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# Use of termitarium soil as a viable source for biofertilizer and biocontrol

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## Abstract

**Background:** Environmental deterioration arising from the misuse of pesticides and chemical fertilizers in agriculture has resulted in the pursuit of eco-friendly means of growing crop. Evidence has shown that biofertilizers and biocontrol can boost soil fertility and suppress soil pathogens without compromising the safety of the environment. Hence, the study investigated the use of termitarium soil as a viable source for biofertilizer and biocontrol.

**Results:** Twenty-seven soil samples were collected from nine different mound soil (household, farm and water bodies in a sterile sample bag). Aliquots of serially diluted samples were plated on nutrient agar, plate count agar, eosin methylene blue agar and MacConkey agar plates. Isolates were identified using standard microbiological techniques. Identified isolates were screened for plant growth-promoting properties using phosphate solubilization test, potassium solubilization test and indole acetic acid production test. Activities of the plant growth-promoting bacteria were carried out using antagonism by diffusible substance method and antagonistic activity of cell-free culture filtrate of bacterial isolates against *Ralstonia solanacearum* and *Fusarium oxysporum*. Two hundred bacterial isolates were recovered from the 27 soil samples. The most predominant isolate was *Bacillus* spp. Out of the 200 bacterial isolates, 57 were positive for phosphate solubilization test, potassium solubilization test and indole acetic acid production test. Out of the 57 isolates, six bacterial isolates had antagonistic activities against *Fusarium oxysporum*, while seven bacterial isolates antagonized *Ralstonia solanacearum*.

**Conclusion:** The result showed that termite mound soil contains some useful bacteria that are capable of solubilizing phosphate and potassium and producing indole acetic acid which are the plant growth-promoting potentials and as well suppressing plant soil pathogen.

**Keywords:** Termites, Phosphate–potassium solubilization, Indole acetic acid, Biocontrol

## Background

The rapid increase in population has resulted in a drastic increase in the use of pesticides and inorganic fertilizers to control plant pests and boost soil fertility in order to increase food production that will meet up with the rising demand for food worldwide. This drastic increase in the use of pesticides and inorganic fertilizers had led to destruction of food chain (due to eutrophication), air pollution and groundwater contamination, thus affecting

human and environmental health (Savci 2012; Alori et al. 2017). Also, prolonged use of these chemicals affects soil by reducing its water-holding capacity, increasing soil salt content, inequality of soil nutrient distribution and ultimately affecting the structure and fertility of soil (Savci 2012). Observing these negative influences of chemical fertilizers and pesticides, the use of eco-friendly methods like the use of biofertilizer and biocontrol to boost soil fertility and suppress soil plant pathogens becomes imperative (Pathak et al. 2018; Igiehon and Babalola 2017).

Biofertilizers are substances that are made up of live microorganisms which could be plant growth-promoting

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rhizobacteria (PGPR) which, when applied to soil, plant or seeds, inhabit the rhizosphere of plants and stimulate plant growth (Malusa and Vassilev 2014). Plant growth-promoting rhizobacteria enrich the soil through potassium solubilization, phosphate mineralization, nitrogen fixation and breaking down organic substances to forms that plants can utilize. The structures and functions of soil microorganisms are widely used as a pointer to assess the degree of soil health (Zhu et al. 2017). This is because soil microorganisms function as a means of transforming carbon-based materials, minerals and energy cycling while also performing further roles that could advance soil health and agricultural sustainability (Choudhary et al. 2018).

Structures in several tropical ecosystems that are primarily built by termites are known as termite mound (Jouquet et al. 2015). Termite mound soil is rich in mineral nutrients and organic matter which make it a suitable habitat for microorganisms (Nithyatharani and Kavitha 2018). Due to this nutrient richness of termite mound soil, small-scale farmers often improve the soil condition of their farmland by using termite mound soil, which they believe can increase crop yield (Deke et al. 2016). A termite mound is built by a mixture of clay components and organic carbon cemented by secretions, excreta or saliva deposited by termites (Sujada et al. 2014). The architectural shapes of termite mounds include cathedral, dome, conical, lenticular and mushroom-like (Abe et al. 2011). These variations in shape depend on species type, ecological temperature conditions, clay availability and the level of termite disturbance in the environment (Arhin et al. 2015). Jouquet et al. (2015) also reported that soil nutrients are accumulated in a termite mound and their turnover plays an important role in the ecosystem.

