Nig. J. Biotech. Vol. 33 (2017) 72-77
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/v33i1.10

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Nitrogen fixation and nodulation of soybean as affected by rhizobial inoculation using different seed adhesives in a sandy clay loam soil.

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(Received: 14:05:2017; Accepted:30 :07:2017)

Abstract

The study evaluated the efficacy of different adhesives added to rhizobial seed inoculum on soybean nodulation and biological nitrogen fixation in a screen house and under field conditions. The experiment was a 6×3 factorial arranged in Completely Randomized Design and Randomized Complete Block Design for the pot and field trials, respectively, with 3 replications. Cassava starch (CSV), corn starch (CS), honey (H), gelatine (G), gum arabic (GA) and water (W) were mixed with Rhizo-fix® at three ratios: 1: 1, 1:2 and 1:3 (inoculants: adhesive).Viable rhizobial cells on coated seeds were determined using plate count technique from serially diluted 10^{-4} aliquot. Nitrogen fixation (NF) was determined using ureide method. Gelatine had most rhizobial cells on seeds (CFU/seed). It had 88, 87 and 84% significant increment when compared with CSV, corn starch CS and GA, respectively. However, CSV and CS considerably had more N fixed than G and was positively correlated with nodule fresh weight. Mixing ratios had no effect on parameters observed. Generally, results obtained by locally sourced adhesives averagely equalled those obtained by the conventional adhesives that had better adhering capability. Thus, locally sourced adhesives could be an alternative to conventional adhesives which are often more expensive and not readily available. Moreover, since mixing ratio had no significant effect on most parameters measured, then the least ratio (1:1) which is probably the most economical should be adopted for rhizobial inoculation.

Keywords: adhesives, inoculation, *Bradyrhizobium japonicum*, nitrogen fixation

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Introduction

Seed rhizobial inoculation is a technology that helps provide N to soil biologically and indirectly reduces the problems associated with the use of inorganic sources of N and its benefits to both soil and the environment cannot be overemphasized. However, many factors such as desiccation are militating against the benefits of seed rhizobial inoculation. Ghalamboran and Ramsden (2009) reported that death of rhizobial cells on seed is majorly attributed to desiccation. This is considered a major problem for African farmers owing to the fact that temperature in the tropics are very high and further expedite the rate of desiccation and death of rhizobial cells on seeds.

The success of Biological Nitrogen Fixation (BNF) in a bid to tackle the insufficiency of N in most agricultural lands, especially in the tropics can only be achieved if the appropriate amount of viable rhizobial cells are been preserved and kept active before the onset of nodulation and nitrogen fixation in order to positively affect plant growth. To improve soybean yield, biological nitrogen fixation, contribution to soil fertility restoration, inoculation with efficient strains of Bradyrhizobia has been tested (Hussain et. al., 2011; Tairo and Ndakidem, 2014). As the use of efficient strains of inoculants is important in BNF studies (Green et.al., 1979, Streeter, 2007) so also is the use of adhesive agents (e.g., gum arabic, , methyl cellulose, and oil) as it helps in increasing the amount of rhizobial cell that adheres to seed (Rugheim, and Abdelgani, 2012).

However, most of these adhesive agents are not readily available and are expensive for local farmers. Hence, there is need to assess and validate the use of other locally available materials which can serve as substitute and also enhance (BNF) and most importantly are readily available and relatively cheap for the resource-poor small holders famers. Therefore this study was undertaken to evaluate the suitability of locally available material used as adhesives for rhizobium seed inoculation in terms of soybean growth, nodulation and biological nitrogen fixation.

Materials and methods

The experiment was carried out at the Federal University of Agriculture Abeokuta, in south-western Nigeria between August 2013 and March, 2014. The study site lies between latitude 7° 12' to 7° 20' N and longitude 3° 20' to 3°28' E with a bimodal rainfall distribution with peak in July and October. The experiment was first carried out as the pot trial and the result obtained was validated on the field.

Seed Inoculation

Carrier-based peat inoculant (Rhizo-fix®) was used, and applied at the rate of 10 g inoculant/kg seed (Rice et. al., 2001) using various adhesives. All adhesives were prepared at 20 % w/v except honey which was already slurry. Rhizo-fix® and the prepared adhesives were applied on seeds at 3 different mixing ratios, first ratio (r_1) 10 g of Rhizo-fix® to 10 ml of adhesive, second ratio (r_2), 10 g of Rhizo-fix® to 20 ml of adhesive and the third ratio (r_3), 10 g of Rhizo-fix® to 30 ml of adhesive. After inoculation the seeds were exposed to air under room condition so as to allow the inoculants to properly stick on seeds. Inoculated seeds were kept in labeled bags and planted immediately after inoculation.

