

PROSPECTING FOR PLANT GROWTH PROMOTING FUNGI AND BACTERIA IN THE RHIZOSPHERE MICROBIOME OF MAIZE FOR THE DEVELOPMENT OF BIOFERTILIZERS AND BIOPESTICIDES

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Abstract

The rapid increase in the global population has led to a greater demand for agricultural production to ensure food security. To meet this demand, commercial fertilizers, which are known to pose health and environmental risks, are often used to achieve high crop yields. However, recent research has shown that the rhizosphere microbiome of maize contains beneficial plant growth-promoting fungi (PGPF) and bacteria (PGPB) that can be utilized for sustainable agriculture, potentially reducing the need for harmful chemical fertilizers. This study aimed to identify and characterize PGPF and PGPB in the rhizosphere microbiome of maize to develop biofertilizers and biopesticides. The researcher employed a culture-dependent approach to analyze the growth promoting microbial communities associated with maize roots. The results revealed a variety of beneficial microorganisms, including species of *Bacillus* and *Trichoderma*, known for their ability to enhance plant growth and suppress pathogens. Furthermore, the researcher evaluated the biofertilizer and biopesticide potential of selected isolates in screen house trials using a complete randomized design with four treatments (Control, NPK, Fungi inoculum, and Bacterial inoculum) and three replicates. The effects of these treatments on growth parameters (plant height and leaf length) was compared using a composite bar chart and the potential for controlling leaf spot disease (caused by *Bipolaris* spp.) was assessed and establishing the significant difference between the treatment means using the least significant difference method. The results indicated that the application of these microbial strains significantly improved maize growth and provided protection against phytopathogens. This research highlights the potential of using PGPF and PGPB from the maize rhizosphere microbiome as an alternative to commercial fertilizers, offering a more sustainable approach to agriculture.

Key words: Plant Growth Promoting fungi, Plant Growth Promoting Bacteria, microbiome, Biofertilizer and Biopesticides

1. Introduction

The burgeoning global population has spurred the need to enhance agricultural productivity for greater crop yields and overall production, aiming to ensure food security (FAO, 2017). The ecosystem, soil fertility, and the growth of cultivated crops are adversely affected by the overuse of chemical pesticides (Rahman, 2018). Continuous use of synthetic chemicals for crop protection also gives rise to additional problems such as the loss of topsoil, soil infertility, reduction in plant growth, lower yield indices, and a gradual decline in native microbial diversity are challenges that must be addressed for the realization of sustainable agriculture. To attain this goal, crops require attributes such as disease resistance, tolerance to salt, drought, and heavy metal stress, and enhanced nutritional value. A promising avenue for achieving these objectives involves the utilization of plant growth-promoting microorganisms (PGPMs), particularly bacteria and fungi. These microorganisms have the ability to enhance a plant's nutrient absorption and water use efficiency, as well as induce resistance against various plant diseases (Armada, 2014; Kumar, 2018).

Studies have highlighted the potential of plant growth-promoting fungi (PGPF) and plant growth-promoting rhizobacteria (PGPR) sourced from soil or the plant rhizosphere (Vessey, 2003 Perotto and Bonfante, 1997). These microorganisms can serve as biofertilizers, bio-stimulants, and inducers of resistance against both abiotic and biotic stresses (Hossain, 2017). However, the interactions among microorganisms, crops, and the soil environment—especially in the rhizosphere—are intricate and can impact the efficacy of PGPM application. Various factors influencing these interactions include environmental conditions like soil temperature and pH, as well as crop genotype and existing microorganisms present in the soil.

Maize (*Zea mays*) holds significant importance globally as a major cereal crop for both food and feed, ranking third in total world production after wheat and rice, maize is a staple food in many countries, particularly those in the tropics and sub-tropics (Mohammadi, 2017). In recent years, there has been a significant 59.60% increase in the use of nitrogen fertilizers in the cultivation of new maize plant varieties, leading to notable environmental concerns (Abdel, 2000). To address this issue, there is a growing emphasis on maximizing the economic use of fertilizers while minimizing the environmental impact of chemical fertilizers. In this context, biofertilizers are emerging as a promising alternative for enhancing maize and other crop species production.

Biofertilizers offer an environmentally friendly and cost-effective solution, allowing for on-farm bulk production if needed. They contribute to a 10–40% increase in crop yield, with up to 50% of nitrogen fixation (Kumar 2018). Continuous application over 3–4 years can sustain fertility due to the efficiency of parental inoculums, effectively supporting plant growth and multiplication.

