COMPARATIVE STUDY OF BIOINOCULANT WITH INORGANIC FERTILIZER ON THE GROWTH OF GUINEA CORN (*Sorghum bicolor*)

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Abstract

Growing awareness on health challenges posed by consuming poor-quality crops has prompted the search for new and improved technologies to increase the quality and quantity of crops without endangering human health. A reliable alternative to using chemical inputs is a microbial inoculant that can act as a biofertilizer. The ability of bacteria and fungi isolated from the rhizosphere of guinea corn to solubilize phosphate was investigated in this study. Phosphate solubilization was quantified using Pikovskaya's medium on standardized isolates using a standard curve. *Seratia marcesceus* and *Aspegillus niger* had the highest phosphate solubilization effect (547.52 and 679.31 ug/ml respectively), therefore the isolates with highest phosphate solubilization were used as bionoculant for *Sorghum bicolor* in comparison with inorganic fertilizer and control using some parameters. The Sorghum applied with bioinoculant was statistically significant (p<0.05) in both varieties (V_1 & V_2) and had the highest observations of all the parameters compared to inorganic fertilizer, and control had the least. It is concluded that the *Seratia marcescens* and *Aspergillus niger* isolated in this study would improve the growth of the *Sorghum bicolor* plant, reducing the need for chemical fertilizer and increasing field application potential. However, additional research into other conditions, location, and crop variety is required in the future.

Keywords: Bio-inoculant, Inorganic fertilizer, *Sorghum bicolor*, rhizophere soil.

Introduction

Plant growth promotion, pest and weed control are all capabilities of microorganisms. Microbial inoculants are beneficial microorganisms that are applied to soil or plants in order to increase crop productivity and health. Inoculants are natural products that are widely used to control pests and improve soil and crop quality, resulting in better human health (Ponge, 2015). Microorganisms metabolites are mixtures that work with soil and soil life to improve soil fertility and health, and thus human health. The inoculants have the potential to reduce the negative effects of chemical inputs, increasing the quality and quantity of agricultural products.
(Elizabeth and Olubukola, 2018).
Microbial inoculants are less harmful to the environment and provide plant nutrients in a more sustainable manner. Microbial inoculants can aid in the reduction of fertilizer application. Bacteria, fungi, and algae are examples of microbial inoculants (Elizabeth and Olubukola, 2018).
Soil microbes play an important role in improving soil health in both cultivated and natural soil environments by promoting plant growth (Ponge, 2015). The number of microorganisms in soil at any given time is determined by soil conditions such as temperature, humidity, pH, salt concentration, and soil type. The rhizosphere microbiome is formed when root exudates released from the rhizosphere act as chemical signals for various microbial communities (Amit and Satish, 2015). The composition of the microbial community promotes plant growth as a direct result of competition through properties such as phosphate solubilization, auxin synthesis, and siderophore production (Matulich and Martiny, 2015). Plant growth-promoting microorganisms (PGPMs) from the genera *Bacillus, Pseudomonas, Clostridium, Rhizobium, Penicillium,* and *Trichoderma* have been shown to improve crop vegetative growth, photosynthetic capacity, and micronutrients by improving crop productivity (Matulich and Martiny, 2015).
Although phosphate is one of the most abundant nutrients in soil, phosphate deficiency can still have an impact on plant health and growth because most phosphates are insoluble. Certain bacteria and fungi are capable of mineralizing and dissolving organic and inorganic phosphorus in soil (Alonso-Ramirez *et al*., 2009). Phosphate dissolution has also been reported under ambient stress conditions involving high levels of aluminum, iron, and dryness. Phosphorus-enhancing agents can be injected into nutrient-deficient soils to reduce the need for external fertilizers (Ahmad *et al*.,2011).
The synthesis of auxins by microbes is a well-known phenomenon, and this property is one of the direct mechanisms by which PGPM influences plant growth. In addition to being naturally occurring, microorganisms commonly produce the auxin, indole acetic acid (IAA). In plant-microbe interactions, IAAs are known to act as reciprocal signaling molecules (Bhattacharyya and Jha, 2019). Furthermore, IAA has been shown to promote plant growth and cell proliferation in roots and shoots, resulting in elongation and vegetative growth. It aids in the development of plant pathogen resistance and increases survivability under stressful conditions such as biotic and abiotic stresses (Bhattacharyya and Jha, 2019).

