Effect of varied nutrient solution concentration of P,Mo,S,Al and Mn supply to soil on the yield nitrogen fixation of two leguminous species pigeon pea (Cajanus cajan) and white leadtree (Leucaena leucocephala) at Nyaruguru district, southern Rwanda.

Rukangantambara, H[*1]. Maniriho [1], G. and Habumugisha Jean de Dieu [2]

1. National University of Rwanda, Faculty of agriculture, Soil and Environment management
2. Institut Polytechnique de Byumba, Faculty of agriculture, environmental management and renewable energy

[*]Corresponding Author: ruhamudu99@yahoo.fr

ABSTRACT:

Many agriculturally important plants in the legumes family can use nitrogen (N) from atmosphere for growth through biological nitrogen fixation (BNF). Legume-nitrogen fixation is dramatically effected under mineral nutrients deficiencies or excess and other environmental constraints. However, it has yet to be established as to whether such process of nitrogen fixation is affected at which sensitivity level parameters.

A pot experiment was conducted on a tree nursery at Nyagisozi sector of Nyaruguru district, southern Rwanda in field to evaluate the effect of varied nutrient solution concentrations of phosphorus, molybdenum or sulfur adequate or limitation, and aluminum or manganese toxicities on the yield nitrogen fixation of two leguminous species pigeon pea (Cajanus cajan) and white leadtree (Leucaena leucocephala). The experiment was laid out in a randomized complete block design (RCBD) with three replications. The 50 seedlings of each species were taken as a reference and fertilized with 50 kg of triple superphosphate (TSP), $Ca(H_2PO_4)_2$, 46% /ha in order to avoid the deficit in phosphorus. Four elements Mo, S, Al and Mn were applied on soil and leaf at different level doses for each block.

In block1, soil application four elements before planting by four different levels doses of each element, Molybdenum: 0; 0.54; 1.08 and 2.16 mg/stem of $Na_2MoO_4.2H_2O$, 39%; Sulfur: 0; 200; 400 and 800 mg/stem of K_2SO_4, 18.4%; Manganese: 0; 10; 20 and 40 mg/stem of $KMnO_4$, 34.8% and Aluminum: 0; 0.5; 1.5 and 13.5 mg/stem of $Al_2(SO_4)_3.18H_2O$,8.12%

In block 2, Leaf application for four elements in five factorials -Molybdenum: 0; 0.27; 0.54; 1.08 and 2.16 mg/stem of $Na_2MoO_4.2H_2O$, 39%; Sulfur: 0; 100; 200; 300 and 400 mg/stem of K_2SO_4, Manganese: 0; 5; 10; 20 and 40 mg/stem of $KMnO_4$, 34.8% and Aluminum : 0; 0.5; 1,0, 1.5 and 13.5 mg/stem of $Al_2(SO_4)_3.18H_2O$,8.12%

After five weeks of applying to the soil, the N fixation was determined by measured the wet biomass weight, volume of nodule and the rate of acetylene (C_2H_2) reduction activity (ARA). The results showed that legumes differ significantly (P=0.05) in their ability to fixed nitrogen. Application of P, Mo, S, Al and Mn elements in general significantly increased wet biomass weight, volume nodulation and the rate of acetylene (C_2H_2) reduction activity (ARA) especially by sulfur application to the leaf at 200,400 and 800 mg/stem doses at second trial and the maximum effect was observed at the dose of 200 mg/stem with increased of 14% comparing to the control.

Keys words: Acetylene Reduction activity (ARA), *Cajanus cajan* and *Leucaena, leucocephala,, Rhisobia ,DAP*

Introduction

At Nyagisozi sector in Nyaruguru district, southern Rwanda, over 80% of cultivable land presents limitations of agricultural production due low pH (<5, 5) and deficiencies of nutrients availability such as N, P and others nutrients elements (Mutwewingabo and Rutunga, 1988). Sustainable N-replenishment strategies as the biological N fixation (BNF) could play an important role in land remediation, with limited need for chemical fertilizer (Chenn, 1999). Interest in BNF has focused on the symbiotic system of leguminous plants with special bacteria, rhizobia which live in the root nodules. Rhizobia infect root hairs of the leguminous plants and produce the nodules. The nodule become the home for bacteria where they obtain energy from the host plant and take free nitrogen from the soil air and process it into combined nitrogen and in return, the plant receives the fixed nitrogen from nodule(Rondon,M., et,2009)

