Vermicompost Effects on Bulbing Dynamics, Nonstructural Carbohydrate Content, Yield, and Quality of 'Rosado Paraguayo' Garlic Bulbs

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Abstract. The objectives of this work were to a) determine vermicompost effect on bulbification dynamics in terms of garlic (*Allium sativum* L.) bulb dry weight and sucrose metabolism and b) evaluate the impact of vermicompost on garlic bulb yield and quality. The treatments were soil (control) and 1 soil : 1 vermicompost (by volume). The use of vermicompost as a substrate caused early bulbing (18 to 20 days) and lengthened bulb filling period. Bulb filling period corresponded to an increase in the total soluble carbohydrates and a later modification in nonstructural carbohydrate distribution patterns regarding fructan (scorodose) metabolism. The vermicompost treatment increased scorodose accumulation, which was directly related to the harvest index, resulting in greater yield and bulb quality. Bulb quality was not modified in terms of bulb pungency and soluble solids content by the use of vermicompost.

In the current agronomical context, the use of solid agricultural waste (vermicompost) in garlic production constitutes a valid alternative, though the physiological and/or agronomical implications of such use are unknown. Argüello et al., (1997) determined the critical period for fertilizing garlic may be between the initiation of shoot growth and the beginning of bulb filling. In particular, previous work on traditional fertilization, such as urea on garlic, show the fertilization advances the onset of bulbification by 5 to 6 d, which favorably lengthens the period of bulb filling, increases the bulb's dry weight by 10%, and increases yield and quality (Ledesma et al., 2000). Vermicompost effect on dry weight, bulbing, yield and quality of garlic bulbs is not known.

The use of vermicompost on onions increased growth and yield from 2.72 g/plant to 38.05 g/plant (Thanunathan et al., 1997). In other vegetables, such as cucumber and lettuce, adding 20% of pig manure vermicompost increased the aboveground dry weight of seedlings as compared to the traditional commercial crop medium (Atiyeh et al., 2000). In tomatoes, adding 20% of pig manure vermicompost resulted in a 12.4% increase in fruit weight; and adding between 20% and 40% of this compost increased commercial fruit size (Atiyeh et al., 2001). Lettuce seedlings treated with a 50% NutriLomb

vermicompost showed more vigorous growth as a consequence of the optimization of the use of water and carbon (Ledesma et al., 2001)

In onions, dry weight is mainly accounted for by nonstructural carbohydrates, such as fructose, glucose, sucrose and fructans (Darbishire and Henry, 1979). Free sugar content in onions varies from 41% to 63% of dry weight and is represented by glucose (17% to 32%), fructose (11% to 25%) and sucrose (7% to 26%) (Fenwick and Hanley, 1985). Glucose (1.2%), fructose (1.4%) and sucrose (7%) were found in garlic bulbs (Arime and Deky, 1983).

Both garlic and onion use polysaccharides as reserve substances. The fructans are the most relevant ones since they are considered to be the most important storage carbohydrates (Fenwick and Hanley, 1985). In garlic, the fructan polysaccharide is the scorodose, which accounts for 53% of garlic dry weight (Fenwick and Hanley, 1985). Ledesma et al. (1997b) found significantly higher amounts of this polysaccharide as compared to glucose and fructose in garlic bulbs at harvest time. Sucrose accumulation is a prerequisite for fructan synthesis (Pontis, 2004).

It is hypothesized that the use of vermicompost on garlic causes early bulbing, lengthens the bulb filling stage, increases dry weight and, therefore, increases yield and quality.

The objectives of this work were to a) determine the vermicompost effect on bulbing dynamics in terms of garlic bulb dry weight and sucrose metabolism and b) evaluate the impact of vermicompost on garlic bulb yield and quality.

Materials and Methods

Medium-sized 'Rosado Paraguayo' garlic

(*Allium sativum* L.) clove seeds taken from the second and third fertile leaves of bulbs sown in the Villa Dolores area (Province of Cordoba, Argentina) were used in this study.

