Assessing the Chemical and Biological Effectiveness of Nano-Engineered Factors in Enhancing Resistance in tomato against *Xanthomonas campestris* pv. *vesicatoria*

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DOI: https://doi.org/10.36077/kjas/2024/v16i1.12536

Received date: 25/6/2023

Accepted date: 10/9/2023

Abstract

Bacterial spot a significant disease that affects tomato crops, despite limited studies and scientific research on this disease, So This study aimed to assess the efficacy of various biological and chemical factors in controlling bacterial spot disease in the field. A field survey was conducted in Najaf Al-Ashraf, specifically in areas such as Al-Haidariya, Al-Rahima, Al-Abbasia, and 20 plastic houses. The disease incidence was observed in these locations, with infection rates from 24% to 60%. Among them, Al-Haidariya had the highest infection rate at 60%, while Al-Abbasia had the lowest at 24%. Regarding to the impact of chemical and biological inducing factors on infection severity, the treatment using nanoscale zinc oxide demonstrated superior effectiveness in reducing the severity of infection caused by *Xanthomonas campestris* pv. *vesicatoria,* In reducing the severity of the infection, which amounted to 11.6%, compared with the treatment of *X.campestris* 2 (control 2) only, which amounted to 74.2%.Field results also indicated that the nanoscale zinc oxide treatment was highly effective in increasing chemical indicators such as chlorophyll content, peroxidase enzyme and catalase enzyme. The values for these indicators were 12.69 mg g^{-1}, 183.25-unit min^{-1} g^{-1} FW and 177.84-unit min^{-1} g^{-1} FW, respectively, compared to the control treatment, which recorded values of 7.79 mg g^{-1}, 88.3-unit min^{-1} g^{-1} FW, and 74.17-unit min^{-1} g^{-1} FW, respectively. Furthermore, the treatment using *Pseudomonas putida* demonstrated superiority in increasing the percentage of TSS, vitamin C content, and acid percentage. The values for these parameters were 8.45%, 20.86 mg g^{-1}, and 0.862%, respectively, compared to the control treatment, which recorded values of 4.78%, 11.09 mg g^{-1} and 0.462%, respectively. Moreover, the nanoscale zinc oxide treatment exhibited superior effectiveness in increasing the yield per plant, early fruit yield per plant, and total yield. The values for these parameters were 7.54 kg $plant^{-1}$, 10.261 kg. $plant^{-1}$ and 8.591 tons $plastichouse^{-1}$ (500 m²), respectively, compared to the control treatment, which recorded values of 4.15 kg $plant^{-1}$, 3.752 kg $plant^{-1}$, and 4.929 tons/plastic house (500 m²), respectively.

Keywords: Tomato, nanoscale zinc oxide, *Xanthomonas campestris* pv. *vesicatoria*, *P. putida*, Bacterial spot disease.

Introduction

Tomato (*Solanum lycopersicum* L.) is a highly valuable crop grown worldwide and belongs to the *Solanaceae* family. It originally thrived in the western region of South America. Tomatoes contain essential minerals, antioxidants like lycopene and beta-carotene, and vitamins C, E, and B, making them nutritionally rich (22). However, tomatoes, like other crops, are vulnerable to various pathogens, including *Xanthomonas campestris* pv. *vesicatory,* which causes bacterial spot disease and leads to significant financial losses for farmers globally (30). Initially, the disease symptoms appear on the lower leaves and gradually spread to all parts of the plant, resulting in oily brown spots under moist conditions the fruits also develop dark raised spots (34). leading to reduced production due to leaf drop and severe blemishes that render them unsuitable for sale (23).

Inducing resistance in plants involves stimulating their defense mechanisms through exposure to environmental factors, prior exposure to pathogens, or the use of chemical or biological substances (24, 40). While controlling plant diseases is important, excessive use of chemical pesticides presents several issues, including disruption of the natural environment, toxicity to beneficial organisms, and the weakening of natural control mechanisms (25). Copper-based compounds or pesticides have been widely used to combat various pathogens, including bacteria, by applying them as sprays on the leaves (20).

Alternative control methods, such as biological control, involve the use of safe and environmentally friendly natural substance one such approach involves using *Pseudomonas* bacteria, which possess numerous beneficial properties and have garnered significant interest in biological control (19). *P. putida*, known for its effectiveness in inhibiting pathogenic activity, promotes plant growth, leading to increased yield. It employs mechanisms like nutrient competition, systemic resistance stimulation, and metabolite production (4).

The using of nanomaterials in agriculture has greatly contributed to pest control advancements Bacterial and fungal diseases are major threats to crops, and nanoparticles have been employed to hinder bacterial growth and enhance plant disease resistance (21). Zinc oxide nanoparticles have gained importance for their ability to stimulate plant growth, seed germination, and provide protection against pests by inhibiting pathogen growth (37). Therefore, this study aims to investigate the use of chemical and biological inducing factors as safe alternatives to combat bacterial spot disease in tomatoes.

