

ENHANCEMENT OF GROWTH OF POTTED MAIZE PLANTS USING *ASPERGILLUS NIGER* AND *LYSINIBACILLUS SPHAERICUS*

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Abstract

Phosphorus is one of the essential nutrients needed for the growth of plants. It is the least mobile nutrient in the soil. Some microorganisms have ability to mineralize and solubilize phosphate in the soil thereby making it available for absorption through the plant roots. *Aspergillus niger* and *Lysinibacillus sphaericus* were isolated from the rhizosphere of some plants and their effects on the growth of potted maize plants were assessed by using these microorganisms as biofertilizer. The experiment was set up using three treatments with two replicates each. These treatments were sterile soil plus maize seed plus microbial inoculum; non-sterile soil plus maize seed plus microbial inoculum; and non-sterile soil plus seed only. The potted maize plants were arranged in a complete randomized block design outdoor so as to receive sunlight. The potted plants were watered at regular interval. *Lysinibacillus sphaericus* and *Aspergillus niger* have phosphate solubilization index 2.50 and 4.1 respectively. There was significant difference in the growth of maize plants containing sterile soil plus microorganism and non-sterile soil plus microorganism over the periods of growth in terms of length and width of the broadest leaves as well as length and girth of the stem base when compared with growth of the maize plants that were not inoculated with microorganism. It is concluded that *Lysinibacillus sphaericus* and *Aspergillus niger* have promoted the growth of the maize plants and they are recommended for use as biofertilizer in agricultural systems and plant healthy growth.

Keywords: Bio-inoculants, Growth parameters, Microorganisms, Phosphate solubilization, Rhizosphere

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INTRODUCTION

Phosphorus is one of the important elements for the growth of plants. It helps in root developments and metabolic activities, particularly in the synthesis of protein (Khan *et al.*, 2010). Microorganisms that can make phosphate soluble for easy absorption by the plants are called phosphate solubilizing microorganisms. They can be found in the rhizosphere of plants.

Phosphate is precipitated in soil or adsorbed by iron and aluminium oxides through ligand exchange. Phosphorus solubilizing microorganisms play role in phosphorus nutrition by enhancing its availability to the plants through release from inorganic and organic materials in the soil. The use of phosphorus solubilizing microorganisms as inoculants increase phosphorus uptake. These microorganisms also increase prospects of using phosphatic rocks in crop production (Balemi and Negisho, 2012). Strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* along with fungi such as *Penicillium* and *Aspergillus* are among

the efficient phosphate solubilizers (Whitelaw, 2000).

Some of the prominent organic acids that have been reported to be released by phosphate solubilizing microorganisms are gluconic acid, oxalic acid, citric acid, lactic acid, tartaric acid and aspartic acid (Venkateswarlu *et al.*, 1984; Kim *et al.*, 1997).

Studies have shown that chemical fertilizers have adverse effects on the soil. These deleterious effects include soil acidity, and eutrophication of the aquatic environment. Hence, this study will provide information on the efficacy of some isolates in phosphate solubilization in the soil. The aims of this study were to determine the physicochemical characteristics of soils from the rhizosphere of some plants; isolate phosphate solubilizing bacteria and fungi; determine the phosphate solubilization index of the isolates; identify the best bacterium and fungus with phosphate solubilization potential using molecular technique; determine the effect of these isolates on the growth of potted maize plant; and

determine the metabolite elaborated by the isolates in Pikovskaya broth medium.

MATERIALS AND METHODS

Collection of the soil samples

A total of 6 soil samples were collected using sterile hand trowel from the rhizosphere of spinach, maize, pumpkin, pepper, cassava, rice and non-rhizosphere soil for the potted maize plants at the Botanical garden, University of Ilorin, Ilorin, Kwara State, Nigeria. The soil samples were collected into sterile labeled polythene bags. The top layers of the soils were cleared of debris and clean sterile trowel was used to dig into the rhizosphere around 20cm deep (Pande *et al.*, 2017).

