



IN-VITRO EVALUATION OF *TRICHODERMA* SPP. ISOLATES FOR THE BIO-CONTROL OF GUAVA WILT PATHOGENS

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ABSTRACT

Guava (*Psidium guajava* L.) is an important fruit crop cultivated widely in tropical and subtropical regions. Among the various diseases affecting guava production, wilt disease caused by different species of *Fusarium* is one of the most destructive. The present investigation was carried out to evaluate the antagonistic potential of different *Trichoderma* isolates against guava wilt pathogens under in vitro conditions. Five *Trichoderma* species viz., *Trichoderma harzianum*, *T. viride*, *T. reesei*, *T. hamatum* and *T. asperellum* were tested against *Fusarium oxysporum*, *F. verticillioides*, *F. solani* and *Fusarium* spp. using the dual culture technique. The results revealed that all the tested *Trichoderma* isolates significantly inhibited the mycelial growth of the pathogens. Maximum inhibition against *F. oxysporum* (50.00%), *F. verticillioides* (55.93%) and *Fusarium* spp. (58.52%) was recorded with *T. harzianum*. In contrast, *T. hamatum* exhibited the highest inhibition against *F. solani* (44.44%). The antagonistic effect of *Trichoderma* species may be attributed to mechanisms such as competition for nutrients and space, mycoparasitism and production of antifungal metabolites. The results suggest that *Trichoderma* spp. can be effectively utilized as biological control agents for the management of guava wilt disease.

Key words: Guava wilt, *Fusarium*, *Trichoderma*, biological control, antagonism, dual culture technique

Introduction

Guava (*Psidium guajava* L.) is one of the most important fruit crops grown in tropical and subtropical regions of the world. India is the leading producer of guava and the crop plays an important role in nutritional security due to its high vitamin C content, dietary fiber and medicinal properties (Singh, 2019; Mitra and Bose, 2020). Despite its economic importance, guava production is severely constrained by several diseases, among which wilt disease is considered the most devastating (Pandey and Dwivedi, 2018).

Guava wilt is mainly caused by soil-borne pathogens belonging to the genus *Fusarium*, particularly *Fusarium oxysporum*, *Fusarium solani* and *Fusarium verticillioides* (Misra and Pandey, 2017; Sharma *et al.*, 2021). The disease is characterized by yellowing of leaves, drooping, vascular discoloration and ultimately plant death.

Once established in an orchard, the disease spreads rapidly and results in severe economic losses to guava growers (Dwivedi and Pandey, 2016).

Chemical control of soil-borne pathogens is often ineffective due to the persistence of pathogens in the soil and may also lead to environmental pollution and development of resistant pathogen strains (Harman *et al.*, 2004; Mukhopadhyay, 2015). Therefore, the use of biological control agents has gained considerable attention as an environmentally safe and sustainable disease management strategy.

Among the various microbial antagonists, species of the genus *Trichoderma* have been widely studied due to their strong antagonistic activity against a wide range of plant pathogenic fungi (Verma *et al.*, 2007; Woo *et al.*, 2014). These fungi are commonly found in soil and rhizosphere environments and play a significant role in

improving plant health.

Trichoderma species suppress plant pathogens through several mechanisms including competition for nutrients and space, antibiosis, mycoparasitism and induction of systemic resistance in host plants (Howell, 2003; Harman, 2006; Kubicek *et al.*, 2019). Therefore, the present investigation was undertaken to evaluate the antagonistic potential of selected *Trichoderma* isolates against major guava wilt pathogens under *in vitro* conditions.

Materials and Methods

Collection and Isolation of Fungal Species

Diseased guava plants showing typical wilt symptoms were collected from orchards of Prayagraj and Kaushambi districts, Uttar Pradesh, India. Infected plant parts (roots and stems) were brought to the laboratory in sterile polythene bags for pathogen isolation. Small tissue pieces along with healthy margins were cut with a sterile scalpel, surface sterilized with 1% sodium hypochlorite for 60 seconds, rinsed three times with sterile distilled water, and aseptically placed on Potato Dextrose Agar (PDA) plates. The plates were incubated at $27 \pm 2^\circ\text{C}$ for fungal growth (Aneja, 2005).

