SUMMARY

of the doctoral thesis entitled:

PLANT BIOSTIMULANTS BASED ON (BIO)MIMETICS OF STRIGOLACTONES

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Strigolactones (SLs) are natural derivatives of carotenoids, released by plants into the soil as signaling molecules. Strigolactones (SLs) serve as signaling molecules that initiate the germination of parasitic plant seeds and stimulate the branching of arbuscular mycorrhizal (AM) fungal hyphae. They function as active phytohormones, regulating the structure of various plant organs and playing a vital role in in plant responses to both biological and environmental stresses.

SLs hold promise for sustainable agriculture by helping plants cope with different stresses. However, obtaining natural SLs or creating synthetic alternatives in sufficient quantities for practical use is challenging. Fluorescent SLs could also aid in studying their mechanisms through bio-imaging or spectroscopy techniques.

Chapter I, titled "**Strigolactones - multifunctional signaling factors**," provides a bibliographic analysis of strigolactones as essential phytohormones in regulating plant development and interactions with the environment. These molecules function as chemical signals that control various processes, including stem branching, root growth, and stress responses. Strigolactones are essential for mycorrhizal symbiosis, facilitating the colonization of roots by beneficial fungi. Additionally, these compounds play a role in protecting plants from pathogens and helping them adapt to challenging environmental conditions. Thus, strigolactones are key factors with multiple functions that contribute to optimizing plant growth and health.

Chapter II, "**The uses of synthetic strigolactones in agriculture**," aims to understand that synthetic strigolactones represent a significant innovation in agriculture, offering solutions to improve crop growth and productivity. These molecules promote branching and root system growth, enhancing the plant's ability to absorb nutrients and water more effectively. Moreover, synthetic strigolactones can boost plant resilience against biotic and abiotic stresses, including drought and pathogen invasions.

Furthermore, they facilitate mycorrhizal symbiosis, promoting a beneficial relationship between plant roots and fungi. By using synthetic strigolactones, farmers

can achieve more robust and resilient crops, contributing to sustainable and efficient agriculture.

In **Chapter III,** titled **"Testing the biological effects of new strigolactone mimics,"** the effects of different strigolactone mimics on the branching of hyphae and radial growth of several plant pathogenic fungi were examined. As far as we know, there is no existing information on the effects of these mimics on colony growth and morphology. The initial fungi tested were *Colletotrichum acutatum* and *Sclerotinia minor*. The results showed that the mimic compound SL6, which is simpler to produce, than the synthetic analog GR24, produced a similar response by inducing hyphal branching and inhibiting the growth of phytopathogens. These results have significance for both fundamental research, including insights into the function and receptor of the D-ring of SLs in plant pathogenic fungi, as well as practical uses such as simulating interactions within the plant rhizomicrobiome.

In a related study, new fluorescent SL mimics (SL20, SL21, SL26, and SL27) were developed. Their structural, spectroscopic, and biological properties in relation to phytopathogens were examined and contrasted with those of previously synthesized fluorescent SL mimics. All SL mimics exhibited effects effects comparable to those of GR24 on phytopathogens *C. acutatum*, *F. graminearum*, *R. solani*, and *S. sclerotium*, suggesting their potential for practical use. The biological activity varied depending on the fungal species, the type and concentration of the SL mimic, and the structure of the hyphae. These differences are likely due to the specific interactions between each receptor and fungal species, warranting further detailed investigation. Considering their biological and spectroscopic characteristics, these SL mimics could be promising candidates for microscopic and spectroscopic studies.

In another experiment, synthetic biomimetic strigolactones were prepared in multi-gram amounts from commercially. The newly developed strigolactone mimics were evaluated against the phytopathogenic strains *Fusarium graminearum* and *C. acutatum*. Most of the treatments using the strigolactone mimics FG-30, FG-31, FG-33, and FG-42 demonstrated a notable suppressive impact on the growth of both *F. graminearum* and *C. acutatum*. Qualitative analysis revealed that both GR24 and the SL mimics influenced the hyphal branching of these fungi. The results suggest that SL mimics, especially FG-33 and FG-31, could be used as control agents for phytopathogens due to their inhibitory effects on hyphal growth and branching. Future research should aim to clarify how these compounds affect fungal growth and development, while also refining concentrations for effective use in sustainable agriculture.

