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Possible induction of systemic resistance to *Lasiodiplodia theobromae* in avocado (*Persea americana* Mill.) under semi-controlled conditions at La Molina

Posible inducción de resistencia sistémica a *Lasiodiplodia theobromae* en aguacate (*Persea americana* Mill.) en condiciones semicontroladas en La Molina

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Abstract

The production and export of avocado fruits, cultivar Hass, has grown exponentially in recent years in Peru, however, a high incidence of symptoms such as regressive death, cankers on plant branches, and fruit rot have been observed affecting optimal crop production and fruit quality. These symptoms are mainly generated by *Lasiodiplodia theobromae* fungus, whose control is limited to a few active ingredients that tend to generate pathogen resistance. The present investigation had as objective to evaluate the inducing effect of resistance in the control of *Lasiodiplodia theobromae* in avocado (*Persea americana*) cultivar Hass of five bioproducts: ProtecSea, Barrera, Timorex Gold, T 22, Vacun Q Pro and water as a control treatment, with a total of 6 treatments with 4 replications, 3 avocado trees per replication. One-year-old avocado trees cultivar Hass were used. Treatments were applied three times via drench at ten-day intervals. Five days after the last application L. *theobromae* was inoculated in the avocado trees cultivar ' Hass' and 40 days after the inoculation, the parameters were evaluated: length of the disease lesion, percentage of dry matter of the roots and leaves, and length of the roots. It was observed that treatments, with the exception of treatment T1 (Control), reduced the length of the disease lesion infected by *L. theobromae* inside the stem. Treatment T6 (T.22) resulted in a higher percentage of root dry matter (46.52 %) compared to the control treatment (41.21 %). On the other hand, there were no significant differences in leaf dry matter content among treatments. Additionally, it was observed that treatment T2 (ProtecSea) reported a root length of 56.80 cm, followed by treatment T6 (T.22) with 54.90 cm. These results are important to continue with investigations into inducing systemic resistance in plants.

Keywords: *Lasiodiplodia theobromae*, *Persea americana*, Barrera, Vacun Qpro, Timorex gold, *Trichoderma harzianum*, ProtecSea.

Resumen

La producción y exportación de frutos de palta, cultivar Hass, ha crecido exponencialmente en los últimos años en el Perú, sin embargo, se ha observado una alta incidencia de síntomas como muerte regresiva, cancros en ramas de la planta y pudrición del fruto que afectan la producción óptima del cultivo y la calidad del

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fruto. Estos síntomas son generados principalmente por el hongo *Lasiodiplodia theobromae*, cuyo control está limitado a unos pocos principios activos que tienden a generar resistencia al patógeno. La presente investigación tuvo como objetivo evaluar el efecto inductor de resistencia en el control de *Lasiodiplodia theobromae* en aguacate (*Persea americana)* cultivar Hass de cinco bioproductos: ProtecSea, Barrera, Timorex Gold, T 22, Vacun Q Pro y agua como tratamiento control, con un total de 6 tratamientos con 4 repeticiones, 3 árboles de aguacate por repetición. Se utilizaron árboles de aguacate cultivar Hass de un año de edad. Los tratamientos se aplicaron tres veces mediante drench a intervalos de diez días. Cinco días después de la última aplicación se inoculó *L. theobromae* en los aguacateros cultivar 'Hass' y 40 días después de la inoculación se evaluaron los parámetros: longitud de la lesión de la enfermedad, porcentaje de materia seca de las raíces y hojas, y longitud de las raíces. Se observó que los tratamientos, a excepción del tratamiento T1 (Control), redujeron la longitud de la lesión de la enfermedad infectada por *L. theobromae* en el interior del tallo. El tratamiento T6 (T.22) dio lugar a un mayor porcentaje de materia seca radicular (46,52 %) en comparación con el tratamiento testigo (41,21 %). Por otro lado, no hubo diferencias significativas en el contenido de materia seca foliar entre los tratamientos. Adicionalmente, se observó que el tratamiento T2 (ProtecSea) reportó una longitud de raíz de 56.80 cm, seguido del tratamiento T6 (T.22) con 54.90 cm. Estos resultados son importantes para continuar con las investigaciones para inducir resistencia sistémica en las plantas.

