

# SEEDS INOCULATION AND NITROGEN FERTILIZATION FOR COWPEA PRODUCTION ON LATOSOL IN THE WESTERN AMAZON

## PRODUTIVIDADE DE FEIJÃO-CAUPI COM INOCULANTE E FONTE MINERAL NITROGENADA EM LATOSSOLO DA AMAZÔNIA OCIDENTAL

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**ABSTRACT:** The cowpea is an important food crop in the North and Northeast regions of Brazil, where the beans are consumed either green or ripe. Yet, considering its socio-economic importance and its tropical origin, cowpea yields are low in those regions, due to inadequate cultivation practices and incorrect soil management. Therefore, the objective of this study was to study the development of cowpea crop inoculated and fertilized with two different nitrogen (N) sources. The experiment was conducted in the municipality of Rorainópolis (RR). The experimental design was randomized blocks with four treatments and five replications. The plant material was ‘BRS Guariba’ cowpea cultivar, and the treatments were: (i) control (no nitrogen fertilization), (ii) seeds inoculated with *Bradyrhizobium elkanii*, (iii) urea as N source (60 kg N ha<sup>-1</sup>), and (iv) ammonium sulphate as N source (60 kg N ha<sup>-1</sup>). The following crop traits were evaluated: foliar macronutrients (N, P, K, Ca, Mg and S), green and dry weight of shoots, dry beans per plant, pod yield and bean yield. Urea and ammonium sulphate promoted high accumulation of nutrients in leaves. Ammonium sulphate also stood out regarding productivity traits, thus proving to be a viable N source for cowpea in the Amazon region. The nodulation with *Bradyrhizobium elkanii* wasn't efficient to replace the fertilization with nitrogen fertilizers for cowpea BRS Guariba.

**KEYWORDS:** Fertilization. *Bradyrhizobium elkanii*. *Vigna unguiculata*.

### INTRODUCTION

The cowpea (*Vigna unguiculata*) plays a major social and economic role for the population in the North and Northeast regions of Brazil (FREIRE FILHO et al., 2005), where it is the main low-cost protein source and offers various employment opportunities (CHAGAS JUNIOR et al., 2010).

According to the fifth Conab (2016) survey, an estimated cowpea yield in Brazil for the 2015/16 production season was 570 kg ha<sup>-1</sup>, and total production 224,900 t. In the North, the estimated yield for the same season was 655 kg ha<sup>-1</sup>, and total production 2,500 t.

The cowpea is considered a tropical crop, adaptable to soil and weather conditions in the Amazon region, but it does not produce good yields there. Among various factors that contribute to this state, the management of soil fertility, particularly low supply of nitrogen (BRITO et al., 2011), and also the use of low-yielding traditional crops play a major role.

In the Amazon region, one major obstacle for agricultural production is low availability of nitrogen in the soil, which is further aggravated by high mineralization rates of organic matter due to

high humidity and temperature (NOVAIS, 2007). Low fertility of soils may lead to incorrect and indiscriminate use of chemical fertilizers by farmers (VALE JÚNIOR et al., 2011). Alternatives, such as sustainable soil management with no-till, minimum tillage, split doses of nitrogen fertilizers, different nitrogen inoculants, and plant coverage may be adopted to diminish these problems (TAGLIAFERRE et al., 2013).

Therefore, due to weather conditions in the Amazon region and physical and chemical characteristics of soils, which are mostly sandy with low fertility, adequate soil fertility management practices—nitrogen in particular—for the cowpea crop are necessary.

Considering the importance of nitrogen fertilization, as well as the environmental and socio-economic conditions in the region, the objective of this study was to evaluate two fertilizers N sources and *Bradyrhizobium elkanii* as a seed inoculant for the production of cowpea BRS Guariba.

### MATERIAL AND METHODS

The experiment was carried on the rural property “Alvorada” (01°14'56"N and 60°9'02"W),

located in Rorainópolis, RR, in the southern region of Roraima at an altitude of 80 m. The average annual rainfall in the local is 2,500 mm and the average annual temperature 25°C. According to the Köppen-Geige climatic classification, the weather is classified as Af— equatorial, hot and humid. Chemical and physical analysis of soil from layer 0–20 cm revealed the following characteristics: pH (H<sub>2</sub>O) 5.15; P (mg dm<sup>-3</sup>) 1.3; K (mg dm<sup>-3</sup>) 54; Ca (cmol<sub>c</sub> dm<sup>-3</sup>) 1.7; Mg (cmol<sub>c</sub> dm<sup>-3</sup>) 0.6; Al (cmol<sub>c</sub> dm<sup>-3</sup>) 0.02; H+Al (cmol<sub>c</sub> dm<sup>-3</sup>) 2.54; m (%) 1.3; V (%) 49.6; MO (dag kg<sup>-1</sup>) 19.6; sand (g kg<sup>-1</sup>) 520; silt (g kg<sup>-1</sup>) 303; and clay (g kg<sup>-1</sup>) 177. The soil was classified as dystrophic yellow latosol.

