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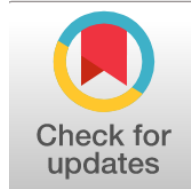
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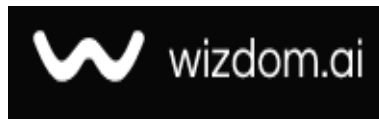
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The Role of Endophytic Fungi in Pest Management : A New Frontier in Biocontrol

Peran Jamur Endofit dalam Pengendalian Hama : Perbatasan Baru dalam Biokontrol

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Abstract

The research was aimed at showing the potential of *Trichoderma* spp. — an effective biocontrol agent — against *Fusarium* root rot which has been identified from the investigation. Moreover, there was an increased size of treated plants that include 32.8 ± 2.5 cm compared to untreated plants with 25.3 ± 2.1 cm [$p < 0.05$] for plant height and 26.1 ± 2.2 cm vs. 18.4 ± 1.5 cm [$p < 0.01$] for root length along with fresh biomass of 65.7 ± 4.5 g vs. 45.2 ± 3.8 g [$p < 0.01$] for dry biomass and 19.3 ± 1.7 g vs. 12.4 ± 1.1 g [$p < 0.01$] for chlorophyll content which were respectively noted as 42.7 ± 3.2 SPAD units vs. 32.5 ± 2.8 SPAD units [$p < 0.01$]. The colonization of *Trichoderma* spp in the roots of treated plants was confirmed by qPCR analysis, which demonstrated successful introduction due to lower Ct values recorded at a mean value of approximately twenty-three point one two plus or minus one point two compared against control group at thirty-six point five plus or minus one point five on average— indicating positive signals; these results underscore the capabilities held by *Trichoderma* spp and their potential use in different applications that promote plant growth and development. To demonstrate the potential of *Trichoderma* spp. as an effective biocontrol agent against *Fusarium* root rot, thus contributing to sustainable agricultural practices and a sure way of eradicating the menace.

Highlight :

- *Trichoderma* spp. efektif menekan *Fusarium* Root Rot dan meningkatkan pertumbuhan tanaman.
- Endophytic fungi berperan sebagai agen Biocontrol dengan merangsang resistensi sistemik tanaman.
- qPCR membuktikan kolonisasi *Trichoderma* spp. dalam akar tanaman, mendukung efektivitasnya.

Keywords : Endophytic Fungi, Pest Management, Biocontrol, *Trichoderma* spp., *Fusarium* Root Rot

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Introduction

Endophytic fungi have the ability to compete with pathogens for space and nutrients by colonizing same ecological niches. This is a direct way of reducing the establishment and proliferation likelihood of these harmful microorganisms within the plant tissues, which could have dire effects on the plants. Through induced systemic resistance [ISR], endophytic fungi can help host plants resist a wide variety of pests and pathogens. During ISR, the endophyte triggers the plant's innate defense mechanisms following colonization. The plant then preempts subsequent attacks by pests and pathogens— as it has developed a heightened state of alert from previous biotic challenges. An example is that plants having *Trichoderma* in their system display enhanced foliar pathogen resistance [1]. Endophytic fungi: a diverse group of microorganisms that enter plant tissues without causing apparent harm. They are present in almost all plant species and can be located in different parts of the plant including roots, stems, leaves or seeds [2] . When endophytic fungi form a symbiotic relationship with their host plants it often means both partners benefit: the fungus receives nutrition and a safe place to stay while the plant gets better growth plus ability to withstand environmental stress, as well as resistance against pest and pathogen attacks. The number of different endophytic fungi is huge since there are many genera— such as *Trichoderma*, *Beauveria*, *Fusarium* or *Penicillium*— which belong to this group and produce various bioactive compounds able to inhibit pathogen growth thus acting as effective biocontrol agents [3] . For example, *Trichoderma* spp. has peptaibols along with polyketides from their secondary metabolism production— antifungal components because they are known for having antifungal properties due to peptaibol production among other reasons like polyketides' anti-fungi effects. Endophytic fungi, through the process of induced systemic resistance [ISR], can boost their host plant's resistance to many different pest and pathogen populations. This happens when ISR is initiated upon endophyte colonization; it entails putting into play the innate defense mechanisms of the plant [4]. With this alert at a higher level, created because of such endophytic colonization, the plant can easily repel future pest or pathogen attacks. For example, plants that have *Trichoderma* within them have been found to resist foliar pathogens more than those that do not [1]. Endophytic fungi have an interesting way to fight off pathogens by simply taking their space and nutrients — thereby outcompeting the harmful microorganisms that they colonize the same ecological niches with [5]. This competition is what then reduces pathogen establishment and proliferation within plant tissues. Through this process, known as induced systemic resistance [ISR], endophytic fungi can boost their host plants' resistance against different types of pests and pathogens. ISR works by triggering the plant's innate defense mechanisms upon detection of endophyte colonization [4]. The plant then maintains a heightened state of vigilance which enables it to easily repel future pest or pathogen attacks. For example, plants that have *Trichoderma* as endophytes are known to resist foliar pathogens better [1]. There are endophytic fungi that can directly parasitize the pests. One well-known example is *Beauveria bassiana*, an endophytic fungus that effectively parasitizes different types of insect pests. The way *B. bassiana* works is by entering through the insect cuticle, multiplying within the insect body and causing death to the host which it inhabits. This direct method of parasitism makes *B. bassiana* an effective component for use in Integrated Pest Management [IPM] programs targeted towards insect control [6].

