The effect of three liquid bio-fertilizers in the production of lettuce (*Lactuca sativa* L.) and cabbage (*Brassica* oleracea L. var. capitata)

Efecto de tres biofertilizantes líquidos en la producción de lechuga (*Lactuca sativa* L.) y repollo (*Brassica* oleracea L. var. capitata)

Hernando Criollo[1, 2], Tulio Lagos[1], Edwin Piarpuezan[1], and Ruth Pérez[1]

ABSTRACT

In modern agriculture, the use of agrochemicals has grown considerably, increasing production costs and causing serious problems for the environment. The use of bio-fertilizers is a viable alternative to improve the profitability of crops, particularly for agriculture on medium and small-sized farms with intensive production systems, such as vegetables. Given that bio-fertilizers can be produced on the farm and used successfully in crop production, this research focused on the effect of three bio-fertilizers on the production of lettuce and cabbage, biweekly applications were made with liquid fertilizers produced from the manure of cows (BFC), guinea pigs (BFGp) and pigs (BFPi) and compared to a commercial foliar fertilizer (CFF) and a control without an application. We observed the presence of *Lactobacillus* and *Saccharomyces* in the BFC and BFGp fertilizers and *Bacillus* in the BFPi fertilizer. The weight and head diameter and yield of lettuce and cabbage favored the bio-fertilizer applications compared to the control, but no statistical differences were found compared to the commercial foliar fertilizer (CFF). This behavior is attributed not only to the mineral content, but also to the presence of metabolite regulators of plant physiology, produced by the microbial action of the bio-fertilizers.

Key words: alternative agriculture, microbial liquids, bioassay, *Saccharomyces* sp., *Lactobacillus* sp., *Bacillus* sp.

RESUMEN

En la agricultura moderna el uso de agroquímicos ha crecido ostensiblemente, incrementando los costos de producción y causando problemas serios en el medio ambiente. El uso de biofertilizantes es una alternativa viable para mejorar la rentabilidad de los cultivos, particularmente en la agricultura de medianos y pequeños agricultores con sistemas intensivos de producción, como las hortalizas. Teniendo en cuenta que los biofertilizantes pueden ser producidos en la misma finca y utilizados con éxito en la producción de cultivos, este trabajo se planteó con el objeto de estudiar el efecto de tres biofertilizantes sobre la producción de lechuga y repollo; se hicieron aplicaciones quincenales de caldos preparados con estiércol de vaca (BFV), cuy (BFCu) y cerdo (BFCe) y se compararon con un fertilizante foliar comercial (FFC) y un testigo sin aplicación. Se determinó la presencia de *Lactobacillus* y *Saccharomyces* en los caldos BFV y BFCu y de *Bacillus* en el caldo BFCe. El peso y el diámetro de cabeza y el rendimiento de lechuga y repollo fueron favorecidos por las aplicaciones de los biofertilizantes comparados con el testigo, pero sin diferencias estadísticas con FFC. Este comportamiento se atribuye no solo al contenido mineral, sino también a la presencia de metabolitos reguladores de la fisiología vegetal, producidos por la acción microbial de los biofertilizantes.

Palabras clave: agricultura alternativa, caldos microbiales, bioensayo, *Saccharomyces* sp., *Lactobacillus* sp., *Bacillus* sp.

Introduction

In recent decades, Colombian agriculture has been affected by the reduction of productivity in horticultural areas, increased production costs and dependence on external inputs, which is reflected in the declining quality of life of farmers and irreversible damage to the environment, due to the indiscriminate use of chemicals (Luna, 2001). Vegetable production is mainly based on the use of technology dependent on synthetic chemical inputs, which in recent years have increased in price by 80%, which is why vegetable crops have been displaced or have dramatically decreased in yield, reducing the income of farmers (Gliessman, 2002). Moreover, indiscriminate pesticide use poses risks to the health of farmers, their families and consumers (Uozumi, 2002).