Furthermore, some bacteria isolated from termite mound soil could be utilized in an eco-friendly way as a potential material for antimicrobial production, biofertilizers and biocontrol which can increase soil fertility and enhance crop production, thereby guaranteeing environmental sustainability (Enagbonma and Babalola 2019). The nutrient richness of termite mounds not only contributes to plant growth but also supports soil beneficial bacteria living in termite mound soil (Chauhan et al. 2017).

Termite mound soil predominantly contains three different phyla of culturable bacteria, i.e., *Firmicutes* (74%), such as *Bacillus subtilis*; *Proteobacteria* (22%), such as *Azotobacter*; and *Actinobacter* (3%), such as *Streptomyces* (Manjula et al. 2014). The organic matter present in the termite mound is highly rich in nitrogen (N), phosphorus (P) and sulfur (S), which facilitate the growth of beneficial microorganisms such as nitrogen fixers, decomposers and sulfur oxidizers (Miyagawa et al. 2011).

The occurrence of these beneficial soil organisms and the high nutrient content in termite mound soil have been shown to enhance crop yield and thus can be used as biofertilizers (Fall et al. 2004).

Several studies have reported different plant growth-promoting activities of bacteria, but there is dearth of information on plant growth-promoting activities of bacteria recovered from different habitats of termite mound soil. Hence, this study is designed to isolate bacteria with biocontrol and plant growth-promoting properties from termite mound soil habitats.

## Methods

### Sample collection

Twenty-seven soil samples were collected from nine different termite mound at different depths (top, middle and down layers) thrice from each location which were household, farmland and near water bodies in a sterile sample bag.

### Isolation and identification of isolates

One gram of the termite mound soil sample was serially diluted up to the fifth dilution. Aliquot was inoculated into sterile agar plate containing nutrient agar, plate count agar, eosin methylene blue agar and MacConkey agar plates which were prepared according to the manufacturer's instructions, and inoculated plates were incubated. Discrete colonies were selected based on their morphological characteristics and were subcultured to obtain pure cultures for further analysis.

Bacterial isolates were characterized on the basis of their morphological and biochemical features. Gram staining and other biochemical such as methyl red test, Voges-Proskauer test, indole test, catalase test, oxidase test, urease test, coagulase test, citrate utilization test and triple sugar iron test were carried out (Tandogan et al. 2014).

### Screening of termitarium bacteria for plant growth-promoting properties

#### Phosphate solubilization test

The bacterial isolate was spot inoculated at the center of the prepared sterile Pikovskaya agar plate and incubated for 72 h at 30 °C. The zones of phosphate solubilization formed around the colonies were recorded after 72 h. The solubilization index was determined by dividing the total diameter of the halo with the diameter of the colony (Tan et al. 2014).

#### Potassium solubilization test

The bacterial isolates were each streaked on a sterile Aleksandrov agar medium and incubated at 30 °C for 3 days. The ability to solubilize mica powder as a source

of insoluble form of potassium was observed by the formation of a clear halo zone around the colony. The solubilization indexes of the isolates were determined by dividing total diameter of the halo with the diameter of the colony (Tan et al. 2014).

#### **Indole acetic acid production**

Bacterial isolate was inoculated into 5 ml of nutrient broth and was shaken on an orbital shaker for 24 h. One milliliter of the bacterial culture was transferred into fresh 5 ml of nutrient broth with the addition of 0.25 ml of L-tryptophan as precursor of indole acetic acid. Nutrient broth without bacterial inoculums served as control. A portion of the bacterial culture was transferred into a sterile tube and was centrifuged at 7000 rpm for 7 min. The supernatant (1 ml) was mixed with 2 ml of Salkowski reagent. The solution was allowed to stand for 25 min, and the development of a pink color indicated IAA production (Tan et al. 2014).

