

## International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693  
ISSN Online: 2617-4707  
IJABR 2024; 8(12): 895-899  
[www.biochemjournal.com](http://www.biochemjournal.com)  
Received: 02-09-2024  
Accepted: 05-10-2024

**Suthar YM**  
Ph.D. Scholar, Department of  
Plant Pathology, B. A. College  
of Agriculture, Anand  
Agricultural University,  
Anand, Gujarat, India

**Gohel NM**  
Associate Professor,  
Department of Plant  
Pathology, B. A. College of  
Agriculture, Anand  
Agricultural University,  
Anand, Gujarat, India

**Patel NB**  
Principle Research Scientist,  
AICRP on Biological Control  
of Crop Pests, ICAR Unit-9,  
Anand Agricultural  
University, Anand, Gujarat,  
India

## Influence of abiotic factors on *in vitro* growth of indigenous *Trichoderma* isolates of Gujarat

Suthar YM, Gohel NM and Patel NB

DOI: <https://doi.org/10.33545/26174693.2024.v8.i12l.3326>

### Abstract

*Trichoderma* can reduce the reliance on agrochemicals while boosting plant growth and resilience to both biotic and abiotic challenges. Ideally, *Trichoderma* should be able to endure various environmental conditions to effectively thrive and protect plants from stressors. However, as living microorganisms, their survival and effectiveness can be compromised by extreme conditions. So, the abiotic stress tolerance of five efficient indigenous *Trichoderma* isolates was evaluated under *in-vitro* conditions. The isolates exhibited maximum growth at pH 6.0 and 30 °C, with growth decreasing at lower and higher pH levels and temperatures. Optimal growth was observed at 100 mM salt concentration, while growth significantly decreased above 500 mM, with the least growth at 1250 mM. The isolates also tolerated 10 and 20 percent PEG concentrations but showed a drastic reduction in mycelial growth at 30 and 40 percent PEG.

**Keywords:** *Trichoderma*, pH, temperature, salt, PEG

### Introduction

*Trichoderma* is a genus of filamentous fungi widely recognized for its role in agriculture and biotechnology due to its potent biocontrol properties and ability to enhance plant growth (Samuels, 2006). These fungi are known for their antagonistic activity against a wide range of plant pathogens, making them valuable in integrated pest management and sustainable agriculture. *Trichoderma* species achieve this through various mechanisms, including the mycoparasitism, antibiosis, production of hydrolytic enzymes, secondary metabolites and competition for nutrients and space (Benitez *et al.*, 2004) [2]. Additionally, they form symbiotic relationships with plants, promoting growth and provide resistance to biotic and abiotic stresses (Gamalero and Glick, 2011) [3].

Native *Trichoderma* isolates refer to strains of the *Trichoderma* genus that are indigenous to specific geographical regions or local environments. These isolates have adapted to the unique climatic and soil conditions of their native habitats, often displaying superior resilience and effectiveness in controlling local plant pathogens compared to non-native or commercially available strains (Harman *et al.*, 2004) [4].

These isolates often exhibit higher efficacy in their natural environment due to their evolutionary adaptation to local biotic and abiotic factors. Additionally, native isolates may possess unique traits such as resistance to local pathogens, tolerance to specific soil pH levels, or enhanced enzyme production, making them well-suited for use in biofertilizers and soil conditioners (Singh *et al.*, 2014) [15].

Understanding the growth and effectiveness of *Trichoderma* strains under various environmental conditions is crucial for optimizing their application in agricultural practices. The growth and effectiveness of *Trichoderma* strains are significantly influenced by abiotic factors, which include temperature, pH, salinity and water availability (Papavizas, 1985) [8]. These factors not only affect the *in vitro* growth rates of *Trichoderma* but also determine their antagonistic potential and ability to colonize various substrates. Understanding how these environmental parameters influence *Trichoderma* growth is essential for optimizing their application in different agro-ecological conditions (Gamalero and Glick, 2011) [3].

