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Effect of essential oils of medicinal plants on leaf blotch in Tanzania grass¹

Efeito de óleos essenciais de plantas medicinais sobre a helmintosporiose do capim Tanzânia

Gil Rodrigues dos Santos^{2*}, Rúbia Borges Cruz Sarmiento Brum³, Henrique Guilhon de Castro⁴, Clebson Gomes Gonçalves⁵ e Rodrigo Ribeiro Fidelis⁴

ABSTRACT - Leaf spots caused by phyto-pathogenic fungi, can reduce the production of forage plants. The essential oils of medicinal plants have antimicrobial potential. The objective here was to evaluate the fungotoxicity *in vitro* of the essential oils of lemongrass (*Cymbopogon citratus* (DC.) Stapf (Poaceae)), citronella (*Cymbopogon nardus* (L.) Rendle (Poaceae)), lemon balm (*Lippia alba* (Mill.) NE Br ex Britton & P. Wilson (Verbenaceae)) and peppermint (*Mentha piperita* L. (Lamiaceae)) on the fungus *Helminthosporium* sp. and the *in vivo* effect of these oils and of commercial neem oil (*Azadirachta indica* A. Juss. (Meliaceae)) on leaf blotch in *Panicum maximum* Jacq. cv. Tanzania-1. The mycelial growth of the fungus was evaluated over five periods (2; 4; 6; 8 and 10 days from incubation) and with five concentrations of essential oils ($C_1 = 250$ ppm; $C_2 = 500$ ppm; $C_3 = 750$ ppm; $C_4 = 1,000$ ppm e $C_5 = 1,250$ ppm). As an alternative control, the preventative and curative effect on leaf blotch of five oil-based treatments were evaluated: lemongrass, citronella, lemon balm, peppermint and neem in four concentrations (2,500; 5,000; 7,500 and 10,000 ppm). The essential oils of lemongrass and citronella were the most effective in reducing mycelial growth of *Helminthosporium* sp. With the essential oil of lemongrass, the pathogen presented the highest growth concentration (1.250 ppm). The results obtained showed that all the essential oils and concentrations tested presented a preventive and curative effect, reducing the severity of leaf blotch.

Key words: Forage plants. Essential oils. *Helminthosporium* sp..

RESUMO - Manchas foliares, causadas por fungos fitopatogênicos, podem reduzir a produção de forrageiras. Óleos essenciais de plantas medicinais apresentam potencial antimicrobiano. Objetivou-se avaliar a fungitoxicidade *in vitro* dos óleos essenciais de capim-limão (*Cymbopogon citratus* (DC.) Stapf (Poaceae)), citronela (*Cymbopogon nardus* (L.) Rendle (Poaceae)), erva-cidreira (*Lippia alba* (Mill.) N.E. Br. ex Britton & P. Wilson (Verbenaceae)) e hortelã-pimenta (*Mentha piperita* L. (Lamiaceae)) sobre o fungo *Helminthosporium* sp. e o efeito *in vivo* desses óleos e do óleo comercial de nim (*Azadirachta indica* A. Juss. (Meliaceae)) sobre a helmintosporiose de *Panicum maximum* Jacq. cv. Tanzânia-1. Foi avaliado, em cinco períodos (dois, quatro, seis, oito e dez dias de incubação), o crescimento micelial do fungo sob cinco concentrações dos óleos essenciais ($C_1 = 250$ ppm; $C_2 = 500$ ppm; $C_3 = 750$ ppm; $C_4 = 1000$ ppm e $C_5 = 1250$ ppm). Como controle alternativo, foi avaliado o efeito preventivo e curativo sobre a helmintosporiose de cinco tratamentos à base de óleo: capim-limão, citronela, erva-cidreira, hortelã-pimenta e nim em quatro concentrações (2500; 5000; 7500 e 10000 ppm). Os óleos essenciais de capim-limão e citronela foram os mais eficientes na redução do crescimento micelial do *Helminthosporium* sp. Sob o óleo essencial de erva-cidreira, o patógeno apresentou crescimento até na maior concentração (1250 ppm). Pelos resultados obtidos verificou-se que todos os óleos essenciais e concentrações avaliadas apresentaram efeito preventivo e curativo, reduzindo a severidade da helmintosporiose.

