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Original article

Fusarium fruit rot of banana in Iran

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Abstract

Banana fruit rot by *Fusarium verticillioides* is one of the most important diseases of banana in Iran. In 2021-2022 this species was identified by both morphological and molecular methods in Iran, also its phylogenetic relationship with the reference species in the GenBank was investigated. Pathogenicity tests revealed that all isolates induced symptoms of banana fruit rot disease that were consistent with those observed in the field. In the second step of this study, control of *F. verticillioides* was evaluated in vitro and in vivo. Nowadays, the current trend is shifting from chemical pesticides toward safer methods. Antagonist microorganisms as biocontrol agents are useful substitutes. Some bacteria and fungi have been found to have inhibitory effects on *F. verticillioides*. In this research we examined bacterial and yeast antagonist isolates, Neem extract and finally a chemical fungicide (Thiabendazole) on mycelial growth of *F. verticillioides*. Our results showed that two bacterial isolates and two isolates of yeasts inhibited the pathogen growth. The methanol leaf extract of Neem at 4000 ppm concentration, showed the most antifungal activity. Integration of bacterial and yeast antagonists with Thiabendazole showed the best activity at 600 ppm concentration of Thiabendazole.

Keywords: antagonist microorganisms, banana, *Fusarium verticillioides*, neem, Thiabendazole

Introduction

Banana is considered one of the earliest cultivated fruit crops (Denham *et al*., 2003), with its origins in tropical India, Southeast Asia, and northern Australia and is now an important food crop because of its nutritional value(Roy *et al*., 2023). Asia accounts for approximately 54% of global banana production (Zakaria, 2023). In Iran, banana is mainly cultivated in the southern and southeastern regions, with Sistan and Baluchestan Province being the leading banana-producing area.

Fusarium diseases, particularly banana Fusarium wilt, have historically caused substantial losses in banana production (Solorzano *et al*., 2025). Members of the *F. fujikuroi* (*F. verticillioides)* can produce mycotoxins and contribute to significant yield losses. *F. musae*, a closely related species, is also an important pathogen associated with banana crown rot (Tava *et al*., 2021). Although fruit rot caused by *F. verticillioides* has received less attention than Panama disease caused by *F. oxysporum* f.sp. *cubense*, it is an important postharvest disease of banana. Previous studies have confirmed *F. verticillioides* as a causal agent of banana fruit rot in Africa and Jordan (Salem *et al.*, 2020).

Effective management of banana postharvest diseases depends on accurate pathogen identification and appropriate control strategies. Biological control methods, including use of endophytic bacteria and plant extracts, have shown potential against *F. verticillioides*(Kara and Soylo, 2022). Several plant extracts have demonstrated antifungal activity and may help reduce postharvest losses(Murad *et al.*, 2021). Neem (*Azadirachta indica*), a medicinal plant with well-documented biochemical and antimicrobial properties, is a promising candidate for controlling fungal pathogens (Rai, 2024).

The general and excessive application of chemical pesticides can adversely affecte humans, animals and the environment because of toxic residues, the development of pathogen resistance… . Therefore, safer and more sustainable alternatives, including plant extracts, biological control agents and microbial antagonists should be investigated. An integrated management strategy, may provide an effective approach for controlling *F. verticillioides* on bananas.

Therefore, this study aimed to evaluate the antifungal efficacy of bacterial, fungal, and yeast antagonists, as well as aqueous, ethanolic, and methanolic extracts of neem, and the fungicide thiabendazole against the important banana pathogen *F. verticillioides* in Iran.

Materials and methods

Study site and sample collection: The study was conducted at Velayat University,Sistan and Baluchestan, Iran. During 2021-2022 banana fruits showing symptoms of fruit rot were

collected from local fruit shops. The collected samples were examined for the presence of *Fusarium* species (Nelson *et al*., 1983; Leslie and Summerell, 2006).

Pathogenicity tests:

a)On banana fruits in laboratory. Fruits were inoculated with a *Fusarium* spore suspension at a concentration of 10^6 spores mL^{-1} using the wound-inoculation method. Disease severity was evaluated using 0-5 scale based on the percentage of infected fruit tissue following Kahawattage *et al*. (2023).

b)On banana seedlings. After preparing seven-month-old 'Harichal' banana seedlings, and pathogen isolates, three inoculation methods were used: sorghum grains method (Sneh *et al*., 1991), Stem injection method (Hagen and Dunwiddie, 2008), and Spray method (Zhang *et al*., 2018). Disease severity (DS) was assessed 4 months after inoculation (Moore *et al.,* 1993) as follows:

$$DS\ (\%) = [\sum (n \times v)/6\ N] \times 100 \qquad \text{Equation 1}$$

Where, n = numbers of seedlings, v: disease score, N = total number of evaluated plants, and 6 = highest disease severity rate.

Statistical analyses were performed using SAS 9.0. Disease index data were analyzed using two-way ANOVA. When significant differences were detected, means were compared using Tukey's multiple comparison test.

Molecular identification. Genomic DNA of isolates was extracted according to the method described by Zhong and Steffenson (2001). The *TEF1-α* and *RPB2* were amplified using the EF-1 and EF-2 (O'Donnell and Cigelnik, 1997), and rpb2-5F2 and rpb2-7CR primers (Sung *et al*., 2007) respectively. The PCR products were sequenced, and the resulting sequences were deposited in the GenBank database.

