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# Essential oils for the control of bacterial speck in tomato crop

**Érika Oliveira da Silva, Samuel Julio Martins* and Eduardo Alves**

Department of Plant Pathology, Universidade Federal de Lavras, CP 3037, CEP 37200000 Lavras, MG, Brazil.

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**Bacterial speck (*Pseudomonas syringae* pv. *tomato*) is considered one of the major diseases of tomato crop worldwide and alternative methods to control it are desirable. The objective of this study was to evaluate the effect of essential oils (EOs) on *P. syringae* pv. *tomato* strain ufv-1 growth, in controlling bacterial speck in tomato plants, as well as to find the best application time of the EOs. The EOs used in this study were thyme (TH), eucalyptus (EU), tea tree (TT), clove (CL), cinnamon (CN), citronella (CI), and lemon grass (LE). An *in vitro* test using EOs were conducted to verify the ufv-1 inhibition, and two tests were carried out in a greenhouse to evaluate the effect of EOs before and after inoculation with *Pst*. Inhibition zones were observed for EU, CI, CL, and CN at a concentration of 1%. Plants pre-treated with EOs showed lower disease severity than that in plants post-treated with EOs ($P<0.05$), whereas higher efficacy was observed using acibenzolar-*S*-methyl and CI (91 and 83%, respectively). Regarding post-treatment, TT, TH, CI, EU, LE, and commercial fungicide, resulted in reducing disease severity by 8 to 40% compared to control (water). Results from this study showed the potential use of EOs in controlling bacterial speck in tomato and suggest the induced resistance as the major mode of action.**

**Key words:** *Pseudomonas syringae* pv. *tomato,* tomato diseases, alternative control of plant diseases, *Solanum lycopersicum.*

## INTRODUCTION

Bacterial speck (*Pseudomonas syringae* pv. *tomato*, *Pst*) is considered one of the major diseases of tomato crop in many countries around the world (Cai et al., 2011), including some located in North and South America (Junior, 2013), in Europe (Milijašević et al., 2009b; Quattrucci et al., 2013), as well as in Africa (Shenge et al., 2008a, b). Dark lesions may be present in infected plant parts, such as leaves, stems, and fruits resulting in depreciation of fruit quality and consequently a decrease in commercialization (Herman et al., 2008; Pietrarelli et al., 2006).

Although there are some tomato cultivars resistant to bacterial speck there is still the chance of disease overcome by new strains of *P. syringae* pv. *tomato*, which continue evolving (Milijasevic et al., 2009a). The use of antibiotics has also been considered as ineffective against several pathogens and many countries do not permit its use. On the other hand, the use of chemicals such as copper-based products may result in phytotoxicity, as well as require frequent applications, causing accumulation in the soil due to its continuous use (Balestra et al., 2009). The use of chemicals products are

*Corresponding author. E-mail: samueljmt@yahoo.com.br, Tel: 1 3022903286.

also a risk to human health because frequently, the post chemical application waiting period is not respected by many growers before harvest, which means that tomato could be carrying high levels of chemicals residues. For instance, high levels of Cu was found in tomato samples after exposing them to different levels of the metal, indicating a food contamination as a result of the metal exposure (Granata et al., 2011).

In this context, the use of essential oils (EOs) for disease control may serve as an alternative method for decreasing or replacing the use of chemical products in agricultural crops. According to Bakkali et al. (2008), EOs are volatile, natural, complex compounds coming from secondary metabolites of aromatic plants and characterized by a strong odor. EOs control bacteria by directly imparting toxic effects which inhibit bacterial growth (Vigo et al., 2009; Lucas et al., 2012), as well as promote plant resistance (Vigo et al., 2009). However, there are only few studies focused on the best time to apply EOs for controlling plant diseases, and some of them show the application of EOs as a preventative method (that is, applied before pathogen inoculation) (Da Silva et al., 2012).

To identify an alternative and environmentally friendly method of managing bacterial speck in tomato plant, we aimed at: (i) evaluating the *in vitro* inhibition of *Pst* when using EOs; (ii) evaluating the effect of EOs in controlling bacterial speck in tomato plants under greenhouse conditions; and (iii) assessing the best time of application of EOs: pre-treatment (before pathogen inoculation) or post-treatment (after pathogen inoculation).

