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Biocontrol and organic management strategies against *Alternaria brassicae* using native *Trichoderma* spp. in mustard crop

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Abstract
Trichoderma spp. were isolated from soils of Chhattisgarh and screened for antagonistic activity against *Alternaria brassicae* using dual culture and inverted plate methods. Among the isolates, *Trichoderma harzianum* (T_6) showed the strongest inhibition of *A. brassicae*, with 53.80% inhibition in dual culture and 42.83% through volatile compounds. For mass application, *T. harzianum* was multiplied and combined with organic amendments (Karanj, Mahua, and Mustard cakes at 3% w/w) and applied to both pot and field soils. The effectiveness of treatments was evaluated by assessing percent disease index (PDI) on mustard at 75 and 90 days after sowing (DAS). The integrated treatment of *Trichoderma* with organic amendments significantly reduced disease severity compared to *Trichoderma* alone, demonstrating a synergistic effect. The native isolates T6 and T4 exhibited strong antagonism via mycoparasitism and volatile compounds, highlighting their potential as effective biocontrol agents. Overall, the integrated use of *Trichoderma* and organic amendments offers a promising strategy for managing *Alternaria* leaf spot in mustard.

Keywords: *Trichoderma harzianum*, *Alternaria brassicae*, biocontrol, organic amendments, mustard leaf spot, volatile compounds

Introduction
Mustard (*Brassica* spp.) is an ancient crop of culinary, medicinal, and nutritional significance, with key species including *Sinapis alba*, *Brassica juncea*, and *Brassica nigra* (Thomas *et al.*, 2012) [23]. Despite being nutrient-rich (Tyagi and Singh, 2019) [24], India faces yield gaps due to biotic and abiotic stresses, notably diseases like Alternaria blight, white rust, and Sclerotinia stem rot (Gupta *et al.*, 2016) [11]. Dark leaf spot caused by *Alternaria brassicae* is a major constraint, and current cultivars lack resistance (Sharma *et al.*, 2013) [21]. Although India's mustard production reached 13.26 million tons in 2023-24 (Anonymous, 2024) [2], regions like Bastar require localized disease management. Chemical control raises environmental concerns, prompting the need for eco-friendly solutions. *Trichoderma* species, such as *T. harzianum* and *T. asperellum*, exhibit mycoparasitic behavior by coiling around and penetrating the hyphae of plant pathogens. This process involves the secretion of cell wall-degrading enzymes (CWDEs) like chitinases, glucanases, and proteases, which break down the pathogen's cell walls, leading to their inhibition or death (Sood *et al.*, 2020) [22]

Organic amendments are considered along with *Trichoderma* spp. because they serve as substrates that improve soil health and microbial activity, aiding in the decomposition of agricultural waste and contributing to a cleaner environment. Moreover, *Trichoderma* spp. also help in the bioremediation of pesticide residues. Additionally, *Trichoderma* spp. help improve root architecture, enhancing nutrient uptake and plant vigor which indirectly increases plant resistance to diseases like Alternaria blight (Zin and Badaluddin 2020) [30]. Previous research has consistently demonstrated the potential of *Trichoderma* species as effective biocontrol agents against a range of plant pathogens, including *Alternaria brassicae*. Studies have shown that *Trichoderma harzianum* exhibits strong antagonistic activity through mechanisms such as competition, mycoparasitism, and production of volatile and non-volatile antifungal compounds (Harman *et al.*, 2004; Vinale *et al.*, 2008) [12, 26].

This study aimed to isolate native *Trichoderma* from Bastar and evaluate their biocontrol potential against *A. brassicae*, including integration with organic amendments for sustainable mustard health.

Materials and Methods

The field experiment was conducted during the *rabi* season 2023-24 at the Research cum Instructional Farm of S. G. College of Agriculture and Research Station, while the laboratory experiment was carried out at the Plant Pathology Laboratory of the same institution, located in Jagdalpur, Chhattisgarh. The experimental site is in the high-rainfall, relatively cool, and humid Southern Bastar Plateau agro-climatic zone of Chhattisgarh, classified as moist and sub-humid.