The term 'biopesticide' refers to the use of beneficial microorganisms to control insects, but a key challenge is the availability of biopesticides relative to the total cropped area. Biopesticides, derived from natural materials such as plants, animals, bacteria, and certain minerals, fall into three main categories: biochemical, plant, and microbial pesticides. Worldwide, 90% of all biopesticides are in use, with *Bacillus thuringiensis* (Bt) being the most commercially successful in the market (Valicente, 2010). Modern agriculture demands the use of biopesticides and biofertilizers to ensure safe and residue-free crop production (Swapna, 2013).

2 Materials and methods

2.1 Experimental Sites

The three experimental sites used for the purpose of the study were: the field, laboratory and the screen house in the College of Agricultural Sciences, Olabisi Onabanjo University, Ayetoro Campus, Ogun State, Nigeria.

i.) **Field:** The preliminary stage of the study was carried out on the Demonstration Farm of the College of Agricultural Sciences, Olabisi Onabanjo University, Ayetoro Campus, which lies on the latitude 7° 12' N and longitude 3° 3' E of Ogun state. The Demonstration farm was ploughed and harrowed while a portion of the farm measuring 10m x 10m was carved out and cropped with maize (Hybrid maize variety Oba super) with spacing of 75 cm x 25cm. Agronomic practices of thinning, supplying and weeding were carried out on the carved plot and the root samples of the maize plants were collected 6 weeks after planting as input for the next stage of the study

ii.) **Laboratory:** The second stage of the study took place in the soil laboratory of the Crop production department in the College of Agricultural Science, Olabisi Onabanjo University Ayetoro Campus for the purpose of extraction, culturing and identification of microorganisms from the maize root samples collected from the field. The microbes were extracted from the maize plant roots, cultured, subcultured and thereafter incubated. The growth media for the growing of the microorganisms prepared in the laboratory were Potato Dextrose Agar (for Fungi), Nutrient Agar (for Bacteria) while Starch Agar was also prepared and used during the Starch hydrolysis test for the confirmation the bacteria strain.

iii.) **Screen House:** The final and major stage of the study which involved testing the effects of identified fungi and bacteria as biofertilizers and biopesticides was conducted on potted maize plant (Hybrid maize variety Oba super) in the screen house of the Crop Production Department of Olabisi Onabanjo University, Ayetoro Campus

2.2 SOIL TESTS

Composite soil samples of the field were collected at an early hour of the day before sunrise. The sample was air dried, packed and sent to the laboratory for nutrient status analysis. In the same vein the composite soil samples for potted plants grown in the screen house was also air dried, packed and sent to the laboratory for nutrient status analysis.

2.3 Extraction and Inoculation of Microorganisms in the Laboratory

Maize root samples collected from field were taken to the Laboratory, washed with running tap water, followed by sterile distilled water and sterilized with absolute ethanol. Then roots were air dried and pounded in a mortar. 10g of the pounded gel was collected with the aid of spatula and placed in a sterilized test tube. 10ml of sterile distilled water was pipetted to the test tube. The suspension was serially diluted to 10^{-2} and 10^{-5} for extraction of bacteria and fungi respectively. 1 ml of the serially diluted suspensions (10^{-2} and 10^{-5}) were inoculated into plates of Nutrient Agar(NA) and Potato Dextrose Agar(PDA) respectively with the aid of sterilized pipette. The inoculated plates were kept in the inoculating chamber. Bacterial growth was observed in the nutrient agar plates at 24 hours while fungal growth was observed in the PDA plates at 48 hours.

2.3 Laboratory Preparation of Potato Dextrose Agar (PDA)

The Kunze and Zipfel (1999) procedures for preparation of PDA was adopted in the preparation of PDA in the laboratory. 100g of clean Irish potato was rinsed with water and peeled. The boiled in 500 ml of distilled water for 45minutes till it softened, allowed to cool and the potato water mixture was strained through a cheesecloth to obtain potato extract. 15g of Agar and 20g of Glucose was added to the potato extract and mixed thoroughly. The mixture was autoclaved at 121°C for 20 minutes and allowed to cool. It was then poured into four sterilized petri dishes to solidify in readiness for inoculation of fungi.

