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Full Length Research Article

ASSESSMENT OF THE POTENTIALS OF AZOTOBACTER SPP. AS BIOINOCULANTS ON THE GROWTH OF POTTED MAIZE PLANTS

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ABSTRACT
The use of chemical fertilizers in crop production has detrimental effects such as soil acidity and eutrophication. There is need for environmentally friendly approach in improving soil nutrients and agricultural productivity. This study aimed at the isolation of *Azotobacter* spp from the rhizosphere of crops and the use of these isolates as biofertilizer for the growth of potted maize plants under 5 treatments. These treatments were maize plant only (control), plant with once application of NPK fertilizer, plants with once, weekly and fortnights application of *Azotobacter* spp. as bio-inoculants. The counts of *Azotobacter* obtained from the rhizosphere of the crops ranged from $4.0 \times 10^4 – 1.0 \times 10^6$ CFU/g. The three high ranking in-vitro biological nitrogen fixing and phosphate solubilization isolates were identified *A. chroococcum*, *A. vinelandii* and *A. beijerinckii*. At the 7th weeks of growth, 83.3 and 100% of the plants that received different levels of bio-inoculants had the perimeter of their girths greater than and significantly different from the controls when cultivated in 8.1 and 12.0 litre pots respectively. At least 91.7% of the plants that received bio-inoculants had higher heights than the controls. It is concluded that the application of *Azotobacter* bio-inoculants enhanced the growth of maize plants compared to the controls.

Keywords: Pot cultivation, Plant growth promoting, Phosphate solubilization, Nitrogen fixation.

INTRODUCTION

Nitrogen is one of the key elements required for the crop and available in abundant quantity in nature (Leghari *et al.*, 2016). The use of chemical fertilizers come with certain disadvantages such as pollution of large water resources, destruction of certain microorganisms, acidity of the soil etc. There are certain microorganisms which can convert this unavailable nitrogen (molecular nitrogen) into available form by fixing it into the soil. These microorganisms allow the conversion of gaseous nitrogen (N_2) to the other forms of available nitrogen for example nitrite, nitrate, and ammonium which are required for the development of metabolic processes of plants (Grzyb *et al*., 2021). This conversion process of gaseous nitrogen takes place as a result of the action of microorganisms in the soil. These microorganisms include: *Azotobacter, Azospirillum*, *Azolla*, *Cyanobacteria*, *Beijerinckia* etc. (Aasfar *et al*., 2021).

The beneficial effects of *Azotobacter* are not only due to its ability to fix atmospheric nitrogen, but also able to secrete growth substances and antifungal antibiotics, which improve plant stand in inoculated field by inhibiting root pathogens (Jimenez *et al*., 2011). It has been also shown that strains of *Azotobacter* could be usefully employed in biofertilizers production, due to their ability of fixing nitrogen and solubilizing phosphates (Wani *et al*., 2016). Beneficial microbes such as plant growth promoting rhizobacteria (PGPR) are being used for sustainable agriculture**.** Biological nitrogen fixation is considered a key process in the biosphere (Padmavathi *et al*., 2015). These microorganisms actively colonize the plant root regions. They possess several physiological features such as production of indole acetic acid (IAA), siderophore, HCN etc. These characteristics are directly involved in plant growth and promotion (Iqbal and Hasnain, 2013).

There is need to look for alternatives to chemical fertilizers in agricultural productivity. This study was undertaken to isolate indigenous *Azotobacter* spp. from soils; and evaluate the effects of the isolates as bio-inoculants on potted maize plants.

MATERIALS AND METHODS

Collection of soil samples

The soil samples were collected from the rhizosphere of different crops into sterile polythene bag and transferred immediately to the laboratory for further analysis (Pant and Agrawal, 2014; Chandra *et al*., 2018).

