Effect of animal manure on population dynamics of indigenous soil *Bacillus spp.*

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Received: June 02, 2024 DOI: 10.14295/bjs.v3i9.628

Accepted: July 22, 2024 URL: https://doi.org/10.14295/bjs.v3i9.628

Abstract

Bacillus spp. are soil inhabitants, many of which play vital roles as biofertilizers and biopesticides in plant production. These bacteria derive their nourishment from soil organic carbon and nitrogen provided by organic matter. Reports indicate that animal manure increases the population and diversity of *Bacillus* spp. in the soil. However, there is limited information on which of the three commonly used animal manure (cattle, goat, and chicken) is more effective in multiplying *Bacillus* cells. This study evaluated the effectiveness of cattle, goat, and chicken manures, standard nutrient broth, and soil, as growth media, on the multiplication of 22 indigenous soil *Bacillus* spp. strains previously isolated from the cabbage rhizosphere. A Completely Randomized Design with five treatments replicated three times was used and the conditions were kept at room temperature. Both standard nutrient broth and sterilized soil media were used as controls. Colony-forming unit counts of *Bacillus* spp. were subjected to Log (x+1) transformation. One-way analysis of variance was used to generate mean differences and means separated using *Duncan's* LSD test ($p = 0.05$). Linear curves were drawn to compare the growth trends for each Bacillus strain. The findings indicate that the growth of *Bacillus* spp. strains in animal manure were significantly higher than in the soil media. Growth in the soil media was significantly lower than in standard nutrient broth media growth. Results further show that the growth of the *Bacillus* spp. in all the media followed the normal growth curve of bacterial cells. Results from this study, therefore, suggest that amendment of soil with cattle, goat, and chicken manures enhances the growth and multiplication of soil *Bacillus* spp. and this has a positive effect on soil fertility. These manures can also be used in the commercial production of the bacillus bacteria as a biofertilizer and biopesticide. Biofertilizers are cheap, have long-term effects on soil fertility, and are health-friendly to the environment and the user.

Keywords: *Bacillus*, growth media, biofertilizer, soil fertility.

Efeito do esterco animal na dinâmica populacional do solo indígena *Bacillus* spp.

Resumo

Bacillus spp. são habitantes do solo, muitos dos quais desempenham papéis vitais como biofertilizantes e biopesticidas na produção vegetal. Essas bactérias obtêm sua nutrição do carbono orgânico e do nitrogênio do solo fornecidos pela matéria orgânica. Relatórios indicam que o esterco animal aumenta a população e a diversidade de *Bacillus* spp. no solo. No entanto, há informações limitadas sobre qual dos três estercos animais comumente usados (bovinos, caprinos e frangos) é mais eficaz na multiplicação de células de *Bacillus* spp. Este estudo avaliou a eficácia de estercos bovinos, caprinos e de galinha, caldo nutriente padrão e solo, como meio de crescimento, na multiplicação de 22 cepas indígenas de Bacillus do solo previamente isoladas da rizosfera do repolho. Foi utilizado um delineamento inteiramente casualizado com cinco tratamentos replicados três vezes e as condições foram mantidas em temperatura ambiente. Tanto o caldo nutriente padrão quanto o meio de solo

esterilizado foram utilizados como controles. As contagens de unidades formadoras de colônias de Bacillus pp. foram submetidas à transformação Log (x+1). A análise de variância unidirecional foi utilizada para gerar diferenças de médias e médias separadas pelo teste LSD de *Duncan* (p = 0,05). Curvas lineares foram desenhadas para comparar as tendências de crescimento para cada cepa de *Bacillus* spp. Os resultados indicam que o crescimento de cepas de *Bacillus* spp. no esterco animal foi significativamente maior do que no meio do solo. O crescimento no meio de solo foi significativamente menor em comparação com o crescimento em meio de caldo nutriente padrão. Os resultados mostram ainda que o crescimento do *Bacillus* spp. em todos os meios seguiu a curva normal de crescimento das células bacterianas. Os resultados deste estudo, portanto, sugerem que a alteração do solo com esterco de gado, cabra e galinha aumenta o crescimento e a multiplicação de *Bacillus* spp. e isso tem um efeito positivo na fertilidade do solo. Esses adubos também podem ser utilizados na produção comercial da bactéria bacilo como biofertilizante e biopesticida. Os biofertilizantes são baratos, têm efeitos a longo prazo na fertilidade do solo e são amigos da saúde do ambiente e do utilizador.