For phylogenetic analysis, *Macroconia leptosphaeriae* (CBS100001) was used as the outgroup (Fig. 2; Kumar *et al*. 2018)

Evaluation of the antagonists effects

42 bacterial isolates and 6 fungal isolates were obtained from the Mycology laboratory, University of Tehran and used as antagonists against *F. verticillioides* (Sipiczki, 2023) (Table 1).

Table 1. Antagonistic isolates

Isolate NO.	Species	Isolate NO.	Species
P_{19}	*Pseudomonas fluorescens* bioI	B_{29}	*B. subtilis*
P_{61}	*P. fluorescens* bioII	B_{28}	*B. subtilis*
P_{46}	*P. fluorescens* bioIII	B_{48}	*B. subtilis*
P_{11}	*P. fluorescens* bioIII	B_5	*B. subtilis*
P_{34}	*P. fluorescens* bioIII	B_{33}	*B. subtilis*
P_{43}	*P. fluorescens* bioIII	B_{16}	*B. subtilis*
P_{10}	*P. fluorescens* bioIII	B_9	*B. subtilis*
P_{21}	*P. fluorescens* bioIII	B_{31}	*B. subtilis*
P_{44}	*P. fluorescens* bioIII	B_{49}	*B. subtilis*
P_6	*P. fluorescens* bioIII	B_{50}	*B. subtilis*
P_{53}	*P. fluorescens* bioIII	B_{26}	*B. subtilis*
P_{38}	*P. fluorescens* bioIII	B_{30}	*B. subtilis*
P_{30}	*P. fluorescens* bioV	B_4	*B. subtilis*
P_{39}	*P. fluorescens* bioV	B_{41}	*B. subtilis*
P_{48}	*P. fluorescens* bioV	B_{49}	*B. subtilis*
P_{18}	*P. fluorescens* bioV	M_{18}	*Pichia guilliermondii*
P_{23}	*P. fluorescens* bioV	M_{45}	*P. kluyveri*
		(M_{48})	*Cryptococcus flavescensi*
P_{50}	*P. fluorescens* bioV	A_5	*Candida membranifuciens*
P_{26}	*P. fluorescens* bioV	A_2	*C. membranifuciens*
P_{28}	*P. fluorescens* bioV	A_4	*C. membranifuciens*
P_{62}	*P. fluorescens* bioV	T_2	*Trichoderma harzianum*
P_{49}	*P. fluorescens* bioV	T_5	*T. viridi*
P_{22}	*P. fluorescens* Chao	T_8	*T. harzianum*
P_{31}	*P. putida* bioA	T_9	*T. hamatum*
P_{35}	*P. aeruginosa*	T_{18}	*T. longibrachiatum*
B_{15}	*Bacillus subtilis*	T_6	*T. virens*
B_{24}	*B. subtilis*		

Three methods (Four-spotted culture (Weller, 1988), dual culture (Eq. 2, Acharya, 2021), and Volatile metabolite assay (Eq.3, Elbouazaoui*et al*., 2022)) were used to evaluate the antagonist effects of different bacterial and yeast isolates.

$PGI\ (\%) = \frac{R_1 - R_2}{R_1} \times 100$ Equation 2

PGI: percentage growth inhibition

R_1andR_2: diameter of pathogen colonies in control and in all treatments

$PIRG = \frac{R_2 - R_1}{R_2} \times 100$Equation 3(Stracquadanio et al., 2020)

R_1: the growth of pathogen in control plates

R_2: the halo of reduced growth around the pathogen after 6 days

PIRG:The average of percentage inhibition

Bioassay of Neem extract

Aqueous, methanolic, and ethanolic extracts of neem (*Azadirachta indica* J) were prepared according to the methods described by Rai (2024), Kiran *et al*. (2023), and Bahraminejad *et al.* (2008). The antifungal activity of the neem extracts against *F. verticillioides* was evaluated using the poisoned food technique (Kiran *et al*., 2023) at concentrations of 0, 500, 1000, 1500, 2000, and 4000 ppm (Eq.4, Kiran *et al*., 2023).

$$percentage\ of\ growth\ inhibition = \frac{dc-dt}{dc} \times 100 \quad \text{Equation 4}$$

Where dc =average increase in mycelial growth in control

dt =average increase in mycelial growth in treated plates.

Evaluation of thiabendazole fungicide

The efficacy of thiabendazole against *Fusarium* isolates was evaluated at concentrations of 100, 300, and 600 ppm using PDA amended with the fungicide (Eq.5, Payan-Arzapalo *et al*., 2024). PDA plates without thiabendazole served as the control treatment. The experiment was arranged in a completely randomized design with 4 treatments and 3 replicates and was repeated twice.

$$MGIP = \frac{Diameter\ of\ colony\ in\ control - Diameter\ of\ colony\ at\ each\ concenntration}{Diameter\ of\ colony\ in\ control} \times 100 \quad \text{Equation 5}$$

MGIP= mycelial growth inhibition percentages

ANOVA was performed on MGIP obtained from fungicide treatments, and the means were compared using Tukey's multiple comparison test.

