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RESEARCH ARTICLE

Enhanced antagonistic potential of gamma-irradiated *Trichoderma* spp. against *Phytophthora capsici* : An *in vitro* study

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Abstract

Enhancing the biocontrol efficacy of microorganisms is a crucial element in advancing sustainable agricultural practices, particularly in the management of soilborne fungal phytopathogens. Among the various methods employed to augment the effectiveness of biocontrol agents, gamma radiation has emerged as a particularly promising approach due to its ability to induce genetic mutations that can enhance antagonistic properties. In the present study, an efficient gamma irradiation technique was employed to induce genetic modifications in 3 biocontrol agents: *Trichoderma guizhouense*, *T. koningiopsis*, and *T. asperellum*. The efficacy of these genetically altered strains was then evaluated against the pathogen *Phytophthora capsici*. Mutant isolates were generated through exposure to varying doses of gamma radiation and were initially screened based on their growth rates. These were subsequently subjected to a series of *in vitro* antagonistic tests. The results demonstrated that five selected mutants Ta 200-1, Ta 250-2, Tg 200-1, Tg 250-2, and Ta 150-3 exhibited significantly enhanced antagonistic potential compared to their parent strains. Importantly, these mutants retained their improved traits even after ten rounds of subculturing, with growth inhibition percentages ranging from 72.11 % - 68.11 %. The findings from the *in vitro* antagonistic studies suggest that gamma irradiation represents an effective strategy for enhancing the antagonistic capabilities of *Trichoderma* species, thereby contributing to the development of more robust biocontrol agents in plant health management.

Keywords

antagonistic; gamma irradiation; *Phytophthora capsici*; *Trichoderma* spp.

Introduction

The idea of biological control has gained momentum in recent decades as a plant health management strategy and is considered a promising alternative for achieving sustainable agriculture with lower ecological costs. Among the various biocontrol agents identified, the discovery of the genus *Trichoderma* by Persoon in the 17th century marked a significant breakthrough in the history of biological control. The success of *Trichoderma* owes to its high reproductive capacity, nutrient utilization efficiency, rhizosphere modifying capacity, plant growth promotion and strong aggressiveness against phytopathogens through diverse defense mechanisms (1). This filamentous soil borne ascomycetous fungus, effectively combats many

soils borne plant pathogens through various antagonistic actions, including antibiosis, mycoparasitism, competition and induced systemic resistance (2). Despite these benefits, the inconsistency and low efficacy of *Trichoderma* spp. under varying agroecological conditions remain major challenges in fully realizing their potential.

Mutagenesis is a promising approach for enhancing the antifungal production and antagonistic potential of beneficial microbes against a broad spectrum of phytopathogens (3). Gamma radiation, a physical mutagenesis technique, induces chromosomal rearrangements and creates variations in the genetic structure of the targeted organism (4). For instance, gamma irradiated *Trichoderma reesei* exhibited enhanced lytic enzyme activity, *T. viride* mutants showed increased cellulase production (5, 6). Additionally, *T. harzianum* mutants demonstrated enhanced chitinase and lipase activities and *T. aureoviride,* showed augmented antifungal activity against soil-borne pathogens *Fusarium graminearum*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* (7, 8).

In the present study, gamma irradiation was used to induce beneficial mutations to enhance the biocontrol characteristics of three isolates of *Trichoderma* spp. The screened mutants were further evaluated through various *in vitro* assays against the pathogen *Phytophthora capsici* causing foot rot of black pepper and the stability of their traits was confirmed via subculturing. Through careful screening and analysis, the mutants displayed significant improvements in key biocontrol traits.

Materials and Methods

Molecular Characterization of *Trichoderma* spp.

Two promising *Trichoderma* cultures, TR PN3 and TR KR2, which were previously isolated from forest soils in the southern zone of Kerala and *Trichoderma asperellum* the reference culture of Kerala Agricultural University was used as parent strains (Fig. 1). Earlier reports on the cultural and morphological characterization of these selected strains indicated that they exhibited significant antagonistic activity against soil borne fungal pathogens *Pythium aphanidermatum*, *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *tracheiphilum* (9-11).

Molecular characterization of *Trichoderma* isolates TR PN3 and TR KR2 was performed for species level identification. DNA was extracted using the CTAB (Cetyltrimethylammonium bromide) method, followed by PCR amplification of the ITS and *tef1* regions (12). Approximately 650 bp of the *Trichoderma* ITS region in the DNA fragments were amplified using universal primers ITS-1F (5′ TCCGTAGGTGAACCTTGCGG 3′) and ITS-4R (5′ TCCTCCGCT-TATTGATATGC 3′) (13). The translation elongation factor, tef 1 gene was amplified using the forward primer EF1 (5´ CATCGAGAAGTTCGAGAAGG 3′) and reverse primer TEF1rev (5′AACTTGCAGGCAATGTGG 3′). Purified PCR products of *Trichoderma* species were sequenced for tef1 using ABI 3730 XL DNA Analyser 3 (Applied Biosystems).

The soil-borne fungal pathogen *Phytophthora capsici* was isolated from black pepper leaves with quick wilt symptoms. This isolation was conducted at the College of Agriculture, Vellayani, Kerala, and Koch's postulates were confirmed. Finally, the identified cultures of *Trichoderma* spp. and the pure culture of the *P. capsici* were preserved on potato dextrose agar (PDA) slants at 4 °C.

