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Original Research Paper

In vitro interaction of Trichoderma harzianum filtrate only and jointly with Lantana camara extract contra Trichophyton rubrum

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Abstract

The research sought to assess *T. harzianum* antifungal efficacy, both independently and in conjunction with Lantana camara extract, against *Trichophyton rubrum*. The study results showed a strong inhibitory effect of Trichoderma filtrate against Trichophyton fungus using the dispersed disc method, and this effect increases with increasing concentration. The most extensive inhibition zone occurred at a concentration. of 50%, while the minimal zone of inhibition was seen at a concentration of 10%. The study results showed that combining Trichoderma fungal filtrate with the Lantana camara plant gives a higher inhibition zone than the effect of the fungal filtrate alone. The inhibition zone measured 30.5 mm when the fungal filtrate was mixed at a 50% concentration with Lantana camara extract at 25 mg/ml, in contrast to the inhibition zone of the fungal filtrate alone, which was 24.5 mm.

Keyword: *Lantana camara* extract, biocontrol, Trichoderma, *Trichophyton rubrum*

1. Introduction

Many superficial fungal infections globally are attributed to the dermatophyte Trichophyton rubrum (T. rubrum). Dermatophytes, a category of fungi, can infiltrate keratinized tissues, including skin, hair, and nails. This species of fungus can infect any area of the skin; the most often afflicted regions are the scalp, nails, feet, axillae, and inguinal area. The illness has a minor to severe cutaneous disorder, differing in severity. Among the most common types of tinea are tinea pedis, tinea cruris, and tinea corporis. resulting from *T. rubrum* infestations. Topical antifungal treatment is successful in around 80% of persons with acute dermatophytosis. Nonetheless, the remaining 20% manifest dermatophytosis, a chronic illness that is refractory to antimycotic treatment. (Blutfield et al., 2015).

A variety of Trichoderma strains were investigated for their possible antagonistic effects on Trichophyton species. We observed a wide range of antagonistic activity, from significant overgrowth to total absence of response, with *Trichoderma vernis* exhibiting the highest efficacy against the evaluated Trichophyton strains.

This strain, which was cultured using Trichophyton rubrum hyphae as a source of carbon, had an enhanced synthesis of extracellular chitinases and beta-glucosidases, which had an effect on the lysis and spore formation that occurred on the Trichophyton rubrum hyphae. Furthermore, the proliferation of Trichoderma in a medium that was lacking in nutrients resulted in the excretion of antibiotics, which successfully restricted the growth of the Trichophyton rubrum inoculum. It is possible that our results will make it easier to create new treatments for onychomycosis, either in conjunction with the medications that are now available or as a potential "natural" therapy strategy. (Omero et al., 2004).

2-Methods and Materials

2-1-a source of Trichoderma samples

The T. harzianum samples were procured from the Directorate of Agricultural Research under the Department of Biotechnology, Ministry of Science and Technology, for use purposes. Subsequently, it was cultivated on PDA medium.

2-2-Source of *Lantana camara* plant

Lantana camara plants were obtained from a nursery in Wasit province it was sent to Prof. Dr. Majid Hanoon Sharhan, who examined and verified them. After being cleaned with tap water to get rid of debris, Plant leaves were collected, desiccated in the shade at ambient temperature, and subsequently ground into a powder before being preserved in glass jars for future utilization.

2-3-Sample collection

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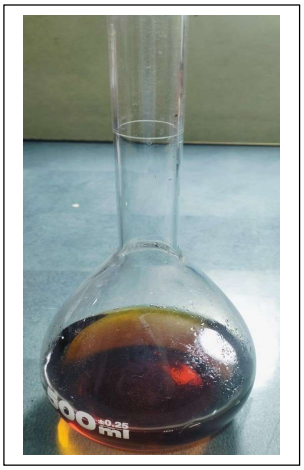
100 samples were collected from Patients in the Al-karama teaching hospital and medical clinics in the Wasit Province region. Skin scrapings, hair, and nails were among the samples gathered. All samples were collected after cleaning the infected area with 70% alcohol. Hair samples were obtained from the hair follicle utilizing a sterilized scalpel. or by plucking infected hairs with sterile forceps; skin scraping samples were collected from the edge of the infected area using a sterile scalpel or the edge of a sterile glass slide; nail samples were collected by scraping the accumulation above and below the infected nail with a sterile scalpel or by cutting off a portion of the infected nail. All samples were thereafter transferred in sterile containers and inoculated into SDA medium supplemented with 0.5 mL/L Cycloheximide and 0.05 g/L Chloramphenicol to suppress the proliferation of saprophytic fungi and bacteria, respectively. (Jartakar et al., 2021).