Palabras clave: *Lasiodiplodia theobromae, Persea americana, Barrera, Vacun Qpro, Timorex gold, Trichoderma harzianum, ProtecSea.*

Introduction

Planning a healthy diet, avocado consumption can fit into a full range of dietary plans (Segovia-Siapco et al., 2021). Avocado fruit has a nutritional profile that contains a high content of unsaturated fatty acids and provides vitamins A, B1, B2, B6, C, D, E, K, folic acid, biotin and elements such as calcium, iron, phosphorus, copper, magnesium, sodium and potassium (Romero, 2019). In addition, its consumption reduces the risk of developing cardiovascular diseases (Fonseca et al., 2016).

Approximately 33 % of the avocado world production is exported and almost 80 % of the volume traded belongs to the Hass cultivar (Romero, 2019) because of the high quality of the pulp, skin thickness and its ability to be stored (Crane et al., 2013 as cited in Apaza, 2019).

Peru is the second worldwide exporter of avocado fruits, after Mexico, with 247 thousand tons (12.4 % of the total), and is one of the most dynamic countries in the export sector (Romero, 2019).

Hass avocado producers in Peru have reported a high incidence of symptoms such as dieback and presence of cankers in their plantations, which shorten the three life span and affect optimal crop production. These symptoms are mainly generated by the infection of *Lasiodiplodia theobromae*, which is a saprophytic, endophytic fungus and latent pathogen (Flores et al., 2021) that manifests itself when the host is stressed or weak (Picos-Muñoz et al., 2015). It generates regressive death in the branches, cankers and rot in the fruit or fruit peduncle (García et al., 2021).

Control of this disease is mainly through the use of fungicides, which are limited to a few active ingredients, which, due to their specific mechanism of action, could induce resistance to the fungus. In addition, their use is restricted to certain periods of crop phenology, due to the maximum residue limits (MRL) and the lack of pesticide period (Martínez, 2017). An alternative to reduce *L. theobromae* infections may be the use of resistance inducer products that stimulate the activation of plant defense genes which have the potential to reduce and/or avoid the risk of pathogen populations or pests resistant to chemical products, therefore, counteracting the chemical damage caused to the plant by pesticides and finally increase the yield of the crops; avoiding a negative impact on the agroecosystem and the health of people (Gómez & Reis, 2011).

The objective of the present research was to evaluate the resistance inducing effect in the control of *Lasiodiplodia theobromae* in avocado (*Persea americana*) of the products Vacun Qpro, Barrera, ProtecSea, T.22 and Timorex, in order to develop control strategies that reduce the use of chemical products.

Materials and methods

Laboratory phase

Location

The trial was carried out in the laboratory of the Plant Pathology Department at La Molina National Agrarian University, in Lima-Peru.

Obtaining the inoculum

The inoculum was obtained from an isolate of *L. theobromae* planted in Potato Dextrose Agar (PDA) medium, provided by the mycotheque of the Phytopathology Diagnosis Clinic of La Molina National Agrarian University, which comes from avocado trees with symptoms. characteristic of *L. theobromae* infections.

Preparation of the conidial suspension of L. theobromae

Once the inoculum was obtained, this was sown in Petri dishes with PDA medium and fragments of pine stems. They were incubated at 25 °C for 3 days, then placed under white fluorescent light at room temperature until reproductive structures were formed during a period of 30 days (Soto, 2018).

A suspension of conidia was obtained from each fungal colony and continuous dilutions were made with distilled water, until obtaining a concentration of 10^5 conidia/mL (Alama et al., 2006).