Isolation of *Azotobacter* from the soils

Ashby's mannitol agar (AMA) was used for the isolation of *Azotobacter* spp. (Ponmurugan *et al.*, 2012). The soil was serially diluted, plated, incubated and the number of colonies formed were counted and expressed in CFU/g. Different discrete colonies were picked from the plates and subcultured on another AMA plate until pure cultures were obtained (Fawole and Oso, 2007). The colonies on AMA could be white, light brown, dark brown or cream with circular or irregular shape. They could be translucent or opaque.

In vitro Biological Nitrogen Fixation (BNF)

Jensen broth (JB) was prepared; 15mL each was dispensed into each MacCartney bottle and sterilized by autoclaving. The bacterial cultures were standardized (Cheesbrough, 2006) and 0.75mL (5%) of it was added into the JB. The broth was placed on a shaker at 120 rpm for 14 days. Thereafter, optical density (OD) was taken using spectrophotometer at 630 nm (Sharma and Saharan, 2015). In-vitro BNF (%) was calculated as follows:

$$\text{In-vitro BNF (\%)} = \frac{\text{OD of reference} - \text{OD of inoculated culture}}{\text{OD of reference}} \times 100$$

Phosphate Solubilization

Pikovskaya's medium (PVK) was prepared as adopted by Nasr *et al.* (2021). The PVK broth was inoculated with 3% standardized culture of the isolate and the mixture was placed on a rotary shaker at 120 rpm. On the 17th day of the commencement of the study, the PVK-bacterial mixture was filtered through Whatman filter paper No 11. The filtrate was then centrifuged at 10,000 rpm for 10 minutes.

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The mixture was carefully decanted and its filtrate used to quantify the solubilized phosphate.
One millilitre of supernatant from phosphate solubilization study was added to 9mL of distilled water and shaken to obtain 1:10 dilution. Then, 1mL from this dilution was introduced into 50mL volumetric flask. This was followed by the addition of 8mL of the combined reagent and made up to its volume with distilled water. Blue colouration developed and its absorbance was read at wavelength of 880 nm using Spectrophotometer. The amount of soluble phosphate was extrapolated from the standard curve and were multiplied by the dilution factor of 500 (APHA, 1999; Ruangsanka, 2014).

Molecular identification of the bacterial isolates
Zymogen DNA extraction kit along with vortexer and microcentrifuge were used for the bacterial DNA extraction. The extracted DNA was subjected to PCR amplification using GeneAmp PCR system 9700 with forward and reverse primers being 27F: AGAGTTTGATCMTGGCTCAG and 1525R: AAGGAGGTGWTCCARCCGCA rspectively. The PCR cocktail mix comprised 1.0µl of 10x PCR buffer, 1.0µl of DMSO, 0.8µl of 2.5mM DNTPs 0.1µl of Taq 5µ/µl and 3.1µl of ultrapure distilled H_2O. The PCR conditions used were initial denaturation at 94°C for 5 minutes followed by denaturation at 94°C for 30 seconds. Annealing temperature was done at 56°C for 30 seconds and extension at 72°C for 45 seconds. The above processes were repeated for 36 cycles. Final extension was done at 72°C for 7 minutes. The product was held at holding temperature at 10°C. The amplicon from the reaction above was loaded on 1.5% agarose gel and the gel picture viewed. The expected base pair of the amplicon is around 850bp.
The PCR product was purified and loaded into 3130XL genetic analyzer along with the components of the sequencing kit by following the manufacturer's instructions in order to obtain the sequence of the organism. Consensus sequence was generated using seqTrace. The most probable identity of the isolates was obtained using the online search tool at the website of National Center for Biotechnology Information (Dashti *et al*., 2009).

Collection and assessment of soils for pot experiments
Soil sample was collected from an uncultivated land at Botanical Garden of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria on latitude 8° 28' 9" N and longitude 4° 39' 39" E (Olayinka *et al*., 2021). The vegetation on the land was cleared and removed. The top soils were gathered at a spot and thoroughly mixed several times using shovel. The soil was packed into 8.1 litre capacity plastic buckets until the net weight of 8.0 kg obtained. Each bucket has 6 perforations at its bottom to allow the drainage of excess water. The tare weight of each bucket was 0.14 kg.