Materials and Methods

Investigating the spread of bacterial spot disease on tomatoes in some areas of Najaf Al-Ashraf:

The surveys were conducted in certain areas of Najaf Al-Ashraf (Al-Haidariya, Al-Abbasia, Al-Rahima) and in twenty plastic houses. Samples were randomly collected based on visible symptoms on leaves and fruits during the agricultural season of 2022 for isolating the causative pathogen. The percentage of infection in the surveyed fields was calculated using the following Percentage of infection % = (Number of infected plants / Total number of plants) × 100

As for the biological bacteria, they were isolated from the soil surrounding the roots of healthy tomato plants for bacterial isolation. The efficiency of biological and chemical inducing factors in determining the severity of infection caused by *X. campestris* pv. *vesicatoria*, the causative agent of bacterial spot disease on tomato, was evaluated. The percentage of infection

Testing the Efficacy of Some Biological Factors (*P. putida* 1) and Chemical Factors (Nano -Engineered Zinc Oxide from Bacteria *P.putida*,-NPsZnPp,Nano-Fertilizer

equation:

severity was studied according to the disease index mentioned by (39). (1) No symptoms on leaves, (2) Mild to moderate yellowing and slight leaf spotting, (3) Widespread yellowing and moderate leaf spotting, and (4) Complete leaf spotting. The percentage of infection severity was calculated using the following equation: Percentage of disease severity % = (Sum of [number of leaves for each degree × degree

number) / (Total number of leaves × highest degree) × 100

Loenergy plus, and Pesticide Champion for Controlling the Bacterial Spot disease on Tomato.

The field experiments

The field experiment took place in a plastic greenhouse at the Agricultural Research Station affiliated with Kufa University. On October 9, 2022, seeds of the Ala variety of tomatoes were sown in Petri dishes containing the T: S1 growth medium, which was produced by the German company "Klasmann." Once the seeds germinated and the second true leaf appeared, the seedlings were transplanted to their permanent location in the greenhouse on November 1, 2022. The land was prepared by plowing, leveling, and clearing weeds. It was divided into 3 plots, each plot divided into 3 experimental units for each treatment, with buffer of 40 cm between units. After 30 days of cultivation, the pathogenic strain *X. campestris* 2 was sprayed over the entire green area at a concentration of 9×10^{-8} CFU/ml water until saturation. Five days later, the biological resistance factor *P. putida* 1 was added at a concentration of 2.7×10^{-7} CFU/ml water. In terms of chemical factors, Nano-Engineered Zinc Oxide from Bacteria (*P. putida* 1) -NPsZnPp was applied at a concentration of 20 ppm. Additionally, the Nano-Fertilizer Loenergy plus and the Champion pesticide were used following the manufacturer's recommendations. The control treatment only received water. Throughout the growing season, crop management tasks were performed, starting from seedling care and continuing until April 16, 2023.Irrigation was carried out as required, and weed removal service was done when necessary. The field experiments were conducted using a Randomized Complete

Block Design (R.C.B.D) with three replicates for each treatment. The means were compared using the Least-Significant-Difference (L.S.D) test at a significance level of 0.05. The statistical software GenStat (5) was used for data analysis.

Indicators studied in the experiment:

Chemical Indicators in Leaves (the growth indicators of tomato were measured in the third pound)

Chlorophyll content in leaves (mg g^{-1} fresh weight):

Total chlorophyll pigment in plant leaves measured using a spectrophotometer at wavelengths 645 nm and 663 nm, and calculated using the equation by (16). Total chlorophyll = [20.2×D (645)] + [8.02 × D (663)] (μg g^{-1})

Estimation of the Total Activity of Peroxidase (POX) Enzyme:

Total activity of POX enzyme measured using a spectrophotometer at wavelength 420 nm, following the method described by (27).

Estimation of the Total Activity of Catalase (CAT) Enzyme:

Total activity of CAT enzyme estimated according to the method described by (1). using a spectrophotometer at wavelength 240 nm.

Percentage of Total Soluble Solid (TSS) (Brix%) in fruits, measured using a hand Refractometer according to the method described by (31).

Chemical Indicators in Fruits:

Determination of the concentration Vitamin C (Ascorbic Acid) in fruits Estimation of Ascorbic Acid: (Vitamin C) concentration in tomato fruits according to the method provided by (31).

The percentage of total neutralizable acidity the percentage of total neutralizable acidity in tomato fruits was calculated according to (9).

Yield indicators and its components

Average fruit yield per plant (kg. $plant^{-1}$) calculated by dividing the total yield of the experimental unit by the number of plants in the experimental unit.

Early plant yield (kg $plant^{-1}$): calculated by dividing the early yield of the experimental unit by the number of plants in the experimental unit.

Total yield of the plastic house (tons/plastic house with an area of $500m^2$) calculated cumulatively from the first to the last germination of each experimental unit using the equation: Yield per plant (kg) × (1150) × Number of plants in the plastic house.

