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A Comparative Study on Different Methods of Inoculating Cowpea Using Microbial Fertilisers

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ABSTRACT

This study was based on setting up a method of inoculating cow pea using microbial fertilizers. the study was designed to evaluate the effects of inoculating bio fertilizers made from rhizobium bacterial with cowpea seeds so as to propose strategies for sustainable management of it. After putting in place six experimental set up made up of mixtures of: cowpea and rhizobium alone, cowpea with rhizobium substrate, cowpea plus rhizobium and gray powder, cowpea with rhizobium and phosphor solubilizing microbes (PSM), with two control experiment of which one set up had no inoculants and the other had just gray powder. The following parameters were verified: the height of plants after two months, average number of nodules per treatment and identification of various diseases attack on the different treated seedlings. Primary data were subjected to analysis of variance (ANOVA). The following results were obtained: the set up made of cowpea with rhizobium and grey powder gave best results with 39.70 cm as average height, 48 as an average number of nodules on these treated plants, no diseases attack due to the fact that Rhizobium which is a bacterium is used alongside with another ingredient. Cowpea with rhizobium and PSM also gave interesting results with average nodules of 41; 36.11cm as average height of plants and no disease. Apart from cowpea treated just with rhizobium alone which also showed average results with 27cm as average height and 27 as average number of nodules with no disease incidence. The control experiments together with cowpea treated with no rhizobium showed negative results with various diseases attack such as angular leave spots disease on seeds treated with grey powder, damping off of seedling for seeds treated with rhizobium substrate, and yellow cercosporiose for seeds without treatment. As recommendation, it is advisable to inoculate cow pea seeds with other ingredients as grey powder before planting.

Keywords: Inoculation, Rhizobium, Soya bens, Seed

INTRODUCTION

Right behind cereals, legumes are the second most important source of human food and animal forage [1, 2]. It is through nitrogen fixation that legumes provide plant tissues which is high in protein leguminous plant are important in maintaining the health of soils, legumes are plant that have nodules on their root which fix the nitrogen from the atmosphere to help the plant. Legume plant such as beans, peas, pulses have rhizobium bacteria in their roots [3]. these bacteria have the ability to fix atmospheric nitrogen in to plants usable form of nitrogen, hence requires less in organic fertilizers [4, 5]. For some year now, our soils are becoming infected and even very poor in microorganism causing cowpea plant not to attain maximum yield [6]. With the pre occupation of knowing if inoculation could be a helping factor in this situation, we had as general objective:

Testing different methods and the effects of Inoculating bio fertilizers made from rhizobial bacterial with cow pea seeds

MATERIALS AND METHODS

Localisation of the Study Area

This study was carried out in the lowlands of Cameroon, specifically in the center region. It has and average rain fall ranging between 700 and 2200 throughout the year and varied temperatures with altitude.

Relief and Soil Type

Various types of reliefs were observed in the area of experimental plots such as: hills, plains, plateaus, this relief brings about a good and equilibrium exchange of temperature in the center compared to other towns. This atmospheric condition gives the soil a two-profile structure which is: ferralitic and hydro morphic soils which are good for Agriculture

Technical Materials

- Cow pea seeds: this is the basic item which will be planted with inoculated material.
- Rhizobium bacterial: it is the leaving organism which is inoculated with the seeds.
- Rhizobium substrate: this are dry plants which are used as substrate for the bacterial.
- Grey powder: it is a dust made by grinding shells of snales.
- Phosphorus solubilizing microbes: this are microorganism which are use alongside with bacterial during inoculation
- Canopy: this is an instrument used as a support material on which the soil is dry and weight.
- A rake: it is an instrument used when drying the soil.
- Plastics bags: this are small plastics bags of 10 kg in which soil is mixed and put for planting of seeds. An electronic balance to measure the different weights of treatment material.
- A table: this is used as a support for the plastics bags with growing seeds, to avoid the direct contact of the plastics bags with the ground.
- Watering can: it is an instrument in which liquid are put and use, when necessary, on the field.

- Trowel: it used to facility the filing of plastics bags with soils.
- Plastics cups: these are smalls cups in which the mixing of seeds with ingredients is done.
- Weighing machine: it is use to weigh the various ingredients to be use.

Methods

Seeds Rhizobium Substrate:

5g of seeds were used for 2 g of Rhizobium substrate. They were put in a plastic cup and mix with 1 ml of water.

