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Effect of *Trichoderma* Species on Seed Borne Fungi of Pearl Millet (*Pennisetum glaucum* L.R. Br) Using Dual Culture Technique

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ABSTRACT

Background and Objective: Millets are susceptible to fungi diseases resulting in high postharvest losses. To minimize these losses, there is a need for effective and eco-friendly control measures towards these pathogens. Thus, the study was carried out to investigate the effect of *Trichoderma* species on seed-borne fungi of pearl millet (*Pennisetum glaucum*) in Makurdi. **Materials and Methods:** Pearl millet seeds were collected from three markets in the Makurdi metropolis namely, Wurukum, Railway, and High-Level Markets, and taken to the Botany Laboratory of the Benue State University for the isolation of fungi. Soil samples from millet plantations in the Apir area of Makurdi were collected in polythene bags and taken to the Laboratory for the isolation of *Trichoderma* species. The standard blotter method was employed for the detection and isolation of seed-borne fungi in pearl millet and the serial dilution technique was used for the isolation of *Trichoderma* from soil samples. The effect of *Trichoderma koningii* on the radial growth of isolated fungi was carried out using a dual culture technique. Data collected from the study were analyzed using Chi-square and t-test. Means were separated using Duncan's Multiple Range Test (DMRT) at a 5% level of significance. **Results:** The study identified four seed-borne fungi in pearl millet: *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus stolonifer*, and *Fusarium* sp. A significant location-based variation in fungal occurrence was observed, with *Aspergillus flavus* being the most prevalent. Seed germination showed no significant difference across locations. Fungal growth was significantly reduced when paired with *Trichoderma koningii*, with *Rhizopus stolonifer* showing the highest reduction in growth compared to other fungi. **Conclusion:** *Trichoderma koningii* proved to be an effective bio-control agent as it inhibited the radial growth of fungi isolates and can be employed in plant disease management. Therefore, *T. koningii* should be employed for seed treatment and industries should be created by the Government and relevant stakeholders where *Trichoderma* isolates can be formulated and produced on a commercial scale for farmers.

KEYWORDS

Trichoderma species, dual culture, seed borne fungi, pearl millet, bio-control agent, plant disease management

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INTRODUCTION

Pearl millet (*Pennisetum glaucum* L. R. Br.), also known as bajra in Hindu or 'yadi' in the Marghi language of North Eastern Nigeria, is a cereal crop of paramount importance, exhibiting considerable potential among millet varieties[1]. Characterized by its robust growth habit, pearl millet is a dual-purpose crop, offering both grain and fodder, with exceptional yielding potentials. As a staple food, it plays a vital role in supporting millions of people in arid and semi-arid regions worldwide[2]. According to the Food and Agriculture Organization (FAO), pearl millet is the sixth most important cereal crop globally, with significant annual cultivation in Africa and the Indian Sub-Continent[3]. India is the largest producer of pearl millet in Asia, with an average productivity of 930 kg/ha. Nigeria ranks as the fifth largest producer of millet worldwide, with an annual production of 1.5 million tonnes as of 2016[4].

Pearl millet is a staple food in many Nigerian households, particularly among low-income families in Northern Nigeria, with millions of tonnes consumed annually[3]. The crop is also utilized in the production of various traditional food products, including "masa" (a fried cake) and "tuwo" (a thick binding paste). Furthermore, pearl millet flour is used to prepare flatbreads, leveraging its gluten-free properties.

Nutritionally, pearl millet is a valuable source of protein (18%), vitamins B, particularly niacin, B6, and folic acid. Its importance extends across the Sahel Region, serving as a primary staple in Northern Nigeria, Niger, Mali, and Burkina Faso. In some regions, pearl millet is processed into a beverage called "fura" (Hausa) or "tukura" (Marghi), which involves grinding the grain into flour, rolling it into balls, parboiling, and mixing it with fermented milk to create a watery paste[1].

Millet crops are susceptible to various diseases, which can manifest severely under diverse climatic conditions, resulting in substantial economic losses[5]. While some diseases occur sporadically in specific climates with minimal impact, others are more prevalent and detrimental. Notably, fungal diseases predominate over bacterial and viral diseases in millets[5]. Key fungal diseases affecting millets include grain mold, ergot, smut, anthracnose, downy mildew, blast, rust, charcoal rot, foot rot, banded sheath blight, and sheath rot. These diseases infect various plant parts, such as roots, stems, leaves, peduncles, and grains, ultimately compromising yield and quality[5]. Furthermore, these fungal diseases not only cause significant qualitative and quantitative yield reductions but also produce mycotoxins, posing serious health risks to humans and animals that consume contaminated grains[6].

The various methods used for managing disease include the use of resistant varieties, cultural practices, chemical control, and biological control. Breeding for disease-resistant varieties has been long used for managing diseases[7]. The evolution of pathogens into newer, more aggressive biotypes poses a significant challenge to disease management in crops[7]. This phenomenon can lead to the breakdown of resistance in previously resistant varieties, rendering them susceptible to infection.

