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BIOCONTROL EFFICACY OF *TRICHODERMA* SPECIES AGAINST *FUSARIUM* PATHOGENS: AN IN VITRO STUDY

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Abstract

Fusarium species are recognized as some of the most destructive soil-borne pathogens affecting a wide range of agricultural crops. These pathogens cause serious diseases such as wilt, root rot, and damping-off in economically important crops, leading to heavy yield losses. The use of antagonistic *Trichoderma* species as biological control agents provides an environmentally safe and sustainable alternative to chemical fungicides. In the present study, the antagonistic potential of selected *Trichoderma* species (*Trichoderma atroviride*, *T. viride*, and *T. harzianum*) was evaluated against different pathogenic *Fusarium* species under in vitro conditions. Antagonistic activity was determined using dual culture assays, and percent radial growth inhibition (PRGI) along with mycelial growth reduction was calculated. The findings showed that the *Trichoderma* species exhibited strong antagonistic effects through mechanisms such as mycoparasitism, competition for nutrients and space, and the production of antifungal compounds. Among the tested species, *Trichoderma viride* demonstrated the highest antagonistic activity, with PRGI values exceeding 87.08% and a significant reduction in *Fusarium* sporulation. Overall, the results indicate that the selected *Trichoderma* species have strong biocontrol potential and can be effectively used in sustainable management of *Fusarium*-induced plant diseases.

Keyword: *Trichoderma* spp., Antagonistic, Biocontrol, *Fusarium* spp.

I. Introduction

Soil-borne fungal pathogens represent a major threat to global agriculture, severely affecting crop growth, productivity, and quality. Among these pathogens, species belonging to the genus *Fusarium* are regarded as highly destructive due to their wide host range, ability to colonize plant vascular tissues, and production of mycotoxins that compromise plant and food safety (Leslie, J. F. and Summerell, B. A., 2006). *Fusarium* species such as *F. oxysporum*, *F. solani*, *F. equiseti*, and *F. verticillioides* cause economically significant diseases including wilt, root rot, damping-off, stem rot, and grain contamination in numerous crops like Soybean, Chickpea, Cotton, Maize, Cereals and horticultural plants (Dean, R., et al., 2012). These pathogens are difficult to manage because they survive in soil for long periods through chlamydospores, their adaptability to varying climatic conditions make disease management extremely challenging (Gordon, T. R., 2017). Traditional methods such as chemical fungicides, resistant cultivars, and cultural practices offer only partial success in managing *Fusarium* diseases. The overuse of fungicides has led to environmental concerns, pathogen resistance, and negative impacts on beneficial soil microbiota (Ghazalibiglar, H., 2016). Hence, there is an urgent need for eco-friendly, sustainable, and effective biocontrol alternatives. Biological control using beneficial microorganisms has emerged as a promising strategy for managing soil-borne pathogens in an environmentally compatible manner (Berg, G., 2021).