Palavras-chave: Plantas forrageiras. Óleos essenciais. *Helminthosporium* sp..

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INTRODUCTION

Leaf spots are very common in important agricultural grasses, with forage grasses being worthy of note among the species of the Poaceae family. Species of the genus *Panicum*, are among those grasses most used as forage for cattle, principally the 'Vendor' and 'Tanzania' cultivars of *Panicum maximum* (BATISTOTI *et al.*, 2011). Leaf spots caused by phytopathogenic fungi impair the rate of photosynthesis in the leaves, which can reduce crop production (GODOY; AMORIM; BERGAMIN FILHO, 2001; MARTINEZ; FRANZENER; STANGARLIN, 2010) with considerable infections by the fungus *Bipolaris maydis* (syn. *Helminthosporium maydis*) in the cv. 'Vendedor' and 'Tanzania' having been reported (MARTINEZ; FRANZENER; STANGARLIN, 2010).

Over recent years, attempts to prevent, control and eradicate plant disease have been centered on using synthetic chemicals. However, the continued and indiscriminate use of pesticides causes a host of environmental and human-health problems, such as the interruption of natural biological control (SOYLU; KURT; SOYLU, 2010), resistance by pathogens causing outbreaks of disease (LEE *et al.*, 2008) and contamination of ground and surface water (FERNANDES NETO; SARCINELLI, 2009).

Many plants have a natural resistance to different pathogens, and this mechanism may be related to fungicidal compounds produced naturally by the plant (SALGADO *et al.*, 2003). The identification of these chemical compounds from medicinal plants makes it possible to obtain substances which can control or inhibit the growth of phytopathogens (SILVA *et al.*, 2009).

Originating from the secondary metabolism in plants, essential oils have a complex chemical composition, the presence of terpenes and phenylpropanoids being of note, and are considered sources of biologically active substances, mainly against microorganisms (OLIVEIRA *et al.*, 2011; SIANI *et al.*, 2000).

The essential oil of the peppermint (*Mentha piperita* L. (Lamiaceae)) has high antimicrobial potential against phytopathogens *Aspergillus flavus* and *A. niger* (HUSSAIN *et al.*, 2010). The antimicrobial action of the oils from the neem (*Azadirachta indica* A. Juss. (Meliaceae)) and citronella (*Cymbopogon nardus* (L.) Rendle (Poaceae)) on soybean rust, caused by *Phakopsora pachyrhizi*, was observed by Medice *et al.* (2007). The essential oil of lemon grass (*Cymbopogon citratus* (DC.) Stapf (Poaceae)) inhibited the growth of the fungus *Colletotrichum gloeosporioides* (SILVA *et al.*, 2009). The main constituents of the essential oil of *C. citratus* already identified, are geranial and neral, while citronellal, geraniol and citronellol are the major constituents of citronella (OLIVEIRA *et al.*, 2011). Tagami *et al.* (2009) highlighted

the antifungal potential of lemon balm (*Lippia alba* (Mill.) N.E. Br ex Britton & P. Wilson (Verbenaceae)) on the mycelial growth of the fungus *Colletotrichum graminicola*.

Antifungal activity of essential oils is related to their hydrophobicity, which allows for interaction between the essential oil and lipids of the wall, cell membrane and mitochondria, this interaction altering permeability and causing disturbances in these structures (COSTA *et al.*, 2011). Soyulu, Kurt and Soyulu (2010) observed morphological changes in the hyphae of *Botrytis cinerea* when treated with the essential oil of the oregano. This cytotoxic property of essential oils is very important in the application of these plant extracts in agriculture, since they can be effective in controlling phytopathogens (BAKKALI, 2008).

