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# Hrp proteins as bioinducers for the biocontrol of bacterial diseases in tomato and pepper plants in greenhouse

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## ABSTRACT

**Background/Objective.** Diseases such as bacterial spot in tomato (*Solanum lycopersicum*) and bacterial spot in chili pepper (*Capsicum annuum*) cause significant global economic losses. A sustainable alternative for their control is the use of protein inducers (Harpin proteins = Hrp) that activate plant defense responses by being recognized by the plant immune system, inducing defense mechanisms against pathogens. The objective of this research was to evaluate the biological effectiveness and optimal application dose of the biological inducer BioFensa (based on Hrp proteins), produced in a pilot plant, to control these diseases.

**Materials and Methods.** Three greenhouse experiments were conducted to evaluate the biological effectiveness of BioFensa (1 µg $mL^{-1}$). The protein inducer was tested for controlling bacterial spot (*X. euvesicatoria* strain BV865 [1] and BV801 [2]), as well as bacterial speck (*P. syringae* pv. *tomato*, strain DC3000 [3]). Each experiment included 5 treatments and 11 replicates. Additionally, an experiment was conducted to determine the optimal dose of BioFensa (0.01, 0.1, and 1.0 µg $mL^{-1}$) against *X. euvesicatoria* strain BV801, with 7 treatments and 8 replicates [4]. In the four experiments in total, plants were sprayed with BioFensa (3 mL per plant) 24 hours before infection, and symptoms were evaluated after 30 days by counting spots on the foliar tissue.

**Results.** BioFensa was effective in significantly reducing damage in chili and tomato plants (LSD, $p \leq 0.05$). At a high concentration (1 µg $mL^{-1}$), it prevented the

appearance of spots on tomato plants by 53%, while for chili plants against strain BV865, it prevented spots by 60%. On the other hand, for chili plants against strain BV801, at low concentrations (0.01 and 0.1 μg $mL^{-1}$), symptoms were significantly reduced by 38-41%, whereas at a higher concentration (1 μg $mL^{-1}$), this effect was not maintained, suggesting a limit in the perception of inducers by the plants.

**Conclusion.** The results suggest that BioFensa has the potential to be an effective alternative to control diseases in horticultural crops such as tomatoes and chili peppers.

**Keywords.** Biological control, resistance inducer, bacterial spot, plant defense system, bacterial speck.

## Introduction

Agricultural crops are vulnerable to diverse phytopathogen attacks, which lead to large reductions in yield and productivity. Once the pathogen establishes in the cultivated areas, it is difficult to eradicate and preventive measures are implemented to prevent pathogens from spreading (Catara and Bella, 2020). The use of bactericides and antibiotics with chemical formulations containing copper commonly are inefficient due to the accumulation of chemical products in the environment and resistance, making these alternatives scarcely profitable, polluting for soils and water, as well as inefficient. This causes significant losses in economically valuable crops, leading to negative impacts on agriculture, the economy and food security (Khan and Ahma, 2024). Among these pathogens are the bacteria *Pseudomonas syringae* and *Xanthomonas euvesicatoria*, both of which are Gram negative and include diverse pathovars with the ability to infect a characteristic group of host plants, being able to overcome many of the host's defenses in different stages of the infection, multiply and cause diseases, mainly due to having a type III secretion system, which is highly relevant in terms of virulence (Cardoso *et al*., 2020). These phytopathogens affect crops such as tomato (*Solanum lycopersicum*) and chili peppers (*Capsicum annuum*), displaying symptoms in leaves, fruits and stems in the form of chlorotic halos (yellow) and/or necrotic spots, as well as causing the premature loss of leaves, affecting the growth and development of the plant, and even the reduction in photosynthesis (Fang and Yang, 2013; Cardoso *et al*., 2020). *P. syringae* includes approximately 60 pathovars with a variety of hosts; however, it affects the tomato (*P. syringae* pv. *tomato*) and is known for causing the speck disease or bacterial smut (Fang and Yang, 2013). On the other hand, *Xanthomonas* cause numerous diseases in over 400 host plant species, being dominant pathogens

of the tomato and chili crops around the world due to the leaf spot or bacterial spot disease (Larrahondo *et al*., 2022; Cardonso *et al*., 2020). Both bacterial species share the type of colonization, so they initially grow epiphytically, colonizing the surface of healthy plants before entering the tissues of the leaves through stomatal or lesions, and once inside the apoplast (endophytic stage), they suppress the basic defense responses of the host by introducing virulence proteins or effectors directly in the plant cells (Xin *et al*., 2018). Many of these effector proteins inhibit the immune pathways of the host, while others modify cell signaling and physiology in different ways, including the suppression of the expression of immunity genes, making the plant tissue more susceptible to the growth of the phytopathogen and favoring the growth of diseases (Raffeiner *et al*., 2022; Zhang *et al*., 2022). However, the elicitors or inducers can activate defensive and adaptive responses in plants when administered in reduced amounts, stimulating the production of secondary metabolites, allowing plants to develop tole minimal changes in their physical structure (Caicedo *et al*., 2021). These biologically derived compounds promote plant health, which can enhance plant development, growth and production. This is beneficial for agriculture in both the pre- and pos-harvest phases. In some cases, they can confer resistance to new generations of plants (Todd *et al.,* 2022, 2023). Therefore, the use of protein inducers for the control of diseases in agricultural crops are an alternative to implement a green strategy, managing to reduce the dangers related to chemical pesticides (Khan and Ahma, 2024).