Postgate,(1998) clearly defined the biological nitrogen (N2) fixation as the process whereby atmospheric nitrogen (N=N) is reduced to ammonia in the presence of nitrogenase which is a biological catalytic found naturally only in certain microorganism such as the symbiotic as rhizobium(leguminous) and frankia (non-leguminous), or the three –living Azospirillum and Azotobacter. Burns and Hardy (1975) reported that these microorganism have the greatest quantitative impact on nitrogen cycle that permits the recycling of the lost amount of nitrogen in atmosphere via the volatilization and denitrification phenomenon and contribute substantially to the overall N economy of the system and increase the productivity of the crops when incorporated into agroecosystems, either as intercrops or as a crop within a rotation (Mafongoya et al., 2006). Various estimates of the contribution on BNF to the soil N fall in the range of 44 – 200 10^{12} N^{-1}, with an average of about 122 10^{12} N^{-1} and legumes contribute an estimated 35 out of 75 10^{12} tones of fixed N annually.

The amount of symbiosis is 100-200 times greater than that fixed by free organisms like Azotobacter (Mulder et al., 1969). As example, the total annual terrestrial inputs of N from BNF range from 139 million to 175 million tons of N, with symbiotic associations accounting for 55 to 60% (80 million to 99 million tons of N (Martins, 2004) .the efficiency of nitrogen fixing are governed not only by genetic determinants owned by rhizobia species but also with the surrounding conditions, generally, restrictions or scarcity of mineral nutrients concentration in soil (Dommergues et Mangenot, 1970). The effect of mineral on biological nitrogen fixation (BNF) has been widely reported (Zahran, 1999) and is considered to be by far the most important environmental factor resulting in crop yield loss. For example,application of large quantities of fertilizer N inhibits N_2 fixation, but low doses (<30 kg N ha^{-1}) of fertilizer N can stimulate early growth of legumes and increase their overall N_2 fixation., the amount of this starter N must be defined in relation to available soil N(Allen,1981)

As it has been reported by Holliday (1981), in much tropical soil, deficient in P was observed the limitation of nodulation and N fixation and the growth of NF on the L. Leucocephala. Similar observations have been reported by the same authors for acacia holoccericea, and acacia sepium used in alley cropping in P- deficient soil. A low and limiting P supply eventually reduces plant growth and thus reduces N demand and N2fixation, but evidence concerning the regulating mechanisms is not clear (RAGOTHAMA, 1999).Soil acidity in tropical region is known to be highly correlated with calcium deficiency, and with aluminium or manganese toxicity (FAO. 1985). Information of the effects of high aluminium concentration either on plant growth or on rhizobium activity in association with nitrogen fixation transformation is available (Paul, 2000). But effect of alumunium on nitrogen fixation in nodules does not appear to have been studied.

The effectiveness of a N2 – fixing symbiosis is greatly dependent on the ability of the host plant to supply photosynthate to bacteria (Mahon, 1977). It has been reported that the bacteria live in compartments, up to 10,000 in a nodule, called bacteroids bathed in nutrients from the host plant with various microelements (Cu, Mo, Co, B) necessary for N_2 fixation (FRANCO, 1978 and Mendel, 2002) for example Mo which consists of one Fe-Mo (iron-molybdenum) based protein and iron deficiencies can therefore interfere with nitrogen fixation, Molybdenum deficiencies is manifested as deficiency of plant N (Pollock, 2002).

Currently, there is a lack of studies comparing the effect of Mo added at different levels and on how plants access molybdate from the soil solution by selected enzymes to carry out redox reactions.

Although it has been established that mineral nutrients elements factors may influence nitrogen fixation, only a few work research have been conducted. Hence, this study focused to investigate how various nutrients concentration of phosphorus, molybdenum, sulfur, aluminum and manganese affects symbiotic N_2-fixation of two leguminous species Cajanus cajan and Leucaena leucocephala.

MATERIALS AND METHODS

Field experiment was conducted on a tree nursery established in 2006 by DUHAMIC ADRI in Nyagisozi sector of Nyaruguru district, southern Rwanda in ceramic pot. Site climate belongs to the AW3 index; the mean annual rainfall amount is 1200 mm, whereas the annual temperatures vary between the minimum of 10.5 ^{o}C and a maximum of 30.8 ^{o}C.