Organic farming is emerging as an alternative to reduce pollution, and is a strategy in the dissemination process and used by farmers around the world who have shown interest in using it as an alternative to conventional practices (FAO, 2008). In fact, the Argentine Movement for Organic Production (MAPO) states that worldwide organic farming has been developing at an annual growth rate of 20% for the last 20 years, this represents a figure of 40 billion dollars in organic products , with markets in Europe, the USA and

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1 Faculty of Agricultural Sciences, Universidad de Nariño. Pasto (Colombia).
2 Corresponding author. hcriollo@udenar.edu.co

Japan (Roca, 2007). Chemical fertilizers are being replaced by organic fertilizers and fertilizers produced through organic farming, also farmers are gaining access to a market in which their products receive higher prices than products grown using synthetic chemical inputs (Udagawa, 1999).

Bio-fertilizers are an important option for agricultural sustainability, as they are conducive to long-term beneficial effects on the physical, chemical and biological aspects of soils (Méndez and Viteri, 2007); the levels of N, P and K in the plant tissues of soybeans, and the availability of P and K in soil were significantly improved by the application of composted rice chaff (Ngoc Son *et al*., 2008). Similarly, Mahfouz and Sharaf-Eldin (2007) obtained higher growth and higher yields of essential oils and productivity in *Foeniculum vulgare* plants treated with bio-fertilizers and half the recommended dose of chemical fertilizer, the applied bio-fertilizer contained *Azotobacter chroococcum*, *Azospirillum liboferum* and *Bacillus megatherium*. With *Stevia rebaudiana,* Das *et al*. (2007) found very significant increases in the levels of N, P, K and the production of biomass, with liquids containing *Azospirillum*, solubilizing bacteria with P and mycorrhizal VA.

Bio-fertilizers are products of the fermentation of organic materials such as manure, green plants and fruits, commonly called microbial liquids or biofermenters (Restrepo, 2001), generally applied foliarly or radically at the time of planting. Generally, to prepare bio-fertilizers, water is mixed with a nitrogen source such as manure or legumes and an energy source such as molasses or cane juice (Restrepo, 1998). This mixture can be enriched with phosphoric powders and other salt minerals (Restrepo, 2002). Finally, for the manufacture of bio-fertilizers, a source of microorganisms (yeast, milk, whey) responsible for transforming organic materials is added (Restrepo, 2001). With an increase in the heterotrophic microbial population achieved, the release of nutrients, enzymes, hormones, organic acids, amino acids, vitamins and relative enrichment of solid organic substrates is reached (Arévalo, 2003).

The presence of bacteria, molds, yeasts and viable misofilos allow for the processing and conversion of organic compounds in bio-fertilizers into simple substances such as minerals, which when supplied to the plant contribute to normal physiological development (Gallardo and Timana, 2002); accelerate the synthesis or transformation of nutrients, making them more assimilative to the plant, leaving no toxic waste in the system (Cortes and Josa, 2006). Moreover, the efficiency of bio-fertilizers depends on the raw materials used (manure, plant waste), the type of fermentation and the microorganisms involved (Ngampimol and Kunathigan, 2008).

Bio-fertilizers contain phototrophic bacteria, lactic acid bacteria and yeast known as PGPR, which are highly efficient agents in promoting plant growth as well as increasing tolerance to disease-causing microorganisms (Esquivel, 2008). These bacteria are applied to seeds, tubers or roots, stimulating growth and yield of crops (Agrios, 2004). The mechanisms of growth-promoting bacteria are not well understood, however, a wide range of possibilities has been suggested that include both direct and indirect effects.

The direct effect consists of an increase in the mobilization of soluble nutrients, followed by improved uptake by plants, increased N_2 fixation and production of phytohormones. Indirect effects include the production of siderophores, antibiotics against fungi, bacteria and viruses, increased number of root nodules and nitrogenase activity, which induce systemic resistance in the plant (Mantilla, 2007).