Harpin proteins (Hrp), according to Liu *et al*. (2020) and Joshi *et al.* (2022), are codified by the *hrp* (hipersensibility and pathogenicity response) genes of Gram-negative bacteria released through the type III secretion system during interactions between pathogens and plants. These proteins have various properties in common (rich in glycine, cysteine-free, they are acidic and thermostable) and are classified into five groups, depending on the similarity between proteins and the domain structures (group HrpN, HrpZ1, HrpW1, Hpa1). Given the importance of Hrp proteins in the response of plants to bacterial infections, a method has been developed to induce, isolate and select these proteins from phytopathological bacteria. Valerio-Landa *et al.* (2021a) proved that the use of these proteins can reduce the formation of necrotic lesions in the treated foliar tissue in tomato plants. Valerio-Landa (2021) and Valerio-Landa *et al.* (2021b) advanced in this line of investigation, developing a specific method to induce, isolate and select Hrp proteins from *Xanthomonas*, using lyophilized tissue from host plants as the only source of carbon and nutrients, according to patent application MX/a/2020/013638 (Valerio-Landa *et al.,* 2020). Due to this, the aim of this work was to evaluate the biological effectiveness of Hrp protein inducers of *X. euvesicatoria* (strain BV801) produced in a pilot plant (solid fermentation), for the control of the bacterial speck in tomato plants and bacterial spot in chili pepper plants under controlled greenhouse conditions. Applying these

inducers may offer a practical solution for the management of bacterial diseases in agricultural crops, making the most of the unique properties of Hrp proteins.

## Materials and Methods

### Establishing the experiment

**Plant material.** We placed 166 Ancho chili pepper plants (*C. annuum*) of the San Luis variety (Pacific Seed Company©) and 55 tomato plants (*S. lycopersicum*) of the Rio Grande variety (CIIASA Seeds©) in 2 L pots with a mixture of substrate (55% sand, 40% soil and 5% agrolite) previously sterilized in an autoclave (120 °C / 103421.4 Pa / 6 h) and kept under greenhouse conditions.

**Microbiological material.** The phytopathogenic bacteria BV801 and BV865, isolated simultaneously from the diseased tissue of chili pepper plants affected by bacterial spot (López-Vielma *et al.,* 2016) and determined as the cause of the disease (Rico-Aguilar *et al.,* 2019) were obtained from the collection of microorganisms of the Phytopathology Laboratory of the Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. (CIATEJ). The bacteria were reactivated from cryoconservation at -80 °C in liquid culture media. For *X. euvesicatoria*, strains BV801 and BV865, medium NYG (0.5% peptone, 0.3% yeast extract, 2 % glycerol, 7.0 pH, agar 1.6% when required) was used. On the other hand, *P. syringae* pv. *tomato* strain DC3000, was placed in a King (KB) liquid medium (2% peptone, 0.8% $K_2HPO_4$ [1 M], 0.6% $MgSO_4 \bullet 7H_2O$ [1 M], 1.2% glycerol, 7.2 pH, agar 1.6% as needed). Subsequently, the media were incubated at 28 °C, for 24 h, in the dark and shaken at 250 rpm. The bacteria were cultivated in liquid media all night (18-20 h), then planted by centrifugation at 10000 rpm for 5 min. Later, the supernatant culture medium was discarded and sterile deionized water was used to wash, repeating the process of centrifugation and washing three times. Next, the concentration of bacterial cells was adjusted to an optic density (OD) of 600 nanometers (nm), OD600 of 1.0 (OD600=1.0≈$1x10^8$ UFC $mL^{-1}$) with a spectrophotometer (Eppendorf®, BioPhotometer).

**Production of Hrp proteins in the pilot plant:** The Hrp proteins were produced in a pilot plant based on the methodology by Valerio-Landa *et al*. (2021a), extrapolated for applying in a tray-style solid fermentation bioreactor (45x30x3.5 cm). The bacterium *X. euvesicatoria* strain BV801 previously grown in a rich NYG medium and adjusted to $OD_{600}$ = 1.0 (≈$1x10^8$ UFC $mL^{-1}$), was applied at a volume of 2.5 mL per tray. The bacteria were then spread onto the induction medium for subsequent

cultivation (0.2% of pulverized *C. annuum* tissue, 1.5% food-grade agar, pH of 7.0). The tray was closed using Kraft paper and sealed with tape, the tray was sterilized with the induction medium; after bacterial inoculation, they were placed in the solid fermentation bioreactor, and incubated at 28 °C +/-3 °C in the dark. The harvest of the bacterial cells was carried out after 48 h by scraping and collecting the bacterial lawn with sterile deionized water. Finally, sterile deionized water was used to wash away the excess xanthan at 10000 rpm for 30 min at 4 °C. Bacterial lysis was carried out using sonication in an exposure time of 15 min with an ultrasonic liquid processor (Sonics VCX 130), using a frequency of 20 Khz, an amplitude of 40%, 30 s on / 30 s off cycles at 130 W, placing the samples in ice. After bacterial lysis, the protocol for protein extraction according to Valerio-Landa *et al*. (2021a) continued; the extracted proteins constituted the product named BioFensa (invention patent application MX/a/2020/013638; Valerio-Landa *et al.,* 2023).

