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EFFECT OF FERMENTATION AGENTS ON THE pH, TTA AND MICROBIAL COMPOSITION OF *FUFU* DOUGH

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ABSTRACT

Fufu, a product of cassava has been adulterated by processors. They add toxic substances to the soaked roots to fasten the fermentation days and make quick money. There is need to evaluate the effect of these fermentation agents on the microbial, pH and total titratable acidity (TTA) of the fufu dough. Fufu dough was produced with fermentation agents; kerosene, detergent and palm ash and also with a control without agent. They were wrapped in polyethylene bags, stored at ambient temperature and evaluated for storage and microbial quality in the Biochemistry Laboratory of National Root Crops Research Institute, Umudike, Nigeria. These samples were assayed for chemical and microbial qualities which include pH and TTA. The results showed that the pH values ranged from 3.70 - 6.80, and Total Titratable Acidity (TTA) values ranged from 0.004 - 0.048 %. The microbial analysis showed increase in fungal (2.1×10^8(cfu/g)) and bacterial (1.0×10^6(cfu/g)) counts as the storage time increased with the control having the least microbial load. The fungal isolates from the samples are Aspergillus niger, Aspergillus flavus and Penicillium spp, while the bacteria isolates from the samples include Bacillus spp and Staphylococcus aureus. Statistically, there were significant differences ($p<0.05$) in appearance of the fufu as storage time increased. The results from this study showed that the fufu with fermenting agents had higher microbial load than the control. It therefore encourages healthy practices among the fufu producers by stopping the use of fermenting agents to reduce the proliferation of pathogenic microorganisms in processed fufu.

Keywords: *Fufu*, microorganisms, pH, fermentation agents, total titratable acidity

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INTRODUCTION

About 800 million people in Africa, Asia as well as Latin America have been using cassava (*Manihot esculenta Crantz*) as a cheap source of carbohydrate (Montagnac *et al*, 2009). Because cassava is found all year round, and also have the capacity to survive in a dry and low moisture soils, it has been regarded as a crop that can be relied on to feed low income farmers and their household. Also, because the eatable roots can be left in the soil for about 3 years, it is usually used as a fallback crop for survival by many household. Therefore, it serves as a food reserve that may be used when unfavorable weather conditions restrict the production of other foods.

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Cassava helps African nations' food security challenges due to it's capacity to provide energy efficiently throughout the year, withstand harsh conditions by adapting the continent's current farming and food systems (Martin and Ejike, 2018).

Cassava roots may be eaten without being cooked or processed into different finished goods. Depending on the preferred mode of consumption and processing, different products are produced. There are different varieties which are bitter in taste, and these undergoes intense grinding and fermentation before they can be consumed while the sweet varieties which contains low cyanide requires little process before they can be eaten such as boiling, roasting or frying(Nweke, 1994). Different processing methods are employed to enhance the products' value, increase their shelf life, and remove the toxin contained in the roots, and this is achieved by eliminating thecyanogenic glucosides inherent in the crop (Westby, 2002, Cardoso *et al*, 2005). Flour, starch, and various fermented items are among the processed goods made from cassava roots. In Africa, the prominent and commonest products from cassava roots are *gari*, *fufu*, *tapioca*, *lafun* as well as *attieke* according toEzemenari *et al*, (1998).Awoyale *et al*, (2021), also submitted that thefinal products of cassava can as well be prepared in form of thick paste, semolina – like particles and in form of flour.

Fufu is a local fermented food that is being consumed in Nigeria, especially in the Southern, Eastern and Western regions even in other West African nations (Rosales-soto *et al*, 2016). In the southern region of Nigeria, it is listed right behind *gari* as a typical native fermented food for many families (Egwim *et al*, 2013). Due to different cultures obtainable in different regions, many families and processors produce *fufu* using diverse techniques (chijioke *et al*, 2020). In recent times, *fufu* has been faced with safety challenges as the processors who engage in it's production now adulterate the food product so as to get enough money.They accomplish this by reducing the number of allowable fermentation days required for the correct retting of the soaked roots.To speed up the fermentation process and ret faster than it should be, they add toxic substances like kerosene, detergent, and palm ash to the already fermenting, soaked roots (Ogbete *et al*, 2022).The idea that these fermentation agents serve to boost the fufu yield is another justification for their use.According to them, the cassava would not produce high *fufu* mash yield if the fermentation isn't done properly (Ogbete *et al*, 2022).Therefore, the aim of this research work is to analyse and evaluate the effect of the substances they add to the fermenting or soaked cassava roots on it's microbial load, pH as well as the total titratable acidity (TTA) of the *fufu* dough produced. The knowledge of microbiological quality of these *fufu* will help to ascertain the level of safety involved with the use of the fermentation agents in *fufu* production thereby make better recommendation to the processors.The aim of this research work was to analyse and evaluate the effect of fermenting substances (powdered detergent, kerosene and ash) on the microbial load, pH and total titratable acidity (TTA) of*fufu* dough**.**

The specific objectives of this research work were to:

i. produce *fufu* using different fermentation agents (detergent, kerosene and palm ash).
ii. determine the microbial load of the *fufu* dough.

iii. determine the pH and TTA of the *fufu* dough.

MATERIALS AND METHODS

Source of Experimental Materials

Fresh roots from cassava (TME 419) were sourced from Cassava Programme in National Root Crops Research Institute (NRCI), Umudike Nigeria. The cassava roots were of 12 months of age when they were harvested.

Preparation of the *fufu*materials

Sixty kilogramme (60 kg) of TME 419 cassava variety was used to produce the *fufu* samples. The fresh roots of the cassavawere peeled, washed, and divided to four equal portions of fifteen kilograms each. The first portion was added 5 ml of kerosene, the second portion was added 5 g. of powdered detergent (Omo) which contains sodium silicate, sodium tripolyphosphate, sodium hydroxide, sulphonic benzene acid of the linear alkyl group as well as lauryl ether sulfate of sodium; the third portion was added 4 g. of palm ash while the fourth portion had nothing added to it which thereby served as the control. They were sliced to small chunk sizes of 7 cm, washed and soaked in a two different bowls which were poured 30 litres of clean water and had equal volume and diameter of 30 litre and 64cm respectively. They were properly labeled as K (*fufu*with kerosene), D (*fufu* with detergent), P (*fufu* with palm ash) and C (control), left to ferment and ret for 72 hrs (3 days) at ambient temperature. After the 72 hr retting, the soft root pulp was sieved to give resulting sediment; the wet*fufu*mash (Ogbete *et al*, 2022). To make *fufu* dough, the procedure outlined by Ogbete*et al*, (2022) was adopted. 100g of the dewatered, sieved mash was formed into balls, cooked in a 700 ml of water using a modern gas cooker for 15 minutes at 100 ^{o}C, then taken out and pounded to make the *fufu* dough. To finish the final*fufu* dough making, the previously pounded dough was re-molded, cooked for another 10 minutes at 180 ^{o}C, and then pounded again. It was pounded finally and then given 45 minutes to cool. The *fufu* dough samples were collected using containers that have been previously sterilized, promptly covered, and brought in the lab to be conducted analysis on. 50 g of each of the cooked *fufu* was packaged in clear polyethylene material typically involved in the packaging and selling ofcooked *fufu*. The fufu samples were properly wrapped, put in a dried clean trays, allowed for an 8-day storage while maintaining an ambient temperature of 28±2^{o}C.After the 8-day storage, analysis of pH, total titratable acidity (TTA) and also the microbial count were carried out on the *fufu* samples daily throughout the period of their storage. Duplicates of the sampleswere made while they were being collected for the analysis.

Microbiological Analysis

One gram (1 g) of the *fufu*samples each were individually homogenized in nine mlof already distilled water. Each sample was diluted serially ten times until a dilution level of 10-4 was reached. For the purpose of determining the load of microbes present in each of the agar media,

the lowest dilution of 1ml as well as other dilutions were poured on the plates of nutrient agar withSabouraud dextrose agar inclusive. Thereafter, at ambient temperatures for 48 and 72 hrs for the proper growth of bacteria and fungi, laboratory incubation were done followed by the determination of the total counts of bacteria and fungi which are viable. This was done by counting the units that formsthe colony (cfu/g) by the end of the time of incubation with this formular by Jideani and Jideani, (2006):

$c = n/vd$

Where,

c: colony forming unit per gram

n: number of the colonies formed

d: dilution blank

v: volume of colonies transferred to the plate

Until pure cultures were obtained, the isolated microorganisms were sub-cultured by streaking them onto sterile nutritional agar in a repeated manner, MacConkey agar slants were for bacteria, while Sabouraud dextrose agar slants were for fungi.

Characterizing and identifying the microbial isolates.

To characterize and identify the isolated microorganisms, the procedure as described by Ogbulie *et al*, (2005) was used. The morphology of colonies based on their characteristics as well as theirchemical tests (such as gram stain, oxidase etc.)were used to define and identify bacterial isolates. On the other hand, the morphological observations made with a low-power objective lens and cultural characteristics stained with cotton-blue lacto phenol solution were used to identify the fungal isolates.

pH of the *fufu* sampes

This was determined according to the method ofOgiehor and Ikenebomeh (2005). 10 geachof the *fufu* dough wasmixed together in distilled water (10 ml) while the pH of the mixture was read with a reference glass electrode; a HANNA pH meter (made by HANNA Instruments, model HI96107, Italy).

Titratable acidity

This was determined according to Obilie *et al* (2004). Exactly 10 grams of sample was mixed in 200 ml of distilled water that was filtered with Whatman filter paper.A 0.1 M NaOH titration of 80 ml of the filtrate was performed with 1% phenolphthalein serving as the indicator. Equation (1) was used to calculate the titratable acidity based on the fact that lactic acid was the predominant fermentation product.

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$$Titratable\ acidity = \frac{\text{Vb x Nb x 0.09}}{\text{Vs}}\ X\ 100\%$$

Where:

Vb = volume of the base;

0.09 = milli-equivalent factor of the lactic acid;

Nb = Normality of the base

Vs = volume of the sample

RESULTS AND DISCUSSION

The pH and Total Titratable Acidity (TTA) of the*fufu* dough samples

The values for the pH of the *fufu*dough samples after an 8 day storage are shown in table 1. The values down of the column ranged from 3.60 (Day 0) of sample K to 6.80 (Day 7) of sample C. No significant ($p>0.05$) difference was observed on pH value of Day 0 (3.60) to Day 7 (4.40) of *fufu* sample K down the column while there was significant ($p<0.05$) difference with the rest of the days of the other samples down the column. The *fufu* samples had pH values that are significantly ($p<0.05$) different from each other across the row for all of the days except for Day 2 where no significant ($p>0.05$) difference was seen among them. This pH ranges agrees with the values from Odo *et al*, (2016), who gotthe pH range of 3.70 - 6.80 for 'Cassava *Fufu* Sold in Abakaliki Metropolis', as well as Omafuvbe *et al*, (2007) who got the pH range of 3.65 – 5.12 for 'Ready-to-eat *Fufu* and *Lafun* sold in Ile-Ife, Nigeria'. The raising pH of food during storage has been associated with the removal of ammonia by spoiling bacteria as reported by Olawepo and Akoma, (2001). The results indicates that the added fermentation agents had no effect on thepH of the *fufu* samples.

