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Effects of Organic and Inorganic Fertilizers on Nitrogen Mineralization Processes Under Bell Pepper (*Capsicum annuum* L.) Production

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Abstract: This study aimed to evaluate the effects of organic and inorganic fertilizers on nitrogen mineralization processes in bell pepper production. The experiment was conducted in a screen house at the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria, using a Completely Randomized Design with three replications. Six fertilizer treatments were applied to the soil: poultry manure, cow dung, neem seed-based fertilizer, single-super phosphate (SSP), urea, and a control. Data were collected on plant height, number of leaves, fruit count, and fruit weight over 12 weeks. Nitrogen mineralization processes, including arginine ammonification, nitrification, and denitrification, were assessed in the Crop, Soil, and Pest Management Laboratory. Soil physical and chemical properties were also analyzed. Analysis of Variance (ANOVA) revealed significant differences in growth parameters among treatments. Plants treated with organomineral fertilizer (NSBF) exhibited the highest plant height, while SSP treatment resulted in higher leaf numbers. Control plants showed the lowest growth metrics. Poultry manure and neem seed-based fertilizer significantly enhanced fruit number and weight, with control plants yielding the least. The results indicate that applying organic fertilizers at recommended rates significantly improved ammonification, nitrification, and carbon content, while reducing denitrification rates in the soil. The integration of organomineral and organic fertilizers not only increased plant height but also positively influenced microbial activity, including ammonifying and nitrifying bacteria. In conclusion, the application of organic fertilizers, particularly neem seed-based fertilizer, is crucial for enhancing soil health and optimizing sustainable bell pepper production.

Keywords: *Bell Pepper, Arginine Ammonification, Nitrification, Denitrification, Neem-Seed Based Fertilizer, Mineralization.*

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I. INTRODUCTION

Bell pepper (Capsicum annuum L.), a member of the Solanaceae family, is one of the most widely cultivated vegetables globally, renowned for its rich nutritional content, particularly antioxidants and ascorbic acid (Leja et al., 2008). Native to Mexico and Central America, bell peppers were first encountered by Christopher Columbus in 1493 (Kelley et al., 2009). Today, they are cultivated on all continents and serve not only as a staple in culinary traditions but also have significant pharmaceutical applications. Bell pepper fruits are commonly used in a variety of dishes, including soups, stews, and sauces (Grubben and El-Tahir, 2004).

The growth and development of bell peppers depend very much on the availability of soil basic essential nutrients, and a couple of fertilizer formulations are handy to supply these nutriemnts. However, soil fertility can be threatened by the continuous depletion of essential nutrients, unless replenished through the use of fertilizers, both organic and inorganic. Fertilizer application is essential to maintaining soil fertility, which plays a critical role in sustaining global food production (Abdul-Elkader et al., 2010). Organic and chemical fertilizers have been shown to enhance both yield and crop quality (Zhang et al., 2019; Serri et al., 2021). Organic amendments such as compost and manure not only improve nutrient availability but also stimulate soil microbial activity and nitrogen (N) mineralization, contributing to healthier plant growth (Smith et al., 2019).

Soil amendments are pivotal in enhancing soil fertility and structure, both of which are critical for root development. Fertilizers provide essential nutrients that are necessary for bell pepper growth, and research has demonstrated that balanced fertilization strategies lead to improved marketable yields (Jones et al., 2021). Bell peppers, in particular, require adequate levels of nitrogen, phosphorus, and potassium, as well as micronutrients like calcium and magnesium, to

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support healthy growth. Fertilizer application also significantly impacts the microbial community composition of the soil, which can influence microbial diversity and functionality (Khamis et al., 1990).

Soil microorganisms play an integral role in maintaining essential soil functions, such as nutrient cycling, organic matter stabilization, and soil aggregation (Dangi et al., 2012). Organic fertilizers contribute various carbon compounds with different chemical compositions, ranging from readily degradable to stable forms, which are utilized by soil microorganisms during mineralization, enhancing their growth and biomass (Lazcano et al., 2020). Studies have indicated that organic fertilizers can substantially increase the organic carbon content in soil when compared to chemical fertilizers (Wang et al., 2019). Therefore, a comprehensive understanding of how organic and inorganic fertilizers impact soil health is critical for the sustainable production of bell peppers.

The current study aims to investigate the effects of organic and inorganic fertilizers on nitrogen mineralization processes during bell pepper cultivation. Specifically, the study will examine ammonifying bacteria, nitrifying bacteria, arginine ammonification, nitrification, and denitrification as indicators of nitrogen mineralization rates. Assessing these parameters will provide valuable insights into the nitrogen dynamics and microbial community composition in the soil, contributing to a better understanding of soil nitrogen status and its influence on bell pepper growth.