Traditionally, chemical pesticides, fungicides, and microbicides, such as fthalide, edifenphos, iprobenfos, tricyclazole, isoprothiolane, probenazole, pyroquilon, felimzone (meferimzone), and diclocymet, have been employed to manage diseases in crops. While these chemicals are effective, their use is often accompanied by significant economic and environmental costs, as well as potential human health risks[8]. The use of alternate methods instead of seed-treating chemicals is of great concern to save our environment. Seed treatment with different plant extracts has been shown effective in controlling seed-borne fungal pathogen[9]. However, this study seeks to evaluate a biological control method where antagonistic organisms will be used to control fungal pathogens of millet *in vitro*. Therefore, the effect of *Trichoderma* species on seed-borne fungi of pearl millet in Makurdi using the dual culture technique was investigated.

MATERIALS AND METHODS

Study area: The study was carried out at the Plant Science and Biotechnology Laboratory of the Benue State University between November, 2023 to May, 2024.

Collection of pearl millet samples: Pearl millet was collected from three major markets in Makurdi namely; Wurukum, Railway, and High-level markets. The millet seeds were collected from three different sale points in each market. They were packaged in polythene bags, labeled properly, and taken to the Botany Laboratory of Benue State University for isolation of the seed-borne fungi.

Preparation of culture media: Potato dextrose agar (PDA) media was used for the isolation of the fungal pathogens for further studies. The media was prepared according to the manufacturer's recommended procedures where 39.6 g of powdered PDA medium were dissolved in 1000 mL of sterile distilled water and stirred vigorously to homogenize. The content of the flask was heated on a heating mantle until the solution became clear and all the PDA powder dissolved. After heating, the mouth of the flask was covered with cotton wool and autoclaved at 121°C for 15 min at 760 mmHg. The sterile medium was allowed to cool to a temperature at which it could be held with hands and two to three drops of streptomycin sulphate were added to inhibit bacterial growth. The medium was dispensed into the Petri dishes and allowed to solidify for further studies.

Detection of fungi on millet seeds: The detection of seed-borne fungi was done using the blotter method[10]. Three layers of Whatman filter papers were soaked in sterile distilled water and placed at the bottom of 9 cm diameter Petri dishes. The seeds were surface sterilized in 5% sodium hypochlorite for 30 sec to 1 min and rinsed in three successive changes of sterile distilled water[11]. Fifteen sterilized seeds were placed on the moist filter paper in the Petri dishes and incubated at ambient temperature for 5-7 days. After 5-7 days of incubation, the seeds were examined for fungal growth and germination of seeds. The data collected include.

Percentage occurrence of fungi: Plates were observed for growth and the occurrence of fungi was determined by counting the number of fungi per market divided by the total number of fungi and expressed as a percentage[12]:

$$\frac{\text{Number of fungi per market}}{\text{Total number of fungi}} \times 100$$

Percentage occurrence of specific fungi: Seeds were observed for growth and the occurrence of specific fungi was determined by counting the number of times each fungus occurred divided by the total number of fungi and expressed as a percentage using the formula[12]:

$$\frac{\text{Number of times each fungus occurred}}{\text{Total number of fungi per plate}} \times 100$$

Percentage of seed germination: The percentage of seed germination was calculated by counting the number of seeds with seed leaves divided by the total number of seeds per plate[12]:

$$\frac{\text{Number of seeds with seed leaf}}{\text{Total number of seeds per plate}} \times 100$$

Isolation and sub-culturing of fungi from millet seeds: On the appearance of fungi, a small quantity of each fungi colony was picked with the aid of an inoculation needle and inoculated on prepared potato dextrose agar. The Petri plates were incubated at ambient conditions of light and temperature for 5-7 days and observed daily for fungal growth. After 5-7 days, sub-culturing was done to obtain the pure culture

of the isolates. To subculture, a sterilized inoculation needle was used to pick a small quantity of the fungal growth on the old culture and transfer it to the center of a freshly prepared PDA in another Petri dish. Sub-culturing was done repeatedly until pure cultures of each fungal organism were obtained as reported by Kator *et al.*[12].

Identification of fungi: The identification of fungi was done by observing the color, nature of growth, and growth rate of the fungi macroscopically in Petri plates. Microscopic identification was done by staining a glass slide with a drop of Lactophenol in cotton blue and with the aid of an inoculation needle, a small quantity of the fungal colony was placed on the stained-glass slide. This was covered with a cover slip and viewed under the 40× objective lens of the light microscope. The observed characteristic of the fungi was compared with standard text for identification[12,13].