Materials and Methods

Study Area

The study was conducted in Niger State Polytechnic, Zungeru, located at Wushishi local government, Niger State. Niger State lies on longtitude 3.20° East and 11.30° North. Kaduna State and FCT, borders to the North-East and South-East of Niger State respectively; Zamfara State to the North, Kebbi State to West, Kogi State to the South and Kwara

State to South West, while the republic of Benin along Agwara LGA borders her North West (Mohammed, 2002).

Collection of Guinea corn seed samples

The red guinea corn variety was obtained from the Ahmadu Bello University Institute of Agricultural Research, Zaria, Mokwa branch, Niger State, Nigeria. Before planting, it was placed in a clean polythene bag at an ambient temperature of $25^0C \pm 3^0C$ and transported unopened to the Federal University of Technology Minna's microbiology laboratory.

Collection of rhizosphere soil samples

Rhizosphere soil samples were carefully collected by uprooting each plant with a sterile trowel and shaking the roots to obtain soil adhering to the roots into clean polythene bags, labeled, and immediately transported from the field (farm) to the laboratory for analysis.

Isolation of rhizosphere microorganisms

Serial dilution technique was employed for the rhizosphere soils (10^{-3}, 10^{-4} and 10^{-5}) in test tube containing 9ml of sterile distilled water. Bacteria and fungi were isolated using nutrient agar and potato dextrose agar, respectively. The inoculated plates were incubated and subcultured to obtain pure isolates (Chessbrough, 2012).

Isolation and enumeration of bacteria from the soil samples

Guinea corn, bacteria were isolated during the cultivation using the pour plate technique from unplanted soil. One gram of soil was weighed and placed in a test tube with 9ml of sterile distilled water. To achieve dilutions, the soil suspension was shaken and serially diluted. A 0.1ml aliquot from each dilution was placed in a separate plate. Following that, sterile molten nutrient agar was added and cooled to 43ºC before pouring. To homogenize the inoculum and medium, the plate was swirled allowed to cool and incubated upside down at 37ºC in an incubator for 24 to 48 hours. At the end of incubation period, the number of colonies on each plate was counted and expressed in cfu/g (Cowan and Steel's, 2001).

Screening of Phosphate Solubilizing organisms

One gram (1g) of rhizosphere soil was suspended into 9 ml sterile distilled water in a tube and make serial dilutions up to 10^{-5}. Aliquot of 1ml suspension from 10^{-3} and 10^{-4} dilutions was added into agar plates containing Pikovskaya's agar medium which is supplemented with phosphate (calcium phosphate 15%). The plates were incubated at 25ºC for 4-5 days.

Transparent zones of clearing around microorganism colonies indicate phosphate solubilization, or the presence of a halo zone indicates phosphate solubilization (Imam, 2008). The culture was then isolated, purified, and identified in order to quantify the extent of solubilisation (Fawole and Oso, 2001).

Quantitative measurement of Phosphate Solubilization

Pikovskaya's liquid medium(100ml) was inoculated with 5ml of the standardized bacterial culture or 2 inoculum plugs from the advanced edge of 4 days old fungal culture. The broth culture was agitated at 120rmp for 15 days. Following that, the microbial culture was filtered through a Whatman's filer No 42, and the filtrate was centrifuged at 10,000 rpm. According to Dubey and Mashehwari (2005): Barton's reagent was used for phosphate quantification. A spectrophotometer with a wavelength of 430nm was used to measure the optical density of the yellow-colored solution. Using the standard curve, the amount of phosphate solubilized in culture medium was calculated.