Determination of physicochemical characteristics of the soils

The soil pH was determined using a standardized pH meter. The moisture content was determined by evaluating the loss in weight of the fresh soil on drying at 105°C in an oven until constant weight was obtained. The soil organic content was determined by further heating of the oven dried soil in a furnace at 600°C for 2 hours and the process repeated until a constant weight (Sule and Oyeyiola, 2012). The water holding capacity of the oven dried soil was determined using the method of Oyeyiola (2009).

Isolation of phosphate solubilizing microorganisms (PSM)

Pikovskaya's agar medium (PVK) was prepared with the composition as described by Kolekaret *al.* (2017) and Chinakwe *et al.* (2019). Ten grams (10g) of the soil sample was weighed and dispersed into 90 ml of sterile distilled water and it was thoroughly shaken. After the preparation and sterilization of the medium, streptomycin was then added to 100ml of the cooled PVK in a conical flask to inhibit bacteria and isolate fungi, while nystatin was added to another 100ml of PVK to inhibit fungi and isolate bacteria. Aliquot (0.1ml) was taken from the different dilutions and inoculated at the centre of sterile solidified PVK agar medium. The inoculum was spread by means of L-shaped glass spreader. Incubation was

done at 37°C for 7 days for the bacterial plates while the fungal plates were incubated at room temperature for the same duration. Colonies showing halo zones were taken as evidence of phosphate solubilization.

Purification and reservation of isolates

The bacteria and fungi colonies showing halo-zones on the PVK medium were subcultured on nutrient agar and potato dextrose agar respectively. The plates were incubated and stored in a refrigerator at 4°C until they are needed (Fawole and Oso, 2007).

Determination of the phosphate solubilization index (PSI) of the isolates

Different sterile set plates of Pikovskaya agar medium were inoculated with the standardized culture of each bacterium while inoculum plugs from 3 days old pure culture were used for the fungal inoculation. The plates were incubated at room temperature for 7 days. The bacterial and fungal isolates were ranked based on the diameter of the halo zone. The best bacterium and fungus were used for the potted plant experiment (Zhen *et al.*, 2016; Pande *et al.*, 2017).

$$\text{Phosphate solubilization index} = \frac{\text{Diameter of colony} + \text{Halo zone}}{\text{Diameter of colony}}$$

Molecular identification of the isolates

This was done using standard methods. This involved the extraction of the bacterial and fungal DNA; amplification of the DNA using PCR; purification of the PCR products; and sequencing of the PCR products. The PCR conditions used were: initial denaturation at 94°C for 5 minutes; further denaturation at 94°C for 30 seconds; annealing at 56°C for 30 seconds; extension at 72°C for 45 seconds; the above cycles were repeated 36 times with final extension at 72°C for 7 minutes; and holding temperature of 10°C. The nucleotide sequence obtained was blasted and it was compared with the data base of National Centre for Biotechnology information (NCBI) in order to get the name of the microorganism.

Preparation of inoculum for the pot experiment

The bacterium, *Lysinibacillus sphaericus* was cultivated on sterile nutrient agar plates and

incubated at 37°C for 24 hours. It was washed with sterile distilled water into a 500ml conical flask aseptically. Then, 0.5 Mc Farland standard was used to standardize the washed culture in the conical flask (Cheesbrough, 2006).

The fungus, *Aspergillus niger* was first cultivated on sterile potato dextrose agar plate and incubated at room temperature for 72–96 hours. Cork borer, 8mm in diameter was used to bore inoculum plugs at the advanced edge of the fungal colony. The inoculum plugs were kept in sterile Petri dish for use to inoculate the soil (Fawole and Oso, 2007).

Cultivation of potted maize plants

Soil sample was collected from a Garden soil and it was thoroughly mixed to form a homogenous sample. Equal amount of the soil (5kg) was distributed into 5 litre plastic buckets. Six evenly spaced holes were drilled at the bottom of each plastic buckets.