Collection of soil samples: Soil samples were collected from millet plantations in the Apir Area of Makurdi, Benue State. The soil samples within the area of the millet plants were collected in polyethylene bags at a depth of 2-3 cm from the rhizosphere of the plants at three different points on the field and pulled together[14]. These were taken to the Botany Laboratory of the Benue State University for isolation of *Trichoderma* species.

Isolation of *Trichoderma* species from soil samples: The serial dilution method of Liamngee *et al.*[14] was employed for this study. For isolation of *Trichoderma* species from the soil samples, a serial dilution technique was employed and a 10^4 dilution of the soil sample was prepared. In this method, a stock suspension was prepared by adding 1 g of the soil samples to 9 mL of sterile distilled water in a sterilized glass tube. One milliliter was further pipetted from the first dilution and introduced into another labelled test tube to obtain 10^{-1} dilution. This was done repeatedly to obtain up to 10^{-4} dilution. A 10^{-4} dilution of the sample was prepared and 1 mL was dispersed in a 9 cm diameter Petri dish. Approximately 15-20 mL of sterilized, molten potato dextrose agar (PDA) was then added to the dish. The agar and inoculum were gently swirled to ensure uniform distribution, and the mixture was allowed to solidify. The culture plates were incubated at room temperature for 7 days. Daily observations were made, and each colony that emerged was considered a single colony-forming unit (CFU). Pure cultures of fungal colonies were obtained through subculturing, following the procedure outlined in section 2.4.4.

Identification of *Trichoderma* isolates: Identification of *Trichoderma* species was conducted through visual observation on Petri dishes and micro-morphological studies using slide culture[14]. Visual observations on PDA-grown isolates focused on growth rates, changes in medium color, and colony morphology, which are considered taxonomically informative characteristics for Trichoderma. The micro-morphological examination involved observing the morphology of conidiophores and conidia, as well as other relevant characteristics, using electronic documentation on the genus *Trichoderma*[14].

***In vitro* antagonistic activity of *Trichoderma* species:** The *in vitro* antagonistic effects of *Trichoderma* species against test fungi (isolated from millet seeds) were evaluated using the dual culture technique on potato dextrose agar (PDA) medium[14]. Mycelial discs (5 mm) of *Trichoderma* species and test pathogens were placed simultaneously, 1 cm from the edge of each Petri dish, in opposite directions. The experiment was conducted in triplicate, with a completely randomized design. Control plates received only the mycelial disc of each test fungus. Plates were incubated at ambient laboratory temperature. The inhibition of test fungi was calculated using the following formula[14]:

$$\frac{R_1 - R_2}{R_1}$$

Where:

R_1 = Mycelia growth of the pathogen without *Trichoderma* (control)

R_2 = Mycelia growth of the pathogen in the presence of *Trichoderma*

Data analysis: Data collected from the study were analyzed using analysis of variance and t-test. Means were separated using Duncan's Multiple Range Test at a 5% level of significance.

RESULTS

The study revealed that four fungi were isolated from pearl millets in Makurdi while *Trichoderma koningii* was isolated from the soil sample. Their macroscopic and microscopic features are presented in Fig. 1-5. The colony of *A. flavus* on PDA had a greenish coloration (Fig. 1a). When viewed under the microscope, *A. flavus* had bluish-green conidia with the conidiophore's hyaline in nature (Fig. 1b). The colonies of *R. stolonifer* were fast growing with cottony like appearance on PDA (Fig. 2a). The sporangiospores were hyaline, smooth-walled and branched forming large terminal globose sporangia (Fig. 2b). Colony of *Aspergillus niger* on PDA was black (Fig. 3a) and the conidiophores were hyaline, inflated at the apex forming globose conidia vesicles when viewed under the microscope (Fig. 3b). The colony color of *Fusarium* sp., was pinkish white a cottony like appearance on PDA (Fig. 4a). The conidia had a cylindrical shape with a septum, single cell, hyaline and a smooth round end when viewed under the light microscope (Fig. 4b). *Trichoderma koningii* on PDA had a rapid growth and produced yellowish-white mycelia with rough surface (Fig. 5a). When viewed under the microscope, the conidia were ellipsoidal, hung at the apex of a long conidiophore, and branched at alternate angles Fig. 5b.

