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Biocontrol of *Fusarium* spp. by soil surface fungi from Mt. Isarog, Camarines Sur

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Abstract

The pathogenic strains of *Fusarium* spp. wilt entire hectares of agriculturally important crops, and biological control is the most advantageous method to halt this problem. In this study, fourteen soil surface fungi were isolated using serial dilution and initial inoculation in synthetic nutrient-poor agar (SNA), Czapek Dox agar (CDA), and malt extract agar (MEA). The soilborne fungal isolates were morphologically identified into four genera: *Aspergillus*, *Curvularia*, *Dictyuchus*, *Trichoderma*, while two were identified as mycelia sterilia. We performed dual culture and best inoculation method to test the biocontrol activity of the 14 soil surface fungi against *Fusarium verticillioides, Fusarium moniliforme,* and *Fusarium* sp. Both methods revealed that soil surface fungi could inhibit the growth of phytopathogenic fungi. *Aspergillus sp*. 3 inhibited the growth of three pathogenic fungi when planted first in best inoculation, while *Trichoderma* sp. inhibited the growth of *Fusarium* sp. in both methods, and *F. verticillioides* when planted first in the best inoculation method.

Keywords – Antagonistic Effect – *Fusarium* – Inhibition – Negative Control – Treatment – Wilt

Introduction

Fusarium spp. are one of the most common and diverse species of filamentous fungi ("*Fusarium* Wilt," 2010) that act either as symbionts or saprobes that provide protection and nutrition for plant roots, or as plant pathogens that cause severe damage to economically important crops (Wang et al. 2015) by inflicting vascular wilts, root rot, cankers, and other afflictions (Nelson et al. 1994) which results in significant losses worldwide (Brunner & Mach 2010). Once an outbreak occurs, the pathogenic strains are very difficult to eliminate, as they require the quarantine of the infected region alongside the inspection of the region's biotic and abiotic factors. Once isolated, the pathogen can be studied to create methods for its containment and elimination (Pocasangre et al. 2011). According to Burgess (2000), *Fusarium* spp. are widely distributed in various substrates of both wet and dry lands in both normal and extreme climate conditions.

Biological control is the most effective pest management method that has been utilized extensively as an economical and cost-effective solution against crop diseases with relatively few side effects. Antagonistic organisms that combat intended pathogenic organisms are applied as biocontrol agents (Alabouvette et al. 2009) while maintaining the quality of the ecosystem to which they were introduced. One of the most commonly used and reliable biological control agents is

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fungi (Thambugala et al., 2020). Terrestrial soil fungi have historically been shown to be effective as they use strategies such as mycoparasitism, competition, induced systemic resistance of their hosts, development of suppressive soils, or abiosis through antagonistic activity (Benhamou et al. 2002) to protect and synergistically improve their designated host plants. Among these, the most common and effective mode of protection is achieved by abiosis through antagonistic activity. Therefore, the researchers prefer to assess the antagonistic activity of isolated soil surface fungi against phytopathogenic *Fusarium* species, including *Fusarium verticillioides*, *F. moniliforme*, and *Fusarium* sp.

Materials and Methods

Preparation of Media

Three different types of media were used to facilitate the growth and isolation of different types of fungi. The SNA media was used due to its inadequate nutrients, which delay fast-growing organisms and allow slow-growing species to grow and to be isolated (Allee 2014). Modified CDA was used as the media with inorganic nitrogen that stimulates the growth of nitrogen-fixing species (Himedia 2018), while chitin (ground shrimp shells), replacing the sucrose, allows chitinase-producing fungi to grow. Finally, MEA was used as it favors the growth of fungi while inhibiting bacterial growth due to its acidic pH (Remel 2010). In the preparation of these media, 1.25 mL of streptomycin is added to every 600 mL medium to suppress the growth of bacteria.

Collection of Samples

The soil surface samples were collected in four different sites in Mt. Isarog, Camarines Sur; intact forest 13.7065°N, 123. 4030°E; demolished forest 13.7066°N, 123.4042°E; low land forest 13.7066°N, 123.4042°E; and the wetland area near the Consocep resort 13.7069°N, 123.4040°E. Five soil samples of approximately 250 grams in total were collected from each site using a trowel, placed in zip lock bags, and stored in a cool, dry place. After the soil samples were cleared of the leaf litter, they were taken to the University of Santo Tomas Science Laboratories for fungal isolation.

Isolation and Purification

All the soil samples collected in each site were mixed to get the collective species of soil fungi per site. Isolation of the soil fungi was based on the procedure mentioned by Rao (1970) with slight modifications. Serial dilution was done by mixing a one-gram soil sample with 9 mL distilled water (10^{-1}). One mL of the mixture was transferred to subsequent test tubes containing 9 mL sterile distilled water (10^{-2} to10^{-6}). One hundred microliters of 10^{-5} and 10^{-6} serial dilutions were evenly spread on each medium in triplicate. The plates were incubated at room temperature for fourteen days and recorded observations and hyphal isolations on the 3rd, 5th, 7th, 9th, 11th, and 14th days. All fungal isolates were transferred to MEA plates to maximize their growth.

The cultures of phytopathogenic fungi were obtained from the Research Center for Natural and Applied Sciences of the University of Santo Tomas.

