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Toleransi Fungisida sebagai Determinan Kompatibilitas Pengendalian Hayati: Studi dari *Streptomyces xiangtanensis* NBSP3F dan *Trichoderma asperellum* G-4274-1

Fungicide Tolerance as a Determinant of Biocontrol Compatibility: Insights from *Streptomyces xiangtanensis* Strain NBSP3F and *Trichoderma asperellum* Strain G-4274-1

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ABSTRACT

Background: The intensive and prolonged use of synthetic fungicides, particularly mancozeb, exerts selective pressure on non-target microbial communities, potentially promoting the emergence of tolerant populations. Therefore, biocontrol agents that remain effective under such chemical stress are needed. **Objective**: This study aimed to evaluate the tolerance responses of *Streptomyces xiangtanensis* NBSP3F and *Trichoderma asperellum* G-4274-1 to mancozeb exposure across a gradient of concentrations. **Methods**: The experiment was conducted in vitro using a poisoned food technique at six concentrations (0%, 1%, 2%, 3%, 4%, and 5%) with six replications. Tolerance of *S. xiangtanensis* was assessed qualitatively based on colony growth, while *T. asperellum* was quantitatively evaluated using mycelial radial growth and growth inhibition metrics. **Results:** Results showed a concentration-dependent suppression of growth in both isolates. *S. xiangtanensis* maintained complete growth (100%) at 0–1% concentrations, decreased to 75% at 2–3%, 50% at 4%, and 25% at 5%. *T. asperellum* exhibited stable growth (90–100%) at 0–2%, moderate reduction (70–72.5%) at 3–4%, and a decline to 45% at 5%. Comparatively, *T. asperellum* showed higher physiological resilience than *S. xiangtanensis* under intermediate to high fungicide stress, though both isolates had reduced adaptability at the highest concentration. **Conclusions:** These findings indicate species-specific differences in adaptive capacity to multisite fungicidal stress, likely due to variation in metabolic flexibility and stress-response mechanisms. Collectively, both isolates demonstrate strong potential as multifunctional biocontrol agents, integrating pathogen suppression, plant growth promotion, and possible roles in pesticide-tolerant biodegradation, offering promising implications for sustainable agroecosystem management.

Keywords**:** *Biocontrol agent; Fungicide tolerance; Mancozeb; Streptomyces xiangtanensis; Trichoderma asperellum*

ABSTRAK

Pendahuluan: Penggunaan fungisida sintetik secara intensif dan berkelanjutan, terutama mankozeb, dapat memberikan tekanan selektif terhadap komunitas mikroba non-target sehingga berpotensi memunculkan populasi yang toleran. Oleh karena itu, diperlukan agen biokontrol yang tetap efektif di bawah tekanan kimia tersebut. **Tujuan**: Penelitian ini bertujuan untuk mengevaluasi respons toleransi *Streptomyces xiangtanensis* strain NBSP3F dan *Trichoderma asperellum* strain G-4274-1 terhadap paparan mankozeb pada gradien konsentrasi. **Metode**: Eksperimen dilakukan secara *in vitro* menggunakan teknik poisoned food pada enam konsentrasi (0%, 1%, 2%, 3%, 4%, dan 5%) dengan enam ulangan. Toleransi *S. xiangtanensis* dinilai secara kualitatif berdasarkan pertumbuhan koloni, sedangkan *T. asperellum* dievaluasi secara kuantitatif menggunakan laju pertumbuhan radial miselium dan inhibisi pertumbuhan relatif. **Hasil**: Hasil penelitian menunjukkan adanya penekanan pertumbuhan yang bergantung pada konsentrasi pada kedua isolat. *S. xiangtanensis* mempertahankan pertumbuhan sempurna (100%) pada konsentrasi 0–1%, menurun menjadi 75% pada 2–3%, 50% pada 4%, dan