Soil-borne fungi were isolated by the serial dilution technique using rhizosphere soil of infected plants (10^{-3} – 10^{-7} dilutions). One milliliter of suspension was spread on PDA plates and incubated at $25 \pm 2^\circ\text{C}$. The resulting fungal colonies were examined microscopically and identified based on cultural and morphological characteristics. Pure cultures were maintained on PDA slants at 27°C for further studies (Barnett and Hunter, 1998; Aneja, 2005).

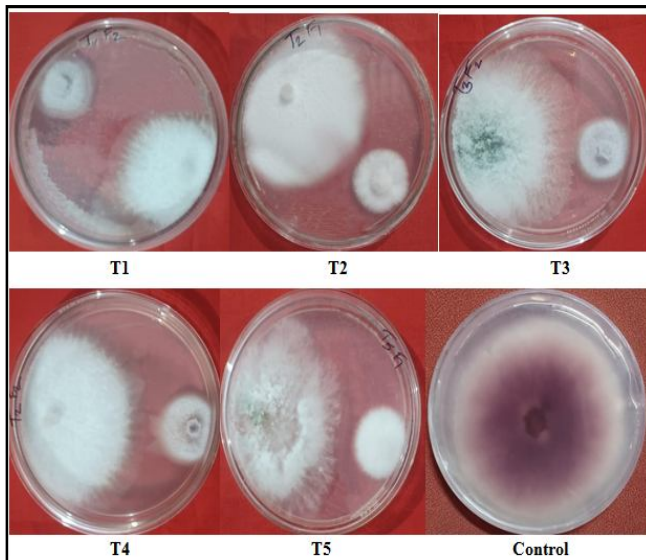


Plate 1: *In-vitro* evaluation of bio-control agent against *Fusarium oxisporium*.

Collection and Procurement of *Trichoderma* spp. Isolates

Soil samples were collected from the rhizosphere region of guava plants from guava-growing orchards. The collected soil samples were subjected to the serial dilution technique ranging from 10^{-3} to 10^{-7} dilutions. From each dilution, 1 ml of the spore suspension was poured onto sterile Petri plates containing Potato Dextrose Agar (PDA) medium and incubated at $25 \pm 2^\circ\text{C}$ for the development of fungal colonies.

In addition, root samples were cut into small fragments and surface sterilized using 1% sodium hypochlorite solution. The sterilized root pieces were washed three times with sterile distilled water to remove traces of the sterilizing agent. The treated segments were then aseptically transferred onto PDA plates and incubated at 25°C to allow the growth of fungal colonies.

The isolates of *Trichoderma* spp. used in the present study were also procured from the Division of Plant Pathology, ICAR–Indian Agricultural Research Institute (ICAR-IARI), Pusa, New Delhi. The obtained cultures were initially maintained on Potato Dextrose Agar (PDA) slants and stored at 4°C for further experimental studies.

Bio-control Agents

Five *Trichoderma* isolates were evaluated:

- *Trichoderma harzianum*
- *Trichoderma viride*
- *Trichoderma reesei*
- *Trichoderma hamatum*
- *Trichoderma asperellum*