In addition, many *Fusarium* species produce mycotoxins that threaten both crop productivity and food safety, making their control a major agricultural challenge (Logrieco, A., et al., 2018). Chemical fungicides have traditionally been used to manage *Fusarium* diseases; however, their effectiveness is limited due to pathogen resistance, environmental toxicity, residue accumulation, and harmful effects on beneficial soil microflora (Pimentel, D., 2005). These limitations have increased interest in eco-friendly and sustainable approaches, among which biological control has emerged as a promising strategy. Among various biocontrol agents, species of *Trichoderma* have gained worldwide recognition for their remarkable antagonistic capabilities against a wide range of plant pathogens, including *Fusarium*. *Trichoderma* species are among the most widely studied and commercially used biocontrol agents due to, it is fast-growing, opportunistic, and strong competitive ability, and capacity to colonize the rhizosphere. They suppress plant pathogenic fungi through several mechanisms, including mycoparasitism, antibiosis, competition for nutrients and space, and induction of systemic resistance in host plants (Harman, G. E., 2004; Druzhinina, I. S., 2011). It is ability to produce hydrolytic enzymes like chitinases, β-glucanase, and proteases facilitates the degradation of pathogen cell walls, thereby inhibiting pathogen growth (Benítez, T., et al., 2004). Additionally, *Trichoderma* synthesizes secondary metabolites such as peptaibols, gliotoxin, and volatile organic compounds that suppress *Fusarium spp*. and other soil-borne fungi (Vinale, F., et al., 2011). Previous studies have demonstrated that *Trichoderma harzianum, T. viride, T. asperellum*, and *T. atroviride* exhibit significant antagonism against many *Fusarium* species such as *F. oxysporum*, *F. solani*, *F. verticillioides*, and *F. equiseti* (Howell, C. R., 2003; Mukherjee, P. K., et al., 2011). Beyond pathogen suppression, *Trichoderma* also enhances plant health by improving nutrient uptake, stimulating root development, and triggering plant defense pathways through induced systemic resistance (ISR) and systemic acquired resistance (SAR) (Yedidia, I., 2001). These unique traits make *Trichoderma* one of the most effective and widely studied biocontrol agents for integrated disease

management. Given the agricultural significance of *Fusarium* pathogens and the growing need for sustainable disease management strategies, evaluating the biocontrol efficiency of different *Trichoderma* species against various *Fusarium* pathogens is critical. Understanding their interactions at morphological, biochemical, and molecular levels will help optimize their application under field conditions and support the development of commercial bio-fungicide formulations. Despite extensive research, the biocontrol efficiency of *Trichoderma* varies depending on the fungal strain, environmental conditions, type of *Fusarium* pathogen, and crop species. Therefore, evaluating and comparing the antagonistic potential of different *Trichoderma* isolates against various *Fusarium* pathogens is essential for selecting superior strains for biological control programs. This study aims to explore the antagonistic potential of multiple *Trichoderma* isolates, compare their inhibitory effects on diverse *Fusarium* species, and contributing valuable information for integrated disease management and sustainable agriculture.

II. Materials and Methods

1. Collection of *Fusarium* Wilted Plant Sample

Fusarium-infected cereals, pulses, vegetable crops, and wilt-affected soil samples were systematically collected from various localities across the Marathwada region of Maharashtra. During collection, special care was taken to ensure that the samples represented diverse agro-climatic conditions and cropping patterns of the region. Each sample was carefully placed in pre-sterilized paper or polythene bags to avoid external contamination and preserve the natural microbial population. After proper labeling, all samples were transported to the laboratory and stored under controlled conditions until further processing and analysis. This standardized sample-handling procedure ensured accuracy and reliability in subsequent isolation and identification of *Fusarium* species

2. Isolation of *Fusarium* Mycoflora

For the detection and isolation of mycoflora associated with wilted plants and infested soil the standard procedures were followed (ISTA, 1966; Neergaard, P., 1973; Agarwal, V. K., 1976). The infected stem portions of wilted plants were first surface-sterilized using 0.1% Mercuric Chloride ($HgCl_2$) for a brief period to eliminate external contaminants. After sterilization, the samples were rinsed thoroughly with sterile distilled water and aseptically transferred onto autoclaved Potato Dextrose Agar (PDA) medium. The inoculated plates were then incubated at room temperature (approximately 25 ± 2°C) for 5 to 7 days to allow fungal growth. Emerging fungal colonies suspected to be *Fusarium* were carefully examined and sub-cultured. All *Fusarium* isolates were purified using the single-spore isolation technique (Hansen, H. N., 1926) ensuring that each culture originated from a single conidium. Throughout the study, strict aseptic conditions were maintained to preserve the purity, reliability, and authenticity of the isolated cultures