Results and Discussion

Investigation of the spread of bacterial spot disease on tomatoes in some areas of Najaf Al-Ashraf revealed, through isolation and field surveys, the appearance of symptoms on leaves and stems in the early stages of disease development as dark oily-looking spots resembling a pinhead, which later turn into wet, greasy black color. As for the fruits, small deep brown ulcer-like spots surrounded by a yellow halo were observed, as shown in Figure 1. This is consistent with findings reported by (28;29). The percentage of infection in the plastic greenhouses surveyed ranged from 24 to 60%. The highest infection rate was recorded in the Al-Haidariyah area, reaching 60%, followed by the Al-Rahimah area with a rate of 50%. The lowest infection rate was recorded in the Al-Abbasia area, which was 24%, as shown in Table 1. The spread of bacterial spot disease on tomatoes is attributed to high humidity, high temperatures, heavy rainfall, strong winds,

plant density and overhead irrigation. All these factors create a suitable environment for the development and spread of the disease. Additionally, the use of infected seeds in agriculture contributes to the spread of the pathogen, as it can be transmitted through seeds. Furthermore, the disease can spread through wounds caused by agricultural operations such as grafting, pruning, harvesting, and pesticide spraying. The bacteria can persist for a long time on infected plant debris and quickly spread to newly planted crops (28;29;34).

Table 1. Percentage of infection of tomato plants by *X. campestris* pv. *vesicatoria* bacteria, the causal agent of bacterial spot disease, in some regions included in the field survey

Region	Variety	Percentage of tomato plants affected by bacterial spot disease (%)
Al-Rahima (Fadak Farm)	Al-Ibrahimi	39
Al-Rahima (Fadak Farm)	Al-Ala	50
Al-Rahima (Fadak Farm)	Al-Huda	35
Al-Rahima (Fadak Farm)	Al-Ala	40
Al-Rahima (Fadak Farm)	Al-Ala	45
Al-Rahima (Fadak Farm)	Al-Ibrahimi	30
Al-Rahima (Fadak Farm)	Al-Huda	40
Al-Haidariya	Al-Ala	60
Al-Haidariya	Al-Ala	45
Al-Haidariya	Al-Huda	36
Al-Haidariya	Al-Zeina	30
Al-Haidariya	Al-Ala	40
Al-Haidariya	Al-Ala	40
Al-Haidariya	Al-Ala	40
Al-Abbasia	Al-Huda	45
Al-Abbasia	Al-Ala	30
Al-Abbasia	Al-Ala	24
Al-Abbasia	Al-Ibrahimi	31
Al-Abbasia	Al-Huda	30
Al-Abbasia	Al-Ala	45

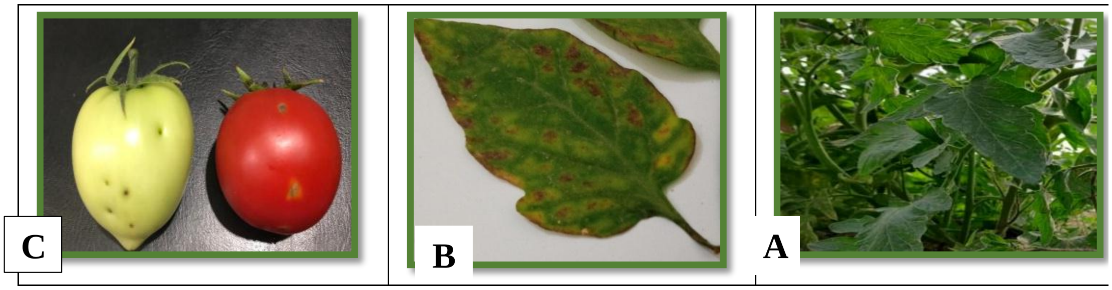

Figure 1. A healthy tomato plant (A), a leaf infected with bacterial spot (B), and fruits infected with bacterial spot (C). Field assessment of the efficiency of biological and chemical inducers in measuring the severity of infection by *X. campestris* pv. *vesicatoria* bacteria.

The results of Table (2) indicated significant differences among all treatments in reducing the severity of infection caused by the bacterial pathogen of bacterial spot disease on tomatoes. The pathogenic bacteria *X. campestris* pv. *vesicatoria* led to an increase in the severity of bacterial spot disease, as observed in control 2 treatment, where the infection severity reached 74.2%. On the other hand, treatments involving *X. campestris* bacteria, nano zinc oxide synthesized biologically, Champion pesticide, and the biocontrol agent *P. putida* resulted in reducing the infection severity of 11.6%, 23.6%, and 27.8% respectively. The treatment with nano zinc oxide showed significant superiority over all other treatments in reducing the severity of infection. This can be attributed to the role of nano zinc oxide particles in affecting the cell wall and plasma membrane of the pathogenic agents, thereby inhibiting proton movement across the cell membrane, leading to the killing and inhibition of the pathogenic microorganism (32; 36). Additionally, some studies have highlighted the role of *P. putida* bacteria in bio-resistance due to its ability to colonize roots and its involvement in the breakdown of toxic compounds that are important for plant protection against pathogens and stimulation of plant growth (26). Furthermore, (17) pointed out the efficacy of the active ingredient copper hydroxide as a pesticide, which plays a role in reducing the severity of infection and suppressing bacterial pathogens. This is attributed to its effective preventive action and better adhesion to plants, preventing the spread of the pathogen.