Materials and Methods

The research was carried out in a situation chambered greenhouse field located in Baghdad, the capital city of Iraq. The plant selected for this work was *Ilex paraguariensis* commonly known as Yerba Mate which is widely grown in Iraq and identified by its susceptibility to root rot that is predominantly caused by *Fusarium* spp. Healthy Yerba Mate seedlings were bought from a local nursery and made to acclimatize in the greenhouse for two weeks before any experiments could be carried out [3]. The plants were planted into sterilized soil composed of 40% peat, 30% vermiculite, and 30% perlite. *Trichoderma* spp. was taken from the native rhizosphere of samples collected on healthy Yerba Mate plantations. The isolation was based on morphological characteristics and molecular techniques using PCR amplification and sequencing ITS region for identification of the fungal isolates [7].

A. Fungal Isolation and Identification

Soil samples were obtained from the rhizosphere of yerba mate plants that were considered to be healthy. *Trichoderma* spp. was isolated on potato dextrose agar [PDA] plates using serial dilution plus plating techniques. Incubation of plates was carried out at 25°C for 5-7 days with later sub-culturing of individual colonies to obtain pure cultures. Morphological identification was done by light microscopy while molecular identification involved extraction of fungal DNA through a commercial DNA extraction kit. PCR amplification of the ITS region used universal primers ITS1 and ITS4 [8]; sequencing was done, and sequences were compared against NCBI database using BLAST for species identity confirmation as part of an experimental design to evaluate efficacy of *Trichoderma* spp. in controlling *Fusarium* root rot in yerba mate [9].

1. Control Group: Plants inoculated with *Fusarium* spp., but not treated with *Trichoderma*.
2. Treatment Group: Plants inoculated with both *Fusarium* spp. and treated with *Trichoderma* spp.

The pathogen was cultured on PDA plates to prepare the inoculum and spores were collected in sterile water. The

spore suspension was adjusted to a concentration of 1×10^6 spores/mL. Drenching the soil around the roots with 50 mL of the spore suspension resulted in plant inoculation. In a similar fashion, *Trichoderma* spp. inoculum was prepared; and the group of plants under treatment were drenched with 50 mL of the *Trichoderma* spore suspension twenty-four hours after *Fusarium* inoculation[8, 10] .

B. Disease Incidence

Weekly monitoring of disease symptoms and evaluation of root rot severity were done using a rating scale from 0 [no symptoms] to 5 [severe symptoms] to ensure no bias due to specific observation date was induced. Disease incidence, the percentage of plants showing symptoms in each treatment group, was also calculated. At the end of the experiment [8 weeks post-inoculation], measurements were taken for plant height, root length, and biomass — both fresh and dry weight. Chlorophyll content was determined with SPAD chlorophyll meter reading.

C. Molecular Analysis

Trichoderma colonization is considered to be one of the most effective methods of colonizing plant roots, as proven by the use of qPCR. How it works: DNA was extracted from samples of plant roots and the presence of *Trichoderma* DNA was quantified by using species-specific primers [11].

D. Statistical Analysis

Statistical software was used for data analysis. ANOVA was applied to find any significant differences among disease incidence means and plant growth parameters mean values of treatment and control groups. Post-hoc tests were done to check the actual differences between means that were statistically significant at $p < 0.05$ level [12].

