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BIOCONTROL POTENTIAL OF *TRICHODERMA HARZIANUM* SPECIES AGAINST SEED-BORNE FUNGI OF FIFTEEN VEGETABLES USING DUAL CULTURE ASSAY

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Abstract

Seed-borne fungi varied significantly across vegetables, with Fusarium *spp. emerging as the most dominant pathogen, particularly in chilli, cucumber, apple gourd, and bottle gourd.* Alternaria *and* Aspergillus *spp. also showed crop-specific prevalence, while* Cladosporium*,* Penicillium*, and* Rhizoctonia solani *were minor contributors. Diversity analysis revealed that Tomato, Bitter Gourd, and Red Chilli harbored the highest fungal diversity, whereas Pumpkin and Apple Gourd recorded the lowest. Percentage occurrence data further confirmed* Fusarium *as the primary seed-borne threat, followed by* Alternaria *and* Aspergillus*. Antagonism assays demonstrated that* Trichoderma harzianum *significantly suppressed the radial growth of* Fusarium oxysporum*,* Alternaria solani*, and* Aspergillus flavus*, while its effect on* Aspergillus niger *was limited. Overall, the study highlights* Fusarium *as the most serious seed-borne pathogen, with* T. harzianum *proving to be an effective biocontrol agent against major fungi, though its efficacy is species-specific.*

Introduction

The quality and viability of vegetable seeds are seriously threatened by fungal infections because contamination can happen both before and after seed preparation. Numerous illnesses that have a detrimental impact on agricultural output and jeopardize food safety are caused by these microorganisms. Because they can cause large financial losses and health risks associated with mycotoxin contamination, seed-borne fungal infections are a major issue for farmers and agricultural stakeholders (Kachhap *et al.,* 2025; Akhtar & Kumar, 2024). Fungi carried by seeds can survive inside the seed coat and are easily passed on to the next generation of plants. Planting, storing, and processing are some of the stages of seed production where they could spread (Kumar & Chaurasiya, 2020; Martín *et al.,* 2022). Fungal pathogen contamination of seeds frequently results in the emergence of severe diseases that impair crop yields by decreasing germination rates and weakening seedling vigor. Because they create mycotoxins, which are toxic secondary metabolites that not only deteriorate the quality of seeds but also present serious health hazards to both humans and animals, some seed-borne fungi are especially hazardous (Ilyas & Manohara, 2023; Biemond *et al.,* 2021). Clarifying their role in disease outbreaks and creating efficient management strategies are the main goals of isolating and characterizing seed-borne fungus. In

order to determine the fungus species causing illnesses, this procedure involves looking at seeds from frequently grown plants. To find and precisely identify these diseases, a variety of approaches are used, such as blotter assays, agar plate techniques, molecular analysis, and seed health testing. Vegetable seeds are often linked to a number of fungus species, including *Aspergillus, Fusarium,* and *Penicillium,* which are known to create a variety of mycotoxins that negatively impact plant health and food safety (Erasto *et al.,* 2023; Hussain *et al.,* 2013). Among these, *Aspergillus niger* and *Aspergillus flavus* are frequently found on the seeds of foods including pepper, tomato, and brinjal. These species produce ochratoxins and aflatoxins, which can infect seeds and provide major health hazards when consumed. In particular, aflatoxins are known to be extremely carcinogenic substances that can cause acute poisoning in humans and animals (Ahmad *et al.,* 2025; El-Dawy *et al.,* 2024; WHO, 2023). Seeds often act as vehicles for pathogens, enabling the dissemination of fungal infections across diverse regions. Certain fungal species can persist either on the seed surface or within the seed tissues for long durations, making them highly resilient and difficult to eradicate. Consequently, investigating seed-borne fungi is essential to understand their distribution, pathogenic potential, and ecological significance (Akhtar & Kumar, 2024; Martín *et al.,* 2022; Singh *et al*., 2021). The production of vegetables is severely hampered by seed-borne fungus, which can degrade seed quality, prevent germination, and have a detrimental effect on crop health. Their presence frequently leads to lower yields and large financial losses. A number of diseases, such as seed rot, damping-off, and seedling blight, are caused by these pathogens and present significant challenges to the implementation of sustainable agricultural methods (Kachhap *et al.,* 2025; Sharma & Jain, 2022; Biology Discussion, 2023). Research has demonstrated that *Fusarium* species, especially *Fusarium oxysporum* and *Fusarium solani,* can infect vegetable seeds and produce mycotoxins such zearalenone and deoxynivalenol (DON). Because of their estrogenic qualities and capacity to interfere with endocrine processes in both humans and animals, these substances are extremely concerning. In addition to lowering seed quality, mycotoxin contamination of vegetable seeds raises health-related expenses associated with foodborne illnesses and causes financial losses for farmers (Shabeer *et al.,* 2023; Ravikumara *et al.,* 2025; Cai *et al.,* 2022). The phytotoxic effects of mycotoxins produced by seed-borne fungi are dose-dependent and can range from acute toxicity to long-term health problems. Their presence in vegetable seeds lowers crop yields, jeopardizes food safety, and causes financial hardships due to lost livestock output and veterinary expenses. To restrict fungal development and toxin generation, effective management necessitates the identification and characterization of fungal infections, the adoption of resistant cultivars, the application of seed treatments, and the maintenance of appropriate storage conditions (Bennett & Klich, 2003; Akhtar & Kumar, 2024; Kachhap *et al.,* 2025).