Another study focused on testing the biological properties of a different strigolactone mimic, SL4. While this compound displays fluorescent characteristics, it did not significantly impact the growth or hyphal branching of *Aspergillus alliaceus* fungi.

Additionally, the effect of SL6 on the *Trametes versicolor* strain was also tested, revealing no significant impact on hyphal branching, with minimal effects observed on colony diameter, primarily at lower concentrations.

In the context of modern agriculture, where diseases caused by phytopathogenic fungi pose a major challenge, the development of new strigolactone mimics and

evaluating their effects on phytopathogenic fungi are of particular importance, along with mitigating various stresses faced by plants.

Chapter IV, "**The effect of laser light and strigolactone mimics on growth and development of *Trichoderma* strains,**" was divided into several subchapters to determine the effects of treatments both separately and in combination. The study of strigolactone mimics and laser light on the growth and development of Trichoderma strains can offer new perspectives on how these molecular signals and environmental factors affect the behavior and effectiveness of this important genus of fungi. The aim was to examine the impact of strigolactone mimics and laser light on the growth and development of *Trichoderma* strains. Through this research, we aimed to gain a deeper understanding of how these factors influence the physiology and behavior of *Trichoderma*, with potential implications for their application in agriculture and biocontrol.

In the first **subchapter 4.1**, we explored how **blue laser light irradiation affects the enzymatic activities and sporulation of *Trichoderma atroviride* when cultivated with reduced amounts of rice bran**. Light is recognized for affecting different aspects of *Trichoderma*, which could have potential applications in industry and agriculture. This study focused on irradiating *Trichoderma atroviride* using a laser-based blue light system. We evaluated the activities of cellulases and proteases, along with the UV-Vis absorption effects of the filtered culture on conidia formation, size, and behavior, as well as the apparent abundance of chlamydospores, considering the duration, dose, and timing of irradiation.

Our results show that the impact on enzymatic activities varies, ranging from positive to neutral or negative. Contrary to previous studies, our light stimulation does not significantly inhibit cellulase activity (CMC-ases) but appears to delay the peak activity over time. The effects on protease activity are partly consistent with the limited existing research on light's influence on proteases. We observed an increase in conidia quantity after irradiation, consistent with expected behavior, and, to our knowledge, we are the first to report an increase in conidial size and chlamydospore numbers

Additionally, our data suggest that the highest irradiation dose led to conidial aggregation at the air-liquid interface, indicating an increase in hydrophobic properties.

The second **subchapter 4.2**, titled "**Production of enzymes by *T. atroviride* in the presence of blue laser light: a large-scale approach,**" aimed to investigate the impact of irradiation on mature mycelium grown on rice bran and scale up the process. Therefore, we first incubated *Trichoderma* with rice bran for 15 days in larger containers, followed by three irradiations at shorter time intervals than in our previous study.

In this study, the bio-control agent *Trichoderma atroviride* was repeatedly exposed to blue laser light and analyzed after different time intervals regarding enzyme production, soluble silicon content, phenolic content, as well as antioxidant activity. Contact angle and interfacial tension were also evaluated. Morphological structures were examined through optical microscopy. We also studied the metabolism of reactive oxygen species (ROS). We investigated the degradation of lignocellulosic matrix using

Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy with energy dispersive X-ray analysis (SEM-EDX).