3. *Fusarium* Pathogen and *Trichoderma* Isolates

In the present study, ten different *Fusarium* species—*Fusarium annulatum, F. chlamydosporum, F. concentricum, F. equiseti, F. foetens, F. fujikuroi, F. hainanense, F. incarnatum, F. pernambucanum,* and *F. pseudocircinatum*—were selected for evaluating their susceptibility to antagonistic fungi. In addition, three *Trichoderma* species, namely *Trichoderma atroviride, T. viride*, and *T. harzianum*, were employed as potential biocontrol agents to assess their antagonistic efficacy against the selected *Fusarium* pathogens. All fungal isolates, including both *Fusarium* pathogens and *Trichoderma* antagonists, were maintained on freshly prepared Potato Dextrose Agar (PDA) medium. The cultures were incubated at 30°C for a period of seven days to allow optimal mycelial growth. Following incubation, actively growing cultures were sub-cultured onto PDA slants to ensure purity and viability. These slants were then stored under refrigerated conditions until further use in antagonism assays.

4. In Vitro Dual Culture Assay for Determining the Antagonistic Efficacy of *Trichoderma* spp. Against Wilt-Causing *Fusarium* Pathogens

Trichoderma atroviride, Trichoderma viride, and *Trichoderma harzianum* were isolated from soil, using Potato Dextrose Agar (PDA) as the growth medium. The antagonistic potential of these *Trichoderma* isolates was evaluated against ten wilt-causing *Fusarium* species, namely *Fusarium annulatum, F. chlamydosporum, F. concentricum, F. equiseti, F. foetens, F. fujikuroi, F. hainanense, F. incarnatum, F. pernambucanum,* and *F. pseudocircinatum,* using the standard dual culture assay technique. For the assay, a 7-day-old mycelial disc (5 mm diameter) from each *Trichoderma* isolate and each *Fusarium* pathogen was aseptically placed on opposite sides of a freshly prepared PDA plate, maintaining a distance of approximately 3 cm from the respective edges of the Petri dish. In the control treatment, PDA plates were inoculated only with the *Fusarium* pathogens to allow uninhibited radial growth. All inoculated plates (treated and control) were incubated at 30°C for 5 days, after which the radial growth of the pathogens was carefully measured. The percentage inhibition of mycelial growth was calculated relative to the control using the following formula (Edington L.V., 1971).

$$\text{I}(\%) = \frac{C-T}{C} X\ 100$$

Were,

I = Percentage inhibition of radial mycelial growth

C = Radial growth of the pathogen in the control plate

T = Radial growth of the pathogen in the presence of *Trichoderma*

III. Result and Discussion

The antagonistic activity of *Trichoderma atroviride*, *Trichoderma viride*, and *Trichoderma harzianum* against different wilt-causing *Fusarium* species under in vitro conditions was evaluated, and the results are summarized in Table 1 (Figures 01, 02 and 03). Among the isolates tested, *T. atroviride* and *T. viride* exhibited the highest antagonistic activity against *Fusarium concentricum*. In contrast, *T. harzianum* showed maximum inhibition against *Fusarium pernambucanum.* Both *T. atroviride* and *T. viride* recorded similar inhibition percentages against *Fusarium fujikuroi* and *Fusarium hainanense*, respectively. Likewise, *T. viride* and *T. harzianum* demonstrated comparable inhibition levels against *Fusarium equiseti*, *Fusarium foetens* and *Fusarium pernambucanum*. On the other hand, the lowest antagonistic activity was observed against *Fusarium foetens* for *T. atroviride*, *Fusarium incarnatum* for *T. viride*, and *Fusarium hainanense* for *T. harzianum* (Figure 04)

Table 1: In Vitro Antagonistic Activity of *Trichoderma atroviride*, *Trichoderma viride* and *Trichoderma harzianum* Against Different *Fusarium* species

