Biological alternatives combined with Naphthaleneacetic Acid (NA) for the management of Plasmodiophora brassicae Woronin in cauliflower (Brassica oleracea L. var. Botrytis)

Alternativas biológicas combinadas con ácido naftalenacético (ANA) para el manejo de Plasmodiophora brassicae Woronin en coliflor (Brassica oleracea L. var. Botrytis)

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Abstract

Introduction— Club root can cause yield losses in crucifers between 70% to 80% and it is a serious problem of crucifers in Colombia.

Objective— To evaluate biological alternatives combined with Naphthaleneacetic Acid (NA) for the management of *P. brassicae* in cauliflower under the conditions of the municipality of Mutiscua.

Methodology— A randomized block experimental design with six treatments and three replications was established in a cauliflower culture, comparing commercial products based on the antagonist microorganisms *Trichoderma harzianum*, *Bacillus subtilis* and *Burkholderia cepacia* + NA, NA alone, a treatment with lime as a production standard and a whiteness. Ten plants were evaluated per experimental unit; height, stem diameter, number of leaves and incidence and severity of the disease. Finally, with the results obtained, a cost benefit analysis was developed.

Results— The results showed that the applications of the biological products *T. harzianum* and *B. subtilis* combined with the growth regulator NA, although they do not curatively control the disease, reduce it when applied preventively. The cost-benefit ratio was more favorable for *T. harzianum* + NA, followed by lime. Although with low effectiveness, lime treatment was the cheapest alternative.

Conclusions— *Trichoderma harziaanum* + NA, as applied in the experiment, and lime constitute non-chemical alternatives feasible for use by farmers for disease management.

Keywords— Antagonists; phytopathogen; club root disease of crucifers; cleaner production

Resumen

Introducción— La raíz del palo puede causar pérdidas de rendimiento en las crucíferas entre el 70% y el 80% y es un grave problema de las crucíferas en Colombia.

Objetivo— Evaluar alternativas biológicas combinadas con Ácido Naftalenoacético (AN) para el manejo de *P. brassicae* en coliflor en las condiciones del municipio de Mutiscua.

Metodología— Se estableció un diseño experimental de bloques al azar con seis tratamientos y tres repeticiones en un cultivo de coliflor, comparando productos comerciales a base de los microorganismos antagonistas *Trichoderma harzianum*, *Bacillus subtilis* y *Burkholderia cepacia* + NA, NA solo, un tratamiento con cal como estándar de producción y un blanco. Se evaluaron diez plantas por unidad experimental; la altura, el diámetro del tallo, el número de hojas y la incidencia y severidad de la enfermedad. Finalmente, con los resultados obtenidos, se desarrolló un análisis coste-beneficio.

Resultados— Los resultados mostraron que las aplicaciones de los productos biológicos *T. harzianum* y *B. subtilis* combinados con el regulador de crecimiento NA, aunque no controlan curativamente la enfermedad, la reducen cuando se aplican preventivamente. La relación coste-beneficio fue más favorable para *T. harzianum* + NA, seguido de la cal. Aunque con baja efectividad, el tratamiento con cal fue la alternativa más económica.

Conclusiones— *Trichoderma harziaanum* + NA, tal y como se aplicó en el experimento, y la cal constituyen alternativas no químicas factibles de ser utilizadas por los agricultores para el manejo de enfermedades.

Palabras clave— Antagonistas; fitopatógeno; enfermedad de la raíz del palo de las crucíferas; producción más limpia

I. Introduction

Club root, caused by the protozoan *Plasmodoophora brassicae* Woronin, affects plants of the Brassicacea family such as cauliflower, broccoli, cabbage, among others, causing root damage and yield losses up to 70% to 80% [1]. The disease produces cell elongation and division in hypocotyls and gall formation in plant roots. As a result, infected plants cannot get enough water and nutrients [2].

Woronin determined the causal agent of the club root, the first report on the morphology and physiology of the pathogen were done in 1878, then the disease has been known for more than 130 years and it is considered the most important of the cruciferous in many agroecosystems and emerging in many places, like Canada [3].

For many years. fungicides have been tested for the control of *P. brassicae*. Studies carried out with the fungicides benomyl and methyl thiophanate were found to reduce the disease under greenhouse conditions, but their efficacy in field trials failed [4]. In an investigation, flusulfamide was not efficient in controlling the pathogen when it was already established in the cortical cells of the host, which was attributed to the fact that the product does not act in the initial stages of infection, only in the germination of resting structures, and in the primary zoospores released by these structures [5].

The fungicides cyazofamid and fluazinam were also compared for the control of this disease. Cyazofamid was more efficient in controlling the disease in various crucifers while fluazinam was phytotoxic to plants [6]. Other researchers also observed the efficiency of the fungicide cyazofamid in inhibiting the germination of the resting structures of *P. brassicae*, when applied in pre-plantation in infected soils [7].

In other results. it was suggested that phosphorous fungicides were effective in the control of the club root applied by irrigation in pre-plantation at 0.21% of the active principle, however, it was considered that this procedure was not economically viable and produced a heavy soil contamination [8].

