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Full Length Research Paper

Microbial production of textile grade pigments

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Biological pigments or biochromes are substances produced by living organisms and have a color resulting from selective color absorption. A total of 37 isolates of *Actinomycetes* and 2 bacteria were isolated from 26 different soil samples collected from different habitats of Kerala. The isolates were screened for pigment production. The effect of pigment production by the strains on various carbon and nitrogen sources were analyzed. The test microorganisms gave maximum pigmentation on glycerol as carbon source and histidine as nitrogen source when they were taken in an optimum concentration of 1:1. For the extraction of microbial pigment, both the organisms were grown on cotton saturated basal medium. The use of these pigments as colouring agents for textile fabrics were demonstrated. Out of the seven different cloth materials (silk, jute, synthetic fiber, satin, shiffon, cotton and polyester) used, silk, jute and synthetic fiber had uptake of the colour of the pigment.

Key words: *Actinomycetes*, pigments, glycerol, histidine, cotton.

INTRODUCTION

Pigments from natural sources have been obtained since long time ago, and their interest has increased due to the toxicity problems caused by those of synthetic origin (Amal et al., 2011). To counteract the harmful effect of synthetic dyes, the pigments from microbial sources are found to be a good alternative (Cañizares-Villanueva et al., 1998; Kramar et al., 2014). In nature, color rich and pigment producing microorganisms (fungi, yeasts and bacteria) are quite common (Dufosse, 2009). Microbial pigments have meaningful advantages over artificial and inorganic colors. Obtaining natural pigments from microbial sources viz. bacteria (Shirata et al., 2000), fungi (Sharmaa et al., 2012), Actinomycetes (Conn, 1943) and algae is the most rewarding perspective (Yeliseev and Kaplan, 1997). Microbial pigments are non-toxic, non-carcinogenic, pharmacological and biodegradable in nature (Venil et al., 2013) and production is one of the evolving area of research and have various industrial applications (Mansi and Gaurav, 2016).

The microbial pigments (except, the photosynthesis pigments) are secondary metabolites being synthesized in idiophase (Barkovich and Liao, 2001). The production of microbial pigments are influenced by pH, temperature, aeration carbon and nitrogen (Amal et al., 2011). Microbial pigment production can be increased in geometric proportions through genetic engineering, as compared to the scaling up methods of chemists (Joshi et al., 2003). Microorganisms produce a large variety of

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stable pigments and the fermentation has higher yields in pigments and lower residues as compared to the use of plants and animals (Duran et al., 2002). Thus, biosynthesis of dyes and pigments via fermentation processes (El-Fouly et al., 2015) have attracted more attention in recent years (Duran, et al., 2002; Hobson et al., 1998). The aim of this study was to isolate pigment-producing microorganisms, extract of the pigments from them and to use them as colouring agents for fabrics in textile industry.

MATERIAL AND METHODS

Isolation of microorganisms from soil

Various pre treatment procedures and selective media were used to assess the optimal conditions for the isolation of pigmented microorganisms, especially actinomycetes. 26 soil samples were collected from different habitats like hills, forests, agricultural fields, rhizosphere, non rhizosphere soil, rubber plantations, river basins, paddy fields, coir pith soils, rock soils, metal sediments, thicket, etc. Pre-treatment procedures for soil samples included air drying, heating at 70°C for 15 min and treating with SDS (0.05%) at 40°C for 20 min. Pre-treated soil samples were serially diluted and plated onto Actinomycetes Isolation Agar, starch casein nitrate agar, nutrient agar and glycerol asparagine agar and the plates were incubated at 25°C for 2 to 3 weeks. After incubation, typically pigmented colonies were selected and maintained on fresh medium of starch casein nitrate (SCN) agar.

Characterisation of the pigment producing microorganisms

Morphological analysis

Morphological identification of isolated strains of microorganisms was done by microscopic observation and standard staining techniques.

Microscopic observation of the isolated strains was done by wet mount and cover slip culture technique and staining techniques used are Gram staining, spore staining and acid fast staining.

Physiological and biochemical test

With minor modifications, these tests were performed by the methods of Gordon et al. (1996). The biochemical test included in the study are decomposition of casein, tyrosine, xanthine, hypoxanthine, urea, esculin and utilization of carbohydrates viz. glucose fructose, lactose, xylose, and sucrose. The inoculums were tested for NaCl resistance and gelatin liquefaction.

The pigmented strains from starch casein nitrate agar were introduced into basal medium in order to standardize the substrate for pigment production

Effect of carbon and nitrogen source on pigment production

The different carbon and nitrogen sources that influence the pigment production in the isolated strains were also investigated using protocol as described in Dastager (2006).

Effect of carbon sources on pigment production: The basal medium of the following composition: 2.0 g of $NaNO_3$, 1.0 g of K_2HPO_4, 0.5 g of $MgSO_4.7H_2O$, 0.01 g of $FeSO_4$, 0.5 g of KCL in 1000 ml distilled water (pH 7.2) was used. The effect of carbon source with 1% *viz* L-glycerol, starch, dextrin, maltose, arabinose, lactose, lactose, galactose, raffinose, glucose on pigment production was studied. The flasks containing 200 ml basal medium along with 1% carbon source were taken and the cultures were inoculated and placed on a shaker for 7 to 10 days at room temperature.

Effect of nitrogen sources on pigment production: The effect of nitrogen sources *viz* L-lysine, L-arginine, L-histidine, L-valine, L-tyrosine, L-asparagine, L-proline, L-glycine L–alanine, on pigment production was studied with same basal medium using 1% L-glycerol as the carbon source.

The basal medium with 1% glycerol as carbon source and 1% aminoacids were taken in conical flasks and cultures were inoculated and placed on a shaker for 7 to 10days at room temperature for pigment production.

Extraction of pigments from broth

The cultures inoculated in a conical flask containing cotton saturated basal medium with 1% glycerol and 1% L-histidine were placed on a shaker at room temperature. The pigment was extracted from the cotton by squeezing. The solution was then centrifuged. The supernatant or the pellet, which retained the pigment, was taken and vaporized in a water bath. The pigment powder obtained was stored for textile coloring. Seven different cloth materials (cotton, polyester, shiffon, satin, silk, jute, and a synthetic Fiber) purchased from the local textile market were cut into squares and presoaked in water. The cloths were then soaked in pigment sample and kept in boiling water bath for 1-2 h and air-dried.

RESULTS AND DISCUSSION

A total of thirty nine isolates were collected from different habitats like hills, forests, agricultural fields, rhizosphere soils, non rhizosphere soils, rubber plantations, river basins, paddy fields, coir pith soils, rock soils, metal sediments, etc under controlled pH condition (set as 7.2). The eight isolates among 39 produced diffusible pigments in starch casein nitrate agar, nutrient agar and glycerol asparagine agar and were labelled as B1, C1, G1, O1, P1, S1, V1 and Y1 (Figure 1). Based on the morphological and biochemical analysis (Berd, 1973), strain B1 and V1 were identified as *Streptomyces* sp, C1 and G1 as *Actinomyces* sp., S1 as *Serratia*, O1 as *Rhodococcus* sp., P1 and Y1as *Nocardia* sp., (Tables 1 and 2). In additon to that, morphological characters like sporulation (Bystrykh et al.,1996), diffusible pigments (Shirling and Gottlieb, 1966) support the conclusion (Tables 1 and 2).