2.4 Laboratory Preparation of Nutrient Agar

The Sherris *et al* (1943) procedures for preparation of Nutrient Agar was adopted in the preparation of nutrient agar in the laboratory. 14g of nutrient agar was added to 500 ml of distilled water. The mixture was boiled in a beaker until it dissolved completely. It was allowed to cool and later autoclaved at 121°C for 20 minutes to sterilize it. The autoclaved mixture was then poured into sterilized petri dishes in readiness for inoculation with 10^{-2} serially diluted suspension of grinded maize root extract.

2.5 Identification and Confirmation of Isolated Bacteria

The methods used in the characterization and confirmation of isolated bacteria in the Laboratory were: Morphology, Gram stain test and Starch hydrolysis test

2.5.1 Morphology

A small drop of bacterial suspension was placed on a sterilized glass slide and covered with a cover slip. The covered glass slide was positioned on the stage of a compound light microscope. The magnification was gradually increased from 10x until a finer detail was obtained. A clear well lit image was then captured.

2.5.2 Gram Stain Test

A small drop of gram stain was pipetted into a plate of the bacterial suspension. The observed colour of the mixture which was blue was recorded.

2.5.3 Starch Agar Preparation and Inoculation of Isolated Bacteria

Benson (2002) procedures for preparation of starch Agar and inoculation of isolated bacteria was adopted. 0.5g of starch powder and 2.5g of Agar powder was dissolved in 100 ml of distilled water. The mixture was sterilized in an autoclave at 121°C for 15 minutes and allowed to cool. It was poured into two sterilized petri dishes and allowed to solidify. A swab of the isolated bacteria was streaked across the plate with an inoculating loop. Bacterial growth was observed after 24 hours and the plate was kept in incubation box at 37°C for 48 hours.

2.5.4 Starch Hydrolysis Confirmatory Test

After 48 hours of incubation of the inoculated Starch Agar plate, 2 drops of 10% iodine solution was added to the edge of the inoculated starch Agar plate, the colour change was observed and recorded after 15 minutes.

2.6 Identification of Fungi

In characterizing the fungi, the morphology, colony growth pattern, density, color mycelia zoning, and diameter of the colonies were observed macroscopically on the sterile PDA medium.

2.7 Experimental Design Used in the Screen House

A Completely Randomized Design (CRD) which consisted of four (4) treatments and three (3) replicates were used in structuring the study conducted in the screen house.

2.8 Experimental Method used in the Screen House

Each treatment consisted of three (3) polyethylene bags (measuring 29cm x 47 cm) filled with loamy soil from valley bottom and placed in the screen house. The loamy soil was air dried, grounded and sieved with 2mm mesh before use. For the control, no treatment was applied to the hybrid maize variety Oba Super seeds before and after planting inside the polyethylene bags and this served as treatment 1. Treatment 2 had NPK 20:10:10 applied to the hybrid maize plants variety Oba super 2 weeks after planting inside the polyethylene bags. Treatment 3 had hybrid maize variety Oba super seeds inoculated with identified fungus before planting inside the polyethylene bags while in treatment 4 the hybrid maize variety Oba super seeds were inoculated with identified bacteria before planting inside the polyethylene bags.

2.9 Data Collection from the Screen House

The plant height (cm) and leaf length (cm) of the maize plant were measured, while number of maize leaves with leaf spot disease were counted four times at an interval of three weeks and recorded for all the four treatments. The Disease scoring was generated based on occurrence of leaf spot on maize plant leaves and graded using Kumar *et al.*, 2013 Disease scoring scale

2.9.1 Method of Data Analysis

The data recorded from the measurements taken from the screen house were analyzed using graphical presentation and one-way ANOVA at 5% level of significance ($P < 0.05$) while the significant difference of the means were analyzed using Least Significant Difference

3. Results

3.1 Chemical Properties of the Soil used for the Experiment

Chemical analyses conducted before the experiment indicated the value of exchangeable bases (Ca, Mg, Na and K), effective Cation Exchange Capacity (ECEC), Electrical conductivity (EC), available nitrogen, organic matter and bulk density measured before the experiment and the result is presented in (Table 3.1).

Nutrient analyses of the soil before the experiment revealed a moderate soil fertility level. The concentration of exchangeable bases; Ca, Mg, Na and K, in the soil were moderate.