Palavras-chave: *Bacillus*, meio de crescimento, biofertilizante, fertilidade do solo.

1. Introduction

Soil fertility and health depend on the chemical composition and nature of the microorganisms inhabiting it (Nkongolo; Narendrula-Kotha, 2020). Many species in the genera *Bacillus* are plant growth-promoting rhizobacteria (PGPR) which mainly perform their role as biopesticides and biofertilizers (Montesinos, 2003; Bhattacharyya and Jha, 2012). These rhizobacteria live in the rhizosphere, rhizoplane, and spaces between cells of the root cortex (Bulgarelli et al., 2013; Lavudi et al., 2023). The antagonistic properties of *Bacillus* spp. make them good biocontrol agents (Saxena et al, 2020).

The major factors that limit the performance of microorganisms in the rhizosphere are soil type, temperature, moisture content, organic matter, nutrient availability, and pH (Banerjee et al., 2006). For example, frequent use of chemical fertilizers impacts negatively on ecological functions, which affects soil pH, nutrient availability, and microbial functions (Yang et al., 2018; Bebber; Richards, 2020; Haq et al., 2021). Reports also indicate that continued use of chemical fertilizers altered the bacterial composition and metabolic activity (Li et al., 2015) and inconsiderate fertilizer applications contribute significantly to changes in the earth's biogeochemical cycles (Amundson et al., 2015). Using inorganic fertilizers alone frequently decreased bacterial richness and diversity, while manure addition increased bacterial diversity (Manching et al., 2014; Chen et al., 2016). Therefore, there is a need to develop alternative methods of managing plant nutrition with lower mineral fertilizer application (Foley et al., 2011, Buddhika et al., 2013).

Many scholars have reported the significant roles of manures when correctly applied in the soil. Previous reports indicate that increasing soil organic matter improves soil physical properties and increases soil microbial communities and biomass (Irshad et al., 2013; Jechalke et al., 2014; Sun et al., 2015; Ling et al., 2016; Gai et al., 2018). Organic fertilizers supply carbon compounds that can be used by soil microorganisms during the process of mineralization (Lazcano et al., 2021). Positive linear regression relating microbial diversity to soil organic carbon, and a positive relationship between fertilization with manure and the total number of bacteria have been reported in different studies (Gomez, et al., 2006 Liina et al., 2012). Similarly, Ge et al. (2008) found that the bacterial diversity was higher in compost and farmyard manure-amended soils regardless of land use patterns or seasons. Higher bacterial diversity was found in soils amended with poultry litter than in those treated with inorganic fertilizers (Jangid, et al.2008).

While previous studies documented the role of animal manures in enhancing bacterial populations and communities, information on how manures from different animals such as goats, cattle, and chickens influence the population growth of different *Bacillus* species in the soil remains scanty. No study has been conducted in Uganda to verify this effect. This information is required for selection of the type of manure that is most appropriate for use to enhance the growth and multiplication of *Bacillus* spp. as biofertilizers and biopesticides in the soil. This study was conducted to determine the effect of the three commonly used manure (goats, cattle, and chicken manure) on the growth and multiplication of *Bacillus* spp.

2. Materials and Methods

2.1 Experimental location

The experiment was conducted in the Research Laboratory of the Department of Plant Sciences, Microbiology and Biotechnology, College of Natural Sciences of Makerere University, Uganda. The laboratory is located along

coordinates 0° 20' 9.9414"N latitude and 32° 33' 59.2374"E longitudes.

