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STUDY ON ENHANCEMENT THE ANTAGONISTIC OF *Trichoderma koningiopsis* AGAINST *Rhizoctonia solani* CAUSING SHEATH BLIGHT IN RICE BY GAMMA IRRADIATION TREATMENT

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ABSTRACT

As targeted mutagenesis techniques become more prevalent for various filamentous fungi, the use of physical mutagen to induce random genetic variations across whole-genome remains an ideal option for genetic improvement. The aim of this study was to investigate the use of gamma radiation to enhance the antagonistic ability of *Trichoderma* against plant pathogenic fungi. The spore suspensions of *Trichoderma koningiopsis* VTCC 31435 was irradiated at doses ranging from 0 to 2500 Gy using a gamma Co-60 source at the Hanoi Irradiation Center. The results showed that the number of surviving spores depends on the irradiation dose. Spore numbers decreased sharply in the dose range of 100 to 1200 Gy, at higher doses, the variation in spore numbers was less pronounced. The required radiation dose to kill 90% of the total number of fungal spores (D10) of the *T. koningiopsis* VTCC 31435 strain was approximately 250 Gy. At a dose of 2500 Gy, the number of fungal but surviving spores decreased by about 7 Log units compared to the non-irradiated. After irradiation, morphological changes, as well as growth characteristics, were observed in some of the radiation-resistant obtained colonies. Using cellophane membrane assay and dual culture methods, five potential radiation-resistant mutants with better antagonistic ability against the fungus *Rhizoctonia solani*, which causes sheath blight in rice, were identified Notably, among these, the potential mutant VTCC(a) I-1 exhibited the highest antifungal activity of media-permeable metabolites (I_{CMA}) and mycelial growth inhibition activity (I_{MG}) against *R. solani*. The I_{CMA} and I_{MG} values of this mutant increased by 154.12% and 148.68%, respectively, compared to the wild-type strain and remained stable for at least six consecutive generations. The research results suggest that gamma irradiation may have potential applications in enhancing the antagonistic abilities against pathogenic fungi and other beneficial biological properties in *Trichoderma* sp.

Keywords: Antagonistic, gamma irradiation, mutant, *Rhizoctonia solani*, *Trichoderma koningiopsis.*

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INTRODUCTION

Sheath blight, caused by the fungus *Rhizoctonia solani*, is among the most economically significant diseases in rice-growing regions worldwide, including Vietnam. This disease significantly affects the quality of harvested rice and reduces yields by up to 30%, with potential losses reaching 45−50% (Senapati et al., 2022). Controlling *R. solani* presents significant challenges due to several characteristics of sclerotia, including the high survival rate, the extremely broad host range, and the ecological behavior (Abbas et al., 2017). The predominant method for managing fungal diseases in rice, including *R. solani*, is the utilisation of chemical fungicides. While these fungicides offer advantages such as broad-spectrum activity, high efficacy, and rapid action, they increasingly reveal limitations. These limitations include reduced effectiveness against soil-borne fungal pathogens, environmental contamination, adverse human health impacts, and the emergence of fungicide-resistant strains (Sharma et al., 2009). Consequently, the use of biological control agents is being explored as an environmentally sustainable alternative to protect rice plants from this pathogenic fungus.

Trichoderma is recognized as a genus of fungi with strong biological control capabilities due to its antagonistic properties against several plant pathogenic fungi, primarily through mechanisms of antibiosis, parasitism, and competition (Harman, 2006). Numerous species within the *Trichoderma* genus exhibit rapid growth rates and resistance to several soil-borne root pathogens such as *Sclerotinia sclerotiorum, Sclerotium rolfsii, Fusarium solani,…* (Zhang et al., 2016; Kotasthane et al., 2015; Rojo et al., 2007). Notably, the efficacy of *Trichoderma* spp. in controlling the sheath blight fungus *R. solani* in rice has been extensively studied. *In vitro* and greenhouse studies have highlighted the superior control capabilities of various strains of *Trichoderma harzianum, Trichoderma virens*, and *Trichoderma atroviride* against *R. solani* (Naeimi et al., 2011). Furthermore, field experiments have demonstrated that treatments with *Trichoderma viride* Talc formulations not only reduce the severity of sheath blight but also enhance root length, dry weight, and plant height in rice (Mathivanan et al., 2005). In tropical wetland rice, the application of *Trichoderma asperellum* has been shown to reduce disease severity by 19%, increase grain weight by 34%, and boost yield by 41% (De Franca et al., 2015).

In Vietnam, numerous studies have highlighted the potential of *Trichoderma* fungi in controlling plant pathogenic fungi (Thanh et al., 2014; Tao & Tuan, 2020; Nguyen et al., 2024). These studies indicate that certain strains of *Trichoderma* are more effective than others against specific diseases. Given the growing emphasis on clean and safe agricultural products, the use of organic microbial fertilizers, formulations containing microbial strains, or bioactive substances from microorganisms with antifungal properties has become a priority. The addition of *Trichoderma*-based biocontrol formulations to cultivated soil provides long-term effectiveness and environmental safety that chemical pesticides cannot match.