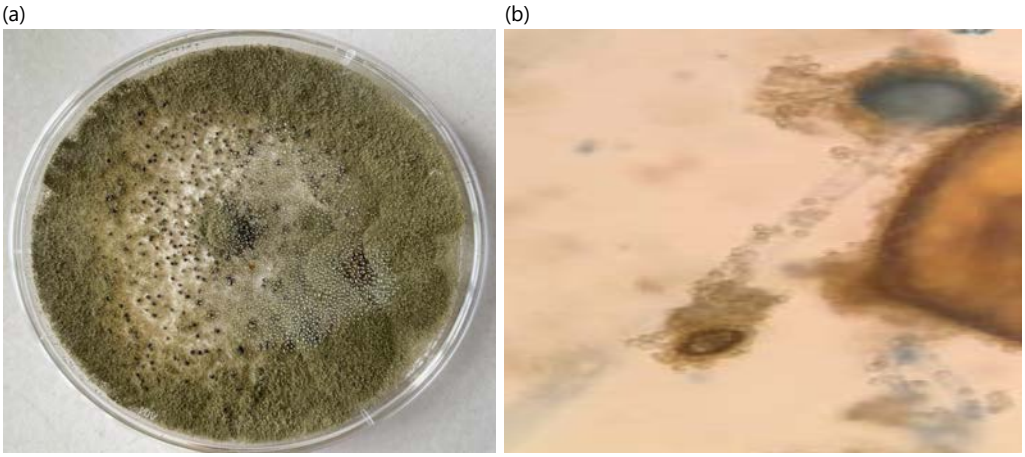

Fig. 1(a-b): View of *A. flavus*, (a) Macroscopic view, colony colour was green and (b) Microscopic view, Conidia were radial in shape and bluish green with transparent conidiophores

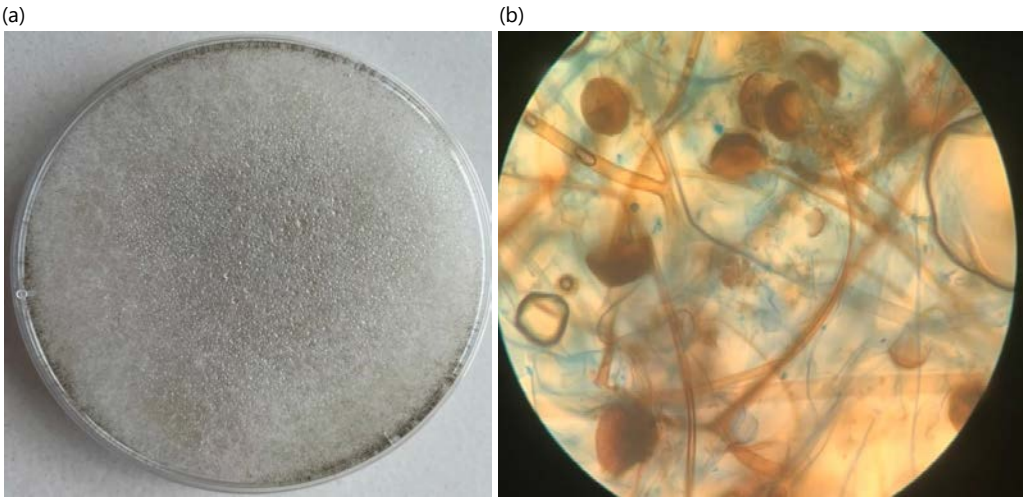

Fig. 2(a-b): View of *R. stolonifer*, (a) Macroscopic view, colony colour was white and cottony in appearance and (b) Microscopic view, Sporangiospores were branched, root like with large globose sporangia

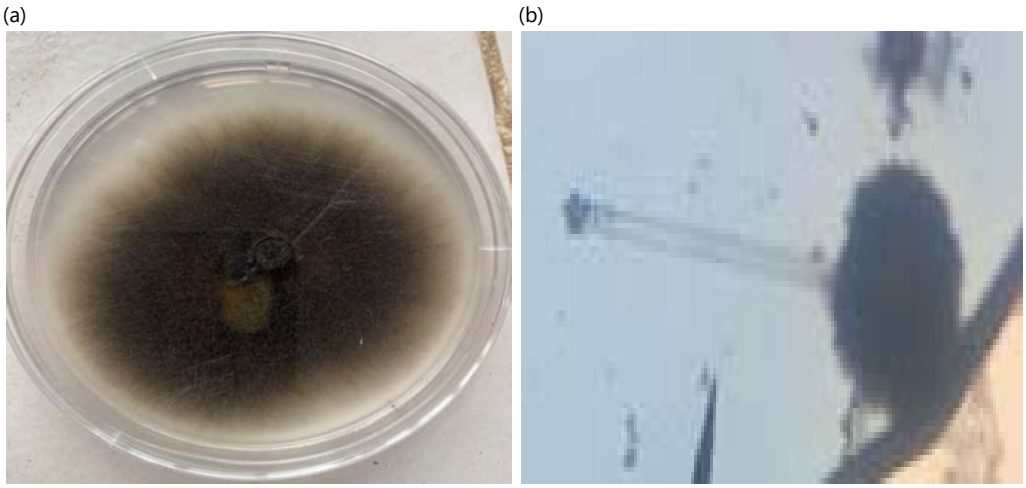

Fig. 3(a-b): View of *A. niger*, (a) Macroscopic view, colony colour was black and (b) Microscopic view, Conidia were dark with transparent conidioph