Dual culture technique

This study was carried out using a selected isolate of

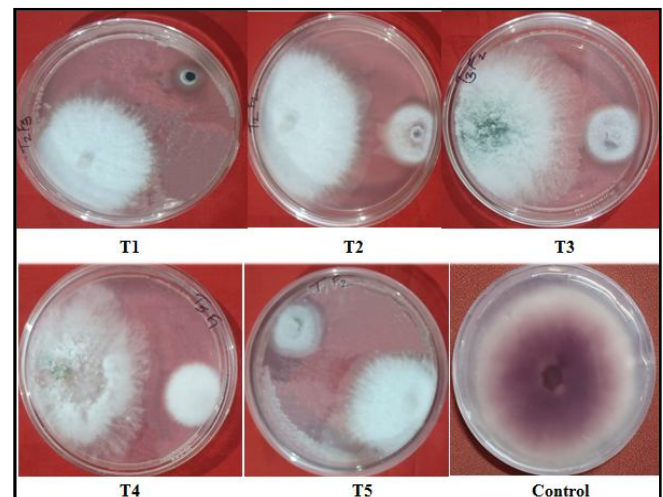


Plate 2: *In-vitro* evaluation of bio-control agent against *F. verticillioides*

Table 1: In-vitro evaluation of bio-control agent against *Fusarium oxysporum*.

Treat-ments	Details	Colony diameter	Inhibition percent
T ₁	<i>Trichoderma harzanium</i>	45.00	50.00
T ₂	<i>Trichoderma viride</i>	49.66	44.82
T ₃	<i>Trichoderma reesei</i>	69.00	23.33
T ₄	<i>Trichoderma hamatum</i>	63.33	29.63
T ₅	<i>Trichoderma asperellum</i>	49.33	45.51
T ₆	Control	90.00	-
	CD at 5%	4.56	
	SE(m)	1.52	

Fusarium spp. The isolate was specifically chosen because of its strong association with guava wilt disease, ensuring that the experimental results would be relevant to practical field conditions affecting guava cultivation. The experiment aimed to evaluate the effectiveness of different bio-agents by studying their interaction with the pathogen and observing the growth behavior of the fungus.

The colony diameter of the test pathogen was measured in millimeters, and the percentage inhibition of mycelial growth was calculated using the formula proposed by Vincent (1947). Observations were recorded at 24-hour intervals. The percent inhibition was calculated using the following formula:

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

Where,

I = Per cent inhibition in growth of test pathogen

C = Radial growth (mm) in control

T = Radial growth (mm) in treatment

Results and Discussion

Evaluate *Trichoderma* spp. isolates for the bio-control of guava wilt against *Fusarium oxysporum*

A total five bio agents were evaluated for their efficacy against *Fusarium oxysporum* through dual culture techniques as explained in material and methods.

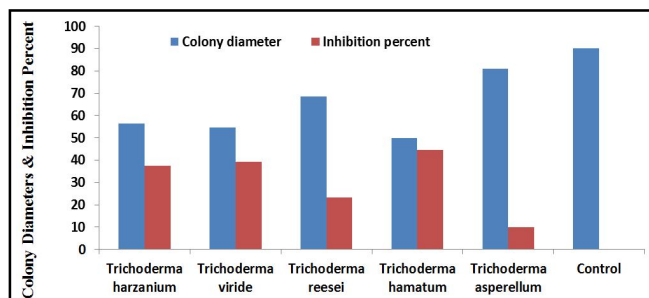


Fig. 1: Effect of *Trichoderma* spp. isolates based on radial growth of *Fusarium oxysporum*.

Table 2: In-vitro evaluation of bio-control agent against *F. verticillioides*

Treat-ments	Details	Colony diameter	Inhibition percent
T ₁	<i>Trichoderma harzanium</i>	39.66	55.93
T ₂	<i>Trichoderma viride</i>	51.66	42.60
T ₃	<i>Trichoderma reesei</i>	64.33	28.52
T ₄	<i>Trichoderma hamatum</i>	56.00	37.77
T ₅	<i>Trichoderma asperellum</i>	44.66	50.37
T ₆	Control	90.00	-
	CD at 5%	3.12	
	SE(m)	1.04	

The result of the study present in the Table 1; Fig. 1. The observation recorded on the inhibition of pathogen in dual culture test revealed that all the five bio agents' viz., *Trichoderma harzanium* (Th-1), *Trichoderma viride* (Tv-1), *Trichoderma reesei* (Tr-1), *Trichoderma hamatum* (Th-1) and *Trichoderma asperillum* (Ta-1) tested inhibited the growth of *Fusarium oxysporum*. The perusal of result showed that *Trichoderma harzanium* (Th-1) gave maximum growth of inhibition of 50.00 % among all the fungal bio agents followed by gave growth inhibition of *Trichoderma asperellum* (Ta-1) gave 45.51 %, *Trichoderma viride* (Tv-1) pathogen give 44.42 % growth inhibition, *Trichoderma hamatum* (Th-1) gave 29.63 % inhibition and least fungal growth inhibition was found in treatment with *Trichoderma reesei* (Tr-1) as 23.33 %.