The results revealed that the pigment production by the strains was affected by carbon and nitrogen sources. The effect of carbon and nitrogen sources on pigment production in the broth was observed from the fourth day of inoculation and reached a maximum in seven days (Table 3). The pigment production started within 4 days in cotton saturated basal medium containing optimal concentrations of carbon (glycerol) and nitrogen

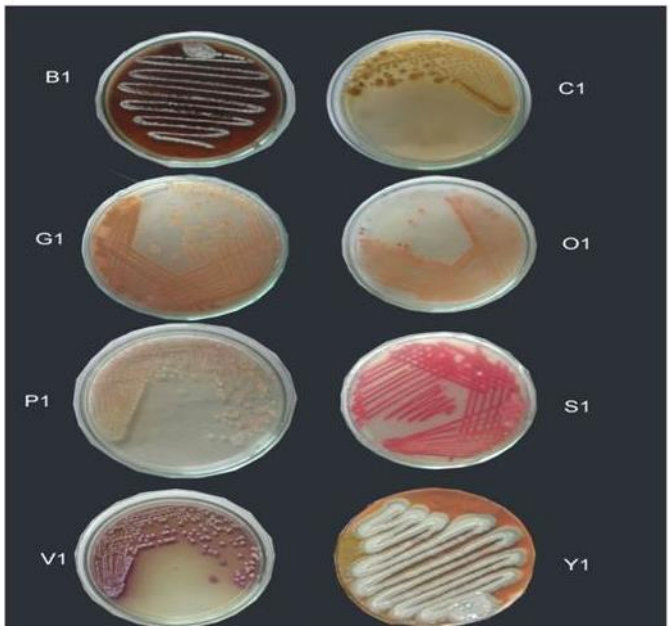

Figure 1. Pigment producing microorganisms.

Table 1. Morphological analysis.

Strain	Location	Gram staining	Spore staining	Acid fast staining	Morphology
B1	Agricultural land	+	+	+	Dry powdery aerial mycelium above, Spores in white and diffusible brown pigment beneath.
C1	Hill	+	+	-	Dry powdery aerial mycelium, septate hyphae, spores in white and diffusible cinnamon colored pigment.
G1	Spinney	+	+		Dry, powdery aerial mycelium, septate, spores in off-white color, golden yellow diffusible pigment beneath.
O1	Forest	-	-	-	Large, circular, mucoid, regular, raised, easily emulsifiable, non-diffusible orange pigmented colonies.
P1	Rice field	+	+	-	Dry, powdery, septate hyphae, spores in off white color, peach colored diffusible pigments beneath the plate.
S1	Rhizosphere soil	-	-	-	Large, circular, regular, raised, opaque, easily emulsifiable non-diffusible rose colored pigment producing colonies.
V1	Spinney	+	+	+	Dry, powdery, aerial mycelium, septate hyphae, grey colored spores with violet colored diffusible pigment.
Y1	Hill	+	+	+	Dry, powdery, septate hyphae, green colored spores with diffusible yellow pigment.

+Positive, -Negative.

(histidine) sources (1:1). The pigment was extracted from the media and the broth was vaporized in boiling water bath. The comparative efficiency of different cloth materials (silk, jute, synthetic fiber, satin, shiffon, cotton and polyester) to uptake the colour of the pigment was checked, in which silk, jute and synthetic fibers were

Table 2. Biochemical analysis.

Isolates	B1	C1	G1	O1	P1	S1	V1	Y1
Pigmentation in solid medium	Brown	Cinnamon	Golden yellow	Orange	Peach	Rose	Violet	Yellow
Solubility	Acetone	Water	Water	n	n	Water	Water	Water
Reduction of nitrate	+	+	+	+	+	+	+	+
Hydrolysis of								
Starch	+	+	+	+	+	+	+	+
Casein	+	+	+	+	+	+	+	+
Gelatin	-	-	-	-	-	+	-	-
Xanthine	+	-	-	+	+	+	+	+
Hypo xanthine	+	+	+	+	+	-	-	+
Tyrosine	+	+	-	+	+	+	+	+
Urea	+	-	+	-	-	-	-	+
Utilization of								
Glucose	+	+	+	+	-	+	+	+
Fructose	+	-	-	+	-	+	-	+
Lactose	-	-	-	-	-	+	-	+
Sucrose	-	-	-	-	-	+	-	+
Xylose	-	-	-	+	-	+	-	+
NaCl resistance	Up to 5%	Up to 5%	Up to 5%	Up to 5%	Up to 5%	Up to 5%	Up to 5%	Up to 5%

+ Positive, –Negative, n- not defined.

Table 3. Effect of carbon and nitrogen source on pigment production.

S/N	Carbon Source	Intensity of pigmentation	Nitrogen source	Intensity of pigmentation
1.	Glycerol	+++	Lysine	++
2.	Glucose	++	Arginine	++
3.	Dextrin	+	Histidine	+++
4.	Maltose	+	Valine	-
5.	Arabinose	++	Tyrosine	-
6.	Lactose	+	Asparagine	-
7.	Galactose	++	Glycine	+
8.	Raffinose	-	Alanine	-
9.	Starch	-	Proline	+

Intensity of pigmentation after incubation for 5 to 7 days was scored as + to +++ (maximal pigment).

found to be most effective in adsorbing the pigment colour (Figure 2). Similarly, Feng et al. (2015) also reported that the pigment production was significantly increased with the use of glycerol as the carbon source support the present work. Tallapragada (2013) suggested that the pigment yield from *Monascus purpureus LPB97* is directly propotional to glycerol concentration. However, other than carbon, nitrogen also play an important role in pigment production. The pigment production in *Bacillus subtilis* (Joshi et al., 2003) was enhanced by the addition of histidine in the medium (Hajjaj et al., 2012; Dufosse et al., 2005). Due to the lack of standard pigment samples, the intensity of the produced pigments were measured by visual observation. Dyeing process is a simple technique in which cloth materials are either dipped in the pigment extract or boiled with the bacterial cells. The difference in color was achieved due to the change in dipping time and temperature. Among the various white cloth materials

Figure 2. Extracted pigments on textiles.

used, silk, jute, synthetic fiber took up the color of the pigment (Figure 2). Others such as satin, shiffon, cotton and polyester showed a light adsorption of the pigments. Venil et al. (2013) suggetested that the dyeing performance vary based on the type of the fibre. Colorfastness of the dyed fabric needs to be ascertained before making any further comments on the nature of the isolated pigments as dyeing component for textile industry.

Conclusion

It is possible with the present study to isolate 8 pigments from microorganisms isolated from soil sample. It is also possible for identification of an appropriate media for pigment production by isolated microorganisms. Further, the use of these pigments as coloring agents for textile fabrics could be demonstrated. Standardization of fermentation conditions for large-scale production of these pigments is another area that needs further study. Extensive studies needs to be conducted to purify and characterize the pigment to identify its structure.

Conflict of Interests

The authors have not declared any conflict of interests.

