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Evaluation of silver nanoparticles and hydrogen peroxide to counteract Huanglongbing (HLB) disease in citrus trees grown in Veracruz

Evaluación de nanopartículas de plata y peróxido de hidrógeno para contrarrestar la enfermedad Huanglongbing (HLB) en cítricos cultivados en Veracruz

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ABSTRACT

Huanglongbing (HLB), also known as "yellow dragon disease", is caused by the bacterium *Candidatus Liberibacter* and represents one of the greatest threats to global citrus production, causing significant economic losses. This study aimed to evaluate the use of silver nanoparticles (NpAg) as a control strategy for HLB in Citrus latifolia (Persian lime). Six experimental treatments were tested: (1) T1: control without treatment, (2) T2: hydrogen peroxide (H_2O_2), (3) T3: NpAg, (4) T4: NpAg + H_2O_2, (5) T5: NpAg + H_2O_2 + organic fertilization, and (6) T6: NpAg + H_2O_2 + chemical fertilization. Treatments T5 and T6 showed the best results, evidenced by the appearance of new shoots, a significant reduction in leaf yellowing, and the absence of thickening in the central leaf vein, which are characteristic symptoms of the disease. These results suggest that the combination of NpAg, H_2O_2, and fertilization has a high potential for HLB control. However, it is necessary to validate these treatments at a larger scale in commercial citrus crops. Furthermore, future studies should include molecular analyses to confirm the efficacy of the proposed approach in suppressing or eradicating *Candidatus Liberibacter*.

KEY WORDS: Silver nanoparticles, HLB, hydrogen peroxide, Persian lemon, and *Diaphorina citri.*

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Revista Bio ciencias
ISSN 2007-3380

RESUMEN

El Huanglongbing (HLB), conocido también como “enfermedad del dragón amarillo,” es causado por la bacteria *Candidatus Liberibacter* y representa una de las mayores amenazas para la citricultura mundial, provocando importantes pérdidas económicas. Este estudio tuvo como objetivo evaluar el uso de nanopartículas de plata (NpAg) como estrategia de control para el HLB en *Citrus latifolia* (limón persa). Se analizaron seis tratamientos experimentales: (1) T1: control sin tratamiento, (2) T2: peróxido de hidrógeno (H_2O_2), (3) T3: NpAg, (4) T4: NpAg + H_2O_2, (5) T5: NpAg + H_2O_2 + fertilización orgánica y (6) T6: NpAg + H_2O_2 + fertilización química. Los tratamientos T5 y T6 mostraron los mejores resultados, evidenciados por la aparición de nuevos brotes, una notable reducción del amarillamiento foliar y la ausencia de engrosamiento en la nervadura central, síntomas distintivos de la enfermedad. Estos hallazgos sugieren que la combinación de NpAg, H_2O_2 y fertilización presenta un alto potencial para el control del HLB. Sin embargo, es necesario validar estos tratamientos a mayor escala en cultivos comerciales de cítricos. Además, estudios futuros deberían incluir análisis moleculares que corroboren la eficacia del enfoque propuesto en la supresión o erradicación de *Candidatus Liberibacter*.

PALABRAS CLAVE: Nanopartículas de plata, HLB, peróxido de hidrógeno, limón persa y *Diaphorina citri*.

Introduction

Huanglongbing (HLB), also known as “citrus greening disease” (Noorizadeh *et al*., 2022), is one of the most significant threats to global citrus production. This disease, caused by the bacterium *Candidatus Liberibacter asiaticus* and transmitted by the psyllid vector *Diaphorina citri* (Boina & Bloomquist, 2015; Pérez-González *et al*., 2022; Ladaniya, 2023), poses a major challenge to citrus cultivation due to the severe symptoms it induces. Once a plant is infected, the disease spreads heterogeneously through the phloem, affecting the central leaf veins of the leaves, roots, flowers, and fruits (Robles-González *et al*., 2013). Young leaves exhibit pale green blotches, chlorosis with thickened green leaf veins, while roots show signs of rot, and the canopy undergoes significant reduction. In fruits, HLB causes premature drop, persistent green coloration at the base, a bitter taste, and deformities, including aborted seeds and reduced size. Additionally, flowering tends to occur prematurely and is accompanied by yellowing (Thakuria *et al*., 2023).

In Mexico, which accounts for 14 % of global citrus production, this disease has a considerable impact in regions such as Veracruz, one of the country’s primary citrus-producing

Revista Bio ciencias ISSN 2007-3380

states. The presence of HLB in this area is significantly affecting both production and the regional economy (Hernández-Landa *et al*., 2017). In response to this situation, citrus growers have implemented various management strategies, including the application of supplemental fertilizers, foliar sprays enriched with micronutrients, and chemical treatments to induce immune responses in plants. However, these approaches have shown limited effectiveness, prompting the search for new alternatives to control the disease (Ghosh *et al*. 2022).

In this context, silver nanoparticles (AgNPs) have emerged as a promising solution due to their exceptional physical, chemical, and biological properties, as well as their well-documented antimicrobial activity (Zhang *et al*., 2016). These nanoparticles have been extensively studied for their bactericidal and fungicidal applications, particularly in biotechnology (Khaydarov *et al*., 2009). On the other hand, hydrogen peroxide (H_2O_2) has also been recognized as a key compound in plants, functioning as a signaling molecule, stress mitigator, and cell wall reinforcement agent (Gill & Tuteja, 2010; Ahmad, 2014). Moreover, H_2O_2 interacts with plant growth regulators such as auxins, gibberellins, and brassinosteroids, playing a crucial role in plant responses to environmental stress (Liheng *et al.,* 2009; Mittler *et al*., 2011; Dietz *et al*., 2016; Nazir *et al*., 2020).