Other publications indicate that attacks by *P. brassicae* can be prevented by treating the soils to be used in the nurseries with a chemical product based on dazomet (Basamid GR) (40 g/m to 60 g/m) and that in addition, the incidence of disease and losses due to this pathogen in cabbage crops can be reduced if healthy plants are transplanted to infested fields with the use of benomyl (Benlate PH 50) (4g/L) (Bezil PH 50 4g/L). For this, the roots must be previously immersed in a suspension of a benomyl-based fungicide with subsequent biweekly sprays of the same product [9].

A more recent investigation reports that cyazofamid was the best fungicide followed by Trichlorodinitrobenzene (TNCB) for the control of club root, not being effective captan, mancozeb, prochloraz + propiconazole, carbendazin, folpep and calcium hydroxide, however. TCNB has as a negative aspect its high toxicity [10].

The municipality of Mutiscua in Norte de Santander, specialized in planting vegetables, including crucifers such as: broccoli, cauliflower and cabbage [11], has been affected for years by the *P. brassicae* pathogen, causing notable losses to farmers, by devastating a large part of these crops, preventing the re-sowing of susceptible species. Due to this, the cruciferous planting area has decreased in the village, causing scarce supply and a rise in prices.

Another cause of the spread of the pathogen *P. brassicae* in the area has to do with the bad agricultural practices of the farmers and the way in which they try to mitigate this problem, performing soil treatments with highly toxic products that threaten against the micro, meso and microbiota of the soil. thus generate a negative impact on its fertility [10].

Deepen on the action of *Trichoderma* spp, is necessary. since it is an antagonistic fungus against pathogenic microorganisms of plants. due to its ability to produce extracellular toxic enzymatic secretions that cause disintegration and death in phytopathogenic fungi [12] and also of nematodes [13]; also on *Bacillus subtilis*, taking into account that it is an antagonistic bacterium which produces secondary metabolites, with antifungal properties. effective suppressants against various phytopathogens [14]; and *Burkholderia cepacia* too, which, although not recommended in the literature against *P. brassicae*, is considered an attractive bacterium due to its antagonistic power to control pathogens, as well as having properties as a plant growth stimulator [15].

In many areas, farmers have chosen to apply agricultural lime in order to raise the pH and reduce the effects of the protozoan *P. brassicae* [16]. This alternative constitutes a common practice in Mutiscua, however, the expected results are not always achieved, for that it is necessary to carry out more research to provide other effective and economically viable alternative solutions to farmers.

The investigation is justified since in tests carried out in the Mutiscua area with commercial products based on *T. harzianum, B. subtilis, B. cepacia* and the rooting Agent Naphthaleneacetic Acid (ANA) separately, a reduction in the disease was observed but not in a significant way [17], however, in other investigations where another method of making the treatments that included treating the postures and the soil before sowing, higher results of disease control were obtained [18], [2].

The objective of this work was to evaluate biological alternatives combined with Naphthaleneacetic Acid (ANA) for the management of *P. brassicae* in cauliflower under the conditions of the municipality of Mutiscua, Norte de Santander.

II. Materiales y Métodos

The research was carried out at the La Aguadita farm with coordinates N 7°18'20.4" W 72°44'58.1", in the Tapaguá village of the Mutiscua municipality (Fig. 1). In this place cauliflower cultivation was implemented in the period from August 16 to November 28, 2019.

Fig. 1. Geographical coordinates of the crop.
Source: Authors.

A. *Experimental design*

An experimental investigation was carried out in cauliflower using the three antagonists (*T. harzianum, B. subtilis* and *B. cepacia*), previously tested under laboratory conditions, they were mixed with the auxinic rooting agent ANA (Hormonagro) compared with agricultural lime and a control No treatment.

Cauliflower cultivation was established with a randomized complete block design, where 6 treatments were implemented, each one with 3 repetitions. for a total of 18 plots (experimental units) of 10 m² each (Table 1).

Table 1.
Treatments evaluated under the conditions of Mutiscua.

Treatments	Description
T1	*T. harzianum* + ANA
T2	*B. subtilis* + ANA
T3	*B. cepacia* + ANA
T4	ANA
T5	Lime
T6	Witness

Source: Authors.

Commercial bioproducts of the antagonists were used in the test, as well as the auxin rooting agent ANA (Hormonagro) [19], as fallow:

1) *T. harzianum (Trichox WP)*

It is a product with a guaranteed composition of 1×10^8 spores/g, with sales registration (ICA): 9121. It has a percentage of microbiological purity ≥ 95%. Spores have a germination of 95% at 24 hours. Its pH ranges between 5.0 - 7.0. Humidity ≤ 5%. Dose of 1cc/L of water. Toxicological category III - Moderately toxic.

It is a bio-ingredient formulated with the fungus *T. harzianum* that controls phytopathogenic fungi through parasitism and antagonism, stimulates plant defense mechanisms and systematic resistance, increases plant vigor and root volume, improves the photosynthetic process which increases production and stimulates the proliferation of beneficial microorganisms in the soil [20].

2) *B. subtilis (Bactox SL)*

It is a product with viability of 5×10^9 spores/mL of product. Contains a percentage of microbiological purity: 95% - 98%. Its pH ranges from 5.0 - 7.0. Sales registration is ICA:9824. Toxicological category IV - Slightly toxic. It is a bioinput formulated with the bacterium *B. subtilis*, that controls diseases caused by fungi and bacteria, solubilizes phosphorus, fixes nitrogen and induces the production of plant growth hormones, reduces plant stress. optimizes crop performance, and stimulates proliferation of beneficial microorganisms [20].

