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Evaluation of biological control agents and fertilizers to control *Plasmodiophora brassicae* in broccoli (*Brassica oleracea* var. italica)

Artículo académico

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RESUMEN

El daño causado en el cultivo de brócoli y la gran pérdida económica, es la razón más importante para definir el mecanismo de control y gestión para *Plasmodiophora brassicae*. Los factores evaluados fueron *Trichoderma* spp. *y Bacillus* sp., Ácidos Húmicos, Inductor de Resistencia, Carbonato de calcio y Nitrato de calcio, distribuidos en tratamiento individual y combinado en dos temporadas (lluvioso y seco) en el año 2018. El $CaCO_3$ evaluado en tratamientos individuales funciona en la estación seca pero no en el estación lluviosa, sin embargo, en tratamientos combinados, fue el mejor tratamiento el que tiene todos los tratamientos tanto en la estación lluviosa como en la seca.

Palabras clave:

Broccoli, control, *Plasmodiophora brassicae.*

ABSTRACT

The damage caused in the cultivation of broccoli by *Plasmodiophora brassicae* and the concomitant large economic losses justify the determination of mechanisms for its control management. The treatments evaluated were *Trichoderma* spp., *Bacillus* sp., humic acids, elicitor, calcium carbonate and calcium nitrate, individually and in combination in two seasonal conditions (rainy and dry) in the year 2018. The $CaCO_3$ evaluated in individual treatments is effective in the dry season but not in the rainy season, however in combined treatments the best treatment was that which has all the treatments in both the rainy and dry season.

Key words:

Broccoli, control, *Plasmodiophora brassicae.*

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Evaluation of biological control agents and fertilizers to control *Plasmodiophora brassicae* in broccoli (*Brassica oleracea* var. italica)

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Abstract

The damage caused in broccoli cultivation by *Plasmodiophora brassicae* and the concomitant large economic losses justify the determination of mechanisms for its control management. Here, the treatments evaluated were soil applications of *Trichoderma* spp., *Bacillus* sp., humic acids, elicitor, calcium carbonate ($CaCO_3$) and calcium nitrate ($Ca(NO_3)_2$), individually and in combination in two seasonal conditions (rainy and dry) in the year 2018. The $CaCO_3$ evaluated in individual treatments was effective in the dry season but not in the rainy season, however under combined treatments the best treatment was the mixture of all treatments when tested in both rainy and dry season.

Key words:

Broccoli, control, *Plasmodiophora brassicae*

Introduction

Broccoli is a source of nutrients and minerals for the human diet. In 100 g of broccoli, there are 56 mg of calcium, 22mg of magnesium, 370 mg of potassium, 87 mg of phosphorus, 87 mg of vitamin C and 69 ug of Vitamin A (Moreiras *et al.* 2013). The consumption of broccoli increases by 4% annually around the world because of its important health benefits. Specifically it has an advantageous effect on various types of cancer, such as lung, prostate, breast, etc, because of its high content of antioxidant nutrients like β - carotene, i.e. 1.9 mg β- carotene in 100g broccoli (Moreiras *et al.* 2013).

The main producers of broccoli are China with 39%, the US with 5% and Mexico with 2%. Together they produce 24. 2 million tons per year (Zilli, 2018). However, the main exporters of broccoli are the European Union with 52%, China with 17% and Mexico with 12% (MCE, 2018). Ecuador participates with 2% of broccoli exports worldwide (MCE, 2018). Broccoli production in Ecuador increased by 5.75% between 2007 and 2011 generating more than 69 million dollars in profits in the agricultural sector (MCE, 2015). In addition, the sale price of a 20 pound box of broccoli increased by 32% from 2015 to 2016 with a current value of 19.67 USD (MAG, 2016). The principal producing provinces are Pichincha, Cotopaxi and Chimborazo with almost 5519 ha (MAGAP, 2016). Thus, the cultivation of broccoli represents an important aspect of the economy and agriculture in the Andean region of Ecuador. The best time to produce broccoli is spring-summer (dry season) since the crops have less phytosanitary problems and in the case of broccoli, the lumps have a better size and less presence of significant diseases (CORFO, 2012).

Tweenty percent of the main production budget is used for pest and disease control. Broccoli's major pests and diseases are: *Hyaloperonospora brassicae, Alternaria brassicae* and *Plasmodiophora brassicae.* Plant diseases are difficult to control during the rainy season, leading to significant losses of production. The main management strategy is based in chemical control.

Plasmodiophora brassicae is a disease that attacks Brassicaceae species. In Broccoli (*Brassica oleracea var. italica*), this disease affects the roots, causing deformation and weakness of the root system which causes a decrease in the absorption of nutrients and can even cause the death of the plant (Haro & Maldonado, 2009). This disease is considered the most dangerous in the broccoli monoculture because it is difficult to recognize during the initial stages of the disease and its aggressiveness (Haro & Maldonado, 2009). According to Galdames (2017), *Plasmodiophora brassicae* decreases the yield by 0.003 ton/ha for every 1% of plants infected worldwide, which causes losses in annual yield between 10% and 15%. Because of the damage caused in broccoli culture and the large economic loss, it is important to define mechanisms of control and management for *Plasmodiophora brassicae.*

Plasmodiophora brassicae is a protozoan with a complex life cycle, that is not completely understood because it consists of different zoosporic stages for the formation of plasmodia within host cells and the formation of spores at rest (Schwelm, 2015). The haploid resting spore releases a zoospore that infects the hairs of the plant root and forms multinucleated plasmodia. These develop several secondary zoospores each with an individual nucleus that are released into the soil. Fusion of these secondary zoospores can occur occasionally. Zoospores invade the root cortex and develop secondary multinucleated plasmodia. This is where meiosis occurs in plasmodia before the formation of resting spores (Schwelm, 2015). The plasmodia cause an abnormal cell enlargement and an uncontrolled cell division that leads to the development of galls, which obstruct the transport of nutrients and water (Schwelm, 2015). In the tissue of infected roots, there are different stages of development of plasmodiophores (Schwelm, 2015). According to molecular and genomic data they are rare and only grow inside living host cells and remain incurable by themselves. The resting spores are extremely resistant to harsh environmental conditions and contaminate arable land for decades which makes it impossible to eradicate the organism through any known chemical or alternative soil treatment (Schwelm, 2015).

Several alternative measures have been proposed for the control and proper management of this disease, such as the application of different sources and concentrations of calcium (Klasse, 1996), nitrogen (Ruaro et al., 2009), and pH changes in the soil. It has been reported that the use of calcium carbonate ($CaCO_3$) and calcium nitrate ($Ca(NO_3)_2$) decreases the percentage of diseased plants by 20% and 10 %, respectively (Ruaro et al., 2009). It has been previously shown that calcium has a growth effect on the crop in the presence of *Plasmodiophora brassicae* (Dixon, 2010). Besides, it has been shown that the use of calcium carbonate can increase the pH of the soil to close to 7, which causes a decrease in the damage of the disease during the culmination of planting and harvest, but not in the long term (Haro & Maldonado, 2009). However, the indiscriminate use of these fertilizers can affect the state of the soil and the plant, lowering the pH of the soil, destroying the microbiological balance, and generating physiological problems for the plant (Agris, 2016).

