academicJournals

Vol. 11(12), pp. 1048-1055, 24 March, 2016
DOI: 10.5897/AJAR2015.9734
Article Number: 654BF4C57705
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Effect of seasons on chemical composition and fungitoxicity of *Cymbopogon citratus* (DC) Staf essential oil

Virlene do Amaral Jardinetti[1]*, Kátia R. Freitas Schwan-Estrada[1], Aline José Maia[2], Willian Ferreira da Costa[1] and Raphaeli Nascimento de Freitas[1]

[1]Universidade Estadual de Maringá, Brazil.
[2]Universidade Estadual do Centro Oeste, Brazil.

Received 19 March, 2015; Accepted 29 October, 2015

The study aimed to evaluate the chemical composition and the fungitoxicity of *Cymbopogon citratus* (DC) Staf essential oil, obtained in different seasons of the year. Therefore, *C. citratus* leaves were harvest at four seasons (summer, fall, winter and spring) and essential oil extracted by hydrodistillation of freshly harvested and dried leaves, with a total of eight samples. Yield was expressed in percentage and the fungitoxicity was evaluated *in vitro* on *Colletotrichum gloeosporiodes* (Penz.) Penz. & Sacc the causal agent of anthracnose at different concentrations (0, 0.25, 0.5, 0.75 and 1 µL mL^{-1}, respectively). The qualitative analysis of essential oil samples was made using gas chromatography coupled to mass spectrometry (GC-MS). The essential oil of freshly harvested leaves had a higher yield (0.25%) in the summer, whereas the essential oil of dried leaves had a better yield (0.48%) in the fall. In i*n vitro* tests, concentrations of 0.75 and 1 µL mL^{-1} significantly inhibited mycelial growth and sporulation, in all samples. In the quantitative analysis, there was a variation in the levels of oil compounds observed in the different seasons. The most abundant compound in essential oil was citral (neral and geranial mixture) and myrcene. However, the variation in the compounds' levels had no influence on the fungitoxicity of *C. gloeosporiodes* (Penz.) Penz. & Sacc.

Key words: Lemongrass, citral, anthracnose, seasons, yield.

INTRODUCTION

Cymbopogon citratus (DC) Staf is an important species of Poaceae Family, considered as a medicinal plant commonly known in Brazil as capim-limão, capim-cidreira, capim-de-cheiro and capim-santo (lemon grass, lemon balm, smelling grass and holy grass). *C. citrates* has an essential oil rich in terpenoids such as neral, geranial and myrcene (Correa et al., 1998; Souza Filho and Alves, 2002; Souza and Lorenzi, 2005; Tyagi and Malik, 2012, Tyagi et al., 2014) and it is widely used in cosmetic, perfumes and pharmaceutical industries (Verma et al., 2013). The analysis of *C. citratus* essential oil from plants of multiple regions determines citral (neral

*Corresponding author. E-mail: virlene@agronoma.eng.br.

Table 1. The experiment results of soil chemical analysis: pH, aluminum concentration, hydrogen, macronutrients and micronutrients.

pH	cmol dm^{-3}				mg dm^{-3}						
$CaCl_2$	$H^+ + Al^{+++}$	Ca^{++}	Mg++	K^+	P	S	Cu	Zn	Fe	Mn	B
6.50	2.64	5.76	1.83	0.51	3.20	6.85	11.30	2.20	19.00	56.00	0.25

From rural laboratory of Maringá– Maringá, Paraná (2011).

and geranial mixture) as the main constituent (Andrade et al., 2009). This terpenes act in the plant defense and can inhibit phytopathogenic fungi growth (Martins et al., 2004; Castro et al., 2007; Adamczyk et al. 2013) presenting no toxicity to humans (Erler et al., 2006).

However, the quality and quantity of essential oil compounds are often affected by environmental conditions (Martins et al., 2006; Blank et al., 2007; Nogueira et al., 2007). Gobbo-Neto and Lopes (2007), confirming that collection time of medicinal plant is one of the most important factors, since the amount and even the nature of active constituents is not constant throughout the year.

According to Costa et al. (2005), the steps of harvest and postharvest affect the chemical compounds' variation in medicinal plants, as well as, assist in obtaining a raw material with quality, resulting in the good quality of the final product. Also, according to Fennell et al. (2004), the chemical changes during postharvest periods of medicinal plants may affect essential oils or crude aqueous extracts' antimicrobial property.

The fungitoxicity of essential oils has been proven in several studies (Carnelossi et al., 2009; Benini et al., 2010; Moura et al., 2012; Sarmento-Brum et al., 2013), and according to Derbalah et al. (2012) the use of essential oils as antimicrobials is considered at low risk, because it is believed that it is difficult for a pathogen to develop resistance to the complex mixture of active components which comprise up the essential oils.

Thus, the current study aimed to assess the effect of harvest on the chemical composition and the fungitoxicity of *C. citratus*' essential oil harvest at different seasons of the year on *Colletotrichum gloeosporiodes* (Penz.) Penz. & Sacc.

MATERIALS AND METHODS

The study was conducted in an experimental field at the State University of Maringá (UEM), Maringá-Paraná, Brazil, with geographic coordinates 23°23'18"S, 51°54'59"O and 526 m of altitude. The regional climate is classified as Cfa, subtropical, with hot summers, uncommonly frosts and a tendency of rainfall concentration during the summer, yet no dry season defined, according to the Köppen's classification (Neto, 2010). Soil chemical analyzes were performed before the experiment. Samples of soil were collected at a depth of 20 to 40 cm at different points, resulting in a composed sample. The pH values and concentrations of chemical elements are found in the soil results in Table 1. The soil is characterized as eutrophic Red Oxisol (Souza and Gasparetto, 2010).