Rhizobial Enumeration

The viability of inoculum was determined using plate count method as described below before the seed were inoculated. Subsamples of 20 intact seeds were randomly picked with a spatula into 20 ml sterile deionised water in a sample bottle, shaken vigorously for 1 – 2 minutes so as to desorb the rhizobial cells from the surface of the seeds into the stock solution. One millilitre from the stock was serially diluted into sequentially labelled 9 ml tubes and 1ml aliquot of appropriate dilutions was spread onto the surface of sterilized plates. Congo Red Yeast Mannitol Agar was aseptically poured into the plates in air-flow (at 60°C); plates were carefully sealed so as to prevent contamination and stored inverted in the incubator at 26oC for 5 days and the numbers of rhizobial colonies were counted. The plates were counted by diving into a quadrant and multiplying the numbers of colonies counted by 4. The mean colony forming unit (CFU) was calculated using the convenient formula (equation 1 and 2) for seed and soil *B. japonicum* count.

For seeds:

$$\text{Mean CFU} = \text{Mean colonies} \times \frac{\text{labeled dilution} \times 10a \times 100b}{20c} \quad \text{........................ Eqn. 1}$$

Where a = Correction value for 1 ml / plate from dilution
b = Correction value for 20 ml in original dilution bottle
c = Correction value for number of seeds in original sample counted.

For soil:

$$\text{Mean CFU} = \text{Mean colonies} \times \text{labeled dilution} \times 10a \times 100b \quad \text{--------- Eqn. 2}$$

Pot Trial

Experimental layout

The experiment was a 6 × 3 factorial combination laid out in Completely Randomized Design with a total of 18 treatment combination replicated 3 times. The treatment were six adhesives (honey, cassava starch, corn starch, water, gelatin and gum arabic) and three mixing ratios of the inoculants and the adhesives (r_1= 10:10, r_2=10:20 and r_3=10:30).

Soil preparation and planting

Soil samples collected from the field location was air-dried and passed through a 2 mm mesh sieve so as to remove debris and stone that could serve as impediments to root growth. The pots were filled with 5 kg of soil. A basal application of Gateway organic fertilizer was added and thoroughly incorporated at the rate of 2 t ha^{-1} to give the starter N nutrition to the soybean plants (Olayinka et. al., 1998). Water was applied to each pot to saturation and the organic fertilizer was left to mineralize for another two weeks before the commencement of planting. This was done so as to ensure that the soil condition was favourable for seed growth. Three inoculated seeds of TGx-1940-2F were planted in each pot; wetting of soil was done every other day while thinning to 2 plants per pot was done at 2 weeks after planting (WAP). Weeding was carried out manually at 3, 7 and 12 WAP.

Field Trial

Experimental layout and soil sampling

The plot size was 3 by 2m with a total of 54 plots. Each plot was separated by a 0.5 m alley, while each replicate was separated by a 1.0 m alley. The field size was 45 by 11m. The treatment involved were the same with that of the pot trial but was laid out using RCBD experimental design. Representative soil samples were taken at random from the field, at 0-15 cm depth using a soil auger. Composite samples were bulked, air dried, gently crushed and passed through a 2 mm and 5 mm sieve based on the type of routine analysis to be determined. The physical and chemical properties of processed soil after processing soil physico-chemical properties was determined using standard methods as described by Agbenin (1995).

Planting and other cultural practices

Experimental plots were fertilized uniformly at the rate of 2 t ha^{-1} with Gateway organic fertilizer to give the starter N effect. Inoculation of seeds took the same trend as that of the screen house. After inoculation the seeds were planted by seed drilling at a space of 60 cm inter row spacing, seeds were covered immediately with soil after sowing to avoid death of cells due to radiation from the sun. Thinning was done to approximately 5 cm between plants in a row at 2 WAP, when the soil was moist and seedlings well established. Weeding was done manually using hoe, on the 3rd and 6th WAP to control weeds.

Data Collection

Five (5) plants were randomly selected and tagged from two middle rows on the field. Data were collected for number and weight of nodules - plants were carefully uprooted from soil within 2 cm of the root so as to obtain intact root and nodules, adhering soil was removed by washing the roots with intact nodules gently with water over a metal sieve. Petioles were used for determination of biological nitrogen fixation using ureide method (Herridge, 1982).