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Microbial quality of retail raw meat in administrative towns of Gojjam area North-West Ethiopia with special reference of Gram positive cocci species

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This study was conducted to determine the bacteriological quality of retail raw meat. Twenty one raw meat samples were randomly collected from four administrative towns: Bure, Debre-Markos, Dejen and Fenoteselam meat-stalls of Gojjam area. Samples were collected and transported kept in cold sterile screw cap bottles with ice contained icebox. It was cultured on plate count agar and mannitol salt agar plates for enumeration of aerobic and pathogenic staphylococci bacteria respectively. Bacterial isolation was identified by culturing on selective medium and biochemical test. The mean total aerobic plate count (APC) ranged 6.325 to 6.477 log cfu/g was not significantly different (P<0.05); but enumeration of pathogenic Staphylococci ranged 3.588 to 4.251 log cfu/g was significantly different (P<0.05) between places. According to international standards microbial quality acceptability of ready-to-eat food and raw meat aerobic plate and pathogenic staphylococci count of almost all samples were categorized in borderline and unsatisfactory quality. A total of 65 gram positive cocci isolates were identified. The dominant bacterial pathogens isolates were *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Streptococcus pyogenes* in a ratio of 0.86, 0.71 and 0.71 respectively. The high bacteria count and isolates of aerobic plate count and pathogenic Staphylococci is an indication of higher risk for retail raw meat consumption. Hence needs improved hygienic practice at all levels in the raw meat production industry.

Key words: Raw meat, aerobic plate count, pathogenic Staphylococci, Gojjama.

INTRODUCTION

Foodborne diseases are an important cause of morbidity and mortality in worldwide but the full extent and cost of unsafe food, and especially the burden arising from contaminants in food, is still unknown. Foodborne

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bacterial pathogens are any bacteria in food with the potential to cause an adverse health effect (WHO, 2015). Normally bacterial pathogens associated with meats can pose risks with food poisoning; and contamination may be associated with the animals themselves, or be introduced to a clean carcass through cross contamination (FSA, 2015).

To supply healthy food for the consumer's microbiological quality guidelines of food have been developed. Microbiological quality of ready-to-eat food in general has limits for consist of aerobic colony count; hygiene indicator organisms; and specific foodborne pathogens (CFS, 2014). According to Commission Regulation EC (2014) and FSANZ (2016), the aerobic colony count of beef of more than 10^5 cfu/cm^2, and ready-to-eat sliced meat of more than 10^7 cfu/g, respectively has unsatisfactory quality standard and also hazards for consumption. In general the aerobic colony count of raw meat microbiological quality is classified into three classes: Satisfactory, if the test results indicating good microbiological quality; borderline or marginal, if the test results are not unsatisfactory and also not satisfactory; and unsatisfactory, if the test results indicate high bacterial count (FAO, 2007; CFS, 2014; Commission Regulation EC, 2014; FSANZ, 2016).

In addition testing pathogenic microorganisms associated with food is significantly important from public health point of view. From these Gram Positive Cocci (GPC) like *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumonia, Streptococcus pyogenes* and *Enterococcus faecalis* are pathogenic microorganisms and responsible for human infections (Todar, 2008; CFS, 2014; FSANZ, 2016). According to CFS (2014) and FSANZ (2016) count of *S. aureus* in ready-to-eat food is more than 10^4 and 10^3 cfu/g respectively has unsatisfactory microbiological quality. In Ethiopia the passion for eating raw meat is at highest and the way it is eaten is sometimes shocking. Meat-stalls are often packed with people who come to eat raw meat; but there is no microbiological standard and also monitoring system for retail raw meat products. On the other hand food consumers in Ethiopia suffer from food-borne bacterial illnesses with the likes of *Staphylococcus aureus* (Ayana et al., 2015).

The trend of raw meat consumption in administrative towns of Gojjam area is known. However there has no microbial quality standard finding of retail raw meat in the study area. Therefore this research was undertaken to check microbial quality of raw meat in meat-stalls; and isolation of *S. aureus* and other pathogenic gram-positive cocci.

MATERIALS AND METHODS

Sampling

The study was conducted in selected four administrative towns of Mirab-Gojjam and Misraq-Gojjam Zones. In each of these zones two administrative towns; Debremarkos and Dejen towns from Misraq-Gojjam zone and Bure and Finoteselam towns from Mirab-Gojjam zone were selected. Twenty-one meat-stalls: Four from Bure, eight from Debremarkos, four from Dejen and five from Fenoteselam were randomly selected. From each meat-stall subsamples were taken from different parts of the available carcass; butchers comminute the subsamples with them knife and thoroughly mixed to form a composite sample. Samples were collected cross-sectionally between April and August 2014; and transported kept in cold sterile screw cap bottles with ice contained icebox to DebreMarkos University College of Agriculture and Natural Resource Management Animal Science Laboratory for analysis.

Preparation of test sample and serial dilution

Blended 25 g of comminuted composite meat sample with 225 ml of peptone water (HIMEDIA) solution. Further decimal dilutions were carried out at 1:10 ratios with peptone water diluents according to EAS (2008).

Aerobic plate counts

Inoculated 0.1 mL of 4th to 2nd (the highest to the lowest dilution) consecutive serial dilutions in duplicate at the center of accordingly labeled pre dried Petri dishes of aerobic Plate Count Agar (PCA) (HIMEDIA) plates. The inoculum spread using sterilized hockey glass spreader and incubated at 35 to 37°C for 48$\pm$2 h. Then plates containing 30 to 300 colonies were selected and recorded average of the colonies counted as number of colony forming units per gram (cfu/g) for each sample.

Enumeration of pathogenic Staphylococci

Inoculated 0.1 mL of 3rd to 1st serial dilutions at the center of accordingly labeled pre dried Petri dishes of Mannitol Salt Agar (MSA) (HIMEDIA) plates into duplicate and incubated at 35 to 37°C for 48$\pm$2 h. Then plates containing 20 to 200 colonies were selected and recorded average of the colonies counted as number of colony forming units per gram (cfu/g) for each sample.

Identification and conformation of gram positive cocci species

From the last dilution inoculum of PCA plates 5 to 10 different colonies were sub-cultured separately on labeled nutrient agar (NA) plates (HIMEDIA) and incubated at 35 to 37°C for 28 to 48 h. Grown colonies on NA plates were subjected for potassium hydroxide test according to PHE (2010) to identify gram positive organisms, and catalase test according to PHE (2014) to identify catalase positive and negative reaction. Only gram positive and catalase negative colonies were sub-cultured on 5% sheep blood enriched Azide Blood Agar Base (HIMEDIA) plates and Bile Esculin Agar (HIMEDIA) slanted test tube medium. Species identifications were undertaken regarding hemolytic and esculin fermentation activity of the inoculum. In addition other gram positive cocci species were isolated on the mannitol fermention character from MSA plates.

Method of data analysis

Microbial count of log10 transformed value was analyzed with mixed procedure using Statistical Analysis Software (SAS) 9.2 to determine the difference between fixed effect of places and random

Table 1. Least squares means difference in log CFU g^{-1} of aerobic plate counts and pathogenic Staphylococci .

Place	Aerobic plate counts		Enumeration of pathogenicStaphylococci	
	Estimate±SE		Estimate±SE	
Bure	6.477±0.113		3.588±0.152^{c}	
DebreMarkose	6.352±0.080		4.251±0.107^{a}	
Dejen	6.477±0.113		3.788±0.152^{bc}	
Fenoteselam	6.477±0.102		4.063±0.136^{ab}	
Effect	**F Value**	**Pr > F**	**F Value**	**Pr > F**
Intercept	15655.6	<.0001	3242.2	<.0001
Place	0.5	0.6899	5.01	0.0225

*Means in a column with the same letter are not significant different (*P≥0.05*).