Due to this, biological control alternatives have been proposed for the management of this disease that increase productivity and have a low environmental impact. Beneficial fungi, such as *Trichoderma spp.*, have been used as an alternative because of their antagonistic effect, phosphate solubilizing activity, growth promotion and ability to increase defense (Camargo & Ávila, 2013). The *Bacillus* species (bacteria) is another alternative, as it has been tested as a biological control against *Plasmodiophora brassicae* (Xing-Yu, et al., 2012). Elicitor has also been tested, as it allows a plant to generate a metabolic response after the infection by a pathogen (Mogollón & Castaño, 2011). Elicitor stimulates the production and accumulation of phytoalexins that are toxic for a broad spectrum of phytopathogenic bacteria and fungi, and is also associated with the induction of defense genes which depend on the salicylic acid and jasmonic acid signaling pathways (Palazon, 2004). Phytoalexins promote the hypersensitivity response that leads to cell death where the pathogen is invading, a rapid and orderly defense reaction is triggered, causing the pathogen to be left without the possibility of continuing with the infection (Palazon, 2004). Additionally, humic acids correspond to a mixture of organic aliphatic and aromatic acids that are not soluble in water when they are in acidic conditions. They are, however, soluble in water in alkaline conditions, which is why humic acids correspond to the proportion of humic substances that precipitate from aqueous solutions at a pH less than 2 (Pettit, 2016). At this low pH, they act as a buffer in the soil, neutralizing the pH and generating an appropriate environment for the development of the root system (Pettit, 2016). All of these alternatives have not been evaluated in combination with a specific concentrations, and thus, making this investigation relevant.

The present investigation was carried out in 2018 in the Province of Cotopaxi, Ecuador to evaluate the effect during two harvest periods (rainy and dry season) of soil application of several low environmental alternatives to increase yield in a field known that have been contaminated previously with *Plasmodiophora brassicae*.

Materials and Methods

Experimental Area

The study was carried out at Agrogana S.A company located in the canton Latacunga of the Province of Cotopaxi at 0°48'10.51 "S and 78°36'58.63" W, with an elevation of 2932 meters above sea level. The experiments were done on 1125 m^2 distributed in 25 m^2 plots of a broccoli field. The plant material used was the cultivar Avenger, which is an important cultivar in the market due to its wide adaptation and constant yield in Ecuador (Sakata, 2016).

Treatments and application

The evaluated factors were: Calcium Carbonate ($CaCO_3$), Calcium Nitrate ($Ca(NO_3)_2$), *Trichoderma* spp., *Bacillus* sp., Humic Acids and a commercial plant defense Elicitor. For Calcium Carbonate ($CaCO_3$) the product Calcium Carbonate was used, which increases phosphorus availability, improves nitrogen fixation and water use, and improves the recovery of nutrients and the root system. The product was in solid form provided from Disensa. For Calcium Nitrate ($Ca(NO_3)_2$), the product Fernical was used, which keeps young plants and increases cell volume. The product was in granulated form from the Fertisa company.

For *Trichoderma* spp. was used the product TRICHOPLUS. *Trichoderma* is a type of facultative anaerobic fungus (can live in the presence or absence of oxygen) and is found naturally in a significant number of agricultural soils and other types of media. The concentration used was of 1.0x 10^8 CFU/g of product. The product was liquid form with a pH of 6.5, and it was obtained from the Microtech Services company. For *Bacillus* sp., the product LINOR was used, which is a product developed from different species and strains of *Bacillus* which produce essential products that are beneficial to the plants. These mixture of amino acids help in nutrition and intervene in primary and secondary plant metabolism and increase yield and crop production. The product was in liquid form and it was obtained from Microtech company.

For Humic Acids the product Robusterra was used which contains complex organic molecules formed by the decomposition of organic matter from leonardite, which is subjected to a chemical activation process to extract the humic and fulvic acids. The product was in soluble powder form and obtained from the company Microtech Services. As an elicitor, DEFENSEPLUS was used, which contains components of biological origin, which potentiate plant defenses (anticipins, phytoalexins, hypersensitive reaction, etc.). The product was in liquid form formulated by the company Microtech Services. The products were applied at the edaphic level using a hoe.

The treatments tested were 15 treatments in total, where 7 were individual treatments and 8 were combined treatments as shown in Table 1.

Table 1. Treatments and dosage per treatment per plot

Nº of Treatment	Treatment	Dosage per Treatment per plot of 25 m^2
1	Control	No treatment
2	$CaCO_3$	10kg
3	$(Ca(NO_3)_2)$	450g
4	*Trichoderma* spp. (T)	25ml
5	*Bacillus* sp. (B)	45ml
6	Humic Acid (HA)	22,5 g
7	Elicitor (E)	45ml
8	*Trichoderma* spp.+ Humic Acid [T+HA]	25ml +22.5g
9	*Bacillus* sp.+ Humic Acid B+ HA]	45ml+22.5g

10	$Ca(NO_3)_2$ + Humic Acid [$Ca(NO_3)_2$ +HA]	450g + 22.5g
11	$CaCO_3$ + Humic Acid [$CaCO_3$ + HA]	10 Kg + 22.5g
12	*Trichoderma* spp. + *Bacillus* sp. + Humic Acid [T+B+HA]	25ml+ 45ml+22.5g
13	*Trichoderma* spp. + *Bacillus* sp. +Humic Acid+ Elicitor [T+B+HA+ E]	25ml+ 45ml+22.5g + 45ml
14	$Ca(NO_3)_2$) + *Trichoderma* spp. + *Bacillus* sp. +Humic Acid+ Elicitor [$Ca(NO_3)_2$)+ T+B+HA+ E]	25ml+ 45ml+22.5g + 45ml +450g
15	$CaCO_3$ + $Ca(NO_3)_2$) + *Trichoderma* spp. + *Bacillus* sp. +Humic Acid+ Elicitor [CaCO3+ $Ca(NO_3)_2$)+ T+B+HA+ E]	25ml+ 45ml+22.5g + 45ml +450g+10kg

Variables under the study

Three response variables were determined: Weight of Lump (kg), Diameter of Lump (cm) and Root Weight (kg).

Frequency and Period of application

During the first period, 2 applications were performed during the rainy season, the initial applications were carried out on February 2 and 9, 2018, the second applications were carried out on March 16th and 29th, 2018, and the harvest was performed on May 3rd, 2018. In the second harvest period 2 application were performed during the dry season, the initial applications were carried out on June 8 and 15, 2018, the second applications were carried out on July 13 and 20, 2018 and the harvest final was performed out on August 15, 2018. The environmental conditions during which the treatments were recorded are shown in Figure 1.

