Protective antifungal activity of *Plantago major* extract against the phytopathogenic fungi *Phytophthora cinnamomi*, *Diplodia corticola* and *Colletotrichum* species

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Introduction

Figure 1. *Plantago major*

Crop-protecting synthetic fungicides raise environmental and human concerns due to accumulation in edible vegetables[1], showing significant toxicity to humans[2], and in soil[3], groundwater and rivers[4], affecting ecological balance. *Plantago major* **(Figure 1)** extract is a rich source of biodegradable secondary metabolites, which have multiple modes of antifungal action and lower probability of development of resistant fungi strains, a very notorious problem with the use of synthetic fungicides[5]. The objective of this work is to evaluate the antifungal activity of *P. major* extract, as a potential replacement of synthetic fungicides, aiming to contribute to sustainable agriculture practices and food safety.

Materials and Methods

To investigate *P. major* inhibition on the mycelial growth of the phytopathogenic fungi *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, *Colletotrichum godetiae*, *Colletotrichum nymphaeae*, *Diplodia corticola* and *Phytophthora cinnamomi,* the dried plant was extracted with 50% (v/v) ethanol, the solution dried by evaporation, and the residue dissolved in water. The aqueous extract was incorporated into PDA medium at different concentrations, 100, 500, 1000 and 2000 µg/mL and mycelial discs of each fungus were placed in the center of each Petri dish. Radial mycelial growth was measured at 3, 6 and 9 days after inoculation. For each treatment, three replicates were performed. The assay ended when the negative control reached full growth. The antifungal activity of the extract was calculated in terms of inhibition percentage of mycelia growth by using the following formula:

$$\text{Inhbition}\ (\%) = \frac{dc - dt}{dc} \times 100$$

, where dc is the average increase in mycelia growth in negative control and dt is the average increase in mycelia growth in treated sets.

References

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Results

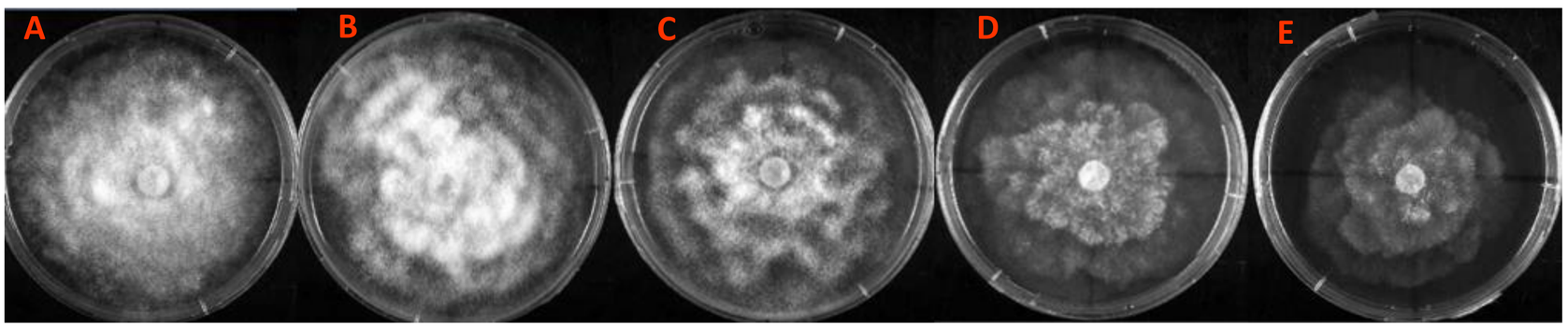

Figure 2. Representative images of *P. major* antifungal activity at different concentrations, 100, 500, 1000 or 2000 µg/mL, against *Phytophthora cinnamomi*, on PDA solid medium, after 6 days of incubation (n=3). Negative control (A), 100 µg/mL (B), 500 µg/mL (C), 1000 µg/mL(D) and 2000 µg/mL (E).

