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BIOLOGICAL CONTROL OF BROWN DISCOLORATION ON *AGARICUS BISPORUS* CAUSED BY *PSEUDOMONAS* WITH OLIVE OIL MILL WASTEWATER.

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KEY WORDS: Biocontrol, olive oil mill wastewater, mushrooms brown discoloration.

ABSTRACT

The preventing bacterial brown blotch on Agaricus bisporus caused by Pseudomonas was investigated. Infected mushrooms were treated with talc formulation of olive oil mill wastewater (olive OMW). The talc formulation of olive OMW significantly reduced the visual symptoms of the disease under laboratory conditions, probably due to the activity of the phenolic compounds which contained on olive OMW.

INTRODUCTION

Agaricus bisporus is well known as a common mushroom, is cultivated in all over the word mainly for used as food item. Brown discoloration (brown blotch disease) caused on *A. bisporus* fruits by *Pseudomonas* species is also well known. Infection with *Pseudomonas* spp. caused a specific colour change. Symptoms are dark brown, often wet and sunken lesions on the mushroom caps and stalks. Economically, it currently is the most important disease of mushrooms. The mushroom market in Greece consumes only white fresh mushrooms, but not when covered with bacterial spots (discolorations). Therefore, the disease causes relatively high losses to growers.

Chemical treatments for preventing bacterial brown blotch using chlorine, or other chemical compounds have not been satisfactory, whereas treatments as biocontrol are still under investigation.

Regarding biocontrol treated mushrooms with various plant extracts or microbial as potential inhibitor of the symptoms of the brown blotch disease of *A. bisporus* could be promising due to enzymes and antibiotics they produced. Other promising products for preventing bacterial brown blotch could be the residues of olive oil mill wastewater (olive OMW). The residues of olive OMW appeared to have effective antifungal activity against soil born plant pathogens (12), against post-harvest diseases (12), and could be a promising solution for preventing discoloration of mushrooms.

The aim of this work was to examine the potential inhibitor effects of olive OMW against brown discoloration on *A. bisporus* caused by *Pseudomonas*.

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MATERIALS AND METHODS

Isolation of the pathogen

Isolation of *Pseudomonas* was made using standard bacteriology techniques following the method of Schaad (1998). Pure *Pseudomonas* colonies was first isolated and grown on King's B medium. Then *Pseudomonas* cultures streaked on Nutrient Agar plates and grown on medium Nutrient Broth at 25 °C for 48 h. Further pathogenicity tests were carried out on mushrooms, as described by Munsch (2000).

Infection process

The potential inhibitor effect of olive OMW against brown discoloration *of A.s bisporus* was performed *in vivo* by using symptom-free mushrooms inoculated with a suspension of the pathogen and symptom-free mushrooms inoculated with a suspension of the pathogen and treated with olive OMW mixed with talc powder 1:4 v:v, or 1:10 v:v, or talc powder only (Fig. 1) and incubated in a humid chamber at 4°C for 5 days. At the end of the experiment typical symptoms on mushrooms were recorded sorting brown discoloration in five classes (0 to 4), where 0 is equal to healthy fruits; 1=slightly brown mushrooms and 4= dark brown mushrooms. All experiments were done in plastic, closed containers; ten mushrooms were placed in each container for each treatment. Each treatment had four replicates.

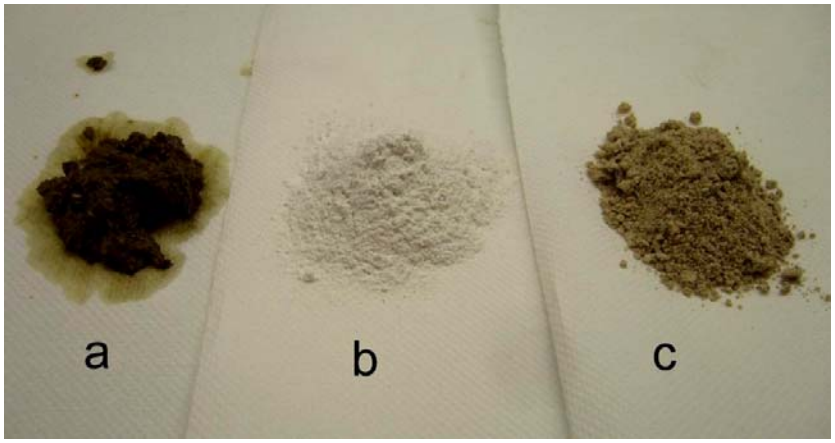

Figure 1. Olive OMW formulation; a: olive OMW mixed with talc powder 1:4 v:v; b: talc powder only and c: olive OMW mixed with talc powder 1:10 v:v

RESULTS AND DISCUSSION

Mushrooms treated with olive OMW in form of talc powder 1:4 v:v significantly reduced Pseudomonas brown discoloration (Figure 2 and 3). A dark brown discoloration was recorded only in treatments with symptom-free mushrooms inoculated with a suspension of the pathogen whereas a slight brown discoloration was recorded only in treatments with symptom-free mushrooms inoculated with a suspension of the pathogen and treated with olive OMW mixed with talc powder 1:10 v:v or with talc powder only.

