

EFFECT OF PHOSPHATE-SOLUBILIZING MICROORGANISMS AND VIVIANITE POWDER ON THE GROWTH AND SEED YIELD OF VIGNA UNGUICULATA (L.) WALP.

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Abstract. This study aimed to evaluate the growth and production parameters of cowpea (*Vigna unguiculata* (L.)) through Phosphate Solubilizing Microorganisms (PSM) associated with vivianite powder used as Rock Phosphate (RP). A randomized complete block design with Sixty (16) treatments (control (no treated plants): C; RKB5 isolate: R0; SSJ7: S0; BNBH2: B0; control with 10 g of vivianite: C10; isolate RKB5 + 10 g vivianite: R10; SSJ7 isolate + 10 g vivianite: S10; BNBH2 isolate + 10 g vivianite : B10; control with 20 g vivianite: C20; RKB5 isolate + 20 g vivianite: R20; SSJ7 isolate + 20 g vivianite: S20; BNBH2 isolate + 20 g vivianite: B20; control with 30 g vivianite: C30; RKB5 isolate + 30 g vivianite: R30; SSJ7 isolate + 30 g vivianite: S30 and BNBH2 isolate + 30 g of vivianite: B30) were used. Each treatment was repeated 20 times and 320 pots were used. The seedling emergence rate, growth (plant height, number of leaves per plant, and leaf area), and production parameters (nodulation and seeds yields) were assessed. Results revealed that S20 fertilizer significantly increased ($P < 0.05$) the dry biomass (23.06 ± 1.97 g) of cowpea plants compared to C20 fertilizer and C plant. The lowest values of studied parameters were from no treated plants. S10 fertilizer increased the plant height by 30.30% compared to BNBH2, SSJ7, and RKB5 fertilizers. Globally, inoculated cowpea plants by S10 fertilizer exhibited the highest seed yields (654.62 ± 19.16 kg/ha). S10 fertilizer increased the cowpea seeds yield by 53.62% and 151.90% respectively compared to C10 fertilizer and no treated plants. According to the results obtained in this study, the combination of SSJ7 isolate + 10 g of vivianite powder per hole is recommended for cowpea grown in Bini-Dang (Adamawa-Cameroon).

Keywords: *Vigna unguiculata*, Phosphate Solubilizing Microorganisms, vivianite, yield, growth, Dang (Adamawa, Cameroon).

INTRODUCTION

In Cameroon, 75% of the staple food is cereals and legumes. Leguminous crops such as groundnut, common bean, soybean, voandzou, and cowpea could be an alternative to improve the nitrogen nutrition of humans and animals and increase the yields of crops used in rotation such as sorghum, cotton, and maize (IRAD, 2013). Cowpea seeds are twice rich in protein than cereal seeds. According to ALZOUMA (1995), this legume is characterized by its much lower price than that of animal proteins (meat, fish, eggs). Its ability to fix atmospheric nitrogen could allow it to enrich the soil with nitrogen. On the other hand, the availability of phosphorus is often a limiting factor in the productivity of legumes on ferralsols (FR) (RABEHARIOSIA, 2004). The soil of the Dang site (Adamawa, Cameroon) is the ferralsol type developed on basalt (MEGUENI *et al.*, 2006). These soils are generally acidic and often deficient in nitrogen and phosphorus with high retention capacities of this last element by heavy metals. Indeed, aluminum (Al), iron (Fe), and calcium (Ca) are present in large quantities in these soils causing the loss of phosphorus by sequestering the free form in the form of precipitates (AlPO_4 , FePO_4 , CaPO_4) not absorbable by plants (GYANESHWAR *et al.*, 2002). Substantial inputs of phosphorus, an essential nutrient for agricultural production, are necessary.

The use of phosphate fertilizers is generally recommended to correct P deficiencies. The phosphorus contained in chemical fertilizers comes from the exploitation of non-renewable resources, which are predictably depleted (CORDELL *et al.*, 2011). However, these fertilizers are not always within the reach of middle farmers and failure to master their use entails environmental risks (VERDURA, 2008). Faced with this situation, agricultural production must instead focus on soil fertilization by biological means that will make it possible to obtain significant agricultural yields and sustainable soil health.

Several soil microorganisms, such as mycorrhizal fungi, rhizobia, and phosphate-solubilizing microorganisms (PSM) improve agricultural production (NWAGA, 2000; NGAKOU *et al.*, 2007; MEGUENI *et al.*, 2011). PSMs are known for their ability to solubilize inorganic phosphates, thus making this element available to plants (GYANESHWAR *et al.*, 2002; MAIMOUNA *et al.*, 2016). In the Adamawa region, several geological materials can be used to improve soil fertility and agricultural production. Hangloa vivianite in Ngaoundere Cameroon is rock phosphate and one of the local geological materials rich in phosphate easily accessible to small farmers. Thus, MSP associated with this rock phosphate provides a cheaper source of fertilizer for crop production (SALMA, 2015; MAIMOUNA, 2017). These PSMs can make phosphorus available to plants and play an important role in leguminous nodule development. Nitrogen-fixing plants require adequate amounts of phosphorus for nodulation (TANG *et al.*, 2001). The objective of this work is to evaluate the growth and production parameters of *Vigna unguiculata* by using PSM and different doses of vivianite used as Rock Phosphate from Hangloa in Bini-Dang. Specifically, it will be a question of studying the effect of the combination of vivianite RP and PSM on the growth, nodulation, and yield of cowpea.

MATERIAL AND METHODS

Description of the study site

The study was carried out in the pot in the experimental farm of the Laboratory of Biodiversity and Sustainable Development of the University of Ngaoundere (Cameroon) during the cropping season in 2019. Ngaoundere Cameroon belongs to agro-ecological zone II known as Sudan-Guinean savannahs with six months raining season (April to October) and six months of the dry season (November to March). Total annual precipitation and mean annual temperature were 1898.6 mm and 25.75°C respectively. The study site was located at latitude North 7°25'119", at longitude East 13°33'415.83", and 1106 m elevation (DEROGOHO *et al.*, 2018).