The experiment was conducted at the Facultad de Ciencias Agropecuarias, Universidad Nacional de Cordoba, Argentina. The material was cultivated in black plastic containers (2 kg). The growth medium was soil taken from the Cordoba Green Belt orchards, unfertilized and intensively worked, with and without commercial vermicompost (NutriLomb) taken from chicken and pig manure.

Treatments were a) soil and b) 1 soil :1 vermicompost (by volume). In both substrates, the physicochemical features evaluated were organic carbon, phosphorus, total nitrogen, electrical conductivity and pH. The content of organic C was determined by Walkley-Black (Nelson and Sommers, 1996). Total N was evaluated by Kjeldahl and extractable P was quantified by Bray and Kurtz Nº1 (Kuo, 1996). The pH values were measured in extracts aquosos 1:2.5 with a pH meter (Orion Research 901). Conductivity electric was measured in satured paste with a conductivity meter (DIST4 of HANNA Instrument).

The following parameters were measured.

Plant height. Ten plants per treatment were measured biweekly from the soil line to the growing point of the plant.

Bulbing index (BI). BI was expressed as neck diameter/bulb diameter ratio (Mann, 1952). Bulbing was considered to start when BI = 0.5

Bulb dry weight. Five plants per treatment were evaluated every 20 d. Bulbs were oven-dried for 7 d at 60 °C.

Total soluble carbohydrates were determined by the Antrona Technique (Scott and Melvin, 1966). Two grams of anthrone is dissolved in 1 L of concentrated H_2SO_4 (95%) Extracts were prepared with 1 g of dry weight 10 mL distilled water, heated for 15 to 20 min in a water bath at 90 °C and cooled (Argüello, 1987). In total, 2 mL of Antrona reagent was mixed with 1 ml extract in a cooled bath (10 to 15 °C), then placed in a water bath at 90 °C for 16 min and cooled. Absorbance was contrasted with glucose solutions of 100 and 200 $\mu g \cdot mL^{-1}$ at 625 nm with a spectophotometer (Shimadzu UV-120).

Sugar types. Carbohydrate composition was carried out in a water extract prepared with 1 g of fresh weight from three bulbs. Samples were analyzed by HPLC in the CEPROCOR (Centro de Excelencia en Productos y Procesos Cordoba) and by gas chromatography in the CEMECO (Centro de Estudios de las Metabolopatias Congenitas). Glucose, fructose, sucrose, and scorodose were quantified.

Chromatography by HPLC. Work was carried out under the following conditions: detector = RID-GA Shimadzu with refraction index; column = Waters Spherisorb S5 NH_2 (4.6 × 250 mm) equilibrated in 80 acetonitrile : 20 H_2O (by volume), room temperature; mobile phase of water = Mili Q with flow = 0.2 mL.

Gas chromatography. 50 μL of garlic water extract was placed in a clean tube with a Teflon seal and 50 μg of rhamnose were added for

internal standard. The extract was carefully dried by a nitrogen current. Overdrying was avoided by placing the tube in lukewarm water. For derivatives, 100 µL Sylon IITP was added; then, the tube was placed in a sand bath for 30 min at 80 °C in a nitrogen atmosphere. After the bath, it was cleaned up by adding 800 µL of hexane and 500 µL of acetyl nitrile. It was vigorously shaken, mixed in a shaker for 5 min and centrifuged at 400 rpm for 5 min. The upper phase was collected and dried again under a nitrogen current and later suspended in 50 µL of hexane. A 3-µL sample was injected in a series II PLUS HP 5890 chromatograph equipped with a HP1 apolar column and HP 35 column of 0.25 mm diameter, 25 m large and 0.5 µm thickness with detector FID. Work program was temperature increase from 70 to 140 °C with 10 °C/min, 140 to 190 °C with 2 °C/min and 190 to 270 °C with 30 °C/min. The sample was run for 80 min.

