Original Research Article

EVALUATION OF THE INFLUENCE OF CULTURE MEDIA AND NEEM CAKE EXTRACT ON THE GROWTH RATE OF THREE SOIL BORNE FUNGI (*Trichoderma harzianum,Trichoderma viride* and *Sclerotiumrolfsii*)

Abstract

Aims: To evaluate the performance and efficacy of three fungi on pure Sabouraud dextrose agar (SDA), Potato dextrose agar(PDA), malt extract agar (MEA); SDA, PDA and MEA modified in combination with different neem cake concentrations.
Study design: The experiment was laid out in a completely randomized design.
Place and Duration of Study: Department of Botany, University of Calabar, between July 2021 and September, 2021.
Methodology: Pathogens were isolated from soil samples and infected fruits of water melon. Different cultured media were used to culture the pathogens. 250mg of Chloramphenicol was added to each medium, autoclaved at 121^0c for 15 minutes, and kept at room temperature of 21 ± 1^0c. After inoculation, colonies were sub cultured to obtain pure culture. Media modification was achieved by adding 10g, 20g, 30g and 40g of extract. 5mm bork borer of fungi were transferred to petri dishes. Linear growth of fungi was recorded after 16 hours. Average linear growth rate was measured at 12 hours interval for five days.
Results: Spore concentration of *S. rolfsii*were significantly higher in pure media than in modified media. Among modified media, concentrations were significantly higher in both PDA and MEA at $1.60x10^6$ spores/ml. *T. viride* on pure SDA produced the highest spore concentration at $4.80x10^6$ spores/ml followed by pure PDA at $4.10x10^6$ spores/ml, whereas on the modified media, MEA had the highest spore concentration at $1.80x10^6$ spores/ml. Pure PDA of *T. harzianum* produced the highest spores at $6.10x10^6$ spores/ml while MEA and SDA had close range of spore production at $4.90x10^6$ spores/ml and $4.30x10^6$ spores/ml respectively. Modified MEA had the highest spore concentration at $1.30x10^6$ spores/ml followed by SDA which had $1.1x10^6$ spores/ml.
Conclusion: The mycelia growth rate reveals that both pure and modified MEA supported the growth of all the fungi better than PDA and SDA.

Keywords: Culture media, mycelial growth, Neem cake extract, *Trichoderma* species, *Sclerotium rolfsii*

1. Introduction

Fungi are one of the most significant group of microorganisms in the microbial world, which perform vital tasks that are sometimes invisible but are critical for hastening up important biological processes. They are ubiquitous and can be found in parasitic relationships with plants and animals, while others exist as free-living microbes in the soil, water, and air. Penicillium, used as an antibiotic, mushrooms and yeast, used for producing bread and beer, are the most common examples of fungi utilized for various economic reasons. However, some fungi infect plants and animals, resulting in a variety of illnesses. For example, Puccinia, which causes wheat rust disease, is pathogenic to both plants and animals [1].

[2] posit that fungi play essential roles in addressing significant global challenges. In many parts of the world, wild mushrooms, particularly if they can be cultivated, constitute a significant non-timber forest resource that provides various benefits, particularly food and income for local communities [1].

Azadiractha indica (Neem tree) is one of the two species in the genus Azadiractha, belonging to the family Maliaceae. Neem fruits, seeds, leaves, stems, and bark contain diverse phytochemicals. Neem seed oil contains glycerides, diverse polyphenols, nimbolide, triterpenes, and beta-sitosterol. The yellow, bitter oil has a garlic-like odor and contains approximately 2% of limonoid compounds. Numerous biological and pharmacological activities neem leave extracts have been reported including antibacterial [3].*A. indica* leave extracts were reported to contain five phytochemicals out of ten screened by [4]. These included alkaloids (13%), saponins (6%), tannins (4.6%), flavonoids (10.00%), and terpenoids (10%).

Neem cake, a byproduct obtained during the extraction of neem oil from neem seeds (kernels), is commonly used as an organic fertilizer and soil conditioner due to its high nutrient content and natural pest-repelling properties[5].Neem seeds can be ground into powder, soaked overnight in water, and sprayed onto crops. However, repeated application is necessary for optimal efficacy, with neem application recommended every ten days. [4] found the ethanolic extract of neem leaves to be instrumental in controlling post-harvest rot of tomato caused by *Aspergillus* species. However, the extract's effectiveness against Aspergillus growth was comparatively low. Among crude plant extracts tested against soil-borne pathogenic fungi, Neemextract was the most effective against *Fusarium oxysporum*, which causes *Fusarium* yellow in common beans [6].

In laboratory studies, growth media such as Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA), and Malt Extract Agar (MEA) are indispensable media for the growth of microorganisms, including pathogenic fungi. PDA particularly, is a general purpose medium for yeasts and moulds and can be supplemented with acid or antibiotics to selectively inhibit bacterial growth [7]. PDA consists of dehydrated potato infusion and dextrose that encourage fungal growth, with agar serving as the solidifying agent. PDA is recommended for growing clinically significant yeast and moulds, given its nutritionally rich base (potato infusion) that stimulates mould sporulation and pigment production.

