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SE06 002

CONTROLLING OLIVE ANTHRACNOSE WITH ANTIFUNGAL METABOLITES FROM BACILLUS SPECIES: A BIOLOGICAL APPROACH

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Anthracnose disease in olive, caused by the fungal pathogen *Colletotrichum acutatum*, is considered one of the most critical issues in olive orchards in Pakistan. This disease poses a significant threat as it results in infections that can lead to the complete damage of olive plants, affecting leaves, stems, and fruits in the field. Controlling this disease is particularly challenging due to the absence of an effective fungicide that does not pose risks to farmer health and the environment. To address this challenge, our study aimed to evaluate the antagonistic activity of a biosurfactant produced by the *Bacillus subtilis* PE-07 strain against the anthracnose-causing agent in olive plants. This strain was selected after screening of forty gram Positive rhizobacteria strains. Additionally, we assessed the heat stability, pH range, and toxicity of the biosurfactant produced by strain PE-07. Our results revealed that the biosurfactant exhibited maximum antifungal activity against *C. acutatum*. In vitro studies indicated that the biosurfactant could reduce fungal activity by inhibiting the spore germination of *C. acutatum*. Furthermore, the biosurfactant demonstrated a wide pH and temperature range, displaying antifungal activity at pH levels ranging from 5 to 10 and a temperature range from room temperature to 110°C. To evaluate the biosurfactant's safety, we conducted toxicity tests on zebra fish (*Danio rerio*). The results showed that the biosurfactant had minimal harmful effects, even at maximum concentrations. In conclusion, our study confirmed that the biosurfactant produced by *B. subtilis* exhibited high pH and heat stability with minimal harmful effects. Therefore, it presents a promising alternative to chemical pesticides for effectively controlling olive anthracnose in Pakistan.

SE06 003

NEW PROMISING "BIOFUNGICIDES" TARGETING THE FUNGAL CELL WALL TO CONTROL THE CUCURBIT POWDERY MILDEW PODOSPHAERA XANTHII

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One of the main limitations of cucurbit production is the powdery mildew disease, caused by *Podosphaera xanthii*. Although different management strategies are used to control it, the application of fungicides is the most effective. However, there are two major problems: the rapid emergence of resistance to fungicides by the pathogen and the strong restrictions on the use and diversity of phytosanitary products imposed at the European level. For this reason, novel targets and strategies are needed to develop new "biofungicides" for sustainable disease control. The fungal cell wall (CW) is a unique and essential structure, not present in human or animal cells, which makes it an ideal target for the development of new phytosanitary products. In this study, three *P. xanthii* cell wall-related genes (coding a GPI-anchored membrane protein and two proteins involved in the synthesis of rhamnose) were studied. To understand their impact on the development of *P. xanthii*, RNA interference (RNAi) technology was used. For this purpose, dsRNA molecules were synthesized and several assays were performed. The infiltration and leaf disc assays on melon and zucchini cotyledons, respectively, showed an effective gene silencing effect, resulting in a significant reduction of gene expression, fungal biomass and area covered by powdery mildew disease. Microscopy analysis confirmed the impact on fungal penetration points. In addition, the application of dsRNAs, through spray-induced gene silencing (SIGS) assays, resulted in a significant decrease in disease development on melon plants. According to the results obtained, these genes could serve as potential targets for the development of new generations of antifungal agents. In addition, SIGS technology could be a promising strategy to incorporate into integrated management programs for this important disease.

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SE06 004

INSECT PARASITOIDS OFFER A NATURAL SOURCE OF NOVEL BIO-INSECTICIDES FOR SUSTAINABLE PLANT PROTECTION IN DIVERSE ECOLOGIES

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Ensuring sustainable and cost-effective food production is an urgent priority for agriculture. While synthetic insecticides are widely used for insect control, they are known to have detrimental effects on the environment and non-target organisms. Heavy reliance on these chemicals poses significant environmental and health risks. One promising alternative is leveraging biotechnology-based natural products or organisms for pest suppression. The diversity of indigenous insect parasitoids presents a promising avenue for discovering new genes and peptides that could serve as alternatives to synthetic insecticides in agriculture. This is particularly important given the escalating issue of insect pests in agriculture, alongside projections of a global population increase to 10 billion in the next four decades. Female parasitoid wasps inject various substances, including venom, ovarian fluid, and polydnavirus, which contribute to the mortality of harmful insects. By analyzing the venom proteins of the wasps species *Aeansius arizonensis* and *Bracon hebetor*, identified potential bioactive compounds through techniques such as SDS-PAGE and protease/trypsin digestion. The genes encoding these proteins were isolated using RT-PCR, and recombinant proteins were produced. The insecticidal activity of these proteins was then assessed on both target and non-target organisms. This foundational research has provided valuable insights that could be leveraged to develop novel insect control strategies, ultimately safeguarding the biodiversity of agricultural crops.