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Effect of the Form and Nature of Nitrogen on Growth and Nodulation of Pigeon Pea (*Cajanus cajan* (L.) Millsp) on Sandy Soil of Mont-Amba, in Democratic Republic of Congo

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Abstract

The study was carried out on the impact assessment of the form and nature of the different nitrogen sources used in culture on growth and nodulation of pigeon pea (*Cajanus cajan* (L.) Millsp) during 3 months of observation on sandy soil of Mount Amba (at the University of Kinshasa in the Democratic Republic of Congo). Height and collar diameter growth, underground and total biomass dry weight, number and dry pea nodules and nodulation index have been evaluated at 4, 6, 8, 10 and 12 weeks of growth to assess the response of the legume to mineral nitrogen and organic nitrogen from chickens manure and pig feces. After observations, it appears that all the treatments soil showed different effects on growth and nodulation of pigeon pea. Therefore, only the soil received 1 kg of pig feces stimulated ($p \leq 0.05$) the development of pigeon pea which resulted in good growth (nodulation 116.8 nodules and nodulation index of 5.0) and biomass production (25.3 gr per plant) while the addition of chicken manure, whatever the dose, reduced them.

Keywords

Mineral and Organic Nitrogen, Growth, Nodulation, Pigeon Pea

1. Introduction

It is widely recognized that nitrogen and phosphorus are often nutritional limiting factors of plant growth in most tropical soils [1] [2] [3] [4] [5]. To raise the level of these elements in such poor acid soils, the use of nitrogen and phosphate fertilizers is a real bargain [4]. Because the increased production recorded the recent decades could be attributed to more half to the increased use of inorganic fertilizers [6]. Among all inputs applied in order to maximize agricultural output, nitrogenous fertilizers rank first. They make up 50% of all nutrient inputs. In addition, among the nutrients brought by these fertilizers, the most important is nitrogen [7], which can be attributed in some measure, 75% of the increase in crop yields recorded [6].

Thus, to meet future food needs, it will obviously continue to make more use of the inorganic nitrogen fertilizer. The efficiency of these fertilizers, however, is one of the lowest among plant nutrients. Therefore, high rates of N fertilization especially when using ammonium sulphate have also been shown to acidify soils. Yet these are severely limited by prohibitive purchasing costs and general lack of availability by the smallholder farmers [8] [9] [10] [11] and may eventually pollute the environment [6], degraded ecosystems and disrupt human health [12]. Nitrogenous fertilizers are subject to losses due to denitrification leading to release of nitrogen N_2 and NO_2 gases, volatilization of ammonia, run-off and leaching which can contaminate surface and underground water. To remedy this situation, technologies that will reduce N fertilizer input by resource-poor farmers in the Nutrient Management Systems (NMS) are urgently needed. Nitrogen input through biological nitrogen fixation (BNF) by grain legumes can help to maintain soil N reserves as well as substitute for N fertilizer requirement for large crop yields. So, researchers in the world exploited the legume *Rhizobium* symbiosis as a substitute for expensive N fertilizers [13] [14] [15] [16] [17]. To enrich soil-plant system in nitrogen and compensate for N potential losses, the phenomenon of BNF is an alternative present in virtually all ecosystems. Through a series of biological systems, nitrogen fixation is considered to have a major role in maintaining the productivity of tropical soils [18]. According to Chris *et al.* [19], Nitrogen (N) contributing by legumes to other crops in the system depends on the species, biological N_2 fixation and growth of legumes as determined by climate and soil, and management of residues.