Bio-inoculation of microbial isolates for Sorghum bicolor

Serratia marcescens and *Aspergillus niger* (bacteria and fungi) were chosen. Sabouraud dextrose broth was prepared for fungi by weighing 15g of powdered broth into 1 litter of distilled water and sterilizing in an autoclave at 121^0C for 15 minutes at a pressure of 15 PSI. After cooling, the fungi were inoculated under aseptic conditions and incubated at room temperature for seven days (Pelczer *et al*., 2005).

For bacteria, nutrient broth was prepared by weighing 13.0g of the powder medium and sterilized at 121^0C and pressured at $1.5NM^{-2}$ for 15 minutes using autoclave. After sterilization, the bacteria, *Serratia marcescens*, was inoculated and incubated at 37^0C for five days. The Macfaland standard was used. (1% H_2SO_4 TO 99ml of distilled H_2O. $Bacl_2$ + 200ml H_20) (Fawole and Oso, 2001).

Therefore, the bio-inoculant produced of both (Fungi and bacteria) was collected in a clean four litter's container separately and taken to the field experiment where *Sorghum bicolor* was planted. One hundred mililitre (100mls) of the bio-inoculant was poured beneath the plant roots every week for seven weeks. However, this was compared with application of inorganic fertilizer and control in field experiment (Gamal-Eldin and Elbanna, 2011).

