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Effects of Biologically Synthesized Iron Oxide Nanoparticles on Rhizospheric Microorganisms Associated with Tomato (*Solanum lycopersicum L.*)

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Abstract

In this study, the effect of iron oxide nanoparticles on soil rhizospheric microbial communities of tomato was investigated. Iron oxide nanoparticles were biologically synthesized using plant extract from *Azadirachta indica*, and characterized using a UV-VIS spectrophotometer. Varying concentrations (25, 50, 75, or 100 %) of biosynthesized iron oxide nanoparticles or precursor solution was rhizoinjected into soils in which tomato plants were grown. Plate count method was used to analyse population size and community structure of test subjects. Quantitative analysis of the bacterial and fungal community was determined and diversity indices were calculated. The results obtained from the analysis revealed that the addition of iron oxide nanoparticles to the soil changed bacterial and fungal community with respect to the control. Also, the bacterial and fungal abundance were changed. Some tolerant microorganisms such as *Micrococcus, Stapylococcus, Aspergillus, Trichoderma* and *Penicillium* could withstand high concentrations of iron oxide nanoparticles. Shannon diversity indices showed that there was difference in the diversity of each concentration of iron oxide nanoparticles for both fungal and bacterial communities. The study's findings showed that high concentration of iron oxide nanoparticles in the soil had adverse effect on both the tomato and the microorganisms associated with the root of the tomato. Further study needs to be conducted to ascertain the magnitude of impact iron oxide nanoparticles will have on plants and rhizosphere microbiome.

1. Introduction

A global rise in human population has increased the demand for food and other materials derived from plants, which has resulted in the search for novel ways to increase plant productivity. One such novel method is nanotechnology using nanoparticles; however, excessive use of nanoparticles may threaten plant health. The overall health of plants is affected by abiotic factors, as well as underground influences. The underground community is colonized by many microorganisms that play essential roles in plant growth, nutrition, and function (**Mendes *et al.,* 2013**). In humans, intestinal microbial communities' effects on health are becoming increasingly apparent; similar functions can be ascribed to microbial communities in the rhizosphere (**Berendsen *et al.,* 2012**).

Nanoparticles are of great interest due to their tiny size, large surface area to volume ratio, catalytic activity, and shape; they have the potential to solve problems in primary production and maximize production in agriculture (**Iravani, 2011**). Other than agriculture, nanoparticles have been applied in various fields such as medicine and pharmacy. More so, medical imaging, drug delivery, cancer therapy, antimicrobial activity, nutrient delivery, space exploration as well as environmental and physiological areas have also benefitted in nanotechnology (**Iravani, 2011**; **Teja and Koh, 2009; Azam *et al.,* 2012; Singh *et al.,* 2016**). Nanoparticles can be synthesized using physical, chemical, and biological methods. The use of biological methods is advancing over physical and chemical methods due to its ecofriendly approach (**Ahmed *et al.,* 2016**).

The underground community of plants, otherwise known as 'Rhizosphere', 'Plant microbiome', or 'Plants' other genome' is the section of soil under the direct influence of plant roots (**Mendes *et al.,* 2013; Katznelson *et al.,* 1948**). Organisms in the rhizosphere range from bacteria, fungi, oomycetes, algae, viruses, archaea to nematodes, protozoa, and arthropods. Many of these are beneficial, some are pathogenic to plants, while others are opportunistic pathogens to man (**Mendes *et al.,* 2013; Raaijmakers, *et al.,* 2008**). The influence of rhizospheric organisms on plants is numerous, so much so that it has been proposed that the underground density or diversity can be an indicator of above-ground diversity and productivity (**Mendes *et al.,* 2013; van der Heiden, *et al.,* 2008; Schnitzer *et al.,* 2011; Wagg *et al.,* 2011**). With the increased use of nanoparticles for various purposes, it is imperative to study nanoparticles' impacts on plant health. This research was aimed at investigating the effects of biologically synthesized iron oxide nanoparticles on rhizospheric bacteria and fungi associated with tomato (*Solanum lycopersicum* L.).