**Experimental design.** To evaluate the biological effectiveness of BioFensa (BF), it was tested against the strain DC3000 of *P. syringae* pv. *tomato* in tomato plants (Experiment 1) and against *X. euvesicatoria* (strains BV801 and BV865) in chili pepper plants (Experiments 2 and 3). Three experiments were performed in totally randomized experimental designs, each one with five treatments (11 repetitions per treatment): (1) diseased plant (PE, with bacterial inoculation); (2) healthy plant (PS, without bacterial inoculation); (3) one commercial chemical inducer (Actigard®, 0.003 g $mL^{-1}$); (4) one commercial αβ Harpin protein inducer (Messenger Gold®, 0.003 g $mL^{-1}$); (5) BioFensa (1 µg $mL^{-1}$). On the other hand, to determine the optimum dose for the application of BioFensa against *X. euvesicatoria* strain BV801 in chili pepper plants (Experiment 4), a bioassay was held in a completely randomized experimental design with seven treatments (eight repetitions): (1) diseased plant (PE); (2) healthy plant (PS); (3) one chemical inducer (Actigard®); (4) one commercial αβ Harpin protein inducer (Messenger Gold®), BioFensa at 0.01 (5); 0.1 (6) and 1 (7) µg $mL^{-1}$ (Table 1). For all induction treatments (Actigard®, Messenger Gold®, BioFensa), they were applied 24 h before the infection with bacteria. The 3 mL treatments were applied by spraying on all of the plants' leaves (adaxial and abaxial).

**Table 1.** Composition of the treatments of Experiment 4 to determine the effect of different doses of the protein inducers under greenhouse conditions for chili pepper plants.

| | **Controls** | | **Inducer** | | |
|---|---|---|---|---|---|
| | **Healthy plant** | **Diseased plant** | **Actigard®** | **Messenger Gold®** | **BioFensa** |
| Ingredient | Without phytopathogen | With phytopathogen | Acibenzolar-S-metilo | Commercial harpin | Protein inducer of *X. euvesicatoria* |
| Dose (3 mL/plant) | Not applicable | $10^8$ UFC/mL | 0.0003 g/mL | 0.03 g/mL | 0.01, 0.1, 1 µg/mL |

**Phytopathological response variables.** To evaluate the severity of the disease, 30 days after the experiment began, response variables were recorded by quantifying the spots: number of chlorotic and necrotic spots, as well as the total number of spots observed on leaves with disease symptoms.

**Statistical analysis of data.** The data obtained from the response variables were analyzed statistically using a one-way analysis of variance and LSD tests: All statistical tests were evaluated at a significance level of 5% ($p \leq 0.05$). The information was processed using the package for statistical analysis Statgraphics Centurion ver. XV.II (http://www.statgraphics.com/).

## Results and Discussion

### Biological effectiveness of BioFensa for the control of *P. syringae* pv. *tomato*, strain DC3000

The results indicate that the symptoms of the bacterial speck (*P. syringae* pv. *tomato*, strain DC3000) in tomato plants, evaluated by the number of leaves with necrotic and chlorotic spots, as well as the number of total spots per plant (Figure 1), significantly decreased with the application of BioFensa (1 µg $mL^{-1}$).

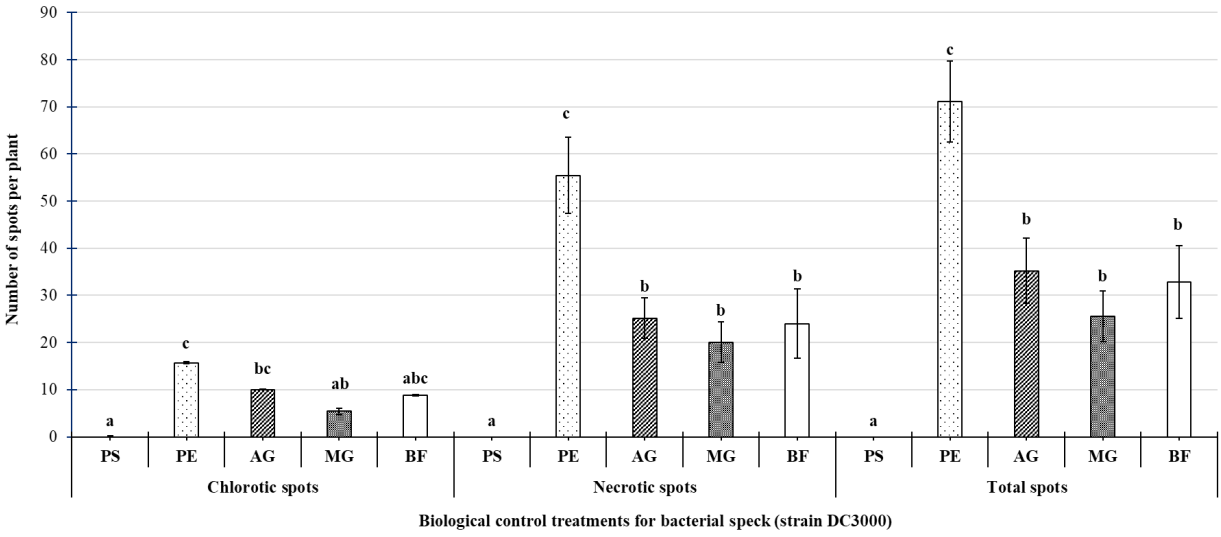


**Figure 1.** Biological effectiveness of different biological treatments for the control of *P. syringae* pv. *tomato*, strain DC3000 (bacterial speck) in tomato plants under greenhouse conditions, depending on the amount of necrotic, chlorotic and total spots per plant. PS=Healthy plant; PE=Diseased plant; AG=Actigard® (0.003 g $mL^{-1}$); MG= Messenger Gold® (0.003 g $mL^{-1}$); BF= BioFensa (1 µg $mL^{-1}$). Different letters in each response variable indicate significant differences according to the LSD test ($p \leq 0.05$). Bars in the rectangle indicate ± the standard error.