Soils used were classified as sandy loam (USAID triangle system) and Tropudalf (soil taxonomy) or humic acrisols (FAO classification); Composite top (0 – 20 cm) soil samples were taken from the trial site and subjected to physical and chemical analysis at the time of planting in 2010. Soil from the experimental fields characterized by: sand % : 70,4; Silt %: 10,9; clay% : 19,1; textural class : sandy loam to sandy clay loam; C % : 0,96; Organic matter % : 1,66; Total nitrogen % : 0,084; pH (soil : water,1:1): 5,3; CEC meq/100g : 14,6; total bases meq/100g : 2,96; Base saturation %: 20,27; Ca meq/100g : 2,4; Mg meq/100 :0.22; K meq/100g : 0,25; Na meq/100g : 0,09 and Al meq/100g : 0,26

Vegetal material: Two leguminous species were used such as - Pigeon pea (Cajanus cajan) is a leguminous species, annual or short-lived perennial shrub or small tree 1-4m tall a component in alley cropping systems. Good nitrogen fixation can be grown on a wide range of soil textures from sands to heavy clays. Pigeon pea prefers pH of 5 -7 but can toleratespH4, 5 – 8, 4 and can handle some aluminum, temperatures of 18–30ºC. Very drought tolerant. Dual purpose crop for grain and fodder. Tolerates low fertility soils. Excellent green manure for improving soil structure and quality

White leadtree (Leucaena leucocephala) is leguminous species, an arborescent deciduous small trees or shrub, to 2m tall. The plant is known for its drought tolerance and thrives on a wide range soils. Its deep root system permits it to tolerate many soil types. It tolerates aluminium and soil low in phosphorus. pH of 4,3 to 8,7 . With its rhizobium, leucaena can fix more than 500kgN/ha

Source of elements used in experiment

Mineral elements used in experimental pot were: Molybdenum in form of Sodium molybdate,$Na_2MoO_4.2H_2O$, 39%; Sulfur in form Potassium sulfate, K_2SO_4, 18.4%; Manganese in form Potassium permanganate, $KMnO_4$, 34.8%; Aluminum in form Aluminum sulfate, $Al_2(SO_4)_3.18H_2O$,8.12%; Phosphorus in Triple superphosphate, $Ca(H_2PO_4)_2$,46%

Methods

The four treatments for soil application block1 and the five treatments for leaf application block2 with tree replicates were laid out under a randomized design.

Block1: Soil application

Four elements were applied in the soil before planting in 4 different doses (i) Molybdenum: 0; 0.54; 1.08 and 2.16 mg/stem, (ii) Sulfur: 0; 200; 400 and 800 mg/stem,(iii) Manganese: 0; 10; 20 and 40 mg/stem and (iv) Aluminum : 0; 0.5; 1.5 and 13.5 mg/stem respectively with side dressing application

In order to avoid the deficit in phosphorus which may occur in tropical countries, 800 mg/pot of P_2O_5 was applied under the chemical fertilizer known as triple superphosphate. In every pot, 6 stems were planted. In 4 weeks, they were diminished until 2 in order to allow their healthy development. The soil was watered every day with 100 ml/pot. Watering tubes were used in order to make the water distribution within the pot uniform and to prevent the destruction of roots during the watering process.

The experimental material was a tree nursery divided in parcels occupied by *Leucaena leucocephala* and *Cajanus cajan* and secondary parcels occupied by treatments. In the first trial, 3 repetitions were carried out whereas 5 repetitions were carried out in the second one. Absolute controls and relative controls were fertilized with phosphorus.

Block2: Leaf application (spray).The doses were fractioned and applied 2 times in 2 to 3 weeks of plant life after each treatment in 5 doses (i) Molybdenum: 0; 0.27; 0.54; 1.08 and 2.16 mg/stes,(ii)Sulfur : 0; 100; 200; 300 and 400 mg/stem,(iii)Manganese: 0; 5; 10; 20 and 40 mg/stem and (iv)Aluminum : 0; 0.5; 1,0, 1.5 and 13.5 mg/stem, respectively with fertigation system

Experimental protocol

Every trial lasted for two months; period at which the vegetative development was maximal. The following parameters were measured: wet biomass weight, nodules volume and Acetylene Reduction Activity (ARA) in order to evaluate the effect of different minerals nutrients applied on nitrogen fixation process of Pigeon pea (Cajanus cajan) White leadtree (Leucaena leucocephala) at Faculty of Agriculture laboratory and in Rwanda Agricultural Board (RAB) laboratory at Rubona station

Acetylene Reduction Activity was determined as follows: Division of roots having nodules into two parts and their introduction in the syringe of 60 ml serving as an incubation side.

Acetylene production by reaction of water on calcium carbure (CaC_2) as following:

$$CaC_2 + 2\,H_2O \longrightarrow Ca\,(OH)_2 + C_2H_2$$

Sampling 10 % of acetylene (V/V) in the syringe followed by the nodules incubation in 30 minutes. Sampling 10 ml of the mixture of C_2H_2 and C_2H_4 in venoject tube.