Considering the impact of leaf blotch disease on grasses destined for pasture, the growing demand for alternative methods of control, and the antifungal potential of the essential oils from medicinal plants, the objectives of this work were to evaluate the fungi-toxicity *in vitro* of the essential oils of lemongrass, citronella, lemon balm and peppermint on the phytopathogen *Helminthosporium* sp., and the *in vivo* effect of these oils and the commercial neem oil (NEEMAX®) on leaf blotch in Tanzania grass.

MATERIAL AND METHODS

Trial I: *In vitro* fungitoxicity of essential oils on *Helminthosporium* sp.

The experiment was carried out at the Plant Pathology Laboratory of the Federal University of Tocantins, Gurupi Campus, in Tocantins.

The fungus *Helminthosporium* sp. was isolated from the leaves of Tanzania grass showing typical symptoms of leaf blotch. For the isolation, Petri dishes with 20 mL of a PDA (potato, dextrose and agar) culture medium were used.

To obtain the essential oils, leaves of lemongrass and citronella 172 days after transplanting, and flowering lemon balm leaves were collected. The leaves were dried at room temperature. Extraction was performed by hydro-distillation (CASTRO *et al.*, 2010), using a Clevenger apparatus. The supernatants were collected and stored in sterile flasks sealed with foil. The peppermint oil (DOKMOS - Cosmetics®) was purchased at the Gurupi Municipal Market.

For each essential oil, a completely randomized factorial design with four replications was used. The treatments consisted of five concentrations of oil (C_1 = 250 ppm, C_2 = 500 ppm, C_3 = 750 ppm, C_4 = 1,000 ppm and C_5 = 1,250 ppm) and a control (dishes containing only the PDA culture medium), and five periods of evaluation (two, four, six, eight and ten days of incubation).

To verify the effect of essential oils on the mycelial growth of phytopathogens, oils were spread over the surface of the culture medium at the above concentrations, with the aid of a Drigalsky handle. Then a disc of PDA, 6 mm in diameter, containing mycelia of the fungus was placed in the center of the dishes. The dishes were sealed with PVC plastic film, identified, and incubated in the dark at a temperature of 27 ± 2 °C.

Considering that the *Helminthosporium* sp. fungus presents slow growth, taking eight to 12 days to occupy the surface of a Petri dish of 90x15 mm, five evaluations were carried out (at two, four, six, eight and ten days of incubation) by measuring the mycelial diameter (the average of two diametrically opposite measurements), using digital calipers.

Trial II: *In vivo* fungitoxicity of essential oils on grass leaf blotch

The experiment was carried out at the Plant Pathology Laboratory and in greenhouses of the Federal University of Tocantins, Gurupi Campus, in Tocantins.

For the cultivation of the plants of *P. maximum* cv. Tanzânia, 42x27x7 cm plastics trays were used, kept at a temperature of 27 ± 5 °C in a greenhouse, with four litres of Germinar® commercial substrate and 10 g de NPK (5-25-15) fertiliser. Four rows were prepared per tray and 30 seeds were planted per row. The trays were watered twice daily with approximately 700 mL of water.

The oils used were obtained as described in Trial I.

For the preparation of the spore suspension, 15ml of distilled and sterile water were added to each dish containing an inoculum of the fungus. Detachment of the mycelium was carried out with the help of a soft-bristle brush. The suspension was filtered through gauze, and the spores were quantified in a Neubauer chamber.

To evaluate the preventive and curative effects on the grass leaf blotch, a completely randomized factorial design with four replications was used, in which the factors were five types of oil (citronella, lemongrass, lemon balm, peppermint and commercial neem - NEENMAX®), and four oil concentrations (2,500; 5,000; 7,500 and 10,000 ppm). As a control, plants sprayed with water (absolute) and plants sprayed with methyl thiophanate at 1,000 ppm (relative), a broad-spectrum fungicide, were used.