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2. Material and methods

2.1 Sources of plant and chemical materials

Neem leaves were obtained from the botanic garden at the University of Benin, Benin City, Edo State, Nigeria. A local variety of tomato seeds were obtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State, Nigeria. All reagents used which included ferric chloride, saffranine, gentian violet, lactophenol cotton blue were purchased from Pyrex Nigeria Limited in Benin City, Edo State, Nigeria.

2.2 Preparation of iron oxide nanoparticles

Iron oxide nanoparticles were prepared in the laboratory using extract from *Azadirachta indica* (Neem tree) leaves and ferric chloride ($FeCl_3$) as a precursor solution. A solution of 0.1 M ferric chloride was prepared by weighing 16.21 g of $FeCl_3$ salt into a 1 L beaker and distilled water was added to make 1 L with a continuous stirring on magnetic stirrer at room temperature. In preparing the leaf extract, A*. indica* leaves were rinsed with distilled water and surface sterilized with absolute ethanol. The leaves were then crushed in a mortar using a pestle, after which 250g of the crushed leaves were weighed, added to 1 L of water and heated for 10 minutes. The mixture was then sieved with a muslin cloth to get the plant extract. To prepare the iron oxide nanoparticles, 500 ml of the precursor solution was mixed with 500 ml of the plant extract using a 1 L beaker. The solution was stirred on a magnetic stirrer at room temperature. An immediate color change was observed (**Makarov *et al.,* 2014**). The synthesized nanoparticles were taken as 100 % concentration and several dilutions were made to obtain 75, 50 and 25 % concentrations.

2.3 Characterization of biologically synthesized iron oxide nanoparticles

The biologically synthesized iron oxide nanoparticles were characterized using a UV-VIS spectrophotometer as described by Martínez *et al.* (2012). An aliquot of 1 ml plant extract was placed into a thoroughly cleaned quartz cuvette, and used as blank for the spectrophotometric readings. Absorbance values for each of the various concentrations of iron oxide nanoparticles were determined at 420 – 780 nm wavelength.

2.4 Planting of tomato seeds

Dried tomato seeds were soaked in water for five minutes in a bowl. The seeds were planted in a nursery at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City. The seedlings were transplanted after 30 days into 30 perforated bowls.

2.5 Rhizoinjection of tomato seedlings with biologically synthesized iron oxide nanoparticles

Rhizoinjection of tomato seedlings with iron oxide nanoparticles started on the 7th week after planting. Nanoparticles were injected into the soil at the rhizospheric region. The experiment had five replicates, and each replicate was rhizoinjected with 75 ml each of water (control), 0.1 M ferric chloride (precursor), 100, 75, 50, and 25 % iron oxide nanoparticles. Rhizoinjection of the experimental plants were done at a 2-day interval.

2.6 Preparation of Potato Dextrose Agar and Nutrient Agar

Potato dextrose agar (PDA) and nutrient agar (NA) used for the study were prepared according to the manufacturers' instructions. The PDA was prepared by dissolving 39 g of powdered PDA in 1 liter of water while the NA was prepared by dissolving 28 g in 1 liter of water. The media were then sterilized by autoclaving at 121 ^{o}C for 15 minutes under pressure. After sterilization, an antibiotic (chloramphenicol) was added to the PDA to inhibit bacterial growth, and an antibiotic (griseofulvin) was added to the NA to inhibit fungal growth.

2.7 Isolation of rhizospheric microorganisms from soil

Soil samples were collected from rhizospheric regions of tomato plants by gently shaking the soils from the roots, and those soil adhering to the roots were used. Nine milliliters of distilled water were dispensed into 6 autoclaved McCartney bottles. Stock solution was prepared by weighing 1 g of the soil sample, which was then transferred into already sterilized bottles containing 9 ml of sterile distilled water for serial dilution preparation; 1 ml from the stock bottles were transferred into McCartney bottles labeled 10^1 to 10^3 containing 9 ml of sterilized distilled water using a sterile pipette. The prepared samples were inoculated onto the PDA and NA using the pour plate method. The PDA cultures were incubated at room temperature (28±2 ˚C) for 72 hours while the NA cultures were incubated at room temperature (28±2˚C) for 24 hours.