Isolation of *Trichoderma*: Rhizospheric soil samples were collected in aseptic poly bags from various agricultural fields in Bastar, Chhattisgarh, India. For serial dilution, 1g of soil was mixed with 9ml of sterilized distilled water. The 10^{-4} and 10^{-5} dilutions were plated on Rose bengal agar to isolate *Trichoderma* spp. Single colonies of each isolate were cultured on PDA plates at 27±2 °C and subcultured every 30 days for further study (Yadav *et al.*, 2018) [29]. Antagonist effect of *Trichoderma* isolates against *Alternaria brassicae* was tested by dual culture technique (Dennis and Webster, 1971[a]) [8]. Inhibitory effect of volatile compounds of *Trichoderma* isolates was tested against the *Alternaria brassicae* by following the "Inverted plate technique" as described by Dennis and Webster (1971[b]) [9]. Mycelial growth of *A. brassicae* was recorded in seven days after inoculation. and percent inhibition was calculated by using the formula as proposed by Vincent (1947) [27].

$$I = \frac{C-T}{C} \times 100$$

Where:
I = Inhibition of mycelial growth (%)
C = Growth of pathogen in control (mm)
T = Growth of pathogen in treatment (mm)

Field and pot experiment, using Randomized Block Design (RBD) and Complete Randomized Design (CRD), respectively, investigated the effectiveness of *Trichoderma harzianum* and various organic cakes (*Karanj*, *Mahua*, Mustard) in controlling *Alternaria brassicae* leaf spot on mustard. Nine treatments, including combinations of the cakes with *T. harzianum*, *T. harzianum* alone, and a control, (Table 3) were evaluated. In the field, plots were 3 × 2 meters with 3 replications, and plants were spaced at 30 × 10 cm. *T. harzianum* was cultured and applied at 0.5% (v/w) after 15 days of multiplication with organic amendments. Amended soil received *T. harzianum* at 3% (w/w) and 200 kg/ha in the field. Disease severity was recorded as percent disease index at 75 and 90 days after sowing (DAS) or on pods before harvest. Data in parenthesis (Table 3) shows Arcsine transformation. The intensity of the disease was calculated by following 0-9 scale on randomly selected plants among the same place as under:

Table 1: Scale (0-9) for rating of *Alternaria* blight will be used

Rating	Reactions	Criteria
0	Immune	No lesion
1	Highly resistance	Non-sporulating pinpoint size or small brown necrotic spots, less than 5% leaf area covered by lesion
3	Resistance	Small roundish slightly sporulating larger brown necrotic spots, about 1-2 mm in diameter with a distinct margin or yellow halo, 5-10% leaf area covered by lesions
5	Moderately resistance	Moderately sporulating, non-coalescing larger brown spots, about 2-4 mm in diameter with a distinct margin or yellow halo, 11-25% leaf area covered by the spots
7	Susceptible	Moderately sporulating, coalescing larger brown spots about 4-5 mm in diameter, 26-50% leaf area covered by the lesions
9	Highly susceptible	Profusely sporulating, rapidly coalescing brown to black spots measuring more than 6mm diameter without margins covering more than 50% leaf area

Percent Disease Index (PDI) was worked out by using formula given by Wheeler (1969) [28].

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of the scores}}{\text{Number of observation} \times \text{Highest number in rating scale}} \times 100$$

Results

In both dual culture and volatile compound assays, the native *Trichoderma* isolate T6 showed the highest antagonistic activity against *Alternaria brassicae*. In the dual culture method, T6 recorded a maximum radial growth inhibition of 53.80%, with the pathogen's mycelial growth restricted to 24.33 mm, and it overgrew the pathogen in just three days. *Trichoderma* isolate T4 followed closely, showing 48.10% inhibition and taking four days to overgrow the pathogen. In the volatile compound assay, isolate T6 again performed best, achieving 42.83% inhibition with pathogen growth limited to 49.83 mm. These results demonstrate T6's strong potential through both direct mycoparasitism and the production of inhibitory volatile compounds.

In pot and field experiments, the combination treatment T7 (Karanj Cake + Mahua Cake + Mustard Cake + *Trichoderma harzianum*) consistently recorded the lowest disease severity. In pots at 75 DAS, T7 achieved a minimum disease severity of 14.07%, reducing disease by 64.49% over control. At 90 DAS, severity remained low at 24.07%, corresponding to a 63.07% reduction. Similarly, in field trials, T7 resulted in 15.56% disease severity at 75 DAS (62.50% reduction) and 23.33% at 90 DAS (64.41% reduction). Treatments involving organic cakes alone or *T. harzianum* alone were less effective compared to the combined application. The integrated application significantly improved disease suppression compared to individual treatments, indicating a synergistic effect between organic amendments and *Trichoderma*. Overall,

Trichoderma isolate T6 and organic amendments, particularly when combined, were highly effective in suppressing Alternaria blight under both laboratory and field conditions.