Table 3.1 Chemical Analysis of the Soil used for the experiment

PARAMETERS	VALUE
BULK DENSITY(g/ml)	0.607
Ca(Cmol/kg)	5.63
Mg(Cmol/kg)	3.72
Na(Cmol/kg)	1.48
K(Cmol/kg)	4.94
EC(mS/M)	315.69
ECEC(Cmol/kg)	15.77
%ORGANIC MATTER	2.137
%AVAILABLE N	0.413

3.2 Identification of plant growth promoting bacteria available from the root extract of maize plant

The morphological examinations of the specimen as shown in plate 1 indicated large circular, circular, white, rough, flat and opaque colony structure which were in line with the standard features of *Bacillus subtilis*. The rod-shaped cells displaying central spore formation are consistent with the recognized traits of this specie. The identification of *Bacillus subtilis* is reinforced by the medium-sized colonies with smooth surfaces. Additionally, the bacterium demonstrated positive characteristics in Gram staining (plate 2). Following the administration of starch hydrolysis test on inoculated starch agar plate, the enzymes (α -amylase and oligo-1-6 glucosidase) secreted by the bacterial cell hydrolyzes the starch thus creating a clear zone around the zone of bacterial growth. Plate 3 revealed a distinct zone around the bacterial growth. The emergence of this clear zone signifies that starch was hydrolyzed and thus the presence of *Bacillus subtilis* was confirmed as positive to starch hydrolysis test.



