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Research article

Impact of biopolymer-based *Trichoderma harzianum* seed coating on disease incidence and yield in oilseed crops

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A B S T R A C T

The use of microbe-based biological control for crop pests is recognized as an environmentally safe substitute for conventional chemical pesticides. However, the practical application of microbial inoculants in large-scale agriculture is underexplored, impeding their widespread commercial adoption. This study addresses the scarcity of research on effective delivery methods for microbial inoculants, particularly through seed coating, which has the potential to be a cost- and time-efficient strategy in crop management. In this research, the *Trichoderma harzianum* strain Th4d, a biological control agent (BCA), was incorporated into specially formulated biopolymeric compositions based on chitosan and cellulose. The efficacy of this seed coating approach was tested against various soil- and seed-borne pathogens in oilseed crops, including soybean, groundnut, and safflower. Results indicate that safflower treated with the biopolymer chitosan-based *T. harzianum* Th4d 1 % liquid formulation blend exhibited a higher seed yield of 793 kg/ha, seed germination of 84.7 %, and a significant reduction in wilt and root rot by 64.7 %. In groundnut crops, the seed coating led to a seed germination rate of 88.6 %, a 72 % reduction in root rot incidence, and a seed yield of 3040 kg/ha. Similarly, soybean crops treated with the biopolymer chitosan and *T. harzianum* Th4d displayed 83.4 % seed germination, a 70.9 % reduction in root rot, and a seed yield of 1239 kg/ha. Further on-farm evaluations demonstrated promising results, with the biopolymer chitosan-based *T. harzianum* Th4d 1 % liquid formulation blend seed treatment showing a high incremental cost-benefit ratio in safflower (1:4.5), soybean (1:2.5), and groundnut crops (1:3.3). This study underscores the potential of microbe-based seed coating as a sustainable and economically viable strategy for pest management in oilseed crops."

1. Introduction

In light of the evolving agricultural landscape, our primary focus should shift towards reducing the reliance on chemical protectants and instead, prioritizing the deployment of microbial biological control agents (BCAs) for managing crop diseases. This approach aims to minimize environmental impact, protect ecosystems, and safeguard human health. Over the past few decades, there has been a growing interest in leveraging microbes to enhance the resilience and yields of agricultural crops [1–5]. Fungi belonging to the genus *Trichoderma* are recognized as natural alternatives to maintain or enhance productivity while reducing the reliance on agrochemicals, restoring soil fertility, and addressing challenges posed by abiotic and biotic stresses [6,7]. Notably, *Trichoderma asperellum* and *Trichoderma harzianum* have been employed in 87 different crops to combat 70 soil-borne diseases and 18 foliar infections, respectively

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[8–10]. To fully harness the potential of biological control agents (BCAs), it is crucial to develop and adopt unique formulations and delivery strategies [11]. However, the extensive application of microorganisms remains a challenge due to the substantial quantity of microbial inoculum required for each plant, making widespread use currently impractical and economically unfeasible [12,13]. Efficient and effective inoculation procedures are critically needed to harness the benefits of microorganisms [14]. Among the various methods for applying beneficial microorganisms to plants, seed inoculation stands out as a precise and cost-effective approach with significant potential for large-scale implementation [15,16].

To ensure that crop growers reap the desired benefits from this technology, it is essential to develop new formulations characterized by extended shelf life, prolonged soil persistence, slow-release capabilities, and high adaptability across a wide range of temperatures. These formulations must also be resilient to local weather variability and exhibit broad-spectrum action with consistent performance under field conditions.

The primary objective of seed treatment is to protect seeds from various threats and constraints within the seedlings' microenvironment, thereby fostering healthy seedlings by creating optimal conditions for enhanced germination and subsequent growth stages [17]. Present challenges in seed coating include developing lower dosage, more efficient, and less harmful active ingredients, enhancing chemical adhesion, controlling pesticide release, and minimizing active ingredient loss. Coating seeds with polymers that improve chemical adhesion can mitigate the risk of environmental contamination, protect against viruses and pests, prevent moisture absorption during storage, and prolong seed viability [18–21]. The recent surge in industrial seed treatment, leveraging advanced machinery and techniques, such as combining fungicides, insecticides, and nematicides, can enhance product efficacy, protect workers, and reduce environmental contamination [22].