In this context, exploring the influence of abiotic factors on the growth of indigenous *Trichoderma* strains under controlled laboratory conditions provides valuable insights. Such studies can help in selecting and developing robust *Trichoderma* strains that are effective

**Corresponding Author:**  
**Suthar YM**  
Ph.D. Scholar, Department of  
Plant Pathology, B. A. College  
of Agriculture, Anand  
Agricultural University,  
Anand, Gujarat, India

under a wide range of environmental conditions. This study aims to investigate the influence of abiotic factors such as temperature, pH, salinity and water availability on the *in vitro* growth of indigenous *Trichoderma* strains. By understanding how these environmental parameters affect indigenous *Trichoderma*, it may be possible to enhance their application as biocontrol agents and improve agricultural productivity in Gujarat.

## Materials and Methods

### Collection of rhizospheric soil sample and isolation of *Trichoderma* isolates

Rhizospheric soil samples from pulse/legume crops *i.e.*, chickpea, pigeonpea, blackgram, greengram, cowpea, soybean and groundnut were collected during *Rabi* 2021 and *Kharif* 2022 from the eight districts *viz.*, Anand, Kheda, Ahmedabad, Vadodara, Panchmahal, Mahisagar, Dahod and Chhota Udepur of middle Gujarat.

The rhizospheric soil samples were processed by using the serial dilution technique for the isolation of *Trichoderma*. The samples were prepared in sterilized distilled water and serially diluted. From the dilution blank, serial dilutions of  $10^{-2}$  to  $10^{-6}$  were prepared. One millilitre of each dilution of  $10^{-3}$  to  $10^{-6}$  was pipetted out to sterile Petri plates containing *Trichoderma* selective media and spread using a glass spreader. The plates were incubated at 28 °C and examined regularly for the development of colonies for five days. Based on the morphological study of the different colonies, selected colonies were sub-cultured in PDA plates by the hyphal tip method.

### Effect of abiotic factors on growth and sporulation of indigenous *Trichoderma* isolates

#### 1. pH

The impact of pH on the radial growth of five indigenous *Trichoderma* isolates was assessed by placing 5 mm discs on potato dextrose agar (PDA) with pH levels of 5, 6, 7, 8 and 9, adjusting pH with 0.1 N NaOH or HCl, following the method of Prabhavathi *et al.* (2014) [9]. The plates were incubated at 28±1 °C for five days, with each treatment replicated four times and mycelial growth was measured after incubation

## 2. Temperature tolerance

The optimum temperature and tolerance limits of five efficient indigenous *Trichoderma* isolates were investigated *in vitro* by incubating 5 mm discs on PDA plates at temperatures of 20, 30, 35, 40 and 45 °C. The experiment, following Shim *et al.* (1997) [14], involved maintaining these temperatures in a BoD incubator with four replicates per treatment. Radial growth was measured after five days of incubation.

## 3. Salinity tolerance

The NaCl tolerance of five efficient indigenous *Trichoderma* isolates was assessed using the poisoned food technique (Sharville, 1961) [13]. Isolates were exposed to PDA amended with NaCl at concentrations of 100, 500, 750, 1000 and 1250 mM. A 5 mm disc of each isolate was placed on the respective media and plates were incubated at 28±1 °C for five days with four replicates per treatment. Radial mycelial growth was measured and analyzed statistically.

## 4. Drought tolerance

Drought tolerance of five efficient indigenous *Trichoderma* isolates was tested by growing them on PDA plates with increasing levels of polyethylene glycol (PEG 6000 Da) at 10, 20, 30, and 40 percent. A 5 mm disc of each isolate was placed on the PEG-amended media, and plates were incubated at 28±1 °C (Kucuk, 2017) [15]. Radial growth was recorded after five days.

## Results and Discussion

### Isolation of indigenous *Trichoderma* isolates

*Trichoderma* isolates were obtained from collected rhizospheric soils using a serial dilution procedure with dilution factors of  $10^{-4}$  to  $10^{-6}$  on ready-made *Trichoderma* selective media (TSM). A total of 130 soil samples were collected from different locations, out of which, 62 soil samples were recovered *Trichoderma* spp.

Out of these 62 indigenous *Trichoderma* isolates, five potent *Trichoderma* isolates were selected based on their *in vitro* antagonistic ability against *Fusarium oxysporum* f. sp. *ciceri*. The further research was conducted using these five potent indigenous *Trichoderma* isolates (Table 1).