The total titratable acidity (TTA) shown in Table 2 ranged from 0.008 (Day 7) in samples K, P and C respectively to 0.058 (Day 0) in sample D. Down the column, a significant ($p<0.05$) difference was observed in the *fufu* dough samples with the exception of sample C which was not seen any significant ($p>0.05$) difference among the days. Across the row, there was no significant ($p>0.05$) difference observed in days 4 and 5 as well as in day 6 of all the samples while significantdifference ($p<0.05$) was observed in days 0, 1, 2, 3 and 7 of all the samples. The TTA obtained were within the range 0.004 to 0.063 reported by Odo *et al*, (2016) for Cassava *Fufu* Sold in Abakaliki Metropolis. From the results, it could be seen that the TTA values of all the *fufu* dough varies across the days. This may be caused by microbial activity, metabolic processes, and environmental factors such as temperature variation, the effect exerted by of carbon (iv) oxide, the absence or effect of oxygen, and humidity under which the samples were stored (Odo *et al*, 2016).

Total Fungal and bacterial Count of the *fufu* dough samples

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Table 3 shows the fungal count (cfu/g) of the *fufu*dough samples. The valuesof thesamples down the column ranged from2.0 x 10^8 cfu/g as seen in Day 0 of sample P to 4.9x10^8cfu/gin Day 6 of sample D. No significant ($p>0.05$) difference was seen on the total fungal count value of Day 0 (4.0x10^4 cfu/g) to Day 6 (4.7x10^6cfu/g) of *fufu* sample K down the column with corresponding significant ($p<0.05$) difference seen with the first three days of the other samples down the column. Across the row was seen no significant ($p>0.05$) difference in the total fungal count at days 0 and 9, while there was seen a significant difference ($p<0.05$) in the remaining days for all of the samples. As the number of days increased, the results showed a rise in microbial count among the samples especially with the *fufu* samples with fermentation agents. The overall fungal count increased from the third day until the ninth day, when there were too many counts to tally.

Table 4 shows the total count for the bacteria. The values for the total bacterial count ofthe*fufu*dough down the column ranged from1.0 x 10^6 cfu/g (Day 0) of sample C to 8.6 x 10^6cfu/g (Day 9) of sample D. Significant ($p>0.05$) difference was observed on the total bacterial countof the*fufu* dough for all the days down the column. No significant ($p>0.05$) difference was observed in the total bacterial count of the*fufu* dough at day 0 across all the samples, while a significant difference ($p<0.05$) was seen for the remaining days for all the samples. From the result, it showed that as storage duration increased, the number of bacteria increased. The high bacterial count found in the *fufu* samples especially with those that contains fermentation agents indicates that the fermentation agents could have aided the proliferation of the bacteria which helped create a conducive environment for the microorganisms.

CONCLUSION

Fufu that has been kept at room temperature for longer than two days is more likely to develop microorganisms and lose its acceptability. From this study, it was found that after the third day of storage, the microorganisms changed the *fufu*sample's pH and titratable acidity (TTA), thereby rendering it unfit for ingestion. Prominently observed, the *fufu* samples with fermenting agents contained larger loads of moulds and bacteria, as was evident from the result. Due to this, using these agents of fermentation should be discouraged totally. Additionally, the sort of microorganisms on the preserved *fufu* may pose some health and food safety risks due to the production of mycotoxins and bacterial toxins. As a result, *fufu* meant for consumption should be adequately prepared without the introduction of fermentation agents and stored for no longer than two days. To reduce the possibility of infection, the *fufu* must be processed in hygienic circumstances to increase its quality.

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APPENDICES

Table 1: pH of the *fufu*dough under8 daysstorage

	SAMPLES	pH		
Days	**K**	**D**	**P**	**C**
0	3.70^{b}±0.01	3.80^{a}±0.01	3.90^{a}±0.01	3.80^{a}±0.01
1	3.80^{b}±0.01	3.80^{a}±0.01	3.80^{b}±0.01	3.90^{a}±0.01
2	3.90^{b}±0.01	3.90^{b}±0.01	3.70^{b}±0.01	4.80^{b}±0.01
3	4.00^{b}±0.1	3.90^{b}±0.1	3.60^{a}±0.1	4.60^{b}±0.01
4	4.10^{b}±0.1	3.95^{c}±0.1	4.80^{c}±0.1	5.90^{c}±0.1
5	4.20^{b}±0.1	4.10^{b}±0.1	4.90^{d}±0.1	5.70^{d}±0.1
6	4.20^{b}±0.1	4.15^{d}±0.1	5.50^{e}±0.1	6.70^{e}±0.1
7	4.40^{b}±0.1	4.20^{d}±0.1	5.80^{e}±0.1	6.40^{f}±0.1

Note: values are means of duplicate determinations. Means with different superscripts along the column are significantly different at ($p < 0.05$).

Key: K = *Fufu* with kerosene, D = *Fufu* with detergent, P = *Fufu* with palm ash, C = *Fufu* Control

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Table 2:TTA of the *fufu* dough under an 8 days storage

	Samples	TTA		
Days	**K**	**D**	**P**	**C**
0	0.038^{d}±0.00	0.058^{c}±0.00	0.048^{d}±0.00	0.042^{b}±0.16
1	0.028^{c}±0.00	0.046^{c}±0.00	0.040^{c}±0.00	0.052^{b}±0.16
2	0.017^{b}±0.00	0.042^{c}±0.00	0.038^{c}±0.00	0.018^{b}±0.16
3	0.012^{b}±0.00	0.034^{b}±0.00	0.038^{c}±0.00	0.021^{b}±0.16
4	0.010^{b}±0.00	0.030^{b}±0.00	0.026^{b}±0.00	0.028^{b}±0.16
5	0.009^{b}±0.00	0.026^{b}±0.00	0.020^{b}±0.00	0.016^{b}±0.16
6	0.009^{b}±0.00	0.020^{b}±0.00	0.018^{b}±0.00	0.010^{b}±0.16
7	0.008^{a}±0.00	0.010^{a}±0.00	0.008^{a}±0.00	0.008^{b}±0.16

Note: values are means of duplicate determinations. Means with different superscripts along the column are significantly different at ($p < 0.05$).

Key: K = *Fufu* with kerosene, D= *Fufu* with detergent, P = *Fufu* with palm ash, C = *Fufu* Control

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Table 3: Total count of the fungi in the stored*fufu* dough (cfu/g)

	Samples		Total fungal count of stored *fufu* (cfu/g)	
Days	**K**	**D**	**P**	**C**
0	$4.0x10^{4a}$	$4.0X10^{4\ a}$	$2.0X10^{8\ a}$	$2.1x10^{8\ a}$
3	$4.0x10^{5\ a}$	$4.5x10^{6\ b}$	$3.0X10^{5\ a}$	$3.0x10^{6\ a}$
6	$4.7x10^{6\ a}$	$4.9x10^{8\ c}$	$4.5X10^{6\ b}$	$4.0x10^{8\ b}$
9	TNTC	TNTC	TNTC	TNTC

Note: values are means of duplicate determinations. Means with different superscripts along the column are significantly different at ($p < 0.05$).

Key: K = *Fufu* with kerosene, D = *Fufu* with detergent, P = *Fufu* with palm ash, C = *Fufu* Control

Table 4: Bacterial count for the stored *fufu* dough (cfu/g)

	Total bacterial count of stored *fufu* (cfu/g)			
Days	**K**	**D**	**P**	**C**
0	$3.0x10^{6\ a}$	$4.0x10^{8\ a}$	$2.0x10^{6\ a}$	$1.0x10^{6\ a}$
3	$4.8x10^{5\ b}$	$5.8x10^{6\ a}$	$4.8x10^{5\ b}$	$2.8x10^{5\ ab}$
6	$5.6x10^{5\ c}$	$6.8x10^{5\ b}$	$6.6x10^{6\ c}$	$4.6x10^{6\ b}$
9	$7.8x10^{6\ d}$	$8.6x10^{6\ b}$	$7.8x10^{6\ d}$	$6.8x10^{4\ c}$

Note: values are means of duplicate determinations. Means with different superscripts along the column are significantly different at ($p < 0.05$).

Key: K = *Fufu* with kerosene, D = *Fufu* with detergent, P = *Fufu* with palm ash, C = *Fufu* Cont

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EXTENSION WORKERS' CAPACITY FOR OUTREACH TO CROP FARMERS ON CLIMATE CHANGE RESILIENCE AND ADAPTATION IN EDO STATE, NIGERIA

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ABSTRACT

The study evaluated capacity for extension workers' outreach to crop farmers on climate change resilience and adaptation in Edo State, Nigeria. The study specifically described socio-economic characteristics of the extension workers; described capacities for outreach by the extension workers; identified constraints to building capacities for outreach by the extension agents and identified strategies to building capacities of the extension workers. A multistage sampling technique was used to select 69 extension workers. Data for this study were obtained through the use of structured interview schedule. Descriptive statistics and factor analysis were employed in the analysis. The results showed that majority of the respondents (50.7%) were Extension Agent (EA) followed by 29.0% who were Block Extension Supervisors (BES). The result reveals that 34.8% of the extension staff to have attended between 1 to 4 conferences in the last three years. About 44.9% of the respondents participate in workshops, training, seminars for extension workers and farmers. Majority (82.6%) of the respondents identified bush burning, massive deforestation and excess use of agro-chemicals in farming as the major causes of climate change. A major constraints to building capacities for outreach by extension agents was absence of well-defined agricultural policy (3.254). Restructuring of extension agents' education and trainings was identified as a major strategy to building the capacities of the extension workers. It is recommended that agricultural extension policies relating to climate change need to be reviewed, among others.

Keywords: Adaptation, extension agent capacity, climate change resilience, outreach.

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INTRODUCTION

Agriculture is one of the most important sectors that contributes immensely to the economies of most African countries including Nigeria (Onoh, Erezi & Clement, 2023). About 70% of the populace depend on agriculture as a source of their livelihood (FAO, 2016; Osuafor & Nwankwo, 2017). In Nigeria, farming depends unreservedly on the quality of the rainy season, which is a situation that makes it susceptible to climate change (Elijah, Osuafor, &

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Edeh 2020; Odjugo, 2010). Climate change is having a greater impact on Nigeria, as seen by rising crop diseases, falling agricultural production, flooding, and the degradation of farmlands that support the country's farming population (Osuafor, Effiong, & Ude, 2021; Onyekwe, Osuafor, Ude & Onwuemelie, et al, 2021; Ojemade, Osuafor, Bankole, Akagbosu & Osifo, 2018). Climate change is a global issue and its impact is evident in the agricultural sector where it has a major influence on agricultural production in most developing countries (Ojemade, Osuafor & Ahaneku,2021; Elum, Modise & Marr, 2017).