Table 2. The covariance parameter estimate of random effects.

Parameter		Estimate± SE	CI (lower, upper)	Z Value	Pr > Z
Aerobic plate counts	Meat-stall	0.000 ±(0.010)	*, *	0	0.5
	Residual	0.051±(0.020)	0.027, 0.135	2.53	0.0057
pathogenic Staphylococci	Meat-stall	0.000±(*)	*, *	*	*
	Residual	0.092±(0.032)	0.058, 0.207	2.92	0.0018

CI = 95% confidence interval; * = cannot be computed.

effect of meat-stalls. For the fixed effect (administrative town), least square difference was used to separate means when the parameter tests were significantly different at P< 0.05. The PROC MIXED estimates the variance components for the meat-stalls and the residual by default used Restricted Maximum Likelihood Estimation (REML). The convergence criteria of colony forming unit bacterial count of PCA plates and MSA plates were met at the first and second iteration respectively. In addition standardization of colony counts and isolates were analyzed with a descriptive statistics.

RESULTS AND DISCUSSION

Bacterial counts and standardization

The mean aerobic plate count was not significantly (*P≥0.05*) different; but the mean count of pathogenic staphylococci was significantly (*P≥0.05*) different between places. The least square mean differences indicate that the highest pathogenic staphylococci count (4.251log cfu/g) was found from Debremarkos but it was not significantly (*P≥0.05*) different from the mean count (4.063 log cfu/g) of Fenoteselam. On the contrary the least count (3.588 log cfu/g) of pathogenic staphylococci was found from Bure however statistically there was no significant (*P≥0.05*) difference with Dejen (3.788log cfu/g) samples. On the other hand there was no significant (*P≥0.05*) difference between Fenoteselam and Dejen (Table 1).

The variance estimate of meat-stalls and residual are presented in Table 2. It indicates that both variance components are significantly (*P≥0.05*) equals to 0. These estimates suggested that no difference in their mean aerobic plate counts and pathogenic Staphylococci among meat-stalls between and within places.

Generally the aerobic plate counts and pathogenic staphylococci were standardized in to three category: Satisfactory, borderline and unsatisfactory illustrated in Figure 1. According to FAO (2007) recommonded microbial criteria for fresh meat, and Commission Regulation EC (2014) process hygine criteria of aerobic colony count for cattle carcass, of all meat samples were categorized in unsatisfactory quality. On the other hand according to aerobic colony count levels in ready-to-eat foods, most (95.24%) of the meat samples were categorized in borderline microbial quality of aerobic colony count; while only few (4.76%) samples had satisfactory quality. In addition according to CFS (2014), *S. aureus* and other coagulase-positive staphylococci criteria in ready-to-eat foods, 47.62 and 52.38% of the samples were categorized in unsatisfactory and borderline quality respectively; but regarding FSANZ (2016), *S. aureus* in ready-to-eat food, all meat samples were categorized in unsatisfactory quality.

Even if, similarly with this finding the mean viable count 4.52×10^{6} cfu/cm^{2} was found from raw meat displayed for sale at Sokoto, Sokoto State, Nigeria (Danlami et al., 2013). Jahan, Mahbub-E-Elahi and Siddique (2015) also found 1.6×10^{7} to 4.23×10^{7}cfu/g mean viable count from fresh raw beef samples collected from seven major markets of Sylhet Sadar. In addition 1.73 × 107 cfu/g of

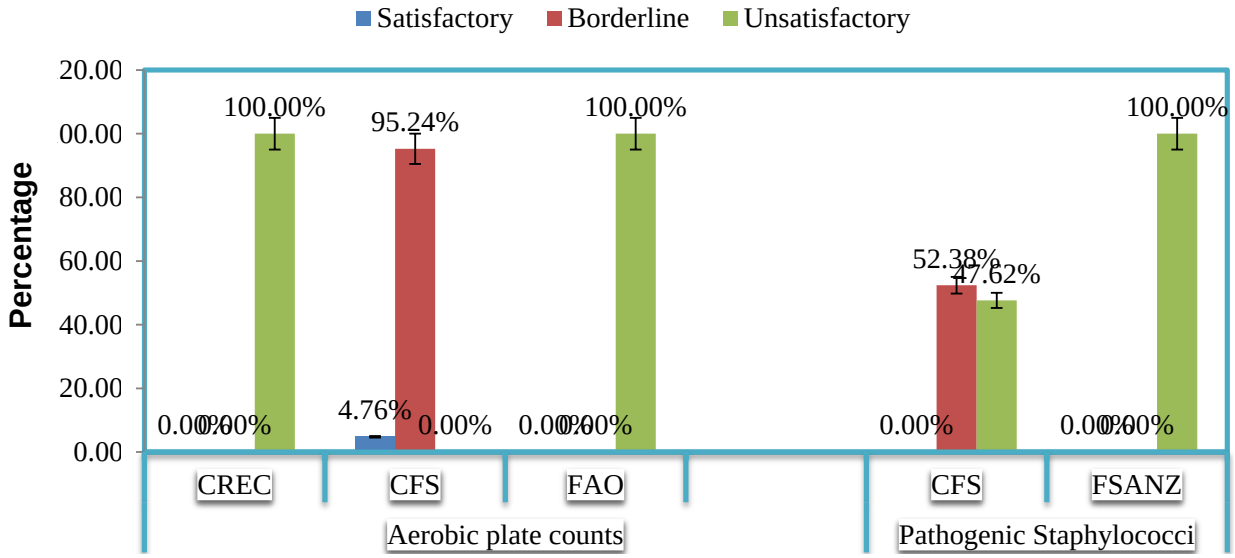

Figure 1. Percentage of the aerobic plate count: according to FAO, 2007 if the count $<10^4$ cfu/cm^2 = satisfactory, between 10^4 and 10^5 cfu/cm^2 = borderline, and $>10^5$ cfu/cm^2 = unsatisfactory; CFS, 2014 if the count $<10^6$ cfu/g = satisfactory, between 10^6 and 10^7 cfu/g = borderline, and $\geq 10^7$ cfu/g = unsatisfactory; and Commission Regulation EC, 2014 if the count $\leq$3.5log cfu/cm^2 = satisfactory, between 3.5log and 5log cfu/cm^2 = borderline, and >5log cfu/cm^2 = unsatisfactory. Count of pathogenic Staphylococci: according to CFS (2014) if the count <20 cfu/g = satisfactory, 20 < to $\leq 10^4$ cfu/g = borderline, and $>10^4$ cfu/g = unsatisfactory; and FSANZ, 2016 if the count $<10^2$ cfu/g = satisfactory, 10^2 to $<10^3$ cfu/g = borderline, and $>10^3$ cfu/g = unsatisfactory.

Staphylococcal count was found from spice used for traditionally dried and grilled meat product at Dandalin Fagge, Kano State, Nigeria (Shamsuddeen, 2009). In all these findings in lined and catagorized in unsatisfactory microbial quality regarding of international standardes.

The most probable reasons of unsatisfactory quality of aerobic plate count and pathogenic staphylococci count in retail raw meat might be transferred from butcher's hands, tools, working surfaces, equipment, water, pests, cleaning equipment, packaging or other meat and/or offal. In addition, the inadequate temperature contro can enhance the microbial load during transportation and storage(Melngaile et al., 2014; FSA, 2015).

