International Multidisciplinary Research Journal 2023, 13: 6-9
doi: 10.25081/imrj.2023.v13.8230
https://updatepublishing.com/journal/index.php/imrj

Research Article

ISSN: 2231-6302

# Morphological variations observed in *Trichoderma asperellum* isolated from commercial products

**Meshu Shewarega Foyate***

College of Agriculture, Department of Plant Science, Wolkite University, Ethiopia

## ABSTRACT

Genus *Trichoderma* contains many species that are of great economic importance because of their ability to suppress pathogens and enhance their biocontrol capabilities against soil borne pathogens. In order to utilize the full potential of *Trichoderma* species in specific applications, precise identification and characterization are vital. The use of molecular markers has given a boost to the analysis of the accurate variation among various isolates of bio-agents. The present study was carried out to estimate the morphological diversity among commercial products of *Trichoderma*. *Trichoderma* based commercial products produced by different firms and made available for sales were collected from Akola and Nagpur markets. A total of eight isolates were isolated from talc powder carrier based samples on TSM. The *Trichoderma* isolates were differentiated by colony characters and morphological features. All isolates showed different growth patterns, colony colour, colony type, pigmentation and morphological characters like phialides, conidial size and shape.

**KEYWORDS:** *Trichoderma*, commercial products of *Trichoderma*, morphological diversity

**Received:** February 01, 2023
**Revised:** March 20, 2023
**Accepted:** March 21, 2023
**Published:** April 01, 2023

***Corresponding Author:**
Meshu Shewarega Foyate
E-mail: meshiti1233@gmail.com

## INTRODUCTION

Many fungi are used as biocontrol agents but *Trichoderma* spp. is widely used as a bio-agent, biological control of plant diseases used to be more successful to date in the rhizosphere than phyllosphere. The genus *Trichoderma* is a ubiquitous soil dweller; that grows rapidly, producing a scintillating array of secondary metabolites and sporulates profusely and has been promoted as a biocontrol agent and plant growth stimulator. Fungal spp. belonging to the genus *Trichoderma* is of worldwide occurrence and has been known since the 1920s for its capability to function as a biocontrol agent against plant pathogens (Samuels, 1996). *Trichoderma* is characterized as rapidly growing colonies bearing tufted or postulate, repeatedly branched conidiophores with lageniform phialides and hyaline or green conidia born in slimy heads and can be easily isolated from soil preferably in acidic soils with pH 3.5 to 4.5 (Shalini *et al.*, 2006).

The potential of *Trichoderma* spp. as a biocontrol agent of plant diseases was first recognized by Weindling (1932). Several reports are available on *Trichoderma* species as important biological control agents in the management of soil borne plant pathogens (Elad *et al.*, 1980; Singh *et al.*, 2007). The study of different mechanisms employed by *Trichoderma* in disease control is antagonism, competition, mycoparasitism, antibiosis and induction of defense responses in plants (Pan & Jash, 2010). *Trichoderma*, reduce growth, survival or infection caused by pathogen by a different mechanism. In addition, some species of the genus are economically important producers of industrial enzymes (*Trichoderma reesei*, Hypocrea jecorina) and antibiotics (Sivasithamparam & Ghisalberti, 1998).

The majority of *Trichoderma*-containing products in the market make claims to be fungicidal, to be used mainly for the biological control of root diseases for preventative and/or curative control of soil borne pathogens such as *Rhizoctonia*, *Pythium*, *Fusarium*, *Verticillium*, *Sclerotinia*, *Phytophthora* etc. It should also be noted that in many instances the use of *Trichoderma* in agricultural production is not limited to those products found on the commercial market, but are a result of in-house or on-the-farm production use, with the localized distribution.

Fungal spores can be produced in solid state fermentation on sterile rice, corn or other grains, and then the biopesticides can be applied directly to the crops or soil by using the *Trichoderma*-colonized substrate or the spores can be separated from the seed support by sifting and re-suspending in water. Another technique involves the production of *Trichoderma* in liquid fermentation, whereby a culture is produced that contains spores, mycelia, lytic enzymes, metabolites etc., and this fungal mixture is directly applied in the field for

biological control. In some countries, such as Venezuela and Cuba, the development and use of *Trichoderma*-based bioproducts are Government-supported and officially endorsed for use in agriculture (Harman, 2010). Rifai (1969) reported Conidiophores and their side branches were long and slender without sterile hyphal elongation, phialides not crowded, rather slender, colonies yellowish, bright, dull to dark green, floccose or with compact conidiophores tufts. Conidia are smooth walled.