[7] posits that Sabouraud Dextrose Agar (SDA) is a growth medium that is suitably employed for the isolation, cultivation, and maintenance of non-pathogenic and pathogenic species of fungi and yeasts. SDA is primarily used for the selective cultivation of yeasts, moulds and aciduric bacteria, and is often used with antibiotics for the isolation of pathogenic fungi from material containing large numbers of other fungi or bacteria. In this study, PDA, SDA, and MEA were each utilized to support the growth of the fungi *Trichoderma harzianum, Trichoderma viride,* and *Sclerotium rolfsii.*

Trichoderma is a well-known filamentous fungus that is widely distributed in soil, plant material, decaying vegetation, and wood [8]. According to [9], the *Trichoderma* genus is related to the order of Hypocreales, family of Hypocreaceae, and the genus comprises of more than 100 phylogenetically defined species. *Trichoderma* is considered an excellent biocontrol agent model due to its high ability to multiply, spread, and easy to isolate and culture [10]. Since *Trichoderma* strains grow rapidly in the soil due to their natural resistance to many toxic compounds,

including herbicides, fungicides, and pesticides, they have a superior ability to colonize, take up soil nutrients, and starve other organisms of nutrition [11,12].

S.rolfsii is a destructive soil-borne plant pathogen that causes Southern blight disease on a wide variety of plants. Despite considerable research, its control remains a problem, and in recent years, it has been particularly damaging on tomatoes in Benue State, Nigeria, peanut, tomato in the southeast USA, and sugar beet in California[13].The difficulty in controlling this pathogen despite concerted efforts might be due to its wide host range, prolific growth, and ability to produce persistent sclerotia[5].

The pathogenicity and virulence of these fungi can be investigated by examining its behavior in various culture media, providing insight into the factors that contribute to its pathogenicity. This research is essential for filling knowledge gaps and contributing to a better understanding of fungal ecology. To this end, our study evaluated the performance of *T. harzianum, T.viride,* and *S.rolfsii*on SDA, PDA, and MEA, as well as their efficacy on SDA, PDA, and MEA modified with different concentrations of neem cake.

2. Methodology

2.1 Experimental site

This research was carried out inthe Department of Botany Research Laboratory, University of Calabar, Calabar between July 2021 and September, 2021.

2.2 Sample Collection, sterilization, isolation and identification of the pathogens

Infected fruits of water melon and soil Samples were gotten from dumpsites in Calabar municipality. The sampleswere isolated and identified in the Department of Botany Post Graduate Laboratory, University of Calabar.

2.3Isolation of pathogens

The collected samples were taken to the laboratory in a sterilized polyethylene bags.Little quantities of the collected soil samples and infected water melon fruits in the polyethylene bags were picked up with spatula and dropped in the plates containing PDA solution and labelled accordingly. The inoculated plates were incubated at room temperature of 27 ± 1^{o} C and daily observations were made for emergence of fungal colonies.Colonies formed were subculture to obtain pure cultures of the isolate.

2.4Identification of the pathogens

A drop of Lacto phenol in cotton blue was used to stain the slide. With a sterilized inoculation needle, the spores of the fungi were picked from culture plates and placed on the slide containing the lacto phenol in cotton blue, then covered with cover slide for observation and subsequent identification under a light microscope (Olympus Optical Philippines) with magnification (x40). The morphological structures of the fungi were compared with those in the Atlas of Imperfect Fungi for identification.

2.5 Preservation of Stock Culture

10ml in each of sterilized PDA medium were poured into sterilized test tubes. Then the test tubes were sterilized in an autoclave at 121°C temperature for 20 minutes. After autoclaving, slanting of test tube was done at 45° angles to increase the surface area of the medium in the test tube. 7

days old fungal hyphae with the help of inoculation needle were placed in the test tube. After inoculation the test tubes were kept in the growth chamber at 27±1ºC temperature.

2.6Multiplication of Fungi Pathogen

PDA medium was poured in sterilized petri dishes, 20ml in each. A 5mm disc of the 3 days old pure culture of the different fungi were placed at the centre of each plate. The inoculated petri dishes were kept in the growth chamber at 27±1ºC temperature. All the works were done under aseptic condition using the lamina air flow bench.

2.7 Experimental design

The experiment was laid out in a completely randomized design (CRD) having 45 treatments with 2 replicates to give a total of 90 experimental units.

2.8 Media Preparation

SDA and MEA were prepared by dissolving 32.5g of Sabouraud Dextrose Agar, and 25g of Malt Dextrose Agar respectively in 500ml of distilled water in separate beakers, stirred and mixed thoroughly to obtain a homogenous mixture. The mixture was then poured into a sterile flask, and sterilized using autoclave at 121^0C for 15minutes. After sterilization the SDA was allowed to cool to 40^0C before adding 250mg of Chloramphenicol then allowed to cool to body temperature before pouring 12 – 15 ml into petri dishes and allowed to cool and solidify (paste) by storing at room temperature.

PDA was prepared by measuring thirty-nine grams (19.5g) of PDA into 500ml of distilled water in a conical flask steer and mixed thoroughly to obtain a homogenous mixture. The flask was corked with non-absorbent cotton wool wrapped with aluminium foil and sterilized using autoclave at 121^0C for 15minutes. After sterilization the PDA was allowed to cool to 40^0C before adding 250mg of Chloramphenicol then allowed to cool to body temperature before pouring about 12 – 15ml into petri dishes. The PDA in the petri dishes were allowed to cool and solidify (gel) by storing at room temperature for 24hours.

2.9 Neem cake extract preparation

Solid Neem cake was broken into smaller pieces to increase the surface area for extraction (using mortar and pestle) and soaked in a container filled with water. Twenty grams (20g) of cake was weighed and soaked in five litres of water after stirring the mixture. After 24hours, a strainer was used to separate the liquid extract from the neem cake solids. The strained liquid contains the beneficial compounds.