Prevalence of Gram positive cocci isolates

From the total meat samples *S. epidermidis* (0.86), *S. aureus* (0.71) and *S. pyogenes* (0.71) were found in higher ratio and followed by *Streptococcus* species (0.48) other than *S. pyogenes* and *S. pneumonia; E. faecalis* (0.24) and *S. pneumonia* (0.10). The occurrence of gram positive cocci bacterial isolate per sample size of the study area, Finoteselam was the leading with 4.20 ratios; and tailed by Bure, DebreMarkos and Dajen with ratio of 3.25, 2.63 and 2.5 respectively. From the total 65 isolates equivalently a ratio of 0.32 was found from DebreMarkos and Fenoteselam; when 0.20 and 0.15 ratios isolated from Bure and Dajen respectively (Table 3).

In line with this finding *S. epidermidis (G*undogan and Ataol, 2013; Igbinosa et al., 2016; Jackson et al., 2013), *S. aureus* (Danlami et al., 2013; Gundogan and Ataol, 2013; Jackson et al., 2013; Adzitey, 2016; Igbinosa et al., 2016; Raji et al., 2016; Martinez, 2017) and *Streptococcus* species (Danlami et al., 2013) were found from retail raw meat and/or meat products. Isolate of *S. epidermidis* in higher ratio of DebreMarkos might be contaminated with skin, conjunctiva, nose, pharynx, mouth, lower intestine, and anterior urethra; because it is highly adapted to the diverse environments of human body (Todar, 2008).

Isolated bacterium might be transmitted from the animals during dressing and/or butcher's hands (FSA, 2015). Concerning the bacteriological culture of the nose and skin of normal humans invariably yields staphylococci. *S. pyogenes* is estimated that between 5 and 15% of normal individuals harbor, usually in the respiratory tract; *S. pneumoniae* also a normal inhabitant of the human upper respiratory tract. *E. faecalis* is a regular component of the intestinal flora, that many European countries use it as the standard indicator of fecal pollution (Todar, 2008).

Staphylococcal food-borne disease is one of the most common food-borne disease and is of major concern in public health programs worldwide (Kadariya et al., 2014). *S. aureus* causes a variety of supportive (pus-forming) infections and toxinoses in humans. In food it causes food poisoning by releasing enterotoxins into food, and toxic shock syndrome by release of superantigens into the blood stream (Todar, 2008; USDA, 2012). *S. pyogenes* is introduced or transmitted to vulnerable

Table 3. Gram positive cocci bacteria species isolates and ratio of isolates occurred from meat samples.

Isolates	Bure (*n=4*)[c]	DebreMarkos (*n=8*)[c]	Dajen (*n=4*)[c]	Finoteselam (*n=5*)[c]	Ratio of *I/N*
Staphylococcus aureus	3	5	3	4	0.71
Staphylococcus epidermidis	3	8	3	4	0.86
Enterococcus faecalis	1	1	-	3	0.24
Streptococcus species	4	1	-	5	0.48
Streptococcus pyogenes	1	6	4	4	0.71
Streptococcus pneumonia	1	-	-	1	0.10
Ratio of *a/n*	3.25	2.63	2.50	4.20	
Ratio of *a*/total isolates	0.20	0.32	0.15	0.32	
Total isolates			**65**		

n, sample size per place; [c], number of isolates occurred per *n*; *I*, is total isolates per *N;* which is total sample size (*N=21*).

tissues, a variety of types of supportive infections can occur. *S. pyogenes* is the leading cause of uncomplicated bacterial pharyngitis and tonsillitis commonly referred to a strep throat. Other respiratory infections include sinusitis, otitis, and pneumonia. *S. pneumonia* can cause pneumonia, usually of the lobar type, paranasal sinusitis and otitis media, or meningitis which is usually secondary to one of the former infections. It is currently the leading cause of invasive bacterial disease in children and the elderly. In recent years, *E. faecalis* has emerged as a significant, antibiotic-resistant, nosocomial pathogen (Todar, 2008).

CONCLUSION AND RECOMMENDATION

High aerobic plate and pathogenic Staphylococci count beyond international microbial standards indicates poor production and handling practices of retail raw meat. Moreover the isolates of *S. aureus* in a higher concentration could result in public health risk for raw meat consumers. This suggests the need for improved hygiene practices at all levels in the raw meat production industry. In addition the responsible body of governmental and non-governmental organizations should strengthen awareness campaigns on improved hygiene practices and the rate of microbial infections with food poisons.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of rates and sources of soluble phosphorus on the behavior of cowpea plants inoculated with rhizobacteria from soils of the north region of Brazil

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Cowpea can obtain N through biological nitrogen fixation (BNF) through symbiosis with rhizobacteria. However, nodulation and BNF are influenced by edaphoclimatic factors that may bring about benefits or damages to the process, and the availability of nutrients is among the main factors that affect BNF and phosphorus (P). Thus, the present study aimed to determine the effectiveness of doses and sources of soluble P on nodulation, accumulation of nutrients, N and P absorption and use efficiency in cowpea plants inoculated with or without rhizobacteria. The experiment was conducted in a greenhouse at the Federal Institute of Education, Science and Technology, Rondônia, Colorado do Oeste-RO Campus, Brazil. The experimental design was randomized blocks in a 5 x 2 x 2 factorial arrangement, corresponding to five P rates (0, 20, 40, 80 and 160 kg ha^{-1} of P_2O_5), two soluble sources of P_2O_5 [single superphosphate (SSP) and thermophosphate], absence and presence of inoculation, with four replication. The findings of this study show that inoculation with *Bradyrhizobium* sp. promotes increment in the dry matter production and increases N and P absorption efficiency in cowpea plants. The single superphosphate led to higher N and P absorption efficiency, production of shoot dry matter and production of nodules, when compared with thermophosphate. Inoculation with *Bradyrhizobium* sp. associated with SSP fertilization promotes higher P absorption efficiency in cowpea plants. Therefore, increase in P rates promoted increments in P concentrations in cowpea leaves.

Key words: *Vigna unguiculata* (L.), *Bradyrhizobium*, phosphorus, fertilization, nutrition.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp], also known as "feijão-de-corda", constitutes one of the main leguminous crops cultivated in Brazil, predominantly in the north and northeast regions. The crop is used for food purposes as

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the main source of protein for low-income populations (Freire et al., 2005). It is estimated that 369.5 thousand hectares of land are used for cowpea cultivation but, mean yield of approximately 207 kg ha^{-1} can be achieved in Brazil (Conab, 2016). The participation of the cowpea crop in the North region in terms of, cultivated area and production is still low, in spite of the fact that, its yield is virtually equal to the national mean. Among the various factors that contribute to the low mean yield of this crop is, management of soil fertility, particularly, insufficient supply of nitrogen (N).

Nitrogen is the nutrient required in largest amount by the bean crop and mineral fertilization is the main form of N supply to plants. However, the application of mineral N in tropical soils usually has low recovery efficiency by plants, normally lower than 50%, and may be limited to 5 to 10% in certain situations in sandy soils, due to the great losses through leaching and volatilization (Duque et al., 1985).