This study proposes the mixed use of silver nanoparticles and hydrogen peroxide as an innovative strategy to combat HLB in citrus crops, specifically in Persian lime (*Citrus x latifolia*) cultivated in Veracruz. The research aims to assess the effectiveness of these tools in mitigating the impact of the disease, ultimately contributing to the development of a viable alternative for the eradication of *Candidatus Liberibacter asiaticus* and the protection of Mexican citrus production.

Material and Methods

Study Area

The study was conducted in the experimental zone of the Universidad Tecnológica de Gutiérrez Zamora, known as “El Cocotero,” located at geographical coordinates 20.48° N and 97.09° W (Figure 1). This region is characterized by a warm and humid climate with well-defined seasonal variations. During the rainy season, the weather is predominantly hot and mostly cloudy, whereas in the dry season, it remains warm, humid, and partially cloudy. The annual temperature ranges from 17 °C to 32 °C, with extreme values rarely dropping below 14 °C or exceeding 35 °C. Additionally, the region records an average annual precipitation of 280 millimeters (INEGI, 2010).

Applied Treatments

The treatments evaluated in Persian lime (*Citrus x latifolia*) plants infected with HLB were distributed into six experimental groups, described as follows: (1) T1: well water as a negative control; (2) T2: hydrogen peroxide (H_2O_2) at 0.3 % (v/v); (3) T3: silver nanoparticles (AgNPs) at a concentration of 0.0685 ppm; (4) T4: a combination of H_2O_2 at 0.3 % and AgNPs at 0.0685 ppm; (5) T5: a combination of H_2O_2 at 0.3 %, AgNPs at 0.0685 ppm, and organic fertilization (57 mL of compost tea); and (6) T6: a combination of H_2O_2 at 0.3 %, AgNPs at 0.0685 ppm, and chemical fertilization (80 g of granular fertilizer with an N-P-K ratio of 17-17-17) (Figure 2a).

Revista Bio ciencias
ISSN 2007-3380

The experimental methodology, along with the application conditions and treatment frequency, is detailed in Table 1. The treatments were designed to evaluate both the individual and combined effects of the applied compounds, considering their antimicrobial action and their potential to enhance the physiological response of plants to HLB infection.

Figure 1. Geographic location of Gutiérrez Zamora, Veracruz, site of the experimental trial in lemon plants (*Citrus x latifolia*) affected by HLB.

Treatment Application in Plants

Each plant received the corresponding treatment via two application methods: root drenching and foliar spraying. A volume of 50 mL of the treatment solution was applied to the root zone to ensure absorption through the root system, while 116 mL was uniformly sprayed onto the foliage and stem, ensuring adequate coverage of the plant surface (Figure 2b). This methodology enabled the assessment of the differential efficacy of the treatments in mitigating the impact of HLB on *Citrus x latifolia*.

Revista Bio ciencias
ISSN 2007-3380

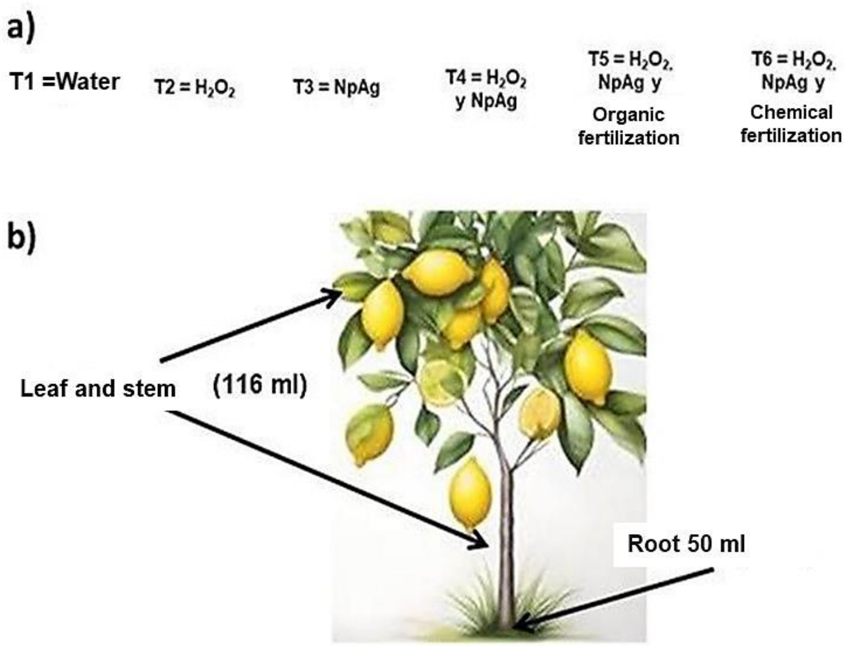

Figure 2. a) Treatments applied to lemon plants. b) Areas of application of treatments on citrus plants.

Synthesis of Silver Nanoparticles

The synthesis of AgNPs was carried out following the protocol described by Frank *et al*. (2010), using ascorbic acid as a reducing agent, citric acid as a stabilizing agent, and silver nitrate ($AgNO_3$) as the precursor of silver ions (Ag^+). The synthesis process was conducted under controlled temperature and agitation conditions to ensure the efficient reduction of Ag^+ and the stabilization of the formed nanoparticles.