2.2 Determination of mineral content

Before the experiment, the amount of Carbon (C), Nitrogen (N), Phosphorus (P), and pH of each manure and soil were determined. Soil texture was also analyzed using the hydrometer method (Boyoucos, 1962), and soil pH was tested using a pH meter. Total N was determined calorimetrically using concentrated sulphuric acid, selenium powder, and salicylic acid. Available P content was determined spectrophotometrically at 882 nm wavelength after its reaction with ammonium molybdate in the presence of ascorbic acid on bray extracts (Murphy; Riley, 1962). Soil organic matter (OM) was analyzed using the Walkley-Black method (Gessesse et al., 2018).

2.3 Analysis of chemical properties of soil, cattle, goat, and chicken manures

The percentage of soil organic Carbon (SOC), N, P, soil texture, and soil pH before the experiment are indicated in (Table 1).

Table 1. Chemical properties of soil, cattle, goat, and chicken manures.

Samples	pH	SOC (%)	N (%)	C : N ratio	P (%)
Chicken manure	6.5	17%	0.697	24	3.585
Goat manure	6.3	11%	0.562	19	3.179
Cattle manure	6.8	13 %	0.453	28	3.262
Soil	5.7	1.5%	0.15	10	0. 1
	The texture of the soil used				
Soil particle	(%)				
Sand	44%				
Clay	42%				
Silt	14%				

Note: Soil organic Carbon (SOC). (C:N) = Carbon : Nitrogen ratio. Source: Authors, 2024.

2.4 Soil samples

Twenty-two (22) indigenous soil bacillus strains were previously isolated from the cabbage rhizosphere of different agro-ecological zones of Uganda and kept in the Laboratory of the Department of Plant Sciences, Microbiology and Biotechnology, College of Natural Sciences of Makerere University, Uganda. The soil for the experiment was obtained from Uganda Martyrs University Demonstration Farm located in Kabale Municipality in South Western Uganda, while cattle, goat, and chicken manures were obtained from farms neighboring Uganda Martyrs University Demonstration Farm. The manures of cattle, goats, and chicken were preferred for use in this experiment because they are available and commonly used organic fertilizers in soil fertility management in Uganda.

The laboratory experiment was designed in a Completely Randomized Design (CRD), with five treatments replicated three times. This design is suitable for controlled experimental conditions. The five treatments were set as follows:

i. *Bacillus* species with sterilized cattle manure.
ii. *Bacillus* species with sterilized goat manure.
iii. *Bacillus* species with sterilized chicken manure.
iv. *Bacillus* species with sterilized soil.
v. *Bacillus* species with standard nutrient broth.

2.5 Sample preparation and inoculation medium

The 100 g of soil, chicken, goat, and cattle manures were separately dissolved in 200 mL of distilled water and allowed to settle overnight. The mixture was then filtered to obtain a liquid extract. The liquid extract was autoclaved at 120 ºC to kill all pathogens and non-spore-forming microorganisms. Then 15 mL of each sterilized manure media were separately inoculated with *Bacillus* spp. at a concentration of $1x10^5$ CFU/mL^{-1} in tubes under aseptic conditions. Standard nutrient broth was used as a control growth media. The growing conditions were kept at room temperature (20-25 ºC) for ten days, taking measurements per day. After ten days, each of the cultures was serially diluted in 0.85% saline water at a 10-6 dilution, and 0.1 mL of this dilution was then cultured on Luria Bertani (LB) medium individually at 37 ºC to determine colony-forming units (Gadhave et al., 2018). The bacterial colonies were counted, and populations were calculated using the formula.

$$\text{Number of CFU/mL}^{-1} = \text{number of colonies} \times \text{dilution factor/Volume used}$$