Cultivation of *C. citratus* seedlings

The seedlings were propagated by stem cuttings using polythene bags (10 × 20 cm), with 500 ml capacity of substrate (Red Oxisoleutroferric + sand + commercial substrate humus-based (2: 1: 1)). They remained in a greenhouse for one week before planting. Then the seedlings were removed from the greenhouse and relocated to an acclimatization area. The seedlings' transplant was conducted at the beginning of June, 2011, in single beds of 50 m^2. The plants leaves were harvested randomly during the different seasons of the year.

Harvesting of *C. citratus* leaves

The harvesting of *C. citratus* leaves were carried out in late summer (March, 2012) nine months after transplanting the seedlings, in late fall (plants with twelve months of age), late winter (plants with fifteen months) and in late spring (plants eighteen months), with a total of four harvesting. The essential oil was extracted from both freshly harvested and dried leaves, in a total of eight extractions. For each sample was weighed 4 kg of leaves and 50% were placed for natural drying in the shade at room temperature for four days to obtain the dried leaves, during this period the leaves lost an average of 57% of its initial content. The other 50% of freshly harvested leaves remained were used for essential oil extraction. All samples were harvested in the late afternoon, between 4:30 and 5 p.m. Climate data (average temperature, relative humidity and rainfall recorded in the previous thirty days of collection) at the moment of harvest and the leaves' drying are described in Table 2. These data were obtained from the Experimental Farm of Iguatemi (FEI / UEM) meteorological station and Seeds analysis and Medicinal plants Laboratory.

Essential oil extraction

The essential oil was obtained using the extraction process by steam distillation, which consists of placing the plant material in the distiller, then by passing the plant material, the steam drags the essential oil (volatile) out of the plant; it passes the condenser where it is collected by a separatory funnel, then the immiscible liquids of different densities separated naturally; removal from the container is made through a stopcock. The extraction process had duration of four hours from the beginning of the first condensate drop (Worwood, 1995). The essential oil was stored in amber glass container in the refrigerator at a temperature of 4°C (+/- 2°C). The essential oil harvested was weighed on an analytical scale for later yield calculation, which was expressed in percentage by the ratio between the amount of used distillable leaves and the amount of essential oil produced(g/g) (Furlan et al., 2010).

Table 2. Climate data (average temperature, rain fall and relative humidity) in the harvest seasons. FEI / UEM laboratories –Maringá, 2012.

Harvest seasons	Temperature (°C)	Rain fall (mm)	Relative humidity (%)
Field			
Summer	26 (+/-1)	90	58 (+/-5)
Fall	15 (+/-1)	344	67 (+/-5)
Winter	27 (+/-1)	4.8	55 (+/-5)
Spring	28 (+/-1)	113	68 (+/-5)
Laboratory			
Summer	27 (+/-2)	-	65 (+/-5)
Fall	18 (+/-2)	-	69 (+/-5)
Winter	30 (+/-2)	-	47 (+/-5)
Spring	30 (+/-2)	-	66 (+/-5)

*Rainfall recorded in the previous 30 days to harvest.

Essential oil qualitative analysis

Qualitative analysis was determined by gas chromatography coupled to mass spectrometry (GC-MS) and determination of their Kovats retention indices.

Gas chromatography coupled with mass spectrometry

The analyses of eight essential oils resulting from the extractions were performed using a GC-MS system in the Department of Analytical Chemistry of the State University of Maringá (UEM), Paraná, Brazil. The chromatograph used was FOCUSGC model ("Thermo Electron"), equipped with a fused silica capillary columnDB-5ms (30 mm x 0.25 mm, 0.25 µm of stationary phase) and mass spectrometer model DSQII ("Thermo Electron"). The operating conditions were: injector temperature 200°C; drag gas flow (helium) 1.0 mL min^{-1}; injection volume of 0.5 µL (dichloromethane solution) withdivision of flux ("split") at a ratio of1:40. The programming of oven temperature was 50°C for 1 min, elevated to 180°C at a rate of 3°C min^{-1} and180°C to 240°C at a rate of 10°Cmin^{-1}, remaining at 240°C for 5 min. Mass spectrum were obtained by ionization of electron impact 70eV, in the range of 50 to 550 mz^{-1}; with an inter face temperature of 250°C and ion source of 200°C (Maia et al., 2013).

Kovats retention index

The calculation of Kovats retention index (KI) was based regarding the homologous series of n-alkanes, analyzed under the same experimental conditions. The retention indices of the compounds were determined according to the equation proposed by Van den Dool and Kratz (1963). The identification of main compounds was performed based on a comparison of its mass spectrum with those calculated from the mass spectrum of the spectral library" NIST MS Search2.0" contained in the soft ware "Xcalibur" accompanying the appliance, comparing their Kovats retention indices obtained experimentally with Kovats retention index data obtained from the literature (Boulanger et al., 1999; Beaulieu and Grimm, 2001; Siegmung et al., 2001; Jordán et al., 2003) for the same compounds analyzed using the same column. Quantitative data of compounds were obtained from percentages of the chromatogram are as through normalization.

Essential oil fungi toxicity analysis

The phytopathogen *C. gloeosporioides* (Penz.) Penz. & Sacc was isolated from mango fruit presenting typical anthracnose symptoms. Fragments from fruit were transferred into petri plates containing PDA growth medium (potato, dextrose, agar) which was maintained at 25°C in biochemical oxygen demand incubator (BOD) for five days. Later the plates with mycelia were transferred to a room with continuous light to stimulate sporulation.