In comparison with the untreated diseased plants, the treatment with BioFensa prevented the appearance of spots by 53% (LSD, $p \leq 0.05$). Specifically, the average number of total spots per treated plant was 15±3, in comparison with 32 ± 4 in the diseased control (PE).

**Biological effectiveness of BioFensa for the control of *X. euvesicatoria* (strains BV801 and BV865)**

On the other hand, for the bacterial spot in ancho chili pepper plants treated with BioFensa (1 µg mL$^{-1}$) and challenged with the *X. euvesicatoria* strain BV865 (Figure 2), they showed a lower number of total spots, similar to those treated with commercial resistance inducers (Messenger Gold® and Actigard®). Specifically, the plants treated with BioFensa showed a significant reduction (LSD, $p \leq 0.05$) of 60% in the total number of spots, compared to the diseased control (PE).

The analyses of variance and the LSD tests LSD ($p \leq 0.05$) indicate that the treatment with BioFensa prevents disease severity by showing a lower occurrence of foliar tissue symptoms, ranging from chlorotic to the presence of necrosis, as

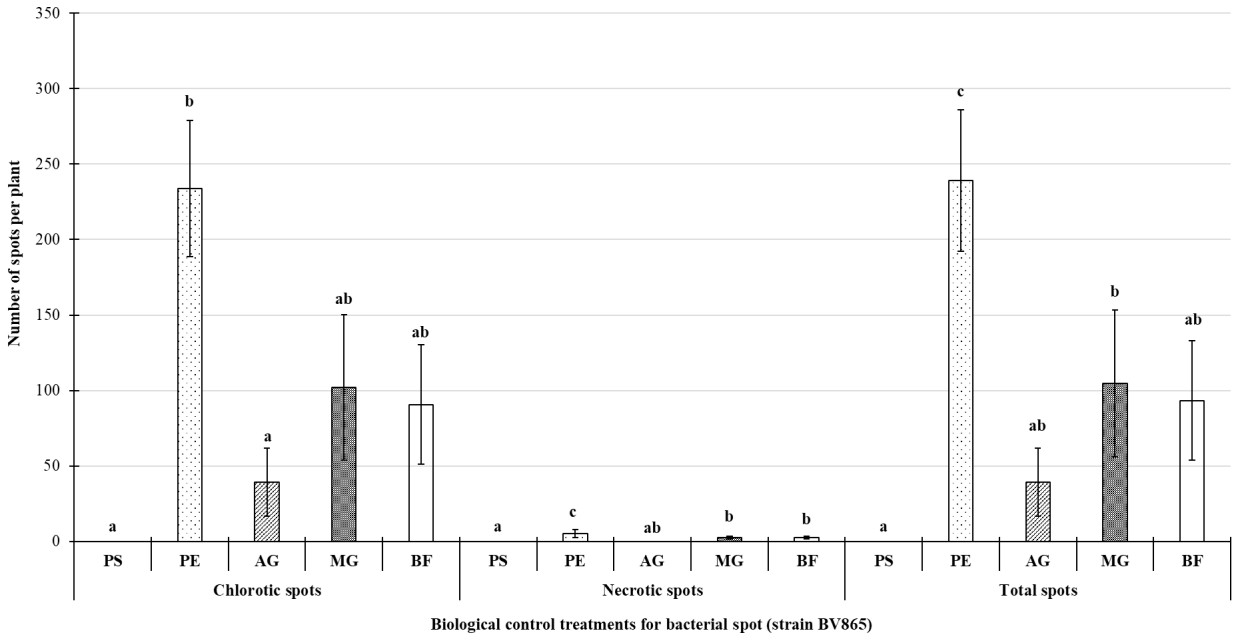


**Figure 2.** Biological effectiveness of different biological treatments for the control of *X. euvesicatoria*, strain BV865 in ancho chili pepper plants, San Luis variety, under greenhouse conditions, depending on the amount of necrotic, chlorotic and total spots per plant. PS=Healthy plant; PE=Diseased plant; AG=Actigard® (0.003 g mL$^{-1}$); MG= Messenger Gold® (0.003 g mL$^{-1}$); BF= BioFensa (1 µg mL$^{-1}$). Different letters in each response variable indicate significant differences according to the LSD test ($p \leq 0.05$). Bars in the rectangle indicate ± the standard error.

observed through visualization, as stated in Rico-Aguilar *et al.* (2019). Therefore, the application of Hrp proteins (BioFensa) significantly prevents the incidence of *P. syringae* pv. *tomato,* DC3000 (bacterial speck) in tomato plants and *X. euvesicatoria,* strain BV865 (bacterial spot) in chili pepper plants.

The concentration of 1 µg $mL^{-1}$ of BioFensa was enough to reduce the level of the spots of the disease, which could be attributed to the activity of the molecular patterns associated to pathogens (PMAP) found in the Hrp proteins of BioFensa. These results are consistent with earlier studies by Valerio-Landa *et al.* (2021b), who suggest that Hrp proteins can be recognized by the immunity receptors of plants, unleashing a new robust defense response by the immunity receptors by the plants.