2-4-Preparation of filtrate

Three 5 mm hyphal agar blocks were perforated from young Trichoderma cultures and inoculated into 300 ml Erlenmeyer flasks with 100 ml of sterile potato dextrose broth (PDB) at 28 ± 2°C. To eliminate hyphal pads, cultures were filtered through a Millipore membrane and sterilized with a 0.2 µm biofilm filter.



(Figure 1): T.harzianum filtrate

2-5- Influence of *Trichoderma. harzianum* on the Trichophyton development

2-5-1-Impact of *T.harzianum* filtrate on the Trichophyton development by the poisoned food technique

Melted PDA medium was mixed with different amounts of fungal filtrate to acquire concentrations of (50, 25, & 10%). Only 20 milliliters of potato dextrose agar medium was administered to the control group on sterile Petri plates.

A mycelial block with a diameter of five millimeters from a seven-day-aged Trichophyton was inoculated into 20 ml culture media inside petri dishes and incubated at (28 ± 2 centigrate). 3 duplicates , Were conducted For each Treatment. Trichophyton colony diameters were measured. The aquation below was used to determine the fungal hyphae growth inhibition percentage. (Sessou et al.,2012).

Mycelial growth inhibition percentage (%) = $(dc - dt)/dc \times 100$, where dc is the average colony diameter in the control sample, and dt is the average colony diameter in the treatment sample (Yassin et al., 2021).

2-5-2-Impact of *T. harzianum* filtrate on Trichophyton proliferation by disc diffusion method

The present investigation evaluated effect of Trichoderma culture filtrate ,On, The hyphal development of Trichophyton using the disc diffusion method. Culture filtrates were prepared at 50%, 25%, and 10% (v/v). At each concentration, Discs of filter paper (5.0 millimeters in diameter) were submerged in the culture filtrates and aseptically placed in the middle of PDA culture dishes pre-inoculated with 1 ml of Trichophyton spore solution. Control was carried out using filter paper discs in sterile distilled water. The antifungal efficacy was evaluated by quantifying the inhibitory zone of fungal proliferation surrounding the filter paper discs. Measurements of these translucent areas were taken with a ruler (Pawan et al. 2012).

2-5-3-Impact of *T. harzianum* on Trichophyton growth in dual culture

A technique that utilized dual cultures was utilized in order to investigate the antagonistic effect that Trichoderma harzianum had on Trichophyton. From the petri plate filled with PDA media, a 5 mm disc of the fungal colony under testing was excised and transferred to the other extreme Of The Petri-dish, & a 5 millimeters Disk of the Trichoderma. harzianum colony was excised and transferred to the other extreme of the plate. The petri plate is kept in an incubator At 28 ± 2 degrees Celsius for a period of seven days. In the control group, a sterile agar plate was placed opposite the Trichophyton plate. which was free of Trichoderma harzianum. all treatment is duplicated three ,Times in a Completely Randomized Design (CRD). The Trichophyton colonies were measured for radial growth after three & seven Days Of culture. The percentage of mycelial growth inhibition of the test fungus was calculated by Sessou et al. (2012) using the following formula. The percentage of inhibition of mycelial growth was calculated as the ratio between the average colony diameter of the control sample (dc)

and the average colony diameter of the treatment sample (dt) multiplied by 100.

2-6- Statistical Analysis

A simultaneous examination Of, Variance (ANOVA) was carried out in addition to the statistical analysis of the data, which was carried out with the help of the software known as the Statistical Package for the Social Sciences (SPSS). In order to compare the differences between the treatments, the Least Significant Difference (LSD) test was utilized, with a probability threshold of less than 0.05.

3- Results and Discussion

3-1: Influence OF *T. harzianum* filtrate ON the development of *T. rubrum* through the use of the poisoned food method

culture filtrate of the fungus *Trichoderma harzianum* had an impact on the radial growth of *Trichophyton rubrum*, and this effect increased as the filtrate concentration increased. After seven days of treatment, the radial growth ranged between 2.25 cm and 5.9 cm, as compared to the control of 8.4 centimeter (Table 1).

The growth of the test fungus *T. rubrum* was strongly suppressed by all of the doses that were supplied, with the 10% filtrate displaying the least amount of action, in Copartion To The control Group. The findings revealed the growth inhibition rates were 73.2% for the 50% solution, 47.14% for the 25% concentration, and 29.76% for the 10% concentration. There were substantial disparities between the assessed therapy and the control group.

