



RESEARCH ARTICLE

# Effects of pH, temperature, water activity and electrical conductivity on the biocontrol activity of *Trichoderma* spp. for enhanced biocontrol efficacy in agriculture

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## Abstract

*Trichoderma* spp. is widely recognized and employed as effective biocontrol agents, providing a natural approach to managing plant diseases. These fungi, commonly found in soil, utilize multiple mechanisms to suppress plant pathogens, making them important tools in sustainable agriculture. However, the biocontrol efficacy of *Trichoderma* spp. is influenced by several environmental factors that impact their growth, sporulation and antagonistic activity against phytopathogens. This research investigates how abiotic factors such as pH, temperature, water activity ( $a_w$ ) and electrical conductivity (EC) affect the efficacy of *Trichoderma* spp. as biocontrol agents against plant pathogens. Controlled laboratory tests were evaluated for conidial production, growth rates and morphological changes under varying environmental conditions. The results indicated that the ideal water activity ( $a_w$  0.985) and temperature (25 °C) considerably increased conidial production and biomass growth. A pH level of 6.5 and an EC of 12.92 dS/m were identified as ideal conditions for optimal growth and sporulation. These results underscore the importance of environmental factors in improving the effectiveness of *Trichoderma* spp., offering actionable suggestions to enhance its use as a biocontrol agent across diverse farming systems, while also recognizing the importance of these factors in improving the large-scale production of *T. harzianum* for both agricultural and industrial purposes, especially in biocontrol application.

**Keywords:** abiotic factor; biocontrol; electrical conductivity (EC); pH; temperature; *Trichoderma* spp.; water activity ( $a_w$ )

## Introduction

*Trichoderma* is a well-known soil-dwelling saprophytic fungus frequently found in environments such as soil, sand and water (1). It is widely recognized fungi that enhance plant growth, regulate various physiological processes and provide protection against numerous plant pathogens (2). It serves as an effective biocontrol agent (BCA) capable of antagonizing several soil-borne plant pathogens, including *Fusarium oxysporum*, *Rhizoctonia* spp. and *Pythium* spp (3). Several studies have shown that *Trichoderma* species employ a wide range of mechanisms for effective biological control (4, 5) such as competing for nutrients and space (6), direct antagonism (7), parasitism (8), antibiosis (9, 10) and the production of hydrolytic enzymes like  $\beta$ -1,3 glucanase, chitinase, protease and cellulase (11). However, its growth and reproduction are significantly influenced by factors like culture age, temperature, EC, water activity ( $a_w$ ) and pH, which collectively determine the quality and quantity of fungal colonies (12).

Although various studies have explored its application methods and efficacy in lab, greenhouse and field conditions (13), limited research exists on how *Trichoderma* spp. survives, grows and responds to stress factors such as EC,  $a_w$  and pH. Abiotic factors, including temperature (12), pH,  $a_w$  and EC, significantly impact its reproduction, colony morphology and proliferation (1). Water, a vital component for cellular activities, is critical for enzymatic function, stability and survival. Water activity, representing the amount of water available in a substrate or culture medium, directly affects microbial growth and cellular processes when suboptimal. Additionally, specific water requirements for fungal growth and metabolism vary among species.

*Trichoderma* can grow over a broad pH range due to its adaptive capabilities; favourable pH conditions promote the expression of genes responsible for metabolite biosynthesis and secretion. Soil moisture influences EC, reflecting dissolved ion concentration, which in turn affects cell wall functionality and enzyme activity (14). Studies indicate *Trichoderma* thrives in soils

with a moisture content of 40 - 80 %, with optimal enzyme activity observed at 50 -70 % moisture (15). However, low water availability can reduce conidial production, impacting its biocontrol efficacy. *Trichoderma* is sensitive to extreme salt and pH conditions, with optimal growth and sporulation at pH 6.0. Deviations to higher pH levels lead to reduced growth, sporulation and colony diameter (16).

This study aims to investigate the impact of abiotic factors, including  $a_w$ , temperature, EC and pH, on *Trichoderma* spp. growth. Recent findings emphasize the critical role of temperature in determining BCA survival and effectiveness. For instance, temperature variations significantly affect *Trichoderma*'s ability to control *Botrytis cinerea* in grape berries and influence its elicitation of plant responses in tomato plants (17, 18). Furthermore, understanding *Trichoderma*'s specific requirements for  $a_w$  and pH is crucial for enhancing its application in biocontrol strategies, ensuring its resilience and efficacy under diverse environmental conditions.

