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A New Calcium Vectoring Technology: Concentration and Distribution of Ca and Agronomic Efficiency in Pepper Plants

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Abstract: Calcium (Ca) is an important macronutrient for plants, although its low mobility through the phloem makes more difficult the translocation to growing tissues, including fruits. The blossom end rot (BER) physiopathy occurs mainly in fruits and is associated with water stress, and especially with low Ca levels, which has a very negative effect on the production of many crops. Currently, through the vectoring process, it is possible to increase the transport of immobile elements to the fruits. The objective of this study is to evaluate the effect of BRANDT® MANNI-PLEX® Ca, which contains Ca with a vector (polyalcohols), provided by the company BRANDT EUROPE S.L. (Carmona, Spain), on Ca accumulation and the production and quality of pepper fruits, both at harvest and post-harvest stage. Pepper plants were grown in a shaded greenhouse and supplied with BRANDT® MANNI-PLEX® Ca and parameters related to biomass, production, and fruit quality were analyzed. The results showed that the product increased shoot biomass, photosynthesis performance, Ca accumulation and quality of pepper fruits both at harvest and post-harvest, while reducing the incidence of Ca physiopathies by 70%. Therefore, this study proves the BRANDT® MANNI-PLEX® Ca efficacy in a crop with a high incidence of Ca physiopathies, such as pepper.

Keywords: antioxidants; blossom end rot; calcium; pepper; pectin; vectorization

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1. Introduction

For 150 years, scientists have studied plant nutrition to understand the processes of absorption, accumulation, transport, and functionality of the different chemical elements necessary for plant growth. From these studies, a lot of information has been obtained on the growth and composition of plants in response to the different growing media, the fertilization programs to be used in the different agricultural areas, as well as the different concentrations of these elements in the nutritive solutions for different plant species [1].

An important macronutrient for plants is calcium (Ca), which is necessary for the integrity and functionality of the cell wall and membranes and, in addition, is involved as a second messenger in the functioning of some hormones and in environmental responses. Most of the Ca is present in cell walls and membranes, as well as inside cell organelles, especially the vacuole. Another characteristic of Ca is its immobility in the phloem, which implies that there is a very limited translocation of Ca from the mature leaves of plants (source organs) to young leaves, shoots, and fruits (sink organs) [1,2].

The functions of Ca^{2+} as a structural element are mainly related to the fact that it confers rigidity and stabilization to the cell wall system. During cell wall biosynthesis, pectins are secreted from cells as methyl esters, which are subsequently de-esterified by pectin methylesterase, thus providing carboxyl groups that bind to Ca^{2+} through covalent and

ionic bonds. The associations of Ca^{2+} with pectins in the cell wall protect the degradation of this structure by the action of polygalacturonase enzymes. Furthermore, Ca^{2+} stabilizes cell membranes through the formation of bridges with phosphate and carboxyl groups between lipids and proteins [3].

In addition to these structural functions, Ca is defined as an essential secondary messenger in signaling and generating physiological responses to stresses, both abiotic and biotic, through specific increases in its cytoplasmic concentration. As such, Ca has also been recently defined as an essential signal in the detection and in the adaptation of plants to variations in the availability of essential nutrients such N, K, Fe, and Mg, through the activation of various Ca-dependent protein kinases [1,2].

As indicated previously, the presence of Ca in the phloem occurs at very low concentrations, so its translocation to different parts of the growing shoot parts such as leaf and fruits will depend on the transpiration rate or pressure in the root to provide Ca through the xylem. For organs with low transpiration rates, such as developing fruits, the root pressure process is insufficient, with symptoms of Ca deficiency appearing in many cases [4]. Ca deficiency is common in horticulture fruits such as tomatoes, peppers, and zucchini, causing rotting and necrotic areas at the distal end of the fruit, a physiopathy called "blossom end rot (BER)" [5].

BER is a physiological disorder that occurs mainly in fruits and is associated with water stress, and especially with the existence of low Ca levels, which has a very negative effect on the production of many crops. The symptoms of this disorder may cover half of the surface of the fruits and begin with the darkening of the tissues that occurs as the cells die. Thus, the appearance of BER is the result of plasma membrane rupture and irregular softening of the cell walls, due to processes of disintegration of the pectin chains by the action of specific degradation enzymes, all caused by Ca deficiency in fruits [5].

One of the strategies commonly used to improve the concentration of nutrients in the upper plant parts is the application of these nutrients via the foliar route combined with standard root fertilization programs [6]. However, this method is not always applicable for Ca because of its limited transport through the phloem and its minimal distribution towards the growing plant tissues (e.g., fruits) [7,8]. Currently, with vectoring process, it is possible to increase the foliar absorption, the concentration, and the distribution of these elements with low phloem mobility through different plant shoot organs including fruit [9–11].

The transport mechanism of agrochemicals through membranes is a key factor for both the absorption and long-distance distribution of these compounds in plants [10,11]. Hence, vectorization of compounds which shows difficulty in plant translocation facilitates and controls their distribution owing to this vector. In general, the vectors most used in this process are amino acids (glutamate, glutamine, lysine, glycine, and alanine), carboxylic acids (citrate, malate, oxalate, acetic, and glucuronic), phenolic acids (salicylic), sugars (glucose and rhamnose), and polyalcohols (sorbitol, mannitol, and xylitol) [9–11].