As the effects of climate change intensify, crop farmers need to build resilience and adaptation to improve their capability to cope. One of the most important issues facing the world today is climate change, which has effects on both natural ecosystems and human societies. Nigeria is especially vulnerable to the effects of climate change because of its heavy reliance on agriculture and its natural resources (Onoh *et al.,* 2023; Osuafor, Ude & Ositanwosu, 2021). The term "adaptation" describes changes brought on by current or expected climatic changes and their repercussions in ecological, social, and economic systems. The ability to ‘adapt’ is one facet of resilience. In the past ten years, the concept of resilience has grown in acceptance as a comprehensive and successful strategy for addressing climate change. In light of climate change, resilience has recently gained importance (Feldmeyer, Wilden, Kind, Kaiser, Goldschmidt, Diller & Birkmann, 2019). The ability to bounce back, move forward, or do both at once to mitigate the effects of climate change are three ways that resilience manifests itself (Folke, 2016). The resilience-adaptation process heavily relies on the work of policy authorities and agricultural extensionists. However, measuring resilience and monitoring adaptation activities have received less attention in the agricultural sector. Agricultural extensionists have a vital role in efforts to increase the resilience and stability of agriculture by assisting farmers in adapting to climate change (Ezike *et al*., 2020; Olorunfemi et al., 2020). Several researchers (such as, Ojemade *et al*., 2020; Akinnagbe & Irohibe, 2018; Ali & Erenstein, 2017) have noted poor levels of acceptance and application of climate change approaches among farmers. This has been attributed to farmers lacking the capability to adapt and develop resilience to the effects of climate change. Agricultural extension agents are responsible for educating farmers about new initiatives and technologies on a global scale. (Ezike *et al*., 2020; Oladele, 2015). According to Olorunfemi *et al.* (2021; Ezike *et al*., 2020), extension agents have a responsibility to share the innovations and best practices now being created by multiple global research projects on how to increase adaptive capacity and assist in enhancing the resilience of those who are vulnerable to the effects of climate change. Consequently, for extension services to crop farmers on climate change resilience to be successful, extension specialists must be included in the process. This is especially true for climate change adaptation initiatives (Onyekwe *et al.,* 2021).

Crop farmers suffer the effects of climate change due to obstacles such as their reliance on rain-fed agriculture, their limited financial resources, their inability to adapt, their inadequate infrastructure, their illiteracy, and their inability to diversify (Anarah *et al.,* 2021, Pipitpukdee *et al.,* 2020). One of the pronounced challenges is the lack of access to crucial information on climate change, which consequently affects their level of awareness and potential for resilience and adaptation. Hence, reaching out to the farmers with the accurate information is

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very central. This study is concerned with community outreach. It typically refers to programs or initiatives where extension agents actively engage with farmers or community to address specific needs or provide support (Ozioko, 2022).

Access to adequate knowledge on climate change situations enhances awareness levels and the adaptive capacities of the farmers (Ojemade *et al.,* 2018). However, for some years now, awareness campaigns on climate change have increased on the radio, television, and through one-on-one visit by extension agents. Several studies (Emenyonu *et al.,* 2020;Olorunfemi *et al.,* 2020; Dimelu, 2016; Oladele, 2013) focused on the factors influencing the knowledge and competence of extension agents on climate change and its adaptation strategies. But little research has been done on the extension agents' capacity for outreach to these farmers. Thus, this paper seeks to evaluate the extension workers' capacity for outreach to crop farmers in Edo State, Nigeria. The aim of the study is to evaluate the extension agents' capacity for outreach to crop producers on climate change resilience and adaptation in Edo State, Nigeria. The specific objectives were to:

i. describe the socioeconomic characteristics of the extension agents;
ii. describe capacities for outreach by the extension agents;
iii. identify constraints to building capacities for outreach by the extension agents; and
iv. identify strategies to building capacities of the extension agents.

MATERIALS AND METHODS

The study was conducted in Edo State. Edo State is in the southern region of Nigeria. Edo State lies within the geographical coordinates of Latitudes $5^0$44'N and $7^0$34'N and Longitude $5^0$04'E and $06^0$43'E (Alakpa *et al*., 2021). The state covers an area of 17,802km^2 and has a population of 3,233,366 (Koyenikan & Omoregie, 2022). Ithas three (3) Agricultural Development Programme (ADP) zones namely Edo North, Edo Central and Edo South zones.The study population consisted of all the extension staff in the three Edo State ADP (ESADP) zones, which was 144, distributed as 41 in Edo North, 46 in Edo Central and 57 in Edo South (Idiake-Ochei, 2016). The three zones comprise of a total of 18 extension blocks or LGAs in the study area. For effective extension coverage, a sample of 50% was randomly selected from each of the three ESADP zones for the study, as follows: Edo North (20), Edo Central (23) and Edo South (28). This gave a total of 71 respondents. Validated questionnaire was used to obtain data from the extension workers. Two questionnaire in Edo South were not completely filled and were dropped. Hence, 69 respondents were used for the data analysis. The objectives were achieved using descriptive statistics.

RESULTS AND DISCUSSION

Socioeconomic Characteristics of the Extension Agents

Table 1 shows that 44 of the respondents are males while 25 are females. This result agrees with Kenneth et al.(2019) which reported that there were more male extension workers than females in Edo State. Also, most of the respondents are within the age range of 31 to 40; and 41 to 50 years. In addition, the results showed that 18.8% of people were single and 76.8% were married, while 55.1% and 36.2% had degrees. 17 years are on average spent in formal education. In terms of household size, the typical household size was 12 people, with 50.6%

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of households consisting of between 6 and 10 people. Based on current rank, 50.7% are extension agents, an average percentage of the respondents (50.7%) were Extension Agents (EA), while 29.0% are Block Extension Supervisors (BES). Majority (52.5%) identity religious group as the social organizations they belong to. In order to reflect the level of personal responsibility and the anticipated physical fitness for both farm and non-farm labor, respondents' ages (measured in years) were categorized. The results indicate that the extension agents were primarily young people, which placed them in the labor force and qualified them for strenuous exercise. For educational level, majority (55.1%) of the extension agents have completed their first degree. Education will guide extension agents to ensure extension messages are prepared in order to take care of all and sundry involved in a particular programme of development.

Capacities for Outreach by the Extension Agent

Conferences, Trainings, Workshops and Funds for Climate Change (CC) Adaptation Activities

Table 2 shows that 44.9% of the respondents participate in workshops, training, seminars for extension workers and farmers. The high degree of participation in conferences on CC might be connected with the hybrid programmes, well-executed activities, and funding of participants. This result corroborates the assertion of Rivera *et al*. (2016) who noted that hybrid workshop encourages more for public and private sector extension staff to participate.

Climate Changes that have taken place in the state within the last Five Years

In Table 3, high temperature is reported by 24.6% (2016-2017), pollution, global warming and drought is reported by 26.1%, 24.6% and 24.6% respectively (2017-2018), change in plant was reported by 24.6% between 2018 and 2019 while between 2019 and 2020, 8.7% reported flood.

Knowledge of Climate Change among the Extension Agents

The knowledge level of Extension Agents on CC is presented in Table 4. On the causes of climate change, majority (92.8%) of the respondents are aware that bush burning leads to climate change. On the effects of climate change, 84.1% are aware that CC will bring about delayed onset of rain fall.

Constraints to Building Capacities for Outreach by Extension Agents

Constraints to building capacities for outreach by extension agents as indicated on Table 5were: lack of distinct agricultural policy (3.254), use of obsolete facilities (3.13), weak staff training on climate change (3.059), meagre funding of rural development program (3.07), and lack of equipment to implement skills learnt at training (2.82).This result agrees with Onoh et al. (2023) who affirmed that limited access to resources, lack of education and training opportunities are key barriers to building capacity of women in agriculture. This result is also in line with the findings of Ozioko *et al.* (2022) who found that absence of well-defined agricultural policy, poor funding of rural development program, lack of human resources and poor staff training are the major constraints to building capacities for outreach by extension agents.

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Strategies to Building Capacities of the Extension Agents

In Table 5, the most accepted strategies identified to strengthen capacities of the extension agents are: appraisal of the agricultural extension policies (3.716), restructuring of extension agents' education and trainings (3.618), adequate resourcing of coordination mechanism and supervision (3.250), appropriate funding of extension activities (3.559), giving incentives to motivate extension workers (3.397), planning of seminars and workshops to enhance the extension agents' proficiency (3.294). This finding corresponds with the report of Ozioko *et al.* (2022) who identified strategies for building capacities as review of agricultural extension policies, reformation of basic education to boost job motivation, and organization of conferences and seminars to increase competence of extension agents.

CONCLUSION AND RECOMMENDATIONS

The study concludes that extension agents were fairly exposed to workshops, training and seminars on climate change resilience and adaptation but there were no known investment on equipment within three years with regards to climate change. Based on the findings of the study, it is recommended that agricultural extension policies relating to climate change need to be reviewed, extension agents' need to be engaged in trainings and workshops in order to increase their skills in order to educate and guide the farmers on climate change resilience and adaptation.

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APPENDICES

Table 1: Percentage distribution of Extension Agents based on Socio-Economic characteristics

Variables	Frequency (N=69)	Percentage (%)	Mean
Sex			
Male	44	63.8	
Female	25	36.2	
Age (years)			40.13
21 – 30	9	13.0	
31 – 40	18	26.1	
41 – 50	29	42.0	
51 – 60	13	18.8	
Marital status			
Single	13	18.8	
Married	53	76.8	
Widowed	1	1.4	
Divorced	1	1.4	
Educational Level			
Secondary	5	7.2	
First Degree	38	55.1	
M.Sc	25	36.2	
Ph.D	1	1.4	
Years spent in formal education			16.61
1 – 6	24	34.6	
7 – 12	44	63.8	
13 – 18	1	1.4	
Household size			5.67
0-5	31	44.8	
6-10	36	50.6	
11 – 12	3	4.3	
Number of years spent in extension work			11.74
0-9	39	56.3	
10-20	11	15.8	
21-30	17	20.1	
31 and above	2	5.7	
Present rank			
Subject matter specialist	13	18.8	
Block extension supervisors	20	29.0	
Block extension agent	1	1.4	
Extension agent	35	50.7	
Belonging to a social organization			
Yes	48	69.6	
Religious group	36	52.2	
Cooperative society	6	8.7	
Political group	6	8.7	
Community association	2	2.9	

Field Survey Data, 2021

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Table 2: Distribution of Extension Agents based on the conferences, trainings, workshops and funds for CC adaptation activities attended

Variables	Frequency N=69	Percentage %	Mean
Number of conferences attended on CC in the last three years			
1 – 4	24	34.8	
5 – 8	4	15.9	
9 and above	2	2.8	
Training, seminars, field trips or farm visit on cc for extension agent	31	44.9	
Workshops, training, seminars on cc adaptation for farmers	31	44.9	
Number of training organized by LGA			
1 – 4	20	28.9	
5 – 8	5	7.2	
9 and above	3	4.3	4.29
Sources of Funds for CC adaptation activities			
Government	21	30.4	
Farmers representatives in the training on CC	30	43.5	
LGA collaboration with CSOs on CC	32	46.4	
LGA representatives in the training on CC	31	44.9	

Field Survey Data, 2021; CC= Climate change; LGA=Local Government Area; CSO=Central Statistical Organisation

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Table 3: Climate Changes that have taken place in the LGA within the last five years

