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Yield, nutrient export and microbiological quality of snap bean grown with swine biofertilizer

Produtividade, exportação de nutrientes e qualidade microbiológica do feijão-vagem cultivado com biofertilizante de suíno

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Abstract

Adequate soil fertilization with organic fertilizer improves plant productivity, allowing healthy food production, besides reducing dependence on mineral fertilizers. This work aimed to evaluate the effect of swine bio-fertilizer doses on plant nutritional status and yield, nutrient extraction and export, and microbiological quality of organically cultivatedsnap-bean, MacarrãoTrepador cultivar. The experiment was developed using a randomized block design with five fertilizer doses (0; 30; 60; 90 and 180 m^3 ha^{-1}), and four repetitions. Each plot contained forty 1.0 m x 0.3 m spaced plants. Greater pod length (14.6 cm), larger number of pods per plant (65), and higher yield (16.5 t ha^{-1}) were obtained when higher bio-fertilizer doses were applied. Fertilization with swine bio-fertilizer improved the plant's nutritional status and increased the culture's yield from 5.9 to 16.5 t ha^{-1}. Macronutrients extracted by plants in the dose that promote the highest yield (kg ha^{-1}) were: 98.1 of N, 10.5 of P; 48.1 of K; 35.0 of Ca; 12.2 of Mg and 6.6 kg ha^{-1} of S. The fertilization with swine biofertilizer increased yield and improves the nutritional status of plants. Biofertilizer application and irrigation with low microbial load water produced microbiological quality pods according to ANVISA standards.

Additional keywords: nutrient extraction; organic fertilization; *Phaseolus vulgaris* L.; plant nutrition.

Resumo

A fertilização adequada do solo com adubos orgânicos melhora a produtividade das plantas e permite obtenção de alimentos saudáveis, além de reduzir a dependência de fertilizantes minerais. Neste trabalho, objetivou-se avaliar o efeito de doses de biofertilizante de suíno sobre o estado nutricional e produtividade das plantas, extração e exportação de nutrientes e qualidade microbiológica das vagens de feijão-vagem, cv. Macarrão Trepador/Favorito, cultivado em sistema orgânico. O experimento foi desenvolvido em delineamento de blocos casualizados com cinco doses de biofertilizante (0; 30; 60; 90 e 180 m^3 ha^{-1}), com quatro repetições. Cada parcela continha 40 plantas espaçadas em 1,0 x 0,3 m. O maior comprimento de vagem (14,6 cm), maior número de vagens por planta (65) e maior produtividade (16,5 t ha^{-1}) foram obtidos com a maior dose de biofertilizante. A produtividade de vagens passou de 5,9 t ha^{-1} para 16,5 t ha^{-1}, em relação à testemunha. Os valores de macronutrientes extraídos pelas plantas, na dose de maior produtividade, foram: 98,1; 10,5; 48,1; 35,0; 12,2 e 6,6 kg ha^{-1} para N, P, K, Ca, Mg e S, respectivamente. A adubação com biofertilizante de suíno promove aumento na produtividade e melhora o estado nutricional das plantas. A aplicação do biofertilizante e água de irrigação com baixa população de microrganismos produz vagens com qualidade microbiológica adequada, conforme padrões da ANVISA.

Palavras-chave adicionais: adubação orgânica; extração de nutrientes; nutrição de plantas; *Phaseolusvulgaris* L.

Introduction

Snap bean (*Phaseolus vulgaris* L.) is a vegetable that stands out for its social, economic and commercial importance and for the pod nutritional value. It is grown mainly by family farmers, using, preferably, indeterminate growth cultivars, in supported system, and spacing of 1.0 x 0.30 m (Peixoto et al., 2002). Harvest of cultivars with indeterminate habit starts about 40 days and pods may be harvested until about 70 days after planting (Vieira et al., 2014). The plant has symbiotic association with N_2-fixing bacteria, what enables soil fertility improvements through fixation of atmospheric N_2 in the plant weight, whichreleases its constituent elements to soil and plants when mineralized (Pelegrinet al.,2009). Moreover, the species responds well to organic fertilization with increased yield and production quality (Araújo et al., 2001).

For a sustainable agriculture, the combination of production systems is important for the use of organic waste, which may be used as a source of nutrients to improve crop yield, in addition to soil enrichment. However, for a proper and safe organic fertilization, some factors linked to crop and soil that influence the plant physiology and nutrition and soil fertility need to be considered in order to achieve success from an agronomic point of view, without environmental risks.

In Minas Gerais, activities related to pig farming have a prominent place in Brazilian agribusiness. This is a very important economic activity; however, it also generates large volumes of waste with pollution potential. The proper disposal of these wastes is very important to the environment, since it is an internal problem of rural properties. Emphasis has been given to the development of research for recycling and use of nutrients of these wastes, considering their effectiveness with regard to soil fertilization and plant nutrition, or complement to mineral fertilizer, due to the low carbon/nitrogen ratio and the existing content of macro and micronutrients (Simioni, 2001; Sediyama et al., 2008; Santos et al., 2012; Sediyama et al.,2014).

The use of pig manure in agriculture can be achieved through a simple process called anaerobic digestion and meet good practices for application and incorporation into the soil, since animal manures can become a source of contamination of food and soil by undesirable microorganisms. Thus, anaerobic digestion of organic matter present in manure is of great importance, before it is applied to the soil, reducing the chances of contamination by pathogenic microorganisms, in addition to improving the quality of manure (Abreu Neto & Oliveira, 2009; Pereira et al., 2009).

Treatment and application of pig manure, in the dose and frequency prescribed by professionals, are needed due to the environmental impacts and the limited soil capacity to recycle the nutrients applied on it. Studies with fertilizer doses and interactions can eliminate waste and avoid phytotoxic effects because very high doses of organic and or chemical fertilizers can cause the imbalance of the relation between nutrients and also cause the salinization of soils and impair crop yields (Araújo et al., 2007; Vidigal et al., 2010). According Menezes Júnior et al. (2013) the addition of nutrients using biofertilizers in soil and leaves is unable to provide required nutrients because they generate imbalances that decrease the growth and development of onion crop.

Research aiming the use of pig manure as a source of nutrients were carried out, including an emphasis on the production of vegetables such as snap beans (Araújo et al., 2001); onion (Vidigal et al., 2010); sweet corn (Santos et al., 2011); pumpkin (Santos et al., 2012), among others. However, there are questions regarding plant nutrition and sanitary quality of food that may come out during its production, when organic waste is used. Unfavorable sanitary conditions in rural and urban areas promote this contamination, turning vegetables in pathogen transmission vehicles. Thus, the implementation of microbiological analysis is important to assess the food quality and make sure of its quality for human consumption (Falavigna et al., 2005; Sousa, 2006).

Coliforms are a group of enterobacteria present in the faeces and the environment, such as soil and surface of plants, animals and utensils. Their search in food is used as a reliable indicator of the sanitary conditions of the product. They are usually divided into two groups: total coliforms (coliforms at 37 °C), derived from the environment, and fecal coliforms (coliforms at 45 °C), which come from fecal contamination, both recently used as indicators of the sanitary quality of food (Rodrigues et al., 2003; Silva et al., 2010).

This study aimed to evaluate the effect of organic fertilization, with swine biofertilizer applied to the soil, on the nutritional status and productivity of the plants, the extraction and export of nutrients and microbiological quality of snap bean pods in organic system.