## Materials and Methods

This study was conducted in the laboratory of Lovely Professional University, Phagwara, Punjab, India (31.2554° N, 75.7049° E). The experiments included fungal inoculation, incubation, conidia counting and the measurement of EC and pH.

### Water activity ( $a_w$ ) and temperature

The water activity ( $a_w$ ) of potato dextrose agar (PDA) was adjusted by adding glycerol at concentrations of 5 g/L, 10 g/L, 50 g/L, 100 g/L and 175 g/L, corresponding to  $a_w$  levels of 0.998, 0.995, 0.985, 0.977 and 0.961, respectively. The  $a_w$  was measured using a Dew point meter (Applied Techno Systems, Mumbai, India). The adjusted water solutions were mixed with nutrient agar and poured into plates, which were inoculated with *Trichoderma harzianum* under aseptic conditions within a laminar airflow cabinet. These plates were incubated at 25 °C in BOD incubator (15). Independent experiments for temperature (15 °C, 25 °C and 35 °C) and  $a_w$  were set up using a completely randomized design (CRD), with 3 replicates for each condition.

### pH

To determine the optimal pH for the growth of *Trichoderma* spp., media with pH levels of 3.5, 4.5, 5.5, 6.5, 7.5 and 8.5 were prepared by adding appropriate amounts of HCl or KOH before sterilization. The pH of the medium was measured with a digital pH meter (Labline Stock Centre, Mumbai, India) before and after fungal inoculation. Plates inoculated with *T. harzianum* were incubated at 28 ± 2 °C for 15 days and fungal growth and sporulation were observed. Growth rate was measured using a scale (19).

### Electrical conductivity (EC)

To assess the impact of EC on the growth and sporulation of *T. harzianum*, NaCl was added to the medium at 3 concentrations (g/l): 1 g (EC: 12.92 dS/m), 2 g (EC: 14.30 dS/m) and 5 g (EC: 17.8 dS/m). The EC of the medium was measured using a pH meter (19). The agar medium was sterilized at 121 °C and 1.5 kg/cm<sup>2</sup> for 15 min before being poured into petri dishes. Mycelium discs (7 mm) of *T. harzianum* were placed at the center of each plate, with 3 replicates for each treatment. Plates were incubated at 28 ± 2°C for 2 weeks and fungal growth and sporulation were monitored daily. Data were analysed statistically using a CRD.

## Results

### Effect of water activity ( $a_w$ ) on conidial production and germination of *T. harzianum*

The conidial production and germination of *T. harzianum* were significantly influenced by different water activity ( $a_w$ ) levels (Table 1). The highest conidial production (923×10<sup>6</sup> conidia/mL) was observed at an  $a_w$  of 0.985, with an average germination rate of 68 %. At an  $a_w$  of 0.998, conidial production decreased to 600 ×10<sup>6</sup>conidia/mL, but the germination rate was higher at 82 %. Similarly, an  $a_w$  of 0.995 resulted in 525×10<sup>6</sup> conidia/mL with a 76 % germination rate. Lower water activity levels severely reduced both conidial production and germination. At an  $a_w$  of 0.977, conidial production dropped to 347×10<sup>6</sup> conidia/mL with a germination rate of only 21 %. The data clearly show that fungal reproductive activity is significantly inhibited by reducing water activity ( $a_w$ ). There was barely any germination and very little conidial production at the lowest  $a_w$  measured (0.961). These results highlight the critical role of water activity in promoting optimal conidial production and germination of *T. harzianum*. Moderate  $a_w$  levels (0.985-0.995) appeared most favourable for fungal growth and reproductive capacity, while extremely low  $a_w$  levels significantly inhibit these processes (Fig. 1 & Fig. 2).

### Effect of temperature on mycelial biomass production of *Trichoderma* spp.

All tested *Trichoderma* species produced substantial biomass across a range of temperatures. *T. harzianum* produced the highest biomass of 1.42 g when incubated at 25 °C, compared to 0.87 g and 0.73 g at 15 °C and 35 °C, respectively presented in (Table 2) and indicated in (Fig. 3).

### Effect of pH on conidial production of *Trichoderma* spp.

The production of conidia by *T. harzianum* was notably influenced by varying pH levels, as indicated by alterations in colony morphology (Table 3). The maximum conidia count was reached at pH 6.5. Conversely, there was a significant decrease in production at pH 5.5 and an even lower yield at pH 8.5. No substantial difference in conidia numbers was observed between pH 3.5 and 4.5. A consistent reduction in conidial production was noted as pH levels rose above the optimal range, with significant drops at pH 7.5 and 8.5 (Fig. 4).