After the formation of their productive structures (spores mass with rose color) *C. gloeosporioides* were transferred to new plates and incubated at 25°C in BOD. After seven days of incubation, *C. gloeosporioides* isolates were used in separate trials. The essential oil was tested at concentrations of 0 µL mL^{-1}, 0.025 µL mL^{-1}, 0.05 µL mL^{-1}, 0.075 µL mL^{-1} e 0.1 µL mL^{-1}, increased by 0.25µL mL^{-1}Tween 20 (emulsifying agent), to inhibit the growth and sporulation of *C. gloeosporioides*. The essential oils at the different concentrations were added to PDA growth medium after sterilized and still liquefied, poured into petri plates. After PDA solidification, a mycelialdisc (7 mm diameter) of the isolated fungus was placed in the plates center, sealed with plastic wrap and kept in a growth chamber at 25°C and photoperiod of 12 h.

From the second day after replication, evaluations were performed every 24 h until one of the isolates reached the plates edge (144 h). The mycelial growth was measured by the colonies' radius in two perpendicular directions, using a digital paquimeter. With the obtained data it was determined the mycelia growth area of the isolated fungus, from this we calculated the percentage of my celial growth inhibition (MGI), using the formula of Bastos (1997).

For each treatment, five plates were replicated each plate represented a repetition in a completely randomized design. Data were subjected to analysis of variance (ANOVA) and when significant regression equations were applied, adjusted with a coefficient of 5% probability and the coefficient of determination (R^2). Analyses were performed using SISVAR software (Ferreira, 2011).

RESULTS AND DISCUSSION

Essential oil qualitative and quantitative analysis

The highest yield was obtained in the extraction of freshly

Table 3. *Cymbopogon citratus* essential oil yield of freshly harvested and dried leaves harvested in different seasons (Maringá, 2012).

Harvest season	Yield (%)	
	Fresh mass	Dried mass
Summer	0.25	0.43
Fall	0.16	0.48
Winter	0.20	0.44
Spring	0.21	0.29

harvested leaves essential oil in summer harvest (0.25%), followed by yields of essential oils collected in the spring, winter and autumn seasons with income 0.21, 0.20 and 0.16%, respectively (Table 3). According to Nascimento et al. (2003), the essential oil yield of *C. citrates* is 0.28 to 0.50% of freshly harvested weight, higher than those found in this study. They observed that collection during the morning period (between 9 and 11 a.m.) showed higher oil yield compared to other periods. Similar results were observed by Marco et al. (2002). This explains (or justify) the lower values found in this study.

The yield variation between same species plants is attributed mainly to differences in harvest season, soil type, region climate and relative humidity (Burt, 2004). The seasonal variation of secondary metabolites can be caused by physiological demands as growth, defense and plant reproduction, as well as differences in the environment as water stress, light, nutrient deficiency and extreme temperatures. The study of this variation is very important as it allows knowing the exact time, approximately, in which some constituents are in greater proportion (Kumar et al., 2011).

According to Silva and Casali (2000), decreasing the amount of water in the plant, the amount of active ingredients in terms of dry mass increases. Justifying the findings of this study, where the highest yield values were observed in essential oil resulting from dried leaves extracts comparing to freshly harvested leaf extracts (Table 3). Yield was expressed by the ratio between the mass of distillable leaves used and the amount of essential oil produced (g/g), thus decrease the dried leaves mass (57%) has optimized performance values. The results also agree with Martins et al. (2002), who observed an increase in oil yield of 21% when compared to essential oil amount extracted from the dried leaves to that obtained from freshly harvested product of *C. citratus*.

For the variation observed in the essential oil yield of freshly harvested mass, from summer to fall harvest, it might have been caused by the change in micro climate parameters of temperature and rain fall recorded in the thirty days prior to harvest. The excess of rain during the thirty days prior to fall harvest (344 mm) provided a decrease in secondary metabolites accumulation in plants. According to the results of Koshima et al. (2006), when they analyzed the essential oil yield extracted from freshly harvested leaves of *C. citratus* grown with mulch and harvested in all seasons of the year, found a higher yield of essential oil in the period with lower rainfall.

In relation to dry mass to yields, fall presented extraction yield of 0.48% and spring presented the lowest yield of 0.29%. In summer and winter extraction yield was 0.43 and 0.44% respectively (Table 3). The lower essential oil yield extracted from the dried leaves during the spring compared to fall, is possible due to temperature differences. During the drying process in fall, it had mild temperature of 18°C, whereas during the spring it had a higher temperature (30°C) (Table 2). Blanco et al. (2002), studied the drying temperature effect on the essential oil yield of *Mentha piperita* and observed that drying temperature elevation caused a reduction in essential oil content. As explained by Calixto (2000), some plants constituents are labile when exposed to heat, thus plants containing them must be dried at low temperatures. In the drying process, water loss occurs naturally, but can also result in volatiles loss depending on the drying temperature. Also, Ortiz et al. (2002) related the essential oil yields with variations in temperature and found that the increase or decrease on it has a direct influence at essential oil yield. In chromatographic, analysis was possible to identify six mainly compounds in the essential oil of *C. citratus*: myrcene; linalool; verbenol; neral; geranial and 2-undecanone (Table 4).

The most abundant compound in the essential oil of *C. citratus*, obtained in different seasons, was the citral composed by a mixture of neral and geranial isomers, this had a higher area in the essential oil from freshly harvested leaves in spring (65.6%), followed myrcene presented an area of 21.48%. For its part, the essential oil extracted from dried leaves in winter, myrcene compound showed greater area of 41.28% and citral with an area of 44.94% (Table 3). These results agree with other authors, who claim the presence of citral as a major component of *C. citratus* essential oil (Martins et al., 2002; Leal et al., 2003; Costa et al., 2005; Furlan et al., 2010; Kumar et al., 2013; Pinto et al., 2015).