25% pada 5%. *T. asperellum* menunjukkan pertumbuhan stabil (90–100%) pada 0–2%, penurunan sedang (70–72,5%) pada 3–4%, dan menurun hingga 45% pada 5%. Secara komparatif, *T. asperellum* menunjukkan ketahanan fisiologis yang lebih tinggi daripada *S. xiangtanensis* di bawah tekanan fungisida tingkat menengah hingga tinggi, meskipun kedua isolat menunjukkan adaptabilitas yang menurun pada konsentrasi tertinggi. **Kesimpulan**: Temuan ini mengindikasikan perbedaan spesifik spesies dalam kapasitas adaptif terhadap tekanan fungisida multisitus, yang kemungkinan disebabkan oleh variasi fleksibilitas metabolik dan mekanisme respons terhadap cekaman. Secara keseluruhan, kedua isolat menunjukkan potensi kuat sebagai agen biokontrol multifungsional, yang mengintegrasikan aktivitas pengendalian patogen, pemacu pertumbuhan tanaman, serta kemungkinan peran dalam biodegradasi toleran pestisida, sehingga memberikan implikasi yang menjanjikan bagi pengembangan strategi pengelolaan agroekosistem berkelanjutan.

Kata kunci**:** *Agen Biokontrol; Toleransi fungisida; Mankozeb; Streptomyces xiangtanensis; Trichoderma asperellum.*

BACKGROUND

Agriculture plays a central role in sustaining global food security; however, crop production systems are continuously constrained by plant pest organisms, including weeds, insect pests, and pathogens, which can reduce yield by 20–100% depending on disease severity (Rovicky et al., 2024). Consequently, synthetic fungicides are extensively applied as a primary strategy for disease management in seed and crop protection systems (Asrul, 2020).

Intensive and repeated fungicide application imposes strong selective pressure on soil microbial ecosystems, resulting in alterations in microbial community structure and function. This pressure contributes to shifts in pathogen dynamics, including increased virulence and reduced sensitivity to chemical control agents (Kaari et al., 2023). In addition, fungicide residues persist in soil environments due to adsorption to organic matter and limited degradation rates, thereby prolonging exposure of non-target microorganisms across cropping cycles (Ahmed et al., 2014; Polti et al., 2014; Carpio et al., 2021). Under chronic fungicide exposure, microbial communities are filtered based on their ability to maintain growth and physiological activity in chemically stressed environments. This ecological filtering process results in the suppression of sensitive microorganisms, which exhibit reduced growth, impaired enzymatic activity, and decreased viability (Becker et al., 2023). In this context, the concept of fungicide tolerance becomes relevant as a phenotypic trait that determines microbial persistence under chemical stress.

In this study, synthetic fungicide tolerance is defined as the capacity of a microorganism to maintain growth under increasing concentrations of synthetic fungicide relative to an untreated control. The definition is based exclusively on measurable phenotypic growth responses and does not encompass biochemical degradation, detoxification, or chemical neutralization of the synthetic fungicide. Tolerance is quantified using growth-related parameters, including colony expansion and biomass accumulation. In contrast, fungicide resistance refers to active biological mechanisms that reduce synthetic fungicide efficacy, including enzymatic degradation, metabolic detoxification, or target-site modification. Accordingly, tolerance reflects sustained growth under chemical stress, whereas resistance reflects active interference with fungicide activity.

Modern biocontrol applications occur in agroecosystems where fungicide exposure is common, necessitating compatibility between biological control agents and chemical inputs. Without sufficient tolerance, biocontrol organisms may fail to establish and function effectively under field conditions despite strong in vitro antagonistic potential. Therefore, fungicide tolerance screening is a critical step in the selection of field-relevant biocontrol agents. Mancozeb is a contact fungicide with multisite activity that disrupts cellular metabolism through interaction with sulfhydryl groups, thereby affecting multiple enzymatic pathways simultaneously (Corkley et al., 2022). This broad-spectrum mode of action effectively suppresses target pathogens but also impacts non-target microbial communities, thereby restricting microbial activity to taxa with high physiological adaptability under chemical stress (Runkle et al., 2017; Carvalho, 2017).