**Table 1:** List of five efficient indigenous *Trichoderma* isolates

| Sr. No. | Isolate No. | District  | Taluka     | Location |
|---------|-------------|-----------|------------|----------|
| 1       | Tr45        | Mahisagar | Santrampur | Jaldada  |
| 2       | Tr2         | Anand     | Tarapur    | Fatepur  |
| 3       | Tr53        | Dahod     | Limkheda   | Dabhada  |
| 4       | Tr7         | Anand     | Borsad     | Borsad   |
| 5       | Tr18        | Kheda     | Kapadvanj  | Dahiyap  |

### Effect of abiotic factors on growth and sporulation of indigenous *Trichoderma* isolates

#### 1. pH

The pH level significantly affects the growth and efficacy of *Trichoderma* isolates, with optimal growth observed between pH 5 and 7 (Table 2). Maximum growth was recorded at pH 6 (90 mm), while growth decreased as pH deviated from this range. At pH 5, growth ranged from 80.53 to 83.30 mm, with the highest in treatment T<sub>1</sub>. At pH

7, growth varied from 79.16 to 86.86 mm, with T<sub>1</sub> again showing the highest growth. As the pH increased to 8 and 9, growth declined, ranging from 45.00 to 64.94 mm at pH 8 and 32.14 to 45.58 mm at pH 9, with T<sub>1</sub> consistently exhibiting the best performance across pH levels. The present findings are supported by the earlier workers *viz.*, Prabhavathi *et al.* (2014) [9], Mishra *et al.* (2016) [6] and Zehra *et al.* (2017) [17] who revealed that the growth of different *Trichoderma* isolates was highest at pH 6.

**Table 2:** Effect of different pH regimes on mycelial growth of efficient indigenous *Trichoderma* isolates

| Tr. No.        | Treatments                        | Growth (mm) at different pH |       |       |       |       | Mean (mm) |
|----------------|-----------------------------------|-----------------------------|-------|-------|-------|-------|-----------|
|                |                                   | 5 pH                        | 6 pH  | 7 pH  | 8 pH  | 9 pH  |           |
| T <sub>1</sub> | <i>T. harzianum</i> isolate Tr45  | 83.30                       | 90.00 | 86.86 | 64.94 | 45.58 | 74.14     |
| T <sub>2</sub> | <i>T. harzianum</i> isolate Tr2   | 82.80                       | 90.00 | 86.49 | 62.18 | 42.01 | 72.70     |
| T <sub>3</sub> | <i>T. asperellum</i> isolate Tr53 | 82.59                       | 90.00 | 82.32 | 50.74 | 38.03 | 68.74     |
| T <sub>4</sub> | <i>T. harzianum</i> isolate Tr7   | 81.53                       | 90.00 | 86.40 | 53.01 | 41.92 | 70.57     |
| T <sub>5</sub> | <i>T. asperellum</i> isolate Tr18 | 80.53                       | 90.00 | 79.16 | 45.00 | 32.14 | 65.37     |
| S.E.m.±        |                                   | 0.74                        | -     | 0.77  | 0.67  | 0.42  | -         |
| C.D. at 5%     |                                   | 2.24                        | -     | 2.32  | 2.02  | 1.27  | -         |
| C.V. (%)       |                                   | 1.81                        | -     | 1.83  | 2.43  | 2.12  | -         |

## 2. Temperature tolerance

Temperature has a significant impact on the growth, metabolic activity and antagonistic potential of *Trichoderma* isolates. The study examined the growth of isolates at temperatures of 20, 30, 35, 40 and 45 °C (Table 3). Optimal growth occurred at 30 °C (90 mm), indicating it as the ideal temperature for *Trichoderma* growth and sporulation. At 20 °C, growth ranged from 78.00 to 83.78 mm, with the highest growth in treatment T<sub>1</sub>. At 35 °C, growth decreased to

37.71–42.92 mm and at 40 °C, it was further restricted to 10.47–16.17 mm. No growth occurred at 45 °C. Sporulation was abundant at 30 °C, sparse at 20 °C and completely inhibited at temperatures of 35 °C and above. Anwer *et al.* (2020) [1], Singh *et al.* (2021) [16] and Ramatsitsi *et al.* (2023) [11] also determined the effect of temperature on the growth and sporulation of different *Trichoderma* isolates and concluded that the optimum temperature range for the better growth and sporulation of *Trichoderma* is 25 to 30 °C.

