

Biological management of Panama wilt of Banana caused by *Fusarium oxysporum* f.sp. *cubense*

Abstract

Bananas are one of the most important fruit crops grown in tropical and sub-tropical regions of the world. It contributes to the livelihood of many growers and constitutes a larger proportion of fruit orchards in India. Panama wilt of banana is a major fungal disease affecting banana cultivations and farmers facing huge economic losses after the introduction of *Fusarium oxysporum* f.sp. *cubense* FocTR4 strain B2 in Bihar. In the present investigation, native and commercial isolates of *Trichoderma viride* and *Trichoderma harzianum* are tested against the all isolates of *Fusarium oxysporum* f.sp. *cubense*. Both the biocontrol agents were found highly effective against the Iso.M, Iso A and Iso.K under *in vitro* as well as under natural pot conditions. But in the case of Iso.G (Foc TR 4 strain B2), these bioagents were found moderately effective under *in vivo* and *in vitro* conditions.

Keywords: -*Trichoderma*, strain ,TR4, fruit crops

INTRODUCTION

Banana is one of the important tropical fruit crop and economically profitable crop of India having high export potential but diseases are the major limiting factor in the cultivation of bananas and adequate management strategies are necessary to promote the production as well as productivity of banana. Banana provides the most economical source of carbohydrates and provides a much more balanced diet than any other fruit in terms of nutritional point of view with 67 to 137 calories per 100 grams. However, it has low amount of fat and protein, it is a rich source of energy and enclose all essential nutrients including minerals and vitamins A, B₁, B₂ and vitamin C and thus it is called as “Apple of Paradise” and as well as “Adams fig” (Bose and Mitra, 2001). The banana research in India is completely towards the extension in production and productivity. The production restraints also vary in country from region to region. However, numerous issues are identical in nature across the banana producing states of India and also among the various countries of the world. In Bihar state, there are two banana growing region one is old Vaishali belt enriched with tall group of banana cultivars and second new Koshi belt dominated with dwarf Cavendish group of banana cultivars.

“Now a days, global banana production is increasingly constrained by Panama wilt disease and being the most significant” (Ghag *et al.*, 2015;García-Bastidas *et al.*, 2014; Ordonez *et al.*, 2015).“In 2013, TR4 was reported to be in Jordan, the first official report of TR4 outside the Southeast Asia Pacific region and a survey in 2014 revealed another infected area, north of the original outbreak”(Ploetz *et al.*, 2015).

Muhammad *et al.*, (2017) reported that TR4 is a noteworthy issue for Cavendish banana developing zones in Pakistan and detailed that cv. Basrai to be influenced by TR4 in specific zones in the Sindh territory and create havoc to banana industry in Pakistan and would be devastating economically.In 2018, TR4 was confirmed to be in Myanmar. The analysis of isolates from Laos, Vietnam and Myanmar provided evidence that the particular TR4 strain in these countries was likely introduced from China (Zheng *et al.*, 2018), Maymon *et al.*, (2018) detailed TR 4 in the lower Carmel coastal plain (1,200 ha), western Galilee (500 ha), and Jordan valley (800 ha) of Israel.

“In 2015, a banana grower from Barari village in the Bihar State of India reported the wilting of Cavendish banana cv. Robusta (AAA) and Grand Naine (AAA). The incidence of the disease was ranged from 2 to 26.6 per cent” (Thangavelu *et al.*, 2019).“Recently, a highly virulent strain of Foc TR 4 strain B2 identified which was affecting Cavendish group of banana cv. Grand Naine(AAA) and Robusta(AAA) in Koshi belt of Bihar” (Shukla and Singh,2019).