Morphological Identification

Spore tip method and morphological identification were performed according to Fatima (2014) with modifications. The results were used to identify the isolate with the aid of the pictorial atlas of Watanabe T. (2010). Pictures of the specimens were then taken under 1000x magnification.

Dual Culture Assay

The soil surface fungi were tested for their antagonistic activity against three species of *Fusarium* by dual culture technique (Rahman et al. 2009). Agar blocks (5 mm) of the soil fungi and *Fusarium* isolate were placed on the opposite sides of the Petri dish. The plates were incubated at room temperature and were examined on the 7th and 10th days. The percent growth inhibition (PGI)

was calculated relative to the control (plate with pathogen only) to determine the degree of the effect of the opposing species to the pathogen (Belete et al. 2015).

PGI = ((D1 – D2)/D1) × 100
Where:
D1 – diameter of pathogen colony in control plate;
D2 – diameter of pathogen colony in treatment

The interactions between the plant pathogen and the soil fungi were assessed according to Wicklow (1980) as follows: (0) mutual intermingling of the two organisms; (1) mutual inhibition on contact, the space between the two colonies is small but marked; (2) mutual inhibition at a distance; (3) inhibition of one organism on contact, the antagonist continued to grow at an unchanged or reduced rate which would eventually grow over the colony of the inhibited organism; and (4) inhibition of one organism at a distance, the antagonist continued to grow through the resulting clear zone at an unchanged or reduced rate.

Best Inoculation Assay

This method only included species of soil fungi with positive reactions against the pathogen in the dual culture method. The cultural antagonism of soil surface fungal isolates against *Fusarium* spp. was observed under two means of inoculation. The first method required the inoculation of *Fusarium* spp., followed by a two-day incubation before introducing the fungal isolates. Conversely, the second method required the inoculation of the fungal isolates followed by a two-day incubation before introducing the *Fusarium* spp. All plates were examined on the 7^{th} and 10^{th} day after the first inoculation.

Statistical Analysis

The variance test was calculated using Analysis of Variance (ANOVA) and statistical F tests at $P \leq 0.05$ to determine if there was a significant difference between the radial growth of *Fusarium* spp. in control and treatment.

Results

Isolation and identification of soil fungi

From the soil samples obtained from Mt. Isarog, Camarines Sur, 14 soil surface fungi (labeled A to N) were isolated and morphologically identified under four genera, namely *Aspergillus*, *Curvularia*, *Dictyuchus*, and *Trichoderma;* however, two isolates were classified as mycelia sterilia (Table 1).

Table 1 Morphological characteristics and identification of soil surface fungi from Mt. Isarog, Camarines Sur.

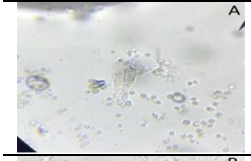
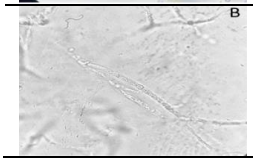
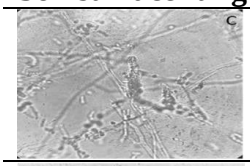
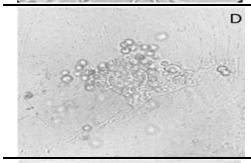
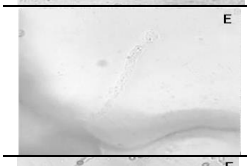
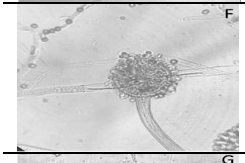
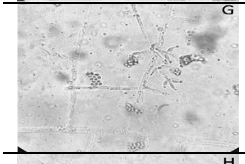
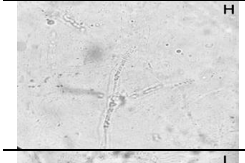
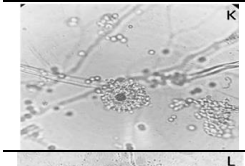
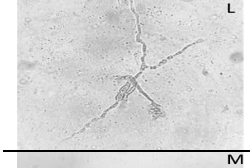
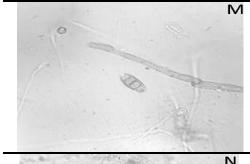
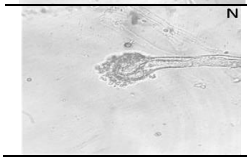
Soil surface fungi	Morphological Characteristics	Identification
	White, fast-growing filamentous colony.	*Aspergillus* sp. 1
	White, circular, slow-growing colony that lacks conidia and conidiophores.	Mycelia sterilia 1

Table 1 Continued.

Soil surface fungi	Morphological Characteristics	Identification
	White, fast-growing filamentous colony.	Dictyuchus sp.
	White, fast-growing filamentous colony with brown-green spores.	*Aspergillus* sp. 2
	White, fast-growing filamentous colony that develops green spore masses as it matures.	*Aspergillus* sp. 3
	White, slow-growing filamentous colony that develops black spores on and near its center as it matures.	*Aspergillus* sp. 4
	White, fast-growing filamentous colony that eventually turns forest green.	*Trichoderma* sp.
	White, fast-growing filamentous colony that lacks conidia and conidiophores.	Mycelia sterilia 2
	White, slow-growing circular colony but produced orange spores as it matures.	*Aspergillus* sp. 5
	Dark green, slow-growing irregular colony that develops white-colored margins as it matures.	*Aspergillus* sp. 6
	White, slow-growing circular colony, which is covered in yellow conidia as it matures.	*Aspergillus* sp. 7
	Forest green, slow-growing circular colony that develops a white margin as it matures.	*Aspergillus* sp. 8
	Black, slow-growing circular colony with a center turning white as it matures.	*Curvularia* sp.
	White, slow-growing circular colony characterized by a color transition from light to dark brown near the center.	*Aspergillus* sp. 9