Streptomyces xiangtanensis strain NBSP3F is a Gram-positive actinobacterium reported to exhibit biocontrol and plant growth-promoting traits through hydrolytic

enzyme production and phytohormone biosynthesis (Iskandar et al., 2026). Members of the genus Streptomyces possess metabolic versatility that enables survival under xenobiotic stress through diverse adaptive responses. Previous studies have reported survival of *Streptomyces* spp. in pesticide-contaminated soils and sustained growth under herbicide exposure, indicating adaptive physiological responses under chemical stress (Pang et al., 2022; Pedó et al., 2025; Chen et al., 2024). However, these responses primarily reflect broad xenobiotic adaptability, and tolerance specifically toward multisite fungicides such as mancozeb remains insufficiently characterized.

Trichoderma asperellum strain G-4274-1 is an antagonistic fungus involved in suppression of soil-borne pathogens through secondary metabolite production and competitive interactions (Albana et al., 2026). Members of *Trichoderma* spp. have demonstrated variable levels of adaptability to agrochemicals, including herbicides and insecticides, which are associated with stress-responsive metabolic adjustments (Tripathi et al., 2013; Abadi et al., 2022). Nevertheless, strain-specific tolerance to multisite fungicides such as mancozeb remains poorly understood under graded exposure conditions.

The lack of detailed information regarding the fungicide tolerance of *S. xiangtanensis* strain NBSP3F and *T. asperellum* strain G-4274-1 represents a critical gap in the selection of pesticide-compatible biocontrol agents. Therefore, this study evaluated their tolerance to mancozeb under graded concentrations and characterized their growth responses under chemical stress. The findings are expected to contribute to the selection of adaptive biocontrol agents capable of maintaining functional stability in fungicide-exposed agricultural systems.

METHODS

Study Site and Experimental Period

The study was conducted at the Microbiology and Mycology Laboratory, Department of Plant Protection, Faculty of Agriculture, Universitas Andalas, Indonesia, from September to December 2025 under controlled laboratory conditions (room temperature 27–28 °C unless otherwise stated).

Experimental Design and Isolates

A completely randomized in vitro experimental design was employed using two independent biological systems: a bacterium (*S. xiangtanensis* strain NBSP3F) and a fungus (*T. asperellum* strain G-4274-1). *S. xiangtanensis* strain NBSP3F (GenBank accession PV809792.1) was obtained from the culture collection of Mhd. Fiqry Iskandar, S. P., M. P (Iskandar, 2025), originally isolated from healthy onion leaf surfaces collected in Solok District, West Sumatra, Indonesia. *T. asperellum* strain G-4274-1 (GenBank accession in submission process) was obtained from Hasan Albana, S. P., M. P and originally isolated from bamboo rhizosphere soil (Albana et al., 2026). Both isolates were maintained on ISP2 agar (*S. xiangtanensis* strain NBSP3F) and PDA (*T. asperellum* strain G-4274-1) and subcultured prior to experimentation to ensure physiological activity and culture purity.

Fungicide Preparation and Treatment

Mancozeb (technical grade, expressed on an active ingredient basis) was employed as the test fungicide to evaluate its inhibitory effects under controlled in vitro conditions. The fungicide was incorporated into the growth medium using the stock incorporation method, which involved aseptically mixing sterilized and cooled molten medium (45–50 °C) with a pre-prepared fungicide solution to achieve final concentrations of 0% (untreated control), 1%, 2%, 3%, 4%, and 5% (w/v). This concentration range was selected to capture both sublethal and inhibitory thresholds of the active compound.