In order to evaluate the preventive effects, the plants were sprayed with 20 mL of the treatments using a hand spray 30 days after planting, and were inoculated after one hour with 20 mL of the spore suspension (1.1×10^5 spores mL⁻¹). After inoculation with the pathogen, the plants were kept in a moist chamber in the dark for 48 hours in the laboratory. After the incubation period, the plants were placed in a natural

environment at a temperature of 30 ± 5 °C, without any control over relative humidity or photoperiod, to enable the disease to develop. Severity assessment was performed nine days after inoculation, the period required for symptoms of blotch to have presented.

For the curative effect, the plants were inoculated with 20 mL of spore solution (1.09×10^5 spores mL⁻¹), the evaluation of the severity of the leaf blot being made after four days. After this period, the plants were sprayed with 20 mL of the treatments and kept in a natural environment, being re-evaluated after 15 days.

Disease severity was assessed using a rating scale: 0 = healthy plant; 1 = less than 1% diseased leaf area; 3 = 1-5% diseased leaf area; 5 = 6 to 25% diseased leaf area; 7 = 26 to 50% diseased leaf area; 9 = more than 50% diseased leaf area (SANTOS *et al.*, 2005).

Statistical Design

The data were subjected to variance (ANOVA) and regression analysis. For the qualitative factor, the means were compared by the Tukey test at 5% probability, and for the quantitative factor, regression equations were adjusted based on the t-test of the coefficients at 5% probability and of the coefficient of determination (R²). The analyses were performed by the SAEG computer system (RIBEIRO JÚNIOR, MELO, 2008).

RESULTS AND DISCUSSION

Trial I: *In vitro* fungitoxicity of essential oils on *Helminthosporium* sp.

There was no mycelial growth of the *Helminthosporium* sp. fungus with the treatments with the lemongrass and citronella oils at concentrations of ≥ 750 ppm (C₃, C₄ and C₅), but under the action of the lemon balm and peppermint oils, the pathogen presented growth at all the doses evaluated (Table 1). After 10 days of incubation, only the concentrations $\geq C_2$ (500 ppm) and $\geq C_3$ (750 ppm) of the lemon balm and peppermint oils respectively, had significantly reduced the mycelial diameter of the *Helminthosporium* sp. Variance analysis showed that the concentrations, evaluation periods and interaction between these factors was significant at 1% for the estimates of mycelial growth of the *Helminthosporium* sp. fungus under the treatments being represented by the regression equations (Table 1).

Concentration C₂ (500 ppm) of lemongrass oil reduced the daily mycelial growth of *Helminthosporium* sp. from 7.22 mm day⁻¹ (control) to 1.63 mm day⁻¹. The main component of the essential oil of lemongrass is citral, and antifungal activity is a prominent property

Table 1 - Mycelial growth (mm) of *Helminthosporium* sp. subjected to different concentrations of the essential oils of lemongrass, citronella, lemon balm and peppermint, for five evaluation periods (2; 4; 6; 8 and 10 days of incubation)