Although these vectors have been most used in the vectorization of pesticides, with great success, in other studies they ease the absorption and distribution of immobile ionic elements in plants, such as Ca^{2+}, Fe^{2+}, and BO_3^- [12–14]. In the case of Ca, the foliar application of Ca chelated with carboxylic acids (CALHARD®) increased this nutrient and the concentration of pectins and ascorbic acid in strawberry fruits [12]. For micronutrients such as B and Fe, it was proved that the combined application of these micronutrients with polyalcohols (sorbitol and mannitol) and the amino acid glutamate, respectively, improved phloem mobility and the concentration of these micronutrients in fruits [13,14].

The exact mechanism of these types of compounds in vectorization is not deeply understood. However, it has been observed that polyols may dissolve mineral nutrients and act as reducing agents and stabilizers that prevent particle aggregation [15]. In addition, vectoring compounds such as mannitol and perseitol may be transported across the phloem through specific transporters carrying immobile nutrients and easing its supply to sink

organs such as fruits. Likewise, plasma membranes are permeable to mannitol and thereby these compounds may promote the entry of mineral nutrients to cells [16,17].

The BRANDT® S.L. company (Carmona, Spain) has developed a vectoring product called MANNI-PLEX®Ca. Therefore, the objective of the present study was to evaluate the effect of BRANDT® MANNI-PLEX®Ca, which contains Ca with a vector (polyalcohols). Specifically, this study assesses its efficiency in the distribution and accumulation of Ca in the different parts of the plant and analyzes the effect on the production and quality of pepper fruits, both at harvest and post-harvest.

2. Materials and Methods

2.1. Experiment Localization

The study was carried out in a greenhouse with a mesh cover of 960 m^2, built in the IFAPA Center "Camino de Purchil" in Granada (Latitude: 37°10′ N; Longitude: 3°38′ W; Altitude: 600 m). The greenhouse is a multi-modular metal structure of gable roof type. The cover of the greenhouse structure was made with a natural white-black mono-filament mesh of 6 × 9 cm^{-2} threads. The bands of the greenhouse structure were made with a mesh of 10 × 16 cm^{-2} black threads around the perimeter and plasticized raffia impermeable to air. The soil was covered with black polyfiber to prevent the emergence of weeds. The microclimate conditions during the experiment are shown in Table S1.

2.2. Plant Material and Treatments Description

The plants used in this study were pepper plants (*Capsicum annuum* cv. Alicum). It is a sweet red Italian cultivar. It has cone-shaped fruit, 22 cm long, and 4 or 5 cm wide. This cultivar was developed by Fitó company (Barcelona, Spain). The experiment was carried out during the months of May–July 2021. The pepper seeds were sown in flat trays (cell size 3 cm × 3 cm × 10 cm, with 100 boxes per tray) filled with a mixture of peat and perlite 50% (*v*/*v*), and they were kept in a nursery for 25 days (HortoPlan S.L.; Motril, Granada). After, in mid-May, the seedlings were transplanted into the shaded greenhouse. Plants grew in the soil at a density of 2.2 plants/m^2 in paired lines with a distance of 0.5 m between paired lines and 1.30 m between adjacent unpaired lines, and 0.5 m between plants. Plants were trellised with nylon thread and trimmed to three stems.

Fertilization was carried out by fertigation. The composition of the nutrient solution (mmol L^{-1}) was: 15 NO_3^-; 1.8 $H_2PO_4^{2-}$; 2 SO_4^{2-}; 0.5 HCO_3^-; 0.5 NH_4^+; 5.5 K^+; 5 Ca^{2+}; 2 Mg^{2+}. The EC values were kept around 2 mS cm^{-1} and the pH at 6. The stock solution was prepared in four tanks of 1000 L each, where the fertilizers were concentrated 100 times to avoid having to recharge the tanks with much frequency. The nutrient solution was injected with a fertilizer injection irrigation controller on demand. The doses of fertilizer applied were calculated according to the characteristics of each commercial formula, the contributions of irrigation water, and the needs of the crop [18]. The pH and electrical conductivity of the nutrient solution, as well as the proportion of the different fertilizers, was automatically adjusted according to the pre-established values. The pH of the nutrient solution was measured daily and, when necessary, it was corrected with 2 mM phosphoric acid to maintain the pH at 6.

Irrigation in all treatments was automated drip. Two drop-holder branches were installed in each cultivation line, with emitters in line of 3 L h^{-1} every 0.25 m (4.44 emitters m^{-2}). The working pressure was 1.8 atm. The irrigation scheduling was completed by means of humidity sensors (ECH_2O EC-5, Decagon Devices) maintaining values of 20–30% of volumetric amount of water in the root zone, allowing depletion of soil water of 30% as irrigation criteria.