So, in this practice, the choice of legumes varieties that nodules easily with indigenous rhizobia, is of capital importance [13]. Among these species, pigeon pea (*Cajanus cajan* L. (J. Millop)) has the capacity of growing in low soils in nitrogen without the addition of nitrogen fertilizer while improving the economy in fixed nitrogen for subsequent cereal crops [20], and provided a quality of produced food for humans [21] [22] [23] and a forage appreciated the cattle (Otero cited by Skerman) [22]. Although, the use of legumes seems to solve the problem due to lack of N in the soil; therefore, the amount of the nitrogen fixed by legumes is dependent not only to generic features of bacteria and the host plant, but also to environmental and agricultural practices [24]. Thus, the fertilizer phosphorus and nitrogen plays an important role [6] [25]. Mont Amba soils, however, have characteristically low available P making application of P through fertilizers necessary. Apart from P deficiency limiting plant growth, it can also limit symbiotic N_2 fixation as the latter has been noted to have a higher P requirement for optimal functioning than either plant growth or nitrate assimilation. If this P constraint is overcome, grain legumes in the cereal based cropping system of NMS should be able to fix a greater amount of N_2. For a mineral concentration of P is required for Nod factors (having an important role in the process of recognizing bacteria-plant) can occur (Mckay and Djodievic, 1993 quoted by Dommergues *et al.* [14]). Thus, in the case of deficient soils, it is certain that the application of P improved nodulation (nodule number and weight) and increases the amount of N_2 fixed [24]. This confirmation is supported by the results of Sanginga *et al.* [26] on *Leucaena leucocephala* and Sanginga *et al.* [27] on cowpeas. Thus the use of organic manure instead of mineral fertilizers seems advantageous because of the disadvantages of mineral fertilizers recorded. Because these appear to be balanced organic fertilizer in essential elements (NPK) and to a lesser extent, S, Ca, Mg and other trace elements for plant growth [28]. This study then attempts to assess the impact of the form and nature of the different sources of nitrogen on growth and modulation of pigeon pea.

2 Material and Methods

2.1. Location and Site Characterization

The experimental site was located on the University of Kinshasa in the Mont-Amba site. According to Köppen classification, the Mont-Amba falls within the Aw4 climate type characterized by 4 months of dry season (from mid-may to august) coupled with 8 months of rainy season, sometimes interrupted by a short dry season in January/February. Daily temperature averages 25˚C and annual rainfall is close to 1500 mm. The average daily temperature is estimated at 24, 5˚C. The relative air humidity is maximum in august, September and November [29].

2.2. Experimental Design and Trial Implementation

Seeds of pigeon pea (*C. cajan* L. (J. Millop)) var flavus DC, were harvested on the university site. The seeds were then dried for 3 days at the sun and stored in polyethylene bags white, tightly closed until their used.

The trial was conducted in vegetation pots from June to august 2003, following the completely randomized design in which six treatments were subjected to four replications (**Figure 1**). The different treatments used were: T1 or Control, T2 or 0.5 kg of pig excrement, T3 or 1 kg of pig excrement, T4 or 0.5 kg of chicken manure, 1 kg or T5 droppings of chickens and T6 or 0.5 g of urea.

The black polyethylene bags of an intern volume of 0.00691 m^3 were used in this experiment and have each received 10 kg of rhizosphere soil collected at a depth of 15 cm in the same site having as composition: 0.651% organic C; 0.069% N; 1.1206% organic matter; 9.4347 C/N; 0.258 Mg^{2+} meq/100 gr; 0.139 meq Ca^{2+}/100 gr; 1.4136 meq K_2O/100 gr; 25.55 ppm of P_2O_5 and pH = 4.58; plus a corresponding treatment dose of fertilizer. Chicken droppings and feces of pigs