## MATERIALS AND METHODS

Three experiments were performed at the Federal University of Lavras (UFLA) in Lavras, Minas Gerais, Brazil. The first experiment comprised an *in vitro* test to evaluate *Pst* growth inhibition and the two other experiments were conducted in a greenhouse to evaluate the effect of EOs in the control of bacterial speck in tomato plants and to determine the best time of application of the natural product.

The pathogen used in this work was the strain ufv-1 of *P. syringae* pv. *tomato,* (hereafter ufv-1), provided by the Laboratory of Plant Pathology of Universidade Federal de Viçosa, Minas Gerais, Brazil. This strain was isolated from tomato leaves and preserved in mineral water, where it was recorded from before the experiments. For the *in vitro* inhibition experiment, the pathogen was cultured on medium 523 (Kado and Heskett, 1970) by using the parallel streak method and incubating at 28°C for 48 h. The inoculum was prepared by suspending the bacterial colonies in autoclaved distilled water, followed by adjustment of the concentration of the bacterial suspension by using a spectrophotometer to $A_{540nm}$ = 0.20, which corresponded approximately to $10^8$ CFU $mL^{-1}$ (Silva et al., 2008). Approximately 200 µL of bacterial suspension was inoculated onto 9-cm diameter Petri dishes containing 20 mL of culture medium 523 and spread using a sterilized Drigalski spatula.

The EOs used in this study includes thyme (TH; *Thymus vulgaris* L.), clove (CL; *Syzygium aromaticum* (Linne) Merril), eucalyptus (EU; *Corymbia citriodora* Hill & Johnson), cinnamon (CN; *Cinnamomum zeylanicum* Blume), citronella (CI; *Cymbopogon nardus* (L.) Rendle.), tea tree (TT; *Melaleuca alternifólia* Cheel), and lemon grass (LE; *Cymbopogon citratus* (D.C.) Stapf) at concentrations (v/v) of 0.1, 1 and 10% (essential oil/powdered milk solution at 1%). To prepare the powdered milk emulsifier 1% (w/v), 1 g of powdered milk was added in 99 mL of water. As positive controls, streptomycin sulfate (25 mg $mL^{-1}$) and Recop® (copper oxychloride; 2.0 mg $mL^{-1}$), were used. Sterilized water was used as negative control. The EOs obtained from Brasil Portrait (Sorocaba, SP, Brazil) were separated by steam drag distillation from plant leaves.

Autoclaved filter paper discs of 6-mm diameter were soaked in 20 µL of each of the tested oils, dried at room temperature, and placed over ufv-1 suspension. Plates were incubated in BOD at 28°C, and after 24 h of incubation, the presence of inhibition halos were evaluated. The experiment was conducted using a completely randomized design (CRD) with six replicates, with each experimental unit consisting of one Petri dish containing four filter paper discs.

In the second experiment, two tests were prepared under greenhouse conditions to evaluate the plant's defense response to EOs when applied before (pre-treatment) or after (post-treatment) pathogen inoculation. Treatments included in this experiment comprised the EOs previously described at a concentration of 0.1%. Two commercial products were used as standard substances for the induction of plant resistance: Bion® (acibenzolar-S-methyl, ASM; 0.2 mg·$mL^{-1}$) and Recop® (copper oxychloride; 2.0 mg·$mL^{-1}$). Powdered milk (PM, 1%) and water were used as inoculated and non-inoculated controls. For all EO-based treatments, 1% PM was used as the emulsifier.

Tomato seeds of cultivar Santa Cruz Kada, which is susceptible to *Pst*, were sown in trays with 128 cells filled with a commercial substrate Plantmax® HT. Approximately 20 days after sowing (DAS), two transplants were placed in 5-kg pots containing the same substrate. The plants were maintained under greenhouse conditions and watered whenever necessary until the end of the trials. After 30 DAS, tomato plants previously placed in a moist chamber for 24 h were spray inoculated with ufv-1 until soaked, and returned to the moist chamber for 24 h to facilitate bacterial infection (Kado and Heskett, 1970).

For the pre-treatment trial, spraying with the previously mentioned 11 products was performed one week before inoculation with *Pst* (23 DAS), whereas for the post-treatment, spraying was performed one week after inoculation (37 DAS), as described by Lucas et al. (2012).