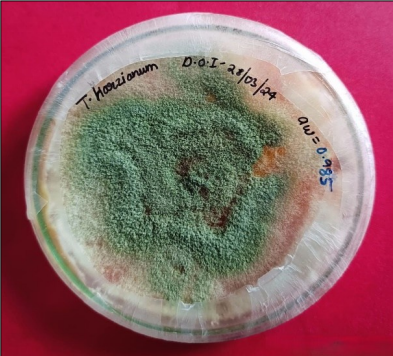
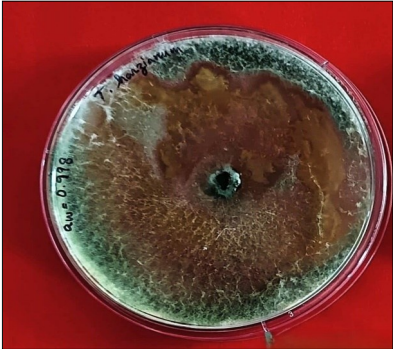
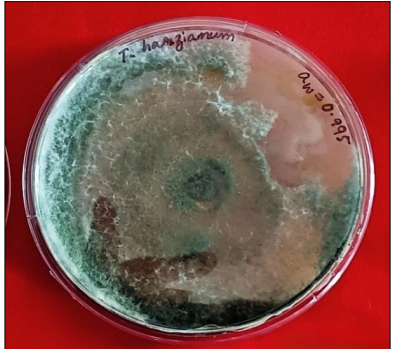


**Table 1.** Effect of different  $a_w$  on conidial production and germination of *Trichoderma* spp

Treatment (Water activity)	Number of conidia (×10 <sup>6</sup> conidia/mL)	Average germination %
$a_w$ 0.985	923	68
$a_w$ 0.998	600	82
$a_w$ 0.995	525	76
$a_w$ 0.977	347	21
$a_w$ 0.961	0.04	N.D*

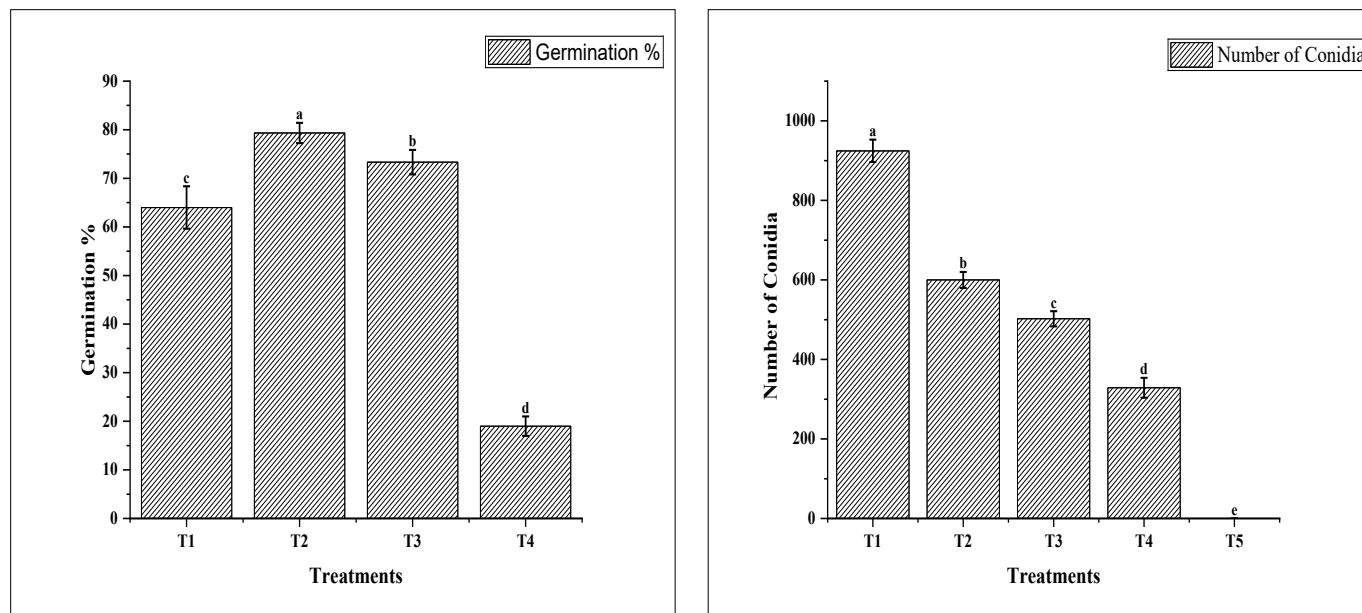
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**Table 2.** Effect of temperature level on mycelial biomass production of *Trichoderma* spp.