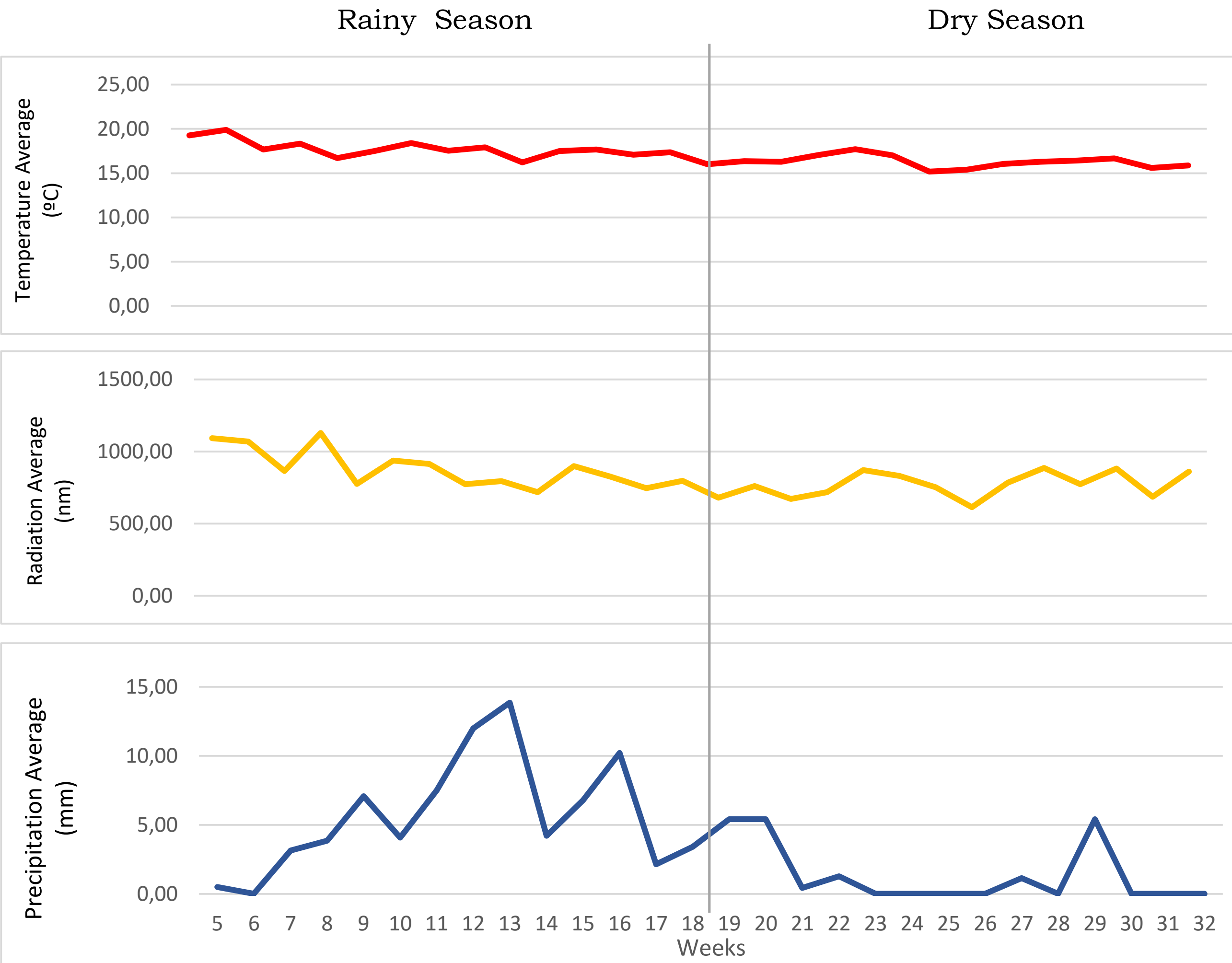

Figure 1. Average of Temperature, Relative Humidity, Radiation and Precipitation from weeks 5 to 18 in the rainy season and from weeks 19 to 32 week in the dry season.

Statistical analysis

A completely randomized experimental design was carried out in plots of 25 m^2 for individual or combinate treatments. Only inner plants per plot were used in the analysis to avoid border effect. The analysis was performed with SPSS software within each harvest and between crops with an analysis of variance (ANOVA) + Tuckey test with 95% confidence. Five (5) plants were taken randomly from each plot immediately after harvesting for analysis. For all treatments, three plots per treatment were analyzed per season, however, in the rainy season only two plots were analyzed since a single plot did not formed lumps.

Results and Discussion

In the piece of ground in which the experimental part was carried out, it was possible to demonstrate what was the symptomatology of the plants prior to the trial (Figure 2.), where it was observed that the plants had a 90% infection by *Plasmodiophora brassicae* compared to a control. In the morphology of the diseased the plant showed large malformations that affect the root system, which was observed better under the microscope, observing clear differences with the healthy plant (control) where in the root system it does not have damage by this disease.

Figure 2. (a) Healthy Plant Morphology, (b) Healthy plant Microscopy (100X), (c) Sick Plant Morphology, (d) Sick Plant Microscopy.

According to the results, it had found that there were differences both when evaluating individual and combined treatments and when evaluating between the two sowing seasons (rainy and dry).

Figure 3. shows the analyzed parameters for both the rainy and dry season comparing the control treatment (farm management) with the individual treatments.

For the lump weight, lump diameter and root weight there were significant differences between $CaCO_3$ treatment and the other individual treatments. The lump weight, diameter and root weight with the $CaCO_3$ treatment were smaller compared to the control. It was observed that in the rainy season the control presented a bimodal distribution reflecting the heterogeneity of the terrain. In addition, $CaCO_3$ was lower in all variables and this was due to the heterogeneity of the terrain, but does not have a deleterious effect (Figure 3).

According to Haro and Maldonado (2009), rain causes water to percolate in the soil which leads to an outflow of basic nutrients such as calcium, therefore it is recommended that $CaCO_3$ can be applied before the onset of the rains.

In the dry season, the lump weight was significantly different between $CaCO_3$ treatment and the other individual treatments, but in lump diameter and root weight there were no significant differences between $CaCO_3$ treatment and the other individual treatments. The average lump weight was 0.60 kg, the average lump diameter was approximately 22 cm and the average root weight was 0,20kg.

It was observed that in the dry season $CaCO_3$ worked better to increase statistically the lump weight. Thus, the same compound in two different situations will not necessarily work the same, so it is important to take into account climatic variables. According to Dixon (2010), calcium provides a long-term boost of soil microbial activity and offers a rapid series of opportunities for crop growth to improve during the growing season. In addition, Donald et al. (2004) demonstrates that the use of calcium in broccoli improves from 40% to 64% when it is in the presence of *Plasmodiophora brassicae* but only in the dry season.

In the rainy season there was no optimal individual treatment, therefore it was important to compare the combined treatments to see if there was a synergy with the treatments.