Utilizing a cross-linking approach, we developed biopolymers embedded with beneficial bioagents such as *Trichoderma*, and evaluated their viability and performance across various laboratory and greenhouse environments [23,24]. The current study focuses on assessing the efficacy of the novel biopolymer blend *Trichoderma harzianum* Th4d as seed coating, in comparison to other commercially available chemicals, for managing plant diseases in oilseed crops. This evaluation was conducted under experimental field conditions over two consecutive years, 2019 and 2020, and through demonstrations in growers' fields.

2. Materials and methods

2.1. Seed material

Groundnut, *Arachis hypogaea* (Linn.) (cv. Kadiri-6), Safflower, *Carthamus tinctorius* (Linn.) (cv. PBNS 12) and Soybean, *Glycine* max (Linn.) Merr. (cv. Basara), and were all procured from the Seed Unit of the ICAR-Indian Institute of Oilseeds Research (ICAR-IIOR), Hyderabad, India, and used in the study.

2.2. Microbial cultures

The fungal bioagent *Trichoderma harzianum* Th4d, fungal pathogens used in the study *viz*., *Macrophomina phaseolina* (Tassi.) Goid causing dry root rot in soybean and safflower, *Fusarium oxysporum* f. sp. *carthami* (Klis. & Houston) the causal organism of wilt disease in safflower and *Aspergillus niger* (Van tieghem) the collar rot pathogen in groundnut were obtained from culture collection of pathology laboratory, ICAR-IIOR.

2.3. Biopolymers and Trichoderma blend preparation

The synthesis of biopolymer blend (chitosan and cellulose) and entrapment of *Trichoderma harzianum* Th4d spores in the polymer matrix was carried out based on the procedures outlined in Refs. [23–25]. To synthesize the chitosan biopolymer blend, chitosan (Cts) and polyethylene glycol (co-polymer) were mixed in distilled water at a ratio of 1:0.33 % w/v under acidic conditions. Glycerol was added as a plasticizer at a concentration of 0.66 % w/v. This mixture was placed on a magnetic stirrer, continuously stirred at 300 rpm, and maintained at a temperature range of 60–80 °C overnight.

For the cellulose biopolymer blend, carboxymethyl cellulose (CMC) and polyvinyl alcohol (PVA) were dissolved in distilled water at a ratio of 1:1 % w/v, with guar gum added as a copolymer at 0.66 % w/v. Triethyl citrate was used as a plasticizer at 0.66 % v/v, and manganese sulfate ($MnSO4$) was used as a crosslinker at 0.32 % w/v. This solution was also stirred continuously on a magnetic stirrer at 300 rpm and maintained at 60–80 °C overnight. The *Trichoderma harzianum* Th4d was entrapped in the synthesized biopolymers.

2.4. Seed treatment

The two biopolymer treatments involving chitosan and cellulose-based *Trichoderma harzianum* Th4d were prepared as per the methodologies of Prasad et al. [24] and Chandrika et al. [23]. The seeds were immersed in a beaker containing a biopolymer blend of *Trichoderma harzianum* Th4d. This mixture was subjected to agitation on a shaker at 150 rpm for 15 min to ensure uniform distribution of the biopolymer blend on the seed surface. After shaking, the treated seeds were air-dried at room temperature for 1 h.

For chemical fungicide treatment, 1 kg seed, carboxin 37.5 % + thiram 37.5 % DS at 2 gm/kg seed and tebuconazole 2 % DS at 1g/kg seed, *Trichoderma harzianum*, Th4d WP (10^7 CFU) at 10g/kg seed were utilized. Control treatments included untreated seeds.

2.5. Experimental field trials (research station) in soybean, safflower and groundnut crops

Safflower was sown in 5 × 2.25 m plots at 45 × 20 cm spacing and soybean was sown in 5 × 2.4 cm plots at a spacing of 30 x 10 cm. The pathogen load in field plots were maintained at 200–300 cfu/g of soil by artificially inoculating the pathogens mass multiplied on sorghum grains. For soybean and safflower crops, 400 g of *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *carthami* were inoculated separately in the respective field plots. Groundnut seed was sown at 30 × 10 cm spacing in a 5 × 2.4 m^2 plots. *Aspergillus niger* inoculum was added at the rate of 200 g/plot. All agronomic practices were followed as per standard recommendations. Field experiments were conducted in a randomized block design with four replications during the years 2019 and 2020.