Sr. No.	Name of *Fusarium* species	T_1	T_2	T_3	I_1	I_2	I_3	C
1	*Fusarium annulatum*	1.1	1.5	1.6	55.99%	77.04%	82.31%	1.9
2	*Fusarium chlamydosporum*	1.2	1.4	1.1	68.88%	80.65%	63.00%	1.7
3	*Fusarium concentricum*	1.5	1.6	1.4	86.53%	87.08%	75.97%	1.8
4	*Fusarium equiseti*	1.3	1.4	1.4	74.77%	80.65%	80.65%	1.7
5	*Fusarium fujikuroi*	1.2	1.2	1.3	73.4%	73.4%	79.65%	1.6
6	*Fusarium foetens*	1.0	1.5	1.5	53.75%	81.53%	81.53%	1.8
7	*Fusarium hainanense*	1.6	1.6	1.1	82.31%	82.31%	55.99%	1.9
8	*Fusarium incarnatum*	1.2	1.0	1.2	68.88%	57.12%	68.88%	1.7
9	*Fusarium pernambucanum*	1.1	1.3	1.3	71.83%	85.16%	85.16%	1.5
10	*Fusarium pseudocircinatum*	1.4	1.7	1.8	61.43%	75.07%	79.81%	2.2

T_1 = Activity of *Trichoderma atroviride* Against *Fusarium* species Length (cm)

T_2 = Activity of *Trichoderma viride* Against *Fusarium* species Length (cm)

T_3 = Activity of *Trichoderma harzianum* Against *Fusarium* species Length (cm)

I_1 = Inhibition percentage (%) of *Trichoderma atroviride*

I_2 = Inhibition percentage (%) of *Trichoderma viride*

I_3 = Inhibition percentage (%) of *Trichoderma harzianum*

C = Control of *Fusarium* species Length (cm)

IV. Conclusion

Trichoderma atroviride, Trichoderma viride and *Trichoderma harzianum* exhibited strong antagonistic potential against various wilt-causing *Fusarium* species, as revealed through the analysis of their effects on mycelial growth and sporulation. Among the tested isolates, all three *Trichoderma* species demonstrated high levels of antagonism, indicating their effectiveness as promising biological control agents for managing *Fusarium*-induced wilt diseases. The ability of these *Trichoderma* species to suppress pathogen growth through mechanisms such as competition, mycoparasitism, and inhibition of sporulation highlights their potential role in sustainable disease management. These findings provide a valuable foundation for developing innovative, eco-friendly strategies to combat major soil-borne pathogens and reduce dependence on chemical fungicides.