The experimental design was randomized blocks with four treatments and five replications. The treatments were: control (no nitrogen fertilization), inoculant (*Bradyrhizobium elkanii*), urea (60 kg ha<sup>-1</sup> N), and ammonium sulphate (60 kg ha<sup>-1</sup> N). The plots consisted of four rows, each 5-m-long, 2-m-wide, and 0.5 m apart. The total area of each plot was 10 m<sup>2</sup>, on which 4.0 m<sup>2</sup> was the experimental area with two central lines, disregarding 0.5 m from both ends.

Soil, which was left fallow after corn, was prepared before planting by disc harrowing. Soil fertilization (350 kg ha<sup>-1</sup> superphosphate and 66.66 kg ha<sup>-1</sup> potassium chloride) was based on soil analysis, second Embrapa Roraima (2009). Sowing was carried out on September 13, 2012 using a 4-row planter and a conventional system with row spacing of 0.5 m and 12 seeds per meter. Rows were thinned out down to 8 plants per meter, or 160,000 plants ha<sup>-1</sup>, 10 days after emergence (days after plant emergence).

The plant material was cowpea cultivar BRS Guariba, which is characterized by semi-erect posture, white beans, black hilum, and good adaptability to the conditions in the North regions (GONÇALVES et al., 2009). Cowpea seeds were inoculated with *Bradyrhizobium elkanii* strain BR 3262 at concentration 10<sup>8</sup> cells g<sup>-1</sup>. Inoculant dosage was 500 g per 50 kg of seeds. Inoculation was carried out after wetting the seeds with 6 ml kg<sup>-1</sup> of sugar solution (10% pv<sup>-1</sup>) (HUNGRIA et al., 2001). Nitrogen sources used in this experiment were urea (46% N) and ammonium sulphate (20% N + 22% S). Nitrogen sources used in this experiment were urea (46% N) and ammonium sulphate (20% N + 22% S). Nitrogen dose was split into two equal applications, one 12 days after plant emergence and the other 25 days after plant emergence. To

compensate for the lack of S in urea, elemental S was applied in 2 equal applications.

The volume of accumulated rainfall between planting and harvest (65-day cycle) was 440 mm. Weed control was done by spraying 50 g of fenoxaprop-p-ethyl + 50 g of clethodim per hectare 15 days after plant emergence, and cucurbit beetle and aphids control by spraying 30 g ha<sup>-1</sup> of cypermethrin. Diseases control was not necessary due to low levels of severity.

Macronutrient status was measured in leaves collected in two central lines on each plot at the beginning of flowering 35 days after plant emergence. The third trifoliate leaf of the apical tuft was collected from 30 plants on all plots, according to recommendations by Ambrosano et al. (1997) and methodology according to Malavolta et al. (1997). Also, green pods per plant were counted and collected 45, 49 and 53 days after plant emergence. The first time, the pods were collected from 10 plants chosen at random per plot, later the pods were collected from the same plants. Dry pods were harvested from ten plants chosen at random from two central rows to obtain the number of dry beans per plant 60 days after plant emergence. Beans from 100 pods were also counted. One hundred beans with 20% humidity were weighed. The yield of dry beans was expressed in kg ha<sup>-1</sup>. Ten plants were collected at random during flowering to evaluate dry matter production of shoots (kg ha<sup>-1</sup>).

The results were submitted to analysis of variance by F test at 5% probability. Later, they were compared by Tukey test at 5% significance using SISVAR 5.3 software (FERREIRA, 2011).

## RESULTS AND DISCUSSION

Accumulation of macronutrients varied depending on N source (Table 1). Ammonium sulphate caused the highest N accumulation. Both urea and ammonium sulphate promoted high accumulation of K relative to the control, but didn't differ from *B.elkanii*. Regarding Mg, all treatments promoted higher accumulation than the control. Also, urea and ammonium sulphate promoted high S accumulation relative to the inoculant, but didn't differ from the control. For P and Ca, there wasn't difference among the treatments.