Gamma mutagenesis

Spore suspensions of *Trichoderma* spp. were harvested from one-week-old cultures by immersing the surface of sporulated PDA plates in 1 % sterile saline solution. The liquid was gently agitated to release the spores, which were then diluted to a concentration of 10^6 spores/mL and transferred into sterile 5 mL glass vials. Mutagenesis was conducted using Co^{60} gamma rays in a Gamma Chamber 5000 at the Radiotracer Laboratory, College of Agriculture, Vellanikkara, Thrissur. The spore suspensions were exposed to gamma radiation at target doses of 150, 200, 250

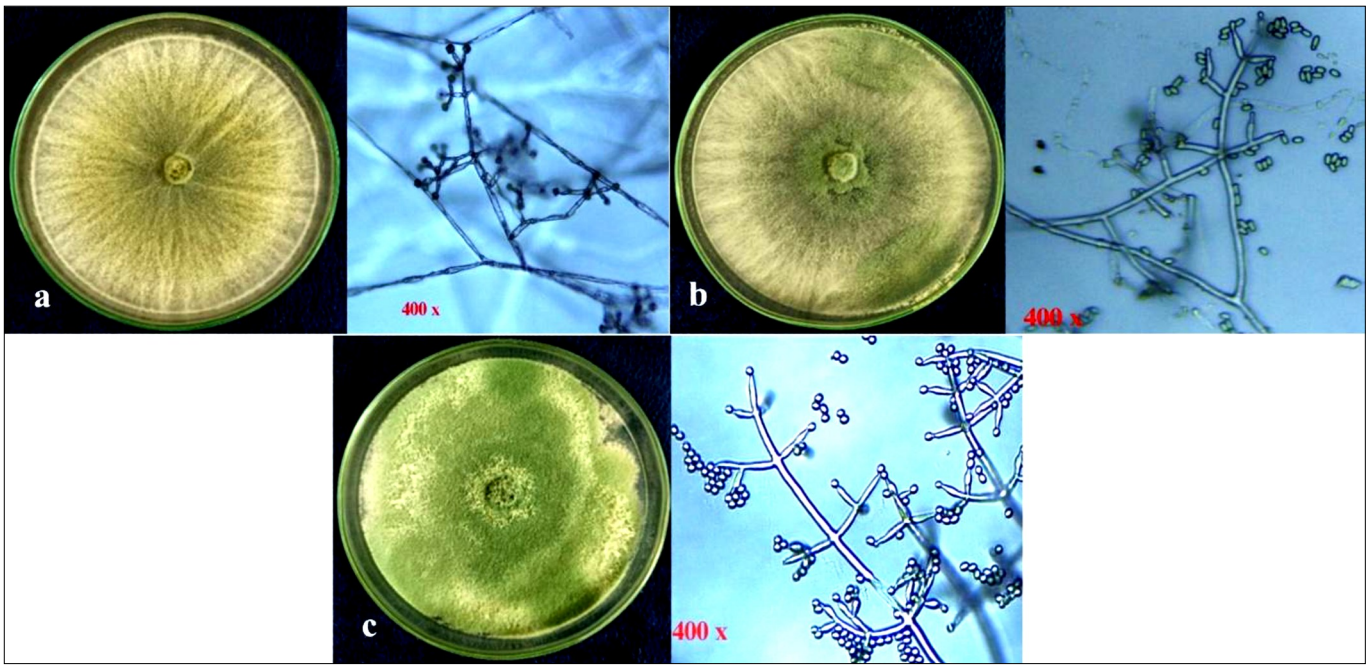

Fig. 1. Parent strains used for gamma irradiation: **a**. *T. guizhouense*, **b**. *T. koningiopsis*, **c**. *T. asperellum.*

and 300 Gy. Following irradiation, the spore suspensions were serially diluted to a concentration of 10^2 spores/mL and plated on PDA medium with 0.1 % Triton X-100, which restricted fungal colony size. The plates were incubated overnight at 28 ± 2 °C in darkness, and the number of germinated spores was subsequently recorded. All germinated spores that developed into small mutant colonies were transferred to PDA plates and incubated at 28 ± 2 °C. The resulting colonies were sub-cultured, and their characteristics, including radial growth, growth pattern on PDA, and colony color, were observed. The mutants obtained were then screened for antimicrobial activity (8).

In vitro antagonistic assays for mutant screening and selection

Dual culture test

The antagonistic property of the selected mutants and parent strains against the pathogenic fungus *P. capsici* was evaluated under *in vitro* conditions using the dual culture technique (14). Mycelial disc of 8 mm diameter was cut from the growing region of both the antagonist and the pathogen. These discs were placed on PDA-containing Petri dishes in opposite directions from each other, with equal distance from the edge of the dishes, and incubated at 28 ± 2 °C. Three replications were maintained for each treatment, with *P. capsici* grown alone serving as the control. The percentage of pathogen growth inhibition (I) was calculated using the formula (C-T)/C X 100, where C represents the growth of the pathogen in the control plate, and T represents the growth of the pathogen in the treatment plate with *Trichoderma*.

Inverted plate technique for volatile interactions

The effect of mutagenic treatment on the release of volatile antifungal metabolites was studied using the inverted plate technique (14). PDA plates were centrally inoculated with 8 mm mycelial discs from 4-day-old cultures of the antagonist and pathogen separately. The plate containing the pathogen *P. capsici* was inverted and placed on the plate containing the antagonistic isolate. Plate without *Trichoderma* isolate served as control. The paired plates were then sealed together using parafilm tape and incubated at 28 ± 2 °C. The radial growth of the pathogen was recorded after 6 days of incubation and growth inhibition percentage was noted as previously described.

