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ISSN: 2320-5407 Int. J. Adv. Res. 7(6), XX-XX

ISSN NO. 2320-5407

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Article DOI: 10.21474/IJAR01/xxx
DOI URL: http://dx.doi.org/10.21474/IJAR01/xxx
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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

ISOLATION OF TRICHODERMA SPP. IN RURAL AREAS OF FIVE DISTRICTS IN ITAPÚA, PARAGUAY

Manuscript Info

Manuscript History
Received:
Final Accepted:
Published:

Key words:
Trichoderma spp., biological control, native strains.

Abstract

Fungal species of the Trichoderma genus are widely recognized for their effectiveness as biological control agents against phytopathogenic fungi of significant agricultural importance. However, Paraguay lacks commercially available products based on native isolates. In this context, the present study focused on obtaining native Trichoderma spp. strains from the districts of Trinidad, Mayor Julio Dionisio Otaño, Fram, Carmen del Paraná, and Natalio, in the Itapúa region. The results suggest the possible presence of Trichoderma spp. in all five analyzed districts, as the isolated samples initially exhibited a greenish-white coloration, which later evolved into an olive-green hue, a macroscopic characteristic distinctive of this fungus. Further identification and characterization of native Trichoderma spp. strains will not only enhance disease control efficiency but also reduce dependence on agrochemicals.

Introduction:-

Trichoderma spp. species have been studied as biological control agents for fungal diseases for approximately 70 years. However, their commercial use has gained increasing relevance in recent times. Studies indicate that *Trichoderma spp.* is one of the most widely used microorganisms in biological disease control (Flores et al., 2018), accounting for more than 60% of biofungicides registered worldwide (Michel et al., 2008). Currently, it is available on the market as a biopesticide, biofertilizer, plant growth promoter, and nutrient solubilizer in agricultural soils, in addition to playing a key role in organic matter decomposition. It is commercialized in solid and liquid formulations containing viable inoculum (hyphae, chlamydospores, and conidia) for biocontrol applications (Michel et al., 2008).

The production of this agent is carried out through various methods. The growth versatility and beneficial properties of *Trichoderma spp.* strains have enabled their use in the development of phytopathogen biocontrol products. Moreover, their application contributes to the advancement of sustainable agriculture by promoting the conservation of natural resources such as soil and water, while simultaneously reducing the environmental impact caused by agrochemical use. *Trichoderma spp.* species are abundant in terrestrial ecosystems (forests and agricultural soils) and have low nutritional requirements. Additionally, they exhibit high adaptability to ecological conditions, can grow saprophytically, interact with animals and plants, and develop on a wide range of substrates, which facilitates their mass production for horticultural applications (Agamez et al., 2008). Therefore, studying the diversity of

Trichoderma species, particularly native strains in various natural habitats, expands knowledge about their biotechnological potential and horticultural significance.

Trichoderma spp. could represent a highly valuable resource for horticulturists in Itapúa, given that its production is cost-effective and has demonstrated favorable results upon application. Furthermore, it poses no health risks to farmers and does not require complex knowledge or handling practices, making it a highly viable and relevant option for small- and medium-scale horticultural farms (Sivila et al., 2013). This study aimed to obtain native *Trichoderma spp*. strains that could be used by small-scale farmers in the districts of Trinidad, Mayor Julio Dionisio Otaño, Fram, Carmen del Paraná, and Natalio in Itapúa, to sustainably enhance the quality of their agricultural products.

Material and Method:-

Preparation of Rice Traps

According to Navarro (2024), rice is considered a suitable substrate for microorganism reproduction. For the preparation of rice traps, the necessary materials were gathered, including glass jars, porous fabric, rubber bands, scissors, and rice. The glass jars underwent a thorough cleaning process to eliminate potential contaminants. Simultaneously, the porous fabric was cut into appropriately sized squares for later use in sealing the jars. Subsequently, the rice was hydrated in distilled water to ensure sufficient liquid absorption, promoting microorganism development. Once hydrated, it was evenly distributed in the glass jars. Finally, the traps were sterilized in an autoclave at 121°C for 15 minutes to guarantee asepsis before field deployment.

Placement and Collection of Rice Traps

The traps were buried at an approximate depth of 20 cm in five districts of Paraguay: Carmen del Paraná, Mayor Julio Dionisio Otaño, Trinidad, Natalio, and Fram. Five traps were placed in each district, totaling 25. To facilitate identification and subsequent retrieval, stakes and GPS georeferencing were used. The traps were collected between five and eight days after placement (Zamorra et al., 2023). The samples were stored in thermal containers to preserve their integrity and later transported to the laboratory at the Faculty of Science and Technology, National University of Itapúa.