Field phase

Location

It took place at the shed house of the Plant Pathology Department of La Molina National Agrarian University, La Molina district, Lima province, Peru. Located at the coordinates 12º04'07"S 76º56'56"W. Temperature conditions were between 18 °C -23 °C and 70 % -81 % relative humidity.

Plant material

84 young trees of approximately 1 year of avocado cultivar Hass grafted on Zutano rootstock were used, without no characteristic symptoms of infection by fungi that affect xylem.

Treatments

Five products were used: ProtecSea (Marine extracts), Barrera (*Origanum vulgare*), Timorex Gold (*Melaleuca alternifolia*), T22 (*Trichoderma harzianum*), Vacun Q Pro (Phosphites) and water as a control treatment, with a total of 6 treatments with 4 replications, 3 plants per replication (Table 1).

These products were selected for their positive effects on plant defense induction. Marine extracts can induce defense mechanisms in plants and promote plant growth (Espinosa-Antón et al., 2020). *Melaleuca alternifolia* oil induces systemic resistance against *Fusarium* wilt in banana and Xanthomonas infection in tomato Plants (Dalio et al., 2020). Terpenes from *Origanum vulgare* inhibited the growth of the fungi *Colletotrichum acutatum* and *Botryodiplodia theobromae* (Numpaque et al., 2011). *Trichoderma* stimulates the production of callose deposits within the cell wall and increases peroxidase and chitinase activity in the plant, as reported by Yedidia et al. (1999 as cited in Jaimes, 2009). Phosphites act as stimulants in the production of natural defenses against pathogen attacks (Intagri, 2023).

Application of products

The application of the products was preventive. The phytosanitary solution was prepared in 3 L of water per treatment, according to the doses recommended in the technical data sheets of the products. Immediately, 250 mL of the solution

Table 1. Treatments used for the effectiveness test against *L. theobromae* in avocado cv Hass under nursery conditions.

Treatment	Tradename	Active ingredient	Commercial dose
T1	Test	-	-
T2	Protecsea	Marine extracts, organic acids, K_2O	0.5 L/200L
T3	Timorex Gold	Tea tree oil (*Melaleuca alternifolia*)	1,5 L/ha
T4	Vacun Q Pro	N, , Cu, Fosfito de Cu	0.5 L/ha
T5	Barrera	Origanum extract	0.8 L/200L
T6	T.22	*Trichoderma harzianum*	2L/ha

was applied to each plant via drench, on three occasions with intervals of ten days.

Inoculation
The conidia spray method was used, which simulated an infection in the field. Five days after the last application, the conidia suspension of the pathogen was inoculated. The stem bark of the Hass cultivar was washed with distilled water and superficially disinfected with 96° ethyl alcohol (Álvarez, 2015). In the stem of the Hass cultivar, a circular wound of 5 mm in diameter was made 5 cm away from the graft, then 20 µl of solution with a concentration of 10^5 conidia/mL was applied. Immediately, the inoculated area was covered with moistened cotton and Parafilm tape to protect from drying out (Chaupín, 2018).

Evaluation
Data was recorded 40 days after inoculation, which was a destructive evaluation (Soto & Cadenas, 2018). The established parameters were: length of the lesion, length of the roots and dry matter of the leaves and roots.

Statistical design
A completely randomized design was used, 6 treatments with 4 replications and 3 young trees in each replication. Analysis of variance (ANVA) and Tukey's means comparison tests were performed, considering a confidence level of 95 %, processed with the INFOSTAT software.

Results and discussion

The induction of systemic resistance generates systemic protection or a decrease in the severity of the disease in the plant (Trinidad-Cruz et al., 2019) against a wide variety of pathogens and is characterized by the accumulation of PR proteins, salicylic acid or jasmonic acid at a local and systemic level (Días, 2012), through the stimulation of biological or chemical inducers.
In this study, there was no contact of the applied product with the inoculated area. Neither were the plants subjected to any environmental stress. In addition, the inoculation was carried out after the applications of the products in the roots of the plant.