Integrated solutions that integrate chemical, biological, and cultural methods are necessary for the effective management of seed-borne fungal diseases. Fungal incidence and mycotoxin contamination can be decreased by practices such crop rotation, cleanliness, biological control with beneficial bacteria, and prudent fungicide treatment. To reduce hazards, farmer education, safe seed handling, and better storage conditions are all crucial. Developing sustainable

solutions that protect crop quality, food safety, and agricultural productivity requires ongoing research into pathogen biology and ecology as well as cooperation between researchers, extension services, and farmers (Cook *et al.,* 2015; Akhtar & Kumar, 2024; Kachhap *et al.,* 2025). Chemical pesticides, fungicides, and microbicides such fthalide, edifenpop diseases.
Despite the effectiveness of these chemicals, their use is frequently linked to significant financial expenses, environmental issues, and possible health hazards.

As a result, alternative strategies that lessen dependency on synthetic chemicals are gaining popu larity. Plant extracts have demonstrated promise in reducing seed borne fungal infections, providing environmentally acceptable remedies. hos, iprobenfos, tricyclazole, isoprothiolane, probenazole, pyroquilon, meferimzone, and diclocymet have historically been used to control it.

In this context, biological control methods particularly the use of antagonistic organisms like *Trichoderma* species are being explored (Kator *et al.,* 2023; Yao *et al.,* 2023; Zhang *et al.,* 2023).

Material and Method

Vegetable seed collection: Samples of vegetable seeds were gathered at the nearby AL-Safa market. They were appropriately tagged and placed in polythene bags.

Preparation of Potato Dextrose Agar: Potato Dextrose Agar (PDA) was prepared by suspending 39 g of dehydrated medium in 1000 mL of distilled water. The mixture was heated to boiling with constant stirring until the medium was completely dissolved. It was then sterilized in an autoclave at 121 °C under 15 lbs pressure for 15 minutes. After sterilization, the medium was mixed thoroughly and allowed to cool to 45–50 °C before being dispensed aseptically into sterile Petri plates, where it was left to solidify. The prepared plates were stored at 4 °C until required for fungal isolation and growth studies. To minimize bacterial contamination, antibiotics such as penicillin or streptomycin were added to the medium after cooling.

Fungal identification on vegetable seeds: The agar plate method is a standardized approach for detecting and identifying internal seed-borne fungi. In this technique, seeds are first surface-sterilized, commonly with sodium hypochlorite, to remove saprophytic contaminants and expose internal pathogenic fungi. A set of 4–6 treated seeds is then placed onto solid nutrient agar, typically Potato Dextrose Agar (PDA) or 2% Water Agar, within sterile Petri plates. The plates are incubated under alternating cycles of light and darkness (commonly 12 h/12 h or 16 h/8 h) for a period of 5–7 days. Following incubation, fungal growth emerging from the seeds is examined and identified based on colony characteristics such as color and texture, as well as microscopic features of sporulating structures observed under a compound microscope. This method provides a reliable means of isolating and characterizing seed-borne fungal pathogens.

Fungal pathogen identification: A compound microscope was used to identify the fungus that grew on the vegetable seed. It was accomplished by looking at the colors of the spores, hyphae, and fungal development on Petri dishes. A glass slide was stained with a drop of lactophenol in cotton blue for microscopic identification. A tiny amount of the fungal colony was then applied to the stained glass slide using an inoculation needle. This was examined under the light microscope's 10x and 40x objective lenses while covered with a cover slip.

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Percentage occurrence of fungi: Plates were observed for growth and the occurrence of fungi was determined by counting the number of fungi per market divided by the total number of fungi and expressed as a percentage.

$$:\frac{Number\ of\ Fungi}{Total\ number\ of\ fungi} \times 100$$

Trichoderma harzianum **isolation from soil sample:** *Trichoderma harzianum* were isolated from soil samples using a serial dilution technique, in accordance with Liamngee *et al.* (2014)'s protocol. First, 1 g of the soil sample was mixed with 9 mL of sterile distilled water in a glass tube that had been sterilized to create a stock solution. To achieve a dilution, 1 mL of this original suspension was moved into a subsequent test tube; this process was continued until a final concentration was obtained. After plating a 1 mL aliquot of the dilution onto a 9 cm diameter Petri dish, 15–20 mL of sterile, melted Potato Dextrose Agar (PDA) was added. After gently swirling the liquid to make sure the inoculum was distributed evenly, it was left to harden. For seven days, the resulting culture plates were incubated at room temperature, and any growing colonies were observed every day. Every unique colony was identified as a Colony-Forming Unit (CFU). Sub culturing was used to isolate pure fungal cultures. Using computerized documentation on the genus Trichoderma14, the micro-morphological analysis involved examining the morphology of conidiophores and conidia as well as other pertinent features.

Antagonistic activity of *Trichoderma harzianum in vitro*: The antagonistic potential of *Trichoderma harzianum* was evaluated using the dual culture technique on potato dextrose agar (PDA) medium against fungal pathogens isolated from vegetable seeds. Mycelial discs (5 mm) of both the test pathogens and *Trichoderma* isolates were placed simultaneously on the plates. The experiment followed a completely randomized design and was replicated three times. For the control treatment, only the mycelial disc of each test pathogen was inoculated. All plates were incubated under controlled temperature conditions.

Trichoderma harzianum **in vitro antagonistic activity:** The following formula was used to determine the inhibition of test fungus.

