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DETERMINATION OF ANTIBIOSIS OF *Trichoderma* species AGAINST FUNGI ASSOCIATED WITH CORN (*Zea Mays* L.) SPOILAGE

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ABSTRACT

Biological control of food spoilage microorganisms is gaining more attention because it is a safe and cheap technique. This study evaluated the antagonistic potential of *Trichoderma* species against corn spoilage fungi by plate co-culture technique for seven days. Fungal isolates from corn spoilage were examined morphologically and microscopically. The percentage growth inhibition (PGI) of *Trichoderma* species against the corn spoilage fungi were *A. flavus* (15%), *A. niger* (14%), *A. terreus* (62.9%), *Fusarium* spp. (5.9%), *Nigrospora* spp. (61.4%) and *Penicillium* spp. (62.5%). The result obtained in this study revealed that *Trichoderma* spp. had significant inhibitory effects against the growth of fungal pathogens associated with corn spoilage. Therefore, it could be explored for control of post-harvest fungal spoilage of corn. It is recommended that in order to compare the antagonistic strength of the Trichoderma species, different species of Trichoderma should be tested against the same spoilage fungi.

Keywords: Biological control, Trichoderma Species, Corn Spoilage. Antagonistic, *Penicillium* spp.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops worldwide, ranking first with a total global production of over 1.15 billion tons (Oli Awoke, 2023). It is an important oily and economic crop and has been utilized as human food and animal feed (Kaul *et al*., 2019). However, maize is highly susceptible to fungal spoilage and consequently mycotoxin contaminants, including aflatoxins (AFs) (Mendoza *et al*., 2017).

Biological control is defined as the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms accomplished naturally or through manipulation of the environment or host or antagonist or by mass introduction of one or more antagonists. Biological control is found to be both environmentally and economically sound (Dukare *et al*., 2019: Zhang *et al*., 2021a). Various biocontrol agents such as fungi and bacteria have been identified for the control of postharvest diseases of many fruits and play an important role in sustainable agriculture and management of plant pathogens (Dukare *et al*., 2019; Mukherjee *et al*., 2020; Morales-Cedeno *et al*., 2021; Zhang *et al*., 2021a; Abd-Rabboh *et al*., 2021; Yassin *et al*., 2022). It has also been investigated that the potential of antagonistic microorganisms to control decay of fruits and vegetables depends on their ability to colonise fruit surfaces and adapt to various environmental conditions (Dukare *et al*., 2019). Biological control using antagonistic microbes to control postharvest disease and decay caused by pathogens is nowadays an emerging and attractive option. The use of antagonist microbes in postharvest disease is advantageous over synthetic fungicides in that antagonist microbes do not produce toxic residues, environmentally friendly, safer application method, easy to deliver and economical to produce (Zhang *et al*., 2021a) .The study aimed to determine the inhibitory activity of Trichoderma species against fungi associated with post-harvest spoilage. Its objectives were;(i) Isolation and identification of Trichoderma species from soil sample. (ii) Isolation and identification of spoilage fungi from corn. (iii) Antibiosis test of Trichoderma species against isolated spoilage fungi.

MATERIALS AND METHODS

Sample collection

Fresh corns (*Zea mays* L.) were purchased from two traders in Choba Market. The corns were kept for five days until sings of fungal spoilage was seen. Then the spoilt parts of each corn were cut for isolation of the spoilage organisms.Topsoil of farmland in University of Port Harcourt was collected for the isolation of *Trichoderma* species.

Isolationand identification of *Trichoderma* species from soil

A modified method of isolation and identification of *Trichoderma* spp. as demonstrated by Siddiquee (2017) was adopted. Soil sample of 10g was dissolved in 100mL of 0.1 % (w/v) Tween 20 sterile solutions and re-suspended by agitation at 150rpm and room temperature for 20min. The suspension was left on the bench of laminar flow (aseptically covered flask or tube of suspension) for 10min to decant insoluble particles and four serial dilutions was carried out with 1mL aliquot in 9mL of distilled water. From these solution (10^{-3} and 10^{-4}), 1mL aliquots were seeded in Petri dishes with Trichoderma selective agar medium (TSM) by the pour plate method. The plates were incubated at 28ºC for 4-7 days until appearance of green fungal colonies showing *Trichoderma* features. These potential *Trichoderma* colonies were sub-cultured in Petri dishes containing Potato Dextrose Agar (PDA) medium and incubated at 28ºC for 7 days. *Trichoderma* strains were identified by analysis of morphology of colonies and visualization of structures of conidiophores with microscope.