Presumptive Test for the Presence of HLB

The presumptive detection of HLB was performed using the methodology proposed by Takushi *et al*. (2007), based on iodine as a reagent. This qualitative test enables the preliminary identification of the disease by assessing the reaction between the starch in the leaves and the iodine solution, producing a characteristic brown coloration in the leaf veins. The presence of this coloration is considered a positive indicator of infection by *Candidatus Liberibacter* spp., allowing the selection of potentially affected plants for further confirmatory analysis using molecular techniques.

Application Frequency

The application of each treatment was carried out as specified in Table 1. The treatment schedule was designed to eliminate the presence of Huanglongbing (HLB) in the plants during the initial treatment stages. Subsequent applications, performed every 15 days, aimed to strengthen the plant's nutritional status and contribute to the complete eradication of *Candidatus Liberibacter* spp.

Plant development and response were monitored through a systematic photographic record (Nikon camera). These images were used to evaluate and document physiological and morphological changes, including modifications in leaf vein structure, variations in leaf coloration, and the emergence of new shoots, providing a detailed visual analysis of the impact of the applied treatments.

Table 1. Development of the treatments applied to the plants

Week	Applications	Days
1	2	Wednesday and Friday
2	2	Wednesday and Friday
3	1	Friday
4	1	Friday
5	0	Without treatment
6	1	Friday
7	0	Without treatment
8	1	Friday

New shoot number

Was quantified through direct counting on each plant. Monitoring was conducted weekly over a period of eight weeks, recording the total number of emerging shoots in each treatment. To assess leaf greenness intensity, an SPAD (Soil Plant Analysis Development) meter was used, which determines the percentage of green color through both visual and digital leaf analysis. This device measures light absorbance at specific wavelengths, which is directly correlated with chlorophyll content in the leaf tissue (Uddling *et al*., 2007).

Additionally, plant height was measured using a tape measure, determining the distance from the base of the stem to the tip of the tallest shoot. These measurements were recorded after experiment's conclusion to assess the treatments' impact on plant growth (Habibullah *et al*., 2019).

Revista Bio ciencias ISSN 2007-3380

Chlorophyll content

In the leaves was determined through an acetone extraction method (80 %), following the protocol described by Arnon (1949). To this end, 0.5 g of fresh leaf tissue was weighed and macerated in acetone to facilitate the release of photosynthetic pigments. The resulting solution was then centrifuged at 10,000 rpm for 10 minutes to separate cellular components. Chlorophyll quantification was performed through spectrophotometry, measuring absorbance at 645 and 663 nm, which allowed for the calculation of total chlorophyll, chlorophyll a, and chlorophyll b concentrations based on the established equations for this method.

Statistical analysis

The data obtained were processed using SPSS statistical software, version 26. An analysis of variance (ANOVA) was performed to determine the presence of significant differences between the evaluated treatments. Subsequently, Tukey's multiple comparison test was applied with a 5 % significance level ($p < 0.05$) to identify statistical differences between experimental groups.

Results and Discussion

In the lemon seedlings assigned to the control treatment (T1), which received only tap water, an improvement in leaf color was observed. However, no reduction in leaf vein thickening or early induction of new shoots was evident (Figure 3). Additionally, one of the plants exhibited defoliation attributed to damage caused by chewing insects. The emergence of new shoots was recorded only at the end of the experiment, after 35 days.

Treatment 2, based on the application of hydrogen peroxide (H_2O_2), significantly promoted the new shoots induction. On average, each plant developed three shoots within the first two weeks after application. In addition, an improvement in foliar coloration was also observed, indicating a possible optimization of photosynthetic photosynthetic activity and physiological state of the plants, as showed in Figure 4. illustrated in Figure 4.

Revista Bio ciencias
ISSN 2007-3380

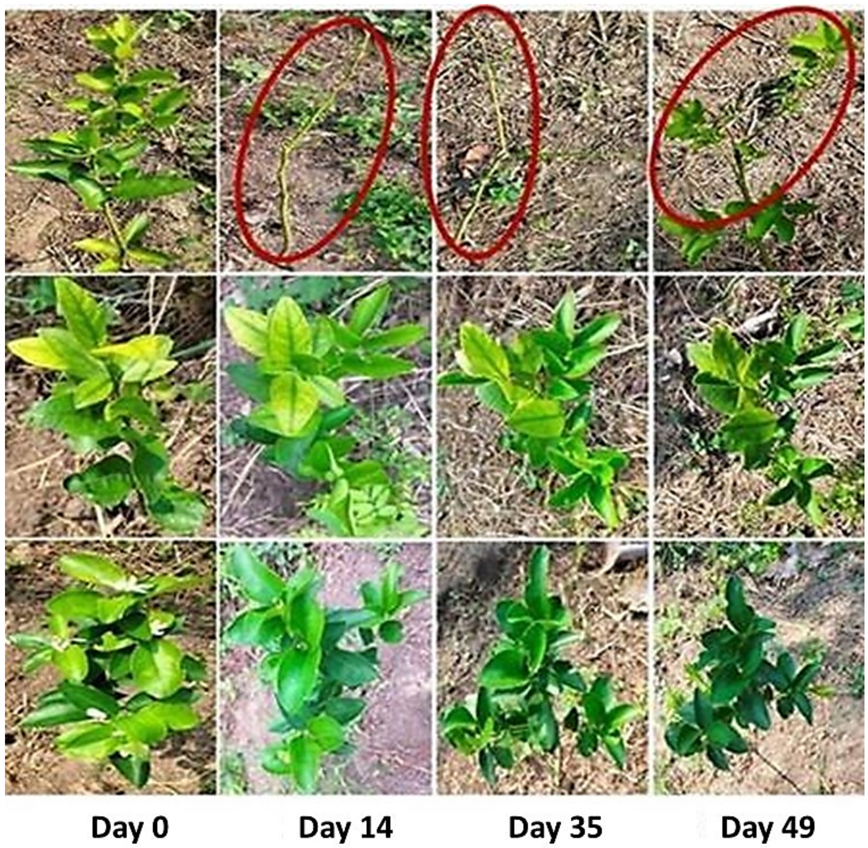

Figure 3. Development of T1 (tap water) plants throughout the study. Day 0, plants are one month old and approximately 40 cm in size. The red ovals mark the defoliated area and the time at which the new foliage appeared.