#### **Screening termitarium bacteria for antagonistic activities**

##### **Antagonistic activities of plant growth-promoting bacteria against *Ralstonia solanacearum***

The antagonistic effect of diffusible compounds on the pathogenic bacterium was evaluated in vitro by dual culture techniques. *Ralstonia solanacearum* was circularly streaked on nutrient agar plates, and 24-h-old culture of isolated bacterial strains was streaked about 2.5 cm away from the pathogenic bacterium. The plates were incubated at 37 °C for 24 h, and the result was recorded by measuring the clear zones around the bacterial colony. Inhibition of bacterial growth was calculated using the formula:  $\frac{R_1 - R_2}{R_1} \times 100$ , where  $R_1$  (a control value) represents the largest radial distance grown by the fungus in the direction of the antagonist and  $R_2$  represents the distance on a line between the inoculation positions of the fungus and the bacteria (Singh et al. 2008).

##### **Antagonistic activities of plant growth-promoting bacteria against *Fusarium oxysporum***

The antagonistic effect of diffusible compounds on the pathogenic fungus was evaluated in vitro by dual culture techniques. *Fusarium oxysporum* was grown on Sabouraud dextrose agar (SDA) plates, disc of 7 mm diameter was cut from the actively growing lawn and inoculated at the center of the SDA plates, and 24-h-old culture of isolated bacterial strains was streaked about 2.5 cm away from *F. oxysporum*. The plates were incubated at 28 °C for 5 days, and the result was recorded by measuring the clear zones around the bacterial colony. Inhibition of fungal growth was calculated using the formula:  $\frac{R_1 - R_2}{R_1} \times 100$ , where  $R_1$  (a control value) represents

the largest radial distance grown by the fungus in the direction of the antagonist and  $R_2$  represents the distance on a line between the inoculation positions of the fungus and the bacteria (Singh et al. 2008).

#### **Results**

Two hundred bacteria were isolated from the nine different termite mound soil at different depths (top, middle and down layer). Additional file 1: Table S1 shows the total bacterial counts obtained from the different termite mound soil. Overall highest bacterial count (12.9 cfu/g  $\pm$  1.1) was obtained from the third sample of termite mound soil, household down layer while the lowest bacterial count (0.2 cfu/g  $\pm$  0.0) was recovered from first farmland top layer. Figure 1 shows the percentage occurrences of the bacteria isolated from the different termite mound soil. *Bacillus* spp. had the highest percentage occurrences of 29.82%.