Isolation and identification of corn spoilage fungi

A modified method of isolation and identification by Madbouly *et al*. (2023) was adopted. The corn samples with fungal growth were surface-sterilized using 70% ethanol for 30s and a 1% NaOCl for 3min and washed 3 times with distilled water. The samples were cut into small pieces ($1cm^2$) with a sterile scalpel, mashed using a sterilized laboratory mortar and pestle. Then 1g of the mashed corn was diluted in 9mL distilled water, ImL of the dilution was placed on potato dextrose agar (PDA) plates, supplemented with 150µgmL^{-1} of

streptomycin to inhibit bacterial contamination and spread out using a sterile spread rod. After incubation for 72–96h at 28°C, the emerging fungal hyphae from the segments were picked up onto new plates and then purified using the single spore technique. The pure fungal cultures were kept on PDA slants at 4°C for further studies.

Antibiosis test of *Trichoderma* species against spoilage fungal isolates

Antibiosis (antagonistic activity) of isolated *Trichoderma* species against isolated fungal pathogens of corn were tested *in vitro* following the dual culture/co-culture method of Ferreira *et al*. (2020). A sterile cork borer (diameter 10mm) was used to transfer test fungus (pathogen) onto two sterile PDA plates halfway between the centre and edge of the plates. Then the antagonist (*Trichorderma* sp.) was transferred with the sterilized cork borer onto one of the plates halfway between the centre and edge the plate, opposite the test fungus. Same set up was done for all the pathogens isolated. Then the culture plates were incubated for seven (7) days at 28°C, colony growth of both biocontrol agent (*Trichoderma* sp.) and pathogen were observed constantly and the radial growth of the pathogens in the control plates and dual culture plates (in the direction of the antagonist *Trichoderma* sp.) were recorded daily up to the seventh (7^{th}) day (day 7) after inoculation, the percentage inhibition of radial growth of pathogens (antagonism of *Trichoderma* sp. against pathogens) was calculated using the formula below.

$$P.G.I\ (\%) = \frac{C-F}{C} \times 100$$ (Gwa and Ekefan, 2017)

Where:

P.G.I = Percentage growth inhibition.

C = the distance (mm) from the point of inoculation to the colony margin of control plate on day 7 after inoculation.

F = the distance (mm) of fungal growth from the point of inoculation to the colony margin in the treated plate in the direction of the antagonist (*Trichoderma* sp.) on day 7 after inoculation.

RESULTS AND DISCUSSION

The result aspresented in table 1 is the identified antagonist (*Trichoderma* spp.) and corn spoilage fungal genera used as test organisms in this study. The identified genera includes *Aspergillus flavus* (Plate count) (Pc1), *A. niger* (Pc2), *A. terreus* (Pc3), *Fusarium* spp. (Pc4), *Nigrospora* spp. (Pc5) and *Penicillium* spp. (Pc6).

The result as presented in Table 2 shows the growth in millimetre (mm) of the control (*Trichoderma* spp.), and spoilage fungi in co-culture with *Trichoderma* spp. for seven days with the calculated percentage growth inhibition (PGI). The result revealed that *Fusarium* spp (Pc4) grew well despite the presence of *Trichoderma* spp. while *Aspergillus niger* (Pc3) and *Penicillium* spp. growths were inhibited by *Trichoderma* spp.

The result as presented in Figure (1) and (2) shows the antagonism of *Trichoderma* spp. against corn spoilage fungi. The result revealed that *Trichoderma* spp. was most antagonistic against *Aspergillus terreus* (62.9%), followed by *Penicillium* spp. (62.5%) and the least antagonism was on *Fusarium* spp. (5.9%).