Revista Bio ciencias ISSN 2007-3380

Figure 4. Lemon seedlings of treatment 2. The ovals indicate the improvement in leaf coloration, as well as a decrease in the ribbing.

The treatment with NpAg (T3) significantly promoted the induction of new shoots, with an average of three shoots per plant recorded between the first and second week following application (Figure 5). A case of defoliation was observed on day 13 in one of the treated plants; however, this plant showed accelerated recovery with the emergence of new shoots within a week. In contrast, in the control group, a plant also exhibited defoliation but without showing comparable recovery within the same period, suggesting a positive effect of NpAg on plant tissue regeneration.

Revista Bio ciencias
ISSN 2007-3380

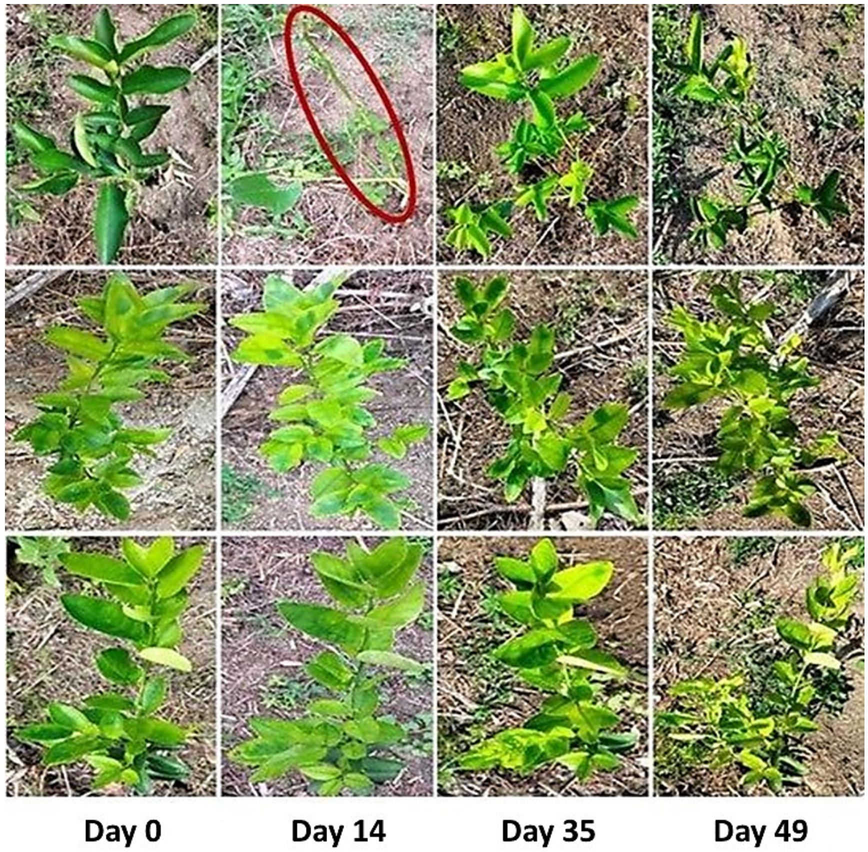

Figure 5. Lemon plants of T3.

The red oval marks the defoliated zone of a plant, which showed recovery in less time than that of T1.

Treatment 4 (T4), which combined the application of NpAg with H_2O_2, induced favorable physiological effects in the plants, evidenced by the appearance of new shoots starting from the fourth week. During the first three weeks, a progressive reduction in leaf vein thickening and an improvement in leaf coloration were observed, suggesting structural and functional recovery of the plant tissue (Figure 6). Although one of the treated plants exhibited defoliation, its regenerative capacity was notably superior to that of the control group, where no comparable recovery was recorded. Additionally, Figure 6 shows that all three plants subjected to this treatment developed homogeneous foliage, indicating a uniform and effective response to the applied protocol.