Table 1. Substrate chemical properties.

Property	Vermicompost	Soil	Soil to vermicompost ratio (1:1, v/v)
Organic carbon (%)	7.79	0.64	1.79
P-Bray (ppm)	290	59.7	283.2
Total N (%)	0.79	0.04	0.14
Electrical conductivity (dS·m⁻¹)	4.90	0.04	0.14
pH	7.20	7.90	7.33

Assimilate partition index. The harvest index (bulb dry weight/total dry weight) was measured in 10 plants as a physiological indicator of yield at harvest. The yield of garlic was evaluated as fresh weight per bulb (g) in 10 randomly chosen plants at harvest. *Bulb quality* measurements consisted of bulb caliber, pungency, and soluble solid content.

Caliber. The equatorial diameter of 10 bulbs was measured and classified into caliber 3 (26 to 35 mm), caliber 4 (36 to 45 mm), and caliber 5 (46 to 55 mm) (Burba 1993).

Pungency. The juice from 100 g of fresh weight was extracted with vegetable juicer, was filtered with Whatman No. 3 filter paper, and was placed glass flask in refrigerator until used for determination of pyruvic acid. Samples of 1 mL were diluted to 100 mL distilled water. A 2-mL sample of the diluted juice was used for determination of pyruvic acid. The method was calibrated with sodium pyruvate as standard, and the results are expressed as µmoles of pyruvic acid per ml of juice (Schwimmer and Weston, 1961).
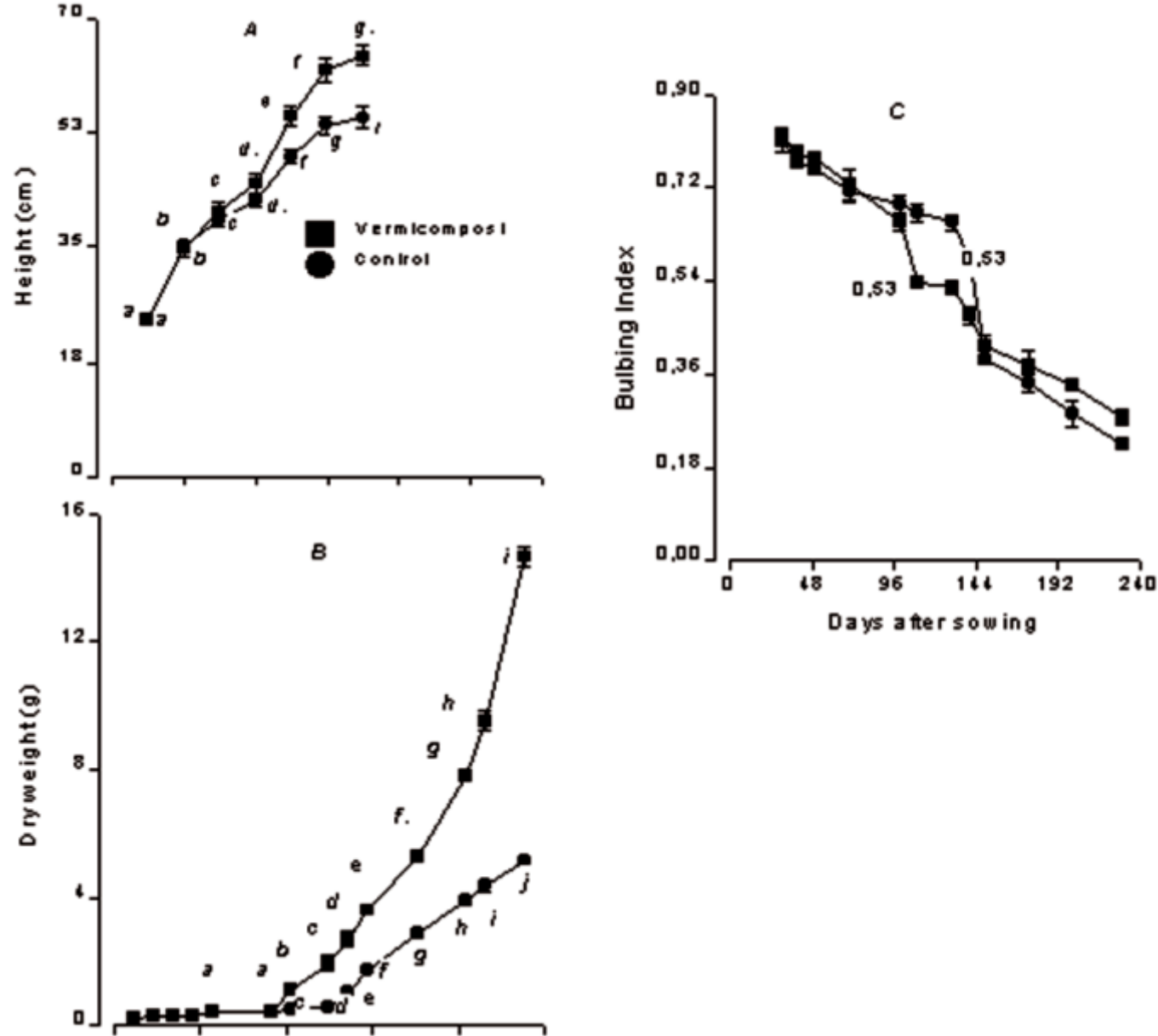