2.6 Data analysis

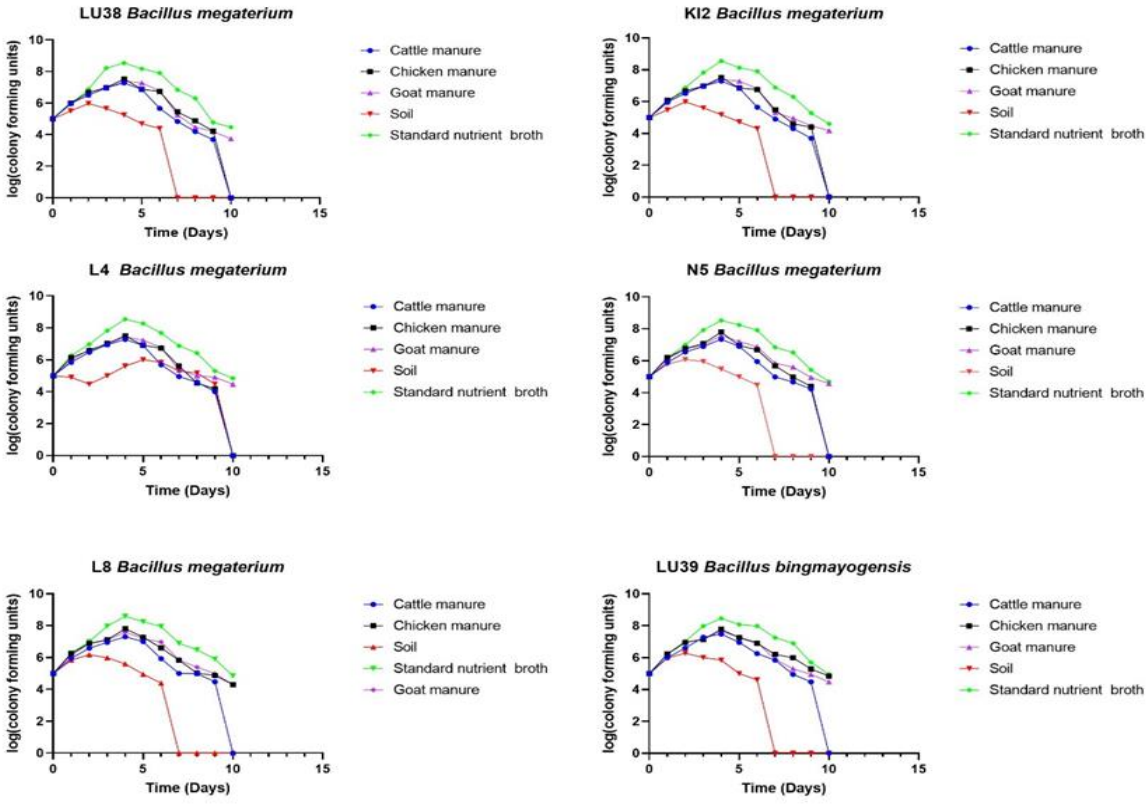
The data were entered into Excel, a computer software, and then transferred to IBM SPSS statistics version 20 for analysis. The colony forming units (CFU) counts of *Bacillus* spp. were subjected to Log (x+1) transformation. One-way analysis of variance (ANOVA) was used to obtain means which were separated using *Duncan's* LSD test ($p = 0.05$). The results with $p \leq 0.05\%$ were statistically significant. Linear curves were drawn to compare the growth trends for each *Bacillus* spp. strain.