Statistical analysis

Statistical analysis was done using SAS (Statistical Analysis System Inc., 2000). The data collected was subjected to analysis of variance (ANOVA) to determine treatment effect at 5% level of significance. Duncan's Multiple Range Test was used for mean separation. Pearson's Correlation analysis was also employed to test relationship between components.

Results

The soil used for this study is sandy clay loam containing less than 250 g kg^{-1} of clay and silt (Table 1). The pH signifies that the soil reaction is near neutral with low P and N.

Table 1. Physico-chemical properties of the experimental soil

Properties	Values
Sand (g kg^{-1})	758.80
Silt (g kg^{-1})	7.00
Clay (g kg^{-1})	234.20
Textural class	Sandy day loam
pH in H_20 (1:1:5)	6.8
Organic carbon (g kg^{-1})	1.17
Total nitrogen (g kg^{-1})	0.142
Available P (mg kg^{-1})	9.51
Calcium (cmol kg^{-1})	3.60

The different adhesives varied in their adhering ability. The result for the rhizioal load on selected seed prior to planting (Figure 1), gelatine had the highest cfu/g of rhizobial cells on seed surface while cassava starch adhered the least cfu/g of seed.

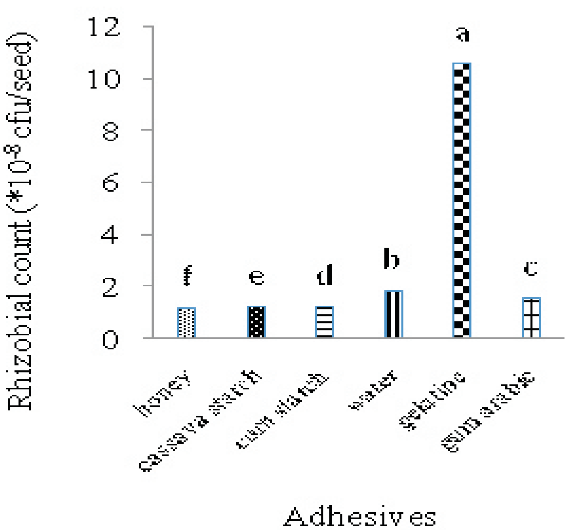
Nodulation

Nodules were formed with all the treatments (Table 2), but nodule formation at 4 WAP in the pot trial was delayed. In the pot trial for nodule count at 8WAP, the various adhesives used had no significant effect ($p<0.05$) on the number of nodule formed but across the adhesives there was significant difference on fresh weight of nodule.

On the field, cassava starch and gum arabic treated plots recorded the highest number of nodules at 8WAP ($p<0.05$). The two treatments also significantly increased the fresh weight of nodule so as corn starch. The trend changed at 12 WAP, as water and cornstarch significantly increased number of nodules taking the place of cassava starch and gum arabic. However, cassava starch and corn starch treated plot recorded nodules with bigger fresh weight when compared to other conventional adhesive tested in this trial. The mixing ratio had no significant effect on all nodulation parameters observed.

Biological Nitrogen fixation

Nitrogen fixation was detected in all treatment examined (Table 3). All treatments had no significant effect on the amount of nitrogen fixed in both pot and field trial. However, cassava starch treated pots fixed an average amount of N that was higher than the amount fixed by other treatment observed. Regardless been the one that adhered the least rhizobial load prior to planting, pots with cassava starch had % increment of 20, 29 , 0.6 , 18 and 7 over honey, corn starch, water, gelatine and gum arabic while on the field, cassava starch had % increment of 6, 11, 15, 18 and 21 in the same sequence as mentioned above.



(Materials with similar alphabets are not statistically different (p <0.05)

Table 2: Effects of adhesives on nodulation and number of pods per plant at 4, 8 and 12 WAP in a pot and field trial.