Duration (years)	Frequency	Percentage
2016 – 2017		
Irregular rainfall	1	1.4
Solar intensity	1	1.4
High temperature	1	24.6
Change of ecosystem	1	1.4
Drought	2	2.9
Heat wave	1.4	1.4
Higher ocean temp	16	23.2
Increase in heavy precipitation	1.4	1.4
Heavy rainfall and hail	4	5.8
2017 -2018		
Pollution	1	26.1
No rainfall	1	1.4
Irrigation	1	1.4
Prolong rainfall	1	1.4
Global warming	1	24.6
Drought	2	24.6
2018-2019		
Striking glacier	17	24.6
Drought	11	15.9
Rising maximum temp	7	10.1
2019-2020	1	1.4
Environmental degradation	1	1.4
Soil degradation	2	2.9
Carbondioxide accumulation in our atmosphere	1	1.4
No rainfall	1	1.4
Flood	6	8.7
Heavy rain and hail	1	1.4
Late planting due delay in rainfall pattern	3	4.3
Irrigation	1.4	1.4
Training received on CC	23	33.3
List of training received		
Weather forecast	9	13.0
Global warming	3	4.3
Adaptation to climate change	4	5.8
Control of floods	4	5.8
Climate change mitigation	5	7.2
Excess use of agro chemicals	3	4.3

Field Survey Data, 2021

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Table 4: Knowledge Level of Causes and Effects of climate change (CC) among the Extension Agents

Variables	Frequency N=69	Percentage (%)
Causes of Climate Change		
Bush burning	64	92.8
Massive deforestation	57	82.6
Excess use of agro-chemicals in farming	57	82.6
Massive urbanization	51	73.9
Human population explosion	50	72.5
Release of chlorofluoro carbons (Green House gases)	51	73.9
Exploration of natural resources such as petroleum, coal burning etc.	46	66.7
Massive agricultural mechanization	39	56.5
Massive industrialization	49	71.0
Effects of Climate Change		
Prolonged rainfall	53	76.8
Irregular rainfall pattern and distribution	51	75.0
Delayed onset of rain fall	58	84.1
Increased erosion of soil	48	69.6
Loss of farmlands and houses due to flooding	45	65.2
Rivers and streams are drying up. These resources initially provided water for agricultural and household use	43	62.3
Low rain fall intensity	48	69.6
High rain fall intensity	50	72.5
Increased temperature (solar intensity)	48	69.6
Too much wind storms	55	79.7
Increased wild fires	57	82.6
Proliferation of crop pests and animal diseases	41	59.4
Increase in invasive weed infestation	37	53.6
Increased postharvest losses of crops	32	46.4
Increased mortality rates	28	40.6

Field Survey Data, 2021; *Multiple responses recorded

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Table 5: **Constraints to building capacities for outreach by extension agents**

Constraints	Mean	Standard deviation
Lack of distinct agricultural policy	3.25	1.06
Poor knowledge and skills on climate change management	2.76	1.08
Weak staff training on climate change	3.05	1.00
Lack of professionalism(certification and regulation) in extension service	2.88	1.09
Failure to pay training allowances	2.70	1.15
Lack of equipment to implement skills learnt at training	2.82	1.07
Meagre funding of rural development program	3.07	1.07
Weak linkages among extension organizations	2.72	1.01
Lack of reliable weather forecasts/climate information	2.53	1.06
Poor harmonization and supervision of extension staffs	2.44	1.09
Use of obsolete facilities	3.13	1.08
Inconsistent policies and programs	2.47	1.08

Field Survey Data, 2021; Mean cut off point ≥3.0=Accept; <3.0=Reject

Table 6: Strategies to Building Capacities of the Extension Agents

Strategies	Mean	Standard deviation	Decision
Appraisal of the agricultural extension policies	3.716	0.6921	Accept
restructuring of extension agents' education and trainings	3.618	0.6236	Accept
Adequate resourcing of coordination mechanism and supervision	3.250	0.8530	Accept
Appropriate funding of extension activities	3.559	0.7408	Accept
planning of seminars and workshops to enhance the extension agents' proficiency	3.294	0.7929	Accept
Giving incentives to motivate extension workers	3.397	0.7153	Accept
Creation of operative linkages between extension and research activities	3.288	0.7798	Accept
Privatizing extension service	2.523	1.1607	Reject
Regular assessment of extension services	3.059	1.0911	Accept
Proper staffing of extension personnel	3.588	0.6519	Accept

Field Survey Data, 2021. Mean cut off point ≥3.0=Accept; <3.0=Reject

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RESPONSE OF ROOT GROWTH PARAMETERS AND NUTRIENT UPTAKE OF COWPEA (*Vigna unguculata L.)*TO RATES OF ORGANIC MANURE

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ABSTRACT

This study was carried out to investigate the effect of different organic manure sources on Root growth parameters and nutrient uptake of cowpeas (Vigna unguiculata L.). The experiment was conducted at the Teaching and Research Farm of the Faculty of Agriculture, Imo State University, Owerri. The experiment was laid out in a Randomized Complete Block Design (RCBD), with five treatments replicated four times. The treatments are 0 ton T_1 (Control), T_2 (10tons and pig manure), T_3 (15tons of pig manure), T_4 (10tons of poultry manure), and T_5 (15 tons of pig manure). From the result of the experiment, the application of poultry manure significantly improved, the number of roots, root length, root dry matter, phosphorus, and potassium uptake at various growth stages of data collection. The result also showed that pig manure significantly improved the percentage of emergence, plant height, number of leaves, Nitrogen uptake, and high number of pods (9.75), seed weight (101.47g), and yield 924.16kg/ha). The cowpea responded significantly in both root growth parameters and nutrient uptake to pig manure and poultry manure at the rates used.

Keywords: Cowpea, Growth, Yield, Nutrient uptake and Organic manure.

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INTRODUCTION

Cowpea (vigna unguiculata) is a legume crop that has been widely cultivated in tropical and subtropical regions of the world due to its high protein content and adaptability to poor soil conditions.However, the productivity of cowpea can be limited by factors such as low soil , fertlity,drought and poor management practices.one way to improve the productivity of cowpea is using organic amendments such pig maunre and poultry manure , can provide essential nutrients and improve soil healthOver the years traditional farmers have perfected the act of using manure soorces from animals especially, poultry,pigs,gaots and crop residues as source of enriching the soil thereby making food available for optimum plant growth.The use of organic inputs such as crop residues and manures has great potential for improving soil productivity and crop yield through improvement of the soil physical, chemical, microbiological properties and nutrient supply (Abbasi *et al.,* 2009). Inputs from organic sources (e.g.animal dropings, compost and sewage sludge) play a central role in the productivity of many tropical farming systems by providing nutrients through decomposition

and substrate for the synthesis of soil organic matter.Imbalanced use of chemical fertilizers by farmers has deteriorated soil health and declined soil organic carbon content. It is essential to adopt a strategy of using organic manures. Organic manures enhances the soil fertility and yield of crops by rendering unviable sources of elemental nitrogen bound, phosphate and decomposed plant residues into available form in order to facilitate the plant to absorb the nutrients (Timsina Jagadi,2018).Moreover, Cowpea is a valuable component of farming systems in many areas because of its ability to restore soil fertility for succeeding cereal crops grown in rotation with it (Carsky *et al.,* 2002; Tarawali *et al.,* 2002; Sanginga *et al.,* 2003). Atmospheric nitrogen fixing ability is extremely valuable when it is cultivated with cereal crops in crop rotation system (Timko *et al.,* 2007). Cowpea crop increases soil nitrogen up to 40–80 kg per hectare (Quin, 1997 and Anil *et al*., 2019).

In Nigeria, loss of soil fertility and rainfall variability are among the factors that contributed to low yields. Studies on the predominantly sandy soils have shown the complexity of soil fertility problems (Giller, 2001).There are slim chances of building soil organic matter in the dry tropics and, hence, nutrient stocks (Giller *et al*., 1997), rendering farmers to rely heavily on external nutrient inputs on a seasonal basis. However, most of the smallholder farmers use sub-optimal amounts of fertilizers due to cash limitations and poor access to fertilizer markets. Therefore, it is important to recycle both endogenous and exogenous nutrient pools. Moreover, continuous use of fertilizer alone cannot sustain crop yield and maintain soil fertility in the long term (Shoko *et al*., 2007; Tisdale *et al*., 1999).

Gupta and Sharma (2006).have reported that organic sources of nutrients improved soil aeration, root development and increase microbial and biological activities in the rhizosphere. Mahatele and Kushwaha (2011), reported that addition of FYM at $10tha^{-1}$ to soil improved the supply of available nutrient to the plant and brought about favorable soil environment which ultimately increased nutrient and water holding capacity of soil for longer period and that resulted in better growth, yield attributes and yield of Pigeon pea. The application of organic amendments can potentially stimulate crop growth and development through the actions of plant growth-promoting hormones, including cytokinins, auxins, and gibberellins (Quilty and Cattle, 2011). Pradeep *et al*. (2012), stated that different bio-composts could enhance seed germination and seedling vigour in four different crops such as maize, green gram, soybean and okra

Organic fertilizer play vital role as a major contributor of plant nutrients. Many workers have tried to assess the importance of organic manures in crop production. Senjobi *et al.* (2010), reported that the use of poultry, sheep/goat manures improved all the growth parameters of the leaf vegetable they worked with.

The addition of organic fertilizers efficiently ensures high production and continuous crops by improving soil properties and increase roots development and soil micro organisms activity (Abou EL-Magd *et al*., 2006; Ayoola and Maknide, 2009).John *et al*. (2004) who reported that poultry manure contains essential nutrients which are associated with high photosynthetic activities that promote root and vegetative growth. The addition of organic fertilizers efficiently ensures high production and continuous crops by improving soil properties and increase roots development and soil micro organisms activity (Abou EL-Magd

et al., 2006; Ayoola and Maknide, 2009), Dademel *et al*. (2004) reported that the nitrogen content in both organic fertilizers has been known to enhance leaf production, flowering, seed formation and root formation, this will lead to higher metabolic activities and consequently higher fresh fruit yield in okra.The response of root growth parameters to organic manure is also important, as it can affect the uptake of nutrients by the plant. Studies have shown that organic manure can increase the length and diameter of cowpea roots, which can improve the uptake of nutrients from the soil (Oke et al., 2017). Additionally, organic manure can increase the number of lateral roots and root hairs, which can also improve nutrient uptake (Akpa et al.,2017).The nutrient uptake of cowpea in response to organic manure is also an important area of research. Studies have shown that organic manure can increase the uptake of nutrients such as nitrogen, phosphorus, and potassium by cowpea (Kumar *et al*., 2018).

Most of research work on cowpea has always centred on above ground parameters without much work on root growth indices,this could be attributed to a labourios nature on the study of root architecture and structure of legumes by Researchers. pig manure and poultry manure have bean shown to increase rootlegnth and diameters in other crops, but effects on cowpea root growth parameters are not well understoodHence this study is done to add to knowledge on information on root growth paramters and its importants in the growth of crops .

MATERIALS AND METHODS

Location

This study was carried out in the Teaching and Research Farm of the Faculty of Agriculture and Veterinary Medicine, Imo State University,Owerri, during late planting season of 2021. Owerri lies between the latitudes $5^{o}10'$N and $6^{o}0'$N and longitudes $6^{o}35'$E and $7^{o}0'$E with an altitude of 91.0m within the Southeast rain forest agricultural zone of Nigeria. The area maintains an average annual rainfall of 2,500 mm, mean minimum and maximum temperature of 23.5^{o}C and 32.1^{o}C respectively, with relative humidity ranging from 70-85% and the annual evapotranspiration is 1450 mm (NIMET, 2010).