Lima et al. (2008), found that essential oil of *C. citratus* presented geranial (43.8%), neral (34.5%) and myrcene (14.6%) as major compounds. Barbosa et al. (2008), reported a variation in citral levels of the essential oil marketed in Brazil from 40.7 to 75.4% and myrcene from 0.24 to 7.29%, being the myrcene levels below what is found in the essential oils studied. Early investigation of the leaf essential oil of *C. citrates* from Rio de Janeiro were also identified and quantified geranial with 53.2% and neral with 36.37% as the major components but the monoterpene myrcene was not observed (Pinto et al., 2015).

Still analyzing the compounds content of the essential oil from freshly harvested leaves it can be observed that, citral presented a larger area (65.56 and 65.49%) in the

Table 4. The main components contents of *C. citratus* essential oil coming from the freshly harvested and dried leaves extractions, harvested at four seasons, identified by GC-MS and expressed as normalization area (%).

Extractions seasons		Compounds (KI cal / Yield (%))					
		Myrcene	**Linalool**	**Verbenol**	**Neral**	**Geranial**	**2-Undecanone**
Summer	FL	992 / 20.15	1101 / 1.15	1184 / 2.34	12411 / 28.94	1271 / 36.55	1294 / 0.19
	DL	991 / 25.70	1101 / 1.20	1184 / 2.21	1241 / 27.02	1271 / 34.90	1294 / 0.27
Fall	FL	991 / 28.90	1100 / 1.28	1183 / 1.67	1241 / 25.57	1271 / 32.58	1294 / 0.80
	DL	991 / 28.23	1101 / 1.25	1184 / 2.02	1241 / 26.31	1271 / 33,87	1294 / 0.74
Winter	FL	991 / 25.35	1100 / 1.41	1183 / 2.28	1241 / 26.30	1271 / 34.71	1294 / 0.60
	DL	992 / 41.28	1101 / 2.21	1184 / 1.70	1241 / 19.29	1271 / 25.65	1294 / 0.89
Spring	FL	991 / 21.48	1100 / 1.12	1183 / 2.25	1241 / 28.40	1271 / 37.16	1294 / 0.27
	DL	992 / 32.25	1102 / 1.24	1184 / 1.33	1242 / 16.75	1272 / 22.28	1295 / 0.25
KItab	-	991	1101	1188	1240	1270	1294

KIcal: Kovats retention index obtained experimentally; KI tab: Kovats retention index data obtained from the literature. FL: freshly harvested leaves; DL: dried leaves.

spring and summer seasons, respectively, when compared to fall and winter (58.15 and 61%). However, there was not just the increase of citral content in these conditions, but also the decrease of myrcene content (21.48 and 20.15%), while in fall and winter presented area of 28.9 and 25.35%, respectively (Table 4).

This change may be caused by micro climate factors (Gobbo and Lopes,2007) and mostly rainfall, since in the climatologic seasons rainfall records presented median (113 and 90 mm) in the thirty days prior to harvest, while in the fall and winter the indexes present significant variation (344 and 4.8mm) respectively (Table 2).

Essential oil fungitoxicity

The essential oil extracted from freshly harvested and shaded dried leaves harvested in all the four seasons, presented significant inhibition on the mycelial growth of *C. gloeosporioides* with a 29% coefficient of variation (Figures 1, 2, 3 and 4). According to Pereira et al. (2011), essential oils can inhibit or reduce phytopathogens growth due to the action of substances present in its composition.

Guerra et al. (2000), attribute the antimicrobial and antifungal activities of *C. citrates* essential oil to citral monoterpene. Guimarães et al. (2011), observed that myrcene compound has no fungi toxic activity, since its celial inhibition was equal to zero for all phytopathogens. The essential oils obtained from the eight extractions, it was observed that the variation in the chemicals levels, mainly citral (neral and geranial mixture) to which the anti fungal action is assigned, had no effect at the fungitoxicity of the essential oil on *C. gloeosporioides.* It was demonstrated that even the lowest citral content (39.03%), found in the essential oil from the dried leaves in spring, showed high fungi toxicity (99%) (Figure 4B). In relation to citral, Guimarães et al. (2011) also demonstrate the citral fitotoxicity and its importance activity in the *C. citrates* essential oil. Lee et al. (2008), observed that compounds as citronellal, neral, geraniol and geranial at a concentration of 28 x 10^{-3} mg $mL^{-1}$of air are able to inhibit 100% mycelial growth of *Phytophthora cactorum.*

It was observed that the essential oil obtained in all extraction seasons demonstrated to be efficient in the control of the phytopathogen mycelial growth. The effect expressed as quadratic for the majority of samples with mycelial growth inhibition (MGI) of 102% at a 0.8 µL mL^{-1} concentration (Figure 1A), of 103% ata 1 µL mL^{-1} concentration (Figure 1B), of 113% at a 0.7µL mL^{-1}concentration (Figure 3A) and 99% at a 0.9 µL mL^{-1} concentration. The essential oil of freshly harvested leaves harvested in fall, although it had a cubic effect, also obtained MGI maximum point of 97% at a 0.9 µL mL^{-1}concentration and with minimum inhibition (1%) at a 0.078 µL mL^{-1}concentration 1 (Figure 2A). The mycelia growth rate of *C. gloeosporioides* in the presence of *C. citrates* essential oil from dried leaves, harvested in the fall and winter, and freshly harvested leaves, harvested in the spring, has expressed positively linear form depending on the essential oil doses, in other words, it presented dose-dependent effect (Figure 2B, 3B and 4A).