It was found that exposing *Trichoderma* atroviride to moderate blue laser light for three rounds of 60 seconds each, at different time intervals, enhances enzymatic activity. The highest levels of cellulase and protease production were observed after three rounds of irradiation. Some effects appear to follow a hormetic behavior for α-amylase production. After determining LPMO activity, the best results were obtained in a small quantity before the third irradiation, but the values were under control. Additionally, the increase in soluble Si content in *Trichoderma* culture after irradiation highlighted an improvement in the breakdown of rice bran lignocellulosic matrix, evidenced by FTIR analysis through reduced vibrations of amide and hydrogen bonds, as well as XRD analysis through increased crystallinity of rice bran incubated with irradiated *T. atroviride*. Moreover, irradiation enhances sporulation of *Trichoderma* and modifies the cohesion and adhesion forces of the growth medium.

Subchapter 4.3 aimed to investigate **the effects of blue laser light irradiation of the enzymatic cocktail from *Trichoderma atroviride* and *Trichoderma harzianum* cultivated in the presence of rice bran at reduced amounts on Mung bean seeds**. Following treatments, no statistically significant changes were observed in the length of Mung bean seedling radicles compared to the control, both in the absence and presence of saline stress. In the absence of saline stress, the non-irradiated control significantly reduced the length of hypocotyls. Under saline stress conditions, the culture medium caused a significant decrease in hypocotyl length, especially in the case of the irradiated *T. harzianum* strain culture medium. Plantlet height was significantly reduced in the presence of saline stress, particularly in the case of the irradiated *T. harzianum* strain culture medium. Treatments with *Trichoderma* strain culture media significantly influenced α-amylase activity in Mung bean seedlings. In the absence of saline stress, the irradiated *T. harzianum* strain culture medium significantly increased α-amylase activity. In the presence of saline stress, both the irradiated control and treatments with *T. harzianum* and *T. atroviride* strain culture media led to significant increases in α-amylase activity, with maximum values observed in irradiated *T. harzianum*. Proton Pump Activity (PPP): Treatments with *Trichoderma* strain culture media significantly influenced proton pump activity in Mung bean seedlings. In the absence of saline stress, an increase in extracellular H^+ levels was observed with irradiated and non-irradiated control treatments, *T. atroviride* non-irradiated, irradiated *T. atroviride*, and non-irradiated *T. harzianum*. In the presence of saline stress, the highest extracellular H^+ levels were recorded in treatments with irradiated *T. atroviride* and *T. harzianum* strain culture media. In the absence of saline stress, treatments with *Trichoderma* strain culture media significantly increased L-proline content in seedling plants. Under saline stress conditions, most experimental variants maintained similar levels of L-proline compared to the absence of stress. Oxidative stress, measured by MDA levels, increased following treatments with *Trichoderma* strain culture media, while irradiation significantly reduced MDA in irradiated *T. harzianum*, indicating a potential adaptation to saline stress. Analysis of ROS fluorescence intensity with H_2DCFDA showed that, in the absence of saline stress, both

irradiated and non-irradiated control treatments increased intracellular ROS levels. Under saline stress conditions, strain irradiation had a biostimulant effect, significantly reducing ROS, especially in irradiated *T. atroviride* and *T. harzianum*. Analysis of Mung bean roots treated with salt and culture media containing strains of interest indicated a reduction in reactive oxygen species ($^{\bullet}O_2^-$) formation, evidenced by decreased intensity of the color formed by NBT. Treatments with fungi of interest had a biostimulant effect, mitigating the negative impact of saline stress, suggesting the beneficial potential of these treatments in managing salt-induced oxidative stress.

Subchapter 4.4 aimed to investigate **the effects of fluorescent strigolactone mimics on *Trichoderma* strains**. Six Trichoderma strains were tested. All tested SLs affected hyphal branching abundance, except SL 21 on *T. harzianum* and *T. atroviride* at higher concentrations. Regarding colony diameter, it was observed that certain strigolactones, especially mimic SL 21, slightly stimulated growth for *T. asperellum* T36 and *T. harzianum* Td50b strains. Unfortunately, measurement for the diameter of the remaining 4 strains, *Trichoderma atroviride* P1 ATCC 74058, *Trichoderma harzianum*, *Trichoderma harzianum* T22 (ATCC 2084), *Trichoderma harzianum* T95 (ATCC 6085), could not be performed as the hyphae covered almost the entire plate on the 3rd day of incubation. Fungal testing was conducted in 2 stages, and for the latter strains (*T. asperellum* T36 and *T. harzianum* Td50b), radial growth was measured on the 2nd day of incubation.