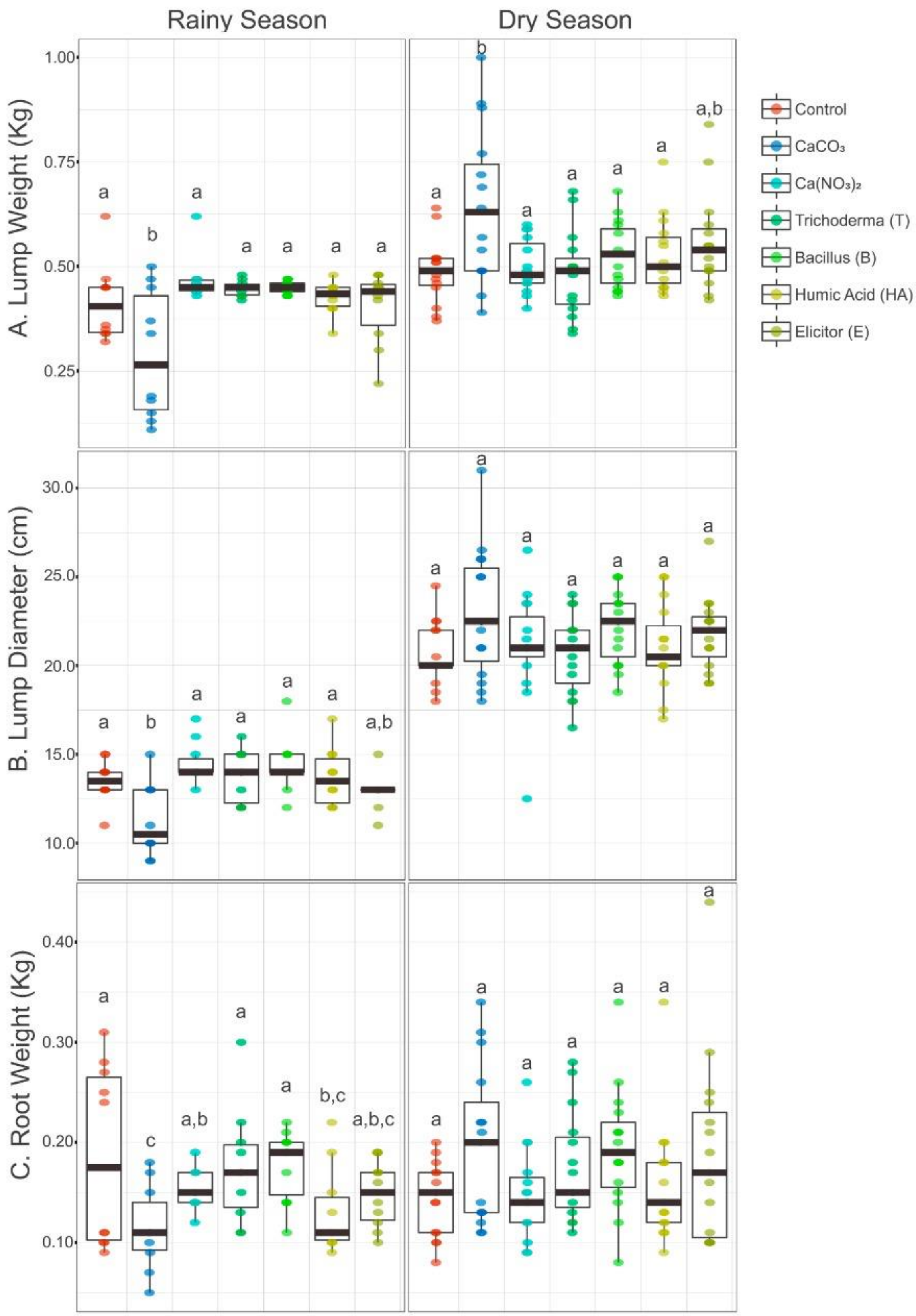

Figure 3: Lump weight, lump diameter and root weight for individual treatments in comparison with the control treatments for the rainy and dry season.

The Figure 4. shows the analyzed parameters for both the rainy and dry season comparing the control treatment (no treatment) with the combined treatments.

In the rainy season, for lump weight there was a significant difference between $CaCO_3$ + ($Ca(NO_3)_2$) + T+ B+ HA+ E ($CaCO_3$ +($Ca(NO_3)_2$) + *Trichoderma* spp. + *Bacillus* sp.+ Humic Acid + Elicitor) treatment and the control treatment. The average lump weight was approximately 0,62kg for the between $CaCO_3$ + ($Ca(NO_3)_2$) T+ B+ HA+ E treatment and 0.40kg for the Control treatment. For lump diameter there were a significant difference between ($Ca(NO_3)_2$) +HA and with control treatment. The average lump diameter was 15 cm for the between ($Ca(NO_3)_2$) T+ B+ HA+ E treatment and 14 cm with control treatment. For the root weight there was a significant difference between the B+HA (*Bacillus* sp.+ Humic Acid) treatment and with Control treatment. The average root weight was approximately 0,12kg for the B+HA treatment and 0,17kg for the control treatment B+ HA treatment is smaller in comparison with the other treatments.

In the dry season, for lump weight there was a significant difference between $CaCO_3$ + ($Ca(NO_3)_2$) + T+ B+ HA+ E ($CaCO_3$ +($Ca(NO_3)_2$) + *Trichoderma* spp. + *Bacillus* sp.+ Humic Acid + Elicitor) treatment and the control treatment. The average lump weight was approximately 0,62kg for the between $CaCO_3$ + ($Ca(NO_3)_2$) T+ B+ HA+ E treatment and 0.48kg for the Control treatment. For lump diameter there were a significant difference between $CaCO_3$ + ($Ca(NO_3)_2$) + T+ B+ HA+ E and with control treatment. The average lump diameter was 24 cm for the between ($CaCO_3$ + ($Ca(NO_3)_2$) + T+ B+ HA+ E treatment and 20 cm with control treatment. For the root weight there was a significant difference between the B+HA (*Bacillus* sp.+ Humic Acid) treatment and with Control treatment. The average root weight was approximately 0,17kg for the B+HA treatment and 0,15kg for the control treatment B+ HA treatment.

Therefore, it was found that in combined treatments $CaCO_3$ + ($Ca(NO_3)_2$) T+ B+ HA+ E increase consistently lump weight and lump diameter in the two seasons (rainy and dry). As already mentioned, $CaCO_3$ has effects on the broccoli plant.

Trichoderma spp. works in all broccoli system, it made that the plant growth with better size like lump weight, lump diameter and root weight. According to Camargo-Cepeda and Ávila (2013), *Trichoderma* spp. had the effect of pathogen biocontroller. That is the reason that *Trichoderma* spp. is capable of promoting the growth of broccoli plant, also has the ability to stimulate the processes of plant development.

Bacillus sp., however, can increase stem size when it is in contact with the plant surface like in the rainy and in the dry season. In addition, Xing-Yu et al. (2013) states that Bacillus sp. showed biocontrol activity against pathogens and that its fundamental role is to promote the growth of the plant because it also has functions on broccoli root and lump since it has control effects on *Plasmodiophora brassicae,* this is due to the production of FTCPs (Cyclopeptides tigo fegicin) that showed strong antifungal.

Humic Acids had an effect on the growth and weight not only of roots but also of stems and inflorescence, with a growth stimulation in some cases of 25% (Oliver, 2009). Regarding Humic Acid , Oliver (2009) talks about the effect it has on the growth in roots. Oliver (2009) states that Humic Acids have physiological functions that increase the size of the inflorescence.

Elicitors caused an increase in cellulose microfibrils that results in an increase in the lignification of cell walls that leads to the synthesis of proteins related to pathogenesis and the hypersensitivity response to the *Plasmodiophora brassicae* (Mogollón and Castaño, 2011). The impact of elicitors on lump weight is not proven, however, Mogollón and Castaño (2011), argue that the elicitors can generate a control response to pathogenesis.