2.10Media modification

The culture media was modified by dissolving 32.5 g of SDA,19.5 g of PDA, 25 g of MEA respectively, and 250mg of Chloramphenicol and 10g of neem cake extract in 500ml of distilled water, in separate flask and autoclaved at 121^0C for 15minutes. After sterilization each modified medium was allowed to cool to body temperature before pouring about 12 – 15ml into petri dishes. The media in the petri dishes were allowed to cool and solidify (paste) by storing at room temperature for 24hours. Other concentrations were obtained by varying extract content accordingly (20g, 30g, and 40g).

2.11 Incorporation of the Fungus Culture

Two (2) centimetre disc of pure culture of the different fungi grown on Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) were selected from the periphery of actively growing colony obtained from fresh 7 days old culture under aseptic conditions. These discs were transferred to the centre of the petri dishes using sterilized inoculating needle. Inoculations were done under perfect aseptic condition inside an inoculation chamber which was sterilized previously by spraying 3% hypochlorite, or 70% alcohol and ultra violet (U. V.) radiation.

2.12 Measurement of Growth Rate

Linear growth (cm) of the fungiwere recorded after 16 hours of inoculation with the help of fine transparent plastic scale in centimetre and by averaging two diameters taken from each colony. While average linear growth rate (ALGR) was measured at 12 hours interval for five days by using the following formula [14];

ALGR (mm/day) = (C2-C0)/2

Where:

C3 = Colony diameter after 52 hour, 64 hours … 160 hours of inoculation

C0 = Initial colony diameter of inoculation.

2.13 Observation on Characteristics of Growth

Colour/pigmentation of colony and substrate, margin/border of colony, topography/elevation of mycelium, surface of mycelium, opacity, size, form and texture of colony were observed by simpleinspection.

2.14 Characterization and Determination of Spore Concentration

For measuring sporulation on different fungi on different modified media, a single disc of 5 mm diameter of a ten days old pure culture was cut out from the fungal colony near the margin with a sterile cork borer and was transferred to 20 ml sterile distilled water in a test tube, where it was mixed thoroughly to make a uniform spore suspension. For each sample, 1µl of spore suspension were drawn into the counting chambers of the haemocytometer using a clean pipette tip. Before pipetting, the flask containing the suspension was vortexed to gently and thoroughly mix. Samples were injected slowly and steadily to avoid bubbles getting into the chambers. Spores were counted in the four corner squares (counting chamber) microscopic fields, recorded under low power (10x) objective of the microscope and concentration was determined using the equation:

Spore/ml = (n) x 10^4

Where: n = the average cell count per square of the four corner squares counted.

The spores were expressed as millions of spore/ ml of sample according to [15].

2.15 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and significant means were separated using Duncan New Multiple range test (DNMRT) at 5% probability level.

3. Results

3.1. Fungal Characterisation

The fungi isolated and identified for this study were *T. harzianum,T. viride and S.rolfsii* (Plate 1; a, b, and c). The inoculated plates were incubated at room temperature of 27±1^{o} C and daily observations were made for emergence of fungal colonies and the following cultural characteristics were observed for the three isolates;

*T.harzianum*formed 1- 2 concentric rings with green conidial production. The conidia production was denser in centre than towards the margins (Plate 2).

T.viride, appears to be a bit granular on Potato Dextrose Agar (PDA) with green conidia distributed throughout. An irregular yellow zone without conidia was present around the inoculum. Some white pustules were also found growing on the green mat of conidia (Plate 3).

*S.rolfsii*white myceliaturns brown in colour and develops Sclerotia similar to a size of a mustard seed (Plate 4).

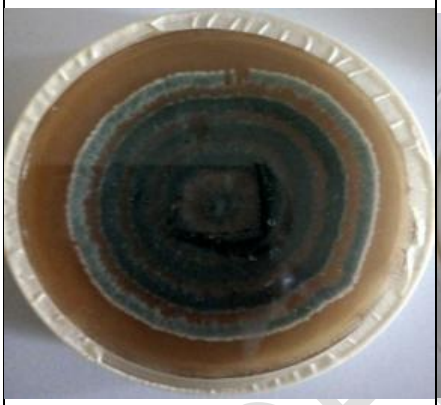
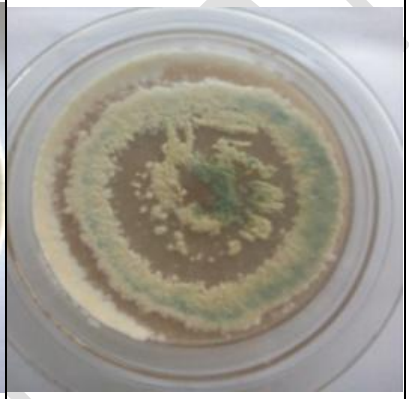
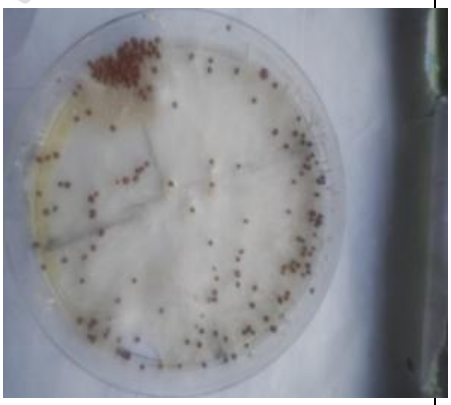
	B	C
		
A		

Plate 1: pure cultures of the three soil-borne fungi used for this research (a) *Trichodermahazianum*. (b) *Trichodermaviride* and (c) *Sclerotiumrolfsii*