R1-R2/R1

Where:

R1 = Mycelia growth of the pathogen without *Trichoderma* (control)
R2 = Mycelia growth of the pathogen in the presence of *Trichoderma*

Statistics analysis: All data were subjected to rigorous statistical evaluation. One-way Analysis of Variance (ANOVA) was performed to determine the significance of differences. To further separate means, Duncan's Multiple Range Test (DMRT) and the Least Significant Difference (LSD) test at the 5% probability level (Zar, 2009) were applied, enabling identification of statistically distinct groups. In addition, Species diversity is also calculated using indices like Shannon–Wiener and Simpson's.

Results

The agar plate assay revealed considerable variation in the incidence of seed-borne fungi among the tested vegetables (Table 1). *Fusarium sp*. was consistently recorded at high levels, particularly in red chilli (5.65), cucumber (5.33), apple gourd (5.33), and bottle gourd (5.0), confirming its dominance across cucurbits and chilli crops. *Alternaria sp*. was also prevalent, with maximum incidence in onion (4.0) and bitter gourd (4.0), while moderate levels were observed in coriander and green chilli. *Aspergillus niger* showed notable growth in lemon (4.0) and red chilli (4.3), whereas *Aspergillus flavus* was more prominent in capsicum (3.15) and bitter gourd (3.67). *Rhizopus sp*. contributed significantly in pea (3.15) and lemon (3.67), while *Cladosporium sp*. was detected at lower levels in tomato (1.5), green chilli (2.67), and lemon (1.5). *Penicillium* and *Rhizoctonia solani* were rarely encountered, with *Rhizoctonia* appearing only in sponge gourd (2.3). Overall, *Fusarium* emerged as the most dominant seed-borne pathogen across vegetables, followed by *Alternaria* and *Aspergillus spp.,* while *Cladosporium, Penicillium*, and *Rhizoctonia solani* remained minor contributors. These findings highlight crop-specific fungal associations, with chili, cucumber, apple gourd, onion, and bitter gourd identified as particularly vulnerable hosts. Overall, the data indicate that *Fusariu*m is the most serious seed-borne threat across vegetables, followed by *Alternari*a. Crops such as red chilli, cucumber, and apple gourd showed the highest *Fusarium* loads, while bitter gourd and onion exhibited broader fungal diversity. This suggests that management strategies should prioritize *Fusarium* control, with crop-specific interventions for *Alternari*a and *Aspergillus* where they occur at significant levels.

The one-way ANOVA conducted across vegetables and fungi revealed significant host-specific variation in seed-borne fungal incidence. *Fusarium sp*. consistently showed highly significant differences ($p < 0.01$), with red chilli, cucumber, apple gourd, and bottle gourd recording markedly higher means compared to tomato, capsicum, and pea. *Alternaria* sp. also varied significantly ($p < 0.05$), with onion and bitter gourd exhibiting higher infection levels than apple gourd and brinjal. *Aspergillus* niger displayed significant variation ($p < 0.05$), being most prevalent in lemon and red chilli, while *Aspergillus flavus* was significantly higher in bitter gourd and capsicum compared to tomato. *Rhizopus* sp. showed moderate but significant differences ($p < 0.05$), with lemon and pea recording higher values than sponge gourd. In contrast, *Cladosporium sp*. did not show significant variation, while *Penicillium* and *Rhizoctonia solani* were restricted to

sponge gourd, making statistical comparison irrelevant. LSD values at 0.05 confirmed these differences, with critical differences ranging from 1.05 to 1.25 depending on the host. Overall, the analysis highlights *Fusarium* as the most dominant and statistically variable seed-borne fungus, followed by *Alternaria* and *Aspergillus spp*., with clear host-specific susceptibility patterns that identify chilli, cucumber, apple gourd, onion, and bitter gourd as the most vulnerable crops.

Table 1: Incidence of seed-borne fungi values represent the mean number of fungal colonies per seed sample followed by the standard error (Mean ± SE). Least Significant Difference (LSD) values are provided to indicate statistical significance among treatments. Means followed by the same letter in DMRT analysis are not significantly different at the 5% probability level.

SN	Vegetable	*Fusarium sp.*	*Alternaria sp.*	*Cladosporium sp.*	*Aspergillus niger*	*Aspergillus flavus*	*Rhizopous sp.*	*Penicillium*	*Rhizotocnia solani*	LSD *5%*
1-	**Tomato** ***(Solanum lycoperscium)***	3±1	2.5± 0.78	1.5± 0.71	1.67± 0.58	1 ± 1	2.3±0.78	-	-	1.05
2-	**Onion** ***(Allium cepa)***	3.67±0.47	4± 11	-	2± 1	1 ± 0	2.38±0.58	-	-	1.12
3-	**Green Chilli** ***(Capsicum anuum)***	-	3±1	2.67±0.58	2.9±0.58	1.65± 0.92	-	-	-	1.08
4-	**Pea** **(*Pistum sativum)***	3.16±1.63	3±1	-	2.38±0.58	2.16±0.58	3.15±1.63	-	-	1.15
5-	**Bitter guard** ***Momordica charantia)***	4±1	4±11		3.6±1.53	2.67±1.53	3.67±1.53	3.667±1.53	-	1.20
6-	**Lemon** ***(Citrus sativus)***	-	3±1	1.5±0.58		4±1	-	3.67±1.53	-	1.18
7-	**Brinjial** ***(Solanum melongena)***	3.67±0.47	2.33±0.58	-	1.68±0.58	2.67±0.58	-	-	-	1.10
8-	**Coriander** ***(coriander sativum)***	3.67±1.53	3.56±1.53	-	3±1	-	-	-	-	1.12
9-	**Sponge guard** **(Luffa cylindrical)**	3.33±1.53	-	-	1.5±0.58	1.56±0.59	2.3±0.78	-	2.3±0.78	1.15
10-	**Cucumber** **(cucumis sativus)**	5.33±2.49	2.33±0.567	-	3±0.82	-	2.38±0.58	-	-	1.25
11-	**Capsicum** ***(Capsicum annum)***	2.6±0.58	3±1	1.5±0.71	-	1.65±0.92	3.15±1.63	-	-	1.10
12-	**Bottle guard** **(*Lagenaria siceraria)***	5±0.82	1.57±0.5	-	2±0.82	3±0.81	-	-	-	1.18
13-	**Pumpkin** ***(Cucurbita pepo)***	4±1.63	2.5±0.78	-	-	-	3±0.82	-	-	1.15
14-	**Apple guard** **(*Benincasa fistulosa*)**	5.33±1.70	1.56±1.25	-	-	-	-	-	-	1.20
15-	**Red Chilli** **(*Capsicum annum)***	5.65±1.25	3.33±1.28	-	4.3±1.73	1.65±0.92	3.15±1.63	-	-	1.22