Concentration	Evaluation period (days of incubation)					Regression	R ²
	2	4	6	8	10		
Lemongrass							
Test	24.5 a	58.6 a	80.9 a	84.0 a	84.0 a	$\hat{y} = 23.04 + 7.22 \text{ EA}^{**}$	0.79
C1	0.0 b	2.6 b	19.0 b	38.9 b	62.9 b	$\hat{y} = -23.04 + 8.11 \text{ EA}^{**}$	0.93
C2	0.0 b	0.0 b	0.0 c	0.0 c	16.3 c	$\hat{y} = -6.52 + 1.63 \text{ EA}^{**}$	0.49
Citronella							
Test	24.5 a	58.6 a	80.9 a	84.0 a	84.0 a	$\hat{y} = 23.04 + 7.22 \text{ EA}^{**}$	0.79
C1	0.0 b	11.5 b	25.7 b	42.2 b	55.5 b	$\hat{y} = -15.52 + 7.08 \text{ EA}^{**}$	0.96
C2	0.0 b	0.7 c	8.5 c	19.4 c	31.8 c	$\hat{y} = -12.59 + 4.11 \text{ EA}^{**}$	0.79
Lemon balm							
Test	24.5 a	58.6 a	80.9 a	84.0 a	84.0 a	$\hat{y} = 23.04 + 7.22 \text{ EA}^{**}$	0.79
C1	5.5 b	23.5 b	44.6 b	65.3 b	79.1 a	$\hat{y} = -13.12 + 9.45 \text{ EA}^{**}$	0.94
C2	0.0 b	3.1 c	14.0 c	28.0 c	45.0 b	$\hat{y} = -16.46 + 5.75 \text{ EA}^{**}$	0.88
C3	0.0 b	0.0 c	3.2 d	12.3 d	23.1 c	$\hat{y} = -9.84 + 2.93 \text{ EA}^{**}$	0.80
C4	0.0 b	0.0 c	0.0 d	4.8 de	13.6 d	$\hat{y} = -5.93 + 1.60 \text{ EA}^{**}$	0.70
C5	0.0 b	0.0 c	0.0 d	1.4 e	6.0 d	$\hat{y} = -2.56 + 0.67 \text{ EA}^{**}$	0.49
Peppermint							
Test	24.5 a	58.6 a	80.9 a	84.0 a	84.0 a	$\hat{y} = 23.04 + 7.22 \text{ EA}^{**}$	0.79
C1	5.8 b	24.3 b	47.7 b	70.2 b	79.2 a	$\hat{y} = -12.18 + 9.59 \text{ EA}^{**}$	0.91
C2	0.0 b	12.2 c	33.4 c	60.4 b	78.8 a	$\hat{y} = -24.96 + 10.33 \text{ EA}^{**}$	0.95
C3	0.0 b	1.1 d	7.0 d	22.8 c	48.6 b	$\hat{y} = -19.73 + 5.94 \text{ EA}^{**}$	0.83
C4	0.0 b	0.0 d	0.3 d	6.8 d	17.9 c	$\hat{y} = -7.76 + 2.13 \text{ EA}^{**}$	0.73
C5	0.0 b	0.0 d	0.0 d	1.7 d	9.8 c	$\hat{y} = -4.11 + 1.07 \text{ EA}^{**}$	0.62

Averages followed by the same letter in a column for each type of oil, do not differ by the Tukey test at 5% significance; ** Significant at 1% probability by t-test

of this oil (SILVA *et al.*, 2009). Oliveira *et al.* (2011) relate the antimicrobial potential of lemongrass oil to its major components, the monoterpene aldehydes, neral and geranial.

The essential oil of citronella reduced the rate of growth of *Helminthosporium* sp. and slowed its development. Under the concentrations C₁ (250 ppm) and C₂ (500 ppm) the fungus presented a growth rate of 7.08 mm and 4.11 day⁻¹ respectively. Development of the pathogen was only observed under these concentrations at the second evaluation after four days of incubation (Table 1). Medice *et al.* (2007), using the essential oil of citronella, observed inhibition of spore germination of the fungus *Phakopsora pachyrhizi*, the causal agent of Asian soybean rust. Oliveira *et al.* (2011), evaluating the chemical composition of the essential oil of citronella, highlighted as major compounds

the aldehyde citronellal and the alcohols geraniol and citronellol, all compounds responsible for the antimicrobial activity of the oil.

The lowest rate of daily mycelial growth, observed when the *Helminthosporium* sp. was subjected to the lemongrass oil, was 0.67 mm day⁻¹ at concentration C₅ (1,250 ppm) (Table 1). After 10 days of incubation, this growth rate gave the fungus a 4.14 mm mycelial diameter, a size 95.6% smaller than that obtained at the same evaluation by the control, where the mycelial diameter was 94.24 mm. When subjected to the essential oil of peppermint, estimates of the growth rates of the *Helminthosporium* sp. under the concentrations C₁ and C₂, were 9.59 and 10.33 mm days⁻¹ respectively. Despite being greater than the growth rate obtained by the control (7.22 mm day⁻¹), the mycelial diameters of

the fungus at the last evaluation were smaller. This is because there was a delay in the development of the pathogen. Under C₂, growth of the *Helminthosporium* sp. was observed after four days of incubation (Table 1). The antifungal activity of the peppermint oil is probably due to the presence of menthol and carvone, both antifungal compounds (FREIRE, 2006).