Material and methods

The experiment was installed at the Vale do Piranga Experimental Farm (FEVP), belonging to EPAMIG, in Oratórios-MG, in the period from 04/23 to 07/03/2012. The local unit had an average annual high temperature of 21.8 °C and average annual low temperature of 19.5 °C; average annual rainfall of 1,250 mm, with higher concentration in the period from October to March, and average altitude of 400 m. Soil used was Cambic Red-Yellow Argisol, terrace phase, clay like texture, and presented the following characteristics at 0-20 cm layer: pH (water 1:2.5)=6.0; organic matter=21 g kg^{-1}; P=13.4 mg dm^{-3}; K=142; Zn=7.5; Fe=173.2; Mn=5.5; Cu= 5.9 and B=0.5 in mg dm^{-3}; Ca^{2+}=2.0 $cmol_c$ dm^{-3}; Mg^{2+}=1.0 $cmol_c$ dm^{-3}; Al^{3+}=0.0 $cmol_c$ dm^{-3}; H+Al=2.48 $cmol_c$ dm^{-3}; CTC(t)=3.36 $cmol_c$ dm^{-3}; CTC(T)=5.84 $cmol_c$ dm^{-3}; V=58% and P-rem=35 mg L^{-1}.

A randomized block design with five treatments consisting of doses of swine biofertilizer (0, 30, 60, 90 and 180 m m^3 ha^{-1}) and four replications was used. The biofertilizer was obtained from the collection of liquid pig manure, from the washing of stalls, and passed through anaerobic digestion for 30 days in capped glass fiber box. Afterwards, manure collection and anaerobic digestion were repeated for the same period for top-dressing. At the time of the first application, the biofertilizer presented the following characteristics, in (g L^{-1}): N=1.90; P=0.30; K=0.40; Ca=0.80; Mg=0.20; S=0.10, in (mg L^{-1}): Zn=32.15; Fe=561.50; Mn=11.20; Cu=11.05 and C. Org.=1.60 dag kg^{-1}, density=1.02 g cm^{-3}, pH=6.8 and C:N=8.4; analyzed according to the EMBRAPA procedures (2009).

Biofertilizer doses were divided into two applications; half of them were applied two weeks before sowing and incorporated into the soil, 20 cm deep using a hoe for each dose, and the rest of them in coverage at 30 days after sowing.

Each plot, with four rows of 3.0 m in length, contained 40 plants spaced 1.0 x 0.3 m, being considered useful the 16 central plants. Sowing was made with two seeds per hole, using the cultivar MacarrãoFavorito with indeterminate growth. Pruning was made at 15 days after sowing (DAS), leaving one plant, conducted in supported system in inverted "V".

Weed management was carried out through two manual weeding with hoe in the planting lines and, externally, through mowing. Drip irrigation was performed,as needed, using perforated strips with 10 cm intervals, arranged in each plant row. Before plant flowering, two sprays with cow urine (fermented for two months), at 1.0% (v/v) and with the following characteristics: in (%) N=6.96; P=0.0; K= 0.89; Ca=0.00; Mg=0.04; S=0.03; C.Org=0.17, in ppm: Zn=0.0; Fe=1.0; Mn=0.0; Cu=0.0 and pH=8.5 were used.

When plants were in full flowering, the collection of the fourth fully expanded leaf from the plant apex was made at the useful area of the plot. The material was placed in paper bags and dried in an oven with air circulation at 65 °C for 72 h. It was later ground and taken to the laboratory for chemical analysis for levels of macronutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), Magnesium (Mg) and sulfur (S) andmicronutrientes: boron (B), copper (Cu), iron (Fe), manganese (Mn) and Zinc (Zn), according to the EMBRAPA procedures (2009).

Microbiological analyses were performed on soil and pod samples according to the methods indicated by Silva et al. (2010) for counting of total coliforms and thermotolerant coliforms. Soil samples were collected at 5.0 cm deep in each treatment, being one single sample collected from each treatment by repetition. Samples were later homogenized and a composite sample was taken. Soil samples were collected with sterile spoons and placed in plastic bags and polystyrene boxes and transported to the laboratory.

Collection of soil samples was conducted in five different seasons: S1 - On 04-19-2012, shortly after fertilization at planting, a week before sowing; S2 - On 05-07-2012, fifteen days after sowing; S3 - On 05-22-2012, thirty days after sowing, before top-dressing; S4 - On 06-06-2012, one week after top-dressing; and S5 - On 07-03-2012, on the day of the first harvesting of pods. At this season, pods samples were also collected for microbiological analyses, irrigation water, cow urine and pure swine biofertilizer. In each season, samples were taken to the microbiology laboratory of EPAMIG and subjected to analysis of bacteria from the group of total coliforms and fecal or thermotolerant coliforms, using the technique of Most Probable Number - MPN (Silva et al*.,* 2010).

Harvesting of pods was held weekly, from 58 DAS. Production components: diameter (mm), length (cm), number, fresh and dry weight of pods were evaluated in each harvest using samples of 20 pods per harvest. The total number and total fresh weight of commercial pods were evaluated. At the end of the harvest of pods in each treatment, six useful plants were cut close to the ground and the shoot was used to determine the weight of fresh and dry matter and analysis of mineral composition. Plant samples were placed in kraft paper bags, identified and placed to dry in a forced air circulating oven at 65 °C for 72 h until reaching constant weight. After determination of dry weight, samples were taken for determination of macro- and micronutrient content.

The accumulation of nutrients was obtained by multiplying the content of each nutrient by the weight of sample dry matter. Pod samples were also collected and dried in an oven with air circulation at 65 °C for 72 h and ground to determine the nutrient content according to EMBRAPA procedure (2009). Yield was obtained by the sum of fresh weight of pods, converted into t ha^{-1}. Data were submitted to analysis of variance, with significance tested by F test and to regression analysis, and the models were chosen based on the significance of the coefficients of determination (R^2), adopting the level of up to 10% probability, using the SAEG (Statistical Analysis System. Version 9.1) software.

Results and discussions

Leaf contents of N showed positive linear effect for biofertilizer doses (Figure 1A). In all applied doses, leaf content of N was in the range considered appropiate to the snap bean crop, which is 40-60 g kg^{-1} (Trani & Raij, 1996). This has happened not only because of the increase in applied biofertilizer doses, but probably because the snap bean plant presents symbiotic association with N_2, what enables soil fertility improvements through fixation of atmospheric N_2 in the plant weight, which provides N required for the plant and also enriches the soil (Pelegrin et al., 2009). In addition, sprays with cow urine (6.96% N) throughout the experiment may have contributed to the appropriate leaf contents.

Leaf contents of P and K were not affected by biofertilizer doses, being the average values 4.6 and 18.4 g kg^{-1}, respectively. P levels were in the appropriate range for culture (3 to 7 g kg^{-1}) and K levels were below the mentioned range, which is 25 to 40 g kg^{-1}, according to Trani & Raij (1996).

There was linear and positive effect for leaf content of Ca and Mg according to the biofertilizer doses, being estimated the highest values 13.8 and 4.3 g kg^{-1}, respectively (Figures 1B and 1C).

There was a significant effect for S, but it was not possible to adjust to the models tested, and the average value was 2.3 g kg^{-1}.

