ESSENTIAL OILS FOR RUST CONTROL ON COFFEE PLANTS

Óleos essenciais no controle da ferrugem em cafeeiro

Ricardo Borges Pereira[1], Gilvaine Ciavareli Lucas[2], Fabiano José Perina[2], Eduardo Alves[2]

ABSTRACT

Rust is considered the most important disease in coffee because it causes severe defoliation in plants and, consequently, reduction in productivity. This study evaluated the *in vitro* effect of essential oils of cinnamon, citronella, lemongrass, clove, tea tree, thyme, neem and eucalyptus on the germination of urediniospores of *Hemileia vastatrix*; the effectiveness of these oils to control rust on seedlings of coffee cultivars Catucaí 2SL, Catuaí IAC 62 and Mundo Novo 379/19 in the greenhouse; and the effect of more promising oils on urediniospores of *H. vastatrix* by transmission electron microscopy (TEM). All the essential oils inhibited the germination of urediniospores with increasing concentrations. All oils promoted partial control of the disease in the greenhouse. However, the oils of thyme, clove and citronella, at a concentration of 1000 µL L^{-1}, were most effective in controlling the disease on cultivars Catucaí 2SL, Catuaí IAC 62 and Mundo Novo 379/19, respectively. The images generated in TEM showed that urediniospores exposed to oils of clove, citronella and thyme promoted cellular disorganization and cytoplasmic vacuolization, which was more pronounced in urediniospores exposed to citronella oil. The oils of thyme, clove and citronella are promising for the control of rust in coffee.

Index terms: *Hemileia vastatrix*, alternative control, transmission electron microscopy.

RESUMO

A ferrugem é considerada a doença de maior importância no cafeeiro, pois causa acentuada desfolha nas plantas e, consequentemente, redução na produtividade. Este trabalho avaliou o efeito *in vitro* de óleos essenciais de canela, citronela, capim-limão, cravo-da-índia, árvore-de-chá, tomilho, nim e eucalipto na germinação de urediniósporos de *Hemileia vastatrix*; a eficácia desses óleos no controle da ferrugem em mudas de cafeeiro das cultivares Catucaí 2SL, Catuaí IAC 62 e Mundo Novo 379/19 em casa de vegetação; e o efeito dos óleos mais promissores sobre urediniósporos de *H. vastatrix* por meio de microscopia eletrônica de transmissão (MET). Todos os óleos essenciais inibiram a germinação dos urediniósporos com o aumento das concentrações. Todos os óleos promoveram controle parcial da doença em casa de vegetação. No entanto, os óleos de tomilho, cravo-da-índia e citronela, na dose de 1000 µL L^{-1}, foram os mais eficazes no controle da doença nas cultivares Catucaí 2SL, Catuaí IAC 62 e Mundo Novo 379/19, respectivamente. Nas imagens geradas em MET, observou-se que urediniósporos expostos aos óleos de cravo-da-índia, citronela e tomilho apresentaram desorganização citoplasmática e vacuolização celular, sendo esta mais pronunciada em urediniósporos expostos ao óleo de citronela. Os óleos de tomilho, cravo-da-índia e citronela são promissores no controle da ferrugem em cafeeiro.

Termos para indexação: *Hemileia vastatrix*, controle alternativo, microscopia eletrônica de transmissão.

(Received in february 5, 2012 and approved in february 28, 2012)

INTRODUCTION

Coffee (*Coffea arabica* L.) is one of the most important sources of foreign currency for Brazil, the world's largest producer and exporter of coffee. According to the Companhia Nacional de Abastecimento (2011), Arabica coffee production in 2011 was 32.18 million bags. In Brazil, it is estimated that losses due to rust (*Hemileia vastatrix* Berk. & Br.) are in the order of 30% of production, mostly due to lack of control measures (POZZA, 2008).

According to Zambolim et al. (2005), the severity of rust and consequent damage to production varies from region to region and from year to year, due to the pending charge and prevailing climatic conditions. Damage caused by rust is mostly indirect, inducing loss of leaves leading to a poor harvest. The early fall of leaves results in fewer fruit set at flowering, and also in dry pellets on primary branches, affecting production. Moreover, having constantly dry branches reduces the longevity of the trees, gradually making the crop uneconomic (POZZA, 2008).

Most cultivars grown in Brazil are susceptible to rust (ZAMBOLIM et al., 2005). The disease is difficult to control, but satisfactory results are obtained with the use of cupric and systemic fungicides in foliar sprays or through soil applications of systemic fungicides, associated or not with insecticides. However, the inappropriate use of these fungicides can select new resistant physiological races of the pathogen (AGRIOS,

[1]Empresa Brasileira de Pesquisa Agropecuária/Embrapa Hortaliças – Centro Nacional de Pesquisa de Hortaliças – BR 060 – Km 09 – Cx. P. 218 – 70359-970 – Gama – DF – Brasil – ricardobp@cnph.embrapa.br
[2]Universidade Federal de Lavras/UFLA – Departamento de Fitopatologia/DFP – Lavras – MG – Brasil

2005) and cause irreversible damage to the environment and to human health.