Following the addition of the fungicide, the medium was homogenized using a vortex mixer for 2 min to ensure uniform dispersion of the active ingredient throughout the matrix, thereby minimizing concentration gradients that could bias fungal growth responses. The homogenized medium was subsequently dispensed into sterile 90 mm Petri dishes at a standardized volume of approximately 9 mL per plate to maintain consistency in medium thickness and diffusion dynamics.

All plates were allowed to solidify at ambient room temperature under aseptic conditions within a laminar airflow cabinet to prevent contamination. The prepared media were used within 24 h of preparation to preserve fungicide stability and efficacy. Each treatment was arranged in a completely

randomized design, comprising six biological replicates (n = 6 plates per concentration per isolate), thereby ensuring statistical robustness and reproducibility of the experimental outcomes.

Culture Standardization and Inoculum Preparation

For *S. xiangtanensis* strain NBSP3F, a 14-day-old actively growing culture on ISP2 agar was used. Inoculation was performed using a sterile inoculation loop via quadrant streak method to allow standardized spatial growth assessment. For *T. asperellum* strain G-4274-1, 7-day-old actively growing cultures on PDA were used. Mycelial plugs (5 mm diameter) were excised from the colony margin using a sterile cork borer to ensure uniform physiological age and metabolic activity. All inoculations were performed under aseptic conditions inside a laminar airflow cabinet to minimize contamination bias.

Morphological Confirmation of *S. xiangtanensis* Strain NBSP3F

The isolate was streaked onto ISP2 agar using the quadrant streak method and incubated at 28 ± 1 °C for 14 days. Colony morphology was documented daily, including aerial and substrate mycelium pigmentation, texture, and growth rate. Microscopic observation was performed using light microscopy at 100× magnification to confirm filamentous and sporulation characteristics consistent with *Streptomyces* spp.

Gram Reaction of *S. xiangtanensis* Strain NBSP3F

Gram reaction was determined using the potassium hydroxide (KOH) string test. A fresh colony was emulsified in a drop of 3% KOH on a clean glass slide and mixed for 30–45 seconds using a sterile loop. Formation of a viscous thread was interpreted as Gram-negative, while absence of viscosity indicated Gram-positive reaction (Schaad et al., 2001). Each test was performed in triplicate.

Hypersensitive Reaction of *S. xiangtanensis* Strain NBSP3F

Pathogenicity exclusion was assessed using *Nicotiana tabacum* (tobacco) leaves. A bacterial suspension adjusted to 10^8 CFU mL^{-1} (OD600 calibrated) was infiltrated into the abaxial leaf surface using a sterile needleless syringe. Plants were maintained under high-humidity conditions (>90% RH) at room temperature (27–28 °C). Lesion development was observed at 24-48 h post-inoculation. Absence of necrosis across all replicates was interpreted as a non-pathogenic behavior (Schaad et al., 2001).

Fungal Culture Maintenance *T. asperellum* Strain G-4274-1

Mycelial plugs (5 mm) were transferred onto PDA plates and incubated at 28 ± 1 °C for 7 days under continuous dark conditions to minimize photomorphogenic variation. Colony morphology (growth rate, pigmentation, sporulation density) was recorded.

Preparation of Fungicide-Amended Media

ISP2 agar and PDA media were sterilized at 121 °C, 15 psi for 15 min using an autoclave (Hirayama, Japan model). After cooling to 45–50 °C, sterile mancozeb solution was aseptically incorporated into media to achieve final concentrations of 0%, 1%, 2%, 3%, 4%, and 5% (w/v). Homogenization was standardized by vortex mixer for 120 seconds per batch. Media were poured (9 ± 1 mL) into sterile 90 mm Petri plates inside a laminar flow cabinet and left to solidify for 30–40 min before use.

In Vitro Tolerance Assay of *S. xiangtanensis* Strain NBSP3F

Tolerance of *S. xiangtanensis* strain NBSP3F to mancozeb was evaluated using a poisoned medium assay on ISP2 agar in Petri dishes. A standardized inoculum derived from a 14-day-old culture was streak-inoculated in a quadrant pattern onto fungicide-amended media. The Petri dishes were incubated at 28 ± 1 °C for 14 days, and colony development was assessed at the end of the incubation period. Growth performance was evaluated based on colony coverage, aerial mycelium development, and substrate pigmentation relative to the untreated control.