The cowpea crop, through the symbiosis of bacteria of the genus *Bradyrhizobium*, can obtain N through the process of biological N fixation (BNF), and is one of the forms to increase the yield of leguminous plants; thus, avoiding costs with soluble N fertilizers (Franco et al., 2002). BNF is shown to be efficient in cowpea and if well nodulated, can achieve high yield levels (Rumjanek et al., 2005). Guimarães et al. (2015) reported that cowpea responded significantly to inoculation, with increase in height of plants, number of pods, grain number, number of nodules, length of pods, dry mass of nodules, grain dry mass and dry mass of aerial part which is equal to or greater than the nitrogen fertilization. However, nodulation and BNF are influenced by edaphoclimatic factors that can bring benefits or damages to the process. The availability of nutrients is among the main factors that affect BNF and phosphorus (P) is among the main nutrients that influence this process. Although, P is extracted in smaller amounts when compared with other macronutrients, it is important for the establishment of nodulation, because it increases the number of root hairs and promotes more sites of infection for N_2-fixing bacteria (Nkaa et al., 2014). The efficiency of N_2 fixation is dependent on the availability of P due to its participation in the symbiotic process (Burity et al., 2000). Among the existing sources of soluble P, single superphosphate (SSP), triple superphosphate and thermophosphate are considerably used and high doses are applied in highly weathered soils, due to the process of adsorption to clay minerals and iron and aluminum oxides. SSP has the advantage of adding S to the soil and, consequently, meeting the requirements of the plants regarding this element, while thermophosphate adds Ca and Mg to the soil.

However, due to the high costs of N fertilizers which resource rural poor farmers cannot afford and coupled with losses of N in the soil, contributing to environmental pollution, it becomes necessary to search for techniques that can maximize its use efficiency. The use of atmospheric N through BNF, performed by diazotrophic bacteria in symbiosis with various leguminous species, such as cowpea, can be an important alternative in the total or partial substitution of N fertilizers (Bonilla and Bolanos, 2009). Although, the application of N_2-fixing bacteria in cowpea is important, studies on its behavior in the Amazon region of Rondônia are scarce. Thus, due to the lack of conclusive results, further studies are necessary to evaluate the efficiency of diazotrophic bacteria and P sources and doses in this region.

Therefore, the study aimed to determine the effective rates and sources of soluble P to enhance nodulation, accumulation of nutrients and N and P absorption and use efficiency in cowpea plants inoculated with or without rhizobacteria from soils of the North region of Brazil.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse at the Federal Institute of Education, Science and Technology, Rondônia, Colorado do Oeste-RO Campus, Brazil, at the geographic coordinates of 13° 06' S and 60° 29' W, with mean altitude of 407 m. The climate of the region, according to Köppen's classification, is Awa, tropical hot and humid with two well defined seasons. The soil sample used in the study was classified as Red Yellow Argisol with very clayey texture (EMBRAPA, 2013), collected in the layer of 0 to 20 cm. The chemical analysis of the soil before the experiment resulted in the following values: organic matter (OM): 10.00 g dm^{-3}; pH ($CaCl_2$): 5.30; P: 1.10 mg dm^{-3}; K: 0.14 cmolc dm^{-3}; Ca: 5.56 cmolc dm^{-3}; Mg: 1.15 cmolc dm^{-3}; Al: 0.0 cmmolc dm^{-3}; H+Al: 2.25 cmolc dm^{-3}; SB: 6.90 cmolc dm^{-3}; cation-exchange capacity (CEC): 9.10 cmolc dm^{-3} and base saturation: 75.30%. Granulometric analysis showed 199 g kg^{-1} of sand, 166 g kg^{-1} of silt and 635 g kg^{-1} of clay. The experimental design was randomized blocks in a 5 x 2 x 2 factorial scheme, corresponding to five doses of P, two soluble sources of P_2O_5 and absence and presence of inoculation, with four replicates. P doses were 0, 20, 40, 80 and 160 kg ha^{-1} of P_2O_5, applied at planting. The utilized sources of P_2O_5 were SSP (21% P_2O_5) and thermophosphate (MG Yoorin) in powder (19% P_2O_5).

Based on the results of the soil chemical analysis, basal fertilization was performed to guarantee the establishment of the crop. The 60 kg ha^{-1} of K_2O was applied in the form of potassium chloride. P and K doses were converted to mg kg^{-1} using values of soil density. The micronutrients were applied according to crop demand, in the form of a solution, using deionized water and salts (p.a.), according to Epstein and Bloom (2006). The experimental units consisted of plastic pots with capacity of 10 dm^{-3} filled with air-dried soil and passed through a 4 mm-mesh sieve. The moisture content in the pots was controlled daily by weighing in order to maintain the soil with 60% of field capacity. Irrigation was performed with distilled water.

The seeds of cowpea, cultivar 'BRS Tumucumaque', were inoculated with the commercial inoculant 'TotalNitro Feijão Caupi' (concentration of 10^9 cells g^{-1}) of the strain *Bradyrhizobium* sp. (Semia 6462 and Semia 6463), produced by the company, Total Biotechnology. The utilized dose was 150 mL of liquid inoculant for every 5 kg of seeds. For increase in the diazotrophic bacteria population introduced in the plant and, and consequently, increase in the beneficial effects on the host plant, humic acids were applied

Table 1. F values, mean and CV for plant height (PH), stem diameter (SD), number of nodules (NNOD), mass of nodules (MNOD), root length (RL), root volume (RV), shoot dry matter (SDM) and root dry matter (RDM) of cowpea plants inoculated with rhizobacteria in response to different rates and sources of soluble P. Colorado do Oeste-RO, Brazil (2016).

Source of variation	GL	Pr > F							
		PH	SD	NNOD	MNOD	RL	RV	SDM	RDM
Inoculation (I)	1	0.00*	0.57	0.02*	0.05	0.97	0.61	0.00*	0.00*
Source of P (F)	2	0.75	0.90	0.00*	0.32	0.13	0.31	0.00*	0.06
Rates of P (D)	4	0.62	0.87	0.35	0.28	0.36	0.31	0.07	0.92
I*F	1	0.23	0.78	0.99	0.34	0.06	0.58	0.99	0.99
I*D	4	0.18	0.98	0.24	0.01	0.39	0.84	0.14	0.44
F*D	3	0.71	0.98	0.15	0.24	0.13	0.58	0.19	0.28
I*F*D	3	0.90	0.88	0.19	0.29	0.63	0.68	0.18	0.76
Residue	57								
Medium		76.61	0.65	155.52	2.83	47.19	19.16	12.49	3.36
CV (%)		19.39	20.73	25.24	26.98	26.31	33.41	12.26	16.23

*Significant by Tukey test at 5% probability. CV, coefficient of variation.

together with the inoculation, which were extracted and provided by the Laboratory of Biotechnology of the University of Norte Fluminense – UENF, established in the municipality of Campos dos Goytacazes-RJ, and isolated from vermicompost (Canellas et al., 2005). The material was previously dissolved in water at 50 mg L^{-1}. The humic substance was directly applied on the seeds, inside plastic bags with a volumetric pipette. After application, the plastic bags were closed and agitated vigorously for 2 min for a homogeneous distribution of the product on the seeds. The seeds were placed to germinate directly in the pots and thinning was performed five days after emergence (DAE), leaving only one plant in each experimental unit.