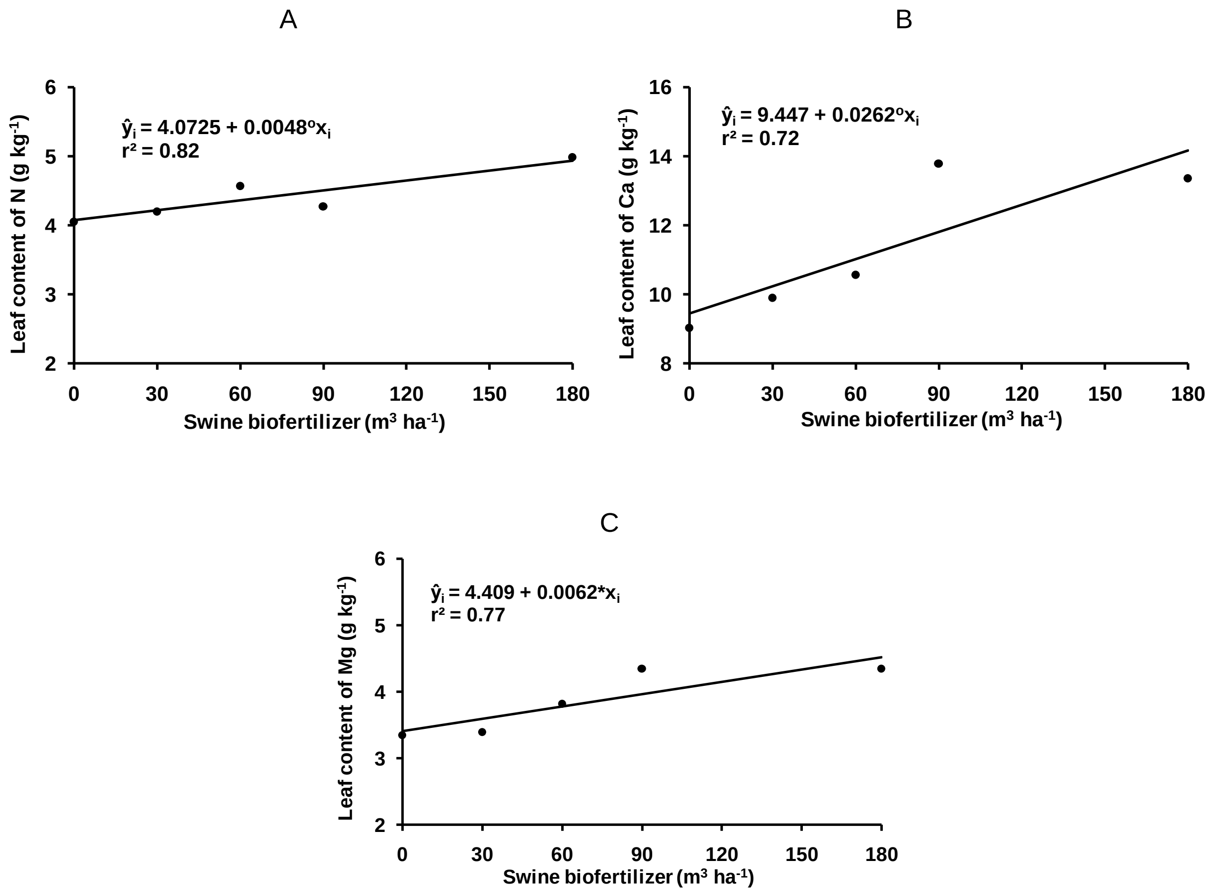

Figure 1 - Leaf contents of nitrogen (A), calcium (B) and magnesium (C) in snap beans, cultivar MacarrãoFavorito, fertilized with swine biofertilizer. Oratórios, EPAMIG, 2012. * and º - significant at 5 and 10% probability by F test, respectively.

Leaf contents of Mg and S were in the appropriate range for snap bean crop, which is from 3 to 8 g kg^{-1} and 2 to 5 g kg^{-1}, respectively (Trani & Raij, 1996). The average contents of Ca (11.3 g kg^{-1}) were below the appropriate range, which is from 15 to 30 g kg^{-1}, according to Trani & Raij (1996). This result may be related to the reduced absorption of Ca due to the high content of K in the soil, together with the amount of K applied through biofertilizer. For the production of pods, the probability of snap bean response to potassium employment is minimized when the available content of K exceeds 176 mg dm^{-3} (Oliveira et al., 2007). These authors evaluated the yield of snap beans according to increasing doses of K_2O and found that the most economic dose was 165 kg ha^{-1} K_2O, with production of 25.3 t ha^{-1}pods for the regional conditions of Areia-PB. The dose of 165 kg ha^{-1} K_2O extrapolates the recommendation of 60 kg ha^{-1} K_2O for this vegetable in soil of good fertility (CFSEMG, 1999) and the provided by the highest applied dose of swine biofertilizer (78 kg ha^{-1} K_2O). The importance of the relation between contents of K, Ca and Mg in the soil on the response of sweet sorghum production to potassium fertilization was observed by Rosolem et al. (1984).

In order not to occur excess supply of some nutrients, such as N, other nutrients, such as K and Ca may be complemented by other sources to balance relations and leaf content, satisfying the nutritional needs of plants. Araújo et al. (2007), working with organic fertilization associated with the use of biofertilizers in sweet pepper crop, concluded that high doses of organic fertilization promoted nutritional imbalances to plants due to nutritional imbalance with consequent reduction in crop yield.

Regarding leaf contents of micronutrients, there was no significant difference in the biofertilizer doses applied, being the average contents in mg kg^{-1}: 47.7; 134.2; 171.6; 9.9 and 42.2 for Zn, Fe, Mn, Cu and B, respectively. Brazilian legislation does not provide maximum permitted values for heavy metals in organic fertilizers, thus, there is not a national reference for the limits of Cu, Zn, Fe and Mn (Higarashi et al., 2008). In addition to the nutritional imbalance of plants, due to the imbalance of nutrients, other aspects such as potential for soil waste clearance, changes, especially in the long term, in pH, cation exchange capacity, macro- and micronutrient accumulation in the soil made on the microbial community, and water quality should be assessed (Simioni, 2001).

Regarding the component production, there was a significant effect on pod length, with increasing linear response according to the biofertilizer doses (Figure 2A). The greatest pod length (14.7 cm) was estimated with the highest applied biofertilizer dose (180 m^3 ha^{-1}). This value is close to that obtained by Araújo et al. (2001), who worked with pig manure doses of up to 40 t ha^{-1} in the presence and absence of mineral fertilization and found average values of 15.43 cm and 10.8 mm for pod length and diameter, respectively, in the absence of mineral fertilization. Pod length is a characteristic influenced by genotype. Peixoto et al. (2002) studied thegenetic divergence among 20 snap bean genotypes with indeterminate growth and found that pod length was one of the characteristics that most contributed to the divergence among the genotypes, together with the number of days for the beginning of flowering, with 58.1% of the total.

In this study, there was no effect of biofertilizer doses on the pod diameter, whose average value was 9.96 mm. Araújo et al. (2001) worked with the same cultivar and also found no answer to the pod diameter as result of pig manure doses in the presence and absence of mineral fertilization, with average values of 10.8 and 10.6 mm, very close to that obtained in this work. The authors also found no differences in length and average weight of pods, explaining that high natural soil fertility, together with the concentration of nutrients in swine manure, may have been responsible for the lack of response of organic and mineral fertilization on these characteristics.

Pod diameter is an easily measured characteristic and is an indicative of the grain development stage and point of harvest, that is to say, immature pods and tender grains, which was kept in all treatments. Although pod diameter have not changed with biofertilizer doses, they showed the commercial standard required by the market, i.e. rated with numbers 4 (8.3 to 9.4 mm diameter) and 5 (9.5 to 10.7 mm diameter).

There was a significant and quadratic effect for pod dry matter, being the highest value 9.25%, estimated at a dose of 122.34 m^3ha^{-1} of biofertilizer (Figure 2B). The number of pods per plant and the pod yield presented a quadratic effect, whose maximum estimated values were 64.2 pods and 16.6 t ha^{-1}, respectively. Both were obtained with the highest biofertilizer dose (180 m^3 ha^{-1}), i.e. there was an increase of 40 pods/plant and 10.6 t ha^{-1}, by passing from dose 0 to 180 m^3 ha^{-1} of biofertilizer (Figures 2C and 2D). These productivity gains results were certainly encouraged by better plant nutrition, which in turn contributed to higher the number of pods and increased productivity. These results corroborate those obtained by Araújo et al. (2001), who worked with the same cultivar and found linear increase to the number of pods, yield per plant and yield of pods fertilized with pig manure, both in the presence and absence of mineral fertilizer.