Two different treatments were carried out as follows: control without application of any product, and foliar application of BRANDT® MANNI-PLEX®Ca product, with three repetitions of each treatment with 8 pepper plants per repetition. The product was composed of Ca complexed with polyalcohols. The water-soluble Ca content was 10%. The amount of product used in each foliar application was 3 mL/L. The products were

applied every 15 days, with the first application on 27 May 2021, the second application on 11 June 2021, the third application on 26 June 2021, and the fourth application on 11 July 2021.

Sampling of plants and fruits was carried out on 19 July 2021 (8 days after the fourth foliar application of the products). Two post-harvest conditions were applied to the fruits as follows: one at room temperature (20–23 °C) that lasted 7 days, and another in a cold room at 4 °C with a duration of 14 days. The different post-harvest times depended on the duration of control fruits in a marketable state.

2.3. Sampling of Plant and Fruit

In the sampling of the plant material (19 July 2021), the shoot of 4 plants was cut per repetition for fresh weight determination. Furthermore, 4 leaves of each plant were sampled from the basal, middle, and apical zones. The leaves sampled from the different parts (basal, intermediate, and apical) were taken from all plants at the same height and with the same degree of development. In addition to the leaves, the marketable fruits at green stadium of each plant were sampled for the analysis at harvest.

The production components and fruit characteristics were analyzed as follows: the number of marketable fruits, the weight of marketable fruits, percentage of fruits with respect to the total presenting physiopathies related to Ca deficiency, length, and firmness.

Additionally, using fruits at green stadium harvested in each treatment, a post-harvest study was carried out both at room temperature (20–23 °C) to mimic shelf life, and in a cold room at 4 °C. Nine fruits (three fruits per repetition) were selected randomly and used for post-harvest experiment. Fruits at room temperature were sampled after 7 days of storage and fruits at 4 °C were sampled after 14 days of storage. The analyzes were carried out on the fruits at the beginning and at the end of post-harvest. All fruits were used to obtain fresh weight and dry weight, water loss percentage, firmness. A subsample from each of the 9 fruits was randomly obtained and independently measured to determine °BRIX titratable acidity, malondialdehyde (MDA) and ascorbate (AsA) concentrations, and antioxidant capacity tests. MDA concentration was determined at the end of post-harvest.

2.4. Analysis of Plant Material

2.4.1. Chl a Fluorescence Analysis

The plants were adapted to 30 min to darkness before taking measurements using a special leaf clip that was placed on each of the leaves. Chl *a* fluorescence kinetics was determined using the Handy PEA Chlorophyll Fluorimeter (Hansatech Ltd., King's Lynn, Norfolk, UK); OJIP phases were induced by red light (650 nm) with a light intensity of 3000 µmol photons $m^{-2}s^{-1}$. The phases of the OJIP fluorescence were analyzed by the JIP test. Measurements were made on fully developed leaves in the middle position of the plant of five plants per treatment. To study energy fluxes and photosynthetic activity, the following parameters obtained from the JIP test were used: variable fluorescence/maximum fluorescence ratio Fv/Fm, the proportion of active reaction centers (RC) (RC/ABS), and performance index (PI_{ABS}) [19].

2.4.2. Determination of the Concentration of Total Ca and Ca Bound to Pectins

The concentration of total Ca and Ca bound to pectins was determined in the leaves taken from the different parts of the plant and in marketable fruits. The determination of total Ca was carried out by ICP-OES, for which 0.2 g of dry and ground plant material was taken and was subjected to digestion with HNO_3 and H_2O_2 at 30% at 300 °C [20]. To determine the concentration of Ca bound to pectins, 0.1 g of plant material was weighed to which 1 mL of extraction buffer was added (50 mM Tris-HCl Buffer, pH = 7.5 + 0.25 mM Sucrose + 1 mM DTT). The mixture was centrifuged at 5000× *g* for 15 min and the precipitate obtained was dried, and was subsequently subjected to digestion with HNO_3 and 30% H_2O_2 at 300 °C [20]. Finally, the concentration of Ca bound to pectins in the

resulting mineralization was analyzed using ICP-OES [21]. The results were expressed as mg Ca g^{-1} DW.

2.4.3. Fruit Quality Parameters

For MDA extraction 0.5 g of fresh plant material was homogenized with 5 mL of 50 mM buffer (0.07% $NaH_2PO_4 \cdots 2\ H_2O$ and 1.6% $Na_2HPO_4 \cdots 12\ H_2O$) in a mortar and subsequently centrifuged at 20,000× *g* for 25 min in a refrigerated centrifuge (4 °C). Subsequently, 1 mL of aliquot of supernatant was mixed in test tubes with 4 mL of 20% trichloroacetic acid containing 0.5% thiobarbituric acid. The resulting mixture was heated at 95 °C for 30 min and then quenched in an ice bath. The samples were then centrifuged at 10,000× *g* for 10 min and the absorbance of the supernatant was measured at 532 nm. The value for non-specific absorption at 600 nm was subtracted from the reading obtained at 532 nm [22]. The results were expressed as μM g^{-1} DW.

Firmness was performed on whole fruit using a GY-1 analog penetrometer fitted with a 4 mm diameter flat probe, which measures the force in kg to penetrate the fruit tissue. Nine firmness measures were obtained per treatment (one per fruit). The results were expressed as Kg cm^{-2}.