2.6. Season, soil and location

The experimental field trials were carried out at the ICAR- Indian Institute of Oilseeds Research (IIOR) plots situated at International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad in the state of Telangana, India, and at Amaravati, in the state of Maharashtra, India during the rainy season of the year 2019 (sown during the month of July). The safflower field experiments, at ICRISAT were conducted from October to February in the post-rainy seasons of 2019 and 2020. The groundnut field experiment at ICAR-IIOR was carried out in 2020 and 2021. Table 1 lists the physic-chemical parameters of the soil at the testing site. Surface (0–15 cm) soils were gathered from the field experimental sites for analysis of nutrient status.

The grower's field experiments were conducted during the year 2019–2020, at Adilabad for soybean, Nagarkurnul for groundnut, and Tandur for safflower. The characteristic black cotton soils (vertisols) are present at the field sites in Adilabad and Tandur, whereas the red soils (alfisols) are present in Mahabubnagar.

2.7. On farm evaluation in grower's fields

The field experiments were laid out with treatments including T1- Cellulose polymer + *T. harzianum* Th4d (10 ml/kg seed); T2- Chitosan polymer + *T. harzianum* Th4d (10 ml/kg seed); T3-carboxin 37.5 % + thiram 37.5 % DS (2 gm/kg seed)/tebuconazole 2 % DS (1 g/kg seed); T4- *T. harzianum* Th4d WP 10 g/kg seed; T5- Control. The sowings were carried out in large plots (0.4 ha) in a contiguous area with adequate spacing between each treatment plot. On-farm trials were undertaken in each area in a randomized block design with four replications.

2.8. Statistical analysis

All treatments were replicated four times in all *in vivo* field studies, the data is presented as mean of all replications. Using SPSS software 16.0, analysis of variance (ANOVA) was performed on the data. Tukey's HSD test critical difference was used to compare the treatment averages ($p = 0.05$).

3. Results

The data on impact of seed coating with biopolymer-based *Trichoderma* blend on the germination, disease incidence, and yield of three oilseed crops (safflower, groundnut, and soybean) are presented in Tables 2–4 and Fig. 1. High levels of significance ($P \leq 0.05$) were found in the analysis between treatments for germination %, disease incidence, and seed yield. When compared to *T. harzianum* Th4d WP treatment, fungicide and control treatments, the seed coating treatments including biopolymers chitosan and cellulose based *T. harzianum* Th4d have significantly influenced improvement in seed germination, lowering disease incidence and enhancing seed yield in all crops over the years.

Table 1
Physicochemical properties of the experimental soil.

	Rajendranagar Soil (Red)	ICRISAT Soil (Black)
pH	7.8	8.2
EC (dS/m)	0.17	0.24
OC (%)	0.6	0.46
Av. N (kg/ha)	265	207
Av. P (k/ha)	15	16.4
Av. K (kg/ha)	180	1152
Av. S (mg/kg)	8	20.6
Boron (mg/kg)	0.46	0.5
DTPA-Zn (mg/kg)	0.54	0.8
DTPA-Cu (mg/kg)	0.12	2.87
DTPA-Fe (mg/kg)	3.3	5.82
DTPA-Mn (mg/kg)	1.2	7.14

*DTPA refers to Diethylenetriaminepentaacetic acid.

Table 2
Effect of different treatments on Fusarium wilt incidence in safflower under field condition at ICRISAT, Hyderabad during 2019, 2020.

Treatments	2019		2020		Disease reduction (Mean)	2019		2020		Yield increase (Mean)
	Wilt incidence (%)	Disease reduction (%)	Wilt incidence (%)	Disease reduction (%)		Seed yield (kg/ ha)	Increase in seed yield (%)	Seed yield (kg/ha)	Increase in seed yield (%)	
Cellulose polymer + *T. harzianum* Th4d (10 ml/ kg seed)	8.4[d]	50.3[b]	27.5[b]	37.9[c]	44.1	796[b]	46.5[ab]	728.5[a]	46.0[a]	46.3
Chitosan + *T. harzianum* Th4d (10 ml/ kg seed)	5.5[e]	67.5[a]	16.9[d]	61.9[a]	64.7	850[a]	50.0[a]	735.0[a]	46.6[a]	48.3
Carboxin 37.5 % + Thiram 37.5 % DS (2 g/kg seed)	11.2[b]	33.7[d]	28.5[b]	35.7[c]	34.7	725[c]	41.4[c]	688.5[b]	42.9[ab]	42.2
***T. harzianum* Th4d WP (10 g/kg seed)**	9.7[c]	42.6[c]	23.3[c]	47.4[b]	45	782[b]	45.6[b]	650.7[b]	39.4[b]	42.5
Control (untreated)	16.9[a]	–	44.3[a]	–	–	425[d]	–	392.7[c]	–	–
C.D. (P=0.05)	3.4	1.7	2.5	4.1		65.0	5.6	59.5	5.5	
C.V. (%)	15.1	1.2	1.3	2.8		6.8	3.9	18.5	3.7	

*No. of replications = 4; C.D = Critical difference; C.V= Coefficient of variation; Values in the superscript indicate Tukey HSD test (p = 0.05).