Therefore, the possible activation of induction of systemic resistance in avocado young trees was determined through the phenotypic evaluation that consisted of observing and quantifying the decrease in the progression of the disease.

Lesion advance length
When making the longitudinal cut of the stem, the vascular infection that interferes with the conduction of water was observed (Delgado et al., 2019). This is shown by necrosis in the vascular tissues (Fig. 1), showing progress of the infection in both directions from the point of inoculation, symptoms that Soto and Cadenas (2018) and Úrbez and Gubler (2011) point out in the experiment they carried out.

Through the analysis of variance (ANVA) carried out, it was shown that there are significant differences between the evaluated treatments, this is due to the fact that the p-value is less than the selected significance level (5 %). It implies that the treatments had affected the progression of the disease.

Likewise, with the Tukey comparison test, (Figure 2) it was determined that the T4 treatment (Vacun Qpro) showed a shorter length of the disease damage progression inside the stem with a value of 0.55 cm, however, statistically it is not different from the rest of the treatments with the exception of T1 (Control) that showed a length advancement of the lesion which reach 3.04 cm.

According to Picos (2017) temperature conditions 18-23°C measured in the present investigation, were within the range for the development of the disease. However, Ali et al. (2005) indicate that temperatures above 30° C, environmental, nutritional and water stress favor the development of *L. theobromae*. However, Apaza (2019) mentions that the temperature range of 20-30°C is optimal for the development and incidence of the pathogen.

The final value of any chemical compound as a disease control agent depends on the mode of action of its molecule in one or more stages of the life cycle of the pathogen (Reuveni &

Figure 1. Comparison of the control T1 with the treatments: T2 (ProtecSea), T3 (Timorex), T4 (Vacun Qpro), T5 (Barrera) and T6 (T.22) in the lesion progression test, of the damage caused by *L. theobromae* in avocado stems cv. Hass.