Discussion

The observed inhibition may be attributed to mechanisms such as mycoparasitism (as evidenced by dual culture assays) and the production of inhibitory volatile compound (Dennis and Webster, 1971c) [10]. Additionally, the research identified key volatile compounds produced by the *Trichoderma* isolates, such as glacial acetic acid and propyl-benzene, which exhibited antifungal properties. These findings support the use of *Trichoderma* spp. in the biological control of fungal pathogens affecting crops (Meena *et al.*, 2017) [16]. *Trichoderma* species produce volatile organic compounds (VOCs) like 6-pentyl-α-pyrone (6-PP), alcohols, aldehydes, aromatics, esters, heterocycles, ketones, terpenes and thiols, which inhibit Alternaria growth and sporulation. These VOCs disrupt cell membranes, respiration, and induce oxidative stress in the pathogen. Among the tested isolates, T_6 exhibited the highest efficacy in both mycoparasitism and volatile compound production. Similarly, efficacy of various species of *Trichoderma* against *A. brassicae* was also observed by several workers when they evaluated *Trichoderma* sp. by various ways, supports the present findings (Kanwar *et al.*, 2016; Kumari and Sharfuddin, 2022; Meena *et al.*, 2017; Nagamani *et al.*, 2017; Raju *et al.*, 2008; Amen *et al.*, 2010; Arzanlou *et al.*, 2013) [14, 15, 16, 18, 20, 3, 4].

Incorporating oil cakes into the soil enhances cation exchange capacity, improves water retention, and contributes to better soil structure, promoting plant growth while also effectively reducing harmful soil-borne pathogens (Ayyandurai *et al.*, 2022) [5]. *Mahua* and *Neem* cakes, in particular, have shown strong antimicrobial properties and disease-suppressing effects. Notably, oil cakes rich in secondary metabolites such as phenolic and flavonoid compounds demonstrated significant antimicrobial activity (Karmelreetha and Muthukumar, 2020) [13]. Additionally, the breakdown of organic amendments or the activity of microorganisms involved in their decomposition leads to the release or production of various antimicrobial by-products, including organic acids, hydrogen sulfide, phenols, tannins, and nitrogenous compounds (Anis *et al.*, 2010) [1]. These combined effects are largely responsible for the suppression of Alternaria disease.

Addition of various types of organic amendments in soil either alone or in combination with antagonists shows effectiveness in reduction of pathogen propagules, improved plant vigour etc. were also reported by earlier worker against *A. brassicae* (Azim *et al.*, 2019; Choudary and Ashraf 2019; Neeraj and Singh 2011; Rafi *et al.*, 2016 and Udupa *et al.*, 2022) [6, 7, 17, 19, 25] agreeing with the present findings.

Table 2: Effect of native *Trichoderma* isolates on mycelial growth of *Alternaria brassicae*

***Trichoderma* isolates**	**Dual culture**			**Volatile compounds**	
	Mycelial growth mm*	**percent inhibition of radial growth**	**Time taken(day) to completely overgrow the pathogen**	**Mycelial growth mm***	**Percent inhibition of radial growth**
T_1	31.33	40.51	5	63.67	26.96
T_2	30.00	43.04	5	64.33	26.2
T_3	29.67	43.67	5	62.67	28.11
T_4	27.33	48.1	4	55.33	36.52
T_5	35.67	32.28	6	70.17	19.5
T_6	24.33	53.8	3	49.83	42.83
T_7	29.33	44.3	5	58.17	33.27
Control	52.67	-	-	87.17	-
C.D.	1.553			1.437	
SE(m)±	0.514			0.475	
C.V.	2.734			1.287	

*Mean of three replications

Table 3: Effect of organic rich amendments against *Alternaria* leaf blight disease of Mustard