“Management of FocTR 4 is a matter of serious concern and different type of approaches such as chemical,biological and manipulation of cultural practices have been attempted by different research workers.The ability of FocTR4 is survive in absence of host is a significant factor limiting the successful management through agronomic practices like crop rotation. Therefore,the only alternative approach for management is by use of antagonistic and growth-promoting fungi which have been successfully demonstrated in biological control of soil borne pathogen in different crop plants” (Tan *et al.*, 2015,Khanet *al.*, 2018).This study on the biological management of Panama wilt in bananas caused by *Fusarium oxysporum* f.sp. *cubense*(Foc) holds significant scientific relevance due to its implications for sustainable disease management in one of the most economically vital fruit crops globally (Pitti *et al.*, 2021; Montenegro *et al.*, 2021; Campos, 2023). The emergence

of the Foc TR4 strain B2 in Bihar has escalated the economic impact of Panama wilt, emphasizing the urgent need for effective control strategies. The utilization of native and commercial isolates of *Trichoderma viride* and *Trichoderma harzianum* as biocontrol agents presents a promising avenue for integrated disease management, contributing to the expanding field of biocontrol research. The study's in-depth assessment, both *in vitro* and *in vivo*, of the efficacy of these biocontrol agents against different isolates of Foc, including the highly virulent Iso. G, provides valuable insights into the specificity and effectiveness of biological interventions in combatting Panama wilt.

MATERIAL AND METHODS

Isolation and purification the pathogen

The infected plants parts of banana cultivars like pseudostem, roots and leaves were collected from the field. These infected plant parts were cut in to small pieces in 2-3 mm and surface sterilized with 0.1% mercuric chloride solution for 30 seconds after that washing with distilled water to 2-3 times. These small pieces were placed aseptically on PDA slants with the help of an inoculating needle and incubated at 28±°C. All the isolates were categorised in to five different groups *viz.*, Iso.M, Iso.A, Iso.K, Iso.G and Iso.R on the basis of host differential of banana cultivars.

Evaluation of antagonistic efficacy of bio-control agents against Foc isolates

Isolation and identification of native and commercial fungal bioagents

Rhizospheric soil from healthy banana plants were collected in poly ethylene bags and brought to the research laboratory of Department of Plant Pathology, RPCAU, Pusa, Samastipur. Serial dilution technique (Johnson and Curl, 1972) was used to isolate fungal antagonist from the rhizospheric soil of healthy banana plants and shade dried. The mycoflora was isolated on rose Bengal agar medium by utilizing a dilution of 10³ and 10⁴. One ml of soil suspension was poured into sterilized Petri plates containing the melted and cooled medium and then rotated gently to get uniform distribution of soil into the medium. At that point, the plates were incubated at 27±2°C and observed frequently for the development of colonies.

The developed colonies were picked and recognised based on mycological keys described by Gilman (1957) and Nelson *et al.* (1983) for identification of *Trichoderma viride* and *Trichoderma harzianum*.

Bioagents were also isolated from two commercial formulation of biocides, *i.e.* Nisarga (Multiplex Agricare Pvt. Ltd. 1% W.P.) and Antagon (Arihant Nature crop Pvt.Ltd.1% W.P.) according to method described in native bioagents isolation.

Evaluation of antagonistic efficacy of *Trichoderma viride* and *Trichoderma harzianum* (native and commercial isolates) against *Focin vitro*

“For the testing the antagonistic effect of *T. harzianum* and *T. viride* (Commercial and native isolate) the PDA plate divided into equal halves” (Karunanithi and Usman, 1999). “The first half was independently inoculated with 7 days old culture disc (5mm in diameter) of each fungal bio-control agent, while the later was also independently inoculated with one disc (5 mm in diameter) of 7 days old culture of pathogenic fungus in the opposite side. Control plate was inculcated with disc of test fungus on PDA medium instead the fungal bio control agents. Three plates were used as replicates for each treatment” [Shukla and Singh, 2019].

All plates were incubated for $28 \pm 2^{\circ}\text{C}$ until the growth of Focisolates in the control treatment reached to the edge of Petri dish. The per cent reduction of linear mycelial growth of pathogenic fungi were calculated using formula as given by Vincent (1927).

$$I = \left(\frac{C - T}{C} \right) \times 100$$

Where,

I = Per cent growth inhibition

C = Colony diameter in control Petri plate;

T = Colony diameter in the treated Petri plate.

Data were subjected to proper statistical analysis of variance according to Snedecor and Cochran (1980). Mean of treatments were compared with F-test and L.S.D. at level of 0.05%.