Four healthy maize seeds were planted in each plastic bucket followed by the addition of 60ml of the standardized bacterial culture. For the fungal inoculation, 4 inoculum plugs from the advanced edge of 72 hours old culture of *Aspergillus niger* was used to inoculate the plastic bucket containing the soil. The inocula were covered with some soils in each plastic bucket. The initial inoculation of the plastic buckets was done on the day of planting of the seeds. The second inoculation of the pots was done at the second week of planting.

Experimental design of the potted maize plant

The cultivation of the potted maize plant was done under these treatments: sterile soil plus bacteria plus maize seeds; non-sterile soil plus bacteria plus maize seeds; sterile soil plus fungi plus maize seeds; non-sterile soil plus fungi plus maize seeds; and non-sterile soil plus maize seeds only. Each set up was done in duplicate.

The potted maize plants were arranged in a complete randomized design in the opening so that it can receive sunlight. All the pots received equal amount (250ml) of water at 2 days interval. There was no addition of

chemical fertilizer or any herbicide to any of the pot. The germinated maize seedlings were thinned to 2 seedlings at one week of planting (Niazi *et al.*, 2015).

Measurement of growth parameters of the potted maize plants

The maize seedlings were allowed to grow for 1 – 2 weeks before the commencement of the measurement of length of the stem, girth of the stem base, length and width of the broadest leaves (Nwanyanwu *et al.*, 2015; Kolekar *et al.*, 2017).

Identification of the functional group of the metabolite

The young pure cultures of each isolates were inoculated into Pikosvkaya's broth medium and then incubated at 30°C for 7 days. The suspensions were filtered with Whatman filter paper No 11 and the filtrate analyzed using Fourier Transform Infrared spectrophotometer.

Data analyses

The statistical package used was SPSS version 16.0. Descriptive statistics in form of means and standard deviation were used to analyze the data. The data obtained were analyzed using ANOVA while Duncan's multiple range was used to compare the mean values of the data (SPSS, 2010).

RESULTS

Physicochemical properties of the soil

The physicochemical properties of the soil samples are shown in Table 1. The pH, moisture content, organic matter, and water holding capacity of the rhizosphere soils ranged from 6.7 - 8.6; 0.4 - 7.32%, 1.39 - 5.9%, and 0.34 - 0.53ml/g respectively.

Phosphate solubilization index of the microorganisms

The phosphate solubilization index of the highest ranked bacterium and fungus was 2.5 and 4.1 respectively (Table 2).

Identification of the isolates

Based on molecular characterization, the isolates were identified as *Lysinibacillus sphaericus* and *Aspergillus niger* (Table 3).

Table 1: Physicochemical properties of the rhizosphere soils

Rhizosphere soils	pH	Moisture content (%)	Organic matter content (%)	Water holding capacity (ml/g)
Pepper	6.7	0.4	1.39	0.39
Rice	7.8	6.47	1.82	0.47
Cassava	8.1	7.32	4.7	0.53
Maize	8.6	2.1	5.34	0.34
Spinach	7.2	0.6	2.37	0.42
Pumpkin	6.7	1.5	3.9	0.35
Potted soil	7.1	1.6	5.9	0.43

Table 2: Phosphate Solubilization index of the microbial isolates

Microbial isolates	Source of microbial isolation	Diameter of colony (cm)	Diameter of halo zone and colony (cm)	Phosphate solubilization index
B1	Bulk soil from abattoir	3.45	5.4	1.57
B2	Bulk soil from abattoir	3.2	4.4	1.38
B3	Bulk soil from abattoir	2.8	4.2	1.50
B4	Rhizosphere soil of Fluted Pumpkin	1.0	2.5	2.50
F1	Rhizosphere soil of Pawpaw	1.1	4.5	4.10
F2	Rhizosphere soil of Fluted Pumpkin	3.4	4.2	1.24
F3	Rhizosphere soil of Pawpaw	5.5	6.0	1.09