3.1. Efficacy of biopolymer-based Trichoderma against safflower wilt in experimental fields

Over the course of two years of field trials, significant improvements were observed in safflower crops when treated with biopolymers. The chitosan-based *T. harzianum* Th4d blend led to a remarkable 23.8 % increase in germination, a 48.3 % boost in seed yield, and a substantial 64.7 % reduction in disease incidence (Table 2; Figs. 1 and 2). When compared with the cellulose biopolymer-based *T. harzianum* Th4d treatment, there was no significant difference in germination rates, which saw a 24.8 % increase. However, the cellulose-based treatment achieved a 44.1 % decrease in disease incidence and a 46.3 % increase in seed yield. Chemical fungicides, specifically carboxin 37.5 % + thiram 37.5 % DS at a concentration of 2 g/kg, resulted in a 21 % increase in seed germination, a 34.7 % reduction in wilt disease incidence, and a 42.2 % improvement in seed yield. Additionally, seeds treated with *T. harzianum* Th4d WP at 10 g/kg powder formulation demonstrated a 16.2 % increase in germination, a 45 % reduction in disease incidence, and a 42.5 % increase in seed yield. In contrast, the untreated control group exhibited the highest disease incidence, the lowest seed germination, and the poorest seed yield outcomes.

3.2. Efficacy of biopolymer-based Trichoderma against collar rot in groundnut in experimental fields

Based on pooled data from two years of field trials, seed treatments using cellulose and chitosan polymer-based *T. harzianum* Th4d blends demonstrated significant improvements in groundnut. The cellulose polymer-based treatment increased germination by 25 %, reduced disease incidence by 59.4 %, and boosted seed yield by 31.1 %. The chitosan polymer-based treatment yielded similar benefits with a 24 % increase in germination, a 72 % reduction in disease incidence, and a 34.5 % increase in seed yield. Notably, the chitosan-based *T. harzianum* Th4d treatment was particularly effective in reducing the incidence of collar rot. The cellulose polymer-based *T. harzianum* Th4d treatment also managed to reduce disease incidence by 60 %. The *T. harzianum* Th4d WP formulation, applied at 10 g/kg, significantly increased seed germination by 11.5 %, reduced disease incidence by 55.7 %, and improved seed yield by 28.2 % compared to the fungicide treatment and control. The chemical fungicide tebuconazole 2 % DS resulted in a 14.5 % increase in seed germination, a 46 % reduction in disease incidence, and a 26.8 % increase in seed yield. In contrast, the control plots exhibited very low seed germination and yield (Table 3, Figs. 1 and 2).

3.3. Efficacy of biopolymer-based Trichoderma against Macrophomina root rot in soybean in experimental fields

Data compiled from soybean trials at two different locations revealed that seed treatments with biopolymer chitosan-based *T. harzianum* Th4d significantly improved seed germination by 20.5 %, reduced root rot incidence by 70.9 %, and increased seed yield by 24.3 %. In comparison, the cellulose-based *T. harzianum* Th4d treatment achieved an 18.5 % increase in seed germination, a 63.6 % decrease in root rot incidence, and a 24.6 % rise in seed yield. The *T. harzianum* Th4d WP formulation at 10 g/kg enhanced seed germination by 11.3 %, reduced disease incidence by 45 %, and increased seed yield by 14.6 %. Similarly, the chemical fungicide treatment of carboxin 37.5 % + thiram 37.5 % DS at 2 g/kg resulted in a 10.7 % increase in seed germination, a 32.5 % reduction in

Table 3
Effect different treatments on *Aspergillus* root rot incidence in groundnut under field condition at ICAR-IIOR, Hyderabad during 2019, 2020.