Table 2. The effect of biological and chemical inducers on the percentage of infection and severity of bacterial spot disease on tomatoes in the field

Treatment	Infection Severity (%)
Control 1	0
X. campestris 2 (Control 2)	74.2
X. campestris 2 + *P. putida*	27.8
Nano zinc oxide (manufactured) + *X. campestris* 2	11.6
Nano fertilizer Loenergy plus + *X. campestris* 2	37.5
Pesticide *Champion* + *X. campestris* 2	23.6
L.S.D (0.05)	2.61

Evaluation of the efficiency of some biological inducers (*P. putida* 1) and chemical inducers (Nano fertilizer Loenergy plus and pesticide Champion and Nano zinc oxide manufactured biologically - NPsZnPp) in controlling the bacteria causing bacterial spot disease on tomatoes in the field.

Chemical Indicators in Leaves

Chlorophyll Content (mg g^{-1})

The results of Table (3) indicated significant differences in the effect of treatments on the chlorophyll content in the leaves. The highest level was observed in both biological and chemical treatments. Specifically, the treatment with Nano zinc oxide manufactured biologically NPsZnPp and the treatment with *P. putida* showed significant superiority, with chlorophyll contents of 12.69 and 10.97 mg g^{-1}, respectively, compared to the control treatment, which had a chlorophyll content of 7.79 mg g^{-1}. The results also demonstrated the impact of additions on the chlorophyll content in the leaves. The treatment without the addition of the bacteria *X. campestris* outperformed the treatment with the addition of *X. campestris*, with chlorophyll contents of 9.5 and 9.3 mg g^{-1}, respectively. Furthermore, the interaction between the biological treatments, chemical treatments, and additions revealed that the highest chlorophyll content was recorded in the treatment with NPsZnPp without the addition of *X. campestris*, with chlorophyll contents of 13.33 and 12.06 mg g^{-1}, respectively, surpassing the other treatments significantly. On the other hand, the lowest chlorophyll content was observed in the treatment with the pesticide without the addition of *X. campestris* and the control treatment with the addition of *X. campestris*, with chlorophyll contents of 4.21 and 6.79 mg g^{-1}, respectively.

Table 3. Efficiency test of biological and chemical inducers against *X. campestris* pv. *vesicatoria* bacteria, the causal agent of bacterial spot disease in tomatoes, in terms of total chlorophyll content (field-based)

	Total Chlorophyll Content in Leaves (mg g^{-1})		
Treatment	**Without the addition of bacteria *X.campestris***	**With the addition of bacteria *X.campestris***	**Treatments average**
Control	8.79	6.79	7.79
P. putida	11.15	10.78	10.97
NPsZnPp	13.33	12.06	12.69
Nano fertilizer	9.96	9.36	9.66
Champion	4.21	7.51	5.86
Additives Average	9.5	9.3	
L.S.D 0.05	Treatments = 0.925	For Additives = 0.585	Interaction = 1.308

The overall activity estimation of Peroxidase (POX) enzyme (unit min^{-1} g^{-1} fresh weight)

The field experiment results indicated significant differences in the effects of treatments on the measurement of total activity of peroxidase enzyme, as shown in Table (4). The highest level was observed for both biological and chemical treatments, where the treatment of Nano Zinc Oxide (NPsZnPp) and the treatment of *P. putida* bacteria significantly outperformed the control treatment. Their total activity levels were 183.25- and 182.2-unit min^{-1} g^{-1} fresh weight, respectively, compared to 88.3-unit min-1 g-1 fresh weight for the control treatment. Regarding to the effects of additives, the results in measuring the total activity of peroxidase enzyme showed that the treatment without the addition of *X. campestris* bacteria had a higher level of 154.34-unit min^{-1} g^{-1} fresh weight than for the addition of *X. campestris* bacteria, which was 144.88-unit min^{-1} g^{-1} fresh weight. Furthermore, the results revealed the interaction between biological and chemical treatments and additives, where the highest percentage in measuring the total activity of peroxidase enzyme was observed in the treatment of NPsZnPp without the addition of *X. campestris* bacteria and the treatment of *P. putida* bacteria without the addition of *X. campestris* bacteria, with values of 186.3- and 184.3-unit min^{-1} g^{-1} fresh weight, respectively. These treatments significantly outperformed the other treatments. On the other hand, the lowest percentage in measuring the total activity of peroxidase enzyme was observed in the control treatment with the addition of *X. campestris* bacteria and the control treatment without the addition of *X. campestris* bacteria, with values of 80 and 96.6 unit. min. g^{-1} fresh weight, respectively.

Table 4. Efficiency test of biological and chemical inducers against *X. campestris* pv. *vesicatoria* bacteria, the causal agent of bacterial spot disease in tomatoes, in measuring the total activity of peroxidase enzyme (field-based)

Treatments	Without the addition of bacteria *X.campestris*	With the addition of bacteria *X.campestris*	Treatments average
Control	96.6	80	88.3
P. putida	184.3	180.1	182.2
NPsZnPp	186.3	180.2	183.25
Nano Fertilizer	160.9	151.4	156.15
Champion Pesticide	143.6	132.7	138.15
Mean of Additions	154.34	144.88	
L.S.D 0.05	Treatments = 10.40	Additions = 6.58	Interaction = 14.71

Total activity estimation of Catalase enzyme (CAT) (unit min^{-1} g^{-1} fresh weight)