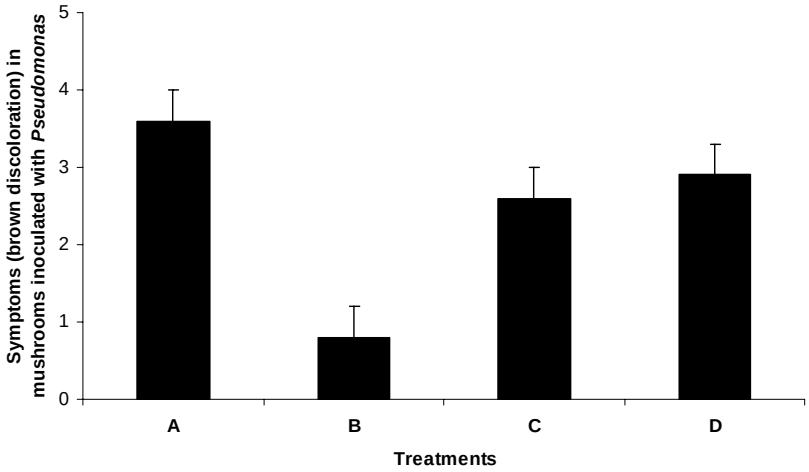

Figure 2. Effect of olive OMW on mushrooms brown discoloration caused by *Pseudomonas* where A: symptom-free mushrooms inoculated with a suspension of the pathogen; B symptom-free mushrooms inoculated with a suspension of the pathogen and treated with olive OMW mixed with talc powder 1:4 v:v; C: symptom-free mushrooms inoculated with a suspension of the pathogen and treated with olive OMW mixed with talc powder 1:10 v:v and D: symptom-free mushrooms inoculated with a suspension of the pathogen and treated with talc powder only.

Olive oil mill wastewater (olive OMW) contains a number of biologically active substances capable of inhibiting the growth of microorganisms (11) and even plants (7). Many phenolic, free fatty acids and aromatic compounds have been detected (5, 11) in olive oil mill residues associated with phytotoxic and antimicrobial properties of these residues (10). Several investigators reported that the inhibition of microbial growth and the toxic activity of olive OMW caused by different chemical compounds of olive residues (1, 2, 5).

Low moleculal-weight phenolic compounds seem to be the main determinants of the anti-microbial effect of olive residues (4, 6) while high molecular-mass polyphenols, organic acid, lipids, oligosaccharides and glycoproteins can contribute to the phytotoxic potential of the waste (3). Thus, several methods have been developed to degrade phenols in olive oil residues.

In the current study brown discoloration on *A. bisporus* caused by *Pseudomonas* significantly inhibited by olive OMW. We assume that the presence of phenolic compounds on olive OMW suppresses bacteria and possible could offer a protection on mushrooms from post-harvest diseases such as brown discoloration. Overall the results of this work demonstrated the high potential of olive OMW as a potential inhibitor against brown discoloration on *A. bisporus* caused by *Pseudomonas*.

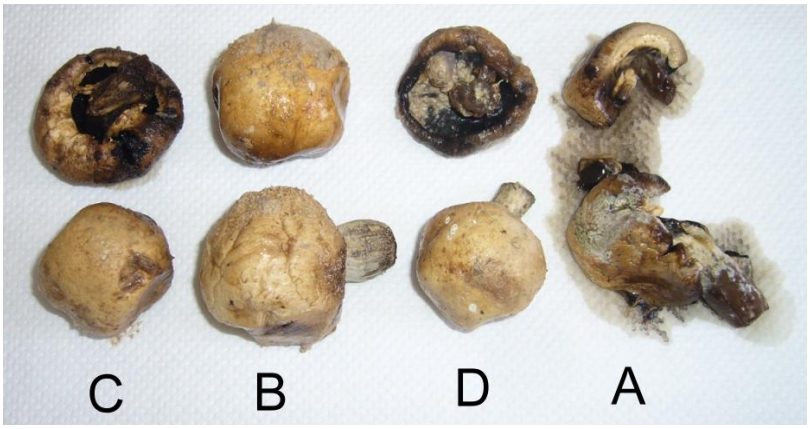

Figure 3. Effect of olive OMW on mushrooms brown discoloration caused by *Pseudomonas* where A: symptom-free mushrooms inoculated with a suspension of the pathogen; B symptom-free mushrooms inoculated with a suspension of the pathogen and treated with olive OMW mixed with talc powder 1:4 v:v; C: symptom-free mushrooms inoculated with a suspension of the pathogen and treated with olive OMW mixed with talc powder 1:10 v:v and D: symptom-free mushrooms inoculated with a suspension of the pathogen and treated with olive OMW mixed with talc powder only.

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