The final **subchapter 4.5**, "**Effects of blue laser light and fluorescent strigolactone mimics on *Trichoderma* strains**," describes how cultures under different wavelengths of light, as well as in the presence of strigolactones, affect the behavior of *Trichoderma* strains, providing data on light ± strigolactone-dependent radial growth, conidiation behavior, and mycoparasitic activities. Additionally, sporule wetting behavior was evaluated. This study showed that both *T. asperellum* T36 and *T. harzianum* Td50b are capable of sensing and responding to different stimuli such as strigolactone mimics or blue laser light treatments. These stimuli had an impact on differentiation, mycoparasitic activity, and spore wetting characteristics. While toluene testing amplified sporule hydrophobicity for both treatments, analyses based on contact angle determination and interfacial tension suggested that spores were rather amphiphilic.

Chapter V, entitled **"Biostimulant effects of *Trichoderma* chlamydospores, applied alone or in combination with strigolactone mimics,"** is dedicated to investigating the biostimulant effects of *Trichoderma* chlamydospores on plants when applied alone or in combination with strigolactone mimics. The study aims to evaluate the influence of various factors on plant growth and development, as well as the interactions between plants and soil microorganisms. Several approaches are used to encourage the colonization of the rhizosphere by beneficial *Trichoderma* strains native to the soil. One method involves foliar application of suspensions rich in chlamydospores—spores with robust cell walls that confer substantial resistance to extreme environmental conditions. *Trichoderma* biomass was cultivated on cornmeal agar and then compared to biomass grown in Potato Dextrose Broth (PDB) through microscopic and thermogravimetric analyses. These analyses showed that biomass

produced on cornmeal agar had a higher chlamydospore content and greater thermal stability. The chlamydospore-rich *Trichoderma* suspension was applied to the leaves of bitter melon (*Momordica charantia*) at two inoculant concentrations. The effects of these treatments on plant were compared to controls and an experimental treatment with propagules produced in PDB. The results showed that the chlamydospore-rich suspension had a more pronounced and longer-lasting effect on plant physiological parameters compared to other treatments. This treatment led to a significant increase in polyphenol and flavonoid levels in leaves (by 17% and 50%) and fruits (by 18% and 31%), as well as enhanced antioxidant activity. Additionally, *Trichoderma* treatment resulted in a yield increase of 25.33–53.07%. The foliar application of *Trichoderma* suspensions did not affect the cytocompatibility of fruit extracts tested on L929 cells.

This chapter also examined the impact of strigolactone mimic solutions on the growth and development of *Arabidopsis thaliana*. The experiment revealed that the two fluorescent SLs (SL20 and SL21) had a stronger stimulating effect on plant growth compared to GR24.

Chapter VI presents the main conclusions, identified innovations, and possible directions for applying the results obtained in this thesis. The following innovative aspects are highlighted: the investigation of the activity of several strigolactone mimics, for which no data are available regarding their effect on the morphology and growth of colonies of phytopathogenic fungi, as well as *Trichoderma*, to our knowledge.

The study seeks to assess the impact of the effects of blue laser irradiation on cellulase and protease activities, as well as conidia size, in *Trichoderma*. The irradiation also enhances the formation of *Trichoderma* chlamydospores and modifies the cohesion and adhesion properties of the growth medium.

Another innovative aspect of the research was the foliar application of a *Trichoderma* chlamydospore consortium on *Momordica charantia*, which fosters phyllosphere colonization and triggers a long-lasting biostimulant effect. This effect is notably enhanced when using *Trichoderma* biomass with a higher concentration of chlamydospores.