3) *B. cepacia (Botrycid)*

Product based on strains of the bacterium *B. cepacia* with a concentration of 1×10^8 colony-forming units per cubic centimeter (CFU/cc), which inhibit the growth and development of phytopathogenic microorganisms. This endophytic bacterium colonizes the interior of plant tissues, stimulating their growth, in addition, fixes atmospheric nitrogen, increases and modifies root hairs, increasing the absorption capacity of nutritive elements, ICA:6204 registration. Toxicological category III - Moderately toxic [21].

4) *Naphthaleneacetic Acid (ANA) - Hormonagro*

Plant growth regulator product with ICA:1966 sales registration. It is a soluble concentrate (SL) of toxicological category III - Moderately toxic. Recommended dose 15cc/20L pump.

It is an enzymatic activator of the following physiological processes in plants: activates cell division, regulates maturation, keeps seeds in a latent germination state, promotes root emission. flowering and fruiting, prevents bud drop. flowers and fruits [19].

The doses of the commercial products recommended by the suppliers were used. Cauliflower seedlings of the Bola de Nieve variety, whose characteristic is its precocity and its easy adaptability to adverse environmental conditions [22], were sown from a seedling producing unit in the municipality. The agronomic and phytosanitary management for the agents different than *P. brassicae* was the same used by the farmers in the area (Table 2).

TABLE 2.
DOSAGE AND FREQUENCY OF APPLICATION USED IN THE INVESTIGATION.

Treatments	Composition	Doses/pump 20L	Number of treatments
1. *T. harzianum* + ANA	1 × 10^8 spores/g	1 g/L + 15 cc	Sowing + 5
2. *B. subtilis* + ANA	5 × 10^9 spores /ml	1 cc/L + 15 cc	Sowing + 5.
3. *B. cepacia* + ANA	1 × 10^8 UFC/cm^3	1 cc/ + 15 c L	Sowing + 5.
4. ANA	17.2 g/L	15 cc/	5
5. Lime	21.6% Ca y 13.1% Mg	1 t/ha	
6. Witness	---------------------	----------------	

Fuente: [23].

Data collection was carried out weekly and 10 plants were randomly sampled per experimental unit, measuring morphometric, phytosanitary and yield variables.

B. *Morphological variables*

1) Plant height

It was determined by measuring from the base of the soil to the apex of the last leaf.

2) Stem diameter

Using a digital caliper (vernier caliper), the stem diameters were taken from each plant at 1 cm above the ground.

3) Number of leaves

A leaf count was carried out in the ten plants selected at random in each plot.

C. *Phytosanitary variables*

1) Disease incidence

The foliar incidence of 10 plants was recorded using (1) [24]:

$$\%\ \text{Incidence} = \frac{\text{Number of affected plant}}{\text{Number of evaluated plant}} \times 100 \qquad (1)$$

Records began eight days after transplanting and were determined every seven days.

2) Severity of the disease

The affectation of diseased leaf tissue was evaluated through the severity of the disease over time using a scale proposed by MSU scientists [25] and modified by researchers at UAlberta [26] (Table 3).

TABLE 3.
SEVERITY SCALE FOR THE FOLIAGE OF THE PLANT AFFECTED THE DISEASE.

Scale	Description
0	Healthy Plant.
1	Symptoms in leaf. incipient wilt.
2	Advanced symptoms. yellowing of leaves, advanced wilting.
3	Deft plant.

Source: Authors.

The severity was estimated with (2) (Townsend y Heuberger) [27]:

$$\text{Severity } (\%) = \frac{\sum \text{axb}}{\text{kxN}} \times 100 \qquad (2)$$

A = The severity classes of symptoms (0. 1. 2. and 3).
B = Number of plants with each grade.
k = Maximum scale degree = 3.
N = The total number of plants in an experimental unit = 10.

3) Incidence in roots

This variable was determined two days after harvest of each of the evaluated plants, applying the formula presented above (1) by visual observation of the deformations of the roots.

4) Root severity

The affectation of diseased root tissue was evaluated using the scale proposed by MSU scientists [25] and modified by researchers at UAlberta [26] (Table 4).

Table 4.
Scale for severity in roots affected by the disease.

Scale	Description
0	Healthy root.
1	Small club roots, less than 25% of the affected root.
2	Club roots of medium size in 50% of the root.
3	Club roots of medium to large size in more than 50% of the root.

Source: [26].

The disease severity index was calculated using the Townsend and Heuberger formula (2) [27] described previously.

5) Area Under the Disease Progress Curve (AUDPC)

At the end of the experiment. AUDPC was determined in each plot according to the method of Campbell and Madden [28], for which the following formula was used (3):

$$\mathbf{ABCPE} = \sum [(\boldsymbol{X}_{¡} + \boldsymbol{X}_{¡} + \mathbf{1})/\mathbf{2}] * (\mathrm{T}_{¡} + \mathbf{1} - \mathrm{Ti}) \qquad (3)$$

Where:

X ¡ = Incidence or severity of the disease in the sample i.
X ¡ + 1 = Incidence or severity of the disease in the sample i + 1.
T ¡ = Time 1.
T ¡ + 1 = Time 2.