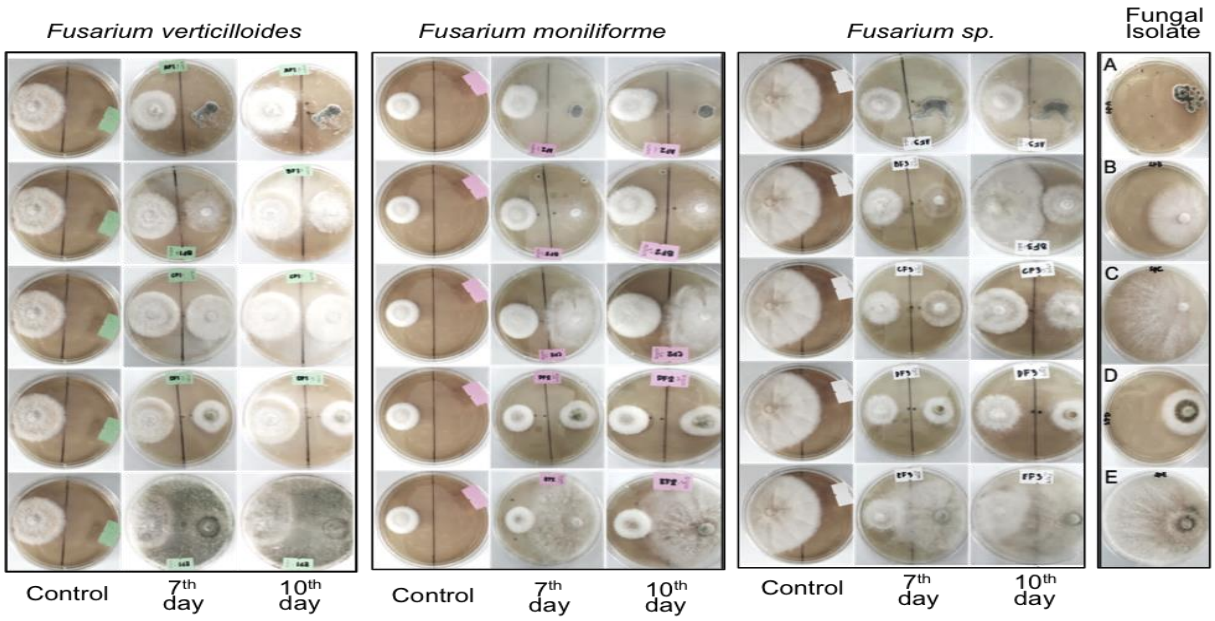


Fig. 1a – Dual culture assay of isolated soil surface fungi (A–E) against *Fusarium verticillioides*, *Fusarium moniliforme*, & *Fusarium* sp. at 7th and 10th day of incubation.

