Scientia Horticulturae 162 (2013) 374–379

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effects of biofertilizer with diazotrophic bacteria and mycorrhizal fungi in soil attribute, cowpea nodulation yield and nutrient uptake in field conditions

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ARTICLE INFO

Article history:
Received 1 July 2013
Received in revised form 8 August 2013
Accepted 8 August 2013

Keywords:
Acidithiobacillus
Glomus etunicatum
AMF
Free living diazotrophic bacteria
Nutritional status
Organic matter

ABSTRACT

Biofertilizer made from rocks with *Acidithiobacillus* releases nutrients for plants and reduces the environmental impact of fertilizer on the soil. The aim of this study is to evaluate the effects of PK rock biofertilizers mixed with earthworm compound and inoculated with free living diazotrophic bacteria and arbuscular mycorrhizal fungi (AMF) on the nodulation, yield and nutrient uptake of cowpeas (*Vigna unguiculata*) and on the soil attributes in field conditions. In a greenhouse experiment, three rates (4.0 t ha^{-1}, 6.0 t ha^{-1}, and 8.0 t ha^{-1}) of biofertilizer were compared with the addition of AMF (*Glomus etunicatum* and *Gigaspora albida*). A treatment with earthworm compound (20 t ha^{-1}) without AMF was used as a control. In the field experiment, the treatments were as follows: three rates of biofertilizer (4.0, 6.0 and 8.0 t ha^{-1}); mineral fertilizer at the recommended rate (RR) and two times RR; and earthworm compound (20 t ha^{-1}). *G. etunicatum* was applied to all treatments. In both experiments, the seeds were inoculated with effective *Bradyrhizobium* strains. In the greenhouse experiment, the highest rate of biofertilizer showed the best results for all analyzed parameters. *G. etunicatum* produced the higher biomass yield of cowpeas but was not competitive with the indigenous AMF in the soil. In the field experiment, the highest rate of mixed biofertilizer effectively increased the grain and shoot biomass yield and increased the available P and K in soil. The mixed biofertilizer showed potential as an alternative for mineral NPK fertilization and seems to be an important factor for soil quality and long-term fertilization.

1. Introduction

The growing world population and the demands for fertilizers have led to changes in agricultural cropping systems and have intensified the research on new techniques to produce maximum yields from field crops. This is especially true in Brazil, where the soil is acidic and deficient in mineral nutrients, particularly phosphorus (P) and potassium (K).

Several studies of the acidic soils in the Brazilian rainforest region have shown the effects of nutrient unavailability on the plant yield and have shown that is necessary to control this problem to improve the productivity and the quality of crops (Stamford et al., 2007, 2008, 2009, 2011). Therefore, it is necessary to find strategies to increase the nutrient availability for plants grown in soils with low content of P and K. Rooney et al. (2011) reported that AMF is beneficial for plant growth by exploiting more nutrients from the soil minerals.

Powdered rocks are not commonly applied in fertilization, and to be used as fertilizer is necessary to increase the availability of the nutrients contained in the minerals, by physical, chemical or microbiological processes, because in general rocks present low available nutrients to promote satisfactory plant growth and yield (Van Straaten, 2007). Recent studies have described the potential of rock biofertilizers from natural phosphate and potassium bearing rocks to increase nutrient availability (Stamford et al., 2006, 2007). However, the P and K rock biofertilizers contain no nitrogen and do not supply N to improve plant growth and yield.

The incorporation of organic matter increases the microbiological activity and enhances the physical and chemical conditions of the soil, even though these materials have a low N content. Biological nitrogen fixation (BNF) and mycorrhizal fungi are included in the most fundamental processes that increase N in the soil and, in combination with organic matter decomposition, influence soil life (Stamford et al., 2007; Aryal et al., 2003). Thus, it is important to

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http://dx.doi.org/10.1016/j.scienta.2013.08.019

enrich in N in the organic matter to produce a complete biofertilizer. The N content can be increased by inoculation with effective free living diazotrophic bacteria, as reported by Lima et al. (2010).