Therefore, research into the potential of essential oils to control plant diseases has been conducted in several pathosystems. Medice et al. (2007) observed that essential oils of eucalyptus (*Corymbia citriodora* Hill & Johnson), thyme (*Thymus vulgaris* L.), neem (*Azadirachta indica* A. Juss.) and citronella (*Cymbopogon nardus* (L.) Rendle) promoted complete inhibition of germination in urediniospores of *Phakopsora pachyrhizi* Syd. & P. Syd. and reduced the severity of Asian rust of soybean (*Glycine max* (L.) Merrill) in the greenhouse. Ranasingue et al. (2002) reported the fungistatic and fungitoxic activity of essential oils from clove (*Syzygium aromaticum* (L.) Merr. & L. M. Perry) and cinnamon (*Cinnamomum zeylanicum* Blume) on *Colletotrichum musae* (Berk. & M.A. Curtis) Arx, *Lasiodiplodia theobromae* (Pat.) Griff & Maubl. and *Fusarium proliferatum* (Matsuhima) Nirenberg. The efficiency of essential oils has also been reported in other pathosystems, such as *Cercospora coffeicola* Berk. & Cooke on coffee plants (PEREIRA et al., 2008; 2011).

The goals of this study were to evaluate the *in vitro* effect of medicinal plant essential oils on the urediniospores germination of *H. vastatrix*, their efficacy in the control of leaf rust on three coffee plant cultivars, and their effects on the urediniospores ultrastructure.

MATERIAL AND METHODS

The trials were conducted at the Federal University of Lavras (Lavras, Minas Gerais State, Brazil), in the period from January to November of 2008.

To obtain the *H. vastatrix* inoculum, naturally infected leaves from coffee plants were collected in the field. Urediniospores were removed from the leaves using a soft bristle brush and stored in microtubes for a maximum period of 48 hours, until use. For inoculation, a suspension of urediniospores of *H. vastatrix* was prepared at a concentration of 0.5 g L^{-1} urediniospores in distilled water containing Tween 80 (0.025%). This concentration was used in all the experiments. The essential oils of all species used in the experiments were acquired from Professor Accorsi Medicinal Plants® (Piracicaba, São Paulo State, Brazil).

To evaluate the toxicity on *H. vastatrix* germination, the essential oils extracted from tea tree (*Melaleuca alternifolia* Cheel), cinnamon (*C. zeylanicum*), lemongrass (*Cymbopogon citratus* (DC) Stapf), citronella (*C. nardus* (L.) Rendle.), clove (*S. aromaticum*), eucalyptus (*C. citriodora*), neem (*A. indica*) and thyme (*T. vulgaris*) were tested at a concentrations of 0, 250, 500, 1000, 1500 and 2000 µL L^{-1} in distilled and sterilized water. Powdered milk, 10 g L^{-1} in distilled and sterilized water, was added as a natural emulsifier at a concentration of 2000 µL L^{-1}, from which came the other dilutions. In order to isolate the effect of the powdered milk, a treatment composed only with this substance was added to the experiment at a concentration of 2000 µL L^{-1}.

Petri dishes of 6.0 cm in diameter were used with 2.0% (w/v) agar-water medium (AW). The treatments were added to the medium, after the temperature had dropped to 40º C, before it was poured on the dishes, so that the final dilutions reached those pre-established. After the solidification of the medium, 500 µL of the conidial suspension of the pathogen was deposited on its surface and spread with a Drigalski spatula. Next, the dishes were incubated at 23º C, in the dark for 24 hours. The experiment was conducted in a completely randomized design, with two dishes for each treatment, and each one divided into four quadrants, where 50 urediniospores per quadrant were appraised, to a total of eight replications. After incubation, germination was stopped by the addition of four drops of lactoglycerol solution and the urediniospore germination percentage appraised under a light microscope.

With the objective of evaluating the efficiency of the essential oils in the control of the leaf rust, three coffee plant cultivars, susceptible to *H. vastatrix*, were chosen: Mundo Novo 379/19, Catuaí IAC 62 and Catucaí 2SL. Their seedlings were acquired at six months of age from the Experimental Station of EPAMIG - South Minas Research Center (Lavras, Minas Gerais State, Brazil) - and transplanted to polystyrene bags containing 3.0 L of substrate composed of expanded vermiculite, organic materials, macro-and micronutrients. The plants were maintained in a greenhouse during the whole experimental period, where they were periodically irrigated and fertilized according to the recommendations (RIBEIRO et al., 1999).

At one year old, the coffee plants were sprayed until dripping with the essential oils of tea tree, cinnamon, lemongrass, citronella, clove, eucalyptus, neem and thyme at a concentration of 1000 µL L^{-1}, powdered milk 10 g L^{-1}, fungicide tebuconazol (Folicur 200 EC®) 200 µL L^{-1}, acibenzolar-S-methyl (ASM) (Bion®) 200 mg L^{-1} (used as resistance induction standard), and distilled water (control), using a manual sprayer. After 30 days, these treatments were repeated. Seven days after the first application, the plants were inoculated with *H. vastatrix* urediniospores suspension. Soon afterwards, the plants were maintained in a humid chamber, in the dark, for 60 hours. The experiment was conducted in a randomized block design, with three replications, and the plot was composed of six plants. Five evaluations of the leaf rust were made, starting from the 30th day after inoculation, at 11-day intervals, according to

the diagrammatic scale of Cunha et al. (2001). Soon afterwards, the areas under the incidence progress curve (AUIPC) and severity of leaf rust progress curve (AUSPC) were calculated, according to Shaner and Finney (1977).