The statistical analysis results indicated significant differences in the effect of treatments on measuring the Catalase enzyme, as shown in Table (5). The highest level was observed in the biological and chemical treatments, where the nanoscale zinc oxide manufactured biologically (NPsZnPp) treatment and *P. putida* bacteria treatment significantly outperformed with enzyme activity levels of 177.84- and 171.98-unit min^{-1} g^{-1} fresh weight, respectively, compared to the control treatment with an enzyme activity level of 74.17-unit min^{-1} g^{-1} fresh weight. Regarding to the effect of additions, the table results showed that the treatment without the addition of *X. campestris* bacteria had a higher total enzyme activity of Catalase, reaching 174.27-unit min^{-1} g^{-1}fresh weight than for the addition of *X. campestris* bacteria, which had an enzyme activity level of 138.91-unit min^{-1} g^{-1} fresh weight. The results also revealed the interaction between biological treatments, chemical treatments, and additions, where the highest percentage of total Catalase enzyme activity measurement was observed in the NPsZnPp treatment without the addition of *X. campestris* bacteria, and with the addition of *X. campestris* bacteria, the enzyme activity levels were 179.74- and 175.95-unit min^{-1} g^{-1} fresh weight, respectively, surpassing significantly the other treatments. The lowest percentage of total Catalase enzyme activity measurement was found in the control treatment with the addition of *X. campestris* bacteria and the control treatment without the addition of *X. campestris* bacteria, with enzyme activity levels of 57.87- and 90.48-unit min^{-1} g^{-1} fresh weight, respectively.

Table 5. Presents the evaluation of the effectiveness of biological and chemical agents in combating *X. campestris* pv. *vesicatoria* bacteria, which is responsible for causing bacterial spot disease in tomatoes. The table specifically focuses on assessing the overall activity of the Catalase enzyme in different field conditions

Treatments	Catalase enzyme (CAT) unit min^{-1} g^{-1} fresh weight		Treatments average
	Without the addition of bacteria *X. campestris*	with the addition of bacteria *X. campestris*	
Control	90.48	57.87	74.17
P. putida	174.93	169.03	171.98
NPsZnPp	179.74	175.95	177.84
Nano Fertilizer	158.99	160.52	159.75
Champion Pesticide	132.25	131.19	131.72
Mean of Additions	147.27	138.91	
L.S.D 0.05	Treatments =6.417	Additions =4.059	Interaction = 9.076

According to the results, it was revealed that the increase in chlorophyll content in the leaves, as shown in Table (3), and the percentage of the Peroxidase enzyme in Table (4), and the percentage of Catalase in Table (5) for plants treated with biologically synthesized zinc oxide nanoparticles can be attributed to the role of zinc. Zinc acts as an assistant factor in the oxidation process of plant cells, which regulates sugar consumption and increases the energy required for chlorophyll production (33). Some studies have also indicated that using smaller quantities of nanoparticles improves plant growth in various crop types. Plant growth and development depend on the interaction between plant cells and nanoparticles, which induce changes in biochemical pathways that affect plant gene regulation. In response to oxidative stress caused by these processes, nanoparticles stimulate plants to produce defensive enzymes and antioxidants, such as increased activity of peroxidase and catalase enzymes (38). Moreover, nanoscale zinc oxide has shown efficacy in improving growth indicators, photosynthetic properties, and **chemical indicators in fruits** increasing the activity of enzymes such as carbonic anhydrase, which plays a crucial role in modifying the hydrogen ion concentration in green plastids. It also enhances the peroxidase, catalase, and superoxide dismutase enzymes, increasing their activity, as well as increasing the protein, carotene, and lycopene contents in tomato crops treated with zinc oxide nanoparticles (15). These findings are supported by a study conducted by (35), which emphasized that the beneficial bacterium *P. putida*, living freely in the soil, promotes plant growth through various mechanisms, stimulates systemic resistance, and plays a role in reducing the density of pathogenic organisms that infect plants. It enhances ISR (induced systemic resistance) by increasing the levels of defensive enzymes such as peroxidase and chitinase, thus strengthening the plant's defenses against pathogenic bacteria. Catalase enzyme, being common in stimulating the efficiency of photosynthesis and respiration, leads to increased growth under parasitic conditions.

The percentage of total soluble solids (TSS %)

The results of the field experiment indicated significant differences in the effect of treatments on the fruit content of total soluble solids (TSS %). As shown in Table 6, the highest level was observed in the organic and chemical treatments. Specifically, the bacterial treatment *P. putida* and the nano fertilizer Loenergy plus significantly outperformed the control treatment, reaching 8.45% and 7.31% respectively, compared to 4.78% in the control treatment. Regarding to the effect of additives, the results in the percentage of total soluble solids (TSS) in tomato fruits showed that the treatment without the addition of bacteria *X. campestris* had a higher percentage at 6.804% compared to the treatment with the addition of bacteria *X. campestris* at 6.436%. Furthermore, the results of the interaction between biological and chemical treatments and additives revealed that the highest percentage of TSS was observed in the treatment of the biological bacteria *P. putida* without the pathogenic bacteria *X. campestris*, with an increase of 8.76% and 8.14% respectively, significantly surpassing the other treatments. The lowest percentage of TSS was observed in the control treatment with the addition of bacteria *X. campestris* and the control treatment without the addition of bacteria *X. campestris*, with values of 4.42 and 5.05% respectively.

