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THYME AND ROSEMARY ESSENTIAL OILS AS AN ALTERNATIVE CONTROL OF PLANT-PARASITIC NEMATODES.

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INTRODUCTION

Phytoparasitic nematodes have proven to be natural competitors of agricultural crops, and cause economic losses in a wide variety of crops. The plant-parasitic nematodes reduce the normal development of plants and make them susceptible to attack by pathogens such as fungi or bacteria (Hooks). In Ecuador, crop losses due to these pest range from 11% to 14%, and in interaction with Fusarium spp., the losses can reach values up to 90% of production (Ramírez, Grijalva, Navarrete, & Guerrero, 2016). Although phytoparasitic nematodes can be controlled by synthetic pesticides (carbamates and organophosphates), the excessive use of these pesticides produce resistance in nematodes and may be persistent in the environment and highly harmful to human health (Opperman & Chang, 1990).

The effort is carried out in the search for natural products with nematicidal activity, which represent an alternative and replace the synthetic chemicals and do not cause toxicological effects on the environment and the human being (Gupta, Sharma, & Naik, 2011). It has been found that several essential oils and their main compounds possess biological, antioxidant, antimicrobial, antifungal, insecticidal and nematicidal activity (Batish, Singh, Kohli, & Kaur, 2008; Espitia Yanes, 2011; Oka et al., 2000). However, only a few essential oils have been evaluated for their nematicidal effects. The objective of the present study was to evaluate the in vitro nematicidal activity of thyme (Thymus vulgaris) and rosemary (Rosmarinus officinalis) essential oil at three concentrations (0.5, 1.0 and 1.5% v/v), against phytoparasitic nematodes.

MATERIALS AND METHODS

Preparation of tested samples: Thyme (Thymus vulgaris) and rosemary (Rosmarinus officinalis) essential oils were purchased from Isabrubotanik S.A (Ambato-Ecuador). Essential oils, at three different concentrations (0.5, 1.0 and 1.5% v/v) were diluted in aqueous solution of Tween 80 (0.5%).

Nematode: The juveniles used in the experiment were provided by the Plant health laboratory (Department of Agriculture, Technical University of Ambato), using roots of tomato tree (Solanum betaceum Cav.) disease-infected by *Meloidogyne* sp., which were isolated by using the Baermann funnel Technique (Gonzales, 2013).

Nematicidal activity: two milliliter suspension containing mean number of ~ 60 juveniles of root-knot nematodes were added to the Petri dishes with the essential oil suspension at the rates of 0.5%, 1% and 1.5%, respectively. Two-milliliter suspension containing an average of 60 juveniles, were kept in a Petri dish with 2 ml sterile aqueous solution of Tween 80 used as control and sterile water used as blank. The experiment was conducted at ~20 °C. The number of motile juveniles was recorded at 0.25, 0.50, 0.75 1, 2, 4, 6, 8, 24 and 48 h after treatment application, using stereoscopic microscope. Juveniles were considered paralyzed (non-mobile) if they did not move when probed with a needle. Data were corrected by Abbott formula (Kong, Lee, Moon, Lee, & Ahn, 2006). Effects of the EO in the nematode were observed by light microscopy (EVOS cell Thermo Scientific, USA.).

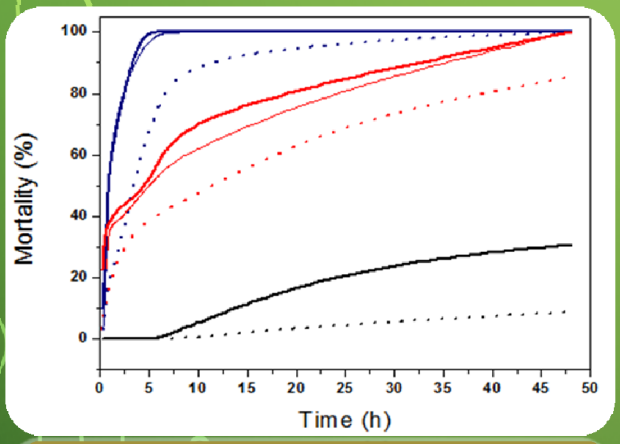

Fig. 1. Effect of thyme (blue) and rosemary (red) essential oils at different concentrations: 0.5% (dot line), 1.0% (fine line) and 1.5% (thick line), control (dot black line) and blank (black line) against *Meloidogyne sp.*

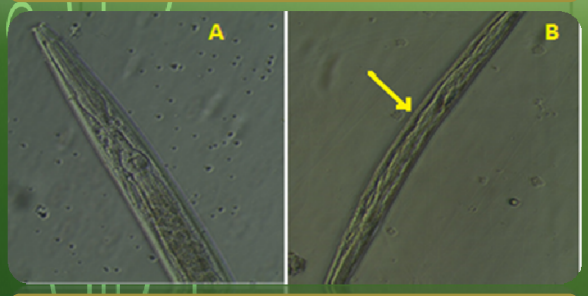

Fig. 2. Morphological changes caused by the thyme EO. **A**) Control and **B**) treated with 1.5% of thyme EO. Arrow indicates the wrinkled surface.

RESULTS

Toxic effects of the essential oils against juvenile nematodes, after 48 h of exposure, are shown in Figure 1. The results varying according the type and concentration of the essential oil. In the present study, the thyme EO show higher nematicidal effect than the rosemary EO even among the concentrations assayed.

These results could be related to the presence of thymol (~46.1%), p-cymene (~33.8%), gamma terpinene (~8.4%), caryophyllene (~5.4%), linalool (~3.4%) and carvacrol (~2.8%) as the main components. Under the microscope, the control appear as a smooth surface (Fig. 2A) and the treated with the thyme EO appears wrinkled (Fig. 2B).

This study evaluated the nematicidal effect of rosemary and thyme essential oils against phytoparasitic nematodes. The strong nematicidal activity of thyme EO was observed at 1.5% (v/v) which indicates that it has potential as a biological nematode control.

CONCLUSION

This study evaluated the nematicidal effect of rosemary and thyme essential oils against phytoparasitic nematodes. The strong nematicidal activity of thyme EO was observed at 1.5% (v/v) which indicates that it has potential as a biological.

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