Biological material

Fekem variety seeds of *Vigna Unguiculata* from IRAD of Garoua were used (Figure 1). This variety was chosen for its great adaptability to the rainy season and its reproduction cycle is 80 to 85 days. It is one of the varieties popularized by IRAD of Cameroon. These seeds are white and their pods are rolled up.



Figure 1: Fekem variety seeds of *Vigna unguiculata* (L.)

Biofertilizers

Phosphate Solubilizing Microorganisms (PSM) used in our study were isolated from the soil and roots of maize and sorghum rhizosphere in the Sudano-Guinean (RKB5 and SSJ7)

and Sudano-Sahelian (BNBH2) zones of Cameroon. They are tolerant to pH ranges between 3.5 and 6.5 (MAIMOUNA, 2017). The ability of these microorganisms to solubilize inorganic phosphates was evaluated in solid media (observation of a halo zone around the colonies) and liquid media (determination of the solubilized phosphorus concentration) containing vivianite Rock Phosphate (RP), Calcium Phosphate (CaPO_4), Iron Phosphate (FePO_4) and Aluminum Phosphate (AlPO_4). The strains selected according to their ability to solubilize insoluble phosphate were grown in Erlenmeyer flasks containing 500 ml of liquid medium (distilled water, 3 g of NaCl, 3 g of yeast extract, and 5 g of peptone.) on a rotating shaker. Then the inoculum was transferred into bottles (Figure 2).



Figure 2: Inoculum of Phosphate Solubilizing Microorganisms

Rock Phosphate

The Rock Phosphate was collected in Hangloa (Adamawa, Cameroon) which is located between $7^{\circ}20$ and $7^{\circ}30$ North latitude and between $13^{\circ}20$ and $13^{\circ}25$ East longitude. This Rock Phosphate is name vivianite (Figure 3) with a mineralogical composition of Fe_2O_3 (68.72%), P_2O_5 (9.17%), Al_2O_3 (7.72%), and SiO_2 (9.67%) (YAYA *et al.*, 2015). This natural Rock Phosphate was powdered with a hammer and then sieved (2 mm mesh sieve) before use.

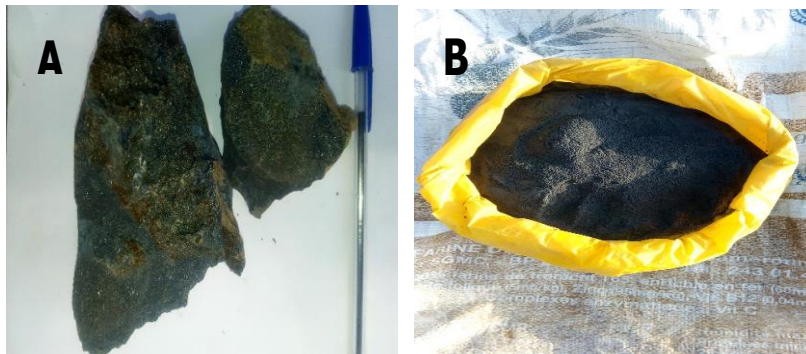


Figure 3: Rock of Vivianite from Hangloa (A: hard rock, B: rock powder)

Experimental design

A randomized complete block design with sixty (16) treatments control (no treated plants): C; RKB5 isolate: R0; SSJ7: S0; BNBH2: B0; control with 10 g of vivianite: C10; isolate RKB5 + 10 g vivianite: R10; SSJ7 isolate + 10 g vivianite: S10; BNBH2 isolate + 10 g vivianite : B10; control with 20 g vivianite: C20; RKB5 isolate + 20 g vivianite: R20; SSJ7 isolate + 20 g vivianite: S20; BNBH2 isolate + 20 g vivianite: B20; control with 30 g vivianite:

C30; RKB5 isolate + 30 g vivianite: R30; SJJ7 isolate + 30 g vivianite: S30 and BNBH2 isolate + 30 g of vivianite: B30) were used. Each treatment was repeated 20 times and 320 pots were used. These pots were arranged in two blocks and the space in both blocks was 1 m. Each block was subdivided into 4 sub-blocks with an area of 4.95 m² (3.3 m x 1.5 m). two consecutive sub-blocks spaced at 1 m. space between two consecutive pots for the same treatment was 20 cm.

Data collection and statistical analyzes

The plant's emergence rate was evaluated 10 days after sowing on all treatments. Growth parameters (plant height, number of leaves per plant, and leaf area) and production parameters (nodulation and seeds yields) were assessed.

The yield is calculated by the formula: average weight of grain (AWg/grams) multiplied by the average number of grains per plant (ANg) and the number of plants per hectare (NP/ha):

$$\text{Fresh yield} = (\text{AWg}) \times (\text{ANg}) \times (\text{NP/ha}) / 1000000. \text{ NP/ha} = (\text{X} \times \text{Y}) / 10000$$

Statistical analyzes were carried out using the "Stratigraphic plus version 5.0" software, which performs the analysis of variance (ANOVA) to determine the interactions between the treatments. Duncan's test was used to judge the difference between treatment means.