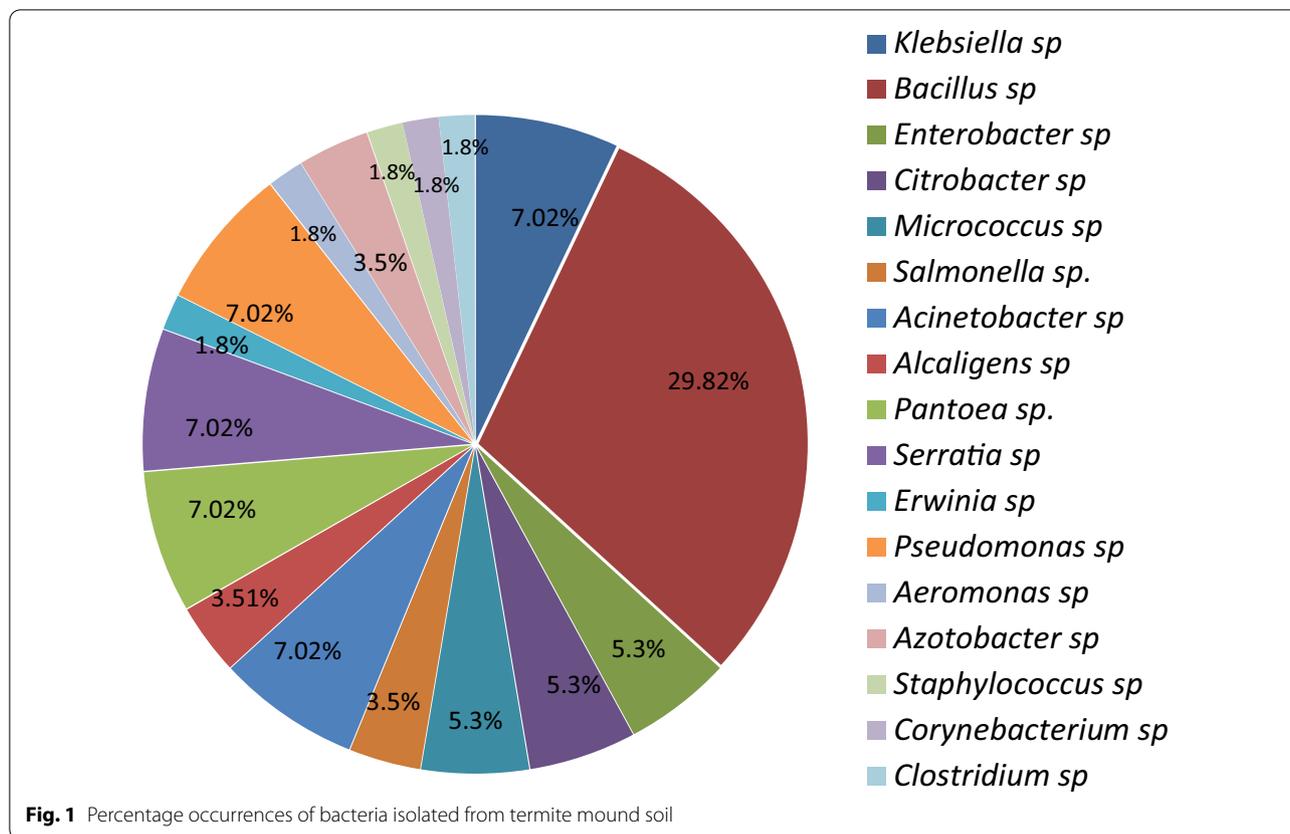
#### **Plant growth-promoting activities**

Out of the 200 bacterial isolates, 117 bacterial isolates were positive for phosphate solubilization assay, 107 isolates were positive for potassium solubilization and 57 isolates were able to produce indole acetic acid. In all, 57 bacterial isolates were positive for all the plant growth-promoting properties investigated (Additional file 1: Table S2).

*Bacillus* sp. isolated from FD recorded the highest phosphate solubilization and indole acetic acid activities, while *Clostridium tertium* from HT had the least phosphorus activity and *Salmonella arizonae* isolated from FT gave the least indole acetic acid activity. *Acinetobacter* sp. obtained from FD showed the highest potassium activity, and *Salmonella arizonae* isolated from WT recorded the least potassium activity. The average phosphorus, potassium solubilization index as well as indole acetic acid production by termite mound isolates from different habitats are presented in Table 1, while the percentage prevalence of the isolates with these essential attributes is presented in Fig. 2.

#### **Antagonistic activities of termitarium bacteria against *R. solanacearum***

All the 57 bacterial isolates that were positive for the plant growth-promoting assay were further screened for biocontrol activity against *R. solanacearum*. Among the 57 isolates, five isolates showed antagonism against *Ralstonia solanacearum* which was evaluated by the reduced radial growth of the test bacterium (*R. solanacearum*). The percentage inhibition (P.I.) ranged from 5.7 to 66.0%. Highest P.I. was shown by *Bacillus* sp. (66.0%) from household down layer, and lowest P.I. was exhibited by



**Table 1** Average solubilization index of P, K and IAA

Habitat	Av. P-solubilization index	Av K-solubilization index	Av IAA produced
House hold	0.288 ± 0.01	0.392 ± 0.05	0.509 ± 0.10
Water	0.221 ± 1.20	0.325 ± 0.14	0.458 ± 1.01
Farm	0.327 ± 0.30	0.323 ± 0.10	0.461 ± 2.02

Mean values with different superscripts (alphabets) in a column are significantly different ( $p < 0.05$ )

*Micrococcus luteus* from farmland middle layer termite mound soil (Fig. 3).