Colony growth was scored using a semi-quantitative scale ranging from 0 to 4, representing progressive levels of growth under fungicide stress. Score 0 indicates complete absence of visible growth. Score 1 indicates restricted growth confined to the inoculation line without radial expansion. Score 2 denotes limited radial growth covering

less than 50% of the Petri dish surface. Score 3 represents substantial colony development covering 50–75% of the Petri dish surface with reduced but evident mycelial density. Score 4 indicates growth comparable to the untreated control, characterized by near-complete coverage of the Petri dish surface with normal aerial mycelium formation and pigmentation. Relative growth was calculated as the ratio of the treatment score to the control score multiplied by 100. Relative growth was expressed as:

$$\text{Relative growth (\%)} = \frac{\text{Treatment score}}{\text{Control score}} \times 100$$

Scoring was based on a predefined growth intensity scale (0–4), where 0 indicates no growth and 4 indicates full colony coverage comparable to control (Table 1).

Table 1. Tolerance classification of *S. xiangtanensis* strain NBSP3F to mancozeb

Score	Colony Growth Description	Relative Growth (%)	Tolerance Category	Interpretation
0	No growth	0	Non-tolerant	–
1	Very low growth	≤ 25	Very low	+
2	Low growth	26–50	Low	++
3	Moderate growth	51–75	Moderate	+++
4	High growth	76–100	High	++++

In Vitro Tolerance Assay of *T. asperellum* Strain G-4274-1

Tolerance of *T. asperellum* strain G-4274-1 to mancozeb was evaluated using a poisoned medium assay on Potato Dextrose Agar (PDA) in Petri dishes. A 5 mm mycelial plug derived from a 7-day-old actively growing culture was placed at the center of each Petri dish with the mycelial surface oriented downward. The Petri dishes were incubated at 28 ± 1 °C for 7 days, and colony development was assessed at the end of the incubation period. Growth performance was evaluated based on colony diameter and radial expansion relative to the untreated control.

Colony growth was quantified by measuring diameter along two perpendicular axes (D_1 and D_2) using a digital caliper with a precision of ±0.01 mm, and the mean colony diameter (D) was calculated as $D = (D_1 + D_2)/2$. Relative growth was determined as the ratio of colony diameter under treatment (D_t) to that of the untreated control (D) and expressed as a percentage using the following equation:

$$\text{Relative growth (\%)} = \frac{D_t}{D_c} \times 100$$

D_t= colony diameter under treatment

D_c= colony diameter under control

Relative growth values were used as the basis for tolerance classification. The scoring system was not applied to this assay; instead, tolerance was expressed directly as continuous quantitative growth response under fungicide exposure (Table 2).

Table 2. Tolerance classification of *T. asperellum* strain G-4274-1 to mancozeb

Relative Growth (%)	Tolerance Category	Data Interpretation
0	No growth	–
>0–25	Very low	+
>25–50	Low	++
>50–75	Moderate	+++
>75–100	High	++++

Statistical Analysis

Data on tolerance to mancozeb fungicide were analyzed using one-way analysis of variance (ANOVA) at a 5% significance level. Measurements were based on six independent replicates per treatment, and results are presented as mean ± standard deviation (SD). Statistical analyses were performed using SPSS version 27.0. When significant differences were detected, means were separated using Duncan Multiple Range Test (DNMRT) at the 5% level.