Treatments	2019		2020		Disease reduction (Mean)	2019		2020		Yield increase (Mean)
	Root rot incidence (%)	Disease reduction (%)	Root rot incidence (%)	Disease reduction (%)		Seed yield (Kg/ha)	Increase in seed yield (%)	Seed yield (Kg/ha)	Increase in seed yield (%)	
Cellulose polymer + *T. harzianum* Th4d (10 ml/kg seed)	6.7^{c}	51.1^{b}	5.7^{d}	67.6^{b}	59.4	2927.5^{a}	17.8^{a}	2900.0^{a}	44.4^{a}	31.1
Chitosan polymer+ *T. harzianum* Th4d (10 ml/kg seed)	4.3^{d}	70.1^{a}	4.6^{e}	73.9^{a}	72	3193.5^{a}	24.9^{a}	2887.5^{a}	44.1^{a}	34.5
Tebuconazole 2 % DS (1 g/kg seed)	7.5^{b}	46.0^{c}	9.5^{b}	46.0^{d}	46	2960.8^{a}	18.8^{a}	2475.0^{b}	34.7^{a}	26.8
***T. harzianum* Th4d WP (10 g/kg seed)**	6.8^{c}	48.2^{b}	6.7^{c}	61.9^{c}	55.7	2932.5^{a}	17.9^{a}	2625.0^{ab}	38.4^{a}	28.2
Control (untreated)	13.6^{a}	–	17.6^{a}	–	–	2398.3^{b}	–	1622.5^{c}	–	–
C.D. (P=0.05)	2.4	2.8	1.0	1.2		5.7	28.2	7.1	19.4	
C.V. (%)	0.4	2.3	0.2	1.2		310.3	8.4	333.6	11.8	

*No. of replications = 4; C.D = Critical difference; C.V= Coefficient of variation; Values in the superscript indicate Tukey HSD test (p = 0.05).

Table 4
Effect of different treatments on *Macrophomina* root rot incidence in soybean under field condition at ICAR-IIOR, Hyderabad (2019) and Amaravati (2020).

Treatments	2019 IIOR		2020 Amaravati		Disease reduction (Mean)	2019 IIOR		2020 Amaravati		Yield increase (Mean)
	Root rot incidence (%)	Disease reduction (%)	Root rot incidence (%)	Disease reduction (%)		Seed yield (kg/ha)	Increase in seed yield (%)	Seed yield (kg/ha)	Increase in seed yield (%)	
Cellulose polymer + *T. harzianum* Th4d (10 ml/kg seed)	3^{d}	62.5^{b}	6.9^{c}	64.6^{b}	63.6	818^{a}	24.2^{a}	1684^{a}	25.0^{a}	24.6
Chitosan polymer + *T. harzianum* Th4d (10 ml/kg seed)	2^{e}	75.0^{a}	6.5^{d}	66.7^{a}	70.9	830^{a}	25.2^{a}	1647^{a}	23.3^{a}	24.3
Carboxin 37.5 % + Thiram 37.5 % DS (2 g/kg seed)	6^{b}	25.0^{d}	11.7^{b}	40.0^{c}	32.5	791^{a}	21.6^{a}	1514^{b}	16.6^{b}	19.1
***T. harzianum* Th4d WP (10 g/kg seed)**	4^{c}	50.0^{c}	11.7^{b}	40.0^{c}	45.0	812^{a}	23.5^{a}	1338^{c}	5.6^{c}	14.6
Control (untreated)	8^{a}	–	19.5^{a}	–	–	620^{b}	–	1263^{d}	–	–
C. D (p=0.05)	2.6	2.6	4.1	1.8		164.3	12.2	171.2	12.9	
C. V (%)	4.3	2.1	9.4	1.4		16.6	4.4	13.4	3.4	

*No. of replications = 4; C.D = Critical difference; C.V= Coefficient of variation; Values in the superscript indicate Tukey HSD test (p = 0.05).

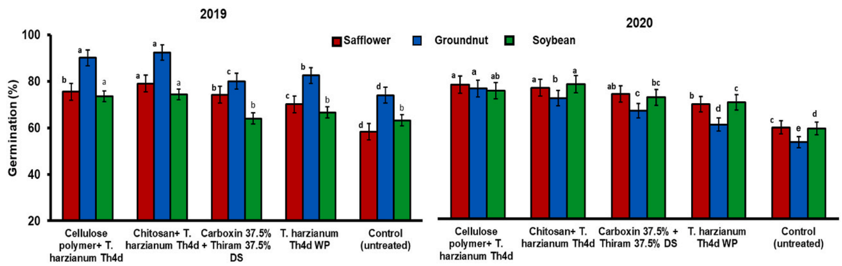

Fig. 1. Germination (%) of oilseed crops under different seed coating treatments at field level evaluation during two years. Error bars represent the standard deviation. Alphabet above the bars indicate Tukey HSD test (p = 0.05).