The statistical evaluation of seed-borne fungi across vegetables demonstrated clear host-specific differences in pathogen incidence. *Fusarium sp*. consistently emerged as the most dominant fungus, forming the highest statistical group in most crops (Fig; 1), particularly red chilli, cucumber, apple gourd, and bottle gourd.)*Alternaria* sp. showed significant prevalence in onion, bitter gourd, and coriander, while *Aspergillus niger* was most prominent in lemon and red chilli, and *Aspergillus flavus* dominated capsicum and bitter gourd. *Rhizopus sp*. contributed moderately in pea and lemon, whereas *Cladosporium, Penicillium*, and *Rhizoctonia solani* remained minor. ANOVA, LSD, and DMRT analyses confirmed these differences, establishing *Fusarium* as the primary seed-borne pathogen with *Alternaria* and *Aspergillus spp*. showing crop-specific significance. These findings highlight the need for targeted disease management strategies, prioritizing *Fusarium* control in cucurbits and chilli crops, and tailored interventions for *Alternaria* and *Aspergillus* in susceptible hosts.

DMRT Groupings of Fungi Across Vegetables

a = High (Red) b = Medium (Yellow) c = Low) Other colors

	Fusarium sp.	*Alternaria sp.*	*Cladosporium sp.*	*Aspergillus niger*	*Aspergillus flavus*	*Phinicillium*	*Rhizocotonia solani*
Tomato	a	a	b	c	c	–	
Onion	a	a	–	b	c		
Green Chilli	a	a	b	a	b		
Pea	a	a	b	b	a		
Bitter Gourd	a	a	b	b	a		
Lemon	a	a	c	a	a		
Brinjal	a	b	c	a	a	a	
Coriander	a	b	b	c			
Sponge Gourd	a	a	c	c	c	b	
Cucumber	a	b	b				
Capsicum	b	c	c	a			
Bottle Gourd	a	c	b				
Pumpkin	a	b	b				
Apple Gourd	a	b					
Red Chilli	a	b	a	c	b		

Fig 1:DMRT value mean followed by the same letter in DMRT analysis are not significantly different at the 5% probability level.

The diversity analysis shows that fungal colonization varies widely across vegetables (Table;2). Tomato, Bitter Gourd, and Red Chilli exhibited the **highest diversity**, with multiple fungi present and balanced distributions, reflected in high Shannon and Simpson indices. Onion, Pea, and

Capsicum showed **moderate diversity**, while vegetables such as Green Chilli, Lemon, Brinjal, Sponge Gourd, Cucumber, and Bottle Gourd had **lower diversity scores**, indicating fewer species or less balanced colonization. The **lowest diversity** was observed in Pumpkin and Apple Gourd, where only two to three fungi were detected. Despite these differences, **evenness values were consistently high**, suggesting that when fungi were present, they tended to be distributed relatively evenly across species. Overall, crops like Tomato, Bitter Gourd, and Red Chilli are more vulnerable to diverse fungal colonization, while Pumpkin and Apple Gourd are comparatively less affected.

Vegetable	Richness (S)	Shannon (H')	Simpson (D)	Evenness (E)
Tomato	6	1.73	0.84	0.96
Onion	5	1.52	0.78	0.95
Green Chilli	4	1.34	0.72	0.97
Pea	5	1.56	0.80	0.97
Bitter Gourd	6	1.70	0.83	0.95
Lemon	4	1.41	0.75	0.98
Brinjal	4	1.36	0.73	0.98
Coriander	3	1.10	0.65	1.00
Sponge Gourd	4	1.32	0.71	0.95
Cucumber	4	1.39	0.74	0.97
Capsicum	5	1.55	0.79	0.96
Bottle Gourd	4	1.33	0.72	0.96
Pumpkin	3	1.05	0.62	0.96
Apple Gourd	2	0.69	0.50	1.00
Red Chilli	5	1.61	0.81	0.97

Table 2: Species Diversity of fungal colonization across vegetables.