Injection of the mixture sampled with the venoject tube by a syringe of 1 ml in the chromatograph for the analysis of reduced quantity of acetylene or produced ethylene by a chromatograph in gaseous phase) which expresses Nitrogenase Activity.

The machine was calibrated with pure ethylene. The concentrations of calibration were respectively 200 and 800 ppm. The apparatus indicated directly ethylene concentration in ppm (evolution time=35-40 seconds) which must be converted in nanomols/hour/stem. When it is already converted in such a form, it is now called Acetylene Reduction Activity (ARA) which expresses Nitrogenase Activity.

After incubation and injection in chromatograph, nodules were detached from roots and their volume was measured with graduated tubes. Results were submitted to an analysis of variance (ANOVA) and tested at 95% degree of freedom

RESULTS

The mean of three repetition of variables measured parameters of N fixation such as the wet biomass weight (WBW) of aerial parts of the plant, nodule volume (NV) and acetylene reduction activity (ARA) for the Leucaena leucocephala and Cajanus cajan are represented in tables below in responses of varied concentration nutrient of phosphorus, molybdenum, sulfur, Manganese, and Aluminum

Table1. The effect of phosphorus on the wet biomass weight (WBW) of aerial parts of the plant, nodule volume (NV) and acetylene reduction activity (ARA) to *Leucaena leucocephala* and *Cajanus cajan.*

Treatment	Cajanus cajan			Leucaena Leucocaphala		
	WBW (g/stem)	NV (ml/stem)	ARA (nmols, C2H4/h/stem)	WBW g/stem	NV (ml/stem)	ARA, (nmols, C2H4/h/stem)
50kgP205/ha	7.35	0.5	2498.6	5,45	0,30	1225,6
Absolute control	6.91	0.4	3100.6	5,62	0,22	1565,1
Mean	7.06	0.43	2556.5	5,34	0,24	1382,3
Standard error	0.24	0.05	451.5	0,29	0,05	337,6
Diference	NDS	NSD	NSD	NSD	NSD	NSD
C.V	8.8	38.2	46.7	17,0	64,4	64,4

N.S.D. No significant difference; WBW: wet biomass weigh;
NV: nodulation volume; ARA: acetylene reduction activity; VSSD: very small significant difference at 5% degree of freedom; CV: coefficient of variation.The rate of P addition to the soil very little significantly affected wet biomass and nodulation development and decreased acetylene reduction activity in 19,4% comparing the control to Cajanus cajan. About the Leucaena leucocephala, decreased were observed on wet biomass weight and acetylene reduction activity 3% and 21, 7% respectively

.

<u>Block1</u>: Soil application

Table2. The effect of Molybdenum applied on the soil on growth wet biomass weight (WBW), nodulation volume (NV) and acetylene reduction activity (ARA) to *Leucaena leucocephala* and *Cajanus*

Treatment	Cajanus cajan			Leucaena Leucocaphala		
	WBW (g/stem)	NV (ml/stem)	ARA,(nmols, C2H4/h/stem)	WBW (g/stem)	NV (ml/stem)	ARA,(nmols C2H4/h/stem)
0.54	9.70	0.43	1302,5	7.83	0.25	176
1.08	9.47	0.60	1302.5	8.73	0.40	525
2.16	9.47	0.48	381.7	9.07	0.28	377
Control	10.13	0.38	858.5	8.23	0.37	1415
Mean	9.67	0.48	7212	8.47	0.32	623
Standard deviation error	1.10	0.09	617.3	1.00	0.10	589
Difference	NS	NS	NS	NS	SN	SN
C.V%	19.7	33.1	148.0	20.5	51.5	164

The results (table2) showed that soil application of molybdenum to Cajanus cajan were no effect on wet biomass comparing to control. The maximum increased were observed on nodulation volume where were supplied the1.08 doses.

The effects of 0, 54 and 1, 08 doses were the same on acetylene activity. About to Leucaena Leucocephala, application of molybdenum effect were observed only on nodulation volume where applied 1, 08 doses.