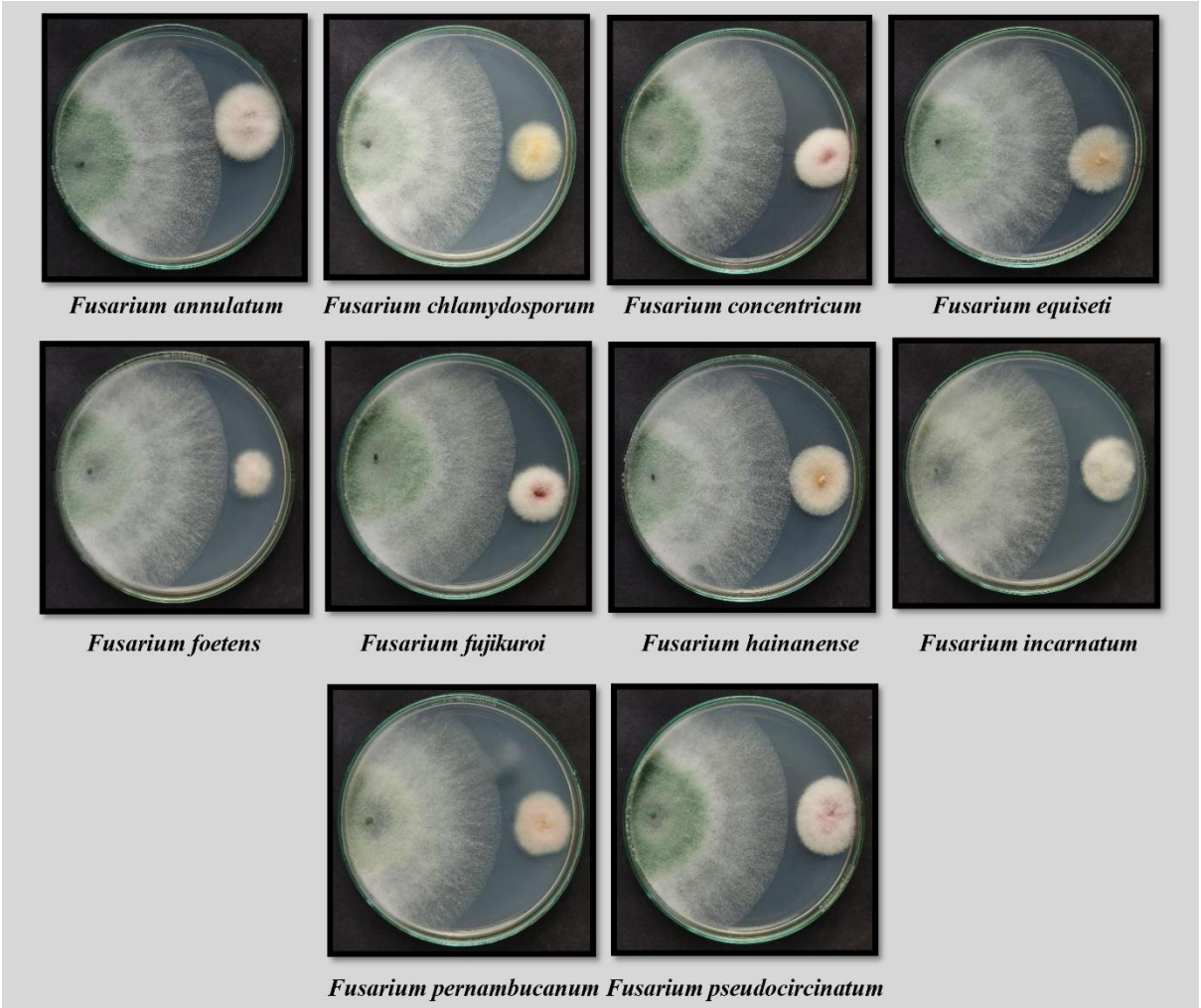

Figure-01: Antagonistic Activity of *Trichoderma atroviride* Against Different *Fusarium* species