RESULTS AND DISCUSSION

Tolerance Response of *S. xiangtanensis* Strain NBSP3F to Mancozeb Fungicide

The results demonstrated that *S. xiangtanensis* strain NBSP3F maintained maximal growth at mancozeb concentrations of 0% and 1%, with relative growth values of 100.00 ± 0.00%. Statistical analysis using Duncan's New Multiple Range Test (DNMRT) at $p \leq 0.05$ indicated no significant difference between these treatments. These findings indicate that the isolate exhibits optimal physiological performance under non-stress and low-stress conditions. Increasing the mancozeb concentration to 2% and 3% resulted in a significant reduction in growth to 75.00 ± 1.41% and 75.00 ± 0.75%, respectively. Further increases to 4% and 5% led to additional declines to 50.00 ± 0.63% and 25.00 ± 2.24%. This pattern clearly demonstrates a concentration-dependent inhibitory effect of mancozeb on bacterial growth (Table 3).

Table 3. Tolerance of *S. xiangtanensis* strain NBSP3F to mancozeb fungicide

Mancozeb Concentration (%)	Colony Growth Score	Relative Growth (%) (Mean ± SD)	Tolerance Category	Data Interpretation
0	4	100.00 ± 0.00 a	High	++++
1	4	100.00 ± 0.00 a	High	++++
2	3	75.00 ± 1.41 b	Moderate	+++
3	3	75.00 ± 0.75 b	Moderate	+++
4	2	50.00 ± 0.63 c	Low	++
5	1	25.00 ± 2.24 d	Very low	+

*Values followed by the same letter within a column are not significantly different according to Duncan's New Multiple Range Test (DNMRT) at $p \leq 0.05$.

Morphological observations revealed progressive alterations in colony characteristics at concentrations of 3–5%, including reduced mycelial density and the appearance of viscous exudates on the medium surface (Figure 1). These changes indicate a disruption of physiological homeostasis rather than a mere reduction in growth rate, suggesting that mancozeb exposure induces metabolic stress that affects colony stability. The physiological profile of *S. xiangtanensis* strain NBSP3F includes nitrogen fixation, phosphate solubilization (halo diameter of 1.41 mm), indole-3-acetic acid (IAA) production (115.08 ppm), hydrolytic enzyme activities (protease and amylase indices of 1 mm), and hydrogen cyanide (HCN) production. These traits indicate substantial plant growth-promoting and antagonistic potential through nutrient mobilization and hormonal regulation (Iskandar et al., 2026). However, these functional traits are not directly associated with mechanisms of fungicide tolerance.

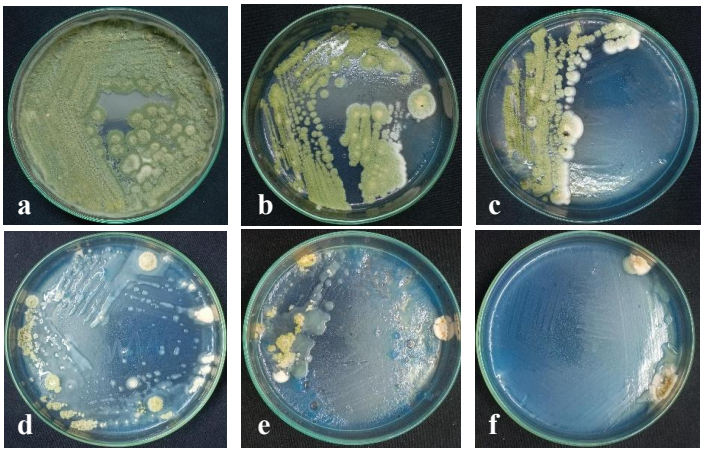

Figure 1. Visual growth of *S. xiangtanensis* strain NBSP3F on ISP2 agar amended with different concentrations of mancozeb after 14 days of incubation: (a) 0% (control), (b) 1%, (c) 2%, (d) 3%, (e) 4%, and (f) 5%.