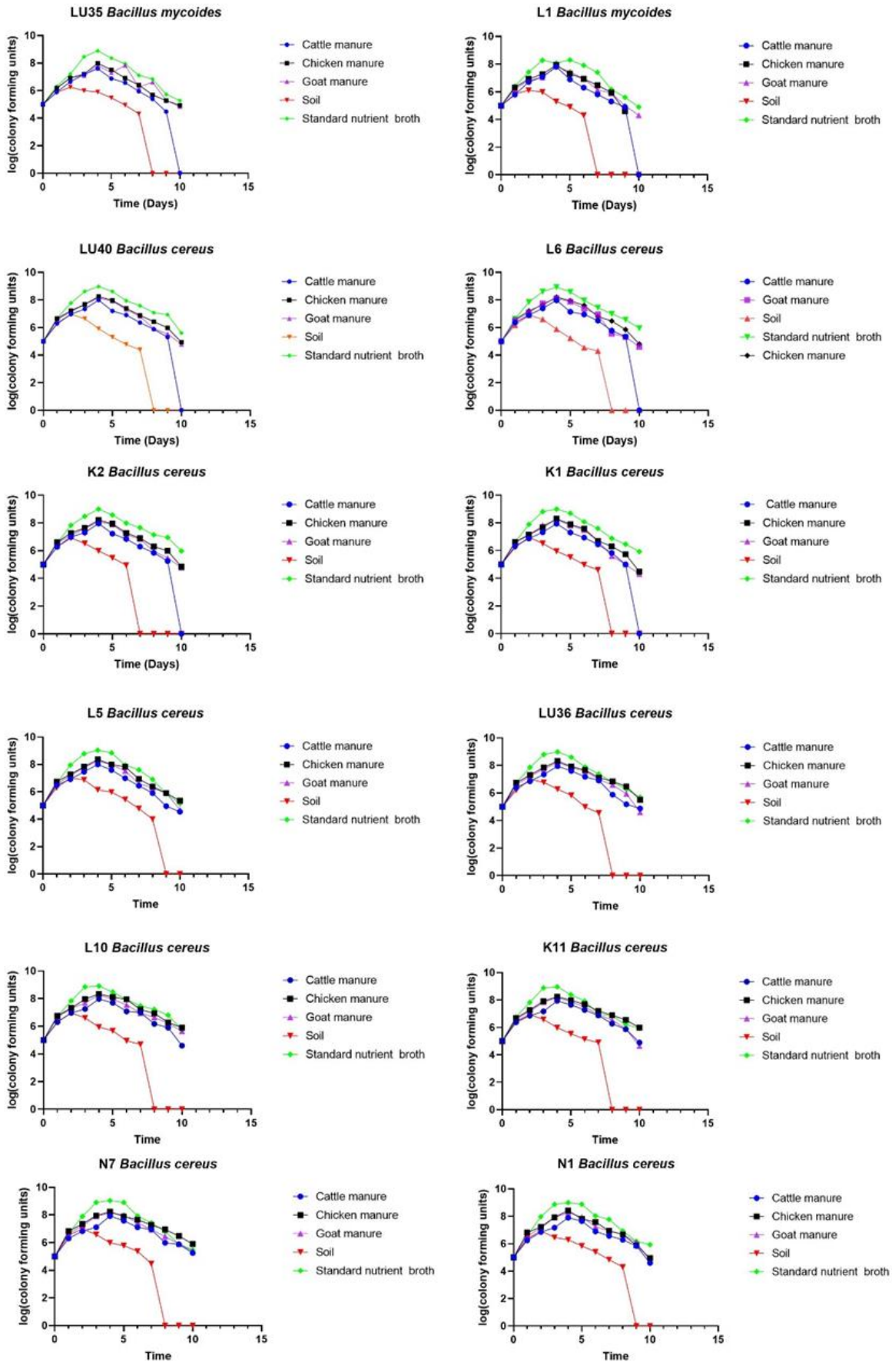
3. Results

3.1 Population growth of Bacillus strains in cattle, goat and chicken manures, and soil media

The results indicate that all *Bacillus* spp. grew in chicken, goat, and cattle manures, standard nutrient broth, and soil culture media following the normal bacterial growth curve (Figure 1). Among the animal manures, chicken manure recorded the highest bacillus growth over the culture period (10^8 CFU). Growth reached maximum and then dropped at different times for all the animal manures, soil, and standard nutrient broth media. The least growth for all *Bacillus* spp. occurred in soil media (from 10^5 to 10^6 CFUs).

The maximum growth attained for manures and standard nutrient broth was recorded on the fourth day of culturing while in soil culture, it was recorded on the second day. The population of bacteria started declining for all the growth media after the fourth day of culturing in manure and standard nutrient broth. On the other hand, all the bacteria in the soil culture died on the eighth day while in other media, they died on the ninth or tenth day.