Evaluation of antagonistic efficacy of *Trichoderma viride* and *Trichoderma harzianum* (native and commercial isolates) against FocTR 4 strain B₂ under pot condition

The efficacy of all the bioagents were tested in poly house under pot condition against the isolates FocTR4 strain B₂. The mass culture of Foc isolates were prepared as per method described by Haware (1980). “The mass culture of FocTR4 strain B₂ isolate was blended in steam sterilized soil separately @50 g/kg soil having 10 kg sterile loamy soil / plastic pots (50cm diameter). Each bioagent with 50 g /pot was applied in pathogen-inoculated pots after 15 days of transplanting. Inoculation of pot with sterilized distilled water was served as control. Each treatment was replicated three times with different banana cultivars independently. The disease incidence (%) was calculated by dividing the total number of transplanted plants showing Panama wilt disease symptoms by the total number of plants transplanted and then multiplied by hundred. The data on inhibition per cent over control also recorded in each treatment and data was recorded up to 90 days after transplanting” [Shukla and Singh, 2019].

Per cent wilt index (PWI, external symptoms):

The intensity of Panama wilt of banana was recorded as per International Musa Testing Program (IMTP) rating in 1-5 scale and per cent wilt index (PWI) was determined in each plot.

Category	Reaction
1	Healthy
2	Slight chlorosis and wilting with no petiole buckling
3	Moderate chlorosis and wilting with some petiole buckling and or splitting of leaf base
4	Severe chlorosis, severe wilting, petiole buckling and dwarfing of newly emerged leaf
5	Dead

$$\text{Per cent wilt index} = \frac{\text{Total sum of numerical rating}}{\text{Total number of plants observed} \times \text{maximum category in the score chart}} \times 100$$

The data was analysed by CRD design.

RESULT AND DISCUSSION

Antagonistic effect of *Trichoderma viride* and *Trichoderma harzianum* on different isolates of *Focin vitro*

Effect of native isolate of *Trichoderma viride* and *Trichoderma harzianum* on different isolates of *Foc in vitro*

The antagonistic activity of native isolates of *Trichoderma viride* and *Trichoderma harzianum* against the various isolates of *Foc* (Iso. M, Iso. A, Iso. K, Iso. R and Iso. G) were determined by the dual culture technique *in vitro*. Five mm disc of seven days old cultures of bioagent (*T. viride* and *T. harzianum*) and isolates of *Foc* were placed equidistant in sterilized Petri plate containing PDA medium. Suitable control was also maintained without antagonist. The growth of the pathogen was measured at 24 hrs interval up to 240 hrs of inoculation. Percent inhibition of mycelial growth of pathogen was calculated. Data are presented in (Table 1).

In case of native isolate of *Trichoderma viride* maximum inhibition was observed in Iso. K (64.6%) followed by Iso. A (62.2%), Iso. M (59.8%) and Iso. R (51.0%). The minimum inhibition was recorded in Iso. G (50.2%) over control after 240 hrs. While in case of native isolate of *T. harzianum*, the maximum inhibition was observed in Iso. K (57.6%) followed by Iso. A (56.4%), Iso. M. (55.2%) and Iso. R (44.0%). The minimum inhibition was recorded in Iso. G (43.6%). The result clearly showed that the native isolates of *T. viride* and *T. harzianum* were highly effective against the Iso. M, Iso. A and Iso. K. But these native isolates of bioagents were found moderately effective against the Iso. R and Iso. G (TR4 strain B₂) of *Foc*. Data pertaining to inhibition per cent over control revealed that the radial growth of Iso. M, Iso. A and Iso. K were significantly inhibited against the native isolate of *T. viride* and *T. harzianum*. However, the differences between Iso. R and Iso. G was found nonsignificant or remained significantly at par. The maximum inhibition was recorded in Iso. K closely followed by Iso. A or the Iso. A and Iso. K varied significantly.

Antagonistic effect of *Trichoderma viride* (commercial) and *Trichoderma harzianum* (commercial) on different isolates of *Focin vitro*

The antagonistic effect of *Trichoderma viride* (commercial) and *Trichoderma harzianum* (commercial) on *Foc* isolates (Iso. M, Iso. A, Iso. K, Iso. G and Iso. R) were studied by dual culture method and results presented in the (Table 2).