The percentage occurrence data reveal clear dominance patterns among seed-borne fungi across the tested vegetables (Table 3). *Fusarium oxysporam* were the most prevalent overall, reaching very high percentages in apple gourd (88.83%), cucumber (44.41%), bottle gourd (45.45%), and pumpkin (44.41%). *Alternaria* **s**pecie**s** were the second most frequent, with notable values in onion (33.33%), coriander (32.36%), and pumpkin (27.77%). *Cladosporium* **species** were relatively rare, detected only in green chilli (24.27%), tomato (13.64%), and capsicum (12.5%). *Aspergillus niger* showed significant occurrence in lemon (36.67%), cucumber (25.1%), and red chilli (23.88%), while *A. flavus* was most prominent in capsicum (26.25%), bottle gourd (25.1%), and pumpkin

(33.33%). *Rhizopus* species were sporadic but reached moderate levels in lemon (33.36%), pea (24.23%), and sponge gourd (25.5). In contrast, *Penicillium* and *Rhizoctonia solani* were detected only in sponge gourd (25.5 each), indicating their limited distribution.

Overall, the results highlight *Fusarium* as the most dominant seed-borne fungus across vegetables, with particularly high percentages in cucurbits and chilli crops. *Alternaria* and *Aspergillus* species also contribute significantly to seed contamination, while Cladosporium, Rhizopus, Penicillium, and *Rhizoctonia* occur less frequently and in fewer hosts. This pattern suggests that management strategies should prioritize *Fusarium* control, with targeted interventions for *Alternaria* and *Aspergillus* in crops where they show high occurrence.

Table 3: Percentage occurrence of specific fungi isolated from seed of vegetable.

SN	Vegetable	*Fusarium sp.* %	*Alternaria sp.* %	*Cladosporium sp.* %	*Aspergillus niger* %	*Aspergillus flavus* %	*Rhizopous sp.* %	*Penicillium* %	*Rhizotocnia solani* %
1-	Tomato	27.7	22.73	13.64	13.51	9.09	20.91	-	-
2-	Onion	30.58	33.33%	-	16.67	8.35	19.83	-	-
3-	Green Chilli	-	27.27	24.27	26.36	15.0%	-	-	-
4-	Pea	24.31	24.08	-	18.31	16.62	24.23	-	-
5-	Bitter guard	17.93	17.39	-	15.96	11.61	15.93	15.96	-
6-	Lemon	-	27.7	13.64	-	36.67%	-	33.36	-
7-	Brinjial	36.70$	23.80	-	16.80	26.70	-	-	-
8-	Coriander	33.67	32.36	-	27.7	-	-	-	-
9-	Sponge guard	36.66	-	-	16.66	17.33	25.5	-	25.5
10-	Cucumber	44.41	19.41	-	25.1	-	19.83	-	-
11-	Capsicum	21.66	25.1	12.5	-	13.75	26.25	-	-
12-	Bottle guard	45.45	12.25	-	16.66	25.1	-	-	-
13-	Pumpkin	44.41	27.77	-	-	-	33.33	-	-
14-	Apple guard	88.83	26.1	-	-	-	-	-	-
15-	RedChilli	30.88	18.51	-	23.88	9.166	17.53	-	-

The antagonistic effect of *Trichoderma harzianum* on the radial growth of seed-borne fungi was evident across all tested pathogens. In the case of *Fusarium oxysporum* (Table 4), radial growth was significantly reduced at all incubation periods compared to the control, with t-values ranging from 3.15 to 6.05 and corresponding p-values below 0.05, confirming strong inhibition. Similarly, *Alternaria solani* (Table 5) exhibited marked suppression, particularly after 72 hr, where the difference was highly significant ($t = 16.2$, $p = 0.001$). Growth inhibition remained consistent through 96 hr and 120 hr, demonstrating the sustained antagonistic activity of *T. harzianum*. For *Aspergillus niger* (Table 6), however, no significant differences were observed between treatment and control at any time point ($p > 0.05$), indicating that *T. harzianum* did not exert a consistent inhibitory effect against this species under the tested conditions. In contrast, *Aspergillus flavus* (Table 7) showed highly significant reductions in radial growth at all intervals, with exceptionally high t-values (18.7–28.6) and p-values well below 0.01, highlighting the potent suppressive effect of *T. harzianum* against this pathogen. Overall, these findings demonstrate that *Trichoderma harzianum* is an effective biocontrol agent against *Fusarium oxysporum*, *Alternaria solani*, and *Aspergillus flavus*, while its effect on *Aspergillus niger* appears limited. The variability in antagonistic response suggests species-specific interactions, emphasizing the importance of pathogen identity in determining the efficacy of biocontrol strategies.

Table 4 : Effect of *Trichoderma harzianum* on radial growth of *Fusarium oxysporam* isolated from seed of vegetables. Values are Mean followed by Standard Error in triplicates.

Radial growth(cm)				
Treatment	48hr	72hr	96hr	120hr
Fusarium oxysporum +Trichoderma harzianum	1.5±0.32	4.2±0.21	5.3±0.5	6.64±0.3
Control	3±0.5	5±0.14	7±0.21	8±0.42
T - value	4.21	3.15	5.62	6.05
P-value	0.004	0.012	0.001	0.001

Table 5 : Effect of *Trichoderma harzianum* on radial growth of *Alternaria solani* isolated from seed of vegetables. Values are Mean followed by Standard Error in triplicates

Radial growth (cm)				
Treatment	48hr	72hr	96hr	120hr
Alternaria solani+Trichoderma harzainum	2 ±0.42	4.055±0.2	5.15±0.4	5±0.31
Control	3±0.14	6±0.16	7.2±0.21	8±0.34
T- value	3.16	16.2	8.36	12.9
P-value	0.021	0.001	0.001	0.001

Table 6 : Effect of *Trichoderma harzianum* on radial growth of *Aspergillus niger* isolated from seed of vegetables. Values are Mean followed by Standard Error in triplicates