Culture filtrate assay

The antifungal activity of culture filtrates (CF) from *Trichoderma* mutants against *P. capsici* was assessed using a culture filtrate assay (14). In this experiment, 100 mL of potato dextrose broth (PDB) was inoculated with mycelial discs taken from the periphery of actively growing *Trichoderma* cultures. The flasks were incubated at 28 °C with shaking at 140 RPM for 7 days. Uninoculated PDB flasks, maintained under identical conditions, were used as controls. Following the incubation period, the culture broth was centrifuged at 5,000 RPM for 10 min to remove mycelial fragments, and the supernatant was filtered through Whatman No. 1 filter paper. To eliminate fungal spores, the filtrate was further passed through 0.22 µm Whatman syringe filters. Under sterile conditions, 10 mL of the culture filtrate was added to a sterile 9 cm Petri dish and mixed with 10 mL of PDA medium, respectively, to achieve a final concentration of 50 % (V/V). The plates were then inoculated with an 8 mm mycelial disc of *P. capsici* and incubated at 28 ± 2 °C. Mycelial growth inhibition was assessed by comparing the growth in treated plates with that in control plates once the control plate had been fully colonized by the pathogen.

Statistical Analysis

Each experiment included 3 replications, and the data obtained were used to perform an Analysis of Variance (ANOVA). The analysis was conducted using KAU GRAPES version 1.0.0 (15).

Results and Discussion

Molecular identification of *Trichoderma* spp.

The cultural and morphological characteristics of the isolated *Trichoderma* strains TR PN3 and TR KR2 were previously studied and reported to possess exemplary antagonistic potential (9). Consequently, there was an urge to further understand the diversity and relationships among species within the genus *Trichoderma*. With ITS primers an amplicon size of ~ 625 bp was obtained for the isolates studied (Fig. 2). The ITS rRNA region is widely used for fungal identification in most fungal databases. However, reliance on the ITS rRNA region alone is inadequate for distinguishing *Trichoderma* species, as it exhibits identical sequences in some closely related species (16). Consequent-

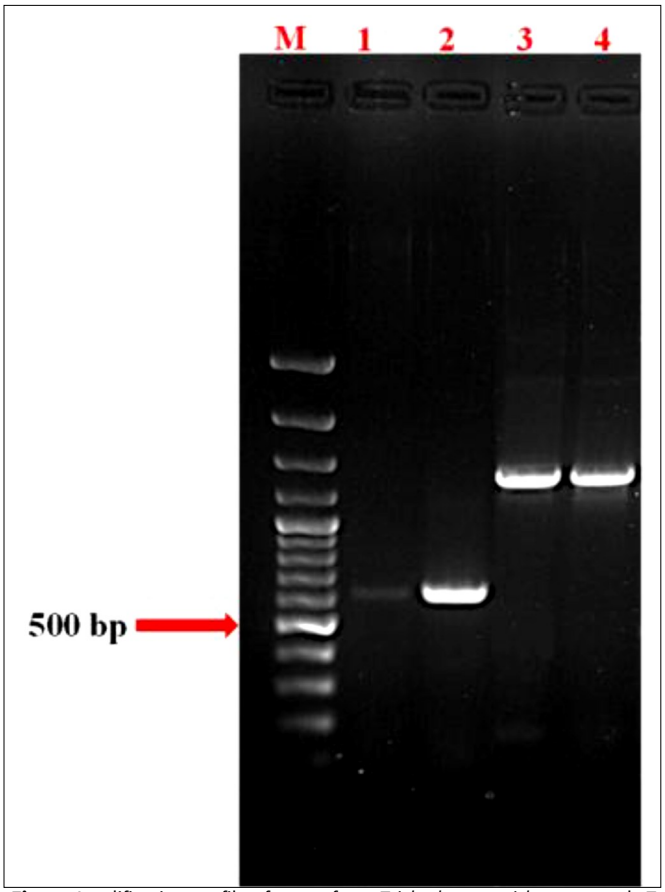

Fig. 2. Amplification profile of genes from *Trichoderma guizhouense* and *T. koningiopsis* **M** - 1 Kb DNA ladder; **Lane 1** – ITS TRPN3; **Lane 2** – ITS TRKR2; **Lane 3** – tef 1 TRPN3 and **Lane 4** – tef 1 TRKR2.

ly, the *tef1* sequences of amplicon size 1200 bp were further analysed through phylogenetic analysis. Phylogenetic trees were generated using Maximum Parsimony method to visually represent the genetic relationship among the isolates. TR PN3 was identified as *T. guizhouense* and TR