Plate 1 Nutrient Agar plate



Plate 2: Gram staining

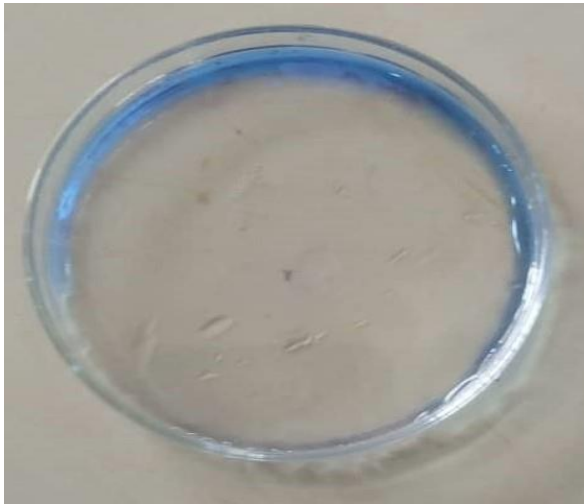


Plate 3: Starch Hydrolysis

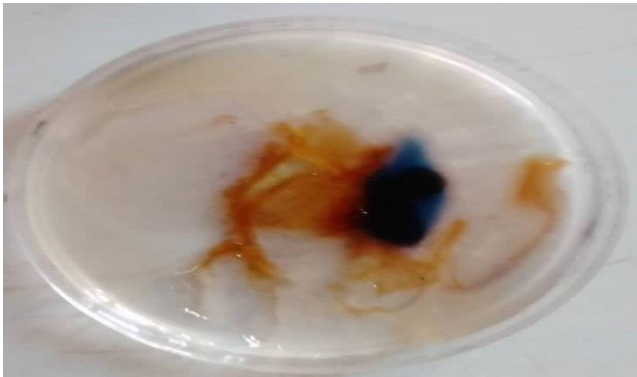


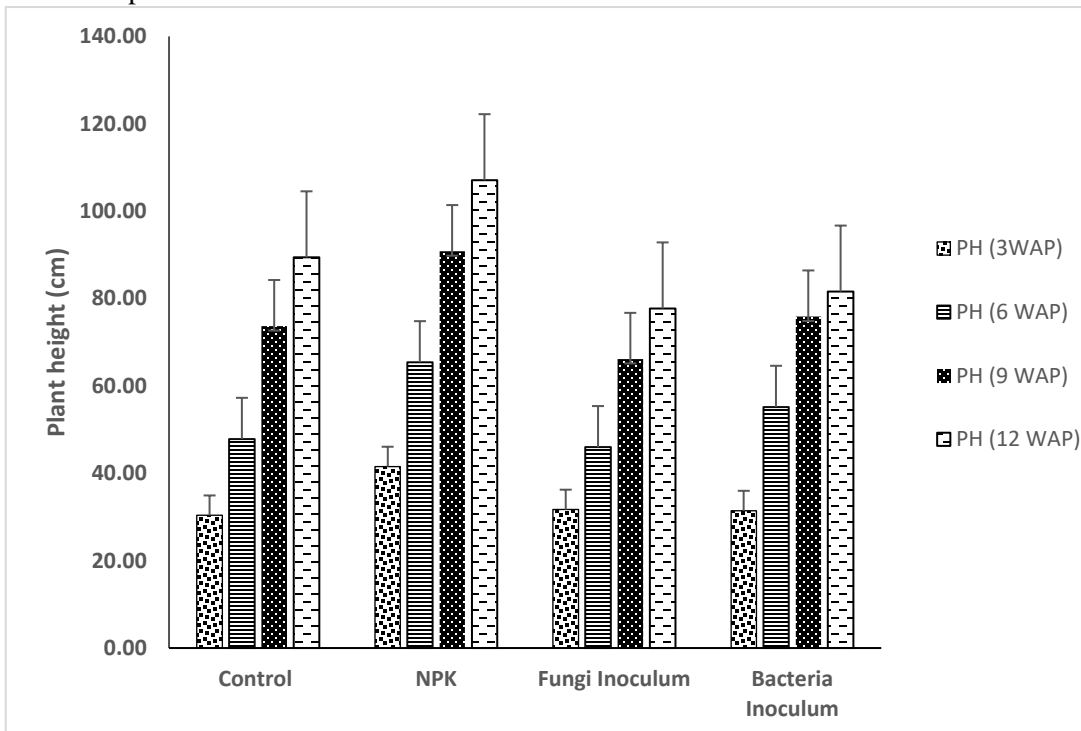
Plate 4: Trichoderma on PDA

3.3 Identification of plant growth promoting Fungi from the root extract of maize plant

The morphological examination of the specimen indicated colonies with thick and velvety texture and dark green color which were standard features of *Trichoderma*. The colonies grew covering the media. Upper and lower surface show a dark green color with a ring pattern evident in green. At 48 hours, the mycelia began to thicken as cotton and white mycelia and colony size increased up to 60 mm. Colony showed a radial growth pattern with a concentric rings of green and white color. Subglobose form of conidia, conidiophores with many branch, phyalid shape of flask, ampulliform and lageniform were observed. These further confirmed *Trichoderma*.

3.4 Comparism of the effect of the identified organisms with the use of inorganic fertilizer on the growth of maize.

Fig 3.4a overleaf shows the effect of the applied inoculum and inorganic fertilizer(NPK) on Plant Height(cm) at different stages of maize plant growth. At 3 weeks after planting the effect of NPK on maize plant indicated the plant height of 41.52 cm while the fungi inoculum indicated plant height of 31.62 cm. The Bacteria inoculum indicated the plant height of 31.43cm while the control experiment indicated plant height of 30.37cm. The highest plant height (41.52cm) value was recorded in NPK while the lowest plant height (30.37cm) was recorded in the control experiment. At 6 weeks after planting, the highest plant height(65.40cm) was recorded in NPK treated maize plant while the lowest plant height (45.97cm) was recorded in fungi inoculated maize plant, the bacteria inoculated maize recorded the plant height of 55.18 cm while the control experiment recorded 47.85cm. At 9 weeks after planting, the highest plant height(90.81cm) was recorded in NPK treated maize plant while the lowest plant height (66.14cm) was recorded in fungi inoculated maize plant, the bacteria inoculated maize recorded the plant height of 75.85 cm while the control experiment recorded 73.66cm. At 12 weeks after planting, the highest plant height (107.08cm) was recorded in NPK treated maize plant while the lowest plant height (77.73cm) was recorded in fungi inoculated maize plant, the bacteria inoculated maize recorded the plant height of 81.57 cm while the control experiment recorded 89.42cm.