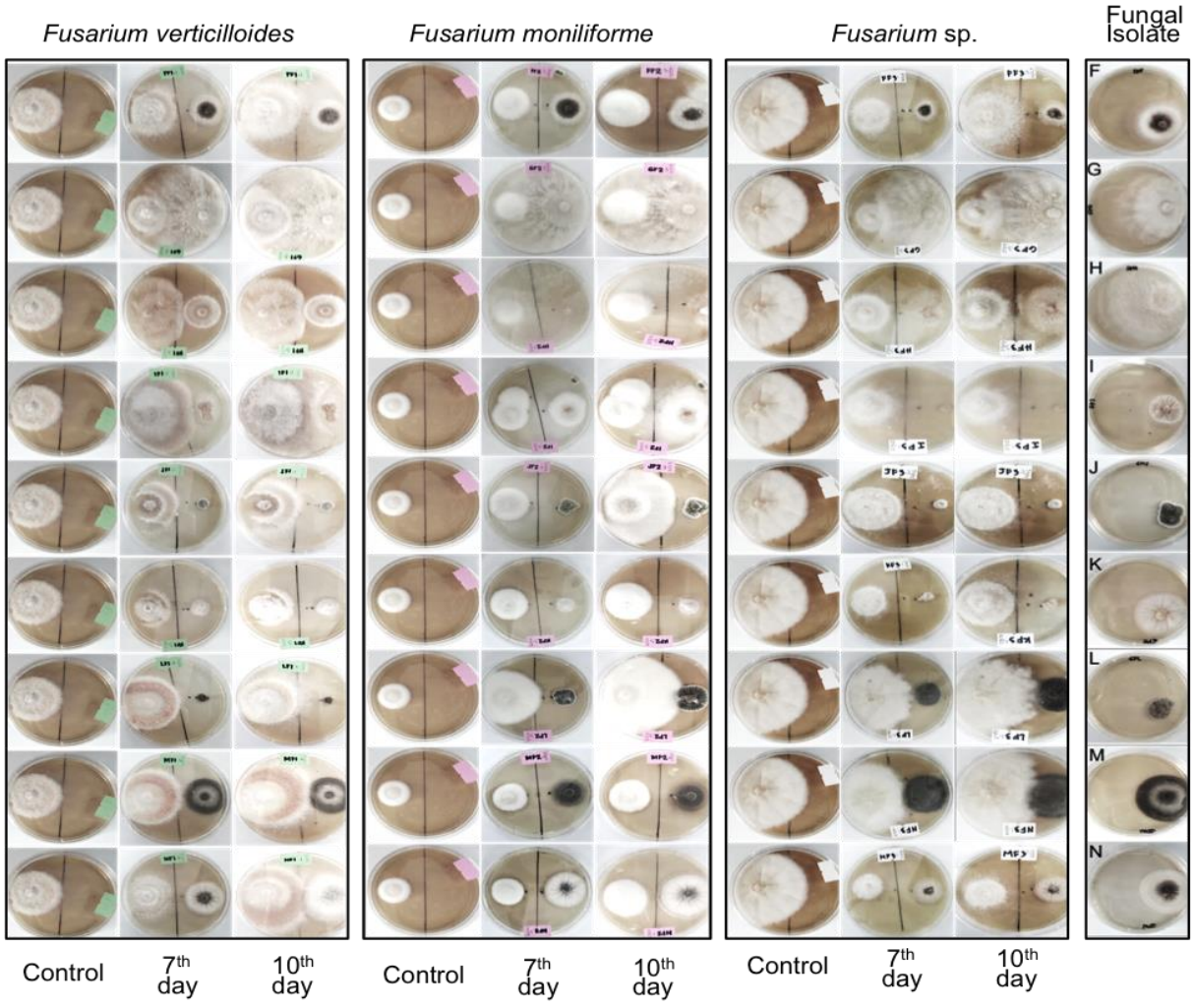


Fig. 1b – Dual culture assay of isolated soil surface fungi (F–N) against *Fusarium verticillioides*, *Fusarium moniliforme*, & *Fusarium* sp. at 7th and 10th day of incubation.

The antagonistic potential of soil fungi species against *Fusarium* spp.

As seen in Figs 1a, b, *Aspergillus* sp. 2, 3, and 9 (D, E, N) and *Trichoderma* sp. (G) inhibited the growth of *F. verticillioides*. *Fusarium moniliforme* was inhibited by *Aspergillus* sp. 3 alone, while *Fusarium* sp. was inhibited by all *Aspergillus* species, *Curvularia*, *Trichoderma*, *Dictyuchus*, and mycelia sterilia 1 & 2.

Table 2 Effect of the soil fungi on the growth of pathogenic *Fusarium* spp. on malt extract agar (MEA) after 7th and 10th days of incubation. Note: The values are expressed as mean ± standard error (SE) (n= 3).

	Fusarium verticillioides			***Fusarium moniliforme***		
Fungal Isolates	Width of inhibition zone (cm)		Wicklow's Interactions	Width of inhibition zone (cm)		Wicklow's Interactions
	7th DAY	10th DAY		7th DAY	10th DAY	
Aspergillus sp.1	4.70±0.06	5.33±0.06	4	2.47±0.12	3.07±0.07	4
Mycelia sterilia 1	4.53±0.30	4.93±0.28	3	3.17±0.18	3.40±0.15	2
Dictyuchus sp.	4.73±0.20	4.97±0.19	3	3.07±0.09	3.50±0.15	3
Aspergillus sp. 2	4.17±0.20	4.30±0.22	4	2.77±0.09	3.03±0.18	4
Aspergillus sp. 3	3.77±1.00	3.10±0.62	3	2.57±0.15	2.67±0.07	4
Aspergillus sp. 4	4.73±0.30	4.97±0.18	4	3.17±0.12	3.67±0.12	2
Trichoderma sp.	3.00±0.30	3.17±0.15	0	2.70±0.32	3.17±0.29	0
Mycelia sterilia 2	4.83±0.30	5.17±0.17	3	3.70±0.90	3.90±0.80	2
Aspergillus sp. 5	5.13±0.50	5.63±0.26	3	4.27±0.35	4.63±0.27	0
Aspergillus sp. 6	5.37±0.30	5.80±0.15	4	3.40±0.25	4.42±0.52	4
Aspergillus sp. 7	5.10±0.10	5.13±0.06	2	3.63±0.33	4.20±0.35	4
Aspergillus sp. 8	5.23±0.20	5.77±0.09	4	3.57±0.68	4.02±0.87	0
Curvularia sp.	4.57±0.40	4.83±0.22	3	2.83±0.18	3.30±0.12	2
Aspergillus sp. 9	4.10±0.40	4.60±0.25	3	2.87±0.17	3.13±0.23	2
Control	4.63±0.33	6.27±0.66	-	2.60±0.10	2.93±0.15	-

	***Fusarium* sp.**		
Fungal Isolates	Width of inhibition zone (cm)		Wicklow's Interactions
	7th DAY	10th DAY	
Aspergillus sp.1	4.83±0.07	4.73±0.20	2
Mycelia sterilia 1	4.47±0.03	4.87±0.09	3
Dictyuchus sp.	4.23±0.03	4.70±0.00	2
Aspergillus sp. 2	4.33±0.12	4.67±0.09	2
Aspergillus sp. 3	3.47±0.15	3.50±0.15	3
Aspergillus sp. 4	4.93±0.03	5.20±0.00	4
Trichoderma sp.	3.17±0.24	3.20±0.21	0
Mycelia sterilia 2	4.43±0.12	4.60±0.20	2
Aspergillus sp. 5	4.60±0.36	5.23±0.33	4
Aspergillus sp. 6	4.73±0.50	4.93±0.67	4
Aspergillus sp. 7	5.13±0.20	5.50±0.17	4
Aspergillus sp. 8	5.37±0.03	6.20±0.40	3
Curvularia sp.	4.07±0.23	4.30±0.35	0
Aspergillus sp. 9	4.27±0.47	4.80±0.17	4
Control	6.47±0.61	7.00±0.40	-