The soluble solids content or °BRIX was determined using a traditional destructive interference test. Juice was extracted from a subsample of each of the 9 fruits that were measured separately. An Abbé-type refractometer (Zeiss, Oberkochen, Würt, Germany, Model B) was used. The results were expressed as % of soluble solid content.

The titratable acidity was determined using a GLP 21 pH meter. A subsample of 5 g from each of the 9 fruits was obtained and macerated independently with 50 mL of distilled water. Then, the pH meter was calibrated and the electrode was introduced into the fruit juice liquor, to which 5 mL of distilled water had previously been added. Titratable acidity was determined using the volume of NaOH 0.1 N required to reach a pH of 8.2. The results were expressed as % of citric acid.

For the extraction and quantification of AsA, the method of Law et al. [23] was followed. This method is based on the reduction in Fe^{3+} to Fe^{2+} by AsA in acid solution. The absorbance at 525 nm was measured against an AsA standard curve. The results were expressed as mg AsA g^{-1} FW.

The ferric-reducing antioxidant power (FRAP) assay was performed with the FRAP reagent, composed of 1 mM 2,4,6-tripyryldyl-2-triazine (TPTZ) and 20 mM $FeCl_3$ in 0.25 M CH_3COONa, pH 3.6. A 100 μL extract obtained from the homogenization of leaves in 10 mL of methanol was added to 2 mL of FRAP reagent. Subsequently, the mixture was incubated at room temperature (20 °C) for 5 min. Absorbance was measured at 593 nm against a standard curve of 25–1600 μM Fe^{3+} prepared using a 25 mM ferrous sulfate stock solution [24]. The results were expressed as mg ferrous sulfate g^{-1} FW.

The Trolox Equivalent Antioxidant Activity (TEAC) test was carried out using a modified version of the method of Cai et al. [25]. First, 7 mM of 2,2′-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was mixed with 2.45 mM of potassium persulfate to produce the ABTS + cation, for which the mixture resulting was incubated for 16 h in the dark at room temperature. Subsequently, the resulting $ABTS^{\cdot+}$ solution was diluted. A 100 μL aliquot of leaf extract (0.5 g/10 mL methanol) was vigorously mixed with 3.9 mL of diluted ABTS solution, and the absorbance at 734 nm was recorded. A standard curve of 0–15 μM of trolox was used. The results were expressed as mmol trolox g^{-1} FW.

2.5. Statistical Analysis

All analyses were repeated in triplicate and statistical comparison between the two treatments were conducted using Student's t-test. Differences between treatment means were compared using Tukey's honestly significant difference (HSD) test at a 95% probability level. The levels of significance were expressed as: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS (not significant).

3. Results and Discussion

3.1. Plant Biomass and Photosynthesis

One of the parameters that more reliably define the nutritional status of plants is the production of biomass expressed in fresh or dry biomass, which means that these parameters are used regularly as indicators to define the efficacy of any agrochemical product in plants [1,26]. Considering the obtained data, the growth of the pepper plants was stimulated by the application of the product tested in comparison to the growth of the control plants, in which the minimum values were obtained (Figure 1). Thereby, BRANDT® MANNI-PLEX® Ca application is useful to increase plant biomass. Similar results were observed in lemongrass plants whose growth was stimulated by the application of nutrients combined with polyols [24].

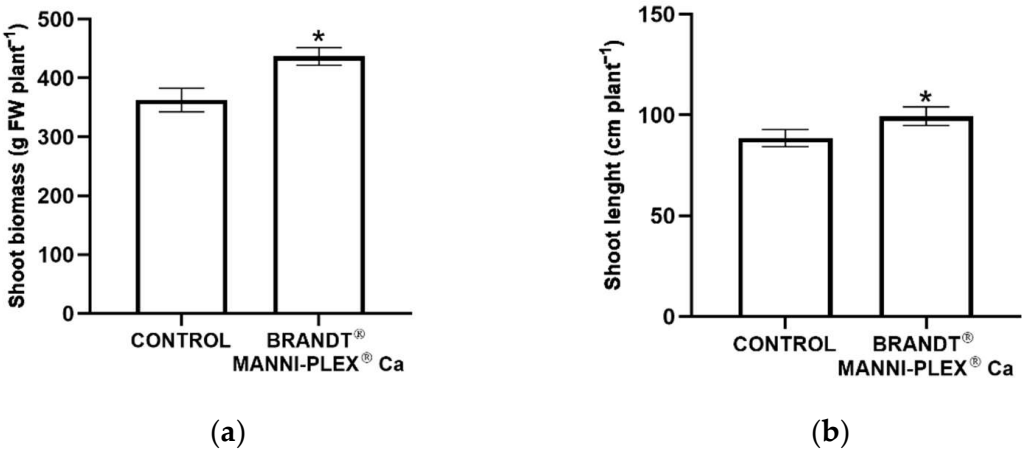


Figure 1. Effect of BRANDT® MANNI-PLEX® Ca application on the vegetative biomass (**a**) and length of the shoot part (**b**) of pepper plants. Values are expressed as means ± standard error (n = 12). The level of significance was represented by $p < 0.05$ (*).