Integrated test

In this experimentant, antagonist suspensions were prepared at a concentration of 1×10^8 CFU mL^{-1}(Wang *et al*., 2022) while pathogen suspension was adjusted to 1×10^5 spores mL^{-1}(Dionio *et al*., 2020).Surface-sterilized banana fruits were wounded at two sites and treated as follows:

a) 30µl of each antagonist suspension (B_{29},M_{18},T_9) as three treatments(T_1,T_2,T_3). Control fruits(T_0) were treated similarly with sterile distilled water.
b) Methanolic neem extract was injected in to the wounds at a concentration of 4000 ppm(T_4).
c) Thiabendazole was injected in to the wounds at a concentration of 600 ppm(T_5).
d) Methanolic neem extract and the antagonist suspension were injected in to the wounds at the above concentrations(T_6)(Table 2).

All of the fruits were kept in 13ºC for 24h(Hu *et al*., 2015). Subsequently, 40µl of the pathogen suspension was inoculated into each wound. After 20 days, the diameter and area of each lesion were measured, and the inhibitory effect of each treatment was calculated according to Eq. 5.

Table 2. Treatments of management experiment

treatment	method
T_1	B_{29} injection
T_2	M_{18} injection
T_3	T_9 injection
T_4	methanol leaf extract injection
T_5	TBZ injection
T_6	antagonists+methanol leaf extract injection

A completely randomized design with 6 treatments and 4 replications was used. The data were subjected to one-way ANOVA and treatment means were compared using Student's *t*-test at $p \leq 0.01$.

Results

Pathogen isolates: *Fusarium* isolates were consistently recovered from banana fruits exhibiting typical rot symptoms, characterized by dark brown lesions surrounded by light-brown halos. Morphological examination revealed white colonies with purple pigmentation on the reverse side, branched and unbranched conidiophores, monophialides, slender and almost straight macroconidia, and clavate microconidia arranged in long chains, with 1-3 septa. Based on these characteristics, the pathogen was identified as *F. verticillioides.*

Pathogenicity test of identified species: In the pathogenicity test on banana, fruits inoculated with *F. verticillioides* developed typical fruit rot symptoms. Disease severity scores for all inoculated fruits ranged from 1 to 2 (Fig.1 A).

Greenhouse experiments: Greenhouse pathogenicity tests confirmed *F. verticillioides* as the causal pathogen (Table 3). Symptoms mainly affected the corm, pseudostem, and leaves(severe tip rot and brown-to-black lesions on young central leaves)(Fig. 1B). Infected pseudostems often broke or toppled at severely rotted areas near the soil surface and corm (Fig. 1C,F). Leaf spraying and stem injection 10cm above the corm ded not produce any symptoms(Table 3).Therefore, the most effective inoculation method was injection within 5cm above the soil surface, which resulted in severe internal pseudostem decay and destruction of the growing point(Fig. 1D,E). The pathogen was successfully re-isolated from symptomatic plants, thereby confirming its pathogenicity.

Table 3. Pathogenicity tests of *F. verticillioides* on banana seedlings

Inoculation method	DS (%)
Soil inoculation	37.03%
Stem injection (5 cm above the corm)	22%
Corm infestation	1%
Death of central leaf	33%
Injection in 10 cm above corm	0.0
Spraying the leaves	0.0
Control	0.0

Fig. 1

Molecular Identification: PCR analysis resulted in the amplification of aproximately 800-bp and 600-bp fragments of the *RPB2* and *TEF1-α* regions, respectively from all tested isolates.The obtained *RPB2* and *TEF1-α* sequences(GenBank accession Nos. PV068616 and PV124597, respectively) showed 100% sequence identity with the corresponding sequences of *F. verticillioides*. In the phylogenetic analysis, the combined *RPB2* and *TEF1-α dataset* placed the isolates within the *F.verticillioides* clade with strong statistical support (100% bootstrap support for both loci).

Fig 2.

Antagonistic effects of yeast and bacterial isolates. P_{26} and B_{29} showed the highest antagonistic activity against *F. verticillioides*, inhibiting mycelial growth by 48.85% and 41.76% respectively and producing antibiotics in four-spotted cultured method (Table 4).

Table 4. Inhibition percent of bacterial strains against pathogen

Bacterial isolates	four spotted	antibiotic production
P_{49}	21.22^{c}	
P_{53}	23.64^{c}	
P_{50}	29.24^{b}	
P_{26}	48.85^{a}*	77.74^{ab}
P_{19}	30.18^{b}	
P_{10}	31.48^{b}	
P_{6}	32.74^{b}	
B_{29}	41.76^{ab}*	95.78^{a}
B_{15}	40.28^{b}	
B_{11}	12.88^{d}	

*The most effective isolates

Numbers within a column followed by the same letter are not significantly different at $p \leq 0.01$ according to T-test analysis

Two yeast isolates, M_{18} and A_4, significantly inhibited the growth of pathogen ($p \leq 0.01$;Table 5). Among the fungal antagonists, T_9, T_8, and T_{18} showed the highest inhibitory effects, with 82.19%, 80.35%, and 80.25% inhibition, respectively, despite their low antibiotic-producing activity (Table 6).