Table 6. Evaluates the efficacy of biological and chemical agents in combating *X. campestris* pv. *vesicatoria* bacteria, which is responsible for causing bacterial spot disease in tomatoes. The assessment focuses on the level of total soluble solids (TSS) present in the fruits, and the testing is conducted in a field setting

Treatments	The percentage of total soluble solids (TSS %)		Treatments average
	Without the addition of bacteria *X.camestris*	with the addition of bacteria *X.camestris*	
Control	5.05	4.42	4.78
P. putida	8.76	8.14	8.45
NPsZnPp	7.32	6.88	7.1
Nano Fertilizer	7.74	6.88	7.31
Champion Pesticide	5.15	5.86	5.51
Mean of Additions	6.804	6.436	
L.S.D 0.05	Treatments = 0.729	Additions = 0.461	Interaction = 1.031

Vitamin C concentration (mg/100ml)

The results of the statistical analysis indicated significant differences in the effect of treatments on the fruit content of vitamin C. As shown in Table 7, the highest level was observed in the organic and chemical treatments. Specifically, the bacterial treatment *P. putida* and the nano-fertilizer Loenergy plus significantly outperformed the Control treatment, reaching 20.86 mg $ml^{-1}$and 18.53 mg ml^{-1} respectively, compared to 11.09 mg ml^{-1} in the control treatment. Regarding to the effect of additives, the results in the vitamin C content in tomato fruits showed that the treatment without the addition of bacteria *X.*

campestris had a higher concentration at 17.05 mg ml^{-1} than for the treatment with the addition of bacteria *X. campestris* at 15.06 mg ml^{-1}. Furthermore, the results of the interaction between biological and chemical treatments and additives revealed that the highest percentage of vitamin C measurement was observed in the treatment of the biological bacteria *P. putida* without the pathogenic bacteria *X. campestris*, with concentrations of 21.51 mg ml^{-1} and 20.22 mg ml^{-1} respectively, significantly surpassing the other treatments. The lowest concentration of vitamin C was observed in the control treatment with the addition of bacteria *X. campestris* and the pesticide treatment with the addition of the pathogen, with values of 9.81 mg ml^{-1} and 9.93 mg ml^{-1} respectively.

Table 7. Presents the evaluation of the efficiency of biological and chemical elicitors in combating *X. campestris* pv. *vesicatoria* bacteria, which is responsible for bacterial spot disease in tomatoes. The assessment was conducted in the field, focusing on the impact on the vitamin C percentage in tomato fruits

Treatments	Vitamin C (mg ml^{-1}) Without the addition of bacteria *X.campestris*	with the addition of bacteria *X. campestris*	Treatments average
Control	12.38	9.81	11.09
P. putida	21.51	20.22	20.86
NPsZnPp	18.82	17.36	18.09
Nano Fertilizer	19.1	17.97	18.53
Champion Pesticide	13.44	9.93	11.68
Mean of Additions	17.05	15.06	
L.S.D 0.05	Treatments =1.975	Additions =1.249	Interaction =2.793

The percentage of acidity that can be neutralized (%)

The results of the field experiment revealed significant variations in the impact of treatments on the percentage of acidity that can be neutralized. The findings presented in Table 8 demonstrated that the organic and chemical treatments exhibited the highest levels. Specifically, both the bacterial treatment *P. putida* and the nano-fertilizer Loenergy plus significantly outperformed the control treatment, with percentages reaching 0.862% and 0.810% respectively, whereas the control treatment registered by 0.462%. In terms of additives, the results concerning the percentage of acidity that can be neutralized in tomato fruits indicated that the treatment without the addition of bacteria *X. campestris* had a higher percentage at 0.704% compared to the treatment with the inclusion of bacteria *X. campestris* at 0.662%. Moreover, the interaction between biological and chemical treatments, as well as additives, revealed that the treatment involving the biological bacteria *P. putida* without the pathogenic bacteria *X. campestris* exhibited the highest percentage of neutralizable acidity, showing increases of 0.893% and 0.835% respectively, which were significantly higher than the other treatments. Conversely, the lowest percentage of neutralizable acidity was observed in the control treatment with the addition of bacteria *X. campestris*, as well as in the control treatment without the inclusion of bacteria *X. campestris*, yielding values by 0.403% and by 0.522% respectively.

Table 8. Presents the results of the field experiment evaluating the efficiency of biological and chemical inducers against *X. campestris* pv. *vesicatoria* the bacterium responsible for bacterial spot disease in tomatoes, specifically in terms of acidity percentage

Treatments	The percentage of acidity that can be neutralized. Without the addition of bacteria *X.campestris*	With the addition of bacteria *X. campestris*	Treatments average
Control	0.522	0.403	0.462
P. putida	0.893	0.832	0.862
NPsZnPp	0.783	0.723	0.753
Nano Fertilizer	0.835	0.785	0.81
Champion Pesticide	0.487	0.567	0.527
Mean of Additions	0.704	0.662	
L.S.D 0.05	Treatments =0.080	Additions =0.051	Interaction =0.114