This study was undertaken to gain knowledge of bio-fertilizers, determine the main chemical and biological characteristics and their effect on the production of crops of lettuce and cabbage.

Materials and methods

The study was conducted in the Centro Multisectorial LOPE-SENA Regional Nariño, located in the municipality of Pasto, Nariño, 2,700 m a.s.l., average temperature of 14°C, average annual rainfall of 841 mm and relative humidity of 73% (Ideam, 2008).

The bio-fertilizers were prepared in 20 L cans. First, 15 L of water, 250 mL of white yogurt, 5 kg manure, 1 L raw milk and 1.5 L molasses were mixed, then borax (150 g) and Huila rock phosphate (260 g) were added; the mixture was stirred for 20 min and resulted in a water volume of 18 L. After two months, the contents of each can were filtered to extract the bio-fertilizer and kept in dark containers at room temperature.

The seedlings were set in trays with peat, using lettuce seeds of the variety Great Lakes and the cabbage Quisto hybrid. The lettuce and cabbage plants were transplanted at a distance of 0.40 x 0.40 m. We performed a background composting with a 10-30-10 fertilizer compound, at a rate of 260 kg ha^{-1} and there were three hand weedings at 30, 60 and 90 days after planting in the field. We also carried out hilling when the plants began to close their leaves, which occurred at 45 d for lettuce and 55 d for cabbage after transplantation. The crops were watered using drip system irrigation. The harvest was done manually, lettuce at 91 d and cabbage at 112 d, when the compaction of the heads was firm to the touch.

The treatments were distributed in a randomized block design with four replications. In each crop five treatments were analyzed, bio-fertilizers produced with manure from cow (BFC), pig (BFPI) and guinea pig (BFGp), a commercial foliar fertilizer (CFF) and a control without foliar application. The results were interpreted by analysis of variance and Tukey mean comparison test ($P \leq 0.05$).

The first application of the treatments was made at the time of transplantation to the roots using a bio-fertilizer solution 50 mL L^{-1} of water. During the crop cycle, biweekly applications of the bio-fertilizers and the CFF were utilized up until fifteen days before harvest, using a dose of 5 mL L^{-1} of water.

From each bio-fertilizer, 500 mL samples were taken in sterilized glass containers for analysis at the Microbiology Laboratory at the Universidad de Nariño, the chemical analysis was done at the Specialized Laboratories, Universidad de Nariño, according to the method proposed by Carreño and Unigarro (2005).

The evaluations of the crop weight were determined individually for each head of cabbage and lettuce, removing damaged outer leaves and curst using a penetrometer (Banco de Normas en Alimentos, 1982); the diameter heads and performance of each crop were evaluated in t ha^{-1} (Muñoz and Ortega, 1995).

Results and discussion

Microbiological characteristics of the bio-fertilizers used

Microbiological testing of the bio-fertilizers determined the presence of *Lactobacillus* and *Saccharomyces* in BFC and BFGp and *Bacillus* in the BFPi microbial liquid (Tab. 1). The presence of *Saccharomyces* is of great importance, because it stimulates the synthesis of antibiotics and other useful substances for plant growth from amino acids and sugars secreted by photosynthetic bacteria, organic matter and plant roots; these secretions are substrates useful to lactic acid bacteria and actinomycetes, which are closely related to the production of *Lactobacillus* and with the contributions of raw milk and white yogurt in the preparation of bio-fertilizers (Mantilla, 2007).

TABLE 1. Microbiological analysis of bio-fertilizers produced with fresh manure from pigs, guinea pigs and cows.

Parameters	Microbial liquid cow manure	Microbial liquid pig manure	Microbial liquid guinea pig manure
Lactobacillus sp.	+	-	+
Saccharomyces sp.	+	-	+
Bacillus sp.	-	+	-

+ Presence of the microorganism.
- Absence of the microorganism.

