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EVALUATING THE EFFICACY OF CHEMICALS AND ANTIBIOTICS IN COMBATING BACTERIAL LEAF BLIGHT OF TOMATO

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ABSTRACT

Bacterial leaf spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* is one of the most destructive biotic stresses that significantly affects the quality and productivity of tomatoes. In the current study, we investigated the effects of ten chemicals, including Score (24.51%), Topsim-M (70%), Cabrio (60%), Fossil (29%), Milvet (80%), Forum Top (53%), Excel (80%), Evcin (80%), Electus Super (30%), and Copper hydroxide (52.4%), as well as ten antibiotics, namely Quinocel (10%), Neflox (30%), Gentam (20%), Velocef (29%), Rithmo (44%), Cefcom (52.4%), Cefstar (52.41%), Novamox-LA (15%), Trisulpha (53%), and Inocef (80%), at different concentrations under laboratory conditions, using the inhibition zone technique. In greenhouse conditions, the most effective chemicals and antibiotics, both individually and in combination, were evaluated at various concentrations against bacterial leaf spot of tomatoes. A Completely Randomized Design was employed for both laboratory and greenhouse experiments. The results from the laboratory conditions indicated that Score exhibited the maximum inhibition zone (34.83 mm), followed by Neflox (29.07 mm) against *X. campestris* pv. *vesicatoria*, compared to the control. Disease severity was assessed using a disease rating scale at specific time intervals. The results showed that the combination of Score and Neflox exhibited the least disease severity (7.50%), followed by other treatments.

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INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is the most predominant vegetable crop worldwide, well-known for its rich source of Vitamins A, B, and C (Imran et al., 2012). Tomato cultivation is exposed to numerous biotic stresses, including bacteria, fungi, nematodes, and viruses, as well as abiotic stress factors such as temperature, light, and humidity. These stressors adversely affect the quality and yield of the crop (Majumder et al., 2020). Among all the biotic constraints faced by the tomato crop, bacterial leaf spot, caused by *Xanthomonas campestris* pv. *vesicatoria*, is the most devastating (Kebede et al., 2014; Potnis et al., 2015). This disease is responsible for a 50% reduction in tomato crop yield, with disease incidence ranging from 22% to 50% (Kavitha and Umesha, 2007; Abrahamian et al., 2019). Typical symptoms of bacterial leaf spot in tomatoes include the expansion of small, dark-colored

spots (1-3 mm in size) with or without yellow halos, appearing on leaves, fruits, and stems. Over time, these spots expand and merge, leading to browning or withering of the entire leaflet and causing defoliation (Stall et al., 2009).

Xanthomonas can survive on a wide range of hosts from the Solanaceae family, such as tomatoes, cherry tomatoes, currant tomatoes, chili peppers, and peppers. The pathogen is gram-negative, cylindrical in shape, aerophilic, motile, and contains a single polar flagellum (Baker et al., 2014). The growth of *X. campestris* is favored by a high temperature range (25-30°C) and high relative humidity. The bacterium can survive in seeds, plant remains, and volunteer plants. It is dispersed by wind-driven rain and enters the plant through injuries and natural openings, such as stomata and hydathodes. Bacterium can also live epiphytically in the tomato phyllosphere (Momol et al., 2002).

Researchers have employed numerous management tactics to control bacterial leaf spot of tomatoes, including the application of chemicals, the use of biocontrol agents, antibiotics, and resistant cultivars (Horvath et al., 2012). Among these strategies, resistant varieties have proven to be the most reliable method for disease management. However, when the disease appears in epidemic proportions, farmers have no choice but to resort to chemical treatments due to their quick action and ready availability (Trueman et al., 2019). Chemotherapeutic management of tomato bacterial spot has involved the use of multiple products, and its success can be attributed to the efficacy of these products in practice, as well as the timing and frequency of spraying. Furthermore, the sensitivity of *Xanthomonas* spp. to the chemicals tested is a crucial factor. Copper-based chemicals, antibiotics, and acibenzolar-S-methyl have shown potential as viable control options for tomato bacterial leaf spot, with the latter having the ability to induce resistance in plants.