Table 1: Macroscopic and microscopic identification of possible fungal isolates

Samples/Isolates code	Macroscopy on MEA	Microscopy with lactophenol cotton blue	Tentative fungi
(Pc1).	Greenish sporing surface and yellow cracked reverse.	Vesicles are globose and phialides are produced directly from vesicle surface.	*Aspergillus flavus*
(Pc2)	Black sporing/granular surface and light cracked reverse.	Septate hyphae with long conidiophores that support spherical vesicles that give rise to large metulae.	*Aspergillus niger*
(Pc3)	Brown/dark-brown sporing/granular surface and light cracked reverse.	Vesicles are hemispherical and phialides cover the entire surface and are produced from a primary row of metulae.	*Aspergillus terreus*
(Pc4)	White fluffy surface and light reverse.	Hyphae are small and septate and give rise to phialides that produced single-celled microconidia.	*Fusarrium* spp.
(Pc5)	Gray wooly surface and dark reverse.	Hyphae are septate with phialides bearing microconidia.	*Nigrospora* spp.
(Pc6)	Blue-green velvet/powdery surface and light reverse.	Hyphae are hyaline and septate and produced brush-like conidiophores.	*Penicillium* spp.
Pc	Two concentric rings with green conidial production.	Septate hyaline hyphae, conidiophores, phialides, and conidia	*Trichoderma* spp.

Key: Pc = Plate count

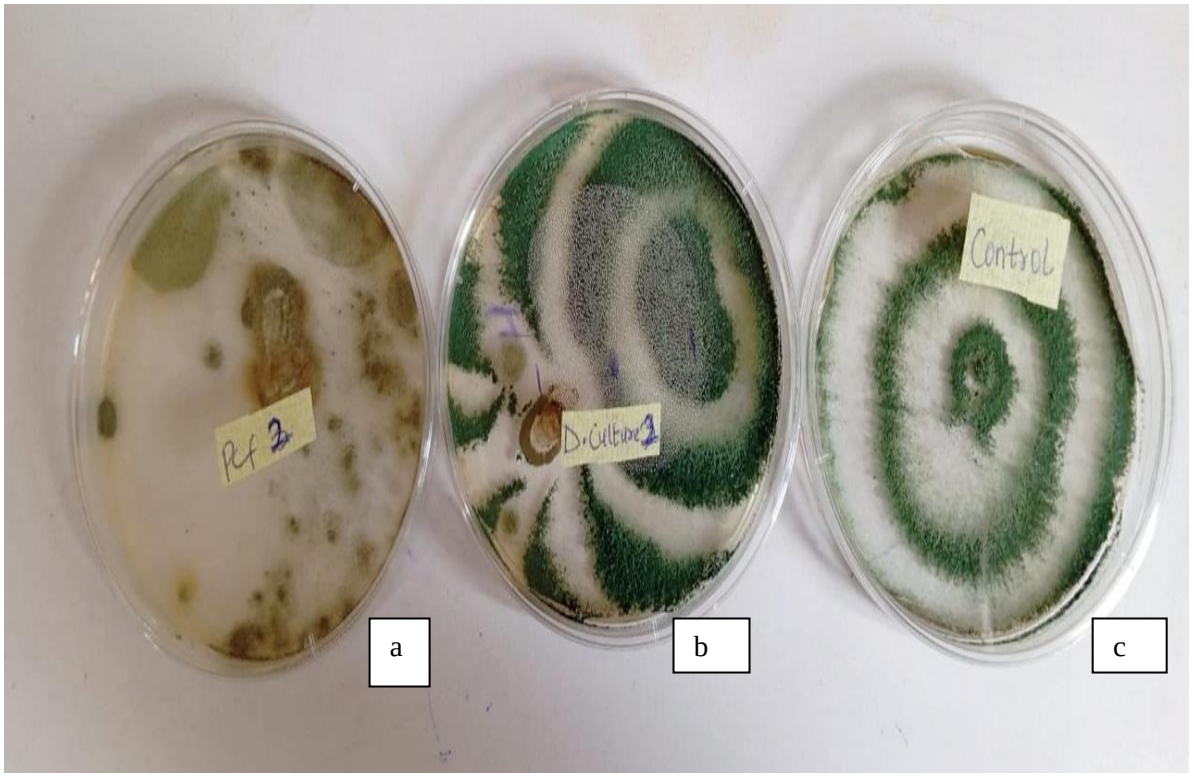

Figure.1:*AspergillusNiger* only(a) and *Trichoderma +A.niger(b)*,Control(of *Trichoderma* only(c)