2.8 Sub-culturing of rhizospheric fungal and bacterial isolates

Single isolated mycelia of fungi were picked up using a sterilized wire loop and placed on a fresh PDA medium. The cultures were incubated at room temperature for 72 hours. A single isolated colony of bacteria was picked up using a sterilized wire loop and was streaked on a fresh NA medium. Incubation of cultures were carried out at room temperature for 24 hours.

2.9 Characterization and identification of rhizospheric bacteria

The bacterial isolates isolated from the tomato rhizosphere were characterized and identified using both cultural and biochemical tests. Cultural characteristics were observed on nutrient agar plates. The cultural characteristics included size, shape, surface opacity, texture, elevation, and pigmentation. All characteristics were observed by visibly observing the plates. Biochemical tests were also carried out to further confirm the identity of the bacteria isolates. Gram staining technique was used for differentiation between Gram-positive and Gram-negative bacteria. A catalase test was performed to differentiate those bacteria that produce the enzyme catalase; immediate active bubbling confirmed positive test. Urease test was performed at identifying *Enterobacteria* that produced urease enzyme, which hydrolyzes urea to give ammonia and carbon dioxide. An indole test was carried out for indole production by test organisms, vital in identifying *Enterobacteria*. A motility test was carried out to test the mobility of the isolates. Movement in different directions gave a positive test. A citrate test was carried out to test the isolates' ability to use citrate as a source of carbon; a bright blue color in the medium gave a positive test. A Carbohydrate fermentation test was conducted to determine the ability of bacterial isolates to utilize different sugars. A change in color from pink to yellow indicated positive results.

2.10 Characterization and Identification of rhizospheric fungal isolates

The fungal isolates were identified using macroscopy and microscopy. The morphological characteristics were noted and described. The isolates were stained with lactophenol and studied on a microscope. A sterile wire loop was used to pick the mycelium unto a clean, grease-free sterile glass slide, and then a

drop of lactophenol blue stain was added. The mycelium was spread evenly on the slide and teased to obtain a homogenous mixture before a glass coverslip was placed on the slide. Observations on the slide was made at x40 magnification. The micrograph was then compared with a manual for fungal identification.

2.11 Statistical analysis

Statistical Package for the Social Sciences (SPSS) and Paleontological Statistics software package for education and data analysis (PAST) were used to determine descriptive statistics as well as diversity indices.

3. Results

Synthesis and characterization of iron oxide nanoparticles

Iron oxide nanoparticles were synthesized using neem plant extract. Different concentrations of the biosynthesized iron oxide nanoparticles were subjected to spectrophotometric analysis at different wavelengths. Figure 1 shows the absorbance values of the different concentrations of the biologically synthesized iron oxide nanoparticles measured at different wavelengths. The maximum absorption values were recorded at wavelengths of 460 nm and 660 nm.

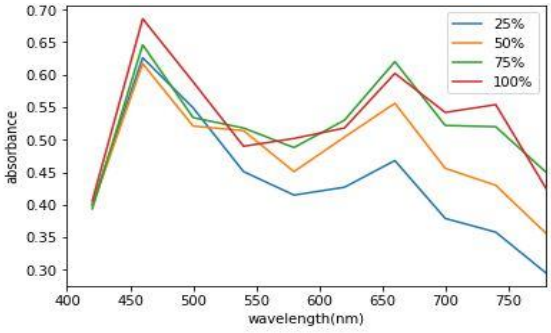

Figure 1: Absorbance values of different concentrations (25, 50, 75, and 100 %) of biosynthesized iron oxide nanoparticles