Seeds Rhizobium Alone:

In this set up, we used 5g of seeds were used for 2 g of Rhizobium substrate. They were put in a plastic cup and mix with 1 ml of water.

Seeds Rhizobium and Grey Powder:

In this set up, we used 5g of seeds were used for 10 g of grey powder. They were put in a plastic cup and mix with 1 ml of water.

Seeds Rhizobium and PSM (Phosphor Solubilizing Microbes):

5g of seeds were used for 2 g of PSM. They were put in a plastic cup and mix with 1 ml of water.

Seeds Alone:

5g of seeds were measured and planted directly in the plastic bags containing treated soil.

Seeds and Grey Powder:

In the last set up, we used 5g of seeds were used for 10 g of grey powder. They were put in a plastic cup and mix with 1 ml of water. Below is a figure showing the inoculated seeds;

Procedure for Setting Up Trial:

Six treatments were carried out with 8 seeds per plastic bag making a total of 48 seeds. The cow pea seeds were weight (5g of seeds for 2 g of substrate were mixed in plastic cups with the absence and presence of various inoculated material, so as to see the different effects at growth.

The soil was prepared by taking 200g of black soil and mixing with 50kg of Sanaga sand giving us a total amount of 250 kg of prepared and treated soil. Each plastic bag, was fill with 5 kg of soil.

Experimental Design

The trial was arranged in an orderly manner with six factors of origin. Each cow pea set up and treatment had as experimental unit the gram (g).

Data Processing and Statistical Analysis

The graphics were produced with the Excel spreadsheet. Primary data were subjected to analysis of variance (ANOVA). The calculated mean values of the variables were subjected to the T-multiple comparison test ($p \leq 0.0001$)

RESULTS AND DISCUSSION

Results

Height of Plants:

The growth of cow pea was evaluated every 2 weeks after inoculation and planting throughout a period of 2 months. The figures below show the growth of plants in relation with the various set up.

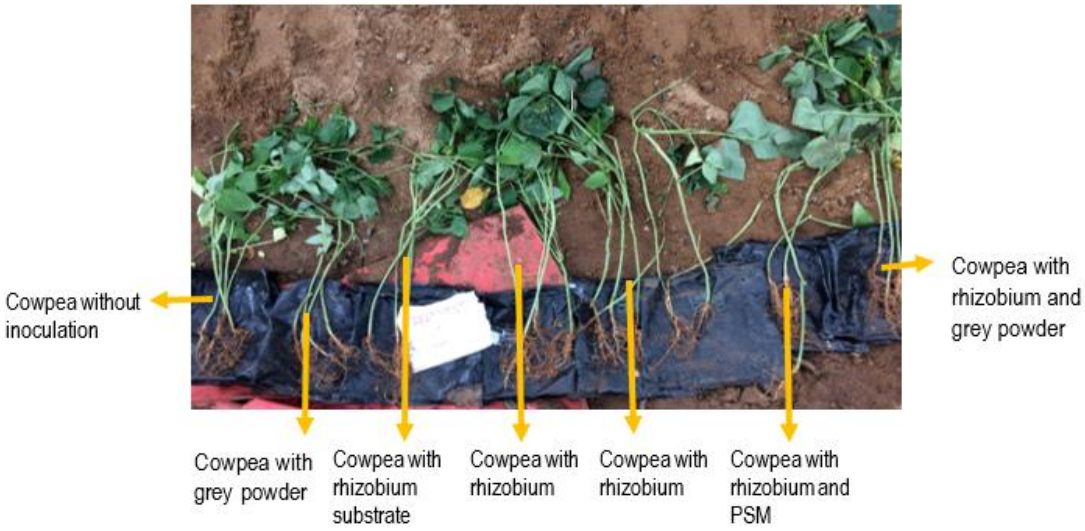

Figure 1: Cowpea seeds showing height of treated plants after two months

Looking at the various set up, the set up made up of Rhizobium and Grey powder has a greatest number of plants with the tallest height (36,61 cm). That shows that, grey powder favours the growth of Rhizobium bacterial hence stimulating rapid growth of plants. Standard deviation varies from 1,83 and 3,36. This shows a large range difference between cowpea without rhizobium and cowpea inoculated with rhizobium and other nutritive elements.