A requirement or mechanism of action that the different biofertilizers and/or agrochemical products must meet is the induction or at least the maintenance of photosynthesis [27]. To test this effect with the Ca product studied, we analyzed different parameters that indicate the photosynthetic activity of the plants. Thus, in this study, we analyzed fluorescence parameters related to the activity and integrity of photosystem II (Fv/Fm, RC/ABS, and PI_{ABS}). Photosynthesis is closely related to plant growth and tolerance to different types of stress, such as the limitation of certain macronutrients such as Ca, since this nutrient is key in stabilizing the cofactor Mn_4CaO_5 cluster required for oxidation of H_2O to O_2 and the transport of electrons [2,28]. Chl *a* fluorescence reflects the photosynthetic state of the plant and the photosynthetic changes produced under the effects of stress. One of the parameters derived from the analysis of the fluorescence of Chl *a* is the quantum yield of primary photosynthesis (Fv/Fm), which is a good indicator of the photosynthetic yield of plants [29]. In the present experiment, no different Fv/Fm values were observed between the plants of the two different treatments. Similarly, apple tree plants supplied with nutrient supplied by MANNI-PLEX® technology did not show difference in Fv/Fm values [30]. Chl *a* fluorescence analysis also provides us with a series of indices that define the vitality of the plant. Thus, high values of the RC/ABS ratio indicate a higher proportion of active reaction centers, making this an essential parameter in the functioning of the electron transport chain in photosystems. In addition, the PI_{ABS} index is an index of photosynthetic performance and represents the overall functionality of the electron flow through PSII [29]. The values of these two parameters were higher in the plants treated with the biostimulant than those of control plants (Table 1), which suggests a positive effect of BRANDT® MANNI-PLEX® Ca on photosynthetic performance that could also contribute to the higher biomass observed in these plants (Figure 1).

Table 1. Effect of BRANDT® MANNI-PLEX® Ca application on some parameters of Chl *a* fluorescence analyzed in leaves of pepper plants.

	Control	BRANDT® MANNI-PLEX® Ca	*p*-Value	$HSD_{0.05}$
Fv/Fm	0.827 ± 0.004	0.824 ± 0.004	NS	0.03
RC/ABS	0.86 ± 0.03	1.31 ± 0.09	**	0.21
PI_{ABS}	8.36 ± 1.09	11.74 ± 1.18	*	2.57

Values are means ± standard error (n = 9). Values with different letters indicate significant differences among treatments. The levels of significance were represented as $p > 0.05$ (NS), $p < 0.05$ (*), and $p < 0.01$ (**).

3.2. Ca Concentration in the Different Plant Organs

An important characteristic of Ca is its immobility in the phloem, which implies that there is a very limited translocation of Ca from mature leaves of plants (source organs) to young leaves, shoots, and fruits (sink organs) [1,2]. Currently, one of the strategies to increase foliar absorption, concentration, and distribution of substances with low mobility through the phloem by the different plant organs of the shoot, including fruits, is the so-called vectorization process [9–11]. The vectorization process of the different agrochemicals that show difficulty in their distribution by the plant consists of facilitating and controlling the distribution of these compounds within the plant through their association with a vector [12–14]. The efficacy of the vectorization process was analyzed in the present experiment measuring Ca concentration in the different plant organs. Thus, the total Ca concentration in basal, intermediate, young leaves, and fruits were higher than those of control plants in plants supplied with BRANDT® MANNI-PLEX® Ca (Table 2). Indeed, this product produced increases concerning the total Ca concentrations obtained in the control plants of 34% in basal leaves, 42% in intermediate leaves, 35% in young leaves, and 76% in fruits (Table 2). Supporting these results, other studies also observed an enhancement of Ca content in tomato and honeydew fruit supply with a mannitol-Ca compound applied at preharvest to leaves [31,32].

Table 2. Effect of BRANDT® MANNI-PLEX® Ca application on the concentration of total Ca and Ca bound to pectins (mg g^{-1} DW) in pepper plants.

		Control	BRANDT® MANNI-PLEX® Ca	*p*-Value	$HSD_{0.05}$
Basal leaves	[Ca] tot	33.92 ± 2.49	41.30 ± 2.52	*	5.67
	[Ca] pec	23.74 ± 1.58	30.97 ± 2.09	*	3.98
Intermediate leaves	[Ca] tot	30.35 ± 2.09	37.63 ± 2.20	*	4.32
	[Ca] pec	23.06 ± 1.77	30.85 ± 2.98	*	3.05
Young leaves	[Ca] tot	16.57 ± 0.73	20.12 ± 0.83	**	1.47
	[Ca] pec	11.23 ± 0.60	15.43 ± 0.62	**	1.23
Fruit	[Ca] tot	1.33 ± 0.11	2.02 ± 0.16	**	0.27
	[Ca] pec	0.94 ± 0.07	1.57 ± 0.13	**	0.18

[Ca] tot: Total Ca concentration, [Ca] pec: Ca bind to pectins. Values are means ± standard error (n = 9). Values with different letters indicate significant differences among treatments. The levels of significance were represented as $p < 0.05$ (*), and $p < 0.01$ (**).