Fig

3.4a: Effect of the applied inoculums and inorganic fertilizer on Plant Height at different growth stages of maize

3.4b Effect of the applied inoculums and inorganic fertilizer (NPK) on Leaf Length at different growth stages of maize

Fig 3.4b overleaf shows the effect of the applied inoculums and inorganic fertilizer(NPK) on leaf length(cm) at different stages of maize plant growth. At 3 weeks after planting the effect of NPK on maize plant indicated the leaf length of 32.36cm while the fungi inoculum indicated leaf length of 24.77cm. The Bacteria inoculum indicated the leaf length of 23.77cm while the control experiment indicated leaf length of 24.73cm. At 6 weeks after planting the effect of NPK on maize plant indicated the leaf length of 48.32cm while the fungi inoculum indicated leaf length of 35.00cm. The Bacteria inoculum indicated the leaf length of 41.51cm while the control experiment indicated leaf length of 36.92cm. At 9 weeks after planting the effect of NPK on maize plant indicated the leaf length of 64.54cm while the fungi inoculum indicated leaf length of 49.57cm. The Bacteria inoculum indicated the leaf length of 55.86cm while the control experiment indicated leaf length of 54.15cm. At 12 weeks after planting the effect of NPK on maize plant indicated the leaf length of 59.56cm while the fungi inoculum indicated leaf length of 51.79cm. The Bacteria inoculum indicated the leaf length of 46.43 while the control experiment indicated leaf length of 59.41cm.

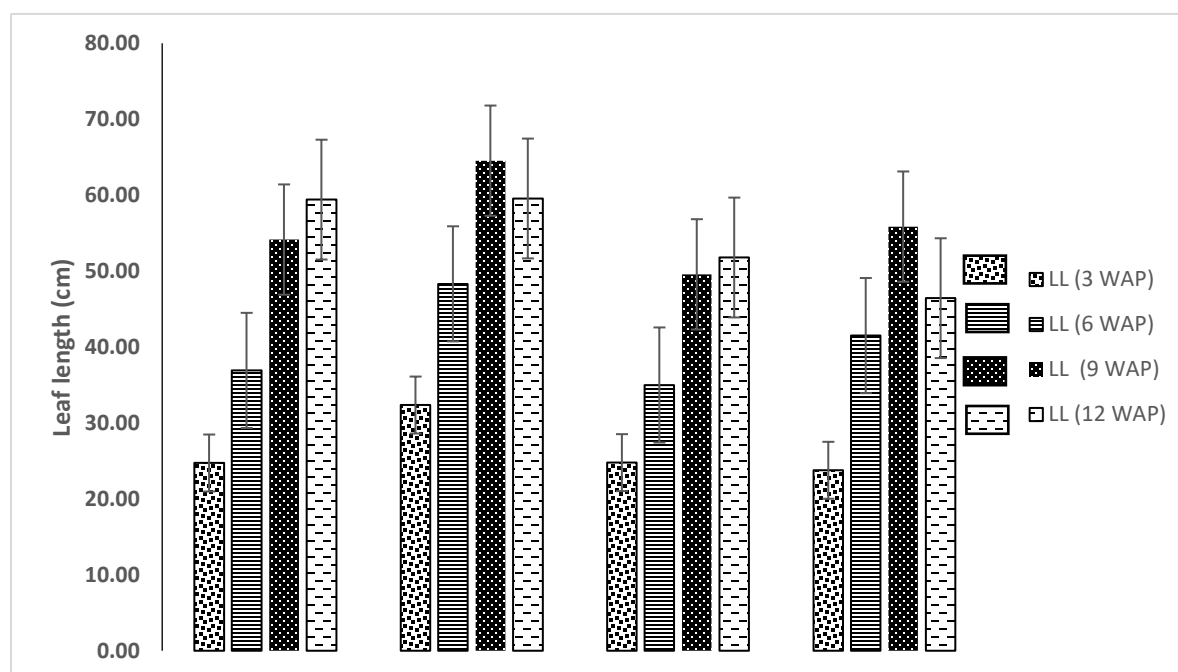


Fig 3.4b: Effect of the applied inoculums and inorganic fertilizer on Leaf Length at different growth stages of maize

3.5 Effects of identified organisms as biopesticide for disease control

Table 3.5 overleaf shows the effect of the applied inoculums and inorganic fertilizer (NPK) on leaf spot disease occurrence at the different stages of maize plant growth. At 3 and 6 weeks after planting, all treatments, including the control, show a disease score of 0.00. At 9 weeks after planting, the disease scores of 2.00 was recorded while a slightly higher disease score of 2.20 was recorded for the NPK treated maize. However, a lower disease score of 1.67 was recorded for the maize plant treated with fungi inoculum while disease score of 0.00 was recorded for maize treated with bacteria inoculum. At 12 weeks after planting, the disease score of 2.33 was recorded for control treatment while the disease for NPK treated maize was 2.27. The disease score of 2.27 was recorded for maize treated with fungi inoculum while the disease score of 1.73 was recorded for maize treated with bacteria inoculum. The Least Significant Difference (LSD)

value indicated 'Ns' (not significant) for the 3 and 6 WAP stages while for the 9 and 12 WAP stages, the LSD is 0.39. This suggested that differences in disease scores of 0.39 or more between treatments are statistically significant.