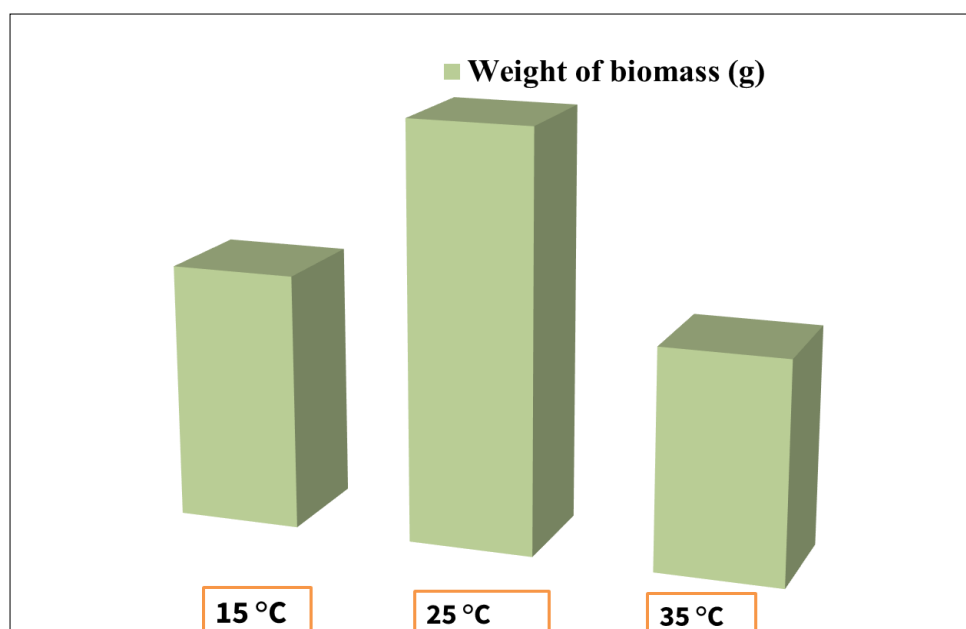
Treatments (Temperature °C)	Weight of biomass (g)
15	0.87
25	1.42
35	0.75

S.no.	Treatments	Number of conidia ( $\times 10^6$ conidia/mL)	Pictures
1.	$a_w = 0.985$	923	
2.	$a_w = 0.998$	600	
3.	$a_w = 0.995$	525	
4.	$a_w = 0.977$	347	
5.	$a_w = 0.961$	0.04	

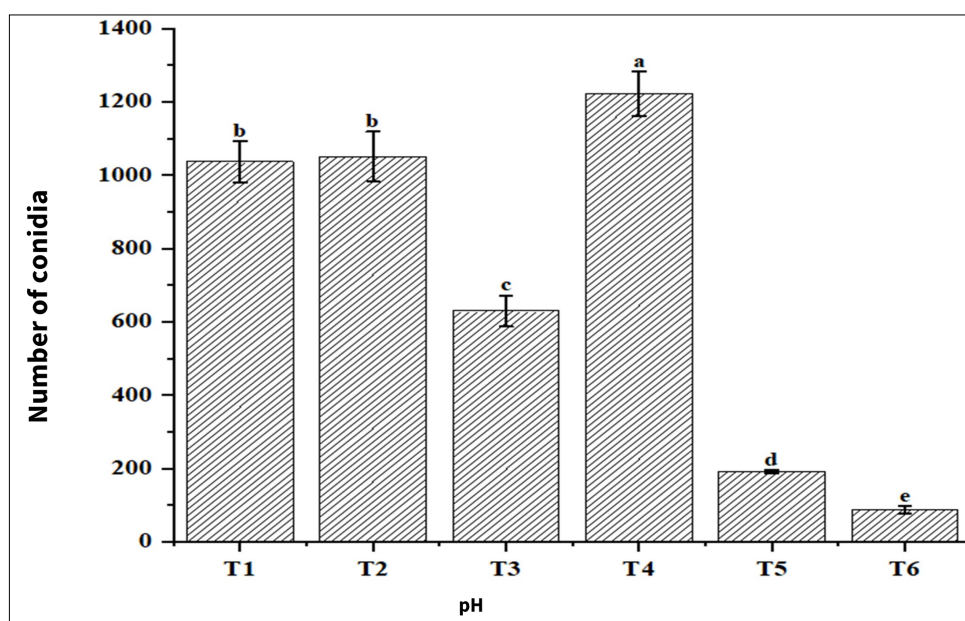
**Fig. 1.** Effect of  $a_w$  on the morphological characteristics and conidial production of *Trichoderma harzianum*.