The *Bacillus* sp. present in BFPi, is a Gram positive sporulated bacillus, listed as a PGPR, due to its ability to fix atmospheric nitrogen and produce phytohormones such as gibberellic acid and indole acetic acid, is considered a disease-suppressive agent, present in organic fertilizers (Mantilla, 2007). It is important in the production of a series of metabolic substances with antagonistic effects that are easily dispersed in the environment (Pacheco, 2006).

Chemical characteristics of the bio-fertilizers used

The results of the chemical analysis (Tab. 2), place BFPi with the highest content of N (0.45%), P (0.16%), K (0.71%), Ca (0.55%), Mg (0.13%) and S (0.29%), Cu (2 mg L^{-1}), Zn (14 mg L^{-1}) and Fe (228 mg L^{-1}), and BFC with lower values in N (0.17%), P (0.03%), K (0.36%) Ca (0.26%), Mg (0.07%), S (0.014%), Cu (0 mg L^{-1}), Zn (3 mg L^{-1}) and Fe (36 mg L^{-1}), the mineral contents of BFC were very similar to BFGp.

TABLE 2. Chemical composition of the bio-fertilizers produced with manure from Guinea pigs (BFGp), pigs (BFPi), cows (BFC) and the commercial foliar fertilizer (CFF).

Parameter	BFGp	BFPi	BFC	CFF
pH	4.7	4.1	3.9	2.5
C/N	9.27	16.52	17.92	-
Carbon	2.16*	7.5*	3.12*	-
Nitrogen	0.23*	0.45*	0.17*	180.0 g L^{-1}
Phosphorus	0.05*	0.16*	0.03*	100.0 g L^{-1}
Potassium	0.63*	0.71*	0.36*	40.0 g L^{-1}
Calcium	0.29*	0.55*	0.26*	0.2 g L^{-1}
Magnesium	0.08*	0.13*	0.07*	12.5 g L^{-1}
Sulfur	0.19*	0.29*	0.14*	33.0 g L^{-1}
Manganese	14.0**	12.0**	15.0**	2.3 g L^{-1}
Copper	0**	2.0**	0.0**	2.7 g L^{-1}
Zinc	4.0**	14.0**	3.0**	7.8 g L^{-1}
Iron	45.0**	228.0**	36.0**	0.32 g L^{-1}

* C, K, P, N, S, Mg, Ca as a percentage.
** Mn, Zn, Cu, Fe as mg L^{-1}.

These results assume that the mineral contents of a bio-fertilizer are closely related to the diet of the animals. Hinestrosa *et al.* (1997) claim that the diet of guinea pigs is based on young grass and easy to digest plant species, similar to that of dairy cattle, excepting mature grasses that are palatable, unlike pigs that are omnivores, with a diet poor in cellulose and richer nutrition in their feed (Sisson, 1978).

As for the C/N ratio, BFC was the highest (17.92), due to a diet rich in cellulose, followed by BFPi (16.52), possibly because of incomplete digestion, since only between 40% and 60% of its nutritional value is utilized (Kolb, 1975), BFGp had the lowest C/N ratio (9.27), due to further digestion and better nutritional balance with plants with tender succulents (Duran, 2003; Hinestrosa *et al*., 1997).

The mineral content of a fertilizer is not enough to determine its quality, it is necessary to take into account other factors. The C/N ratio plays a role in the mineralization of N and can be used as a parameter of quality of the fertilizer in the real contribution of N (Stevenson, 1986; Paul and Clark, 1996; Epstein, 1997; Foth and Ellis, 1997). According to Leblanc *et al.* (2007), if the C/N ratio of a fertilizer is less than 20, it is easily degraded, initially immobilized by microbes when they die, which are released to the environment. In addition, the C/N ratio is important for the requirements of the microorganisms that use carbon as an energy source and nitrogen as a basic element for the formation of proteins and other constituents of the cell protoplasm.