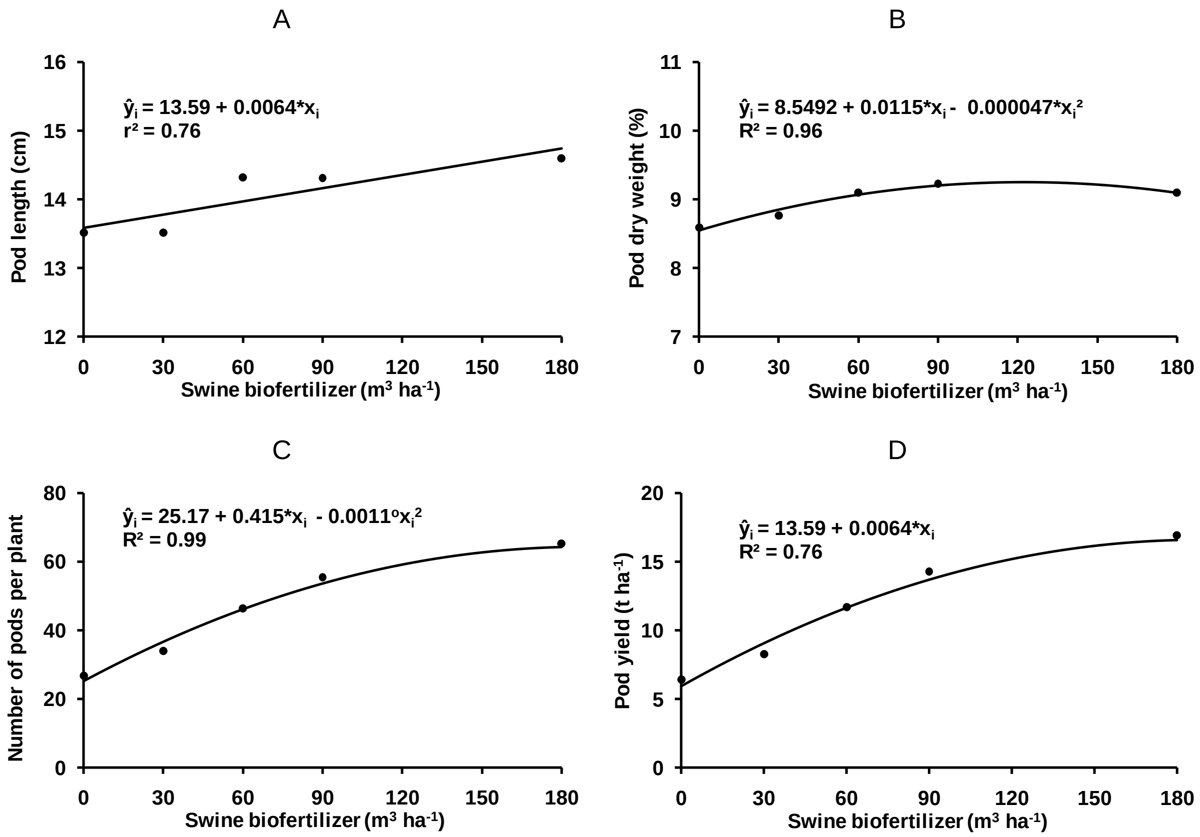

Figure 2 - Pod length (A), pod dry weight (B), average number of pods per plant (C) and pod yield (D) on snap beans, cultivar MacarrãoFavorito, fertilized with swine biofertilizer. Oratórios, EPAMIG, 2012. * and ^{o} - significant at 5 and 10% probability by F test, respectively.

Snap bean yields above 30 t ha^{-1} were achieved in conventional system with the same cultivar MacarrãoTrepador, in studies with pig manure doses in the presence of mineral fertilization (Araújo et al., 2001); doses ofP_2O_5 (Oliveira et al., 2005) and of 2 doses of K_2O (Oliveira et al., 2007).

In this research, the maximum yield obtained was 16.6 t ha^{-1}, higher than that observed by Santos et al. (2014) with the same cultivar in organic system, which was 9.6 t ha^{-1}. For cultivation in organic system, yield of 16.6 t ha^{-1}, is considered satisfactory, since there was no spending on the application of mineral fertilizers and agricultural pesticides.

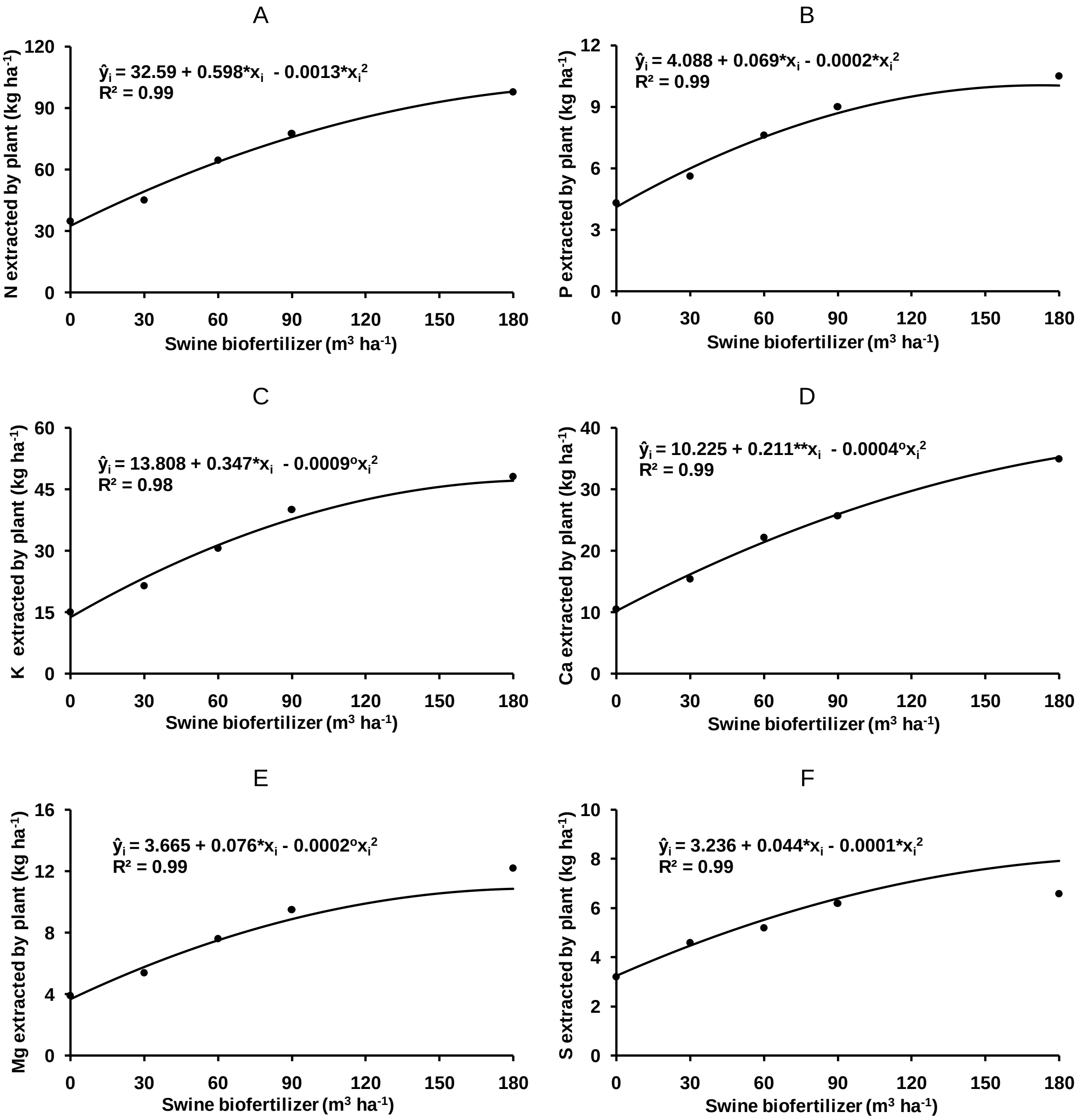

Figure 3 - Extraction of macronutrients by snap bean plants, cultivar MacarrãoFavorito, fertilized with swine biofertilizer. Oratórios, EPAMIG, 2012. **, * and ^{o} - significant at 1, 5 and 10% probability by F test, respectively.

