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ISOLATION, MOLECULAR CHARACTERIZATION AND APPLICATION OF *PENICILLIUM CITRINUM* AS BIOFERTILIZER POTENTIALS TO ENHANCE COWPEA GROWTH

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ABSTRACT

Chemical fertilizers are linked to a persistent decline in soil fertility, posing health hazards. This study investigated *P. citrinum* as a biofertilizer for cowpea (*Vigna unguiculata* growth. The test fungus was identified using molecular techniques from alligator pepper. Using a pot experimental method, an in-situ experiment was conducted on cowpea (TVX-3236) in the greenhouse to screen for the isolate's mycofertilizer potential. For six weeks, the cowpea was planted in six replicates in loamy soil. *P. citrinum* treatment was applied to the cowpea leaves in the following amounts 20 ml, 35 ml, 50 ml, 65 ml and 80 ml per bucket at a concentration of 0.08 spores per milliliter (mL) with no inoculation on the control. *P. citrinum* was applied on the second week and data was collected for agronomic traits (plant height, leaf number, leaf area, and root length) and leaf color. The impact of *P. citrinum* on cowpea height, leaf number, leaf area, and root length revealed that these agronomic parameters rose with increasing *P. citrinum* concentration. The cowpea in the pot that received the 80 ml *P. citrinum* inoculation performed the best, indicating that the plant benefited from this treatment. The experiment's results suggest that the isolate can be utilized as a mycofertilizer to promote cowpea growth. For that reason, this study offers some initial data for further investigation into the application of *P. citrinum* as a biofertilizer in agriculture. This fungus strain's capacity to stimulate plant development may aid in the preservation and revegetation of some vegetations.

Keywords: Fungi, Molecular techniques, *Penicillium citrinum*, Biofertilizer, Rhizosphere, Cowpea

INTRODUCTION

Agriculture is vital to a country's economy and there are numerous issues confronting contemporary intensive agricultural methods around the world, all of which represent serious risks to food security. According to Santos *et al*. (2012), conventional agriculture plays a significant role in meeting the food demands of a growing human population, which has also led to an increasing dependence on chemical fertilizers and pesticides. Chemical fertilizers are industrially manipulated, substances composed of known quantities of nitrogen, phosphorus and potassium, and their exploitation causes air and ground water pollution by eutrophication of water bodies (Youssef and Eissa, 2014). According to Bisht and Singh (2021), chemical fertilizers are responsible for the accumulation of toxic compounds in soil. Large numbers of chemical fertilizers have carcinogenic effects, while some contains acid radicals that increased the acidity of soil, thus adversely affecting the soil, plant, and human health. Large numbers of chemical fertilizers have carcinogenic effects, while some contains acid radicals that increased the acidity of soil, thus adversely affecting the soil, plant, and human health. In this regard, recent efforts have been channeled more towards the production of 'nutrient-rich high-quality food' in sustainable comportment to ensure biosafety. A reliable substitute to synthetic fertilizers which are the utmost threat to the environment and deteriorate the soil fertility and its health are plant growth-promoting rhizobacteria (PGPRs) which are the microbial inoculants and can be used as biofertilizers, bio-pesticides, bio-herbicides, and biocontrol agents. These microbes are innocuous and effective in less quantity, have more targeted activity and faster breakdown process, and induce the protection mechanism to plants (Alori *et al*., 2017a; Babalola, 2010).

It is necessary to apply fungal biofertilizers because they play key roles in increasing production, promoting plant growth, strengthening plant health, and improving soil fertility (Itelima *et al.*, 2018). Research has indicated that biofertilizers, including vesicular-arbuscular mycorrhizae (VAM) and AMF, enhance soil quality by improving nutrient availability and soil structure.

Also, David *et al.* (2023), stated that *Aspergillus niger* and *Penicillium chrysogenum* proved to be the best candidates among the isolates with mycofertilizer potentials. Since many fungal strains, especially *Aspergillus niger* and *Penicillium sp*., have been identified as phosphate solubilizers, plants can be inoculated with these mycorrhizal fungi that can produce up to 80% of the phosphorus needed by the plants for optimal development and production while also reducing the amount of phosphate (Khan and Bano, 2016).

According to Kumar (2018), biofertilizers are microorganisms that improve the availability of nutrients to the host plant—support the growth of plants. They enhance agricultural sustainability, soil health, and soil fertility. These active strains of bacteria, algae, and fungi increase soil fertility, mineralize elements, and move nutrients from soil to plants, improving crop productivity eco-friendlily.

Biofertilizers are a safe and effective way to increase output since they quickly break down and multiply while transmitting inert nutrients. They can be applied through soil, roots, or seeds (Vejan *et al*., 2016). Soil microbes used as biofertilizers include free-living nitrogen-fixing bacteria like *Azotobacter*, *Beijerinckia*, *Clostridium*, *Nostoc*, *Klebsiella*, and *Anabaena*; symbiotic bacteria such as *Rhizobia*, *Frankia*, and *Azospirillum*; phosphorus-solubilizing biofertilizers, viz., *Bacillus megaterium var. phosphaticum*, *Bacillus subtilis*, *Bacillus circulans*, and *Pseudomonas striata*; and fungi like *Penicillium sp*. and *Aspergillus awamori*, *Glomus species*, *Rhizoctonia*, *Pezizella*, etc. Biofertilizers are cheap and renewable sources of plant nutrients (Ammar *et al.*, 2023).

In view of the above stated facts, the long-term use of biofertilizers proves to be economical, eco-friendly, more

efficient, productive, and accessible to marginal and small farmers over chemical fertilizers.