The potentiometric evaluation of the pH of the bio-fertilizers showed values of 4.7 for BFGp, 4.1 for BFPi and 3.9 for BFC; the food consumed and its fiber content are responsible for generating higher bacterial populations and therefore a higher content of carbon dioxide, responsible for acidification of microbial media from the HCO_3^- (Good *et al.,* 1966).

Ito (2006) and Segura (2002), argue that when the pH remains close to 4.2, fermentation tends to stabilize the solubility of the nutritional elements for the plant, allowing better nutrient availability. Similarly, Molina (2002) states that the pH influences the solubility of the products and the availability of nutrients to be absorbed and that with slightly acidic pH values there is a greater availability of elements such as N, P, S, Cu , Zn and Fe, whereas when conditions are moderate or basic, precipitates that are difficult to absorb form. The correlation between *Lactobacillus* sp. and pH is important, as it can cause a decrease in pH by producing lactic acid and short chain fatty acids (Ito, 2006).

Agronomic evaluation of lettuce and cabbage crops

The analysis of variance for the effect of the bio-fertilizers on cabbage and lettuce crops showed highly significant differences ($P\leq0.01$) for the variables head weight, diameter and yield, the variable hardness, showed no differential response to the application of treatments in both crops.

Head weight

The comparison test of means for lettuce head weight (Tab. 3) showed that the treatments with BFGp and BFC had the highest weights (969.34 and 880.47 g, respectively) with statistically significant differences from the control (522.79 g), the CFF and BFPi treatments (747.73 g and 668.13 g, respectively) had statistically similar heads of lettuce for the bio-fertilizers and the control.

The cabbage heads had the highest weight with the BFGp and CFF treatments (1615.75 and 1525.0 g, respectively) with statistical differences compared to the control (1066.48 g); the BFPi (1246.25 g) and BFC (1428.75 g) treatments behaved statistically similarly with no statistical differences from the control.

These results show that bio-fertilizers have an effect similar to commercial foliar products, which despite having a higher mineral content, lack facilitators that potentialize the assimilation of nutrients like bacteria, which can induce the formation of metabolites that favor foliar penetration and improve the physiological processes of plants (Gajdos, 1992).

The positive effects produced by bio-fertilizers must bring a great amount of available nutrients to plants (Restrepo, 2002). They also contain amino acids produced by microorganisms in highly variable amounts, forming molecules such as thiamine, which plays an important role in enhancing

TABLE 3. Observation of the variables: head weight, hardness, diameter and yield of lettuce and cabbage.

Treatment	Head weight (g)		Hardness (psi)		Diameter(cm)		Yield (t ha^{-1})	
	Lettuce	Cabbage	Lettuce	Cabbage	Lettuce	Cabbage	Lettuce	Cabbage
BFPi	668.13 ab	1246.25 ab	7.29 a	19.93 a	13.06 c	20.45 ab	19.05 b	38.40 b
BFGp	969.34 a	1615.75 a	10.47 a	21.02 a	15.32 ab	22.99 a	24.68 a	4840 a
BFC	880.47 a	1428.75 ab	9.70 a	22.12 a	16.04 a	22.53 ab	23.80 a	48.68 a
CFF	747.73 ab	1525.00 a	8.97 a	20.96 a	14.29 bc	21.90 ab	20.58 ab	41.45 b
Control	522.79 b	1066.48 b	7.46 a	19.34 a	12.66 c	19.29 b	16.30 b	30.58 c

Means with different letters indicate significant differences, Tukey test ($P\leq0.05$).

the acquired immunity in plants; nicotinic acid, pantothenic acid, ascorbic acid and folic acid are also produced by microorganisms and act in the synthesis of essential enzymes and coenzymes that are essential for metabolic processes (Andrew, 2002; Martínez, 2002).

Head hardness

The different applications did not affect hardness (Tab. 3), possibly because it is a variable little affected by applications of nutritional elements; in this regard Jaramillo and Leyva (2002) argue that the hardness and compaction in vegetables are more related to the genetic and climatic conditions in the area to be cultivated. Similarly, Cubero (2003) argues that the physical properties of some vegetables are more influenced by environmental variations than by agronomic and management practices.