II. MATERIALS AND METHODS

The screen house experiment was conducted at the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria (Latitude: 7.2872°N; Longitude: 5.1361°E). The Nirmida F1 variety was sourced from a reputable agro-store in Akure. Chemicals used in the laboratory were obtained from Pascal Chemical Company Ltd. (Akure, Nigeria). The experiment followed a completely randomized design (CRD) with six treatments, each replicated three times.

A. Soil Amendments:

Soil amendments included poultry manure at 5 t/ha, cow dung at 5 t/ha, organomineral (neem-seed based fertilizer) at 300 kg/ha, single-super phosphate at 200 kg/ha, urea at 150 kg/ha, and a control with no fertilizer application.

Containers were filled with 20 kg of soil and arranged in triplicate. Organic fertilizers were applied 2 weeks before transplanting, while the neem-seed-based and mineral fertilizers were applied 2 weeks after transplanting.

B. Data Collection:

Data collection began 2 weeks after transplanting and continued at 2-week intervals. Growth and yield parameters, including plant height and number of leaves, were measured throughout the experiment.

Soil samples from each container were collected using foil paper, packed in sealed polythene bags, and analyzed for physico-chemical properties., as well as soil microbiological parameters, specifically parameters connected with soil N mineralization.

C. Determination of Physico-Chemical Properties of Soil:

Soil texture, pH, organic matter content, and nutrient status of air-dried soil samples were determined following standard methods (AOAC, 1990). The soil samples were analyzed as follows:

Total nitrogen (N) was determined using the Kjeldahl digestion and distillation method, available phosphorus (P) was measured by the Bray 1 method, exchangeable potassium (K), calcium (Ca), and magnesium (Mg) were extracted with 1M ammonium acetate at pH 7.0 and quantified using a flame photometer, soil pH was measured in a 1:2 soil-water suspension using a pH meter, and organic matter (OM) was determined by the dichromate oxidation method.

III. DETERMINATION OF SOIL N MINERALIZATION INDICES

A. Determination of Ammonifying Bacteria:

Ammonifying bacteria were quantified using the method described by Alexander (1977). Nutrient medium was prepared with the following composition: peptone (10 g), NaCl (5 g), ammonium ferric acetate (0.30 g), agar (15 g), yeast extract (2 g), and tap water (pH 7.0). To isolate ammonifying bacteria, 1 g of soil was added to 100 mL of distilled water, shaken, and serially diluted. A 2-mL aliquot from each dilution was transferred to sterile bottles, which were placed in a mechanical shaker. After vigorous shaking, the resulting suspension was used to inoculate nutrient agar plates, which were incubated at 28°C for 48 hours. Colony-forming units (CFUs) were counted to estimate bacterial density.

B. Determination of Nitrifying Bacteria:

Nitrifying bacteria were isolated using the method outlined by Alexander (1977). An ammonium-based medium was prepared and autoclaved, then allowed to solidify. Soil samples were mixed with sterile water, and serial dilutions were performed until a 1:10 dilution was achieved. The diluted soil samples (1 mL) were added to 20 mL of the prepared medium in sterile petri dishes. Incubation was performed at 25-30°C for 7–10 days. Colony growth was monitored to estimate the presence and density of nitrifying bacteria.

C. Determination of Arginine Ammonification:

Arginine ammonification was measured according to the method outlined by Alef and Kleiner (1987). Two grams of moist soil were treated with 2% L-arginine for 3 hours, then stored in a freezer at -4°C for an additional 3 hours. Ammonium (NH_4^+) production was measured as an indicator of arginine ammonification. Untreated soil samples were kept in the dark for the same duration as controls. After thawing, the soil was shaken with 40 mL of 2M KCl for 30 minutes.

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The filtrate was analyzed for ammonium-N using distillation after adding 0.2 g of MgO and $CaCl_2$.

D. Determination of Nitrification:

Nitrification was assessed following the method of Alef and Nannipieri (1995). Soil (30 g) was mixed with 20 g of washed sharp sand in a beaker, and 2 g of urea dissolved in distilled water was added. The mixture was incubated at room temperature for 21 days, with sampling intervals at 14 and 21 days. After incubation, 2 mL of 2M KCl was added to the soil sample, shaken, and left for 2 hours before filtration through Whatman No. 42 filter paper. The filtrate was analyzed for available nitrogen (N) using the Kjeldahl distillation method. The distillate was collected in 5 mL of 2% boric acid and titrated with 0.01 M HCl to determine ammonium-N concentration.