As seen in Table 2, the width of the zone of inhibition and the interactions were noted and interpreted, referring to Wicklow's table of interactions. Table 2 shows that the growth of *F. verticillioides* and *Fusarium* sp. were faster than *F. moniliforme*. Mycelia sterilia 1, *Aspergillus* spp. 2, 3, 9, and *Trichoderma* sp. inhibited the growth of *F. verticillioides* on day 7, while the rest

of the species showed no significant inhibition. On day ten, all soil surface fungi isolates inhibited the growth of the pathogen. *Aspergillus* spp. 2 and 3 exhibited inhibition at a distance, while *Aspergillus* sp. 3 eventually covered the pathogen. *Aspergillus* sp. 9 displayed inhibition at a distance while *Trichoderma* sp. intermingled with the pathogen. Although *Trichoderma* sp. inhibited the growth of *F. verticillioides* on day 7, there was a minimal increase in the size of the pathogen on day 10. For *F. moniliforme*, both *Aspergillus* spp. 1 and 3 displayed inhibitions at a distance. For *Fusarium* sp., *Aspergillus* sp. 3 and mycelia sterilia 1 both displayed inhibition on contact with the pathogen, while *Trichoderma* sp. exhibited mutual intermingling. *Aspergillus* spp.1 and 4 displayed inhibitions at a distance. *Curvularia* sp. and *Trichoderma* sp. showed mutual intermingling, while *Dictyuchus* sp. and mycelia sterilia 2 displayed mutual inhibition at a distance.

Table 3 Analysis of variance (ANOVA) table of dual culture. Having a $p<0.05$ indicates that there is a significant difference between the radial growth of the *Fusarium* in treatment and control.

Fungal Isolates	***Fusarium verticillioides***				***Fusarium moniliforme***			
	7th DAY		10th DAY		7th DAY		10th DAY	
	PGI	p-value	PGI	p-value	PGI	p-value	PGI	p-value
Aspergillus sp.1	-2.17	0.85	14.80	0.25	5.12	0.44	-4.66	0.45
Mycelia sterilia 1	1.35	0.82	21.19	0.15	-21.79	0.05	-16.04	0.09
Dictyuchus sp.	-2.90	0.80	20.66	0.12	-17.95	0.02	-19.45	0.05
Aspergillus sp. 2	9.42	0.30	31.30	0.04	-6.41	0.28	-3.53	0.68
Aspergillus sp. 3	18.11	0.28	50.48	0.01	1.28	0.86	8.98	0.17
Aspergillus sp. 4	-2.90	0.80	20.66	0.14	-21.79	0.02	-25.14	0.01
Trichoderma sp.	34.78	0.01	49.41	0.01	-3.86	0.78	-8.08	0.51
Mycelia sterilia 2	-5.07	0.61	17.46	0.17	-42.31	0.29	-33.11	0.30
Aspergillus sp. 5	-11.59	0.29	10.01	0.40	-64.10	0.01	-58.13	0.01
Aspergillus sp. 6	-16.66	0.11	7.34	0.51	-30.77	0.04	-50.74	0.05
Aspergillus sp. 7	-10.87	0.23	17.99	0.16	-39.74	0.04	-43.35	0.03
Aspergillus sp. 8	-13.77	0.15	7.88	0.49	-37.18	0.23	-37.09	0.29
Curvularia sp.	0.72	0.87	22.79	0.14	-8.98	0.31	-12.63	0.12
Aspergillus sp. 9	10.87	0.26	26.51	0.09	-10.26	0.24	-6.94	0.51

Fungal Isolates	***Fusarium sp.***			
	7th DAY		10th DAY	
	PGI	p-value	PGI	p-value
Aspergillus sp.1	25.18	0.05	32.38	0.01
Mycelia sterilia 1	30.85	0.03	30.47	0.01
Dictyuchus sp.	34.47	0.02	32.86	0.01
Aspergillus sp. 2	32.92	0.02	33.33	0.01
Aspergillus sp. 3	46.33	0.01	50	0
Aspergillus sp. 4	23.63	0.06	25.71	0.01
Trichoderma sp.	50.98	0.01	54.29	0
Mycelia sterilia 2	31.37	0.03	32.39	0.01
Aspergillus sp. 5	28.79	0.05	25.24	0.03
Aspergillus sp. 6	26.72	0.09	29.53	0.06
Aspergillus sp. 7	20.53	0.11	21.43	0.03
Aspergillus sp. 8	16.92	0.14	11.43	0.23
Curvularia sp.	37.05	0.02	38.57	0.01
Aspergillus sp. 9	33.95	0.05	31.43	0.01