Identification of rhizospheric bacterial and fungal isolates

Morphological description of the bacteria isolated from the tomato rhizosphere is shown in Table 1. The shape, margin, size, elevation, and optical property of the bacterial isolates were represented. From the observed morphological characteristics of the bacterial isolates, the observed shapes were circular, irregular, and rhizoid; entire, lobate, and undulate margin was observed, flat and raised elevation were observed, and opacity was observed across all isolates. Biochemical tests carried out to determine the bacterial isolates (Table 2) revealed that the suspected bacteria were *Staphylococcus, Micrococcus, Pseudomonas*, and *Salmonella* spp. The tests also revealed that all isolates were positive for the catalase test and negative to the urease test. All except *Pseudomonas* and *Salmonella* spp were Gram positive. More so, the fungal isolates observed in the study were *Trichoderma, Aspergillus, Penicillium, Rhizopus, Pythium*, and *Fusarium* spp (Table 3). The shape, margin, size, elevation, pigmentation, texture, and optical property were observed. All isolates were large in size and opaque to light. An even distribution of flat and raised elevation in the colonies was observed. A similar observation was seen in the margin of the various colonies.

Growth response of tomato plant to different concentrations of iron oxide nanoparticles

Table 4 shows the incremental height of *Solanum lycopersicum* rhizoinjected with different concentrations of nanoparticles and the precursor solution. Values are presented as mean ± standard error. The control showed the highest incremental growth (14.9±0.8 cm) compared with the precursor treatment, which showed the least growth (-13.4±1.6 cm) after 20 days of rhizoinjection. The changes in plant height after 20 days of rhizoinjection with 75 and, 100 % iron oxide nanoparticles were -6.38 cm, and -3.14 cm, respectively. The highest change in plant height was observed in the order of control (14.86 cm) > 25 % (11.72 cm) > 50 % (9.8 cm) iron oxide nanoparticles as shown in Figures 2 and 3. The effects of iron oxide nanoparticles on the number of leaves of *Solanum lycopersicum* are presented in Table 5. Each value is s mean ± standard error of 5 replicates. The number of leaves was found to decrease with an increase in nanoparticle concentrations (Figure 4).

Quantitative analysis of bacteria and fungi isolated from tomato rhizosphere

The quantitative analysis of the bacteria isolated from the tomato root rhizosphere (Table 6) indicated a progressive reduction in the number of isolates with an increase in iron oxide nanoparticles' concentration. The precursor and the 100 % concentration of nanoparticles were observed to have the least amount of bacterial species, and the control has the highest number of bacterial species. However, *Staphylococcus* and *Micrococcus* were able to maintain a relatively high number across higher concentrations of iron oxide nanoparticles (Table 6). A similar observation was recorded when the quantitative analysis of fungi isolated from tomato rhizosphere was made. The relationship between the number of fungal colonies and iron oxide nanoparticles' concentration was inversely proportional; with *Aspergillus, Penicillium*, and *Trichoderma* surviving across all treatments. Rhizopus also displayed some level of tolerance. (Table 7).

Diversity indices of bacteria and fungi isolated from tomato rhizosphere

The diversity indices of the bacterial populations isolated from tomato roots are represented in Table 7. The 100 % concentration and precursor showed the highest dominance values (0.3689). On the contrary, the control showed the lowest value for dominance (0.234), highest values for Simpson's diversity index (0.767), and Shannon's index (1.527), and 75 % concentration showed the lowest Shannon's index values (1.043). There was a gradual decrease in the diversity with an increase in iron oxide nanoparticles' concentration, with the control being the most diverse and the precursor treatment being the least diverse (Table 7). A similar observation was made when diversity indices of fungi isolated from tomato rhizosphere was calculated. However, it was observed that the 50 % treatment showed the highest diversity with the lowest dominance values (0.262), highest Simpson's index (0.738) and Shannon's index (1.491), and the 100 % treatment displaying least diversity with the highest dominance (0.431), lowest Simpson's index (0.569) and lowest Shannon's index (0.918).