Mancozeb acts as a multisite contact fungicide by interacting with sulfhydryl (-SH) groups of essential enzymes and proteins, thereby disrupting multiple metabolic pathways simultaneously. This nonspecific mode of action explains the observed growth suppression in *S. xiangtanensis* strain NBSP3F as a generalized physiological stress response rather than a specific detoxification mechanism. Consequently, plant growth-promoting traits such as nitrogen fixation and phosphate solubilization primarily reflect ecological fitness and rhizosphere competence, rather than adaptive resistance to fungicide exposure. This distinction is essential for accurately interpreting performance under chemically stressed conditions.

These findings are consistent with previous reports. Briceño et al. (2016) demonstrated that *Streptomyces* spp. are capable of degrading organophosphate pesticides by more than 80% through specific enzymatic pathways, indicating that high tolerance within this genus is often associated with active biodegradation rather than passive resistance. Fuentes et al. (2018) further reported that *Streptomyces* consortia can reduce pesticide toxicity through synergistic metabolic interactions. In addition, Pedó et al. (2025) observed that plant growth-promoting *Streptomyces* spp. maintained growth above 70% under glyphosate exposure, while Chen et al. (2024) reported that *S. saraceticus* retained more than 60% viability under combined pesticide stress. In contrast, *S. xiangtanensis* strain NBSP3F exhibited only 75.00% growth at 2–3% mancozeb and declined sharply to 25.00% at 5%, indicating relatively limited tolerance to multisite fungicides. This discrepancy suggests that tolerance capacity may vary substantially among species and depends on both the mode of action and chemical complexity of the applied pesticide.

Tolerance Response of *T. asperellum* Strain G-4274-1 to Mancozeb Fungicide

T. asperellum strain G-4274-1 maintained high relative growth at mancozeb concentrations of 0–2%, reaching 100.00 ± 0.50%, 95.00 ± 0.90%, and 90.00 ± 2.75%, respectively. No significant differences were detected among these treatments according to DNMRT at $p \leq 0.05$ (Table 4), indicating stable physiological performance under low chemical stress. Growth declined significantly at higher concentrations. Relative growth decreased to 72.50 ± 4.40% and 70.00 ± 3.10% at 3% and 4% mancozeb, respectively, and dropped sharply to 45.00 ± 5.60% at 5%, which differed significantly from all lower treatments. This trend demonstrates a clear concentration-dependent inhibitory effect. A consistent pattern was observed in radial growth. Colony diameter decreased from 8.00 ± 0.04 cm in the control to 7.60 ± 0.07 cm, 7.20 ± 0.22 cm, 5.80 ± 0.35 cm, 5.60 ± 0.25 cm, and 3.60 ± 0.45 cm with increasing mancozeb concentration. This progressive reduction reflects restricted hyphal extension under fungicide stress.

Table 4. Tolerance of *T. asperellum* strain G-4274-1 to mancozeb fungicide

Mancozeb Concentration (%)	Mycelial Diameter (cm) (Mean ± SD)	Relative Growth (%) (Mean ± SD)	Tolerance Category	Data Interpretation
0	8.00 ± 0.04 a	100.00 ± 0.50 a	High	++++
1	7.60 ± 0.07 a	95.00 ± 0.90 a	High	++++
2	7.20 ± 0.22 a	90.00 ± 2.75 a	High	++++
3	5.80 ± 0.35 b	72.50 ± 4.40 b	Moderate	+++
4	5.60 ± 0.25 b	70.00 ± 3.10 b	Moderate	+++
5	3.60 ± 0.45 c	45.00 ± 5.60 c	Low	++

*Means followed by the same letter within a column are not significantly different according to Duncan's New Multiple Range Test (DNMRT) at $p \leq 0.05$.

Morphological changes became evident at 3–5% mancozeb. Colonies exhibited reduced peripheral expansion while maintaining relatively dense central regions. This pattern indicates that growth inhibition primarily affected hyphal extension rather than total biomass formation, suggesting disruption of colony development processes (Figure 2). Overall, the response of T. asperellum strain G-4274-1 was strongly concentration-dependent. Growth remained stable at 0–2% but declined significantly at ≥3%, reaching minimal levels at 5%. This response indicates that the isolate

tolerates sublethal stress but cannot sustain optimal growth under high fungicide pressure.