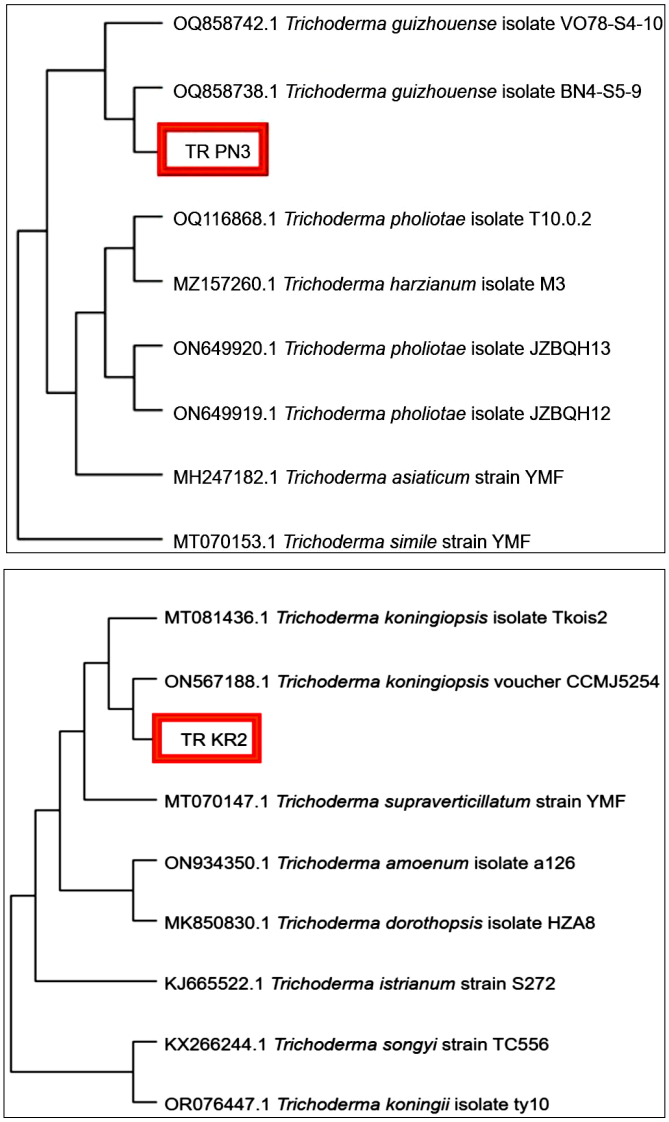

Fig. 3. Single gene phylogenetic tree of TR PN3 and TR KR2 from Maximum Parsimony analysis.

KR2 as *T. koningiopsis* (Fig. 3).

Identifying *Trichoderma* species has proven to be a difficult task, primarily due to significant homoplasy in phenetic traits. This has driven a shift in identification methods from those based solely on phenotypic characteristics to DNA-based approaches, which are further supported by phenotypic observations (17, 18). Among the molecular markers used for species identification, ITS, *tef1*, and *rpb2* (RNA polymerase subunit 2) are indispensable, as each provides unique information that cannot be substituted by other markers. Specifically, *tef1* and *rpb2* serve as complementary markers for accurate species identification (19).

Mutagens obtained through gamma irradiation

To introduce genetic variation in *T. guizhouense*, *T. koningiopsis*, and *T. asperellum*, spore suspensions of the isolates were subjected to varying doses of gamma radiation (150–300 Gy). The findings revealed an inverse relationship between spore germination and the irradiation dose. As the dose increased from 150 Gy - 300 Gy, the number of germinated spores decreased correspondingly, with the inhibitory effect on germination ranging from 71.68 -13.5 % (Fig. 4). The number of mutants obtained after irradiation for each isolate was 173 for *T. guizhouense*, 189 for *T. koningiopsis* and 208 for *T. asperellum*. All distinct and prominent mutants were sub-cultured, and their growth rates were recorded. From the sub-cultured isolates, 10 fast-growing mutant strains, each with a growth of 9 cm by the 3rd day, were selected from each parent strain. Consequently, further *in vitro* studies were conducted with the 30 mutant isolates. Substantial changes in morphological characteristics were observed in the mutant colonies with

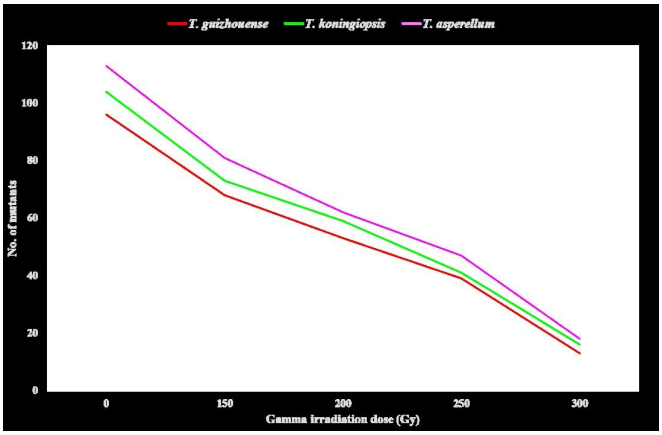

Fig. 4. Correlation between gamma irradiation dose and number of mutants obtained.

respect to cultural traits, sporulation rate, and pigmentation.

Mutation or strain improvement of biocontrol agents is a key strategy for enhancing their longevity and antagonistic effectiveness against phytopathogens (20). When radiation beams interact with water molecules in *Trichoderma* cells, they generate free radicals that can damage DNA and disrupt cellular functions (21). Multicellular or bicellular spores are more resistant to γ-radiation compared to unicellular spores. This might explain why the unicellular spores of *Trichoderma* spp. are more sensitive to γ-radiation. Additionally, factors such as spore density can also influence sensitivity of fungi to gamma radiation (22). Gamma radiation of high energy disrupts the DNA of microorganisms by inducing cross-linkages and other structural modifications, which may inhibit their growth or reproduction. However, when cellular alterations are less severe and fail to completely inhibit these processes, they may instead result in induced mutations. (23).

Previous research has demonstrated a negative correlation between increased gamma radiation levels and the survival of irradiated spores, as well as the growth of *Trichoderma* species. *Trichoderma* spores exposed to radiation doses ranging from 0-2500 Gy demonstrated a decline in survival rate as the dose increased (24). At 400 Gy, 90 % of fungal spores were killed, and at doses above 450 Gy, complete spore mortality was observed. The opti-

mal γ-radiation dose for inducing mutations was identified based on approximately 40-50 % inhibition of spore germination on PDA medium. For instance, a dose of 250 Gy did not completely kill *T. viride* but resulted in around 40-50% inhibition of spore germination (25). Moreover, irradiation at a dose of 250 Gy did not negatively impact the growth rate or sporulation of *T. aureoviride* (8). The enhanced effectiveness of the mutants following gamma radiation exposure may result from mutations occurring in specific regions of the genome, leading to phenotypic changes that can be identified through selection (26).