Table3: The effect of sulfur applied on the soil on the growth, nodulation volume (NV) and Acetylene reduction activity (ARA)

Treatment	Cajanus cajan			Leucaena Leucocaphala		
	WBW (g/stem)	NV (ml/stem)	ARA,(nmols C2H4/h/stem)	WBW (g/stem)	N.V (ml/stem)	ARA, (nmols C2H4/h/stem)
100	9.87	0.15	1396.3	7.13	0.28	2507.6
200	8.80	0.37	703.3	6.93	0.27	1468.6
400	8.27	0.15	277.8	6.67	0.08	703.1
Control	7.07	0.12	1094.3	6.60	0.12	1278.4
Standard deviation errror	0.78	0.09	154.4	6.83	0.19	1489.4
Difference	NS	NS	NS	SN	NS	NS
VSSD at 5%	-	-	-	-	-	-
C.V %	15.1	77.4	31.8	7.9	24.9	42.3

WBW: wet biomass weight; NV: nodulation volume; ARA: acetylene reduction activity; VSSD: very small significant difference; NSD: Non-significant difference at 5 % degree of freedom; CV: coefficient of variation

Effect of sulfur soil application on Cajanus cajan were observed on the all parameters studies. About to wet biomass weight and acetylene reduction activity, the maximum increased were observed at 100 doses application comparing to control (table3). The doses 200 and 400 decreased the activity of acetylene activity comparing to the control.

About the Leucaena leucocephala, the 100 doses increased the wet biomass, nodulation and acetylene reduction activity, the 200 increased wet biomass and acetylene only. The 400 doses increased wet biomass and nodulation only to comparing to control respectively.

Table4. The effect of manganese applied on soil on growth wet biomass weight (WBW) nodulation volume (NV), and acetylene reduction activity (ARA) to *Cajanus cajan* and *Leucaena leucocephala*

Treatment	Cajanus cajan			Leucaena Leucocaphala		
	WBW (g/stem)	NV (ml/stem)	ARA,(nmols C2H4/h/stem)	WBW (g/stem)	NV (ml/stem)	ARA,(nmols C2H4/h/stem)
10	8.00	0.35	2069.8	6.27	0.27	2871.3
20	7.43	0.27	1273.9	6.13	0.17	1220.3
40	7.43	0.20	1915.3	6.27	0.15	1449.0
Control	6.73	0.22	2110.9	5.07	0.16	3214.2
Mean	7.40	0.26	1842.5	5.93	0.19	3214.2
Standard deviation error	0.40	0.07	637.1	0.32	0.05	635.6
Difference	NS	NS	NS	NS	NS	NS
C.V%	9.40	44.1	59.9	9.2	41.5	50.3

WBW: wet biomass weight; NV: nodulation volume (NV), and ARA: acetylene reduction activity
CV: Coefficient of variation

Effect of Manganese application to Cajanus Cajan was positively. The 10, 20 and 40 doses increased the wet biomass, then only 10 and 20 doses increased the nodulation volume, finally 10 doses increase acetylene reduction activity comparing to the control. About Leucaena Leucocephala, the positively affect were on wet biomass weight were observed from to all doses. On nodulation volume, the negative effect was observed where applied 40 doses. And all doses had negative effect on acetylene reduction activity respectively comparing to control.

Table5. The effect of Aluminum applied on the soil on the growth wet biomass weight (WBW), nodulation volume (NV) and acetylene reduction activity (ARA) to *Leucaena leucocephala* and *Cajanus cajan*.

Treatment	Cajanus cajan			Leucaena Leucocephala		
	WBW (g/stem)	N V (ml/stem)	ARA, (nmols C2H4/h/stem)	WBW (g/stem)	NV (ml/stem)	ARA,(nmols C2H4/h/stem)
0.5	10.33	0.35	1037.4	7.93	0.27	1164.0
1.5	9.77	0.62	1203	7.27	0.35	688.3
13.5	10.17	0.63	305.7	6.83	0.18	748
Control	9.27	0.41	480	9.77	0.18	667
Mean	9.07	0.50	787	7.95	0.24	816.3
Standard deviation	0.70	0.13	762.1	1.05	0.08	3867
Difference	NS	NS	NS	NS	NS	NS
CV%	12.3	45.2	22.9	22.9	53.7	82

WBW: wet biomass weight; NV: nodule volume; ARA: acetylene reduction activity NS: Non significant; CV: coefficient of variation

Response of Cajanus cajan and Leucaena leucocephal to aluminium application were negative to cajanus cajan with 0,5 doses on nodulation volume and with 13,5 doses on acetylene activity. About leucaena leucocephala, negative effect was observed with all doses on wet biomass weight.