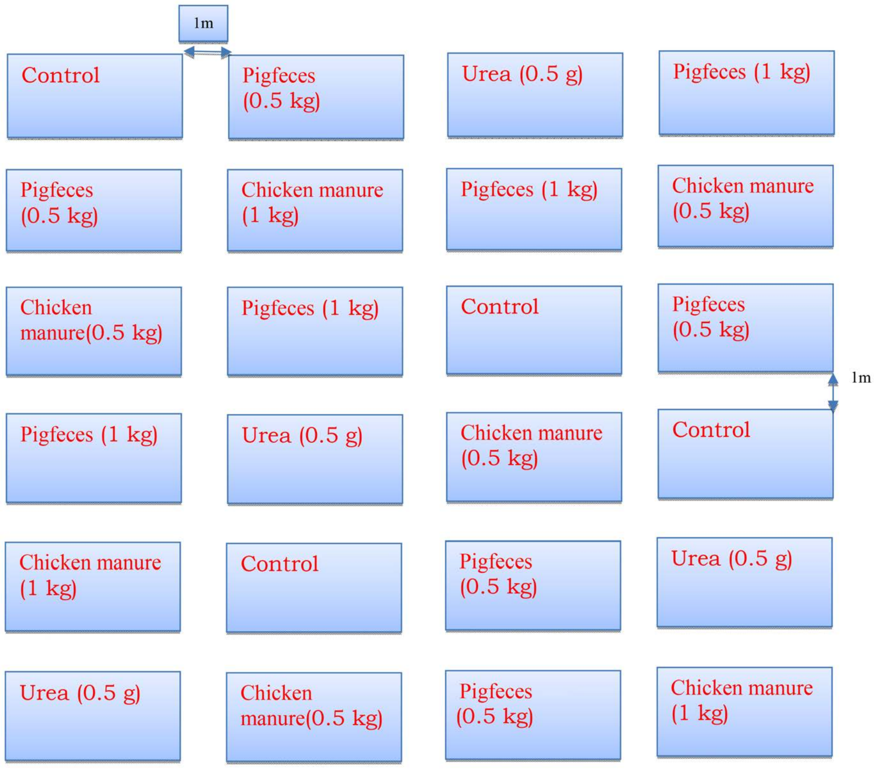

Figure 1. Experimental design plan.

were applied 3 days before sowing (DBS), while urea was applied two weeks after sowing (WAS) by fractionation in three steps, at intervals of two weeks. Manual seeding was carried in bags and used as experimental units at a rate of eight seeds per package to prevent the irregular germination. Once raised, the thinning has occurred for only two remain with vigorous plants per bag. The nitrogen has been used in organic form (nature of Pig feces and Chicken manure) and in inorganic form (urea nature) to evaluate its effect on pigeon pea.

2.3. Data Collection and Statistical Analyses

To evaluate the effectiveness of treatments applied, the height and collar diameter growth of seedlings of *C. cajan* was assessed at 4, 6, 8, 10 and 12 weeks of growth after sowing (WAS). The dry weight of aboveground, underground and total biomass; number and dry weight of nodules; and nodulation index have thus been evaluated at the end (12 WAS) of trial after harvest. Biomass and nodules were dried separately following the treatment, firstly in the sun for three days and after in stove at 69˚C for 48 hours to obtain constant weight, and then weighed. In the end, the index of nodulation which measure the percentage ratio of the dry weight of nodules on the dry weight of aboveground biomass of the legume was calculated. The more is the index of nodulation, the greater nodulation is good [30]. Statistical analysis of data was obtained by the treatment at IRRISTAT software (version 92-1). Duncan Multiple Range Test (DMRT) was used to compare the averages of all the treatments at 5% probability level.

3. Results

3.1. Growth in Height and Collar Diameter

Growth in height and collar diameter of pigeon pea is presented at Table 1 and Table 2. It appear that the control in which no fertilizer is provided gave an average of greater height compared to other treatments throughout the observation period ranging from 4 to 12 WAS (Table 1). However, the difference of the heights of plants following treatments was significant (p = 5%) not only to the sixth and eighth WAS. Indeed, the application of 1 kg of chicken manure on the ground has been mediocre compared to the control where a plant height has significantly lower (p = 0.05) at the 4th, 10th and 12th WAS (Table 1). The application of 0.5 kg of chicken manure was second in ascending order on the height of plants.

To evaluate the Table 2 in which the collar diameter of seedlings of pigeon pea is presented, it appears that the application of 1 kg and 0.5 kg of pig manure, 1 kg of chicken droppings emerge with diameter the collar average of pigeon pea very higher than those of plants have received nothing (control). It emerged significant differences (p = 0.05) at the 4th and 6th SAS for the 1 kg of pigs feces application; at the sixth and eighth WAS for the 1 kg Chicken manure; and at the 12th WAS for 0.5 kg pig feces application (Table 2).

3.2. Biomass Production

Table 3 shows the production of dry weight biomass per plant of pigeon pea. It turns out that the plants ender 1 kg of pig excrement application gave an average and below-ground biomass apparently higher than the control followed by those ender 0.5 g of urea. However, the differences are not significant (p = 0.05).

The lowest dry biomass obtained ender treatment with 0.05 kg of chicken manure. Therefore, particular the plants ender treatment at 1 kg of chicken manure showed yield of total biomass significantly (p = 0.05) lower than the

Table 1. Changes in plant height (cm) of pigeon pea in the 4th to the 12th WAS under the effect of different treatments applied to him.