Source of Materials

Plant materials that were used in this study were collected from Imo State University Teaching and Research Farm. Cowpea beans seeds were source from Imo ADP.

Experimental Design

The experimental design was a randomized complete block design with 5 treatments replicated four times. Treatments used consist of 0 ton T_1 (Control), T_2 (10tons of pig manure), T_3 (15tons of pig manure), T_4 (10tons of poultry manure), and T_5 (15 tons of pig manure)

Planting and Agronomic operations

cowpea seeds were planted at a depth of 2-3cm, spacing of 50cm × 50cm and four seeds were sown per hole on each 1m × 1m bed. After germination, the seeds were thinned to one seedling per stand. Weeding was done regularly by handpicking throughout the period of research, to keep the beds weed free.

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Data Collection

The following parameters were collected:

Root length: Five plants were randomly selected for measurement of root length and this was done using centimetre ruler.

Number of Roots: The numbers of roots were counted visually by carefully separating them from the soil by gently pinching and washing the soil particles

Root Dry Weight: To measure root dry matter, in every step, roots were put in an oven at 57 C for 48 hrs; then they were weighed with a scale with measurement

Root Nodules: The number of root nodules was counted by carefully separating them from the soil by gently pinching and washing the soil particles

Total uptake of N/P/K will be calculated separately by the following formula:

$$\text{Uptake of N/P/K (kg ha}^{-1}) = \frac{\text{N\% P\% K\%} \times \text{dry matter kgha}^{-1}}{100}$$

Statistical Analysis

Data obtained was subjected to statistical analysis using Analysis of Variance (ANOVA) to determine if the treatments have any significant effect on parameters measured. All data were analyzed according to One-Way ANOVA using SPSS software version 20.0.

RESULTS

Effect of Treatments on Number of Roots

The response of number of roots to application of organic manure is presented in Table 1. The result reveals that source of manure and rate significantly ($P<0.05$) influenced number of roots. The results showed that At 2 and 4WAP, T_2 recorded significantly highest number of roots (8.500 and 15.500 respectively) compare to the lowest (5 and 11.5 respectively) number of roots obtained from T_1. At 6 WAP, showed that T_3 recorded the highest number of roots (18.75) which was significantly different ($P<0.05$) from the lowest number of roots (13.250) observed from T_5. This was followed by control with 18.50 T_2 with mean number of roots (17.50), while at 8 WAP T_3 recorded significantly highest (27.500) number of roots compare to the lowest (19) observed in T_2. Control was able to give better result than T_2 and T_5 as shown in Table 6. Whereas at 10WAP, T_4 recorded highest number of roots (38.750) which was significantly different ($P<0.05$) compare to the control with 24.25 number of roots. This was followed with T_3 with number of roots as 30.250, T_2 with number of root as 27.250 while T_5 recorded 24.500 of number of roots. They results are statistically different. It was observed at the end of experiment that 10tons of poultry manure performed better than chicken manure.

Effect of Treatment on Root Length of Cowpea

The influence of organic manure source and rate of application on root length at different growth stage of cowpea is presented in Table 2. There was no significant different ($P<0.05$) on the result shown except at 10WAP. It was evident from Table 2, that T_3 at 2WAP recorded the longest root length (4cm) which was not significantly different ($P<0.05$) from the shortest root length (3.125cm) observed from T_2. At 4WAP, T_4 had the highest root length (7.475cm) compare to the lowest root length (6.150cm) obtained in T_1. This was followed by T_3 with root length of 7.425cm and T_4 with root length of 7.0750cm while T_2 gave 6.475cm of root length. At 6WAP there was no significant different among treatments as T_4 recorded root length of 11.725cm, followed by T_3 with 11.425cm, T_2 gave 11.1750cm of root length, among the treated plots T_5 gave the lowest root length (10.825cm) compare to control with 10.975cm of root length. Also at 8WAP, T_3 gave the highest root length (15.700cm) which was statistically similar to other root lengths 14.400cm, 14.650cm, 14.700cm and 14.800cm respectively from T_1, T_5, T_4 and T_2 in that order whereas at 10WAP T_5 produced significantly maximum root length (21.950cm) compare to the minimum (18.500cm) recorded from control. This was closely followed by T_3 with root length of 21.925cm which was statistically similar to those of T_4 (20.850cm) and T_2 (20.375cm) as shown in Table 2.

Effect of Treatment on Number of Root Nodules

Table 3, showed that effect of manure source and rate of application did not have significant different ($P<0.05$) on number of root nodules. It was observed that nodules did not form at 2 and 4WAP as shown in Table 3. However, at 6WAP,8WAP and 10WAP there was no statistical diffference observeed in the number of root nodules for all observed treatment.It was observed that type of manure and rate applied different on number of root nodules recorded.

Effect of Treatment on Root Dry Matter

The effect of sources of organic manure and rates of application on root dry matter is presented in Table 4. There was significant different ($P<0.05$) among the treatment levels and manure source on root dry matter. At 2WAP T_4, significantly recorded maximum root dry weight (2.0568g) compare to the minimum root dry weight (0.9303g) observed from T_2. At 4, 6, 8 and 10WAP T_5 produced maximum root dry matters (4.082g, 6.4988g, 8.8375g and 9.105g respectively) which were significantly different ($P<0.05$) from the minimum root dry matters (1.3665g, 2.5623g, 3.6765g and 4.3215g). This was followed by T_4 with second highest maximum root dry matter compare to T_2 and T_3 as shown in Table4. Poultry manure at 15 tons and 10tons significantly improved root dry matter when compare with pig manure.

Effect of Treatments on Nitrogen Uptake

The response of Nitrogen uptake by the root of cowpea influence by manure source and rate of application is presented in Table 5. There was significant different ($P<0.05$) among the source and treatment level on nitrogen uptake as weeks increases. At 2WAP T_4 recorded

significantly maximum Nitrogen uptake (0.059%) which was significantly different (P<0.05) from minimum (0.0875%) Nitrogen uptake observed in T_1. This was followed by T_5 with Nitrogen uptake of 0.05675g while T_3 had 0.0405% and 0.02475% of Nitrogen uptake. At 4, 6, and 8WAP, T_5 (15 tons of poultry manure) significantly recorded the maximum Root Nitrogen uptakes (0.12325%, 0.3135% and 0.4105% respectively). Compare to the minimum Nitrogen uptakes (0.02425%, 0.049% and 0.11875%) observed in T_1. This was followed by T_4 with Nitrogen uptake 0.085%, 0.1915% and 0.30375% respectively, which was significantly different (P<0.05) from other intermediate levels. At 10 WAP, T_2, recorded the maximum Nitrogen uptake (0.5850%) which was significantly different (P<0.05) from the lowest (0.21675%) obtained in T_1. It is observed that among treatment level, T_3 at 10WAP recorded significantly lowest Nitrogen uptake (0.37025%) compared to other treated plots.

Effect of Treatment on Phosphorus Uptake

The results of the influence of manure type and rates on phosphorus uptake of cowpea is presented in Table 6. Organic manure treatments significantly (P<0.05) influenced phosphorus uptake as weeks increases. The values of phosphorus uptake was maximum (11.3675ppm) from T_5 at 2WAP compare to the minimum observed in control. Also at 4WAP, 15ton/ha rate of poultry manure (T_5) recorded the highest Phosphorous uptake (29.2785ppm) which was significantly different (P<0.05) from the lowest (6.2823ppm) Phosphorous uptake from control. 10tons/ha rate (T_4) gave higher Phosphorous uptake (22.763ppm) than 15tons/ha rate of pig manure with Phosphorous uptake of 13.695ppm and T_2 (10ton/ha rate of pig manure) with Phosphorous uptake of 10.8778ppm.

Also values of Phosphorous uptake at 10ton/ha rate (T_4) and 6WAP growth stage shows that poultry manure (T_4) treatment recorded the highest (51.067ppm) P uptake while the least (13.6835ppm) was observed in control. Among the treated plots, 10ton/ha rate (T_2) of pig manure recorded significantly least (27.6693ppm) P uptake compared to T_3, and T_5. Similarly, at 8 and 10WAP T_5 (15ton/ha of poultry manure) significantly recorded the maximum phosphorous uptake (77.308ppm and 88.8075ppm respectively) compare to the least P uptake (21.4658ppm and 31.156ppm) obtained in control. However, it was observed that poultry manure both 15ton/ha and 10ton/ha rates significantly stimulated the uptake of Phosphorous more than pig manure as shown in Table 7.

Effect of Treatments on Potassium Uptake

The response of potassium (K) uptake to source and rates of organic manure application is shown in Table 7. There were significant differences (P<0.05) in potassium (K) uptake as affected by source and rates applied organic manure. At 2WAP and 4WAP growth stages, plants that received 15t/ha^{-1} of poultry manure (T_3) recorded the highest K uptake (0.621% and 1.5103% respectively) which were significantly different (P<0.05) from the least K uptake (0.0823% and 0.160% respectively) observed in T_1. Among the treated plots 10t/ha^{-1} rate (T_2) of pig manure recorded the least followed by 15t/ha^{-1} of pig manure (T_3) as shown in table 7.Similar trend was observed at 6, 8 and 10WAP were 15t/ha^{-1} of poultry manure (T_5) recorded the highest K uptake (2.6923%, 5.4563% and 7.3908% respectively) compare to the K uptake (0.3587%, 1.659% and 2.696%) observed T_2 (10t/ha of pig manure) recorded

higher K uptake (2.854% and 5.444%) than K uptake (1.8915% and 4.3183% respectively) by 10tha^{-1} + poultry manure. In all, T_5 influenced K uptake than any other treatment level.

DISCUSSION

The result showed that the application of Pig manure and Poultry manure could improve root length, number root, number of nodules and root dry matter compare to control, although, the response is dependent on manure type, rate and environmental factors. This improvement could be due to fact that application of pig manure and poultry manure to soils provide more nutrients to maintain root growth parameters thereby maintained the supporting function of the cowpea plant. This is in conformity with Udom *et al.* (2007), and Waniyo *et al.* (2013), who reported that, organic manures supply nutrients to plants, improves soil structure, aeration and encourages good root growth which may invariably had resulted in increased growth and yield of the maize plant.

Similarly, Abou El-Magd *et al.* (2006), and Ayoola and Makinde, (2009), have reported that addition of organic fertilizers efficiently increase roots development, high production and continuous crops by improving soil properties and soil microorganisms. Also Gupta and *S*harma(2006) have reported that organic sources of nutrient improved soil aeration root development and increase microbial and biological activities the rhizosphere.

It was observed that application of organic manure (pig manure and poultry manure) could enhanced the soil organic matters which improve soil structure and availability of essential nutrient that could contribute to cowpea growth and yield by directly supplying essential nutrients and indirectly modifying soil physical structures that can improve the root growth, rhizosphere and enhanced plant growth. Our observation is in agreement with work of Akinrinde et al. (2017) who investigated the effect of pig and poultry manure on the growth of cowpea. Their results showed that cowpea plants treated with pig and poultry manure exhibited significantly higher shoot length, root length, leaf area, and biomass compared to control plants without any fertilizer application. This indicates that both pig and poultry manure can promote the growth of cowpea roots.