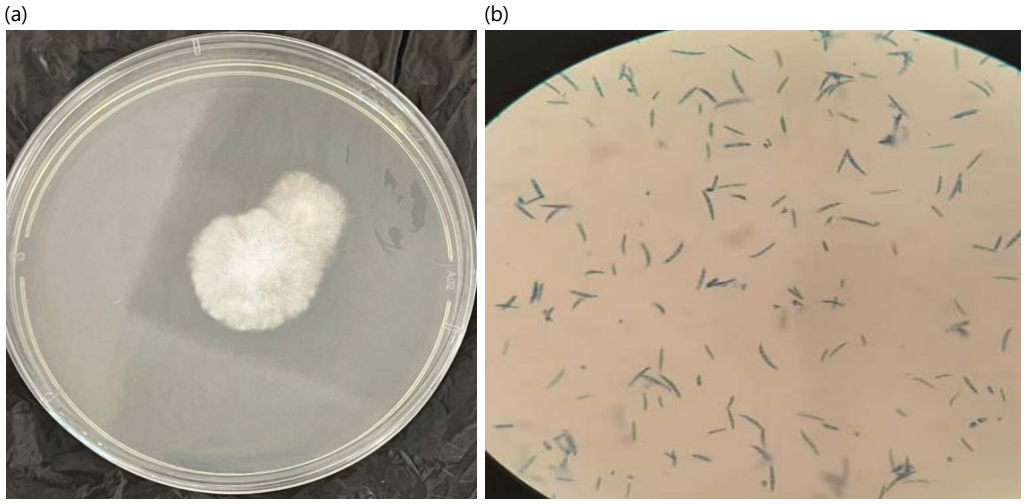

Fig. 4(a-b): View of *Fusarium* sp., (a) Macroscopic view, colony colour was whitish cream with a cottony like appearance and (b) Microscopic view, Conidia were hyaline, oval and curved with smooth tapered ends

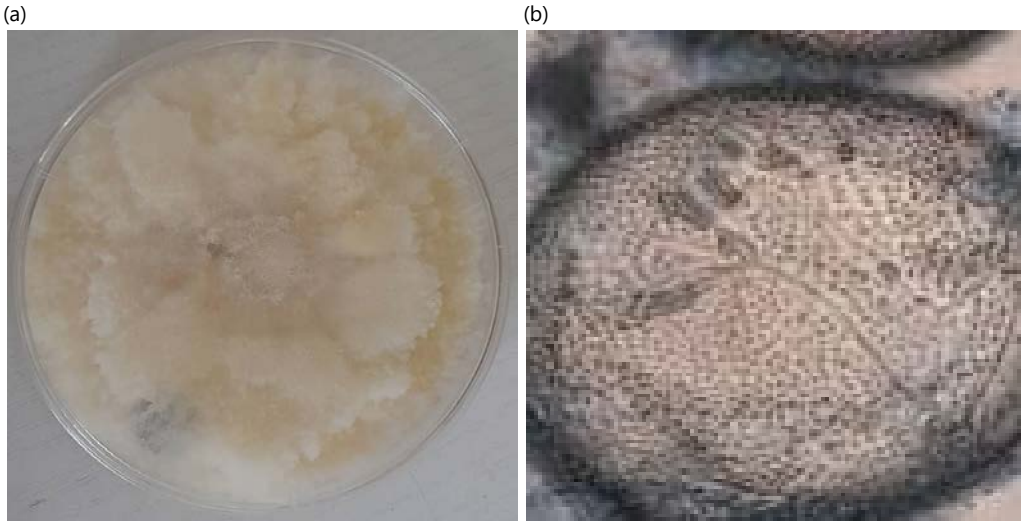

Fig. 5(a-b): View of *T. koningii*, (a) Macroscopic view, colony colour was yellowish white with rough surface and (b) Microscopic view, Conidia were ellipsoidal and at alternate angles on a long conidiophores

Occurrence of seed borne fungi on pearl millet based on location: The occurrence of seed-borne fungi on pearl millet is shown in Table 1. There was a significant relationship in fungi occurrence based on location (χ^2 = 14.262, df = 6, p = 0.027). *Aspergillus flavus* had significantly higher occurrence with 16 (47.06%), followed by *Aspergillus niger* 9 (26.47%), *Fusarium* sp., 7 (20.59%) and the least was *Rhizopus stolonifer* 2 (5.88%). Pearl millet seeds obtained from High Level had significantly higher fungi occurrence with 13 (38.24%) followed by Railway 11 (32.35%) and the least was Wurukum 10 (29.41%).

Percentage seed germination of pearl millet in Makurdi: The percentage of seed germination of pearl millet is presented in Table 2. Out of the 135 seeds that were plated, 103 seeds representing 76.30% notably germinated. There was no significant seed germination in pearl millet collected from Wurukum, Railway, and high-level markets (χ^2 = 3.522, df = 2, p = 0.172). Pearl millet seeds obtained from high levels had the highest seed germination with 37 (27.41%) followed by Wurukum at 36 (26.67%) and the least was from Railway with 30 (22.22%).