Table 5. Inhibitory effect(%) of yeasts against pathogen

Yeast isolates	four spotted	antibiotic production
A_5	28.1[c]	
A_4	50.01[a]*	69.5[ab]
A_2	26.3[c]	
M_{48}	42.33[b]	
M_{45}	39.24[b]	
M_{18}	60.04[a]*	64.8[b]

*The most effective isolates
Numbers within a column followed by the same letter are not significantly different at ($p \leq 0.01$) according to T-test analysis

In addition, many yeast strains exhibited fungicidal activity through the production of volatile antifungal metabolites. Among them, M_{18} showed the highest inhibitory effect, reducing the radial mycelial growth of *F. verticillioides* by up to 97% (Table 6).

Table 6. Antagonistic inhibitory effects by dual cultured method and volatile metabolite production

isolates	dual culture (%)	volatile metabolite production (%)
T_6	74.08[b]	27.05[d]
T_{18}	80.25[a]	54.92[b]
T_5	74.08[b]	54.75[b]
T_2	56.77[cd]	37.39[c]
T_8	80.35[a]	26.76[d]
T_9	82.19[a]*	59.91[b]
B_{29}	60.43[c]	29.08[d]
P_{26}	60.99[c]	53.61[b]
A_4	61.53[c]	59.44[b]
M_{18}	58.17[cd]	97.83[a]*

*The most effective isolate
Numbers within a column followed by the same letter are not significantly different at ($p \leq 0.01$) according to T-test analysis

Dual-culture assays showed significant inhibition of *F. verticillioides*. T_9, T_8, and T_{18} showed the highest inhibition (82.19%, 80.35%, and 80.25%, respectively). T_9 volatile metabolites inhibited growth by 59%, while M_{18} showed the strongest combined volatile and antibiotic activity (97.83%). P_{26} produced more effective volatiles than B_{29}, whereas *B. subtilis* showed the highest antibiotic activity.

Neem extracts showed variable antifungal activity. Methanolic leaf extract (4000 ppm) was most effective (77.76%), followed by ethanolic leaf extract (4000 ppm;74.38%). Methanolic and ethanolic seed extracts showed moderate, while aqueous leaf and seed extracts showed the lowest activity (Table 7).

Table 7. Effect of different neem extracts on *F. verticillioides*

Type of extract	500 ppm		1000 ppm		2000 ppm		4000 ppm	
	L	S	L	S	L	S	L	S
aqueous	4.63^{f}	4.13^{f}	6.33^{f}	5.06^{f}	8.83^{f}	8.26^{f}	9.55^{f}	9.04^{f}
ethanol	16.65^{e}	9.73^{f}	26.1^{d}	12.3^{e}	39.2^{bc}	19.66	74.38^{a}	31.07^{c}
methanol	19.56^{de}	16^{e}	35.2^{c}	15.63^{e}	43.04^{b}	24.16	77.76^{a}	38.9^{bc}

L:leaf; S:seed

*The most effective isolate

Numbers within a column followed by the same letter are not significantly different at $p \leq 0.01$ according to T-test analysis

According to Table 8, isolate A_4, alone and in combination with the methanolic leaf extract, showed excellent antifungal activity.

Table 8. Efficacy of combined treatments against *F. verticillioides*

Isolate NO.	Inhibition(%)	
	With extracts of Neem	Without extract of Neem
B_{29}	43.77^{d}	60.43^{a}
P_{26}	47.56^{b}	60.99^{a}
M_{18}	45.27^{c}	58.17^{b}
A_4	50.32^{a}	61.53^{a}

Numbers within a column followed by the same letter are not significantly different at $p \leq 0.01$ according to T-test analysis

In vitro fungicide assays showed significant inhibition of *F verticillioides* mycelial growth at 600 ppm (Table 9).

Table 9. The effect of different concentrations of thiabendazole on *F. verticillioides*

	MGIP %		
	100 ppm	300 ppm	600 ppm
Thiabendazole	49.16[b]	53.47[b]	92.46[a]

Numbers within a column followed by the same letter are not significantly different at p≤ 0.01 according to T-test analysis

The methanolic neem leaf extract-antagonist combination inhibited *F verticillioides* by 88.70%, compared with 37.24% for the 4000 ppm methanolic extract alone.

Fig. 3

Table 10. The effects of treatments on banana fruit rot

treatment	spot area $(mm)^2$	Inhibitory effect (%)
B_{29}	200.389[b]	45.43[b]
T_9	193.478[b]	51.30[b]
M_{18}	134.971[b]	54.26[b]
Methanol leaf Extract	302.380[b]	37.24[b]
TBZ	0.000[a]	100.00[a]
Methanol leaf Extract+ antagonists	75.000[a]	88.70[a]

Numbers within a column followed by the same letter are not significantly different at p≤ 0.01 according to T-test analysis