The pressing need for sustainable agricultural methods has sparked interest in alternative approaches, such as biofertilizers. Among them, *Penicillium citrinum*, a filamentous fungus, has gained recognition for its potential to enhance to promote growth and elevate crop yield. The study is aimed at establishing the effectiveness of *Penicillium citrinum* as a biofertilizer in the growth and development of cowpea.

MATERIALS AND METHODS

Study Area

The study was conducted in Mycology/ Pathology laboratory of Plant Science and Biotechnology and Regional Centre for Biotechnology and Bio-fuel Research Laboratory where the DNA extraction was carried out,. Amplification. While sequencing of the PCR products were done at Inqaba Biotechnological, Pretoria South Africa.

Materials

Cowpea seeds TVX-3236 were obtained from International Institute for Tropical Agriculture (IITA) Ibadan in March 2023. Other materials include decayed alligator pepper Sabouraud Dextrose Agar (SDA), ethanol, spatula, cotton wool, forceps, foil, masking tape, marker, surgical blade, Petri dishes, sodium hypochlorite, chloramphenicol, weighing scale, distilled water, autoclave, laminar flow cabinet, cooling bath, Bunsen burner, stirrer, light microscope, slides, test tubes, volumetric flask, dropper, wash bottles, lab coat, hand gloves, nose mask, , hair cover, Erlenmeyer flask, peptone, inoculation loop, refrigerator, microwave, cotton blue lactophenol stain.

Methods

This study was carried out in different phases- collection of alligator pepper from local markets, preparation of SDA, isolation of fungi species from decayed infected alligator pepper using direct plating method, subculturing of fungi to get pure culture, transferring to slant, microscopy, molecular analysis, production of biofertilizer, planting and measuring of growth parameters.

Collection of Fruits

Alligator pepper (Plate 1) was gotten from a local market in Port-Harcourt on the 10th of September 2023. The alligator pepper was kept in a damp place for 3 weeks to decay.

Plate 1: Fungi infected alligator pepper pods

Isolation of Fungi from Aframomum melegueta Pods

Fungal organisms were isolated from *Aframomum melegueta pods* using a modified Streaking Plating Method of Sanders (2012). The *Aframomum melegueta* pods were surface sterilized in 70% ethanol for 3 minutes and rinsed with sterile distilled water for three consecutive times. The already prepared SDA medium was dispensed into Petri dishes and allowed to solidify at room temperature. Then a loop was flamed until it became red hot, allowed to cool, after which, it was used to pick the colony of *A. melegueta pods* aseptically near the flame and placed on the Petri dishes. Then the loop was flamed again to sterilize. The petri dishes were sealed using a cling film, labelled correctly, placed upside down to prevent contamination and incubated at 25$\pm$2^{o}C for 3-7 days. At the end of the incubation period, the fungal organisms observed growing on the SDA medium were sub cultured and incubated at 25$\pm$2^{o}C for 7 days to obtain pure cultures.

Fungal DNA Extraction and Gel Electrophoresis

Fungal DNA was extracted using Fungal/Bacterial MiniPrepKit (Zymo Research Group, California, and USA). The kit protocol was used with modifications. Fungal DNA was extracted from the mycelium of the pure culture. fungal DNA quantity and purity was determined using NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific Inc. Wilmington, Delaware, USA).

Molecular characterization using the Internal Transcribed Spacer (ITS) marker and identification

The Genomic DNA of the isolate coded RCBBR- P12 was extracted following the protocol of Quick-DNA^{TM}Fungal/Bacterial MiniPrepKit (Zymo Research Group, California, USA) as described by the manufacturer, with modifications at the Regional Center for Biotechnology and Bioresources (RCBB), University of Port Harcourt, Rivers State, Nigeria. The RCBBR- P12 isolate DNA quantity and concentration were measured using the Nanodrop 2000c spectrophotometer (Thermo fisher Scientific Inc. Wilmington, Delaware, USA). The DNA purity was measured as a ratio of absorbance at 280 nanometer (nm) to that of 260 nanometer. The quality of the DNA of the isolate RCBBR- P12 was further quantified using the Agarose gel

electrophoresis performed according to the modified method of Saghai-Maroof *et al.* (1984). The DNA sample of the RCBBR- P12 isolate shipped to the International Institute of Tropical Agriculture (IITA) Bioscience Centre, Ibadan, Nigeria for amplification and sequencing. The primers used to amplify fragments of the nuclear ribosomal DNA (rDNA) of the RCBBR- P12 isolate were the Internal Transcribed Spacer 1 (ITS1) with the sequence ITS-1 TCCGTAGGTGAACCTGCGG and ITS4 with the sequence TCCTCCGCTTATTGATATGC. The amplicons were sequenced using the ABI 3500 capillary electrophoresis sequencer. The DNA sequence file was saved in the Bioedit file with extension.ab1. The sequence was analysed using the Molecular Evolutionary Genetics Analysis (MEGA) version 7.0.26 software, and aligned using the Basic Local Alignment Search Tool for nucleotide (BLASTN) 2.8.0 version of the National Centre for Biotechnology Information (NCBI) database.

Preparation of Biofertilizer

Preparation Of Broth

Biofertilizer was prepared according to the modified method of Anubrata and Rajendra (2014). To prepare broth, A sterilized 1000ml conical flask was filled with 500 ml of distilled water and 5g of peptone and 20g of dextrose were measured and dissolved in the conical flask containing 500 ml of water. The flask was labelled accordingly and the flask containing the broth was autoclaved and allowed to cool (plate 2). The 7 days old *Penicillium* gotten from *Aframomum melegueta* (alligator pepper) was then inoculated into the broth and shook continuously from time to time. The broth was left to ferment for 18 days. After 18 days the *P. citrinum* in the broth had multiplied. A biomass count was done afterwards to determine the quantity of *P. citrinum* present in the broth.