RESULTS AND DISCUSSIONS

Cowpea plant emergence rate

Figure 4 shows the seedling emergence rate of cowpea plants according to treatment at 10 days after sowing. The analysis of variance (ANOVA) revealed a highly significant difference (P<0.001) between treatments (control (no treated plants): C; RKB5 isolate: R0; SJJ7: S0; BNBH2: B0; control with 10 g of vivianite: C10; isolate RKB5 + 10 g vivianite: R10; SJJ7 isolate + 10 g vivianite: S10; BNBH2 isolate + 10 g vivianite : B10; control with 20 g vivianite: C20; RKB5 isolate + 20 g vivianite: R20; SJJ7 isolate + 20 g vivianite: S20; BNBH2 isolate + 20 g vivianite: B20; control with 30 g vivianite: C30; RKB5 isolate + 30 g vivianite: R30; SJJ7 isolate + 30 g vivianite: S30 and BNBH2 isolate + 30 g of vivianite: B30) relative to cowpea seedling emergence rate. S10 (85.5 ± 3.53%) and B10 (85.5 ± 2.82%) fertilizers exhibited the highest seedling emergence rates and respectively. The lowest seedling emergence rates were from C plots and the inoculated pots with only isolates (B0, S0, and R0). S10 fertilizer increased the seedling emergence rate by 28.26% compared to the C treatment.

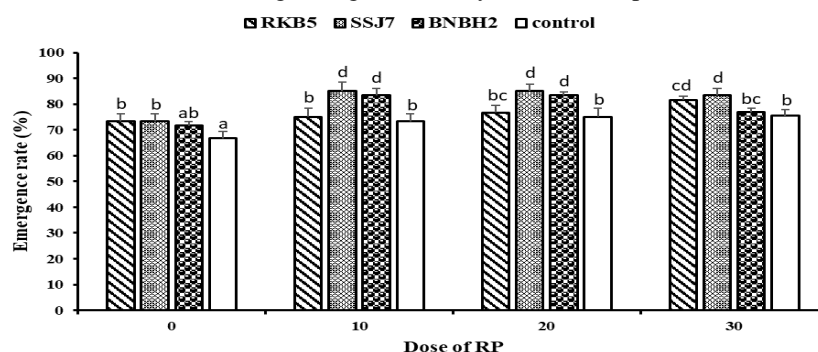


Figure 4. Seed emergence rate of *Vigna unguiculata* 10 days after sowing. NB: the values with the same letters are not significantly different at the 5% level.

Cowpea plant height

Table 1 recorded the average height of cowpea plants according to the isolates and the levels of vivianite powder (control (no treated plants): C; RKB5 isolate: R0; SSJ7: S0; BNBH2: B0; control with 10 g of vivianite: C10; isolate RKB5 + 10 g vivianite: R10; SSJ7 isolate + 10 g vivianite: S10; BNBH2 isolate + 10 g vivianite : B10; control with 20 g vivianite: C20; RKB5 isolate + 20 g vivianite: R20; SSJ7 isolate + 20 g vivianite: S20; BNBH2 isolate + 20 g vivianite: B20; control with 30 g vivianite: C30; RKB5 isolate + 30 g vivianite: R30; SSJ7 isolate + 30 g vivianite: S30 and BNBH2 isolate + 30 g of vivianite: B30). It appears that the evolution of plant's height is continuous for all the treatments and the tallest were observed at 70 days after sowing. Cowpea plants from B10 (15.63 ± 0.93 cm) and S10 (15.50 ± 1.26 cm) were the tallest while the shortest was the C plants as well as the fertilized plants with BNBH2, SSJ7, and RKB5. B10 and S10 fertilizers increased the cowpea plant height by 15.26% and 14.30% respectively compared to no treated pot. The analysis of variance at the 70 days after sowing showed a significant difference (P<0.05) between treatments on plants height.

Table 1

Treatment	Plants height				
	Cowpea plant's height (cm)				
	14 DAS	28 DAS	42 DAS	56 DAS	70 DAS
C	7.16 ± 0.99 ^a	10.26±1.33 ^a	11.26±1.03 ^a	12.26±1.3 ^a	13.56±1.61 ^a
R0	7.73 ± 1.19 ^{abc}	10.9±0.66 ^{abc}	11.86±0.74 ^{ab}	13.3±1.26 ^{bc}	14.54±1.76 ^{bc}
S0	7.73 ± 1.36 ^{abc}	11.06±1.23 ^{abc}	11.9±1.18 ^{ab}	12.8±0.97 ^{ab}	14.16±2.13 ^{ab}
B0	7.43 ± 1.4 ^{abc}	11.30 ± 1.42 ^{bcd}	12.30 ± 1.19 ^{bc}	13.16±1.01 ^{bc}	14.13±0.91 ^{ab}
C10	8.03 ± 1.14 ^{bcd}	11.6±0.89 ^{cd}	12.86±0.61 ^{cde}	13.7±0.52 ^{cd}	14.53±0.99 ^{cd}
R10	8.13 ± 1.19 ^d	12.56±0.75 ^e	12.36±0.85 ^{efg}	14.6±0.71 ^e	15.06±1.03 ^{bcde}
S10	8.83 ± 1.23 ^d	12.83±0.71 ^e	13.8±0.7 ^{fg}	14.76±0.96 ^e	15.5±1.26 ^{de}
B10	8.80±1.2 ^d	12.7±1.38 ^e	13.93±1.14 ^g	14.9±0.94 ^e	15.63±0.93 ^e
C20	7.06±1.06 ^a	10.96±1.65 ^{abc}	12.63±0.93 ^{bc}	13.4±1.18 ^{bc}	14.66±1.63 ^{bc}
R20	7.26±0.86 ^{ab}	11.26±1.43 ^{bcd}	13.33±1.39 ^{efg}	14.7±1.38 ^e	15.4±1.76 ^{de}
S20	7.63±0.89 ^{abc}	10.66±1.23 ^{ab}	12.93±0.77 ^{cde}	14.76±0.84 ^e	15.16±0.87 ^{cde}
B20	8.13±1.23 ^{cd}	11.2±1.26 ^{bc}	13.16±1.15 ^{def}	15.03±1.42 ^e	15.4±1.4 ^{cde}
C30	7.50±1.19 ^{abc}	11.3±0.86 ^{bcd}	12.63±0.71 ^{cd}	14.6±0.73 ^e	14.83±0.72 ^{bcde}
R30	7.53±1.32 ^{abc}	11.46±1.17 ^{bcd}	12.63±1 ^{cd}	14.66±1.12 ^e	15.06±0.96 ^{bcde}
S30	7.70±1.11 ^{abc}	12.03±0.69 ^{de}	13.16±0.48 ^{def}	14.33±0.85 ^{de}	15.03±0.89 ^{bcde}
B30	8.16±1.27 ^{cd}	11.36±1.07 ^{bcd}	11.26±1.03 ^{cde}	14.63±1.14 ^e	15.13±1.2 ^{cde}

