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Ammonium Consumption by Nitrifying Bacteria in Soil Sample Treated with Ethanol Extracts of Velvet beans (*Mucuna pruriens*).

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Ammonium nitrogen in soil is necessary for plant growth and development. This study investigated the ability of ethanol extracts of medicinal plant *Mucuna pruriens* (velvet bean) to reduce ammonium consumption by nitrifying bacteria in the soil. Soil samples were treated and subjected to exposure to 1%, 2% and 4% of the plant extracts for a period of 5 weeks. The extracts were able to reduce ammonium consumption in the soil sample. Ammonium consumption was reduced by as much as 85.18% using the dose-response model. The results were significant at χ^2(p0.05). The effects of the extracts in reducing ammonium consumption are of significance to agriculture in substituting chemical compounds used as inhibitors that could result in environmental pollution.

Keywords:*Mucuna pruriens*, ammonium, extracts, nitrification.

INTRODUCTION

Ammonium nitrogen in soil provides a source of nitrogen for plant growth and development. Soil organisms including bacteria and fungi convert organic nitrogen to ammonia, which is further converted to nitrite or nitrate by the ammonia oxidizing bacteria (Hunter *et al*., 1988). The ammonia oxidizing bacteria include *Nitrosomonas*, *Nitrosovibrio* and *Nitrosococcus* spp.

Soil nitrogen in the form of ammonium salts serves as electron acceptors and nutrients exploited by microbial biomass for the growth and production of degradative enzymes (Dani and Pennickx, 1999). Through the nitrification pathway, some ammonium may be transformed to nitrite and nitrate through the catabolic action of *ammonia monooxygenase* in the first and second steps respectively of the nitrification process (Stein and Arp, 1998; Soliman and Eldyasti, 2018.).

The process of denitrification which results in the loss of soil ammonium is however undesirable for agricultural practices (Celen and Kilic, 2004). The loss of ammonia from the soil negatively impacts environmental quality and human health (Li *et al*, 2017). Nitrifying bacterial population in the soil determines the nitrification potential of the soil. Factors that affect the oxidation of ammonium in the soil include handling, temperature, moisture content, soil electrical conductivity, pH etc. (Zhang and Wienhold, 2002).

Nitrifying potential of the soil is dependent on amendment with ammonium. Under favourable conditions of temperature and moisture, nitrate is formed during incubation. In soils not amended, the rate of nitrate formation is limited by the rate at which ammonium is formed from the mineralization of organic soil nitrogen (Schmidt and Belser, 1994). The nitrification potential of the soil significantly affects the application of nitrification inhibitors (Mukhtar and Lin, 2019).

Mucuna pruriens (Velvet bean) is a climbing legume indigenous to China, India, West Indies and Africa, usually applied by farmers to restore soil fertility due to its ability to smother rampant weeds (Manyong *et al*., 1999). The plant exhibits high tolerance to abiotic stress like drought, low soil fertility and high soil activity. It is high in protein, carbohydrates, lipids, fibre, minerals, alkaloids, saponins, serotonin and sterols. It is used extensively as a medical plant for the treatment of various diseases (Thyaga Raju *et al*, 2017). The seeds contain dihydroxy phenylalanine (L-DoPa) used to treat symptoms associated with Parkinson's disease and the pod contains *mucunain* that cause severe itching irritation when in contoct with the skin (Stansley and Yamamoto, 2015).

METHODOLOGY

Sample collection

Soil samples used for the study were obtained from the Campus of the Federal University of Technology,

Owerri, Nigeria (5.4850^{0}N, 7.0350^{0}E). Samples were collected randomly from the surface (0-15 cm) using a spade (Haile *et al*., 2006; Archarya, 2018). The whole plant sample (leaves, stems and roots) were collected from an agricultural farm at Agudama-Epie, Yenagoa, Nigeria (5.0333^{0}N, 6.3333^{0}E).

Sample preparation

Soil samples were homogenized, air-dried and sieved with a 2mm mesh (Archarya, 2018). The sieved soil samples were preserved in polythene bags and kept in the laboratory at room temperature (28 ± 2^{0}C) until further use. One hundred grams (100g) of soil was weighed into plastic cans and moistened to 60% water holding capacity (WHC) by capillary porosity (Hunter, 2004; Haile *et al*., 2006). Moistened soil samples were incubated in the laboratory for 2 weeks to stabilize (Murray *et al*., 1990).

The plant samples were dried in the dark in the laboratory at room temperature for 8 weeks (Azwanida, 2015). The dried plant materials were finely ground to powder (Haile e*t al*., 2006) and used to prepare extracts. One hundred grams (100g) ofthe plant was mixed with 500ml of 95% ethanol (1:5 w/v). This was shaken thoroughly and allowed to stand for 48 hours. The extract was then filtered using Whatmann filter paper No. 1. Ethanol was removed by evaporation (Haile et al, 2006). The extract was then diluted in 100ml of distilled water to serve as the stock as described by James *et al*, 2014.