The evaluation of strain BV801 of *X. euvesicatoria* in chili plants treated with BioFensa (1 µg $mL^{-1}$) displayed a reduction of only 13% in the total number of spots, which was not statistically significant (LSD, $p \leq 0.05$) with the diseased treatment (PE) (Figure 3). This means that the concentration of 1 µg $mL^{-1}$ of BioFensa may not have been optimal for the case of strain BV801, in comparison with strain BV865 (Figure 2). The variability in the efficiency observed may be due to the intrinsic virulence of strain BV801 and the saturation of the PMAP receptors in the plants, which coincides with studies that indicate that high concentrations of inducers can lead to the desensitization of defense receptors (Boller and Felix, 2009; Abdul *et al.,* 2020). When comparing the different treatments, a greater

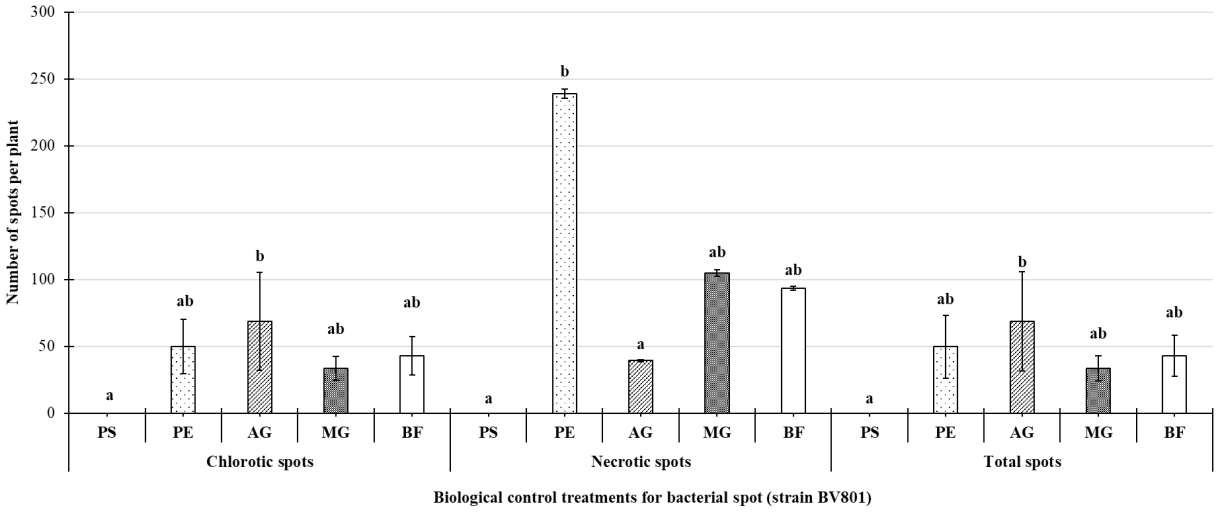


**Figure 3.** Biological effectiveness of different biological treatments for the control of *X. euvesicatoria*, BV801, in ancho chili peppers San Luis variety under greenhouse conditions, according to the number of necrotic, chlorotic and total spots per plant. PS=Healthy plant; PE=Diseased plant; AG=Actigard® (0.003 g $mL^{-1}$); MG= Messenger Gold® (0.003 g $mL^{-1}$); BF= BioFensa (1 µg $mL^{-1}$). Different letters in each response variable indicate significant differences according to the LSD test ($p \leq 0.05$). Bars in the rectangle indicate ± the standard error.

reduction in foliar spots was found in tomato plants with strain DC3000 and in chili plants with strain BV865, since strain BV801 displayed a greater degree of disease virulence. Likewise, when comparing the effect of the commercial inducers, a similar behavior was observed between Messenger Gold® and BioFensa, in relation to the necrotic spots (Figure 3). In addition, the treatment with Actigard® displayed a greater degree of the disease than the diseased control (PE) in relation to the total stains (Figure 3). The plants that were pretreated with BioFensa (1 µg $mL^{-1}$) displayed the same degree of disease than the diseased control plants (PE). This result may be due to the fact that the ability of chili plants to perceive PMAP inducers and activate their physiological state of resistance (PSR) seems to have been affected by the high concentrations of Hrp proteins during this investigation. In order to prove this hypothesis, an experiment was carried out to evaluate the effect of different doses of BioFensa in chili plants infected with the strain BV801 of *X. euvesicatoria* (Figure 4).

The plants treated with different concentrations of protein solutions (0.01, 0.1 and 1 µg $mL^{-1}$) for the control of *X. euvesicatoria* strain BV801 in ancho chili plants (Figure 4) displayed few symptoms of the disease when the concentration of 0.01 µg $mL^{-1}$ was used, in relation to the total spots on the leaves, achieving a 41% reduction of the disease. For necrotic lesions on leaves, a reduction of 51% was found, for the concentration of 0.1 µg $mL^{-1}$, 38% was found, and at 1 µg