In addition, organic manure application also affects the accumulation of macro and micro elements in the soil. It was observed that plant growth rates are influenced by elements of Nitrogen (N), Phosphorous (P) and Potassium (K). N element as a constituent of chlorophyll, division and cell enlargement in the apical meristem, activity of the apical meristem generated the shoot growth thereby enhancing overall plant height (Purberjanti *et al.*, 2019).

Superiority of poultry manure on root parameters (length of root, number of roots, root nodules and root dry matter) over pig manure could be because it increases macroelement content in the cells and enhanced their division due to high organic matter and micronutrients present in it.

In this study, it was observed that organic manure types and rate significantly enhanced nutrient uptake by the root of cowpea. This could be due to fact that organic manure increases organic matter content, nutrients bioavaiability and increased microorganisms activities that improved solubility, mineralization and absorption of these essential nutrient thereby improves root growth parameters and above ground parameter.Adewole and Ilesam, (2011),

concluded that organic-based fertilizer enhanced the bioavailability of plant nutrients, and thus, improved the uptake nutrients by the okra plant roots. Uptake of N, P, K and Ca, were enhanced significantly by the rate and types of manure applied, Adewole and Ilesam, (2011), reported that organic fertilizer enhanced the uptake of nutrients (N, K, Na and Cu, Ca) more than any other soil amendments. The study by Kumar etal.(2018) provides valuable insights into the effects of organic manure on nutrient uptake in cowpea.Pig and Poultry manure when applied to cowpea cultivation can enhance the availability of N,P and K which are crucial elements for plant growth and development.

Pig and poultry manure not only provide essential nutrients but also enhance nutrient availability in the soil. The organic matter in the manure improves soil structure, water-holding capacity, and nutrient retention, facilitating better nutrient uptake by plant roots. A study by Ojeniyi *et al*. (2018) evaluated the effect of pig and poultry manure on nutrient uptake in cowpea. The results revealed that plants treated with pig and poultry manure had significantly higher concentrations of nitrogen, phosphorus, potassium, calcium, magnesium, iron, zinc, copper, and manganese in their roots compared to control plants. This indicates that the application of pig and poultry manure enhances the nutrient uptake capacity of cowpea roots.

The maximum production of root growth parameters as determined by root length, number of root and root dry matter when poultry manure and pig manure were applied could be attributed to the steady release of nutrients (N, P, K and Ca) upon decomposition of organic manure that enhanced growth and yield of cowpea. This is consistent with work of Waniyo *et al.* (2013), who reported the same findings in maize.

In this study, it was observed that N, P and K uptake at later growth stage (10WAP) of cowpea plant were higher in the root evaluated, this could be attributed to steady availability of macro element and micro nutrients in the soil which were slowly release due to microbial action on decomposition of organic manure. This is consistent with findings of Akintonye and Olaniya, (2012) and Waniyo *et al.* (2013), that organic fertilizers release nutrients slowly.

In this study poultry manure significantly improved more root dry matter, phosphorus and potassium uptake, number of roots, root length and number of nodules compare to the pig manure.

CONCLUSION

In conclusion, the application of pig and poultry manure can have a significant impact on the growth and nutrient uptake of cowpea roots. Poultry manure application rate of 10tons and 15tonha^{-1} exerted significant influence on number of roots, root length, root dry matter number of nodules, uptake of phosphorous and potassium by cowpea compare to control and pig manure. 15tonha^{-1} of both organic manure improve more of root parameters and nutrients uptake These organic fertilizers provide essential nutrients, enhance nutrient availability in the soil, increase organic matter content, and improve soil structure. These studies referenced above demonstrate the positive effects of pig and poultry manure on cowpea root growth and nutrient uptake. Further studies are needed to fully understand the effects of organic manure

on cowpea root growth parameters and nutrient uptake, and to develop effective management strategies for improving the productivity of cowpea production systems.

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APPENDICES

Table 1: Effect of Treatments on Number of Roots

Treatments	2WAP	4WAP	6WAP	8WAP	10WAP
$T_{1\text{-control}}$	5.000^{b}	11.500^{b}	18.500^{a}	23.750^{ab}	24.250^{b}
$T_{2\text{-10tns of pig manure}}$	8.500^{a}	15.500^{a}	17.500^{bc}	19.000^{ab}	27.250^{b}
$T_{3\text{-15tns of pig manure}}$	6.000^{ab}	12.000^{ab}	18.7500^{a}	27.500^{a}	30.250^{ab}
$T_{4\text{-10tns of poultry manure}}$	7.750^{ab}	11.750^{ab}	13.750^{c}	27.250^{a}	38.750^{a}
$T_{5\text{-15tns of poutry manure}}$	8.000^{a}	11.750^{ab}	13.250^{b}	22.500^{a}	24.500^{b}

Mean in the column, having the same letter(s) are not significantly different at P≤0.05, according to Least Significant Difference (LSD) method.

Table 2: Effect of Treatment on Root Length of Cowpea

Treatments	2WAP	4WAP	6WAP	8WAP	10WAP
$T_{1\text{-control}}$	3.6750^{a}	6.1500^{a}	10.9750^{a}	14.4000^{a}	18.550^{b}
$T_{2\text{-10tns of pig manure}}$	3.1250^{a}	6.4750^{a}	11.1750^{a}	14.8000^{a}	20.375^{ab}
$T_{3\text{-15tns of pig manure}}$	4.0000^{a}	7.4250^{a}	11.4250^{a}	15.7000^{a}	21.925^{ab}
$T_{4\text{-10tns of poultry manure}}$	3.1750^{a}	7.0750^{a}	11.7250^{a}	14.6500^{a}	20.850^{a}
$T_{5\text{-15tns of poutry manure}}$	3.7250^{a}	7.4750^{a}	10.8250^{a}	14.7000^{a}	21.950^{a}

Mean in the column, having the same letter(s) are not significantly different at P≤0.05, according to Least Significant Difference (LSD) method.

Table 3: Effect of Treatment on Number of Root Nodules

Treatments	2WAP	4WAP	6WAP	8WAP	10WAP
$T_{1\text{-control}}$	-	-	0.7500^{a}	3.7500^{a}	6.0000^{a}
$T_{2\text{-10tns of pig manure}}$	-	-	1.5000^{a}	4.5000^{a}	7.2500^{a}
$T_{3\text{-15tns of pig manure}}$	-	-	2.0000^{a}	4.0000^{a}	7.2500^{a}
$T_{4\text{-10tns of poultry manure}}$	-	-	1.2500^{a}	3.5000^{a}	7.5000^{a}

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$T_{5\text{-}15tns\ of\ poutry\ manure}$	-	-	1.2500[a]	3.7500[a]	6.5000[a]

Mean in the column, having the same letter(s) are not significantly different at P≤0.05, according to Least Significant Difference (LSD) method.

Table 4: Effect of Treatment on Root Dry Matter(g)

Treatments	2WAP	4WAP	6WAP	8WAP	10WAP
$T_{1\text{-}control}$	1.0400[d]	1.3665[e]	2.5623[e]	3.6765[e]	4.3215[e]
$T_{2\text{-}10tns\ of\ pig\ manure}$	0.9303[e]	1.8303[d]	3.4788[d]	5.7700[c]	7.625[c]
$T_{3\text{-}15tns\ of\ pig\ manure}$	1.7368[c]	2.2123[c]	4.3075[c]	4.8895[d]	5.7213[d]
$T_{4\text{-}10tns\ of\ poultry\ manure}$	2.0568[a]	3.2890[b]	5.8493[b]	6.1313[b]	8.2333[b]
$T_{5\text{-}15tns\ of\ poutry\ manure}$	1.9083[b]	4.0828[a]	6.4988[a]	8.8375[a]	9.105[a]

Mean in the column, having the same letter(s) are not significantly different at P≤0.05, according to Least Significant Difference (LSD) method

Table 5: Effect of Treatments on Nitrogen Uptake

Treatments	2WAP	4WAP	6WAP	8WAP	10WAP
$T_{1\text{-}control}$	0.01875[e]	0.02425[c]	0.0490[e]	0.11875[e]	0.21675[d]
$T_{2\text{-}10tns\ of\ pig\ manure}$	0.2475[d]	0.05475[bc]	0.11175[d]	0.23775[c]	0.5850[a]
$T_{3\text{-}15tns\ of\ pig\ manure}$	0.0405[c]	0.07125[b]	0.12875[c]	0.23575[d]	0.37025[d]
$T_{4\text{-}10tns\ of\ poultry\ manure}$	0.059[a]	0.085[b]	0.1915[b]	0.30375[c]	0.4675[c]
$T_{5\text{-}15tns\ of\ poutry\ manure}$	0.05675[b]	0.12325[a]	0.3135[a]	0.4105[a]	0.5680[b]

Mean in the column, having the same letter(s) are not significantly different at P≤0.05, according to Least Significant Difference (LSD) method.

Table 6: Effect of Treatment on Phosphorus Uptake

Treatments	2WAP	4WAP	6WAP	8WAP	10WAP
$T_{1\text{-}control}$	3.5605[e]	6.2823[e]	13.68350[e]	21.4658[e]	31.1560[e]
$T_{2\text{-}10tns\ of\ pig\ manure}$	4.0365[d]	10.8778[d]	27.66930[d]	45.785[c]	61.8348[c]
$T_{3\text{-}15tns\ of\ pig\ manure}$	9.0198[c]	13.695[c]	35.8168[c]	40.0255[d]	50.6185[d]
$T_{4\text{-}10tns\ of\ poultry\ manure}$	10.9925[b]	22.763[b]	51.067[b]	58.343[a]	75.2848[b]

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$T_{5\text{-}15tns\ of\ poutry\ manure}$	11.3675[a]	29.2785[a]	44.1143[b]	77.308[a]	88.8078[a]

Mean in the column, having the same letter(s) are not significantly different at P≤0.05, according to Least Significant Difference (LSD) method.

Table 7: Effect of Treatments on Potassium Uptake

Treatments	2WAP	4WAP	6WAP	8WAP	10WAP
$T_{1\text{-}control}$	0.0823[e]	0.160[e]	0.3588[e]	1.659[e]	2.6968[e]
$T_{2\text{-}10tns\ of\ pig\ manure}$	0.1508[d]	0.433[d]	0.9338[d]	2.854[b]	5.444[b]
$T_{3\text{-}15tns\ of\ pig\ manure}$	0.4138[c]	0.674[c]	1.3528[c]	2.485[c]	4.3183[c]
$T_{4\text{-}10tns\ of\ poultry\ manure}$	0.5798[b]	1.117[b]	1.8915[d]	1.8915[d]	4.3183[c]
$T_{5\text{-}15tns\ of\ poutry\ manure}$	0.621[a]	1.5103[a]	2.6923[a]	5.4563[a]	7.3908[a]

Mean in the column, having the same letter(s) are not significantly different at P≤0.05, according to Least Significant Difference (LSD) method.

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EVALUATION OF BIOCONTROL EFFICACY OF TRICHODERMA HARZIANUM AGAINST FUSARIUM OXYSPORIUM IN TOMATOES (SOLANUM ESCULENTUM L.)