Legend: C: control; R0: RKB5 isolate with 0 g of vivianite; S0: isolate SSJ7 with 0 g of vivianite; B0: BNBH2 isolate with 0 g of vivianite; C10: control with 10 g vivianite; R10: RKB5 isolate + 10 g of vivianite; S10: SSJ7 isolate + 10 g of vivianite; B10: BNBH2 isolate + 10 g of vivianite; C20: control with 20 g vivianite; R20: RKB5 isolate + 20 g of vivianite; S20: SSJ7 isolate + 20 g of vivianite; B20: BNBH2 isolate + 20 g of vivianite; C30: control with 30 g vivianite; R30: RKB5 isolate + 30 g of vivianite; S30: SSJ7 isolate + 30 g of vivianite; B30: BNBH2 isolate + 30 g of vivianite. DAS: days after sowing. The values of the column affected by the same letters are not significantly different at the 5% level.

Evolution of cowpea leaves production

The analysis of variance performed at 70th DAS (Figure 5) shows a significant difference (P <0.05) between fertilizer and leaf production (number of leaves per plant and leaf area). Figure 6 shows the average number of leaves per plant at 70th days after sowing according to treatments (control – no treated plants): C; RKB5 isolate: R0; SSJ7: S0; BNBH2: B0; control with 10 g of vivianite: C10; isolate RKB5 + 10 g vivianite: R10; SSJ7 isolate + 10 g vivianite: S10; BNBH2 isolate + 10 g vivianite : B10; control with 20 g vivianite: C20; RKB5 isolate + 20 g vivianite: R20; SSJ7 isolate + 20 g vivianite: S20; BNBH2 isolate + 20 g

vivianite: B20; control with 30 g vivianite: C30; RKB5 isolate + 30 g vivianite: R30; SSJ7 isolate + 30 g vivianite: S30 and BNBH2 isolate + 30 g of vivianite: B30). It appears from this figure that the vivianite powder associated with isolates increased cowpea leaves production. Plants from S10 fertilizers exhibited the greatest leaves (8.60 ± 2.89 leaves per plant) and the lowest was from control plants (6.60 ± 1.68 leaves per plant). S10 fertilizer increased the plant height by 30.30% compared to BNBH2, SSJ7, and RKB5 fertilizers.

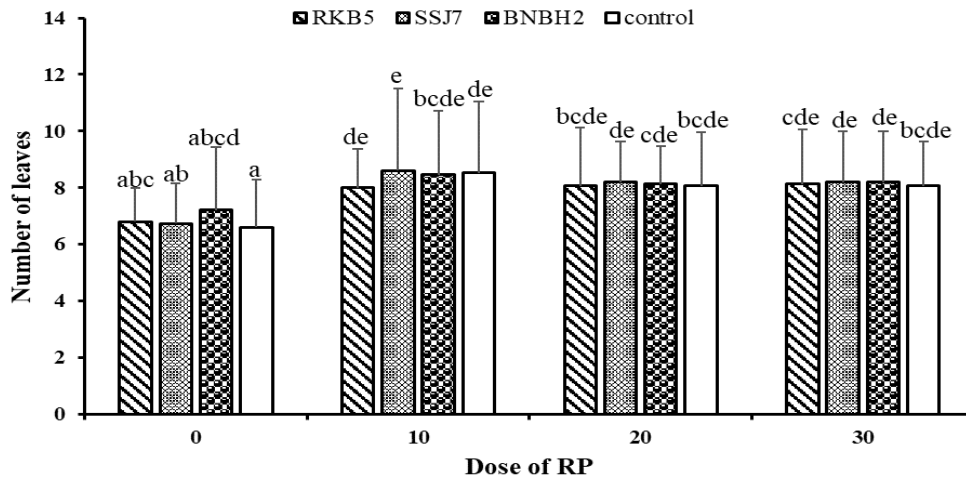


Figure 5. Number of cowpea leaves

Figure 6 shows the leaf area of cowpea plants depending on the treatment. It appears that the combination of vivianite powder with isolates SSJ7 or BNBH2 increased the leaf area of cowpea compared to the treatment without vivianite. The greatest leaf area (25.04 ± 2.43 cm²) was obtained on treated plants with S30 while the lowest value (16.47 ± 3.75 cm²) of this parameter was from no treated plant (C). S30 fertilizer increased leaf area by 52.03%. The combination of vivianite powder and MSP isolates improves leaf area compared to vivianite powder alone.