Soil analysis

The physicochemical analysis of the soil samples was carried out using standard methods to determine nitrate-nitrogen, NO_3-N (Keeney and Nelson, 1982); ammonium-nitrogen, NH_4-N (Keeney and Nelson, 1982); total nitrogen (Yong *et* al, 2005); organic matter (Yong *et al*., 2005); and organic carbon (Pereira *et al*., 2006).

The total heterotrophic bacterial count was determined using spread plate technique (Pelczar *et al*., 2003; Ameh and Kawo, 2017).One gram (1g) of treated soil sample kept in the laboratory for 1 week for the activation of soil bacteria was weighed into 9ml of sterile distilled water (Oyeyiola *et al*., 2013). This was serially diluted using micropipette to give 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions. Using micropipette, 0.1ml of the diluent was plated onto nutrient agar medium in duplicates and spread using a sterile spreader. The plates were then incubated at 37^{0}C for 48 hrs after which, colonies were counted.

The ammonia oxidizing bacterial count was determined using the modified Schmidt and Belser (1994) medium. Aliquots (0.1ml) of the serially diluted samples were plated on selective agar medium containing keto-konazole to inhibit fungal growth (Elumalai *et al*., 2009). The plates were incubated at 37^{0}C for 9 days and the colonies counted.

Determination of NH_4-N

Dried extract-treated soil sample (0.5g) was weighed into a polythene bottle and0.25gof activated charcoal added. To this, 20ml of Morgan extracting solution was added, covered and shaken in a mechanical shaker for 60 mins. The mixture was filtered into a test tube using Whatmann No. 1 filter paper and used for the determination of NH_4-N. The method described by Keeney and Nelson (1982) and Pansu (2006) was used for the determination of NH_4-N in the extracts from the treated soil samples based on the determination of the absorbance after treatment.

Three millilitres (3ml) of the filtrate was mixed thoroughly with 1.8ml 15% Sodium tartarate in a clean test tube. Aliquots of Alkaline sodium phenate (0.6ml) were added and mixed thoroughly by shaking. To this, 0.3ml 40% Sodium hypochlorite followed by 1.5ml of distilled water was added. The mixture was shaken properly and the absorbance value read at 630nm against the reagent blank in a Unicam Spectrophotometer. Standard calibration curve for NH_4-N was obtained by plotting absorbance values against concentration. NH_4-N in the samples was determined from the regression analysis of the standards.

RESULTS AND DISCUSSION

The results of the physicochemical analysis of the soil sample and microbial analysis are presented in Table 1 and Table 2 respectively. The total heterotrophic bacterial count was 9.8 X10^{6}cfu/ml.

The results showed that the ammonium-nitrogen content of the soil samples reduced gradually over a period of Five (5) weeks. This ammonium consumption is mainly due to the activities of ammonium oxidizing bacteria in the soil. The control sample showed a reduction of ammonium-nitrogen of 83.33 mg/kg soil. The various samples treated with ethanol plant extracts of *Mucuna pruriens* also showed reduction in ammonium-nitrogen. The soil samples treated with 1% plant extracts showed ammonium consumption of 12.35 mg/kg and those treated with 2% plant extracts showed ammonium consumption of 12.34 mg/kg soil over 5 weeks. Soil samples treated with 4% plant extracts showed ammonium consumption of 46.30 mg/kg soil. Soil samples treated with 1% and 2%plant extracts have lower ammonium-nitrogen consumption rates compared to soil samples treated with 4% plant extracts.

Using the formula described by Sahrawat (1980), it was determined that the plant extracts were able to inhibit

the process of nitrogen mineralization in the soil sample. Fitting the results into the dose-response model proposed by Haanstra *et al.* (1985), the results obtained showed that the extracts affect net nitrification with coefficient of determination (r^2) > 0.7. Compared to the control, the 1% plant extracts reduced ammonium consumption by 85.18%, the 2% plant extracts by 85.19% and the 4% plant extracts by 44.44%. The ability of 1% and 2% plant extracts to inhibit the process of nitrogen mineralization by nitrifying bacteria in the soil samples will improve soil fertility by making ammonium-nitrogen in the soil available for plant use. The results were significant at χ^2(p0.05), where χ^2_{cal} (10.134) < χ^2_{tab} (18.307). Fig 1 shows the expected NH_4-NO_3 content of the soil sample over an extended period after application of the various treatments.