In nature, the mutation rate of microorganisms depends on their growth conditions and typically ranges from 10^{-10} to 10^{-6}. This rate can increase significantly when experimental mutagenic agents are used, reaching levels between 10^{-5} and 10^{-1} (Davati et al., 2013). The application of physical agents represents one of the traditional mutagenesis techniques that have been successfully employed to enhance the biological activity of various microbial species. Our recent studies indicated that gamma irradiation offers several advantages, including a broad mutation spectrum, high mutation frequency, efficiency, and rapid application, in some strains of bacteria and filamentous fungi (Diep et al., 2016; Diep et al., 2020).

Aiming to develop a high-quality and stable strain for the experimental production of a *Trichoderma*-based microbial formulation capable of preventing fungal diseases in rice, this study initially investigated the use of gamma radiation to generate *Trichoderma*

strains with enhanced antagonistic properties against *R. solani*, derived from the original *Trichoderma koningiopsis* VTCC 31435 strain.

MATERIALS AND METHODS

Microbial strains and culture medium

The fungal strain *T. koningiopsis* VTCC 31435 was obtained from the Institute of Microbiology and Biotechnology, Vietnam National University, Hanoi. The pathogenic strain *R. solani* RHN1, responsible for rice sheath blight, was provided by the Division of Pathology and Phyto - Immunology at the Plant Protection Research Institute.

Potato dextrose agar (PDA) media, NaCl, Triton X-100 and other chemicals at analytical grade were purchased from Merck, Germany.

Preparation of spore suspension and irradiation treatment

Spore suspensions of *Trichoderma* were prepared as described by Darabzadeh et al. (2018). The fungi were cultured on PDA plates and incubated at 25 ± 1 ^{o}C for one week. Sterile 0.9% NaCl was added, and the spores were scraped off. The suspensions were homogenized in test tubes, and the spore concentration was adjusted to 10^8–10^9 CFU/mL.

Sterile test tubes containing 10 mL of the *Trichoderma* spore suspension were irradiated in duplicate at the same dose rate (0.23 Gy/second) with the radiation doses ranging from 100 to 2,500 Gy under gamma-ray ^{60}Co source at the Hanoi Irradiation Center. Actual absorbed doses were measured by Gafchromic dosimeters (for doses ≤ 1,000 Gy) and B3 Dose Stix dosimeters (for doses > 1,000 Gy).

Screening potential mutants with high antagonistic ability against *Rhizoctonia solani*

Cellophane membrance assay

The method was modified from the one previously described by Alfiky (2019) for the preliminary assessment of antifungal activity against *R. solani* in this study. The assay involved quantifying the metabolites and/or enzymes produced by the mutant strains of *Trichoderma*.

After irradiation, *Trichoderma* spore suspensions at varying doses were promptly spread onto PDA plates with 0.1% Triton X-100 to isolate radiation-resistant colonies, which were then individually cultured on the same medium. Sterile cellophane membranes (GelAir Cellophane, Bio-Rad) with diameters matching those of the petri dishes were placed on the new PDA plates. 5 mm mycelial discs from four-day-old radiation-resistant *Trichoderma* colonies were inoculated at the center and incubated at 25 ± 1 ^{o}C for 48 hours. After incubation, the *Trichoderma* colonies and membranes were removed, and four-day-old *R. solani* was inoculated at the center of these plates. The plates were incubated at 25 ± 1 ^{o}C for another 72 hours, and the *R. solani* colony diameter was measured with an electronic calliper and compared to the control (*R. solani* on fresh PDA). The antifungal activity of media-permeable metabolites produced by the radiation-resistant *Trichoderma* colonies was calculated using formula (1) and compared to that of the original *T. koningiopsis* VTCC 31435 strain.

$$I_{CMA} = (1 - T/C) \times 100 \quad (1)$$

where: I_{CMA} represents the antifungal activity against *R. solani* of media-permeable metabolites (%); C is the diameter of the pathogen colony on fresh PDA plate (mm); and T is the diameter of the pathogen colony on PDA plate that has been previously inoculated with *Trichoderma* on cellophane membranes (mm).

The five (05) radiation-resistant mutants with the highest I_{CMA} will be selected for further testing using the dual culture test.