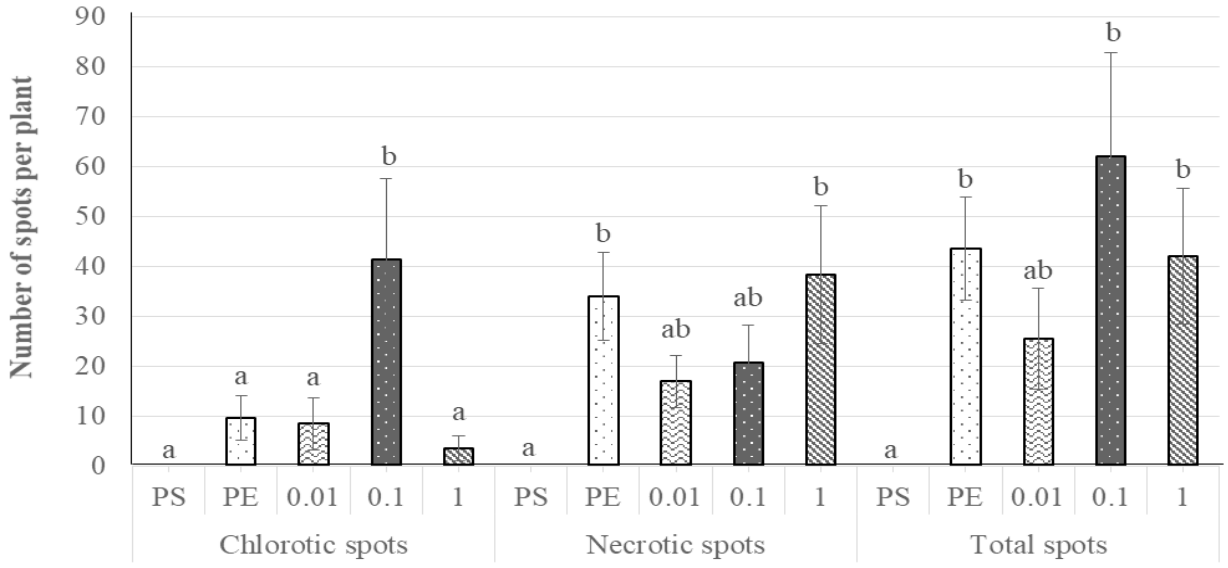


**Figure 4.** Effect of different doses of BioFensa (0.01, 0.1 and 1 µg $mL^{-1}$) on the control of *X. euvesicatoria*, strain BV801 on ancho chili plants, San Luis variety, under greenhouse conditions. PS=Healthy plant; PE=Diseased plant. Different letters indicate, within each response variable, significant differences according to the LSD test ($p \leq 0.05$). Bars in the rectangle indicate ± the standard error.

$mL^{-1}$ there were no significant differences were found in comparison with the diseased control (-15% the 1 μg $mL^{-1}$ treatment in comparison with PE), therefore no biological effectiveness was registered, causing a negative effect on the plant with this concentration (Figure 4, necrotic spots). In this regard, Zehra *et al.* (2021) mention that the effectiveness of the inducers can vary, depending on a series of factors, including the type of inducer, the plant species and environmental conditions. This coincides with the results of Figure 4, and could therefore be due to the inducers of effectors in plants working better at lower concentrations, since plants have a limited number of receptors, and at low concentrations, they allow for the activation of the adequate defense response. At higher concentrations, receptors saturate and can cause negative regulation mechanisms, cause toxicity disorganize signaling and divert energy resources from other vital processes such as growth, leading to energy depletion (Boller y Felix, 2009; Abdul *et al.,* 2020).

The findings of this study show that a concentration of 0.01 or 0.1 μg $mL^{-1}$ of BioFensa was enough to induce PSR and significantly reduce the severity of the bacterial speck and spot in tomato and chili plants, respectively (LSD, $p \leq 0.05$). This response could be related to the activity of the activity of molecular patterns associated to pathogens (PMAP) found in Hrp proteins, which are recognized by the innate immunity receptors in plants, thus activating the defense responses. Previous studies such as those by Boller and Felix (2009) have proven that similar proteins, such as flagellin (*flg22*) and even peptides such as systemin (18 amino acids) can induce defense responses in sub-nanomolar concentrations. Diverse studies, including the revision by Zehra *et al.* (2021) have proven that microbial elicitors, such as proteins and peptides, can unleash an Induced Systemic Resistance (ISR, mediated by jasmonic acid and ethylene). The phenomenon, characterized by a generalized and long-lasting state of alert, prepares the plant to respond faster and more effectively to a wide spectrum of pathogens. The activation of ISR is unleashed by the perception of elicitors, which act as danger signals and set the plant's defense machinery on motion (PMAP) when recognized by specific receptors in the surface of plant cells, starting a cascade of intracellular signaling that culminates in the activation of defense genes, as well as the establishment of local and systemic resistance, and the production of effector proteins. In this context, harpin protein Hpa1 has proven to have several beneficial effects on plants, including the promotion of growth and the induction of resistance to phytopathogens. Earlier investigations such as thos by Wang *et al.* (2020) revealed that Hpa1 can significantly mitigate the symptoms of the tobacco mosaic virus (TMV) in *Pinellia ternata*, suggesting an effective stimulation of the resistance to the disease, showing that Hpa1 has a significantly superior antiviral activity than the control and that is protective effect surpasses it curative effect. Additionally, Hpa1 induced a notorious increase in the activity of enzymes related to the defense (polyphenol oxidase, peroxidase, catalase

and superoxide dismutase), as well as in the expression of genes associated to the resistance of diseases by the plant (PR1, PR3, PR5 and PDF1.2).

This suggests that the Hrp proteins in BioFensa may be acting through a similar mechanism, unleashing the production of antimicrobial defenses and strengthening cell barriers. Additionally, the variability in the response to different concentrations and types of pathogens underscores the importance of optimizing the dose of resistance inducers to maximize their effectiveness. In this sense, literature also suggests that the combination of resistance inducers with other biological or chemical treatments may offer a more robust strategy for the management of diseases (Walters *et al.,* 2013).

The results from this study on the effectiveness of the biological inducer BioFensa (Hrp proteins) in the reduction of the bacterial speck in tomato and bacterial spot in ancho chili peppers coincide with the existing literature on the use of resistance inducers in plants. Boller and Felix (2009) pointed out that PMAPs such as flagellin are recognized by specific receptors and act as powerful elicitors of defense responses at sub-nanomolar concentrations, a mechanism similar to the one observed with BioFensa, which displayed a greater effectiveness at low concentrations (0.01 μg $mL^{-1}$). This phenomenon has also been documented by Abdul *et al.* (2020), who pointed out that the efficiency of resistance inducers may depend on the concentration used and the type of pathogen, reflecting the findings of this study, where higher concentrations did not always improve the defensive response. In addition, Walters *et al.* (2013) discussed the challenges and need to optimize the doses of inducers to maximize their effectiveness, a recommendation that resonates with the results regarding BioFensa being more effective at low concentrations (0.01 μg $mL^{-1}$). The capacity of resistance inducers to activate systemic defense responses in plants has been well documented (Ryals *et al.,* 1996; Durrant and Dong, 2004) and the results provide additional evidence that BioFensa can induce a physiological state of resistance, significantly reducing the severity of diseases. These findings suggest that BioFensa may be a valuable tool in the biological control of pathogens that cause diseases, promoting a more sustainable agriculture and reducing the dependency on chemical pesticides (Pieterse *et al.*, 2012; Van Loon *et al*., 2006).