Biomass Count

An empty mortar was measured and filled with 25ml of *Penicillium* broth. The mortar was then placed in an oven to dry off the liquid content and the solid content left was measured. To get the biomass, the measurement of the empty mortar was minus from the measurement of the mortar filled with dried mass of *Penicillium*. The biomass count showed that in every 25ml of broth, we have 2g of *penicillium citrinum* i.e in every 1ml of broth, we have 0.08g of *P. citrinum.*

Planting of Vigna unguiculata (L.) Walp.) (TVX- 3236 variety)

This experiment was carried out at the Centre for Ecological Studies University of Port Harcourt (Greenhouse).

Cowpea seeds (Plate 2) with variety number TVX-3236 were gotten from IITA Ibadan, and the loamy soil used was gotten from Faculty of Agriculture Farm University of Port Harcourt. The variety of cowpea was planted in a loamy soil in 6 replicates and labelled accordingly:

One (1) replicate for control.
One (1) replicate for 20ml of *P. citrinum* broth
One (1) replicate for 35ml of *P. citrinum* broth
One (1) replicate for 50ml of *P. citrinum* broth
One (1) replicate for 65ml of *P. citrinum* broth
One (1) replicate for 80ml of *P. citrinum* broth

The seeds germinated 4 days after planting (plate 3). After one week of planting some of the seeds were thinned out, remaining three (3) seedlings in each bucket (replicate). Three (3) seedlings were later left in each bucket to avoid overcrowding and competition. True leaves were seen 5 days later.

Plate 2: Cowpea seeds

Plate 3: *Vigna unguiculata* plant before the application of *P. citrinum*

Application of *P. citrinum* for Plant Growth

A modified method described by Khan *et al.* (2008) was adopted for the application of *P. citrinum*. Eighteen days old broth containing *Penicillium sp.* was applied as shown in plate 4, to the foliage *Vigna unguiculata* at week 2 at a concentration of 0.08 spores per milliliter (mL)spores/ml at a rate of 20 ml, 35 ml, 50 ml, 65 ml, 80ml/bucket. Planting medium without the addition of *P. citrinum* was used as control.

Plate 4: Application of *Penicillium citrinum* broth

Agronomic Assessment

After emergence of true leaves at week 2, measurement of plant height, leaf number and leaf area were taken every week for 5 weeks, starting from week 2 after application of *P. citrinum* on true leaves. Root length was taken after harvesting. The plants were harvested and the roots were carefully washed to remove soil particles before taking measurement using a 30cm meter rule. The plant height and leaf length and breadth was measured using a 30cm meter rule. The number of leaves were counted manually while the leaf discoloration was determined with visual observation of the leaves. In other to get the average of each parameter, the different parameters of each plant were added together and was then divided by the total number of plants in the bucket. All data were obtained statistically and are presented in bar charts.

RESULTS AND DISCUSSION

Isolation, morphological and microscopic identification of fungi associated with *Aframomum melegueta*

The result of the fungal isolation is presented in Plate 5. The isolated fungus RCBBR- P12 was found to be associated with *Aframomum melegueta*. Colony with white diffusing pigment, showed presence of white mycelium that later becomes blue-green as shown in Plate 5. It has a threadlike, short, tiny, branched hyphae. When examined under a microscope, it resembles a paint brush. The macro- and microscopic features of the isolates were examined and compared with those of Samson *et al.,* (2010).

Plate 5: A pure culture of fungal isolate

Molecular characterization using the Internal Transcribed Spacer (ITS) marker and identification

The genomic DNA of the isolates RCBBR- P12 was successfully extracted. The NanoDrop result showed that the concentration of the DNA of the isolates was 148.1ng/µl. While the absorption peak of the 260nm/280nm readings was 1.562 and the 260nm/ 230nm readings was 2.963. The result of the Amplified PCR product generated from RCBBR- P12 isolate is shown in Plate 2 below. The amplified DNA showed a band on gel when observed under UV light. From the result, the ladder used indicated that the RCBBR- P12 isolate sequence had 518 base pairs.

The result of the RCBBR- P12 isolate sequence alignment is presented in figure 1. The result indicated that RCBBR- P12 isolate sequence aligned with 100 sequences deposited in the composite biological database of National Center Biotechnology Information (NCBI). The RCBBR- P12 isolate sequence was 99.80 % identical to *Penicillium citrinum.* These findings showed that isolate is a *Penicillium citrinum*. From the results, the micrographs are shown in plate 2 below and microscopic description, macroscopic description and inference of the pure culture of the fungi isolate used in biofertilizer production observed is shown in table 1

Table 1: Macroscopic Description and Microscopic Description

Specimen Identity	Cultural Characteristics	Microscopic Characteristics	Inference
RCBBR_P12	A white mycelium that later becomes Blue green.	Threadlike, short, tiny, branched hyphae. The Conidiophores are clustered while some are single.	*Penicillium sp.*

Micrograph of Test Organism

The micrograph of the pure culture isolated from alligator pepper is shown in Plate 2a and b:

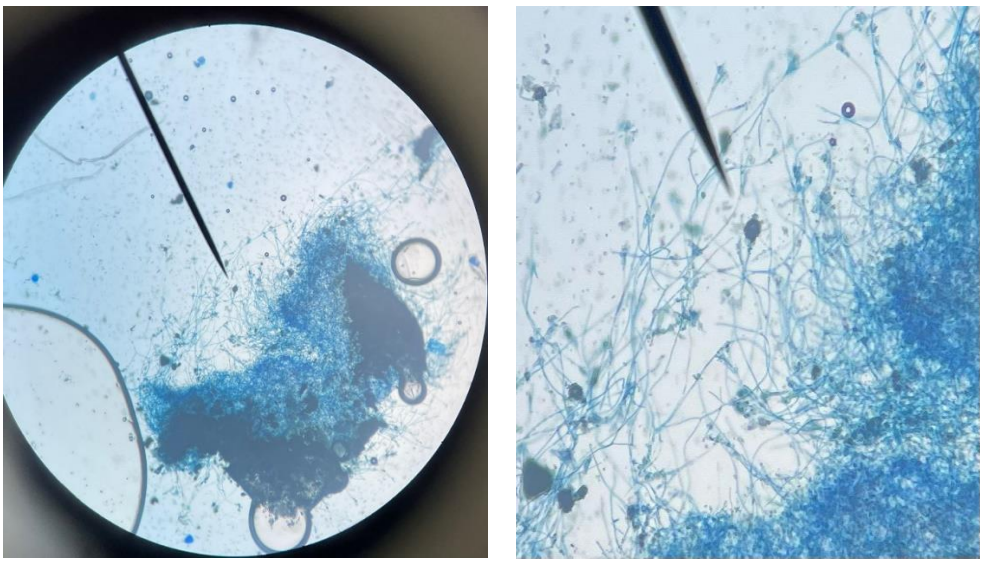

Plate 6: Microscopic Appearance of RCBBR_P12 using a light microscope

DNA Extraction and Concentration Determination

The genomic DNA of the isolate RCBBR_P12 was successfully extracted and showed good quality. The Nanodrop result, is shown in Table 2 and Figure 1.

Table 2: NanoDrop spectrometry characteristics of the DNA from the isolates

S/N	Isolate code	A260	A280	Purity ($\frac{A260}{A280}$)	DNA Concentration (ng/µl)
1	RCBBR_P12	2.963	1.562	1.9	148.1

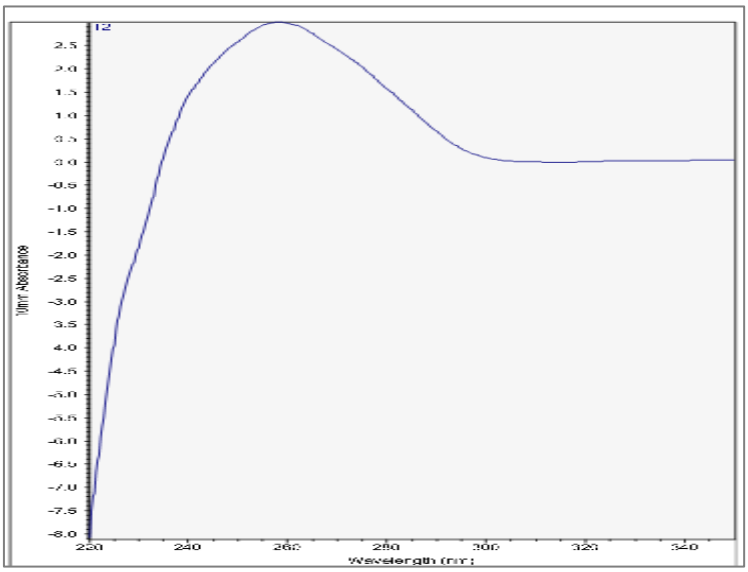

Figure 1: Absorption spectrum of gDNA from the fungal isolate

Polymerase Chain Reaction (PCR) and Gel Electrophoresis

The result of the amplified DNA or PCR band of the isolate RCBBR_P12 is presented in figure 2a and 2b. The amplified DNA showed bands on gel when observed under UV light. From the result, the ladder used indicated that the RCBBR_P12 isolate sequence had over 600 base pairs.

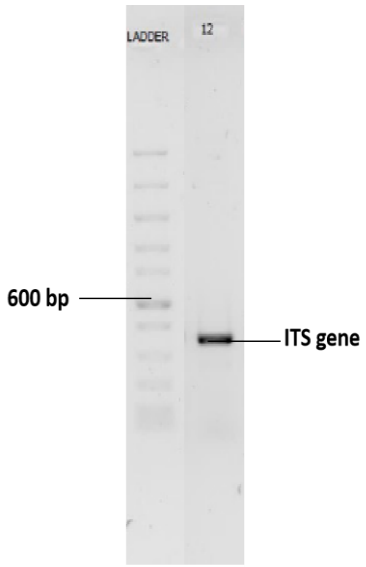

Figure 2a: Agarose gel electrophoresis of the gDNA from the fungal isolate

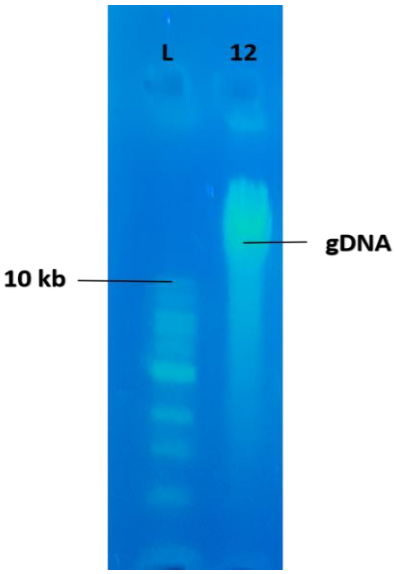

Figure 2b: Agarose gel electrophoresis of the ITS gene amplicons from the fungal isolate

DNA Sequencing

The sequencing result after alignment of the DNA sequence of the isolate RCBBR_P12 are shown in Figure 3 and it was specified that the sequence length was 518 base pairs. This result authenticated the DNA amplification result as shown in figure 2 above. Also, from the results, it was noticed that the colours of the bases of the nucleotides were existing in four colours [green: adenine (A), red: thymine (T), blue: cytosine (C), black: guanine (G). These diverse colours allow for easy interpretation of the sequence.