Dual culture test

The antagonistic efficacy of the top five potential mutants of *Trichoderma*, preliminarily selected using the cellophane membrane assay, was evaluated *in vitro* against *R. solani* using a dual culture test. A 5 mm diameter mycelial disc of *Trichoderma* was excised from the periphery of a four-day-

old actively growing culture and inoculated opposite to *R. solani* on the same plate, 2 cm away from the edge. These paired cultures of the antagonist and the test pathogen were placed equidistantly from the periphery to ensure equal growth opportunities. On the control plates, the test pathogen was inoculated alone 2 cm from the edge of the plate. After a 3−5 days incubation period at 25 ± 1 ^{o}C, the radial growth of *R. solani* was measured in both control and treatment plates, and the mycelial growth inhibition activity was calculated using the following formula:

$$I_{MG} = (1 - T/C) \times 100 \quad (2)$$

where: I_{MG} represents the mycelial growth inhibition activity (%); C is the radial growth of *R. solani* (mm) in the control; and T is the radial growth of *R. solani* (mm) in the treatment.

Data analysis

The experimental data were analyzed using analysis of variance (ANOVA) followed by Duncan's test, with significance defined at $p < 0.05$. All statistical analyses were conducted using SPSS software (Version 22.0), performed in triplicate, and results were presented as mean values with standard deviation (±SD).

RESULTS AND DISCUSSION

Induced mutagenesis by gamma irradiation

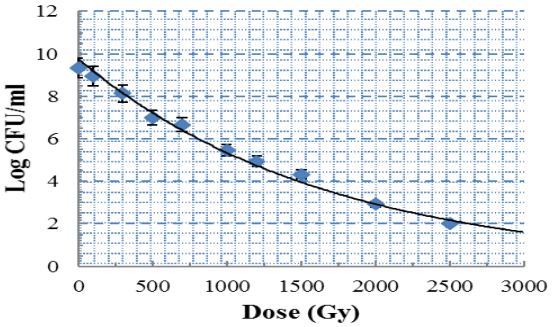

Figure 1. Effects of gamma irradiation on the viability of *Trichoderma koningiopsis* VTCC 31435

Gamma radiation has been employed to induce genetic diversity in microorganisms in general, and *Trichoderma* spp. specifically, due to its ability to alter DNA structure (Hoe et al., 2018). The effect of gamma radiation on the *T. koningiopsis* VTCC 31435 strain was evaluated by measuring the number of surviving spores in the irradiated spore suspensions. The correlation between the logarithm of the number of surviving *T. koningiopsis* VTCC 31435 spores (CFU/ml) and the radiation dose is shown in Figure 1.

In a study examining the impact of gamma irradiation on the morphological characteristics and antagonistic properties of *T. viride* against the pathogenic fungus *Macrophomina phaseolina*, Baharvand et al. (2014) found that the survival rate of *T. viride* was 9.7% at a dose of 400 Gy, and no spore germination was observed at a dose of 450 Gy. In another study, Soufi et al. reported that Co-60 gamma radiation at a dose of 250 Gy inhibited 43.4% of the germination rate of *Trichoderma aureoviride* spores, and a dose of 450 Gy resulted in the complete death of *T. aureoviride* spores in the solution (Soufi et al., 2021). Our research demonstrates that the number of surviving spores is dose-dependent. A significant reduction in survival spore was observed in the dose range of 100 to 1,200 Gy, with less variation in spore numbers at higher doses. The D10 value for the *T. koningiopsis* strain was approximately 250 Gy. This D10 value is the radiation dose necessary to achieve a 10-fold reduction in the number of *T. koningiopsis* spores or, equivalently, to eliminate 90% of the total spore population of this microorganism. At a dose of 2,500 Gy, the number of surviving *T. koningiopsis* spores were decreased by about 7 log units compared to the non-irradiated control. The discrepancies in the aforementioned studies can be attributed to factors such as temperature, chemical composition of the culture medium, density of the spore suspension, fungal growth phase, as well as the physiological conditions and repair capabilities of the fungal cells, all of which influence their post-irradiation survival (Diep et al., 2020).

After irradiation, clear morphological changes and growth characteristics were

observed in some of the radiation-resistant colonies obtained, including alterations in colony shape and color, sporulation rate, and pigment production (Fig. 2). Similar findings have been reported in several studies utilizing gamma radiation as a mutagenic agent on various *Trichoderma* spp. (Soufi et al., 2021; Abbasi et al., 2016; Baharvad et al., 2014).