At the same time, rainfall was measured at a meteorological station of the Plantar Project in the town of Mutiscua, 1 km from the place of the experiment. At five-week time, pH was measured in al plots in order to see the effect of the lime on soil pH.

D. *Yiels*

In the experiment. the total production per plot was estimated and the yield in t/ha was determined. For this, the plants that were in the experimental units and the percentage of flowering plants were counted, then 10 heads were weighed at random in each plot and the weight in grams was averaged. With these data, the average weight/plot affected by the percentage of flowering plants in 10 m² was obtained and the yield in t/ha of each experimental unit was determined.

E. *Statistical analysis*

With the information obtained about height, stem diameter, number of leaves, disease incidence and severity of foliage and roots, as well as, the yield, an analysis of variance was carried out after checking the assumption of normality (Shapiro Wilk test) and homogeneity of the variances.

The means were compared by the Tukey test with a 5% probability of error. The SPSS version 21 package for Windows was used [38].

F. *Assessment of the cost benefit of new alternatives versus traditional and untreated ones*

The necessary analyzes were performed to assess the economic effectiveness of each biological or alternative treatment using the CIMMYT-recommended partial increment methodology [29].

In it, the analysis is simplified and the determination of the increase in the value of production is sought, once the amount of the cost of each biological or alternative treatment has been deducted from the witness and the traditional variant with the use of lime.

It was raised from the following formula (4):

$$EE = Vipn - Ct \quad (4)$$

Where:

EE	=	Economic effectiveness.
Vipn	=	Value of the increase in the production of new variants (biological or alternative).
Ct	=	Additional cost of the new variant, with respect to the traditional control (lime variant), and without treatment (witness).

To estimate the cash flows of each new treatment, a planning period of sixty days was taken into account, conditioned by the time it takes to convert the cauliflower from planting to harvest.

The following items were taken into account as operating costs:

- Salary expenses for phytosanitary treatments.
- Direct material expenses for fundamental treatments. that is. those incurred with lime, alternative biologicals and ANA.

All the treatments carried out during the crop cycle were considered from the purchase prices of the following products:

- Commercial *T. harzianum*.
- Commercial *B subtilis*.
- Commercial *B. cepacia*.
- Commercial ANA.

G. *Agricultural lime*

For each treatment. the recommended dose in each case was taken into account. The increase in the cost per salary in the phytosanitary activities to the positions in the variants with treatment was calculated as the increase in the monetary value of the production of each plot in relation to the control treatment for each case, as well as its significance in value. The marketing price of cauliflower for the end of November of the study year was taken into account.

III. Results

A. *Determination of the effect of biological alternatives combined with naphthaleneacetic acid (ANA) for the control of P. brassicae in cauliflower*

1) *Plant height and stem diameter*

At week one there was statistical differences between the *T. harzianum* + ANA treatment (10.11 cm) and the ANA treatment (8.08 cm). At week two there was also statistical difference between the treatment *T. harzianum* + ANA (11.25) and ANA (8.79 cm). At week three there was statistical difference between the treatments *T. harzianum* + ANA and *B. subtilis* + ANA (14.05 cm and 11.87 cm) with respect to the treatments *B. cepacia* + ANA (11.01 cm), ANA (11.53 cm), lime (12.41 cm), and control (12.32 cm). At week four there was no statistical difference between the treatments. At week five, the *T. harzianum* + ANA treatment differed from the other treatments, presenting the highest height (20.45 cm). From week six to week seven there was no statistical difference between the treatments, however, the highest relative values during these weeks was the *T. harzianum* + ANA treatment (20.71 cm and 23.18 cm). From week eight to week eleven there was no statistical difference between the treatments, however, the highest relative values during these weeks was the ANA treatment (25.23 cm to 31.45 cm) (Table 5).

Table 5.
Description of the results in the variable height of the plant.

Treatments	Hight (cm)										
	Weeks										
	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11
T. harzianum + ANA	10.11 a	11.25 a	14.05 a	16.79 a	20.45 a	20.71 a	23.18 a	24.83 a	25.56 a	26.46 a	30.06 a
B. subtilis + ANA	9.29 ab	10.23 ab	11.87 a	15.04 a	17.87 ab	19.90 a	20.89 a	23.23 a	24.15 a	25.38 a	26.82 a
B. cepacia + ANA	8.63 ab	9.49 bc	11.01 b	13.95 a	17.90 ab	19.25 a	20.05 a	23.70 a	24.18 a	27.82 a	28.02 a
ANA	8.08 b	8.79 c	11.53 b	14.91 a	17.83 ab	20.22 a	20.93 a	25.23 a	26.65 a	29.16 a	31.45 a
Lime	9.49 ab	10.46ab	12.41 b	15.58 a	18.43 ab	20.64 a	21.64 a	23.36 a	24.91 a	25.80 a	28.07 a
Witness	8.51 ab	10.00abc	12.32 b	14.14 a	17.50 b	20.08 a	21.45 a	23.46 a	23.95 a	24.84 a	24.93 a
Coef. of Variation (%)	7.09	4.98	4.42	8.02	5.45	10.12	9.18	14.9	15.38	14.28	12.26
Typic Error*	0.37	0.28	0.31	0.69	0.57	0.1	1.13	2.06	2.21	2.19	1.99

Unequal letters in the columns differ for $p \leq 0.05$ by Tukey's test.

Source: Authors.