Antagonistic potential of selected mutants

Dual culture test

Dual culture test was performed on 30 selected mutant isolates and their parent strains,*T. guizhouense*, *T. koningiopsis*, and *T. asperellum*. The test exhibited significant growth inhibition against *P. capsici*, ranging from 61.55 % to 75.34 %, as shown in Table 1. Ten mutants -Ta 250-2, Tg 250-2, Tk 200-1, Ta 300-1, Tk 150-1, Tg 200-1, Ta 150-3, Tk 200-2, Ta 200-1, and Tg 300-1 demonstrated a more effective antagonistic response than their parent isolates, with the maximum inhibition recorded for the mutant isolate Ta 250-2 (75.34 %). Meanwhile, mutants Tg 150-1, Tg 250-1, Ta 200-3, Tk 250-1, Ta 250-1, Ta 300-2, Tg 250-3, Tg 300-2, Tk 150-3, Tg 200-2, Tk 250-3, Tk 150-2, and Tg 150-2 exhibited no significant difference in inhibitory activity compared to their parent strains. The mutant Tg 150-2 exhibited the lowest growth reduction of the pathogen (61.55 %). Based on these findings, further *in vitro* tests were conducted with the ten most effective mutant isolates.

Table 1. Dual culture test of *T. guizhouense*, *T. koningiopsis*, *T. asperellum* and selected mutants at different doses against *Phytophthora capsici*

***Trichoderma* isolate**	**Mycelial growth inhibition (%) ***
T. guizhouense	68.41^{gh}
Tg 150-1	67.15^{i}
Tg 150-2	61.55^{q}
Tg 200-1	71.04^{d}
Tg 200-2	62.74^{no}
Tg 200-3	68.67^{g}
Tg 250-1	66.33^{j}
Tg 250-2	73.37^{b}
Tg 250-3	65.11^{lm}
Tg 300-1	69.04^{fg}
Tg 300-2	64.52^{m}
T. koningiopsis	67.22^{i}
Tk 150-1	72.11^{c}
Tk 150-2	61.96^{pq}
Tk 150-3	63.33^{n}
Tk 200-1	73.18^{b}
Tk 200-2	69.96^{e}
Tk 200-3	67.56^{i}
Tk 200-4	68.78^{g}
Tk 250-1	65.33^{kl}
Tk 250-2	67.85^{hi}
Tk 250-3	62.29^{op}
T. asperellum	68.78^{g}
Ta 150-1	68.44^{gh}
Ta 150-2	67.33^{i}
Ta 150-3	70.81^{d}
Ta 200-1	69.55^{ef}
Ta 200-2	67.70^{i}
Ta 200-3	65.96^{jk}
Ta 250-1	65.26^{l}
Ta 250-2	75.34^{a}
Ta 300-1	72.19^{c}
Ta 300-2	65.11^{lm}

***** Means of three replications marked with the same superscripts are not significantly different according to Duncan's multiple range test at P < 0.05.

Effect of volatile metabolites

To further investigate the volatile interactions between the biocontrol agent and pathogen, the inverted plate technique was employed. This approach effectively prevents direct physical contact and minimizes exposure to both secreted metabolites and those diffused through the medium between the antagonist and its interacting partner (14). Among the ten screened mutant isolates of *Trichoderma* spp., the highest growth inhibition was observed in Tg 250-2, with a rate of 74.11%. Statistical analysis revealed a significant difference in the inhibitory effects of volatiles from mutant isolates of *Trichoderma* spp. compared to their parental strains on *P. capsici,* as illustrated in Fig. 5. Notably, seven isolates—Tg 250-2, Tg 200-1, Ta 250-2, Ta 200-1, Ta 150-3, Ta 300-1, and Tg 300-1 outperformed their parental strains, exhibiting inhibition percentages ranging from 66.15 % - 74.11 %. In contrast, the mutant strains of *T. koningiopsis* (Tk 150-1, Tk 200-1, and Tk 200-2) demonstrated relatively poor growth suppression of the pathogen.

Interaction of culture filtrate of Trichoderma spp. against P. capsici

To study the interaction between non-volatile metabolites of mutant isolates of *Trichoderma* spp. against *P. capsici*, a culture filtrate assay was conducted at a 50 % concentration. The results, illustrated in Fig. 6, demonstrate a significant difference in the inhibitory effects of *T. guizhouense* and its mutants compared to the other isolates. The highest growth inhibition was observed in Tg 250-2, at 84.18 %, followed by Tg 300-1 (82 %) and Tg 200-1 (78.71 %). Conversely, the growth of the pathogen in the mutant isolates Tk 150-1, Tk 200-1, and Tk 200-2 was faster than in the parent *T. koningiopsis*, indicating a lower antagonistic potential. Among the three *in vitro* tests performed, this assay exhibited the highest antagonistic potential.

