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Research Article

Agronomical Response of Three Fodder Legumes with the Application of Rhizobacteria

Ricardo Luna Murillo[1]*, Ringo John López Bustamante[1], Raúl Trávez Travez[1], Ana Espinoza Coronel[2], Guadalupe Murillo Campuzano[3], Luis Bravo Minda[4], Dolores Troy[4], Juan José Palomo[5], Byron Pallo Toaquiza[5], Kimberly Cedeno Magaly Magaly Lopez Cedeno[6] and Edilberto Chacón Marcheco[1]

[1]*Researcher – Professor, Technical University of Cotopaxi - UTC, Office La Maná, La Maná, Ecuador*

[2]*IndependentConsultantConsultAR, Quevedo, Ecuador*

[3]*Researcher – Professor, State Technical University of Quevedo - UTEQ, Quevedo, Ecuador*

[4]*Researcher – Professor, Autonomous Regional University of the Andes – UNIANDES, Quevedo, Ecuador*

[5]*Students, Academic Unit of Agricultural Sciences and Natural Resources, Career - Agricultural Engineering, Technical University of Cotopaxi - UTC, Office La Maná,, La Maná, Ecuador*

[6]*Student, Career –Chemical Engineering, University of Guayaquil - UG, Guayaquil, Ecuador*

***Corresponding Author:** Ricardo Luna Murillo, Researcher – Professor, Technical University of Cotopaxi - UTC, Office La Maná, La Maná, Ecuador.

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Abstract

The rapidly growing legumes may be especially useful as cover crops to restore soil fertility. The objective of this research was to evaluate the agronomic response of the legume kudzu (*Pueraria phaseoloides*), centrosema (*Centrosema acutifolium*), mucuna (*Mucunapruriens*) with application of rhizobacteria (*Azotobacter chroocucum*). Using two substrates for planting (cover and ground), with and without use of rhizobacteria as vegetable growth promoters. The data collection was performed at 30, 45, 60 and 75 days. The variable plant height; forage biomass; root length; weight; number of nodules; colonization rate; endophyte density; total populations and functional groups were evaluated. The design was random block. For the comparison between means the multiple range test of Tukey was used. The analyses of the data were carried out using the INFOSTAT program. For both experiments the highest plants was the centrosema. The highest values of forage biomass, root length and weight were registered in the mucuna. The inoculant with greater effect was the *Azotobacter chroococum*. The increased presence of total aerobic, fungi and yeasts are presented in the legume kudzu, with or without the application of inoculants.

Keywords: *Legumes; Rhizobacteria; Inoculants; agronomic performance; nodules; chemical composition*

Introduction

In Ecuador, the removal of forests for the implementation of monocultures for export and agribusiness has meant a drastic reduction in biodiversity and consequently soil fertility depletion. Only in the last 40 years, 50% of forests have been destroyed; on the Sierra, the forest practically no longer exists, and in the Costa only there is 10% of primary forest [1].

Some crops such as legumes are the key of the sustainable development of agriculture, due to its ability to fix atmospheric nitrogen in symbiosis with *Rhizobium* allowing inter alia a considerable saving of nitrogen fertilizers to increase soil fertility and develop sustainable agro-ecosystems with minimal use of agrochemicals [2,3].

Legume species of fast-growing such as mucuna and kudzu may be especially useful as cover crops to control erosion, weed suppression and restoration of soil fertility [4].

The ability of plants like mucuma have to fix atmospheric nitrogen is due to it establish symbiotic relationship with soil microorganisms (rhizobia), becoming an efficient source of nitrogen; moreover, they accumulate more than 10 tons of dry matter per hectare (tDMha[1]), and a source of protein and minerals which make them useful in animal feed[5].

Due the above, this paper is to evaluate the agronomic response of the legume kudzu (*Pueraria phaseoloides*), centrosema (*Centrosema acutifolium*), and mucuna (*Mucunapruriens*) with application of rhizobacteria (*Azotobacter chroococum*).

Materials and Methods

The research was conducted at the Experimental Center "La Playita", belonging to the Technical University of Cotopaxi, Extension La Maná. The agro-meteorological conditions were: annual average temperature 23°C, relative humidity 82, 00%, half annual rainfall 1000-2000 mm, heliofania [hours sun per year] 757,00.