## MATERIAL AND METHODS

Talc based *Trichoderma* based commercial products available in the Akola and Nagpur markets were collected and stored in cool and dry places in the laboratory for further use. The details of the samples collected are given in the Table 1.

For the isolation of *Trichoderma* from commercial products Serial dilution technique (Johnson & Curl, 1972) was used. One ml of Trichoderma suspension from dilutions ($10^{-3}$ and $10^{-4}$) was aseptically poured onto sterilized Petri plates and then Trichoderma selective medium (TSM) was poured. Plates were rotated gently to get uniform distribution of Trichoderma suspension in the medium. The plates were incubated at $28 \pm 1^{o}$C and observed at frequent intervals for the development of colonies.

Morphological identification was done based on cultural (colony and growth rate) characterization and microscopic observation.

Measurement of different conidia was carried out 100 times to find the particular range of conidia. The number of divisions of the stage micrometer coinciding with the division of the ocular micrometer was noted, and the ocular index (μ) was calculated by the formula:

$$\text{Ocular index } (\mu) = \frac{\text{No. of division of stage micrometer coinciding with ocular division}}{\text{No. of division of ocular micrometer coinciding with stage division}} \times 10\mu$$

## RESULT

The morphological studies were based on different parameters like colony growth, colony colour, pigmentation, phialides, conidia size and shape. Colony characters of *Trichoderma* isolates were studied using 7 days old cultures that were incubated at 28°C ± 2°C. All *Trichoderma isolates* grew well and formed conidia within 4 to 6 days. The radial growth (mm) was measured at 7$^{th}$ day after inoculation. Also the spore size, spore shape, spore count and phialides were recorded. The *Trichoderma* isolates maintained on the PDA medium showed variation.

TPPAK-1: The colony was milky white colour at initial stage latter turned to the light green. Scattered and compact growth was observed with smooth edge. Light yellow pigmentation on PDA medium. Phialides were elongated and cylindrical. Conidial shape was oval or round with discreet and scattered sporulation. Few black conidia were developed at the tip of phialides (Figure 1 and 2). Average size (L x B) of conidia was 1.96 - 2.87 x 0.95 -1.50 μm (Table 2).

TPPAK-2: As shown in table 2, the cottony was cottony white at initial stage latter turned to green colour. Mycelium growth rate was fast with dense sporulation. Pale yellow pigmentation on PDA media was observed. Phialides were lageniform along long main branch and pushed by the phialides tips. Conidial shape was oval to round and conidia ornamentation was smooth and black in colour (Figure 1 and 2). Average size (L x B) was 2.91 - 3.10 x 1.86 - 2.59.

TPPAK-3: Fast growing cottony growth was observed. Colony with white mycelial growth at initial stage later turned to pale greenish colour. Yellow pigmentation was observed on PDA. Phialides were cylindrical, slightly inflated and crowded at the tip. Oval shaped conidia's were observed under microscope (Figure 1 and 2). The average conidial size (L x B) was 2.20 - 2.86 x 1.45 - 2.32 μm (Table 2).

TPPAK-4: The colony was white at initial stage latter changed to deep green. Yellowish pigmentation was observed on PDA. Phialides were cylindrical or slightly inflated and compacted. Conidia shape was globose having black colour (Figure 1 and 2).