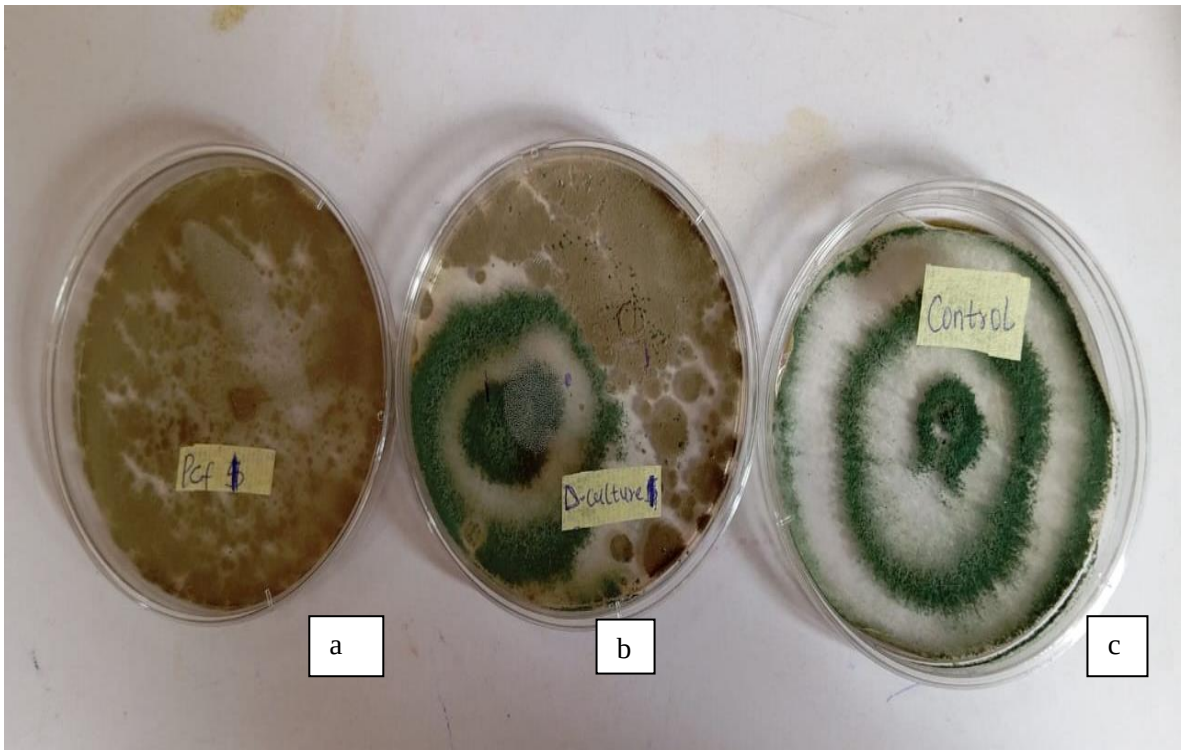

Figure.2: *Aspergillus flavus* only(a) and *Trichoderma+A.flavus*,(b)Control(of *Trichoderma* only(c)

Table 2: The mean values of the control plates (C), treated plates (F) and PGI of *Trichoderma* spp. against the fungal pathogens

Isolate	Day 1 (mm)		Day 2 (mm)		Day 3 (mm)		Day 4 (mm)		Day 5 (mm)		Day 6 (mm)		Day 7 (mm)		PGI (%)
	C_1	F_1	C_2	F_2	C_3	F_3	C_4	F_4	C_5	F_5	C_6	F_6	C_7	F_7	
Pc1	6.0	6.0	7.0	9.5	16.0	16.5	29.0	18.0	40.0	14.0	21.0	15.0	22.0	18.5	15
Pc2	6.0	6.0	13.5	15.5	16.0	21.5	20.0	22.5	21.5	18.5	23.0	20.0	25.0	21.5	14
Pc3	6.0	6.0	10.0	18.5	59.0	30.5	80.0	25.5	83.0	30.0	84.0	30.5	81.0	30.0	62.9
Pc4	6.0	6.0	12.5	15.0	21.5	26.5	32.0	26.0	37.0	32.0	42.5	35.0	67.5	36.5	5.9
Pc5	6.0	6.0	12.0	19.0	47.5	35.0	62.0	35.5	76.5	33.0	78.0	34.5	72.5	28.0	61.4
Pc6	6.0	6.0	51.0	28.0	47.0	39.0	82.0	40.0	82.0	40.0	83.0	40.5	80.0	30.0	62.5