Statistical analyses were conducted using the Sisvar (v. 4.5) statistical software. The qualitative means were grouped using the Scott-Knott test ($p \leq 0.05$) and regression graphs were generated for quantitative means. The urediniospores germination percentage data were transformed to $\sqrt{(x + 1)}$.

To elucidate the action mechanism of the most promising essential oils on the ultra-structure of urediniospores of *H. vastatrix*, the essential oils of cinnamon, citronella, clove and thyme were tested at a concentration of 1000 µL L^{-1}. Tebuconazol fungicide at 200 µL L^{-1} and distilled and sterilized water were used as standard treatments.

The essential oils were added separately in Erlenmeyer flasks containing distilled and sterilized water and *H. vastatrix* urediniospores. The Erlenmeyers were maintained in a shaker at 100 rpm, at 23° C, in the dark, for 24-hour period. After this, 2.0 mL aliquots of these solutions were put in microtubes and pre-fixed in a modified Karnovsky solution (glutaraldehyde 2.5%, paraformaldehyde 2.0% in sodium cacodylate buffer 0.05M, $CaCl_2$ 0.001 M, pH 7.2), for 24 hours. The microtubes were centrifuged for five minutes at 6000 rpm and the supernatant discarded. Next, 0.5 mL of 1.0% agarose gel was added to the microtubes, to form a pellet, on which the urediniospores adhered. These fragments were transferred to 0.05M sodium cacodylate buffer solution, washed three times for 10 minutes and post-fixed in 1.0% osmium tetroxide for one hour. The same fragments were then washed twice in distilled water for 15 minutes, and en-bloc stained in 0.5% uranyl acetate solution, for 12 hours, at 4° C, and washed again in distilled water. The samples were dehydrated in a graded acetone series (once at 25%, 50%, 75%, 90% and three times at 100%) and infiltrated in an increasing Spurr/acetone gradient of 30% (8 hours), 70% (12 hours) and 100% twice, for 24 hours each. The fragments were mounted in molds, embedded in pure Spurr resin and put to polymerize in an oven at 70° C, for 48 hours.

For the ultramicrotomy, the blocks were taken to the trimming apparatus to remove the excess. Soon afterwards, thick (0.85 µm) and thin (<100 nm) sections were cut, using a Reichrt-jung (ultracut) ultramicrotome equipped with diamond knife. Thick sections were collected with a gold ring, put on glass slides, stained with toluidine blue (1.0 g toluidine blue, 1.0 g sodium borate and 100 mL distilled water), filtered in a 0.22 µm cellulose membrane and permanently mounted in Permalt medium. Thin sections were picked up on gold slot grids, and allowed to dry on Formvar-coated aluminum racks (Rowley; Moran, 1975). These sections were post-stained with uranyl acetate, followed by lead citrate for three minutes each. Soon afterwards, the samples were examined using a Zeiss EM 109 transmission electron microscope operating at 80kv and 9.0 mm distance. The images generated were digitally recorded and edited in the Photo-Paint software of the Corel Draw 12 package.

RESULTS AND DISCUSSION

The germination of the *H. vastatrix* urediniospores decreased with increased concentrations of essentials oils (Figure 1). The cinnamon, citronella, lemongrass, tea tree and thyme oils totally inhibited the germination of the urediniospores starting from 1000 µL L^{-1}, with DL_{50} (concentration able to inhibit or promote the death of 50% of the viable urediniospores) estimated at 77, 116, 173, 57 and 58 µL L^{-1}, respectively. The clove oil totally inhibited the germination of the urediniospores starting from 1500 µL L^{-1}, with DL_{50} estimated at 175 µL L^{-1}, while neem and eucalyptus oils totally inhibited the germination at a concentration of 2000 µL L^{-1}, with DL_{50} estimated at 845 and 935 µL L^{-1}, respectively. The powdered milk treatment did not differ from the control.

Similarly, Pereira et al. (2008) observed reduction of *Coffea coffeicola* conidial germination when subjected to increasing concentrations of the essential oil of thyme. This effect is probably due to the presence of compounds such as thymol and carvacrol, already known for their fungicidal and bactericidal properties (ZAMBONELLI et al., 2004). Similary, the effect of cinnamon oil is attributed to cinnamaldehyde and eugenol compounds present in large concentrations (MONTES-BELMONT; CARVAJAL, 1998).

Ponce et al. (2003) reported the antimicrobial power of essential oils of tea-tree and clove, when used at a concentration of 500 mL L^{-1} on bacterial growth by concentration minimal method. In this case, the toxicity of oils on the pathogens is attributed to the antimicrobial compounds terpinen-4-ol and eugenol present in high concentrations in oils (VIEIRA et al., 2004). Essential oils of *Cymbopogon* sp., *Thymus* sp. and *Cynamomum* sp. have large amounts of monoterpenes (dlimonene, cineole, b-myrcene, anethole, p-anisaldehyde, carvacrol, carvone, limonene, felandrene, pinene, etc.), which are responsible for inhibiting germination of several pathogens (WILSON et al., 1997). When in direct contact with microorganisms these compounds alter the permeability of cell membranes, causing leakage of their constituents (PIPER et al., 2001; RASOOLI et al., 2006).