Evaluation of *Trichoderma* spp. isolates for the bio-control of guava wilt against *F. verticillioides*

A total five bio agents were evaluated for their efficacy against *Fusarium verticillioides* through dual culture techniques as explained in material and methods. The result of the study present in the Table 2; Fig. 2. The observation recorded on the inhibition of pathogen in dual culture test revealed that all the five bio agents' viz., *Trichoderma harzanium* (Th-1), *Trichoderma viride* (Tv-1), *Trichoderma reesei* (Tr-1), *Trichoderma hamatum* (Th-1) and *Trichoderma asperellum* (Ta-1) tested inhibited the growth of *Fusarium oxisporium*. The

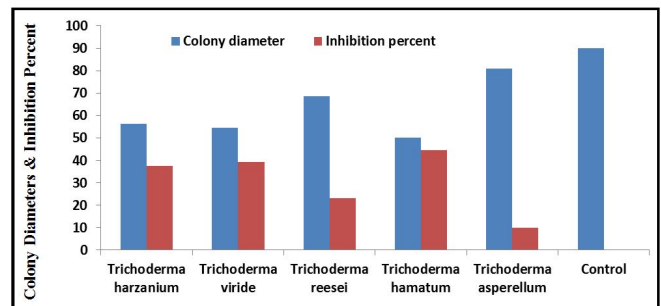


Fig. 2: Effect of *Trichoderma* spp. isolates based on radial growth of *F. verticillioides*.

Table 3: *In-vitro* evaluation of bio-control agent against *Fusarium* spp.

Treat-ments	Details	Colony diameter	Inhibition percent
T ₁	<i>Trichoderma harzanium</i>	37.33	58.52
T ₂	<i>Trichoderma viride</i>	39.00	56.66
T ₃	<i>Trichoderma reesei</i>	83.33	7.41
T ₄	<i>Trichoderma hamatum</i>	52.66	41.48
T ₅	<i>Trichoderma asperellum</i>	60.33	32.96
T ₆	Control	90.00	-
	CD at 5%	4.09	
	SE(m)	1.36	

perusal of result showed that *Trichoderma harzanium* (Th-1) gave maximum growth of inhibition of 55.93 % among all the fungal bio agents followed by gave growth inhibition of *Trichoderma asperellum* (Ta-1) gave 50.37 %, *Trichoderma viride* (Tv-1) pathogen gave 42.60 % growth inhibition, *Trichoderma hamatum* (Th-1) gave 37.77 % inhibition and least fungal growth inhibition was found in treatment with *Trichoderma reesei* (Tr-1) as 28.52 %.

Evaluation of *Trichoderma* spp. isolates for the bio-control of guava wilt against *Fusarium* spp.

A total five bio agents were evaluated for their efficacy against *Fusarium* spp. through dual culture techniques as explained in material and methods. The result of the study present in the Table 3; Fig.3. The observation recorded on the inhibition of pathogen in dual culture test revealed that all the five bio agents' viz., *Trichoderma harzanium* (Th-1), *Trichoderma viride* (Tv-1), *Trichoderma reesei* (Tr-1), *Trichoderma hamatum* (Th-1) and *Trichoderma asperellum* (Ta-1) tested inhibited the growth of *Fusarium oxysporium*. The perusal of result showed that *Trichoderma harzanium* (Th-1) gave maximum growth of inhibition of 58.52% among all the fungal bio agents followed by gave growth inhibition of *Trichoderma viride* (Tv-1) pathogen gave 56.66 % growth inhibition, *Trichoderma hamatum* (Th-1) gave 41.48 % inhibition, *Trichoderma*