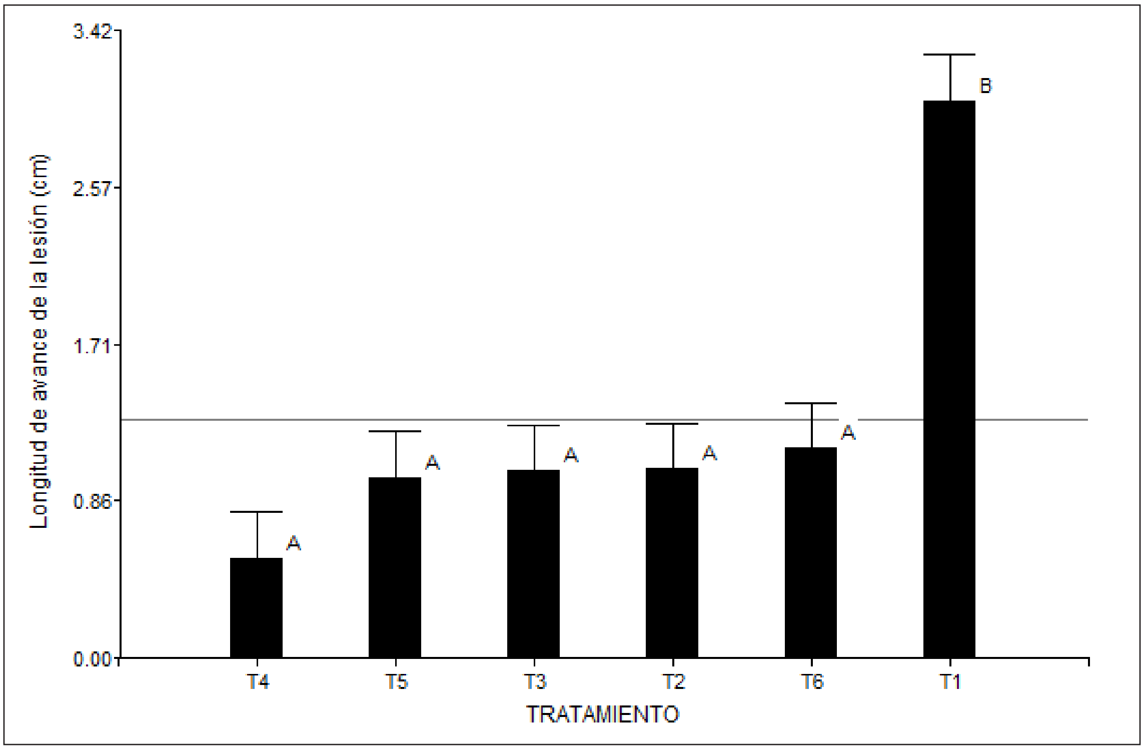

Figure 2. Length of disease lesion progression (cm) of treatments T4 (Vacun Qpro), T5 (Barrera), T3 (Timorex Gold), T2 (ProtecSea), T6 (T.22) and T1 (control) used in the cultivation of avocado cv. Hass. With the presence of the cutting line on the Y axis that represents the average length of the lesion (cm) and the bar above the column symbolizes the standard error.

Cohen, 2020). Important stages in the life cycle of *L. theobromae* involved in host infection and disease development include conidial germination, penetration into the host, growth of the mycelium on the host, and sporulation.

According to the results obtained, (Sánchez & Rincón, 2021) reported in *Botrytis cinerea* that copper, the active ingredient of Vacun Qpro(T4), shares the inhibitory power on germination and its organic component activates the production of phytoalexins, these are synthesized in healthy cells close to damaged cells and accumulate in necrotic tissues. That is, they are produced strictly around the area of infection and the resistance of the plant to the pathogen is carried out when the phytoalexins reach a sufficient concentration to inhibit the development of the pathogen (Agrios, 2015).

Regarding oregano oil, the active ingredient of Barrera (T5), it can be used as an alternative to the traditional use of fungicides in the preventive control of *L. theobromae* in avocado. Pérez-Alfonso (2012, as cited in Zhang et al., 2019) reported that the phenolic compounds thymol and carvacrol from oregano had antifungal activity by inhibiting postharvest-associated pathogens such as *P. digitatum, P. italicum, Fusarium spp.* and *Aspergillus spp.* Likewise, Rienth et al. (2019), reported that these compounds prevented the development of *Plamopara viticola* in grapevine. This antifungal activity could be due mainly to the properties of the compounds that, due to their highly lipid nature and low molecular weight, are capable of breaking the cell membrane, causing cell death or inhibiting the sporulation and germination of fungi (Nazzaro et al., 2017).

Melaleuca alternifolia, active ingredient of Timorex (T3), reduced the progression of the lesion in the stem of the avocado plant, possibly due to its inhibitory activity on mycelial growth and destruction of the cytoplasmic membrane that it possesses. Likewise, Reuveni & Cohen, (2020) point out that the fungicidal activity of Timorex against fungal pathogens and oomycetes arises from its ability to alter the permeability of the cell membrane of hyphae in *Bremia lactucae*.

Trichoderma harzianum (T6) presented less inhibition of disease progression. Soto & Cadenas (2018) specify that this biological controller was not effective in inducing the defense of grape plants against the attack by *L. theobromae*. However, Pani and Kumar (2021) point out that *T. harzianum* is effective against a variety of soil plant pathogens.

Dry matter percentage – roots

The induction of resistance by reducing or eliminating the infection of the pathogen in the plant favors the accumulation of dry matter in the aerial and root parts, due to the greater efficiency of use of the available nutrients that is probably related to the increase in activity of photosynthetic enzymes and nitrogen assimilation (Domingues et al., 2020).

In the evaluation of the percentage of dry matter of the roots, the analysis of variance carried out indicates that there are significant differences between the evaluated treatments, this is due to the fact that the p - value was lower than the selected significance level (5 %).

