Scientia Horticulturae 209 (2016) 236–240

Contents lists available at ScienceDirect

Scientia Horticulturae

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

The impact of biofertilizers with diazotrophic bacteria and fungi chitosan on melon characteristics and nutrient uptake as an alternative for conventional fertilizers

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ARTICLE INFO

Article history:
Received 12 April 2016
Received in revised form 15 June 2016
Accepted 18 June 2016
Available online 7 July 2016

Keywords:
Cucumis melon
Nutrient uptake
Organic agriculture
Organic matter
Rock biofertilizers

ABSTRACT

Fertilization is one of the most important factors to improve plant characteristics and nutrient uptake. Biological N2 Fixation (BNF) is a process of great importance in crop production systems, to enhance sustainability and prevent land degradation in modern agriculture. The impact of the biofertilizer (NPKB) produced from PK rock biofertilizer mixed with earthworm compound enriched in N by free living diazotrophic bacteria and of the bioprotector (NPKP) with chitosan from *Cunninghamella elegans* were investigated in a field experiment with melon. The study compared the influence of biofertilizers with soluble fertilizers (NPKF) on the melon characteristics and nutrient uptake. The experiment was conducted (February–April 2012) in the Bahia state, Brazil, and the treatments were NPKP (4 t ha^{-1}); NPKP (8 t ha^{-1}); NPKP (12 t ha^{-1}); NPKB (8 t ha^{-1}); NPKB (12 t ha^{-1}); and NPKF (soluble mineral fertilizers) at the recommended rate for irrigated melon. A control with an earthworm compound (20 t ha^{-1}) that was not inoculated with diazotrophic bacteria was added as a comparison. The results demonstrated a positive effect of NPKP and NPKB, especially when applied at a higher rate (12 t ha^{-1}), which increased the commercial characteristics of melon compared with the soluble fertilizer applied in the recommended rate. Nutrient uptake in the melon fruits indicated a significant difference among the different fertilizer sources and the best results were displayed with NPKB and NPKP applied at the highest rate (12 t ha^{-1}), especially for total S-$SO4^{-2}$. Therefore, NPKP and the NPKB are potential alternatives to soluble fertilizers (NPKF).

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1. Introduction

Increasing world population and demands for fertilizers and pesticides have led to sensible changes in agricultural systems and intensification of the use of new techniques to maximize yields (Lima et al., 2010). Fertilization with NPK is important factor to attend plant productivity and nutrient uptake. The addition of NPK fertilizers in recommended rate may increase yield, plant characteristics and food quality, especially in tropical soils (Stamford et al., 2008).

Soluble fertilizers are of great importance for plant yield but their use by low-income farmers is prohibitive due to cost. Furthermore, soluble nutrients may lixiviate to the deeper soil layers and promote plant damage (Van Straaten, 2007). In modern and sustainable agriculture, the application of soluble fertilizers and soil amendments may increase food production and meet needed economic criteria to increment soil fertility while minimizing environmental damage (Stamford et al., 2008).

Despite these facts, Brazilian soils are generally low in available P, and renewable and natural sources of phosphate are absolutely necessary and important for the efficient use of these products in agriculture (Van Straaten, 2007; Oliveira et al., 2014). The great demand for fertilizers and the public's desire to reduce environmental problems, especially the scarcity of primary material to produce soluble fertilizers, has resulted in an increasing drive for research to study alternative fertilizer sources for use in sustainable agriculture (Lima et al., 2007; Moura et al., 2007; Stamford et al., 2009).

An effective and economic fertilization is the production of new biofertilizers from phosphate and potash rocks with the addition of

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http://dx.doi.org/10.1016/j.scienta.2016.06.027
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elemental sulfur inoculated with *Acidithiobacillus*; because sulfur-oxidizing bacteria are important in recycling nutrients in the soil and some species are important for the release of elements from rocks (Van Straaten, 2007; Stamford et al., 2006, 2008).

Nitrogen is one of the most important nutrients for increasing plant growth and yield, and due to its role in some chemical compounds as proteins, nucleic acids and many other components, it is necessary for all life. However, P and K rock biofertilizers do not provide N to be utilized by plants and microbial organisms in the soil. However, the mixture of rock biofertilizers with organic matter, such as an earthworm compound inoculated with free-living diazotrophic bacteria, was shown to be effective in the enrichment of N by the process of biologic nitrogen fixation (BNF) as reported by Lima et al. (2010), and this process may be important for increases in soil fertility.

Mixed biofertilizer (NPKB) provides nutrients for plants, especially when inoculated with fungi that contain chitosan in their biomass (cell walls) such as *Cunninghamella elegans* (Franco et al., 2004). However, a bioprotector (NPKP) expresses antimicrobial properties for plant protection against pathogens and increases the availability of nutrients (Franco et al., 2011).

The aim of this study was to evaluate the effectiveness of a biofertilizer enriched in N (NPKB) and a bioprotector (NPKP) with the addition of fungi chitosan from *C. elegans* on the characteristics and nutrient uptake by melon (*Cucumis melo*) grown in an Argisoil from Southwestern Bahia, Brazil. The biofertilizers with NPKB and NPKP were compared with the mineral soluble fertilizer (NPKF) and the earthworm compound (which was not enriched in N by diazotrophic bacteria) to verify the possibility of their use as an alternative for conventional NPK soluble fertilizer.

2. Materials and methods

2.1. Production of biofertilizers (BNPK) and the bioprotector (NPKP)

The PK rock biofertilizers were produced at the Federal Agricultural University of Pernambuco (UFRPE), Horticultural Experimental Station, using two furrows (10 m long, 1 m wide and 0.5 m deep). To produce the P and K biofertilizer, 4000 kg of natural phosphate for a total P = 110 (g kg^{-1}) was purchased from Irecê (Bahia), Brazil and was applied along with 4000 kg of potash rock (biotite), purchased from Santa Luzia (Paraiba), Brazil for a total K = 100 (g kg^{-1}) and following the procedure described by Stamford et al. (2007).