E. Determination of Denitrification:

Denitrification was determined following Tiedje's (1988) method. A 1:10 dilution of soil (10 g) was prepared in 90 mL distilled water. A nitrate broth was used as the nutrient medium, consisting of KNO_3 (5 g), peptone (5 g), and yeast extract (2 g), with the pH adjusted to 7.0. The broth was sterilized by autoclaving at 121°C for 15 minutes. The presence of ammonium ions (NH_4^+) was detected using Nessler's reagent, which forms a yellow to brown-colored complex with ammonia.

F. Data Analysis:

The data collected from soil analysis and yield parameters were subjected to statistical analysis using Analysis of Variance (ANOVA). All statistical analyses were performed using Minitab 17 software. Treatment means were separated using Tukey's Honest Significant Difference (HSD) test at the 5% level of significance.

IV. RESULTS AND DISCUSSION

The effects of organic and inorganic fertilizers on the height of bell pepper are shown in Table 1. Significant differences ($P \leq 0.05$) in height were observed among the different fertilizer treatments used. Bell pepper planted in containers treated with organic fertilizers (cow dung and poultry manure) gave highest plant height across all weeks while the control (zero fertilizer) container recorded the lowest heights. This aligns with research indicating that inorganic fertilizers can enhance pepper height significantly over zero fertilizer or organic fertilizer (Oladitan, 2018). Plants treated with urea fertilizer recorded the highest height while the application of zero fertilizer had the lowest height. This suggests that nutrient from mineral fertilizers enhances the establishment of pepper.

Table 1: Effects of Organic and Inorganic Fertilizers on Plant Height (cm)

Treatment	Weeks after transplanting					
	2	4	6	8	10	12
PM	21.00ab	28.63b	39.60b	46.83b	53.07bc	62.13bc
CD	19.36b	30.60ab	36.97b	49.23ab	53.77bc	64.20bc
NSBF	17.40b	29.60ab	39.07b	52.10ab	61.90ab	76.57a
SSP	25.30a	26.36b	35.47b	48.83ab	62.77ab	71.83ab
UREA	16.93b	35.40a	55.57a	58.83a	66.23a	76.87a
CONTROL	22.53ab	26.93ab	32.87b	44.83b	50.83c	56.73c

Means in the same column that do not share a letter are significantly ($P<0.05$) different using Tukey's HSD

Results summarizing the effects of fertilizer treatments on number of leaves from 2 to 12 weeks after transplanting (WAT) of bell pepper are shown in Table 2. Significant differences ($P \leq 0.05$) in leaf number were observed. Across all the weeks after transplant, soil treated with single-super phosphate had the highest impact on number of leaves. At 4 WAT, poultry manure and SSP gave equal leaf numbers, however, some variability were observed among treatment with respect to their effects on leaf number. This suggests that SSP can significantly enhance vegetative growth when used in crop production (Kareem et al., 2021).

Table 2: Effects of Organic and Inorganic Fertilizers on Leaf Number

Treatment	Weeks after Transplanting					
	2	4	6	8	10	12
PM	6.44ab	28.67a	26.33ab	33.00a	38.67a	40.67ab
CD	7.33a	11.67b	17.00b	26.00b	33.67b	36.00b
NSBF	6.30ab	25.00a	27.33ab	28.33b	35.67a	39.33ab
SSP	7.23ab	28.67a	32.33a	34.67a	41.67a	42.67a
UREA	6.67ab	18.33b	28.67ab	33.00a	39.00a	37.00b
CONTROL	5.54b	21.33a	26.33ab	27.00b	32.00b	34.00b

Means in the same column that do not share a letter are significantly ($P<0.05$) different using Tukey's HSD

Significant variations were observed among the treatment regarding their influences on fruit number and weight (Table 3). The presence of fertilizer (either organic and inorganic) significantly increased fruit weight and

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numbers compared to the control. This positive response and increased yield of bell pepper after application of treatments can be attributed to the initial low status of nutrient in used soil. Olowokere (2004) observed that organic and organomineral fertilizer increase significantly the yield of bell pepper.