The previous soil analysis at baseline showed pH 5,8; nitrogen 18 ppm, phosphorus 8 ppm; potassium 0,6 meq/100 ml; calcium 7meq/100 ml; magnesium 1,10m eq/100 ml; sulfur 14 ppm; 1,70 ppm zinc, copper 6,90 ppm; iron 108 ppm, manganese 4,00 ppm, boron 0,24 ppm, organic matter 4,2% with a 49% of sand texture, silt 43% and clay 8%.

Three kudzu legumes (*Pueraria phaseoloides*), centrosema (*Centrosema acutifolium*) and mucuna (*Mucuna pruriens*) were used.

Two consecutive experiments were performed, in a first phase, the behavior of the species studied in two types of substrates (bag and soil), with three replicates and two plants as experimental unit was evaluated. A second experiment was to apply two bacterial inoculants (*Azotobacter chroococum* and *Pseudomonas fluorescens*) at the start of planting, 15 and 30 days after planting in both cases bagas in soil, with three replicates and two plants as experimental unit. The investigation lasted 120 days of field work, experimental work 75 days and 45 days of establishment of the trial. Periodically was performed control of undesirable plants.

Once established the legume varieties was proceeded to take the experimental data at 30, 45, 60 and 75 days, separating the roots of forage biomass. The variables under study were: plant height (cm), forage biomass (BF), root length (cm), weight (g), number of nodules, percentage of colonization, endophyte density, total populations and functional groups. Once weighed the plants parts, proceeded to lead them to the laboratories (Laboratory of Microbiology Laboratory UTEQ and ANCUPA-CIPAL) to determine the agronomic indicators and the amount of bacteria and fungi existing in each legume.

A Completely Randomized Design (CRD) was used. To determine differences between average, the multiple range test of Tukey, ($p < 0, 05$) was used. The INFOSTAT statistical package was used.

Results and Discussion

Experiment-1

The variable plant height, showed higher values for centrosema legume both bag and soil with 35,93 and 34,90 cm at 30 days; 52,70 and 52,04 at 45 days; 64,84 and 65,97 cm at 60 days and 83,28 and 81,80 cm at 75 days (Figure 1).

As forage biomass (Figure 2)shows that the legume mucuma indicates the highest value in interaction with 177,57and 180,67 g/m^2, however centrosema described the max in this variable studied with 206.67 g/m^2, higher than 14,23 g reported in the kudzu legume [6].

The greatest value in length and root weight appears in mucuna with 54, 11 cm and 12,65 g in bag(Figure 2). While the number of nodes is greater in the kudzu legume with 23, 93 and 23, 40, also exceeding the nodulation values reported in the roots of legumes in the southern area of Sancti Spiritus, Cuba with (2-8) nodes in *Centrosema* sp., *C.virginianum, C.molleandStylosanthesviscous* [7].

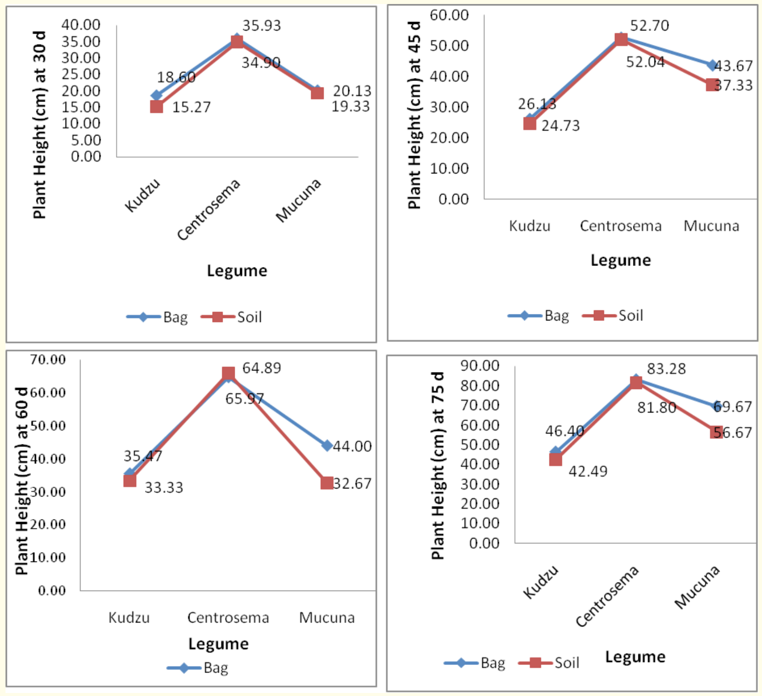

Figure 1: *Interaction of plant height at 30, 45, 60 and 75 days in the kudzu, mucuna and centrosema legumes.*