Another set of soil was collected from an uncultivated land behind Central research laboratory, University of Ilorin, Ilorin, Nigeria on latitude 8° 28' 50" N and longitude 4° 40' 42" E into 12.0 litre capacity plastic buckets until the net wet of the soil was 12 kg with a tare weight of 0.33 kg. The soils from the 2 locations were used for 2 sets of maize planting. The plastic buckets containing the soils were taken to the Department of Microbiology, University of Ilorin, Nigeria on latitude 8° 28' 50" N and longitude of 4° 40' 34" E for the pot experiments.

Sowing of maize in the pots
Seeds of Samas-27 variety of maize was purchased from the Institute of Agricultural Research, Zaria, Nigeria. Two seeds were planted per pot and the number of seedlings were thinned to one at week one of the cultivation. Each of the potted soil received equal amount of water (250 mL) on a daily basis. The maize plants were cultivated in 8.1 and 12.0 litre capacity plastic buckets between mid-March to mid-June and July to September 2020 respectively.

Application of treatments to the cultivated maize plants
Five treatments were used in this study. These treatments were maize plant only (without any addition of bio-inoculant or NKP fertilizer), maize plant with once application of NPK fertilizer, maize plant with: once, weekly and fortnight application of bio-inoculant. Two pots were used for each treatment. The pots were arranged in a complete randomized block design (Ramzi and Alsalim, 2019).

For the bio-inoculant, the isolate was first transferred from its stock culture slant and streaked to cover the entire plates of nutrient agar. The plates were incubated at 30°C for 48 hours. The culture was aseptically washed into 100 mL of sterile physiological saline until its turbidity matched that of 4.0 McFarland standard which is equivalent to 1.2×10^9 CFU/mL (Bhattacharjee *et al*., 2014). One hundred millilitre of the standardized culture was then applied to the pots designated to contain the bio-inoculant for the required duration. Once application of NPK fertilizer was done by adding 5.0 g of its granules using spot placement on the 14th days after cultivation.

Assessment of effects of treatments on the growth of maize plant
At 3rd, 5th and 7th week of cultivation of the maize plants, girth of stem and plant height were measured and recorded for each treatment (Prajapati *et al*., 2008; Ramzi and Alsalim, 2019).

Data analysis
The data were means of 2 replicates. The standard deviation (SD) of each mean was determined and the means were separated by one way analysis of variance (ANOVA) using Duncan's multiple range test at $\alpha = 0.05$ (Dutta and Thaker, 2017; Dashti *et al*., 2021).

RESULTS

Counts of *Azotobacter* spp. isolated from the soils
The population of *Azotobacter* across the rhizosphere soils of the different plants ranged from $4.0 \times 10^4 - 1.0 \times 10^6$ CFU/g (Table 1)

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Table 1: Counts of *Azotobacter* isolated from the soils

Rhizosphere soils	Count (CFU/g)
Okro	6.0×10^5
Rice	1.0×10^5
Cassava	4.0×10^4
Moringa	2.5×10^5
Sorghum	7.0×10^5
Teak	1.0×10^5
Date palm	1.0×10^5
Pawpaw	1.5×10^5
Potato	1.0×10^6
Jatropha	1.2×10^5

Plant growth promoting characteristics of the isolates
The phosphate solubilized and in-vitro biological nitrogen fixation of the isolates ranged from 0.09 - 215 µg/mL and 6.22 – 18.90% respectively (Table 2).

Table 2: Plant growth promoting characteristics of bacterial isolates

S/No	Bacterial isolates	Phosphate solubilization (µg/mL)	In vitro BNF (%)
1	AZ 1	1.59	7.89
2	AZ 2	1.94	15.31
3	AZ 3	1.06	12.44
4	AZ 4	105.00	14.83
5	AZ 5	2.09	6.70
6	AZ 6	45.00	6.22
7	AZ 7	0.80	12.44
8	AZ 8	85.00	11.96
9	AZ 9	1.35	17.22
10	AZ 10	0.69	16.99
11	AZ 11	0.09	17.22
12	AZ 12	215.00	18.90

BNF=Biological nitrogen fixation

Identification of bacterial isolates
The three isolates that ranked high in term of in-vitro biological nitrogen fixation and phosphate solubilization were AZ4, AZ8 and AZ12 and were identified as *A. chroococcum, A. beijerinckii* and *A. vinelandii* respectively (Table 3).