In summary, the *in vitro* results evidently demonstrate that the mutant isolates of *Trichoderma* spp. exhibited varying degrees of growth inhibition against *P. capsici*. Notably, 7 mutant isolates Tg 200-1, Tg 250-2, Tg 300-1, Ta 150-3, Ta 200-1, Ta 250-2, and Ta 300-1 derived from the parent strains *T. guizhouense* and *T. asperellum*, displayed

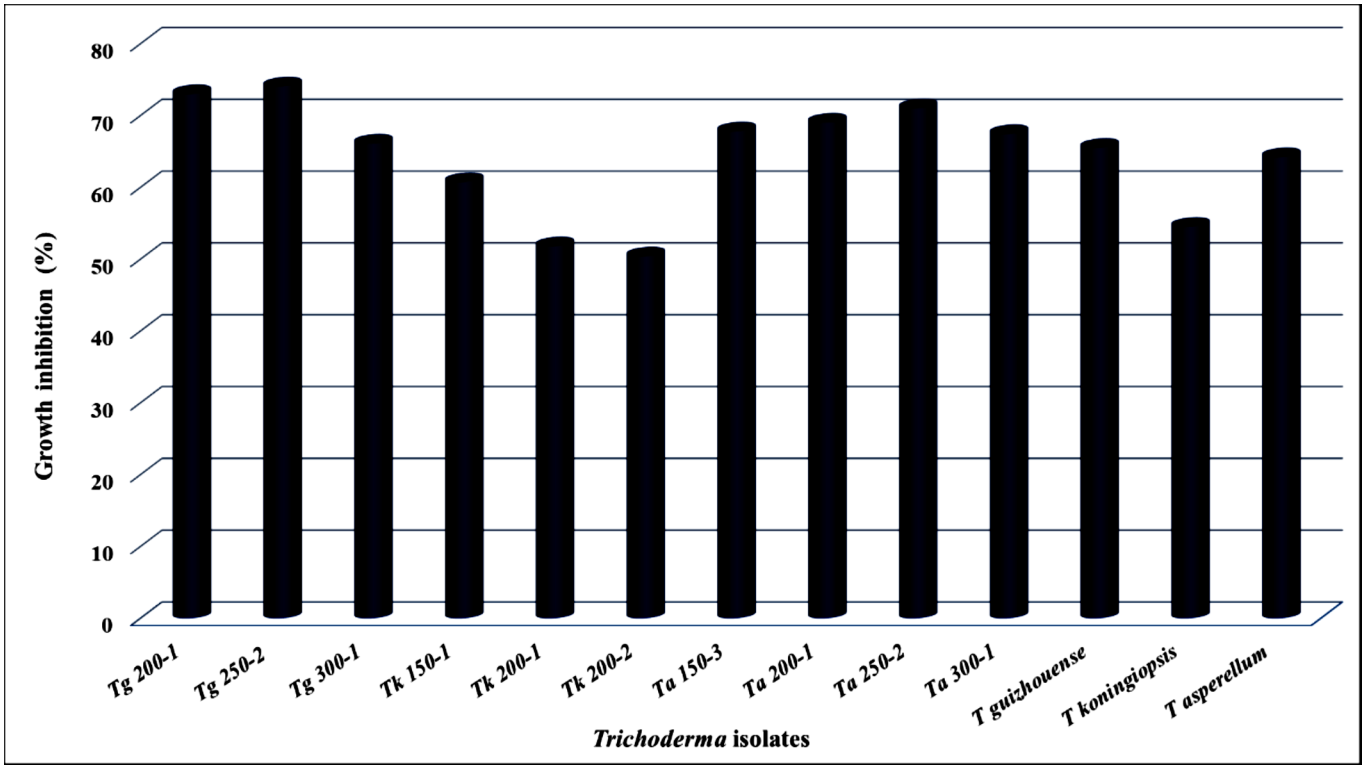

Fig. 5. Effect of volatile metabolites from *Trichoderma* mutant isolates against *Phytophthora capsici.*