Table 3: Effects of Organic and Inorganic Fertilizer on Fruit Number and Weight (kg)

TREATMENT	FRUIT NUMBER	FRUIT WEIGHT
PM	4.33ab	12.33ab
CD	2.66ab	6.46bc
NSBF	4.67a	17.56a
SSP	2.33ab	5.11bc
UREA	2.66ab	5.88bc
CONTROL	2.00b	3.71c

Means in the same column that do not share a letter are significantly ($P<0.05$) different using Tukey's HSD

The results of daily counting of the colony forming units (CFU) number of bacteria capable of the ammonification and of the AMM process using ammonium concentrations after induction of the ammonification by peptone in soil samples (Figure 1) indicates that fertilizer treatments significantly ($P\leq0.05$) impact nitrifying bacteria and ammonifying bacteria activity in the soil.

Soil pH is known to be greatly affected by Soil treated with poultry manure showed higher ammonifying bacterial counts compared to soil amended with with cow dung. This aligns with the findings of

The highest amounts of ammonifying bacteria (CFU) was observed in NSBF and poultry manure amended pot. Urea ammonification bacterial activity was close to the figure obtained from pots treated with cow dungs. All treatment types significantly ($P\leq0.05$) increased the activity of ammonifiers compared to the soil with zero fertilizer application. Soil is affected, either positively or negatively by the pattern of soil use that reflects directly on the fertility of the soil (Marinkovic et al, 2012).

Categorically, change in substrate availability (NH_4^+, NO_3^-), physicochemical properties of the soil, such as pH or temperature regulates the overall community structure of nitrifying microorganisms (Huang et al, 2020).

Nitrifying bacteria activities was higher in soil treated with organ mineral fertilizers.

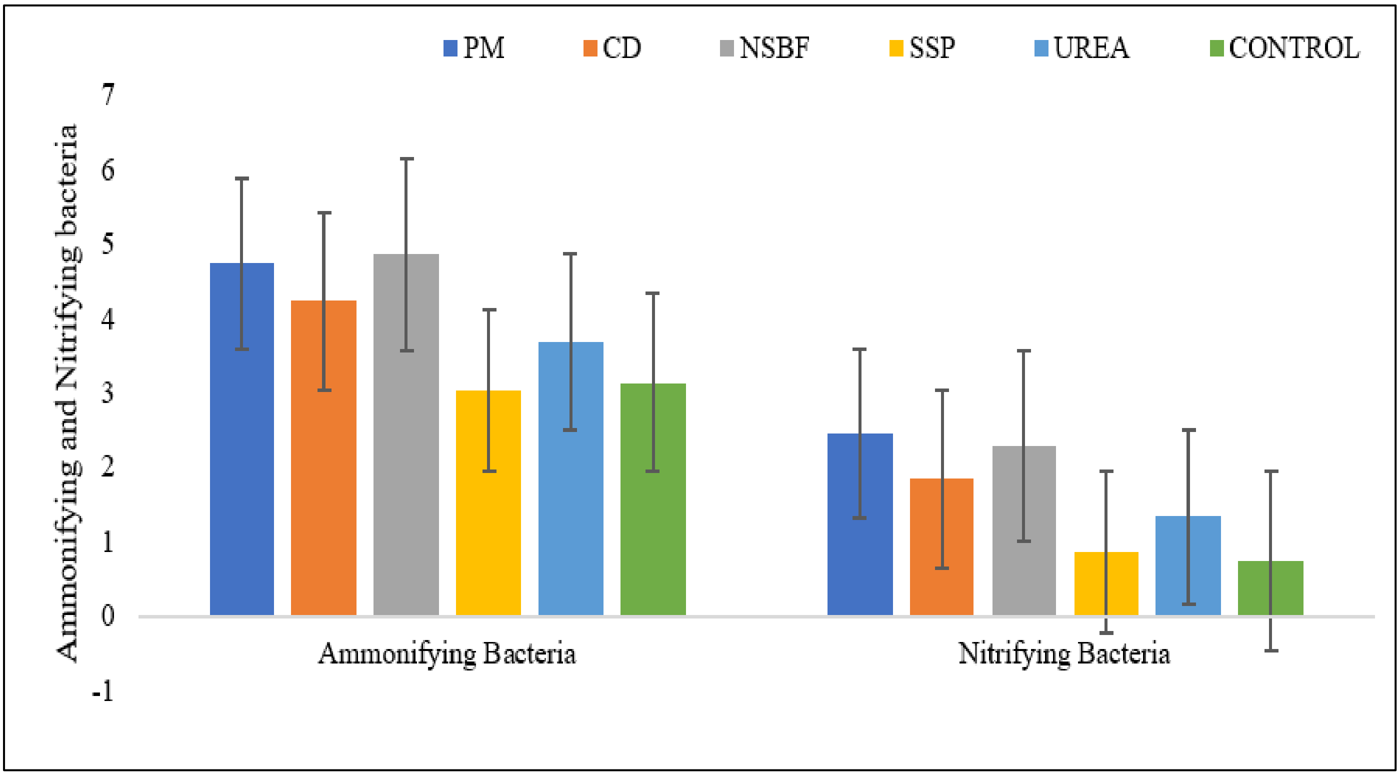

Fig 1: Effects of Fertilizer Treatments on Ammonifying (10^{-9}) (cfu g^{-1} soil) and Nitrifying Bactria (10^{-3}) (cfu g^{-1} Soil)