Table 1 Morphological description of bacterial isolates from *Solanum lycopersicum* rhizosphere

Morphology	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Shape	Circular	Circular	Irregular	Irregular	Rhizoid
Margin	Undulate	Entire	Lobate	Undulate	Lobate
Elevation	Flat	Raised	Flat	Flat	Flat
Size	Large	Small	Small	Large	Large
Optical property	Opaque	Opaque	Opaque	Opaque	Opaque
Suspected bacteria	*Micrococcus* spp	*Staphylococcus* spp	*Pseudomonas* spp	*Staphylococcus* spp	*Salmonella* spp

Table 2 Biochemical characteristics of bacterial isolates from *Solanum lycopersicum* rhizosphere

Biochemical test	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Gram staining	+	-	-	+	+
Cell type	Cocci	Baccili	Baccili	Cocci	Cocci
Mannitol	+	-	+	-	+
Lactose	-	-	-	-	+
Sucrose	-	-	-	-	+
Glucose	+	+	+	+	+
H_2S	-	-	-	-	+
Motility	+	+	-	-	-
Urease	-	-	-	-	-
Catalase	+	+	+	+	+
Indole	-	-	-	+	+
Citrate	+	+	+	+	-
Suspected Bacteria	*Micrococcus* spp	*Pseudomonas* spp	*Salmonella* spp	*Staphylococcus* spp	*Staphylococcus* spp

+: Positive to test; -: Negative to test

Table 3: Morphological description of fungal isolates from *Solanum lycopersicum* rhizosphere

Morphology	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
Margin	Entire	Entire	Rough	Rough	Entire	Rough
Elevation	Raised	Flat	Raised	Flat	Raised	Flat
Size	Large	Large	Large	Large	Large	Large
Texture	Smooth	Rough	Rough	Smooth	Smooth	Rough
Pigmentation	Green	Light green	Dark green	White	White	White
Optical property	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Suspected Fungi	*Trichoderma* spp	*Aspergillus* spp	*Penicillium* spp	*Rhizopus* spp	*Pythium* spp	*Fusarium* spp

Table 4: Incremental height of *Solanum lycopersicum* rhizoinjected with different concentrations of iron oxide nanoparticles

	Plant Height (cm)					
DAR	**CTRL**	**25 %**	**50 %**	**75 %**	**100 %**	**PREC**
5	3.7±0.7	2.9±0.5	2.4±0.3	2.9±0.6	3.7±0.8	3.1±0.4
10	6.6±0.9	4.8±1.1	3.5±0.3	4.3±0.7	5.2±0.9	-5.2±4.0
15	10.3±0.8	7.3±1.4	5.8±0.4	-1.8±5.1	3.6±4.4	-4.8±4.2
20	14.9±0.8	11.7±2.6	9.8±0.9	-6.4±5.0	-3.1±6.0	-13±0.0

R: Days after rhizoinjection; CTRL: Control; PREC: Precursor solution

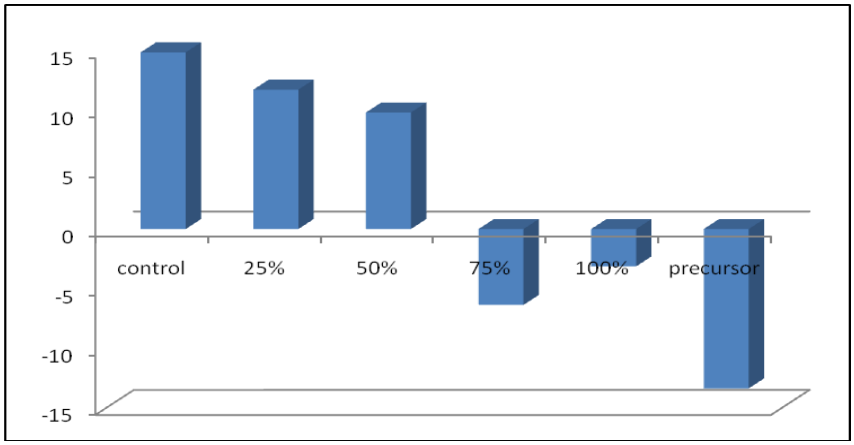

Figure 2: Change in plant height of *Solanum lycopersicum* after 20 days of rhizoinjection with iron oxide nanoparticles

Figure 3: Height of *Solanum lycopersicum* rhizoinjected with different concentrations of iron oxide nanoparticles after 20 days
A: Control; B: 25 % iron oxide nanoparticles, C: 50 % iron oxide nanoparticles; D: 75 iron oxide nanoparticles %; E: 100 iron oxide nanoparticles %; F: Precursor solution