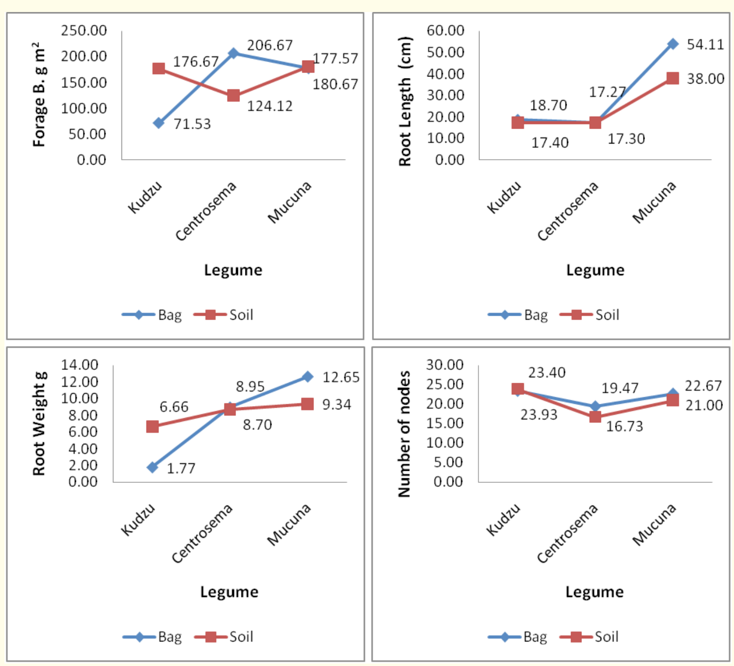

Figure 2: *Interaction of root length, weight and number of root nodules on kudzu, mucuna and centrosema legumes.*

The greatest presence of total aerobes occurred in the kudzu legume with 4,00 x 10^7 Colony Forming Units [CFU] at 30 days, 4,10 x 10^7 CFU at 45 days; 3,10 x 10^7 CFUat 60 days and 4,50 x 10^6 CFU at 75 days [Table 1], higher than indicated by other authors in this legume 1,0 x 10^6 CFU [8].

Legumes	Total Aerobes CFU/cm^3			
	30 days	45 days	60 days	75 days
Kudzu	4,00 x 10^7	4,10 x 10^7	3,10 x 10^7	4,50 x 10^6
Centrosema	5,10 x 10^6	3,80 x 10^6	2,00 x 10^6	1,60 x 10^6
Mucuna	3,70 x 10^6	1,00 x 10^7	4,67 x 10^6	2,90 x 10^6

Table 1: *Total aerobes population in Colony Forming Units.*

The kudzu legume shows in fungi and yeasts present 4, 20 x 10^7 Colony Forming Units (CFU) at 30 days and 3,50 x 10^6 CFU at 60 days; mucuna 1,90 x 10^7 CFU at 45 days and centrosema4,20 x 10^6 CFUat 75 days (Table 2), these results are better than those reported in research on kudzu and centrosema with 7,5 x 10^4 and 4,1 x 10^6 in fungi [6,8].

Legumes	Fungi and Yeasts CFU/cm^3			
	30 days	45 days	60 days	75 days
Kudzu	4,20 x 10^7	3,40 x 10^5	3,50 x 10^6	1,60 x 10^6
Centrosema	1,10 x 10^6	5,00 x 10^6	1,60 x 10^6	4,20 x 10^6
Mucuna	1,00 x 10^7	1,90 x 10^7	1,21 x 10^6	8,59 x 10^5

Table 2: *Fungi and Yeasts Population in Colony Forming Units.*

In the microbiological analysis of soil, mucuna legume indicates the highest values obtained in colonization 78, 05%; endophyte density with 5, 46%; viable spores obtained 92,00; and at the suggested morpho species, all of the legumes show *Glomus, Acaulospora* reddish brown (Table 3).