(Table 1): The potential influence of *T. harzianum* filtrate on the development of *T. rubrum* through the use of the poisoned food method

Treatment		Radial growth (cm)	Inhibition (%)
Trichoderma	Concentration (%)		
Filtrate	50	2.25	73.2%
	25	4.44	47.14 %
	10	5.9	29.76%
Control		8.4	
LSD (0.05)		0.283	

In each case, the value represents the average of 3 tests.

D

Fig 2: The impact of T. harzianum filtrate on the T. rubrum growth was determined by employing the poisoned food method. (A: 50%, B: 25%, C: 10%, D:control)

According to Patil & Prajapati (2017), a number of research have been carried out in order to examine the efficiency of Trichoderma species as an antimycotic or antifungal agent against a wide range of pathogenic fungi.

Trichoderma produces approximately 180 secondary metabolites from several chemical compound families. The substances can be categorized as peptaibols, hydrophilic compounds, and volatile antibiotics. *Trichoderma.Viride, Trichoderma. harzianum,* and *trichoderma. koningii* can synthesize 6PAP (6-pentyl-α-pyrone), a volatile antibiotic implicated in biocontrol, as well as hydrolytic enzymes such glucanases and chitinases, and antibiotics that may suppress the proliferation of dangerous fungi. (Kubicek et al., 2001; Reino et al., 2008 ;Kubicek et al., 2011)

Trichoderma is a group Of fungi that is found everywhere in the soil, on plants, in waste, on decaying wood, in organic fertilizers and in the air. In recent decades, several species of Trichoderma have been used as biological control agents to mitigate the occurrence of fungal diseases in plants of various kinds. (Deang et al., 2006; Ye et al., 2018).

Biological control mechanisms include competition, hyperparasitism, antibiosis, and development of resistance in plants. (Ruan & Liu, 2020).

The study showed that the Trichoderma filtrate suppressed the growth of Trichophyton, which could be due to the bioactive metabolites. This finding is in concurring with the Xiao et al. (2022), Who reported That ,Trichoderma taxi was effective In, inhibiting the growth of Trichophyton mentagrophytes through the production of trichodermin.

An investigation of the antifungal activity of T. viride, T. virens, T. harzianum, and T. lixii against the dermatophyte fungus was also carried out by Waleed et al. (2022). This investigation was likewise identical to the one described above. *Trichophyton tonsurans* and *Microsporum canis*, which exhibited different degrees of suppression of these fungi. The growth values of colony diameter varied from 0.46 to 5.40 cm across all concentrations and isolates. *T. harzianum* demonstrated the most potent antifungal activity, suppressing *T. tonsurans* and *M. canis* by 50% and 46% cm, respectively.

3-2-The impact of *T.harzianum* filtrate on the Growth of *Trichophyton rubrum* by the Disk Diffusion Technique

culture filtrate of *Trichoderma harzianum* showed inhibitory activities against *T. rubrum*, with inhibition zones spanning between 14.2 and 24.5 mm, according to the study's results, which are shown in (Table 3-7). The outcome also showed that when filtrate concentration grew, so did the effect of filtrate. Figure (3–12) shows that the highest inhibition zone, measuring 24.5 mm at a concentration 50%, and the lowest inhibition zone, measuring 14.2 mm at a concentration 10%.

(Table 2):The influence of *T. harzianum* filtrate on the development of *Trichophyton rubrum* as measured by the disc diffusion method

Treatment	Concentration (%)	Inhibition zones (mm)
Trichoderma Filtrate	50	24.5
	25	18.15
	10	14.2
Control		0
LSD (0.05)		0.199

The mean of three replicates is used to calculate each value.

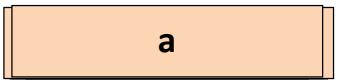


Figure 3: Impact of *T. harzianum filtrate* on Trichophyton rubrum development as measured By the Disk Diffusion technique

(a: filtrate10%, b: filtrate25%, c: filtrate 50%, d: control)

The inhibitory effects of *Trichoderma harzianum* filtrate on T. *rubrum* appear to be connected with poisonous substances that are produced by *T. harzianum* such as acetic acid, phenylethyl alcohol, harzianic acid, and other antifungal compounds (Vinale et al., 2009; Khan et al., 2020).

It is without any reasonable question that Trichoderma Spp are effective As a bio-control agent contra plant pathogenic fungus. This has been shown beyond any reasonable doubt. Antagonistic behavior of Trichoderma fungus toward phytopathogenic fungi & dermatophytes can be partly attributed to a basic mechanism. including those causing infections in nails, skin, and hair, is posited to be their direct mycoparasitic activity (Chet, 1990; Omero et al., 2004).