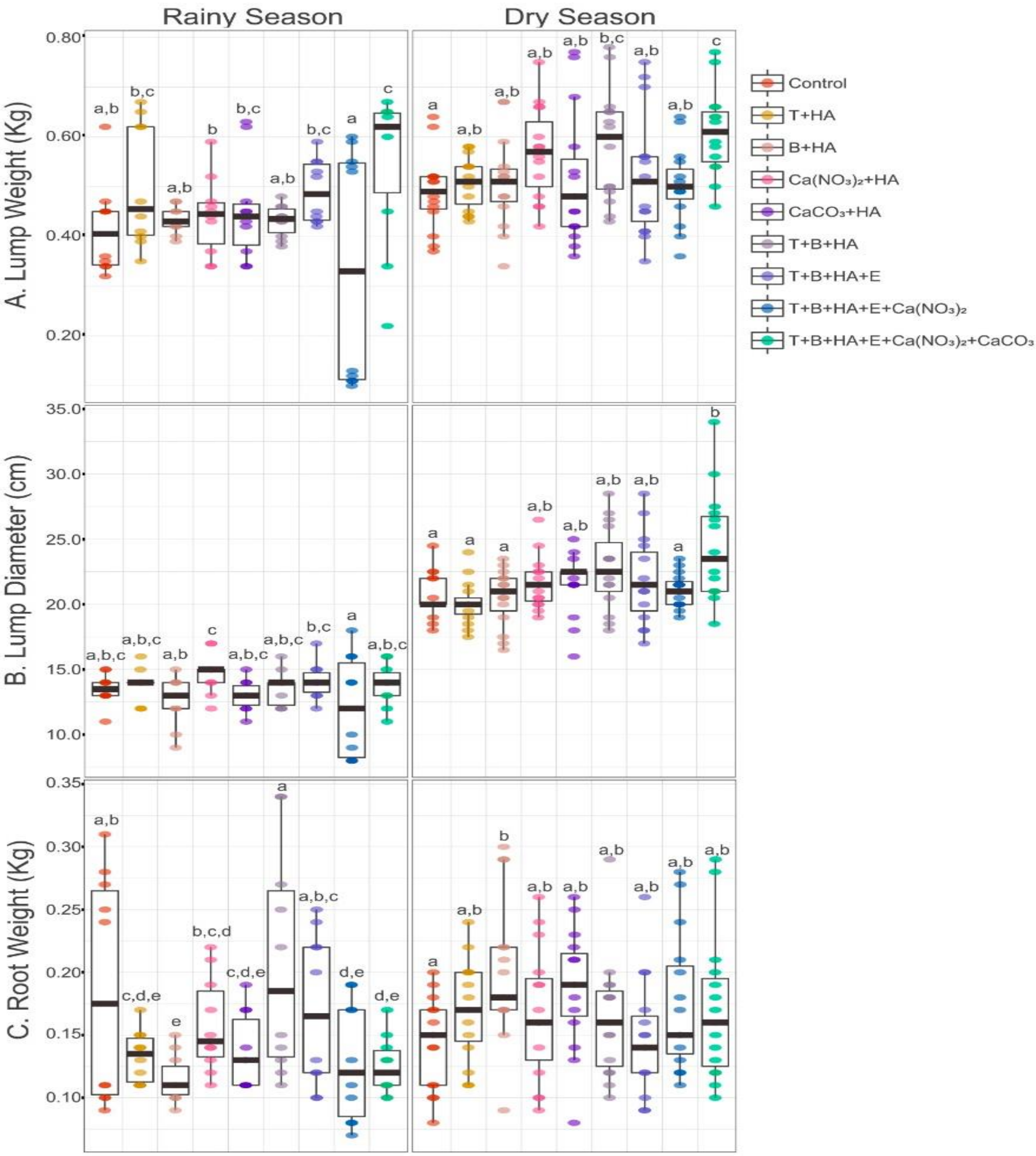

Figure 4.Lump weight, lump diameter and root weight for combined treatments in comparison with the control treatment for the rainy and dry seasons.

So, to control *Plasmodiophora brassicae* in broccoli the combined treatment was the best option found in our study: T+ B+ HA+ E+ $(Ca(NO_3)_2)$ + $CaCO_3$. The combination of these compounds act on not only to increase the weight of the lump, but also on the diameter of the lump and root weight, making the disease not attack treated broccoli plant so aggressively. The application of Calcium Carbonate ($CaCO_3$) increased the soil pH from 5.7 to 7.2 throughout the investigation.

The economic analysis was carried out considering the average weight of pellets for $CaCO_3$ (0.60kg) and the average pellet weight for $CaCO_3$ + Ca $(NO_3)_2$) + T + B + HA + E (0.62kg). Table 2. shows the costs of the treatments per hectare and in Table 3. the net benefit and the utility are presented according to the 2 best treatments that were obtained, both individually and combined in the environmental conditions of the trial.

Table 2. Price for all treatments and dosage per Ha

Treatment	Dosage/Ha	Cost/unit	Total (US) per ha
$CaCO_3$	4000kg	0.09kg	360
$Ca(NO_3)_2$	180kg	0,7kg	126
Trichoplus	30l	8l	240
Linor	6l	12l	72
Robusterra	9l	12.50l	112.50
Defenseplus	6l	17l	102
		Total	1012.50

Table 3. Economic analysis for $CaCO_3$ and for all treatments

	Treatments	
Variables	$CaCO_3$	$CaCO_3$+$Ca(NO_3)_2$) +T+B+HA+E
Cost US/ha (farm managment)	4500	4500
Lump cost (US/kg)	0.31	0.31
Treatment cost (US/ha)	360	1012.50
Harvest treatment (kg/ha)	24000	24800
Total income (US/ha)	7440	7688
Net profit (US/ha)	2580	2175.5
Utility (%)	53	39

According to the economic analysis, the individual treatment $CaCO_3$ is recommended, which presented a utility of 53% per hectare on average only in dry season. Regarding the economic analysis, the combined treatment T+ B+ HA+ E+ ($Ca(NO_3)_2$) + $CaCO_3$ is recommended, which presented a utility of 39% per hectare on average both in the rainy season and in the dry season of the crop cycle.

In conclusion, the best treatment to control *Plasmodiophora brassicae* in both rainy and dry seasons was that which contains all the treatments analyzed since not only did it act on the lump weight, on the lump diameter but also on the root weight, giving as a result a treatment that helps in all the physiological part of the plant. This treatment should continue to be applied in all the cycles corresponding to the monoculture of broccoli and at the same time to be proving different doses. In the future, research should continue on alternatives that are friendly to the environment that help in the management of this disease by minimizing the use of chemicals that damage the soil. The application of T15 treatment with the following doses per Hectare is recommended: *Trichoderma* spp. (30 liters) + *Bacillus s*p. (6 liters) + Humic Acid (9 kilograms) + Resistance Inductor (6 liters) + Calcium Nitrate ($CaNO_3)_2$) (180 kilograms) + Calcium Carbonate ($CaCO_3$) (4 Tons) as a control measure for *Plasmodiophora brassicae.*

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Annex 1

Table 4. Lump Weight (Individual Treatments Rainy Season)

Factores inter-sujetos

		Etiqueta de valor	N
Tratamiento	1,00	Control	30
	2,00	CaCO3	30
	3,00	Ca(NO3)2	30
	4,00	Trichoderma (T)	30
	5,00	Bacillus (B)	30
	6,00	Humic Acid (HA)	30
	7,00	Elicitor (E)	30
	8,00	T+HA	30
	9,00	B+HA	30
	10,00	Ca(NO3)2+HA	30
	11,00	CaCO3+HA	30
	12,00	T+B+HA	30
	13,00	T+B+HA+E	30
	14,00	T+B+HA+E+Ca(NO3)2	30
	15,00	T+B+HA+E+Ca(NO3)2+CaCO3	30
Bloque	1,00	1	150
	2,00	2	150
	3,00	3	150
Cosecha	1,00	Cosecha 1	225
	2,00	Cosecha 2	225
cosecha = 1 & bloque ~= 3 & (Tratamiento = 1 | Tratamiento <= 7) (FILTER)	0	Not Selected	380
	1	Selected	70

Table 5. Lump Weight (Individual Treatments Rainy Season)

Peso Pella (kg)

HSD Tukey[a,b]

Tratamiento	N	Subconjunto 1	2	3	4	5	6
Control	30	,3833					
Humic Acid (HA)	30	,4060	,4060				
CaCO3+HA	30	,4067	,4067				
Bacillus (B)	30	,4137	,4137	,4137			
T+B+HA+E	30	,4237	,4237	,4237	,4237		
Ca(NO3)2+HA	30	,4310	,4310	,4310	,4310	,4310	
T+B+HA	30	,4357	,4357	,4357	,4357	,4357	
T+B+HA+E+Ca(NO3)2	30	,4383	,4383	,4383	,4383	,4383	
Ca(NO3)2	30		,4608	,4608	,4608	,4608	
Trichoderma (T)	30		,4653	,4653	,4653	,4653	
T+HA	30		,4667	,4667	,4667	,4667	
CaCO3	30			,4739	,4739	,4739	
B+HA	30				,4790	,4790	
Elicitor (E)	30					,4923	
T+B+HA+E+Ca(NO3)2+CaCO3	30						,5853
Sig.		,187	,086	,092	,180	,077	1,000

Se visualizan las medias para los grupos en los subconjuntos homogéneos.

Se basa en las medias observadas.

El término de error es la media cuadrática(Error) = ,005.

a. Utiliza el tamaño de la muestra de la media armónica = 30,000.

b. Alfa = 0,05.