Discussion

Effective management of *F. verticillioides*, an important pathogen of various crops, may involve the use of biological agents and plant-derived products such as neem extracts. In the present study, the antagonistic acrivities of fungal, bacterial and yeast isolates, as well as neem extracts, were evaluated against *F. verticillioides* infecting banana. Our findings showed that *T. harzianum* strains significantly inhibited the mycelial growth of the pathogen and volatile antifungal metabolites. The inhibitory activity of *T. harzianum* against *F. verticillioides* is consistent with previous findings, with mechanisms of suppression including mycoparasitism, competition for nutrients and space, and production of antifungal compounds (Sobowale *et al*., 2009). In a research the ethanol leave extracts of neem notably enhanced seed germination and seedling vitality in maize, indicating possible advantages in *F. verticillioides* control (Fandohan *et al*., 2004). Additionally, Neem seed powder has been shown to mitigate the severity of diseases caused by *F. oxysporum* and root-knot nematodes in tomato plants, suggesting its

wider applicability in the management of soil-borne pathogens (Hadian *et al*., 2011). In our study, Neem extracts have been assessed for their antifungal efficacy against *F. verticillioides*, showed good findings.We used different Neem leaf extracts against *F. verticillioides* and an excellent antifungal activity against mycelial growth were observedby the methanol leaf extract of Neem at 4000ppm, which is in accordance with results of Kiran *et al*. (2023). Ismaila *et al*. (2022) also examined a safe method to manage fusarium wilt in banana. They used natural products especially essential oils of some plants such as ginger. Their results showed a good bioactive antifungal effect on pathogen too.In the study of Attia *et al*. (2025) *Bacillus subtilis* showed significant effects against *F. verticillioides* on *Vicia faba*. In our experiments this bacterial antagonistproduced antibiotic metabolite and inhibited the mycelial growth of *F. verticillioides*. Matic *et al*. (2014) used some antagonist yeasts including *Pichia guilliermondii,* to control of *F. fujikuroi* on rice, and their results revealed a 95% decrease in pathogenicity of this species. In the present study, two yeast species, *Pichia guilliermondii* and *Candida membranifuciens*, showed 60.04% and 50.01% inhibition against growth of *F. verticillioides* respectively.

Chemical control as the last advised method in plant pathology, to be employed only after exploring more sustainable and integrated pest management (IPM) strategies. This tendency toward non-chemical approaches is due tothe environmental consequences, the emergence of resistance, and the possible detrimental effects on beneficial organisms. Nevertheless, there are circumstances in which chemical control is essential and effective, particularly when alternative methods do not yield adequate results or in instances of severe and widespread infections. For such conditions a low toxic, and effective fungicide was selected against the test pathogen fungi; Thiabendazole treatment at 600 ppm were more effective in reducing mycelial growth by 92.46%. Tava *et al*. (2021) studied the effect of prochloraz and tebuconazole on fruit rot of banana caused by *F. verticillioides*, and their results showed a well inhibitory effect in vitro against this species. We also evaluated one of imidazole fungicides; Thiabendazole, on *F. verticillioides*. In vivo evaluations showed that between all treatments, Thiabendazole at 600 ppm concentration wasthe best effective treatment against the pathogen.

According to our findings in the current study, the leaf extract of *A. indica* demonstrates good efficacy against the *F. verticillioides* as the banana fruit rot pathogen. Finally, we found that after chemical fungicide, combination of bacterial and yeast antagonists with methanol leaf extract of neem showed the best inhibition against *F. verticillioides*. Additionally, it is necessary to isolate and characterize the bioactive compounds within the Neem extract, as well as conduct field experiments, to endorse this herbal remedy for combating Banana wilt.

References:

1. Acharya S. 2021.Dual Culture Approach for the Evaluation of Antagonistic Activity of Native *Trichoderma* Isolates against *Bipolaris sorokiniana*. International Journal of Advanced Research 9 (12): 1002-1008.
2. Attia M.S., El-Wakil D.A., Hashem A.H., Al-Askar A.A., Abdelgawad H., Alotaibi R.S., Abdel-Kader S.A., Abdelaziz A.M. 2025. Investigating the activity of *Bacillus subtilis* and *Trichoderma harzianum* to mitigate Fusarium wilt disease of diverse cultivars of *Vicia faba*. Scientific Reports 15: 16093. 10.1038/s41598-025-99381-2
3. Bahraminejad S., Asenntorfer R.E., Riley I.T., Zwer P., Schultz C.J. and Schmidt O. 2008. genetic variation of flavonoid defense compound concentration in oat (*avena sativa*L.) entries and testing of their biological activity. 1127-1132. In: Proceedings of Australasian plant breeding conference.18-21 Jan 2008, Christchurch, New Zealand.
4. Denham T.P., Haberle S.G.,Fullagar C.R.,Field J.,TherinM.,PorchM. Winsborough B. 2003. Origins of agriculture at Kuk swamp in the Highlands of New Guinea. Science 301:189-193.
5. Dionio B.T., Bacoba C.J.M., Puig C.G., Ramos F.R.A. 2020. Fusarium Heart Rot:First Report on Pineapplein South Cotabato and Davao City, Philippines.Southeastern Philippines Journal of Research and Development 25 (1): 183-196. dx.doi.org/10.53899/spjrd.v25i1.65
6. Elbouazaoui A., Sijilmassi B., Maafa I., Allal D., Ahmed S. 2022.Biocontrol activity of *Bacillus*, *Paenibacillus* and *Pseudomonas* against Fusarium wilt of chickpea in Morocco.Acta Agriculturae Scandinavica, Section B- Soil & Plant Science 72 (1): 847-859, 10.1080/09064710.2022.2100819
7. Fandohan P., Gbenou J.D., Gnonlonfin B., Hell K., Marasas W.F., Wingfield M.J. 2004. Effect of essential oils on the growth of *Fusarium verticillioides* and fumonisin contamination in corn. Journal of Agricultural and Food Chemistry52(22):6824-6829. 10.1021/jf040043p.
8. Hadian Sh., Rahnama K., Jamali S., Eskandari A.2011. Comparing Neem Extract with Chemical Control on *Fusarium oxysporum* and *Meloidogyne incognita* Complex of Tomato. Advances in Environmental Biology. 5(8): 2052-2057.
9. Hagen E.N., Dunwiddie P.W. 2008. Does stem injection of glyphosate control invasive knotweeds (*Polygonum* spp.)? A comparison of four methods. Invasive Plant Science and Management 1(1):31-35.
10. Hu H., Xu Y., Lu H-P., Xiao R., Zheng X., Yu T. 2015. Evaluation of yeasts from Tibetan fermented products as agents for biocontrol of blue mold of Nashi pear fruits. Journal of Zhejiang University-Science B16 (4): 275-285.