**Fig. 2.** Means effect for conidium germination and conidia production of *T. harzianum* grown in cultures at different  $a_w$ .



**Fig. 3.** The effect of temperature on the growth of *T. harzianum*.



**Fig. 4.** Main effect means for conidium production of *T. harzianum* at different pH levels.

**Table 3.** Effect of different pH levels on conidial production of *Trichoderma* spp.

Treatment (pH)	Number of conidia ( $\times 10^6$ conidia/mL)
3.5	1010
4.5	1041
5.5	663
6.5	1293
7.5	197
8.5	98

### Effect of electrical conductivity (EC)

The results indicated significant differences in the effects of 3 levels of EC 12.92, 14.30 and 17.8 ds/m with 3 replications (R-1, R-2 and R-3) of each, on the growth and sporulation of *T. harzianum*. The result showed optimal growth at an EC of 12.92 ds/m presented in (Fig. 5).

## Discussion

The results of the present study provide valuable insights into the influence of various environmental factors, such as water activity ( $a_w$ ), temperature, pH and electrical conductivity (EC), on the growth and reproductive capacity of *Trichoderma harzianum*. These factors are critical in optimizing the conditions for conidial production and germination, which are important for both research and industrial applications, such as biocontrol agent formulation.

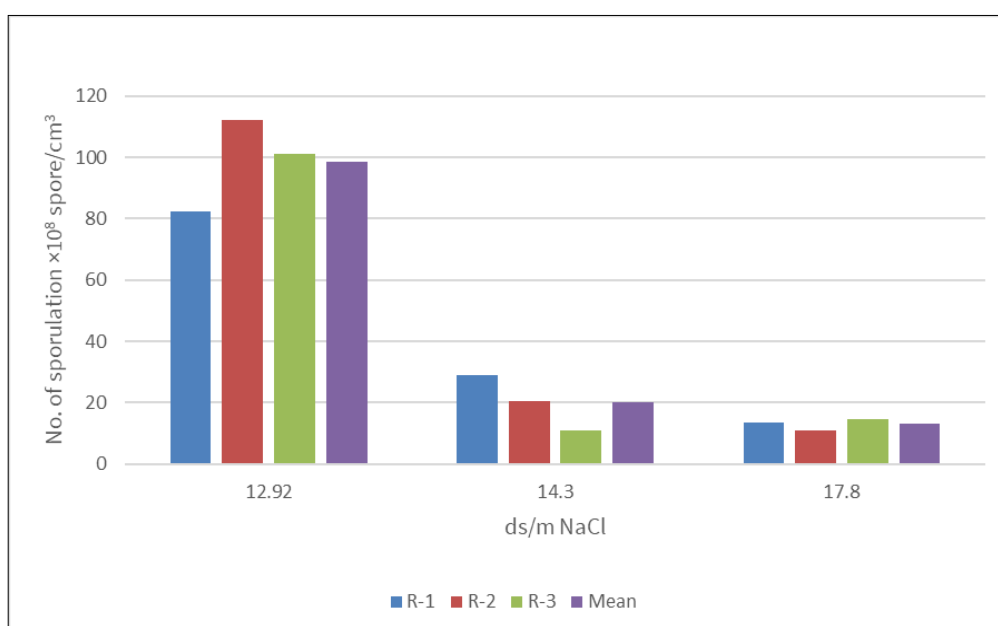
Water activity ( $a_w$ ) has a profound impact on the conidial production and germination of *T. harzianum*. The highest conidial production ( $923 \times 10^6$  conidia/mL) was observed at an  $a_w$  of 0.985, with a germination rate of 68 %. These results are consistent with findings that moderate water activity levels significantly enhance fungal growth and reproduction (20). The reduced conidial production observed at higher  $a_w$  levels (0.998) with an increase in germination (82 %) suggests that while high water availability favors germination, it may inhibit sporulation due to potential alterations in metabolic activity or osmotic stress (21).