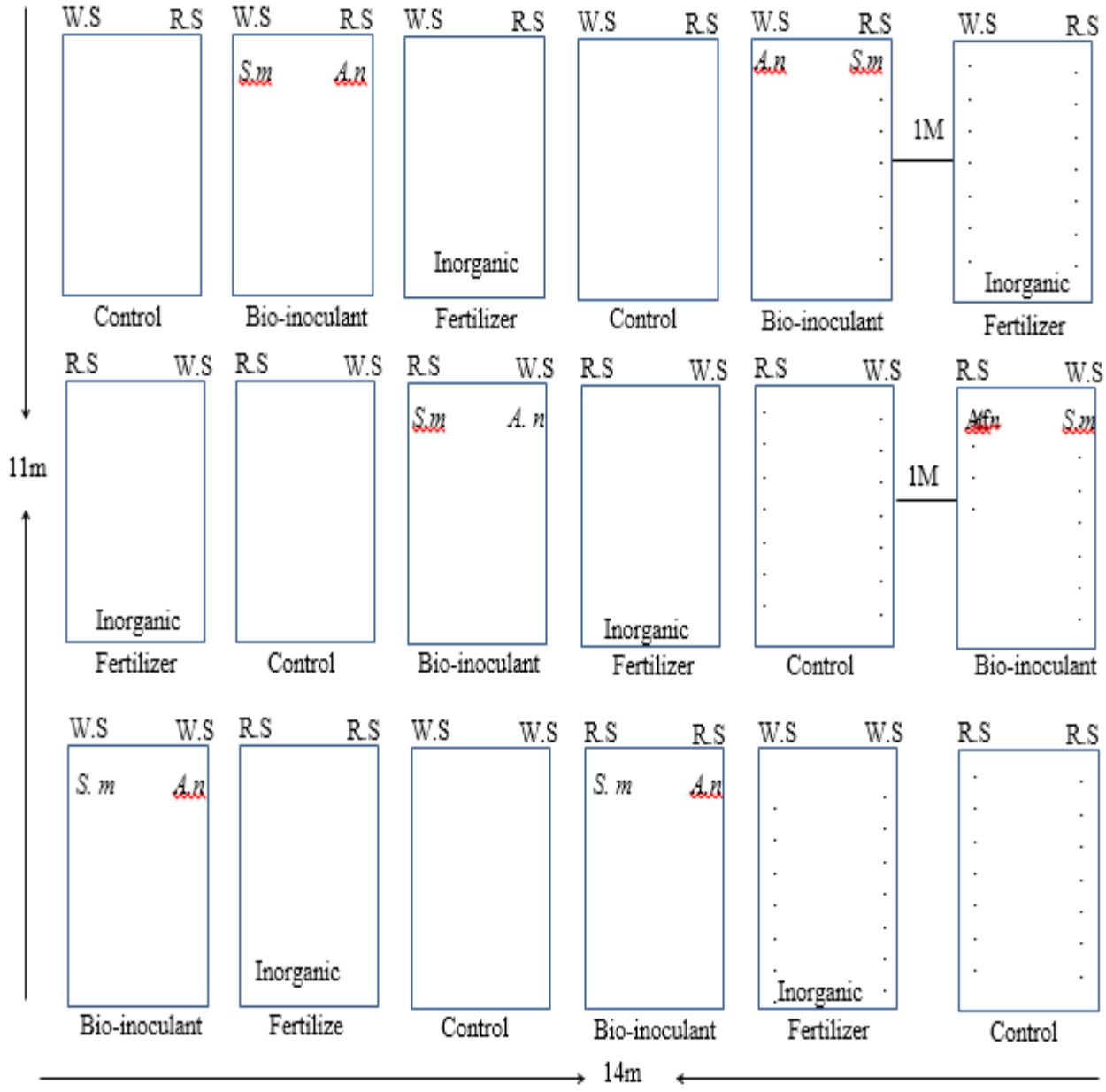

Figure 1: Experimental field layout for the varieties of *Sorghum bicolor*

KEY: W.S = White sorghum (V_1) R.S = Red sorghum (V_2)
S. m = Serratia marcescens *A.n = Aspergillus niger*

Results

Phosphate solubilization effect of fungal isolates

Among the fungi isolates from the guinea corn rhizopheric soil *Aspergillus niger, Aspergillus fumigatus, Trichoderma person, Aspergillus flavus, Verticillium tenerium, Mucor pusillus, Trichophyton verrucosum, Trichophyton persoon* and *Trichophyton quinckeneum* exhibited phosphate solubilization effect (Table 1). The Aspergillus *niger*, had the highest phosphate solubilization effects (679.31ug/ml), while *Mucor pusillus* had the least ((1.82 ug/ml).

Table 1: Phosphate solubilization by the fungal isolates

Fungal isolates	Phosphate solubilized (μg/ml)
Trycophyton verrucosum	3.63
Mucor pucillus	1.82
T. quickenum	6.16
A. fumigatus	13.08
Trichoderma persoon	252.71
Aspergilus flavus	125.17
Verticillium tenerium	154.36
Aspergilus niger	679.31
T. verrucosum	0.00

Phosphate solubilization effect of bacteria isolate

The qualitative phosphate solubilization effects of bacterial and fungi isolates from the guinea corn rhizopheric soil are shown in Table 2. The quantitative phosphate solubilization effects of bacterial isolates from the guinea corn rhizopheric soil are shown in Table 2. These include *Escherichia coli, Bacillus licheniformis, Serratia marcescens, Micrococcus luteus Bacillus subtilis* and *Lactobacillus bulgaricus*, exhibited phosphate solubilization effect. *Serratia marcescens*, exhibited the highest phosphate solubilization activity (547.52 ug/ml), while *Micrococcus luteus*, exhibited the least (1.45 ug/ml) phosphate solubilization effect.

Table 2: Phosphate solubilization of Bacterial isolates.

Bacteria isolates	Phosphate solubilization μg/ml
Bacillus subtilis	2.82
Micrococcus luteus	1.45
Serratia marcescens	547.52
Escherichia coli	455.57
Lactobacillus bulgaricus	22.17
Bacillus licheniformis	121.30

Table 3: Growth parameters (cm) of *Sorghum bicolor* red variety (V_1) treated with inorganic and bio-inoculants.