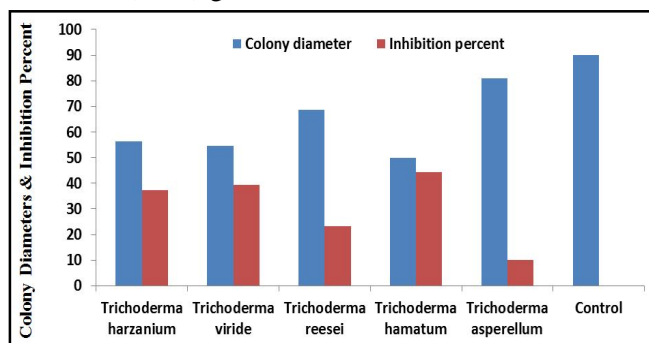


Fig. 3: Effect of *Trichoderma* spp. isolates based on radial growth of *Fusarium* spp.

Table 4: *In-vitro* evaluation of bio-control agent against *F. solani*.

Treat-ments	Details	Colony diameter	Inhibition percent
T ₁	<i>Trichoderma harzanium</i>	56.33	37.41
T ₂	<i>Trichoderma viride</i>	54.66	39.26
T ₃	<i>Trichoderma reesei</i>	68.66	23.21
T ₄	<i>Trichoderma hamatum</i>	50.00	44.44
T ₅	<i>Trichoderma asperellum</i>	81.00	10.00
T ₆	Control	90.00	-
	CD at 5%	2.11	
	SE(m)	0.70	

asperellum (Ta-1) gave 32.96 %, and least fungal growth inhibition was found in treatment with *Trichoderma reesei* (Tr-1) as 7.41 %.

Evaluation of *Trichoderma* spp. isolates for the bio-control of guava wilt against *F. solani*

A total five bio agents were evaluated for their efficacy against *F. solani* through dual culture techniques as explained in material and methods. The result of the study present in the Table 4.; Fig. 4. The observation recorded on the inhibition of pathogen in dual culture test revealed that all the five bio agents' viz., *Trichoderma harzanium* (Th-1), *Trichoderma viride* (Tv-1), *Trichoderma reesei* (Tr-1), *Trichoderma hamatum* (Th-1) and *Trichoderma asperillum* (Ta-1) tested inhibited the growth of *Fusarium oxysporium*. The perusal of result showed that *Trichoderma hamatum* (Th-1) gave maximum growth of inhibition of 44.44% among all the fungal bio agents followed by gave growth inhibition of *Trichoderma viride* (Tv-1) pathogen gave 39.26 % growth inhibition, *Trichoderma harzanium* (Th-1) gave 37.41 % inhibition, *Trichoderma reesei* (Tr-1) gave 23.21 %, and least fungal growth inhibition was found in treatment with *Trichoderma asperellum* (Ta-1) as 10.00 %.

A total five bio agents were evaluated for their efficacy against *Fusarium* spp. through dual culture techniques. The observation recorded on the inhibition of

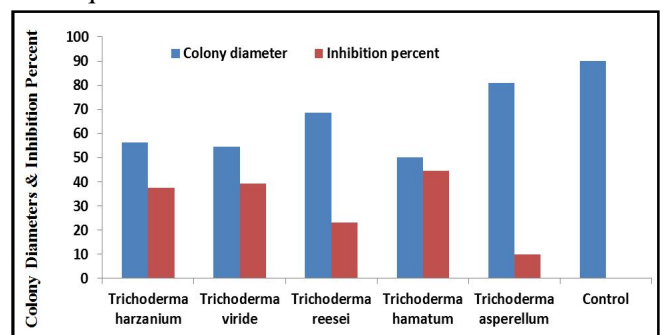


Fig. 4: Effect of *Trichoderma* spp. isolates based on radial growth of *F. solani*.