Evaluations were performed at 45 DAE, which corresponds to the phenological stage of full flowering of the crop. Plant height was measured from the base of the plant to the apical meristem, using a graduated ruler, while stem diameter was determined using a digital caliper, at the height of 2 cm from the base of the plant. Plants were later collected and divided into roots and shoots. After that, all the collected plant materials were washed in running water and deionized water, respectively. Root length was determined with a graduated ruler and root volume through the volumetric cylinder method, in which the roots were submerged in a graduated cylinder with a known volume of distilled water and root volume was determined by the difference between the initial and final volumes of the cylinder. During the period, the nodules were removed, counted and dried in an oven at 65°C for 72 h, for later determination of their mass. After drying the plant material, the dry matter was weighed and ground in a Wiley-type mill, and the samples were subjected to sulfuric and nitric-perchloric digestion, for the determination of N and P contents in the different plant parts (roots and shoots), according to the methodology described by EMBRAPA (2009).

The absorption efficiency index, the ratio between the total content of nutrient in the plant and root dry matter, was calculated according to Swiader et al. (1994), while N and P use indices, ratio between the total dry matter produced and the total accumulation of nutrient in the plant were calculated according to Siddiqi and Glass (1981).

The data were subjected to normality test (Shapiro Wilk) and analysis of variance using the computational program for statistical analysis SISVAR. The effects of inoculation, for each source of the nutrient, were evaluated by Tukey test at 0.05 probability level. For the variable with statistical significance as a function of P doses, regression analysis was used for the Student's t-test.

RESULTS AND DISCUSSION

There were significant effects ($p \leq 0.05$) of the interaction of inoculation (I) and P sources (P) on the P content in the shoots, total P in the plant and P absorption efficiency. The other results had no significant effect of interaction and are independently presented for each source, dose and inoculation (Tables 1 and 2).

Inoculation with rhizobacteria in the presence of humic acids influenced plant height, shoot dry matter, root dry matter, N content in the roots, P content in the roots, total N in the plant, N absorption efficiency and P absorption efficiency (Table 3). Plants inoculated with *Bradyrhizobium* sp. (150 mL of liquid inoculant for every 5 kg of seeds) showed increase in plant height, shoot dry matter production and root dry matter production in the order: 20.44, 12.55 and 23.74%. This occurred because, normally, plants accumulate biomass until reproduction stage and, from this stage on, senescence begins, with consequent gradual decrease in biomass. Corroborating the results found in the present study, Zilli et al. (2009, 2011) cultivated cowpea under field conditions and in greenhouse, respectively, and observed higher results of shoot dry matter in treatments inoculated with strains recommended for the cowpea crop. In a similar study, Araújo et al. (2010) evaluating cowpea plants inoculated with *Bradyrhizobium* strain BR3262 in red latosol, observed higher values of root dry matter in inoculated treatments, when compared with the control (without inoculant or fertilization) and the treatment fertilized with N, P and K. Frigo (2013) reported that the production of root dry matter in the treatment inoculated with the strain

Table 2. F values, mean and CV for the N content in the shoots (NCS), N content in the roots (NCR), P content in the shoots (PCS), P content in the roots (PCR), total N in the plant (TNP), total P in the plant (TPP), N absorption efficiency (NAE), N use efficiency (NUE), P absorption efficiency (PAE) and P use efficiency (PUE) of cowpea plants inoculated with rhizobacteria in response to different rates and sources of soluble P (Colorado do Oeste-RO, Brazil, 2016).

Source of variation	GL	Pr > F									
		NCS	NCR	PCS	PCR	TNP	TPP	NAE	NUE	PAE	PUE
Inoculation (I)	1	0.85	0.00*	0.11	0.00*	0.00*	0.08	0.00*	0.39	0.00*	0.49
Source of P (F)	2	0.02	0.00*	0.00*	0.11	0.00*	0.00*	0.00*	0.62	0.00*	0.45
Rates of P (D)	4	0.21	0.67	0.00*	0.94	0.27	0.00*	0.60	0.63	0.05	0.45
I*F	1	0.07	0.99	0.00*	0.99	0.99	0.01*	0.99	0.33	0.01*	0.64
I*D	4	0.20	0.38	0.06	0.13	0.03	0.33	0.14	0.67	0.99	0.56
F*D	3	1.00	0.75	0.06	0.20	0.55	0.08	1.00	0.62	0.16	0.32
I*F*D	3	0.62	0.53	0.42	0.21	0.63	0.12	0.68	0.56	0.59	0.56
Residue	57										
Medium		27.62	20.61	2.89	1.95	408.49	42.23	125.89	0.64	13.01	6.35
CV (%)		10.60	17.49	7.85	18.33	15.98	15.20	20.19	39.97	19.20	58.26

*Significant by Tukey test at 5% probability. CV, coefficient of variation.

Table 3. Effect of rhizobacteria inoculation in the presence of humic acids on plant height (PH), shoot dry matter (SDM), root dry matter (RDM), number of nodules (NNOD), N content in the roots (NCR), P content in the roots (PCR), total N in the plant (TNP), N absorption efficiency (NAE) and P absorption efficiency (PAE) of cowpea plants cultivated in greenhouse and harvested at 45 days after planting in Colorado do Oeste-RO, Brazil (2016).

Inoculation	PH (cm)	SDM (g)	RDM (g)	NNOD (g kg^{-1})	NCR (g kg^{-1})	PCR (g kg^{-1})	TNP (mg)	NAE	PAE
Without	69.85^{b}	11.79^{b}	2.99^{b}	149.75^{b}	17.41^{b}	1.68^{b}	386.49^{b}	105.71^{b}	11.05^{b}
With	84.13^{a}	13.27^{a}	3.70^{a}	160.72^{a}	24.17^{a}	2.25^{a}	432.94^{a}	148.31^{a}	15.19^{a}
Increase (%)	20.44	12.55	23.74	7.32	38.82	33.92	12.01	40.29	37.46

Medium followed by the same letter in the column, do not differ statistically between themselves by Tukey test at 5% probability.

BR 3267 was approximately 3% higher than that in the fertilized treatment. The distribution of dry matter in the plant is a variable that allows discussion on a little-studied process, which is the translocation of photoassimilates, and which often facilitates the understanding of the response of plants in terms of yield (Benincasa, 2003). Gualter et al. (2011) pointed out that N-fixing bacteria can significantly contribute to great supply of N to the plant and, consequently, with increase in plant dry matter.

The number of nodules in the inoculated treatment was approximately 161, showing an increase of 7.32% in relation to the control performed at 45 DAE. These values for number of nodules demonstrate a satisfactory nodulation for the utilization of cowpea cultivar. Xavier et al. (2007) reported a significant increase in the number of nodules up to 50 DAE, but the number reduced after 60 DAE, while the dry mass of the nodules and specific mass of nodules significantly increased up to 70 DAE. This is explained by the fact that the older nodules began to undergo senescence and then decomposition, resulting in

Table 4. Effect of different sources of soluble P on the number of nodules (NNOD), shoot dry matter (SDM), N content in the roots (NCR), P content in the shoots (PCS), total N in the plant (TNP), N absorption efficiency (NAE) and P absorption efficiency (PAE) in cowpea plants cultivated in greenhouse and harvested 45 days after planting (Colorado do Oeste-RO, Brazil , 2016).

Source of P	NNOD (un.)	SDM (g)	NCR (g kg^{-1})	PCR (g kg^{-1})	TNP (mg)	NAE (mg g^{-1})	PAE (mg g^{-1})
Control	138.19[b]	11.95[b]	19.37[b]	2.57[b]	379.35[b]	114.17[b]	11.54[b]
SSP	173.91[a]	14.12[a]	26.34[a]	3.04[a]	488.43[a]	177.52[a]	15.48[a]
Thermophosphate	146.23[b]	12.85[ab]	21.21[b]	2.70[b]	428.75[ab]	131.87[b]	14.21[ab]

Medium followed by the same letter in the column, do not differ statistically by Tukey test at 5% probability.

the decrease of the number of nodules after 56 days.