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ABSTRACT

Utilizing biological techniques to manage plant diseases has demonstrated efficacy in fostering ecosystem sustainability and augmenting agricultural output and quality. A study was undertaken at the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria to evaluate the suppressive impact of T. harzianum on the proliferation of F. oxysporusm f. sp. lycopersici, and the advancement of F. oxysporum infection in tomato plants. The trial comprised culturing only T. harzianum in the Petri dish, culturing T. harzianum and F. oxysporum in the Petri dish (dual culture), and culturing only F. oxysporum in the petri dish, and with Mancozeb. The influence of T. harzianum on the advancement of F. oxysporum infection in tomato plants comprises of four distinct concentrations (1g, 2g, 3g, and 4g) of T. harzianum extract. The results revealed that the dual culture of T. harzianum and F. oxysporum successfully suppressed the mycelial growth of F. oxysporum. On the seventh day, the level of antagonistic activity exhibited by T. harzianum against F. oxysporum peaked at 9.05mm. Leaf yellowing and severe wilting, indicative of Fusarium wilt, were seen during the monitoring period. Tomato disease incidence and severity exhibited a consistent linear decline with increasing concentrations of T. harzianum. The disease severity reached its maximum level, (31.6%) on week four. Mancozeb treatment compared favorably with 4g T. harzianum in decreasing the mycelial growth of F. oxysporium, and reducing wilt incidence and severity. Therefore, T. harzianum can function as a biocontrol agent, providing a sustainable substitute for synthetic fungicides in the control ofFusariumoxysporium wilt disease.

Keywords: Biofungicides, fungal pathogens, Fusarium, Trichoderma, biocontrol

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INTRODUCTION

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Tomato plant, (*Solanum esculentum* L.), is widely recognized for its strong market demand, economic significance (Barari, 2016), medicinal properties, and abundance of essential elements such as vitamin B, vitamin C, phosphorus, potassium, and magnesium (Ali *et al*., 2020).*Fusarium oxysporium* is a significant soil-borne disease that has economic implications as it diminishes both the quality and quantity of tomatoes (Singh and Kamal, 2012). *F. oxysporum* f. sp. lycopersici is the primary cause of fusarium wilt disease in tomatoes. This disease poses a significant danger as it can lead to substantial economic losses, ranging from 10 to 90%, depending on environmental conditions (Singh and Kamal, 2012). The signs of the disease caused by *F. oxysporum* include leaf yellowing; stem browning, wilting, root browning, reduced root system, and necrosis. These symptoms lead to a decrease in photosynthesis in infected plants (Tomar *et al*.,2017). *F. oxysporum* spores (chlamydospores) in the soil make cultural and chemical control methods less successful in combating the

Traditional control methods are ineffective, and synthetic fungicides are toxic, expensive, and polluting (Senthilkumaret al., 2011; Sundaramoorthy and Balabaskar, 2013).

Biological control methods, such as Trichoderma, a mycoparasitic fungus, are cost-effective and risk-free alternatives to chemical pesticides for plant diseases (Zin and Badaluddin 2020; Ferreira and Musumeci 2021).Pathogenic antagonists in the biological control of diseases are environmentally and economically beneficial because they do not affect humans or animals (Alwathnani et al., 2012; Ghazalibiglar et al. 2016).

T. harzianum reproduces rapidly and outcompetes fungal pathogens for resources and space.

It exhibits antibiosis, fungistasis, and myco-parasitism (Howell, 2003; Ramezani, 2009; Soodet al., 2020). Trichoderma inhibits plant pathogenic fungi such Rhizoctonia, Fusarium, and Pythium, with varying genetics (Zin and Badaluddin 2020; Barbosa et al., 2022).Trichoderma directly affects pathogenic fungi by producing antibiotics and lytic enzymes like cellulases, hemicellulases, xylases, and chitinases (Qin et al.,2021). T. harzianum and T. virens produce Trichodermamine B and viridin, virone, and trichosetin, which may block the polygalacturonase enzyme released by F. oxysporum f. sp. lycopersici (Howell, 2003; Singh et al., 2022; Harman et al., 2004). The study aimed at evaluating the inhibitory effects of T. harzianum against F. oxysporium f. sp. lycopersici mycelial growth, and its wilt incidence and severity reduction for long- term sustainable disease management in Nigeria.

MATERIALS AND METHODS

Location of the study area

The study was conducted at the botanical garden of Imo State University, Owerri situated in the humid rainforest ecosystem of Nigeria, during the 2020/2021 cropping seasons. The latitude and longitude coordinates of the Imo State University, Owerri are:5.4891° N, 7.0177° E. This region is predominantly inhabited by farmers, and the ecosystem is known to contribute to the higher occurrence and intensity of tomato wilt (Terna et al., 2017). The laboratory experiment was conducted in the Department of Plant Science and Biotechnology, Imo State University Owerri.

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Soil analysis

The top 0- 3cm soil profile of the study area, at the start of the experiment, was processed and analyzed for some physical and chemical properties following standard procedures (Page, 1983; White, 1988) (See Table 1).

Experimental Design

The impact of *T. harzianum* on the mycelium growth of *F.oxysporium* was examined using a completely randomized block design (CRBD) consisting of four blocks and four treatments replicated three times. The treatments consisted of the following: *T. harzianum* in the Petri dish, culturing *T. harzianum* and *F. oxysporum* in the Petri dish (dual culture), and culturing only *F. oxysporum* in the Petri dish, supplemented with the positive control,(Mancozeb). Each treatment was replicated three times. The influence of *T. harzianum* on the advancement of *F. oxysporum* infection in tomato plants comprises of four distinct concentrations (1g, 2g, 3g, and 4g) of *T. harzianum* extract.

Isolation and identification of *Trichoderma harzianum*

The *T. harzianum* was isolated using the plate dilution method as described by Mendoza-Mendoza et al. (2016) and Brito-Vega (2020). Ten grammes of soil material, was individually suspended in 500 mL conical flasks containing 100 mL of sterilised distilled water. The mixture was then agitated for a duration of 5 minutes. Subsequently, one-milliliter portions were extracted using a graded micropipette (100-1000 µL) and transferred to test tubes containing 4 mL of sterile distilled water. Each sample was subjected to serial dilutions (1/10 w/v), and 0.5 mL portions from each dilution were evenly spread on Petri dishes containing PDA culture medium. The culture medium was supplemented with ampicillin and amoxicillin (1 mg mL-1 of each). A glass was used to uniformly distribute the sample material on the surface of the culture medium. Each dilution was replicated three times. The petri dishes were placed in an incubator set at a temperature of 25 °C ± 1 for a duration of eight days. Subsequently, the T. harzianum was isolated again to obtain pure culture. After a duration of eight days, samples of mycelium were extracted from the colonies using a platinum loop and applied onto PDA culture media using streaking. *T. harzianum* was identified by its quick and extensive development and its white-to-green coloration. A compound microscope was used to observe the morphological features of conidia and conidiophores.

Isolation and identification of *Fusariumoxysporium*

Following the methods outlined by Suwandi *et al.* (2022), *F.oxysporium* obtained from tomato roots exhibiting wilt symptoms in the field and from soil contaminated with *F. oxysporium* were transported to the laboratory. The diseased tomato root samples were cut into one-centimeter-long with a steriled sharp knife, and then washed under running water

and their surfaces were sterilised by immersing them in a solution containing 1% sodium hypochlorite for 2 minutes. Afterward, the segments were rinsed three times with distilled water and dried on filter paper in a laminar airflow. Subsequently, the samples were placed on a Petri dish containing 2% (w/v) agar and 0.1% streptomycin sulphate, which served to suppress bacterial growth. The samples were then kept in an incubator for a period of 48

hours. The mycelium was transferred to the Potato Dextrose Agar (PDA) medium using the single hyphae technique. The obtained isolation results were utilised for subsequent research. The fungus was subsequently identified by examining its culture characteristics and microscopic traits, as described by Leslie and Summerell (2006) and Jidda (2017).

Seed collection, sowing, and experimental procedures

Certified tomato seeds were obtained from the Imo State Agricultural Development Programme, Owerri in October 2022. The seeds were sun-dried and stored in a fabric bag, where they were maintained at an ambient temperature of 25°C. Prior to sowing, tomato seed were primed with sterile water for a duration of 10 hours. Aseptic technique was employed to gather spores from 14-day old cultures by scraping the surface of the Potato Dextrose Agar (PDA) using a sterilised spatula. The spore count was determined using a hemocytometer. The spores were diluted in sterile distilled water to achieve a final spore concentration of 1.0 x 106 colony-forming units per millilitre (CFU/ml). *T.harzianum* was measured at various concentrations of 1g, 2g, 3g, and 4g, derived from cultures that were 14 days old.

Pots with a top diameter of 24 cm, bottom diameter of 18 cm, and height of 20.5 cm were sterilised using a 10% solution of sodium hypochlorite in order to minimise the presence of unwanted germs. The pots were filled to a three-quarter capacity with loam soil, and was sterilised in the oven at a temperature of 80°C for a duration of 48 hours. Three tomato seeds were planted at a depth of 0.5 cm in each pot. Each pot received an identical volume of 300 ml of sterilised water, which had been treated in an autoclave. The tomato seedlings were reduced to one seedling per pot 10 days after they first appeared. *T. harzianum* was given at concentrations of 1g, 2g, 3g, and 4g, along with Mancozeb treatments, on the 14th day after emergence (DAE). Fourteen days after applying Trichoderma and Mancozeb treatments, ten millilitres of Fusarium oxysporium was administered to the pots. Each pot received a weekly application of 250 cc of sterile water, repeated four times.

Preparation of crude extracts of *Trichoderma harzianum*

The *T. harzianum*were cultivated on PDA medium and subjected to incubation at a temperature of 25 ± 2°C for a duration of 7 days. Subsequently, the fungal growth was carefully removed from the agar surface in order to get a mycelial sample weighing 5 g. Next, the mycelial growth was introduced into a solution of 200 mL of 80% ethanol containing 0.2 M HCl. The mixture was then placed on a rotating shaker and incubated at a temperature of 25 ± 2°C for a duration of 24 hours. The specimens were subsequently subjected to centrifugation at a speed of 9000 rpm in order to isolate the solid constituents. The solid components were subjected to a repeated extraction operation using ethyl acetate as the solvent. Ultimately, the extracts were amalgamated and subjected to partial evaporation utilising a rotatory evaporator.

The inhibitory effect of *T. harzianum*on *F. oxysporum f. Spplycospesici*

Following the procedure (Takudzwa*et al*.,2022), two distinct media were formulated, Potato dextrose agar (PDA) and PDA enriched with Mancozeb (75% WP). The two media were prepared individually in accordance with the manufacturer's instructions. The purified strains of *T. harzianum* and *F. oxysporum* were cultivated in large quantities using aseptic

techniques on potato dextrose agar (PDA) in Petri dishes with a diameter of 90 mm. Aseptic cork-borer was utilised to excise a mycelial disc with a diameter of 5 mm from pure cultures of *T. harzianum and F. oxysporum.* Subsequently, the two fungal discs were positioned 45 mm apart in dual cultures. The control experiments include 5 mm mycelial disc from pure cultures of *T. harzianum and F. oxysporum* cultivated alone, and *F. oxysporum* cultivated in PDA supplemented with Mancozeb (positive control). The Petri dishes were arranged in a Completely Randomised Block Design (CRBD) consisting of four blocks, with each treatment being reproduced three times. The plates were placed in an incubator set at a temperature of 25 ± 2°C for a duration of seven days. Sterile conditions were maintained throughout the whole procedure.