After 37 DAS for the pre-treatment and 44 DAS for the post-treatment, five evaluations of disease severity for both trials were conducted weekly according to an index scale described by Yunis et al. (1980), in which 0 = no symptoms; 1 = 2–5 specks together or spread all over the leaf; 2 = 6–10 specks; and 3 = more than 11 specks per leaf. For the controls, disease severity data were transformed using MCKINNEY's index (Mckinney, 1923) to calculate the area under the disease progress curve (AUDPC) (Shaner and Finney, 1977).

The experiment was conducted using a random block design with five replicates, and three plants per pot, being the mean of these three plants the experimental unit. Data of AUDPC was subjected to one-way ANOVA analysis and the means were compared using Tukey's test, with 5% probability. The Shapiro–Wilk test was used to verify normality of the data; the transformation of data was deemed not necessary. For statistical analysis, software Sisvar was used (Build 72) Copyright Daniel Furtado Ferreira 1999–2007 version 5.1 (Ferreira, 2011).

## RESULTS AND DISCUSSION

No inhibition halos were observed for any of the products under study at the 0.1% concentration. At a concentration of 1%, EOs from EU, CN, CI, and LE were observed to be toxic to ufv-1. At a concentration of 10% or higher, all

**Table 1.** *In vitro* inhibition of *Pseudomonas syringae* pv. *tomato* growth by using various concentrations of essential oils, powdered milk, copper oxychloride, or streptomycin sulfate.

| Treatments | Concentration of essential oils (%) | | | |
|---|---|---|---|---|
| | 0.1 | 1.0 | 10 | 100 |
| Citronella | -[1] | + | +[2] | + |
| Lemon grass | - | + | + | + |
| Eucalyptus | - | + | + | + |
| Cinnamon | - | + | + | + |
| Thyme | - | - | + | + |
| Tea tree | - | - | + | + |
| Clove | - | - | + | + |
| Powdered milk (1 mg·mL$^{-1}$)[3] | - | - | - | - |
| Copper oxychloride (2 mg·mL$^{-1}$)[3] | - | - | - | - |
| Streptomycin sulfate (25 mg·mL$^{-1}$)[3] | - | - | - | + |
| Sterile water (control) | - | - | - | - |

[1] Absence of inhibition halo. [2] Presence of inhibition halo. [3] The same concentration was used in all treatments in which essential oils were not added.

EOs inhibited the pathogen's growth, whereas bacterial proliferation was observed in treatments using sterile water, PM or copper oxychloride, a contact fungicide which are known to show toxic effect against phytobacteria in general. On the other hand, streptomycin sulfate also inhibited bacterial growth at 100% concentration (Table 1).

Previous studies have also shown *in vitro* antimicrobial activity of substances obtained from medicinal plants. Zabka et al. (2009) found an *in vitro* activity of some EOs, being *Carum carvi*, *Cymbopogonnardus*, *Pelargonium roseum*, *Pimenta dioica*, and *Thymus vulgaris* the most effective ones against six important pathogenic and toxinogenic fungal species: *Fusarium oxysporum*, *Fusarium verticillioides*, *Penicillium expansum*, *Penicillium brevicompactum*, *Aspergillus flavus,* and *Aspergillus fumigatus*. In addition, Lucas et al. (2012) reported *in vitro* inhibition of *Xanthomonas vesicatoria* by using EOs of CI, CL, CN, LE, EU, TH, and TT at a concentration of 10%.

When the two time points of EOs application against bacterial speck in tomato were analyzed under greenhouse conditions we found a significant statistic effect for pre-treatment trial (a week before pathogen inoculation) compared to post-treatment ($P$<0.05) (a week after pathogen inoculation), with the use of EOs showing high disease control (low AUDPC value).

When treatments means of pre-treatment trial were compared using Tukey's test, ASM and CI were found to be the most effective ones in controlling bacterial speck in the tomato plant, with disease controls of 91% and 83%, respectively (Figure 1).

In addition, EOs of LE, TH, TT, EU, and CN were also statistically different from the inoculated controls treated with water and PM, resulting in disease controls of 63, 56, 55, 32 and 30%, respectively.