The observed pattern reflects partial tolerance rather than true resistance. Tolerance, in this context, refers to the ability to maintain reduced growth under stress, whereas resistance implies minimal inhibition. The absence of sustained growth at higher concentrations suggests that the isolate relies on general physiological adaptation rather than specific detoxification mechanisms. Previous studies support this interpretation. Mendarte-Alquisira et al. (2024) reported that *Trichoderma* spp. maintained growth above 65% under pyrethroid exposure, although activity declined at higher concentrations. Linta et al. (2024) showed that growth responses depend strongly on pesticide type and concentration. Khore et al. (2025) further demonstrated that *T. koningii* maintained growth under systemic fungicides, with progressive inhibition at increasing concentrations. Taken together, these findings indicate that fungicide tolerance in Trichoderma is strain-specific and concentration-dependent. In the present study, *T. asperellum* strain G-4274-1 maintained ≥70–90% growth at low to moderate concentrations but declined to 45.00% at 5% mancozeb, indicating moderate tolerance with limited performance under high multisite fungicide stress.

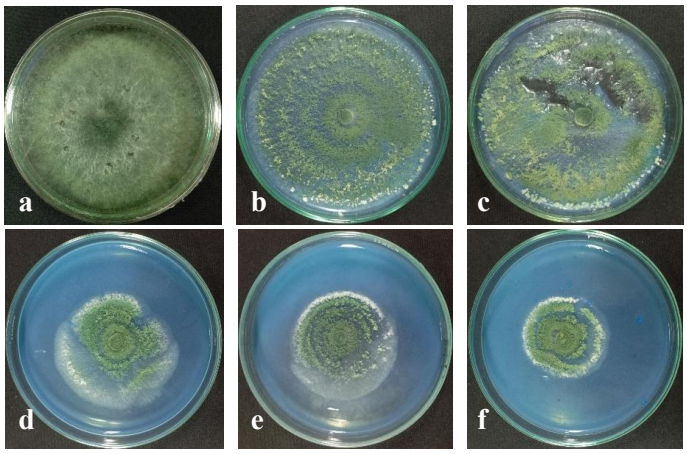

Figure 2. Growth of *T. asperellum* strain G-4274-1 on PDA amended with different concentrations of mancozeb after 7 days of incubation: (a) 0%, (b) 1%, (c) 2%, (d) 3%, (e) 4%, and (f) 5%.

CONCLUSION

Mancozeb exposure induced a clear concentration-dependent reduction in growth in both *S. xiangtanensis* strain NBSP3F and *T. asperellum* strain G-4274-1, confirming the presence of a defined physiological tolerance threshold under multisite fungicide stress. *S. xiangtanensis* strain NBSP3F maintained maximal growth (100.00%) at 0–1% mancozeb, which decreased to 75.00% at 2–3%, declined further to 50.00% at 4%, and reached 25.00% at 5%. In contrast, *T. asperellum* strain G-4274-1 exhibited 100.00% growth in the control, followed by gradual reductions to 95.00%, 90.00%, 72.50%, 70.00%, and 45.00% at 1%, 2%, 3%, 4%, and 5%, respectively. Overall, *T. asperellum* strain G-4274-1 demonstrated greater physiological stability under low to moderate fungicide pressure (0–2%) than *S. xiangtanensis* strain NBSP3F, suggesting a more robust adaptive response during early exposure phases. However, both isolates exhibited substantial growth inhibition at 5% mancozeb, indicating a clear upper limit of physiological tolerance under multisite fungicide exposure and confirming strain-dependent variability in adaptive capacity. This pattern highlights that tolerance is strongly dose-dependent and likely governed by differential cellular resilience and detoxification capacity between isolates.

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