There are studies that describe the direct activity of essential oils from plants on phytopathogens, or indirectly by activating the defense mechanisms of the plants against pathogens (FRANZENER *et al.*, 2003; SALGADO *et al.*, 2003; ZACARONI *et al.*, 2009; ZANANDREA *et al.*, 2004).

Trial II: *In vivo* fungitoxicity of essential oils on grass leaf blotch

For the preventive effect on Tanzania grass leaf blotch, those plants treated with the fungicide methyl thiophanate showed no symptoms of the disease.

The results for the preventive effect of essential oils on Tanzania grass leaf blotch (Table 2) showed that all the oils evaluated reduced the severity of disease.

Under the concentration of 2,500 ppm of commercial Neem oil, the plants showed a significantly lower severity rating than those treated with the essential oils of lemongrass and lemon balm (Table 2). The preventive action of the Neem oil was efficient in controlling angular blight in the bean, whose causal agent is *Phaeoisariopsis griseola*, (CARNEIRO; PIGNONI; GOMES, 2008), however in other studies (MARTINEZ, 2002; PIGNONI; CARNEIRO, 2005), it was found that neem showed low efficiency in the control of disease, due to the environmental conditions, the inoculum pressure being extremely favourable to the development of symptoms of disease, the decomposition of azadirachtin over time, and the small effect of the active compounds from neem on the fungi tested.

The essential oil of peppermint, at a concentration of 5000 ppm was effective in reducing the severity of leaf blotch in the grass, an increase in concentration not reducing the grade of severity (Table 2). The *in vivo* antimicrobial activity of the essential oil of peppermint was observed by Fatemi *et al.* (2011), where the essential oil was effective in reducing the decomposition of oranges (*Citrus sinensis*) caused by species of *Penicillium*.

There was no significant difference between the average ratings of disease severity when plants were exposed to concentrations of 7,500 and 10,000 ppm of the essential oils tested (Table 2).

For the curative effect, before application of the treatments, the plants presented between 1 and 5% diseased leaf area, thereby receiving a rating of three for the severity of the leaf spot.

As there was no interaction between the essential oils and treatments used, the effects were studied separately (Table 3). The results observed showed that there was no significant difference between the evaluated essential oils in the healing of leaf blotch.

When comparing concentrations it can be seen that the essential oils of citronella, lemongrass, lemon balm, peppermint and neem oil, at concentrations of 2,500; 5,000; 7,500 and 10,000 ppm, and of the fungicide methyl thiophanate, significantly reduced the severity ratings of the grass leaf blotch, since only in the absolute control did the disease progress to rating 7 (Table 3).

Perini *et al.* (2011) verifying the curative effect of citronella oil on rice blast, caused by *Pyricularia grisea*, found a reduction of up to 50% in the number of plants with symptoms of the disease. The curative effect of neem oil was verified by Carneiro (2003). The author observed controlling of powdery mildew in the tomato, *Oidium lycopersici*, using concentrations of 0.25 to 2% of neem

Table 2 - Severity of leaf blotch under the preventive effect of essential oils applied in four dosages in Tanzania grass plants inoculated with the *Helminthosporium* sp. fungus