Preparation of Culture Medium

According to Rodríguez (2019), Potato Dextrose Agar (PDA) is widely used for fungal isolation, making it an essential medium for this study. The process began by measuring the appropriate quantity of dehydrated PDA medium, following the manufacturer's specifications. The PDA was then mixed with 500 ml of distilled water and sterilized in an autoclave at 121°C for 15 minutes. After sterilization, the mixture was cooled to a safe handling temperature. It was then poured into Petri dishes inside a laminar flow hood to maintain sterile conditions and prevent cross-contamination. Finally, the plates were left undisturbed until the medium was fully solidified.

Inoculation and Sample Isolation

For microorganism isolation, four rice grains were extracted from each trap using sterilized tweezers, ensuring aseptic conditions. These grains were inoculated in Petri dishes containing PDA medium, designed to promote the growth of fungi and bacteria of interest. The samples were incubated at 24°C for seven days to allow microbial colony development. After the incubation period, sample purification was carried out by selecting a single conidium, thereby avoiding contamination with other strains. Subsequently, the hyphal tip method was applied to obtain homogeneous and representative cultures, ensuring accuracy in further analyses.

Results and Discussions:-

The detection of the possible presence of *Trichoderma spp*. in the five studied districts suggests that the soil conditions in these areas favor its development. This represents a positive factor for agricultural productivity, given the well-established potential of this fungus as a biocontrol agent and plant growth promoter. Specifically, among the 25 installed traps, velvety colonies were observed in Carmen del Paraná, Mayor Julio Dionisio Otaño, Trinidad, and Fram (Fig. 1). These colonies initially exhibited a greenish-white coloration, later evolving into an olive-green hue, a macroscopic characteristic distinctive of *Trichoderma spp*. (Ávila et al., 2014).

ISSN: 2320-5407 Int. J. Adv. Res. 7(6), XX-XX

Districts	P1	P2	P3	P4	P5
Trinidad					
Mayor Julio Dionisio Otaño					
Fram					
Carmen del Paraná					
Natalio					

Fig. 1. Possible presence of *Trichoderma spp*. in different districts of Itapúa.

Although molecular identification of the collected and isolated native strains could not be performed due to logistical reasons, previous studies have demonstrated the presence of *Trichoderma spp*. in various regions with similar agroecological conditions. For instance, a study conducted in Matanzas Province, Cuba, successfully isolated and identified native strains such as *Trichoderma* 2T-isolate, *T. harzianum* 6(A-53)2, and T. viride 4(TS-3)1. These strains exhibited different levels of in vitro antagonistic activity against the phytopathogenic fungi *Rhizoctonia solani*, *Fusarium spp*., and *Sclerotium rolfsii*, which cause diseases in agricultural crops characteristic of the region (Samaniego et al., 2018).

Similarly, in the San Pedro Department of Paraguay, seven *Trichoderma spp*. strains were evaluated, six of which were molecularly identified at the species level as *T. asperellum*, while the remaining strain was classified as *T. erinaceum* (Fernández et al., 2017). Likewise, Delosantos (2018) conducted a study in San Pedro, Paraguay, evaluating seven *Trichoderma spp*. strains, of which six were molecularly identified as T. *asperellum* and one as *T. erinaceum*. These findings reinforce the presence and diversity of *Trichoderma* in Paraguayan soils, highlighting its potential in the biological control of agricultural pathogens.

Conclusion:-

The positive biological effects of the antagonistic fungus *Trichoderma spp*. on plants have been demonstrated both in laboratory environments and in greenhouse crop production. Numerous studies support the benefits offered by this fungus, facilitating the development of environmentally friendly biological products. Therefore, it is essential to promote knowledge about the use and availability of *Trichoderma spp*. species, as well as encourage their adoption and further research on the management of native strains in agriculturally significant crops.

The study of soil microbiology and its interaction with agricultural practices is crucial for developing sustainable strategies in horticulture. Further identification and characterization of native *Trichoderma spp*. strains will not only improve disease control efficiency but also reduce dependency on agrochemicals, thereby promoting a more sustainable approach to agricultural production. In this context, agricultural biotechnology presents itself as a viable and promising alternative for sustainable production, offering new opportunities to enhance soil health and crop productivity, ultimately benefiting both farmers and the entire ecosystem.

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ISSN: 2320-5407 ***Int. J. Adv. Res. 7(6), XX-XX***

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