At weeks one and two there was no statistical difference between the treatments, however, the highest relative value of diameter at week 1 was for *B. subtilis* + ANA (2.59 mm), and at week 2 for *T. harzianum* + ANA. At week 3 there was a statistical difference between the treatment of *T. harzianum* + ANA and *B. subtilis* + ANA (4.42 mm and 4.25 mm) with respect to the treatment of *B. cepacia* (3.46 mm). From week 4 to week 11 there was no statistical difference between the treatments, however, the highest relative values from weeks 4 to 11 was the treatment *T. harzianum* + ANA (6.88 mm to 16.29 mm) (Table 6).

Table 6.
Description of the results in the stem diameter variable.

Treatments	Diameter of the stalks (mm)										
	Weeks										
	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11
T. harzianum + ANA	2.49 a	2.99 a	4.42 a	6.88 a	9.27 a	9.95 a	10.83 a	12.21 a	13.09 a	14.15 a	16.29 a
B. subtilis + ANA	2.59 a	2.86 a	4.25 a	6.20 a	7.76 ab	9.28 a	9.98 a	10.88 a	11.66 a	12.90 a	13.82 a
B. cepacia + ANA	2.31 a	2.54 a	3.46 b	5.39 a	7.07 b	8.20 a	9.11 a	10.26 a	11.42 a	12.47 a	13.69 a
ANA	2.20 a	2.50 a	3.58 ab	5.62 a	7.53 ab	8.76 a	9.93 a	11.07 a	12.15 a	13.10 a	14.88 a
Lime	2.51 a	2.93 a	4.24 ab	6.58 a	8.19 ab	9.76 a	10.34 a	11.15 a	12.60 a	12.86 a	14.05 a
Witness	2.43 a	2.78 a	4.08 ab	5.44 a	8.41 ab	9.77 a	10.20 a	11.01 a	11.97 a	12.68 a	14.56 a
Coef. de Variación (%)	8.95	9.3	8.57	14.77	8.74	7.6	9	10.7	10.4	12.42	11.43
Error Típico*	0.12	0.14	0.19	0.51	0.4	0.4	0.52	0.68	0.73	0.93	0.96

* Unequal letters in the columns differ for $p \leq 0.05$ by Tukey's test.

Source: Authors.

2) *Number of leaves*

During week one and two there was no statistical difference. however. the absolute relative values of the lime treatment were higher (with 3.26 and 3.76 leaves). At week three, the *T. harzianum* treatment (with 6.43 leaves) had statistical difference with respect to the ANA treatment (5.43 leaves). Likewise, the relative values of the *T. harzianum* treatment were higher from weeks four to eleven (with 7.60 to 11.81 leaves) (Table 7).

Table 7.
Descripción de los resultados de la variable número de hojas.

Treatments	Number of leaves										
	Weeks										
	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11
T. harzianum + ANA	3.03 a	3.70 a	6.43 a	7.60 a	8.43 a	9.43 a	9.76 a	9.76 a	10.26 a	11.03 a	11.81 a
B. subtilis + ANA	3.06 a	3.50 a	6.06 ab	7.40 a	7.50 a	8.60 a	8.76 a	9.33 a	9.56 a	9.83 a	10.23 a
B. cepacia + ANA	2.93 a	3.33 a	5.50 ab	6.80 a	7.33 a	8.53 a	9.10 a	9.70 a	9.80 a	10.13 a	10.73 a
ANA	2.86 a	3.06 a	5.43 b	6.90 a	7.66 a	8.53 a	9.33 a	9.70 a	10.00 a	10.16 a	11.13 a
Lime	3.26 a	3.76 a	6.16 ab	7.43 a	7.73 a	8.76 a	9.46 a	9.60 a	9.70 a	10.20 a	10.17 a
Witness	2.86 a	3.46 a	6.01 ab	6.83 a	7.31 a	8.41 a	8.63 a	8.83 a	9.46 a	9.56 a	10.46 a
Coef. of Variation (%)	31.97	28.23	5.76	4.48	5.5	5.1	6.86	6.88	7.61	7.39	8.26
Typic Error*	0.55	0.56	0.19	0.18	0.24	0.25	0.36	0.37	0.43	0.43	0.51

* Unequal letters in the columns differ for $p \leq 0.05$ by Tukey's test.

Source: Authors.

B. *Incidence, severity and Area under the disease progress curve (AUDPC) in the foliage*

During week four there was no statistical difference ($p > 0.05$) for the incidence. At week five there was a statistical difference between the *T. harzianum* + ANA treatment (0.60) and the Lime treatment (1.80), the rest of the treatments were with intermediate values between these two. From week six to week eleven there was no statistical difference, however, at weeks six, eight, nine, and eleven the *T. harzianum* + ANA treatment reported lower relative incidence levels (Table 8).

Table 8.
Percentage of incidence of the disease in the foliage.