The inhibition by the isolates against *F. verticillioides* was calculated based on the Percent Growth of Inhibition (PGI). *Aspergillus* sp. 2 showed 9.42 PGI against the pathogen while *Aspergillus* spp. 3 and 9 exhibited 18.11 and 10.87 PGI, respectively. *Trichoderma* sp. with 34.78 PGI also showed inhibition against the pathogen on day 7. On the 10th day, *Aspergillus* sp. 3 and

Trichoderma sp. showed the highest PGI of 50.48 and 49.41, respectively. For *F. moniliforme*, only *Aspergillus* sp. 3, with a PGI of 1.28 and 8.98, inhibited the pathogen on days 7 and 10, respectively. All species inhibited the growth of *Fusarium* sp. with *Aspergillus* sp. 2 and 3; *Dictyuchus* sp., mycelia sterilia 1 and 2; *Trichoderma* sp. and *Curvularia* sp. showing the significant difference in the growth of the pathogen in control.

For the results of best inoculation, we decided to disregard species *Aspergillus* sp. 1, 4, 5, 6, 7, 8, and 9 due to contamination during incubation and their negative results in the dual culture. The results in the ANOVA table further support all data in this method.

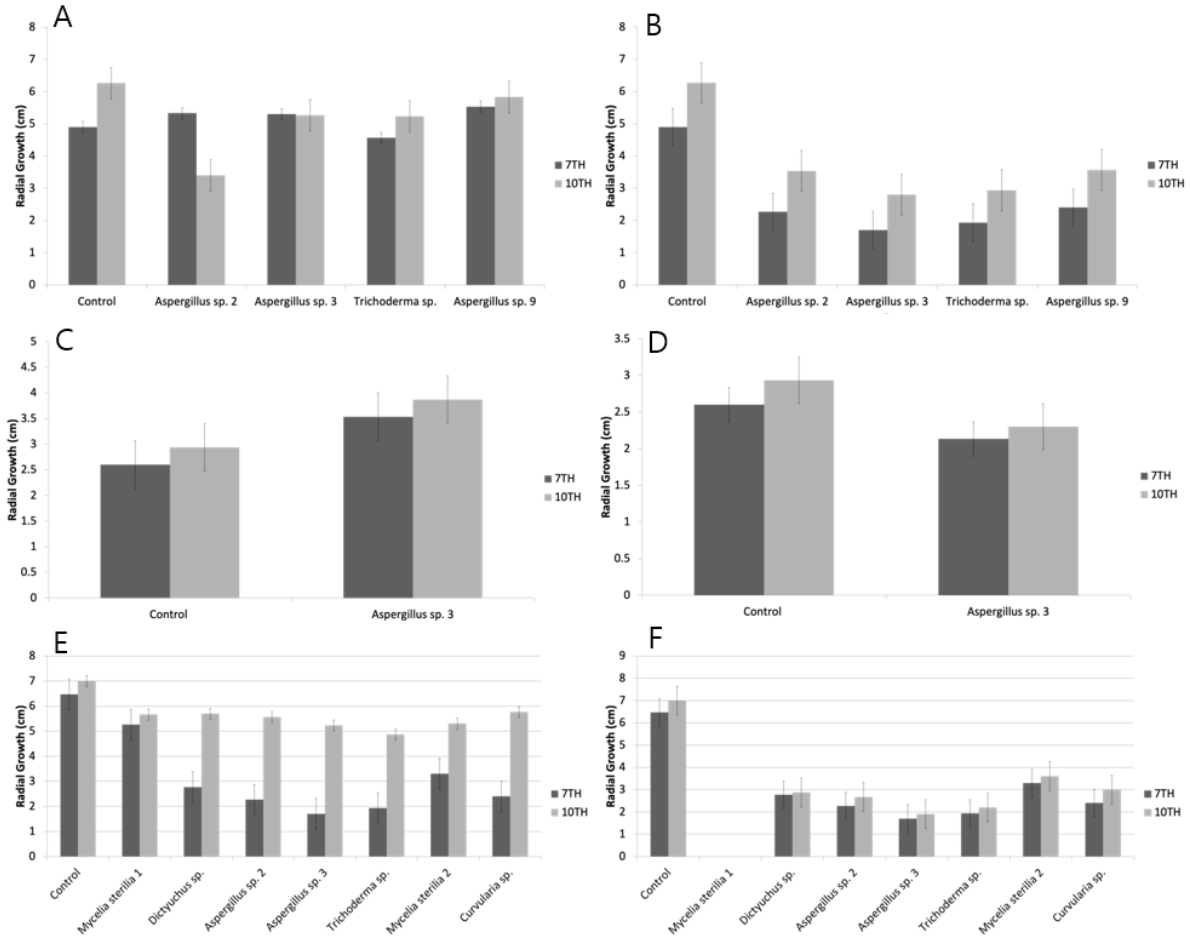


Fig. 2 – Radial growth of *Fusarium verticillioides.* A, B *Fusarium moniliforme.* C, D and *Fusarium* sp. E, F in best inoculation method. The first column (A, C, E) show the pathogenic fungi initially grown in culture before the soil surface fungi. The other column (B, D, F) show the soil surface fungi initially grown in culture.