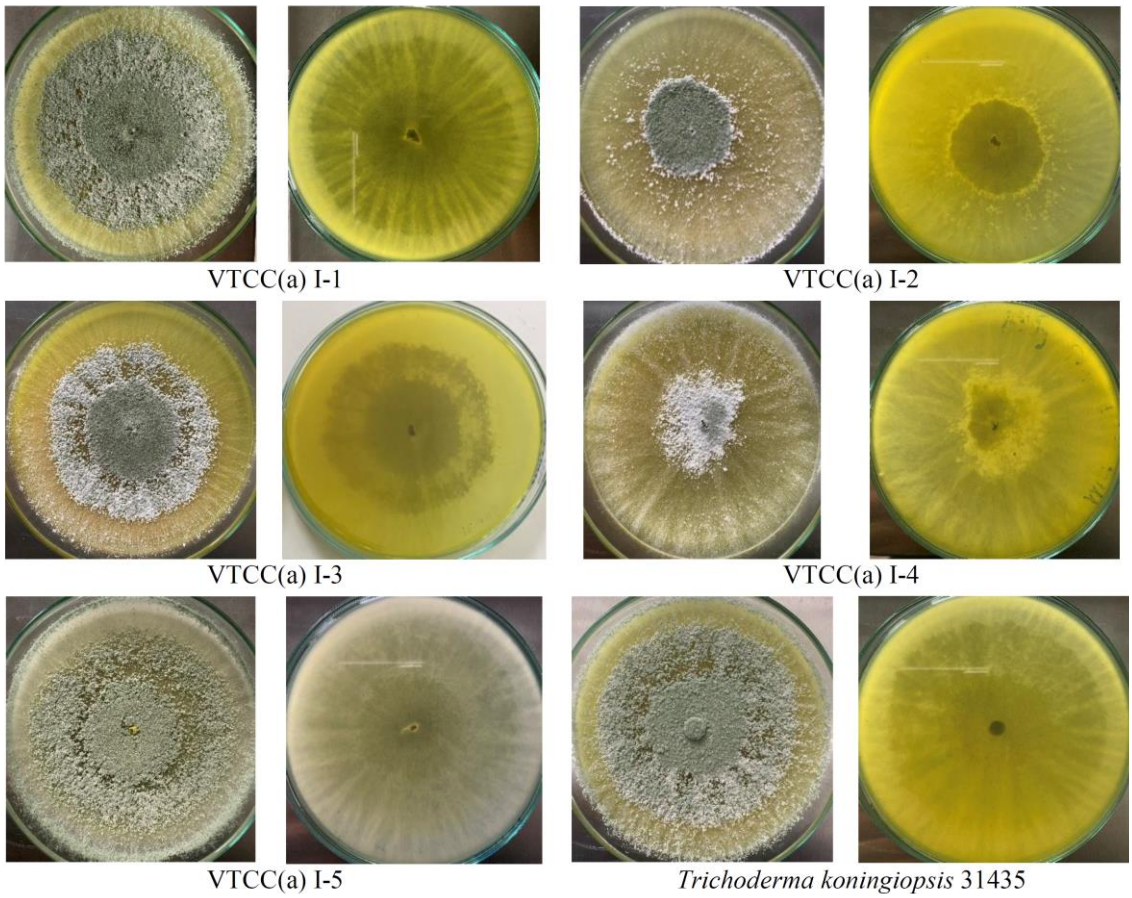

Figure 2. Irradiation treatment with Co-60 resulted in morphological changes and altered pigment production in radiation-resistant colonies cultured on PDA medium after 5 days of incubation at 25 ± 1 ^{o}C

Potential mutants with high antagonistic ability against *Rhizoctonia solani*

The antifungal activity of media-permeable metabolites

Parasitism and antibiotic production are two of the primary biocontrol mechanisms proposed for *Trichoderma* spp. (Harman, 2006). These mechanisms often function synergistically (Schirmböck et al., 1994), and their efficacy in biocontrol varies depending on the *Trichoderma* strains, the pathogen, the crop, and environmental conditions. In both mechanisms, hydrolytic enzymes and metabolites secreted by *Trichoderma* play a major role, enabling them to effectively control pathogens.

The cellophane membrane assay was performed on the radiation-resistant colonies obtained, prioritizing those with morphological or growth characteristic changes. The antifungal activity against *R. solani* of metabolites from the top five potential mutants of *T. koningiopsis* VTCC 31435 is presented in Table 1 and Figure 3A.

Table 1. The antifungal activity of media-permeable metabolites produced by wild type and the top five potential mutants of *Trichoderma koningiopsis* VTCC 31435

No.	Potential mutants	I_{CMA} (%)	Increase compared to the wild-type strain (%)
1	*Trichoderma koningiopsis* VTCC 31435	$51.49^{a} \pm 1.35$	100^{a}
2	VTCC(a) I-1	$79.25^{c} \pm 4.01$	$154.12^{c} \pm 11.84$
3	VTTC(a) I-2	$63.52^{b} \pm 3.33$	$123.54^{b} \pm 9.72$
4	VTTC(a) I-3	$60.66^{b} \pm 3.33$	$117.76^{b} \pm 3.43$
5	VTCC(a) I-4	$61.36^{b} \pm 0.47$	$119.22^{b} \pm 2.26$
6	VTCC(a) I-5	$75.61^{c} \pm 1.26$	$146.91^{c} \pm 4.87$

Note: Means with different superscript letters in the same column are significantly different (Duncan's test, one-way ANOVA, $p < 0.05$).