Block2: Leaf application

Table 6.The molybdenum effect applied on leaves on the growth wet biomass weight (WBW), nodulation volume (NV) and acetylene reduction activity

Treatment	Cajanus cajan			Leucaena Leucocaphala		
	WBW (g/stem)	NV (ml/stem)	ARA,(nmols C2H4/h/stem)	WBW (g/stem)	NV (ml/stem)	ARA,(nmols C2H4/h/stem)
0,25	6,07	0,40	1082,7	5,53	0,17	126
0,54	6,20	0,35	1362,6	4,27	0,34	1361
1,08	6,47	0,35	296,5	5,63	0,21	304
2,16	6,60	0,38	1785,8	5,63	0,21	287
Control	5,20	0,48	2354,8	5,10	0,23	469
Mean	6,11	0,42	1376,7	5,23	0,05	363
Standard	0,31	0,06	290,7	0,53	0,05	363
Difference	NSD	NSD	HS	NSD	NSD	NSD
VSSD	-	-	948,2	-	-	-
CV	8,7	26,4	36,6	17,4	39,2	134

WBW: wet biomass weight; NV: nodule volume; ARA: acetylene reduction activity CV: coefficient of variation

Considering the results presented in the table above illustrated that the wet biomass weight were increased, nodulation volume and acetylene activity decreased comparing to control for all doses. About leucaena leucocephala, all doses showed the positive effect on wet biomass weight, and only 1.08 doses applied on nodulation volume and acetylene reduction activity comparing to control.

Table7. The effect of sulfur applied on the leaves on the growth wet biomass weight (WBW), nodulation volume (NV) and acetylene reduction activity (ARA) to *Leucaena leucocephala* and *Cajanus cajan*.

Treatment	Cajanus cajan			Leucaena Leucocephala		
	WBW (g/stem)	NV (ml/stem)	ARA,(nmols C2H4/h/stem)	WBW (g/stem)	NV (ml/stem)	ARA, (nmols C2H4/h/stem)
100	7,20	0,53	714,7	5,53	0,23	199
200	6,87	0,55	7 14,5	5,43	0,23	643
300	6,63	0,57	1049,7	4,97	0,15	286
400	6,83	0,82	346,7	5,27	0,30	146
Control	6,10	0,43	1174,7	4,87	0,13	186
Mean	6,73	0,58	803,6	5,21	0,21	292
Standard	0,44	0,10	499,1	0,36	0,06	75
Difference	NSD	NSD	NSD	NSD	NSD	SND
VSSD at 5%	-	-	-	-	-	-

WBW: wet biomass weight; NV: nodule volume; ARA: acetylene reduction activity VSSD: very small significant difference; CV: coefficient of variation

For Leucaena leucocephala, the sulfur input caused an amelioration of ARA. This parameter increased significantly at the dose of 200 mg/stem with 244% of increase comparing to the control. For Cajanus cajan, significantly increased at the dose 300mg/stem

Table 8: The effect of Manganese applied on leaves on growth wet biomass weight (WBW), nodulation volume (NV) and acetylene reduction activity (ARA) to *Cajanus cajan* and *Leucaena leucocephala*

Treatment	Cajanus Cajan			Leucaena Leucocaena		
	WBW (g/stem)	NV (ml/stem)	ARA, (nmols C2H4/h/stem)	WBW (g/stem)	N.V (ml/stem)	ARA,(nmols C2H4/h/stem)
5	6,87	0,63	786,1	5.00	0.23	124.1
10	7,00	0,60	231,4	5.70	0.44	829.5
20	7,23	0,62	1001,5	5.07	0.32	433.3
40	6,27	0,23	419,8	5.33	0.22	554.7
Control	6,79	0,55	503,9	3.67	0.27	118.8
Standard error	0,60	0,16	344,8	0.77	0.30	412.1
Difference	-	-	-	-	-	-
CV%	152	49,4	118,5	27.0	66.5	87.4

NV: Nodule volume; WBW: wet biomass weight; ARA: Acetylene reduction activity
NS Non-significant; CV: Coefficient of variation
Effect negative was illustrated with 5 and 40 doses on wet biomass weight, 40 doses on the nodulation volume and acetylene reduction activity of Cajanus cajan. About leucaena leucocephala, negative effect was illustrated on 5 and 40 doses on nodulation volume, then positive effect was obtained with 5 dose on acetylene reduction activity.

Table 9: Effect of Aluminum applied on the leaves on the growth wet biomass weight (WBW), nodulation volume (NV) and acetylene reduction activity (ARA) to *Cajanus cajan* and *Leucaena leucocephala*.