**Table 1: Trichoderma based products collected from different firm**

| Code | Name of firm and Address | Trade name | CFU | Formulation | Mfg. Date |
|---|---|---|---|---|---|
| TPPAK-1 | Krishi Vigyan Kendra, Sisa (Udegaon),Post: dongargaon, Tq. & Dist. Akola, Maharashtra, India | KVK *Trichoderma* | $2x10^{6}$CFU | 1.5% WP | May., 2018 |
| TPPAK-2 | Maharashtra State Seeds Corporation Ltd. Mahabeej Bhavan", Krishi Nagar | MSSCL *Trichoderma* | $2x10^{8}$CFU | 1% WP | Aug., 2018 |
| TPPAK-3 | Patanjali headquarters, industrial area of Haridwar is located at New Delhi | Patanjali *Trichoderma* | $2x10^{6}$CFU | 1.5% WP | Sep., 2018 |
| TPPAK-4 | International panacea Ltd. Connaught citrus, New Delhi-110001, India | Sanjeevni | $2x10^{9}$CFU | 1% WP | Oct., 2018 |
| TPPAK-5 | Neem India products pvt. Ltd. Sidarth Arcade, Opp. MTDC Railway station road, Maharashtra, India | TRISTAR | $2x10^{8}$CFU | 5% WP | Mar., 2018 |
| TPPAK-6 | Margo Biocontrol pvt. Ltd Antarasarahally industrial area Tumkur-572106, Karnataka | Ecoderma | $2x10^{8}$CFU | 1% WP | June, 2018 |
| TPPAK-7 | Anand Agro care Nashik, Maharashtra | Nicoderma | $2x10^{9}$CFU | 1% WP | Sep., 2018 |
| TPPAK-8 | Abhinav Biotech pvt. Ltd. Ranjit Nagar, New Delhi-110008 | Abhinav *Trichoderma* | $2x10^{6}$CFU | 1% WP | Feb., 2018 |

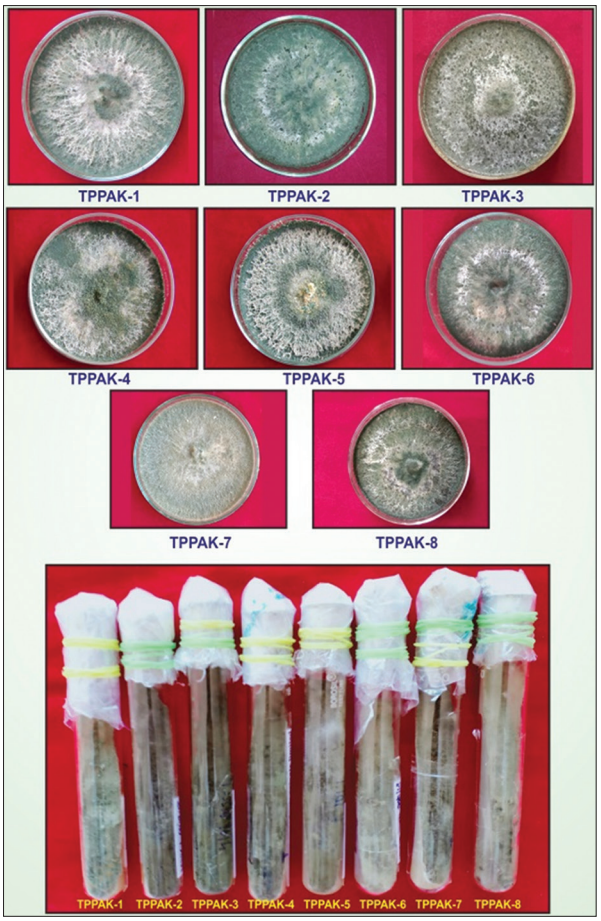


**Figure 1:** The pure culture of *Trichoderma isolates*

The average conidial size (L x B) was 1.95 - 2.03 x 0.95 - 1.11 µm (Table 2).

TPPAK-5: Fast colony growth was observed. The Colony was light olive green with yellow tinge colour. Colorless yellowish pigmentation was observed on PDA. Phialides were less branched size with long and thin structure. Conidia shape was oval to round (Figure 1 and 2). The average conidial size (L x B) was 2.25 - 2.75 x 1.75 - 2.32 µm (Table 2).

TPPAK-6: The cottony was cottony light white at initial stage latter turned to green colour with flat and ring like growth. Mycelium growth rate was fast with dense sporulation. Whitish to pale green pigmentation was observed on PDA. Phialides were lageniform along long main branch and contains much more conidia's at the tips (Figure 1 and 2). Conidia shape was oval with black ball. The average conidial size (L x B) was 2.20 - 2.95 x 1.65 - 2.25 µm (Table 2).

TPPAK-7: The colony was white at initial stage later slowly changed light green. Mycelia growth rate was very fast. Dark yellow to dirty yellow pigmentation was observed on PDA. Phialides were cylindrical and sharply constricted at the tips. Conidia shape was roundish and compacted at the tips (Figure 1 & 2). The average conidial size was 1.75 - 2.20 x1.74 - 2.20 µm (Table 2).