Table 6. Lump Weight (Combined Treatments Rainy Season)

Factores inter-sujetos

		Etiqueta de valor	N
Bloque	1,00	1	150
	2,00	2	150
	3,00	3	150
cosecha = 1 & bloque ~= 3 & (Tratamiento = 8 | Tratamiento <= 15) (FILTER)	0	Not Selected	380
	1	Selected	70
Cosecha	1,00	Cosecha 1	225
	2,00	Cosecha 2	225
Tratamiento	1,00	Control	30
	2,00	CaCO3	30
	3,00	Ca(NO3)2	30
	4,00	Trichoderma (T)	30
	5,00	Bacillus (B)	30
	6,00	Humic Acid (HA)	30
	7,00	Elicitor (E)	30
	8,00	T+HA	30
	9,00	B+HA	30
	10,00	Ca(NO3)2+HA	30
	11,00	CaCO3+HA	30
	12,00	T+B+HA	30
	13,00	T+B+HA+E	30
	14,00	T+B+HA+E+Ca(NO3)2	30
	15,00	T+B+HA+E+Ca(NO3)2+CaCO3	30

Table 7. Lump Weight (Combined Treatments Rainy Season)

Peso Pella (kg)

HSD Tukey[a,b]

Tratamiento	N	Subconjunto 1	2	3	4	5	6
Control	30	,3833					
Humic Acid (HA)	30	,4060	,4060				
CaCO3+HA	30	,4067	,4067				
Bacillus (B)	30	,4137	,4137	,4137			
T+B+HA+E	30	,4237	,4237	,4237	,4237		
Ca(NO3)2+HA	30	,4310	,4310	,4310	,4310	,4310	
T+B+HA	30	,4357	,4357	,4357	,4357	,4357	
T+B+HA+E+Ca(NO3)2	30	,4383	,4383	,4383	,4383	,4383	
Ca(NO3)2	30		,4608	,4608	,4608	,4608	
Trichoderma (T)	30		,4653	,4653	,4653	,4653	
T+HA	30		,4667	,4667	,4667	,4667	
CaCO3	30			,4739	,4739	,4739	
B+HA	30				,4790	,4790	
Elicitor (E)	30					,4923	
T+B+HA+E+Ca(NO3)2+CaCO3	30						,5853
Sig.		,187	,086	,092	,180	,077	1,000

Se visualizan las medias para los grupos en los subconjuntos homogéneos.

Se basa en las medias observadas.

El término de error es la media cuadrática(Error) = ,005.

a. Utiliza el tamaño de la muestra de la media armónica = 30,000.

b. Alfa = 0,05.

Table 8. Lump Diameter (Individual Treatments Rainy Season)

Factores inter-sujetos

		Etiqueta de valor	N
Bloque	1,00	1	150
	2,00	2	150
	3,00	3	150
Tratamiento	1,00	Control	30
	2,00	CaCO3	30
	3,00	Ca(NO3)2	30
	4,00	Trichoderma (T)	30
	5,00	Bacillus (B)	30
	6,00	Humic Acid (HA)	30
	7,00	Elicitor (E)	30
	8,00	T+HA	30
	9,00	B+HA	30
	10,00	Ca(NO3)2+HA	30
	11,00	CaCO3+HA	30
	12,00	T+B+HA	30
	13,00	T+B+HA+E	30
	14,00	T+B+HA+E+Ca(NO3)2	30
	15,00	T+B+HA+E+Ca(NO3)2+CaCO3	30
Cosecha	1,00	Cosecha 1	225
	2,00	Cosecha 2	225
cosecha = 1 & bloque ~= 3 & (Tratamiento = 1 | Tratamiento <= 7) (FILTER)	0	Not Selected	374
	1	Selected	76

Table 9. Lump Diameter (Individual Treatments Rainy Season)

Diametro de Pella (cm)

HSD Tukey[a,b]

Tratamiento	N	Subconjunto					
		1	2	3	4	5	6
Control	30	14,8167					
Humic Acid (HA)	30	15,0833	15,0833				
CaCO3+HA	30	15,1500	15,1500				
Ca(NO3)2+HA	30	15,7167	15,7167	15,7167			
Bacillus (B)	30	15,8500	15,8500	15,8500	15,8500		
T+B+HA	30	15,9333	15,9333	15,9333	15,9333		
T+B+HA+E+Ca(NO3)2	30		16,6500	16,6500	16,6500	16,6500	
T+HA	30		16,6833	16,6833	16,6833	16,6833	
B+HA	30		16,7333	16,7333	16,7333	16,7333	
Trichoderma (T)	30			17,1833	17,1833	17,1833	
CaCO3	30			17,2833	17,2833	17,2833	
Elicitor (E)	30				17,4500	17,4500	17,4500
Ca(NO3)2	30					17,7667	17,7667
T+B+HA+E	30					18,0833	18,0833
T+B+HA+E+Ca(NO3)2+CaCO3	30						19,0667
Sig.		,624	,064	,103	,086	,203	,078

Se visualizan las medias para los grupos en los subconjuntos homogéneos.

Se basa en las medias observadas.

El término de error es la media cuadrática(Error) = 3,674.

a. Utiliza el tamaño de la muestra de la media armónica = 30,000.

b. Alfa = 0,05.

Table 10. Lump Diameter (Combined Treatments Rainy Season)

Factores inter-sujetos

		Etiqueta de valor	N
Tratamiento	1,00	Control	10
	2,00	CaCO3	10
	3,00	Ca(NO3)2	10
	4,00	Trichoderma (T)	10
	5,00	Bacillus (B)	10
	6,00	Humic Acid (HA)	10
	7,00	Elicitor (E)	10
	8,00	T+HA	10
	9,00	B+HA	10
	10,00	Ca(NO3)2+HA	10
	11,00	CaCO3+HA	10
	12,00	T+B+HA	10
	13,00	T+B+HA+E	10
	14,00	T+B+HA+E+Ca(NO3)2	10
	15,00	T+B+HA+E+Ca(NO3)2+CaCO3	10
Bloque	1,00	1	75
	2,00	2	75
Cosecha	1,00	Cosecha 1	150
cosecha = 1 & bloque ~= 3 & (Tratamiento = 8 | Tratamiento <= 15) (FILTER)	1	Selected	150

Table 11. Lump Diameter (Combined Treatments Rainy Season)

Diametro Pella (cm)

HSD Tukey[a,b]

Tratamiento	N	Subconjunto		
		1	2	3
CaCO3	10	11,3000		
T+B+HA+E+Ca(NO3)2	10	12,1000	12,1000	
B+HA	10	12,6000	12,6000	12,6000
Elicitor (E)	10	12,9000	12,9000	12,9000
CaCO3+HA	10	13,0000	13,0000	13,0000
Control	10		13,5000	13,5000
T+B+HA	10		13,6000	13,6000
Humic Acid (HA)	10		13,7000	13,7000
Trichoderma (T)	10		13,8000	13,8000
T+B+HA+E+Ca(NO3)2+CaCO3	10		13,8000	13,8000
T+HA	10		13,9000	13,9000
T+B+HA+E	10		14,1000	14,1000
Bacillus (B)	10			14,4000
Ca(NO3)2	10			14,5000
Ca(NO3)2+HA	10			14,7000
Sig.		,261	,081	,051

Se visualizan las medias para los grupos en los subconjuntos homogéneos.

Se basa en las medias observadas.

El término de error es la media cuadrática(Error) = 1,843.

a. Utiliza el tamaño de la muestra de la media armónica = 10,000.

b. Alfa = 0,05.