Fig. 1. Plant height (**A**), bulb dry weight (**B**) and bulbing index (BI) (**C**) during garlic growth. BI = 0.53 = Start of bulbing. Different letters within a treatment date reflect statistical differences at $P \leq 0.05$. The bars represent standard errors.
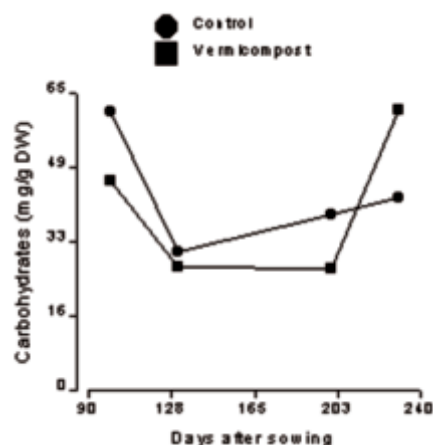
Fig. 2. Total soluble carbohydrates in garlic bulbs as influenced by vermicompost.

Total solid soluble content was evaluated in the same juice diluted in 50% of distilled water, by using a hand refractometer (Atago ATC-1, Japan).

Experimental design. The experiments were conducted in 3 consecutive years, following a completely random design with three repetitions per treatment. Data were analyzed by using the ANOVA and Tukey tests ($P \leq 0.05$) by INFOSTAT 2004 (Facultad de Ciencias Agropecuarias, Universidad Nacional de Cordoba, Argentina).

Results and Discussion

Vermicompost amendment application increased organic C content, P, and N. The pH of mixture soil and vermicompost decreased lightly and the conductivity electrical increased without produce effect of salinity (Table 1). This increase in substrate fertility correlates with an increase in plant growth, as shown in other horticultural species (Atiyeh et al., 2000, 2001; Bityutskii et al., 1997; Ledesma et al., 2001). Similar findings are reported in this study (Fig. 1).

Figure 1 shows the effect of vermicompost on the bulb dry weight, bulbing index, and garlic growth. Plant height did not differ during the first 60 d after sowing (Fig. 1A). These results can be explained by the fact that clove seed sprouting and the start of the aboveground growth were supported by carbohydrate reserves in the clove seed (Ledesma et al., 1981; Núñez et al., 1997). After 60 d after sowing, plant height was increased by vermicompost.