Treatment	Cajanus cajan			Leucaena Leucocaphala		
g/stem	WBW (g/stem)	NV (ml/stem)	ARA,(nmols C2H4/h/stem)	WBW (g/stem)	NV (ml/stem)	ARA,(nmols C2H4/h/stem)
0.5	6.70	0.57	1038.0	5.90	0.27	1164.0
1.5	6.83	0.45	1203.3	5.40	0.33	689,7
4.5	6.83	0.45	306.4	6.03	0.22	747.7
13..5	6.53	0.42	479.7	4.77	0.38	914.7
Control	6.23	0.30	787.0	5.60	0.22	665.5
Mean	6.63	0.42	762.9	5.56	0.38	816.3
Standard deviation	0.56	0.10	196.4	0.41	0.22	386.7
Difference	NSD	NSD	NSD	NSD	0.10	NSD
C.V	6.63	4.2.2	129	12.9	58.8	82.0

WBW: wet biomass weight; NV: nodule volume; ARA: acetylene reduction activity
Negative effect was on acetylene reduction activity of Cajanus cajan with doses 4, 5 and 13, 5. About leucaena on wet biomass weight, leucocephala, negative effect with 13,5 doses

DISCUSSION

This study showed that the response of *Leucaena leucocephala and* pigeon pea (*Cajanus cajan)* to phosphorus application was very slightly and non significant table1. This should be explained by rapidly formed insoluble complexes with aluminum and incorporated into organic matter by microbes (Bieleski, 1973; Ragothama, 1999).This finding is consistent with the results obtained by Kuo and Lotse (1972), who reported that soil solution P released from fertilizer particles can react with soil component in different ways, adsorption, precipitation and immobilization by microorganisms and plant roots.

The same way, Tisdale et al. (1993) reported that phosphorus fertilizer application supplies crop P requirement,however, crop utilization of inorganic fertilizer of application is only 10 – 15% of P applied because there is limited soil volume occupied by plant root system and relatively short diffusion distance of phosphate anion in soil solution and the conversion of fertilizer P to sparingly soluble inorganic and organic reduces plant availability

The response of *Leucaena* and *Cajanus cajan to application of molybdenum* showed an slightly increase on its aerial part, nodule volume and acetylene reduction activity. The results presented in the table 6, showed that ARA was higher to the control of *Cajanus cajan* than where we applied 0.27, 0.54 and 1.08 mg/stem by leaves application. The same situation of decreasing the Nitrogen fixation were observed by Anderson and Oertel(1946) on lucerne and possible causes the decrease ARA where molybdenum was applied was associated with an increase the direct inhibiting effect of molybdenum on rhizobium

The sulfur application showed that in the first experiment, no effect on Cajanus cajan as far as three measured parameters: when the dose of sulfur was beyond 100mg/stem. Between the doses of 100 mg/stem and 200mg /stem, nodule volume increased from 125% to 133% and decreased beyond this dose (200 mg/stem). Results showed that the sulfur application in200, 400 and 800 mg/stem dose stimulated an increase of wet biomass of *Cajanus cajan*. Effect of 200mg/stem, increase of about 14% comparing to the control. And the acetylene reduction activity reduced as long as the dose of sulfur increased.

And those leguminous species did not react at the same ways face to sulfur application. The potassium sulfate used as the source of sulfur is a chemical fertilizer commonly used in agricultural fertilization. It contains 50 % of K_2O and 18% of sulfur. I n our trial, apart from sulfur, we brought a great quantity of potassium to the used species. We may affirm that the obtained results were influenced by potassium application.

The Manganese treatments did not cause a decrease of wet biomass weight, volume of nodule and acetylene reduction activity. The two first parameters tended to increase when doses were a bit elevated. The tolerance of Leucaena leucocephala to Manganese toxicity was stated by Loue the wet biomass weight, nodule volume and acetylene reduction activity. For Leucaena leucocephala, the sulfur input caused an amelioration of acetylene reduction activity with increased significantly at the dose of 200 mg/stem with 244% of increased comparing to the control

The sulfur supplied to the soil enhanced an increase of nodule volume of *Leucaena leucocephala*. At the doses of 100 and 200 mg/stem respectively, the nodule volume increased from 125 to 133% comparing to the control. And was observed the decrease of acetylene reduction (1987).This might be the cause of no response of those species to manganese in terms of nodulation, wet biomass weight and acetylene reduction activity. According to White (1970), manganese toxicity symptoms may be observed at the content of about 1000 ppm to Leucaena. Manganese toxicity is highly detected in soils with a very low pH and rich in soluble manganese.