In regard to post-treatment trial, fungicide treatment was most effective in the disease control, with a control of 40%, followed by the EOs of LE, EU, CI, TH, and TT, with disease controls of 25, 19, 13, 9 and 8%, respectively (Figure 2).

No plants showed any signs of phytotoxicity that may be attributable to the use of the products in the greenhouse test, including copper oxychloride or ASM, although such effects have been reported in previous studies that used the same products on tomato plants (Gilardi et al., 2010).

Not all previous studies have shown an association between *in vitro* inhibition tests and *in vivo* treatment tests (Fravel, 2005; Medeiros et al., 2012). However, in the current study, the EOs of LE and CI showed *in vitro* inhibition of ufv-1 at the second lowest concentration tested (1%) and were also efficient in the *in vivo* control of the disease, in both preventive and curative forms. On the other hand, the treatments using ASM and CN showed control of the disease when applied before the inoculation of ufv-1 but were not statistically different from the control when applied after pathogen inoculation.

Results from this study showed that EOs of these specific medicinal plants have characteristics that resemble products with plant resistance induction activity, because best results were obtained when treatments were applied before inoculation of pathogen. For instance, the EO of CI decreased bacterial speck severity in tomato plants when applied before pathogen inoculation (Figure 1), suggesting that induction of resistance is the main mechanism of action in the study pathosystem once these two EOs showed characteristics similar to the commercial standard product ASM, a benzothiadiazole (BTH) known to mimic the pathogen–host interaction and then resulting in systemic acquired resistance in plants. Also, Veloso et al. (2012) identified 24 chemical compounds