Essential oil	Concentrations (ppm)					Regressão equation	R ²
	0	2500	5000	7500	10000		
Lemongrass	7 a	4.5 a	3.5 ab	3 a	3 a	$\hat{y} = 6.10 - 0.019 C^{**}$	0.70
Citronella	7 a	3.5 ab	3.5 ab	3.5 a	3 a	$\hat{y} = 5.70 - 0.016 C^{**}$	0.49
Lemon balm	7 a	4.5 a	4.5 a	3.5 a	3 a	$\hat{y} = 6.30 - 0.018 C^{**}$	0.69
Peppermint	7 a	3.5 ab	3 b	3 a	3 a	$\hat{y} = 5.60 - 0.017 C^{**}$	0.56
Neem	7 a	3 b	3.5 ab	3 a	3.5 a	$\hat{y} = \bar{x} = 4$	-

Averages followed by the same letter in any one column do not differ by the Tukey test ($P > 0.05$). ** Significant at 1 by the t-test; Ratings for severity: 0 = healthy plant; 1 = less than 1% diseased leaf area; 3 = 1-5% diseased leaf area; 5 = 6 to 25% diseased leaf area; 7 = 26 to 50% diseased leaf area; 9 = more than 50% diseased leaf area

Table 3 - Severity of leaf blotch under the curative effect of essential oils applied in four dosages in Tanzania grass plants inoculated with the *Helminthosporium* sp. fungus

Essential oil	Concentration - OE (ppm)				T1	T2	Comparisons
	2500	5000	7500	10000			
Lemongrass	3	3	3	3	7	3.5	3.75 a
Citronella	3.5	3.5	3	3	7	3.5	3.92 a
Lemon balm	3.5	3.5	3.5	3.5	7	3.5	4.08 a
Peppermint	3.5	3.5	3	3	7	3.5	3.92 a
Neem	3	3.5	3	3	7	3.5	3.83 a
Comparisons	3.3 B	3.4 B	3.1 B	3.1 B	7 A	3.5 B	

Averages followed by the same letter, lowercase in a column and uppercase on a line, do not differ by the Tukey test ($P > 0.05$); T1 - water, T2 - Methyl tiophanate at 1000 ppm.; Ratings for severity: 0 = healthy plant; 1 = less than 1% diseased leaf area; 3 = 1-5% diseased leaf area; 5 = 6 to 25% diseased leaf area; 7 = 26 to 50% diseased leaf area; 9 = more than 50% diseased leaf area

oil. The antimicrobial effect of the neem may be related to the compound azadirachtin, considered to be the main active component of the plant (ALVES *et al.*, 2009).

The *in vivo* action of plant extracts on another species of *Helminthosporium* was observed by Franzener *et al.* (2003). The authors examined the action of the aqueous extract of camphor on wheat brown spot, caused by the fungus *Bipolaris sorokiniana* (syn. *Helminthosporium sorokiniana*), and observed a significant reduction in the number and size of the lesions.

Essential oils inhibit or reduce the growth of phytopathogens due to the action of substances in their composition. These substances may affect the integrity of cell membranes, causing spillage of cellular contents (PEREIRA *et al.*, 2011). Costa *et al.* (2011), evaluating the effect of the essential oil of the clove (*Syzygium aromaticum*) on the hyphae of *R. solani*, observed different morphological changes, such as the presence of vacuoles, disruption of cellular contents, decrease in the sharpness of the cell wall, intense fragmentation and less turgescence of the hyphae.

The use of essential oils as antimicrobial agents is considered low risk, since it is believed that it is difficult for a pathogen to develop resistance to the complex mixture of active components that make up these oils (DERBALAH; DEWIR, EL-SAYED, 2012). According to Al-Reza *et al.* (2010), essential oils are antifungal agents with promising potential for agro-industry, since their active compounds may exhibit different forms of invasion in order to inhibit the growth of phytopathogens.

CONCLUSIONS

1. The essential oils of lemongrass and citronella showed potential in the control of *Heminthosporium* sp., significantly reducing the mycelial growth of the pathogen;

2. The essential oils of lemongrass, citronella, lemon balm, peppermint and neem may be an alternative to synthetic fungicides and a prominent tool for integrated disease management, since they significantly reduced leaf blotch in Tanzania grass.

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