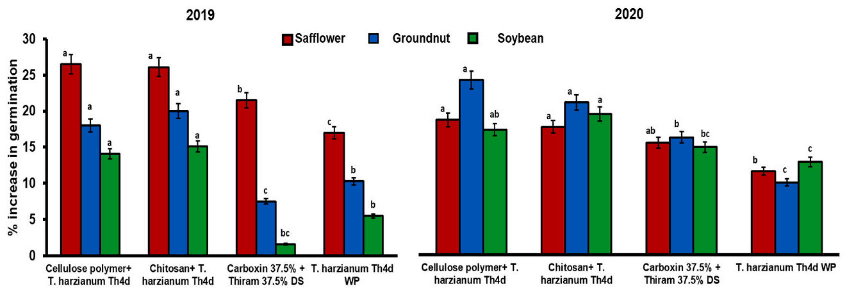

Fig. 2. Increase in germination (%) of oilseed crops under different seed coating treatments at field level evaluation during two years. Error bars represent the standard deviation. Alphabet above the bars indicate Tukey HSD test (p = 0.05).

disease incidence, and a 19.1 % improvement in seed yield (Table 4, Figs. 1 and 2).

3.4. On farm evaluation of biopolymer-based T. harzianum, Th4d seed coating technology in soybean grower's fields

During the rainy season of 2019, on-farm trials were conducted in growers' fields in Adilabad, Telangana, India. The cumulative results from these trials revealed a 26.6 % increase in seed yield with the application of a biopolymer chitosan-based *T. harzianum* Th4d blend. This treatment also notably improved germination to 85 %, reduced disease incidence to 2.0 %, and achieved a seed yield of 1900 kg/ha, compared to the control. In comparison, the cellulose-based *T. harzianum* Th4d treatment resulted in 80 % seed germination, a reduced root rot incidence of 3.5 %, and a similar seed yield of 1900 kg/ha. The chitosan-based *T. harzianum* Th4d seed treatment demonstrated a high incremental cost-benefit ratio (ICBR) of 1:2.5 (Table 5).

3.5. On farm evaluation of biopolymer-based T. harzianum, Th4d seed coating technology in groundnut grower's fields

In groundnut growers' fields, the application of a biopolymer chitosan-based *T. harzianum* Th4d seed treatment significantly enhanced seed germination to 81.5 %, collar rot disease incidence of 3.6 %, and achieved a 26.6 % increase in seed yield. This treatment also provided a high incremental cost-benefit ratio (ICBR) of 1:3.3. Likewise, the cellulose-based *T. harzianum* Th4d seed treatment resulted in 78.1 % seed germination, a collar rot incidence of 5.6 %, and a 12.5 % increase in seed yield, with an ICBR of 1:1.1 (Table 6).

3.6. On farm evaluation of biopolymer-based T. harzianum, Th4d seed coating technology in safflower grower's fields

In on-farm evaluations conducted in safflower growers' fields, the seed treatment with a biopolymer chitosan-based *T. harzianum* Th4d led to impressive results. This treatment achieved a seed germination of 85.5 %, a wilt incidence of 5.5 %, and a root rot incidence of 3.0 %. In contrast, the control treatment, which received no seed treatment, exhibited a 28.5 % incidence of Fusarium wilt and a 14.5 % incidence of Macrophomina root rot. Additionally, the chitosan-based *T. harzianum* treatment increased safflower yields by 26.5 % and demonstrated an exceptional incremental cost-benefit ratio (ICBR) of 1:4.5. Similarly, the cellulose-based *T. harzianum* Th4d treatment resulted in 80.5 % seed germination, with wilt and root rot incidences of 8.5 % and 6 %, respectively. This treatment also led to a 30 % increase in seed yield and an ICBR of 1:4.7 (Table 7). In comparison to conventional practices, the use of *Trichoderma harzianum* in combination with biopolymer chitosan or cellulose seed coatings significantly reduced disease incidence and enhanced seed yield.

4. Discussion

The physiological explanation for the higher germination percentage in polymer-based seed coating treatments when compared to other treatments might be due the initiation of metabolic processes, which usually occur after imbibition of the seed coat. The fact that seed coat imbibition happens faster with polymer-based seed treatment than with other treatments could be attributed to the hydrophilic groups present in biopolymeric film-based seed coating. This aligns with earlier studies [26–28].

