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Achieving Natural control of the phytopathogenic fungus *Cylindrocladium* sp. using two different thyme essential oils applied under *in vitro* conditions

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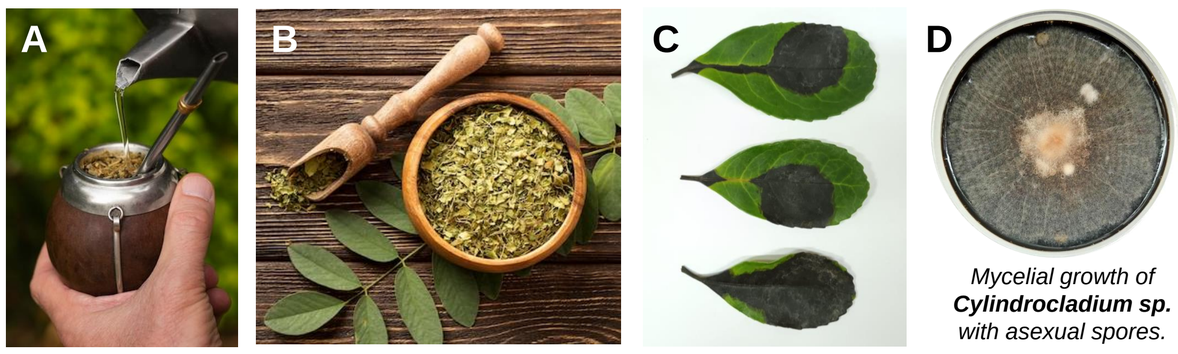
INTRODUCTION & AIM

Ilex paraguariensis (*Ip*) is a crop with economic and cultural importance in South America, where its leaves are used to prepare an infusion called “mate” (Figure 1-A & 1-B). This crop is affected by different phytopathogenic microorganisms.

Cylindrocladium (*Cy*) (Figure 1-D) are the causal agent of the black spot of *Ip* (Figure 1-C).



*Mycelial growth of **Cylindrocladium sp.** with asexual spores.*

Figure 1. Yerba Mate: use and disease. A) Mate drink, B) Yerba mate leaves, c) Symptoms of Black spot, D) Mycelial growth of *Cylindrocladium* sp.

Natural products, as essential oils (EOs) are complex mixtures of molecules that are considered safe for the environment and can be used to control microorganisms in crops.

The objective of this work was to evaluate the use of two thyme (*Thymus vulgaris*) EOs as control agents of *Cy*.

METHOD

The thyme EOs (T1 and T2) were characterized by GC-MS. *Cy* was isolated from infected leaves of *Ip*. *A* 14-day culture of *Cy* was used to obtain the work solution (100,000 spores/ml).

The EOs were diluted in dimethyl sulfóxide at a rate of 1:4, and nine two-fold serial dilutions were performed. Treatments consisted of the addition of 15 µl of work solution to 165 µl broth with *Ip* and 20 µl of each EO dilution, which were then incubated for 72 h. Four replications were carried out. Negative (*Ip* broth) and positive controls (without EOs) were performed. The minimum inhibitory concentration (MIC) was determined as the minimal concentration in which no micelial growth was observed. Tubes whitout micelial growth were sowed in *Ip* medium and incubated for 48 h. The minimum fungicide concentration (MFC) was defined as the minimal EO concentration at which no *Cy* colonies were grown.

RESULTS & DISCUSSION

T1 was mainly composed of thymol (40.32%) and p-cymene (36.43%), while T2 comprised p-cymene (59.65%), thymol (11.78%), and eucalyptol (5.35%).

Both EOs exhibited fungistatic and fungicidal activity, with MIC values of 2.35 µl/ml for T1 and 31.25 µl/ml for T2 (Figure 2), and MFCs of 2.93 µl/ml (Figure 3) and 50 µl/ml, respectively.

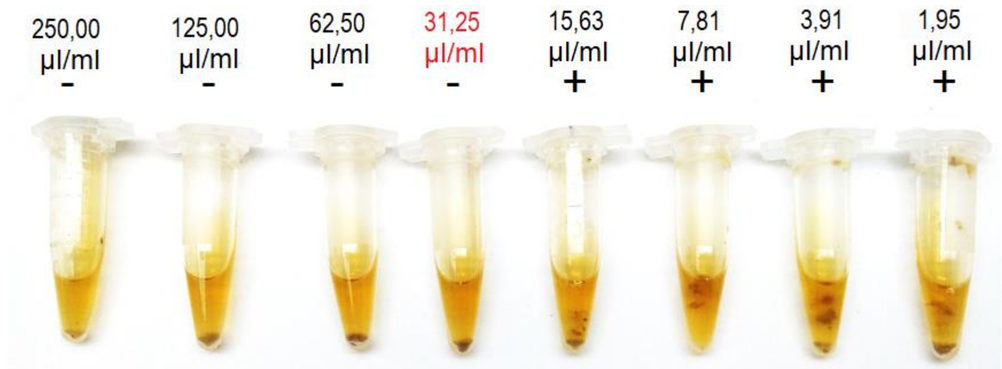


Figure 2. Fungistatic activity of *Thymus vulgaris* EO (T2) against *Cylindrocladium* sp. Values in red font indicate the minimum inhibitory concentration of T2.

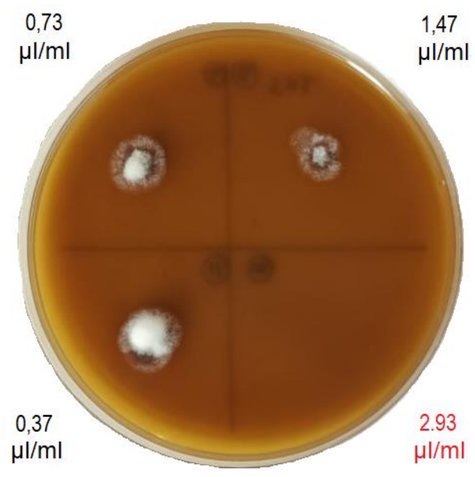


Figure 3. Fungicidal activity of *Thymus vulgaris* EO (T1) against *Cylindrocladium* sp. Values in red font indicate the minimum fungicidal concentration of T1.

Despite both EOs showing good antifungic activity, T1 showed a greater bioactivity, which was correlated with the higher relative amount of thymol compared with T2. This agree with Burt (2004), who indicate that terpenes with an aromatic ring as thymol, have strong antimicrobial activity.

CONCLUSION

These results demonstrate the effectiveness of thyme EOs as natural control agents against *Cy*.

FUTURE WORK / REFERENCES

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