Conversely, at lower water activity levels, the study demonstrates a sharp decline in both conidial production and germination. At an  $a_w$  of 0.977, conidial production was reduced to  $347 \times 10^6$  conidia/mL with a germination rate of only 21 %, which highlights the critical need for sufficient water availability for optimal fungal development. The drastic reduction in conidial production at the lowest  $a_w$  (0.961) was consistent with findings where very low water activity inhibited fungal spore production and viability (22).

Overall, these findings underscore the importance of maintaining moderate water activity levels (0.985 - 0.995) for fostering favorable conditions for *T. harzianum* growth and conidial production, which agrees with previous studies.

Temperature is a key factor influencing the mycelial growth of *Trichoderma* species. In this study, *T. harzianum* produced the highest biomass (1.42 g) at 25 °C, with a significant reduction in biomass at both lower (15 °C) and higher (35 °C) temperatures. This result is in line with studies that demonstrate the optimal growth of *Trichoderma* species at moderate temperatures. The 25 °C is typically the ideal temperature for biomass production in *Trichoderma*, as extreme temperatures may stress the fungal cells, leading to reduced metabolic activity and growth (23).

The reduced biomass production at 15 °C and 35 °C in our study is consistent with the notion that *T. harzianum* has a relatively narrow optimal temperature range for robust growth. Enzyme kinetics are probably greatly retarded at the lower temperature of 15 °C. On the other hand, the initiation of thermal stress at the higher temperature of 35 °C is responsible for the observed decrease. Important proteins and enzymes may become denatured because of high temperatures. When taken as a whole, these physiological reactions highlight the fact that *T. harzianum* grows best in a more limited temperature range, with variations affecting essential cellular functions needed for strong growth, with optimal growth occurring at moderate temperatures. The results also suggest that both lower and higher temperatures could impair the energy allocation toward mycelial growth, possibly diverting resources to stress responses rather than vegetative growth.



**Fig. 5.** The influence of EC on *T. harzianum* growth.

The impact of pH on conidial production in *T. harzianum* was significant, with the highest conidial production occurring at pH 6.5. These studies are supported by similar findings, which demonstrated that *Trichoderma* species exhibit optimal growth and sporulation under slightly acidic to neutral conditions (24, 25). At pH values below 6.5, particularly at pH 3.5 and 4.5, conidial production decreased sharply, which may be due to the acidic stress that can impair cellular functions and inhibit proper metabolic activity (26).

The decline in conidial production at pH 7.5 and 8.5 in this study corroborates the findings that alkaline conditions can negatively affect fungal growth and conidial production in *Trichoderma* species (27). The optimal pH for *T. harzianum* conidial production in this study, around 6.5, suggests that slightly acidic environments are most conducive to the formation of viable spores and the overall health of the fungus.

The EC of the medium also played a crucial role in the growth and sporulation of *T. harzianum*. The highest biomass and optimal sporulation were observed at an EC level of 12.92 ds/m. This result suggests that moderate salinity levels favor fungal growth, which could be attributed to the osmotic balance maintained within the fungal cells at this conductivity level (28).

At higher EC levels (14.30 and 17.8 ds/m), the study observed reduced growth and sporulation, likely due to osmotic stress caused by high salt concentrations, which can disrupt cellular integrity and metabolic processes. The optimal EC for *T. harzianum* growth observed in this study is in agreement with earlier findings that indicate moderate salinity levels promote fungal metabolism, while high salinity can cause cellular dehydration and inhibit fungal reproduction (29).

## Conclusion

The present study highlights the significant role of environmental factors such as water activity, temperature, pH and electrical conductivity in influencing the growth, conidial production and germination of *T. harzianum*. The results suggested that optimal conditions for conidial production and germination lie within moderate ranges of water activity (0.985-0.995), temperature (25 °C), pH (6.5) and EC (12.92 ds/m). A strong scientific foundation for refining large-scale production procedures and guaranteeing the stability and effectiveness of bio fungicide formulations is provided by these exact characteristics, which are essential for optimizing conidia yield and viability. Manufacturers can improve sporulation efficiency, product shelf-life and ultimately the field performance of *Trichoderma*-based biocontrol agents by preserving these conditions during fermentation and product development for industrial and agricultural applications.

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## Authors' contributions

NS designing of experiments, carried out the field experiment and drafted the manuscript. VK conceptualization of research. MKM analysis of the data and interpretation. GS, DKU, AK, preparation of the final manuscript. AKS, PKD conceived of the study and reviewed the manuscript data. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** The authors declare that there is no conflict of interest.

**Ethical issues:** None

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