Radial growth (cm)				
Treatment	48hr	72hr	96hr	120 hr
Aspergillus niger+ Trichoderma harzianum	3.10±2.1	4.3±5	4.72±3.2	5±4
Control	4±0.24	6±0.36	3±0.19	5±0.23
T- value	0.74	0.59	0.093	0.00
P-VALUE	0.50	0.58	0.40	1.00

Table 7 : Effect of *Trichoderma harzianum* on radial growth of *Aspergillus flavus* isolated from seed of vegetables. Values are Mean followed by Standard Error in triplicates

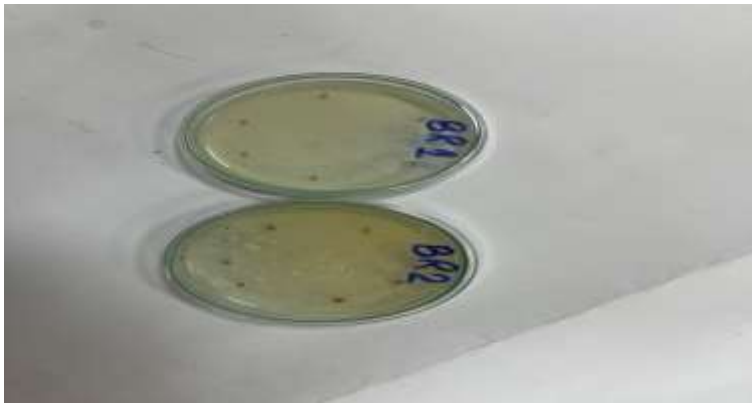
Radial growth (cm)				
Treatment	48hr	72hr	96hr	120hr
Aspergillus flavus+ Trichoderma harzianum	1.28±0.34	1.94±0.32	3.78±0.5	4.21±0.61
Control	5±0.15	7±0.23	6± 0.17	8±0.41
T-value	18.7	28.6	8.3	9.1
P- value	0.001	0.001	0.001	0.0008

Discussion:

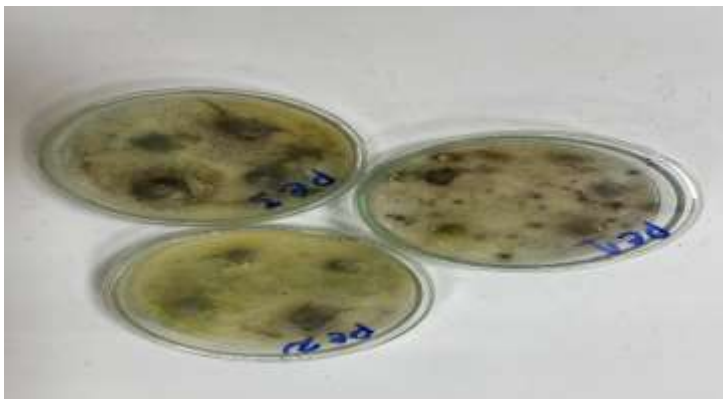
Identifying and characterizing seed-borne fungal infections linked to a variety of vegetables, such as tomato, lemon, coriander, sponge gourd, brinjal, bitter gourd, okra, and onion seeds, was the goal of the current study. A wide range of fungal genera with possible pathogenic and mycotoxigenic implications were discovered through the isolation and identification of fungi using normal agar plating and microscopic techniques. *Alternaria* was the most prevalent fungus species found in tomato seeds, which is consistent with earlier research showing *Alternaria spp.* as frequent seed-borne diseases in solanaceous crops (Smith *et al.,* 2018; Kumar & Singh, 2020). The output and quality of tomato crops are greatly impacted by *Alternaria* species, which are known to cause leaf spots, fruit rot, and seed decay (Jones *et al*., 2017). The finding of *Alternaria* on tomato seeds highlights the necessity of efficient seed treatment and storage procedures to reduce the spread of disease by pointing to a possible source of inoculum for field infections.*Penicillium* species were mostly isolated from lemon seeds. This result is consistent with research by Patel and Desai (2019), who identified *Penicillium* as a prevalent post-harvest pathogen in citrus fruits and seeds. *Penicillium* species are known to create mycotoxins such patulin and induce blue mold, both of which are harmful to consumers' health (Miller *et al*., 2016). The existence of *Penicillium* in lemon seeds emphasizes how crucial it is to keep an eye out for fungal contamination in citrus seed lots, particularly in humid storage environments that encourage fungal growth/*Alternaria* and *Fusarium* species, which are known for their pathogenicity and mycotoxin production, were found in coriander seeds (Gupta *et al*., 2021). *Fusarium* and *Aspergillus* species were found in mung bean and chickpea seeds, which is in line with research by Reddy *et al*. (2017) that identified these fungi as significant seed-borne diseases that impact legume crops. Because *Aspergillus* species have the ability to produce aflatoxin, which offers significant hazards to food safety, their presence is especially alarming (Williams *et al.,* 2019).The growth of seed-borne fungi is greatly influenced by environmental conditions such temperature, humidity, and storage time. Similar to the results of the maize study, warm, humid weather, which is typical in tropical and subtropical areas where these crops are grown and stored, promotes fungal growth and mycotoxin generation (Reddy *et al.,* 2017). Inadequate drying and storing procedures worsen fungal contamination, which increases seed deterioration and may pose health risks because of mycotoxin buildup.Concerns regarding the safety of these seeds for planting and consumption are raised by the discovery of mycotoxigenic fungi like *Aspergillus*, *Penicillium*, and *Fusarium* in a variety of seed varieties. These fungi create mycotoxins such fumonisins, ochratoxins, and aflatoxins that can be harmful to both human and animal health (Williams *et al*., 2019). To reduce the dangers of fungal contaminationiand mycotoxin, it is crucial to regularly assess and apply excellent farming and storage practices.The impacts of *Fusarium*, *Macrophomina*, and *Trichoderma* on seeds are especially significant in this regard. *Trichoderma* species are well known for being effective biocontrol agents against fungal diseases carried by seeds. They produce antifungal metabolites, compete for nutrients and space, and trigger plant defense mechanisms, among other hostile activities (Harman *et al*., 2004). It has been demonstrated that applying *Trichoderm*a to seeds lowers the frequency of *Fusarium* and *Macrophomina* infections, increasing seed germination and

