Induction of natural defenses by *Mimosa tenuiflora* and *Quercus robur* extract in lettuce against *Sclerotinia sclerotiorum*

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Abstract

The induction of plant innate defenses is one of the most promising alternatives to achieve a sustainable plant protection against pathogens. Natural compounds such as plant extracts have been reported enhancing the natural defensive responses of the plant. The extract of Mimosa tenuiflora *and* Quercus robur *is a natural product with potential efficacy reducing the incidence of a wide range of phytopathogenic fungi in a great variety of hosts. In this study, we aimed to elucidate the protective effect and the mode of action of this extract using the* Lactuca sativa *vs.* Sclerotinia sclerotiorum *pathosystem. To do that, 4 weeks-old lettuce plants cv. Romana were treated with 2 cc/L of the plant extract either by soil drench or foliar applications. All treatments were applied 72 h before the inoculation. Treatments were able to reduce significantly the progression of the pathogen, decreasing the diameter of the infection by 32% and 17% in the foliar and soil drench applications, respectively. ROS levels were significantly lower in treated and inoculated plants compared with non-treated plants, but no differences were detected between foliar and soil drench treatments. Analysis of callose deposition showed that the treatment induced significantly higher accumulation of this molecule in inoculated plants with both foliar and radicular treatments compared with non-treated and inoculated plants. As a conclusion, it is noteworthy that treatments with* M. tenuiflora *and* Q. robur *extract reduce significantly the infection of* S. sclerotiorum *in lettuce leaves, with the strengthening of the wall through callose deposition and the release of ROS being the two mechanisms involved in the defense of the plant against the pathogen. These two processes are defense mechanisms that plants use against different pathogenic organisms since they prevent colonization in early phases. The fact that the treatment enhances them indicates that it could be effective against different microorganisms.*

Keywords: Induced resistance, *Lactuca Sativa, Mimosa tenuiflora, Quercus robur*, *Sclerotinia sclerotiorum*

Introduction

The growing concern for the environment and human health is increasing the demand of fruits and vegetables without toxic waste. In this sense, in recent years there has been a significant increase in the implementation of formulations based on botanical extracts from plants such as *Mimosa tenuiflora* and *Quercus robur*, which also increase the yield of the crops, and induce the defense mechanisms of the plants. These extracts would provide an eco-friendly alternative to the classical chemical treatments. For this reason, the main goal of this study was to evaluate the efficacy of *M. tenuiflora* and *Q. robur* extract enhancing the plant immune system in Lettuce against *Sclerotinia sclerotiorum.*

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Material and Methods

Applications of *M. tenuiflora* and *Q. robur* extract were performed on 4-week old lettuce plants either by soil drench or foliar applications using a concentration of 2 ml l^{-1}, 72 h before inoculation. Pathogen inoculation was performed using 3 mycelial plugs grown on PDA per plant, laying the mycelia in contact with the leaf. Additionally, lots of plants treated with plant extract and non-inoculated, non-treated and inoculated, and non-treated and non-inoculated were included for comparative purposes. Infection development and physiological parameters such as plant growth, and shoot and root dry weight were evaluated. Parameters involved in plant resistance were monitored through determination and quantification of reactive oxygen species (ROS) response (i.e. H_2O_2), and evaluation of hormones and phenolic compounds levels by chromatographic analysis. Evaluation methods described in Llorens et al. 2013.

Results and Conclusions

In this study, the effectiveness of a mixture of *M. tenuiflora* and *Q. robur* extract was evaluated. The results obtained showed that both soil drench and foliar treatments are able to reduce the diameter of infection by 30 and 17%, respectively. Moreover, a single preventive treatment induced the deposition of callose in the site of the inoculation as well as the induction of H_2O_2 in treated and inoculated plants. In the same way, the treatment with the botanical extracts induced accumulation of Jasmonic acid and Jasmonic isoleucine (Table 1). Our results suggested that the application of *M. tenuiflora* and *Q. robur* extract is able to activate several mechanisms of plant innate immunity in lettuce plants which results in a protection against *S. sclerotiorum*.

Table 1: Effect of treatment with *Mimosa tenuiflora* and *Quercus robur* extract on parameters involved in plant resistance. The data show the average of three independent experiments. Different letters represent statistically significant differences ($P < 0.05$ least-significant difference test).

Treatment	Peroxide (% pixels per image)	Callose (% pixels per image)	JA (ng/g)	JA-Ile (ng/g)
Control non-inoculated	0 ± 0	799.9 ± 66.6 a	349.4 ± 262.5 a	881.2 ± 20.6 b
Control inoculated	21111 ± 3333.3 b	4666.2 ± 799.9 bc	5543.1 ± 2333.4 b	78969.4 ± 25771.0 a
Soil non-inoculated	222.2 ± 777.7 a	1333.2 ± 333.3 ab	2613.5 ± 169.6 a	6173.2 ± 969.5 b
Soil inoculated	35555.2 ± 4444.4 c	8665.8 ± 999.9 d	1461.3 ± 1098.0 a	34502.1 ± 27098.5 a
Leaf non-inoculated	0 ± 0	7332.6 ± 1666.5 cd	474.7 ± 308.9 ab	1636.8 ± 1151.5 b
Leaf inoculated	43332.9 ± 5555.5 c	19664.8 ± 2333.1 e	701.6 ± 478.7 ab	43830.3 ± 342.5 a

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References

Llorens, E., Fernández-Crespo, E., Vicedo, B., Lapeña, L. & García-Agustín, P (2013). Enhancement of the citrus immune system provides effective resistance against Alternaria brown spot disease. *J. Plant Physiol.* 170:146-154.

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