Fig. 2A shows that the growth of *F. verticillioides* was inhibited by *Trichoderma* sp. on day seven, but all soil fungi showed inhibition on day 10, with *Aspergillus* sp. 2, showing the most significant inhibition. However, in Fig. 2B, the introduction of the soil fungus first leads to higher inhibition of the growth of the pathogen compared to the previous method. The growth of *F. moniliforme* can only be inhibited if the soil fungi are planted first (Fig. 2C, D). In the case of *Fusarium* sp., inhibition was observed on day seven, but an abrupt growth spike was observed on day ten. However, growth inhibition was also evident in all other samples except mycelia sterilia 1, which was contaminated and was, therefore, not included in the analysis.

Based on the analysis of variance when the pathogenic fungi are planted first, the values show that there is no significant difference between the size of the *F. verticillioides* in control and

treatment on day seven, but a significant difference was observed on day ten with *Aspergillus* sp. 2 (p=0.02). *Aspergillus* sp. 3 (p=0.02) shows a significant difference in the growth of *F. moniliforme* on both day seven and day ten (p=0.01). In *Fusarium* sp., *Trichoderma* sp. (p=0.04) showed a significant difference in day 7 while all samples including Mycelia sterilia1 (p=0.04), *Dictyuchus* sp. (p=0.05), *Aspergillus* sp. 2 (p=0.03), *Aspergillus* sp. 3 (p=0.01), *Trichoderma* sp. (p=0.01), Mycelia sterilia 2 (p=0.01), and *Curvularia* sp. (p=0.04) showed significant growth inhibition on day 10.

Table 4 Analysis of variance (ANOVA) for best inoculation when the pathogenic fungus is inoculated first.

Fungal Isolates	***Fusarium verticillioides***				***Fusarium moniliforme***			
	7th DAY		10th DAY		7th DAY		10th DAY	
	PGI	p-value	PGI	p-value	PGI	p-value	PGI	p-value
Aspergillus sp. 2	-6.48	0.19	45.69	0.02				
Aspergillus sp. 3	-5.76	0.19	15.87	0.21	-35.89	0.02	-31.81	0.01
Trichoderma sp.	-2.88	0.29	16.40	0.19				
Mycelia sterilia 2								
Curvularia sp.								
Aspergillus sp. 9	-10.80	0.17	0.16	0.97				

Fungal Isolates	***Fusarium sp.***			
	7th DAY		10th DAY	
	PGI	p-value	PGI	p-value
Mycelia sterilia 1	18.50	0.12	19.05	0.04
Dictyuchus sp.	20.02	0.10	18.57	0.05
Aspergillus sp. 2	17.44	0.15	20.48	0.03
Aspergillus sp. 3	17.95	0.13	25.23	0.01
Trichoderma sp.	29.30	0.04	30.48	0.01
Mycelia sterilia 2	22.08	0.08	24.29	0.01
Curvularia sp.	14.34	0.20	17.61	0.04

Table 5 Analysis of variance (ANOVA) for best inoculation when soil surface fungus is inoculated first.

Fungal Isolates	***Fusarium verticillioides***				***Fusarium moniliforme***			
	7th DAY		10th DAY		7th DAY		10th DAY	
	PGI	p-value	PGI	p-value	PGI	p-value	PGI	p-value
Aspergillus sp. 2	38.87	0.01	43.56	0.02				
Aspergillus sp. 3	53.28	0.01	55.27	0.01	17.95	0.45	0.22	21.59
Trichoderma sp.	54.72	0.01	53.14	0.01				
Aspergillus sp. 9	55.07	0.01	56.07	0.03				

Fungal Isolates	***Fusarium sp.***			
	7th DAY		10th DAY	
	PGI	p-value	PGI	p-value
Mycelia sterilia 1				
Dictyuchus sp.	57.17	0.01	55.62	0.01
Aspergillus sp. 2	64.91	0.01	58.72	0.01
Aspergillus sp. 3	73.68	0.01	70.59	0.01
Trichoderma sp.	70.07	0.01	65.94	0.01
Mycelia sterilia 2	48.91	0.01	44.27	0.01
Curvularia sp.	62.84	0.01	53.56	0.01

If the soil fungi were planted first, the fungal isolates significantly reduce the growth of the pathogens. In *F. verticillioides*, all soil fungi significantly inhibited the growth of the pathogen on

day seven and day 10. *Aspergillus* sp. 3 did not affect the growth of *F. moniliforme*, but *Fusarium* sp. was inhibited by all soil fungi on both day seven and day 10.

Discussion

Fungi are ubiquitous microscopic organisms that may grow rapidly and usually push their way between soil particles with the aid of favorable conditions such as moisture and temperature (Abdin et al. 2010). They perform important functions such as decomposers in the soil and other activities related to water dynamics, nutrient cycling, and disease suppression. The majority of the known 80,000 fungal species are likely to occur in soil environments at some stage in the life cycle (Bridge & Spooner 2001). Since soil is the primary source of these microorganisms (Waksman 1961), several filamentous fungi with bioactivity were derived. So far, antibiotics have been identified from the culture of these filamentous fungi, which are used to treat animal and plant diseases (Bulock & Kristiansen 1997).