B1 – B4 = Bacterial isolates; F1 – F3 = Fungal isolates

Table 3: Molecular identification of the microbial isolates

S/No	Isolate	Total Score	Percentage Identity	Accession Code	Closest relative
1	F2	3144	99%	AM0270052.1	<i>Aspergillus niger</i>
2	B4	17641	94.38%	CP014643.1	<i>Lysinibacillus sphaericus</i>

Growth parameter of the potted maize plants

The length of the stem and its girth as well as the width and length of the widest leaves were measured using a string and ruler (Tables 4 - 11). It was observed that the maize plant in the pots that have received microbial inoculum showed better growth than the uninoculated pots (control).

Fourier transform analysis of the metabolite in PVK broth

The absorption peaks of the metabolites in the PVK broth of the isolates ranged from 1397.36 cm^{-1} to 1637.99 cm^{-1} regions and this correspond to the presence of carboxyl groups. The details of the spectra are shown in Figures 1 - 2.

Table 4: Effect of *A.niger* inoculants on the length of the broadest leaves of maize plant

Treatments	Length of leaves(cm)			
	Period of plant growth (week)			
	2	3	4	5
Sterile soil, plus <i>A. niger</i> and maize plant	24.25±0.35 ^a	49.0±1.41 ^b	66.7±0.71 ^b	75.85±1.91 ^a
Non-sterile soil, plus <i>A. niger</i> and maize plant	31.05±1.48 ^b	54.6±0.71 ^c	71.2±1.70 ^c	85.2±0.28 ^b
Non-sterile soil plus maize plant	26.8±2.55 ^{ab}	42±0.00 ^a	60.1±0.42 ^a	76.9±1.56 ^a

Means followed by the same alphabet(s) in the same column are not significantly different at $\alpha = 0.05$ based on Duncan's multiple range test

Table 5: Effect of *A.niger* inoculants on the width of the broadest leaves of maize plant

Treatments	Width of leaves(cm)			
	Period of plant growth (week)			
	2	3	4	5
Sterile soil, plus <i>A. niger</i> and maize plant	1.55±0.07 ^a	3.5±0.14 ^b	4.9±0.14 ^b	5.45±0.35 ^b
Non-sterile soil, plus <i>A. niger</i> and maize plant	2.30±0.00 ^b	3.3±0.14 ^b	4.8±0.28 ^b	6.25±0.21 ^b
Non-sterile soil plus maize plant	2.15±0.07 ^b	2.4±0.00 ^a	3.6±0.14 ^a	4.25±0.35 ^a

Means followed by the same alphabet(s) in the same column are not significantly different at $\alpha = 0.05$ based on Duncan's multiple range test

Table 6: Effect of *A.niger* inoculants on the length of stem of maize plant

Treatments	Length of stems (cm)			
	Period of plant growth (week)			
	2	3	4	5
Sterile soil, plus <i>A. niger</i> and maize plant	4.5±0.14 ^a	12.0±0.42 ^b	15.1±0.42 ^a	18.8±0.42 ^b
Non-sterile soil, plus <i>A. niger</i> and maize plant	7.3±0.00 ^b	12.5±0.07 ^b	16±1.41 ^a	18.9±0.28 ^b
Non-sterile soil plus maize plant	7.5±0.71 ^b	10.6±0.42 ^a	13.6±0.57 ^a	17.35±0.21 ^b

Means followed by the same alphabet(s) in the same column are not significantly different at $\alpha = 0.05$ based on Duncan's multiple range test

Table 7: Effect of *A.niger* inoculants on the girth of maize plant

Treatments	Perimeter of girth of stem base (cm)			
	Period of plant growth(week)			
	1	2	3	4
Sterile soil, plus <i>A. niger</i> and maize plant	1.7±0.14 ^a	3.6±0.28 ^b	5.9±0.28 ^b	6.4±0.14 ^b
Non-sterile soil, plus <i>A. niger</i> and maize plant	2.2±0.28 ^a	2.75±0.35 ^{ab}	4.5±0.28 ^b	6.2±0.28 ^b
Non-sterile soil plus maize plant	1.65±0.07 ^a	2.60±0.28 ^a	3.9±0.00 ^a	4.8±0.28 ^a