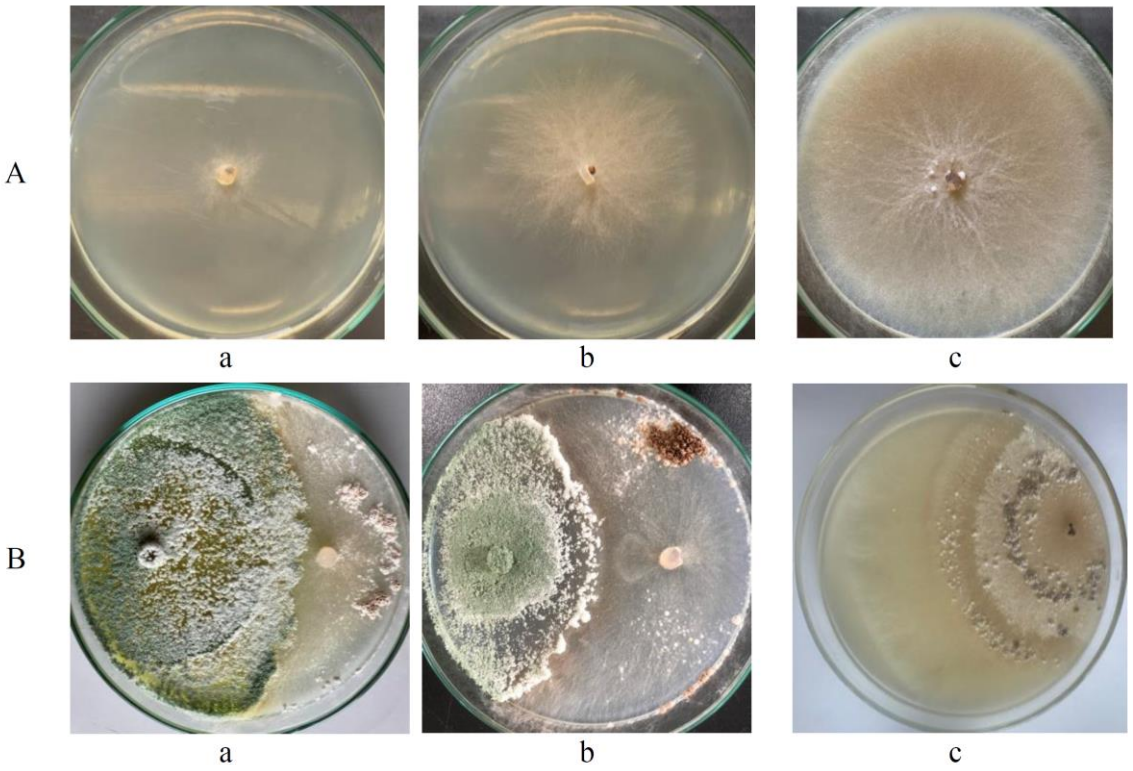

Figure 3. A-Cellophane membrane assay: (a) *Rhizoctonia solani* on PDA previously inoculated with VTCC(a) I-1, (b) on PDA previously inoculated with *Trichoderma koningiopsis* VTCC 31435, and (c) *Rhizoctonia solani* on fresh PDA; B-Dual culture test on PDA medium: (a) *Rhizoctonia solani* with VTCC(a) I-1, (b) *Rhizoctonia solani* with *Trichoderma koningiopsis* VTCC 31435, and (c) *Rhizoctonia solani* alone

The results in Table 1 indicate that the antifungal activity of metabolites from the five potential mutants against *R. solani* was higher than that of the wild-type strain. Their I_{CMA} values were 79.25% and 75.61% (an increase of 154.12% and 146.91% compared to the wild strain), although these values were not statistically significant. The other three mutants, VTTC(a) I-2, VTTC(a) I-3, and VTCC(a) I-4, exhibited similar antifungal activity, with I_{CMA} values of 63.52%, 60.66%, and 61.36%, respectively,

which were not statistically different from each other.

The use of cellophane membranes to determine the activity of various enzymes secreted by filamentous fungi, and to assess the antagonistic ability against plant pathogenic fungi in certain *Trichoderma* strains, has been well-documented (Liu et al., 2010; Alfiky, 2019). The small pore size of the cellophane membrane allows molecules such as proteins and other bioactive substances to pass through while retaining spores and hyphae. Consequently, using cellophane membranes facilitates the easy detection of bioactive molecules secreted by filamentous fungi without interference from fungal hyphae, which are removed along with the membrane before conducting the assay (Liu et al., 2010).

In Vietnam, various methods such as dual culture, agar diffusion, and culture filtrate evaluation have been employed to assess the antagonistic ability of filamentous fungi, particularly *Trichoderma* spp., against plant pathogenic microorganisms. However, no studies have been published on the use of cellophane membranes for this purpose. Our results indicate that using cellophane membranes is a rapid, easy-to-perform, and highly flexible technique for evaluating the antifungal activity of metabolites from a large number of *Trichoderma* strains. This observation aligns with the findings of Alfiky et al. (2019), who utilized the cellophane membrane assay to screen for UV-irradiated mutants of *Trichoderma virens* and *Trichoderma asperellum* with higher antagonistic activity against *Sclerotium rolfsii* and *R. solani*.