These results confirmed the *C. citratus* essential oil potential in control of the phytopathogen that causes anthracnose in mango fruits. Santos et al. (2013), also verified that the *C. citrates* essential oil was effective in reducing the mycelia growth of *Helminthosporium* sp.

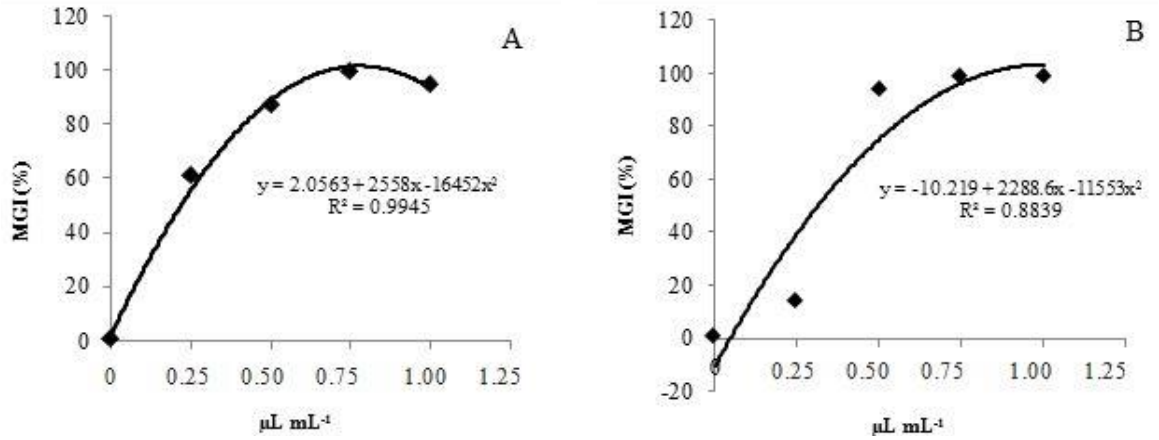

Figure 1. *C. gloeosporioides* mycelial growth inhibition (MGI %) in presence of *C. citratus* essential oil obtained from freshly harvested (A) or dried (B) leaves harvested in summer.

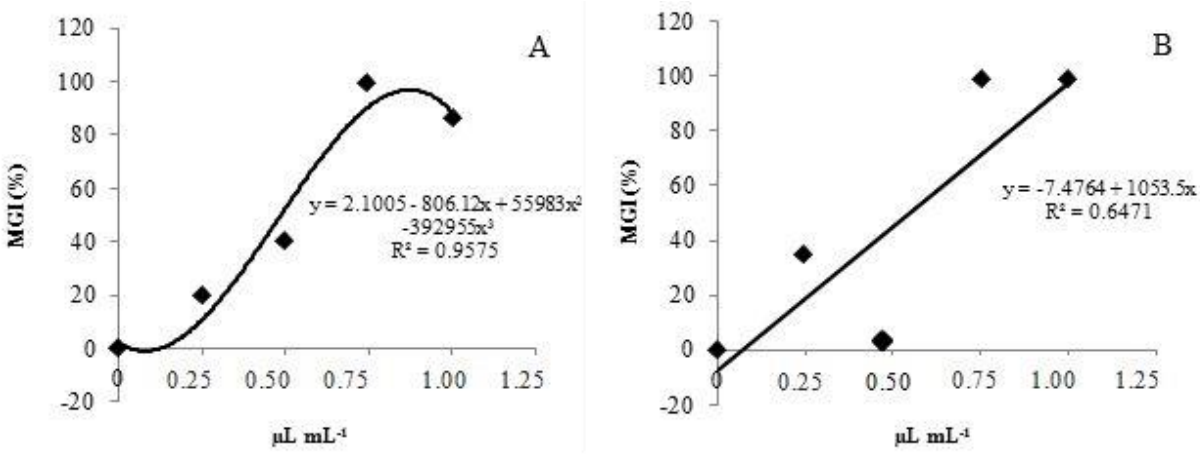

Figure 2. *C. gloeosporioides* mycelial growth inhibition (MGI %) in presence of *C. citratus* essential oil obtained from freshly harvested (A) or dried (B) leaves harvested in fall.

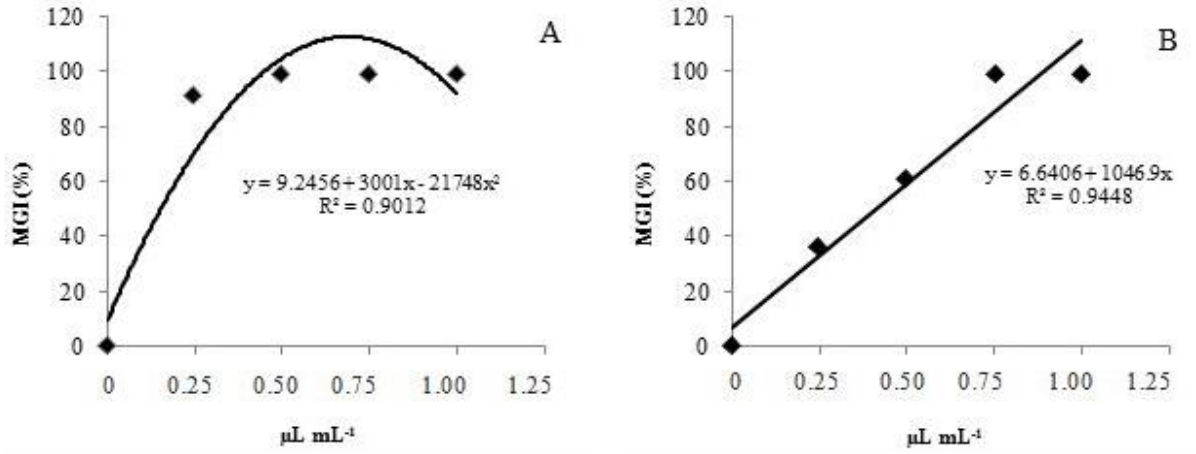

Figure 3. *C. gloeosporioides* mycelial growth inhibition (MGI %) in presence of *C. citratus* essential oil obtained from freshly harvested (A) or dried (B) leaves harvested in winter.