In case of *Trichoderma viride* (commercial) isolate, maximum inhibition was recorded in Iso. K (55.1%) followed by Iso. A (54.2%), Iso. M (53.2%), Iso. R (42.8%) while minimum inhibition was noticed in Iso. G (42.3%) over control. But in case of commercial isolate of *Trichoderma harzianum*, the inhibition per cent was found maximum in Iso. K (46.1%) followed by Iso. A (45.5%), Iso. M (43.9%) and Iso. R (33.7%). The minimum inhibition was recorded in Iso. G (TR4 strain B₂) i.e. 33.2% over control. Both the commercial isolates of *T. harzianum* and *T. viride* were found less effective against the Iso. M (race 1), Iso. A (race 1) and Iso. K (race 2). Data pertaining to inhibition per cent over control against the commercial isolate of *T. viride* and *T. harzianum* showed that considerable variation in all the isolates of Foc. The differences between Iso. R and Iso G was found non significant or Iso. G at par with Iso. R and minimum inhibition were recorded in Iso. G.

But in case of Iso. R (race 4) and Iso. G (TR4 strain B₂) showed ineffectiveness against the commercial isolates because these isolates were showed only little amount of mycelial growth reduction in dual culture techniques.

Effectiveness of native and commercial isolates of *T. viride* and *T. harzianum* against the FocTR 4 strain B₂ (Iso. G) under pot condition

Mass culture of FocTR4 strain B₂ (Iso. G) was multiplied on sand maize medium and added to steam sterilized soil (15 psi for 30 minutes) in pots @ 5% (w/w). Soil mixture without inoculums served as control. Each pot was planted with one-month old banana sucker of different cultivars viz., Malbhog (AAB), Alpan (AAB), Kothia (ABB), Robusta (AAA) and Grand Naine (AAA). After 15 days of transplanting different native and commercial isolates of *Trichoderma harzianum* and *Trichoderma viride* were multiplied on sorghum sand medium mixed in each inoculum containing pots @ 50 gm/pot. Observation was done based on the first appearance of symptom of disease, per cent incidence of the disease and per cent inhibition over control. Results are presented in (Table 3).

Results revealed that the native and commercial isolates of *T. viride* and *T. harzianum* were significantly lower in comparison to the control.

Among the different isolates of *T. viride* and *T. harzianum*, the native isolate of *T. viride* and *T. harzianum* were found highly and moderately effective against the Foc TR4 strain B₂, respectively. Because Panama wilt disease symptom was not observed up to 59 days in cv. Alpan followed by cv. Kothia (58 DAT), cv. Malbhog

(56 DAT), cv. Robusta (54 DAT) and cv. Grand Naine (51 DAT) against the native isolate of *T. viride*. The native isolate of *T. viride* was found significantly effective in disease suppression against the FocTR4 strain B₂ followed by native isolate of *T. harzianum*. Commercial isolate of *T. viride* and *T. harzianum* were found less effective against the FocTR4 strain B₂. Data pertaining to inhibition over control under pot condition revealed that the T₂ and T₃ was found non significant or significantly at par in cultivar Malbhog (AAB), Grand Naine (AAA) and Robusta (AAA). However, the differences between T₁ and T₃ in cultivar Alpan (AAB) was found non significant or T₃ at par with T₁. In comparing this study with existing research on soil quality and Fusarium wilt in bananas from Latin America, an intricate interplay between biological management (Araya-Alman *et al.*, 2020; Rey *et al.*, 2022) and environmental factors becomes apparent (Olivares *et al.*, 2021a). Soil quality studies often elucidate the role of soil microbiota and physicochemical properties in influencing plant health (Olivares *et al.*, 2021b; Olivares *et al.*, 2022a). The integration of biocontrol agents, such as *Trichoderma viride* and *Trichoderma harzianum*, in the present investigation aligns with the broader understanding that soil health directly impacts plant-pathogen interactions. Additionally, the moderate efficacy observed against the highly virulent Iso. G underscores the complexity of Fusarium wilt dynamics, suggesting that soil quality may influence the success of biocontrol strategies. Comparative analyses with Latin American studies could shed light on regional variations in soil microbiota, offering nuanced insights into the adaptability and efficacy of biological management practices in diverse agroecosystems (Olivares *et al.*, 2022b; Rodriguez *et al.*, 2023a).