The mycelial growth inhibition activity

To evaluate the effectiveness of the screening method using the cellophane membrane assay, the antagonistic abilities against *R. solani* of the top five potential mutants with the highest I_{CMA} values were tested in vitro using the dual culture test.

Table 2. The mycelial growth inhibition activity of wild type and the top five potential mutants of *Trichoderma koningiopsis* VTCC 31435

No.	Potential mutants	I_{MG} (%)	Increase compared to the wild-type strain (%)
1	*Trichoderma koningiopsis* VTCC 31435	$56.37^{a} \pm 2.70$	100^{a}
2	VTCC(a) I-1	$83.74^{c} \pm 1.85$	$148.68^{c} \pm 4.13$
3	VTTC(a) I-2	$83.03^{c} \pm 1.02$	$147.57^{c} \pm 8.83$
4	VTTC(a) I-3	$67.08^{b} \pm 0.62$	$119.15^{b} \pm 4.76$
5	VTCC(a) I-4	$67.57^{b} \pm 0.51$	$120.04^{b} \pm 5.33$
6	VTCC(a) I-5	$68.45^{b} \pm 0.85$	$121.63^{b} \pm 6.22$

Note: Means with different superscript letters in the same column are significantly different (Duncan's test, one-way ANOVA, $p < 0.05$).

The results presented in Table 2 and Figure 3B indicate that all five selected potential mutants exhibited higher or significantly higher inhibitory activity against the growth of *R. solani* mycelium (I_{MG}) compared to the original strain. The mutants VTCC(a) I-1 and VTCC(a) I-2 demonstrated the highest inhibitory activity against *R. solani*, with I_{MG} values of 83.74% and 83.03%, respectively, and these differences were not statistically significant. VTCC(a) I-1 also displayed the highest antifungal activity of the metabolites, as indicated by the highest I_{CMA} value. While the mutant VTCC(a) I-5 had an I_{CMA} value not statistically different from VTCC(a) I-1 in the cellophane membrane assay, its inhibitory activity against *R. solani* mycelial growth was lower than that of VTCC(a) I-1 and VTCC(a) I-2, and it was not statistically different from the average I_{MG} of mutants VTCC(a) I-3 and VTCC(a) I-4.

According to Soufi et al. (2021), mutants exhibiting greater efficacy through specific

antagonistic mechanisms may simultaneously be considered inefficient regarding other antagonistic properties. This characteristic clearly indicates the semi-specificity in the interaction between *Trichoderma* and its hosts. This also explains why VTCC(a) I-5 exhibits high antifungal activity of media-permeable metabolites against *R. solani*, with I_{CMA} reaching 75.61% in the cellophane membrane test. However, its mycelial growth inhibition activity (I_{MG}) is not as high compared to other mutants in the dual culture test.

Random mutagenesis by gamma irradiation is one of the strategies to enhance the antifungal activity of various *Trichoderma* species. In the study by Wash et al. (2015), 10-day-old spores of *T. viride* were irradiated with gamma cobalt-60 at doses of 0, 20, 30, 40, and 50 krad to induce mutations. *In vitro* testing of 16 selected mutants revealed that the mutant TVGM1 exhibited high antagonistic efficacy against *S. rolfsii*, *Rhizoctonia bataticola* and *Fusarium oxysporum*, had the highest chitinase activity, reaching 0.62 units/mg. Abbasi et al. (2016) used gamma radiation at a dose of 250 Gy to mutate *Trichoderma harzianum*, resulting in mutants with increased spore production, invasion rate, and enhanced antagonistic ability compared to the original wild strain. Dual culture and culture filtrate tests, along with molecular markers, demonstrated that some mutants had superior biocontrol efficacy against *Macrophomina phaolina*, *R. solani*, *Sclerotinia sclerotiorum* and *Fusarium graminearum*, and exhibited phenotypic and genotypic differences from the wild strain. In another study, Soufi et al. (2021) investigated the antagonistic efficacy of gamma-irradiated *T. aureoviride* mutants against three soil-borne pathogens (*F. graminearum, S. sclerotiorum*, and *R. solani*) under in vitro conditions. They used rep-PCR to analyze the genetic variation of the mutant and wild strains. The results indicated that the mutants exhibited varying inhibitory effects on the pathogens, with higher antagonistic activity against *F. graminearum* compared to *R. solani* and *S. sclerotiorum*. Five mutants with the lowest genetic similarity to the wild strain showed significantly improved antagonistic efficacy.