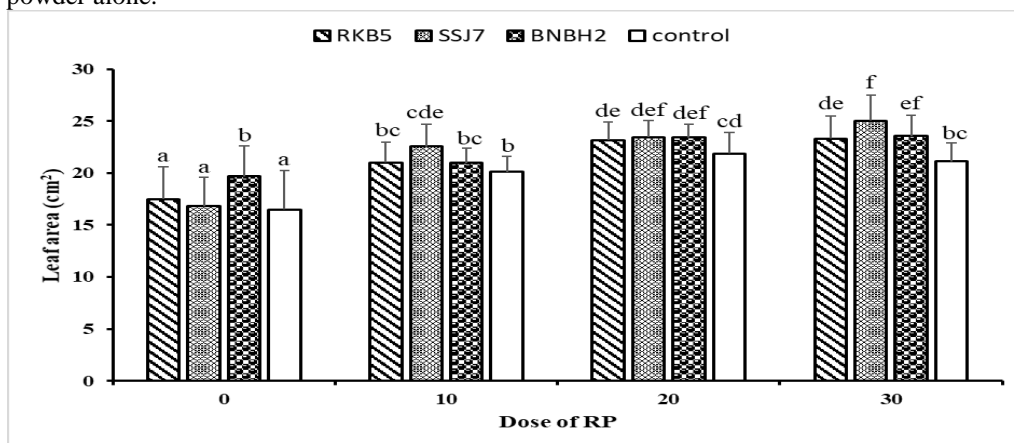


Figure 6. Vivianite Rock Phosphate and PSM on leaf area

Cowpea nodulation

In the present study, the highest number of nodules was obtained by the combination of 20 g of RP with BNBH2 isolate (B20: 17.66 ± 2.08), and the lowest value was obtained by the control (C: 6.66 ± 1.15) (Table 2). This B20 treatment increased the number of nodules by 165.16% compared to the control. The analysis of variance shows that the association of vivianite RP with PSM isolates presented a very high significant difference ($P < 0.001$).

It is also recorded in this table that the combination of vivianite RP with PSM isolates increases the efficiency of the nodules compared to the treatments amended with only RP and not inoculated with PSM and compared to the control. The highest value was obtained by the R20 treatment ($86.52 \pm 8.65\%$), and the lowest value by the control ($74.81 \pm 7.14\%$). This R20 treatment increased the efficiency of nodules by 15.65% compared to the control. The analysis of variance shows a highly significant difference ($P < 0.001$) for this parameter.

Table 2

Effect of Rock Phosphate vivianite and PSM by different treatments on nodulation

Treatment	Number of nodules	The efficiency of nodules (%)
C	6.66 ± 0.57^a	74.81 ± 1.06^a
R0	10.00 ± 1.00^{bcd}	75.34 ± 2.84^{ab}
S0	9.00 ± 1.73^{abc}	76.40 ± 3.69^{abc}
B0	8.00 ± 1.00^{ab}	77.32 ± 1.59^{abc}
C10	10.66 ± 0.57^{bcde}	74.88 ± 2.10^a
R10	13.33 ± 1.05^{efgh}	78.69 ± 2.75^{abcd}
S10	13.66 ± 1.15^{efgh}	78.88 ± 1.92^{abcd}
B10	12.00 ± 1.64^{defg}	83.75 ± 3.69^{ef}
C20	11.33 ± 1.52^{cdef}	77.97 ± 2.29^{abc}
R20	14.33 ± 1.52^{gh}	79.17 ± 2.14^{bcde}
S20	15.00 ± 2.00^{hi}	80.59 ± 2.80^{cdef}
B20	17.66 ± 2.08^i	82.89 ± 2.07^{efg}
C30	11.00 ± 2.00^{cdef}	77.74 ± 2.41^{abc}
R30	12.66 ± 3.51^{defgh}	86.52 ± 2.59^g
S30	12.66 ± 2.51^{defgh}	82.81 ± 2.55^{defg}
B30	11.33 ± 2.08^{cdef}	83.15 ± 3.29^{efg}

Legend: C: control treatment with 0 g of vivianite; R0: RKB5 isolate with 0 g of vivianite; S0: isolate SSJ7 with 0 g of vivianite; B0: BNBH2 isolate with 0 g of vivianite; C10: control with 10 g vivianite; R10: RKB5 isolate + 10 g of vivianite; S10: SSJ7 isolate + 10 g of vivianite; B10: BNBH2 isolate + 10 g of vivianite; C20: control with 20 g vivianite; R20: RKB5 isolate + 20 g of vivianite; S20: SSJ7 isolate + 20 g of vivianite; B20: BNBH2 isolate + 20 g of vivianite; C30: control with 30 g vivianite; R30: RKB5 isolate + 30 g of vivianite; S30: SSJ7 isolate + 30 g of vivianite; B30: BNBH2 isolate + 30 g of vivianite. The values with the same letters are not significantly different at the 5% level.

Effect of Rock Phosphate and PSM on the biomass of cowpea plants

Figure 7 reveals that fertilized plants with vivianite powder and PSM isolates significantly produced the highest biomass per plant and the control gave the lowest values compared to the other treatments. This highest value was obtained by the combination of 20 g of vivianite with SSJ7 isolate (34.13 ± 3.19 g) and the lowest value was obtained with the control (18.48 ± 3.04 g). Furthermore, S20 treatment increased the biomass of plants by 84.68% compared to the control. It is also noted that the treatments amended with RP and inoculated with PSM improve this parameter compared with the treatments compared to the control C. It appears from the statistical analyzes that the addition of Rock Phosphate associated with the isolates presents high significant values ($P < 0.001$) for the dry biomass.

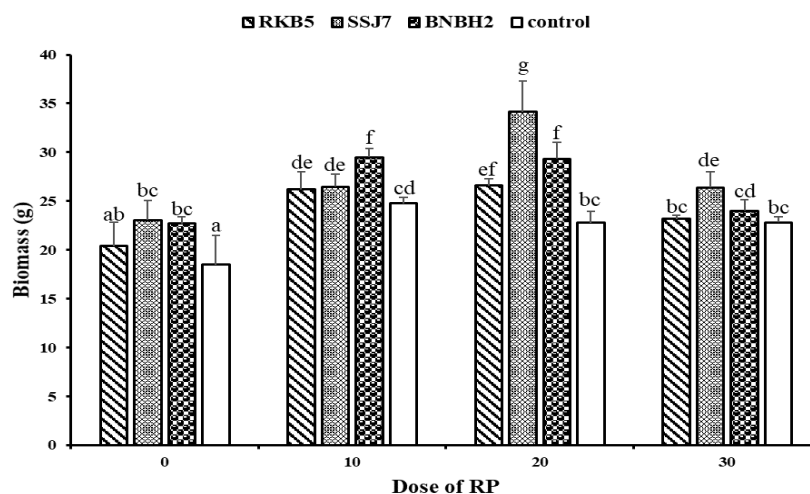


Figure 7. Dry biomass
The values with the same letters are not significantly different at the 5% level.