A	B	C

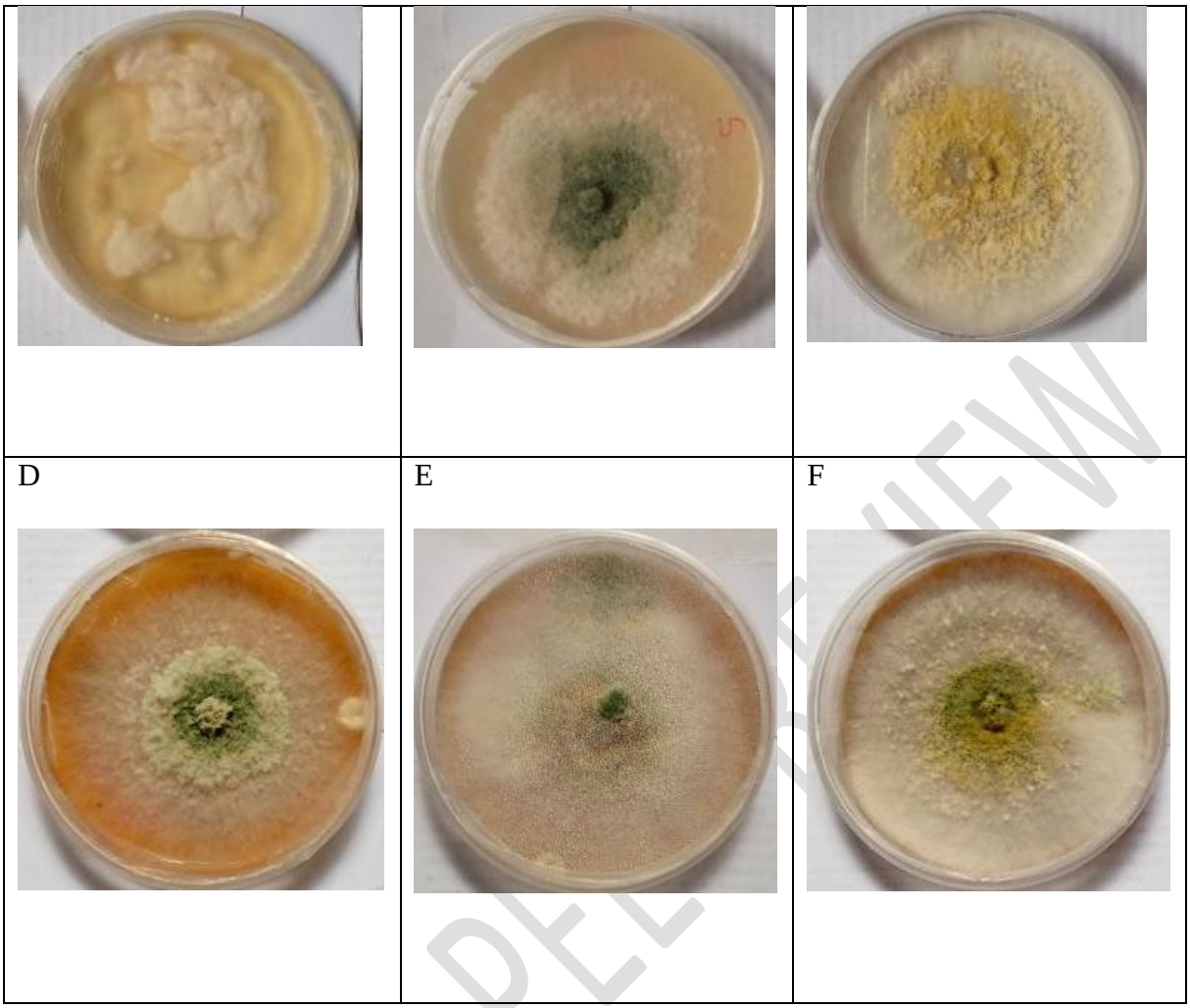
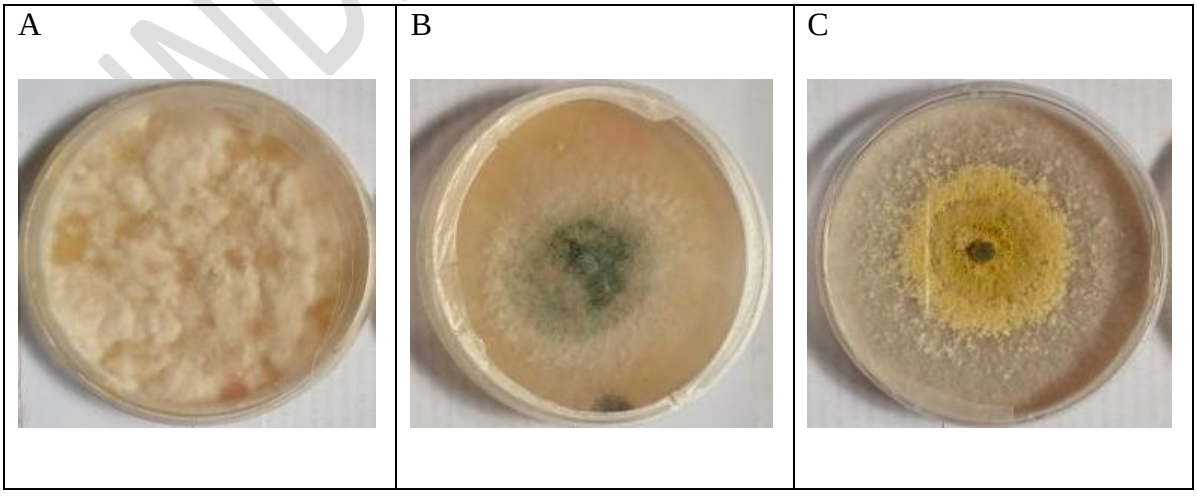