The N content of the roots, P content of the roots, total N of the plant, N absorption efficiency and P absorption efficiency increased by approximately 38.82, 33.92, 12.01, 40.29 and 37.46%, respectively, with the inoculation of *Bradyrhizobium* sp. in relation to the non-inoculated control as a result of the beneficial effects of rhizobacteria on N and P assimilation by cowpea plants (Table 3). The accumulated amounts of N and P tended to follow the production of dry matter. Hence, it is proved that the population of rhizobium inoculated in the cowpea was able to perform symbiosis and provide the N necessary for the initial development of the crop, and that the inoculant was effective in the process of biological fixation, promoted higher N and P fixation by the roots, when compared with the control treatment (without P or inoculation), and presented itself as a viable alternative for N fertilization in the crop. Corroborating the observed results, Brito et al. (2011) reported that BNF provided the largest part of the N accumulated in cowpea plants.

The effect of the different sources of soluble P was significant ($p<0.05$) on the number of nodules, shoot dry matter, N content in the roots, P content in the shoots, total N in the plant, N absorption efficiency and P absorption efficiency in cowpea plants (Table 4). The single superphosphate (SSP) showed significant difference in relation to the control and the thermophosphate. With regards to the number of nodules, the source SSP contributed to the nodulation which is satisfactory and these results are similar to those obtained by Xavier et al. (2008) and Gualter et al. (2011) in cowpea. These results demonstrated that, besides promoting higher P absorption, the application of SSP can also promote a better use of this element by the plant.

The effect of interaction between inoculation and P sources on P content of the shoots, total P in the plant and P absorption efficiency is presented in Table 5. It is observed that the available SSP in the presence of inoculation with *Bradyrhizobium* sp. promoted increase in P content of the shoots, total P in the plant and P absorption efficiency in cowpea plants. These increases were approximately 21.87, 18.77 and 52.07%, respectively, and there was no significant difference for the source thermophosphate in the presence or absence of inoculation. The source, SSP was also superior to the thermophosphate, because it is more efficient in increasing the P contents of the plant and its absorption efficiency.

The cowpea crop has the characteristic of low P requirement, but it has demonstrated higher and frequent response when cultivated in soil with good availability of the nutrient. In the present study, the P content in the soil was equal to 1.10 mg dm^{-3}, a content considered as very low, which justifies the absence of response to most variables as a function of the increasing doses of P in the soil. Only leaf P contents and the total P in the plant were significantly ($p<0.05$) influenced by the P doses (Figure 1). This occurred due to the greater availability of this nutrient. The highest contents of P in cowpea leaves were obtained with the application of 160 kg $ha^{-1}$of P_2O_5, a rate which is more than that recommended for maintenance fertilization of cowpea in soils of Rondônia. These contents are within the required range considered as adequate for cowpea, which is from 2.6 to 5.0 mg kg^{-1} (Malavolta et al., 1997). Increases in P contents of cowpea leaves in response to P doses were also observed by Silva et al. (2010) that studied doses and forms of application of P in yellow latosol in the state of Roraima. Besides promoting higher P absorption, the application of P also promotes increases in the total P in the plant and, consequently provided better use of the nutrient by the plant. This increase in leaf P content with the application of phosphate fertilization can lead to future increase in grain production observed when the crop is

Table 5. Phosphorus content in the air (PCA), total P in the plant (TPP) and P absorption efficiency (PAE) in cowpea plants inoculated or not with rhizobacteria and fertilized with different sources of soluble P. (Colorado do Oeste-RO, Brazil, 2016).

Source	PCA (g kg^{-1})		TPP (mg)		PAE (mg)	
	Inoculation					
	Without	With	Without	With	Without	With
SSP	2.56^{bB}	3.12^{aA}	42.35^{bA}	50.30^{aA}	11.54^{bA}	17.55^{aA}
Thermophosphate	2.81^{aA}	2.98^{aA}	38.29^{aA}	38.76^{aB}	10.56^{bA}	12.76^{aB}

The lowercase letters separate averages in each column and the case separate the medium inside the line. Same letters do not differ by Tukey test at 5% probability.

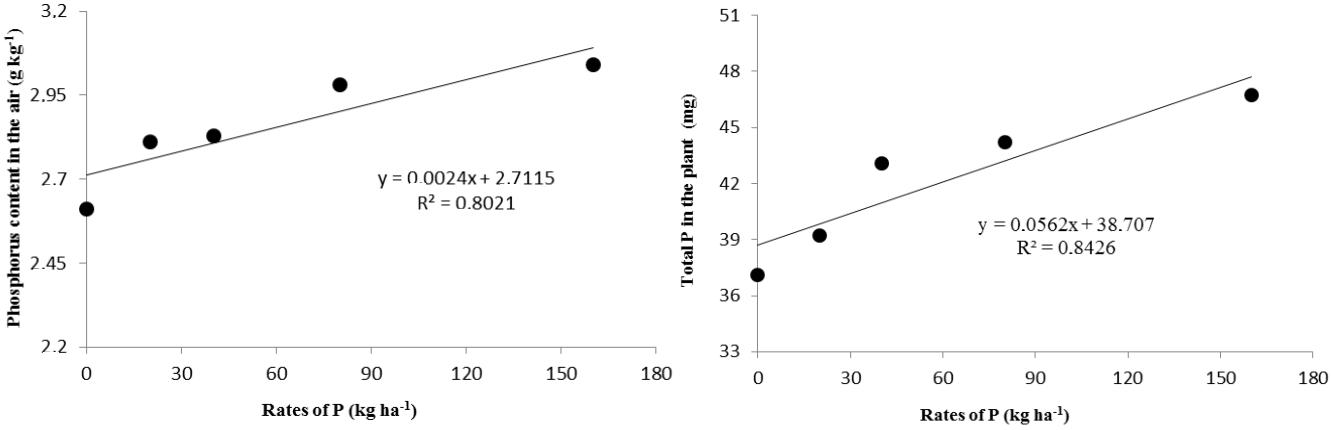

Figure 1. Phosphorus content of the air and total P in cowpea plants fertilized with different doses of soluble P. Colorado do Oeste-RO, Brazil (2016).

cultivated until its phenological stage, considering the previous findings on correlation between leaf contents of nutrients and yields of crops.

Although, there are some experimental results, the studies on the relationships between N, P and inoculation in cowpea in the presence of humic acids in the Western Amazon are in the initial stage, and there is a lot to be studied in tests conducted both in greenhouse and under field conditions for comparisons, and to further strengthen the scientific basis for a new technological process for agricultural production.

Conclusions

Inoculation with *Bradyrhizobium* sp. promotes increase in dry matter production and increase in N and P absorption efficiency in cowpea plants. Single superphosphate leads to higher N and P absorption efficiency, production of shoot dry matter and production of nodules, in comparison with thermophosphate. The inoculation with *Bradyrhizobium* sp. associated with single superphosphate fertilization promotes higher P absorption efficiency in cowpea plants. The increase in P doses promotes increase in the P contents of cowpea leaves, which will contribute to improvement in cowpea productivity in Brazil.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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