Treatments	Pot (75 DAS)		Pot (90 DAS)		Field (75 DAS)		Field (90 DAS)	
	% disease severity*	% decrease over control	% disease severity*	% decrease over control	% disease severity*	% decrease over control	% disease severity*	% decrease over control
T_1-*Karanj* Cake + *T. harzianum*	27.04 (31.31)	31.78	44.81 (42.00)	31.25	27.41 (31.43)	33.93	44.07 (41.32)	32.77
T_2-*Mahua* Cake + *T. harzianum*	24.81 (29.84)	37.38	42.22 (40.50)	35.23	25.56 (30.22)	38.39	41.48 (40.00)	36.72
T_3-Mustard Cake + *T. harzianum*	28.15 (32.00)	28.97	46.67 (43.07)	28.41	28.89 (32.37)	30.36	45.56 (42.39)	30.51
T_4-*Karanj* Cake + *Mahua* Cake + *T. harzianum*	20.74 (27.06)	47.66	34.07 (35.69)	47.73	21.11 (27.05)	49.11	34.81 (36.02)	46.33
T_5-*Karanj* Cake + Mustard Cake + *T. harzianum*	24.07 (29.34)	47.66	41.48 (40.07)	36.36	26.93 (30.53)	37.50	41.85 (40.23)	36.72
T_6-*Mahua* Cake + Mustard Cake + *T. harzianum*	23.70 (29.10)	40.19	39.26 (38.78)	39.77	25.19 (30.00)	39.29	40.00 (39.14)	38.98
T_7-*Karanj* Cake + *Mahua* Cake + Mustard Cake + *T. harzianum*	14.07 (22.02)	64.49	24.07 (29.34)	63.07	15.56 (23.27)	62.50	23.33 (28.60)	64.41
T_8-*T. harzianum* without cake	31.48 (34.11)	20.56	51.85 (46.05)	20.45	33.33 (34.96)	19.64	52.59 (46.49)	20.34
T_9-Control (No use of *Trichoderma*)	39.63 (39.00)		65.19 (53.83)		41.48 (40.04)		65.56 (54.15)	
C.D.	4.10		6.06		7.32		10.42	
SE(m)±	1.36		2.00		2.42		3.44	
C.V.	9.05		8.02		13.48		14.58	

*Mean of three replications; Data in parenthesis shows Arcsine transformation

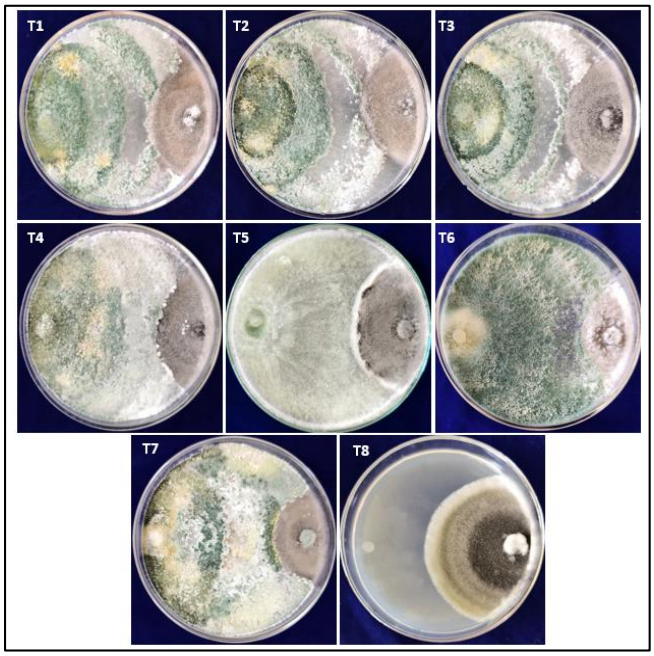

Plate 1: Shows Dual culture of *Trichoderma* strains against *Alternaria brassicae*