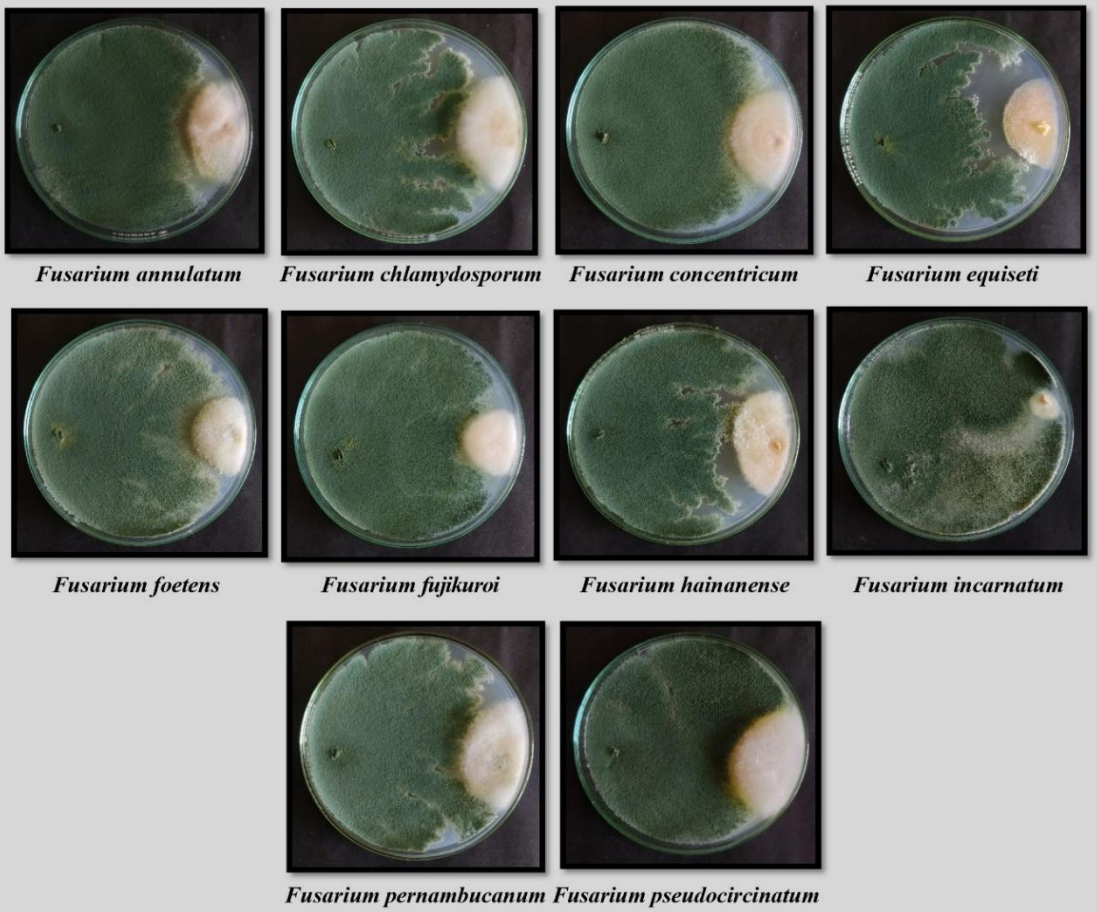

Figure-02: Antagonistic Activity of *Trichoderma viride* Against Different *Fusarium* species