**Table 3:** Effect of temperature on mycelial growth of efficient indigenous *Trichoderma* isolates

| Tr. No.        | Treatments                        | Growth (mm) at various temperature |       |       |       |       | Mean (mm) |
|----------------|-----------------------------------|------------------------------------|-------|-------|-------|-------|-----------|
|                |                                   | 20 °C                              | 30 °C | 35 °C | 40 °C | 45 °C |           |
| T <sub>1</sub> | <i>T. harzianum</i> isolate Tr45  | 83.78                              | 90.00 | 42.92 | 16.17 | 0.00  | 46.57     |
| T <sub>2</sub> | <i>T. harzianum</i> isolate Tr2   | 82.01                              | 90.00 | 42.75 | 14.34 | 0.00  | 45.82     |
| T <sub>3</sub> | <i>T. asperellum</i> isolate Tr53 | 80.13                              | 90.00 | 39.59 | 13.90 | 0.00  | 44.72     |
| T <sub>4</sub> | <i>T. harzianum</i> isolate Tr7   | 81.13                              | 90.00 | 41.92 | 12.86 | 0.00  | 45.18     |
| T <sub>5</sub> | <i>T. asperellum</i> isolate Tr18 | 78.00                              | 90.00 | 37.71 | 10.47 | 0.00  | 43.24     |
| S.E.m.±        |                                   | 0.99                               | -     | 0.48  | 0.17  | -     | -         |
| C.D. at 5%     |                                   | 2.98                               | -     | 1.45  | 0.51  | -     | -         |
| C.V. (%)       |                                   | 2.44                               | -     | 2.35  | 2.52  | -     | -         |

## 3. Salinity tolerance

*Trichoderma* species, known for their adaptability in diverse environments, are significantly affected by salinity, which influences their physiological processes such as osmotic regulation, enzyme activity and nutrient uptake. A study examined the growth of *Trichoderma* isolates at varying salt concentrations (100, 500, 750, 1000, and 1250 mM) (Table 4). At 100 mM, all isolates grew normally, reaching 90 mm in five days. Growth declined as salinity increased, with growth ranging from 80.60 to 89.47 mm at 500 mM and a

significant drop was observed at 750 mM (45.00 to 51.31 mm). At 1000 mM, growth was reduced further to 25.06–38.40 mm and at 1250 mM, it was minimal (10.34–29.62 mm). Sporulation was profuse at 100 mM, sparse at 500 mM in treatment T<sub>1</sub> and absent in all isolates at concentrations of 500 mM and above. A similar result was also found by Ramanagouda *et al.* (2021) [10] who noticed a drastic reduction in mycelial growth and morphological variations at and above 700 mM salt concentration.

**Table 4:** Effect of salt concentration on mycelial growth of efficient indigenous *Trichoderma* isolates

| Tr. No.        | Treatments                         | Growth (mm) at different salt concentrations |        |        |         |         | Mean (mm) |
|----------------|------------------------------------|----------------------------------------------|--------|--------|---------|---------|-----------|
|                |                                    | 100 mM                                       | 500 mM | 750 mM | 1000 mM | 1250 mM |           |
| T <sub>1</sub> | <i>T. harzianum</i> isolates Tr45  | 90.00                                        | 89.47  | 51.31  | 38.40   | 29.62   | 59.76     |
| T <sub>2</sub> | <i>T. harzianum</i> isolates Tr2   | 90.00                                        | 89.18  | 49.15  | 36.25   | 25.41   | 58.00     |
| T <sub>3</sub> | <i>T. asperellum</i> isolates Tr53 | 90.00                                        | 83.07  | 50.87  | 31.97   | 14.40   | 54.06     |
| T <sub>4</sub> | <i>T. harzianum</i> isolates Tr7   | 85.00                                        | 84.34  | 49.06  | 30.39   | 19.76   | 53.71     |
| T <sub>5</sub> | <i>T. asperellum</i> isolates Tr18 | 90.00                                        | 80.60  | 45.00  | 25.06   | 10.34   | 50.20     |
| S.E.m.±        |                                    | -                                            | 0.93   | 0.93   | 0.30    | 0.37    | -         |
| C.D. at 5%     |                                    | -                                            | 2.81   | 2.80   | 0.90    | 1.11    | -         |
| C.V. (%)       |                                    | -                                            | 2.19   | 3.79   | 1.85    | 3.69    | -         |