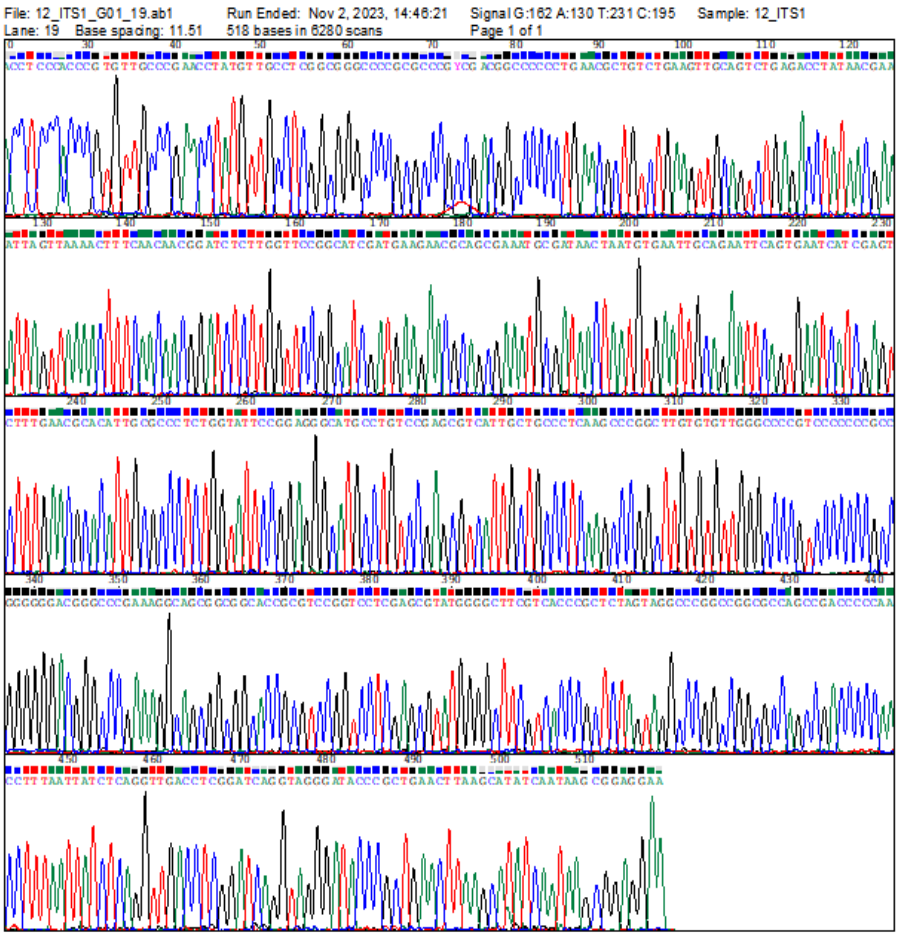

Figure 3: Sequence alignment of the DNA of isolate RCBBR_P12 after alignment

Sequence Alignment

The alignment results are presented in figure 4, displayed the alignment scores presented as red lines. The scores of the alignments of all aligned sequences were greater than 200.

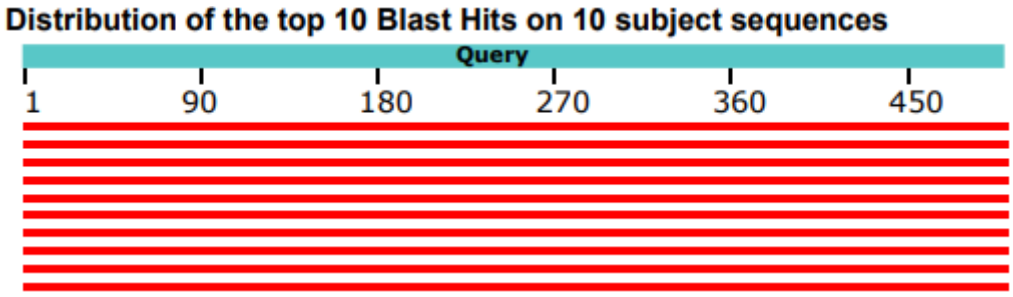

Figure 4: Alignment scores of all aligned sequences

Figure 5 indicated that the RCBBR_P12 isolate sequence shows that the sequence was 99.80% identical to the partial sequence of the small subunit of ribosomal RNA gene of *Penicillium citrinum* isolate ET34. These findings showed that isolate RCBBR_P12 is a *Penicillium sp.*

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident
subunit ribosomal RNA gene, partial sequence						
Penicillium citrinum strain VT65 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	Penicillium citrinum	898	898	100%	0.0	99.80%
Penicillium citrinum isolate ET34 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	Penicillium citrinum	898	898	100%	0.0	99.80%
Penicillium citrinum strain VT76 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	Penicillium citrinum	898	898	100%	0.0	99.80%
Penicillium citrinum strain VT54 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	Penicillium citrinum	898	898	100%	0.0	99.80%
Penicillium sp. isolate M1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	Penicillium sp.	898	898	100%	0.0	99.80%
Aspergillus sp. isolate M24 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	Aspergillus sp.	898	898	100%	0.0	99.80%
Penicillium sp. strain Xia1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	Penicillium sp.	898	898	100%	0.0	99.80%
Penicillium citrinum isolate ICL-PG-FUN-SG-2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	Penicillium citrinum	898	898	100%	0.0	99.80%

Figure 5: Graphical summary of the RCBBR_P12 isolate sequence

The GenBank closest matches and percentage similarity of the fungal isolate RCBBR_P12 is shown in table 3 and it shows that the sequence was 99.80% identical to the partial sequence of the small subunit of ribosomal RNA gene of *Penicillium citrinum* isolate ET34.