A number of studies (Aseel et al., 2025; Khalil et al., 2025; Saini et al., 2025) have demonstrated that Trichoderma species and plant extracts has The capability to effectively suppress the development of phytopathogens through Parasitism, Antibiosis, & Induced Systemic Resistance.

3-3--the impact Of *Trichoderma harzianum* on *Trichophyton* growth *In* dual culture

Findings of the investigation into how *T. harzianum* affected Trichophyton in a dual culture test (Table 3). shown how *T. harzianum* prevented *T. rubrum* radial mycelial development. T. rubrum had 2.2 and 2.4 cm mean radial growth after three and seven days of therapy, whereas the control was 2.8 and 7.8 cm. Radial growth inhibition percentages (PIRG) ranged from 21.43 to 64.1%.

The test pathogen *T. rubrum* was totally overgrown by the fungus *Trichoderma harzianum*, which also showed strong antagonistic activity and grew all over the petri plate. (Figure 4).

(Table 3): T. harzianum's antagonistic activity against T. rubrum in paired culture assay

reatmen t	adial growt h of *T.rub rum* after 3 days cm)	nhibit ion (%)	adial growt h of *T.rub rum* fter 7 days cm)	nhibit ion (%)	vergro wth after 8 days
.harzia num and T. rubrum .	.2	1.43	.4	9.2	++
ontrol	.8		.8		
SD (0.05)		0.163			

Each number is the three-replica average. +++ denotes strong antagonistic activity (61-75 PIRG), where PIRG is radial development inhibition (SoyTong 1988).

(Figure 4) Trichoderma and Trichophyton strains grown in a dual culture

(a- Trichophyton alone, b- *Trichophyton* with *Trichoderma*, c- overgrowth of *Trichoderma*)

The result of our study indicated that the *T. harzianum* excised antimycotic property against *Trichophyton rubrum* in dual culture, and this is due to the antifungal metabolite produced by this Trichoderma that was released in the growth medium, which possessed the ability to impede the formation of Trichophyton colonies.

Studies indicate that the mycelia of Trichoderma Spp develop On, The infected surface . They are responsible for the development of the pathogens. consistently encircle their mycelia before penetrating their cell walls. The enzymatic activity may lead to the disintegration of the pathogens' mycelia.

Assay for antagonistic cultures indicates Trichoderma typically interacts with the infectious agent 2 days post-vaccination, subsequently enveloping or infiltrating the microbial colony to inhibit pathogen proliferation. Trichoderma can grow alongside the pathogen's mycelium, intertwine with it, or encircle it. Moreover, throughout our investigation, we recognized that T. mentagrophytes and T. taxi were concurrently interconnected and associated with each other (Gao et al., 2002).

The findings of the antagonistic culture test suggested that Trichoderma typically takes two days following the inoculation, contact with the pathogen. Subsequently, Trichoderma wrapped or penetrated the microbial colony in order to restrict the growth of the microbial colony.

Trichoderma can grow along with the mycelium of the pathogen, coil around it, or thread around it. Furthermore, study result showed *T. mentagrophytes* and *T. taxi* were related and linked to each other. (Gao et al., 2002).

The findings of this study were in line with those of Mohamed et al. (2021) who found that the inhibition rates of T. harzianum against Fusarium verticillioides and Fusarium proliferatum were 60.64 and 68.38%, respectively. This investigation was also successful.

Yelena et al. (2023) in the dual culture found that 2 spp of trichoderma, T. pararogersonii & T. rossicum, showed antagonistic activity against soil pathogens such as Alternaria sp., Aspergillus niger, and Fusarium sp. the activity of suppression the two trichoderma species. was found to be considerably higher than their antagonistic activity.

Druzhinina et al. (2011) provide several reasons for the superior biological control ability of Trichoderma spp. There are processes going on Like as Mycoparasitisum, Antibiosis & Competition For blank & resources.

Many Spp Of trichoderma are able to generate enzymes that can destroy

The Wall of cell wall. These enzymes are β_(1,6)-glucanases, Chitinases & Protease, which may be involved in the lysis of the fusarial mycelium (Sood et al., 2020).

trichoderma is classified As a polyfunctional fungus, as both biofertilizer and biofungicide (biological agent). Trichoderma is used as a biofertilizer because it produces volatile compounds and solubilizes phosphate, thus helping the plant to

access this nutrient. As a biological agent, it lowers illnesses caused by species of Phytophthora, Rhizoctonia and Sclerotinia. Trichoderma viridae and *Trichoderma harzianum* now dominate the global market for Trichoderma-based formulations and products (Sambed and Saugat, 2022).