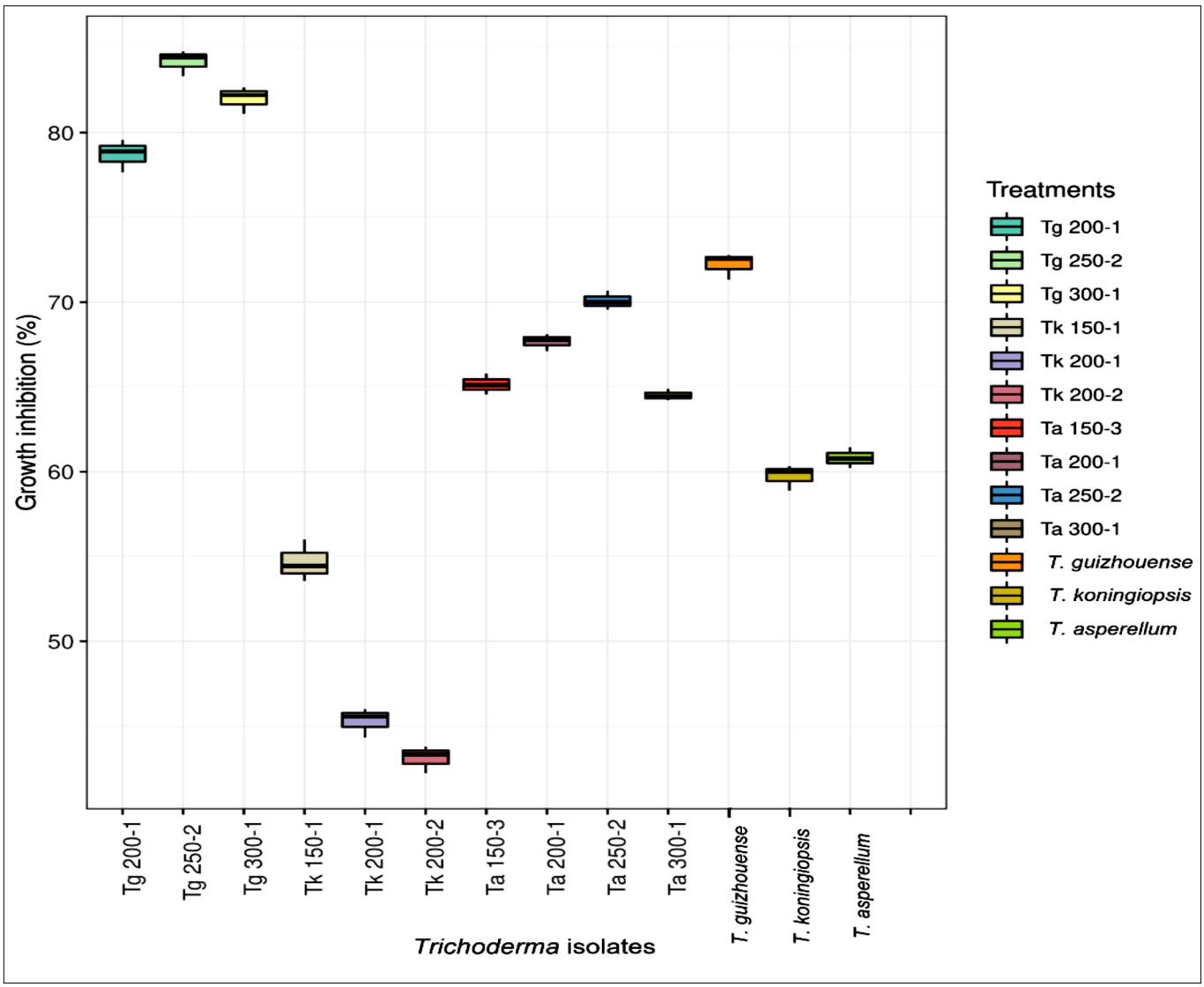

Fig. 6. Interaction of culture filtrates from *Trichoderma* spp. against *P. capsici.*

significantly enhanced antagonistic activity compared to their parent isolates. These mutants were subsequently

screened and sub-cultured ten times, after which a dual culture test was conducted to verify the stability of their mutated traits. As evidenced by the dual culture test results presented in Table 2, 5 mutant isolates retained their antagonistic potential even after 10 rounds of subculturing, outperforming their parent strains with a growth inhibition percentage ranging from 72.11 % -68.11 %. A slight decline in inhibitory effect was noted in all isolates when compared to the initial dual culture results, apart from isolate Ta 200-1. Mutant isolates Tg 300-1 and Ta 300-1 exhibited a lower inhibition percentage compared to their parent strains. Therefore, the final mutant isolates of *Trichoderma* spp. obtained after *in vitro* screening, with exemplary antagonistic potential, were Ta 200-1, Ta 250-2, Tg 200-1, Tg 250-2, and Ta 150-3 (Fig. 7).

Table 2. Antagonistic potential of best mutant isolates against *Phytophthora capsici*

***Trichoderma* isolate**	**Mycelial growth inhibition (%) ***
Tg 200-1	69.93[b]
Tg 250-2	68.59[c]
Tg 300-1	62.11[f]
Ta 150-3	68.11[c]
Ta 200-1	73.30[a]
Ta 250-2	71.70[b]
Ta 300-1	62.26[f]
Tg	66.30[d]
Ta	64.07[e]

***** Means of 3 replications marked with the same superscripts are not significantly different according to Duncan's multiple range test at P < 0.05.

The results of the *in vitro* investigation strongly suggest that exposure to gamma radiation can improve the antagonistic capabilities of *Trichoderma* spp. Consistent with these findings, several studies have employed random mutagenesis through gamma irradiation to enhance the biocontrol efficacy of *Trichoderma* spp. against various plant pathogens. For example, *T. viride* mutants have shown increased biocontrol potency against *Macrophomina phaseolina* (25), while *T. harzianum* mutants have demonstrated increased disease suppression of charcoal rot in melon (27). Additionally, mutants of *T. viride* have exhibited higher levels of antifungal metabolite production and improved biological efficacy against various soil borne pathogens (28). Similarly, *T. aureoviride* mutants have shown increased antifungal activity against *Fusarium graminearum* (8).

Beyond their biocontrol applications, *Trichoderma* species are widely used as host organisms for industrial enzyme production. Physical mutagens, such as gamma radiation, have been successfully applied to enhance the production of large quantities of hydrolytic enzymes from these filamentous fungi. Mutant strains of *T. reesei* and *Trichoderma* sp. VTCC have been reported to produce high levels of cellulase, maintaining this trait even after five generations (5,24). Similarly, irradiation of *T. viride* induced significant beneficial mutations, resulting in a 90.9% increase in chitinase activity among mutants (29).