The plants supplied with the Ca product also presented the highest concentrations of Ca bound to pectins in all parts of the plants analyzed (Table 2). The synthesis of pectins and the binding of Ca to these structures is closely linked to the concentration of total Ca in the different plant tissues [1,2,26]. In short, the product based on Ca vectorization tested in this study represent a valid and effective strategy to increase Ca uptake via the foliage, its concentration, and its distribution through the phloem to the different parts of the plant, including active growth areas such as the young leaves and fruits. The highest concentration of total Ca, and especially of Ca bound to pectins, in plants treated with the

product could mean an increase in the resistance of plants to the presence of both abiotic and biotic stresses, given the protective role of these compounds [33,34]. As such, the higher biomass could be possibly due to an increase in the concentration of Ca linked to pectins, which could act by avoiding and/or reducing the loss of water in these plants thus facilitating their adaptation to environmental stresses.

3.3. Production and Fruit Characteristics

Considering the different components of production, the application of the Ca product was effective in inducing a significant improvement in the production of pepper plants (Table 3). Indeed, plants supplied with the product showed higher number of marketable fruits (+43%), weight of marketable fruit (+46%), and marketable production (+46%) compared to control plants. Therefore, the use of BRANDT® MANNI-PLEX® Ca could be very useful for farmers by considerably increasing the production of pepper fruits.

Table 3. Effect of BRANDT® MANNI-PLEX® Ca application on the production components in pepper plants and some components of the quality of pepper fruits at harvest.

	Control	**BRANDT® MANNI-PLEX® Ca**	***p*-Value**	**$HSD_{0.05}$**
N° of marketable fruits plant^{-1}	3.52 ± 0.66	5.05 ± 0.61	**	1.22
Weight of marketable fruits plant^{-1} (g)	138.17 ± 22.43	202.07 ± 39.61	*	60.53
Marketable production (g m^{-2})	276.34 ± 36.55	404.14 ± 57.32	*	94.21
Length (cm)	11.42 ± 1.35	15.29 ± 1.87	*	3.56
Firmness (Kg cm^{-2})	0.47 ± 0.10	0.77 ± 0.11	*	0.20
°BRIX (%)	4.40 ± 0.41	4.67 ± 0.35	NS	0.82
Titratable acidity (%)	0.38 ± 0.06	0.34 ± 0.04	NS	0.13

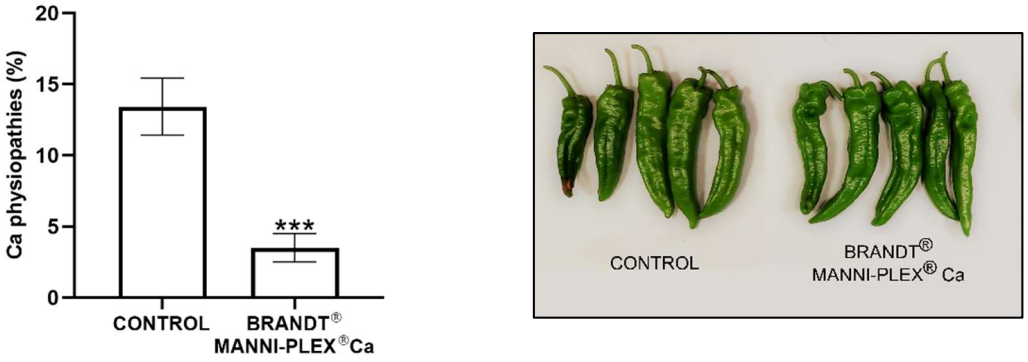
Values are means ± standard error (n = 9). Values with different letters indicate significant differences among treatments. The levels of significance were represented as $p > 0.05$ (NS), $p < 0.05$ (*), and $p < 0.01$ (**).

Regarding the parameters related to the quality of the pepper fruits, it should be noted that peppers of BRANDT® MANNI-PLEX® Ca treatment presented higher values of fruit length (Table 3), which suggests that the application of this product effectively promote the growth and development of the fruits. Besides, firmness is another parameter that shows a directly proportional association with the concentration of Ca linked to pectins in fruits [35–37]. This relationship is clearly observed in this study since the fruits with the highest firmness values were presented in the treated plants and the lowest in control plants (Table 3). These results confirm that Ca product application, by inducing a higher concentration of Ca linked to pectins in the fruits (Table 2), significantly increased the peppers firmness (Table 3). Finally, regarding quality parameters such as °Brix and acidity, no significant differences were obtained between any of the treatments analyzed (Table 3), which suggests that the organoleptic properties of the peppers would not be affected by the product application. These results agree with those observed in other studies applying Ca bound to different chelates [30–32].

