had their roots inoculated with 2 mL of a nematode suspension containing 4000 eggs and eventual second-stage juveniles (J2) of *M. javanica*. The other half of the plants were not inoculated (negative control).

Nematodes were obtained from a single species population maintained in tomato cv. Santa Clara under greenhouse conditions. Nematode extraction was performed according to the method of Hussey and Barker (1973) adapted by Boneti and Ferraz (1982). The concentration of the nematode suspension was adjusted using a Peters counting chamber under a light microscope.

Plants were grown for 60 days in a greenhouse. Irrigation was applied daily as needed.

Leaf chlorophyll index

The leaf chlorophyll index was determined in five fully developed leaves per pot at 60 days after nematode inoculation using a chlorophyll meter (1030 CFL, ClorofiLOG, Falker, Porto Alegre, Brazil).

Shoot height, fresh and dry weight, and P content

After 60 days of inoculation with parasitic nematodes, plants were carefully removed from the pots. The harvested material was separated into shoots and roots and shoot height and fresh weight were determined. Shoots were oven dried at 65°C for 48 h for dry weight determination. Then, the dried material was ground, and the P content determined according to Silva (2009) and Lermen et al. (2017).

Root fresh weight, AMF spore density, and root colonization

Roots were carefully washed, dried with paper towels, and weighed to obtain the fresh weight. AMF spore density and root colonization were determined in 10 g segments according to Lermen et al. (2017) and Gerdemann et al. (1963), respectively. Fine roots were prepared following the protocol of Phillips and Hayman (1970). The number of colonized and non-colonized root segments was counted to estimate root colonization by AMF (Giovanetti and Mosse, 1980).

Nematode count and population density

Nematodes were extracted from fresh roots according to the method of Hussey and Barker (1973), modified by Boneti and Ferraz (1982). Nematode counts were determined using

a Peters chamber under a microscope. Total counts were divided by the root fresh weight to obtain the nematode population density (number of nematodes per g of root).

Extraction, quantification, and characterization of essential oils

Fresh shoots (100 g) were ground in a blender with 1 L of deionized water and hydro-distilled for 3 h using a modified Clevenger apparatus (Lermen et al., 2015; Urcoviche et al., 2015). The extracted essential oil was rotary evaporated in amber flasks and weighed. The yield of essential oil was calculated as the oil weight divided by the shoot weight × 100. After extraction, the essential oil samples were stored at -20°C until analysis. The constituents of the essential oil were identified using gas chromatography-mass spectrometry (GC-MS) on a QP2010 SE system (Shimadzu). Samples were diluted in dichloromethane before they were injected into a SH-RTx-5MS column (Shimadzu, 5% phenyl-methylsiloxane, 30 m × 0.25 mm id, 0.25 µm) using an auto sampler (Shimadzu AOC–20i). Helium was used as the carrier gas at a flow rate of 1.0 ml min^{-1} with split ratio of 20:1, and 2 µL of each sample were injected. The column temperature was initially programmed to 40 °C, heating at 6°C min^{-1} to reach the final temperature of 300°C. The injector and the GC-MS interface temperatures were maintained at 250ºC. Mass spectra were recorded at 70 eV with mass range from m/z 50 to 550 amu.

Statistical analysis

Data were subjected to analysis of variance at $p < 0.05$, and, when appropriate, means were compared by Tukey's test ($p < 0.05$) using Sisvar (Ferreira, 2011).

Conclusion

AMF inoculation increased plant growth, P uptake, and the yield and of essential oil, while decreasing the nematode population density in basil. *R. clarus* was more efficient than *C. etunicatum* in controlling *M. javanica*.

Acknowledgement

To the PIBIC program of the UEM-CNPq-FA, for the scholarship granted to B.A. Silva; to the CAPES for the doctor's scholarship to A. Miamoto and for the research productivity scholarship granted to C. Silva, O. Alberton, and C.R. Dias-Arieira.

References

Anjos ECT, Cavalcante, UMT, Gonçalves DM, Pedrosa EMR, Santos VF, Maia LC (2010) Interactions between an arbuscular mycorrhizal fungus (*Scutellospora heteragama*) and the root-knot nematode (*Meloidogyne incognita*) on sweet passion fruit (*Passiflora alata*). Braz Arch Biol Technol. 53:801-809.

Berry SD, Spaull VW, Cadet P (2009) Field assessment of biologically-based control products against nematodes on sugarcane in South Africa. Afr Plant Prot. 15:1-12.

Blank AF, Souza EM, Arrigoni-Blank MF, Paula JWA, Alves PB (2007) Maria Bonita: a linalool type basil cultivar. Pesqui Agropecu Bras. 42:1811-1813.

Boneti JIS, Ferraz, S (1981) Modificação do método de Hussey e Barker para extração de ovos de *Meloidogyne exigua* de raízes de cafeeiro. Fitopatol Bras. 6:553.

Borowicz VA (2001) Do arbuscular mycorrhizal fungi alter plant-pathogen relations? Ecology. 82:3057-3068.