TPPAK-8: The colony colour was white to light green with. Pale yellow pigmentation was observed on PDA. The phialides were lageniform or bottle shaped (Figure 1 and 2). Shape of conidia was globose to oval. The average conidial size (L x B) was 1.83-2.14 x 1.03-1.23 µm (Table 2).

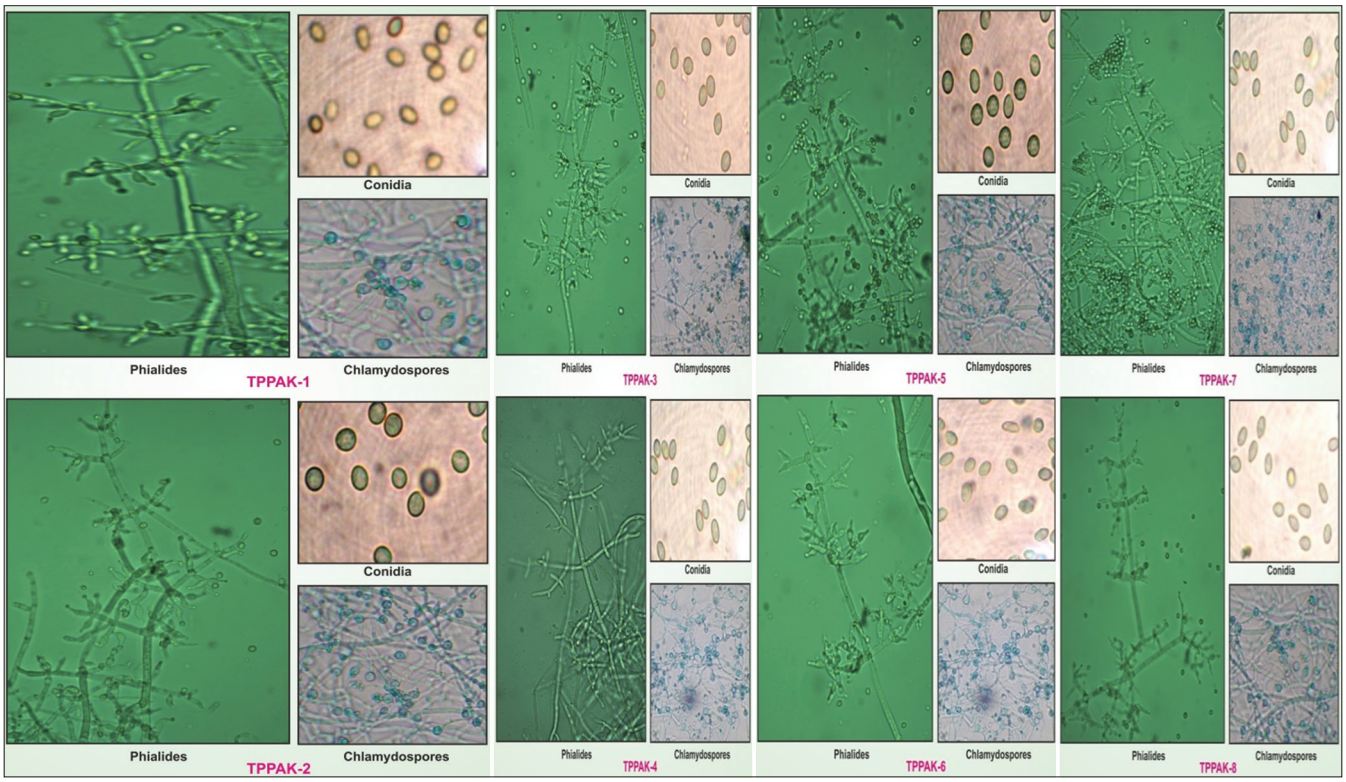


**Figure 2:** Microscopic photograph of Trichoderma isolates

Table 2: Morphological Variations Observed in *Trichoderma Asperellum* isolated from Commercial Products