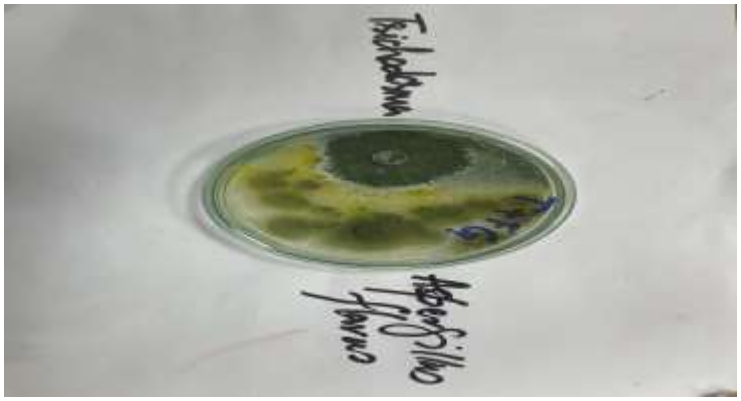
seedling vigor in a variety of vegetable crops, such as cucumber,On the other hand, a harmful soil and seed-borne disease called *Macrophomina phaseolina* causes charcoal rot in a variety of vegetable crops, including cowpea, okra, and bitter gourd. It causes poor germination and seedling death when it infects seeds (Kumar *et al.,* 2018). Management is difficult because the disease creates microsclerotia that thrive in soil and seed coatings. Macrophomina infection has been successfully reduced by treating seeds with fungicides and biocontrol agents like *Trichoderma* (Chaudhary *et al.,* 2020). *Fusarium* species, especially *Fusarium oxysporum* and *Fusarium solani*, are important seed-borne infections that cause root rot and wilt in plants like brinjal, tomatoes, and cucumbers. Significant yield losses result from these fungi's invasion of seeds and seedlings (Smith *et al*., 2018). It has been shown that applying *Trichoderma* and other biocontrol agents to seeds can inhibit *Fusarium* growth and enhance plant health (Reddy *et al*., 2017). To effectively manage *Fusarium*-related infections, crop rotation and resistant seed variants are also advised.This study concludes by highlighting the wide variety of fungal infections that can infect different vegetable seedlings. In order to minimize fungal contamination and guarantee seed quality, the results highlight the significance of seed health testing, appropriate drying, and storage conditions. Promising methods for controlling seed-borne diseases like *Fusarium* and *Macrophomina* are provided by the use of biocontrol agents like *Trichoderma*, which support food safety and sustainable agricultural production. To lessen the influence of these infections on crop productivity and food safety, more study is advised to investigate