Parameter	*A. niger*	*S. marcescens*	Inorganic fertilizer	Control
Lenth of plant	128.33± 7.10 ^{c}	116.33± 7.54^{b}	102.00± 5.33^{a}	102.33± 4.43^{a}
Width of plant	7.83±1. 34 ^{b}	16.50±4. 54 ^{c}	5.00±0.5 5 ^{a}	4.67±0.2 4 ^{a}
Length of leaf	93.33±6 .75 ^{d}	89.00±6. 55 ^{c}	63.33±2. 45 ^{a}	70.33±5. 43 ^{b}
Breadth of leaf	7.27±1. 43 ^{c}	5.87±0.8 7 ^{b}	5.37±0.5 6 ^{b}	4.27±0.1 5 ^{a}

Data are Mean ± SEM of triplicate determination. Values followed by different superscript alphabet were not significantly different (p<0.05).

Table 4: Growth parameters (cm) of *Sorghum bicolor* white variety (V_2) treated with inorganic and bio-inoculants

Parameter	*A. niger*	*S. marcescens*	Inorganic fertilizer	Control
Lenth of plant	110.67± 4.32 ^{b}	108.33±5 .35^{b}	91.67±5 .43^{a}	87.67±7 .64^{a}
Width of plant	5.23±0.3 4 ^{b}	5.70±0.2 3 ^{b}	4.17±0. 32 ^{a}	4.50±0. 26 ^{a}
Length of leaf	73.33±3. 43 ^{c}	85.00±5. 67 ^{d}	54.67±1 .90 ^{a}	68.33±2 .56 ^{b}
Breadth of leaf	5.90±0.3 5 ^{c}	4.90±0.4 5 ^{b}	4.10±0. 23 ^{ab}	3.90±0. 13 ^{a}

Data are Mean ± SEM of triplicate determination. Values followed by different superscript alphabet were not significantly different (p<0.05).

Discussion

Phosphorus is commonly present in forms that are not immediately available to plants. Due to the slow diffusion rate in the soil which often results in a zone of P-depletion around plant roots as P is absorbed faster than it can be made available to replenish the soil around the root (Bagayoko *et al*., 2000).

Soil fungi and bacteria that live near or on the root surface of plants are directly involved in plant growth improvement and protect plants from disease and abiotic stresses by producing various regulatory chemicals in the rhizosphere (Barea *et al*., 2005). One of the alternative biotechnological solutions in sustainable agriculture for meeting plant phosphate demands is the use of phosphate solubilizing microorganisms as inoculants (Liu *et al*., 2014).

In the present study, *Escherichia coli*, *Bacillus licheniformis* and *Lactobacillus bulgaricus* isolated from rhizospheric soil exhibited phosphate solubilization effect (Table 2). The results indicated that *Serratia marcescens* are better phosphate solubilizer (547.52ug/mL) while *Micrococcus luteus* (1.45ug/ml) exhibited the least phosphate solubilizing effect (Table 2) Results of phosphate solubilizer obtained in this study can be compared with those achieved by Baset *et*

al. (2010). These results indicated that rhizospheric bacteria have the ability to solubilize precipitated phosphates as reported by (Solaimam and Anawar, 2015). According to Aishiki *et al.*, (2017). in addition to rhizobacteria, several fungi such as species of *Aspergillus* can efficiently solubilize P. This is consistent with the results obtained in this study as *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Mucor pusillus, Trichophyton verrucosum, Trychophyton megininii* and *Trichophyton quinckeneum* were able to solubilize phosphate (Table 1). However, results obtained in this study indicated that fungal isolates were able to solubilize phosphates better than the bacterial isolates (Table 1). This finding disagrees with the study of (Raaijmakers *et al*., 2001) which reported that even though the fungal isolates were able to solubilize phosphates to some extent; their ability was not comparable to that of the bacterial isolates. All of the above-identified fungi and bacterial species found to be efficient phosphate solubilizers which have a great role in increasing crops productivity and production without contaminating the environment and affecting human health (Verma *et al.*, 2001).