Table 12. Root Weight (Individual Treatments Rainy Season)

Factores inter-sujetos

		Etiqueta de valor	N
Tratamiento	1,00	Control	30
	2,00	CaCO3	30
	3,00	Ca(NO3)2	30
	4,00	Trichoderma (T)	30
	5,00	Bacillus (B)	30
	6,00	Humic Acid (HA)	30
	7,00	Elicitor (E)	30
	8,00	T+HA	30
	9,00	B+HA	30
	10,00	Ca(NO3)2+HA	30
	11,00	CaCO3+HA	30
	12,00	T+B+HA	30
	13,00	T+B+HA+E	30
	14,00	T+B+HA+E+Ca(NO3)2	30
	15,00	T+B+HA+E+Ca(NO3)2+CaCO3	30
Bloque	1,00	1	150
	2,00	2	150
	3,00	3	150
Cosecha	1,00	Cosecha 1	225
	2,00	Cosecha 2	225
cosecha = 1 & bloque ~= 3 & (Tratamiento = 1 | Tratamiento <= 7) (FILTER)	0	Not Selected	380
	1	Selected	70

Table 13. Root Weight (Individual Treatments Rainy Season)

Peso Raiz (kg)

HSD Tukey[a,b]

Tratamiento	N	Subconjunto			
		1	2	3	4
T+B+HA+E	30	,1120			
Humic Acid (HA)	30	,1220	,1220		
Control	30	,1337	,1337	,1337	
CaCO3+HA	30	,1393	,1393	,1393	,1393
T+B+HA+E+Ca(NO3)2+CaCO3	30	,1443	,1443	,1443	,1443
Ca(NO3)2	30		,1483	,1483	,1483
T+HA	30		,1507	,1507	,1507
T+B+HA+E+Ca(NO3)2	30		,1527	,1527	,1527
CaCO3	30		,1550	,1550	,1550
Bacillus (B)	30		,1553	,1553	,1553
B+HA	30			,1577	,1577
Ca(NO3)2+HA	30			,1617	,1617
Elicitor (E)	30			,1633	,1633
T+B+HA	30				,1697
Trichoderma (T)	30				,1703
Sig.		,122	,094	,227	,168

Se visualizan las medias para los grupos en los subconjuntos homogéneos.

Se basa en las medias observadas.

El término de error es la media cuadrática(Error) = ,002.

a. Utiliza el tamaño de la muestra de la media armónica = 30,000.

b. Alfa = 0,05.

Table 14. Root Weight (Combined Treatments Rainy Season)

Factores inter-sujetos

		Etiqueta de valor	N
Tratamiento	1,00	Control	10
	2,00	CaCO3	10
	3,00	Ca(NO3)2	10
	4,00	Trichoderma (T)	10
	5,00	Bacillus (B)	10
	6,00	Humic Acid (HA)	10
	7,00	Elicitor (E)	10
	8,00	T+HA	10
	9,00	B+HA	10
	10,00	Ca(NO3)2+HA	10
	11,00	CaCO3+HA	10
	12,00	T+B+HA	10
	13,00	T+B+HA+E	10
	14,00	T+B+HA+E+Ca(NO3)2	10
	15,00	T+B+HA+E+Ca(NO3)2+CaCO3	10
Bloque	1,00	1	75
	2,00	2	75
Cosecha	1,00	Cosecha 1	150
cosecha = 1 & bloque ~= 3 & (Tratamiento = 8 | Tratamiento <= 15) (FILTER)	1	Selected	150

Table 15. Root Weight (Combined Treatments Rainy Season)

Pruebas de efectos inter-sujetos

Variable dependiente: Peso Raiz (kg)

Origen	Tipo III de suma de cuadrados	gl	Cuadrático promedio	F	Sig.
Modelo corregido	,439[a]	29	,015	19,561	,000
Interceptación	3,233	1	3,233	4181,806	,000
Tratamiento	,116	14	,008	10,723	,000
bloque	,027	1	,027	34,498	,000
cosecha	,000	0	.	.	.
filter_$	,000	0	.	.	.
Tratamiento * bloque	,296	14	,021	27,333	,000
Tratamiento * cosecha	,000	0	.	.	.
Tratamiento * filter_$	,000	0	.	.	.
bloque * cosecha	,000	0	.	.	.
bloque * filter_$	,000	0	.	.	.
cosecha * filter_$	,000	0	.	.	.
Tratamiento * bloque * cosecha	,000	0	.	.	.
Tratamiento * bloque * filter_$	,000	0	.	.	.
Tratamiento * cosecha * filter_$	,000	0	.	.	.
bloque * cosecha * filter_$	,000	0	.	.	.
Tratamiento * bloque * cosecha * filter_$	,000	0	.	.	.
Error	,093	120	,001		
Total	3,764	150			
Total corregido	,531	149			

a. R al cuadrado = ,825 (R al cuadrado ajustada = ,783)

Table 16.Lump Weight (Individual Treatments Dry Season)

Factores inter-sujetos

		Etiqueta de valor	N
Tratamiento	1,00	Control	15
	2,00	CaCO3	15
	3,00	Ca(NO3)2	15
	4,00	Trichoderma (T)	15
	5,00	Bacillus (B)	15
	6,00	Humic Acid (HA)	15
	7,00	Elicitor (E)	15
Cosecha	2,00	Cosecha 2	105
Bloque	1,00	1	35
	2,00	2	35
	3,00	3	35
cosecha = 2 & (Tratamiento = 1 | Tratamiento <= 7) (FILTER)	1	Selected	105

Table 17.Lump Weight (Individual Treatments Dry Season)

Peso Pella (kg)

HSD Tukey[a,b]

Tratamiento	N	Subconjunto 1	Subconjunto 2
Trichoderma (T)	15	,4827	
Control	15	,4893	
Ca(NO3)2	15	,4987	
Bacillus (B)	15	,5273	
Humic Acid (HA)	15	,5280	
Elicitor (E)	15	,5567	,5567
CaCO3	15		,6413
Sig.		,385	,229

Se visualizan las medias para los grupos en los subconjuntos homogéneos.

Se basa en las medias observadas.

El término de error es la media cuadrática(Error) = ,010.

a. Utiliza el tamaño de la muestra de la media armónica = 15,000.

b. Alfa = 0,05.

Table 18. Lump Weight (Combined Treatments Dry Season)

Factores inter-sujetos

		Etiqueta de valor	N
Tratamiento	1,00	Control	15
	2,00	CaCO3	15
	3,00	Ca(NO3)2	15
	4,00	Trichoderma (T)	15
	5,00	Bacillus (B)	15
	6,00	Humic Acid (HA)	15
	7,00	Elicitor (E)	15
	8,00	T+HA	15
	9,00	B+HA	15
	10,00	Ca(NO3)2+HA	15
	11,00	CaCO3+HA	15
	12,00	T+B+HA	15
	13,00	T+B+HA+E	15
	14,00	T+B+HA+E+Ca(NO3)2	15
	15,00	T+B+HA+E+Ca(NO3)2+CaCO3	15
Cosecha	2,00	Cosecha 2	225
Bloque	1,00	1	75
	2,00	2	75
	3,00	3	75
cosecha = 2 & (Tratamiento = 8 | Tratamiento <= 15) (FILTER)	1	Selected	225

Table 19. Lump Weight (Combined Treatments Dry Season)

Peso Pella (kg)

HSD Tukey[a,b]

Tratamiento	N	Subconjunto		
		1	2	3
Trichoderma (T)	15	,4827		
Control	15	,4893		
Ca(NO3)2	15	,4987		
T+B+HA+E+Ca(NO3)2	15	,5020	,5020	
T+HA	15	,5087	,5087	
B+HA	15	,5093	,5093	
CaCO3+HA	15	,5120	,5120	
T+B+HA+E	15	,5200	,5200	
Bacillus (B)	15	,5273	,5273	
Humic Acid (HA)	15	,5280	,5280	
Elicitor (E)	15	,5567	,5567	,5567
Ca(NO3)2+HA	15	,5680	,5680	,5680
T+B+HA	15	,5840	,5840	,5840
T+B+HA+E+Ca(NO3)2+CaCO3	15		,6087	,6087
CaCO3	15			,6413
Sig.		,101	,063	,335

Se visualizan las medias para los grupos en los subconjuntos homogéneos.