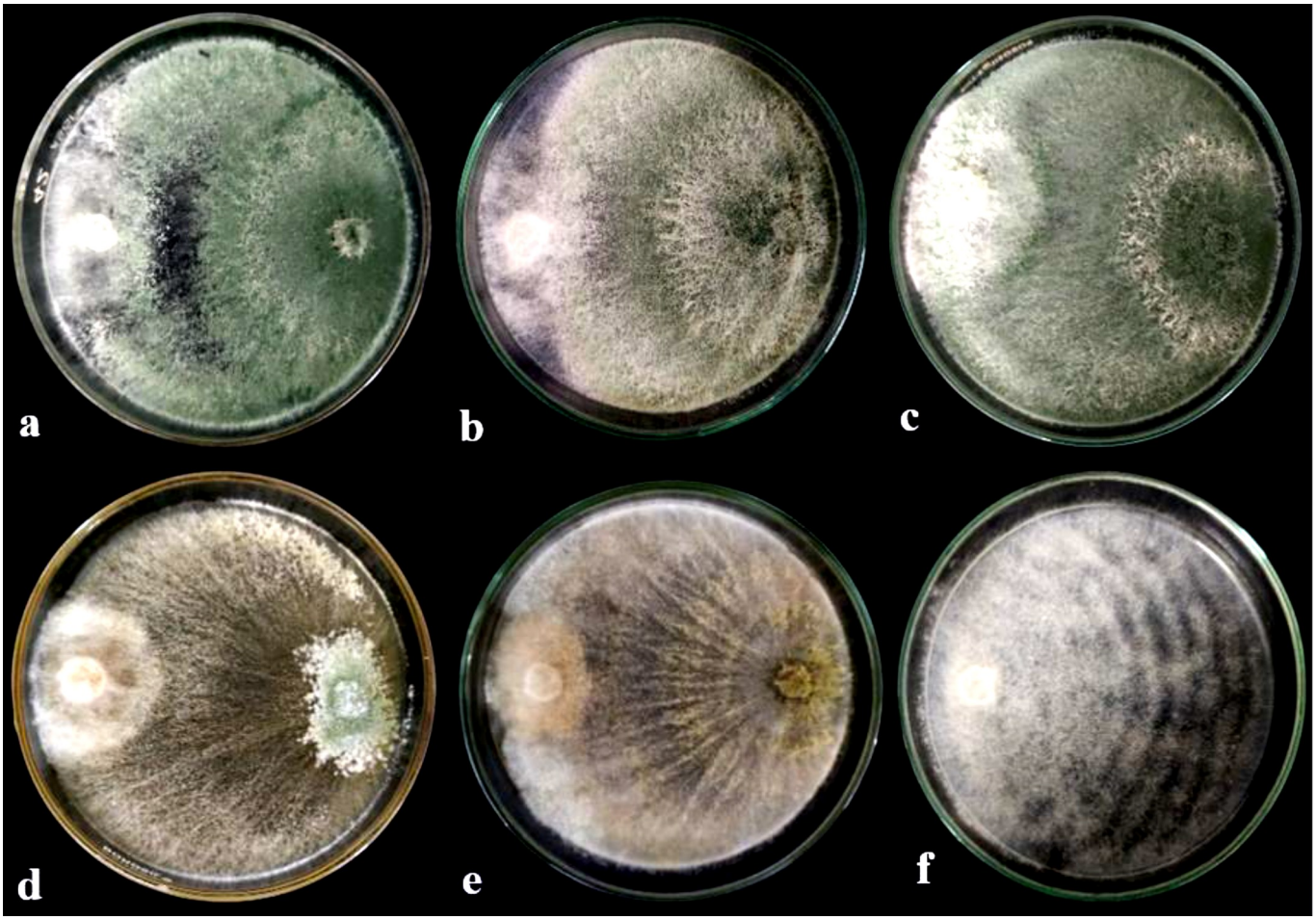

Fig. 7. Effect of screened mutant isolates on the colony growth of *P. capsici* in dual culture test. **a**.Ta 200-1, **b**. Ta 250-2, **c**. Tg 200-1, **d**. Tg 250-2, **e**. Ta 150-3, **f**. Control – *P. capsici.*

Additionally, gamma-irradiated mutants of *T. afroharzianum* NAS107 exhibited the highest cellulase and xylanase activities (30). Mutants of *T. reesei* 2414, generated through gamma radiation, showed a remarkable enhancement in cellulase production during solid-state fermentation using a combination of rice by-products (31). Furthermore, *T. reesei* is classified as a Biosafety Level 1 (BSL-1) microorganism by the American Type Culture Collection (ATCC), indicating that it is not an opportunistic pathogen causing infections in humans (32). Thus, *Trichoderma* spp. acts as safe, low-cost biocontrol agents with wide industrial applications and minimal risk to humans and the environment. The enhancement of hydrolytic enzyme production in mutant strains is a key factor contributing to the effective antagonism of *Trichoderma* against phytopathogens.

It is well documented that various *Trichoderma* species exhibit differential antagonistic activities, and the mechanisms of action among biocontrol agents can vary even within the genus *Trichoderma* (33, 34). Consequently, while certain mutants may demonstrate enhanced efficacy through specific antagonistic mechanisms, they may concurrently exhibit inefficiency in other antagonistic traits and may inadvertently stimulate the radial growth of the tested pathogens. Similar outcomes have been reported in certain gamma induced mutant strains of *T. aureoviride*, which showed weak antagonistic responses against soilborne pathogens such as *Fusarium graminearum*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* (8). This variability is likely due to the stochastic nature of gamma ray-induced mutagenesis, which is expected to produce undesirable mutations with negative effects.

Conclusion

Inducing mutations through ionizing radiation in *Trichoderma* spp. can lead to genetic alterations, resulting in mutants with significantly enhanced antagonistic capabilities, which are valuable for the biocontrol of various soilborne fungal phytopathogens. In this study, *T. guizhouense*, *T. koningiopsis*, and *T. asperellum* were subjected to gamma irradiation, leading to the identification of five superior mutants based on *in vitro* analyses. Considering that these superior mutants demonstrated increased growth inhibition against the tested pathogen *Phytophthora capsici*, it is advisable to assess the combined application of these selected mutants in greenhouse and field trials to improve resistance against multiple pathogenic fungi. However, additional research is needed to clarify the mechanisms utilized by these efficient mutants, particularly through enzymatic assays and gene expression analysis.

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Authors' contributions

AJ carried out the experiment and wrote the manuscript. SST planned the experiment. SGV provided Trichoderma cultures for the research. PSP participated in irradiating Trichoderma samples. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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