Means followed by the same alphabet(s) in the same column are not significantly different at $\alpha = 0.05$ based on Duncan's multiple range test

Table 8: Effect of *L.sphaericus* inoculants on the length of the broadest leaves of maize plant

Treatments	Length of leaves(cm)			
	Period of plant growth (week)			
	2	3	4	5
Sterile soil, plus <i>L. sphaericus</i> and maize plant	6.1±0.00 ^a	35.2±0.42 ^b	45.2±1.70 ^b	63.3±1.83 ^b
Non-sterile soil, plus <i>L. sphaericus</i> and maize plant	6.3±0.28 ^a	36.7±1.27 ^b	43.4±0.84 ^{ab}	58.6±1.83 ^{ab}
Non-sterile soil plus maize plant	6.0±0.14 ^a	30.4±0.28 ^a	40.9±0.57 ^a	54.65±0.35 ^a

Means followed by the same alphabet(s) in the same column are not significantly different at $\alpha = 0.05$ based on Duncan's multiple range test

Table 9: Effect of *L.sphaericus* inoculants on the width of the broadest leaves of maize plant

Treatments	Width of leaves(cm)			
	Period of plant growth(week)			
	1	2	3	4
Sterile soil, plus <i>L. sphaericus</i> and maize plant	1.6±0.14 ^a	3.0±0.28 ^a	4.1±0.00 ^b	5.85±0.21 ^b
Non-sterile soil, plus <i>L. sphaericus</i> and maize plant	1.7±0.14 ^a	2.8±0.14 ^a	3.1±0.28 ^a	4.5±0.28 ^a
Non-sterile soil plus maize plant	1.85±0.07 ^a	2.75±0.07 ^a	3.4±0.14 ^a	4.4±0.28 ^a

Means followed by the same alphabet(s) in the same column are not significantly different at $\alpha = 0.05$ based on Duncan's multiple range test

Table 10: Effect of *L.sphaericus* inoculants on the length of stem of maize plant

Treatments	Length of stem (cm)			
	Period of plant growth (week)			
	1	2	3	4
Sterile soil, plus <i>L. sphaericus</i> and maize plant	2.05±0.07 ^a	8.25±0.21 ^b	14.55±0.49 ^c	17.65±0.21 ^c
Non-sterile soil, plus <i>L. sphaericus</i> and maize plant	3.45±0.21 ^b	8.9±0.28 ^b	12.15±0.21 ^b	14.95±0.35 ^b
Non-sterile soil plus maize plant	2.45±0.07 ^a	7.25±0.21 ^a	11.0±0.28 ^a	13.3±0.28 ^a

Means followed by the same alphabet(s) in the same column are not significantly different at $\alpha = 0.05$ based on Duncan's multiple range test

Table 11: Effect of *L.sphaericus* inoculants on the girth of maize plant

Treatments	Perimeter of girth of stem base (cm)			
	Period of plant growth (week)			
	1	2	3	4
Sterile soil, plus <i>A. niger</i> and maize plant	Nd	2.55±0.35 ^a	4.05±0.07 ^b	5.8±0.28 ^b
Non-sterile soil, plus <i>A. niger</i> and maize plant	Nd	2.10±0.28 ^a	2.75±0.21 ^a	3.8±0.28 ^a
Non-sterile soil plus maize plant	Nd	2.20±0.28 ^a	3.05±0.21 ^a	4.45±0.21 ^a

Nd = Not determined; Means followed by the same alphabet(s) in the same column are not significantly different at $\alpha = 0.05$ based on Duncan's multiple range test