11. Ismaila A.A., Ahmad Kh., Siddique Y., AbdulWahab M.A., Bashir Kutawa A., Abdullahi A., Mohd Zobir S.A., Abdu A., Akmar Abdullah S.N.2023. Fusarium wilt of banana: current update andsustainable disease control using classical and essential oils approaches. Horticultural Plant Journal. 10.1016/j.hpj.2022.02.004
12. Kahawattage A., Hansini N., Daranagama D.A., Ranasinghe Ch. 2023. Effect of pre-treatments with natural compounds for controllinganthracnose in papaya variety Red Lady.Journal of Horticulture and Postharvest Research 6(2): 169-180, 10.22077/jhpr.2023.5762.1292
13. Kara M., Soylu S. 2022. Isolation of endophytic bacterial isolates from healthy banana trees and determination of their in vitro antagonistic activities against crown rot disease agent *Fusarium verticillioides*. Journal of Agricultural Sciences 27 (1): 36-46.
14. Kiran B.L., Nayana K.N., Raveesha K.A. 2023. Evaluation of antifungal effect of medicinal plants against Panama wilt of Banana caused by *Fusarium oxysporum* f. sp. *cubense*. Journal of Applied Biology and Biotechnology 11(1): 73-79, 10.7324/JABB.2023.110110
15. Leslie J.F., Summerell A. 2006. The *Fusarium* Laboratory Manual.Blackwell Publishing, United States. 387 pp. 10.1002/9780470278376
16. Matic S., Spadaro D., Garibaldi A., Lodovica Gullino M. 2014. Antagonistic yeasts and thermotherapy as seed treatments to control *Fusarium fujikuroi* on rice.Biological Control (73): 59-67, 10.1016/j.biocontrol.2014.03.008.
17. Moore N.Y., Pegg K.G., Allen R.N., Irwin A.G. 1993. Vegetative compatibility and distribution of *Fusarium oxysporum* f.sp. *cubense* in Australia. Australian Journal of Experimental Agriculture 33: 797-802.
18. Murad N.B., Mustafa M., Shaari Kh., Zainudin N.A. 2021. Antifungal Activity of Aqueous Plant Extracts and Effects on Morphological and Germination of Fusarium Fruit Rot Pathogens.Sains Malaysiana 50 (6): 1589-1598. 10.17576/jsm-2021-5006-07
19. Nelson P.E., Toussoun T.A., Marasas W.F.O. 1983. *Fusarium* species: an illustrated manual for identification. Pennsylvania State University Press. Pennsylvania. 206 pp.
20. Payan-Arzapalo M.A., Lopez-Avendano J.E., CazarezFlores L.L., Lopez-Inzunza H.d.J., Molina-Cardenas L., Vega-Gutierrez T.A., Tirado-Ramirez M.A. 2024. Effectiveness of Chemical Fungicides for the in vitro Control of *Fusarium* spp. Causing Basal Rot in Onion in Sinaloa, Mexico. Agro Productividad, 10.32854/agrop.v17i11.3115
21. Rai Sh. 2024. Exploring the properties and extraction of neem leaves. International Journal of Toxins and Toxics 01 (02):32-39.