Production parameters

Regarding production parameters, the number of flowers, pods, and seeds yields were assessed.

Table 3 recorded the number of flowers, pods, and yield per hectare of cowpea according to the different treatments. The addition of vivianite Rock Phosphate associated with PSMs increases the number of flowers and pods. The SSJ7 isolate + 10 g of RP (S10) presents the highest value (5.66 ± 3.9) and the control presented the lowest value (3.33 ± 1.34) for the number of flowers. This treatment increased the number of flowers by 69.96% more than the control. The highest number of pods was obtained by the treatment S10 (10 g RP and the SSJ7 isolate) (5.53 ± 1.71) and the lowest value was obtained by the control (3.20 ± 1.14). The analysis of variance shows that the association of PSM and RP significantly improves ($P < 0.05$) the number of flowers and the number of pods. Results also saw a slight drop between the average number of flowers and the number of pods formed per plant. This result shows that not all flowers formed mature pods. Some flowers aborted and some immature pods dropped from the plants. The greatest value of yield was obtained by treatment of 10 g of RP with the SSJ7 isolate (654.62 ± 19.16 Kg/ha), and the lowest value was obtained with the control (259.87 ± 4.65 Kg/ha). This result shows an increase of 151.90% by this treatment compare to the control. The analysis of variance between isolates and dose of vivianite interaction shows a very high significant difference ($P < 0.001$). The contributions of different doses of vivianite combined with PSM had a positive impact on yield compared to the non-inoculated and non-amended control.

Table 3

The number of flowers, pods, and yield per hectare			
Treatment	Number of flower	Number of pods	Yield Kg/ha
C	3.33 ± 1.34^a	3.20 ± 1.14^a	259.87 ± 4.65^a
R0	4.33 ± 1.67^{abcd}	4.13 ± 1.40^{abc}	384.25 ± 6.17^{bc}
S0	4.13 ± 1.50^{abc}	4.00 ± 1.3^{ab}	377.14 ± 4.24^b

B0	4.26 ± 1.48 ^{abcd}	4.13 ± 1.30 ^{abc}	397.12 ± 5.83 ^{cd}
C10	4.46 ± 1.95 ^{abcde}	4.33 ± 1.58 ^{bcd}	426.11 ± 7.29 ^e
R10	4.86 ± 1.64 ^{bcdef}	4.73 ± 1.43 ^{bcd}	569.33 ± 3.89 ⁱ
S10	5.66 ± 1.71 ^f	5.53 ± 1.64 ^e	654.62 ± 19.16 ^k
B10	5.40 ± 1.76 ^{def}	5.13 ± 1.30 ^{de}	621.14 ± 11.25 ^j
C20	4.26 ± 1.27 ^{abcd}	4.13 ± 1.06 ^{abc}	428.93 ± 7.15 ^{ef}
R20	5.60 ± 1.99 ^{ef}	5.46 ± 1.50 ^e	503.76 ± 2.70 ^h
S20	4.66 ± 1.17 ^{bcdef}	4.60 ± 1.05 ^{bcd}	440.34 ± 3.65 ^{fg}
B20	4.80 ± 1.65 ^{bcdef}	4.66 ± 1.44 ^{bcd}	513.16 ± 4.10 ^h
T30	3.93 ± 1.03 ^{ab}	3.86 ± 0.99 ^{ab}	410.82 ± 3.99 ^d
R30	5.53 ± 1.68 ^{ef}	5.40 ± 1.45 ^e	576.50 ± 7.17 ⁱ
S30	5.13 ± 1.59 ^{cdef}	5.00 ± 1.3 ^{cde}	627.11 ± 6.50 ^j
B30	4.13 ± 1.95 ^{abc}	4.06 ± 1.86 ^{abc}	449.60 ± 2.54 ^g

Legend: C: control treatment with 0 g of vivianite; R0: RKB5 isolate with 0 g of vivianite; S0: isolate SSJ7 with 0 g of vivianite; B0: BNBH2 isolate with 0 g of vivianite; C10: control with 10 g vivianite; R10: RKB5 isolate + 10 g of vivianite; S10: SSJ7 isolate + 10 g of vivianite; B10: BNBH2 isolate + 10 g of vivianite; C20: control with 20 g vivianite; R20: RKB5 isolate + 20 g of vivianite; S20: SSJ7 isolate + 20 g of vivianite; B20: BNBH2 isolate + 20 g of vivianite; C30: control with 30 g vivianite; R30: RKB5 isolate + 30 g of vivianite; S30: SSJ7 isolate + 30 g of vivianite; B30: BNBH2 isolate + 30 g of vivianite.

NB: the values with the same letters are not significantly different at the 5% level.