3-4-The influence of T. harzianum filtrate combined with L. camara extract on the development of T. rubrum as measured by the disc diffusion technique. A combination of extract and filtrate was found to have an additive impact on the development of T. rubrum, in accordance with the results of the study. rather than an antagonistic impact on the activity of the filtrate (Table 3-9).

The combination of lantana at a concentration of 25 mg/ml and filtrate at 50% yielded the biggest inhibitory growth zone, measuring 30.5 mm, compared to lantana alone (25.2 mm) and filtrate (24.5 mm).

Table 4: Impact of the combination of *Trichoderma. harzianum* filtrate *and ethanolic extract of Lantana. camara* on the expansion of *T.rubrum* by the disc diffusion method

therapy	Inhibition zones (mm) *T. rubrum*
L.camara extract (25 mg/ml) +*T.harzianum* Filtrate (50 %)	
T.harzianum Filtrate alone (50%)	24.5
Ethanolic extract alone 25 mg/ml	25.2
L.camara extract +*T.harzianum* Filtrate	30.5
LSD (0.05)	0.200

Each number is the average of three replicates, with a total of three, and the significance level is less than 0.05.

Based on the findings of our research, it was determined that the combination of ethanolic lantana extract At Conc. Of (25 Mg/ml) with filtrate of *Trichoderma harzianum* At Conc. OF 50% exhibited an additive response. This was demonstrated by the fact that the calculated Chi-squared value was 1.306, which is lower than the table Chi-squared value of 3.84.

Table 5**:** presents the results of the Chi-squared (χ2) test, which is used to evaluate the level of involvement

therapy and Conc.		Zone of inhibition (mm)				calculated	index of involvement
antana extract 5mg/ml	richoderma filtrate 0%	antana extract (alone)	iltrate alone	xpected I.Z.	bserved .Z.(Comb.)	χ2	
		5.2	4.5	7.5	0. 5	.306	dditive

Antifungal combination therapy is one strategy used to counteract fungal resistance caused by the limited therapeutic efficacy of currently available commercial antifungals.

Mirghani et al. (2018) define synergy as the interaction or collaboration of two or more entities, resulting in a combined effect that exceeds the sum of their individual contributions.

An additive interaction is defined as when two active mixtures are combined and have a potency equivalent to the potency of the individual active components in each mixture, and an antagonistic interaction is defined as when the cumulative impact is fewer than the aggregate efficacy of the discrete elements (Zhang et al., 2017).

This study shows that Trichoderma attacks directly the pathogenic fungi and degrades them (mycoparasitism); the extracts give an initial antimicrobial shock to the pathogen. The present finding is in consistent with Aneta et al. (2023) who reported that *Trichoderma viride* and *Trichophyton tonsurans* exerted a synergistic effect.

The results obtained when extracts of medicinal plants were combined with antifungal drugs or other bioagents for controlling Malassezia pachydermatis (Bohmova et al., 2019), Candida species (Rosato et al., 2009) & Aspergillus species (Sarkhani et al., 2018) ,Were additive to synergistic.

Regarding the control of pathogenic fungi, the present results indicate the importance of the combination of Trichoderma and Lantana extracts as alternatives to synthetic fungicides that are not only cost-effective but also environmentally friendly.

Trichoderma harzianum has been shown to have biocontrol properties (Yao et al., 2023;

Guzmán-Guzmán et al., 2025). Such characteristics are demonstrated in processes like mycoparasitism and antibiosis.

The Lantana camara plant has antifungal constituents which inhibit the growth of mycelial mats. These are Thymol,Cymene ,Carvacrol, alpha-Terpinyl acetate, Pinene & Linalool. Trichoderma can be easily cultured and formulated, resulting in reduced production costs , The combination approach has several benefits. It reduces the overall cost of production. *(Woo et al., 2014; Lodi et al., 2023).*

4-Conclusion

The antifungal activity of *T. harzianum* filtrate combined with *L. camara* extract against *Trichophyton rubrum* fungus from patients was investigated. Results demonstrated that the filtrate ,OF Trichoderma fungus has a very strong inhibition effect against the fungus *Trichophyton rubrum* on the culture medium, and the effect increases with the increase of concentration. Furthermore, combining the fungal filtrate with the extract of the *Lantana camara* gave a stronger inhibitory effect against the *T. rubrum* fungus compared to treatment with the filtrate alone.

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