Table 3.5: Table of Least Significant Difference of Leaf Spot Disease between the treatment means

	Disease scoring for Leaf Spot			
	3 WAP	6 WAP	9 WAP	12 WAP
Control	0.00	0.00	2.00	2.33
NPK	0.00	0.00	2.20	2.27
Fungi Inoculum	0.00	0.00	1.67	2.27
Bacteria Inoculum	0.00	0.00	0.00	1.73
LSD	<i>Ns</i>	<i>Ns</i>	<i>0.39</i>	<i>0.39</i>

Key

WAP: Weeks After Planting 3WAP: Vegetative stage 6 WAP: Vegetative stage 2

9WAP: Reproductive stage 12WAP: Grain filling stage

4.0 Discussion, Conclusion and Recommendation

4.1 Discussion

The differences recorded in the effect of the four treatments on the growth parameters (Plant Height and Leaf Length) is an indication that some of the treatments significantly influenced the growth parameters. NPK treatment consistently resulted in the highest plant height at each stage of measurement (3, 6, 9, and 12 WAP), indicating that NPK fertilizer promotes better growth compared to other treatments. The Control treatment show the lowest growth throughout the observation period, this suggested that the absence of any treatment results in the least growth. The fungi and bacteria inoculum treatments resulted in higher plant heights compared to the control but were generally lower than the NPK treatment. Between the two, Bacteria inoculum showed a slight edge over Fungi Inoculum in terms of plant height, especially noticeable at 6, 9, and 12 WAP. At 3 WAP, the NPK treatment showed a significant increase in height compared to the control and other treatments. At 6 WAP, the trend continues with NPK-treated plants showing the highest growth, followed by Bacteria Inoculum, Control, and Fungi Inoculum. By 9 WAP, all treatments show substantial growth, but NPK still leads, followed by Bacteria Inoculum, Control, and Fungi Inoculum. At 12 WAP, the plants treated with NPK reach over 107 cm, while the Control group just under 90 cm, and the Fungi and Bacteria Inoculum treatments reach around 77 cm and 82 cm, respectively. NPK treatment consistently results in the longest leaf length at 3, 6, and 9 WAP. However, by 12 WAP, the leaf length is similar to that of the Control treatment. Control treatment shows a steady increase in leaf length over time, generally performing better than the Fungi and Bacteria Inoculum treatments at most stages. Fungi Inoculum treatment displayed the shortest leaf lengths at most stages, indicating that it may be the least effective treatment for increasing leaf length. Bacteria Inoculum treatment showed better performance than Fungi Inoculum, especially at 6 and 9 WAP, but falls behind the Control and NPK treatments by 12 WAP. The NPK fertilizer significantly enhances maize leaf length compared to the Control, Fungi Inoculum, and Bacteria Inoculum treatments, particularly at earlier stages (3, 6, and 9 WAP). At 12 WAP, the difference between NPK and Control treatments diminishes. The Bacteria Inoculum treatment showed some beneficial effects on leaf length, especially at 6 and 9 WAP, but was less effective than NPK. Fungi Inoculum was the least effective treatment for promoting leaf length growth. This significant difference in effect of the treatments on the growth parameters was similar to the one observed in a study conducted by Yosefi *et al.*,

(2011) which stated that microbial inoculants or biofertilizers are formulations containing viable algae, fungi, and bacteria either individually or in combinations to support plant growth and enhance crop yield. The constituents of biofertilizers which are beneficial microbes positively impact soil chemical and biological characteristics by nitrogen fixation, cellulolytic activity, or phosphate solubilization. The application of these biofertilizers to seeds, plant surfaces, roots, or soil, they inhabit the rhizosphere, exerting biological activity that improves nutrient bioavailability, fosters plant growth, and enhances soil microflora, thereby elevating soil fertility (Umehsa *et al.*, 2018). In congruence with the significant effect of the biofertilizers on the growth parameters observed in this study Batista *et al.*, (2018) posited that inoculation of *Bacillus spp.* RZ2MS9 and *Burkholderia ambifaria* RZ2MS16 strains promoted corn and soybean growth under greenhouse conditions. Additionally, the plant growth-promoting potential of *Arthrobacter arilaitensis* and *Streptomyces pseudovenezuelae* has been demonstrated in enhancing maize growth under water-stressed conditions (Chukwuneme *et al.*, 2020).