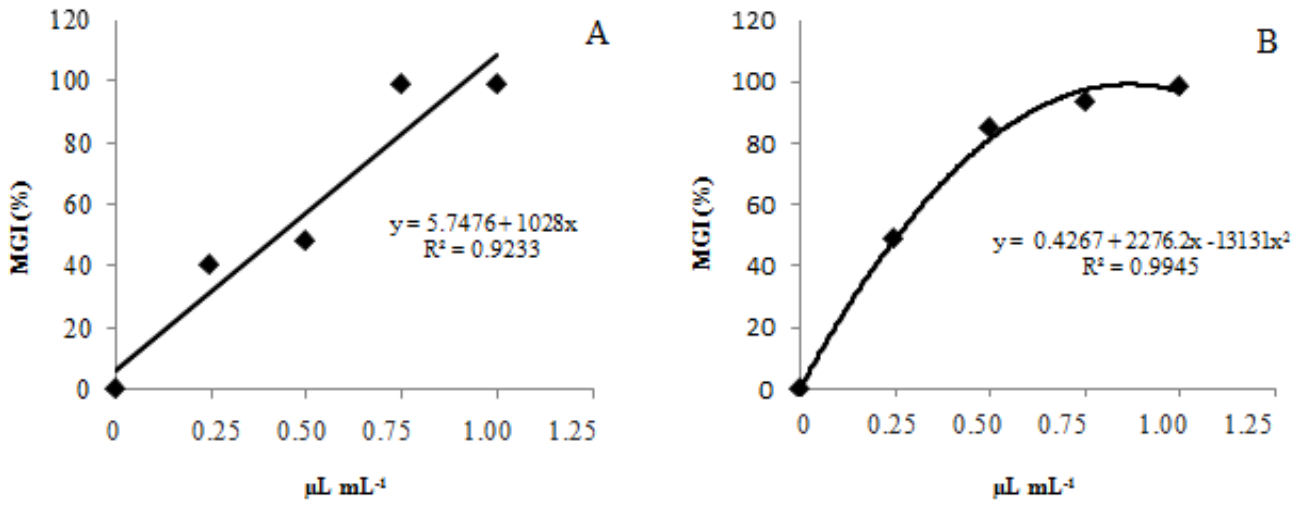

Figure 4. *C. gloeosporioides* mycelial growth inhibition (MGI %) in presence of *C. citratus* essential oil obtained from freshly harvested (A) or dried (B) leaves harvested in spring.

5 and 7 µL mL^{-1}).

Conclusion

The results presented in the current study, corroborated with the cited literature demonstrate that the *C. citratus* essential oil has effective control of *C. gloeosporioides,* due to the activity of chemical compounds present in this oil.

Conflicts of interest

The authors have not declared any conflict of interest.

REFERENCES

Adamczyk S, Kiikkilä O, Kitunen V, Smolander A (2013). Potential response of soil processes to diterpenes, triterpenes and tannins: Nitrification, growth of microorganisms and precipitation of proteins. Appl. Soil Ecol. 67(2013):47-52.

Andrade EHA, Zoghbi MGB, Lima MD (2009).Chemical composition of the essential oils of *Cymbopo goncitratus* (DC.)Stapf cultivated in North of Brazil. J. Essent. Oil-Bear. Plants 12(1):41-45.

Aquino CF, Sales, NLP, Soares EPS, Martins ER, Costa CA (2014). Composição química e atividade *in vitro* de trêsóleos essenciais sobre *Colletotrichum gloeosporioides* do maracujazeiro. Rev. Bras. Plantas Med. 16(2):329-336.

Barbosa LCA, Pereira UA, Martinazzo AP, Maltha CRA, Teixeira RR, Melo EC (2008).Evaluation of the chemical composition of Brazilian commercial *Cymbopo goncitratus* (D. C.) Stapf samples. Molecules 13(8):1864-1874.

Bastos CN (1997). Efeito do óleo de *Piper aduncum* sobre *Crinipellis perniciosa* e outros fungos fitopatogênicos. Fitopatol. Bras. 22(3):441-443.

Beaulieu JC, Grimm CC (2001). Identification of volatile compounds in cantaloupe at various developmental stages using solid phase microextraction. J. Agric. Food Chem. 49(3):1345-1352.

Benini PC, Schwan-Estrada KRF, Klais EC, Cruz MÊS, Itako AT, Mesquini RM, Stangarlin JR, Tolentino Junior JB (2010). Efeito *in vitro* do oleo essencial de *Ocimum gratissimum* colhidona squatroestações do anosobrefitopatógenos. Arq. Inst. Biol. 77(4):677-683.

Blanco MCSG, Ming LC, Marques MOM, Bovi OA (2002). Drying temperature effects in peppermint essential oil content and composition. Acta Hortic. 569(2002):95-98.