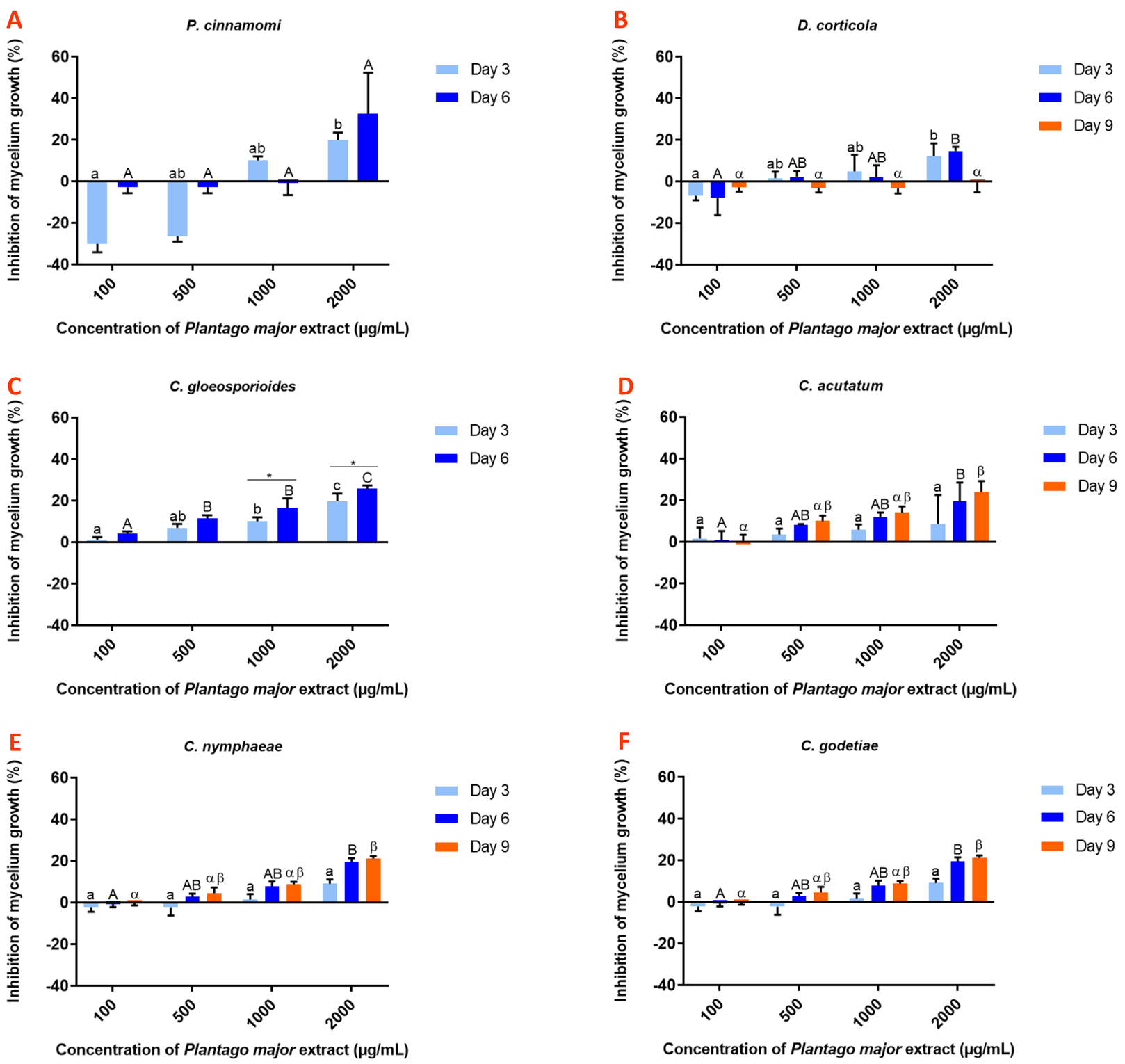

Figure 3. Effect of *P. major* on mycelial growth of *Phytophthora cinnamomi* (A), *Diplodia corticola* (B), *Colletotrichum gloeosporioides* (C), *Colletotrichum acutatum* (D), *Colletotrichum godetiae* (E) and *Colletotrichum nymphaeae* (F) isolates on PDA medium with incorporation of *P. major* extract. Percentage of growth inhibition determined after 3,6 and 9 days of incubation at different concentrations of *P. major* extract, 100, 500, 1000 or 2000 µg/mL. Data are presented as mean of three independent experiments ± SD. One-way ANOVA and Kruskal Wallis test were used for multiple comparisons. Differences were considered statistically significant if P <0,05. Mean values followed by the same letters are not statistically different (lowercase letters for day 3, capital letters for day6 and Greek letters for day 9). Comparisons between different days of the same concentration are only represented if they are significant.

Conclusions

P. cinnamomi
Significant diference between concentrations 100 and 2000 µg/mL on the third day.

D. corticola
Reestablishment of mycelial growth after nine days, suggesting adaptation of the fungus.

C. gloeosporioides
The extract has dose-dependent inhibition on fungal growth on the third and sixth days.

C. acutatum
C. godetiae
C. nymphaeae
Significant differences from day 6 to day 9 between 100 and 2000 µg / mL.

The extract from *P. major* has the potential to replace synthetic fungicides with convenient application programs in crops in order to control and prevent fungal growth. By inhibiting fungal growth by 20 – 32,2%, *P. major* extract would not be likely to promote fungal resistances and would not have impact on the environment.