Little information is available in the literature at national level about the amounts of nutrients to be used aiming the nutrition of snap bean plants to obtain satisfactory yields, especially in organic system. The recommendation for organic fertilization is made based on the nutrient content of the fertilizer, especially on the content of N, since it is normally the nutrient provided in larger quantity. However, according to Trani (2007), the recommendation for nitrogen fertilization for vegetables should be based on the extraction of this nutrient by plants and export by the crops. To Sampaio & Brasil (2009), the amounts of nutrients extracted by the crop and exported with pod harvest provide estimates of the plant nutritional needs and serve as a tool for calibrating the fertilization recommendations. Thus, it is necessary to analyze both plants and harvested parts as food.

There was significant increase in the amounts of macronutrients extracted (absorbed) by snap bean plant according to the swine biofertilizer doses applied. N was the nutrient extracted in greater amounts by plants, followed by K, especially in the highest applied biofertilizer dose (Figures 3A and 3C), as in most cultures. In cowpea, N was also the most extracted nutrient, followed by K and with much higher values than other nutrients (Sampaio & Brasil, 2009).

The extraction of P, Ca, Mg and S by plants also increased with the applied biofertilizers doses (Figures 3B, 3D, 3E and 3F). In a population density of 33,000 plants ha^{-1}, macronutrients values extracted by plants at the dose of the highest yield were: 98.1; 10.5; 48.1; 35.0; 12.2 and 6.6 kg ha^{-1} for N, P, K, Ca, Mg and S, respectively (Figure 3). S was extracted in smaller amounts and these results corroborate those found by Barzan et al. (2014), who evaluated the extraction of macronutrients by the snap bean with determinate growth. Values obtained for the extraction of micronutrients (g ha^{-1}) were: 127.7; 899.1; 293.4; 21.1 and 40.1 for Zn, Fe, Mn, Cu and B, respectively.

The order of macro and micronutrients absorption was similar to that found in cowpea, where it was also found that Fe was the micronutrient absorbed in large amounts by plants (Neves et al., 2009). Usually, concentrations of Fe in pig manure are higher than other micronutrients and, although legumes absorb large amounts of Fe, precautions are advisable when quantifying the dosages to be applied to the soil, with prior analysis of soil and residue, in order not to cause accumulation of micronutrients, particularly regarding Cu and Zn (Higarashi et al., 2008). For micronutrients, there was also significant increase in extracted amounts of Zn, Cu and B, with higher values obtained with the highest biofertilizer doses applied (Figures 4A, 4B and 4C). For Fe and Mn, there was no significant effect regarding the biofertilzer doses, with average values of 1,466.7 and 771.1 g ha^{-1}, respectively.

The natural green color in vegetables is very important, since the appearance of the product increases the consumer acceptance. In the case of snap beans, the pigments responsible for the characteristic bright green color are chlorophyll *a* and *b*, where higher nutrient extraction is extremely important for the vegetable and also improves essential components to thehuman diet (Fávaro et al., 2000; Peres et al., 2011).

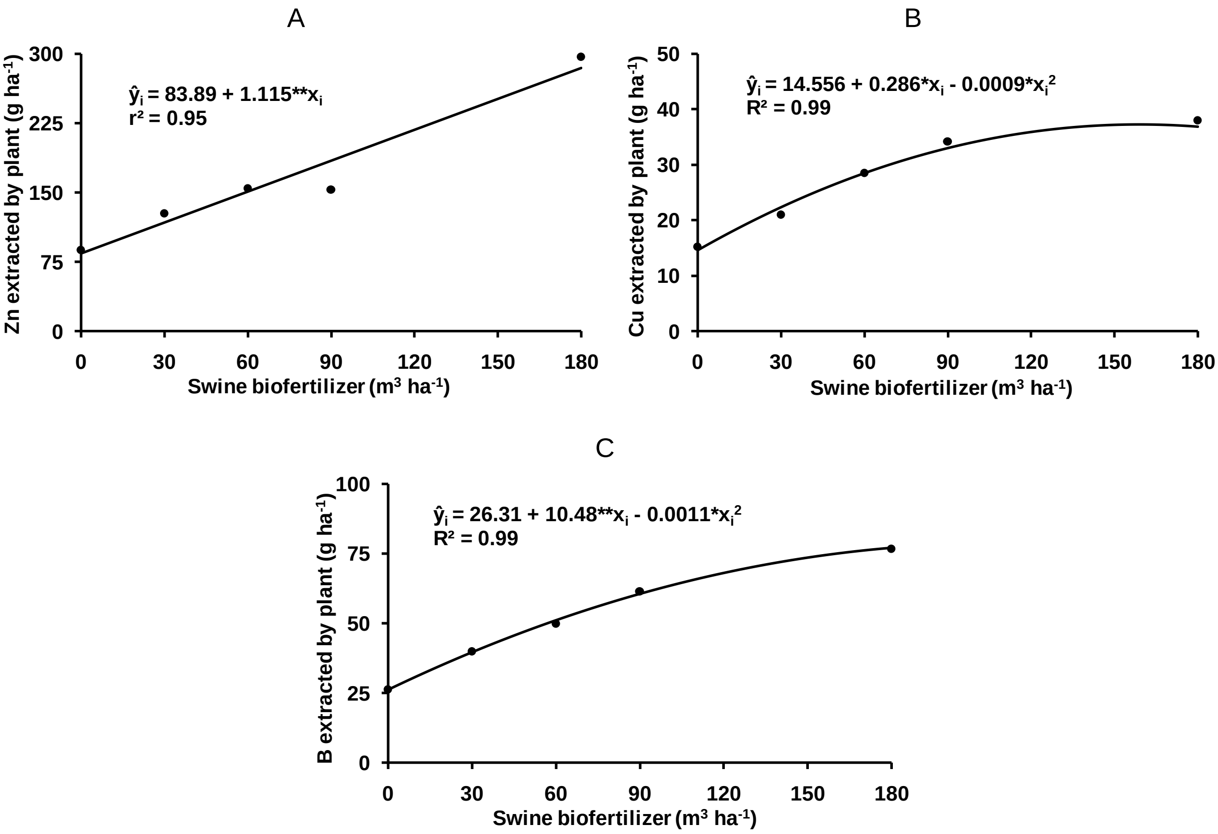

Figure 4 - Extraction of micronutrients by snap bean plants, cultivar MacarrãoFavorito, fertilized with swine biofertilizer. Oratórios, EPAMIG, 2012. ** and * - significant at 1 and 5% probability by F test, respectively.

There was a significant difference for the nutrient export by pods according to the biofertilizer doses applied, with higher exports recorded with the use of the highest doses (Figures 5 and 6). Among the macronutrients, higher values were observed for N and K, followed by P, Ca, Mg and S. The amounts of macronutrients exported at the dose of the highest yield were (kg ha^{-1}): N (54.4), K (21.4), P (6.5), Ca(5.2), Mg (4.5) and S (3.0) (Figure 5), corresponding to 55% N; 45% K; 62% P; 15% Ca; 37% Mg and 46% S extracted by snap bean plant, ie these were the percentages exported in the pods.