22. Roy M., Langthasa S., Hazarika J., Hazarika D.N. 2023. Effects of various indigenous ripening methods on phonological and physical characteristics of banana. The pharma Innovation Journal 12 (9): 871-876.
23. Salem N.M., AlMomany A.M., Tahat M.M., Aldakil H. 2020. First Report of *Fusarium verticillioides* causing Banana Fruit Rot in Jordan.Plant Disease.10.1094/PDIS-05-20-1116-PDN
24. Sipiczki M. 2023. Identification of antagonistic yeasts as potential biocontrol agents: Diverse criteria and strategies. International Journal of Food Microbiology 406: 110360. 10.1016/j.ijfoodmicro.2023.110360
25. Sneh B., Burpee L.L., Ogoshi A. 1991. Identification of *Rhizoctonia* species. The American Phytopathological Society Press, SaintPaul, Minnesota. 133 pp.
26. Sobowale A.A., Odebode A.C., Cardwell K.F., Bandyopadhyay R. 2009. Suppression of growth of *Fusarium verticillioides*(Niren.) using strains of *Trichoderma harzianum* from maize (*Zea mays*) plant parts and its rhizosphere. Journal of Plant Protection Research49(4):452-459. 10.2478/v10045-009-0072-7.
27. Solorzano R., Maguina H.A.R., Johnson L., Sierra C.U., Cruz J. 2025. Current Progress in Microbial Biocontrol of Banana Fusarium Wilt: A Systematic Review. Agronomy 15: 619. 10.3390/agronomy15030619
28. Stracquadanio C., Quiles J.M., Meca G., Cacciola S. 2020. Antifungal activity of bioactive metabolites produced by *Trichoderma asperellum* and *Trichoderma atroviride* in liquid medium. Journal of Fungi 6:263. 10.3390/ jof6040263.
29. Tava V., Prigitano A., Cortesi P., Esposto M.C., Pasquali M. 2021.*Fusarium musae* from Diseased Bananas and Human Patients: Susceptibility to Fungicides Used in Clinical and Agricultural Settings. Journal of Fungi 7(9): 784. 10.3390/jof7090784
30. Wang J., Cai B., Li K., Zhao Y., Li Ch., Xiang D., Zhang L., Xie J., Wang W. 2022. Biological Control of *Fusarium oxysporum f.sp.cubense* Tropical Race 4 in Banana Plantlets Using Newly Isolated *Streptomyces* sp. WHL7 from Marine Soft Coral. Plant Disease 106 (1):254-259.
31. Weller D.M. 1988. Biological control of soil-borne plant pathogens in the rhizosphere with bacteria. Annual Review of Phytopathology 26: 379-407.
32. Zakaria L. 2023. Fusarium Species Associated with Diseases of Major Tropical Fruit Crops. Horticulture 9 (3): 322. 10.3390/horticulturae9030322
33. Zhang M., SunX., Cui L., Yin Y., Zhao X., Pant S., Wang W. 2018. The Plant Infection Test: Spray and Wound-Mediated Inoculation with the Plant Pathogen *Magnaporthe grisea*. Journal of Visualized Experiments 4(138): 57675. 10.3791/57675

34. Zhong S., Steffenson B.J. 2001. Virulence and molecular diversity in *Cochliobolus sativus*. Phytopathology 91: 469-476.

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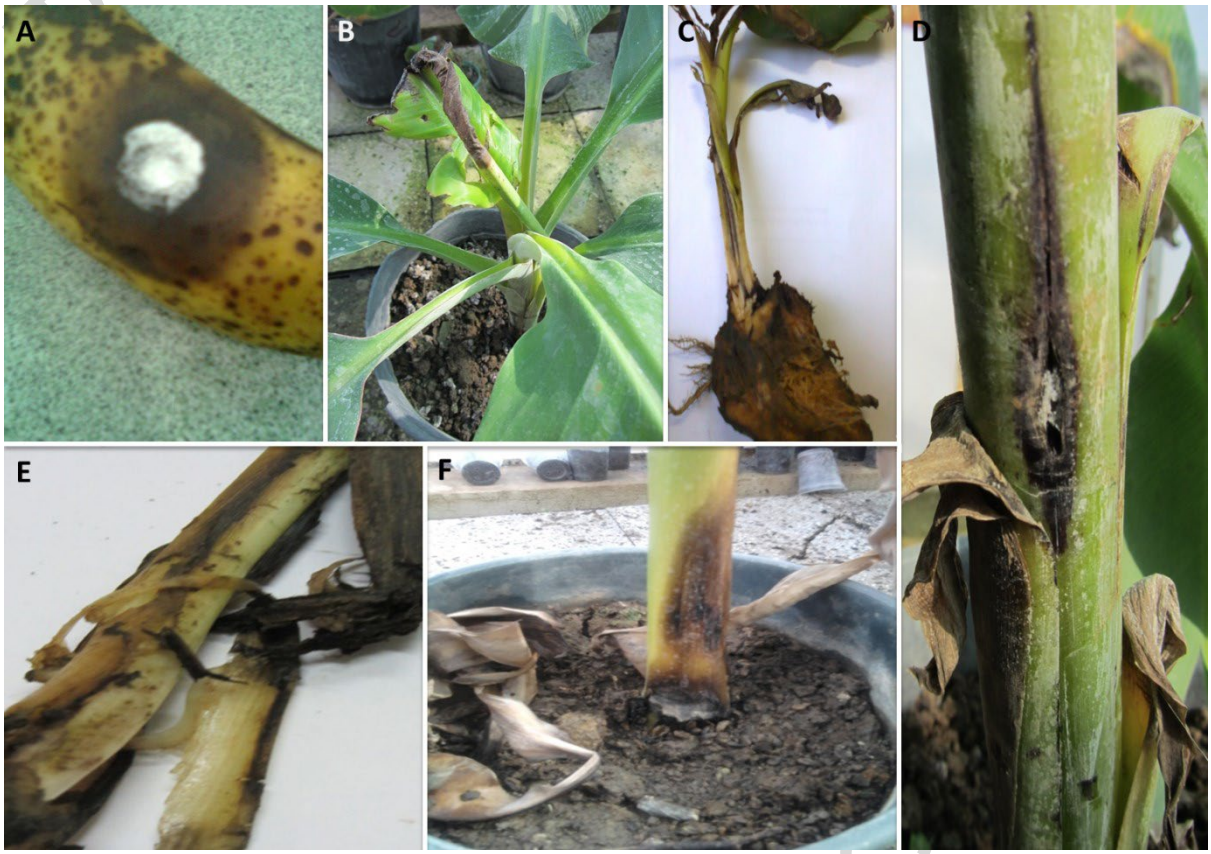