**Table 1.** Mean values of nutrient accumulation in cowpea for different N sources.

Nutrient source	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sulphur
Urea	40,78 b	2,24 a	18,92 a	10,90 a	2,12 a	22,58 a
Ammonium sulphate	44,38 a	2,52 a	20,84 a	12,02 a	2,26 a	22,64 a
<i>Bradyrhizobium elkanii</i>	37,46 c	2,60 a	18,70 ab	10,86 a	2,18 a	14,70 b
Control	37,66 bc	2,60 a	14,76 b	11,44 a	1,82 b	19,34 ab
CV (%)	4,44	8,64	12,34	9,75	7,88	14,60
DMS	3,21	0,39	4,09	1,99	0,30	5,24

Means followed different letters in the column differ by Tukey test at 5% probability.

The availability of nutrients for plant uptake depends on various physical, chemical and biological processes. Several mechanisms in the soil and in the plant may influence nutritional composition of the plant, such as ionic interactions, which can be classified as inhibition when the presence of one ion inhibits or decreases the absorption of another ion, or synergism when one ion increases the absorption of another ion.

Data in table 1 show that N status was higher in the treatment with ammonium sulphate. As this fertilizes contains S, this increase demonstrates the interaction between N and S. Nitrogen uptake may be affected by the presence of other nutrients, including S. According to Malavolta and Moraes (2007), N and S demonstrate synergism, thus affecting the quality of the final product. In plant physiology, the union of the metabolic pathways of assimilation of N and S is represented by the incorporation of sulfide in the O-acetylserine by the OAS-thiol lyase enzyme in the formation of cysteine (CRAWFORD et al., 2000). A nutritional imbalance of these nutrients affects protein synthesis.

Urea was amended with elemental sulfur (S<sup>0</sup>) to compensate for S present in ammonium sulphate. However, more N was found in the treatment with ammonium sulphate. This is probably due to the fact that elemental sulfur is absorbed by plants only after its oxidation to sulphate by means of reactions catalyzed mainly by microorganisms (HOROWITZ; MEURER, 2006). Sulfur is absorbed by plants in the form of SO<sub>4</sub><sup>2-</sup>; therefore, the uptake of SO<sub>4</sub><sup>2-</sup> by plants from ammonium sulphate might have been higher than elemental sulfur.

In cowpea, K is extracted and exported in large quantities. Potassium has many functions in the plant, mainly the activation of several enzymatic

systems, many of which participate in the processes of photosynthesis and respiration. Deficient plants show slow growth, poorly developed roots, weak and very flexible stems, susceptibility to disease, and poor formation of seeds and fruits which are usually smaller and rather faintly colored (SOUZA et al., 2012).

Viana and Kiel (2010), evaluating N and K in wheat, reported that high N doses associated with K increased dry matter production of shoots. Also, Panaullah et al. (2006), evaluating K uptake in rice and wheat in succession, found that N fertilization increased K uptake in wheat. This demonstrates that there is an interaction between N and K. In this study urea and ammonium sulphate increased K content relative to the control, but didn't differ from the treatment with seed inoculation.

Variations were observed regarding plant production traits (Table 2). Ammonium sulphate promoted high production of fresh weight of shoots (FWS) relative to the inoculant, but didn't differ from the other treatments. Ammonium sulphate also increased dry weight of shoots (DWS), more than urea and the inoculant; however, the results didn't differ from the control treatment. No difference among the treatments was observed for the number of green pods per plant (GPP) and dry beans per plant (DBP). Pod yield (PY) in treatments with urea and ammonium sulphate was higher than with the inoculant, but didn't differ from the control. And for the bean yield (BY), ammonium sulphate promoted higher production than the inoculant and the control, but didn't differ from urea.

**Table 2.** Yield components evaluated in cowpea crop for different treatments with N fertilization

Nutrient source	FWS	DWS	GPP	DBP	PY	BY
	-----g plant <sup>-1</sup> -----		--Unit. plant <sup>-1</sup> ---		-----kg ha <sup>-1</sup> -----	
Urea	46,9 ab	6,88 b	3,92 a	48,0 a	6624,0 a	872,6 ab
Ammonium sulphate	65,9 a	9,67 a	4,46 a	60,0 a	6300,0 a	1064,0 a
<i>Bradyrhizobium elkanii</i>	33,6 b	4,93 b	3,72 a	40,0 a	4920,0 b	660,0 b
Control	45,9 ab	6,73 ab	3,92 a	52,0 a	5408,0 ab	650,0 b
CV (%)	29,12	29,14	14,37	29,83	12,80	20,92
DMS	25,34	3,72	1,04	26,99	1346,4	307,42

FWS = fresh weight of shoots; DWS = dry weight of shoots; GPP = number of green pods per plant; DBP = number of dry beans per plant; PY = pod yield; BY = bean yield. Means followed different letters in the column differ by Tukey test at 5% probability.