Brandão JAC, Cavalcante UMT, Pedrosa EMR, Maia, LC (2004) Interação entre fungos micorrizicos arbusculares e *Pratylenchus coffeae* na produção de mudas de gravioleira (*Annona muricata*). Nematol Bras. 28:27-33.

Bressan W, Siqueira JO, Vasconcellos CA, Purcino ACP (2001) Fungos micorrízicos e fósforo, no crescimento, nos teores de nutrientes e na produção do sorgo e soja consorciados. Pesqui Agropecu Bras. 36:315-323.

Brito ODC, Hernandes I, Ferreira JCA, Cardoso MR, Alberton O, Dias-Arieira CR (2018) Association between arbuscular mycorrhizal fungiand *Pratylenchus brachyurus* in maize crop. Chil J Agric Res. 78:521-527.

Calvet C, Estaún V, Campribí A, Hernández-Dorrego A, Pinochet J, Moreno MA (2003) Aptitude for mycorrhizal root colonization in *Prunus* rootstocks. Sci Hortc. 9:1-10.

Camargo SB, Vasconcelos DFSA (2014) Atividades biológicas de Linalol: conceitos atuais e possibilidades futuras deste monoterpeno. Rev Ciênc Méd Biol. 13:381-387.

Chen WC, Kang Y, So PS, Wai CW, Wong MH (2018) Arbuscular mycorrhizal fungi increase the proportion of cellulose and hemicellulose in the root stele of vetiver grass. Plant and Soil. 425:309-319.

Copetta A, Lingua G, Berta G, Bardi L, Masoero G (2007) Influence of arbuscular mycorrhizal fungi on growth and essential oil composition in *Ocimum basilicum* var. Genovese. Caryologia. 60:106-110.

Dias-Arieira CR, Cunha TPL, Chiamolera FM, Puerari HH, Biela F, Santana SM (2012) Reaction of vegetables and aromatic plants to *Meloidogyne javanica* and *M. incognita*. Hortic Bras. 30:322-326.

Elsen A, Gervacio D, Swennen R, De Waele D (2008) AMF-induced biocontrol against plant-parasitic nematodes in *Musa* sp.: a systemic effect. Mycorrhiza. 18:251-256.

Ferreira DF (2014) Sisvar: a guide for its Bootstrap procedures in multiple comparisons. Ciênc Agrotec. 38:109-112.

Freitas MSM, Martins MA, Vieira IJC (2004) Produção e qualidade de óleos essenciais de *Menthaarvensis* em resposta à inoculação de fungos micorrízicos arbusculares. Pesqui Agropecu Bras. 39:887-894.

Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc. 46:235-246.

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84:489-500.

Habte M, Zhang YC, Schmitt D (1999) Effectiveness of *Glomus* species in protecting white clover against nematode damage. Can J Bot. 77:135-139.

Hanif MA, Nawaz H, Ayub MA, Tabassum N, Kanwal N, Rashid N, Saleen M, Ahmad M (2017) Evaluation of the effects of zinc on the chemical compositionand biological activity of basil essential oil by using Raman spectroscopy. Ind Crops Prod. 96:91-101.

Hassiotis CN, Ntana F, Lazari DM, Poulios S, Vlachonasios KE (2014) Environmental and developmental factors affect essential oil production and quality of *Lavandula angustifolia* during flowering period. Ind Crops Prod. 62:359-366

Hussey RS, Barker KR (1973) A comparison of methods colleting inocula of *Meloidogyne* spp. including a new technique. Pant Dis. 57:1025-1028.

Ichimura M, Ikushima M, Miyazaki T, Kimura M (1995) Effect of phosphorus on growth and concentration of mineral elements and essential oils of sweet basil leaves. Acta Hortic. 396:195-202.

Kapoor R, Giri B, Mukerji KG (2002) Mycorrhization of coriander (*Coriandrum sativum* L.) to enhanced he concentration and quality of essential oil. J Sci Food Agric. 82:339-342.

Lermen C, Cruz RMS, Souza JS, Marchi BA, Alberton O (2017) Growth of *Lippia alba* (Mill.) N. E. Brown inoculated with arbuscularmycorrhizal fungi with different levels of humic substances and phosphorusin the soil. J Appl Res Med Aroma. 7:48-53.

Lermen C, Mohr FBM, Alberton O (2015) Growth of *Cymbopogon citratus* inoculated with mycorrizal fungi under different levels of lead. Sci Hortic. 186:239-246.

Lin B, Zhuo K, Wu P, Cui R, Zhang LH, Liao J (2013) A novel effector protein, MJ-NULG1a, targeted to giant cell nuclei plays a rolein *Meloidogyne javanica* parasitism. Mol Plant Microbe Interact. 26:55-66.

Morelli F, Ferarrese L, Munhoz CL, Alberton O (2017) Antimicrobial activity of essential oil and growth of *Ocimum basilicum* (L.) inoculated with mycorrhiza and humic substances applied to soil. Genet Mol Res. 16:1-11.