The mycelial growth inhibition

The formula for calculating mycelial growth inhibition is (C - T) / C, where C represents the growth of mycelium in the control group and T represents the growth of mycelium in the treatment group.

Effects of T. harzianum on Tomato wilt development

The frequency of disease occurrence was assessed on a weekly basis by enumerating the number of plants exhibiting signs of *F. oxysporum* within a period of 1 to 4 weeks following inoculation. The calculation was performed via the formula provided below:

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100$$

The frequency of disease occurrence was assessed on a weekly basis by enumerating the quantity of tomato plants exhibiting wilt symptoms during the time frame of 1 to 4 weeks after inoculation.

The disease severity

The severity of the disease was evaluated on a weekly basis, beginning from 2 weeks after inoculation (WAI), using a grading system ranging from 1 to 5. The rating was determined based on observable symptoms such as yellowing, wilting, and necrosis on both the leaves and stem. The rating scale ranged from 0 to 5, with 0 indicating healthy plants with no symptoms. A rating of 1 indicated 1-2 leaves showing yellowing, wilting, or necrosis, accounting for 1-25% of the plant. A rating of 2 indicated 3-5 leaves showing these symptoms, accounting for 26-50% of the plant. A rating of 3 indicated 6-8 leaves showing symptoms, accounting for 51-75% of the plant. A rating of 4 indicated 9-11 leaves showing symptoms, accounting for 76-100% of the plant. Finally, a rating of 5 indicated that the plant was dead.

DATA ANALYSIS

All data collected underwent statistical analysis using analysis of variance (ANOVA). The significance of differences between means was assessed using Tukey's HSD test ($p < 0.05$) with the use of MINITAB 19 software.

RESULTS

Effect of *T. harzianum* on mycelium growth of *F. oxysporum*.

Notable disparities were observed in the growth of *F. oxysporum* in pure culture, dual culture, and mancozeb on the third and seventh days following inoculation. In a dual culture setting, the presence of *T.harzianum* resulted in a significant ($p < 0.05$) decrease in the growth of *F. oxysporum* mycelium between 3 and 7 days after inoculation (DAI). A significant difference ($p < 0.05$) was seen in the inhibition of *F.oxysporium* mycelial growth between different days of inoculation of *T. harzianum* and control treatments (Table 2). *T. harzianum* exhibited the most pronounced growth inhibitory activity (9.05mm) against *F. oxysporum* on the 7th day of incubation, whereas day 3 demonstrated the least inhibitory activity (17.2mm), (Table 2). The use of Mancozeb resulted in the most significant decrease in mycelial development during the observation periods.

The mycelial diameter exhibited a negative correlation with the inhibition percentage in the dual culture, (Table 2).Our findings indicate that the mycelial growth of *F. oxysporium* differed among the various treatments and observation days.

Field Disease incidence

The symptoms of Fusarium wilt disease showed substantial variation across all treatments during the study periods. During the monitoring period, we saw typical indications of Fusarium wilt, such as leaf yellowing and severe wilting. The onset of yellowing symptoms were observed on the older leaves, subsequently leading to chlorosis and wilting of the foliage. The seedlings from the sterile water exhibited no signs of disease throughout the whole assessment period.

The plants that were treated with Mancozeb had the lowest percentage of disease incidence, (15%). On the other hand, the treatment with sterile water had the greatest percentage of disease incidence (95%),(Fig.1).The impact of varying doses of *T. harzianum* on the rate of tomato wilt disease incidence was statistically significant ($P\leq 0.05$). The occurrence of tomato wilt disease decreased in a linear manner as the concentrations of *T. harzianum* increased (Fig.1).

Efficacy of *Trichoderma harzianum* against *Fusarium oxysporum* wilt disease severity in tomato plants from week 1 to 4 after inoculation.

The severity of the disease progressively escalated from week 1 to week 4. Week 4 exhibited the most notable disease severity, (31.6%) (Table 3). The negative control (sterile water) exhibited the highest disease severity (38.08%), while 4g of *T. harzianum*recorded 6.03%. In contrast, mancozeb had the lowest disease severity at 3.64% across the monitoring periods (Table 3).

The impact of varying concentrations of *T. harzianum* on the severity of tomato wilt disease was statistically significant ($P\leq 0.05$). The severity of tomato wilt disease showed a linear decline as the concentrations of *T. harzianum* increased, (Table 3).

DISCUSSIONS

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The result of the study showed that *Trichoderma harzianum* is a suitable biocontrol agent for the *F. oxysporium* wilt disease due to its effectiveness in inhibiting the growth of *F. oxysporium* and decreasing wilt incidence and severity of tomato.

The inhibition of *F. oxysporium* mycelial growth by *T. harzianum* observed in this study was similar to previous findings of Lakhdari et al.,(2018) who demonstrated the the mycelial growth inhibition of *T. harzianum* against *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Alternaria solani*, and Carvalho et al.,(2014), against *Fusarium oxysporum f. sp. Phaseoli.* In addition, the findings are supported by Alwathnani *et al*.,2012; Sundaramoorthy and Balabaskar,2013) who documented the suppression of *Fusarium oxysporum* spp by Trichoderma spp.

The suppression of *F. oxysporium* f spp lycospesici mycelial growth by *T. harzianum* may be attributed to the synthesis of antibiotics (Bhardwaj and Kumar, 2017), the release of cell wall degrading enzymes leading to cellular damage (Zhao *et al*., 2014), and the high colonisation rates of *T. harzianum*, which suppress the growth of competing microorganisms (Bizos *et al*., 2020).

The decreased tomato wilt disease incidence by the application of *T. harzianum* observed in the present study was consistent with the report by (Harman, 2005; Moosa *et al*.,2017) who documented a decrease in the occurrence of tomato wilt disease incidence due to the application of *T. harzianum*.

The decreased tomato wilt disease severity by *T. harzianum* application observed in the present study collaborates with Khalil, and Shimaa, (2020) who reported decreased foliar yellowing and wilt or vascular browning of tomato, and Haque et al.,2023, who reported drastic reduction of tomato wilt severity and improved tomato growth and biomass.Inaddition, application of *T. harzianum* on peanut seeds was reported to reduce both the incidence and the severity of peanut brown root rot (Erazo et al., 2021).The decrease in wilt disease severity by *T. harzianum* may be attributed to the synthesis of secondary metabolites, increased enzymatic activity, and changes in the pathogen hyphae (Erazo et al., 2021).

CONCLUSION

The strong antagonistic activity of *T. harzianum* against *F. oxysporium* growth, and reduction of the wilt disease incidence and severity, therefore qualify *T. harzianum* to be exploited in the development of natural fungicides to avoid the negative effects of synthetic fungicides.

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APPENDICES

Table 1. Physical and chemical properties of the soil profile of the study area

Sand	**Silt**	**Clay**	**pH**	**Organic matter**	**Organic carbon**	**Nitrate**	**PO_4**	**SO_4**	**Ca^2+**	**Mg^2+**	**K2+**	**Na^2+**	**CEC**
830 kg–1	330 kg–1	90g kg–1	6.01	20 g kg–1	0.96 g kg–1	12.6 mg/kg	4.21 mg/kg	121.73 mg/kg	7.62mg /kg	7.41 mg/kg	6.69 mg/kg	1.59mg /kg	23.32 Ca/mg

Table 2. Effect of *T. harzianum* on mycelium growth inhibition of *F. oxysporum* (mm)

	Mycelium growth (mm) on different days			F- value	P- value
Treatments	Day 3	Day 5	Day 7		
Trichoderma only	17.48(0.47)c	78.54(0.41)b	86.76(0.23)a	29722.68	0.002
Fusarium only	32.14(0.18)c	53.91(0.33)b	69.00(0.31)a	13144.66	0.001
Dual culture	17.32(0.11)a	12.999(0.28)b	9.047(0.27)c	930.90	0.003
Mancozeb **(**400 ppm**)**	12.31(0.58)a	6.51(0.48)b	5.36(0.31)b	187.08	0.001

Means that do not share a letter within a column in a treatment are significantly different (Turkey's HSD test ($p < 0.05$).Numbers in brackets are ± standard deviation.

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Figure1: Effects of *Trichodermaharzianum* at different concentrations on percentage disease incidence.

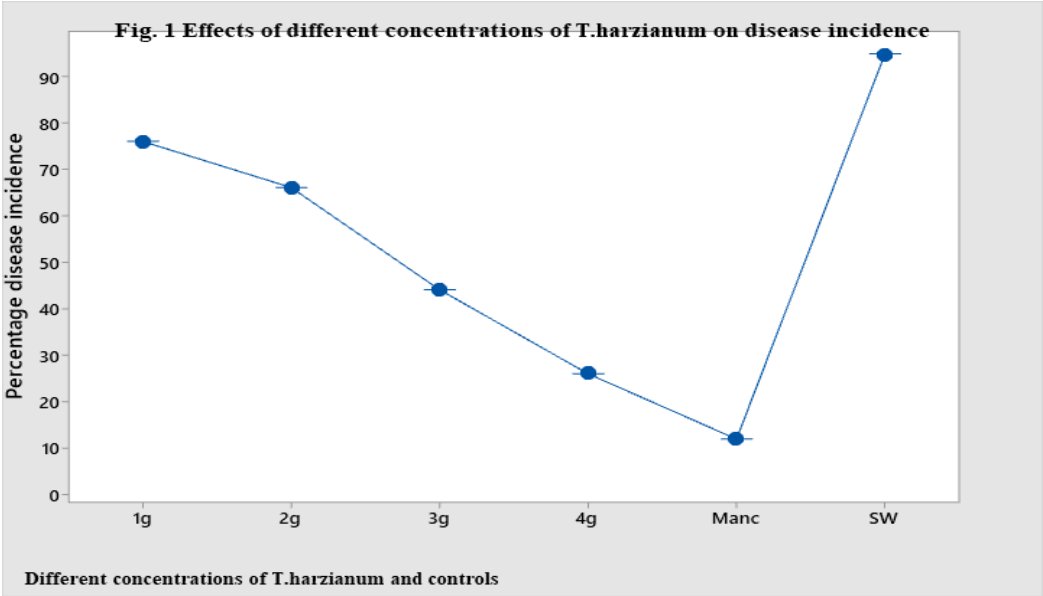

Table 3: Effects of *Trichoderma harzianum* at different concentrations on percentage disease severity from week 1 to 4 after inoculation

Treatments	WK1	WK 2	WK 3	WK 4	F-value	P-value
SW	0.51(0.09)d	20.40(0.53)c	50.60(0.36)b	80.82(0.42)a	24929.38	0.001
1g	0.403(0.025)d	10.40(0.10)c	23.43(0.38)b	36.23(0.25)a	13415.43	0.002
2g	0.33(0.083)d	10.40(0.10)c	30.07(0.12)b	54.3(0.40)a	11691.62	0.000
3g	0.23(0.064)d	3.04(0.05)c	14.40(0.20)b	23.37(0.32)a	9096.37	0.000
4g	0.17(0.12)c	1.27(0.21)c	12.97(1.42)b	15.50(0.10)a	356.96	0.001
Manc.	0.03(0.06)c	0.83(0.47)c	5.43(0.32)b	8.70(0.36)a	431.80	0.000

Means that do not share a letter within a column in a treatment are significantly different (Turkey's HSD test ($p < 0.05$). Numbers in brackets are ± standard deviation.