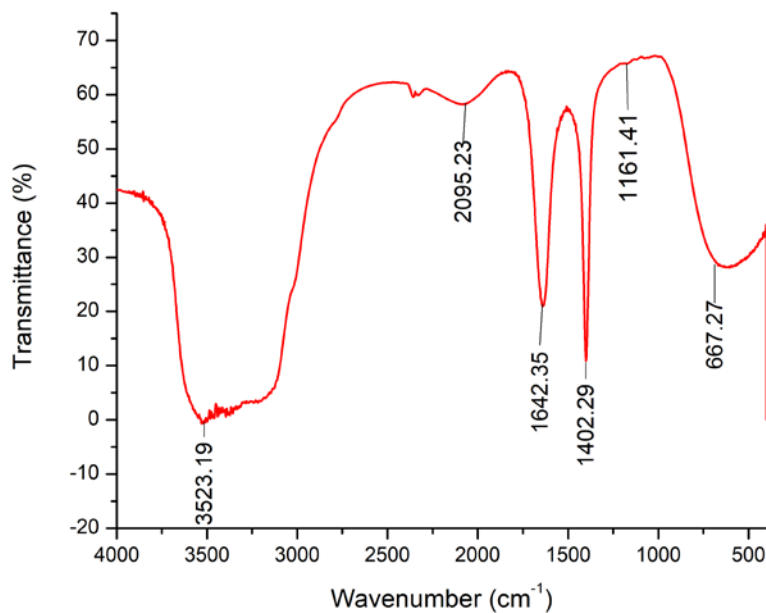


Figure 1: Fourier transform spectra of metabolite elaborated by *A. niger* in PVK broth

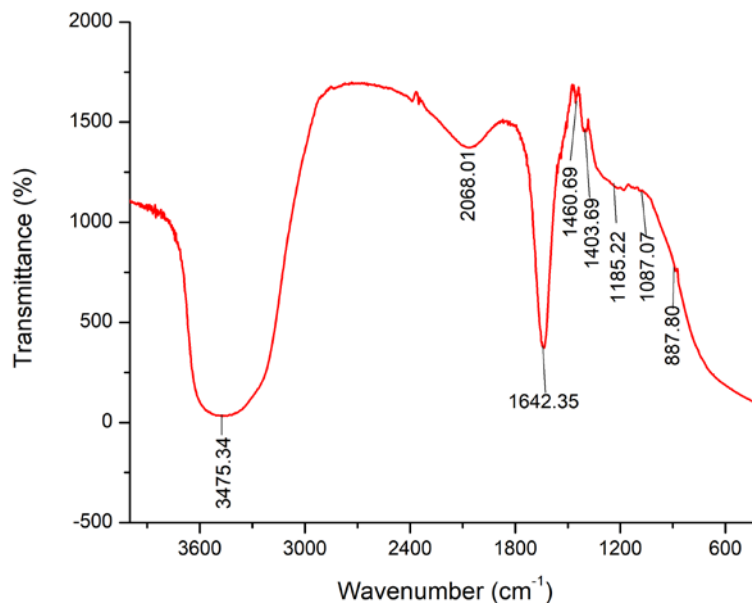


Figure 2: Fourier transform spectra of metabolite elaborated by *L. sphaericus* in PVK broth

DISCUSSION

The pH of the rhizosphere soils from the different plants ranged from 6.7 to 8.6. These soils can be described as being slightly acidic to alkaline. The moisture content, organic matter content and water holding capacity of the soil samples ranged from 0.4 - 7.32%, 1.39 to 5.90%, and 0.34 - 0.53ml/g respectively. Sule and Oyeyiola(2012) reported that the pH, moisture content, organic matter content and water holding capacity of soils from the rhizosphere of cassava cultivar TMS 419 ranged from 4.19 - 6.68, 0.12 - 8.76%, 0.36 - 11.8% and 0.25 - 0.34ml/g respectively. The neutral to alkaline soil will encourage the presence of large bacterial population in comparison with fungal population in the soil. This is because the bacteria thrive better on alkaline soil while fungi thrive best in soil with low pH (acidic).