In our study, the mutant VTCC(a) I-1 exhibited the highest antifungal activity of media-permeable metabolites against *R. solani* (I_{CMA}) as well as the highest mycelial growth inhibition (I_{MG}) among the screened mutants, with these values significantly increased (by 154.12% for I_{CMA}, and 148.68% for I_{MG}) compared to the original *T. koningiopsis* VTCC 31435 strain. Moreover, the average ICMA and/or IMG values of this mutant did not exhibit statistically significant differences and remained stable for at least six consecutive generations (Table 3).

Table 3. The antifungal activity of media-permeable metabolites (I_{CMA}) and mycelial growth inhibition activity (I_{MG}) of VTCC(a) I-1 against *Rhizoctonia solani* after 6 consecutive generations

Generations	I_{CMA} (%)	I_{MG} (%)
1	$79.25^{a} \pm 4.01$	$83.74^{a} \pm 1,85$
2	$79.88^{a} \pm 2.30$	$85.14^{a} \pm 3.96$
3	$81.61^{a} \pm 6.40$	$79.87^{a} \pm 1.46$
4	$78.35^{a} \pm 2.22$	$83.42^{a} \pm 4.20$
5	$81.70^{a} \pm 3.95$	$81.10^{a} \pm 3.24$
6	$80.00^{a} \pm 1.30$	$83.02^{a} \pm 3.82$

Note: Means with the same superscript letters in the same column are not significantly different (Duncan's test, one-way ANOVA, $p < 0.05$).

CONCLUSION

The results of the study demonstrate that gamma irradiation is an effective method to enhance the antagonistic ability of *T. koningiopsis* VTCC 31435 against the pathogenic fungus *R. solani*, which causes sheath blight in rice plants. Gamma irradiation

induced changes in morphological and growth characteristics, as well as increased the antifungal activity of metabolites (I_{CMA}) and the inhibitory effect on the mycelial growth of pathogens (I_{MG}) in some radiation-resistant colonies. These effects may be due to random genetic mutations caused by gamma radiation. However, to confirm this hypothesis, further studies on the molecular characteristics of the potential mutant strains are required. Additionally, field trials should be conducted to evaluate the effectiveness of these mutants before they are applied in the production of biocontrol products for the prevention of fungal diseases in rice plants.

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REFERENCES

Abbas A., Jiang D., Fu Y., 2017. *Trichoderm*a spp. as antagonist of *Rhizoctonia solani*. *J Plant Pathol Microbiol*, 8: 402. https://doi: 10.4172/2157-7471.1000402

Abbasi A., Safaie N., Shams-bakhsh M., Shahbazi S., 2016. Biocontrol activities of gamma induced mutants of *Trichoderma harzianum* against some soilborne fungal pathogens and their DNA fingerprinting. *Iran J Biotech*, 14(4): e1224. http://doi.org/10.15171/ijb.1224

Alfiky A., 2019. Effects of ultraviolet irradiation on the in vitro antagonistic potential of *Trichoderma* spp. against soil-borne fungal pathogens. *Heliyon*, 5(7): e02111. http://doi.org/10.1016/j.heliyon.2019.e02111

Baharvad A., Shahbazi S., Afsharmanesh H., Ebrahimi M. A., Askari H., 2014. Investigation of gamma irradiation on morphological characteristics and antagonist potential of *Trichoderma viride* against *M. phaseolina*. *International Journal of Farming and Allied Sci.*: 1157–1164.

Darabzadeh D., Esfahani Z. H., Hejazi P., 2018. Improvement of cellulase production and its characteristics by inducing mutation on *Trichoderma reesei* 2414 under solid state fermentation on rice by products. *App food Biotechnol.*, 5(1): 11–18. doi: 10.22037/afb.v5i1.18651

Davati N., Najafi M. B., 2013. Overproduction strategies for microbial secondary metabolites: A review. *Life Science*, 3(1): 23–37.

De Franca S. K. S., Cardoso A. F., Lustosa D. C., Ramos E. M. L. S., De Filippi M. C. C., Silva G. B. D., 2015. Biocontrol of sheath blight by *Trichoderma asperellum* in tropical lowland rice. *Agron Sustain Dev*., 35: 317–324. http://doi.org/10.1007/s13593-014-0244-3

Diep T. B., Thom N. T., Sang H. D., Thao H. P., Binh N. V., Thuan T. B., Lan V. T. T., Quynh T. M., 2016. Screening streptomycin resistant mutations from gamma ray irradiated *Bacillus subtilis* B5 for selection of potential mutants with high production of protease. *VNU J Sci Nat Sci Technol*, 32(1S): 170–176.

Diep T. B., Thom N. T., Sang H. D., An T. X., Binh N. V., Quynh T. M., 2020. Effect of gamma irradiation on the viability and cellulase production of some filamentous fungi. *Vietnam Journal of Biotechnology*, 18(2): 341–348. http://doi.org/10.15625/1811-4989/18/2/15280

Harman G. E., 2006. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology*, 96(2): 190–194. https://doi.org/10.1094/PHYTO-96-0190

Hoe P. C. K., Rahim K. A., Saud H. M., 2016. A review on microbial mutagenesis through gamma irradiation for agricultural applications. *JSNM*, 28(2): 20–29.