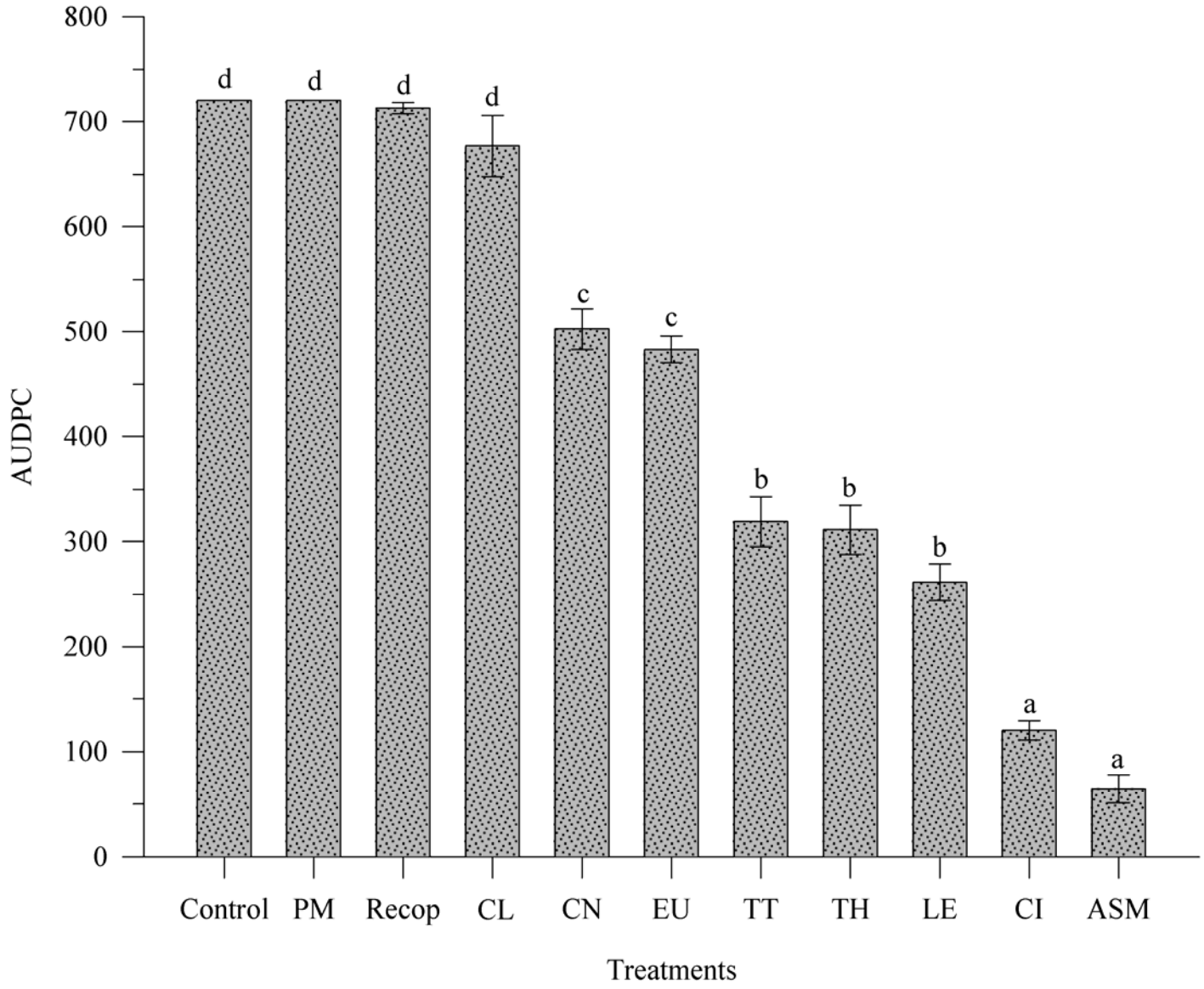


**Figure 1.** Area under the disease progress curve (AUDPC) in tomato plants of the cultivar Santa Cruz Kada with pre-treatment (7 days before pathogen inoculation) by using essential oils of citronella (CI); lemongrass (LE), thyme (TH), tea tree (TT), eucalyptus (EU), cinnamon (CN), clove (CL), and the products: copper oxychloride (Recop®), acibenzolar-*S*-methyl (ASM), powdered milk (PM), and water (control). Coefficient of variation (CV) = 9.31. Treatments followed by the same letter were not significantly different at 5% according to Tukey's test. The vertical bars represent the standard error of the mean.

present in the citronella EO that belonged to the terpene and terpenoid compound groups. Some studies have shown evidence that these compounds, which are produced as secondary metabolites in plants, have an important role in the plant's defense against various pathogens (Attaran et al., 2008; Henriquez et al., 2012).

The curative control of bacterial speck disease was observed by applying EOs, suggesting that direct toxicity mechanism may also be involved in the plant's defense system, thereby optimizing the efficiency of EOs, once one of the possibilities of increasing disease control in plants is using products that have more than one action mechanism (Fravel, 2005).

EOs have been used in the control of not only phytopathogens of the aerial plant parts, as shown in this and other studies (Balestra et al., 2009), but also in the control of pathogens from other pathosystems, such as post-harvested fruits and vegetables, as well as in seed and grain production. Santos et al. (2012) observed that the application of the EO of *Origanum vulgare* L. together with chitosan-inhibited spore germination and growth of *Rhizopus stolonifer* URM 3728 and *Aspergillus niger* URM 5842 fungi in artificially inoculated grapes. The use of *C. citriodora* oil at a concentration of 0.5% resulted in