Revista Bio ciencias
ISSN 2007-3380

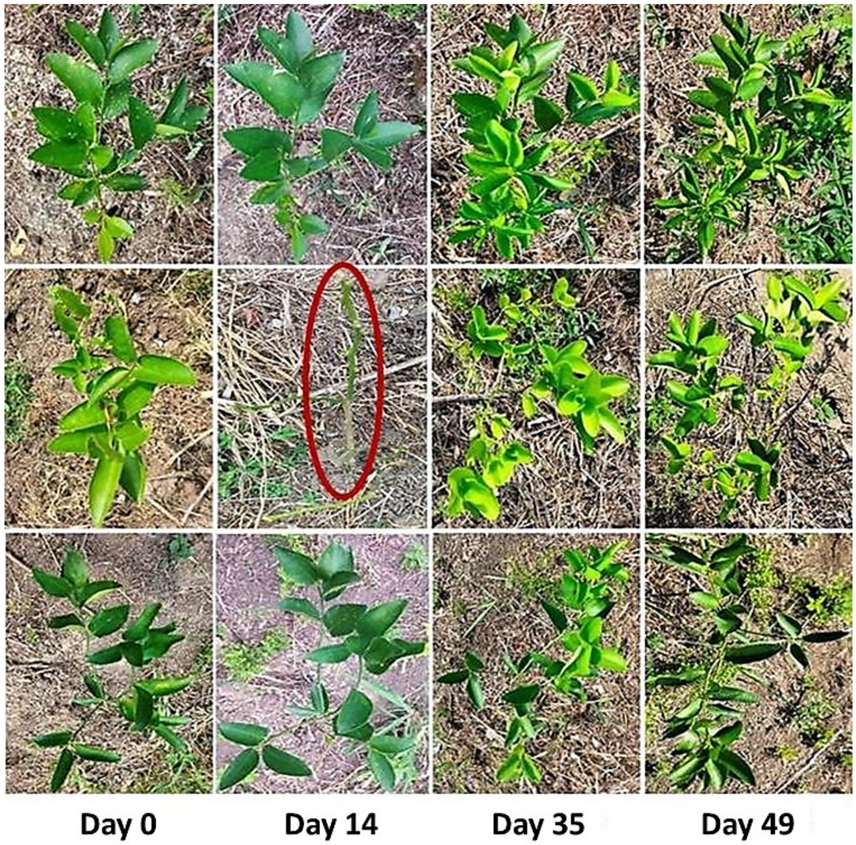

Figure 6. Lemon seedlings of T4. The red oval marks the defoliated zone of a plant, which showed recovery in less time than that of T1.

Treatment 5 (T5), based on the combined application of NpAg, H_2O_2, and organic fertilization with compost infusion, demonstrated a favorable physiological response in the treated plants, significantly accelerating the appearance of new shoots. Plant regeneration was evident within 10 days following fertilization, even in those plants that had suffered mechanical damage, suggesting an interactive effect among the treatment components. These results highlight the potential of combining NpAg, H_2O_2, and organic fertilization to enhance vigor and recovery in affected plants. Figure 7 shows the positive response of the plants to this treatment, confirming the effectiveness of the applied strategy.

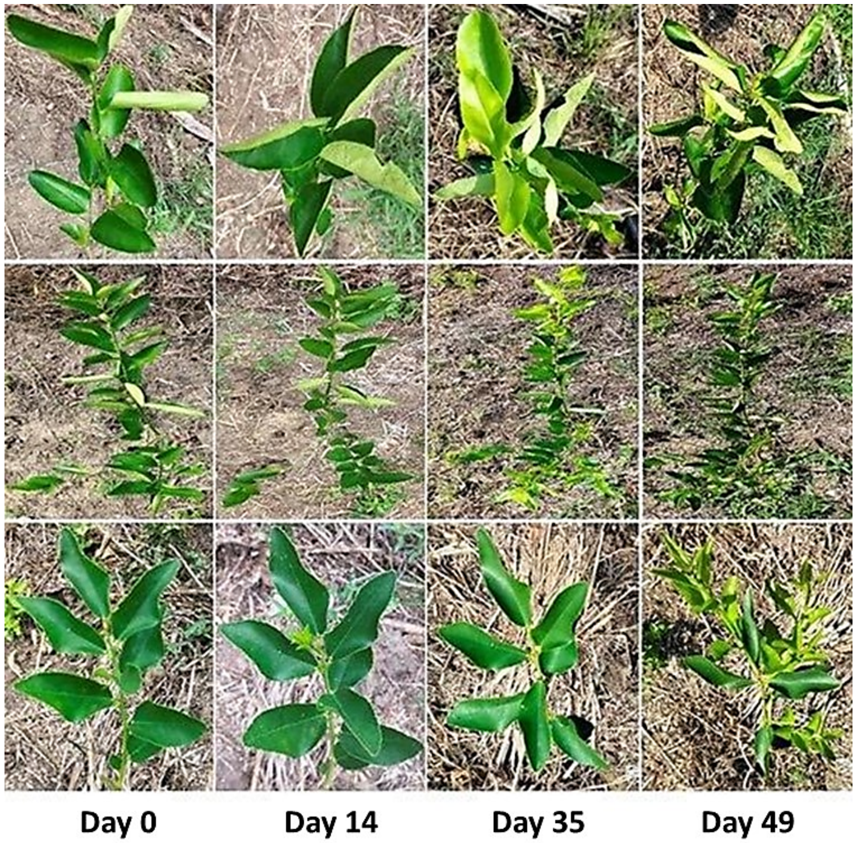

Figure 7. Lemon seedlings with T5.

Treatment 6 (T6), which involved the combined application of NpAg, H_2O_2 and granular chemical fertilization (comprising both fast-release, easily dissolved and slow-release types that have coatings regulating nutrient availability and reducing losses due to leaching (González *et al*., 2005), induced a positive physiological response in the treated plants. A significant increase in the generation of new shoots was observed, accompanied by a progressive reduction in leaf vein thickening and an intensification of leaf coloration, suggesting a more efficient recovery compared to the other treatments evaluated (Figure 8). These results indicate that the synergy between the applied compounds contributes to the restoration of plant functionality and the structural strengthening of the plants. The figure illustrates the formulation of the treatment and its effects on plant growth and vigor, highlighting its potential as a strategy for the recovery of affected citrus plants.

Revista Bio ciencias ISSN 2007-3380

Figure 8. Lemon seedlings with treatment 6 (T6). The treatment consisted of a mixture of NpAg + H_2O_2 + granular chemical fertilization (0.3 %). The red oval marks the defoliated zone of a plant.