Research aimed at identifying alternative management tactics to combat bacterial spot in tomatoes has proven to be a formidable challenge due to the limited availability of effective products. Integrating existing management strategies and research efforts focused on inducing resistance may significantly enhance control efficiency. Therefore, recognizing the importance of chemicals and antibiotics, ten chemicals and ten antibiotics were tested in the current study to evaluate their efficacy against *X. campestris* pv. *vesicatoria* in order to identify effective products with bactericidal potency (Itako et al., 2014).

MATERIALS AND METHODS

Collection of diseased samples

Diseased samples were collected from the field area of Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Collected samples were placed in polythene bags (14×22) and brought to Phytobacteriology lab.

Isolation and purification of pathogen

Nutrient agar media was prepared for the isolation of the pathogen. Diseased samples were washed with tap water to remove soil debris. The diseased samples were then cut into small pieces (2-3 mm) using sterilized scissors, along with some healthy portions. To sterilize the samples, they were dipped into a 1% sodium hypochlorite solution for 30 seconds and then washed three times with distilled water to eliminate the toxic effects of sodium hypochlorite. After washing, the samples were placed on sterilized filter paper to dry. Once the samples were dry, the media was poured into 90 mm Petri plates. After the media solidified, three diseased samples were transferred to each Petri plate. The plates were sealed with parafilm and placed in an incubator at 30°C. They were then incubated at this temperature for 24 hours, and bacterial growth was observed at the end of this period.

For purification, a single colony was selected using a sterilized loop and streaked onto new nutrient agar media plates. These new Petri plates were also sealed with parafilm and placed in a Heraeus incubator at 25°C. This entire procedure was repeated 2-3 times to multiply and purify the bacterial culture.

Identification and preservation of pathogen

The bacterial pathogen was identified based on biochemical tests, which included Gram staining, the KOH test, and the Oxidase test, as well as morphological characteristics such as colony color and appearance under a microscope. For preservation, media was poured into a test tube, and a pure culture was transferred into the test tube, which was then placed in a shaking incubator at 28°C for 24 hours. The bacteria were stored in Eppendorf tubes containing 1 mL of glycerol and 1 mL of bacterial suspension. In 2 mL Eppendorf tubes, 1 mL of glycerol and 1 mL of bacterial growth suspension were added. These Eppendorf tubes were labeled and stored at -18°C in a refrigerator.

Pathogenicity test

Koch's Postulates were employed to confirm the presence of the pathogen. For this purpose, 40 tomato plants were cultivated in 25 cm earthen pots under greenhouse conditions. After one month, a bacterial suspension was applied to the cultivated plants. The suspension was prepared by mixing a bacterial colony with distilled water. Then, the suspension was injected into tomato leaves using a sterilized syringe (0.5 mL), while 10 tomato plants were maintained as controls and treated with distilled water. Disease symptoms appeared 5-7 days after inoculation. Subsequently, the pathogen was re-isolated from the inoculated leaves and compared with the parent plant to confirm its presence.

Preparation of chemical and antibiotics concentrations

Three concentrations (100 ppm, 200 ppm, 250 ppm) were prepared to assess the antibacterial potential at different dosages. To create a 100 ml stock solution, the percentages of active ingredients in the chemicals and antibiotics were divided by 100 and then added to distilled water to reach a total volume of 100 ml. To prepare the 100 ppm, 200 ppm, and 250 ppm concentrations from the stock solution, 1 mL, 2 mL, and 2.5 mL, respectively, were taken from the stock solution and mixed with 100 ml of distilled water.

***In vitro* evaluation of chemicals and antibiotics**

Ten chemicals, such as Score (24.51%), Topsim-M (70%), Cabrio (60%), Fossil (29%), Milvet (80%), Forum Top (53%), Excel (80%), Evcin (80%), Electus super (30%), and Copper hydroxide (52.4%), as well as ten antibiotics, including Quinocel (10%), Neflox (30%), Gentam (20%), Velocef (29%), Rithmo (44%), Cefcom (52.4%), Cefstar (52.41%), Novamox-LA (15%), Trisulpha (53%), and Inocef (80%), with different concentrations, were evaluated using the inhibition zone technique under laboratory conditions. For this purpose, nutrient agar media was poured into Petri plates and allowed to solidify. After that, a sterilized cotton swab was used to spread bacterial culture on the Petri plates within a laminar air chamber (RTVL-1312). Following this, 1 cm circular pieces of filter paper were cut and sterilized in an autoclave (RTA85) at 121°C and 15 Psi for 15 minutes. These sterilized filter paper pieces were then dipped into the prepared concentrations of chemicals and antibiotics. After removing excess moisture, the filter paper was placed in the center of the plates. For the control treatment, pieces of filter paper were dipped in distilled water. The plates were then sealed with wrapping tape and incubated in a Heraeus incubator at 25-30°C for 2-3 days. The experiment was conducted using a Completely Randomized Design with three replications of each treatment. Data were recorded after 24, 48, and 72 *hours*, and the inhibition zone was measured using a digital vernier caliper (VCL-150).