C: control, F: treated plate, PGI: percentage growth inhibition

Discussion

The corn spoilage fungi isolated and identified in this study have also been reported by other researchers to be involved in corn spoilage; *Aspergillus flavus* (Li *et al*., 2021b), *A. niger* (Hurtado *et al*., 2024), *A. terreus* (Pitt and Hocking, 2022), *Fusarium* spp. (Aminu and Keta, 2021), *Nigrospora* spp. (Gomaa, 2021) and *Penicillium* spp. (Aminu and Keta, 2021; Al-Masoodi *et al*., 2023).

Trichoderma spp. showed antagonism against *Aspergillus flavus* with a percentage growth inhibition (PGI) of 15%. This showed inhibition of *A. flavus* by *Trichoderma* spp. In a similar study by Ren *et al*. (2022), they reported a PGI range of 13-65% by *Trichoderma* isolates against*A.* flavus

Trichoderma spp. showed antagonism against *A. niger* with a percentage growth inhibition (PGI) of 14%. This result agrees with the report of Sobowale *et al*., (2022) in the study "Fungitoxicity of *Trichoderma longibrachiatum* (Rifai) metabolites against Fusarium *oxysporum*, *Aspergillus niger* and *Aspergillus tamarii*." In a review by Khan *et al*. (2020), *Trichoderma* spp. was also reported to be antagonistic to *A. niger*.

Trichoderma spp. showed antagonism against *A. terreus* in this study. However the antagonism was weak (14%). This result is not in agreement with the review by Prabhu *et al*. (2022), who reported the co-culturing of *Trichoderma* spp. and *A. terreus* for their by-product in industrial solid state fermentation. Espinosa-Ortiz *et al*. (2022) also reported co-culture of *Trichoderma harzanium* and *A. terreus* for removal of organic pollutant. The antagonism observed in this study could be a situation of strains specific inhibition.

Trichoderma spp. showed the very little antagonism (PGI 5.9%) against *Fusarium* spp. This result does not agree with the results by Abhiram and Masih (2018) when they co-cultured on plate *Trichoderma viride* and *Fusarium oxysporum*, and the results revealed that *Trichoderma viride* showed maximum inhibition 71.0% over *Fusarium oxysporum* strain (E) and minimum inhibition 62.50% over *Fusarium oxysporum* strain (D). Anjum *et al*. (2020) using the dual culture technique evaluated antagonistic potential of *T. atroviride*, *T. hamatum*, *T. harzianum*, *T. longibrachiatum*, and *T. viride* against *Fusarium oxysporum* f. spp. *capsici* and the result was 67.18%, 70.15%, 68.75%, 69.46% and 66.75% inhibition, respectively, which does not agree with the result in this study.

Trichoderma spp. was antagonistic to *Nigrospora* spp. in this study at a percentage growth inhibition of 61.4%. This result corroborates with the report by Zhang *et al*. (2021b) who reported relative inhibitory rate of 89.80% by *Trichoderma* spp. against *Nigrospora sphaerica*.

Trichoderma spp. displayed a high antagonism against *Penicillium* spp. at a percentage growth inhibition of 62.5%. This result agrees with the antagonism of 35.5±1.9% reported by Yuliantoro and Prihatiningrum (2023).

CONCLUSION

The result obtained in this study revealed that Trichoderma spp. antagonized many genera of corn spoilage fungi. Therefore, it could be explored for control of post-harvest fungal spoilage of corn. The use of Trichoderma species as biocontrol agents can reduce the use of chemical fungicides which can have negative impacts on the environment and human health. Further studies are needed to evaluate the efficacy of Trichoderma species under field conditions and to develop practical applications for their use in corn production.

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