Table 3: Identity of the selected bacterial isolates

Code	Identification	Accession number
AZ4	*Azotobacter chroococcum*	MH249629.1
AZ8	*Azotobacter beijerinckii*	MN340240.1
AZ12	*Azotobacter vinelandiii*	LN874283.1

Effect of bioinoculants on growth of potted maize plants cultivated in plastic buckets of different capacities:

Cultivation in 8.1 litre pots
For the 1st set of maize cultivated in 8.1 litre plastic buckets, at the 3rd week of cultivation, all the plants inoculated with the isolates showed significant differences in their girths when compared with the control plants with no inoculant. In addition, some of the plants that received once (33.3%), weekly and fortnight (41.7% each) application of bioinoculants had their girths equal to or higher than those that received once application of NPK fertilizer (Table 4).

At week 5 of cultivation of the maize plants, at least 75% of the plants that received once, weekly and fortnight application of bio-inoculants had their girths bigger and statistically significant than the control plants. For those plants that received weekly bioinoculants, only those that received *A. beijerinckii* and *A. chroococcum* showed no significant difference in the perimeter of their girths when compared with those that received once application of NPK fertilizer (Table 4).

At the 7th week of growth of the maize plants, at least 83.3% of the plants that received bio-inoculants had their girths bigger and significantly different from those plants without any treatment. The maize plants that received once application of NPK showed the highest perimeter of their girths (Table 4).

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Table 4: Effect of bioinoculants on the girth of maize plants cultivated in 8.1 litre plastic buckets