**Antagonistic activities of termitarium bacteria against *F. oxysporum***

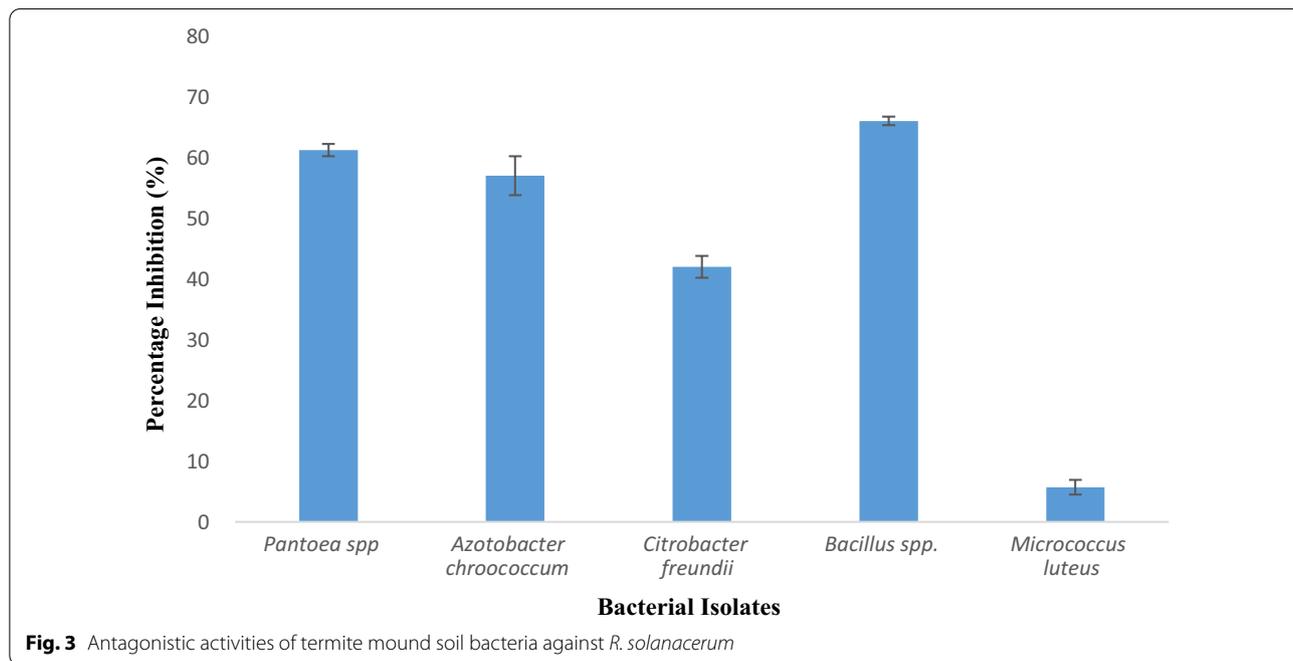
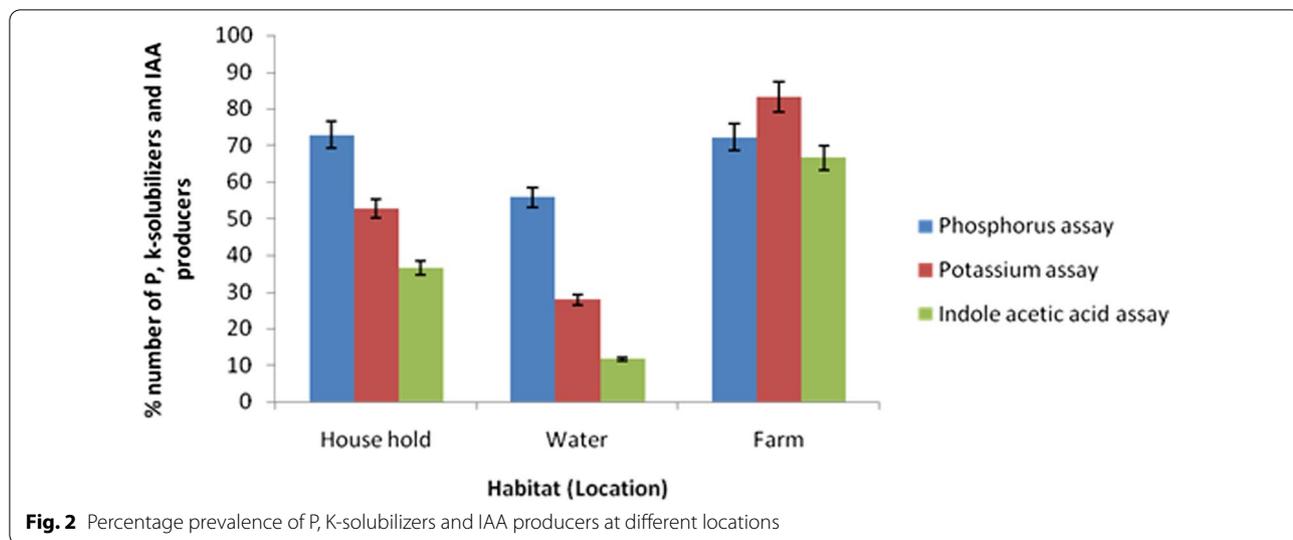
Termitarium biocontrol activity was observed by reduced radial growth of the test fungus (*F. oxysporum*). Three isolates exhibited the potential to control *Fusarium oxysporum* among the 57 plant growth-promoting isolates. The highest P.I. driven by the secretion of diffusible compounds was shown by *Bacillus sp.* (56.1%) from household, down-layer termite mound soil sample, while *Azotobacter chroococcum* from household