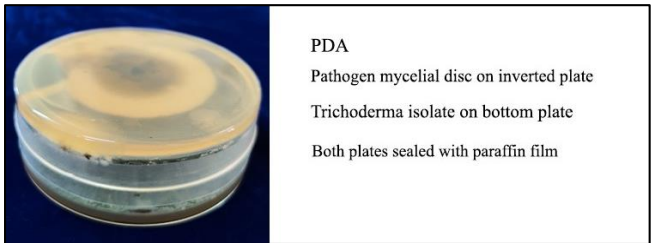

Plate 2: Illustration of double plate technique

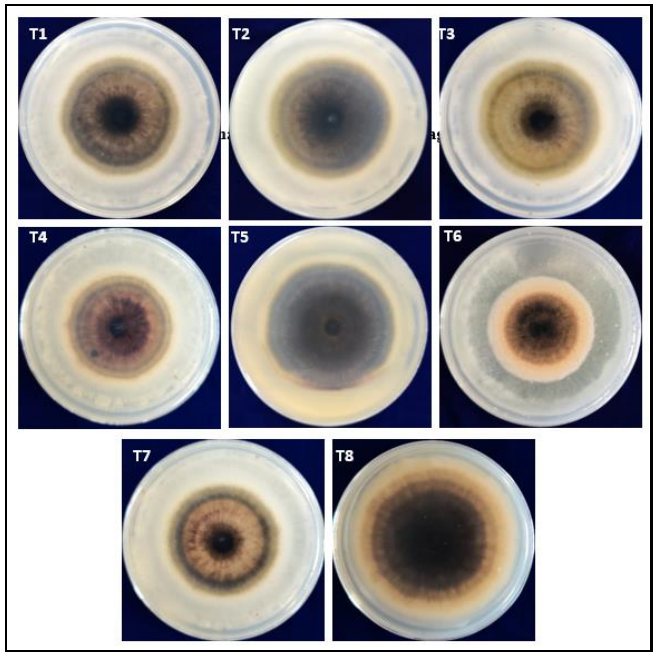

Plate 3: Shows volatile effect of native *Trichoderma* isolates against *Alternaria brassicae*

Conclusion

A *rabi* 2023-24 survey in Bastar district showed varying Alternaria blight incidence on crucifers, linked to environmental and agricultural factors. Isolated *Alternaria* spp. displayed diverse growth and morphology. Native *Trichoderma* isolates, especially T_6 and T_4, showed strong antagonism against *A. brassicae*, highlighting their potential as eco-friendly biocontrol agents. The combination of *Karanj* Cake, *Mahua* Cake, Mustard Cake, and *T.*

harzianum (T_7) effectively reduced Alternaria leaf spot, likely by suppressing the pathogen and boosting plant.