Inoculants	Perimeter of girth (mm)								
	Week 3			Week 5			Week 7		
	A	B	C	A	B	C	A	B	C
AZ1	21.5^{cde} ± 0.85	22^{de} ± 1.41	21.5^{cd} ± 0.71	30.5^{a} ± 0.71	30^{bcd} ± 1.41	33.5^{cd} ±1.41	36.5^{de} ± 0.57	34^{bc} ± 0.85	36.5^{de} ± 1.41
AZ2	24.5^{f} ± 0.71	18^{abc} ± 0.71	20^{bc} ± 1.41	28.5^{ab} ± 1.41	27.5^{ab} ± 0.28	33.5^{cd} ± 0.71	36^{de} ± 0.00	39^{ef} ± 1.41	37^{def} ± 1.41
AZ3	21^{cde} ± 1.41	20.5^{cde} ± 0.71	20^{bc} ± 0.00	26.5^{a} ± 0.42	28^{abc} ± 0.00	31.5^{bc} ± 0.57	30^{a} ± 1.41	36^{cd} ± 0.28	30^{a} ± 0.00
AZ4	20.5^{cd} ± 0.71	22^{de} ± 0.00	22^{cd} ± 0.71	32.5^{de} ± 0.21	38.5^{h} ± 0.57	35.5^{d} ± 0.14	37.5^{de} ± 2.12	41.5^{fg} ± 0.71	39.5^{fg} ± 2.12
AZ5	22.5^{def} ± 0.71	22.5^{e} ± 2.12	22^{cd} ± 1.41	33^{e} ± 1.41	33.5^{ef} ± 1.41	34^{d} ± 1.41	41^{f} ± 1.41	40^{ef} ± 2.82	39^{efg} ± 0.00
AZ6	21^{cde} ± 0.57	19.5^{bcd} ± 0.42	18^{ab} ± 1.41	28.5^{ab} ± 0.71	32^{de} ± 1.41	30^{ab} ± 1.41	31^{ab} ± 0.00	39.5^{ef} ± 0.71	34^{bc} ± 0.71
AZ7	21^{cde} ± 0.00	23^{e} ± 1.41	20^{bc} ± 2.82	27^{b} ± 1.41	28.5^{bc} ± 0.71	28^{a} ± 0.00	37^{de} ± 2.12	40^{ef} ± 0.00	37.5^{defg} ± 0.42
AZ8	23.5^{ef} ±1.41	21^{de} ± 0.28	23^{cd} ± 0.00	35.5^{f} ±0.57	39^{h} ± 2.12	34^{d} ± 0.28	38.5^{ef} ± 0.71	43.5^{gh} ± 1.41	37.5^{defg} ± 1.41
AZ9	22^{def} ±1.41	21^{de} ± 1.41	24^{d} ± 1.41	33.5^{de} ± 1.41	30.5^{cd} ± 0.71	33.5^{cd} ± 0.28	35.5^{cd} ± 0.57	37.5^{de} ± 1.41	40^{g} ± 1.41
AZ10	17^{ab} ± 0.71	23^{e}±0.00	22.5^{cd} ± 0.71	32.5^{de}±0.57	34.5^{fg} ± 0.71	30^{ab} ± 0.00	36.5^{de} ± 0.71	39^{ef} ± 0.00	36^{cd} ± 0.00
AZ11	19^{bc} ± 0.00	17.5^{ab}±0.42	17.5^{ab} ± 0.28	35.5^{f} ± 1.41	25.5^{a} ± 0.57	29.5^{ab}±0.71	35.5^{cd}±0.42	30^{a} ± 0.00	36.5^{de} ± 0.57
AZ12	21.5^{cde}±0.71	20.5^{cde}±1.41	21.5^{cd}±2.12	30^{bc} ± 0.00	36.5^{gh}±0.00	34.5^{d}±1.41	36^{de}±1.41	41.5^{fg}±1.41	38.5^{defg}±0.14
P	16^{a} ± 1.41	16^{a} ± 1.41	16^{a} ± 1.41	28^{ab} ± 1.41	28^{abc} ±1.41	28^{a} ± 1.41	33^{bc} ± 0.71	33^{b} ± 0.71	33^{b} ± 0.71
F	22^{def} ± 2.82	22^{de} ± 2.82	22^{cd} ± 2.82	39^{g} ± 1.41	38^{h} ± 1.41	38^{e} ± 1.41	45^{g} ± 1.41	45^{h} ± 1.41	45^{h} ± 1.41

A= Once inoculated; B= Weekly inoculated; C= Fortnightly inoculated; AZ= *Azotobacter*; P= Maize plant not inoculated (control); F= Once NPK fertilizer application.
Values are means of 2 replicates. Means followed by different superscripts are significantly different.

Table 5 shows the height of maize cultivated in 8.1 litre capacity plastic buckets for the different treatments. The height of 91.7% and 58.3% of the plants that received weekly and fortnight application of bio-inoculants were higher and significantly different from those that received once application of NPK fertilizer at the 3rd week of commencement of planting. The height of 75% of the maize plants that received once application of bio-inoculant showed significant difference when compared with those without any treatment.

At the 5th week of cultivation of maize plants, the highest heights were obtained by those plants that received once application of NPK fertilizer which were significantly different from other treatments. It was observed that 41.7, 91.7 and 50% of the plants that received once, weekly and fortnight application of bio-inoculants showed higher heights which were significantly different from those without any treatment (Table 5).

At the 7th week of growth of the maize plants, between 91.7 to 100% of the maize plants that received different levels of bio-inoculants produced higher heights than the control plants without any treatment. Only 50% of the plants that received once application of bio-inoculants showed higher heights than those that received once application of NPK fertilizer. Furthermore, 91.7% of the plants that received both weekly and fortnight application of bio-inoculants showed higher heights than those that received once application of NPK fertilizer (Table 5).