down layer of the termite mound soil showed the lowest biocontrol activity on *Fusarium oxysporum* of 46.0% (Fig. 4).

**Discussion**

The environmentally friendly plant growth-promoting potential and disease control methods are important in growing crops. The type of termite that colonized the mound and the geographical location of the mound influence the kind of bacteria present in the termite mound soils (Enagbonma and Babalola 2019). Local farmers, especially in Northeast, Thailand, use termite mound soil for improving crop yield (Miyagawa et al. 2011; Bhardwaj et al. 2014), and currently in Africa, some farmers are mixing termite mound soil directly with the field soil to achieve higher yield (Devi and Thakur 2018).

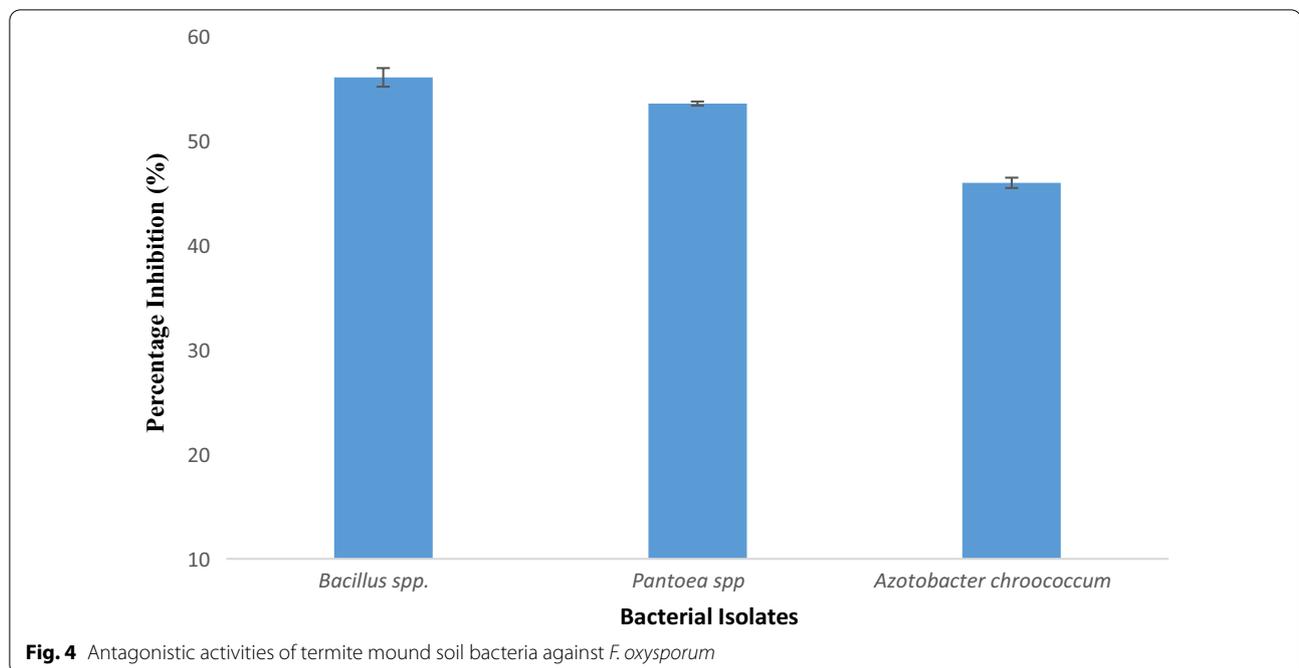
Microbial groups endowed with nitrogen fixation, polysaccharide degradation (Chew et al. 2018), phosphate and solubilization of potassium as well as production of indole acetic acid (IAA) from tryptophan (Miyagawa et al. 2011) have been reported from termite mounds. The high diversity of bacteria in termite mound soil could be as a result of the high amount of organic matter in the termite mound. Chauhan et al. (2017) and Enagbonma and Babalola (2019) reported that microbial activities in