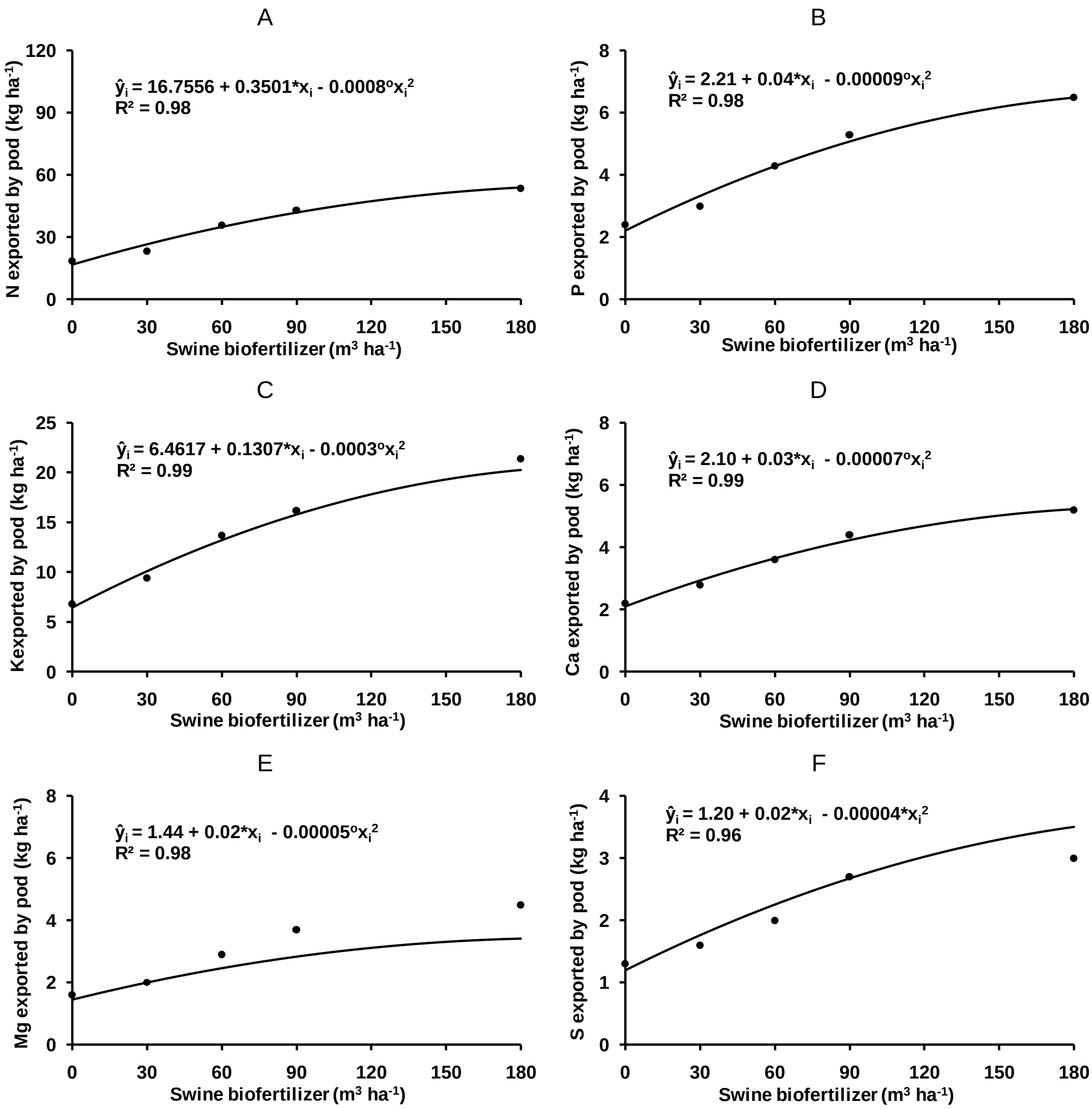

Figure 5 - Export of macronutrients by snap bean pods, cultivar MacarrãoFavorito, fertilized with swine biofertilizer. Oratórios, EPAMIG, 2012. * and ^{o} - significant at 5 and 10% probability by F test, respectively.

A significant difference was observed for the export of micronutrients, except for the Mn, which showed an average content of 89.8 g ha^{-1}. The greatest exports of micronutrients occurred at the highest biofertilizer dose (Figures 6A to 6D). The decreasing export order (g ha^{-1}) was: Fe (91.4), Mn(89.8), Zn (48.4), B (23.1), Cu (12.2), corresponding to 10.16% Fe; 30.60% Mn; 37.90% Zn; 57.60% B and 57.82% Cu extracted by snap bean plant, these being the percentages exported in the pods.

The export order of macro and micronutrients was similar to those found in cultivated cowpea (Neveset al., 2009). Among the micronutrients, Fe was the most exported by the pods and the export increase was proportional to the applied biofertilizer doses. Content of Fe in the pods increased from 44.8 g ha^{-1} in the control to 150.2 g ha^{-1} at the highest applied dose, i.e. it increased approximately 300% (Figure 4B). This increase is due to the crop response to fertilization, because the biofertilizer had considerable content of Fe in its composition. It is difficult to compare the extraction and export of nutrients by snap bean plants with other studies, since the spacing, harvest time and cultivar may influence these characteristics.

According to the results of the microbiological analyses, there was a slight reduction for total coliforms and a more marked reduction in thermotolerant coliforms in the soil during the snap bean cycle, reaching values <3 MPN g^{-1}, especially in the last two samples , S4 and S5, i.e. one week after topdressing and in the first pod harvest, respectively. There was no increase in the number of total coliforms with increase in the biofertilizer dose applied (Table 1).

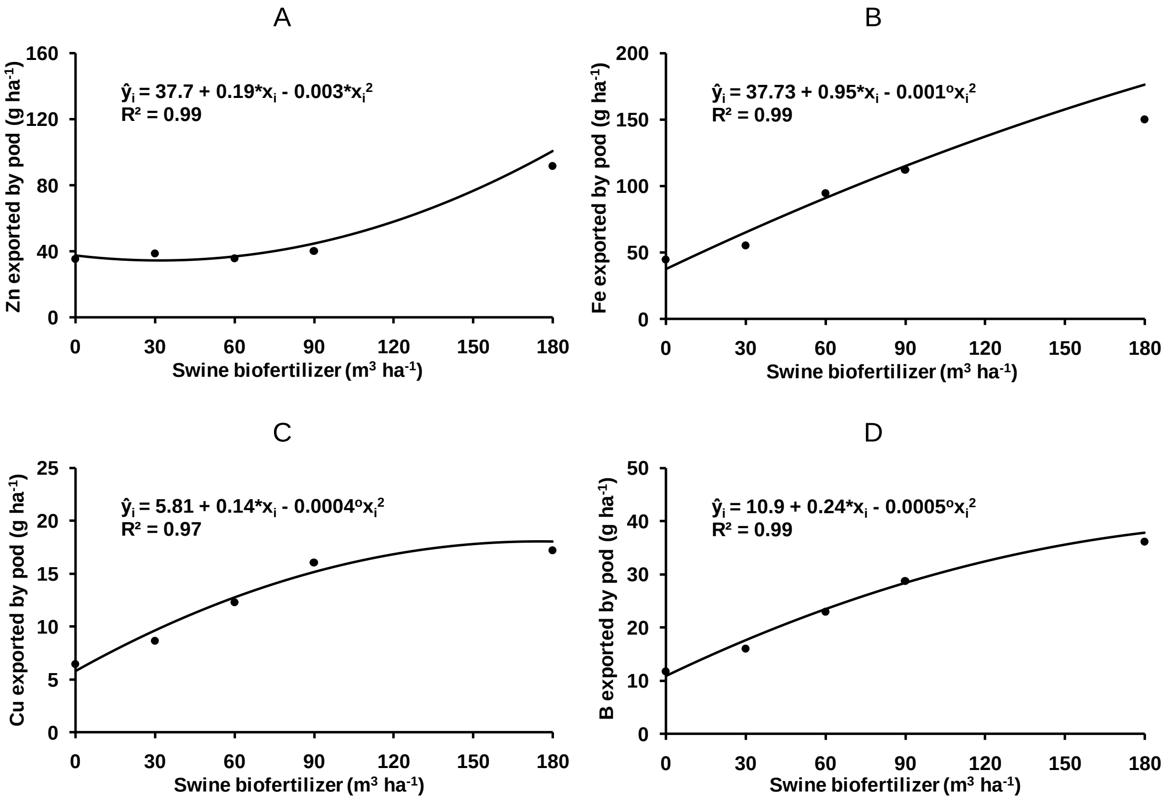

Figure 6 - Export of micronutrients by snap bean pods, cultivar MacarrãoFavorito, fertilized with swine biofertilizer. Oratórios, EPAMIG, 2012. * and ^{o} - significant at 5 and 10% probability by F test, respectively.