Plate 2: Petri dishes of radial mycelia growth of *T. hazianum* after 100 hours on (a) SDA, (b) PDA, (c) MEA, (d) modified SDA, (e) Modified PDA and (f) Modified MEA



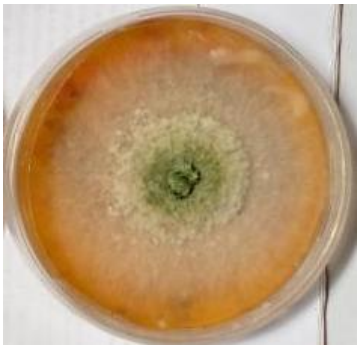
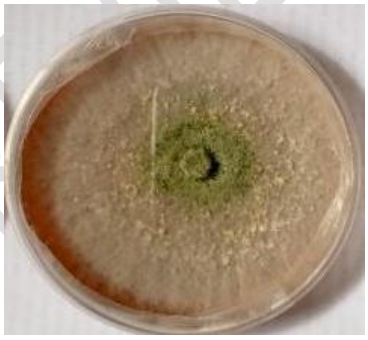
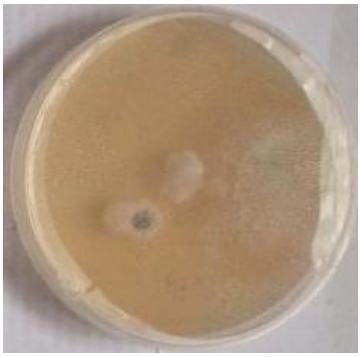
D 	E 	F 

Plate 3: Petri dishes of radial mycelia growth of *T. viride* after 100 hours on (a) SDA, (b) PDA, (c) MEA, (d) modified SDA, (e) Modified PDA and (f) Modified MEA

A 	B 	C 

D	E	F
		

Plate 4: Petri dishes of radial mycelia growth of *S. rolfsii* after 100 hours on (a) SDA, (b) PDA, (c) MEA, (d) modified SDA, (e) Modified PDA and (f) Modified MEA

3.2Spore Concentration

Result obtained from spore concentration of *S. rolfsii* on different growth media reveals that spore concentration were higher in pure media than in modified media. Among modified media, it was observed that the spore concentrations were significantly higher in both PDA and MEA at $1.60x10^6$ spores/ml while SDA had the lowest spore concentration in pure at $3.20x10^6$ spores/ml and modified at $1.0x10^6$ spores/ml (Figure 1).

Figure 1: Effect of Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA), Malt Dextrose Agar (MEA) and modification of Media with different levels of Neem Concentration on the Sporulation of *S. rolfsii.*

Evaluation of spore count of *T. viride* shows that pure SDA produced the highest spore concentration at $4.80x10^6$ spores/ml followed by pure PDA at $4.10x10^6$ spores/ml and the lowest in pure MEA at $2.70x10^6$ spores/ml. Whereas on the modified media, MEA had the highest spore concentration at $1.80x10^6$ spores/ml with the lowest in PDA at $4.0x10^6$ spores/ml (Figure 2).

Figure 2: Effect of Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA), Malt Dextrose Agar (MEA) and modification of Media with different levels of Neem Concentration on the Sporulation of *T. viride.*

In *T. harzianum,* it was observed that pure PDA produced the highest spores at $6.10x10^6$ spores/ml while MEA and SDA had close range of spore production at $4.90x10^6$ spores/ml and $4.30x10^6$ spores/ml respectively. However, on the modified media, MEA had the highest spore

concentration at $1.30x10^6$ spores/ml followed by SDA which had $1.1x10^6$ spores/ml while the lowest spore count was recorded in PDA at $8.0x10^6$ spores/ml (Figure 3).

Figure 3: Effect of Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA), Malt Dextrose Agar (MEA) and modification of Media with different levels of Neem Concentration on the Sporulation of *T. harzianum*.

3.3 Mycelia Growth Rate

All the culture media used for this study supported the mycelia and sporulation of the soil borne-fungi to various degrees. However, MEA modified with 40g/ml of Neem cakeextract was observed to have significant impact of the growth of *S. rolfsii* at 0.7 after 52hours of inoculation. Generally, there was a significant impact of *T. harzianum* and *T. viride* grown on the different media at 52 hours after inoculation except at 30g/ml on PDA and SDA (Plate 2 and Table 1).

The mycelia growth of *S. rolfsii* reveals that neem cake extract had significant impact on fungi when grown on the different growth media at various concentrations. The mycelia growth on PDA at 0 g/ml, 10 g/ml, 30 g/ml, 40 g/ml and SDA at 0 g/ml, 10 g/ml, 20 g/ml were significantly different from other growth media. While, the mycelia growth of *T. harzianum* were significant when grown on SDA at 0 g/ml and 20 g/ml of neem extract concentrations.

After 64 hours the various concentration of neem had no significant effect on the mycelia growth of *S. rolfsii* grown on the different media. However, the various neem concentration significantly improved the growth of *T. harzianum* and *T. viride* except at 30 g/ml in PDA and SDA(Table 1).
The growth of *S. rolfsii* was on MEA the various concentration was significantly higher than PDA and SDA whereas MEA and PDA had significant effect on the mycelia growth was significantly higher in the various concentration except on SDA without neem and SDA with 20g/ml of neem concentration after 88 hours of inoculation. After 100 hours of inoculating the different fungi on the different growth media(Table 1). It was observed that the mycelia growth on MEA at all the concentration and PDA at 20 g/ml were significantly different from growth obtain on other media.
More so, the mycelia growth rate of *T. harzianum* across the different media and neem concentration were not significantly different from each other. Whereas *T. viride* mycelia growth observed in pure MEA was not significantly different from PDA but significant different from SDA. This trend was also observed in 20 g/ml concentration, but differ in 10 g/ml, and 40 g/ml concentration were all the media had no significant impact on the fungi growth after 100 hours of inoculation.
The mycelia growth of *S. rolfsii* reveals that neem cake had significant impact on fungi when grown on the different growth media at various concentration. The mycelia growth on PDA at 0 g/ml, 10 g/ml, 30 g/ml, 40 g/ml and SDA 0 g/ml, 10 g/ml, 20 g/ml were significantly different

from other growth media. While, the mycelia growth of *T. harzianum* were significant when grown on SDA 0 g/ml and 20 g/ml of neem concentration.