Figure 2 shows the responses of arginine ammonification to different fertilizer treatments. This paper considers arginine ammoification activity as a biological that participates in the mineralization of organic N. Some reports earlier suggest that soil microbes catabolize arginine through different pathways, and NH_4^+ emerges as the main final product. There was a strong decline in arginine ammonification among some fertilizer treatments, which attest to the fact that AA is a sensitive factor of of nitrogen mineraliation in the soil. The higher rate of N mineralization in treated soil was observed in organic fertilizers (poultry manure and cow dung), this has been attributed to higher availability of available N. This confirms the result obtained by Zaman et al., (2004) that greater rate of nitrogen

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mineralization has been attributed to higher availability of available N as well as to higher microbial activity. The maximum level of ammonium-N mineralized from arginine (2.2 µg – 2.4 µg) was observed in soil treated with poultry manure and neem-seed based fertilizer. The lowest level of ammonium-N was observed in the control soil, which gave (0.3 µg). Cow dung gave the 1.9 µg as a value of ammonium-N, and low arginine ammonification rate was observed in SSP. This result aligns with the findings of Kresovic and Licina (2002), who gave a report that the arginine ammonification at level 0.1-17.1 µg N g^{-1} will most often be observed in arable soil. They stated that the proportionality of arginine ammonification to the soil microbial biomass may be used as an indicator of microbial activity. Nourbaksh and Alinejadian (2009) described arginine aaammonification as an estimation of soil microbial-population size , therefore it is an inexpensive and very fast method of recommended for the calculation of microbial potential activity (Kresovic and Licina, 2002).