Table 3: GenBank closest matches and percentage similarity of the fungal isolate

S/N	Strain	Organism	Closest GenBank Match	Similarity (%)	Accession No
1	RCBBR_P12	*Penicillium citrinum*	*Penicillium citrinum* isolate ET34	99.80	

Phylogenetic Analysis of Isolate RCBBR_P12

Phylogenetic trees constructed showed the relationship between the isolate from this study and other fungal isolates on GenBank. The phylogenetic analyses showed that *Penicillium citrinum* are closely related to the fungal isolate obtained from alligator pepper as presented in Figure 6

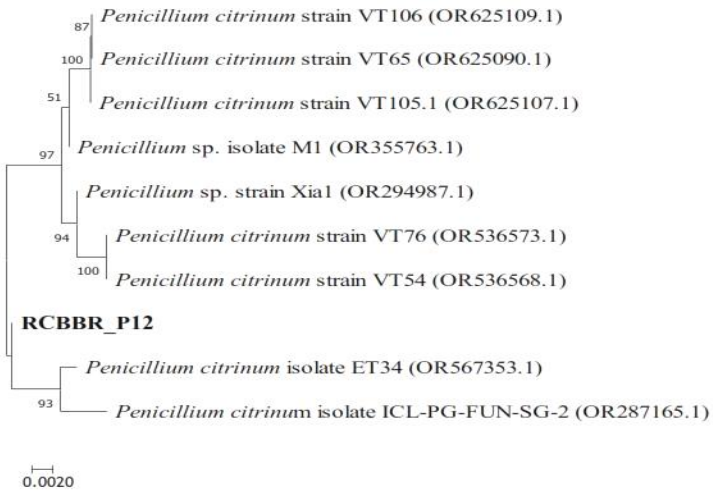

Figure 6: Neighbor-joining evolutionary relationship of the fungal isolate from table 5

Discolorations on Foliage

Pale green discoloration was observed in control.

No discoloration was seen in 20ml replicate.

Pale green with white spot discoloration was observed in 35ml replicate.

Pale green discoloration with yellow spots were seen on the leaves of 50ml, 65ml, and 80ml replicates.

Growth Observation

The improvement in the growth rate of cowpea plants after application of *P. citrinum* is shown in plate 3.

Plate 7: Cowpea plant at week 3 after application of *P. citrinum*

Agronomic Characteristics After Application of Biofertilizer from Week 2 to Week 6

Plant Height (cm)

The result of the plant height of cowpea plants from week 2 to week 6 after application of *P. citrinum* on true leaves are shown in Figure 7. From the result it was observed that the plant height increased over time and with increasing biofertilizer concentration. The maximum plant height was observed in the 80 ml treatment, suggesting that this treatment may have a positive effect on the cowpea plant.

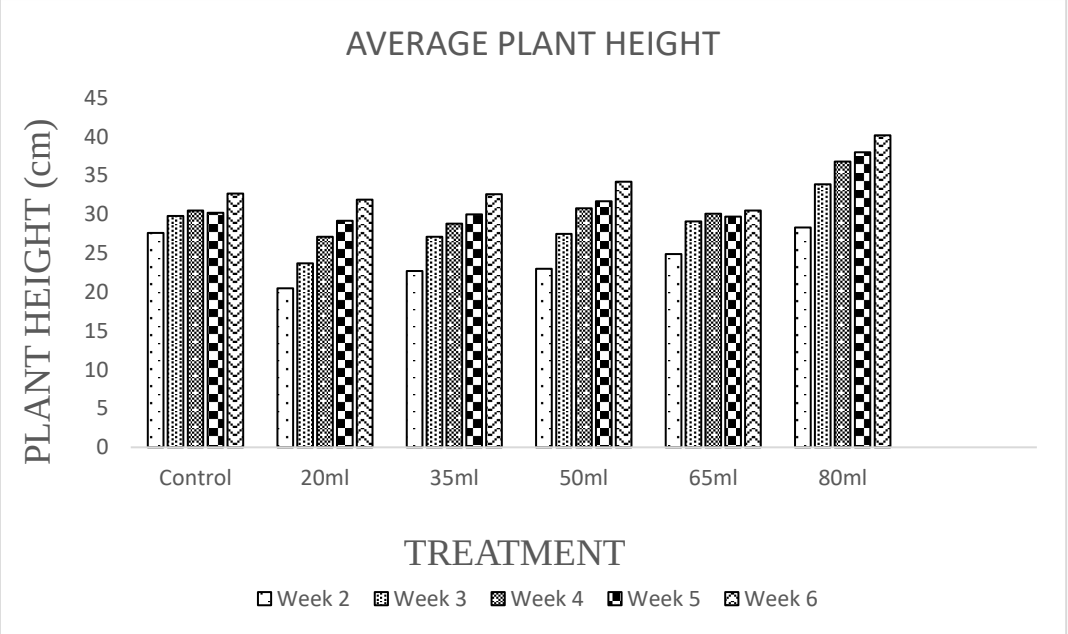

Figure 7: Average plant height

Number of Leaves

The result of the number of leaves of cowpea plants from week 2 to week 6 after application of biofertilizer on true leaves are shown in Figure 8. From the result it was observed that the leaf number increased over time and with increasing biofertilizer concentration. The maximum leaf number was observed in the 80 ml treatment, suggesting that this treatment may have a positive effect on the cowpea plant.