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Table 5: Effect of bioinoculants on the height of maize plants cultivated in 8.1 litre plastic buckets

Inoculants	Height of plants (mm)								
	Week 3			Week 5			Week 7		
	A	B	C	A	B	C	A	B	C
AZ1	70^{a} ± 2.83	83.5^{bcd}±4.95	81^{efgh} ± 0.00	150^{cd} ± 2.83	160^{ab} ± 2.83	155^{b} ± 0.00	255^{bc} ± 0.00	282^{d} ± 2.83	280^{fg} ± 2.12
AZ2	76^{bcde} ± 2.12	80^{bc} ± 1.41	73^{bc} ± 0.00	141.5^{b}± 2.12	168.5^{cd}±1.41	155^{b} ± 1.41	255^{bc} ± 0.71	290^{ef} ± 1.41	280^{fg} ± 2.12
AZ3	70^{a} ± 1.41	85^{cd} ± 3.54	76.5^{cde}±2.12	121.5^{a} ± 3.54	165^{bc} ± 0.00	157^{b} ± 0.00	250^{ab}±1.41	295^{ef} ± 0.00	293.5^{h} ± 2.12
AZ4	80^{ef} ± 2.12	87.5^{d} ± 2.83	85^{h} ± 2.83	169^{g} ± 2.12	181^{fg} ± 4.24	172de ± 0.00	290^{g} ± 3.54	312.5^{h}± 4.24	300^{i} ± 2.83
AZ5	72.5^{abc}±2.12	84^{bcd} ± 2.12	80^{defgh}± 0.00	160^{ef} ± 7.07	172de ± 2.83	167^{cd} ± 2.83	270de± 2.83	284de± 2.12	282^{g} ± 2.83
AZ6	69^{a} ± 1.41	69^{a} ± 0.71	67.5^{a} ± 2.12	146.5^{bc}±3.54	161^{ab} ± 2.83	165^{c} ± 2.12	250^{ab} ± 0.00	275^{c} ± 2.83	270^{cd} ± 0.00
AZ7	77^{bcdef}± 4.24	87.5^{d} ± 2.12	82^{gh} ± 0.71	157.5^{de}±3.54	168^{cd} ± 0.00	162.5^{c}± 2.12	260^{c} ± 1.41	298^{g} ± 2.83	277.5^{efg}±2.12
AZ8	74^{abcd} ± 2.12	85^{cd} ± 2.12	80^{defgh}± 2.83	166^{fg} ± 1.41	180^{fg} ± 2.83	175^{e} ± 2.83	270de ± 2.12	301^{g} ± 2.83	300^{i} ± 0.00
AZ9	82^{f} ± 0.00	87.5^{d} ± 2.12	83.5^{h} ± 3.54	168.5^{g}± 1.41	155^{a} ± 2.83	165.5^{c}± 2.12	280^{f} ± 0.00	290^{ef} ± 0.00	275^{def} ± 0.00
AZ10	72^{ab} ± 2.83	84^{bcd} ± 0.00	75^{cd} ± 2.12	142.5^{bc}±3.54	177^{ef} ± 2.83	146.5^{a}± 2.12	276.5^{f}±3.54	295^{fg} ± 1.41	273.5^{de} ± 2.12
AZ11	73^{abc} ± 2.12	79.5^{bc} ± 2.12	77^{cdef} ± 2.83	139.5^{b}± 3.54	162.5^{bc}±1.41	152.5^{b}±3.54	252.5^{b}±1.41	268.5^{b}± 4.95	255^{b} ± 2.83
AZ12	79^{def} ± 3.54	87.5^{d} ±2.83	82.5^{gh} ± 1.41	167^{fg} ± 0.00	183^{fg} ± 0.00	177.5^{e}± 3.54	275^{ef} ± 2.83	297.5^{g}±2.12	290^{h} ± 3.54
P	70^{a} ± 2.12	70^{a} ± 2.12	70^{ab} ± 2.12	155de ± 2.83	155^{a} ± 2.83	155^{b} ± 2.83	245^{a} ± 3.54	245^{a} ± 3.54	245^{a} ± 3.54
F	78^{cdef} ± 2.83	78^{b} ± 2.83	78^{cdefg}± 2.83	185^{h} ± 4.24	185^{g} ± 4.24	185^{f} ± 4.24	266^{d} ± 2.83	266^{b} ± 2.83	266^{c} ± 2.83