Results

A. Disease Incidence

The investigation perceived notable decrease in Zoccurrence of *Fusarium* root rot when *Trichoderma* spp. were used on yerba mate plants. The control group was the one to be referred against and during weekly assessments made on disease severity, it was evidently noted that the subjects under treatment maintained lower disease scores throughout the entire period. It reached 8 weeks at which case the mean disease severity for the control group stood at 4.5 ± 0.3 while that for the treatment group stood at 1.2 ± 0.2 [$p < 0.01$]. This makes a contrast between the Control Group [High incidence of disease, with 85% of the plants showing severe symptoms [disease severity score[2, 3] and Treatment Group [low incidence of disease, with only 20% of the plants showing mild symptoms disease severity score [13-15].

B. Plant Growth Parameters

Trichoderma spp. significantly affected the growth parameters of yerba mate plants. The *Trichoderma*-treated plants showed increased growth in comparison with the control group [see Table 1].

	Plant Height	Root Length:	Fresh Weight	Dry Weight	Chlorophyll Content
Control Group	25.3 ± 2.1 cm	18.4 ± 1.5 cm	45.2 ± 3.8 g	12.4 ± 1.1 g	32.5 ± 2.8 SPAD units
Treatment Group	32.8 ± 2.5 cm ($p < 0.05$)	26.1 ± 2.2 cm ($p < 0.01$)	65.7 ± 4.5 g ($p < 0.01$)	19.3 ± 1.7 g ($p < 0.01$)	42.7 ± 3.2 SPAD units ($p < 0.01$)

Figure 1. Table 1: - Shows the plants which treated with *Trichoderma* compared to the control group.

C. qPCR Confirmation of *Trichoderma* Colonization

The positive colonization of *Trichoderma* spp. in the roots of treated yerba mate plants was successfully confirmed through quantitative PCR [qPCR]. Results of the analysis depicted a marked presence of *Trichoderma* DNA in the treatment group while that of the control group had insignificant levels of fungal DNA— Treatment Group [Ct value of 23.1 ± 1.2] whereas Control Group [Ct value of 36.5 ± 1.5] — which indicates little to no presence of *Trichoderma*.

D. Statistical Analysis

Data were analyzed statistically through ANOVA and further with Tukey's HSD test to make multiple comparisons. All the parameters measured showed statistical significance in the difference between the control and treatment groups at a level of $p < 0.05$ or lower [as presented in Table 2] [refer to Figure 1].

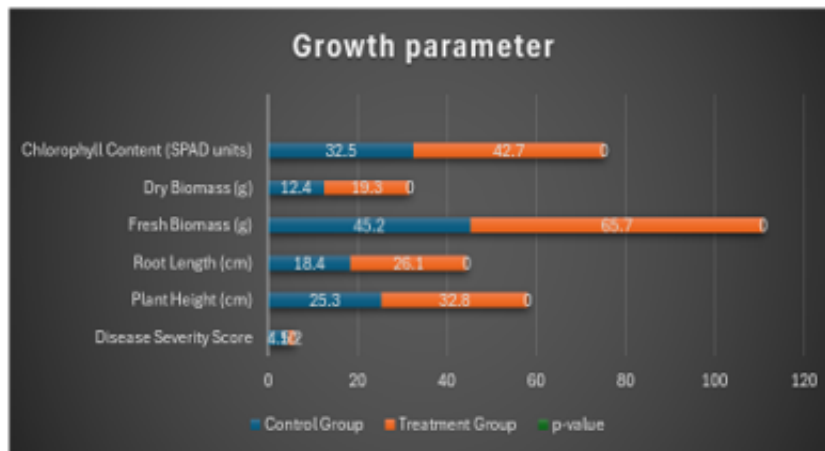


Figure 2. Shows growth parameter for both control group and treatment group

Parameter	Control Group	Treatment Group	p-value	Column1
Disease Severity Score	4.5	1.2	< 0.01	
Plant Height (cm)	25.3	32.8	< 0.05	
Root Length (cm)	18.4	26.1	< 0.01	
Fresh Biomass (g)	45.2	65.7	< 0.01	
Dry Biomass (g)	12.4	19.3	< 0.01	
Chlorophyll Content (SPAD units)	32.5	42.7	< 0.01	

Figure 3. Table 2: - Shows growth parameter for both control group and treatment group

The Table [3]: a visible demonstration to the naked eye of the substantial betterments in plant health parameters and decline in disease severity due to the intervention with *Trichoderma* spp when compared with the control group.