LU35 Bacillus mycoides
L1 Bacillus mycoides
LU40 Bacillus cereus
L6 Bacillus cereus
K2 Bacillus cereus
K1 Bacillus cereus
L5 Bacillus cereus
LU36 Bacillus cereus
L10 Bacillus cereus
K11 Bacillus cereus
N7 Bacillus cereus
N1 Bacillus cereus
log(colony forming units)
Time (Days)
Time
Cattle manure
Chicken manure
Goat manure
Soil
Standard nutrient broth

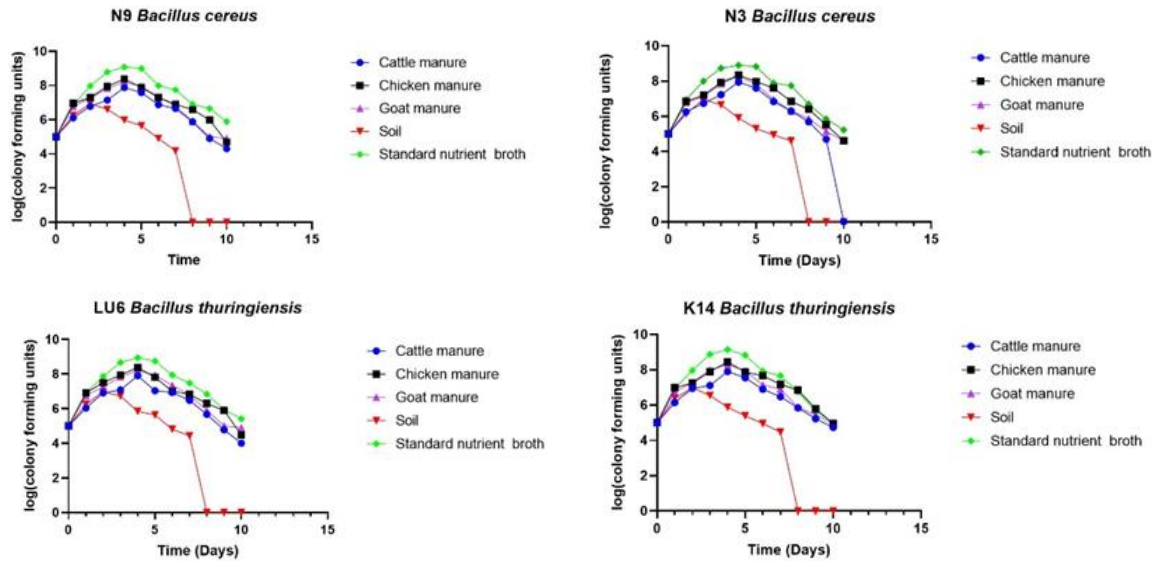


Figure 1. Growth curves for *Bacillus* spp. strains. Source: Authors, 2024.

Although the growth of *Bacillus* spp. in animal manures was highest in chicken manure media (Figure 1), the results in (Table 2) indicate that there is no significant difference in population growth among all the animal manures. The means and standard deviations reveal that the growth population of Bacillus strains cultured in chicken manure, goat manure, cattle manure, and standard nutrient broth were significantly higher than those cultured in the soil media. However, the growth of *Bacillus* spp. in animal manure and standard nutrient broth cultures was not significantly different.

Table 2. Means and standard deviations of growth of *Bacillus* strains in soil, standard nutrient broth, cattle, goat, and chicken manure media.

Bacillus spp.	Mean (S.D)				
	Cattle manure	Chicken manure	Goat Manure	Loam soil	Standard nutrient broth
LU38 –*B.megaterium*	5.18^{a} (2.08)	5.49^{a} (2.09)	5.78^{a} (1.31)	3.32^{b} (2.67)	6.64^{a} (1.47)
K12- *megaterium*	5.20^{a} (2.07)	5.48^{a} (2.09)	5.89^{a} (1.17)	3.30^{b} (2.65)	6.66^{a} (1.35)
L4-*megaterium*	5.25^{a} (2.04)	5.47^{a} (2.11)	5.97^{a} (1.07)	3.40^{b} (2.73)	6.73^{a} (1.30)
N5- *megaterium*	5.31^{a} (2.04)	5.59^{a} (2.13)	6.13^{a} (1.02)	3.43^{b} (2.76)	6.75^{a} (1.33)
L8-*megaterium*	5.38^{a} (2.03)	6.09^{a} (1.16)	6.13^{a} (1.09)	3.45^{b} (2.78)	6.83^{a} (1.28)
LU39 *bingmayogensis*	5.53^{a} (2.08)	6.33^{a} (.98)	6.17^{a} (1.11)	3.52^{b} (2.83)	6.87^{a} (1.25)
LU35-*mycoides*	5.60^{a} (2.08)	6.37^{a} (1.04)	6.47^{a} (1.07)	3.98^{b} (2.61)	7.01^{a} (1.33)
L1-*mycoides*	5.60^{a} (2.07)	5.89^{a} (2.19)	6.24^{a} (1.12)	3.41^{b} (3.02)	6.86^{a} (1.30)
K2-*cereus*	5.91^{a} (2.15)	6.73^{a} (1.11)	6.59^{a} (1.14)	3.74^{b} (3.02)	7.38^{a} (1.20)
LU40-*cereus*	5.93^{a} (2.16)	6.77^{a} (1.11)	6.62^{a} (1.17)	4.12^{b} (2.76)	7.35^{a} (1.26)