These results confirm that ammonium sulphate is an ideal N source for cowpea in that region, and can replace urea. Nitrogen fertilization of this crop in soil should also be maintained to ensure high yield of beans and pods. Reduced amount of N (maximum 20 kg ha<sup>-1</sup>) promoted nodulation and crop yield, according to Xavier et al., 2008.

Cowpea has the ability to establish efficient symbiotic relationship with rhizobia. However, some factors, such as competition with other microorganisms and inhibition by chemicals present in soil, may undermine the effectiveness of inoculants in regions different from their origin (NEVES; RUMJANEK, 1997). Soil pH is one of the main limiting factors for nodulation and nitrogen fixation (HUNGARY AND VARGAS, 2000; RAZA et al., 2001). Some species may tolerate acidity better than others, and this tolerance may vary between strains of the same species (HUNGARY et al., 1997). Bacterial fixation grow in an ideal pH range between 6.0 and 7.0, and few grow well at pH below 5.0 (RODRIGUES et al., 2006; Ali et al., 2009). To maximize the contribution of biological nitrogen fixation (BNF) to common bean in acid soils, it is necessary that the strains used in the inoculants be adapted to this condition, competitive and efficient in the infection process. In general, treatment with *B. elkanii* inoculant did not yield satisfactory results. One of the possible explanations may be low adaptability of the strain used in the inoculation to the environmental conditions of the experiment site, such as the low pH value in water (5.15), which may have reduce *B. elkanii* inoculation and fixation.

In general, *B. elkanii* didn't produce satisfactory results. One possible explanation may be low adaptability of the strain to the environmental conditions of the experimental site. The *B. elkanii* BR 3262 is one of the isolates which

have potential for biological nitrogen fixation (BNF). It comes from the Atlantic Forest region, demonstrates excellent adaptability to the region of Piauí Savannah, and interacts very well with BRS Guariba (GUALTER et al., 2007; ZILLI et al., 2006).

BRS Guariba did nodulate; however, the effect wasn't any better than for ammonium sulphate. Therefore, if the goal is to replace chemical fertilizers, a possible solution would be to establish appropriate inoculum density for the region (SILVA JÚNIOR et al., 2014). Another alternative would be to study the behavior of different cowpea inoculated with different strains of nitrogen fixing bacteria in the Amazon region. Similar work for a semi-arid region was carried out by Marinho et al. (2014), in which several inoculated cultivars showed bean yield similar to plants fertilized with urea.

The table 3 presents arrays of simple linear correlations between leaf macronutrient levels, dry matter, and yield components of cowpea. The following correlations were significant and positive: FWS x DWS ( $r = 0.99^{**}$ ), FWS x GPP ( $r = 0.94^{*}$ ), DWS x GPP ( $r = 0.94^{*}$ ) for urea; FWS x DWS ( $r = 0.99^{**}$ ) for ammonium sulphate; FWS x DWS ( $r = 0.99^{**}$ ) Mg x S ( $r = 0.88^{*}$ ) for *B. elkanii*; and FWS x DWS ( $r = 0.99^{**}$ ) for the control. The correlation between FWS and DWS becomes evident where in dry matter increases with green matter (RODRIGUES et al., 2012). The positive correlation between Mg x S may be associated with higher levels of aminoacid (cystine, cysteine and methionine), favoring production of chlorophyll, in which Mg is present (NEVES et al., 2008).

However, the following correlations were negative: P x Mg ( $r = -0.90^{*}$ ), K x DBP ( $r = -0.92^{*}$ ), Ca x Mg ( $r = -0.95^{*}$ ), S x BY ( $r = -0.96^{**}$ ) for urea; Ca x S ( $r = -0.92^{*}$ ), S x PY ( $r = -0.90^{*}$ ) for ammonium sulphate; N x FWS ( $r = -0.98^{**}$ ), N x

DWS ( $r = -0.98^{**}$ ), S x DBP ( $r = -0.91^*$ ) for *B. elkanii*; and N x PY ( $r = -0.92^*$ ), K x Ca ( $r = -0.90^*$ ), Ca x FWS ( $r = -0.94^*$ ), Ca x DWS ( $r = -0.94^*$ ) for the control. Magnesium uptake may suffer competition with others cations due to low affinity with binding sites on the plasma membrane (NETO et al., 2014). Pegoraro et al. (2014),

studying uptake of nutrients by common bean 36 days after plant emergence, observed high translocation of nutrients from leaves to beans in the following order: P > N > Mg > S > K > Ca. Thus, reduction of P, K, Mg and S may be related to translocation to flowers and fruits when entering the reproductive stage 35 days after plant emergence.