Legumes	Colonization (%)	Endophyte Density (47.5%)	Viable spores	suggested morphospecies	Color
Kudzu	58,97	1,23	75,00	Glomus/Acaulospora	reddish brown
Centrosema	31,91	0,32	76,00	Glomus/Acaulospora	reddish brown
Mucuna	78,05	5,46	92,00	Glomus/Acaulospora	reddish brown

Table 3: *Microbiological Analysis of soil.*

In the total populations of total aerobic dilutions, the kudzu legume expressed in bacteria with greater value 7, 80 x 10^6, higher than achieved in other studies [9], where for the mucuna 2, 04 x 10^6 is obtained. Meanwhile, mucuna was the legume with higher values in mushrooms with 5, 40 x 10^6 (Table 4).

Legumes	Total Populations CFU/gss			Functional Groups CFU/gss		
	Bacteria	Fungi	Actinomycetes	Phosphorus solub	Cellulolytic	N-fixing free living
Kudzu	7,80 x 10^6	3,50 x 10^6	8,10 x 10^3	0,00 x 10^0	1,70 x 10^5	5,40 x 10^3
Centrosema	2,20 x 10^6	3,00 x 10^6	8,00 x 10^4	4,90 x 10^3	1,90 x 10^4	1,30 x 10^4
Mucuna	4,70 x 10^6	5,40 x 10^6	1,80 x 10^4	4,50 x 10^2	9,90 x 10^3	1,30 x 10^4

Table 4: *Microbiological Analysis of soil in total populations and functional groups.*

Experiment-2

Is this experiment, plant height at 45, 60 and 75 days was higher in legume centrosema with values of 35,56; 48,84 and 60,80 cm. As for effect of inoculants at 45, 60 and 75 days in *Azotobacter chroococum* had more effect reaching 30, 04, 41,54 and 55,94 cm. The optimal substrate for plant growth was in bag where 28, 42 was obtained; 39, 47 and 53.72 cm in all ages studied (Table 5).

Factors	Plant height (cm)		
Legume	45 days	60 days	75 days
Kudzu	19,00 c	30,46 c	41,25 c
Centrosema	35,56 a	48,84 a	60,80 a
Mucuna	30,33 b	37,76 b	53,77 b
Inoculants			
Azotobacterchroococum	30,04 a	41,54 a	55,94 a
Pseudomonafluorecens	26,56 b	36,49 b	4793 b
Place			
Bag	28,42 a	39,47 a	53,72 a
Soil	28,18 a	38,57 a	50,16 b
CV (%)	11,09	12,09	6,25

Uneven letters in the same column differ Tukey Test (P < 0, 05).

Table 5: *Plant height (cm) in the use of rhizobacteria as plant growth promoters in three forage legumes.*

Mucuna legume gets the most highlighted length of root with 34,83 cm and the largest number of nodules found into kudzu with 29,85 [Table 6]. *Pseudomona fluorescens* is the inoculant that excels in long root and nodule number 32, 58 and 24, 07 cm. While the most suitable substrate for these legume species was the indicated soil 31, 44 cm and 24, 31 in their respective order. These results differ from other reports which 55,37cm long root in mucuna was obtained without the use of *rhizobacteria* [8].

The best legume in root weight and forage biomass was mucuna, expressed in 4,13g and 43, 33 g/m^2. In weight of root, the inoculant which has the highest effect was *Azotobacter chroococum* with 3.77g and for forage biomass was gotten by *Pseudomona fluorescens* with 27, 49 g/m^2.

The substrate varied by variable, weight of root in bag achieved 3, 45g forage biomass in soil describes 27, 22 g/m^2. Being still lower than that it was described by other authors [8], with values of 8,95g in root weight in mucuma.

Other authors [10] suggest that the activity of these bacteria and fungi is stimulated to a greater extent when the plants are inoculated together with several of them to apply them separately.

Uneven letters in the same column differ Tukey Test (P < 0, 05).

Pahwa [11] found in leucaena that nodulation; yields of biomass, dry matter and protein were significantly higher when inoculated with a specific strain of rhizobia and two strains of Azotobacter (ICM-2001 and S-3). Other authors report 14 nodules with Rhizobium to inoculate leucaena [12].