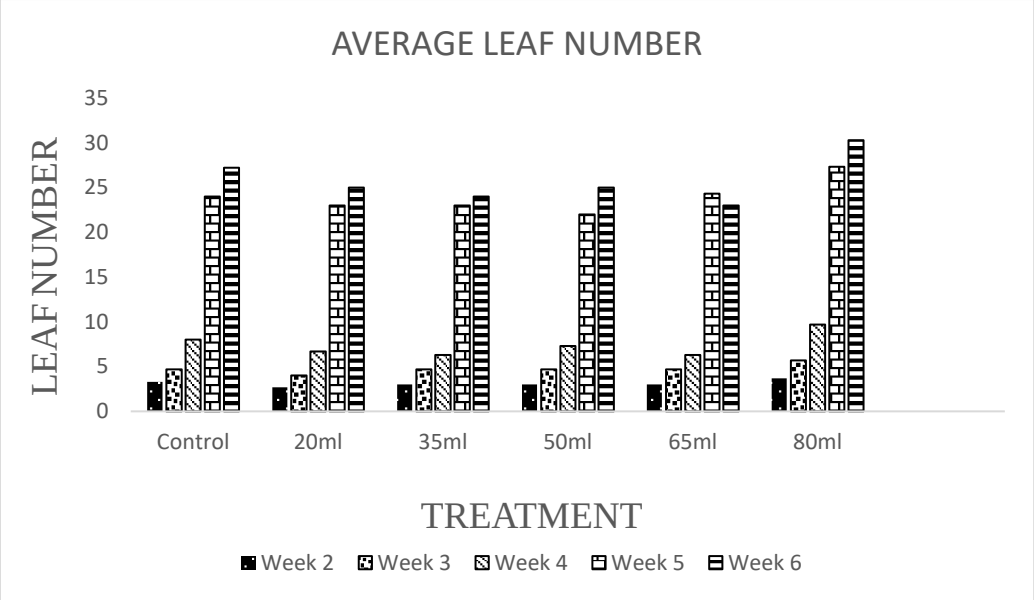

Figure 8: Average leaf number

Leaf Area (cm^2)

The result of the leaf area of cowpea plants from week 2 to week 6 after application of biofertilizer on true leaves are shown in Figure 9. From the result it was observed that the leaf area increased over time and with increasing biofertilizer concentration. The maximum leaf area was observed in the 80 ml treatment, suggesting that this treatment may have a positive effect on the cowpea plant.

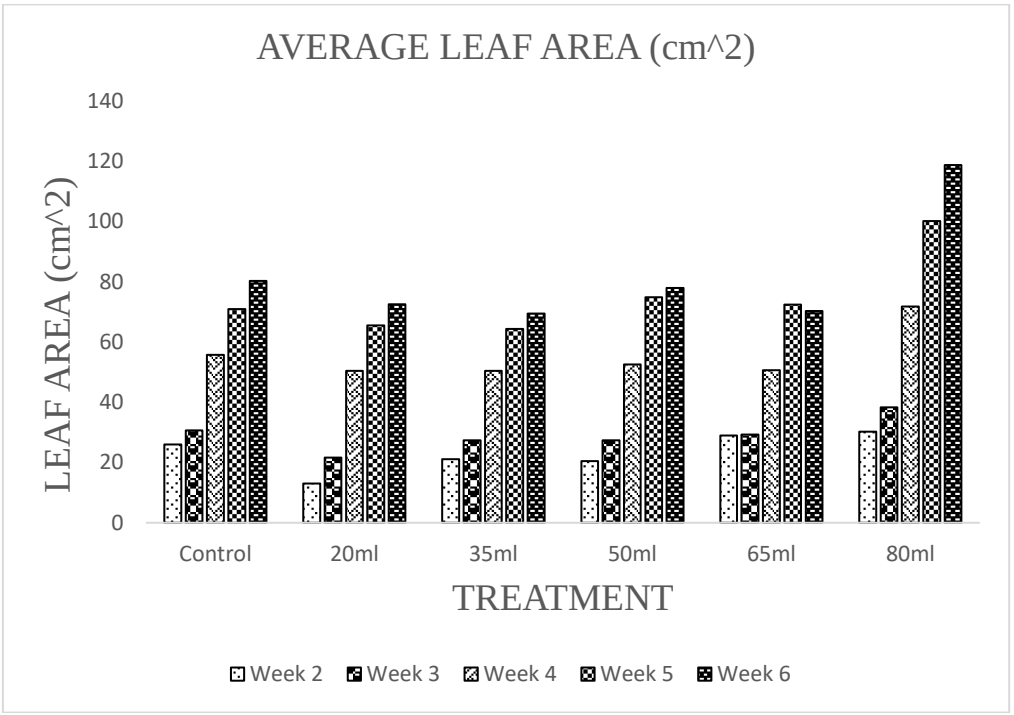

Figure 9: Average leaf area

Root Length (cm)

The result of the root length of cowpea plants at the end of the experiment in week 6 are shown in Figure 10. From the result it was observed that the root length increased over time and with increasing biofertilizer concentration. The highest root length was observed in the 80 ml treatment, suggesting that this treatment may have a positive effect on the cowpea plant root.