Considering the influence of agro-environmental factors on *Fusarium* wilt in bananas from Latin America, the present study contributes to the discourse by addressing the practical applicability of biocontrol agents under different environmental conditions (Rodriguez *et al.*, 2023b; Martínez *et al.*, 2023). The observed efficacy of *Trichoderma viride* and *Trichoderma harzianum* against multiple isolates of *Fusarium oxysporum* f.sp. *cubense* in both controlled (*in vitro*) and natural pot (*in vivo*) conditions indicates a robust potential for biological management across diverse agro-environments. However, the moderate effectiveness against the Iso. G strain highlights the need for a nuanced understanding of the agro-environmental

factors influencing the interaction between biocontrol agents and specific *Fusarium* strains. Collaborative research efforts between regions facing *Fusarium* wilt challenges could yield valuable insights into the adaptability of biocontrol strategies to varying environmental contexts, enhancing the global applicability of such interventions (Olivares *et al.*, 2020; Vega *et al.*, 2022).

CONCLUSION

During the evaluation of biocontrol agents like *Trichoderma viride* and *T. harzianum* (Native and commercial isolates) against the different isolates of Foc under *in vitro* as well as in artificial pot condition. Among the both biocontrol agents, *T. viride* (native) was found highly effective against the Iso. M and Iso. A and Iso. K under *in vitro* as well as under pot condition. Under pot condition, the native isolate of *T. viride* and *T. harzianum* were found highly and moderately effective against the FocTR 4 strain B₂, respectively. Because Panama wilt symptom not develop up to 59 days in cv. Alpan followed by cv. Kothia (58 DAT), cv. Malbhog (56 DAT), cv. Robusta (54 DAT) and cv. Grand Naine (51 DAT) against the *T. viride* (native). Commercial isolate of both bio control agents was found less effective against the FocTR 4 strain B₂.

REFERENCE

- Araya-Alman, M., Olivares, B., Acevedo-Opazo, C. *et al.* (2020). Relationship Between Soil Properties and Banana Productivity in the Two Main Cultivation Areas in Venezuela. *J Soil Sci Plant Nutr.*; 20 (3): 2512-2524. <https://doi.org/10.1007/s42729-020-00317-8>.
- Bose, T. K. and Mitra, S. K. (2001). *Fruits: Tropical and Sub-tropical Vol. 1*, Naya Udyog, Calcutta, pp. 227-318, 721 pp.
- Campos, B. O. (2023). *Banana Production in Venezuela: Novel Solutions to Productivity and Plant Health*. Springer Nature. <https://doi.org/10.1007/978-3-031-34475-6>
- García-Bastidas, F., Ordóñez, N., Konkol, J., Al-Qasim, M., Abdelwali, M., Salem, N., Ploetz, R. C., & Kema, G. H. J. (2014). First report of *Fusarium oxysporum* f. sp. *cubense* TR 4 associated with Panama disease of banana outside Southeast Asia. *Plant Disease*. (98):694–694.