Head diameter

Tukey's test (Tab. 3) established that the larger heads of lettuce were with the applications BFC and BFGp, with average diameter of 16.04 and 15.32 cm, respectively, with statistical differences compared to the lower responses observed with the BFPi application and the control treatment with averages of 13.06 and 12.66 cm, respectively. The CFF treatment with 14.29 cm was statistically exceeded only by the treatment BFC.

In cabbage, BFGp (22.99 cm), BFC (22.53 cm), CFF (21.9 cm) and BFPi (20.45 cm), were statistically similar, the control treatment showed the lowest average (19.29 cm) with statistical differences from the BFGp treatment. In this case, the bio-fertilizers showed a response similar to the commercial foliar fertilizer (CFF), possibly due to the bacterial content and the metabolites excreted and present in these liquids, which may be responsible for the increases in this variable.

Martínez (2002) and Mantilla (2007) claim that *Saccharomyces* sp. and *Lactobacillus* sp., present in BFGp and BFC, stimulate the synthesis of antibiotics and other useful substances for plant growth, from amino acids and sugars secreted by photosynthetic bacteria, this leads to the assumption that the effects produced by these bacteria are responsible for the increase in diameter; in addition, that the *Bacilllus* sp. in the BFPi can be incorporated into the liquid fertilizer similar to the hormones, amino acids and sugars, which are absorbed through the stomata of the leaves.

Crop yields

The Tukey mean comparison test (Tab. 3) showed that the BFGp (24.68 t ha^{-1}) and BFC (23.8 t ha^{-1}) treatments had the highest average production of lettuce with statistical differences compared to the BFPi application (19.05 t ha^{-1}) and the control (16.3 t ha^{-1}), the CFF application (20.58 t ha^{-1}) was statistically similar to all treatments.

Biweekly applications of BFC and BFGp produced the highest yields of cabbage (48.68 and 48.40 t ha^{-1}) with statistical differences compared to the other treatments, the yields with the application of CFF (41.45 t ha^{-1}) and BFPI (38.40 t ha^{-1}) were similar and statistically higher than the results achieved with the control (30.58 t ha^{-1}).

BFC and BFGp had a better nutritional balance for lettuce and cabbage crops, possibly because of the efficient microorganisms *Lactobacillus* sp. and *Saccharomyces* sp., that, in addition to their beneficial effects, support the growth of other efficient microorganisms that improve chemical properties, the C/N ratio, pH and assimilation of nutrients that contribute to better plant growth.

Viteri (2002) and Andrew (2002) state that bio-fertilizers allow higher yields in crops, actively contributing to the improvement of the structure and aggregation of soil particles that increase their ability to absorb water and control soil-borne pathogens by competition and increased microbial biodiversity.

The results in this study allow us to ensure the benefits of bio-fertilizers used in the production of vegetables such as lettuce and cabbage with a significant reduction of costs and additional benefits for the environment, as confirmed by Viteri *et al.* (2008) using bio-fertilizers on the onion.

Conclusions

The presence of *Lactobacillus* sp. and *Saccharomyces* sp. was established in BFC and BFGp; in BFPi mineral content was determined to be the highest with the presence of bacteria of the *Bacillus* sp. type. The lowest mineral content occurred in BFC and BFGp.

The bio-fertilizers applied to the lettuce crop, showed a similar behavior to CFF for the head weight variable; for the head diameter and yield of lettuce variables, the highest averages occurred in the BFC and BFGp treatments, higher than the control and BFPi.

In the cultivation of cabbage, the best performance for head weight was found with BFGp and CFF, which were higher than the control, while only BFGp was higher than the control for the head diameter variable. For the yield variable, the best performance was presented by BFC and BFGp, which were superior to the other studied treatments.

The hardness of the heads of lettuce and cabbage was not affected by the bio-fertilizers or the commercial foliar fertilizer.

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