termite mound are high and assist plant growth. Their findings were attributed to the fact that termite mound soil contains approximately two-to-three times calcium and phosphorus, five times carbon and nitrogen and 50 times ammonia and organic matter compared with their surrounding soil. It has also been reported that the clay content in termite mound soils increases their soil porosity and water-holding capacity (Jouquet et al. 2016). In this study, the total bacterial counts in termite mound sample are not in conformity with the findings of Kumar

et al. (2018) where higher value of  $65.5 \times 10^5$  cfu g<sup>-1</sup> was reported.

This could, however, be due to the different changes in environmental factors of the habitats, microbial diversity and possibly the areas or layers of the termite mound soil collected. The nutrients present in termite mound soil are not only contributing to plant growth but also harness soil beneficial microorganisms such as decomposers. Abundance of bacterial population in termite mound soil sample could be due to the fact that they could metabolize diverse kind of complex compounds, which



are not attacked by other types of microorganisms (Devi and Thakur 2018).

The bacterial isolates recovered in this study revealed higher affinity to solubilize phosphorus (117 bacterial isolates) than potassium. Chakdar et al. (2018) stated that termite mound soils hold higher amount of phosphorus when compared to the surrounding soils due to the presence of highly efficient phosphate-solubilizing bacteria. Ability of these phosphate solubilizing bacteria to solubilize inorganic and organic phosphorus is seen as significant features for increasing soil fertility and their use as inoculants concurrently can increase plant phosphorus uptake and increase crop yield (Hameedaa et al. 2008).

The result of the potassium solubilization potential is in line with the study of Parmar and Sindhu (2013) who also reported the efficiency of potassium solubilization by different bacteria and added that potassium solubilization varies with the nature of potassium bearing minerals and aerobic conditions in soil. A wide range of bacterial genera namely *Pseudomonas*, *Burkholderia*, *Acidithiobacillus*, *Bacillus* and *Paenibacillus* have been shown to release potassium in accessible form from potassium-bearing minerals in soils (Sheng 2005; Liu et al. 2012). There are strong evidences by Meena et al. (2014, 2015) that soil bacteria are capable of transforming soil potassium (K) to the forms available to plant effectively. In addition to increasing plant resistance to diseases, pests and abiotic stresses, K is required to activate over 80 different enzymes responsible for plant and animal processes such as energy metabolism, starch synthesis,

nitrate reduction, photosynthesis and sugar degradation (Almeida et al. 2015; Hussain et al. 2016; Yang et al. 2015).