CONCLUSION

Ammonium consumption in soil by nitrifying bacteria reduces ammonium-nitrogen available for plant growth and development. A reduction of ammonium consumption by bacteria will improve the fertility of the soil. The results indicate that 1% and 2% plant extracts are efficient in the reduction of ammonium consumption Similar to results were obtained by Al-Ansari and Abdulkareem (2014) and Zhao *et al*. (2015). The results were also similar to that reported by Guo*et al*. 2021 using a continuous flow analyzer. The ability to retard ammonium consumption could be used to the advantage of plants. These extracts could serve as substitute for chemical compounds used in nitrification inhibition. This is of great advantage in agriculture because the plant extracts are environmentally friendly. The soil however is a complex ecosystem of different types of organism with their peculiar organs and systems. It is therefore recommended that further study should be carried out on the effects of the extracts on soil organisms to determine their suitability for use.

Table 1. Physicochemical parameters of soil sample

S/N	Property	Quantity
1	Organic carbon (%)	1.821
2	Organic nitrogen (%)	0.116
3	C:N	16:01
4	Organic matter (%)	3.139
5	pH	5.62
6	Ca (meq/100g)	0.6
7	Mg (meq/100g)	0.25
8	Na (meq/100g)	0.022
9	K (meq/100g)	0.042
10	Al (meq/100g)	0.32
11	H (meq/100g)	0.48
12	NO_3-N (mg/kg)	19
13	NH_4-N (mg/kg)	160.49
14	P (ppm)	4.2

Table 2. Microbial load of soil sample

S/N	Microbial count	Load (cfu/ml)
1	Total heterotrophic bacteria	9.8×10^6
2	Ammonium oxidizing bacteria	1.64×10^6
3	Nitrite oxidizing bacteria	4.3×10^6

Table 3. Ammonium-Nitrogen content of soil sample

Week	Control (mg/kg)	1% (mg/kg)	2% (mg/kg)	4% (mg/kg)
0	160.49	160.49	160.49	160.49
1	121.22	158.67	162.41	155.67
2	109.31	160.21	177.30	130.34
3	99.40	161.11	167.17	118.73
4	85.21	158.87	164.7	123.90
5	77.16	148.14	153.15	114.19

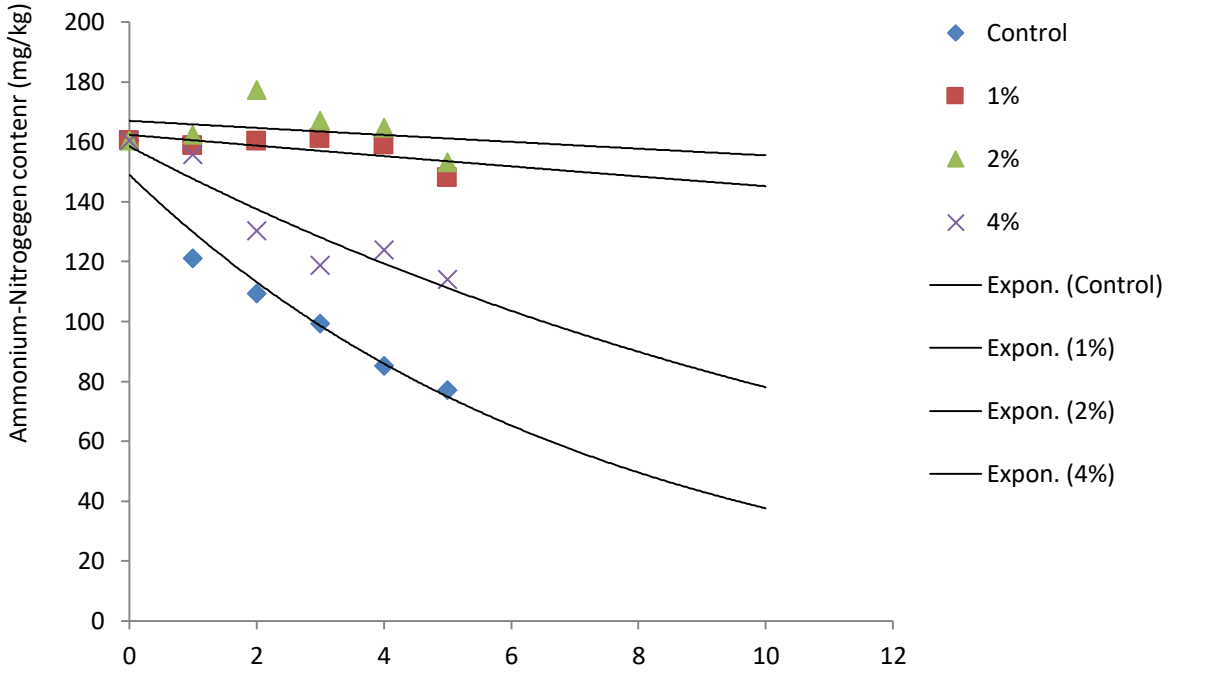

Fig. 1. Projected Ammonium-Nitrogent content of soil

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