Kotasthane A., Agrawal T., Kushwah R. and Rahatkar O. V., 2015. In-vitro antagonism of *Trichoderma* spp. against *Sclerotium rolfsii* and *Rhizoctonia solani* and their response towards growth of cucumber, bottle gourd and bitter gourd. *European J. Plant Pathol*., 141(3): 523–543. https://doi.org/10.1007/s10658-014-0560-0

Liu S. Y., Feng P. C., Lo C. T., Peng K. C., 2010. Plate assay for fungal enzymes using cellophane membranes. *Anal. Biochem*, 397: 121–123. http://doi.org/10.1016/j.ab.2009.09.016

Mathivanan N., Prabavathy V. R., Vijayanandraj V. R., 2005. Application of talc formulations of *Pseudomonas fluorescens* Migula and *Trichoderma viride* Pers. ex SF Gray decrease the sheath blight disease and enhance the plant growth and yield in rice. *J Phytopathol*, 153: 697–701. http://doi.org/10.1111/j.1439-0434.2005.01042.x

Naeimi S., Okhovvat S. M., Javan-Nikkhah M., VágvÖlgyi C., Khosravi V., Kredics L., 2011. Biological control of *Rhizoctonia solani* AG1-1A, the causal agent of rice sheath blight with *Trichoderma* strains. *Phytopathol. Mediterr.*, 49(3): 287–300. http://doi.org/10.14601/Phytopathol_Mediterr-3437

Nguyen T. D., Phan Q. K., Do A. D., 2024. Antagonistic activities of *Trichoderma* spp. isolates against *Neoscytalidium dimidiatum* causing brown spot disease on dragon fruit *Hylocereus undatus*. *J App Biol Biotech*, 12(1): 265–272. https://doi.org/10.7324/JABB.2024.152864

Rojio F. G., Reynoso M. M., Ferez M. and Chulze S. N., 2007. Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions. *Crop Protection*, 26(4): 549–555. http://doi.org/10.1016/j.cropro.2006.05.006

Schirmbock M., Lorito M., Wang Y. L., Hayes C. K., Arisanatac I., Scala F., Harman G. E., Kubicek C. P., 1994. Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. *App. Environl Microbiol*, 60: 4364–4370. http://doi.org/10.1128/AEM.60.12.4364-4370.1994

Senapati M., Tiwari A., Sharma N., Chandra P., Bashyal B. M., Ellur R. K., Bhowmick P. K., Bollinedi H., Vinod K. K., Singh A. K., Krishnan S. G., 2022. *Rhizoctonia solani* Kühn pathophysiology: Status and prospects of sheath blight disease management in rice. *Front. Plant Sci.*, 13: 881116. http://doi.org/10.3389/fpls.2022.881116

Sharma R., Singh D., Singh R., 2009. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. *Biol Control*, 50(3): 205–221. https://doi.org/10.1016/j.biocontrol.2009.05.001

Soufi E., Safaie N., Shahbazi S., Mojerlou S., 2021. Gamma irradiation induces genetic variation and boosting antagonism in *Trichoderma aureoviride*. *Arch Phytopath Plant Protect*, 54(19–20): 1649–1674. http://doi.org/10.1080/03235408.2021.1936377

Tao V. X., Tuan T. V., 2020. Isolation and characterization of *Trichoderma* strains antagonistic against pathogenic fungi on orange crops. *VNU J Sci.*: *Natural Sciences and Technology*, 36(3): 98–104. http://doi.org/10.25073/2588-1140/vnunst.5086 (in Vietnamese with English summary).

Thanh N. T., Nhung H.T., Thuy N. T., Lam T. T. N., Giang P. T., Lan T, N., Viet N. V., Man V. T., 2014. The diversity and antagonistic ability of *Trichoderma* spp. on the *Aspergillus Flavus* pathogen on peanuts in North Center of Vietnam. *World Journal of Agricultural Research*, 2(6): 291–295. http://doi.org/10.12691/wjar-2-6-8

Wash S., Ingle S.T., Dandale S., Mane S. S., 2015. Improvement in biocontrol ability of *Trichoderma viride* through gamma irradiation. *Trends in Biosciences*, 8(20): 5622–5626.

Zhang F., Ge H., Zhang F., Guo N., Wang Y., Chen L., Ji X., Wei Li C., 2016. Biocontrol potential of *Trichoderma harzianum* isolates T-aloe against *Sclerotinia sclerotiorum* in soybean. *Plant Physiol. Biochem.*, 100: 64–74. http://doi.org/10.1016/j.plaphy.2015.12.017