- Ghag, S.B., Shekhawat, U.K.S., Ganapathi, T.R. (2015). *Fusarium* wilt of banana: biology, epidemiology and management. *Pans Pest Articles & News Summaries*. 61: 250-263.
- Gilman, J. C. (1957). A manual of soil fungi. 2nd ed. Ames, Iowa: The Iowa State University Press. p. 450.
- Haware, M P. (1980). Methods of artificial inoculation and disease rating of root pathogens in *Phyto Pathological techniques ed: Chand J N and Sharma G. S.* 32-35 pp.
- Johnson, L. F. and Curl, E. A. (1972). Methods for research on the ecology of soil-borne plant pathogen. 426 So. Sixth St., Minneapolis, MN 55415: Burgess Publishing Company.
- Karunanithi, K. and Usman, K. M. (1999). Screening of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *sesame* causing wilt in seasmum. *Crop research*.18(1): 127-130.
- Khan, N., Hidalgo, P. M., Ice, T. A., Maymon, M., Humm, E. A., Nejat, N., *et al.*(2018). Antifungal activity of *Bacillus* species against *Fusarium* and analysis of the potential mechanisms used in biocontrol. *Front. Microbiol.* 9:2363.
- Martínez, G.; Olivares, B.O.; Rey, J.C.; Rojas, J.; Cardenas, J.; Muentes, C.; Dawson, C.(2023) The Advance of *Fusarium* wilt Tropical Race 4 in Musaceae of Latin America and the Caribbean: Current Situation. *Pathogens* 2023, 12, 277. <https://doi.org/10.3390/pathogens12020277>.
- Maymon, M. (2018). First Report of *Fusarium oxysporum* f. sp. *cubense* TR 4 causing *Fusarium* Wilt of Cavendish Bananas in Israel. *Plant Disease* 59:348.
- Montenegro, E., Pitti-Rodríguez, J, Olivares-Campos, B. (2021). Identification of the main subsistence crops of Teribe: a case study based on multivariate techniques. *Idesia (Arica)*,; 39(3), 83-94. <https://dx.doi.org/10.4067/S0718-34292021000300083>.
- Mushammad, Aish., Hussain, I., Khanzad, K. A., mondo, L., Ali, M., Yasmin, T. and Hyder, M. Z. (2017). Molecular characterization of *Fusarium oxysporum* f.sp. *cubense* TR 4 causing Panama disease in Cavendish banana in *Pakista*. *Pak. J. Agri. Sci.* Vol. 54(1):1-8.
- Nelson, P. E., Toussoun, T. A. and Marasas, W. F. O. (1983). *Fusarium* species: An illustrated manual for identification. Pennsylvania state University Press, University Park.
- Olivares B, Rey JC, Lobo D, Navas-Cortés JA, Gómez JA, Landa BB. (2021a). *Fusarium* Wilt of Bananas: A Review of Agro-Environmental Factors in the Venezuelan Production System Affecting Its Development. *Agronomy*, 11(5):986. <https://doi.org/10.3390/agronomy11050986>.

- Olivares B, Rey JC, Lobo D, Navas-Cortés JA, Gómez JA, Landa BB. (2022a). Machine Learning and the New Sustainable Agriculture: Applications in Banana Production Systems of Venezuela. *Agricultural Research Updates*. 42, 133 - 157. Nova Science Publishers, Inc.
- Olivares B, Vega A, Calderón MAR, Rey JC, Lobo D, Gómez JA, Landa BB. (2022b). Identification of Soil Properties Associated with the Incidence of Banana Wilt Using Supervised Methods. *Plants*, 11(15):2070. <https://doi.org/10.3390/plants11152070>.
- Olivares, B., Paredes, F., Rey, J., Lobo, D., Galvis-Causil, S. (2021b). The relationship between the normalized difference vegetation index, rainfall, and potential evapotranspiration in a banana plantation of Venezuela. *SAINS TANAH - Journal of Soil Science and Agroclimatology*, 18(1), 58-64. <http://dx.doi.org/10.20961/stjssa.v18i1.50379>.
- Olivares, B., Pitti, J., Montenegro, E. (2020). Socioeconomic characterization of Bocas del Toro in Panama: an application of multivariate techniques. *Revista Brasileira de Gestao e Desenvolvimento Regional*, 16(3):59-71. <https://doi.org/10.54399/rbgdr.v16i3.5871>.
- Ordóñez, N., García, F.A., Laghari, H., Akkary, M. and Harfouche, E. N. (2015). First report of *Fusarium oxysporum* f. sp. *cubense* TR 4 causing Panama disease in Cavendish bananas in Pakistan and Lebanon. *Plant Disease*. 99, 1448.
- Pitti, J; Olivares, B; Montenegro, E. (2021). The role of agriculture in the Changuinola District: a case of applied economics in Panama. *Tropical and Subtropical Agroecosystems*. 25 - 1, 1 - 11. <http://dx.doi.org/10.56369/tsaes.3815>.
- Ploetz, R. C. (2015). Management of *Fusarium* wilt of banana: A review with special reference to TR 4. *Crop Protectio*. (73)7 –15.
- Rey, J.C.; Olivares, B.O.; Perichi, G.; Lobo, D. (2022). Relationship of Microbial Activity with Soil Properties in Banana Plantations in Venezuela. *Sustainability* 14, 13531. <https://doi.org/10.3390/su142013531>.
- Rodríguez-Yzquierdo, G.; Olivares, B.O.; González-Ulloa, A.; León-Pacheco, R.; Gómez-Correa, J.C.; Yacomelo-Hernández, M.; Carrascal-Pérez, F.; Florez-Cordero, E.; Soto-Suárez, M.; Dita, M.(2023). Soil Predisposing Factors to *Fusarium oxysporum* f.sp *cubense* Tropical Race 4 on Banana Crops of La Guajira, Colombia. *Agronomy* 2023, 13, 2588. <https://doi.org/10.3390/agronomy13102588>.
- Rodríguez-Yzquierdo, G.; Olivares, B.O.; Silva-Escobar, O.; González-Ulloa, A.; Soto-Suarez, M.; Betancourt-Vásquez, M.(2023) Mapping of the Susceptibility of Colombian Musaceae Lands to a Deadly Disease: *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4. *Horticulturae* 2023, 9, 757. <https://doi.org/10.3390/horticulturae9070757>.
- Shukla, D.N. and Singh, S.K. (2019). First ever report of TR 4 of *Fusarium oxysporum* f.sp. *cubense* in Dwarf Cavendish group of bananas in Bihar state