Effect of *Trichoderma koningii* on radial growth of *Rhizopus stolonifer* isolated from pearl millet: The effect of *Trichoderma koningii* on radial growth of *Rhizopus stolonifer* is presented in Table 3. There was no significant difference in the radial growth of *Rhizopus stolonifer* in the control and radial growth of *Rhizopus stolonifer* paired with *Trichoderma koningii* after 48 hrs of observation. However, there was a significantly higher radial growth in the control (2.13±0.15, 2.83±0.21 and 3.57±0.42) cm at 72, 96, and 120 hrs, respectively compared with radial growth of *Rhizopus stolonifer* paired with *Trichoderma koningii* (1.50±0.10, 1.78±0.10 and 2.15±0.30) at 72 to 120 hrs, respectively.

Effect of *Trichoderma koningii* on radial growth of *Aspergillus flavus* Isolated from pearl millet: The effect of *Trichoderma koningii* on radial growth of *Aspergillus flavus* is presented in Table 4. Radial growth of *Aspergillus flavus* paired with *Trichoderma koningii* was significantly lower at 48 to 120 hrs (0.67±0.13, 0.88±0.38, 0.93±0.38 and 1.17±0.21), respectively compared with the control (1.70±0.43, 2.30±0.52, 2.84±0.80 and 3.47±1.15) at 48 to 120 hrs.

Table 1: Occurrence of seed borne fungi of pearl millet in Makurdi Metropolis

	Location			
Fungi isolate	Wurukum	Railway	High level	Total
Rhizopus stolonifera	1	1	0	2
Aspergillus flavus	7	6	3	16
Fusarium sp.	0	4	3	7
Aspergillus niger	2	0	7	9
Total (%)	10(29.41)	11(32.35)	13(38.24)	34.00

χ^2 = 14.262, df = 6 and p = 0.027

Table 2 Percentage seed germination of pearl millet in Makurdi

Location	Number of seeds germinated (n=135)	Germination (%)
Wurukum	36	26.67
Railway	30	22.22
High level	37	27.41
Total	103	76.30

Table 3: Effect of *Trichoderma koningii* on radial growth of *Rhizopus stolonifer* isolated from pearl millet

Radial growth (cm)				
Treatment	48 hrs	72 hrs	96 hrs	120 hrs
R. stolonifer+T. koningii	1.07±0.12	1.50±0.10	1.78±0.10	2.15±0.30
Control	1.67±0.40	2.13±0.15	2.83±0.21	3.57±0.42
T-value	2.472	6.008	7.814	4.759
p-value	0.069	0.004	0.001	0.009

Values are Mean±Standard Deviation in triplicates

Table 4: Effect of *Trichoderma koningii* on *Aspergillus flavus* isolated from pearl millet

	Radial growth (cm)			
Treatment	48 hrs	72 hrs	96 hrs	120 hrs
A. flavus+T. koningii	0.67±0.31	0.88±0.38	0.93±0.38	1.17±0.21
Control	1.70±0.43	2.30±0.52	2.84±0.80	3.47±1.15
T-value	3.369	3.784	3.708	3.408
p-value	0.028	0.019	0.021	0.027

Values are Mean±Standard Deviation in triplicates

Table 5: Effect of *Trichoderma koningii* on *Fusarium* sp. isolated from pearl millet

	Radial growth (cm)			
Treatment	48 hrs	72 hrs	96 hrs	120 hrs
Fusarium sp.+*T. koningii*	0.15±0.05	0.23±0.11	0.47±0.25	0.68±0.43
Control	2.93±0.90	3.47±0.85	3.83±0.90	4.17±1.26
T-value	5.337	6.539	6.220	4.511
p-value	0.006	0.003	0.003	0.011

Values are Mean±Standard Deviation in Triplicates

Table 6: Effect of *Trichoderma koningii* on *Aspergillus niger* isolated from pearl millet

	Radial growth (cm)			
Treatment	48 hrs	72 hrs	96 hrs	120 hrs
A. niger+T. koningii	0.83±0.29	1.17±0.35	1.47±0.42	1.76±0.25
Control	2.02±0.08	5.00±0.40	5.10±0.53	6.73±1.55
T-value	6.947	12.293	9.347	5.472
p-value	0.002	0.000	0.001	0.005

Values are Mean±Standard Deviation in triplicates

Table 7: Comparative effect of *Trichoderma koningii* on radial growth of fungi species isolated from pearl millet