Blank AF, Costa AG, Arrigoni-Blank MF, Cavalcanti SCH, Alves PB, Innecco R, Ehlert PAD, Sousa IF (2007). Influence of season, harvest time and drying on Java citronella (*Cymbopo gonwinterianus* Jowitt) volatile oil. Rev. Bras. Farmacogn. 17(4):557-564.

Boulanger R, Chassagne D, Crouzet J (1999). Free and bound flavour components of amazonian fruits. 1: Bacuri. Flavour Fragr. J. 14(5):303-311.

Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods: A review. Int. J. Food Microbiol. 94(3):223-53.

Calixto JB (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytoterapeutic agents). Braz. J. Med. Biol. Res. 33(2):179-189.

Carnelossi PR, Schwan-Estrada KRF, Cruz MÊS, Itako AT, Mesquini RM (2009). Óleos essenciais no controlepós-colheita de *Colletotrichum gloeosporioides* emmamão. Rev. Bras. Plant. Med. 11(4):399-406.

Castro HG, Barbosa LCA, Leal TCAB, Souza CM, Nazareno AC (2007). Crescimento, teor e composição do óleoessencial de *Cymbopogonnardus* (L.). Rev. Bras. Plant. Med. 9(4):55-61.

Correa AD, Siqueira-Batista R, Quintas LEM (1998). Plantas Medicinais: do cultivo à terapêutica. Petrópolis, RJ: Vozes. P 246.

Costa LCB, Corrêa RM, Cardoso JCW, Pinto JEBF, Bertolucci K, Ferri PH (2005). Secagem e fragmentação da matériaseca no rendimento e composição do óleoessencial de capim-limão. Hortic. Bras. 23(4):956-959.

Derbalah AS, Dewir YH, El-Sayed AE (2012). Antifungal activity of some plant extracts against sugar beet damping-off caused by *Sclerotiumrolfsii*. Ann. Microbiol. 62(3):1021-1029.

Erler F, Ulug I, Yalcinkaya B (2006). Repellent activity of five essential oils against *Culexpipiens*. Fitoterapia 77(7-8):491-494.

Fennell CW, Light ME, Sparg SG, Stafford GI, Staden JV (2004). Assessing African medicinal plants for efficacy and safety: agricultural and storage practices. J. Ethnopharmacol. 9(95):113-121.

Ferreira DF (2011). Sisvar: a computer statistical analysis system. Ciênc. Agrotec. 35(6):1039-1042.

Furlan MR, Martins RCC, Rodrigues E, Scalco N, Negri G, Lago JHG (2010). Variação dos teores de constituintesvoláteis de