The sulfur-oxidative bacteria were grown in 2000 mL Erlenmeyer flasks that contained 1000 mL of culture specific medium (El Tarabily et al., 2006) and were sterilized for 30 min at 120 °C. The Erlenmeyer flasks were shaken (150 rev min^{-1}) for 5 days at 30 °C. The materials (phosphate and potash rocks plus elemental sulfur) were incubated for 60 days, and the water was maintained at a level near the field holding capacity. To avoid excessive humidity, due to the rain and to increase the efficiency of the oxidative bacteria, the furrows were covered with black plastic. The analysis of the P and K biofertilizer (using methodology) (A) Mehlich 1 and (B) extraction with 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) – pH = 3.3, available K (A) = 10 (g kg-1) and (B) = 5 (g kg^{-1}).

The biofertilizer (NPKB) was processed by mixing PK rock fertilizers with an earthworm compound enriched in N by inoculation with the selected free-living bacteria (NFB 10001) in accordance with Lima et al. (2010). The analysis of the earthworm compound showed: pH 7.95; organic carbon (100.7 g kg^{-1}); total N (8.6 g kg^{-1}); total S (2.98 g kg^{-1}); and total P (1.12 g kg^{-1}). The rock biofertilizer (PKB) and the organic biofertilizer (OB) were mixed in a proportion of 1:4 (PKB: OB), inoculated with free-living bacteria (NFB 10001) and incubated for 30 days. The NPKB was analyzed (Embrapa, 2009) and showed: pH (H_2O) = 5.9; total N = 19 g kg^{-1}; available P = 20 g kg^{-1} and available K = 19.9 g kg^{-1}. The bioprotector (NPKP) represents the biofertilizer (NPKB) inoculated with *Cunninghamella elegans* (UCP 542), which are fungi that contain chitosan in their cellular wall (Franco et al., 2004). The fungus *C. elegans* was purified in Petri dishes with potato dextrose agar (PDA) grown for 10 days at 28 °C. The monosporic culture of *C. elegans* was obtained by growing the Mucorales fungus in Potato - Dextrose (BD) and using 2000 mL Erlenmeyers flasks (containing 1000 mL) that were shaken (180 rotations per minute) for 96 h at 28 °C. The culture was diluted in distilled water (20 L^{-1}) that was applied by manual irrigation.

For the production of the bioprotector (NPKP), the NPKB from PK rocks were mixed with the earthworm compound and incubated for 30 days, and the chemical analyses at the final period of incubation showed: pH = 6.4, total N = 20 g kg^{-1}; available P = 21 g kg^{-1} and available K = 19 g kg^{-1}.

Table 1
Number and average weight of melon fruits as affected by the different fertilization treatments (Bioprotector, Biofertilizers and mineral soluble Fertilizers).

Fertilization treatments (t ha^{-1})	Number of fruits	Weight of fruits (kg $fruit^{-1}$)
Control	6116 ± 114^{C}	0.79 ± 0.04^{C}
NPKP 4	8340 ± 84^{B}	1.21 ± 0.11^{B}
NPKP 8	9452 ± 57^{AB}	1.25 ± 0.05^{B}
NPKP 12	10286 ± 36^{A}	1.43 ± 0.03^{A}
NPKB 8	9074 ± 75^{AB}	1.16 ± 0.04^{B}
NPKB 12	9174 ± 86^{AB}	1.40 ± 0.06^{A}
NPKF*	10842 ± 73^{A}	1.45 ± 0.07^{A}
C.V. (%)	8.61	3.85

* Followed the recommendation for melon (IPA, 2008). Means followed by the same letter in columns are not significant by the Tukey test ($p < 0.05$).

2.2. Site, soil and experimental conditions

The field experiment was conducted at the Experimental Station of the Federal Institute of Education Science and Technology, Bahia State, Brazil. The soil was classified as "Red Yellow Argisoil medium texture" (Embrapa, 2013) with a low availability of P and K and was predominantly cultivated with fruits (melon, mango, and grape), cotton and cowpea legumes for subsistence purposes. The climate was of a BSwh' type in accordance with the Köppen classification. The chemical analyses of soil, collected at 0–20 cm deep, showed: pH (H_2O) = 6.2; organic matter (g kg^{-1}) = 12.31; P (Mehlich 1) = 22 mg dm^{-3}; exchangeable cations (cmolc dm^{-3}) K = 0.46; Ca = 4.05; Mg = 2.6; Na = 0.12; Al = 0.0 and H + Al = 2.72. The physical analyzes showed: particle density (g cm^{-3}) = 2.61; bulk density (g dm^{-3}) = 1.40; sand (g kg^{-1}) = 700; lime (g kg^{-1}) = 90 and clay (g kg^{-1}) = 210.

One month before the field experiment was conducted, the melon seedlings (Siemens hybrid "10.00") were grown in polypropylene trays (450 cells) on the commercial substrate "Vivatto Slim". The seedlings were sown on February 02, 2012 and transplanted manually afterwards.

Before planting (10 DBP), the soil was prepared for melon cultivation by cutting and removing all of the experimental area vegetation and was followed with conventional tillage of one plowing and two diskings. The rows were opened for planting the melon seedlings and applied the fertilization treatments. Each plot have four rows 10 m long and 8 m wide, and plants spaced 2.0 m × 0.5 m. To evaluate the melon yield were harvested 36 plants of the two central rows. Irrigation was based on the tensiometer methodology and was installed in the soil at 20 cm deep and 10 cm distance

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