In garlic, bulb dry weight depends on the growth of the aboveground part (Burba, 1993; Ledesma et al, 1981, 1997a). In this study dry weight remained constant regardless of treatments until 100 d from sowing (Fig. 1B). The inductive and morphogenic stages took place after clove seed sprouting (Ledesma et al., 1981; Núñez et al., 1997). During these stages, there is no photoassimilate accumulation in the bulb. In the first stage, thermal and photoperiodic stimuli are captured by the leaf, thus making it possible for the second stage to begin the bulbification morphogenic process (Núñez et al., 1997). However, the increase in aboveground growth by the use of vermicompost corresponds to a significant advance of the onset of the bulbing stage (about 18 to 20 d earlier than that of the control plants, Fig. 1C). Similar evidence of earlier bulbing (5 to 6 d) was observed when applying urea fertilizer (Ledesma et al., 2000).

The bulb filling stage begins at the same time as the clove seed differentiation in the bulb (Ledesma et al., 1997a). This stage is made evident by an increase in bulb dry weight (Fig. 1B), which suggests greater accumulation of reserve substances. These are the total soluble carbohydrates (Fig. 2) and nonstructural carbohydrate (increased fructans polysaccharide shown by scorodose) (Fig. 3).
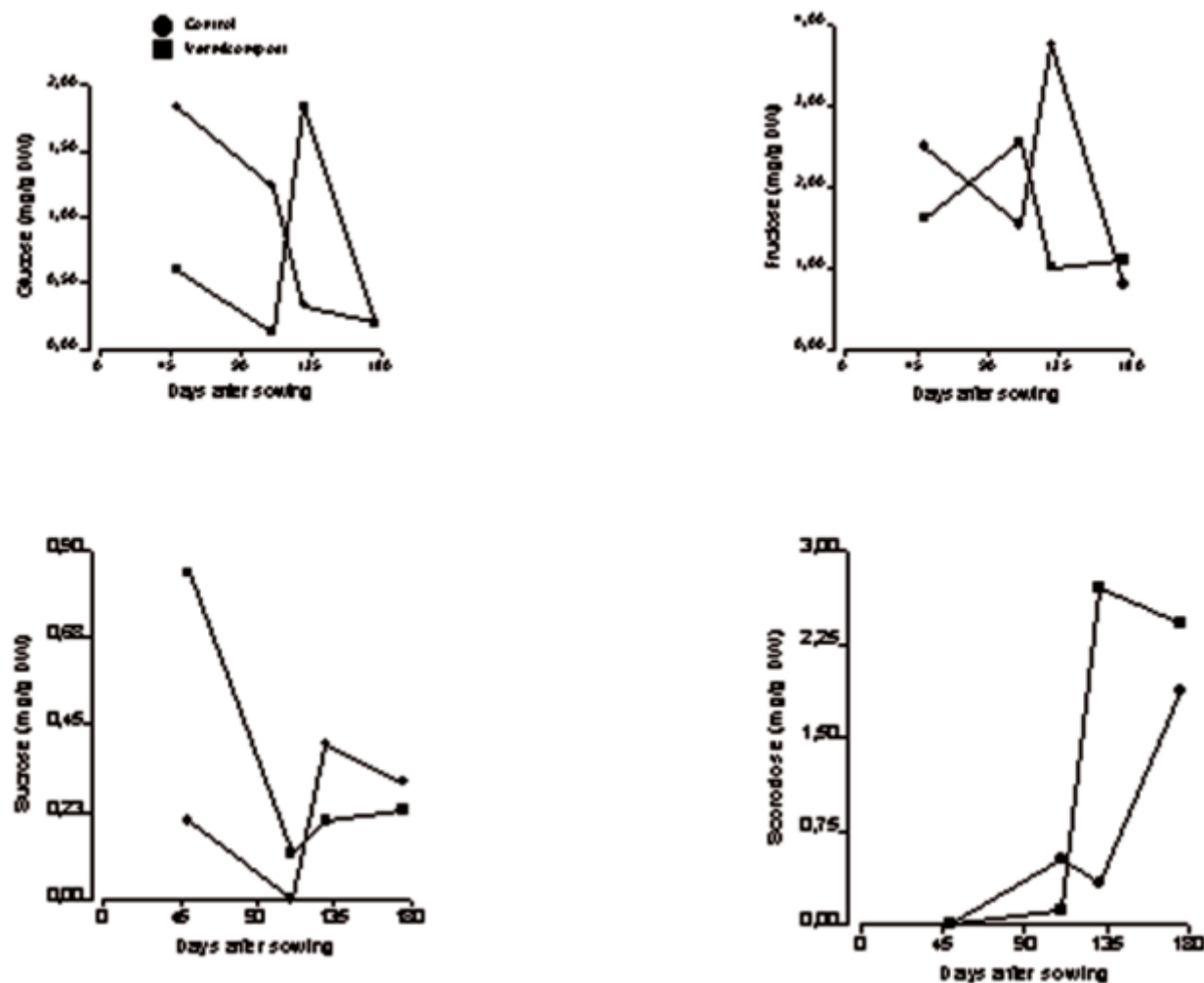