	Radial growth (cm)			
Treatment	48 hrs	72 hrs	96 hrs	120 hrs
R. stolonifer+T. koningii	1.07 ± 0.12^{a}	1.50 ± 0.10^{d}	1.78 ± 0.10^{ac}	2.15 ± 0.30^{g}
A. flavus+T. koningii	0.67 ± 0.31^{b}	0.88 ± 0.38^{e}	0.93 ± 0.38^{c}	1.17 ± 0.21^{i}
Fusarium sp.+*T. koningii*	0.15 ± 0.05^{d}	0.23 ± 0.11^{def}	0.47 ± 0.25^{ab}	0.68 ± 0.43^{gh}
A. niger+T. koningii	0.83 ± 0.29^{c}	1.17 ± 0.35^{f}	1.47 ± 0.42^{b}	1.76 ± 0.25^{h}

Values are Mean±Standard Deviation in triplicates, mean values with similar alphabets are not significant, otherwise they are

Effect of *Trichoderma koningii* on radial growth of *Fusarium* sp., isolated from pearl millet: The effect of *Trichoderma koningii* on radial growth of *Fusarium* sp., is presented in Table 5. Radial growth of *Fusarium* sp., paired with *Trichoderma koningii* was significantly lower at 48 to 120 hrs (0.15±0.05, 0.23±0.11, 0.47±0.25 and 0.068±0.43), respectively compared with the control (2.93±0.90, 3.47±0.85, 3.84±0.90 and 4.17±1.26) at 48 to 120 hrs.

Effect of *Trichoderma koningii* on radial growth of *Aspergillus niger* isolated from pearl millet: The effect of *Trichoderma koningii* on radial growth of *Aspergillus niger* is presented in Table 6. Radial growth of *Aspergillus niger* paired with *Trichoderma koningii* was significantly lower at 48 to 120 hrs (0.83±0.29, 1.17±0.35, 1.47±0.42 and 1.76±0.25), respectively compared with the control (2.02±0.08, 5.00±0.40, 5.10±0.53 and 6.73±1.55) at 48 to 120 hrs.

Comparative effect of *Trichoderma koningii* on radial growth of fungi species isolated from pearl millet: The comparative effect of *Trichoderma koningii* on radial growth of fungi species isolated from pearl millet is presented in Table 7. Radial growth of *Rhizopus stolonifer* paired with *Trichoderma koningii* was significantly higher at 48 to 120 hrs (1.07±0.129, 1.50±0.10, 1.78±0.10 and 2.15±0.30) compared with radial growth of *Aspergillus niger* (0.83±0.29, 1.17±0.35, 1.47±0.42 and 1.76±0.25), *Aspergillus flavus* (0.67±0.31, 0.88±0.38, 0.93±0.38 and 1.17±0.21), and *Fusarium* sp. (0.15±0.05, 0.23±0.11, 0.47±0.25 and 0.68±0.43), respectively.

DISCUSSION

The seed-borne fungi isolated from pearl millet in this study were; *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus stolonifer*, and *Fusarium* sp. A study by Hussain *et al*.[15] isolated similar fungal organisms which include; *Alternaria alternata*, *Aspergillus alba*, *A. flavus*, *A. niger*, *Bipolaris* spp., *C. lunata*, *Drechslera* spp., *Fusarium semitectum*, *F. moniliforme*, *Helminthosporium* spp., *Penicillium* spp., and *Rhizopus* spp., from different varieties of millets in Pakistan. A similar study by Malik *et al*.[3] in Kebbi State Nigeria reported fungi species of *Aspergillus fumigatus*, *Aspergillus niger*, *Grapium* sp., *Microsporum* sp., *Trichophyton* sp., as seed borne fungi from millets. Also in Kano State Nigeria, Fayinminu *et al*.[6] implicated fungi species of *A. flavus*, *A. fumigatus*, *A. niger*, *A. oryzae*, *A. Candida*, and *A. Parasiticus* as seed-borne fungi from millet. Seed-borne pathogens have been involved in seed rots during germination and seedling mortality leading to poor crop stand. Seed-borne pathogens, whether present externally or internally, can have devastating effects on plant growth and productivity. These pathogens can cause a range of symptoms, including seed abortion, seed rot, seed necrosis, and reduced or eliminated germination capacity. Additionally, infected seeds can give rise to damaged seedlings, which can lead to the development of disease at later stages of plant growth through systemic or local infection[16]. The presence of fungal pathogens in millet seeds suggests contamination occurred during field harvesting. Previous studies have reported the association of field and storage fungal pathogens with millet seeds. Fungal pathogens, including *Aspergillus niger*, *Penicillium* sp., *Fusarium* sp., *Rhizopus* sp., and *Helminthosporium* sp., have been isolated from rotted seeds and abnormal seedlings, and have been implicated in seed decay and damping-off of millet seedlings[17].