In this study, *Lysinibacillus sphaericus* and *A. niger* were the best bacterium and fungus that solubilized calcium triphosphate in the PVK agar medium. In a similar study, Pande *et al.*(2017) isolated *Burkholderia cepacia* and *Alcaligenes aquatilis* from rhizosphere soils. Zhen *et al.* (2016) investigated the production of organic acid by *P. oxalicum* and *A. niger* isolated from the rhizosphere soils. Firew *et al.* (2016) isolated *Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp. from the rhizosphere soils of some plants in Addis Ababa, Ethiopia. Dwivedi *et al.* (2004) have used *Aspergillus awamori* to solubilise rock phosphate in order to enhance the growth of rice and wheat.

Studies related to the production of organic acids have shown that citric and oxalic acids were the two major organic acids produced by phosphate solubilizing microorganisms (Zhen *et al.*, 2016; Pande *et al.*, 2017). Maliha *et al.* (2004) reported that *A. niger* produced majorly citric acid and oxalic acid while *A. flavus* and *P. canescens* produced oxalic, citric, gluconic acid and an additional succinic acid in case of *A. flavus*. In this study the Fourier transform infrared spectrophotometry analysis revealed the elaboration of carboxylic acids by the two isolates used.

Extensive researches have been done on *Lysinibacillus sphaericus*. For instance, Sharma and Singh (2015) isolated strain of *lysini bacillus sphaericus* SNCh5 and used it to treat seeds of *Vigna radiata* (mung beans) before they were sown into pots. They observed that *Vigna radiata* (mung beans) seeds treated with a strain *lysini bacillus sphaericus* showed maximum effect on all the growth parameters in comparison to the untreated seeds. This is in concert with the results in this study which showed that maize plant in sterilized pots inoculated with *lysini bacillus sphaericus* showed better growth rate in all parameters compared to that of the untreated soils. Furthermore, Naureen *et al.* (2017) explored the potential of *Lysinibacillus sphaericus* ZA9 on the growth of tomato and cucumber.

Analysis of the potted plant showed that in the first week after inoculation of the isolates (bacteria and fungi) and planting of the seeds in the pots, there were insignificant differences between the various potted plants. In the second week, the unsterilized but uninoculated soil performed better than the sterilized and inoculated soil. This was because the isolates were trying to adjust to the new environment. From the third week and up to the fifth week, data collected showed that the maize plants of the sterilized and inoculated potted soils were catching up and exceeding the control.

Further analysis of the data from this experiment showed that the sterile soils inoculated with *Lysinibacillus sphaericus* did better than the unsterile soils inoculated with the same bacterium. This was because of absence of competition with the inoculum. This gave the inoculated isolates time to establish before contamination from the environment. Nwanyanwu *et al.* (2015) reported the best growth performance of 31.2cm, 38.4 cm² and 2.93cm for the plant height, leaves area, and shoot length respectively in maize inoculated with *Pseudomonas* sp.

For the fungal isolate (*A. niger*), growth parameters of the potted plants were least in the unsterilized and uninoculated soils. However, the growth of the maize plants in the natural

soil inoculated with inoculum plugs of *A. niger* showed better growth than the sterile but inoculated soils in terms of the length of leaves, width of leaves, and length of the stem. There was significant difference in the growth parameters of the maize plant that received microbial inoculum irrespective of whether the soil was sterilized or not when compared with the maize plant planted in sterile but uninoculated soil.

CONCLUSION

From the data obtained in this study, it can be concluded that *Lysinibacillus sphaericus* and *Aspergillus niger* are capable of solubilizing fixed phosphate in the soil, making the nutrient available for the growth of maize and thereby enhancing its growth.

RECOMMENDATION

Lysinibacillus sphaericus and *A. niger* are recommended for use as biofertilizer in order to enhance the growth of maize plants.

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