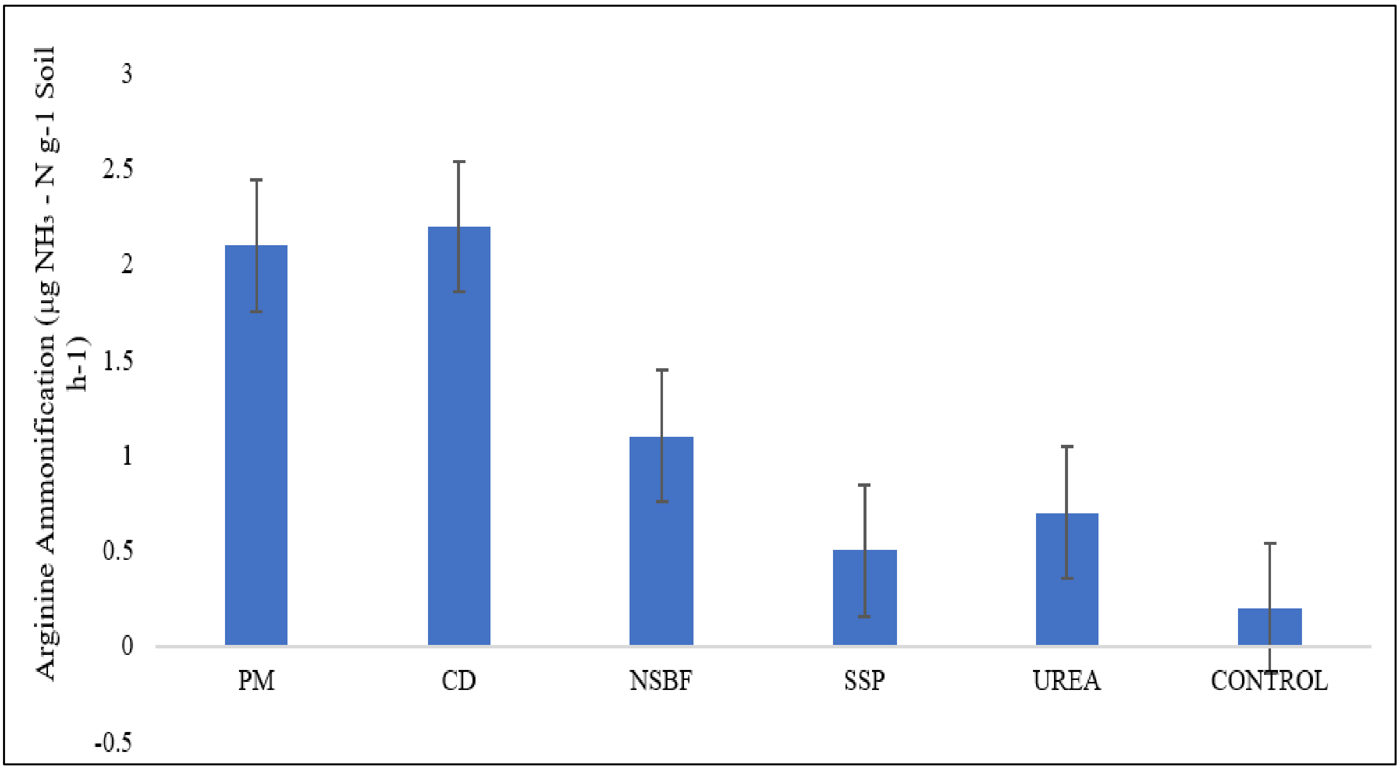

Fig 2: Effects of Fertilizer Treatments on Arginine Ammonification

Figure 3 illustrated the effects of organic and inorganic fertilizers on nitrification. Significant difference (P≤0.05) in soil nitrification were observed among the different treatments. Here, urea ranked the highest ammonia producer at day 14 and 21. For nitrate production, under nitrification, cow dung and poultry manure ranked highest producer of nitrate. Assessment of the global soil nitrification rate across various terrestrial ecosystems was done by Li et al. (2020). Their findings revealed that total soil nitrogen was the primary contributor to nitrificatiion, with a coefficient of 0.29. this was followed by mean annual temperature of 0.25, soil pH of 0.24, and microbial biomass nitrogen of 0.19. Application of mineral fertilizers offers instant alternative to naturally occurring nutrients. However, their long term use impacts conversion of ntrogen process in soil (Verma et al., 2018). NO_3^- excessive level in the soil is caused by the use of increasing fertilizer rate made of synthetic nitrogen in agroecosystems (Zhai et al., 2017).

In this study, NSBF significantly increased (P≤0.05) nitrification rate. Nitrification was inhibited through the application of NSBF compared to the application of organic and inorganic fertilizer that increased the rate of nitrification. This is similar to the findings of Byrnes et al., 2017 and Cantarella et al., 2018 that biological nitrification inhibitors are usually produced by certail plans which include Neem (*Azadirachta indica*).

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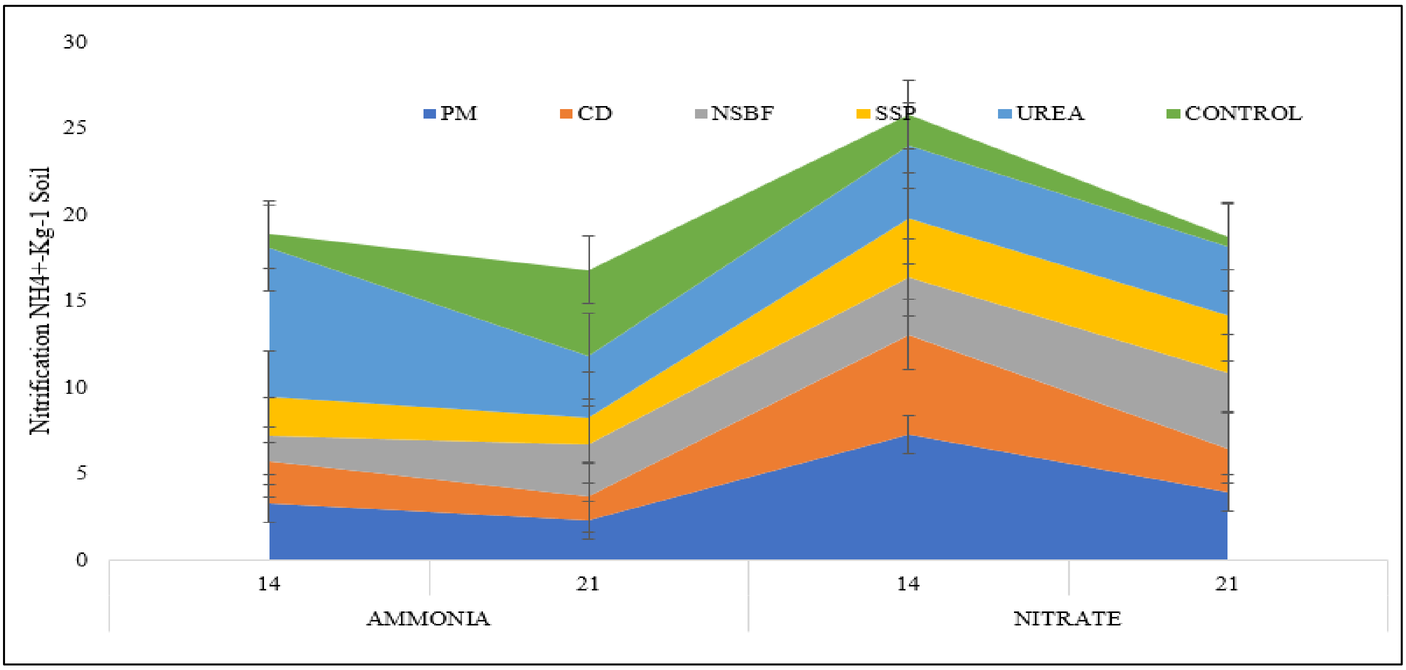