of India. National symposium on Recent challenges and opportunity in sustainable plant health management. February 26-28, 2019, organized by Indian Phyto pathological Society, New Delhi at Institute of Agricultural Sciences, BHU, Varanasi, UP.

- Snedecor, G. W. and Cochran, W. G. (1980). Statistical methods. 5th Ed., Ioawa State University Press, Ames, Iowa, USA. 593 pp.
- Tan, D., Fu, L., Han, B., Sun, X., Zheng, P., and Zhang, J. (2015). Identification of an endophytic antifungal bacterial strain isolated from the rubber tree and its application in the biological control of banana Fusarium wilt. Plos ONE 10:e0131974.
- Thangavelu, R., Mostert, D., Gopi, M., Ganga Devi P., Padmanaban B., Molina A. B. and A. Viljoen (2019). First detection of *Fusarium oxysporum* f. sp. *cubense* TR4 on Cavendish banana in India. *Eur. J. Plant Pathol.* <https://doi.org/10.1007/s10658-019-01701-6>.
- Vega, A.; Olivares, B.O.; Rueda Calderón, M.A.; Montenegro-Gracia, E.; Araya-Almán, M.; Marys, E. (2022). Prediction of Banana Production Using Epidemiological Parameters of Black Sigatoka: An Application with Random Forest. *Sustainability* 14, 14123. <https://doi.org/10.3390/su142114123>
- Vincent, J. M. (1927). Determination of per cent inhibition *in vitro*. *Nature*. 159:850.
- Zheng, S.J. (2018). New Geographical Insights of the Latest Expansion of *Fusarium oxysporum* f.sp. *cubense* TR 4 Into the Greater Mekong Subregion. *Frontiers in Plant Science*. 9:457.
- DN Shukla and SK Singh, Effect of different aqueous plant extracts against the various isolates of *Fusarium oxysporum* f.sp. *cubense* causing Panama wilt of banana. *International Journal of Chemical Studies* 2019; 7(4): 2759-2764

Table 1. Effect of native isolate of *Trichoderma viride* and *Trichoderma harzianum* on different isolates of *Focin vitro*

Isolates	Radial growth(mm)*	
	<i>Trichoderma viride</i> (Native)	<i>Trichoderma harzianum</i> (Native)