Table 1 - Average values for total and thermotolerant coliforms in soil samples (MPN g^{-1}), at different collection seasons in samples of pods, biofertilizer and water used for plant irrigation according to the swine biofertilizer doses. Oratórios, EPAMIG, 2012.

Dose (m^3 ha^{-1})	Collection seasons of soil samples*					Pod	Biofertilizer	Water
	S1	S2	S3	S4	S5			
				MPN - Total Coliforms				
0	2.1×10^3	1.1×10^3	9.3×10^1	2.1×10^2	2.8×10^1	$< 3.0 \times 10^0$	4.3×10^1	-
30	1.1×10^4	4.6×10^2	1.5×10^3	2.1×10^3	2.4×10^3	$< 3.0 \times 10^0$	4.3×10^1	-
60	4.6×10^3	1.1×10^4	4.6×10^2	1.1×10^3	2.4×10^3	$< 3.0 \times 10^0$	4.3×10^1	-
90	1.1×10^4	2.4×10^3	4.3×10^1	1.5×10^3	9.0×10^0	$< 3.0 \times 10^0$	4.3×10^1	-
180	2.4×10^3	2.4×10^3	4.6×10^2	7.0×10^0	2.3×10^1	$< 3.0 \times 10^0$	4.3×10^1	-
				MPN - Thermotolerantcoliforms**				
0	1.1×10^1	9.3×10^1	$< 3 \times 10^0$	4.0×10^0	$< 3.0 \times 10^0$	$< 3.0 \times 10^0$	9.0×10^0	<1.1/100 mL
30	2.4×10^2	2.1×10^1	7.5×10^2	9.3×10^1	$< 3.0 \times 10^0$	$< 3.0 \times 10^0$	9.0×10^0	<1.1/100 mL
60	7.5×10^1	2.3×10^1	1.5×10^2	7.0×10^0	$< 3.0 \times 10^0$	$< 3.0 \times 10^0$	9.0×10^0	<1.1/100 mL
90	7.5×10^1	1.5×10^1	1.5×10^1	4.6×10^2	$< 3.0 \times 10^0$	$< 3.0 \times 10^0$	9.0×10^0	<1.1/100 mL
180	2.8×10^1	1.5×10^2	1.5×10^1	4.0×10^0	4.0×10^0	$< 3.0 \times 10^0$	9.0×10^0	<1.1/100 mL

*S1 = Sample on 04-19-2012, shortly after fertilization at planting, a week before sowing; S2 = On 05-07-2012, fifteen days after sowing; S3 = On 05-22-2012, thirty days after sowing, before top-dressing; S4 = On 06-06-2012, one week after topdressing; and S5 = On 07-03-2012, on the day of the first harvesting of pods. **Upper limit for fecal coliforms is 5.0×10^2 MPN g^{-1}, according to the resolution - RDC No. 12 of January 2, 2001 ANVISA (BRASIL, 2001).

Regarding the microbiological analysis of the pods, in the first harvest, the MPN values were $<3 \times 10^0$ for both total coliforms and thermotolerant C. at all applied biofertilizer doses (Table 1). These values are below the limit allowed by law, defined in Resolution - RDC no. 12 of January 2, 2001 of the Brazilian National Health Surveillance Agency (ANVISA), which is 5×10^2 MPN g^{-1} (Brasil, 2001). The low number of these microorganisms indicates that food is safe, i.e. it is harmless to health.

For swinebiofertilizer, values were 4.3×10^1 and 9.0×10^0 MPN mL^{-1} for total and thermotolerant coliforms, respectively. Regarding the water used for plant irrigation, values were <1.1 / 100 MPN mL^{-1} for thermotolerant coliforms (Table 1). This value is within the limits for drinking water, according to the current legislation (Brasil, 2004). If water showed <1.1 / 100 MPNmL^{-1} for thermotolerant coliforms, values for total C. were negative, according to the identification methodology. Thus, it is possible to produce vegetables with quality appropriate for human consumption, with water used in irrigation, fermented cow urine and swine biofertilizer. This fact is due, probably, to the culture medium conditions less favorable to microorganism survival, ie, low level of total and thermotolerant coliforms in swine biofertilizer, irrigation water and also in fermented cow urine applied during cultivation.

Countings of total and thermotolerant coliforms in the sample of fermented cow urine were less than 3×10^0MPN mL^{-1}. Probably, the animal health and cow urine storage time have helped to eliminate possible pathogens. According to Magalhães (2013), pure cow urine, hermetically sealed in plastic container kept in the dark, shows growth of total coliforms, thermotolerant coliforms and mesophilic aerobic microorganisms until around the 5th week, decreasing drastically and reaching zero, or near that, after the 7th week. In the traditional cultivation of snap bean, the vertical staking is used, where plant grows out of contact with the ground. Allied to this, the fermentation of organic matter present in pig manure before its application to soil was of great importance to ensure the elimination of pathogens, besides promoting the stabilization of organic matter and improving the fertilizing value of the manure. In this case, the application of biofertilizers from pig manure, incorporated into the soil before planting and coverage, followed by ridging, was not a source of soil and pod contamination.

Soil organic fertilization based on swine biofertilizers for snap bean cultivation is a promising technique. However, the actual needs of the soil should be assessed, in order to avoid accumulation or imbalance of nutrients and toxic elements and impairment of crop yield.

Conclusions

Snap bean fertilization with swine biofertilizer promotes increase in productivity and improves the nutritional status of plants.

Macronutrient extraction by plants and export by pods increases with the applied biofertilizer doses.

The application of biofertilizers and irrigation water with low population of microorganisms produces pods with appropriate microbiological quality, according to ANVISA standards.

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References

Abreu Neto SM, Oliveira RA (2009) Remoção de matéria orgânica, de nutrientes e de coliformes no processo anaeróbio em dois estágios (reator compartimentado seguido de reator UASB) para o tratamento de águas residuárias de suinocultura. Engenharia Agrícola 29(1):148-161.

Araújo EM, Oliveira AP, Cavalcante LF, Pereira WE, Brito NM, Neves CML, Silva EE (2007) Produção do pimentão adubado com esterco bovino e biofertilizante. Revista Brasileira de Engenharia Agrícola e Ambiental 11(5):466-470. doi:10.1590/S1415-43662007000500003

Araújo JS, Oliveira AP, Silva, JAL, Ramalho CL, Neto FLC (2001) Rendimento do feijão-vagem cultivado com esterco suíno e adubação mineral. Revista Ceres 5(278):501-510.

Barzan RR, Furlan FF, Martini GFA, Sampaio MLY, Firmano RF, Fregonezi GAF, Takahashi LSA (2014) Extração de macronutrientes do feijão-vagem de crescimento determinado 'UEL-1' na safra da seca. In: CONAFE - CONGRESSO NACIONAL DE PESQUISA DE FEIJÃO, 11, 2014, Londrina. Anais...

Brasil - Agencia Nacional de Vigilância Sanitária - ANVISA (2001) Regulamento técnico sobre os padrões microbiológicos para alimentos. Disponível em: <http://www.anvisa.gov.br>. (Acesso em: 05 dez. 2013).

Brasil - Ministério da Agricultura, Pecuária e Abastecimento (2004). Decreto 4.954 SISLEGIS. Disponível em: <http://www.planalto.gov.br/ccivil_03/_ato2004-2006/2004/decreto/d4954.htm>. (Acesso em: 15 abr. 2014).

CFSEMG - Comissão de Fertilidade do Solo do Estado de Minas Gerais (1999) Adubação orgânica. In: Ribeiro AC, Guimarães PTG, Alvarez V., VH (Ed) Recomendações para o uso de corretivos e fertilizantes em Minas Gerais – 5ª Aproximação. Viçosa. p.87-92.