Fig 3: Effects of Fertilizer Treatments on Nitrification

The effects or organic and inorganic fertilizers on denitrification is shown in figure 4. Denitrification is biochemically limited by the availability and quality of carbon substrates in soil, including soil organic matter, root exudates, and plant litter. These organic substrates not only supports denitrification compared to soil treated with mineral fertilizers. The control pots consistently recorded the lowest rate of denitrification. Significant differences ($P \leq 0.05$) were observed among treatments. Soil treated with poultry manure and cow dung significantly increased denitrification rate compared to soil treated with inorganic fertilizers. This suggests that organic fertilizers can significantly enhance denitrification rate in the soil. Studies show that poultry manure is highly rich in organic matter and nitrogen, thereby promoting microbial activity, which is essential for denitrification processes in the soil (Zhang et al. 2020). Similar to poultry manure is cow dung. Addition of cow dung to soil has been shown to soil microbial conditions necessary for denitrification due to its high organic content (Ogunyemi et al. 2021). Organic fertilizers improving rate of denitrification may be attributed to the mineral fertilizers increase nitrogen loads in soil, which is usually not balanced with organic matter. Inorganic fertilizers (urea and SSP) provides nutrient in available form, urea especially provides available nitrogen, which can lead nitrogen imbalance if not accompanied with organic matter (Li et al. 2018).