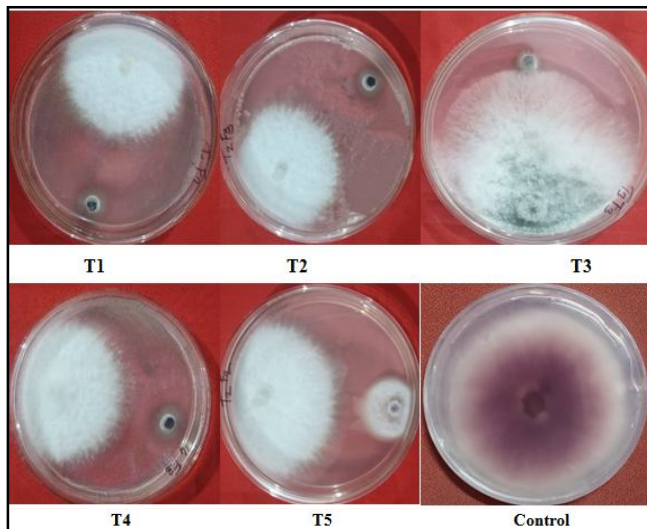


Plate 3: In-vitro evaluation of bio-control agent against *Fusarium* spp.

pathogen in dual culture test revealed that all the five bio agents' viz., *Trichoderma harzianum* (Th-1), *Trichoderma viride* (Tv-1), *Trichoderma reesei* (Tr-1), *Trichoderma hamatum* (Th-1) and *Trichoderma asperellum* (Ts-1) tested inhibited the growth of *Fusarium* spp. The perusal of result showed that *Trichoderma viride* (Tv-1) gave maximum growth of inhibition of 47.36% among all the fungal bio agents followed by *Trichoderma harzianum* (Th-1) gave growth inhibition of 39.63 %, *Trichoderma asperellum* (Ts-1) gave 30.74 % pathogen growth inhibition, *Trichoderma reesei* (Tr-1) gave 28.41 % inhibition and least fungal growth inhibition was found in treatment with *Trichoderma harzianum* (Th-1) as 13.03 %. Hasan *et al.*, (2020) worked on biocontrol efficacy of *Trichoderma asperellum*-enriched coconut fibre against *Fusarium* wilts of cherry tomato. The results showed that the effect of *T. asperellum* on the growth of tomato plant and its capacity to inhibit *Fusarium*

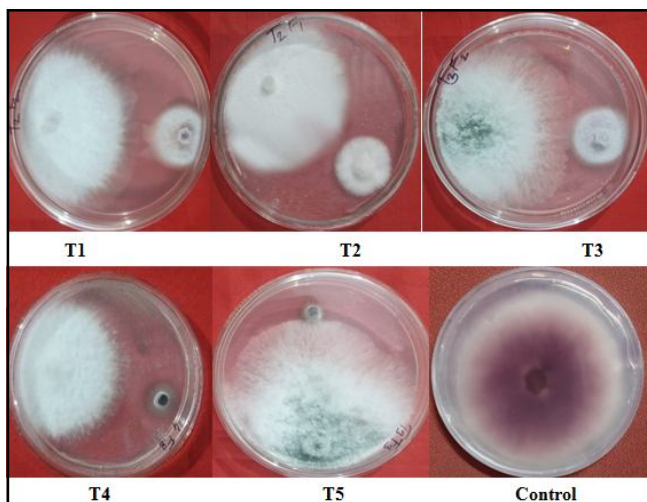


Plate 4: In-vitro evaluation of bio-control agent against *F. solani*.

oxysporum was significantly different compared to positive and negative control.

Conclusion

The present investigation demonstrated the antagonistic potential of different *Trichoderma* species against major pathogens associated with guava wilt under in-vitro conditions. All the tested *Trichoderma* isolates significantly inhibited the mycelial growth of *Fusarium* species, confirming their effectiveness as biological control agents. Among the tested isolates, *Trichoderma harzianum* showed the highest inhibition against *Fusarium oxysporum*, *F. verticillioides* and *Fusarium* spp., whereas *Trichoderma hamatum* was found most effective against *Fusarium solani*.