## 4. Drought tolerance

The effect of Polyethylene glycol 6000D (PEG 6000D) on *Trichoderma* isolates is of interest due to its ability to simulate water stress conditions, prompting adaptive responses in the fungi. A study examined the growth of *Trichoderma* isolates at varying PEG 6000D concentrations (10, 20, 30, and 40%). All isolates grew well at 10 percent

PEG concentration, with a colony diameter of 90 mm. At 20 percent, growth ranged from 77.11 to 89.38 mm, with the highest growth in treatment T<sub>1</sub>. A significant reduction in growth occurred at 30 percent, where colony diameters varied between 45.11 and 58.15 mm. At 40 percent, growth ranged from 35.74 to 42.12 mm. Sporulation was abundant at 10 percent PEG, sparse at 20 percent (except T<sub>5</sub>, which

showed no sporulation) and minimal at 30 percent in treatments T<sub>1</sub> and T<sub>2</sub>. No sporulation occurred at 40 percent PEG concentration. Similar result was also observed by

Muthuvaduganathan *et al.* (2023) [7] who stated that the growth and sporulation of *Trichoderma* isolates were reduced with increasing PEG concentration.

**Table 5:** Effect of PEG on mycelial growth of efficient indigenous *Trichoderma* isolates

| Tr. No.        | Treatments                        | Growth (mm) at different PEG concentrations |       |       |       | Mean (mm) |
|----------------|-----------------------------------|---------------------------------------------|-------|-------|-------|-----------|
|                |                                   | 10%                                         | 20%   | 30%   | 40%   |           |
| T <sub>1</sub> | <i>T. harzianum</i> isolate Tr45  | 90.00                                       | 89.38 | 58.15 | 42.12 | 69.91     |
| T <sub>2</sub> | <i>T. harzianum</i> isolate Tr2   | 90.00                                       | 85.25 | 55.38 | 40.07 | 67.68     |
| T <sub>3</sub> | <i>T. asperellum</i> isolate Tr53 | 90.00                                       | 84.30 | 50.45 | 36.49 | 65.31     |
| T <sub>4</sub> | <i>T. harzianum</i> isolate Tr7   | 90.00                                       | 82.96 | 51.62 | 37.50 | 65.52     |
| T <sub>5</sub> | <i>T. asperellum</i> isolate Tr18 | 90.00                                       | 77.11 | 45.11 | 35.74 | 61.99     |
| S.E.m.±        |                                   | -                                           | 1.10  | 0.64  | 0.59  | -         |
| C.D. at 5%     |                                   | -                                           | 3.31  | 1.94  | 1.79  | -         |
| C.V. (%)       |                                   | -                                           | 2.62  | 2.47  | 3.10  | -         |

## Conclusions

The findings of the present experiment underscore the paramount importance of *Trichoderma* spp. as a potent biocontrol agent due to its remarkable ability to resist various abiotic stresses. The study on abiotic stress tolerance in five efficient indigenous *Trichoderma* isolates revealed that their optimal growth occurred under specific conditions. Maximum growth was observed at pH 6.0 and 30 °C, indicating these as the optimal pH and temperature for *Trichoderma* growth and sporulation. Salt tolerance was highest at 100 mM, with growth decreasing significantly at concentrations above 500 mM, particularly at 1250 mM. The isolates also demonstrated resistance to 10 and 20 percent PEG concentrations, but a drastic reduction in growth occurred at 30 and 40 percent PEG. These findings underscore the adaptability of native indigenous *Trichoderma* isolates to moderate abiotic stress conditions indicating the potential of *Trichoderma* as a promising ally in sustainable farming practices, offering farmers an effective and environmentally friendly solution for managing plant diseases.