The current study revealed an increase in the percentage of total soluble solids (TSS) as shown in Table (6), as well as an increase in vitamin C as shown in Table (7), and an increase in the percentage of acid content as shown in Table (8) in plants that were supplemented with the biological bacteria *P. putida*. This could be attributed to the ability of *P. putida* bacteria to facilitate the absorption of essential nutrients and stimulate growth-regulating substances and metabolic processes, It also produced ethylene, which enhanced the formation of enzymes that reduced the sugar content in fruit walls and promoted the production of simple sugars, thereby increasing the concentration of total soluble solids during the ripening process (18). Furthermore, *P. putida* bacteria had significant importance in the field of agricultural biotechnology due to their stimulating and protective effects on plants. had notable characteristics such as root colonization, promoting plant growth, providing protection against soil-borne pathogens through competition for food and space, inducing systemic resistance, and synthesizing plant hormones. They also played a role in nutrient solubilization and had the ability to adapt to various stress conditions (11). Moreover, *P. putida* bacteria contributed to plant growth by producing certain effective biological inoculants in the soil. This enhanced the plant's ability to absorb important nutrients, thereby improving plant growth through the regulation of various hormones such as ethylene, salicylic acid, gibberellic acid, indoleacetic acid, cytokinin, and several antioxidants (such as peroxidase, catalase, and ascorbic acid, i.e., vitamin C) (8). As for the nano-fertilizer that contained essential nutrients necessary for crop growth, such as nitrogen, phosphorus, and potassium, these elements were crucial in the soil. Nitrogen, which plants usually required in the form of nitric acid or ammonium or synthetic ammonia or urea or sodium nitrate, was provided. Therefore, the use of a nano-fertilizer containing NPK concentrations played a role in the growth and improvement of plants (7).

The yield indicators

The yield per plant (kg. plant^{-1}) was examined as the measured indicator.

Through the current study and the results that appeared in the field experiment, there are significant differences in the effect of the treatments on the average yield of one plant of fruits, and as shown in Table (9), it was the highest level for the biological and chemical treatments. Specifically, the treatment involving bio-synthesized nano zinc oxide particles (NPsZnPp) and the *P. putida* bacteria treatment achieved yields of 7.54 and 6.25 kg plant^{-1}, respectively, surpassing the control treatment, which yielded 4.15 kg plant^{-1}. Regarding the influence of additions, the tabulated results indicated a higher yield per plant for the treatment without the incorporation of *X. campestris* bacteria, reaching 6.16 kg plant^{-1} than for the treatment with *X. campestris* bacteria, which yielded 5.65 kg plant^{-1}. Moreover, the outcomes of

the interaction between biological and chemical treatments and additions revealed that the treatment involving bio-synthesized nano zinc oxide particles (NPsZnPp) without the presence of pathogenic *X. campestris* bacteria resulted in the highest yield per plant. In contrast, with the addition of *X. campestris* bacteria, the yields were 7.92 and 7.16 kg plant^{-1}, respectively, outperforming the other treatments. The lowest yield per plant was observed in the control treatment with the addition of *X. campestris* bacteria and the control treatment without the addition of *X. campestris* bacteria, yielding 3.45 and 4.86 kg plant^{-1}, respectively.

Table 9. Displays the outcomes of field efficiency experiments assessing the impact of biological and chemical factors on *X. campestris* pv. *vesicatoria* the bacterium responsible for causing bacterial spot disease in tomatoes. The results are presented in relation to the yield achieved per individual plant

Treatments	Plant Yield per plant (kg plant^{-1}) Without the addition of bacteria *X.campestris*	With the addition of bacteria *X.camestris*	Treatments average
Control	4.86	3.45	4.15
P. putida	6.36	6.14	6.25
NPsZnPp	7.92	7.16	7.54
Nano Fertilizer	5.73	5.98	5.85
Champion Pesticide	5.93	5.54	5.73
Mean of Additions	6.16	5.65	
L.S.D 0.05	Treatments =0.838	Additions =0530	Interaction =1.185

Early plant yield

The results of the previous study and field experiment revealed significant variations in the impact of treatments on early plant yield. As shown in Table (10), both biological and chemical treatments exhibited the highest level of effectiveness. The treatment involving biologically manufactured nano zinc oxide (NPsZnPp) and the *P. putida* bacteria treatment significantly outperformed others, yielding 10.261 and 9.286 kg. plant^{-1}, respectively, in comparison to the control treatment which yielded 3.752 kg. plant^{-1}. As for the effect of the additions, the results of the table indicated on the early plant yield, as the treatment without the addition of the bacteria *X.campestris*, which amounted to 7.363 kg. plant-1, excelled compared to the treatment of adding the bacteria *X.campestris*, which amounted to 6.278 kg. plant-1. Additionally, the results demonstrated the interaction between biological treatments, chemical treatments, and additives. The treatment combining biologically manufactured nano zinc oxide (NPsZnPp) without the inclusion of *X. campestris* pathogenic bbacteria, and the *P. putida* bacteria treatment exhibited the highest

percentage of early plant yield, reaching 11.403 and 10.408 kg. plant^{-1}, respectively. These treatments significantly outperformed the others. Conversely, the control treatment with *X. campestris* bacteria addition and the control treatment without *X. campestris* bacteria addition showed the lowest percentage of early plant yield, with yields of 3.088 and 4.417 kg. plant^{-1}, respectively.