Table 1: Mean effect of MEA, PDA, SDA and various concentrations of Neem cake on the mycelia growth rate of *S. rolfsiiT. harzianum* and *T. viride* from 52 hours to 100 hours.

52 HOURS															
	S. rolfsii					*T. harzianum*					*T. viride*				
Conc./ Media	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml
MEA	0.2	0.3	0.4	0.4	0.7	0.8	0.7	0.6	0.6	0.8	0.4	0.5	0.6	0.6	0.6
PDA	0.0	0.0	0.0	0.0	0.0	0.3	0.5	0.6	0.0	0.7	0.2	0.3	0.4	0.0	0.6
SDA	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.1	0.3	0.5	0.0	0.5	0.2	0.3	0.3
LSD	0.458														
64 HOURS															
	S. rolfsii					*T. harzianum*					*T. viride*				
Conc./ Media	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml
MEA	0.7	0.8	0.9	0.6	1.2	1.7	1.6	1.1	1.3	1.2	1.5	1.6	1.6	1.3	1.4
PDA	0.0	0.1	0.3	0.0	0.5	1.7	1.2	1.3	0.0	1.3	1.2	1.1	1.2	0.0	1.1
SDA	0.0	0.1	0.0	0.1	0.0	1.7	1.1	0.3	0.3	1.2	0.0	1.2	0.3	0.7	0.6
LSD	0.897														
76 HOURS															
	S. rolfsii					*T. harzianum*					*T. viride*				
Conc./ Media	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml
MEA	1.2	1.2	1.2	1.1	1.6	2.6	2.6	2.1	2.3	2.4	2.4	2.5	2.5	2.2	2.3
PDA	0.1	0.2	0.5	0.0	0.8	1.8	1.8	1.7	0.0	1.7	2.1	1.7	1.8	0.0	1.8
SDA	0.0	0.3	0.0	0.4	0.1	2.0	2.0	0.9	1.0	2.1	0.3	1.8	0.9	1.7	1.7
LSD	1.134														
88 HOURS															

	S. rolfsii					*T. harzianum*					*T. viride*				
Conc./ Media	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml
MEA	2.1	2.3	2.1	1.8	2.6	3.7	3.5	3.0	3.1	3.2	3.3	3.4	3.2	3.1	3.3
PDA	0.3	0.7	1.2	0.0	1.6	3.4	3.2	2.9	0.0	2.7	2.9	2.5	2.6	0.0	3.2
SDA	0.5	0.6	0.0	0.9	0.9	2.2	2.6	1.2	1.7	2.6	0.3	2.7	0.9	1.9	2.4
LSD	1.489														

100 HOURS

	S. rolfsii					*T. harzianum*					*T. viride*				
Conc./ Media	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml
MEA	2.7	2.9	2.8	2.6	3.3	4.3	4.2	3.6	3.8	3.8	4.0	3.9	3.7	3.5	3.7
PDA	0.5	0.9	1.7	0.0	2.1	3.6	3.5	3.3	0.0	3.2	3.6	3.1	3.2	0.0	3.5
SDA	0.6	0.8	0.1	1.2	2.2	2.6	3.2	1.3	1.9	3.1	0.5	3.2	1.1	2.4	3.1
LSD	1.377														

Similarly, *T. viride*showed significant mycelia growth when grown on 0 g/ml and 20 g/ml neem extract concentrations after 112 hours of inoculation (Table 2). Mycelia growth of *S. rolfsii* grown on PDA at 0 g/ml, 10 g/ml, 30 g/ml and 40 g/ml were significantly different from growth observation on MEA, while the growth rate on modified SDA were significant at 0 g/ml, 10 g/ml, 20 g/ml and 40 g/ml after 124 hours of inoculation. Pure SDA, 20 g/ml and 30 g/ml concentration of neem extract were significantly different from other when the mycelia growth of *T. harzianum* was assessed. The mycelia growth of *T. viride* were only significantly different when grown on pure SDA and 20 g/ml after 124 hours of inoculation (Table 2).The growth rate generally observed showed that *S. rolfsii* growth on PDA and SDA were significantly different to MEA after 136 hours, 148 hours and 160 hours of inoculation. It was also generally observed that the mycelia growth of the three fungi in this study were significantly different in SDA at all concentration after 136 hours, 148 hours and 160 hours of inoculation(Table 2).

Table 2: Mean effect of MEA, PDA, SDA and various concentrations of Neem cake on the mycelia growth rate of *S. rolfsii*, *T. harzianum* and *T. viride* from 112 hours to 160 hours.