Fertilization with Hangloa vivianite RP associated with PSM improved the emergence rate. Indeed, phosphorus would have influenced the installation and the start of this plant at emergence. FROSSARD *et al.* (2004) show that natural phosphate improves root development, which leads to an increase in plant emergence. DOMMERGUES *et al.* (1999), report that some rhizosphere microorganisms produce vitamins like thiamin, nicotinic acid, pantothenic acid, and others, which stimulate germination. It would therefore be possible that the stimulation of germination observed with these isolates, combined with vivianite, is due to the production of these substances and the increase in the concentration of assimilable P in the vivianite by these microorganisms. The growth parameters of cowpea were positively affected by the association between PSM and vivianite RP. Indeed, the increase in the growth of cowpea would be due to the phosphorus contained in this rock. The best result obtained by combining PSM and RP could be explained by the fact that, in addition to the phosphate released, these PSM produce vitamin B12 and auxins which play a role in stem expansion. In addition, some PSMs increase the efficiency of nitrogen fixation and the release of certain trace elements (GYANESHWAR *et al.*, 1998). These results agree on many points with certain authors like BABANA & ANTOUN (2005) studying the effect of rock phosphate in the presence of PSM on wheat, and they obtained significant results concerning the height of this plant. ABOU-EL-SEOUD & ABDEL-MEGEED (2012) also showed that the combination of RP and PSM improves maize growth compared to the control. BENAGGAB-AMAR (2011) also showed that the number of leaves and leaf areas of cowpea that received RP and PSM increased compared to the control without inoculation of PSM and RP.

At the same time, nodulation and yield parameters such as the number of flowers, pods, and yield in kg/ha of *Vigna unguiculata* were positively affected by the association of Hangloa vivianite with PSM. This could be explained by the fact that P enhances symbiotic

nitrogen fixation by increasing the size, the number of nodules, and the activity of nitrogenase (TSVETKOVA & GEORGIEV, 2003). This increase in symbiotic nitrogen fixation leads to an improvement in the total biomass of the plant (DAOUI, 2007). These results are in agreement with those obtained by KASONGO *et al.* (2012) who showed that the application of different doses of Kanzi phosphate rocks significantly increased the number of soybean nodules. Studies by FANKEM *et al.* (2015) showed that the combination of PSM and RP significantly increased the number of nodules in soybeans and beans compared to the non-inoculated amended control. MAIMOUNA *et al.* (2016), also showed that PSM and vivianite significantly increased the dry mass of maize and sorghum compared to the control. These PSMs associated with RP, react differently depending on the doses of vivianite. Strains of rhizosphere bacteria such as *Pseudomonas*, *Bacillus* as PSM applied to the leaves and flowers of apple trees (a woody) significantly improved the yield of trunk cross-sectional area (from 13.3 to 118.5%), fruit weight (4.2 to 7.5%), stem length (20.8 to 30.1%), and stem diameter (9.0 to 19.8%) compared to the control (LUTFI *et al.*, 2007). SALMA (2015), showed that several bacterial isolates (including *Pseudomonas*, *Burkholderia*, and *Rhizobium*) having a beneficial effect on plants (biofertilizer) can stimulate growth and improve crop yield. These results corroborate those of BABANA (2003) and ZEHRA (2010); working respectively on wheat and sunflower, these authors have shown that the combination of MSP and PR increases the yield compared to the amended and non-inoculated control.

CONCLUSIONS

The present work aimed to evaluate the growth and production parameters of *Vigna unguiculata* by using PSM and different levels of vivianite powder from Hangloa in Ngaoundere Cameroon. The results of the pot work showed that the doses of 10, 20, and 30 g of vivianite associated with the isolate SSJ7 and the dose of 10 g of RP associated with the isolate BNBH2, improved the emergence rate of the seeds of *Vigna unguiculata*. The height parameter was improved by the 10 g of RP associated with isolates BNBH2 and SSJ7. The number of leaves was improved by the 10 g of RP associated with isolate SSJ7. The greatest number of nodules is noted by the 20 g of RP associated with the BNBH2 isolate. Concerning inoculation with PSMs, the behavior and effectiveness of each of the isolates change according to the doses of vivianite. Cowpea plants grown in control pots were less productive compared to all other treatments. Given the results obtained, the dose of vivianite that ensures the optimal yield of cowpea grains is the combination of 10 g of vivianite plus the SSJ7 isolate. This combination is the most effective for growing cowpea in our study area.

BIBLIOGRAPHY

- ABOU-EL-SEOUD & ABDEL-MEGEED, A., 2012. Impact of rock materials and biofertilizations on P and K availability for maize (*Zea Maize*) under calcareous soil conditions. *Saudi Journal of Biological Sciences*, 19: 55-63.
- ALZOUMA, I., 1995. Connaissance et contrôle des Coléoptères Bruchidae ravageurs des légumineuses alimentaires au Sahel. *Sahel IPM*, 1(4) : 10-11
- BABANA A. H. & ANTOUN H., 2005. Biological system for improving the availability of Tilemsi rock for wheat (*Triticum aestivum* L.) cultivated in Mali. *Nutrient cycling in agroecosystems*. 72: 147-157.
- BABANA A. H., 2003. Mise au point d'un inoculant biologique pour le blé irrigué du Mali. PhD Thesis, Université de Laval.138p.
- BENAGGAB-AMAR R., 2011. Nutrition azotée sous déficience en phosphore chez le niébé (*Vigna unguiculata* Sub. *Sp unguiculata* L. Walp). Mémoire de Magister en Sciences