Mahmoud., *et al*. [13] refer stimulating effects on plant height and dry weight using *Azotobacter sp*., while Pati., *et al*. [14] reported increases in the length of roots and buds to apply A. chroococcum.

Factors	Root Length (cm)	Number of Nodes	Root Weight (g)	Forage Biomass (g/m²)
Kudzu	25,02 b	29,85 a	3,37 a	18,63 b
Centrosema	32,32 ab	27,09 a	2,1 a	18,00 b
Mucuna	34,83 a	11,75 b	4,13 a	43,33 a
Inoculants				
Azotobacterchroococum	28,87 a	21,73 b	3,77 a	25,81 a
Pseudomonafluorecens	32,58 a	24,07 a	3,04 a	27,49 a
Place				
Bag	30,01 a	21,48 b	3,45 a	26,08 a
Soil	31,44 a	24,31 a	3,36 a	27,22 a
CV (%)	30,88	13,94	46,87	62,75

Table 6: *Root length (cm), number of nodes, Root weight (g), Forage biomass(g/m²)in the use of rhizobacteria as plant growth promoters in three forage legumes.*

Legume mucuna reported the greatest value in fungi with 2,30 x 10^7 CFU/cm³ in relation to the inoculant as many fungi and yeasts is shown in *Pseudomonas fluorescens* with 1,64 x 10^7 in the ages of 45 and 75 days with 1,60 x 10^7 CFU/cm³. Mucuna has the highest value with 9,65 x 10^6 CFU/cm³ for bacteria, Azotobacter chroococum inoculant presented the highest average aerobic bacteria with 8,00 x 10^6 and at the age of 45 days with 7,84 x 10^6 (Table 7).

Factors	CFU/cm³	
	Bacteria	**Fungiy Yeasts**
Kudzu	6,65 x 10^6	9,96 x 10^6
Centrosema	496 x 10^6	1,46 x 10^7
Mucuna	9,65 x 10^6	2,30 x 10^7
Inoculant		
Azotobacterchroococum	8,00 x 10^6	1,52 x 10^7
Pseudomonafluorecens	6,18 x 10^6	1,64 x 10^7
Days		
45	7,84 x 10^6	1,60 x 10^7
60	7,20 x 10^6	1,55 x 10^7
75	6,22 x 10^6	1,60 x 10^7

Table 7: *: Bacteria, Fungiand Yeasts (CFU/cm³)in the use of rhizobacteria as plant growth promoters in three forage legumes.*

In chemical composition the highest level of protein was present in mucuna with 22,29% at 75 days without inoculant, kudzu had the lowest levels of protein, and however levels improved at the ages of 45 and 75 days with 21,70 and 20,15 % using the *Azotobacter chroococcum* inoculant (Table 8).

In studies with *Pseudomonas* 7 NSK (apparently a line of P. *aeruginosa*), an increase in the yield of barley, maize and wheat was observed [15].

Legume	Age	Protein (%)		
		without inoculants	*A.chroococum*	*P.fluorecens*
Kudzu	45	7,50	21,70	18,00
	75	6,25	20,15	15,90
Centrosema	45	10,62	15,67	20,45
	75	19,01	16,84	18,15
Mucuna	45	18,12	19,12	20,54
	75	22,29	17,90	18,30

Table 8: *Chemical Composition three forage legumes.*

Conclusions

The *Azotobacter* and *Pseudomona* elevate the yields and also perform stimulators effect on plants and roots growth. However was noted that the symbiosis between nitrogen-fixing microorganisms and legume species studied had an interesting effect on the behavior of variables evaluated, where the tallest plants occurs in centrosema, but the production of biomass, length root and root weight were higher in mucuna and secondly the largest number of nodules was determined in kudzu.

A higher increase in protein levels was observed in the legume kudzu more rhizobacteria combination between (20-21%) compared to plants without rhizobacteria (6-7%).

The results of this investigation show that the biologic fixation of nitrogen is of great importance, justifying the use of biofertilizers for insure the sustainability of the agricultural systems and to give security to the environmental conditions; since they eliminate the damage that produce the continuous applications of mineral fertilizers.

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