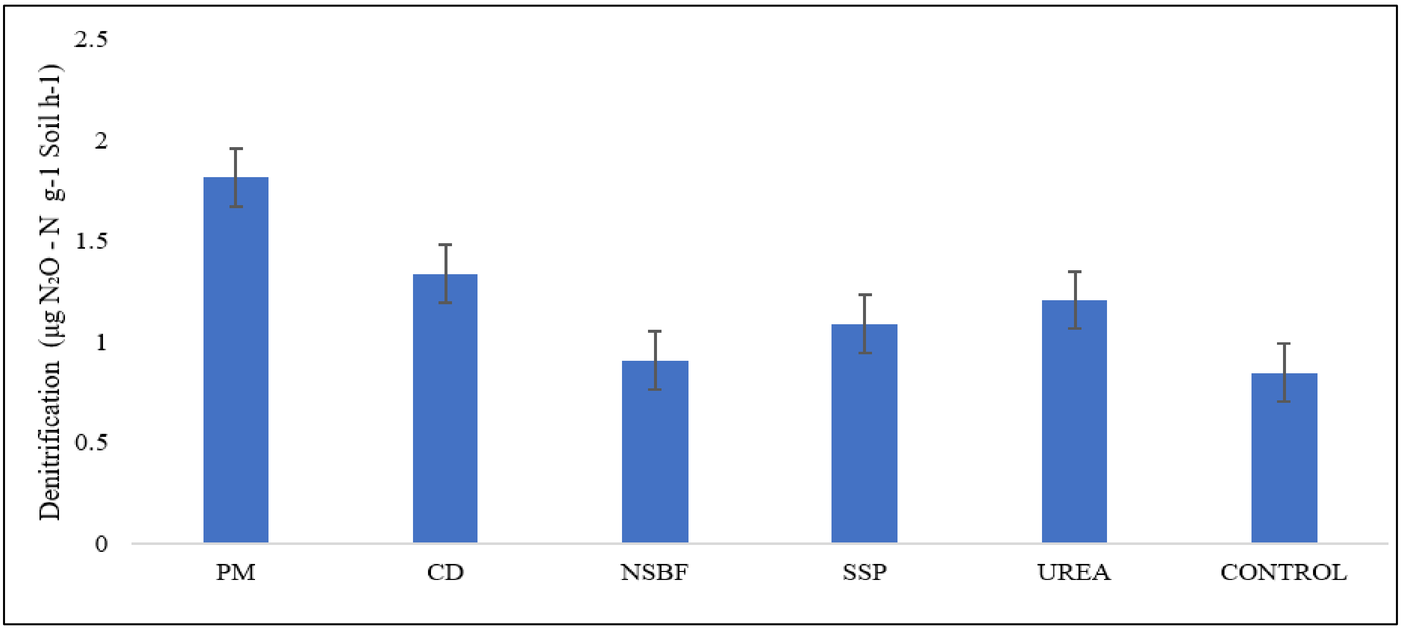

Fig 4: Effect of Fertilizer Treatments on Denitrification

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The results of the analysis for the physico-chemical properties of soil before fertilizer application is shown in figure 4.1 (a). The result of the analysis of the soil characteristics and physical chemistry indicate that the soil contained 48.50% sand, 31.20% silt, and 20.00 % clay. The initial contents of organic carbon and organic matter in the soil was 2.24%, and 3.86%, respectively. The soil was low in N, exhangeable magnesium, available phosphorus, exchangeable potassium, and calcium were marginal.

A. Changes in Soil Nutrient Status

The effects of organic and inorganic fertilizers on physico-chemical properties is shown in figure 4(b). On application of the organic fertilizers to the soil as observed in table 4b), there was an increase was observed in the soil nutrient. The soil in which poultry manure and cow dung was applied had a higher organic carbon of 3.39% and 3.58% respectively compared to organic fertilizers, urea with OC of 1.09% and SSP with 3.39%. This aligns with the research carried out by Lustosa et al., 2017. He mentioned that poultry manure is very concentrated in nitrogen and phosphate and is more effective to both plant and soil. In year 2000, it was stated by Ojeniyi that increase in the organic matter content of the soil was expected because organic fertilizers have the ability of increasing soil organic matter Urea reduced the pH of the soil in this study compared to organic fertilizers. Reduction in the pH of the soil increases the acidity of the soil. Fertilizers made up of ammonia, especially urea, reduce the soil pH; this increases its acidity (Goulding, 2016).

Table 4(a): Soil Physical and Chemical Properties before Treatment Application

Soil Properties	pH 1:2	Organic C (%)	Organic matter (%)	N (%)	P (mg/kg)	K (cmol/g)	Na (cmol/g)	Ca (cmol/g)	Mg (cmol/kg)	Sand (%)	Silt (%)	Clay (%)
	6.23	2.24	3.86	0.26	68.21	0.25	0.37	7.00	3.50	48.50	31.20	20.00

Table 4(b): Soil Chemical Properties after Fertilizer Application

	Soil Properties	pH 1:2	Organic carbon (%)	Organic matter (%)	N (%)	P Mg/kg	K Cmol/kg	Na Cmol/kg	Ca Cmol/kg	Mg Cmol/kg
	PM	6.02	3.39	5.84	0.54	135.26	1.11	1.27	10.00	5.00
	CD	4.08	3.58	6.17	0.58	73.81	0.42	0.50	10.20	5.00
Treatments	NSBF	5.69	3.20	5.51	0.50	82.91	0.92	1.10	10.00	5.00
	SSP	6.08	3.39	5.64	0.52	75.68	0.43	0.48	10.00	4.80
	UREA	4.19	1.09	1.88	0.18	10.97	0.41	0.43	5.00	2.10
	CONTROL	5.95	3.10	5.35	0.50	104.38	0.46	0.58	10.00	4.20

V. CONCLUSION

Results obtained from the study showed that application of mineral fertilizer significantly enhances bell pepper height and leaf number compared to organic fertilizers. Organomineral fertilizer (NSBF) produced the highest fruit weight and numbers. NSBF also improved nitrification rate compared to mineral fertilizers. The application of poultry manure and cow dung resulted in a sustainable environment for soil microbes. Poultry manure enhanced arginine ammonification rates. The application of organic fertilizers promoted higher nitrification rates compared to mineral fertilizers. Organic fertilizers not only improved significantly yield parameters, with the highest fruit weight, also nitrifying bacteria were more active in soil treated with NSBF and organic fertilizers.

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