A= Once inoculated; B= Weekly inoculated; C= Fortnightly inoculated; AZ= *Azotobacter*; P= Maize plant not inoculated (control); F= Once NPK fertilizer application.
Values are means of 2 replicates. Means followed by different superscripts are significantly different.

Cultivation in 12.0 litre pots

Only 3 isolates that showed better effect on the maize plant growth from the 8.1 capacity buckets were used for the 2nd cultivation in 12 litres capacity buckets. These isolates were *Azotobacter chroococcum* (AZ4), *Azotobacter beijerinckii* (AZ8) and *Azotobacter vinelandiii* (AZ12).

When the maize plants were cultivated in 12.0 litre capacity plastic buckets, at week 3, all (100%) of the plants that received bio-inoculants had the perimeter of their girths greater than the control plants that did not receive any treatment and these differences were significant. The plants that received once application of NPK had their girths greater and significantly different from those that received any level of bio-inoculant (Table 6).

At the 5th week, the perimeter of girths of plants that received once inoculation of *A. chroococcum* and *A. beijerinckii* did not show any significant difference when compared with those that did not receive any treatment. However, the perimeter of girths of plants that received once inoculation of *A. vinelandiii* were higher and different significantly from the control. For the plants that received weekly and fortnight application of bio-inoculants, their girths were bigger and showed significant difference when compared with the controls that did not receive any treatment (Table 6).

At week 7, all the maize plants that received bio-inoculants had their girths bigger and showed significant difference when compared with the control that did not receive any treatment. However, the perimeter of the girths of maize plants that received once application of NPK fertilizer were significantly and bigger than those that received different levels of bi-inoculants (Table 6).

Table 6: Effect of bioinoculants on the girth of maize plants cultivated in 12.0 litre plastic buckets

Isolates	Perimeter of girth (mm)								
	Week 3			Week 5			Week 7		
	A	B	C	A	B	C	A	B	C
AZ4	18.5^{ab}± 0.71	22.5^{b} ± 0.71	21.5^{bc} ± 2.12	30^{a} ± 0.00	36.5^{bc} ± 2.12	35^{bc} ± 2.12	45.5^{b} ± 2.12	44.5^{b} ± 2.12	56.5^{c} ± 2.12
AZ8	20.5^{b}± 2.12	21.5^{b} ± 2.12	21.5^{bc} ± 0.71	30.5^{a} ± 2.12	32.5^{ab} ± 2.12	30.5^{ab} ± 2.12	46^{b} ± 1.41	45.5^{b} ± 2.12	46^{b} ± 0.00
AZ12	19^{ab} ± 0.00	23.5^{b} ± 1.41	18.5^{ab} ± 2.12	35^{b} ± 1.41	40^{c} ± 2.83	37^{c} ± 0.00	48^{b} ± 0.71	46.5^{b} ± 0.71	46.5^{b} ± 2.12
P	16.5^{a} ± 0.71	16.5^{a} ± 0.71	16.5 ^{a} ± 0.71	28^{a} ± 0.71	28^{a} ± 0.71	28^{a} ± 0.71	38^{a} ± 0.71	38^{a} ± 0.71	38^{a} ± 0.71
F	24.5^{c}± 2.12	24.5^{b} ±2.12	24.5^{c} ± 2.12	55^{c} ± 2.83	55^{d} ± 2.83	55^{d} ± 2.83	62^{c} ± 4.24	62^{c} ± 4.24	62 ^{c}± 4.24

A= Once inoculated; B= Weekly inoculated; C= Fortnightly inoculated; AZ= *Azotobacter*; P= Maize plant only;
F = Once NPK fertilizer application.
Values are means of 2 replicates. Means followed by different

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superscripts are significantly different

At the 3rd, 5th and 7th week, the heights of maize plants that received bio-inoculants were higher and different significantly when compared with those that did not receive any treatment but were lesser and significantly different from those that received once application of NPK fertilizer (Table 7).