3.4. Fruits Physiopathies

In fruits such as tomatoes, peppers, and zucchini, Ca deficiency is common, generating rot and necrotic areas at the distal end of the fruit, a physiopathy called "blossom end rot" (BER). BER is a physiological disorder that occurs mainly in fruits and is associated with water stress, and especially with a localized Ca deficiency, which has a very negative effect on the production of many crops [5]. In our study, the percentage of fruits that presented pathophysiology related to Ca deficiency was lower in plants supplied with BRANDT® MANNI-PLEX® Ca application, being around 3.5% of the total fruits compared to the 13% observed in control plants (Figure 2a). Thus, the incidence of physiopathies was reduced by 70% because of the product application. In general, the data presented in Table 3 and

Figure 2a are clearly reflected in Figure 2b where the effectiveness of the Ca products is shown.



(**a**) (**b**)

Figure 2. Effect of BRANDT® MANNI-PLEX® Ca application on the percentage of fruits with physiopathies related to Ca deficiency (**a**) and photography showing the appearance of pepper fruits (**b**). Values are expressed as means ± standard error (n = 9). The level of significance was represented by $p < 0.001$ (***).

These results indicate the effectiveness of the product in preventing the appearance of this type of physiopathies, possibly because of better nutritional control of the fruits regarding the Ca concentration, and especially to a higher Ca concentration linked to pectins in the fruits (Table 2). Indeed, different studies showed that the appearance of BER is the result of the rupture of the plasma membranes and irregular softening of the cell walls, due to processes of disintegration of the pectin chains by the action of specific degradation enzymes, all this caused by the deficiency of Ca in fruits. In this regard, an increase in fruit Ca levels was proved to very significantly reduce BER occurrence [35–37].

3.5. Post-Harvest Study

In post-harvest studies, the most important indices that indicate the maintenance of the vigor of the stored plant material (useful life) are the fresh weight loss percentage or the water loss percentage, and the malondialdehyde concentration (MDA) [38]. Thus, throughout the experiment, fruit weight decreased mainly due to water loss. However, this decrease was much smaller in the fruits of plants treated with the Ca product, which was also reflected in a lower water loss percentage compared to the control fruits. MDA concentration is the indicator parameter for membrane lipid peroxidation and an increase in its values suggests the excessive presence of toxic reactive oxygen species (ROS) [39,40]. ROS are derived from the ripening processes that lead to the degradation of structures, such as the cell wall and cell membranes, and organic compounds involved in the quality of the fruits, such as vitamins, amino acids, etc. [41]. In Table 4 and in Figure 3 we verify that post-harvest at room temperature for 7 days was much more stressful and more damaging than that produced at a temperature of 4 °C for 14 days, where the MDA concentration in the fruits was lower. Treated plants showed the fruits with the higher concentrations of total Ca and Ca bound to pectins (Table 2) and were the ones that most effectively maintained the integrity of the pepper fruits during storage, thus delaying the ripening processes and degradation of cellular structures and components.

Table 4. Effect of BRANDT® MANNI-PLEX® Ca application on the initial and final fresh weight and water loss percentage in pepper fruits subjected to post-harvest at room temperature and 4 °C.

Temperature	Treatments	Initial Fresh Weight (g fruit^{-1})	Final Fresh Weight (g fruit^{-1})	Water Loss (%)
Room temperature	Control	29.04 ± 4.72	11.83 ± 1.59	59.26 ± 6.03
	BRANDT® MANNI-PLEX® Ca	35.41 ± 4.78	30.53 ± 3.16	13.78 ± 2.48
	p-value	*	**	***
	$HSD_{0.05}$	5.35	4.31	9.33
4 °C	Control	30.27 ± 3.98	20.75 ± 2.31	31.45 ± 3.17
	BRANDT® MANNI-PLEX® Ca	37.18 ± 4.83	34.98 ± 3.07	5.92 ± 0.46
	p-value	*	**	***
	$HSD_{0.05}$	5.73	4.32	4.96

Values are means ± standard error (n = 9). Values with different letters indicate significant differences among treatments. The levels of significance were represented as $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

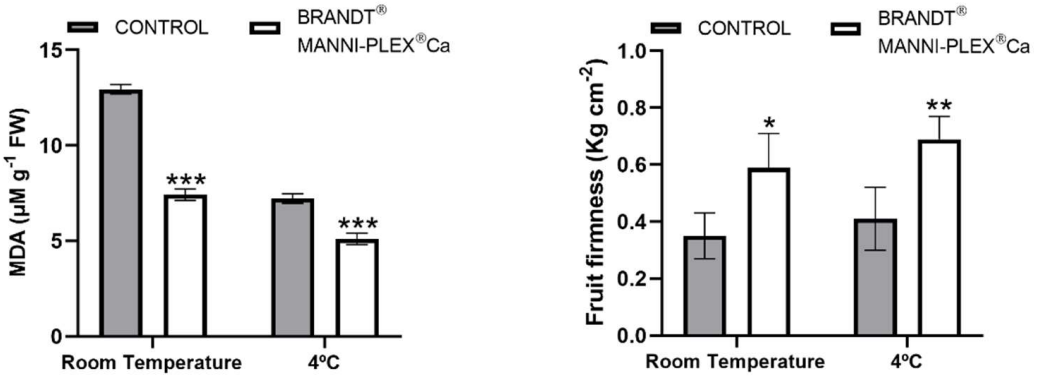


Figure 3. Effect of the application of BRANDT® MANNI-PLEX® Ca on the concentration of MDA (**a**) and on the firmness of pepper (**b**) in pepper fruits subjected to post-harvest at room temperature and 4 °C. Values are expressed as means ± standard error (n = 9). The levels of significance were represented by $p < 0.05$ (*) and $p < 0.001$ (***).