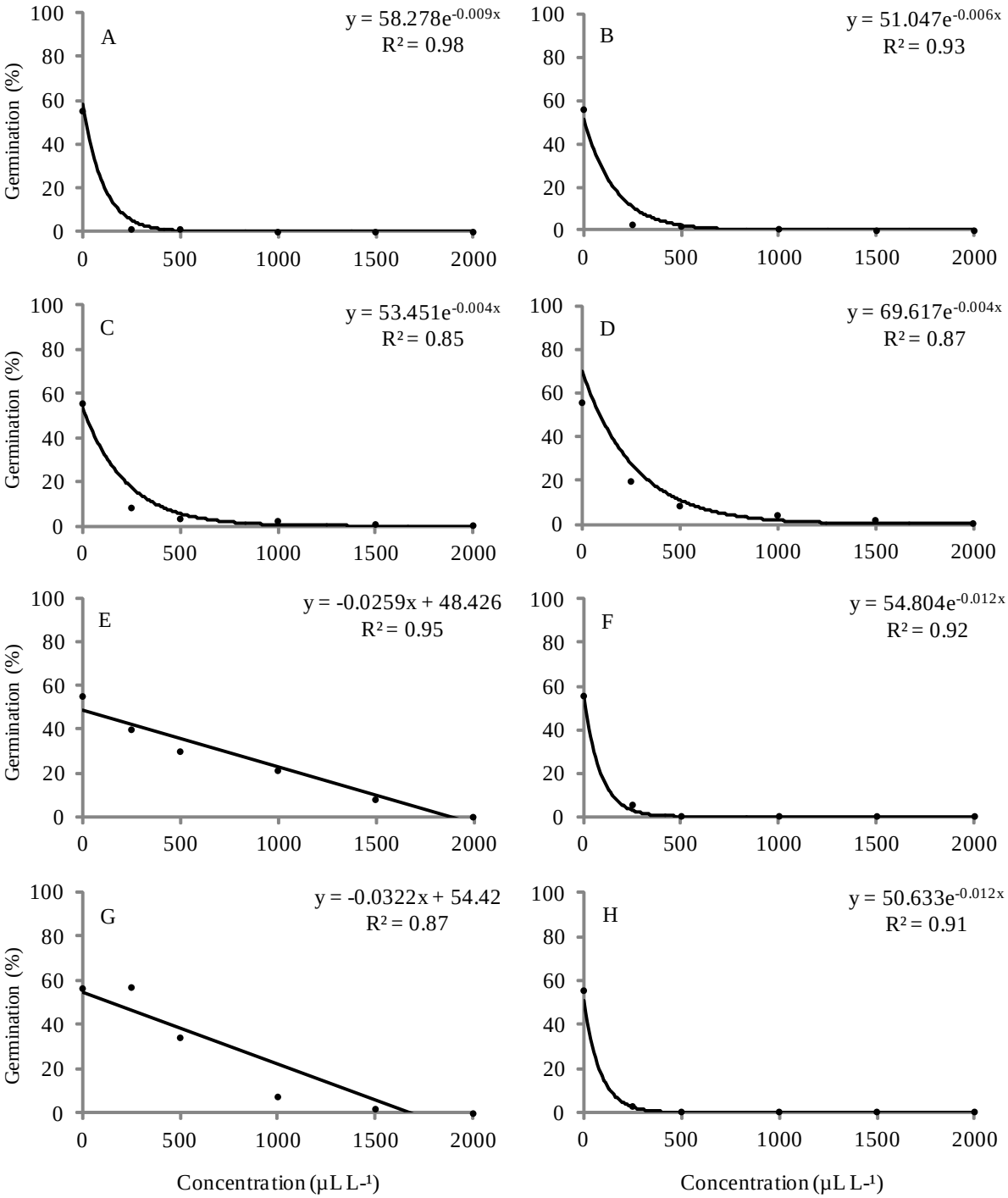

Figure 1 – Percentage of *Hemileia vastatrix* urediniospores germination, submitted to different concentrations (0, 250, 500, 1500 and 2000 µL L^{-1}) of essential oils extracted from cinnamon (A), citronella (B), lemongrass (C), clove (D), eucalyptus (E), tea tree (F), neem (G) and thyme (H). Data transformed to $\sqrt{(x + 1)}$.

In the experiment conducted in the greenhouse, no phytotoxicity symptom was observed due to the application of the essential oils. Significant interactions were observed in the area under the incidence progress curve (AUIPC) and severity of leaf rust (AUSPC) for the cultivars and products or substances. Tebuconazol fungicide presented the highest reduction in AUIPC on Catuaí IAC 62 cultivar, 78.75%, followed by clove oil, with a reduction of 26.3% (Figure 2A). Lemongrass, cinnamon, thyme, tea tree, citronella oils and acibenzolar-S-methyl reduced the disease incidence by 15.5%, 15.0%, 13.2%, 12.1%, 12.0% and 7.9%, respectively. As regards the AUSPC of the disease, the fungicide presented the highest reduction, 94.8% (Figure 2B). Clove and lemongrass essential oils presented reductions of 67.9% and 67.7%, respectively, followed by tea tree, with reductions of 55.4%. The cinnamon oil reduced the AUSPC by 45.3%, followed by thyme and citronella, with controls of 37.5% and 32.7%, respectively. The acibenzolar-S-methyl presented a reduction of severity of 12.1%, while eucalyptus and neem oils did not differ from the control.