EMBRAPA- Empresa Brasileira de Pesquisa Agropecuária (2009) - Manual de análises químicas de solos, plantas e fertilizantes. Brasília: Embrapa Informação Tecnológica, 627p.

Falavigna LM, Freitas CBR, Melo GC, Nishi L, Araújo SM, Falavigna-Guilherme AL (2005) Qualidade de hortaliças comercializadas no noroeste do Paraná, Brasil. Parasitología Latinoamericana 60(3-4):144-149. doi: 10.4067/S0717-77122005000200007

Fávaro SP, Sá MC, Ida EL; Braga Neto JA (2000) Cor de feijão vagem fresco e processado após aplicação de cálcio. Scientia Agricola 57(3):561-563.

Higarashi MM, Coldebella A, Oliveira PAV, Kunz A, Mattei RM, Silva VS, Amaral AL (2008) Concentração de macronutrientes e metais pesados em maravalha de unidade de suínos em cama sobreposta. Revista Brasileira de Engenharia Agrícola e Ambiental 12(3):311--317. doi: 10.1590/S1415-43662008000300013.

Magalhães WG (2013) Crescimento e qualidade microbiológica de alface cultivada com soluções de urina de vaca. UFV (Tese de Doutorado em Fitotecnia).

Menezes Júnior FOG, Gonçalves PAS, Kurtz C (2013) Biomassa e extração de nutrientes da cebola sob adubação orgânica e biofertilizantes. Horticultura Brasileira 31(4):642-648. doi:10.1590/S0102--05362013000400022.

Neves ALR, Lacerda CF, Guimarães FVA, Hernandez FFF, Silva FB, Prisco JT, Gheyi HR (2009) Acumulação de biomassa e extração de nutrientes por plantas de feijão-de-corda irrigada com água salina em diferentes estádios de desenvolvimento. Ciência Rural 39(3):758-765.

Oliveira AP, Cardoso MO, Barbosa LJN, Silva JEL, Morais MS (2005) Resposta do feijão-vagem a P_2O_5 em solo arenoso com baixo teor de fósforo. Horticultura Brasileira 23(1):128-132. doi:10.1590/S0102-05362005000100027.

Oliveira AP, Silva JÁ, Alves AU, Dorneles CSM, Alves AU, Oliveira ANP, Cardoso EA, Cruz IS (2007) Rendimento de feijão-vagem em função de doses de K_2O. Horticultura Brasileira 25(1):29-33. doi:10.1590/S0102-05362007000100007.

Peixoto N, Braz LT, Banzatto DA, Moraes EA, Moreira FM (2002) Características agronômicas, produtividade, qualidade de vagens e divergência genética em feijão-vagem de crescimento indeterminado. Horticultura Brasileira 20(3):447-451. doi:10.1590/S0102-05362002000300010

Pelegrin R, Mercante FM, Otsubo IMN, Otsubo AA (2009) Resposta da cultura do feijoeiro à adubação nitrogenada e à inoculação com rizóbio. Revista Brasileira de Ciência do Solo 33:219-226.

Pereira EL, Campos CMM, Moterani F (2009) Efeitos do pH, acidez e alcalinidade na microbiota de um reator anaeróbio de manta de lodo (UASB) tratando efluentes de suinocultura. Ambiente & Água 4(3):157-168. doi:10.4136/ambi-agua.109.

Peres JE, Arruda MC, Fileti MS, Fischer IH, Simionato, EMRS, Voltan, DS (2011) Qualidade de feijão-vagem minimamente processado em função das operações de enxague e sanificação. Semina: Ciências Agrárias 32(1):173-180. doi: 10.5433/1679--0359.2011v32n1p173

Rodrigues KL, Gomes JP, Conceição RCS, Brod CS, Carvalhal JB, Aleixo JAG (2003) Condições higiênico-sanitárias no comércio de alimentos em Pelotas-RS. Ciência e Tecnologia de Alimentos 23(3):447-452. doi:10.1590/S0101-20612003000300026

Rosolem CA, Machado JR, Brinholi O. (1984) Efeito das relações Ca/Mg, Ca/K e Mg/K do solo na produção de sorgo sacarino. Pesquisa Agropecuária Brasileira 19(12):1443-1448.

Sampaio LS, Brasil EC (2009) Exigência nutricional do feijão-caupi. In: CONGRESSO NACIONAL DE FEIJÃO-CAUPI, 2., 2009, Belém, PA. Da agricultura de subsistência ao agronegócio. Anais... Belém, PA: Embrapa Amazônia Oriental, p. 56-72.

Santos JR, Nunes MUC, Santos MC, Souza IM, Tavares FA (2014) Desempenho de cultivares de feijão-vagem de crescimento indeterminado, em cultivo orgânico, na época de verão no litoral sul de Sergipe. Disponível em <www.abhorticultura.com.br/Eventosx/trabalhos/.../A414_T1048_Comp.pdf> (Acesso em: 26 jul. 2014).

Santos MR, Sediyama MAN, Moreira MA, Megguer CA, Vidigal SM (2012) Rendimento, qualidade e absorção de nutrientes pelos frutos de abóbora em função de doses de biofertilizante. Horticultura Brasileira 30(1):160-167. doi:10.1590/S0102-05362012000100027

Santos MR, Sediyama MAN, Santo IC, Salgado LT, Vidigal SM (2011) Produção de milho-verde em resposta ao efeito residual da adubação orgânica do quiabeiro em cultivo subsequente. Revista Ceres 58(1):77-83. doi: 10.1590/S0034-737X2011000100012

Sediyama MAN, Vidigal SM, Pedrosa MW, Pinto CLO, Salgado LT (2008) Fermentação de esterco de suínos para uso como adubo orgânico. Revista Brasileira de Engenharia Agrícola e Ambiental 12(6):638–644. doi:10.1590/S1415-43662008000600011

Sediyama MAN, Santos MR, Vidigal SM, Pinto CLO, Jacob LL (2014) Nutrição e produtividade de plantas de pimentão colorido, adubadas com biofertilizante de suíno. Revista Brasileira de Engenharia Agrícola e Ambiental 18(6):588-594. doi.org/10.1590/S1415-43662014000600004.

Silva N da, Junqueira VCA, Silveira NFA, Taniwaki MH, Santos RFS, Gomes RAR (2010) Manual de métodos de análise microbiológica de alimentos e água. 4. Ed. São Paulo: Livraria Varela, 632 p.

Simioni J (2001) Avaliação dos riscos ambientais pela acumulação de Cu e Zn nos solos fertilizados com dejetos de suínos. UFSC (Dissertação de Mestrado em Agroecossistemas).

Sousa CP (2006) Segurança alimentar e doenças veiculadas por alimentos: utilização do grupo coliforme como um dos indicadores de qualidade de alimentos. Revista APS 9(1):83-88.

Trani PE(2007) Calagem e adubação para hortaliças sob cultivo protegido. Artigo em Hypertexto. Disponível em: <http://www.infobibos.com/Artigos/2007_1/cp/index.htm>. (Acesso em: 04 ago. 2015).

Trani PE, Raij B Van (1996) Hortaliças. In: Raij B van, Cantarella H, Quaggio JA, Furlani AMC Recomendações de adubação e calagem para o Estado de São Paulo. 2. ed. Campinas: IAC, 285p. (IAC. Boletim técnico, 100).

Vidigal SM, Sediyama MAN, Pedrosa MW, Santos MR (2010) Produtividade de cebola em cultivo orgânico utilizando composto à base de dejetos de suínos. Horticultura Brasileira 28(2):168-173. doi: 10.1590/S0102-05362010000200005.

Vieira JCB, Puiatti M, Cecon PR, Bhering AS, Silva GCC, Colombo JN (2014) Viabilidade agroeconômica da consorciação do taro com feijão-vagem indeterminado em razão da época de plantio. Revista Ceres 61(2):226--233. doi:10.1590/S0034-737X2014000200010.