Treatments	Incidence of the disease							
	Weeks							
	W4	W5	W6	W7	W8	W9	W10	W11
T. harzianum + ANA	0.20 a	0.60 b	1.31 a	1.87 a	2.21 a	2.43 a	2.84 a	2.84 a
B. subtilis + ANA	0.20 a	1.32 ab	1.52 a	1.86 a	2.25 a	2.73 a	3.14 a	3.14 a
B. cepacia + ANA	0.20 a	1.24 ab	1.66 a	2.16 a	2.33 a	2.93 a	3.14 a	3.14 a
ANA	0.20 a	1.45 ab	1.66 a	2.01 a	2.39 a	2.73 a	2.93 a	3.14 a
Lime	0.20 a	1.80 a	1.87 a	2.25 a	2.46 a	2.63 a	2.73 a	3.14 a
Witness	0.3589 a	1.38 ab	1.86 a	2.18 a	2.73 a	3.14 a	3.14 a	3.14 a
Coef. of Variation (%)	50.27	25.66	17.99	13.2	19.76	11.52	9.84	6.86
Typic Error*	0.06	0.19	0.17	0.15	0.27	0.18	0.16	0.12

* Unequal letters in the columns differ for $p \leq 0.05$ by Tukey's test.

Source: Authors.

At four week there was no statistical difference for the severity of the disease between the treatments. At five-week time. there was statistical difference between the *T. harzianum* + ANA treatment (0.39) and the Lime treatment (0.94), the rest of the treatments were intermediate between those two. From six week to eleven week there was no statistical difference either, however, the *T. harzianum* + ANA treatment reported the lowest relative levels of severity during weeks six, eight, nine and ten (Table 9).

Table 9.
Percentage of severity of the disease in the foliage.

Treatments	Foliar severity							
	Weeks							
	W4	W5	W6	W7	W8	W9	W10	W11
T. harzianum + ANA	0.20 a	0.39 b	0.73 a	0.97 a	1.07 a	1.18 a	1.29 a	1.32 a
B. subtilis + ANA	0.20 a	0.74 ab	0.83 a	0.97 a	1.10 a	1.22 a	1.32 a	1.32 a
B. cepacia + ANA	0.20 a	0.70 ab	0.89 a	1.08 a	1.13 a	1.22 a	1.34 a	1.38 a
ANA	0.20 a	0.79 ab	0.89 a	1.03 a	1.10 a	1.27 a	1.31 a	1.38 a
Lime	0.20 a	0.94 a	0.97 a	1.10 a	1.13 a	1.22 a	1.36 a	1.43 a
Witness	0.27 a	0.77 ab	0.97 a	1.08 a	1.20 a	1.27 a	1.41 a	1.50 a
Coef. of Variation (%)	25.77	20.43	14.3	9.1	10.12	4.43	7.8	7.18
Typic Error*	0.03	0.08	0.07	0.05	0.06	0.03	0.06	0.05

* Unequal letters in the columns differ for p ≤ 0.05 by Tukey's test.

Source: Authors.

The AUDPC of *P. brassicae* in cauliflower cultivation in terms of incidence had a statistical difference between the *T. harzianum* + ANA treatment (2660.00) and the Control treatment (3686.66), the rest of the treatments were intermediate between these. Regarding the AUDPC old severity variable, the treatments did not have statistical differences, however, the value of the *T. harzianum* + ANA treatment was relatively lower compared to the others (956.58) (Table 10).

Table 10.
AUDPC for the percentage of the incidence and severity.

Treatments	Production	
	Incidence AUDPC	Severity AUDPC
T. harzianum + ANA	2660.00 b	956.58 a
B. subtilis + ANA	3290.00 ab	1139.43 a
B. cepacia + ANA	3453.33 ab	1205.53 a
ANA	3406.66 ab	1213.33 a
Lime	3628.33 ab	1314.46 a
Witness	3686.66 a	1333.90 a
Coef. of Variation (%)	11.04	12.76
Typic Error*	214.16	88.07

* Unequal letters in the columns differ for p ≤ 0.05 by Tukey's test.

Source: Authors.

1) *Root disease severity*

For the severity variable in roots, there was a statistical difference between the *T. harzianum* + ANA treatment, relates with ANA and witness (84.17 and 87.54) respectively. The rest of the treatments were intermediate between these from the statistical point of view (Table 11).

Table 11.
Percentage of severity in roots affected by disease.

Treatments	Severity in roots
T. harzianum + ANA	47.14 b
B. subtilis + ANA	74.07 ab
B. cepacia + ANA	80.81 ab
ANA	84.17 a
Lime	77.44 ab
Witness	87.54 a
Coef. of Variation (%)	17.7
Typic Error*	0.09

* Unequal letters in the columns differ for $p \leq 0.05$ by Tukey's test.

Source: Authors.

3) *pH*

The pH of the soils did not present statistical differences between the different treatments ($p > 0.05$), observing that the levels were below pH 7, may be for that reason did not significantly favor the reduction of the percentage of incidence and severity of club root in the broccoli plots (Table 12).

Table 12.
Soil pH values in the experimental units.

Treatments	pH
T. harzianum + ANA	6.73 a
B. subtilis + ANA	6.80 a
B. cepacia + ANA	6.73 a
ANA	6.86 a
Lime	6.73 a
Witness	6.70 a
Coef. of Variation (%)	1.55
Typic Error*	0.06

* Unequal letters in the columns differ for $p \leq 0.05$ by Tukey's test.

Source: Authors.

4) *Precipitation during the crop cycle*

Due to the climatic conditions of the municipality of Mutiscua, rainfall increased by week five and six (68.58 mm and 65.53 mm), which was related to the increase in the dynamics of the disease in all treatments and probably explains why after this moment there are no statistical differences between the treatments in both, morphometric and some phytosanitary variables (Fig. 2).