The maintenance of the fruit's vigor and integrity before the post-harvest storages carried out was confirmed with the firmness data (Figure 3b). In Figure 3b we verify that the product, with the maximum concentrations of total Ca and Ca bound to pectins (Table 2), presented the highest firmness values after the conclusion of the storage period both at room temperature and at 4 °C (Figure 3b). These results confirm that the application of the Ca product can be defined as an effective tool to maintain the useful life of fruits and reduce the processes of maturation and degradation of cellular structures and components in post-harvest programs. Different investigations concluded that increases in the proportion of Ca in the pectins of the cell wall of the fruits improves, in addition to the resistance against fungal and bacterial pathogens, the storage and post-harvest quality of fruits [35–37].

Considering parameters related to organoleptic properties, at the end of post-harvest, there were no significant differences regarding °BRIX nor titratable acidity comparing the different treatments (Table 5). Therefore, these results suggest that BRANDT® MANNI-PLEX® Ca application does not change the organoleptic properties of pepper fruit, which could affect its marketability. Similar results were observed in other studies that applied vectoring Ca products [30–32].

Table 5. Effect of BRANDT® MANNI-PLEX® Ca application products on some components of the quality of pepper fruits (organoleptic characteristics and antioxidant capacity) subjected to post-harvest at room temperature and 4 °C.

Temperature	Treatments	°BRIX (%)	Titratable Acidity (%)	Ascorbate (mg g^{-1} FW)	FRAP (mg g^{-1} FW)	TEAC (mmol g^{-1} FW)
Room temperature	Control	4.70 ± 0.45	0.062 ± 0.014	1.12 ± 0.06	3.03 ± 0.16	4.06 ± 0.20
	BRANDT® MANNI-PLEX® Ca	5.33 ± 0.30	0.044 ± 0.014	1.43 ± 0.05	3.77 ± 0.29	5.16 ± 0.26
	p-value	NS	NS	**	**	***
	$HSD_{0.05}$	0.85	0.010	0.13	0.41	0.45
4 °C	Control	4.57 ± 0.36	0.034 ± 0.012	1.18 ± 0.04	3.15 ± 0.29	4.60 ± 0.41
	BRANDT® MANNI-PLEX® Ca	4.90 ± 0.28	0.034 ± 0.011	1.48 ± 0.04	3.85 ± 0.29	5.48 ± 0.17
	p-value	NS	NS	***	*	**
	$HSD_{0.05}$	0.73	0.011	0.09	0.62	0.63

Values are means ± standard error (n = 9). Values with different letters indicate significant differences among treatments. The levels of significance were represented as $p > 0.05$ (NS), $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

In addition to the quality parameters previously exposed, this study also analyzed different phytochemical compounds that define the antioxidant capacity of agricultural products intended for human consumption, such as ascorbate or vitamin C, and the antioxidant tests FRAP and TEAC. The most effective antioxidant of the different plant products is ascorbate, also known as vitamin C. As an antioxidant, ascorbate directly removes toxic reactive oxygen species avoiding and/or reducing cell death characteristic of any stress [42]. Ascorbate can be synthesized by plants and by most mammals, but not by humans [43], where it is essential in the maintenance of a healthy immune system and the prevention of cardiovascular diseases [44]. We analyzed the values of these parameters at the beginning (Table S2) and at the end of post-harvest (Table 5). The results showed higher values of ascorbate and antioxidant tests in peppers from plants supplemented with BRANDT® MANNI-PLEX® Ca, indicating an improvement in their antioxidant properties. Different investigations have shown the positive relationship between an adequate nutritional status of Ca in the fruits and the improvement of the phytochemical properties of the fruits, especially in relation to antioxidant compounds [12,35–37]. Hence, the BRANDT® MANNI-PLEX® Ca also could be useful to obtain fruits with better antioxidant properties, which improves their marketability.

4. Conclusions

The obtained results suggests that BRANDT® MANNI-PLEX® Ca, product based on Ca vectorization, enhance the growth, the concentration and distribution of Ca, as well as the production of pepper fruits in a cultivation with a high incidence of Ca physiopathies. Thus, the application of this product could considerably reduce the prevalence of this problem. Besides, BRANDT® MANNI-PLEX® could be useful to extend post-harvest shelf life of pepper fruit. This last effect could be related to the delaying the degradation processes of cellular structures and components in the fruits giving rise to a higher and antioxidant capacity. However, to confirm the usefulness of the product, it would be necessary to analyze the cost–benefit ratio and test its effectiveness on different cultivars. Further studies would also be necessary to determine its mechanism of action.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12020410/s1, Table S1: Microclimate conditions during

the experiment, Table S2: Effect of BRANDT® MANNI-PLEX® Ca application on some phytochemical components of the quality of pepper fruits at the beginning of harvest period.

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