The inhibitory activity of *Trichoderma* spp. may be attributed to mechanisms such as competition for nutrients and space, mycoparasitism, and production of antifungal metabolites. The results of the study indicate that *Trichoderma* spp., particularly *T. harzianum* and *T. hamatum*, have strong potential for the biological management of guava wilt pathogens. However, further studies under greenhouse and field conditions are necessary to validate their effectiveness and develop suitable bio-formulations for sustainable disease management in guava cultivation.

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Author Contribution

Vivek Kumar Vishwakarma conceived and designed the experiment, carried out laboratory work, collected and analyzed the data, and prepared the manuscript. Abhilasha A. Lal supervised the research work, provided technical guidance, and reviewed and edited the manuscript. Both authors read and approved the final version of the manuscript.

Declaration: Authors do not have any conflict of interest.

References

Aneja, K.R. (2005). *Experiments in Microbiology, Plant Pathology and Biotechnology*. 4th Edition, New Age

- International Publishers, New Delhi, India.
- Barnett, H.L. and Hunter B.B. (1998). *Illustrated Genera of Imperfect Fungi*. 4th Edition, APS Press, St. Paul, Minnesota, USA.
- Dwivedi, S.K. and Pandey V.B. (2016). Studies on guava wilt disease and its management. *Journal of Plant Pathology*, **98(2)**, 345–350.
- Harman, G.E., Howell C.R., Viterbo A., Chet I. and Lorito M. (2004). Trichoderma species—opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, **2**, 43–56.
- Harman, G.E. (2006). Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology*, **96(2)**, 190–194.
- Hasan, M., Rahman M., Islam M. and Hossain M. (2020). Biocontrol efficacy of *Trichoderma asperellum*-enriched coconut fibre against Fusarium wilt of cherry tomato. *Biological Control*, **145**, 104259.
- Howell, C.R. (2003). Mechanisms employed by Trichoderma species in the biological control of plant diseases: The history and evolution of current concepts. *Plant Disease*, **87**, 4–10.
- Kubicek, C.P., Herrera-Estrella A., Seidl-Seiboth V., Martinez D.A., Druzhinina I.S. and Thon M. *et al.* (2019). Comparative genome sequence analysis of Trichoderma species. *Nature Reviews Microbiology*, **17**, 161–174.
- Misra, A.K. and Pandey B.K. (2017). Occurrence and management of guava wilt disease in India. *Indian Phytopathology*, **70(1)**, 1–7.
- Mitra, S.K. and Bose T.K. (2020). *Fruits: Tropical and Subtropical*. Naya Prokash, Kolkata, India.
- Mukhopadhyay, A.N. (2015). Biological control of plant diseases: Current status and future prospects. *Indian Phytopathology*, **68**, 1–9.
- Pandey, S. and Dwivedi S.K. (2018). Management strategies for guava wilt disease in India. *International Journal of Plant Protection*, **11(1)**, 48–55.
- Sharma, P., Sharma S. and Kumar V. (2021). Identification and characterization of Fusarium species associated with guava wilt. *Journal of Applied and Natural Science*, **13(2)**, 456–462.
- Singh, G. (2019). *Guava: Production, Processing and Marketing*. Narendra Publishing House, New Delhi, India.
- Verma, M., Brar S.K., Tyagi R.D., Surampalli R.Y. and Valero J.R. (2007). Antagonistic fungi, Trichoderma spp.: Panoply of biological control. *Biochemical Engineering Journal*, **37**, 1–20.
- Woo, S.L., Ruocco M., Vinale F., Nigro M., Marra R. and Lombardi N. (2014). Trichoderma-based products and their widespread use in agriculture. *Open Mycology Journal*, **8**, 71–126.