Table 5: Effects of iron oxide nanoparticles on the number of leaves of *Solanum. lycopersicum*

DAR	CTRL	25 %	50 %	75 %	100 %	PREC
0	6±0.4	6±0.5	6±0.4	6±0.5	6±0.4	6±0.5
5	7±0.4	6±0.6	6±0.4	6±0.5	6±0.4	6±0.7
10	9±0.4	7±0.5	7±0.5	6±0.5	7±1.0	3±1.9
15	10±1.0	8±0.4	8±0.6	4±1.8	6±2.0	1±0.5
20	10±1.2	8±1.3	4±0.7	3±1.7	4±2.5	0±0.0

DAR: Days after rhizoinjection; CTRL: Control

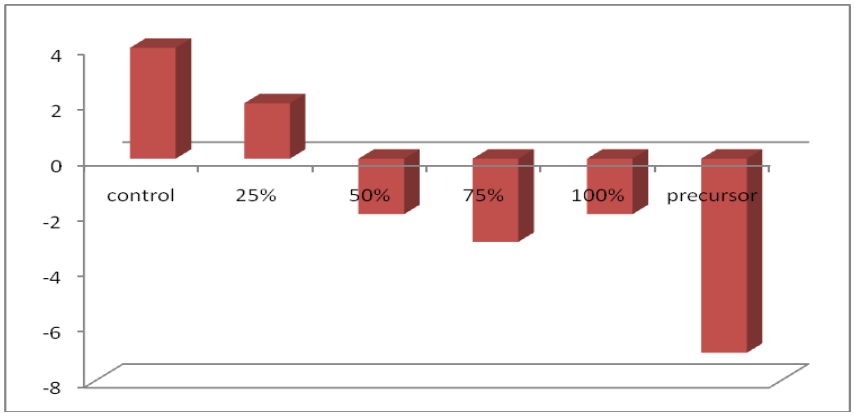

Figure 4: Change in the number of leaves of *Solanum lycopersicum* after 20 days of rhizoinjection with iron oxide nanoparticles

Table 6: Quantitative analysis of rhizospheric bacteria isolated from *Solanum lycopersicum* root.

ISOLATES	CTRL	25 %	50 %	75 %	100 %	PREC
Pseudomonas spp	10	10	0	0	0	0
Micrococcus spp	18	11	9	9	8	5
Salmonella spp	22	15	10	0	0	0
Staphylococcus spp	15	17	11	10	10	8
Staphylococcus spp	15	8	6	6	6	5

CTRL: Control; PREC: Precursor

Table 7: Quantitative analysis of rhizospheric fungi isolated from *Solanum lycopersicum* root

ISOLATES	CTRL	25 %	50 %	75 %	100 %	PREC
Penicillium spp	20	25	17	15	10	7
Aspergillus spp	30	35	20	15	12	10
Fusarium spp	6	7	5	0	0	0
Trichoderma spp	2	3	2	2	2	2
Pythium spp	2	2	2	0	0	0
Rhizopus spp	11	15	10	8	0	0

CTRL: Control; PREC: Precursor

Table 8: Diversity indices of bacterial population isolated from *Solanum lycopersicum* root

INDICES	CTRL	25 %	50 %	75 %	100 %	PREC
Dominance_D	0.234	0.242	0.280	0.367	0.369	0.369
Simpson_1-D	0.767	0.758	0.720	0.633	0.631	0.631
Shannon_H	1.527	1.502	1.321	1.043	1.044	1.044
Evenness_e^H/S	0.921	0.898	0.937	0.946	0.947	0.947
Menhinick	0.737	0.833	0.800	0.750	0.775	0.775
Margalef	1.045	1.116	0.932	0.721	0.739	0.739

CTRL: Control; PREC: Precursor

Table 9: Diversity indices of fungal population isolated from *Solanum lycopersicum* root