| Sr. No | Code | Colony growth after 7th day (mm) | Colony colour | Pigmentation | phialides | Conidia | |
|---|---|---|---|---|---|---|---|
| | | | | | | Shape | Size (L x B) |
| 1 | TPPAK-1 | 83.33 mm | whitish then turns light green with smooth colony edges | whitish to light yellow | elongated and cylindrical | oval or round | 1.96-2.87 x 0.95-1.50 |
| 2 | TPPAK-2 | 94.12 mm | white and deep greenish colour | pale yellowish | lageniform along long main branches | oval to round | 2.91- 3.10 x 1.86-2.59 |
| 3 | TPPAK-3 | 89.5 mm | whitish to pale greenish colour cottony growth | creamy whitish to black | cylindrical or slightly inflated | oval | 2.20 –2.86 x 1.45 –2.32 |
| 4 | TPPAK-4 | 92.24 mm | White & changed to deep green | yellowish | cylindrical or slightly inflated and compacted | globose having black colour | 1.95- 2.03 x 0.95-1.11 |
| 5 | TPPAK-5 | 95.43 mm | Light olive green with yellow tinge colour | colorless to yellowish | less branched size with long & thin structure | round | 2.25-2.75 x 1.75 -2.32 |
| 6 | TPPAK-6 | 89.95 mm | Light white to green with flat and ring like growth | whitish to pale green | along the long main branches lageniform | oval with black ball | 2.20 - 2.95 x 1.65 –2.25 |
| 7 | TPPAK-7 | 91.52 mm | whitish slowly turns light green | dark yellow to dirty yellow | cylindrical shape and sharply constricted at the tips | roundish and compacted at the tip | 1.75- 2.20 x1.74- 2.20 |
| 8 | TPPAK-8 | 90.75 mm | white to light green | pale yellow | lageniform or bottle shaped | sub globose to oval | 1.83-2.14 x 1.03-1.23 |

L= Length, B= Breadth

## CONCLUSSION

Morphological observations were recorded on colony colour, colony growth, pigmentation, conidial shape and size and phialides. Trichoderma isolates grew well and formed conidia within 3-5 days. The radial growth (mm) was recorded in isolate after 7th DAI. Trichoderma isolates exhibited morphological variation such as growth pattern, colour, colony type, pigmentation, phialides, conidia size and shape. The Trichoderma isolates were differentiated by colony characters and morphological features. All isolates showed different growth pattern, colony colour, colony type, pigmentation and morphological characters like phialides, conidial size and shape.

## REFERENCES


Elad, Y., Chet, I., & Katan, J. (1980). *Trichoderma harzianum*: A Biocontrol Agent Effective against *Sclerotium rolfsii* and *Rhizoctonia solani. The American Phytopathological Society, 70*(2), 119-121.

Harman, G. E. (2000). Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Disease, 84*(4), 377-393. https://doi.org/10.1094/PDIS.2000.84.4.377

Johnson, L. F., & Curl, E. A. (1972). *Methods for research on the soil borne plant pathogens.* Minneapolis, US: Burgess Publishing Company.

Pan, S., & Jash, S. (2010). Variability in induction of defense response in Bengal gram against *Trichoderma* species. *Indian Phytopathology, 63*(1), 35-41.

Rifai, M. A. (1969). A revision of the genus *Trichoderma*. *Mycological Papers, 116*, 1-56.

Samuels, G. J. (1996). *Trichoderma*: a review of biology and systematic of the genus. *Mycological Research, 100*(8), 923-935. https://doi.org/10.1016/S0953-7562(96)80043-8

Shalini, Narayan, K. P., Lata, & Kotasthane, A. S. (2006). Genetic relatedness among *Trichoderma* isolates inhibiting a pathogenic fungi *Rhizoctonia solani. African Journal of Biotechnology, 5*(8), 580-584.

Singh, H. B., Singh B. N., Singh S. P., Singh S. R., & Sharma, B. K. (2007). Biological control of plant diseases: current status and future prospects. In J. K. Johri (Eds.), *Recent advances in biopesticides: biotechnological applications*, New Delhi, India: New India Publishing Agency.

Sivasithamparam, K., & Ghisalberti, E. L. (1998). Secondary metabolism *Trichoderma* and *Gliocladium*. In C. P. Kubicek & G. E. Harman (Eds.), *Trichoderma* and *Gliocladium* (Vol. 1, pp. 139-191) London, UK: Taylor and Francis Ltd.

Weindling, R. (1932). *Trichoderma lingorum* as a parasite of other soil fungi. *Phytopathology, 22*(10), 837-845.