Studies of the tableland soils of the Brazilian Northeast showed that elemental sulfur inoculated with *Acidithiobacillus* is a promising material for soil fertilization because *Acidithiobacillus* releases enough P and K from rocks and increases nutrient availability in soils cultivated with different species (Stamford et al., 2008, 2009, 2011). Biofertilizers produced from P and K rocks and elemental sulfur inoculated with oxidizing bacteria produces sulfuric acid and release nutrients (El Tarabily et al., 2006). However, to provide N for plants and to control the effects of the low pH, the rock biofertilizers need to be mixed with organic matter with a high pH (Lima et al., 2010).

The incorporation of organic matter in the soil and the addition of arbuscular mycorrhizal fungi (AMF) are methods that promote soil fertility and plant growth by increasing the nutrient availability and nutrient absorption, especially in reference to P, a nutrient that is found in low levels in tropical soils (Stamford et al., 2007; Aryal et al., 2003).

The aim of this study was to evaluate the influence of PK rock biofertilizer mixed with earthworm compound and enriched in N by inoculation with effective free living diazotrophic bacteria on cowpea nodulation, yield and nutrient uptake. Additionally, we evaluated the effects of the fertilizer conditions on the nutrient availability in an acidic soil from the rainforest region of Pernambuco compared with mineral soluble fertilizers in field conditions. The effect of AMF was also observed in fertilized plants.

2. Materials and methods

2.1. Rock and organic biofertilizer production

The P and K rock biofertilizers were produced at the Horticultural Experimental Station of the Federal University Rural of Pernambuco (UFRPE) using two furrows (each 10.0-m long, 1.0-m wide and 0.5-m deep). For each biofertilizer, 4000 kg of natural phosphate with total P of 240 g kg^{-1}, purchased from Irecê (Bahia), Brazil, and 4000 kg of potash rock (biotite) with total K of 100 g kg^{-1}, from Santa Luzia (Paraiba), Brazil, was mixed with 400 kg of elemental sulfur and inoculated with *Acidithiobacillus* bacteria, following the procedure described by Stamford et al. (2007).

The sulfur oxidizing bacteria were grown in 2000 ml Erlenmeyer flasks that contained 1000 ml of specific culture medium (El Tarabily et al., 2006) and had been sterilized for 30 min at 120 °C. The Erlenmeyer flasks were shaken (150 rpm) for 5 days at 30 °C. The materials (phosphate and potash rocks mixed with elemental sulfur) were incubated for 60 days, and the humidity was maintained at a level that was near the field holding capacity. To avoid the effects of excessive humidity due to rain and to increase the efficiency of the oxidizing bacteria, the furrows were covered daily using black plastic.

Analysis of the P and K rock biofertilizer, extracted by (A) Mehlich 1 solution and (B) citric acid according to Embrapa (2009), yielded the following results: (P-biofertilizer)-pH = 3.8, available P (A) = 60 (g kg^{-1}) and (B) = 48 (g kg^{-1}); (K biofertilizer-BK) – pH = 3.3, available K (A) = 10 (g kg^{-1}) and (B) = 0.5 (g kg^{-1}).

The organic biofertilizer with earthworm compound enriched in N was produced in field conditions with selected free living bacteria (NFB 10001) that was cultured in LG liquid media (50 ml) in 125 ml Erlenmeyer flasks and shaken (180 rpm) for 96 h at 28 ± 5 °C temperature, according to the methodology of Lima et al. (2010). After inoculation, the material was incubated for 30 days following the same process described above for the PK rock biofertilizer, and the humidity was maintained near water holding capacity. Samples were collected, and the total N determined by the Kjeldhal method, using the Kjeltec auto analyzer (1030 Model). The results of the chemical analysis of the mixed biofertilizer (NPKB) are as follows: pH 7.15; organic carbon 100.7 g kg^{-1}; total N 20.6 g kg^{-1}; total sulfur 12.9 g kg^{-1}; total P 11.2 g kg^{-1}; total K 10.1 g kg^{-1}.