L6-*cereus*	5.94[a] (2.16)	6.74[a] (1.14)	6.56[a] (1.24)	4.06[b] (2.72)	7.32[a] (1.22)
K1-*cereus*	5.91[a] (2.17)	6.68[a] (1.22)	6.51[a] (1.34)	4.16[b] (2.76)	7.36[a] (1.29)
L5-*cereus*	6.39[a] (1.16)	6.87[a] (1.12)	6.71[a] (1.18)	4.69[b] (2.48)	7.25[a] (1.45)
LU36-*cereus*	6.47[a] (1.09)	6.98[a] (1.02)	6.76[a] (1.17)	4.24[b] (2.82)	7.28[a] (1.30)
L10-*cereus*	6.54[a] (1.06)	7.07[a] (1.04)	6.90[a] (.99)	4.20[b] (2.78)	7.36[a] (1.24)
K11-*cereus*	6.57[a] (.99)	7.04[a] (.97)	6.75[a] (1.16)	4.23[b] (2.80)	7.25[a] (1.27)
N7-*cereus*	6.54[a] (.94)	7.04[a] (.97)	6.82[a] (1.06)	4.25[b] (2.82)	7.27[a] (1.42)
N1-*cereus*	6.46[a] (1.02)	6.85[a] (1.15)	6.79[a] (1.14)	4.69[b] (2.44)	7.38[a] (1.35)
N9-*cereus*	6.28[a] (1.16)	6.81[a] (1.18)	6.63[a] (1.24)	4.14[b] (2.77)	7.44[a] (1.31)
N3-*cereus*	5.85[a] (2.19)	6.76[a] (1.25)	6.52[a] (1.24)	4.14[b] (2.75)	7.26[a] (1.42)
LU6-*thuringiensis*	6.17[a] (1.19)	6.73[a] (1.23)	6.63[a] (1.24)	4.15[b] (2.78)	7.25[a] (1.36)
K14-*thuringiensis*	6.35[a] (1.05)	6.90[a] (1.17)	6.69[a] (1.21)	4.18[b] (2.77)	7.25[a] (1.51)

Note: Means in the same raw with different superscripts are statistically different at a 5% level of significance. Authors, 2024.

4. Discussion

The study investigated the effect of chicken, goat, and cattle manures on the population of indigenous soil *Bacillus* strains, using standard nutrient broth and soil culture media as control. The results confirm the normal growth of *Bacillus* strains in the different media, suggesting that the media used does not alter the physiological growth behavior of the bacteria. The results further indicate that the growth of *Bacillus* spp. in cattle, goat, and chicken manures was not significantly different, suggesting that the three different manures provide the optimum nutrient requirement for *Bacillus* spp. growth. This means that the test results of manure and soil composition before the experiment had chicken manure with the highest SOC (17%) and N (0.697) (Table 1) did not cause a significant difference in bacillus population growth in the manure media.

On the other hand, growth in the manures was significantly higher than in soil media, confirming that the manures provide organic carbon that is required in substantial amounts for bacillus growth. This is in agreement with previous studies which reported that organic manure enhance bacillus growth through provision of organic carbon (Hindersah et al., 2019).