Pure culture of fungus

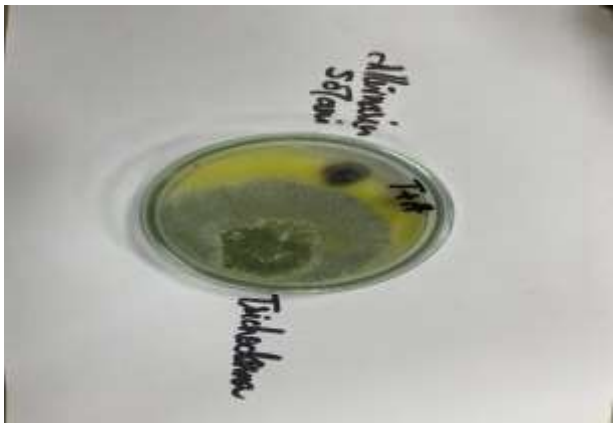


Seed cultured on PDA medium



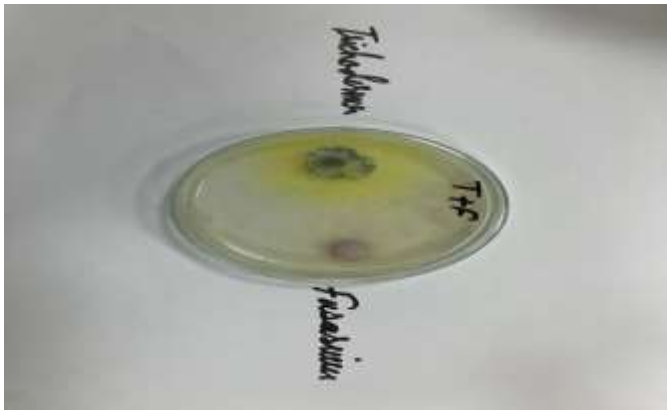


Fungal growth on seeds

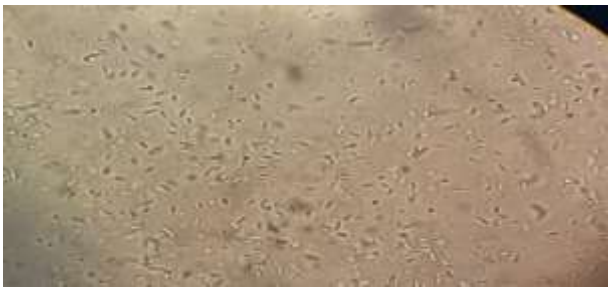


Dual culture: ***Trichoderma*** **vs.** ***A. solani***

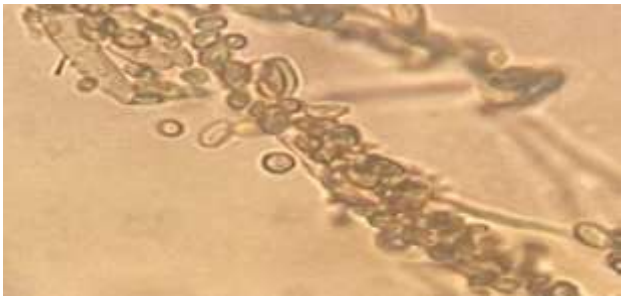
Dual culture: Trichoderma vs ***A. flavus***



Dual culture*: Trichoderma vs Fusarium*

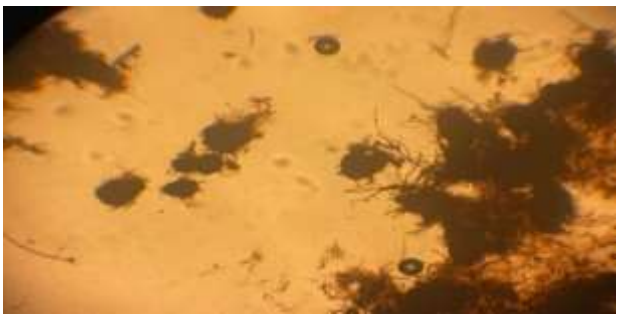


Fusarium **species**



Cladosporoium **species**

Aspergillus **specie**



Macrophamina **species**

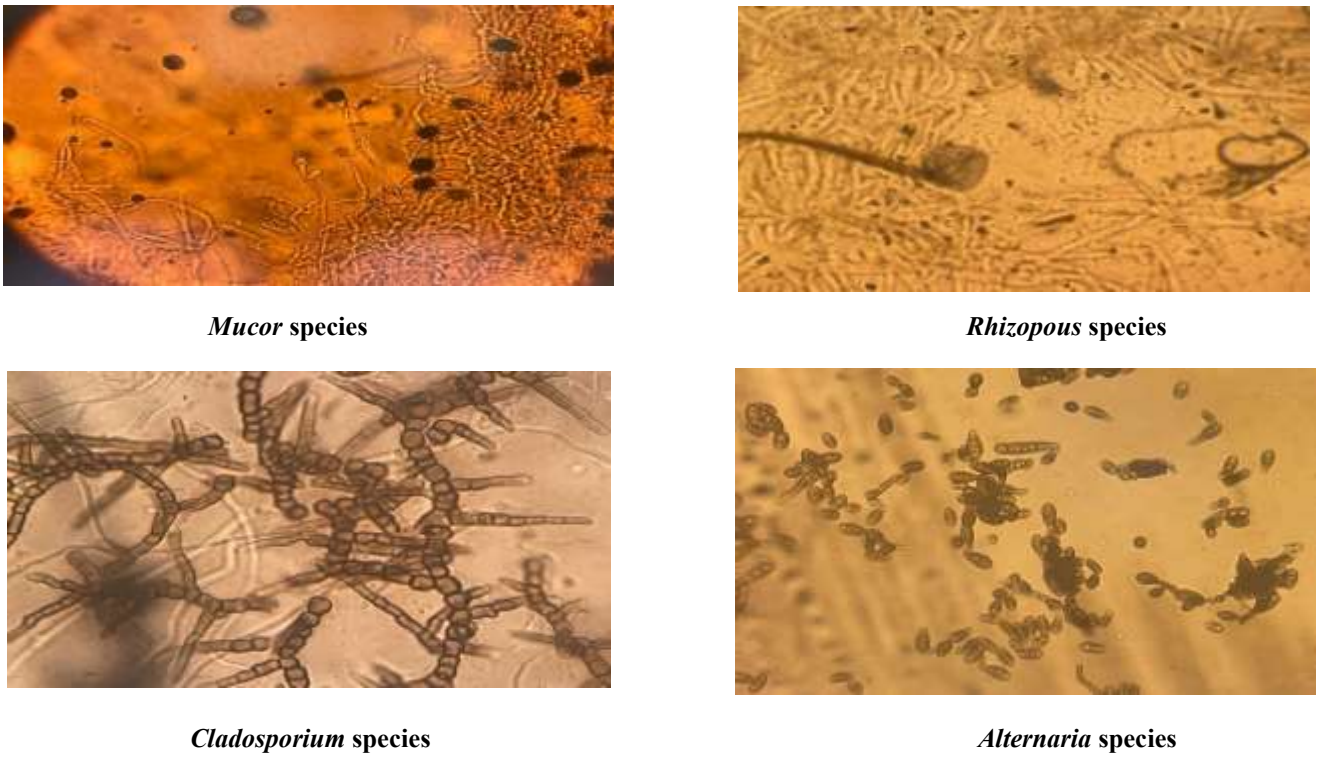


Figure 1 Microscopic view of seed borne fungi

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