Presumptive Tests for HLB Detection

To determine the presence of HLB in the plants, a presumptive iodine test was performed, a qualitative assay based on the detection of brown coloration in the leaf veins, indicating starch accumulation in response to infection. This test, with an accuracy of 80 % (Takushi *et al*., 2007), allowed for the evaluation of the presence or absence of *Candidatus Liberibacter* spp. at both the beginning and end of the experiment (Figure 9).

At the start of the study, all plants from the different treatments showed positive results for the presence of HLB. However, after 60 days of evaluation, variations in the results were observed, suggesting a differential impact of the applied treatments. In particular, the combination

Revista Bio ciencias
ISSN 2007-3380

of NpAg with H_2O_2 and fertilization showed a significant reduction in test positivity, indicating a potentially inhibitory effect on the bacteria. The progressive decrease in positive results by the end of the study highlights the efficacy of these strategies in mitigating the disease, positioning them as a promising alternative for managing HLB in citrus crops.

The results of the presumptive iodine test for HLB detection were as follows (Table 1):

Table 2. Obtained results from the different treatments

Treatment	Description	Plants Positive at the Beginning	Positive Plants at the End
T1 (Control)	No treatment	3/3	3/3
T2 (H_2O_2)	Hydrogen peroxide	3/3	2/3
T3 (NpAg)	Silver nanoparticles	2/3	1/3
T4 (NpAg + H_2O_2)	Silver nanoparticles + Hydrogen peroxide	3/3	2/3
T5 (NpAg + H_2O_2 + Fert. Orgánica)	Silver nanoparticles + Hydrogen Peroxide + Organic Fertilization	2/3	1/3
T6 (NpAg + H_2O_2 + Chemistry Fert.)	Silver Nanoparticles + Hydrogen Peroxide + Chemical Fertilization	3/3	0/3

These results suggest that the combination of NpAg, H_2O_2, and appropriate fertilization has a positive impact on reducing the incidence of HLB, highlighting its potential as a management strategy for this disease in citrus crops.

To eliminate HLB, various strategies have been evaluated to mitigate its impact on citrus crops. Among the most explored options is the removal of infected trees, a method that, while reducing the annual progression rate of the disease, lacks widespread acceptance due to the associated costs and the adverse ecological effects it generates (Bassanezi *et al*., 2013). Another line of research focuses on the use of activators and antibiotics to inhibit disease development, achieving a reduction in HLB incidence of up to 30 % and 36.6 %, respectively (Hu *et al*., 2018). However, the continuous use of antibiotics carries the risk of generating bacterial resistance, which could compromise the long-term efficacy of these treatments (Zhang *et al*., 2014). This issue, along with the potential adverse effects on the productivity of treated plants, underscores the need to explore alternatives that are less likely to produce undesirable side effects.

This study proposes an innovative strategy based on the combined application of NpAg, H_2O_2, and chemical fertilization, aimed at effectively controlling HLB. The results indicate that plants treated with this combination showed significant improvements in their physiology,

Revista Bio ciencias
ISSN 2007-3380

characterized by superior leaf development and the absence of HLB in the presumptive tests at the end of the experiment. Compared to previous studies, such as those by Hu *et al*. (2018), where the application of antibiotics and activators reduced disease incidence, the combined treatment proposed in this work showed even more favorable results, with all three evaluated plants testing negative in the presumptive HLB test. This suggests that the combination of NpAg and H_2O_2 could offer a more sustainable and effective alternative for disease control in citrus crops.