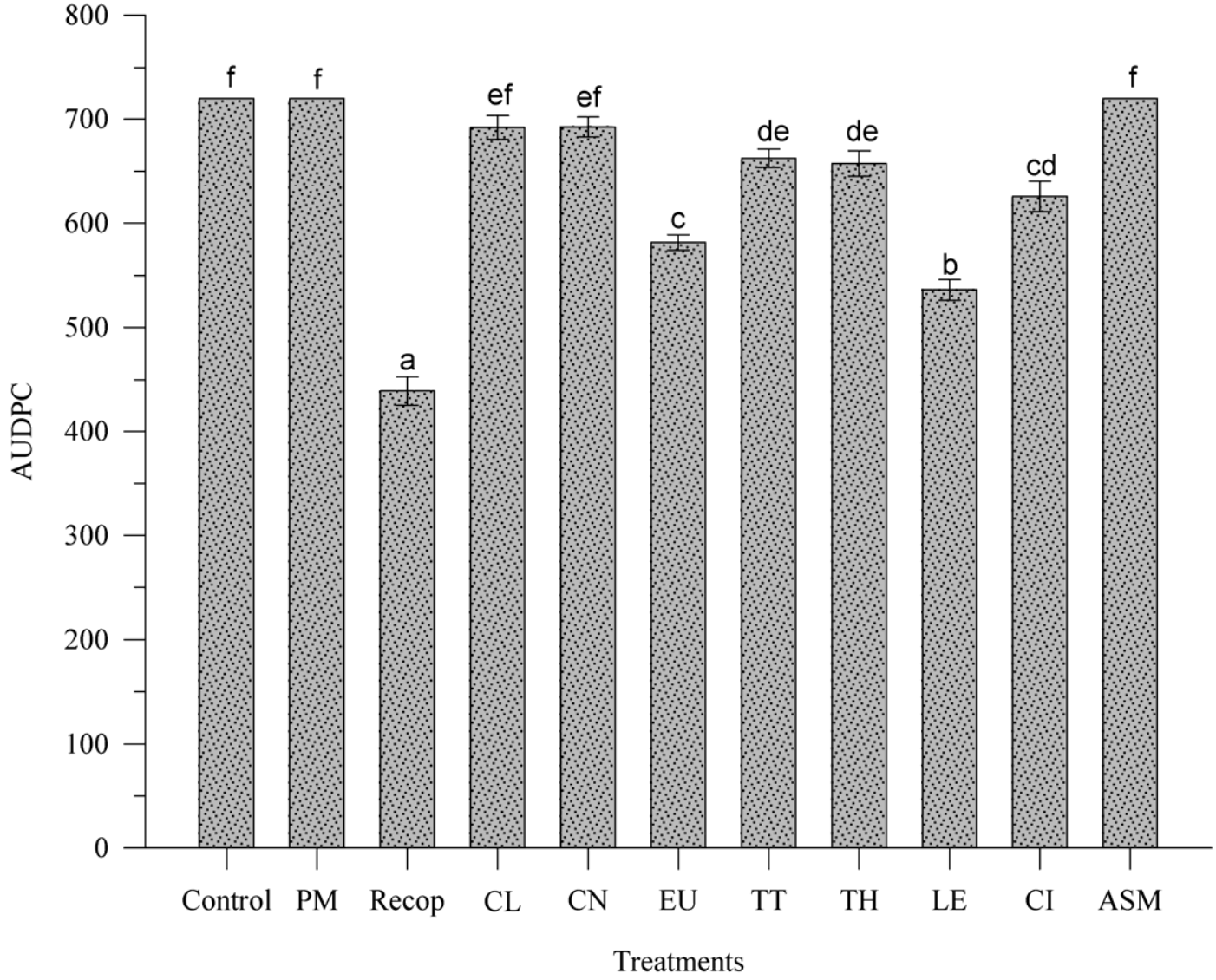


**Figure 2.** Area under the disease progress curve (AUDPC) in tomato plants of the cultivar Santa Cruz Kada with post-treatment (7 days after pathogen inoculation) by using the essential oils of citronella (CI), lemon grass (LE), thyme (TH), tea tree (TT), eucalyptus (EU), cinnamon (CN), clove (CL), and the products: copper oxychloride (Recop®), acibenzolar-*S*-methyl (ASM), powdered milk (PM), and water (control). Coefficient of variation (CV)= 3.53. Treatments followed by the same letter are not significantly different at 5% according to Tukey's test. The vertical bars represent the standard error of the mean.

an 83% decrease in the occurrence of soft-rot fungus (*Pectobacterium carotovorum* subsp. *carotovorum*) in lettuce (Silva et al., 2012). Amaral and Bara (2005) studied the effect of the EO of clove (*S. aromaticum*) on fungal growth in seeds of rice, soybean, corn, and beans and observed that clove oil imparted a fungicidal effect at concentrations of 0.5 to 0.1%.

Our findings suggest the potential role of EOs of medicinal plants in the alternative management of plant diseases and the importance of conducting further research in this area.

The EOs utilized in this study inhibited bacterial growth and the best time point of application identified was before pathogen inoculation being the citronella oil the most effective one. Also, a better disease control was obtained by pre-treatment than post-treatment. The study results showed that the induction of plant resistance may be the main mode of action of EOs in the tomato plant.

## Conflict of Interest

The author(s) have not declared any conflict of interest.

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