INDICIES	CTRL	25 %	50 %	75 %	100 %	PREC
Dominance_D	0.291	0.282	0.262	0.324	0.431	0.424
Simpson_1-D	0.709	0.718	0.738	0.676	0.569	0.576
Shannon_H	1.420	1.433	1.491	1.207	0.918	0.943
Evenness_e^H/S	0.689	0.699	0.740	0.836	0.835	0.856
Menhinick	0.712	0.643	0.802	0.633	0.612	0.688
Margalef	1.173	1.120	1.242	0.813	0.629	0.679

CTRL: Control; PREC: Precursor

4. Discussion

The absorbance values of the biologically synthesized iron oxide nanoparticles for 25, 50, 75, and 100 % concentrations (Figure 1) peaked at 460 nm. The plant extract acted as a reducing and capping agent (**Lopez-Tellez *et al.,* 2018**). This plant-assisted reduction mechanism has been attributed to phytochemicals such as terpenoids, flavones, ketone aldehyde, amines, and carboxylic acid (**Obiazikwor and Shittu, 2018**). Other biological materials like orange peel, plants like *Commelaena benghalensis, Hordeum vulgare,* and *Rumex acetosa* have been used to synthesize iron oxide nanoparticles (**Obiazikwor and Shittu, 2021; Lopez-Tellez *et al.,* 2018; Obiazikwor and Shittu, 2018; Makarov *et al.,* 2014**).

In this study, two growth parameters were considered; incremental plant height and the number of leaves. The effects of iron oxide nanoparticles on the incremental height of the tomato plant (Table 4) showed that some treatments rhizoinjected with ferric chloride precursor solution started to die off after ten days of rhizoinjection. The 100 and 75 % iron oxide nanoparticle treatments started dying off after 15 and 20 days of rhizoinjection, respectively, while the 50 % treatment showed stunted growth. Some replicates' death accounts for the negative values represented after 20 days of rhizoinjection; all replicates of the ferric chloride precursor treatment had died off. The control plants had the highest values of plant height (14.9±0.8) compared to other treatments. This observation may be attributed to iron toxicity. Excess iron has been reported to decrease the absorption of Ca^{2+}, Mg^{2+}, P, and K, which are essential for plant growth (**Rout and Sahoo, 2015**).

The effects of iron oxide nanoparticles on the number of leaves of *Solanum* lycopersicum (Table 5) showed that the precursor treatment showed defoliation after ten days of rhizoinjection. Other concentrations (75 and 100 %) showed similar symptoms after 15 days of rhizoinjection. Defoliation symptoms result from iron toxicity as free iron can generate free radicals, which can damage a wide variety of cellular structures; excess iron reduces the absorption of Mg^{2+}, an essential element in the synthesis of chlorophyll (**Mehraban *et al.,* 2008**). However, the control plants showed a higher degree of chlorosis than the 25 % concentration of iron oxide nanoparticles. This observation is probably because although iron is abundant in the soil, its bioactivity is relatively low, making it not readily available for plants, and iron availability can affect the natural distribution of species as well as limiting the growth of fast-growing economic plants (**Rout and Sahoo, 2015; Colombo *et al.,* 2013**).

The bacterial organisms isolated in this study were *Micrococcus* spp, *Pseudomonas* spp, *Salmonella* spp, *and Staphylococcus* spp (Table 1). The biochemical characteristics (Table 2) of the isolated bacteria indicated that *Micrococcus* spp and *Staphylococcus* spp were Gram-positive, *Pseudomonas* spp, and *Salmonella* spp were Gram-negative. These results have been attributed to the physical and chemical characteristics of bacterial cell walls. Gram-positive bacteria have a thicker peptidoglycan cell wall as compared to Gram-negative bacteria. All bacterial isolates were positive for catalase and glucose fermentation tests and negative to urease test. The fungal organisms isolated in this study are *Trichoderma* spp, *Aspergillus* spp, *Penicillium* spp, *Rhizopus* spp, *Pythium* spp, and *Fusarium* spp (Table 3). All isolates were opaque to light.