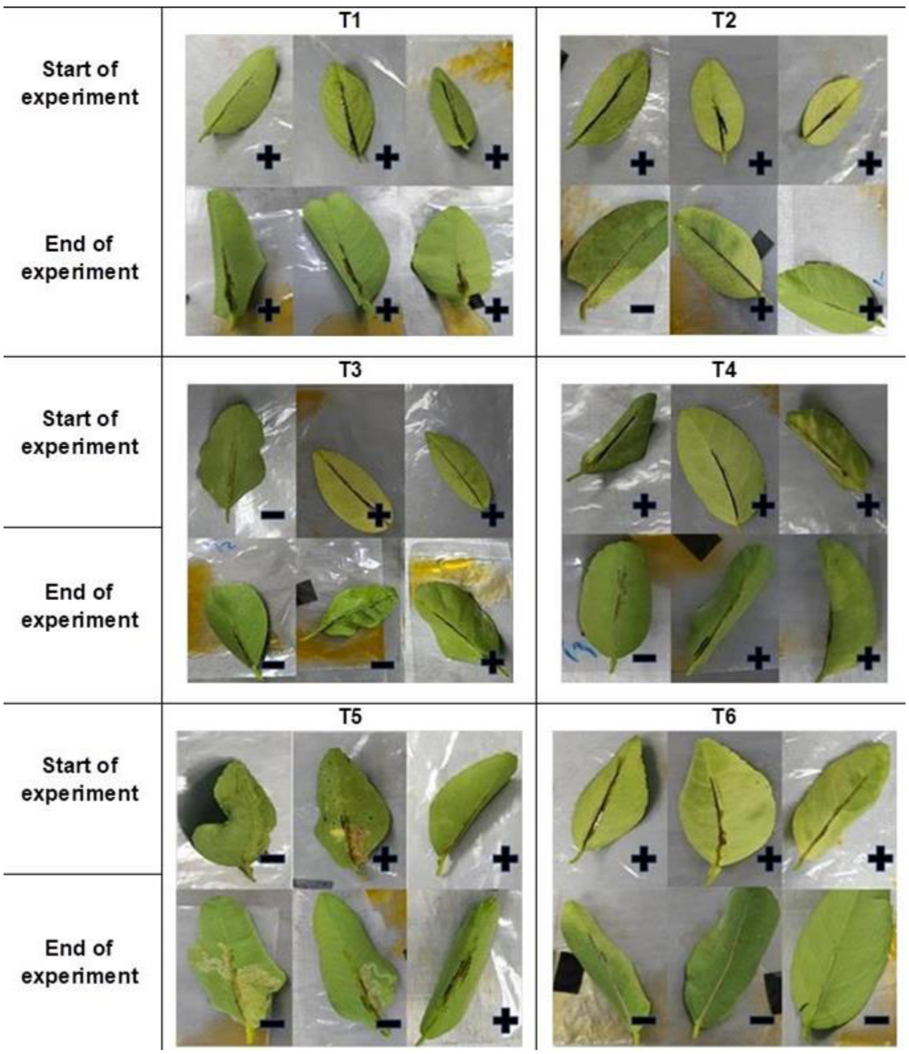

Figure 9. Presumptive test for HLB by iodine test on lemon plants grown for 49 days. Brown coloration on leaf veins, positive test (+), and absence considered negative (-). The experiment was performed in triplicate on each of the treated plants.

The presumptive iodine test, used as a preliminary tool for HLB detection, showed 80 % effectiveness, positioning it as a valuable resource for the early identification of the disease (Etxeberria *et al*., 2008). In this study, the test was successfully used to monitor the response of the treated plants, confirming the efficacy of the proposed combined treatment. The definitive confirmation of the absence of HLB in the treated plants was obtained through PCR testing, which further strengthened the reliability of the results.

The proposed mechanism of action in this study suggests that hydrogen peroxide, when applied at low concentrations and following an accumulation phase, compromises bacterial membrane integrity (Wang *et al*., 2023), thereby facilitating the entry of NpAg. These nanoparticles, due to their small size and high surface-to-volume ratio, can penetrate cellular membranes and exert their bactericidal action. NpAg inhibits enzymatic activity critical for bacterial survival, leading to cell death (Rai *et al*., 2014). Additionally, H_2O_2 could accelerate the oxidation of starch accumulated in the plant's xylem system, improving sap and nutrient transport, which would contribute to the recovery of plants affected by HLB.

The treatment with NpAg and H_2O_2 also demonstrated beneficial effects at the physiological level. As observed in this study, the treated plants began to develop new shoots, which can be attributed to the activating effect of H_2O_2 on the plant's physiological functions. Although H_2O_2 does not me*et al*l the criteria to be considered a phytohormone in the strict sense, its ability to induce changes in plant development is well known, suggesting that its application may have contributed significantly to the improvement of the plants' overall condition.

The bactericidal action of NpAg is attributed to their ability to alter key processes within bacterial cells. The nanoparticles interfere with membrane permeability, inhibit cellular respiration, and may react with important cellular components, such as proteins and DNA, disrupting cellular function and leading to bacterial cell death (Pal *et al*., 2007; Liao *et al*., 2019). This mechanism makes NpAg a highly effective tool for controlling pathogenic bacteria such as *Candidatus Liberibacter*, the causal agent of HLB.

Through this study, it has been demonstrated that the combined treatment of NpAg, H_2O_2, and chemical fertilization constitutes an effective and promising strategy for HLB control. The treated plants not only showed improvements in physical development but also exhibited a significant decrease in bacterial load, highlighting the importance of an integrated strategy combining bacterial control and nutritional management. These findings offer a positive outlook for the future management of HLB in citrus crops, providing producers with a viable and potentially more sustainable alternative to combat the disease.

This approach not only targets the elimination of the bacteria causing HLB but also optimizes the physiological conditions of affected plants, contributing to overall recovery. However, further studies are recommended to explore the long-term effects of the treatment and to analyze in detail the impact on the root system and vascular tissues, such as the xylem and phloem.

Statistical Analysis

Table 3 presents the values obtained for the evaluated variables, which reveal significant differences between the applied treatments. Regarding the number of shoots, it was observed that all treatments differed significantly from each other, with treatment T6 showing the highest number of shoots. This finding suggests that treatment T6 favors greater vegetative regeneration, indicative of a more efficient recovery of plants affected by HLB.

Concerning the percentage of green color, treatment T6 again stood out, showing significant differences compared to the other treatments. This result indicates that T6 not only stimulates the growth of new shoots but also improves the overall health of the plant by promoting a higher intensity of green color in the leaves, reflecting an improvement in photosynthetic activity and the integrity of plant cells.

Regarding plant size, treatments T2, T3, T4, and T6 did not present significant differences among them. However, treatment T5 showed the highest value in this variable. This suggests that, although T6 is highly effective in terms of shoot number and chlorophyll content, treatment T5 has a notable impact on overall plant growth, possibly related to better nutrient utilization and an increase in the biomass of the treated plants.

As for chlorophyll content, treatments T5 and T6 showed the highest values, with no significant differences between them, but with significantly higher values compared to the other treatments. This indicates that both T5 and T6 promote better photosynthesis and greater chlorophyll accumulation in the leaves, which is indicative of an improvement in the overall health of the plants.