2.2. Greenhouse experiment

The greenhouse experiment used soil samples collected from the same area as the field experiment, classified as sand texture Spodosol Ferrocarbic Ortic (Embrapa, 2006). The field experiment was carried out at the Experimental Station of the Institute of Agronomic research (IPA) located in the District of Itapirema (rainforest region of Pernambuco state), Northeast Brazil, at 7°59′0″, of south latitude, 38°19′16″of west longitude. The soil chemical analysis (Embrapa, 2009) showed the following: pH (H_2O 1:2.5) = 6.0; ($cmol_c$ dm^{-3}), A + Al^{+3} 3.18; Al^{+3} 0.05; Na^{+} 0.05; K^{+} 0.09; Ca^{+2} 1.25; Mg^{+2}0.65; P 10.5 (mg dm^{-3}); Organic Carbon 6.72 (g kg^{-1}); N 0.65 (g kg^{-1}).

The greenhouse experiment was set up in a factorial (4 × 3) + 1 and was conducted in a randomized block design with four replicates. The following fertilization treatments were used: (1) NPKB biofertilizer from PK rocks and earthworm compound (4.0 t ha^{-1}); (2) biofertilizer NPKB, (6.0 t ha^{-1}); (3) biofertilizer NPKB (8.0 t ha^{-1}); and (4) Soluble mineral fertilizer (NPKF) applied at recommended rate for cowpeas (IPA, 2008). The following treatments with AMF additions were used: (1) *Glomus etunicatum* (2) *Gigaspora albida* and (3) without AMF. A control treatment without AMF containing earthworm compound (20.0 t ha^{-1}) was used for comparative purposes.

The mycorrhizal fungi (*G. etunicatum* and *Gigaspora margarita*) were purchased from the Institute Agronomic of Pernambuco (IPA), were multiplied in trays (6 L) containing sterilized soil and vermiculite clay in the same proportion (1:1), and were grown in millet grass for 30 days. In roots (10 cm^3) were determined at approximately 386 spores for *G. etunicatum* and 418 spores for *Gigaspora margarita*. Mycorrhizal fungi were added with the application of the mixed substrate (10 cm^3) and millet grass roots (10 cm^3) for each plant. The nodulation and shoot biomass were determined for each plant.

Prior to sowing, the seeds of cowpea (cv. IPA 206) were inoculated with strains NFB 700 and BR 3267, selected for cowpea in field conditions. The inoculant was produced at the Soil Microbiology Laboratory (University Federal Rural of Pernambuco) and the strain grown in a yeast mannitol medium that was prepared at a minimal concentration of 10^8 (UFC mL^{-1}), in accordance with Stamford et al. (2008).

2.3. Field experiment

The field experiment was carried out in a block design with four replicates. The following fertilization treatments were used: (1) biofertilizer (NPKB) 4.0 t ha^{-1}; (2) NPKB 6.0 t ha^{-1}; (3) NPKB 8.0 t ha^{-1}; (4) mineral fertilizers (NPKF) at the recommended rate (RR); (5) NPKF (two times RR); (6) PK rocks (2000 kg ha^{-1}); and (7) a control treatment with earthworm compound (20 t ha^{-1}). All of the treatments were inoculated with *G. etunicatum* mycorrhizal fungi.

Fertilizers treatments were estimated following the recommended rate for irrigated cowpea in Pernambuco state (IPA, 2008), applying the mixed soluble fertilizer (NPKF) with the following: N fertilizer (ammonium sulfate – 400 kg ha^{-1}) + P fertilizer (simple super phosphate – 500 kg ha^{-1}) + K fertilizer (potassium sulfate – 100 kg ha^{-1}). The biofertilizer NPKB was calculated based on the total N analyzed in the NPKB (20 g kg^{-1}) that corresponded to application of 4000 kg ha^{-1} (10 times ammonium sulfate applied).