Results obtained from these this study showed that aluminum was not in excess in sandy loam soil of Nyagisozi sector and that it was not the origin of a small amount of fixed nitrogen to *Leucaena leucocephala* and *Cajanus cajan*, although a small increase statistically significant was observed

CONCLUSION

Results from this investigation show that varied mineral nutrients such as P, Mo, S, Al and Mn applied to the soil could influence the processes of biological nitrogen fixation of Cajanus cajan and Leucaena Leucocenala. The obtained results indicated that the Cajanus cajan shows a good potential to be used as a grain legume in rotation systems under acid soil at Nyagizosi sector of Nyaruguru district, southern Rwanda, and could be recommended to promote the sustainability of agrosystems in this zone due to its high efficiency for N2-fixation which contributes to a positive N-balance.

REFERENCE

Allen, O.N and Allen, E.K., 1981. Leguminosae: A source book of characteristics uses and nodulation. Madison, WI: University of Wisconsin. Press, pp: 812.

Anderson, A.J., 1956. Molybdenum deficiencies in legumes in Austalia. Soil Science 81: 173 – 192.

BIELESKI R. L., 1973. Phosphate pools and phosphate availability. Annual review, Plant physiol.24: 225 – 252.

Burns R C, Hardy R W F. Nitrogen fixation in bacteria and higher plants. New York, N.Y: Springer-Verlag; 1975

Dommergues,Y et Mangenot,F.,1970. *Ecologie microbienne du sol*. Ed.: Masson et Cie, Paris

Chenn, P., 1999. Microorganism in agriculture. Biol.Sci. review. May, Vol.11, pp 2-4

FAO., 1985. *Les inocula des légumineuses et leurs applications*. Rome.

FRANCO,A.A.,1978.*Micronutrientsrequirements of legume-Rhizobium symbiosis. Limitations and Potentials for biological nitrogen fixation in the tropics*. Ed.:

J .Döbereiner,H. R. Burris, A. Kuo, S. and Lotse, E.G., 1972. Kenetics of phosphate adsorption by calcium carbonate and ca-kaolinite. Soil Sci. Am. Proc. 36: 725 – 729

LOUE, A., 1987.Les *oligo-éléments en Agriculture*.Nouvelleédition revue et corrigée, Paris.

Mendel, R.R, Haensch, R., 2002. Molybdoenzymes and Molybdenum cofactor in plant. Journal of experimental botany 53: 1689 – 1698.

Mafongoya, P.C, Bationo, A., Kihara and Waswa, B.S., 2006. Appropriate technologies to replenish soil fertility in southern Africa. Natr. Cycl. Agroecosyst.76: 137 - 151

MULDER, E.G. ETW.L.vVANVEEN, 1960. *The influence of carbon dioxide on symbiotic nitrogen fixation*. Pl. Soil, vol.13, pp.265-278.

Mutwewingabo, B., Rutunga,V., 1988. Etudes des sols des stations d'essais du projet d'intensification de l'agriculture de Gikongoro(PIA). Serie №1, Minagri-PIA

Mulder, E.G., and E.L.Vanveen., 1960. *The influence of carbon dioxide on symbiotic nitrogen fixation*. Pl. Soil, vol.13, pp.265-278

Martins, L.M.V, Xavier,G.R,Rangel, F.W., 2003. Contribution of biological nitrogen fixation to cowpea: a stragy for improving grains yield in semi arid regions of Brasil. Biol. Fertile. Soils. 38 :333 – 339

Mahon,J.D., 1977. Respiration and the energy requirement for nitrogen fixation in modulated pea roots. Plant physiol. 60: 817 – 821.

Pollock,V.V., Conover,R.C., Johnson, M.K., and Barba,M.J.,2002. Bacterial expressions of the molybdenum domain of assimilate nitrate reductase. Arch. Biochem. Biophys., 2, 237 - 248

Postgate, J., 1998. Nitrogen Fixation. 3rd Edition. Cambridge University Press, Cambridge UK.

RAGOTHAMA, K.G. (1999). Phosphate acquisition. Annual rev. Plant physiol., plant Mol. biol. 50: 665 – 693.

Paul E A. Towards the year 2000: directions for future nitrogen research. In: Wilson J R, editor. Advances in nitrogen cycling in agricultural ecosystems. Wallingford, United Kingdom: CAB International; 1988. pp. 417–425

Rondon, M., and Lehmann, 2009. Biological nitrogen fixation by Common beans (Phaseolus Vulgaris L.). Biology and fertility in soil. pp: 67

Tisdale, S.L., Nelson, W.L., Beaton, J.D., and Havlin, J.L., 1993. Soil fertility and fertilizers. 5th Edition. Pp.176-229. Macmillan Published Company, New York.

Zahran, H.H., 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63: 968–989