The presence of storage fungal genera, such as *Aspergillus* and *Penicillium*, on seeds indicates that contamination occurred during storage. The detection of *A. niger* and *A. flavus* on seeds, abnormal seedlings, and rotted seeds confirms that *Aspergillus* species, although often saprophytic, can cause reduced germination in seeds[18]. Furthermore, *A. flavus* produces toxic metabolites that can inhibit shoot and root elongation[18]. *Aspergillus niger* is a damaging storage fungus that can deteriorate seed quality and reduce seed germination. Moreover, molds associated with pearl millet seeds have been reported to be pathogenic to humans, producing toxins that may cause respiratory diseases[15].

The study reported that pearl millet seeds obtained from high-level markets had the highest number of fungi compared to seeds obtained from Wurukum and Railway markets. The disparity observed in the occurrence of fungi in the different locations could be a result of the environmental conditions of the markets, the sanitary condition, the method of storage used, and the mode of display for sale in the market. These pathogens associated with millet seeds must have been present right from the field and after harvest, transferred to storage houses and marketplaces[19]. Also, the study revealed that millet seeds collected from High levels had the highest percentage of germination compared to seeds obtained from other markets. The findings from this study conforms with other research findings of Hussain *et al*.[15] and Makun *et al*.[17] in which they reported different percentages seed germination of cereals obtained from different locations. The variation in percentage seed germination within the locations as observed in this study might be a result of the source of collection of the millet seeds, the storage method employed, and also the possibility of the seed treatment technique used.

In terms of individual fungi occurrence, *Aspergillus flavus* was implicated in this study to be the dominant fungi isolated from millet seeds obtained across the locations. This result was contrary to the previous findings[3,6,15] in which they reported fungi species of *Trichophyton* sp., *Alternaria alternata*, and *Aspergillus niger* as the dominant seed-borne fungi pathogens of millet, respectively. The percentage occurrence of *A. flavus* (26.47%) recorded in this study was high compared to 14.00 and 11.67% reported by Fayinminu *et al*.[6] and Hussain *et al*.[15], respectively. The variability in the frequency of occurrence of these fungi may be attributed to differences in inoculum density in the area or prevailing environmental conditions that favor fungal growth[20]. These fungi likely colonized the grains at various stages, including during production in the field, transportation or storage.

The effect of *Trichoderma koningii* on radial growth of the isolated fungi from pearl millet in this study revealed that the different fungi gave different radial growth but T. *koningii* was able to inhibit their radial growth compared to untreated fungi. The use of *T. koningii* in the control of fungi radial growth in the study is a diversification away from other previous methods of control such as the use of plant extract reported by several researchers. In a similar study carried out by Khalili[21], it was reported that *Trichoderma harzianum* and *Trichoderma virens* were able to significantly inhibit the mycelial growth of *Bipolaris oryzae*. He *et al*.[22] stated that disease reduction by biological control is possible by reducing pathogen inoculum (decreased development and release of viable spores, decreased survival, and reduced spread), reducing pathogen infection of the host, and reducing the severity of the pathogen attack. The ability of *T. koningii* to inhibit the growth of the isolated fungi could be attributed to its ability to grow faster than the other organisms implying the need for more nutrient utilization and space than them. This reason is supported by Khalili *et al*.[21], who explained that inhibition of pathogen growth is due to the production of amylase by *Trichoderma* spp., which is partially responsible for the rapid growth of antagonists in potato dextrose agar medium. Liamngee *et al*.[14] added that in addition to amylase, it has been reported that *Trichoderma* species also produce extracellular cellulose and pectinase enzymes that are capable of hydrolyzing the cell walls of other fungi.

CONCLUSION

The study has shown that *A. niger*, *A. flavus*, *R. stolonifer*, and *Fusarium* sp., were fungi isolated from seeds of pearl millet in Makurdi. The distribution of these fungi in millet seeds varied across locations with seeds obtained from High levels having the highest fungi occurrence. The dominant species was *A. flavus* in pearl millet seeds collected across the locations. *Trichoderma koningii* proved to be an effective biocontrol agent as it inhibited the radial growth of the fungal isolates. Based on the study's findings, it is recommended that additional research be conducted on seed-borne fungi in pearl millet, an area with limited investigation. Further studies should also focus on isolating other Trichoderma species from the rhizosphere of pearl millet plantations for documentation. Additionally, *Trichoderma koningii* should be formulated into powders and produced on a commercial scale for use by farmers.

SIGNIFICANCE STATEMENT

High-quality seed is important for increased crop production and the proper establishment of a sound seed industry in the country. *Trichoderma koningii* used in the study significantly inhibited fungi growth *in vitro*. Therefore, the study's outcome proves relevant to the government, farmers, researchers, and consumers. For researchers, it will provide the basis for more research on other biocontrol agents and how they can be formulated for farmers. For consumers, it will provide them with safe and healthy produce for consumption. For policymakers, it will enable them to enact laws favoring the use of biocontrol agents in plant disease control. The government could also create industries where these biocontrol agents can be formulated and produced in large quantities thereby creating employment.

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