**Table 3.** Simple linear correlation between nitrogen, phosphorus, potassium, calcium, magnesium and foliar sulfur, fresh weight of shoots (FWS), dry weight of shoots (DWS), green pods per plant (GPP), dry beans per plant (DBP); pod yield (PY); and bean yield (BY) for each treatment, Rorainópolis - RR, 2013.

Variables	Ca	Mg	S	FWS	DWS	GPP	DBP	PY	BY
Urea									
P	0,78	-0,90*	-0,21	-0,44	-0,44	-0,14	0,20	-0,01	0,30
K	-0,23	0,50	-0,64	-0,40	-0,40	-0,67	-0,92*	0,35	0,50
Ca	1	-0,95*	-0,44	-0,33	-0,33	-0,17	-0,09	0,51	0,41
S	-	-	1	0,68	0,68	0,73	0,79	-0,61	-0,96**
FWS	-	-	-	1	0,99**	0,94*	0,68	0,08	-0,62
DWS	-	-	-	-	1	0,94*	0,68	0,08	-0,62
Ammonium sulphate									
Ca	1	-0,44	-0,92*	0,11	0,11	0,38	0,38	0,68	0,36
S	-	0,41	1	0,01	0,01	-0,51	-0,51	-0,90*	-0,27
FWS	-	-0,01	-	1	0,99**	0,40	0,41	0,01	0,54
Inoculant ( <i>Bradyrhizobium elkanii</i> )									
N	0,41	0,48	0,71	-0,98**	-0,98**	-0,44	-0,80	-0,50	-0,61
Mg	0,86	1	0,88*	-0,61	-0,61	0,10	-0,63	-0,30	-0,31
S	0,73	-	1	-0,81	-0,81	-0,29	-0,91*	-0,67	-0,56
FWS	-0,50	-	-	1	0,99**	0,37	0,85	0,53	0,64
Control									
N	0,11	-0,58	-0,02	0,01	0,01	0,59	0,80	-0,92*	0,23
K	-0,90*	0,73	0,47	0,78	0,78	0,64	-0,21	0,27	0,08
Ca	1	-0,44	-0,05	-0,94*	-0,94*	-0,45	-0,07	-0,17	-0,30
FWS	-	0,23	-0,14	1	0,99**	0,42	0,19	0,13	0,56

\* significant at 5% probability by T test; \*\* significant at 1% probability by T test

## CONCLUSIONS

Urea and ammonium sulphate promote accumulation of macronutrients in leaves and the development of cowpea.

Ammonium sulphate increases yield of beans and pods, and it is a viable nitrogen and sulfur source for cowpea production in the Amazon region.

The nodulation with *Bradyrhizobium elkanii* wasn't efficient to replace the fertilization with nitrogen fertilizers for cowpea BRS Guariba.

**RESUMO:** O feijão-caupi é uma das principais alternativas alimentares para as populações das regiões norte e nordeste do Brasil, sendo consumido na forma de grãos verdes ou maduros. Apesar da importância social e da origem tropical, a espécie apresenta baixa produtividade na região amazônica, incluindo o estado de Roraima, devido à baixa qualidade agrônômica e manejo incorreto do solo. O objetivo do trabalho foi caracterizar o desenvolvimento da cultura do feijão-de-corda com duas diferentes fontes de nitrogênio (N) tratadas com inoculante. O experimento foi realizado no município de Rorainópolis (RR). O delineamento foi em blocos casualizados, com quatro tratamentos e cinco repetições. Foi utilizada a cultivar de feijão-caupi 'BRS Guariba'. Os tratamentos foram: testemunha (ausência de adubação nitrogenada), inoculante *Bradyrhizobium elkanii*, 60 kg ha<sup>-1</sup> de N provenientes da uréia e 60 kg ha<sup>-1</sup> de N da fonte sulfato de amônio. Foram avaliados os teores foliares de macronutrientes (N, P, K, Ca, Mg e S), peso da massa verde e seca da parte aérea, número de grãos secos por planta, produção de vagens e produção de grãos. Em relação ao acúmulo de nutrientes a uréia e sulfato de amônio foram as fontes que apresentaram desempenho superior. Em relação as

características produtivas, o sulfato de amônio foi o que mais se destacou, sendo uma fonte viável de suprimento de N para o feijão-caupi na região amazônica. A nodulação com *Bradyrhizobium elkanii* não foi eficiente para substituir a adubação com fertilizantes nitrogenados para o feijão-caupi BRS Guariba.

**PALAVRAS-CHAVE:** Adubação. *Bradyrhizobium elkanii*. *Vigna unguiculata*.

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