Sernatia marcescens and *Aspergillus niger* (bio-inoculants) suggested that these isolates could serve as efficient biofertilizer candidates for activating phosphorus and simultaneously promoting crop growth. Therefore, these isolates were evaluated further as bioferterlizer for improving growth of the plant in comparisom with the inorganic fertilizer. Interestingly, it was observed that plants inoculated with the *Serratia marcescens and Aspergillus niger* improve the growth parameters of *Sorghum bicolor* red (V1) and white (V2) varieties when compared with those treated with inorganic fertilizer. Specifically, the length of the plant, width of plant, length of leaves and breadth of leaves were significantly ($p<0.05$) higher in both varieties of *Sorghum bicolor* treated with bio fertilizer from *A. niger* and *Serratia marcescens* when compared with those treated with inorganic fertilizer and the control group without any treatment. Results of the present study can be compared with those obtained by Ravikumar *et al*. (2004), who found that inoculation with *Azotobacter chroococcum* stimulated an increase in leaf area in *Rhizhopora* seedling species. Furthermore, the increased leaf length and breadth in plant treated with bio-fertilizer from *A. niger* and *Serratia marcescens* may directly influence the photosynthesis efficiency of the plant, thereby, contributing towards the better performance of the plants. In line with the findings from the present study, Sharma *et al*. (2012) reported significant improvement in the vegetative growth parameters of 'Royal Delicious' apple saplings by using single and/or dual application of soil inoculation of *Glomus fasciculatum, Glomus mosseae*, and *A chroococcum* strains namely, *A. chroococcum* strain-I (AZ1) and *Azotobacter. chroococcum* strain-II (AZ2) at nursery stage under reduced inorganic fertilization.

It is important to consider that the growth-promoting performance of bacteria will be influenced by biotic and abiotic factors in soil. Ribaudo *et al*. (2006), evaluated parameters associated with the growth of tomato seeds inoculated with *Azospirillum brasilense* FT326 where increases were found in the length of the main root hair, root surface, and root and shoot fresh weight. These authors proposed ethylene as an intermediary in the signal path stimulating plant growth. In other studies, inoculation of seaweed with distinct varieties of rhizospheric bacteria significantly increased the root biomass mean up to 98.2% in relation to the control treatment (Rajad *et al*., 2001).

Results of plant length obtained in this study can be compared with those achieved by Baset *et al*. (2010), who demostrated that inoculation with rhizobacteria (*Azospirillum brasilense* Sp7 and *Bacillus sphaericus* UPMB10) and nitrogen supplements stimulated root length and number in banana plants (*Musa spp*. cv. 'Berangan', type AA) with a 43% root increase in inoculated plants. The authors also compared these results with those obtained when bacterial strains were used without any nitrogen supplement, obtaining significant results. Gamal-Eldin and Elbanna (2011), in their studies of purple, nonsulfur photosynthetic bacteria (*Rhodobacter capsulatus*), achieved beneficial effects in terms of high rice plant yield (*Oryza sativa*). Results of the present study can be compared with those obtained by Ravikumar *et al*. (2004), who found that inoculation with *Azotobacter chroococcum* stimulated an increase in leaf area in *Rhizhopora* seedling species. This effect is explained by the capacity of the microorganism to fix atmospheric nitrogen and make it available to the plant. Furthermore, this microorganism in its culture medium secretes hormones such as auxins, gibberelins, and cytokines which stimulate plant growth (Mehboob *et al*., 2009).

By indication, the rhizopheric *Sernatia marcescens* and *Aspergillus niger* isolated in this study would enhance the growth of *Sorghum bicolor* plant. This would lower the use of chemical fertilizer and have greater application potential in the field.

Conclusion

Findings from this study suggested that the bacteria and fungi isolated from rhizosphere soil of Guinea corm can be used as inoculants to replace agrochemicals for improving crop productivity and production.

Conflict Of Intrest

The authors declare no conflict of interest exist.

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