Table 7: Effect of bioinoculants on the height of maize plants cultivated in 12.0 litre plastic buckets

Isolates	Height of maize plants (mm)								
	Week 3			Week 5			Week 7		
	A	B	C	A	B	C	A	B	C
AZ4	75.5^{a} ±0.71	92.5± 2.12	82 ± 0.71	167.5^{b}± 4.53	190± 7.07	177± 4.24	308^{c} ± 4.24	336.5^{c}± 7.78	330± 7.07
AZ8	80.5^{bc}± 2.12	93.5 ± 2.12	83 ± 1.41	169^{b} ± 2.83	186± 7.07	170± 2.83	277^{b}± 4.24	302.5^{b}± 6.36	290± 8.49
AZ12	82^{c} ± 1.41	90.5^{b}± 0.71	84± 0.00	165^{b}± 4.24	185± 4.24	175± 2.83	287.5^{b}± 2.83	300^{b} ± 4.24	284± 5.66
P	77^{ab} ± 1.41	77^{a} ± 1.41	77± 1.41	150^{a} ± 7.07	150± 7.07	150± 7.07	250^{a}± 5.66	250^{a}± 5.66	250± 5.66
F	91^{d}± 2.83	91^{b} ± 2.83	91^{c}± 2.83	290^{c}± 8.49	290^{c}± 8.49	290^{c}± 8.49	494^{d}± 5.66	494^{d}± 5.66	494^{d}± 5.66

A= Once inoculated; B= Weekly inoculated; C= Fortnightly inoculated; AZ=*Azotobacter*; P= Maize plant only; F = Once NPK fertilizer application.
Values are means of 2 replicates. Means followed by different superscripts are significantly different

DISCUSSION

The range of count of *Azotobacter* obtained in this study (4.0×10^4 - 1.0×10^6) is lower than the 3.0×10^5 - 1.1×10^6 CFU/g reported by Islam *et al.* (2008). Purwaningsih *et al*. (2022) obtained counts of *Azotobacter* in the range of $1.1 \times 10^6 – 4.9 \times 10^6$ CFU/g from the rhizosphere of rice.

In this study out of the 12 *Azotobacter* spp. isolated from rhizosphere of the plants, 3 of them were capable of both high in-vitro biological nitrogen fixation and phosphate solubilization and were identified as *A. chroococcum*, *A. beijerinckii*, and *A. vinelandii*. Chen *et al.* (2018) isolated *A. beijerinckii, A. chroococcum, A. tropicalis* and *A. vinelandii* from the rhizosphere of rice while Sulaiman *et al*. (2019) isolated *A. chroococcum, A. beijerinckii* and *A. salinestris* from the rhizosphere of *Acacia* spp,

Azotobacter spp. have been used in cereal to improve growth and grain when used as biofertilizers or bioinoculants (Ladha *et al*., 2016; Aasfar *et al*., 2021). When applied as bio-inoculants, these organisms multiply rapidly and develop a thick population in the rhizosphere (Wani *et al*., 2016). The plant responses ranged from increase in seed germination rates, root development, enhancement in nutrient uptake, root and shoot biomasses, leaf number and area (Aasfar *et al*., 2021). El-Sorady *et al*. (2022) observed that cultivars of wheat that received 75% urea fertilizer together with *Azotobacter* produced the best results for all the agronomic traits tested. Furthermore, Mrkovacki *et al*. (2016) obtained a positive effect on the grain yield of maize when *Azotobacter* was used as inoculants in pot experiments.

Conclusion

In this study maize plants that were inoculated with weekly application of *Azotobacter* bio-inoculants showed better growth parameters in term of perimeter of girths and height of the plants when compared with the controls that did not receive any treatment.

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