When comparing the treatments using the Tukey test (Figure 3), it was determined that treatment T6 (T.22) resulted in the highest percentage of dry matter (46.52 %) with respect to the control treatment and T4 (Vacun Qpro) with less percentage of dry matter (34.01 %) compared to the control.

From the results obtained, Brandão et al. (2002) mentions that the species *T. harzianum* (T6) guarantees adequate root growth. Likewise, it reduces the need for conventional NPK fertilization and helps in the solubilization of phosphate in the soil, also improves the absorption of micronutrients such as Na, Zn, Cu, Fe among others. Khan (2017), as cited in Pani and Kumar (2021), also reports increases in the growth of the roots that leads to an increase in the dry matter of these in the plant.

On the other hand, Tucuch-Pérez et al. (2021) refer to the thymol and carvacrol metabolites present in the oregano extract (T5) that increase

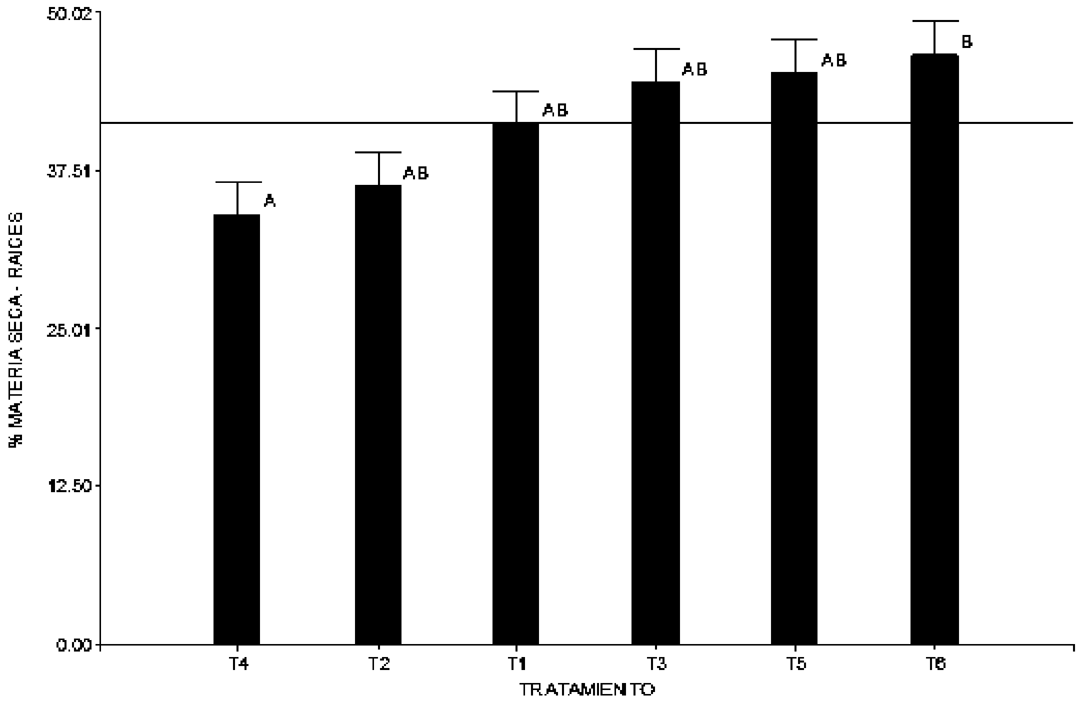

Figure 3. Percentage of dry matter - roots of the treatments T4 (Vacun Qpro), T2 (ProtecSea), T1 (control), T3 (Timorex Gold), T5 (Barrera) and T6 (T.22) used in the crop avocado cv. Hass.

the activity of auxins in the plant. Auxins represent the main regulator of the architecture of the root system, since they influence the formation of the primary root and the emergence of lateral roots. Overvoorde at al. (2010), as cited in Ombrosi (2023) reported greater root development and therefore an increase in root dry matter.