Table 3. Statistical analysis of the variables analyzed

T	NB	CV	Size	Chlorophyll
		%	cm	µg/ml
T1	2.50 ± 0.71^{a}	36.00 ± 1.41 ^{a}	72.50 ± 10.61 ^{a}	12.08 ± 0.11 ^{a}
T2	4.33 ± 0.58 ^{a}	51.33 ± 3.21 ^{b}	78.33 ± 16.07 ^{ab}	16.11 ± 0.28 ^{b}
T3	4.33 ± 0.58 ^{a}	50.33 ± 3.06 ^{b}	91.67 ± 2.89 ^{ab}	16.27 ± 0.38 ^{b}
T4	7.67 ± 0.58 ^{b}	52.50 ± 2.12 ^{bc}	110.00 ± 14.14 ^{ab}	18.15 ± 0.21 ^{c}
T5	11.00 ± 1.00 c	64.00 ± 1.41 ^{cd}	125.00 ± 7.07 ^{c}	22.25 ± 0.35 ^{d}
T6	13.33 ± 0.58 ^{d}	63.00 ± 5.57 ^{d}	98.33 ± 2.89 ^{abc}	23.25 ± 0.35 ^{d}

T = Treatment, NB = Number of shoots, CV = Green color percentage

The results suggest that treatments combining silver nanoparticles, hydrogen peroxide, and chemical fertilization are significantly more effective in the recovery and improvement of Persian lime plants affected by HLB. These treatments not only contribute to better leaf regeneration but also optimize overall plant growth and health, representing a promising alternative for the management of this disease in citrus crops.

Treatments T5 and T6, which exhibited a higher number of new shoots (11.00 ± 1.00 and 13.33 ± 0.58, respectively), suggest that NpAg and H_2O_2 may have reduced bacterial load by inhibiting the enzymatic activity of the bacteria, while simultaneously improving nutrient transport in the plants (Chen & Schluesener, 2008; Nazir *et al*., 2020). Fertilization, especially in treatments T5 and T6, provided essential nutrients that enabled the plants to overcome stress caused by infection, promoting the regeneration of damaged tissue and the production of new shoots.

Leaf yellowing, a characteristic symptom of HLB, results from a disruption in chlorophyll metabolism and nutrient mobility within the plant. Treatments T5 and T6, which showed the highest green color percentages (64.00 ± 1.41 and 63.00 ± 5.57), indicate that H_2O_2, in addition to its bactericidal properties, acts as a signaling molecule that enhances the plant's resistance to pathogens by strengthening cell walls and promoting the repair of damaged tissue (Gill & Tuteja, 2010). The reduction of blockage in the vascular vessels allows for greater circulation of water and nutrients, contributing to the recovery of leaf pigmentation and the restoration of photosynthetic activity in the plants.

Regarding overall plant growth, HLB reduces the plants' ability to transport carbohydrates and other essential nutrients, negatively impacting their development. Treatments T5 and T6, which showed the highest plant sizes (125.00 ± 7.07 and 98.33 ± 2.89 cm), reflect that NpAg improves nutrient availability to the vascular system, reducing bacterial damage in the conducting vessels and facilitating the efficient transport of water and nutrients (Ul Islam *et al*., 2023). The combination with fertilization, especially the chemical fertilization in treatment T6, provides the macro and micronutrients necessary for plant recovery, favoring growth and overall strengthening.

HLB also interferes with chlorophyll production, an essential component for the photosynthesis process. Treatments T5 and T6 showed significantly higher chlorophyll content (23.25 ± 0.35 μg/ml and 22.25 ± 0.35 μg/ml, respectively), indicating an improvement in the photosynthetic function of the treated plants. Both H_2O_2 and NpAg promote the regeneration of photosynthetically active tissue, contributing to the reduction of visible disease symptoms (Gill & Tuteja, 2010).

Treatment T6, which combines silver nanoparticles, H_2O_2, and chemical fertilization, is especially effective against the effects of HLB due to its ability to address multiple aspects of the pathological process. First, NpAg exerts a direct action on bacterial load, inhibiting the proliferation of *Candidatus Liberibacter* within the vascular vessels. On the other hand, H_2O_2 acts as a chemical signal that triggers defense responses in the plant, stimulating the production of protective compounds and improving vascular transport. This synergy between the treatment components not only reduces bacterial load but also promotes the restoration of vascular function and the recovery of the plant at both the structural and physiological levels.

Conclusions

Hydrogen peroxide positively impacted treated plants, significantly enhancing leaf coloration, mitigating central leaf vein thickening, and promoting the emergence of new shoots with a healthier appearance.

The combination of AgNPs and H_2O_2 exhibited a synergistic effect in controlling HLB, accelerating improvements in leaf coloration, promoting the emergence of new shoots, and reducing central leaf vein thickening in a shorter time frame.

Treatments integrating AgNPs and H_2O_2, supplemented with either organic or chemical fertilization, proved to be more effective in HLB management. These treatments facilitated a rapid plant recovery, yielding remarkable results such as early shoot emergence, improved leaf pigmentation, and a noticeable reduction in disease symptoms.

The combination of bactericidal treatments with proper nutrient management, both organic and chemical, substantially enhances the overall health of lemon plants, standing out as a promising strategy for HLB management in citrus cultivation.

Authors' Contributions

"Conceptualization of the work, Author 1 and Author 4; Methodology development, Author 1 and 3; Experimental validation, Author 2 and Author 3; Results analysis, Author 1 and Author 4.; Data management, Author 1; Writing and manuscript, Author 1, Author 2, Author 3, and Author 4.; Writing, reviewing and editing, Author 1, Author 2, Author 3, and Author 4.

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Conflicts of Interest

The authors declare no conflict of interest.

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