112 HOURS

	S. rolfsii					*T. harzianum*					*T. viride*				
Conc./ Media	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml	0 g/ml	10 g/ml	20 g/ml	30 g/ml	40 g/ml
MEA	4.0	3.8	4.0	3.7	4.5	4.5	4.5	4.3	4.5	4.5	4.5	4.5	4.5	4.5	4.5

PDA	0.8	1.3	2.3	0.0	2.7	4.5	4.3	4.1	0.0	4.0	4.3	4.1	4.1	0.0	4.5
SDA	1.4	1.1	0.4	1.7	3.2	3.0	4.2	1.7	2.3	4.1	1.8	4.1	1.5	3.8	4.1
LSD	1.130														

124 HOURS

	S. rolfsii					*T. harzianum*					*T. viride*				
Conc./	0	10	20	30	40	0	10	20	30	40	0	10	20	30	40
Media	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml
MEA	4.5	4.4	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
PDA	1.0	1.7	2.7	0.0	3.1	4.5	4.5	4.5	0.0	4.5	4.5	4.3	4.4	0.0	4.5
SDA	1.2	1.4	0.8	2.3	3.3	3.6	4.5	2.1	2.5	4.4	2.4	4.3	2.2	4.1	4.3
LSD	0.746														

136 HOURS

	S. rolfsii					*T. harzianum*					*T. viride*				
Conc./	0	10	20	30	40	0	10	20	30	40	0	10	20	30	40
Media	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml
MEA	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
PDA	1.6	2.1	3.0	0.0	3.8	4.5	4.5	4.5	0.0	4.5	4.5	4.5	4.5	0.0	4.5
SDA	1.6	1.8	1.2	2.9	3.8	4.0	4.5	2.5	2.9	4.5	2.6	4.5	2.9	4.3	4.5
LSD	0.80														

148 HOURS

	S. rolfsii					*T. harzianum*					*T. viride*				
Conc./	0	10	20	30	40	0	10	20	30	40	0	10	20	30	40
Media	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml
MEA	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
PDA	1.9	2.7	3.4	0.0	4.0	4.5	4.5	4.5	0.0	4.5	4.5	4.5	4.5	0.0	4.5
SDA	2.0	2.3	1.8	3.3	4.1	4.3	4.5	3.0	3.2	4.5	2.9	4.5	3.4	4.5	4.5
LSD	0.641														

160 HOURS															
	S. rolfsii					*T. harzianum*					*T. viride*				
Conc./	0	10	20	30	40	0	10	20	30	40	0	10	20	30	40
Media	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml	g/ml
MEA	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
PDA	2.4	3.1	3.9	0.0	4.3	4.5	4.5	4.5	0.0	4.5	4.5	4.5	4.5	0.0	4.5
SDA	2.6	2.8	2.2	3.8	4.4	4.5	4.5	3.6	3.8	4.5	3.4	4.5	3.7	4.5	4.5
LSD	0.658														

4. Discussion

The research was conducted to check the best media among MEA, PDA and SDA that will support mycelia growth of three soil-borne fungi namely *S. rolfsii*, *T. harzianum* and *T. viride*. Each of these media were modified by adding different concentrations of neem cake extract at 10 g/ml, 20 g/ml, 30 g/ml and 40 g/ml. Similar studies have been carried out by[16] who investigated the growth of *Rhizoctoniasolani*, *Uromycesappendiculatus*, *Cercosporabeticola*, *Aspergillusfumigatus*, *Alternariaalternaata* and *A. helianthin* in various media namely PDA, CZA, CA, NA and SA. The cultural and morphological characteristics of the stock culture of these three soil-borne fungi used for this study reveals that *T. harzianum* grows faster than *T. viride* and *S. rolfsii*.

The outcome of the result of these fungi cultured in MEA, PDA, and SDA reveals that MEA supports the development of mycelia growth better than PDA and SDA. This contradicts the reports of [18, 19, 20]; that PDA supports growth of fungi mycelia than other growth media. However, the research supports the findings of [17] who reported that among the five media used to culture the fungus *Phomopsisspp*. grown on the host leave media perform better while PDA was the second highest.
These different fungi apart from mycelia growth also differed in colony colour/pigment, topography/elevation of the mycelia, surface of mycelia on petri dish, size of colony, form, texture and margin/border of the colony on these different growth media supporting the reports of [18,19] that fungi grown in various nutrient media show variation in vegetative growth and colony morphology with pigmentation and sporulation depending on the composition of specific culture medium.

The use of neem cake at various concentrations of 10 g/ml 20 g/ml, 30g /ml and 40 g/ml to modify the different growth media was assessed to ascertain their impact on the mycelia growth and sporulation of these soil-borne fungi. The result which generally revealed that neem cake enhanced and supported the growth and sporulation of these fungi. This is in agreement with[20] who reported that flamingo medium provided the most effective condition for the isolation of *A. fumigatus* compared to the other media. In this study, MEA recorded the best support for mycelia growth followed by PDA and SDA recording the least mycelia growth.

The sporulation of these different soil-borne fungi grown on different media reveal a trend different from mycelia growth. The results from the spore count revealed that the spores varied in the different media which is in agreement of the findings of [18, 19]that there were variations in vegetative and sporulation as well as pigmentation of fungi grown on various nutrient media. Data generated showed that fungi cultured on pure media (i.e. MEA, PDA and SDA without neem cake) produce more spores than the modified media. The trend suggest that the various concentration of neem cake only support the growth of mycelia and not spore production. This agrees with [21] on a research that revealed a significant reduction in spore formation on a neem extract mediated growth medium. This may mean that if the purpose of cultivation of fungi is spore production modifying the medium with neem cake will not produce good and desirable result.