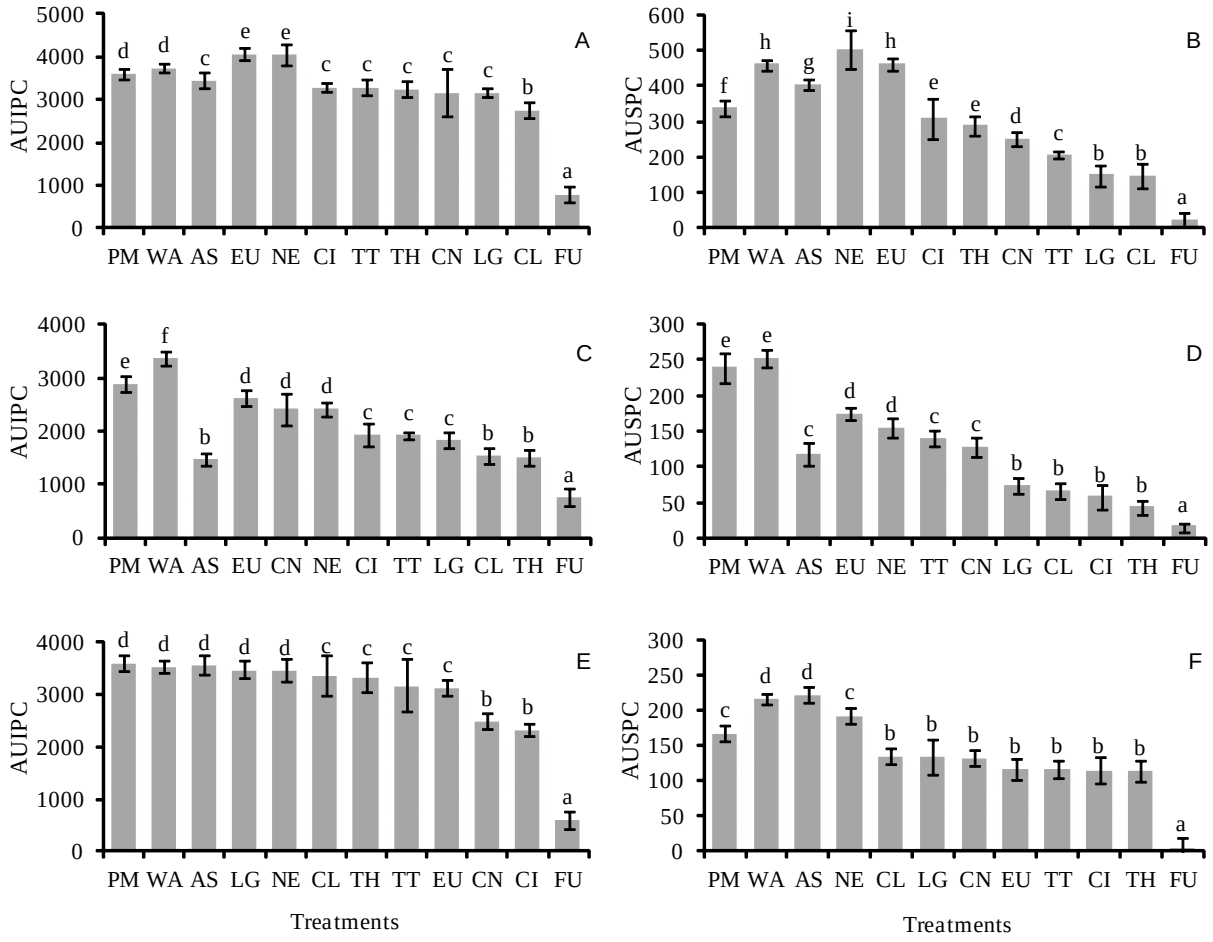

Figure 2 – Areas under the incidence progress curve (AUIPC) (A, C and E) and severity of coffee leaf rust progress curve (AUSPC) (B, D and F) for the coffee plant cultivars Catuaí IAC 62 (A and B), Catucaí 2SL (C and D) and Mundo Novo 379/19 (E and F), treated with essential oils of citronella (CI), cinnamon (CN), lemongrass (LG), neem (NE), tea tree (TT), thyme (TH), eucalyptus (EU) and clove (CL) at a concentration of 1000 µL L^{-1}, acibenzolar-S-methyl (AS) 200 mg L^{1}, fungicide tebuconazol (FU) 200 µL L^{-1}, powdered milk (PM) 10 g L^{-1} and distilled water (WA). Averages followed by same letter do not differ among themselves by the Scott-Knott test ($p \leq 0.05$). Error bars represent the standard deviation of the mean.

For Catucaí 2SL cultivar, tebuconazol fungicide reduced the AUIPC and the AUSPC by 77.6% and 93.9%, respectively, in relation to the control, differing from the other treatments (Figure 2C and D). Acibenzolar-S-methyl and oils of thyme and clove reduced the leaf rust incidence by 56.6%, 55.5% and 54.3%, respectively, followed by lemongrass, tea tree and citronella oils, which reduced the incidence by 45.7%, 43.0% and 42.7%, respectively. The other oils promoted reductions of 22.3% to 28.3%. Regarding the AUSPC, the thyme, citronella, clove and lemongrass oils presented controls of 83.0%, 77.2%, 73.7% and 70.8%, respectively, followed by acibenzolar-S-methyl and oils of cinnamon and tea tree, with controls of 53.3%, 49.1% and 44.8%, respectively. The eucalyptus and neem oils reduced the AUSPC by 38.5% and 30.8%, respectively. During the evaluations of disease in the greenhouse less sporulation of the pathogen was observed on infected leaves of Catucaí 2SL cultivar, due to the horizontal resistance of this cultivar to coffee leaf rust.