Table 10. Displays the results of field-based efficiency tests for biological and chemical elicitors against *X. campestris* pv. *vesicatoria* the bacterium responsible for causing bacterial spot disease in tomatoes. The tests specifically evaluate the early plant yield per fruit as an indicator

Treatments	Yield per plant (kg. plant^{-1}) Without the addition of bacteria *X.campestris*	With the addition of bacteria *X.campestris*	Treatments average
Control	4.417	3.088	3.752
P. putida	10.408	8.165	9.286
NPsZnPp	11.403	9.12	10.261
Nano Fertilizer	5.903	5.76	5.831
Champion Pesticide	4.685	5.257	4.971
Mean of Additions	7.363	6.278	
L.S.D 0.05	Treatments =0.527	Additions =0.333	Interaction =0.745

The total yield of the plastic greenhouse (ton. 500 m^2 greenhouse)

Based on the previous study and the results obtained from the field experiment, significant differences were observed in the impact of treatments on the overall yield rate of the fruits. Table 11 demonstrated that the highest level was recorded for both biological and chemical treatments. Specifically, the treatment involving bio-synthesized nano zinc oxide particles (NPsZnPp) and the *P. putida* bacteria treatment achieved yields of 8.591 and 7.172 tons 500m^2 greenhouse, respectively, surpassing the control treatment, which yielded 4.929 tons 500m^2 greenhouse. Concerning the influence of additions, the tabulated results indicated a higher overall yield rate for the treatment without the incorporation of *X. campestris* bacteria, reaching 7.042 tons 500m^2 greenhouse than for the treatment with *X. campestris* bacteria addition, which yielded 6.531 tons 500m^2 greenhouse. Furthermore, the outcomes of the interaction between biological treatments, chemical treatments, and additions revealed that the treatment involving bio-synthesized nano zinc oxide particles (NPsZnPp) without the presence of pathogenic *X. campestris* bacteria resulted in the highest overall yield rate of the fruits. Conversely, with the addition of *X. campestris* bacteria, the yields were 8.926 and 8.257 tons500m^2 greenhouse, respectively, outperforming the other

treatments. The lowest overall yield rate of the fruits was observed in the control treatment with the addition of *X. campestris* bacteria and the control treatment without the addition of *X. campestris* bacteria, yielding 4.252 and 5.607 tons 500 m^2 greenhouse, respectively.

Table 11. Showcases the efficacy evaluations conducted on biological and chemical elicitors in relation to their impact on the total fruit yield (field-based) against *X. campestris* pv. *vesicatoria* the bacterial culprit responsible for bacterial spot disease in tomatoes

The total yield of the plastic greenhouse (ton. 500m^2 greenhouse)			
Treatments	**Without the addition of bacteria *X.campestris***	**With the addition of bacteria *X.campestris***	**Treatments average**
Control	5.607	4.252	4.929
P. putida	7.559	6.785	7.172
NPsZnPp	8.926	8.257	8.591
Nano Fertilizer	6.497	6.992	6.745
Champion Pesticide	6.620	6.371	6.495
Mean of Additions	7.042	6.531	
L.S.D 0.05	Treatments =0.6537	Additions =0.4135	Interaction =0.9245

The increase in fruit yield per plant, as observed in Table (9), early plant yield in Table (10), and total fruit yield in Table (11), was attributed to the significant importance of nano-zinc oxide. Nano-zinc oxide played a crucial role in protein and enzyme production, as well as in increasing chlorophyll synthesis, which improved the metabolic process and enhanced plant growth, productivity, and yield. Moreover, nano-zinc oxide served as a vital component in physiological and cellular processes, as well as in the formation of plant hormones such as Abscisic acid, Auxin, and Gibberellin. The absorption of these particles by plants played a significant role in increasing fruit production. Therefore, it was concluded that the use of nano-sized particles of mineral elements efficiently increased crop production and improved fruit quality (14). Adequate amounts of essential nutrients, including zinc (Zn) and nano-sized particles of zinc oxide, were necessary to enhance and increase tomato production.

Thus, the aforementioned interpretation of the results emphasized the utmost importance of nano-sized zinc particles. In a previous study, researchers highlighted that foliar spraying of zinc was one of the most effective measures for improving crop quality and productivity (2). Furthermore, the root bacteria *P. putida* enhanced plant growth activity by altering plant hormone concentrations and facilitating nutrient absorption through biological nitrogen fixation, dissolution of inorganic phosphates, formation of iron acid, and stimulation of plant systemic resistance, thereby improving plant growth and productivity (13). The study also indicated that *P. putida* bacteria increased the concentration of signaling compounds such as Ethylene and Jasmonic acid, which stimulated plant defenses at the cellular and molecular levels, along with their existing mechanisms, resulting in increased plant growth, fruit weight, and leaf number (3).

Conclusion

Bacterial spot caused by bacteria *X.campestris* is one of the most common diseasea affecting tomato , and in order to limit its spread .it is necessary to use disease-free seeds ,reduce humidity in greenhouses and use biological synthesized zinc oxide nanoparticles to control it ,as it has proven effective in controlling and improving plant growth.

Conflict of Interest

The authors have no conflict of interest.

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