Fig. 1. A. Symptoms on inoculated fruits with F. verticillioides, B. Browning of the central young leaves, C. corm rot, D. Injection at 5 cm above the corm, E. phloem browning in longitudinal section of pseudostem, E. and F. symptoms of infected seedlings with pathogen isolates

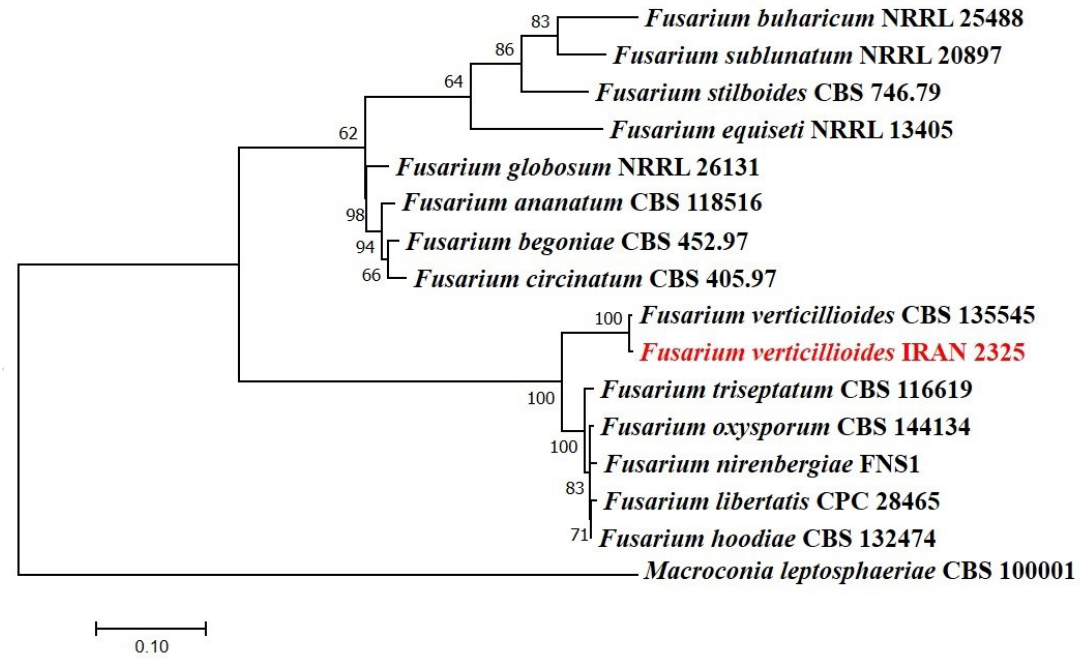

Fig 2. The Maximum Likelihood (ML) tree obtained based on the combination of tef1 and rpb2 sequences alignment of 16 isolates of Fusarium with Macroconia leptosphaeriae (CBS 100001) serving as the outgroup taxon. Values from 1000 replicates are displayed at the nodes. The strain highlighted in red represents the specimen recovered in this study

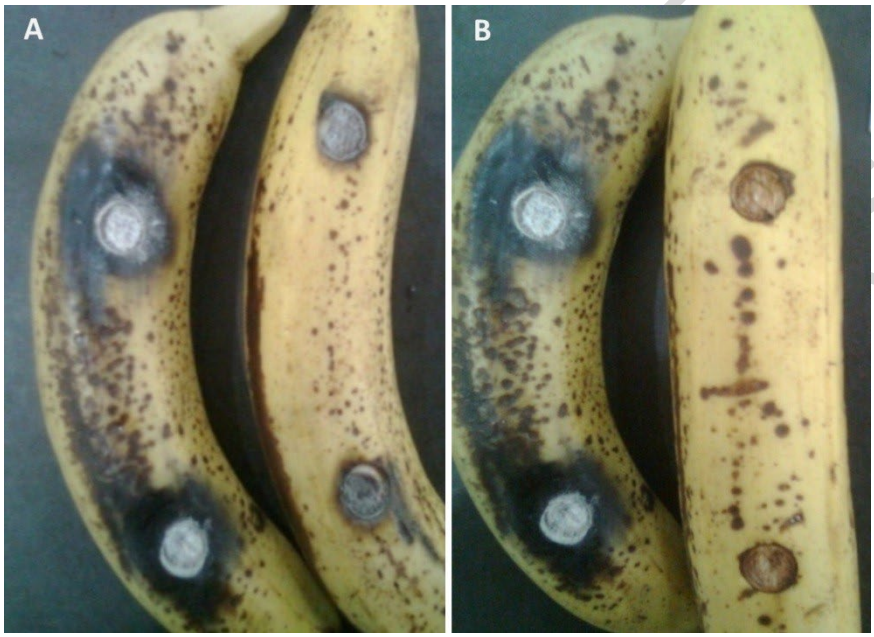

Fig. 3. Inhibition zone 10 days after application of M18 strain (A) and Thiabendazole (B) against F. verticillioides and comparing to control