Therefore, further evaluations are required to analyze the most effective concentrations for the reduction of diseases in tomato plants caused by *P. syringae* pv. *tomato* DC3000, as well as in chili pepper for *X. euvesicatoria* strain BV865 based on the results of *X. euvesicatoria* strain BV801, where a higher concentration of application of Hrp protein-based inducers displayed a greater effectiveness in the reduction of the bacterial spot, making BioFensa an alternative as a possible tool in the control of important phytopathogenic bacteria for agricultural production.

## Conclusions

The application of the biological inducer BioFensa (Hrp proteins) proved to be efficient to reduce the severity of *P. syringae* pv. *tomato* DC3000 (bacterial speck) in tomato plants and *X. euvesicatoria* (strains BV865 and BV801), which causes the bacterial spot in ancho chili pepper plants, displaying an effectiveness comparable to Actigard® y Messenger Gold® commercial inducers. Results suggest that lower concentrations of BioFensa (0.01 μg $mL^{-1}$) are more efficient, particularly in ancho chili pepper plants, whereas high concentrations (1 μg $mL^{-1}$) do not always improve the defensive response. BioFensa has the potential to be a valuable tool for the integrated management of bacterial diseases, promoting a more sustainable agriculture and reducing the dependency on chemical pesticides. However, the study was conducted under controlled greenhouse conditions, which could limit the generalization of the results under field conditions. Therefore, additional studies are required in the field to explore the biological effectiveness of BioFensa, both on its own and in combination with other biological or chemical treatments, to maximize its efficacy and validate its use in the phytosanitary management of bacterial diseases in agriculturally important plants.

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## References

Abdul MNA, Kumar IS and Nadarajah K. 2020. Elicitor and receptor molecules: orchestrators of plant defense and immunity. International Journal of Molecular Sciences 21(3): 963. https://doi.org/10.3390/ijms21030963

Boller T and Felix G. 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annual Review of Plant Biology 60: 379-406. https://doi.org/10.1146/annurev.arplant.57.032905.105346

Caicedo LLH, Aranda ALV, Sáenz de la OD, Gómez CEZ, Márquez EE and Zepeda HR. 2021. Elicitors: bioethical implications for agriculture and human health. Revista Bioética 29: 76-86. https://doi.org/10.1590/1983-80422021291448

Cardoso JLS, Souza AA and Vieira MLC. 2022. Molecular basis for host responses to *Xanthomonas* infection. Planta 256: 84. https://doi.org/10.1007/s00425-022-03994-0

Catara V and Bella P. 2020. Bacterial diseases. *In*: Gullino M, Albajes R, Nicot P. (eds). Integrated pest and disease management in greenhouse crops. Plant Pathology in the 21st Century, vol 9. Springer, Cham. https://doi.org/10.1007/978-3-030-22304-5_2

Conrath U, Beckers GJ, Langenbach CJ and Jaskiewicz MR. 2015. Priming for enhanced defense. Annual Review of Phytopathology 53: 97-119. https://doi.org/10.1146/annurev-phyto-080614-120132

Durrant WE and Dong X. 2004. Systemic acquired resistance. Annual Review of Phytopathology 42: 185-209. https://doi.org/10.1146/annurev.phyto.42.040803.140421

Fang XX and Yang SH. 2013. *Pseudomonas syringae* pv. *tomato* DC3000: a model pathogen for probing disease susceptibility and hormone signaling in plants. Annual Review of Phytopathology 51: 473-98. https://doi.org/10.1146/annurev-phyto-082712-102321

Joshi JB, Senthamilselvi D, Maupin-Furlow JA and Uthandi S. 2022. Microbial protein elicitors in plant defense. *In*: Kumar A. (eds). Microbial biocontrol: sustainable agriculture and phytopathogen management. Springer, Cham. https://doi.org/10.1007/978-3-030-87512-1_10

Khan E and Ahma IZ. 2024. Production and commercialization of biocontrol products. pp. 1665-180. *In*: Kumar A, Santoyo G, Singh J (eds). In plant and soil microbiome, biocontrol agents for improved agriculture, Academic Press Pages. https://doi.org/10.1016/B978-0-443-15199-6.00004-X

Larrahondo V, Villarraga DM and Moreno-Sarmiento N. 2019. Control biológico: camino a la agricultura moderna. Revista Colombiana de Biotecnología 21(1): 2-5. https://doi.org/10.15446/rev.colomb.biote.v21n1.80860

López-Vielma C, Solís-Sánchez A, Quiñones-Aguilar E, Qui-Zapata J y Rincón-Enríquez G. 2016. Aislamiento del agente causal de la mancha bacteriana de chile en las regiones productoras de Jalisco, Zacatecas y Michoacán. Biotecnología y Sustentabilidad 1 (1): 143-146. https://revistas.uaz.edu.mx/index.php/biotecnologiaysust/issue/view/faltante/1ro