Quantitative analysis of bacteria isolated from *Solanum lycopersicum* root (Table 6) revealed that *Pseudomonas* spp only grew on the control and 25 % concentration of iron oxide nanoparticles, *Micrococcus* spp grew on all concentrations as well as *Staphylococcus* spp. Salmonella spp grew on 25 %, 50 %, and control treatments. There seemed to be a progressive reduction in the number of organisms from the control to the precursor treatment. *Micrococcus* and *Staphylococcus*'s ability to grow on all treatments is probably due to their tolerance to heavy metal toxicity (**Muhammad and Ibrahim, 2018; Alnaimat *et al.,* 2017**). This tolerance has been reported since tolerant bacteria can direct the energy budget from growth towards other functions that confer resistance (**Alboghobeish *et al.,* 2014**). However, report has it that most iron tolerant bacteria are Gram negative (**Trevors *et al.,* 1985**). *Pseudomonas* and *Salmonella* probably did not grow under high concentrations because of excess iron's ability to denature proteins. The quantitative analysis of fungi isolated from *Solanum lycopersicum* root (Table 7) was reported. *Penicillium* spp, *Aspergillus* spp, and *Trichoderma* spp grew on all iron oxide nanoparticles and precursor treatment concentrations. *Fusarium* spp and *Pythium* spp grew on the control, 25, and 50 % concentrations of iron oxide nanoparticles. *Rhizopus* spp grew on the control, 25, 50, and 75 % concentrations of iron oxide nanoparticles. It has been reported that *Aspergillus, Penicillium*, and *Trichoderma* exhibit tolerance to excess heavy metals (**Anahid, *et al.,* 2010**). Rhizopus showed some tolerance level to high concentrations of iron oxide nanoparticles, which agrees with previous studies (**Oladipo *et al.,* 2018**). Although it has also been reported that excess iron is toxic to Rhizopus because it can catalyze the formation of O_2 free radicals leading to injury (**Ibrahim *et al.,* 2008**). *Pythium* spp showed the least growth both in number and occurrence. This observation may be because *Trichoderma* has been reported to act as a biological control of *Pythium* (**Hader *et al.,* 1984**), coupled with the effects of excess iron.

Diversity indices of bacterial populations isolated from *Solanum lycopersicum* rhizosphere (Table 8). The precursor treatment showed greater dominance values indicating that a particular taxon dominates the population. The control showed the highest of Shannon's indices values, indicating high diversity in the community. This observation agrees with a study carried out where magnetic iron oxide nanoparticles affected the soil bacterial community. Iron oxide nanoparticles been reported to be toxic to bacteria due to the generation of reactive oxygen species (ROS) and this led to a decrease in the survival of *E. Coli*, mainly due to oxidative stress. The release of Fe^{2+} from Fe_3O_4 results in weaker enhancement of bacterial community richness and community composition (**He *et al.,* 2011**). Investigation of the fungi's diversity indices isolated from the root of *Solanum lycopersicum* (Table 9) revealed that the 50 % concentration of iron oxide nanoparticles showed the lowest dominance, highest Simpson's and Shannon's index values indicating a high diversity in the 50 % treatment compared to other treatments. This value is probably because of fungi's advanced mechanisms, especially *Rhizopus*, to absorb the soil's biologically inactive iron (**Ibrahim *et al.,* 2008**), allowing the fungi in the 50 % treatment to access essential iron in the soil. The high dominance values of 100 % and precursor treatments indicate that a particular taxon dominates the population.

5. Conclusion

The study attempted to evaluate the effects of iron oxide nanoparticles on the bacterial and fungal communities in the rhizosphere of tomato plants. The effects on the number of leaves and height of the tomato plant were also tested. The results obtained suggests a direct connection between high concentration of iron oxide nanoparticles and changes in the community structure of tomato rhizospheric microbiome. Indiscriminate disposal of iron oxide nanoparticles should be discouraged as an accumulation of iron oxide nanoparticles in the soil, and other substrata where plants grow soon may have adverse effects on both above-ground and below-ground diversity in the long run. Further investigations need to be

carried out to better predict the severity and the proximity of such adverse effects.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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