Cymbopogoncitratus (DC) Staf, Poaceae, coletadosem diferent esregiões do Estado de São Paulo. Rev. Bras. Farmacogn. 20(5):686-691.
Gobbo-Neto L, Lopes NP (2007). Plantasmedicinais: fatores de influência no conteúdo de metabólitossecundários. Quim Nova 30(2):374-381.
Guerra MJM, Badell JB, Albajes ARR, Pérez HB, Valencia RM, Azcuy AL (2000). Evaluación toxicological aguda de losextractos fluidos al 30 y 80% de *Cymbopogon citratus* (D.C.) Stapf (Caña Santa). Rev. Cub. Plant. Med. 5(3):97-101.
Guimarães LGL, Cardoso MG, Sousa PE, Andrade J, Vieira SS (2011).Atividades antioxidante e fungitóxica do óleoessencial de capim-limão e do citral. Rev. Cienc. Agron. 42(2):464-472.
Jordán MJ, Martínez RM, Cases MA, Sotomayor JA (2003). Watering level effect on *Thymus hyemalis*lange essential oil yield and composition. J. Agric. Food Chem. 51(18):5420-5427.
Koshima FAT, Ming LC, Marques MOM (2006). Produção de biomassa, rendimento de óleoessencial e de citralemcapim-limão, *Cimbopogon citratus* (D. C.) Stapf, com coberturamortanasestações do ano. Rev. Bras. Plant. Med. 8(4):112-116.
Kumar P, Mishra S, Malik A, Satya S (2011). Insecticidal properties of Mentha species: A review. Ind. Crops Prod. 34(1):802-817.
Kumar P, Mishra S, Malik A, Satya S (2013). Housefly (*Musca domestica* L.) control potential of *Cymbopogon citratus* Stapf. (Poales: Poaceae) essential oil and monoterpenes (citral and 1, 8-cineole). Parasitol. Res. 112(1):69-76.
Leal TCAB, Freitas SP, Silva JF, Carvalho AJC (2003). Produção de biomassa e óleoessencialemplantas de capimcidreira [*Cymbopogon citratus* (DC.)Stapf] emdiferentesidades. Rev. Bras. Plant. Med. 5(2):61-64.
Lee YS, Kim J, Shin SC, Lee SG, Park IK (2008). Antifungal activity of Myrtaceae essential oils and their components against three phytopathogenic fungi. Flavour Fragr. J. 23(1):23-28.
Lima RK, Cardoso MG, Moraes JC, Vieira SS, Melo BA, Filgueiras CC (2008). Composição dos óleosessenciais de Anis-estrelado *Illiciumverum* L. e do Capim-limão *Cymbopogoncitratus* (DC.) Stapf: Avaliação do efeitorepelentesobre *Brevicorryne brassicae* (L.) (Hemiptera: Aphididae). BioAssay 3(8):1-6.
Maia AJ, Schwan-Estrada KRF, Botelho RV, Jardinetti VA, Faria CMDR, Batista AF, Costa WF (2013). Bud break and enzymatic activity in buds of grapevines cv. Ives treated with *Gallesiaintegrifolia*hydrolate. Acta Physiol. Plant. 35(9):2727-2735.
Marco CA, Nascimento IB, Innecco R, Mattos SH, Nagão EO (2002). Efeito de horário de cortenaprodução de óleoessencial de capim-santo (*Cymbopogoncitratus* DC.). Hortic. Bras. 20(2): Supl. 2.
Martins FT, Santos MH dos, Polo M, Barbosa LCA (2006). Variaçãoquímica do óleoessencial de *Hyptissuaveolens* (L.) POIT.sobcondições de cultivo. Quim. Nova 29(6):1203-1209.
Martins MBG, Martins AR, Telascrêa M, Cavalheiro AJ (2004). Caracterizaçãoanatômica da folha de *Cymbopo goncitratus* (CD) Stapf (Poaceae) e perfilquímico do óleoessencial. Rev. Bras. Plant. Med. 6(3):20-29.
Martins PM, Melo EC, Almeida LCB, Santos RHS, Machado MC (2002). Influência da temperatura e velocidade do ar de secagem no teor e composiçãoquímica do óleoessencial de capim-limão. Acta Hortic. 569(2002):155-160.
Moura GS, Schwan-Estrada KRF, Alves APF, Franzener G, Stangarlin JR (2012). Controle da antracnoseemmaracujá-amareloporderivados de capim-limão (*Cymbopogoncitratus*). Arq. Inst. Biol. 79(3):371-379.
Nascimento IB, Nagao EO, Innecco R, Matos SH (2003). Efeito do horário de corte no óleoessencial de capim-santo. Rev. Cienc. Agron. 34(2):169-172.
Neto ARS (2010). Análise da conjunturaagropecuáriasafra 2010/11.Agrometeorologia. Secretaria da agricultura e do abastecimento do estado do Paraná-SEAB. pp. 1-11.
Nogueira MA, Diaz G, Sakumo L (2007). Caracterizaçãoquímica e atividadebiológica do óleoessencial de *Lippiaalba*cultivada no Paraná. Rer. Cienc. Farmac. Basic. Apl. 28(3):273-278. http://serv-bib.fcfar.unesp.br/seer/index.php/Cien_Farm/article/viewFile/239/234
Ortiz RS, Marrero GV, Navarro ALT (2002). Instructivotécnico para el cultivo de *Cymbopogoncitratus* (DC.) Stapf (Caña Santa). Rev. Cub. Plant. Med. 7(2):89-95.
Pereira RB, Lucas GC, Perina FJ, Resende MLV, Alves E (2011). Potential of essential oils for the control of brown eye spot in coffee plants. Cienc. Agrotecnol. 35(1):115-123.
Pinto ZT, Sánchez FF, Santos AR, Amaral ACF, Ferreira JLP, Escalona-Arranz JC, Queiroz MMC (2015). Chemical composition and insecticidal activity of *Cymbopogoncitratus* essential oil from Cuba and Brazil against housefly. Braz. J. Vet. Parasitol. 24(1):36-44.
Santos GR, Brum RBCS, Castro HG, Gonçalves CG, Fidelis RR (2013). Efeito de óleosessenciais de plantasmedicinaissobre a helmintosporiose do capimTanzânia. Rev. Cienc. Agron. 44(3):587-593.
Sarmento-Brum RBC, Santos GR dos, Castro HG, Gonçalves CG, Júnior FC, Nascimento IR do (2013). Effect of essential oils of medicinal plants on the anthracnose of sorghum. Biosci. J. 29(5):1549-1557.
Siegmung B, Derler K, Pfannhauser W (2001). Changes in the aroma of a strawberry drink during storage. J. Agric. Food Chem. 49(7):3244-3252.
Silva F, Casali VWD (2000). Plantasmedicinais e aromáticas: Pós-colheita e óleosessenciais. Viçosa: Arte e Livros. P 135.
Souza de V, Gasparetto NVL (2010). Evaluation Some Classes of Erodibility Soil of Maringá – PR Through Phisical and Geotechnical Analyses. Bol. Geogr. 28(2):5-16.
Souza Filho AP, Alves SM (2002). Alelopatia: Princípiosbásicos e aspectosgerais. Belém: Embrapa Amazônia Oriental. P 260.
Souza VC, Lorenzi H (2005). Botânicasistemática: guiailustrado para identificação das famílias de Angiospermas da flora brasileira, baseadoem APG II. Nova Odessa, São Paulo: Plantarum. P 640.
Tyagi AK, Gottardi D, Malik A, Guerzoni ME (2014). Chemical composition, *in vitro* anti-yeast activity and fruit juice preservation potential of lemon grass oil. Food Sci. Technol. 57(2):731-737.
Tyagi AK, Malik A (2012). Morphostructural Damage in Food-Spoiling Bacteria due to the Lemon Grass Oil and Its Vapour: SEM, TEM, and AFM Investigations. Evid. Based. Complement. Altern. Med. 2012(2012):12.
Van Den Dool H, Kratz DP (1963). A generalization of the retention index system including liner temperature programmed gas-liquid partition chromatography. J. Chromatogr. 11(1963):463-467.
Verma RS, Padalia RC, Chauhan A (2013). Introduction of Cymbopogondistans (Nees ex Steud.) Wats to the sub-tropical India: Evaluation of essential-oil yield and chemicalcomposition during annual growth. Ind. Crops Prod. 49(2013):858-863.
Worwood S (1995). Aromaterapia - um guia de A a Z para o usoterapêutico dos óleosessenciais. São Paulo: Best Seller. P 251.