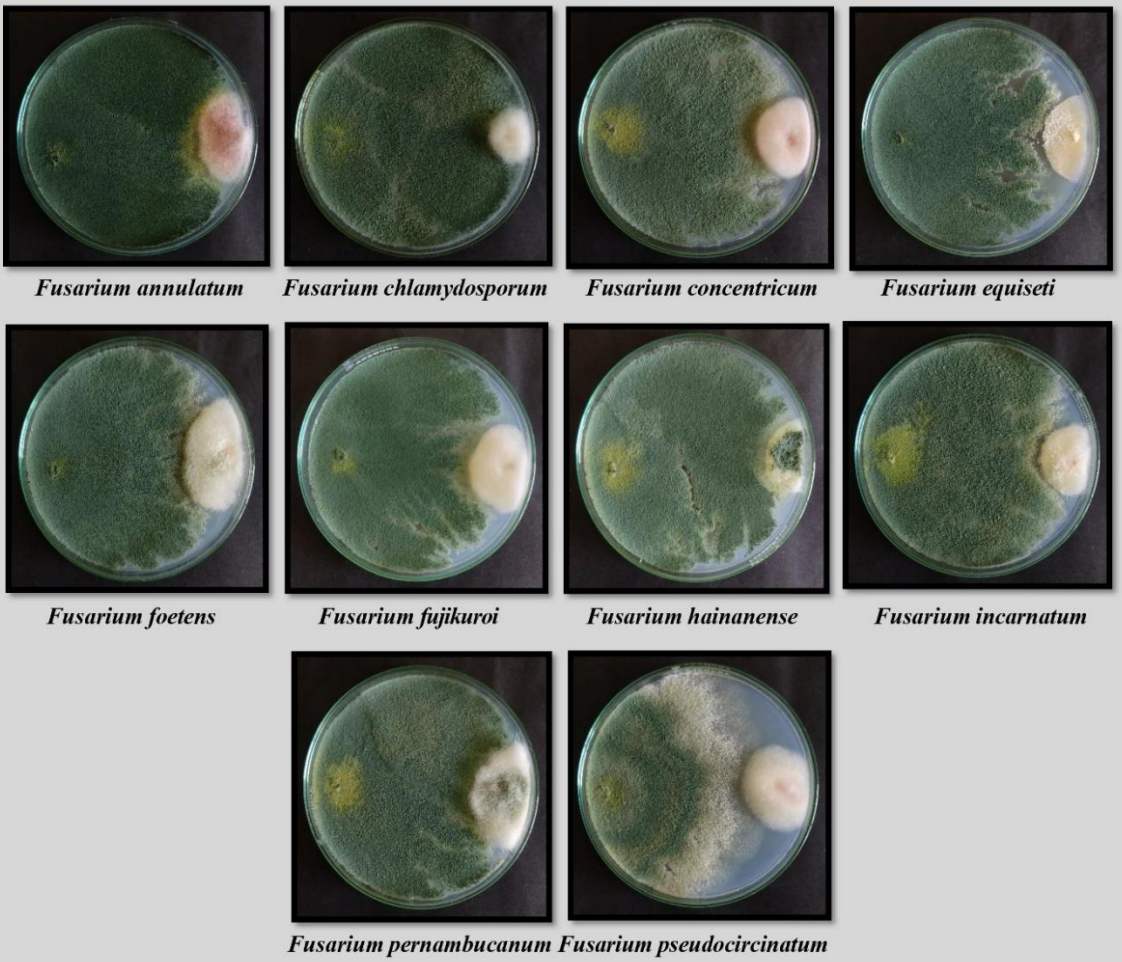

Figure-03: Antagonistic Activity of *Trichoderma harzianum* Against Different *Fusarium* species

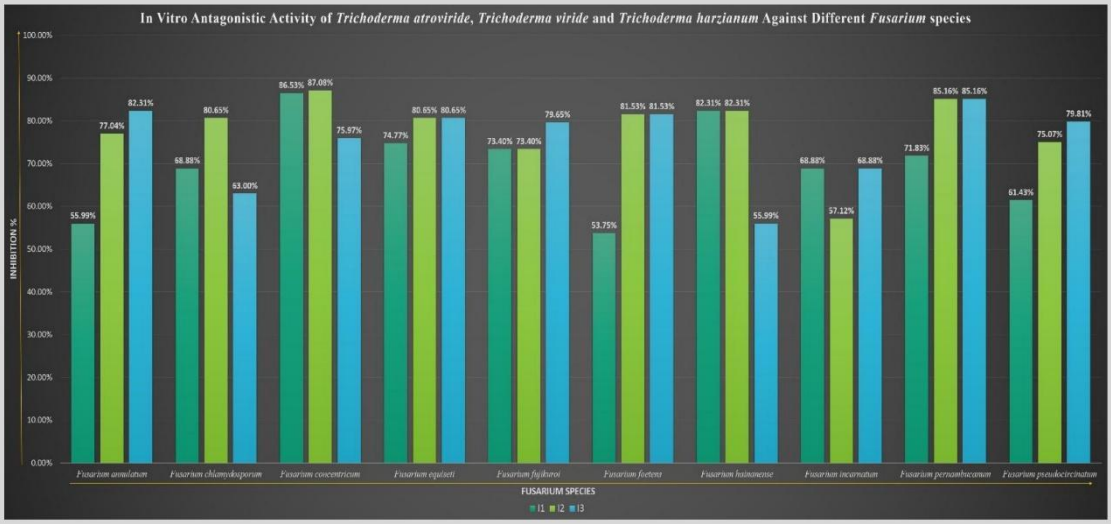

Figure-04: Evaluation of Inhibition Percentage of *Trichoderma* Species Against *Fusarium* Species

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