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References

1. Anis M, Zaki MJ, Dawar S. Effect of oilseed cakes alone or in combination with *Trichoderma* species for the control of charcoal rot of sunflower (*Helianthus annuus* L.). Pak J Bot. 2010;42(6):4329-4333.
2. Anonymous. Agriculture Statistics at a Glance 2023. New Delhi (India): Government of India, Ministry of Agriculture & Farmers Welfare, Department of Agriculture & Farmers Welfare, Economics, Statistics & Evaluation Division; 2024. p. 52.
3. Amen F, Razdan VK, Mohiddin FA, Bhat KA, Sheikh PA. Effect of volatile metabolites of *Trichoderma* species against seven fungal plant pathogens *in vitro*. J Phytol. 2010;2(10):34-37.
4. Arzanlou M, Khodaei S, Narmani A, Babai-Ahari A, Azar AM. Inhibitory effect of *Trichoderma* isolates on growth of *Alternaria alternata*, the causal agent of leaf spot disease on sunflower, under laboratory conditions. Arch Phytopathol Plant Prot. 2013;47(13):1592-1599.
5. Ayyandurai M, Akila R, Mini ML, Manonmani K. Gas chromatography-mass spectrometry (GC-MS) analysis of antimicrobial compounds produced by mahua oil cake against the stem rot pathogen *Sclerotium rolfsii*. J Appl Nat Sci. 2022;14(2):600-606.
6. Azim K. Organic amendments to alleviate plant biotic stress. In: Plant Health Under Biotic Stress. 2019. p. 147-165.
7. Choudhary A, Ashraf S. Utilizing the combined antifungal potential of *Trichoderma* spp. and organic amendments against dry root rot of mungbean. Egypt J Biol Pest Control. 2019;29(1):83.
8. Dennis C, Webster J. Antagonistic properties of species groups of *Trichoderma*. II. Production of volatile antibiotics. Trans Br Mycol Soc. 1971;57:41-58.
9. Dennis C, Webster J. Antagonistic properties of species groups of *Trichoderma*. III. Hyphal interactions. Trans Br Mycol Soc. 1971;57:363-369.
10. Dennis C, Webster J. Antagonistic properties of species groups of *Trichoderma*. I. Production of non-volatile antibiotics. Trans Br Mycol Soc. 1971;57(1):25-39. doi:10.1016/S0007-1536(71)80077-3.
11. Gupta AK, Raj R, Kumari K, Singh SP, Solanki IS, Choudhary R. Management of major diseases of Indian mustard through balanced fertilization, cultural practices and fungicides in calcareous soils. Proc Natl Acad Sci India Sect B Biol Sci. 2016;88(1):229-239.
12. Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. *Trichoderma* species—opportunistic, avirulent plant symbionts. Nat Rev Microbiol. 2004;2(1):43-56.
13. Karmelreetha A, Muthukumar A. Role of oil cakes against soil borne pathogens, an eco-friendly approach. JPS Scientific Publications. 2020;11:120-127.
14. Kanwar H, Trivedi A, Sharma SK. Efficacy of eco-friendly management practices of powdery mildew, *Alternaria* blight and bacterial leaf blight of cluster bean under organic farming. Adv Life Sci. 2016;5(6):2324-2332.
15. Kumari S, Sharfuddin C. Antagonistic potential of *Trichoderma simmonsii* isolated from Bihar against pathogenic fungus *Alternaria brassicae*. J Mycopathol Res. 2022;60(2):245-249.
16. Meena M, Swapnil P, Zehra A, Dubey MK, Upadhyay RS. Antagonistic assessment of *Trichoderma* spp. by producing volatile and non-volatile compounds against different fungal pathogens. Arch Phytopathol Plant Prot. 2017:1-20.
17. Neeraj, Singh K. Organic amendments to soil inoculated arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* treatments reduce the development of root-rot disease and enhance the yield of *Phaseolus vulgaris* L. Eur J Soil Biol. 2011;47(5):288-295.
18. Nagamani P, Bhagat S, Biswas MK, Viswanath K. Effect of volatile and non-volatile compounds of *Trichoderma* spp. against soil borne diseases of chickpea. Int J Curr Microbiol App Sci. 2017;6(7):1486-1491.
19. Rafi H, Dawar S, Tariq M. Combined effect of soil amendment with oil cakes and seed priming in the control of root rot fungi of leguminous and non-leguminous crops. Pak J Bot. 2016;48(3):1305-1311.
20. Raju SK, Kumar KVK, Rajamannar M. *in vitro* efficacy of volatile and non-volatile metabolites of *Trichoderma* species on rice sheath blight pathogen, *Rhizoctonia solani* Kühn. Oryza. 2008;45(1):84-86.
21. Sharma M, Deep S, Bhati DS, Chowdappa P, Selvamani R, Sharma P. Morphological, cultural, pathogenic and molecular studies of *Alternaria brassicae* infecting cauliflower and mustard in India. Afr J Microbiol Res. 2013;7(26):3351-3363.
22. Sood M, Kapoor D, Kumar V, Sheteiwy MS, Ramakrishnan M, Landi M, *et al. Trichoderma*: the "secrets" of a multitalented biocontrol agent. Plants. 2020;9(6):762. doi:10.3390/plants9060762.
23. Thomas J, Kuruvilla KM, Hrideek TK. Mustard. In: Peter KV, editor. Handbook of Herbs and Spices. Cambridge (UK): Woodhead Publishing; 2012. p. 388-398.
24. Tyagi PK, Singh R. Rapeseed and Mustard: Breeding Objectives of Rapeseed and Mustard. Saarbrücken (Germany): Lambert Academic Publishing; 2019. p. 11.
25. Udupa AV, Balakrishna G, Shivanna MB. Influence of non-edible oil cakes and their composts on growth, yield and *Alternaria* leaf spot disease in chilli. Int J Recycl Org Waste Agric. 2022;11:301-318.
26. Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M. *Trichoderma*-plant-pathogen interactions. Soil Biol Biochem. 2008;40(1):1-10.
27. Vincent JM. Distortion of fungal hyphæ in the presence of certain inhibitors. Nature. 1947;159(4051):850.
28. Wheeler BEJ. An Introduction to Plant Diseases. London (UK): John Wiley and Sons Ltd; 1969. p. 301.
29. Yadav RN, Mishra D, Zaidi NW, Singh US, Singh HB. Bio-control efficacy of *Trichoderma* spp. against the major diseases of rice (*Oryza sativa* L.). Int J Agric Environ Biotechnol. 2018;11(3):543-548.
30. Zin NA, Badaluddin NA. Biological functions of *Trichoderma* spp. for agriculture applications. Ann Agric Sci. 2020;65(2):168-178. doi:10.1016/j.aoas.2020.09.003.