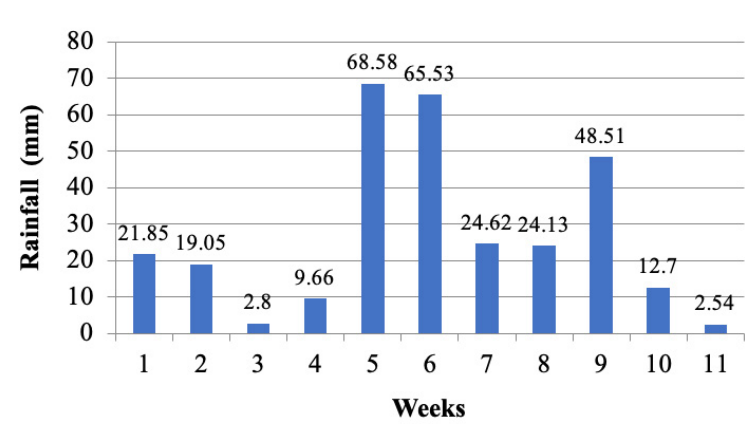

Fig. 2. Distribution of rainfall by sampled week.
Source: Meteorological Station of the Plantar Project in Mutiscua.

C. *Assessment of the cost benefit of the new alternatives versus the traditional and untreated ones*

1) *Productivity*

The production table shows there were no statistical differences for the ave rage percentage of productive plants for the three experimental units, however, the *T. harzianum* + ANA treatment differed with the other for the variable weight of the head, there was a statistical difference treatment (401.00 g) and the ANA and the witness (208.66 g and 164.33 g) (Table 13).

Table 13.
Description of the average percentage in crop production.

Treatments	Production	
	% of productive plant	Media weight/head (g)
T. harzianum + ANA	80.00 a	401.00 a
B. subtilis + ANA	58.33 a	321.66 ab
B. cepacia + ANA	61.66 a	254.33 ab
ANA	56.66 a	208.66 b
Lime	56.66 a	259.33 ab
Witness	58.33 a	164.33 b
Coef. of Variation (%)	14.6	23.49
Typic Error*	5.23	36.43

* Unequal letters in the columns differ for $p \leq 0.05$ by Tukey's test.

Source: Authors.

The treatment with the highest production index was *T. harzianum* + ANA, standing out from the other treatments with a production of 20 kg/10 m², followed by *B. subtilis* + ANA with a production of 11.6 kg/10 m² and finally the treatments *B. cepacia* + ANA and lime did not differ significantly in production with a yield of (9.7 and 9.07 kg/10 m²) (Table 14).

Table 14.
Description of the average crop yield.

Treatments	Yiels (kg)/m²	Yiels (kg)/10 m²	t/ha
T. harzianum + ANA	2.005	20.05 a	20.05
B. subtilis + ANA	1.166	11.66 ab	11.66
B. cepacia + ANA	0.97	9.7 ab	9.7
ANA	0.73	7.3 ab	7.3
Lime	0.907	9.07 ab	9.07
Witness	0.595	5.95 ab	5.95
Coef. of Variation (%)		15	
Typic Error*		1.24	

* Unequal letters in the columns differ for $p \leq 0.05$ by Tukey's test.

Source: Authors.

The treatment that generated the best income was *T. harzianum* + ANA with a gain of $82.126 per treatment equivalent to 82.126 Colombian pesos/ha compared to the other treatments, the lime treatment was the second best with a gain of $61.312 per treatment. However, the treatments with the lowest gain were *B. cepacia* + ANA and *B. subtilis* + ANA (Table 15).

Table 15.
Description of cost benefit generated by the crop expressed in Colombian pesos.

Treatments	Incomes	Costs	Profits	Lime	Witness o
T. harzianum + ANA	125.000	42.874	82.126	20.814	40.606
B. subtilis + ANA	90.625	49.894	40.731	–20.581	–0.789
B. cepacia + ANA	95.312	66.880	28.432	–32.880	–13.088
ANA	87.500	41.750	45.750	–15.562	4.230
Lime	87.500	26.188	61.312	----	19.792
Witness	54.000	12.480	41.520	19.792	----

Source: Authors.

The analysis carried out on the marketable inflorescences showed significant differences in the cost-benefit relationship for the establishment and production of this crop, however, the good price at which cauliflower is found in the city of Bucaramanga can be highlighted, at which. It is carried in boxes with an average of 12 heads. Allowing farmers to obtain higher profits by the end of November.

III. Discussion

Although there was not always a statistically significant difference between the treatments over the weeks. the relative action that the *T. harzianum* + ANA and *B subtilis* treatments had in comparison with the other treatments used on the development of the crop was highlighted. The treatments that included *Trichoderma* and *B. subtilis* showed a tendency to stimulate the variables height and thickness of cauliflower plants, verifying the stimulating properties of these two antagonists, which has been proposed for the case of *Trichoderma* in cauliflower by UNAL [18] and for *B. subtilis* in cauliflower by YNAU [2]. Other authors mention that the antagonist fungus *T. harzianum* acts as a growth stimulator in multiple cultures [30] as the same as *B. subtilis* [14].