Morh FBM, Lermen C, Gazim ZC, Gonçalves JE, Alberton O (2017) Antifungal activity, yield, and composition of *Ocimum gratissimum* essential oil. Genet Mol Red. 16:1-10.

Nair A, Kolet SP, Thulasiram HV, Bhargava S (2015) Systemic jasmonic acid modulation in mycorrhizal tomato plants and its role in induced resistance against *Alternaria alternata*. Plant Biol. 17:625-631.

Nunes JLS, Souza PVD, Marodin GAB, Fachinello JC (2009) Eficiência de fungos micorrízicos arbusculares sobre o crescimento do porta-enxerto de pessegueiro 'Aldrighi'. Bragantia. 68:931-940.

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc. 55:157-160.

Pozo MJ, Azcón-Aguilar C (2007) Unraveling mycorrhiza-induced resistance. Curr Opin Plant Biol. 10:393-398.

Pozo MJ, Cordier C, Dumas-Gaudot E, Gianinazzi S, Barea JM, Azcón-Aguilar C (2002) Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomatoplants. J Exp Bot. 53:525-534.

Pumplin N, Zhang X, Noar RD, Harrison MJ (2012) Polar localization of a symbiosis-specific phosphate transporter is mediated by a transient reorientation of secretion. Proc Natl Acad Sci. 109:665-672.

Rabelo M, Souza EP, Soares PMG, Miranda AV, Matos FJA, Criddle DN (2003) Antinociceptive properties of the essential oil of *Ocimum gratissimum* L. (Labiatae). Braz J Med Biol Res. 36:521-524.

Rizvi AH, Khan MMAA, Verma PC, Saxena G (2014) Biochemical activity of *Ocimum gratissimum* essential oil against fruit-rotting fungi *Penicillium expansum* and *Penicillium digitatum*. In: Kharwar RN, Upadhyay RS, Dubey NK, Raghuwanshi R. *Microbial Diversity and Biotechnology in Food Security*. Springer, New York, pp. 343-348.

Rodrigues CR, Faquin V, Trevisan D, Pinto JEBP, Bertolucci SKV, Rodrigues TM (2004) Mineral nutrition, growth and essential oil content of mint in nutrient solution under different phosphorus concentrations. Hortic Bras. 22:573-578.

Schouteden N, De Waele D, Panis B, Vos CM (2015) Arbuscular mycorrhizal fungi for the biocontrol of plant-parasitic nematodes: a review of the mechanisms involved. Front Microbiol. 6:1-12.

Sharma IP, Sharma AK (2017) Co-inoculation of tomato with an arbuscular mycorrhizal fungus improves plant immunity and reduces root-knot nematode infection. Rhizosphere. 17:81-82.

Siddiqui ZA, Mahmoo I (1996) Role of plant symbionts in nematode management: a review. Bioresour Technol. 54:217-226.

Silva CF (2009) *Manual de Análises Químicas de Solos, Plantas e Fertilizantes*. 2ª. Ed. Embrapa Informação Tecnológica, Brasília, 627p.

Smith SE, Jakobsen I, Grønlund M, Smith FA (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiol. 156:1050-1057.

Talavera M, Itou K, Mizukuno T (2001) Reduction of nematode damage by root colonization with arbuscular mycorrhiza (*Glomus* spp.) in tomato-*Meloidogyne incognita* (Tylenchida: Meloidogynidae) and carrot-*Pratylenchus penetrans* (Tylenchida: Pratylenchidae) pathosystems. Appl Entomol Zool. 36:387-392.

Teixeira JPF, Marques MOM, Furlani PR, Facanali R (2002) Essential oil contents in two cultivars of basil cultivated on NFT-hydroponics. Acta Hortic. 569:203-208.

Tiwari S, Pandey S, Chauhan PS, Pandey R (2017) Biocontrol agents in co-inoculation manages root knot nematode [*Meloidogyne incognita* (Kofoid & White) Chitwood] and enhances essential oil content in *Ocimum basilicum* L. Ind Crop Prod. 97:292–301.

Urcoviche RC, Gazim ZC, Dragunski DC, Barcellos FG, Alberton O (2015) Plant growth and essential oil content of *Mentha crispa* inoculated with arbuscular mycorrhizal fungi under different levels of phosphorus. Ind Crop Prod. 67:103-107.

Vos C, Schouteden N, Van Tuinen D, Chatagnie O, Elsen A, De Waele D (2013) Mycorrhiza-induced resistance against the root-knot nematode *Meloidogyne incognita* involves priming of defense gene responses in tomato. Soil Biol Biochem. 60:45-54.

Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol ofroot pathogens. Can J Bot. 1227:1198-1227.

Zhang L, Zhang J, Christie P, Li X (2008) Pre-inoculationwith arbuscular mycorrhizal fungi suppresses root knot nematode (*Meloidogyne incognita*) on cucumber (*Cucumis sativus*). Biol Fertil Soils. l45:205-211.