Fifty-seven isolates were positive for all the three plant growth-promoting assays (phosphorus, potassium and indole acetic acid assay). Chauhan et al. (2017) also documented the abilities of different bacterial isolates that produced indole and also solubilized phosphate. IAA promotes root development, uptakes nutrients (Carrillo et al. 2002), coordinates demand and acquisition of nitrogen and enhances crop yields (Kiba et al. 2011). Zerihun et al. (2019) reported that IAA is one of the most important phytohormones which may function as an important signal molecule in the regulation of plant growth. IAA values recorded in this study disagree with the report of Devi and Thakur (2018) who recorded IAA potential of the bacterial isolates from their termite mound soil between 0.6 and 47.56  $\mu\text{g/mL}$ . The variations in the values obtained could be due to the presence of different termite types and microbial diversities as well as activities in mound soil of different regions and even countries. Examples of some of the termite types are: *Cubitermes niokoloensis*, *Odontotermes* and *Macrotermes michaelseni* and may have different influence on the plant growth potential.

The four bacterial isolates belonging to the genera (*Bacillus*, *Pantoea*, *Azotobacter* and *Clostridium*) uphold considerable promise for plant growth and biocontrol of plant diseases in this study. The ability of the bacterial isolates with antibacterial and antifungal ability indicated

that they produce extracellular cell wall-degrading enzymes such as chitinase and  $\beta$ -1,3-glucanase and anti-fungal compounds (Solanki et al. 2012). Zhang et al. (2008) were of the opinion that bacteria play significant roles in defending plants against diseases and abiotic stress through an extensive range of mechanisms that involves production of lytic enzymes and antibiotic compounds. *Bacillus* spp. are the most predominant isolates and also showed highest biocontrol activity against *Ralstonia solanacearum* and *Fusarium oxysporum*. This is in accordance with previous study of Manjula et al. (2014) and Choudhary and Johri (2009) who reported that *Bacillus* spp. provides protection to crop plants against diseases caused by fungal pathogen.

More also, Athukorala et al. (2010) added that *Bacillus* spp. play an important role in the biocontrol of fungal diseases because they produce different types of metabolites (volatile and diffusible) and may use multiple mode of action against fungal pathogens. The occurrence of these soil beneficial organisms and high nutrient content in termitarium soil have been shown to enhance crop yield and thus can be used as biofertilizers (Fall et al. 2004).

Menichetti et al. (2014) opined that the daily activities of termites that feed on litter are the key driving factor that circulates nutrients in soil occupied by them. Moreover, *Bacillus* spp., *Citrobacter freundii*, *Azotobacter chroococcum* and *Pseudomonas aeruginosa* solubilized phosphate, phosphorus and IAA which provide additional advantages for their ability to be used as biocontrol agents for agricultural management. This present study showed that termite mound soil bacteria are higher in household samples down layer than in the other habitats under investigation; this could be due to the ability of these termitarium bacteria to assess several decomposed materials aside the wood or leaf litters.

## Conclusion

The result showed that termite mound soil contains some useful bacteria such as *Bacillus* sp., *Citrobacter freundii*, *Azotobacter chroococcum* and *Pseudomonas aeruginosa* that are capable of solubilizing phosphate and potassium as well as producing indole acetic acid which are some of the potentials required of the organisms to promote plant growth and suppress plant–soil pathogen. Therefore, termite mound soil could be adopted and promoted as a viable source for biofertilizers and biocontrol.

## Abbreviations

IAA: Indole acetic acid; P: Phosphorus; K: Potassium; SDA: Sabouraud dextrose agar; T: Top; M: Middle; D: Down; HD: Household down; FD: Farm down; WD: Water down; HM: Household middle; FM: Farm middle; WM: Water middle; HT: Household top; FT: Farm top; WT: Water top; PI: Percentage inhibition.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42269-021-00560-8>.

**Additional file 1. TABLE S1:** Counts of bacterial isolates from different depth of termite soil. **TABLE S2:** Plant Growth Promoting Bacteria Obtained from Termite Mound Soil.

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## Authors' contributions

SO, PO and AE conceived and designed the study. SO, E and OD carried out most of the laboratory work. PO, AE and OD and E analyzed and interpreted the data. SO, E and DU helped in writing—original draft. All authors read and approved the final manuscript.

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## Availability of data and materials

The authors declare that all relevant data supporting the findings of this study are included in this article.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors have no conflict of interest to declare.

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