Treatment	Time in WAS				
	4th	6th	8th	10th	12th
Control	28.5b	46.8a	66.9a	86.0b	101.8a
Pig feces (0.5 kg)	23.7ab	39.6a	58.5a	79.8b	97.4a
Pig feces (1 kg)	24.9ab	45.1a	62.9a	84.0b	100.3a
Chicken manure (0.5 kg)	22.6a	40.1a	53.6a	66.4a	88.8a
Chicken manure (1 kg)	23.8ab	42.0a	56.3a	72.6ab	90.0a
Urea (0.5 g)	25.3ab	39.9a	61.0a	80.6b	96.0a
Mean	24.8	42.3	59.9	78.2	95.7
CV (%)	12	14.3	15.5	10.8	9.0

In the columns, values followed by same letter are not significantly different at the 5% level by the Duncan test.

Table 2. Evolution of collar diameter (mm) pigeon pea plants from 4 to 12 SAS as a result of the different treatments applied to him.

Treatment	Time in WAS				
	4th	6th	8th	10th	12th
Control	2.2a	3.5a	4.6a	5.5a	6.6a
Pig feces (0.5 kg)	2.3a	4.5ab	6.5c	7.9bc	8.2a
Pig feces (1 kg)	2.7a	4.6b	6.2bc	8.2abc	8.8a
Chicken manure (0.5 kg)	2.4a	3.8ab	5.4abc	6.9abc	7.9a
Chicken manure (1 kg)	2.6a	4.5ab	6.0abc	7.5bc	7.7a
Urea (0.5 g)	2.3a	3.6	4.9ab	6.6ab	7.6a
Mean	2.4	4.1	5.6	7.1	7.8
CV (%)	12.6	12.2	16.2	13.1	18.0

In the columns, values followed by same letter are not significantly different at the 5% level by the Duncan test.

Table 3. Dry weight of aboveground, underground and total weight at the 12th WAS pigeon under the effect of different treatments.

Treatment	Biomass dry weight (gr/plant)		
	Below-ground	Underground	Total
Control	13.6ab	7.1ab	21.3abc
Pig feces (0.5 kg)	12.9ab	9.1b	22.6bc
Pig feces (1 kg)	15.4b	9.1b	25.3c
Chicken manure (0.5 kg)	9.0a	4.8a	14.0a
Chicken manure (1 kg)	11.1ab	5.9a	17.3ab
Urea (0.5 g)	14.8b	4.8a	20.0abc
Mean	12.8	6.8	20.1
CV (%)	27.5	25.7	23.8

In the columns, values followed by same letter are not significantly different at the 5% level by the Duncan test.

application of 0.5 kg and 1 kg of pig feces in (**Table 3**). These results reflect the poor intake of 0.5 kg of chicken manure on the soil compared to the control. The same observation was done for this treatment on plant height of pigeon pea (**Table 1**).

3.3. Nodulation Capacity

Symbiotic characteristics of pigeon pea for nodulation are presented in **Table 4**. It follows from the consideration of these results that treatment with 1 kg of pig excrement is revealed significantly ($p = 0.05$) higher number of nodules per plant compared to other treatments applied to the soil. The highest average in both the dry weight of nodules per plant of pigeon pea and in terms of relative

Table 4. Symbiotic characteristics of pigeon pea under the effect of different treatments at harvest (12th WAS).

	Symbiotic characteristics		
Treatment	Number of nodules	Dry weight of nodules (gr)	Nodulation index
Control	37.3a	0.6ab	4.2b
Pig feces (0.5 kg)	43.8a	0.5ab	4.1b
Pig feces (1 kg)	116.8b	0.8b	5.0c
Chicken manure (0.5 kg)	13.5a	0.2a	2.1a
Chicken manure (1 kg)	29.0a	0.3ab	2.7a
Urea (0.5 g)	37.8a	0.4ab	2.8a
Mean	46.3	0.5	3.5
CV (%)	77.5	60.8	53.9

In the columns, values followed by same letter are not significantly different at the 5% level by the Duncan test.

nodulation index were observed at plants which received 1 kg of pig excrement. In storytelling, the lowest values in number and dry weight of nodules, in nodulation index were observed under the application of 0.5 kg of chicken manure consistently (**Table 4**).