The biological function of polymers in conjunction with *Trichoderma* as a protection mechanism against several crop pathogens was assessed in the current work as a seed treatment. Prior research has attempted to modify plant responses using polymers alone and in combination with helpful bacteria and pesticides, with varying degrees of effectiveness depending on the pathogen, the compound's dosage, and the formulation used [29]. Numerous earlier studies have demonstrated that polymer matrices are effective in reducing stress to the biologically derived active ingredients and work synergistically with microbes to provide innate plant immunity against disease in a variety of crops, including sunflower, tobacco, rice, grapevine, orchids, soybean, wheat, and maize [30–36]. Our earlier studies biopolymer seed coating alone or in combination with helpful microbes revealed that either cultivar's germination or vigour

Table 5
On farm evaluation of biopolymer-based *Trichoderma harzianum*, Th4d seed coating technology in soybean.

Treatment	Germination (%)	Root rot incidence (%)	Yield (kg/ha)	Increase in yield over control (kg)	Increase in yield over control (%)	Cost of increased (Rs.) (A)	Plant protection cost * (Rs.) (B)	Net profit (Rs.) A-B	ICBR
Chitosan + *T. harzianum* Th4d (10 ml/kg seed)	85.0	2.0	1900	400	26.6	12800	3600	9200	1:2.5
Control	71.2	12.0	1500	–	–	–	–	–	–
Cellulose + *T. harzianum* Th4d (10 ml/kg seed)	80.9	3.5	1900	300	18.7	9600	3600	6000	1:1.6
Control	70.3	11.0	1600	–	–	–	–	–	–

Market price of soybean: Rs.32/kg; *Labour charges included; ICBR = Net profit/Plant protection cost.

Table 6
On farm evaluation of biopolymer-based *Trichoderma harzianum*, Th4d seed coating technology in groundnut.

Treatment	Germination (%)	Root rot incidence (%)	Yield (kg/ ha)	Increase in yield over control (kg)	Increase in yield over control (%)	Cost of increased yield (Rs.) (A)	Plant protection cost * (Rs.) (B)	Net profit (Rs.) A-B	ICBR
Chitosan + *T. harzianum* Th4d (10 ml/kg seed)	81.5	3.6	1900	400	26.6	18000	4200	13800	1:3.28
Control	72.6	15.2	1500	–	–	–	–	–	–
Cellulose+ *T. harzianum* Th4d (10 ml/kg seed)	78.1	5.6	1800	200	12.5	9000	4200	4800	1:1.14
Control	70.4	12.0	1600	–	–	–	–	–	–

Market price of groundnut: Rs.45/kg; *Labour charges included; ICBR = Net profit/Plant protection cost.

Table 7
On farm evaluation of biopolymer-based *Trichoderma harzianum*, Th4d seed coating technology in safflower.

Treatment	Germination (%)	Disease incidence (%)		Yield (kg/ ha)	Increase in yield over control (kg)	Increase in yield over control (%)	Cost of increased (Rs.) (A)	Plant protection cost * (Rs.) (B)	Net profit (Rs.) A-B	ICBR
		Wilt	Root rot							
Chitosan + *T. harzianum* Th4d (10 ml/kg seed)	85.5	5.5	3.0	1050	220	26.5	9900	1800	8100	1:4.5
Control	80.0	28.5	14.5	830	–	–	–	–	–	–
Cellulose+ *T. harzianum* Th4d (10 ml/kg seed)	80.5	8.5	6.0	995	230	30.0	10350	1800	8550	1:4.7
Control	75.0	23.5	12.5	765	–	–	–	–	–	–

Market rates: 1) Safflower- Rs. 4500 q^{-1} 2) *Trichoderma harzianum* - Rs. 300^{-1} kg.