For Mundo Novo 379/19 cultivar, the fungicide reduced the AUIPC and the AUSPC of the disease by 83.1% and 97.4%, respectively (Figure 2E and F). Citronella and cinnamon oils reduced leaf rust incidence by 34.0% and 29.6%, respectively, followed by eucalyptus, tea tree, thyme and clove oils, with reductions of 11.3%, 10.2%, 5.7% and 4.8%, respectively. The other oils and acibenzolar-S-methyl did not reduce the disease incidence. Regarding the AUSPC for Mundo Novo 379/19 cultivar, it was observed that thyme, citronella, tea tree, eucalyptus, cinnamon, lemongrass and clove oils reduced AUSPC by 47.5%, 42.2%, 46.3%, 46.0%, 38.7%, 38.0% and 37.6%, respectively, while neem oil reduced the disease severity by 10.9%. Acibenzolar-S-methyl did not reduce the severity of leaf rust.

Some authors have previously confirmed the efficiency of acibenzolar-S-methyl and of some essential oils in the control of plant diseases. Costa et al. (2007) observed reductions of 47.14% and 99.6% in the rust severity on Catuaí IAC 144 coffee treated with acibenzolar-S-methyl 200 mg L^{-1} and tetraconazol fungicide, respectively. Pereira et al. (2011) obtained a reduction of 64.94% in the severity of brown eye spot on Catucaí 2SL coffee treated with acibenzolar-S-methyl 200 mg L^{-1}.

Similar works have reported the efficiency of essential oils in controlling diseases in other pathosystems. Pereira et al. (2008) obtained reduction of 16.1% in area under the severity progress curve for brown eye spot on coffee treated with thyme essential oil at 500 µL L^{-1}. Medice et al. (2007) observed reductions of 35% to 62% in the severity of Asian rust of soybean on different cultivars treated with essential oils of thyme, citronella, eucalyptus and neem, at a concentrations of 300, 500, 1000 and 3000 µL L^{-1}, respectively. Recently, Pereira et al. (2011) demonstrated the effectiveness of essential oils in controlling brown eye spot in the greenhouse. According to the authors, citronella oil at 1000 µL L^{-1} decreased disease severity by 43.08% and 29.62% on Catucaí 2SL and Mundo Novo 379/19 cultivars, respectively, while cinnamon oil at 1000 µL L^{-1} reduced the severity of the disease by 58.28% on cultivar Catuaí IAC 62.

It is therefore suggested that the partial control of rust obtained by application of essential oils, possibly due to the presence of toxic compounds in large quantities, provides a protective effect (MONTES-BELMONT; CARVAJAL, 1998). This evidence does not exclude the possibility that other compounds present in oils in smaller amounts may be contributing indirectly to disease control by inducing plant defense response. To prove this, further studies are needed, such as the quantification of enzymes related to plant defense and gene expression, the latter possibly to explore which defense genes were being expressed through the application of oils.

The images generated by the transmission electron microscope showed that urediniospores of *H. vastatrix* exposed to the fungicide tebuconazole (Figures 3B1, B2 and B3) and clove oil (Figures 3D1, D2 and D3) presented cellular vacuolization, whereas untreated cells presented normal ultra-structures (Figures 3A1, A2 and A3). Urediniospores exposed to the citronella (Figures 3C1, C2 and C3) and thyme oils (Figures 3E1, E2 and E3) presented, in most images, cellular vacuolization and cytoplasmic disorganization. However, this was less severe than that observed in urediniospores exposed to citronella oil.

The deleterious effects of essential oils on fungal spores have also been observed in other studies. Rasooli et al. (2006) showed by transmission electron microscopy that *Thymus eriocalyx* (Ronniger) Jalas and *T. x-porlock* essential oils cause severe damage to the cells of *Aspergillus niger* Van Tieghem, such as morphological changes of hyphae during germination, plasma membrane rupture and destruction of the mitochondria and other cellular organelles. Liu et al. (2009) observed that spores of *Geotrichum citri-aurantii* (Ferraris) R. Cif. & F. Cif. treated with thyme oil at 200 µL L^{-1} presented plasma membrane disruption and cytoplasmic and mitochondrial disorganization. De Billerbeck et al. (2001) and Helal et al. (2007) reported that the hyphae walls of *A. niger* and *A. flavus* Link, respectively, disappeared in some regions after treatment with citronella essential oil. According to Piper et al. (2001), the action of essential oils and their substances, when in contact with microorganisms, promotes cell permeability and flow of their constituents.