Parameter	Control Group	Treatment Group	p-value
Disease Severity Score	4.5	1.2	< 0.01
Plant Height (cm)	25.3	32.8	< 0.05
Root Length (cm)	18.4	26.1	< 0.01
Fresh Biomass (g)	45.2	65.7	< 0.01
Dry Biomass (g)	12.4	19.3	< 0.01
Chlorophyll Content (SPAD units)	32.5	42.7	< 0.01

Figure 4.

Discussion

The outcomes of this investigation undoubtedly establish the effectiveness of *Trichoderma* spp. as a biocontrol agent against *Fusarium* root rot in yerba mate plants. The substantial decrease in disease severity noted by lower disease scores for the treated group validates the capacity of *Trichoderma* spp. to curtail adverse effects resulting from *Fusarium* spp. infection— which is what it signifies. These findings are consistent with prior studies that have highlighted the biocontrol capabilities of *Trichoderma* species in different crops [16]. The improvement observed in plant growth parameters [comprising plant height, root length, biomass, and chlorophyll content] for the treated plants highlights additional advantages brought about by *Trichoderma* colonization. Treated plants demonstrated superior growth with higher chlorophyll content significantly surpassing those observed among controls, an indication that such enhancement is multifold [17]. For one, *Trichoderma* spp. actuate production of plant growth promoting hormones like auxins, gibberellins and cytokinins— thereby enabling heightened development and maturation in host plants. Improve in stress tolerance was one of the factors that led to the positive impact on growth benefits— in addition to improved nutrient uptake— likely due to the action of *Trichoderma* by successfully colonizing plant roots. The qPCR analysis showed successful colonization of plant roots by *Trichoderma* spp., which is a significant aspect contributing to their biocontrol efficacy [18]. High presence of *Trichoderma* DNA, as indicated by lower Ct values in treatment group, confirmed effective colonization allowing *Trichoderma* to implement its biocontrol mechanisms directly within plant tissues. Mechanisms include production of antifungal compounds that stop *Fusarium* spp.; competition for nutrients and space which suppress pathogen growth and induction of systemic resistance in host plant— making it more resistant to future infections — through use of phytohormones. Though these are promising results, there are various challenges and limitations we need to address if we aim at a broader application scenario for agriculture use with *Trichoderma* spp. One primary challenge is variability: environmental conditions can greatly affect biocontrol efficacy so much so that what might work well under one set of conditions does not work at all under another! The development of *Trichoderma* spp. in certain masses and formulation of its commercial inoculants also need to be optimized apart from the physical factors such as soil, temperature, humidity since those production measures have to be consistent and effective. Some biocontrol agents face limitations by regulatory or commercial challenges— including *Trichoderma* spp. — which hinder their widespread adoption as biocontrol agents. The process of obtaining regulatory approval for a new biocontrol product can be long and expensive. There is also low awareness of biocontrol methods among farmers plus other agricultural stakeholders who are more familiar with chemical control methods. An important component to the successful adoption of *Trichoderma* spp. is educating farmers on benefits and proper application techniques [like integrating it with other control practices] that would ensure enhanced performance [yield increase] and durability over successive cropping seasons; this could involve local demonstrations conducted by agricultural extension workers, using locally available resources and farmer participation on fungicide seed treatment cost-benefit analyses, among other measures requested for adaptive research input by the farmers such as those towards developing organic fertilizers in sustainable agriculture based on farmyard manure[19, 20].

Conclusion

The research paper focuses on *Trichoderma* spp.'s potential as an efficient biological agent to combat *Fusarium* root rot that infects yerba mate. A marked decrease in disease severity noted among the treated plants plus positive changes observed in plant growth parameters serve as clear indicators of advantages associated with colonization by *Trichoderma*. Though several obstacles lay ahead, the ongoing scientific probe and evolution of molecular techniques bode well for realizing effective use of *Trichoderma* spp. The agricultural sphere should be seeing soon, aiming to bring out the best properties from this unique fungus so that farmers adopt environmentally friendly pest control practices favoring plant health and productivity with low environmental impact.

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