index were affected, proving that the molecule was not toxic [23,25]. Our findings show that the combination of natural chitosan polysaccharide transformed in to a film forming polymers and *Trichoderma*, when used as a seed treatment on safflower, soybean, and groundnut plants, acts as a defense mechanism against soil-seed borne pathogens by inducing a physiological state of enhanced defensive ability. A comparable chitosan-induced disease resistance was reported in treated wheat seedlings against Fusarium blight, lowering the severity of the disease [37]. Similar results were shown by Stanley-Raja et al., 2021 when rice plants were sprayed with chitosan, which significantly shortened the lesion length and successfully controlled the incidence of bacterial leaf blight disease. According to reports, chitosan can alter a plant's defense mechanisms in response to different infections. This action is exemplified by the build-up of proteins associated with pathogenesis, phytoalexins, and proteinase inhibitors [29]. Not only is chitosan directly harmful to pathogens, but it also increases the production of POD (Peroxidases) and PPO (Polyphenol oxidases) in date palms, strengthening their host resistance against the wilt pathogen [38]. Chitosan and *Trichoderma* sp. treated plants showed significantly promoted plant growth, vigor and other physiological parameters in sugarbeet plant and profoundly controlled the pathogen [39]. The disease severity of seedling blight in castor was dramatically reduced in *Trichoderma* treated ones. *Trichoderma harzianum* Th4d colonized castor roots and triggered induced systemic resistance, which is confirmed by the expression profiling of a few signature genes known to be up-regulated during ISR [40]. Agricultural biostimulants include a variety of organic compounds and beneficial microbes, such as biopolymers, protein hydrolysates, humic acid, citric acid, etc. Previous studies Calvo et al. [41] have identified some commonalities in the responses of plants to different biostimulants, such as enhanced nutrient uptake [42], stress tolerance [43,44], increased root growth, and vigour index [45]. The use of bio stimulants through seed treatment has been demonstrated by researchers to have a substantial impact on the changing micro environment of the plant by enhancing nutrient uptake, protecting the plant from stressors by activating defense mechanisms, and increasing metabolic processes, among other things [46,47]. In this study, the biopolymers like chitosan and cellulose, along with a helpful microbe called *Trichoderma*, greatly boosted germination, decreased disease incidence, and increased yield. According to Kachapur et al. [48], the transfer of organic material from the source to sink in the treated plants of biopolymer-based seed coating may be the cause of the higher seed output. Numerous reports on numerous crops' increased yield as a result of integrated seed coating technologies [49].

As with any biological experiment, it is important to determine if the changes in treatment outcomes are attributable to the treatments themselves or merely to the variability of the growth conditions. Every seed treatment must be tested on different crops and larger areas for numerous seasons and at different field locations in order to develop products that are effective on larger areas and under a variety of different growing situations. Hence, on farm validation has been taken up in grower's fields of soybean, groundnut, and safflower crops and the biopolymer-based *Trichoderma* seed treatment effect was documented (Tables 4–6). Demonstrations conducted in grower's fields offer a fantastic chance to test out new technologies and tools in real-world farming settings. In comparison to untreated seed, polymer-based seed treatments with *Trichoderma* performed best in grower's fields in terms of germination improvement, disease reduction and seed yield enhancement. The ICBR increase from 1.6 to 2.5 in soybean, 1.1 to 3.3 in the groundnut and 4.5 to 4.7 in safflower shows economic benefit to grower's due to the technology adopted. This outcome, which includes a greater yield-to-benefit ratio, demonstrates the effectiveness of using biopolymers in conjunction with *Trichoderma* seed treatment to reduce disease incidence, and, ultimately, raise yield and improve produce quality.

5. Conclusion

Experimental field trials and on-farm validation in growers' fields demonstrated that biopolymers combined with *Trichoderma* seed treatment provided effective protection against soil- and seed-borne diseases. The results were comparable to, or even better than, those obtained with fungicide treatments or controls, effectively reducing pathogen damage under real crop production conditions. This combination induced resistance in plants, significantly reducing the need for chemical pesticides and offering an environmentally friendly strategy for controlling soil and seed diseases. Our study showed that biopolymer-*Trichoderma* seed treatment in oilseed crops enhanced the natural defense responses of the plants and reduced disease incidence under field conditions by activating induced resistance mechanisms in seedlings. Furthermore, biopolymers could potentially be used to deliver other compatible microbial or chemical inputs through seeds as co-formulants, thereby increasing their efficacy against disease spread through seeds and soil.

Data statement

The data will be made available on request.

CRediT authorship contribution statement

K.S.V. Poorna Chandrika: Investigation, Data curation, Writing – review & editing, Writing – original draft, Project coordination. **R.D. Prasad:** Conceptualization, Methodology, Supervision, Writing – review & editing, Funding acquisition, Project administration and lead. **S. Lakshmi Prasanna:** Supervision, Writing – original draft. **B. Shrey:** Experimentation, Data compilation. **M. Kavya:** Writing – original draft, data representation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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