The differences observed in the effect of the four treatments on the occurrence of leaf spot disease indicated that some of the treatments showed potentials to control the severity of the disease. At 3 and 6 WAP, all treatments, including the control, showed disease score of 0.00. This indicated that there was no visible leaf spot disease at these early stages across all treatments. At 9 and 12 WAP the control treatment showed a steady increase in disease severity, indicating natural disease progression while NPK treated maize showed similar trend to the control but slightly higher at 9 WAP and slightly lower at 12 WAP, indicating that NPK fertilization does not significantly affect disease progression. The fungi inoculum treated maize showed a lower disease score at 9 WAP compared to control and NPK which indicated some potential for protection against leaf spot disease, but equalized by 12 WAP. The bacteria inoculum treated maize showed the best performance in terms of leaf spot disease control, with no disease spot observed until 9 WAP and a significantly lower score at 12 WAP compared to other treatments. The bacteria inoculum treatment proved to be the most effective in controlling leaf spot disease in the early stages, with a significantly lower disease score even at 12 WAP. The NPK treatment does not significantly alter disease progression compared to the control. The fungi inoculum showed some potential but does not maintain a significant difference by 12 WAP. This result is in tandem with study conducted by Mishra *et al.* (2020) which stated that Microorganisms like *Trichoderma spp.*, *Penicillium*, *Bacillus spp.*, *Rhizobium*, and *Pseudomonas spp.* have been identified as plant growth-promoting fungi (PGPF) and plant growth-promoting rhizobacteria (PGPR), demonstrating their potential to enhance defense mechanisms against plant pathogens. Parker *et al.*, (2019) stated that biopesticides typically impede or disrupt the translation process, thereby affecting protein synthesis through various mechanisms. For instance, some, like blasticidin, bind to prokaryotic 50S ribosomes, hindering peptide transfer and inhibiting chain elongation. According to Kloepper (2004), PGPR inhabit the rhizosphere of numerous plant species, where they confer advantageous effects on the host, such as heightened plant growth and decreased vulnerability to diseases induced by plant pathogens like nematodes, fungi, bacteria, and viruses. PGPR enhanced seed germination rate, root growth, yield, leaf area, chlorophyll content, nutrient uptake, protein content, hydraulic activity, tolerance to abiotic stress, shoot and root weights, and delayed senescence (Adesemoye, 2009).

4.2 Conclusion

Agriculture and soil bear the responsibility of supporting human life on earth. The excessive exploitation of resources has reduced productivity, leading people to seek alternative means for sustaining their livelihoods. PGPR (Plant Growth-Promoting Rhizobacteria) plays a crucial role in promoting plant growth, remediating and managing polluted and degraded wastelands and eutrophied water bodies, and mitigating issues such as pesticide pollution, nitrogen, and phosphorous runoff. However, the excessive reliance on chemical fertilizers and pesticides by the human population has resulted in the circulation of harmful chemicals. These substances pose risks not only to human health but also disrupt ecological balance, entering the food chain through various pathways. Such changes can impact plant-microbe interactions by altering microbial biology and biogeochemical cycles. The application of modern tools and techniques to

enhance PGPR presents a key opportunity for sustainable agriculture, improving soil fertility, plant resilience, crop yield, and maintaining a balanced nutrient cycle.

4.3 Recommendations

Based on the result of this research, it is established that the identified plant growth promoting Rhizobacteria (*Bacillus subtilis*) and fungi (*Trichoderma spp*) revealed clues for plant growth promotion and biocontrol against pests and diseases and are therefore suitable for the development of biofertilizers and biopesticides. Therefore, it is recommended that *Bacillus subtilis* and *Trichoderma spp*. which are endogenous microorganisms can favourably substitute for chemical synthetic fertilizers and pesticides thus preventing health hazards associated with the indiscriminate use of the latter. However, further research on developing microbial communities, and exploring interdisciplinary approaches encompassing biotechnology, nanotechnology, agro biotechnology, chemical engineering, and material science, along with diverse ecological and functional biological strategies, can yield new formulations and opportunities with significant potentials.

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