When analyzing the results of incidence, severity and AUDPC in the different treatments. it can be observed that in some cases they were lower for the treatment of *T. harzianum* + ANA and even in most of the cases in which there was no statistically significant differences with others treatments throughout the weeks. This treatment stood out over the others (with the exception of *B. subtilis* + ANA) for having lower relative results in the variables described above, corroborating the claims of INNIA and UNAH [31] on the effectiveness of species of the genus *Trichoderma* as an antagonist of plant pathogens, specifically on *Plasmodiophora*, reducing the severity of the disease on the root system of the plant. In other studies. a significant decrease in cabbage club root in relation with the control has also been verified using the antagonist *B. subtilis* [32].

The results support those of other investigations carried out in Colombia where the effect of three strains of *Trichoderma* (*T. asperellum* strain Th034. *T. brevicompactum* strain Th201 and *T. koningiopsis* strain Th003) on the reduction of club toot in cabbage where it was applied 1, 7 or 14 times during the cycle, obtaining the reduction of the disease by *T. asperellum* in a proportional way to the number of applications carried out. From these results it could be concluded that *T. brevicompactum* exhibits potential for the management of cruciferous club root when applied frequently [18], as happened in the present investigation.

The results observed with *B. subtilis* correspond to others where a very powerful strain of this antagonist was evaluated in China [2], it had strong inhibitory effects on both survival and germination of *P. brassicae* resting spores where the response to disease suppression increased with the time seedling age in pot and field conditions. On the other hand, the immersion of seeds with *B. subtilis reduced* the incidence rate of the disease and the incidence of the disease [2]. It is emphasized in both investigations that prevention at an early stage is the best way to reduce the damage caused by the disease. and it was the methods fallowed in the present research.

When the rains increased in weeks 5 and 6 an increase of the incidence and severity occurred, therefore, the interference in the absorption and translocation of nutrients, minerals and water in the plant of broccoli as some authors have pointed out [24]. Researchers from the ICA [23] affirm that the spread of the pathogen is favored especially in those areas by rainwater and runoff, which help activate the rested spores and invade the susceptible crops. More recent studies suggest that soil moisture above 60% to 70% substantially favors the development of the disease [33].

Similar results were observed in other investigations where, despite the fact that no statistical differences were observed between the treatments for the control of the disease, it was possible to verify that the progress of this increased after heavy rains with higher levels on different planting dates where there were crowded higher rainfall [1].

The present results in relation to soil pH confirm what was stated by OSU Department of Horticulture [16], who demonstrated that if a pH of 7.1 is not reached in the soils, infection is not reduced. It is also verified what is stated by other authors in relation to the efficiency of this practice depends on other factors such as the quality of the lime used and the soil, since there are soils that easily respond to the application of lime, but others do not [34]. On the other hand, research related to *Trichoderma* should be continued in front of different pH when lime is applied, since it is pointed out that when liming is increased, the effectiveness of the antagonist decreases [35]. It would be advisable to carry out a chemical and microbiological analysis to determine the conditions in which the soil is found. before establishing the cruciferous crops under these conditions and thus decide the dose of lime to apply and monitor the composition of the microbiota in the soil, mainly in the rhizosphere of the plants.

The application of the rooting agent Hormonagro (ANA) when it was applied alone was not effective for the control of hernia in other investigations [17], which is verified again, however, it was demonstrated in the present investigation that mixed with *Trichoderma* is superior than rooter alone and the antagonist alone. It had been reported that another rooter. Fortiraíz rooting agent [36] had shown good encouraging results, by maintaining the incidence of the disease at 10.63%, the plants reaching higher height and higher yields than the controls that reached between 14% and 17% of the incidence of the disease. This is why it was recommended for the management of the disease and increase the productivity of broccoli cultivation in Ecuador [37], however under the edaphoclimatic and precipitation conditions of Mutiscua this has not been verified.

Given the encouraging results obtained. the investigations with *T. harzianum* combined with the growth regulator (ANA) and to a certain extent with the mixture *B. subtilis* + ANA should be continued testing for the control of club root under these conditions, using different species of crucifers, as well as prospecting native strains of antagonists with a higher level of adaptation to these environmental conditions that could favor their efficacy.

It is important to take into account cultural measures such as the elimination of previous harvest residues and the rotation of crops with different species such as legumes (beans, peas, broad beans, string beans) and cereals (corn. grasses) or other vegetables (celery, beets, onion, coriander) [9], since it has been shown that the concentration of the inoculum influences the efficacy of *Trichoderma* [18].

In future research must be done. and a comparison should be made with higher doses of biological products used in this research to assess whether it is possible to obtain greater efficacy with these environmentally friendly treatments.

IV. Conclusiones

The use of biological products *T. harzianum* and *Bacillus subtilis* combined with growth regulators ANA although do not curatively stop the cruciferous club root disease, they generate improvements in morphometric variables and reduce the severity of the pathology both of foliar and in the roots, showing these treatments less degrees of affectation by the protozoan.

Frequent precipitations and higher humidity in the soil favored the spread of the pathogen and increased the variables of the disease, thus interfering with the effect of the antagonists.

The soil pH was not raised enough in the experiment with the application of lime to inhibit the spread of this protozoan in the crop, so the development of the disease was not prevented.

The most favorable benefit / cost ratio was obtained with the application of the antagonistic fungus *T. harzianum* + ANA, which showed higher productivity. followed by lime, which, although with little efficacy, was the cheapest alternative.

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