	120 hrs	240 hrs	Inhibition over control (%) at 240 hrs	120 hrs	240 hrs	Inhibition over control (%) at 240 hrs
Iso. M (race 1)	15.6	35.6	59.8	21.4	39.6	55.2
Control	35.3	88.4		35.4	88.3	
Iso. K (race 2)	13.5	31.4	64.6	19.5	37.6	57.6
Control	33.4	88.5		33.5	88.5	
Iso. A (race 1)	14.2	33.4	62.2	20.5	38.5	56.4
Control	34.3	88.5		34.5	88.4	
Iso. G (race 4)	24.6	44.3	50.2	28.4	50.4	43.6
Control	37.4	89.1		37.5	89.4	
Iso. R (race 4)	23.4	43.3	51.0	27.6	49.7	44.0
Control	36.3	88.4		36.6	88.7	
CD at 5%	1.68	1.29	2.21	1.73	1.27	2.09
S.Em. (\pm)	0.57	0.43	0.70	0.58	0.43	0.66
C.V. (%)	3.67	1.19	2.10	3.41	1.12	2.21

*Mean of three replications

Table 2. Antagonistic effect of *Trichoderma viride* (commercial) and *Trichoderma harzianum* (commercial) on different isolates of Foc *in vitro*

Isolates	Radial growth(mm)*					
	<i>Trichoderma viride</i> (Commercial)			<i>Trichoderma harzianum</i> (Commercial)		
	120 hrs	240 hrs	Inhibition over control (%) at 240 hrs	120 hrs	240 hrs	Inhibition over control (%) at 240 hrs
Iso. M (race 1)	22.5	41.4	53.2	25.8	49.6	43.9
Control	35.5	88.4		35.6	88.5	
Iso. K (race 2)	20.5	39.7	55.1	23.6	47.7	46.1
Control	33.5	88.4		33.6	88.4	
Iso. A (race 1)	21.3	40.5	54.2	24.6	48.6	45.1
Control	34.5	88.4		34.6	88.5	
Iso. G (race 4)	30.5	51.5	42.3	32.7	59.4	33.2
Control	37.5	89.3		37.5	88.9	
Iso. R (race 4)	29.5	50.5	42.8	31.7	58.8	33.7

Control	36.6	88.4		36.7	88.6	
CD at 5%	1.88	1.28	2.13	1.94	1.20	1.98
S.Em. (\pm)	0.63	0.43	0.67	0.65	0.40	0.62
C.V. (%)	3.62	1.12	2.33	3.57	0.99	2.65

*Mean of three replications

Table 3. Effectiveness of native and commercial isolates of *T. viride* and *T. harzianum* against the Foc TR 4 strain B₂ (Iso. G) under pot condition

Bioagent		Malbhog* (Silk Group)			Alpan* (Mysore Group)			Kothia* (Bluggoe group)			Grand Naine* (Dwarf Cavendish Group)			Robusta* (Dwarf Cavendish Group)		
		First appearance of disease (DAT)	Incidence (%)	Inhibition over control (%)	First appearance of disease (DAT)	Incidence (%)	Inhibition over control (%)	First appearance of disease (DAT)	Incidence (%)	Inhibition over control (%)	First appearance of disease (DAT)	Incidence (%)	Inhibition over control (%)	First appearance of disease (DAT)	Incidence (%)	Inhibition over control (%)
T ₁	<i>T. viride</i> (Native)	56	46	52.6	59	33	62.1	58	34	57.5	51	46	52.6	54	42	56.3
T ₂	<i>T. harzianum</i> (native)	49	53	45.4	51	53	39.1	44	47	41.3	34	53	45.4	36	50	47.9
T ₃	<i>T. viride</i> (Commercial)	46	57	41.2	49	35	59.8	48	39	51.3	37	57	41.2	38	52	45.8
T ₄	<i>T. harzianum</i> (Commercial)	40	60	38.1	41	58	33.3	39	57	28.8	41	60	38.1	43	58	39.6
T ₅	Control	26	97		27	87		29	80		22	97		24	96	
CD at 5%			2.27	4.38		1.99	5.82		2.27	5.26		2.27	5.16		2.36	5.29
S.Em. (\pm)			0.71	1.32		0.62	1.76		0.71	1.59		0.71	1.62		0.74	1.60
C.V. (%)			1.96	5.16		1.95	6.26		2.39	6.16		1.96	6.63		2.14	5.84

*Mean of three replications