5. Conclusion

During the studies it was observed that the different media and the modified versions show various diversity in the colour of colony, topography of mycelia, surface and margin of the mycelia on petri dish, form and texture of these fungi colonies. The mycelia growth rate reveals that both pure and modified MEA supported the growth of all the fungi better than PDA and SDA. The study demonstrates that fungi particularly *S. rolfsii*, *T. harzianum* and *T. viride* can be cultivated for faster and better growth on MEA. While cultivation for increased sporulation can be on PDA. Neem cake also impacted significantly on these soil-borne fungi by enhancing mycelia growth. This therefore can be concluded that neem cake is recommended as an additive to different nutrient media particularly MEA, PDA and SDA to modify media for mass production of fungi. It could also be deduced from this research that the recommended concentration for neem cake suitable for the cultivation of *T. harzianum* and *T. viride* to achieve desirable growth on SDA is 20 g/ml and 30 g/ml.

Reference

1. Nikita, P.Economic Importance of Fungi: Definitions, Examples, Fungus, and Fungal Organisms. 2023. Accessed on 11 July, 2023. Available: https://www.collegesearch.in/articles/economic-importance-of-fungi

2. Allen, G. T. Niego, Christopher L., Peter, M., Naritsada, T., Sylvie, R., Miriam, G., Hedda, S., Esteban, C., Arttapon, W., Kevin, D. H., and Marc, S. (2023). The contribution of fungi to the global economy. *Fungal Diversity*

3. Singh, N., Sastry M. S. Antimicrobial activity of Neem oil. *Indian Journal of Pharmacology*. 1997;13:102–106.

4. Ogar, V. B., Ndarake, E. I., Ngele, B. A. and Umana, J. E. Investigating the effect of acqueous and ethanolic extract of *Azadiractha indica* on a fungus causing post-harvest deterioration of *Solanum lycopersicum (*tomato). *Proceedings of the International Society of Pesticidal Plants Nigeria*. 2023;1(1): (In press).

5. Adhikari, P., Shrestha, S. M., Manandhar, H. K. and Marahatta, S. effects of *Trichoderma* isolates on*Sclerotium rolfsii*. J*ournal of Agriculture and Forestry University*. 2022: 5: 299-310.

6. Obongoya1, B. O., Wagai, S. O. and Odhiambo, G. Phytotoxic Effect of Selected Crude Plant Extracts on Soil-Borne Fungi of Common Bean. *African Crop Science Journal*. 2010: 18(1); 15 – 22.

7. Sagar, A. Potato Dextrose Agar (PDA)- Principle, Uses, Composition, Procedure and Colony Characteristics. 2022. Accessed 21 August 2023. Available: https://microbiologyinfo.com/potato-dextrose-agar-pda-principle-uses-composition-procedure-and-colony-characteristics

8. Gajera, H., Domadiya, R., Patel, S., Kapopara, M. &Golakiya, B. Molecular mechanism of *Trichoderma* as bio-control agents against phytopathogen system. *Current Resource Microbiology of Biotechnology*. 2013: 1(4): 133-142.

9.Kumar, S. *Trichoderma*: A biological weapon for managing plant diseases and promoting sustainability. International Journal Agriculture of Science Medicinal Veterinary. 2013:1: 106–121.

10.Pandya, J. R., Sabalpara, A. N. and Chawda, S. K. Trichoderma: a Particular Weapon for Biological Control of Phytopathogens. Journal of Agriculture and Technology. 2011: 7(5):1187-1191.

11.Chet, I. Inbar, J. & Hadar, I. Fungal antagonists and mycoparasites. In: Wicklow DT, Söderström B (eds) The Mycota IV: Environmental and microbial relationships. Springer-Verlag, Berlin;1997.

12.Benitez, T.; Rincon, A. M.; Limon, M. C., & Codon, A. C. Biocontrol mechanisms of *Trichoderma* strains. Int. Microbiol. 2004: 7(4): 249−260.

13. Kator, L., Yula, Z. Y., and Onah, O. D. *Sclerotium rolfsii*; Causative organism of southern blight, stem rot, white mold and sclerotia rot disease. Annals of Biological Research. 2015: 6 (11):78-89

14.Elad, Y. Chet, I., &Henis, Y. A selective medium for improving quantitative isolation of *Trichoderma species* from soil. Phytoparasitica. 1981:9: 59-67.

15. Pitt, D & Poole, P. C. Calcium induced conidiation in penicillin notatum in submerge culture. Transactions British Mycological Society. 1981: 219-230.

16. Hase, V., Nasreen S. Influence of different culture media on growth of plant pathogenic fungi. International Journal of Multidisciplinary Research and Development. 2017: 4: 67-70.

17. Pushpa, D. Patil, A. A. Baikar and R. A. Raut. Performance of different growth media for the Growth and Sporulation of Phomopsis sp. of Cashew. Plant Archives. 2016: 16(1): 275-278.

18. Kuhn and Ghannoum. Indoor mold, toxigenic fungi, and Stachybotryschartarum: infectious disease perspective. Clinical Microbiology Review. 2003:16(1):144-72.

19. Kumara, K. L. W. Influence of carbon, nitrogen, temperature and pH on the growth and sporulation of some Indian isolates of *Colletotrichum gloeosporioides*causing anthracnose disease of papaya. 2008.

20. Zhang, Z. J., Alfons, J. M., Debets, Paul, E., Verweij Sijmen, E. &Schoustra, E. Selective Flamingo Medium for the Isolation of *Aspergillus fumigatus Microorganisms* 9, 1155. 2021.

21. Simone, A. G. Mossini, Carla C. Arrotéia, and Carlos Kemmelmeier. Effect of Neem Leaf Extract and Neem Oil on *Penicillium* Growth, Sporulation, Morphology and Ochratoxin A Production. Toxins (Basel). 2009: 1(1): 3–13.