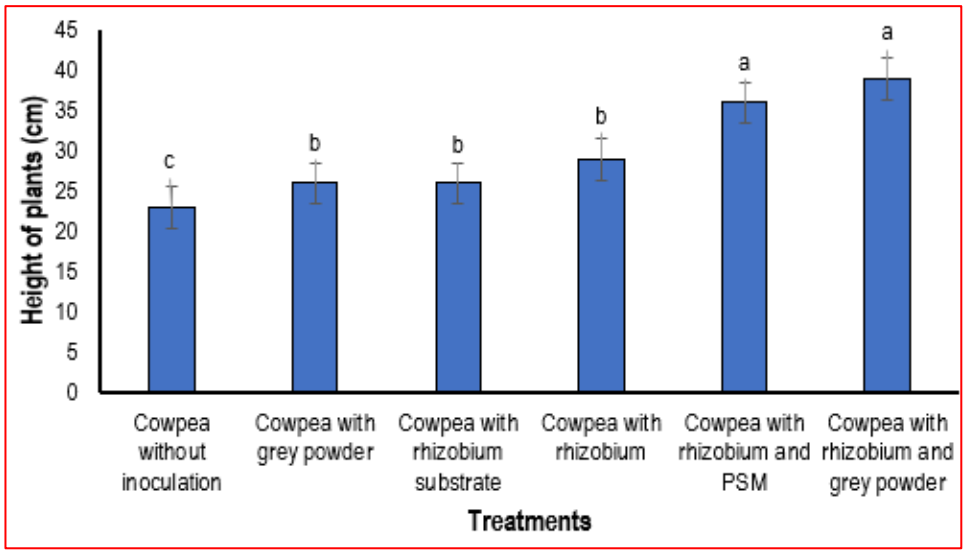

Figure 2: Height of cowpea plants after two months according to treatments

Surface Area of Cowpea Leaves:

The difference in surface area of the treated plants were seen by calculating the standard variation using the formulae below:

$$S = \frac{2}{3}\text{length} + \text{width}$$

With *S: Surface area*

The use of this formula gave us the following table (Table 1). Looking at the table, the set up made up of seed rhizobium plus white powder shows less than one standard deviation indicating maximum size of leaves compared to others. While the absence of rhizobium in some treatments show greater standard deviation hence smaller surface of leaves.

Table 1: Table showing surface area (in cm^2) and standard deviation of cowpea leaves

Treatments	2 weeks	4 weeks	6 weeks	8 weeks	average	Standard Deviation
Cowpea without inoculation	11.5	14.32	13.32	12.99	13,03[b]	1,16
Cowpea with grey powder	10.99	15.76	13.1	13.26	13,27[b]	1,95
Cowpea with rhizobium substrate	14.6	15.6	13.26	12.54	14[b]	1,13
Cowpea with rhizobium	11.94	12.44	13.6	15.26	13,31[b]	1,47
Cowpea with rhizobium and PSM	16.92	17.24	18.92	17.92	17,75[a]	0,88
Cowpea with rhizobium and grey powder	14.6	18.24	15.94	15.6	16,59[a]	1,60

PSM: Phosphor Solubilizing Microbe

Average Number of Nodules in Relation with Each Treatment:

After 2 months of planting, plants from the various set up were harvested and nodules were counted. The pictures below show roots of various treated cowpea plants, followed by a table showing the number of nodules of cowpea after two months.

Figure 3: Roots of various treated cowpea plants (A: Cowpea with rhizobium and PSM; B: Cow pea with rhizobium and grey powder)

Table 2: Table showing number of nodules per treatment

Methods of application of treatments	Average number of nodules after Two months
Cowpea without inoculation	15[c]
Cowpea with grey powder	19[c]
Cowpea with rhizobium substrate	23[b]
Cowpea with rhizobium	27[b]
Cowpea with rhizobium and PSM	41[a]
Cowpea with rhizobium and grey powder	48[a]

PSM : Phosphor Solubilizing Microbe

Aggressiveness of Diseases in The Growing Stages of Cowpea Plants After Inoculation:
Three (03) diseases were observed on some treatments: Cowpea without inoculation, cow pea with grey powder and cow pea with rhizobium substrate, these diseases are:

- Angular leaves spot disease on Seeds and Grey powder;
- Damping off of seedling on Seeds Rhizobium substrate;
- Yellow cercosporiose on cow pea without inoculation.

Figure 4: Cowpea without inoculation, Figure 5: Cowpea with grey powder

Figure 6: Cowpea with rhizobium substrate, Figure 7: Cowpea with rhizobium and grey powder

Looking at the various set up, the set up made up of cowpea without inoculation, cowpea with grey powder and cowpea with rhizobium substrate are attack by yellow cercosporiose, angular leaves spot and damping off respectively. These seeds were vulnerable due the fact that they did not receive Rhizobium to stimulate the other ingredient in order to resist diseases.