Dry matter percentage – leaves

The analysis of variance carried out for the percentage of foliar dry matter, in order to determine the effectiveness of the treatments in increasing the percentage of dry matter, showed that the p-value is higher than the selected significance level (5 %), which implies that there is not enough evidence to affirm real differences among treatments.

Barrera (T5) showed a higher dry matter content value of 54.24 % (Figure 4) than the control treatment, possibly due to the phenolic compounds Timol and Carvacrol that the product contains, since these, in addition to inducing resistance, they modulate the activity of auxins, and induce a greater growth of the plant (Tucuch-Pérez et al., 2021), therefore, an increase in leaves and an increase in dry matter.

Canet et al. (2010) point out that the activation of resistance in the plant generates a depletion of nutrients, which coincides with the results obtained in the treatments T2, T3, T4, and T6 that decrease the percentage of dry matter of the leaves with respect to the control treatment.

Root length

The analysis of variance carried out for root length indicates that there are statistical differences between the treatments because the p-value is less than the selected significance level (5%). Therefore, enough evidence affirms that the treatments influence root development.

When comparing the treatments using the Tukey test (Figure 5) it was observed that values in the T2 treatment (ProtecSea) reported a root length of 56.80 cm, followed by the T6 treatment (T.22) with 54.90 cm.

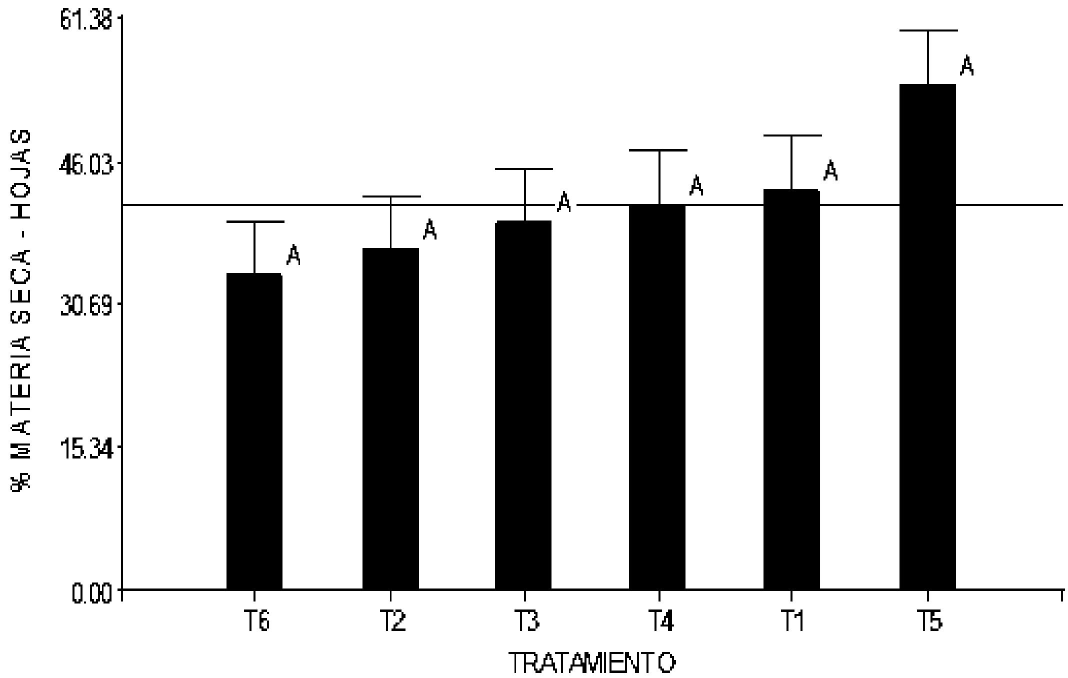

Figure 4. Percentage of dry matter - leaves of the treatments T6 (T.22), T2 (ProtecSea), T3 (Timorex Gold), T4 (Vacun Qpro), T1 (control) and T5 (Barrera) used in the crop avocado cv. Hass.