Se basa en las medias observadas.

El término de error es la media cuadrática(Error) = ,008.

a. Utiliza el tamaño de la muestra de la media armónica = 15,000.

b. Alfa = 0,05.

Table 20. Lump Diameter (Individual Treatments Dry Season)

Factores inter-sujetos

		Etiqueta de valor	N
Tratamiento	1,00	Control	15
	2,00	CaCO3	15
	3,00	Ca(NO3)2	15
	4,00	Trichoderma (T)	15
	5,00	Bacillus (B)	15
	6,00	Humic Acid (HA)	15
	7,00	Elicitor (E)	15
Bloque	1,00	1	35
	2,00	2	35
	3,00	3	35
Cosecha	2,00	Cosecha 2	105
cosecha = 2 & (Tratamiento = 1 | Tratamiento <= 7) (FILTER)	1	Selected	105

Table 21. Lump Diameter (Individual Treatments Dry Season)

Diametro Pella (cm)

HSD Tukey[a,b]

Tratamiento	N	Subconjunto 1
Control	15	20,6333
Trichoderma (T)	15	20,7000
Humic Acid (HA)	15	21,0333
Ca(NO3)2	15	21,0667
Elicitor (E)	15	21,8333
Bacillus (B)	15	22,1000
CaCO3	15	22,9000
Sig.		,111

Se visualizan las medias para los grupos en los subconjuntos homogéneos.
Se basa en las medias observadas.
El término de error es la media cuadrática(Error) = 5,290.
a. Utiliza el tamaño de la muestra de la media armónica = 15,000.
b. Alfa = 0,05.

Table 22. Lump Diameter (Combined Treatments Dry Season)

Factores inter-sujetos

		Etiqueta de valor	N
Tratamiento	1,00	Control	15
	2,00	CaCO3	15
	3,00	Ca(NO3)2	15
	4,00	Trichoderma (T)	15
	5,00	Bacillus (B)	15
	6,00	Humic Acid (HA)	15
	7,00	Elicitor (E)	15
	8,00	T+HA	15
	9,00	B+HA	15
	10,00	Ca(NO3)2+HA	15
	11,00	CaCO3+HA	15
	12,00	T+B+HA	15
	13,00	T+B+HA+E	15
	14,00	T+B+HA+E+Ca(NO3)2	15
	15,00	T+B+HA+E+Ca(NO3)2+CaCO3	15
Bloque	1,00	1	75
	2,00	2	75
	3,00	3	75
Cosecha	2,00	Cosecha 2	225
cosecha = 2 & (Tratamiento = 8 | Tratamiento <= 15) (FILTER)	1	Selected	225

Table 23. Lump Diameter (Combined Treatments Dry Season)

Diametro Pella (cm)

HSD Tukey[a,b]

Tratamiento	N	Subconjunto 1	Subconjunto 2
T+HA	15	20,1000	
B+HA	15	20,5333	
Control	15	20,6333	
Trichoderma (T)	15	20,7000	
Humic Acid (HA)	15	21,0333	
Ca(NO3)2	15	21,0667	
T+B+HA+E+Ca(NO3)2	15	21,1000	
Ca(NO3)2+HA	15	21,6333	21,6333
CaCO3+HA	15	21,6333	21,6333
Elicitor (E)	15	21,8333	21,8333
T+B+HA+E	15	21,8333	21,8333
Bacillus (B)	15	22,1000	22,1000
T+B+HA	15	22,8000	22,8000
CaCO3	15	22,9000	22,9000
T+B+HA+E+Ca(NO3)2+Ca CO3	15		24,3333
Sig.		,091	,123

Se visualizan las medias para los grupos en los subconjuntos homogéneos.

Se basa en las medias observadas.

El término de error es la media cuadrática(Error) = 5,631.

a. Utiliza el tamaño de la muestra de la media armónica = 15,000.

b. Alfa = 0,05.

Table 24. Root Weight (Individual Treatments Dry Season)

Factores inter-sujetos

		Etiqueta de valor	N
Tratamiento	1,00	Control	15
	2,00	CaCO3	15
	3,00	Ca(NO3)2	15
	4,00	Trichoderma (T)	15
	5,00	Bacillus (B)	15
	6,00	Humic Acid (HA)	15
	7,00	Elicitor (E)	15
Bloque	1,00	1	35
	2,00	2	35
	3,00	3	35
Cosecha	2,00	Cosecha 2	105
cosecha = 2 & (Tratamiento = 1 | Tratamiento <= 7) (FILTER)	1	Selected	105

Table 25. Root Weight (Individual Treatments Dry Season)

Peso Raiz (kg)

HSD Tukey[a,b]

Tratamiento	N	Subconjunto 1
Control	15	,1433
Ca(NO3)2	15	,1487
Humic Acid (HA)	15	,1567
Trichoderma (T)	15	,1747
Elicitor (E)	15	,1880
Bacillus (B)	15	,1927
CaCO3	15	,1953
Sig.		,137

Se visualizan las medias para los grupos en los subconjuntos homogéneos.

Se basa en las medias observadas.

El término de error es la media cuadrática(Error) = ,003.

a. Utiliza el tamaño de la muestra de la media armónica = 15,000.

b. Alfa = 0,05.

Table 26. Root Weight (Combined Treatments Dry Season)

Factores inter-sujetos

		Etiqueta de valor	N
Tratamiento	1,00	Control	15
	2,00	CaCO3	15
	3,00	Ca(NO3)2	15
	4,00	Trichoderma (T)	15
	5,00	Bacillus (B)	15
	6,00	Humic Acid (HA)	15
	7,00	Elicitor (E)	15
	8,00	T+HA	15
	9,00	B+HA	15
	10,00	Ca(NO3)2+HA	15
	11,00	CaCO3+HA	15
	12,00	T+B+HA	15
	13,00	T+B+HA+E	15
	14,00	T+B+HA+E+Ca(NO3)2	15
	15,00	T+B+HA+E+Ca(NO3)2+CaCO3	15
Cosecha	2,00	Cosecha 2	225
Bloque	1,00	1	75
	2,00	2	75
	3,00	3	75
cosecha = 2 & (Tratamiento = 8 | Tratamiento <= 15) (FILTER)	1	Selected	225

Table 27. Root Weight (Combined Treatments Dry Season)

Peso Raiz (kg)

HSD Tukey[a,b]

Tratamiento	N	Subconjunto 1
Control	15	,1433
Ca(NO3)2	15	,1487
T+B+HA+E	15	,1487
Humic Acid (HA)	15	,1567
T+B+HA	15	,1633
Ca(NO3)2+HA	15	,1673
T+B+HA+E+Ca(NO3)2+CaCO3	15	,1700
T+HA	15	,1707
Trichoderma (T)	15	,1747
T+B+HA+E+Ca(NO3)2	15	,1747
CaCO3+HA	15	,1873
Elicitor (E)	15	,1880
Bacillus (B)	15	,1927
CaCO3	15	,1953
B+HA	15	,2007
Sig.		,143

Se visualizan las medias para los grupos en los subconjuntos homogéneos.

Se basa en las medias observadas.

El término de error es la media cuadrática(Error) = ,003.

a. Utiliza el tamaño de la muestra de la media armónica = 15,000.

b. Alfa = 0,05.