Table 3: Table showing number of nodules per treatment

Treatments	Diseases		
	Angular leaves spot disease	Damping off of seedling	Yellow cercosporiose
Cowpea without inoculation	(A)	(A)	(P)*
Cowpea with grey powder	(P)*	(A)	(A)
Cowpea with rhizobium substrate	(A)	(P)*	(A)
Cowpea with rhizobium	(A)	(A)	(A)
Cowpea with rhizobium and PSM	(A)	(A)	(A)
Cowpea with rhizobium and grey powder	(A)	(A)	(A)

(P)*: Presence of disease
(A): Absence of disease

Cowpea with rhizobium, cowpea with rhizobium and grey powder and cowpea with rhizobium and PSM (Phosphor Solubilizing Microbes) shows no attack of diseases due to the fact that rhizobium which is a bacterium is use alongside with other ingredients. Some of these ingredients such as PSM and Grey powder supply the plant with nutritive elements hence making them to resist from various diseases.

DISCUSSION

Height of Plants and Surface Area of Leaves

The variations observed in the height and surface area of leaves revealed that the performance of the plant's growth and surface area of leaves depend on other ingredients associated with rhizobium. These results corroborate those obtained by previous studies [1, 7], who explained that seeds inoculated with rhizobium alone showed weak performance than seeds inoculated with rhizobium and other ingredients.

Average Number of Nodules and Aggressiveness of Diseases in The Growing Stages of Cowpea Plants

The variations observed in the number of nodules of cowpea after two months can be explained by the growth of rhizobium bacterial hence stimulating rapid growth of plant. All the same, the aggressiveness of diseases in the growing stages of cowpea plants depend on other ingredients associated with rhizobium. These results corroborate those obtained by previous studies [8, 9, 10], who explained that seeds inoculated with rhizobium alone developped a few numbers of nodules and showed an aggressiveness of diseases than seeds inoculated with rhizobium and other ingredients.

CONCLUSION

This study revealed that, cowpea seeds inoculated with rhizobium and grey powder has a very good performance indicating that the presence of these 2 materials ameliorate the nature of the cow pea plant making it resistant to diseases, having a rapid growth hence producing the greatest number of nodules. Cowpea seeds inoculated with PSM also give better results with the presence of solubilized phosphate. Apart from cow pea inoculated with rhizobium alone which also produce good results, the poor results of cowpea without inoculation indicate the

absence of rhizobium bacterials which contributes to the great performance of the other treatments.

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References

[1] Nwaga, D., 2008. Bio fertilisant myco. Centre de Biotechnologie & Faculté des Sciences. Université de Yaoundé I. 4e édition, février 2008. 2p.

[2] Asadi Rahmani et al. 2005. Helper bacteria: a new dimension to the mycorrizal symbiosis. New Phytol. 128: 197-210

[3] Nwaga, D., 1994. La symbiose Rhizobium-légumineuse: de la recherche au développement. Cam. Biochem. Soc. Vol II: 44-50.

[4] Hoben, 1991. Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes. Microbiol. Mol. Biol. Rev. 68: 280–300.

[5] Giller, 2001.Phosphorus fertilization and symbiotic nitrogen fixation in common bean. Agronomy 1. 71: 925-926.

[6] Obaton, M., 1992. Facteurs pédoclimatiques limitant la fixation biologique de l'azote chez les légumineuses.

[7] Caetano-Anolles, G., Paparozzi, E.T. and Gresshoff, P.M., 1991. Mature nodules and root tips control nodulation in soybean. Journal of Plant Physiology 137: 389-396.

[8] Hosseini, 2008.Plantes oléagineux. Agriculture en Afrique tropicale. DGCI- Goekintgraphics SA, Belgique.

[9] Carsky, R.J., Eteka, A.C., Keatinge, J.D.H. et Manyong, V.M., 1998.Plantes de couverture et gestion des ressources naturelles en Afrique Occidentale. Actes de l'atelier organisé par l'IITA et le CIEPCA. Cotonou (Bénin). 315p.

[10] Nwaga, D. et Ngo Nkot L., 1998. Tolérance à l'acidité in vitro des rhizobia isolés du niébé (Vignaunguiculata) au Cameroun en comparaison avec Bradyrhizobiumjaponicum. Cahiers agricultures 7: 407-410.