One of the most invasive pathogenic organisms in plants with very limited control agents is *Fusarium*. These pathogens can survive in the soil for years in the absence of their host plants and can infect a wide range of hosts under favorable conditions (Bouzoumita et al. 2018). Many studies have demonstrated that various *Fusarium* species are associated with corn *Fusarium* sheath rot (CFSR), which significantly affects the quality and quantity of maize (Li et al. 2014, Zhai 2018). Biological control has gained great interest in many pathosystems due to the large input of pesticides, which causes economic, environmental, and safety concerns (Liu & Prada 2018). Various organisms, including endophytes (Lamo & Takken 2020), Pseudomonas (Gia et al. 2020), and yeasts (Fernandez-San Millan et al. 2021), have been utilized as biocontrol agents against *Fusarium.* Historically, strains with biocontrol potential have been isolated from suppressive soils, studied, and used against different soil pathogens (Köhl et al., 2019), which leads to the idea that many soil surface fungi may also affect the growth of the Fusarium.

Here, we isolated 14 soil surface fungi from the soil samples taken from Mt. Isarog, Camarines Sur. As fungi play a significant role in the decomposition of organic matter in the soil, it is expected to observe several species of fungi in the soil samples. The soil surface fungi were morphologically identified as *Aspergillus* spp. 1 to 9, *Curvularia* sp, *Dictyuchus* sp, and *Trichoderma* sp., with two isolates classified as mycelia sterilia. Haas et al. (2016) initially reported that Aspergillus and Penicillium exhibit a high proportion in potting soil and compost.

To determine the biocontrol activity of the isolated soil surface fungi, dual culture assays were performed. However, most of the fungal isolates were rendered ineffective by *Fusarium* spp. *Fusarium verticillioides* was affected on the seventh day the most by *Aspergillus* spp. 2, 3, and 9, as well as *Trichoderma* sp. On the tenth day, only *Aspergillus* sp. 3 decreased its growth. *Aspergillus* sp.3 inhibited the growth of the pathogen by covering all available space and overgrowing over it. *Fusarium moniliforme* was shown to be resistant against all of the fungal isolates, except for *Aspergillus* sp.3. It should be highlighted that *Aspergillus* sp. 3 on the tenth day caused the lowest increase in growth diameter of the pathogen. The rest of the species have only caused the pathogen to grow significantly more than control growth.

Interestingly, *Aspergillus* spp. 4, 5, 6, and 7; *Dictyuchus* sp., and mycelia sterilia1 had p-values less than 0.05, which indicated a significant difference between the growth of the pathogen in the control and treatment. However, the PGI of the species was negative, which means that the size of the *Fusarium* increased, and therefore, the fungal isolates acted as synergists. The growth of *Fusarium* sp. was affected by all the isolates but *Trichoderma* sp. showed the greatest growth inhibition of the pathogen as the difference between days seven and ten were significantly higher compared to the rest of the isolates. Special emphasis is given to mycelia sterilia 1, *Aspergillus* sp. 3, and *Trichoderma* sp. for causing the most significant reduction and exhibiting the smallest growth difference between the days. This may indicate that these fungal isolates may have the necessary characteristics to be biocontrol agents. These results are in agreement with Israel & Dodha (2005) that some strains of *Aspergillus* can be used against *Fusarium*. Our results are also in parallel with various data on *Trichoderma* sp., which states that the genus *Trichoderma* is effective

in inhibiting the growth of *Fusarium.* This agrees with the data published in previous studies (Belete, et al. 2015, Harman et al. 2004, Rahman et al. 2009, Sivan & Ilan 1989), highlighting its usefulness.

The best inoculation was performed to determine if the fungal isolates could be utilized as either pathogenic preventive or pathogenic treatment biocontrol agents (Nwankiti & Gwa 2018). For *F. verticillioides*, only *Aspergillus* sp. 2 caused the growth of the pathogen to decrease on day ten when *Fusarium* was planted first. When the fungal isolate was planted first, the size of *Fusarium* was significantly reduced on day seven, but with a minute increase on day 10. *Fusarium moniliforme* was not affected by *Aspergillus* sp. 3. *Fusarium* sp. displayed results that showed vulnerability against all of the fungal isolates. When it was introduced first, its growth was significantly reduced on the seventh day. However, on the tenth day, its growth spiked. Special emphasis is placed on mycelia sterilia1 and *Aspergillus* sp. 3 as the former presented the smallest difference between both days, and the latter caused the most significant reduction of growth on the seventh day. Interestingly, all of the fungal isolates were able to inhibit the growth of the pathogen to a third of its size on both days when they were planted first. Problematically, the data of mycelia sterilia 1 was contaminated, which resulted in its loss. This signifies the possibility that the remaining fungal isolates have the potential to become pathogenic preventive agents.

Conclusion

The results of both methods reveal that soil fungi can inhibit the growth of *Fusarium* species. *Aspergillus* sp. 3 was able to inhibit *F. moniliforme* on days seven and ten, while *Trichoderma* sp. was able to inhibit *Fusarium* sp. in best inoculation when the pathogen is planted first. *Aspergillus* spp. 2, 3, and *Trichoderma* sp. were able to inhibit the growth of *F. verticillioides* and *Fusarium* sp. when inoculated first. *Aspergillus* sp. 9 also inhibited *F. verticillioides*, while *Dictyuchus* sp., mycelia sterilis 2, and *Curvularia* sp. inhibited the growth of *Fusarium* sp. This shows that soil surface fungi including *Aspergillus, Trichoderma, Dictyuchus, Curvularia,* and even mycelia sterilia have the potential to be used as biocontrol agents against *Fusarium* species. Finally, these findings encourage further intensive research on using soil surface fungi as alternative biological control agents and sources of biologically active pharmaceutical and agricultural products.

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