4. Discussion

In general, all soil treatments had each a different effect on growth and nodulation of pigeon pea. Indeed, the mineral nitrogen (urea) had an effect on the border between the feces of pigs upstream and downstream chicken droppings both on nodulation, biomass production and on total height growth of plants of pigeon pea. Thus, the quantity of mineral nitrogen (0.5 g) applied to the soil could be treated as starter N for pigeon pea, which should stimulate nodulation as observed elsewhere [24].

Note also that the irregularity of the growth in both diameter and height in plants receiving 0.5 g of urea as a fertilizer, could be due to the split application of this fertilizer, because every time it has been applied, growth was stimulated immediately and then gradually slowed down as N becomes deficient by leaching and other losses (**Table 1** and **Table 2**). These results corroborate the observation by Danso and Eskew [6] and Joly [18]. This is the observation made also for the treatment of chicken manure, the effect on growth was rapid during the first week and gradually slowed down as the age of plants evolved. In fact, the dung being a light organic fertilizer, N would have been quickly released, used in part by the seedlings, and then lost resulting in depth by leaching and through other channels as observed by Danso and Eskew [6] and Joly [18].

This depletion of N would have resulted in the decline in growth of seedlings that received the treatments equivalent to 0.5 kg and 1 kg of chicken manure to the end of the test. By cons, poor nodulation observed in the seedlings have undergone treatments with chicken manure is due to the concentration of N that would have inhibited the nodulation compared to the control and treatment to 0.5 g of urea, given that the excess of available nitrogen inhibits nodulation [24], on one hand. On the other hand, it is thought that the chicken manure has a high concentration of metals (Pb, Cu and Zn) and soluble salts, which thus have inhibited the growth and nodulation by rhizobia by acting on toxicity [31] [32] [33] [34].

We can also note the alternation of several organic substances contained in the fertilizer that are suspected to be toxic to rhizobia as to cause inhibition of nodulation [35] [36]. However, we found that pigeon pea grows on the substrate significantly improved by the application of pig manure (soil application of 1 kg and 0.5 kg) with good symbiotic characteristics (**Table 4**), among others. The conclusive evidence of the pronounced effect compared to other treatments is the rise in number of nodules per plant (**Table 4**). When closely related to the acceleration of the activity of nitrogenase, this parameter determines the concentration of N in plants and therefore the increase in biomass production as reported Abd-Alla *et al.* [37]. As such, we agree with Abd-Alla *et al.* [37] that there was in the feces of pigs a number of beneficial elements including a high concentration of P, with N, supported the good performance of both fertilizer on growth than on the nodulation of pigeon pea in our trial. In addition to P, one can also refer to metals such as Co, Cu, etc., which have a potential to improve the nodulation of legumes (Chatel *et al.* 1978; Bollard, 1983). Indeed, Abd-Alla and Abdel [38] had by the application of Co and Cu (100 mg/kg) significantly increased the number of nodules and the production of dry biomass in faba bean (*Vicia faba*). In this case, the feces of pigs are a good source of major elements (NPK) and other related items.

They are supposed to stimulate nodulation and increase the survival and multiplication of rhizobia in the soil and improve their natural habitat as found by Heckman *et al.* [39] of treated human waste, and therefore they enhance the growth and biomass production of crops. The presence of nodules on the control treatment shows that the legume under study have found a strain of rhizobia indigenous to the same nodules in the soil and pigeon pea nodulation can start even in very poor soils in N such that our test.

5. Conclusion

At the end of our observations, it appears that all the treatments applied to the soil produced, each, different effect on growth and nodulation of pigeon pea. However, treatment of 1 kg of pig manure ensured a smooth behavior of plants in terms of pigeon pea nodulation, growth and biomass production. This result indicates the power of pig feces to serve as fertilizer and organic amendment in improving fertility and soil physical properties of Mont-Amba although its optimum amount to be determined. So pig feces are richer in nutrients that stimulate growth and nodulation of pigeon pea, in contrast to the dung of hens on the contrary, reduced the performance against the observations reported by Zuang [28] on vegetable crops.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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