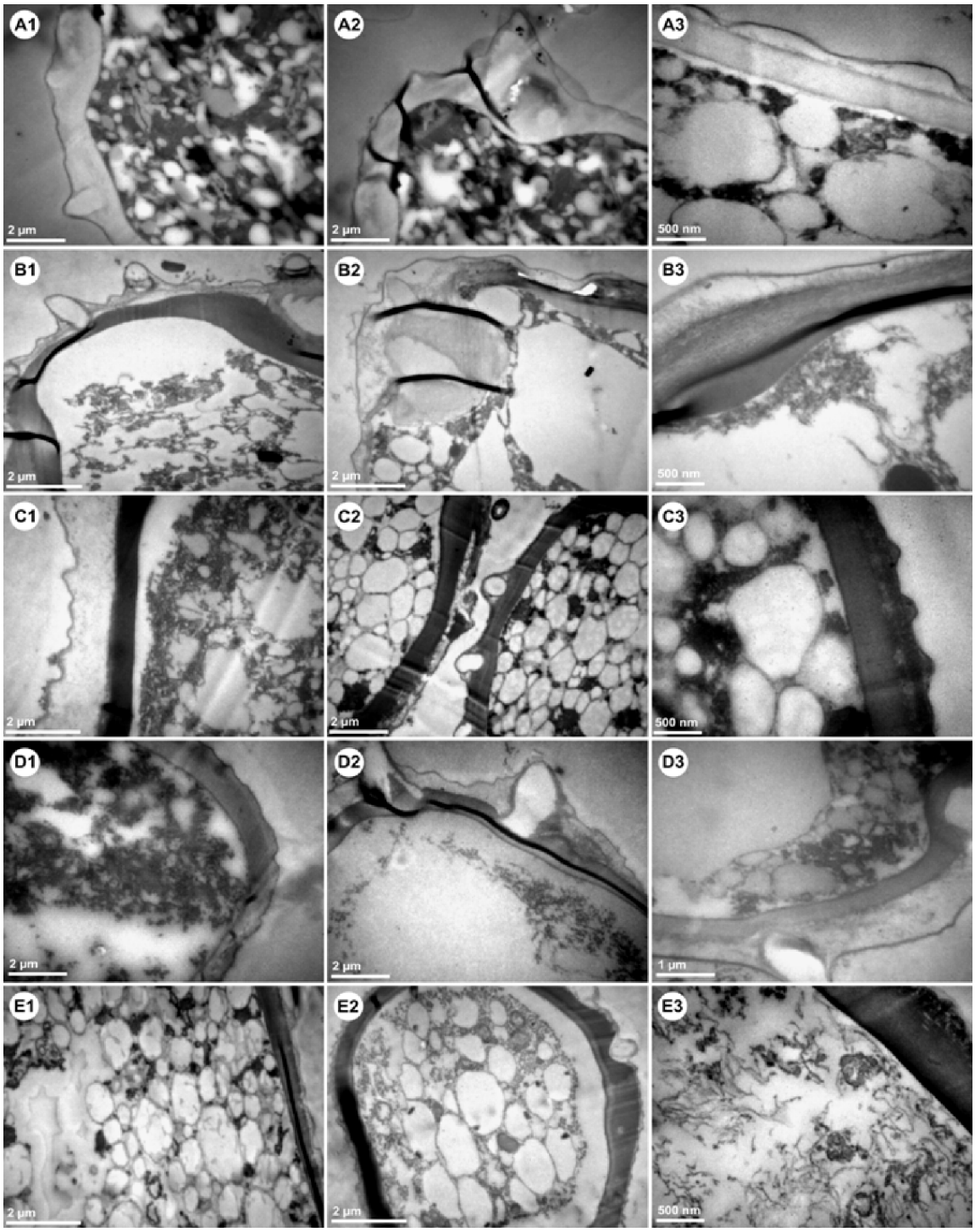

Figure 3 – Transmission electron micrographs of *Hemileia vastatrix* urediniospores exposed to distilled water (A1 to A3) present normal cells, and urediniospores exposed to the fungicide tebuconazole at 200 µL L^{-1} (B1 to B3) and to essential oil of clove at 1000 µL L^{-1} (D1 to D3) present severe cellular vacuolization. Urediniospores submitted to oil of citronella (C1 to C3) and thyme (E1 to E3) present disorganization and moderate cellular vacuolization.

Based on the results obtained by electron microscopy and germination test, it is believed that essential oils act directly on the urediniospores of *H. vastatrix*, reducing the infection rate of the pathogen and, consequently, the disease severity. The alternative use of essential oils has the main purpose of reducing the use of pesticides which, over time, cause irreversible damage to human health and the environment. Thus, the use of essential oils, along with other control strategies used in the integrated management of diseases, can provide satisfactory results in reducing disease in coffee, especially in organic coffee production fields, where pesticides are not used.

CONCLUSIONS

The essential oils of cinnamon, citronella, lemongrass, clove, eucalyptus, tea tree, thyme and neem reduce the germination of *Hemileia vastatrix* urediniospores.

Essential oils promote partial control of the rust on coffee plants in the greenhouse. The oils of thyme, clove and citronella are the most promising for the control of the disease on Catucaí 2SL, Catuaí IAC 62 and Mundo Novo 379/19 coffee plant cultivars.

Essential oils of citronella, thyme and clove promote disorganization and cellular vacuolization in urediniospores of *H. vastatrix*.

REFERENCES

AGRIOS, G.N. **Plant pathology**. 5th ed. New York: Academic, 2005. 922p.

COMPANHIA NACIONAL DE ABASTECIMENTO. **Acompanhamento de safra brasileira:** café, segunda estimativa, maio/2011. Brasília: Conab, 2011. 25p.

COSTA, M.J.N.; ZAMBOLIM, L.; RODRIGUES, F.A. Avaliação de produtos alternativos no controle da ferrugem do cafeeiro. **Fitopatologia Brasileira**, Brasília, v.32, n.2, p.150-155, mar./abr. 2007.