## Acknowledgement

The researchers highly acknowledge the Hon. Vice Chancellor, Dr. K. B. Kathiria; Director of Research and Dean PG studies, Dr. M. K. Jhala, Principal and Dean, Dr. Y. M. Shukla and Professor and Head Department of Plant Pathology Dr. R. G. Parmar for the keen guidance and support throughout the course of study.

## Conflict of Interest

The author declare that they have no conflict of interest.

## Statement on human and animal rights

This article does not contain any studies with human participants or animals performed by any of the authors.

## References

- Anwer MA, Singh BK, Prasad BD, Yadav AK, Kumari P. Abiotic stress tolerant *Trichoderma asperellum* TVB1 from hot spring and its antagonistic potential against soil-borne phytopathogens. *International Archive of Applied Sciences and Technology*. 2020;11(3):70-90.
- Benitez T, Rincon AM, Limon MC, Codon AC. Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology*. 2004;7(4):249-260.
- Gamalero E, Glick BR. Mechanisms used by plant growth-promoting bacteria. In: Maheshwari DK, editor. *Bacteria in Agrobiolgy: Plant Nutrient Management*. Springer; 2011. p. 17-46.
- Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*. 2004;2(1):43-56.
- Kucuk C. *In-vitro* antagonistic activity against *Fusarium* species of local *Trichoderma* spp. isolates. *Journal of Biological and Environmental Sciences*. 2017;11(32):67-74.
- Mishra N, Khan SS, Sundari SK. Native isolate of *Trichoderma*: A biocontrol agent with unique stress tolerance properties. *World Journal of Microbiology and Biotechnology*. 2016;32(8):1-23.
- Muthuvaduganathan N, Peter AJ, Thirumalai S, Maiti S. Isolation of *Trichoderma* spp. from arid and semi-arid conditions and screening for its *in-vitro* bio-efficacy and abiotic stress tolerance. *Journal of Mycopathological Research*. 2023;61(2):217-226.
- Papavizas GC. *Trichoderma* and *Gliocladium*: Biology, ecology and potential for biocontrol. *Annual Review of Phytopathology*. 1985;23(1):23-54.
- Prabhavathi NM, Amaresh YS, Naik MK, Mallesh SB, Kuchanur PH. Study of different temperature levels on radial growth and dry mycelial weight of *Trichoderma* spp. isolated from redgram-based conservation agriculture ecosystem. *International Journal of Plant Protection*. 2014;7(2):424-428.
- Ramanagouda G, Naik MK, Sharma M. Biocontrol potentials of novel indigenous *Trichoderma* isolates against *Fusarium* wilt of chickpea. *Indian Phytopathology*. 2021;75(1):135-146.
- Ramatsitsi MN, Khosa MC, Mashamaite CV, Ramachela K. *In-vitro* assessment of eight selected indigenous fungal isolates tolerance to various abiotic stresses and their effects on seed germination. *Current Microbiology*. 2023;80(12):386.
- Samuels GJ. *Trichoderma*: Systematics, the sexual state and ecology. *Phytopathology*. 2006;96(2):195-206.
- Sharvelle EG. *The Nature and Use of Modern Fungicides*. Minnesota: Burgess Publication Company; 1961. p. 308.
- Shim JO, Son SG, Kim YH, Lee YS, Lee JY, Lee TS, Lee SS, Lee MW. The cultural conditions affecting the mycelial growth of *Grifola umbellata*. *Journal of Mycology*. 1997;25(3):209-218.

15. Singh A, Shahid M, Srivastava M, Pandey S, Sharma A, Kumar V. Optimal physical parameters for growth of *Trichoderma* species at varying pH, temperature and agitation. Journal of Virology and Mycology. 2014;3(1):1-7.
16. Singh J, Rajput RS, Singh P, Ray S, Vaishnav A, Singh SM, Singh HB. Screening, isolation and characterization of heat stress tolerant *Trichoderma* isolates: Sustainable alternative to climate change. Plant Archives. 2021;21(1):1717-1734.
17. Zehra A, Dubey MK, Meena M, Upadhyay RS. Effect of different environmental conditions on growth and sporulation of some *Trichoderma* species. Journal of Environmental Biology. 2017;38(2):197-209.