The growth of bacteria in organic manure from goats, cattle, and chicken is a verification that these organic manures contain essential nutrients for growth and other metabolic functions because the manure used had more Organic carbon and nitrogen than soil media (Table 1). The major nutrients required are nitrogen, carbon, phosphorus, sulfur, oxygen, and hydrogen which are consumed and metabolized for energy and reproduction of new *Bacillus* spp. cells and the major source of nutrients are organic compounds and importantly, manures (Neufeld et al., 2017). Neufeld et al. (2017) reported that manure increases C availability for soil microbes by delivering a high rate of exogenous C into the soil.

Chaudhry et al. (2012) also reported significantly higher bacterial diversity in soils with organic fertilization than in soils with chemical fertilizer application. On the other hand, Pan et al. (2009) demonstrated that manure application increased SMBC and SMBN by 13% and 49% compared with the application of mineral fertilizer. Total N and organic carbon (OC) increased after the application of organic fertilizer (Ozlu; Kumar, 2018). Similarly, other previous studies reported that SOC influenced soil physical, chemical, and biological indicators (He et al., 2015). Billings et al. (2021) noted that SOC provides diverse energy sources for soil microorganisms. Therefore, the result from this research is a confirmation of the reports from previous studies and a suggestion that manures are alternative soil fertility amendments, not only through improvement of soil physical properties but also through enhancement of bacillus growth and multiplication.

The findings suggest that cattle, goat, and chicken manures equally contain a threshold of essential nutrients required by microorganisms for the production of new bacillus cells. *Bacillus* bacteria enhances plant growth and can be used as a biofertilizer. Since manure from different animals enhances bacillus multiplication and growth, these manures can be used as an alternative to inorganic fertilizers which are chemically destructive to the soil (Yang et al., 2018; Bebber; Richards, 2020; Haq et al., 2021). The growth of *Bacillus* spp. was very low in the soil media, and this can be attributed to low levels of both soil organic carbon and nitrogen in the soil samples

used in this study (Table 1). The C:N ratio in the soil sample used in this study was low (mean = 10), yet Howell (2005) reported that microorganisms do best when they are fed on a carbon-nitrogen ratio of 20-30 to maintain an equilibrium between mineralization and immobilization. Furthermore, Kendalynn et al. (2022) reported that soils with low organic matter (OM) had inadequate C, N, and P, and Alexandre et al. (2022) established that there is limited growth of bacteria in soils with little OM. Thus, soil with low organic carbon and nitrogen cannot sustain microbial growth and survival. In this study, all bacillus species in soil media stopped growth on the second day and died on the eighth day.

While previous research compared organic and inorganic fertilizers in soil fertility management, and the role of manures in enhancing microbial diversity, this study has specifically found that using any cattle, goat, or chicken manure equally increases the population of *Bacillus* spp. in the soil.

5. Conclusions

Cattle, goat, and chicken manures enhance indigenous soil *Bacillus* spp. population growth. Thus, cattle, goat, and chicken manures are all suitable for application to fertilize soils through enhancement of soil indigenous *Bacillus* spp. population production and growth. Soil with low carbon and nitrogen content does not support bacillus growth and therefore, plant growth in these soils will always be poor.

6. Recommendations

Mixed farming should be encouraged so that farmers use animal manure to enhance soil fertility of their soils for improved crop performance through increased growth and multiplication of *Bacillus* spp. which are biofertilizers and biopesticides.

7. Acknowledgments

The authors are grateful to the World Bank for providing financial support under the ACALISE Programme. The authors also appreciate Uganda Martyrs University for the technical and career guidance offered in this research.

8. Authors' Contributions

Silver Baryakabona: planning for the study, data collection and preparation, data analysis, presentation, and discussion of findings. *Joseph Ssekandi*: quality control in data collection and analysis and manuscript review. *Laban Frank Turyagyenda*: quality control in data collection and analysis and manuscript review.

9. Conflicts of Interest

No conflicts of interest.

10. Ethics Approval

Yes, applicable. This study was approved by the Ethics Committee of St. Francis Hospital Nsambya. All methods were carried out by the relevant guidelines. Reference number SFHN-2020-10.

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Funding

Yes, applicable. World Bank for providing financial support under the ACALISE Programme.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.