- Agronomiques, Ecole Nationale Supérieure d'Agronomie (ENSA) EL Harrach-Alger, 78p.
- CORDELL D., ROSEMARIN A., SCHRÖDER J. J. & SMIT A. L., 2011. Towards global phosphorus security: A systems framework for phosphorus recovery and reuse options. *Chemosphere*, 84 : 747-758.
- DAOUI K., 2007. Recherche de stratégies d'amélioration de l'efficacité d'utilisation du phosphore chez la fève (*Vicia faba* L.) dans les conditions d'agriculture pluviale au Maroc. Mémoire de thèse, pp : 156-201.
- DEROGO N, MEGUENI C, TCHUENTEU T.L., 2018. Study of intercropping systems castor oil and legumes on seeds yield and yield-related traits in two agroecological zones of Cameroon. *Scholars Journal of Agriculture and Veterinary Sciences.*; 5(6):352-368.
- DOMMARGUES Y., DUHOUX E. & DIEM H. G., 1999. Les arbres fixateurs d'azote. 1375 ref, ORSTOM, FAO, Editions Espaces 34, Montpellier, 501p.
- FANKEM H., TCHUISSEU TCHAKOUNTE G. V., NGO NKOT L., MAFOKOUA H. L., TCHOUOMO DONDJOU D., SIMO C., NWAGA D. & ETOA F. X., 2015. Common bean (*Phaseolus vulgaris* L.) and soya bean (*Glycine max*) growth and nodulation as influenced by rock phosphate solubilizing bacteria under pot-grown conditions. *International Journal of Agricultural Policy and Research*. 3 (5): 242-250.
- FROSSARD E., JULIEN P., NEYROUD, J- A. & SINAJ, S., 2004. Le phosphore dans les sols. État de la situation en Suisse. Cahier de l'environnement no 368. Office fédéral de l'environnement, des forêts et du paysage, Berne, 180p.
- GYANESHWAR P., NARESH KUMAR G., PAREKH L. J. & POOLE P. S., 2002. Role of soil microorganisms in improving P nutrition of plants. *Plant and Soil*, 245: 83-93.
- GYANESHWAR P., NARESHKUMAR G., & PARCKH L. D., 1998. Effect of buffering on the phosphate solubilizing ability of microorganisms. *World Journal of Microbiology and Biotechnology*, 14 : 669-673.
- IRAD, 2013. Contribution de la recherche à l'amélioration de la production et la consommation des légumineuses alimentaires au Cameroun. Projet C2D légumineuses, 57p.
- KASONGO R. K., VAN RANST E., VERDOODT A., KANYANKOGOTE P. & BAERT G. 2012. Réponse du soja (*Glycine max*) à l'application de phosphate de Kanzi et de dolomie rose de Kimpese sur sol sableux en RD Congo. *Canadian Journal of Soil Science*, 92 : 905-916.
- LUTFI, P., METIN, T., FIKRETTIN, S. & AHMET E., 2007. Floral and foliar application of plant growth promoting rhizobacteria (PGPR) to apples increases yield, growth, and nutrient element contents of leaves. *Journal of Sustainable Agriculture* 30 (4), 145-155.
- MAIMOUNA A., 2017. Intérêt des microorganismes solubilisant les phosphates de roche pour la production des céréales (maïs et sorgho) dans la zone septentrionale : aspects écologiques et agronomiques. Thèse doctorat PhD. Université de Ngaoundere . 134p.
- MAIMOUNA, A., MEGUENI, C., NWAGA, D., WASSOUNI, F., NKONGMENECK, B. A. & MANCHANG, K. T., 2016. Impact of selecting phosphate Solubilizing microorganisms on the growth of maize and sorghum using Vivianite as inorganic phosphate supply. *International Journal of Current Research*, 8(10): 39579-39591.
- MEGUENI C., AWONO E. T. & NDJOUENKEU R., 2011. Effet simultané de la dilution et de la Combinaison du Rhizobium et des mycorhizes sur la production foliaire et les propriétés physico-chimiques des jeunes feuilles de *Vigna unguiculata* (L.) Walp. *Journal of Applied Biosciences*, 40: 2668-2676.
- MEGUENI C., NGAKOU A., KALALAO M. M. & KAMENI T. D., 2006. Responses of Soybean (*Glycine max* L.) to Soil Solarization and Rhizobial Field Inoculation at Dang Ngaoundere, Cameroun. *Asian Journal of Plant Sciences*, 5(5): 832-837.
- NGAKOU A., NWAGA D., NEBANE C. L. N., NTONIFOR N. N., TAMO M. & PARH A. I., 2007. Arbuscular Mycorrhizal Fungi, Rhizobia and Metarhizium anisoplae enhance P, N, Mg, K, and Ca accumulation in field grown cowpea. *Journal of Plant Sciences* 5: 518-529.
- NWAGA D., 2000. Agriculture biologique et alternative de la bio fertilisation. In : Utilisation des biofertilisants microbiens pour une agriculture biologique. Acte du premier atelier de

- formation sur l'utilisation des biofertilisants. Yaoundé du 31 mars au 1er avril 2001. pp.23-24.
- RABEHARISOA L., 2004. Gestion de la fertilité et de la fertilisation phosphatée des sols ferrallitiques des Hautes Terres de Madagascar. Thèse de Doctorat, Université d'Antananarivo. 199p.
- SALMA T., 2015. Dissolution biologique des phosphates: Interaction bactéries-mycorhizes. Thèse Doctorat en microbiologie agroalimentaire Philosophiae doctor (Ph.D) Québec, Canada 150p.
- TANG C., HINSINGER P., DREVON J. J., & JAILLARD B., 2001. Phosphorus deficiency impairs early nodule functioning and enhances proton release in roots of *Medicago truncatula* L. *Annals of Botany*, 88p.
- TSVETKOVA G. E. & GEORGIEV G. I., 2003. Effect of phosphorus nutrition on the nodulation, nitrogen fixation, and nutrient-use efficiency of *Bradyrhizobium japonicum* soybean (*Glycine max* L. Merr.) symbiosis, *Bulg. J. Plant Physiol. Special issue* 331–335.
- VERDURA, 2008. Support pédagogique sur le développement durable. *Agriculture bio.*, 89: 89- 145.
- YAYA F., NGUETNKAM J. P., TCHAMENI R., BASGA S. D. & PENAYE J., 2015. Assessment of the Fertilizing effect of Vivianite on the Growth and yield of the Bean “*phaseolus Vulgaris*” on Oxisoils from Ngaoundere (Central North Cameroon). *International Research Journal of Earth Sciences*, 3(4): 18-26.
- ZEHRA E., 2010. Performance of phosphate solubilizing bacteria for improving growth and yield of sunflower (*Helianthus annuus* L.) in the presence of phosphorus fertilizer. *African Journal of Biotechnology*, 9(25): 3794-3800.