Fig. 3. Effect of vermicompost on nonstructural carbohydrate content (glucose, fructose, sucrose, and scorodose) in garlic bulbs before bulbing starts (50 d), during bulbing (100 d), and at the end of the bulb filling period (170 d).

Table 2. Effect vermicompost on assimilate partition Index, garlic yield and bulb quality components at harvest.

Parameter	Soil	Soil : vermicompost ratio
Assimilate partition index		
Harvest index (%)	67.7 a[z]	80.0 b
Garlic yield		
Bulb fresh weight (g)	9.98 a	28.2 b
Bulb quality components		
Caliber 3 bulbs (%)	57.4 a	7.23 b
Caliber 4 bulbs (%)	42.6 a	77.07 b
Caliber 5 bulbs (%)	---	15.7
Total solid soluble content (°Brix)	19 a	16 a
Pungency (μmol pyruvic acid/mL)	2.21 a	2.53 a

[z]Different letters indicate significant differences between treatments ($P < 0.05$).

Glucose, fructose, and sucrose have been reported in garlic (Mizuno et al., 1957; Srinivasan et al., 1953). Similar performance has also been reported in this study. The relative and absolute contents of nonstructural carbohydrates depend upon a range of factors, including agronomic and environmental conditions of growth (Fenwick and Hanley, 1985). As it is the case of vermicompost which modifies the quantity and pattern of sugar accumulation (Figs. 2 and 3). From our results, it can be suggested that there is not sugar accumulation during the inductive stage (Fig. 3). However, in the morphogenic stage, the glucose contents decrease due to the fact that they are used in this process, and there is scorodose accumulation, that increases by vermicompost treatment (Fig. 3). Vermicompost increases the bulb dry weight by the accumulation of nonstructural carbohydrates whose distribution patterns change, thus favoring the metabolism of fructan precursors and accumulating as scorodose (Fig. 3).

Such reserve substance accumulation in the vermicompost treatment, represented by scorodose polysaccharide, occurs for a longer period due to the earlier start of bulbing. This response translates into a 2-fold increase of the bulb's dry weight, increased size, and; therefore, higher quality and yield at harvest (Table 2). Vermicompost resulted in an increase in scorodose accumulation, which is directly related to the Harvest Index (Table 2).

Quality, expressed as bulb pungency and soluble solid concentration (SSC) was not affected by vermicompost (Table 2).

It can be concluded that the use of vermicompost as a substrate produces an earlier start of bulbification and, thus, lengthens the bulb filling period. This corresponds to an increase in the total soluble carbohydrates and a subsequent modification in the nonstructural carbohydrate distribution patterns, and hence, a modification in the pattern of the fructan (scorodose) metabolism. Scorodose accumulation is directly related to the harvest index, and is shown as greater yield and bulb quality.

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