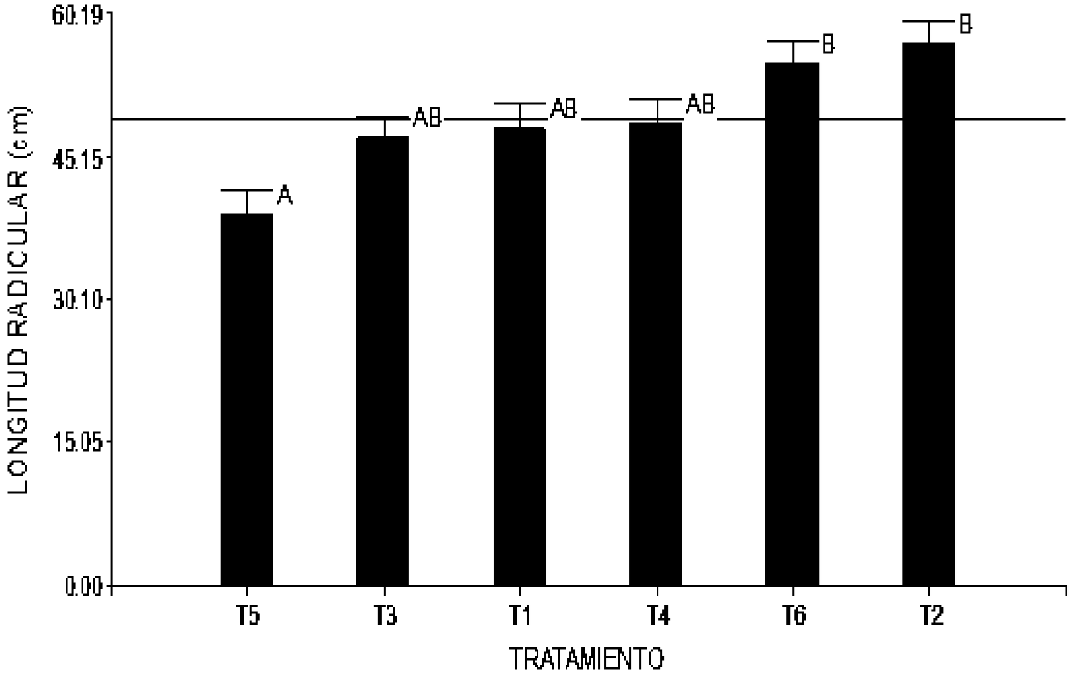

Figure 5. Comparison of root length (cm) of the treatments T5 (Barrera), T3 (Timorex Gold), T1 (control), T4 (Vacun Qpro), T6 (T.22) and T2 (ProtecSea) applied via drench in avocado cv. Hass.

In seaweed extracts (ProtecSea active ingredient), cytokinins and auxins predominate in quantity and activity driving cell division and differentiation of roots and stems of the plants, increasing root growth (Espinosa-Antón et al., 2020).

Likewise, *Trichoderma harzianum* can increase root development and synthesize growth hormones that generate greater root length (Sánchez, 2022).

Conclusion

With the preventive application of the products under nursery conditions, phenotypic evidence of possible systemic resistance induction in the plant is observed when evaluating parameters such as lesion advancement length, dry matter percentage (leaves and roots), and root length. Furthermore, these findings lay the groundwork for conducting molecular research to identify the genes responsible for plant defense.

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Authors contributions

NVJA: Conceptualization of the work, experimental design, and manuscript review, study support, and supervision.
LMAC: Thesis advisor for the university degree.
AVCD: Study supervision.
JMSH: Support in the conceptualization of the work.

Conflict of interest:

The signing authors of this research work declare that they have no potential conflict of personal or economic interest with other people or organizations that could unduly influence this manuscript.

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