	Pot trial						Field trial					
Treatments	Nodule count plant^{-1}	Nodule fresh weight (g plant^{-1})	Nodule count plant^{-1}	Nodule fresh weight (g plant^{-1})	Nodule count plant^{-1}	Nodule fresh weight (g plant^{-1})	Nodule count plant^{-1}	Nodule fresh weight (g plant^{-1})	Nodule count plant^{-1}	Nodule fresh weight (g plant^{-1})	Nodule count plant^{-1}	Nodule fresh weight (g plant^{-1})
	4WAP		8WAP		12WAP		4WAP		8WAP		12WAP	
Honey	NA	NA	3	0.095^{f}	3	0.02	3	0.045	1^{bc}	0.018^{bc}	1^{b}	0.02^{b}
Cassava starch	NA	NA	5	0.60^{a}	1	0.02	5	0.082	3^{a}	0.03^{a}	3^{ab}	0.13^{a}
Corn starch	NA	NA	4	0.25^{cd}	1	0.02	5	0.043	1^{ab}	0.02^{a}	5^{a}	0.16^{a}
Water	NA	NA	12	0.48^{b}	1	0.01	7	0.08	1^{ab}	0.002^{b}	4^{a}	0.06^{ab}
Gelatine	NA	NA	8	0.18^{e}	1	0.02	5	0.04	0^{b}	0.002^{b}	4^{ab}	0.05^{ab}
Gum arabic	NA	NA	10	0.35^{cd}	1	0.01	5	0.06	3^{a}	0.02^{a}	2^{ab}	0.04^{b}
Mixing ratio												
1	NA	NA	7.44	0.28	1	0.01	5	0.06	0.03	0.03	3	0.07
2	NA	NA	5.68	0.19	2	0.02	4	0.05	0.02	0.02	3	0.05
3	NA	NA	7.68	0.25	1	0.01	4	0.06	0.01	0.01	3	0.10

Table 3. Percentage N fixed at 8 WAP in pot and field trial.

	% NF Pot trial	% NF Field Trial
Adhesives		
Honey	45.99	57.45
Cassava starch	55.11	61.02
Corn starch	42.78	54.97
Water	54.78	52.85
Gelatine	46.42	51.76
Gum arabic	51.57	50.48
	Ns	Ns
Mixing ratio		
1	49.06	55.50
2	52.33	54.54
3	46.94	54.24

(Materials with similar alphabets are not statistically different (p <0.05)

Discussion

The soil used for the experiment is sandy clay loam containing less than 30 % of clay and silt; implying that the soil is well aerated and the water and nutrient holding capacity of the soil is low. The available phosphorus was relatively low and this was not considered as a limiting nutrient since soybean is more efficient at producing good yield at low soil phosphorous levels than other major agronomic crops (Abassi et. al., 2008). The soil pH was near neutral while the soil nitrogen content was very low. These conditions were favourable as high N soil content tends to circumvent the benefits of BNF and *B. japonicum* also thrives well under near neutral pH conditions (Lapinskas, 2008). The adhesives studied all differed in their adhering capability which could be as a result of the adhesives tenacity and viscosity at varying concentrations. Among the adhesives tested under this study, gelatine was the best in terms of adherence of peat inoculant to seeds before planting; as this translated to gelatine having lot more of viable rhizobial cells attached to the seed. This could be as a result of gelatine being able to form a film when it is mixed in thin layer over a surface becoming very tenacious in nature as reported by Johnston-Banks (1990).

In this study, nodulation and % nitrogen fixed varied under the different adhesive treatments. One would assume that pots treated with gelatine; that adhered the most cfu/seed of B. japonicum would have a more positive effect on the nodulation parameters observed but cassava starch and corn starch which was among the adhesives that had least cfu/seed of B. japonicum increased these parameters throughout the period of observation. Conversely, nodule fresh weight of cassava starch was the highest as compared to that of water that had the highest number of nodules or with that of gelatine that adhered the most inoculants. Nodule fresh weight per plant is considered the most important nodulation parameter examined as observed by many researchers (Khurana et. al., 2001; Fatima et. al., 2012; Saleh et. al., 2013). The findings also corroborates with that of Zoundji et. al. (2015) and Masresha and Kibebew (2017) that observed a significant and positive correlation existed between nodule number, biomass production, nitrogen uptake and grain yield. So, increases of nodule number involve better nitrogen nutrition which provided a good development of shoot and root of the plant and improved grain yield. In this study, cassava and corn starch considerably had more nitrogen fixed when compared with other conventional adhesives. Cassava and corn starch were least effective in terms of adherence of peat based rhizobium inoculants on the seed but also with the highest percentage of N fixed on the long run, this suggests that the few bacteria that adhered were preserved and active till the onset of nodulation and nitrogen fixation in the plant. The preservative ability of cassava starch can also be linked to its ability to limit the quantity of heat dissipated from the soil and high water activities as reported by Mugnier and Jung (1985).

Conclusion

Cassava and corn starch were strong competitor with conventionally used adhesives, especially in terms of yield and nitrogen fixation making them have an edge over other adhesives studied. Beside these, they are relatively inexpensive and readily available in Nigeria. Since all mixing ratios had no effect then the least ratio (1:1) which is the most economical should be adopted for rhizobial inoculation.

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