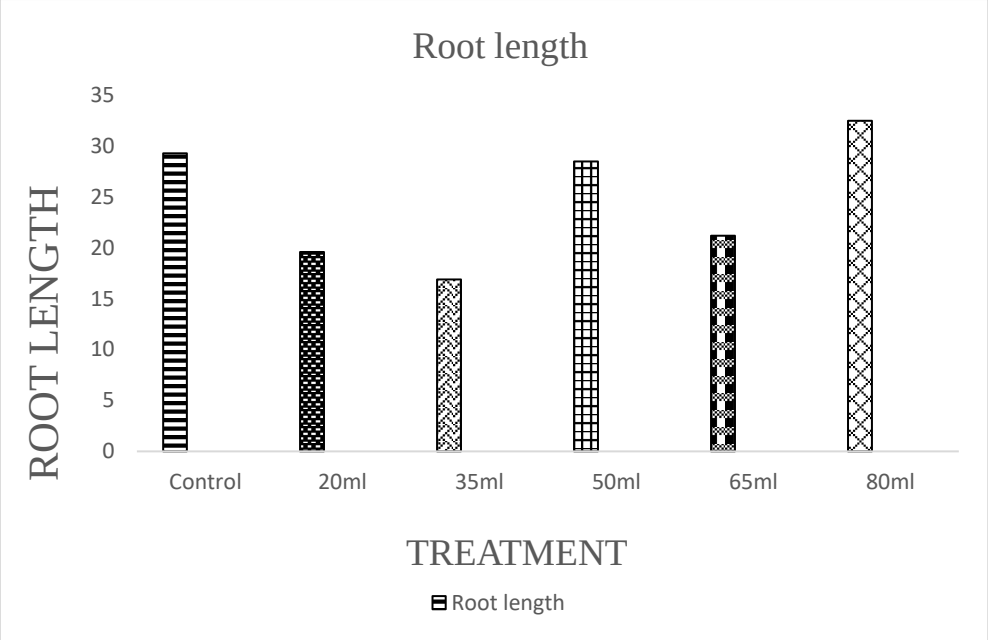

Figure 10: Average root length at week 6

Discussion

An investigation into the fungi linked to the post-harvest spoiling of *A. melegueta* pods revealed the existence of a fungus. The isolates' identification as *Penicillium citrinum* was verified by molecular analysis. This finding is consistent with that of Nmom *et al.* (2007), who isolated *Aspergillus* sp. and *penicillium* sp. from *A. melegueta* pods and found that these pollutants seriously harmed the pods of the plant. According to Keller *et al.* (2005), fungi release a variety of secondary metabolites known as mycotoxins into their surroundings. It is thought that the generation of these potent metabolites increases the relevance of fungal pathogenicity in animals (Seyedmojtaba *et al.,* 2015). According to Bennett and Klich (2013), mycotoxins are regarded as one of the putative virulence factors of fungi since they have the ability to reduce human immunity and hence increase the fungus's infectivity. Despite *P. citrinum*'s ubiquitous occurrence in the environment, infections with the fungus are incredibly rare, despite the potential harm that their mycotoxins can do to human health (Hesse *et al*., 2017).

Fungal biofertilizers, either used alone or in combination, have been shown to have a significant positive influence on plant development in addition to influencing growth and yield in natural field systems (Gentili and Jumpponen, 2006). Numerous research has looked into and recommended using these fungal species to promote plant growth. A portion of the isolated fungi that promote plant development are from the *Penicillium* genus.

In this work, we assessed the growth-promoting characteristics and plant growth of *Penicillium citrinum* that was isolated from alligator pepper. Babu *et al.* (2015) corroborated this conclusion with their research, which showed that *Penicillium citrinum* and other *Penicillium* species effectively stimulate plant development. This is because of their capacity to generate gibberellins (GAs) and indole-3-acetic acid (IAA), claim Khan *et al.* (2018). These phytohormones are essential to the growth and development of plants, and the *Penicillium* species that produce them have made them a viable candidate for use as biofertilizers.

Numerous fungi, including *Penicillium citrinum,* have been identified as endophytic fungi. Additionally, it has been demonstrated that the endophytic fungus *Penicillium* sp. enhances nutrient absorption, which in turn promotes plant development (Gasoni and Gurfinkel, 1997), particularly in legumes.

The research of Hakim *et al.* (2015), is in agreement with the current study as it also show the ability of *P. citrinum* to improve the growth of the plant. The results of our findings, are reconfirmed with the findings of Mittal *et al.* (2008), who investigated the impact of six phosphate-solubilizing fungi on the growth and seed production of chickpea (*Cicer arietinum* L. Cv. GP f2) plants in pot experiments. These fungi included two strains of *A. awamori* and four strains of *P. citrinum*.

Similar findings have been reported by Waqas *et al.* (2015) who indicate that when compared to plants in the control group, one of the *Penicillium* endophyte strains, *Penicillium citrinum* LWL4, was able to increase plant growth regardless of the presence or absence of the *Sclerotium rolfsii*-caused root rot disease in sunflowers.

The result the application of *P. citrinum* as a biofertilizer yielded noteworthy improvements in the growth parameters of cowpea, as evidenced by significant increases in plant height, leaf area, and the number of leaves. These collective results highlight the multifaceted benefits of using *P. citrinum* as a biofertilizer for cowpea cultivation. The positive influence on plant height, leaf area, and the number of leaves underscores the potential of *P. citrinum* to enhance crop productivity and contribute to sustainable agricultural practices.

CONCLUSION

In summary, our study of the state of knowledge regarding endophytic *Penicillium* species with numerous applications revealed that this genus has been heavily used to advance agriculture. The study's experiments demonstrated that the fungal isolates employed in the research may benefit plants. They might enhance plant development and productivity in addition to enhancing soil fertility and health. This study provided more evidence that fungal candidates could be used as biofertilizers. A promising tactic to increase crop yields and decrease the usage of artificial fertilisers and establish environmentally sustainable agriculture is biofertilizer inoculation.

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