Liu Y, Zhou X, Liu W, Huang J, Liu Q, Sun J, Cai X and Miao W. 2020. Hpa Xpm, a novel harpin of *Xanthomonas phaseoli* pv. *manihotis*, acts as an elicitor with high thermal stability, reduces disease, and promotes plant growth. BMC Microbiology 20(1): 4. https://doi.org/doi:10.1186/s12866-019-1691-4

Pieterse CM, Van der Does D, Zamioudis C, Leon-Reyes A and Van Wees SC. 2012. Hormonal modulation of plant immunity. Annual Review of Cell and Developmental Biology 28: 489-521. https://doi.org/10.1146/annurev-cellbio-092910-154055

Raffeiner M, Üstün S, Guerra T, Spinti D, Fitzner M, Sonnewald S, Baldermann S and Börnke F. 2022. The *Xanthomonas* type-III effector XopS stabilizes CaWRKY40 a to regulate defense responses and stomatal immunity in pepper (*Capsicum annuum*). Plant Cell 34(5):1684-1708. https://doi.org/10.1093/plcell/koac032

Rico-Aguilar JC, Quiñones-Aguilar EE, Rincón-Enríquez G, Guizar-Gonzalez C, Morales-García JL, Gómez-Dorantes N y López-Pérez L. 2019. Escala cualitativa de severidad de *Xanthomonas vesicatoria* en plantas de chile cultivar becán. Revista Biotecnología y Sustentabilidad 4(1): 47-54. https://doi.org/10.57737/biotecnologiaysust.v4i1.680

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY and Hunt MD. 1996. Systemic acquired resistance. The Plant Cell 8(10): 1809-1819. https://doi.org/10.1105/tpc.8.10.1809

Sticher L, Mauch-Mani B and Metraux JP. 1997. Systemic acquired resistance. Annual Review of Phytopathology 35: 235-270. https://doi.org/10.1146/annurev.pyto.35.1.235

Todd JNA, Carreón-Anguiano KG, Islas-Flores I and Canto-Canché B. 2022. Microbial effectors: key determinants in plant health and disease. Microorganisms 10: 1980. https://doi.org/10.3390/microorganisms10101980

Todd JNA, Carreón-Anguiano KG, Couoh-Dzul OJ, de los Santos-Briones C and Canto-Canché B. 2023. Effectors: key actors in phytopathology. Revista Mexicana de Fitopatología 41(2). https://doi.org/10.18781/R.MEX.FIT.2210-4

Valerio-Landa SD, Quiñones-Aguilar EE y Rincón-Enríquez G. 2020. Método para la obtención y aplicación de un inductor de resistencia contra agentes fitopatógenos en plantas. Patente de Invención MX/a/2020/013638 ante el Instituto Mexicano de la Propiedad Industrial (Sometido el 14 de diciembre de 2020 ante el IMPI). https://vidoc.impi.gob.mx/visor?d=MX/E/2020/087009

Valerio-Landa SD, Quiñones-Aguilar EE, Enriquez-Vara JN, Hernández-Gutiérrez R, Hernández-Montiel LG y Rincon-Enriquez G. 2021a. Método de inducción, aislamiento y selección de proteínas HRP de bacterias fitopatógenas, una fuente potencial de inductores de resistencia vegetal. Mensaje Bioquímico 45: 70-78. https://10.22201/fm.0188137xe.2021.45.07

Valerio-Landa SD, Quiñones-Aguilar EE, Enriquez-Vara JN, Hernández-Gutiérrez R, Hernández-Montiel LG and Rincon-Enriquez G. 2021b. Protein solutions from *Xanthomonas* as resistance inductors for the control of bacterial spot in tomato. Revista Fitotecnia Mexicana 44:609-616. https://doi.org/10.35196/rfm.2021.4.609

Valerio-Landa SD. 2021. Proteínas Hrp de *Xanthomonas* y su evaluación como inductores para la resistencia fisiológica en plantas contra fitopatógenos. Tesis doctoral. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. Zapopan, Jalisco, México. 307 p.

Valerio-Landa SD, Quiñones-Aguilar EE, Hernández-Gutiérrez R y Rincón-Enríquez G. 2023. Inductor biológico proteico para el control de enfermedades de plantas. Patente de Invención MX-a-2023-008646 ante el Instituto Mexicano de la Propiedad Industrial (Sometido el 21 de julio de 2023 ante el IMPI).

Van Loon LC, Rep M and Pieterse CM. 2006. Significance of inducible defense-related proteins in infected plants. Annual Review of Phytopathology 44: 135-162. https://doi.org/10.1146/annurev.phyto.44.070505.143425

Walters DR, Ratsep J and Havis ND. 2013. Controlling crop diseases using induced resistance: challenges for the future. Journal of Experimental Botany 64(5): 1263-1280. https://doi.org/10.1093/jxb/ert026

Wang D, Wang B, Wang J, Wang S, Wang W and Niu Y. 2020. Exogenous application of harpin protein Hpa1 onto *Pinellia ternata* induces systemic resistance against tobacco mosaic virus. Phytopathology 110(6): 1189-1198. https://doi.org/10.1094/PHYTO-12-19-0463-R

Zehra A, Raytekar NA, Meena M and Swapnil P. 2021. Efficiency of microbial bio-agents as elicitors in plant defense mechanism under biotic stress: a review. Current Research in Microbial Sciences 2: 100054. https://doi.org/10.1016/j.crmicr.2021.100054

Zhang S, Li C, Si J, Han Z and Chen D. 2022. Action mechanisms of effectors in plant-pathogen interaction. International Journal of Molecular Sciences 23(12): 6758. https://doi.org/10.3390/ijms23126758