CUNHA, R.L. et al. Desenvolvimento e validação de uma escala diagramática para avaliar a severidade da ferrugem (*Hemileia vastatrix*) do cafeeiro. In: SIMPÓSIO BRASILEIRO DE PESQUISA DOS CAFÉS DO BRASIL, 2., 2001, Vitória. **Resumos**... Vitória: Embrapa Café, 2001. p. 77-78.

DE BILLERBECK, V.G. et al. Effects of *Cymbopogon nardus* (L.) W. Watson essential oil on the growth and morphogenesis of *Aspergillus niger*. **Canadian Journal of Microbiology** (online), Ottawa, v.47, n.1 , p.9-17, Jan. 2001.

HELAL, G.A. et al. Effects of *Cymbopogon citratus* L. essential oil on the growth, morphogenesis and aflatoxin production of *Aspergillus flavus* ML2-strain. **Journal of Basic Microbiology**, Berlin, v.47, n.1, p.5-15, Feb. 2007.

LIU, X. et al. Antifungal activity of thyme oil against *Geotrichum citri-aurantii in vitro* and *in vivo*. **Journal of Applied Microbiology** (online), Oxford, v.107, n.5, p.1450-1456, Nov. 2009.

MEDICE, R. et al. Óleos essenciais no controle da ferrugem asiática da soja *Phakopsora pachyrhizi* Syd. & P. Syd. **Ciência e Agrotecnologia**, Lavras, v.31, n.1, p.83-90, jan./fev. 2007.

MONTES-BELMONT, R.; CARVAJAL, M. Control of *Aspergillus flavus* in maize with plant essential oils and their components. **Journal of Food Protection**, Ames, v.61, n.5, p.616-619, May 1998.

PEREIRA, R.B. et al. Extrato de casca de café, óleo essencial de tomilho e acibenzolar-S-metil no manejo da cercosporiose-do-cafeeiro. **Pesquisa Agropecuária Brasileira**, Brasília, v.43, n.10, p.1287-1296, 2008.

PEREIRA, R.B. et al. Potencial de óleos essenciais no controle da cercosporiose-do-cafeeiro. **Ciência e Agrotecnologia**, Lavras, v.35, n.1, p.115-123, jan./fev. 2011.

PIPER, P. et al. Weak acid adaptation: the stress response that confers resistance to organic acid food preservatives. **Microbiology**, Reading, v.147, n.10, p.2635-2642, Oct. 2001.

PONCE, A.G. et al. Antmicrobial activity of essential oils on the native microflora of organic Swiss chard. **Lebensmittel-Wissenschaft-Technologie,** Zürich, v.36, n.7, p.679-684, Nov. 2003.

POZZA, E.A.A Importância das doenças foliares do cafeeiro, In: NÚCLEO DE ESTUDOS EM FITOPATOLOGIA. (Org.). **Manejo fitossanitário da cultura do cafeeiro**. Brasília: Sociedade Brasileira de Fitopatologia, 2008. p.81-94.

RANASINGHE, L.; JAYAWARDENA,B.; ABEYWICKRAMA,K. Fungicidal activity of essential oils of *Cinnamomum zeylanicum* (L.) and *Syzygium aromaticum* (L.) Merr et L.M. Perry against crown rot and anthracnose pathogens isolated from banana. **Letters in Applied Microbiology**, Oxford, v.35, n.3, p.208-211, Mar. 2002.

RASOOLI, I.; REZAEI, M.B.; ALLAMEH, A. Growth inhibition and morphological alterations of *Aspergillus niger* by essential oils from *Thymus eriocalyx* and *Thymus x-porlock*. **Food Control**, Vurrey, v.17, n.5, p.359-364, 2006.

RIBEIRO, A.C.; GUIMARÃES, P.T.G.; ALVAREZ, V.V.H. **Recomendações para o uso de corretivos e fertilizantes em Minas Gerais**: 5ª aproximação. Viçosa, MG: CFSEMG, 1999. 359p.

ROWLEY, C.R., MORAN, D.T. A simple procedure for mounting wrinkle-free sections on formvar - coated slot grids. **Ultramicrotomy**, Amsterdam, v.1, n.2, p.151-155, 1975.

SHANER, G.; FINNEY, R.F. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. **Phytopathology**, Ithaca, v.67, p.1051-1056, Aug. 1977.

VIEIRA, T.R. et al. Constituintes químicos de *Melaleuca alternifolia* (Myrtaceae). **Química Nova**, São Paulo, v.27, n.4, p.536-539, jun./jul. 2004.

WILSON, C.L. et al. Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. **Plant Disease**, Beltsville, v.81, n.2, p.204-210, Feb.1997.

ZAMBOLIM, L.; VALE, F.X.R.; ZAMBOLIM, E.M. Doenças do cafeeiro (*C. arabica* e *C. canephora*), In: KIMATI, H. et al. (Ed.). **Manual de fitopatologia:** doenças das plantas cultivadas. 4.ed. São Paulo: Agronômica Ceres, 2005. v.2, p.165-180.

ZAMBONELLI, A. et al. Chemical composition and fungicidal activity of commercial essential oils of *Thymus vulgaris* L. **Journal of Essential Oil Research**, v.16, n.1, p.69-74, 2004.