Evaluation of chemicals and antibiotics under greenhouse conditions

The most effective treatments among chemicals (Score) and antibiotics (Neflox) were evaluated under laboratory conditions, both individually and in combination with three different concentrations, in a glasshouse setting. For this purpose, 36 tomato plants were grown in earthen pots measuring 25 cm in diameter. In the laminar flow cabinet (RTVL-1312, Robus United Kingdom), a bacterial suspension was prepared by mixing bacterial colonies in distilled water. After one month, this prepared bacterial suspension was applied to the matured plants. Once disease symptoms had developed, the specified concentrations of chemicals and antibiotics were applied. The experiment was designed using a Completely Randomized Design with three replications for each treatment. Data regarding disease severity were recorded three times at 5-day intervals using a disease rating scale (Table 1).

Data analysis

Laboratory and greenhouse experiments were conducted using a Completely Randomized Design, and pairwise comparisons were made using the Least Significant Difference (LSD) method (Steel et al., 1997). The recorded data were analyzed using analysis of variance (ANOVA).

RESULTS

Evaluation of different antibiotics against *X. campestris* pv. *vesicatoria* under lab conditions

The results showed that among all tested antibiotics, Neflox exhibited the maximum inhibition zone (29.07 mm), followed by Gentam (27.70 mm), Quinocel (24.31 mm), Trisulpha (23.59 mm), Velocef (18.77 mm), Rithmo (18.01 mm), Novamox-LA (15.22 mm), Inocef (14.55 mm), Cefstar (14.24 mm), and Cefcom (13.57 mm), as compared to the control (Table 2 and Figure 1). The interaction between treatments and concentrations (T×C) indicated that Neflox showed the maximum inhibition zone at 600 ppm (29.61 mm), followed by 400 ppm and 200 ppm, as compared to Gentam (26.38 mm,

27.61 mm, 28.22 mm), Quinocel (23.38 mm, 25.22 mm, 24.33 mm), Trisulpha (20.16 mm, 23.38 mm, 27.22 mm), Velocef (18.11 mm, 17.61 mm, 20.61 mm), Rithmo (14.50 mm, 16.05 mm, 17.50 mm), Novamox-LA (14.00 mm, 15.55 mm, 16.11 mm), Inocef (14.05 mm, 14.22 mm, 15.38 mm), Cefstar (16.16 mm, 13.44 mm, 13.11 mm), and Cefcom (12.22 mm, 12.61 mm, 15.88 mm) (Table 2 and Figure 2).

Table 1. Disease rating scale to evaluate potato cultivars towards *Ralstonia* wilt (Jahanzaib et al., 2017).

Rating	Disease severity (%)	Response
1	0.00	Immune
2	1-9	Highly resistant
3	10-20	Resistant
4	21-35	Moderate resistant
5	36-50	Moderate susceptible
6	51-65	Susceptible
7	66-100	Highly susceptible

Table 2. Impact of different antibiotics on inhibition zone of *X. campestris* pv. *vesicatoria* under lab conditions.

Treatments	Active ingredients (%)	Inhibition zone (mm)
Neflox	Norfloxacin (30%)	29.07 a
Gentam	Gentamicin (20%)	27.70 b
Quinocel	Enrofloxacin (10%)	24.31 c
Trisulpha	Sulfadiazine (53%)	23.59 c
Velocef	Cephradine (29%)	18.77 d
Rithmo	Clarithromycin (44%)	16.01 e
Novamox-LA	Amoxicillin (15%)	15.22 f
Inocef	Ceftriaxone sodium (80%)	14.55 fg
Cefstar	Cefepime (52.41%)	14.24 gh
Cefcom	Ceftazidime (52.4%)	13.57 h
Control	Distil water	0.00 i
LSD		0.7852

*Mean value in the column sharing similar letter does not differ significantly as determined by LSD test (P<0.05)

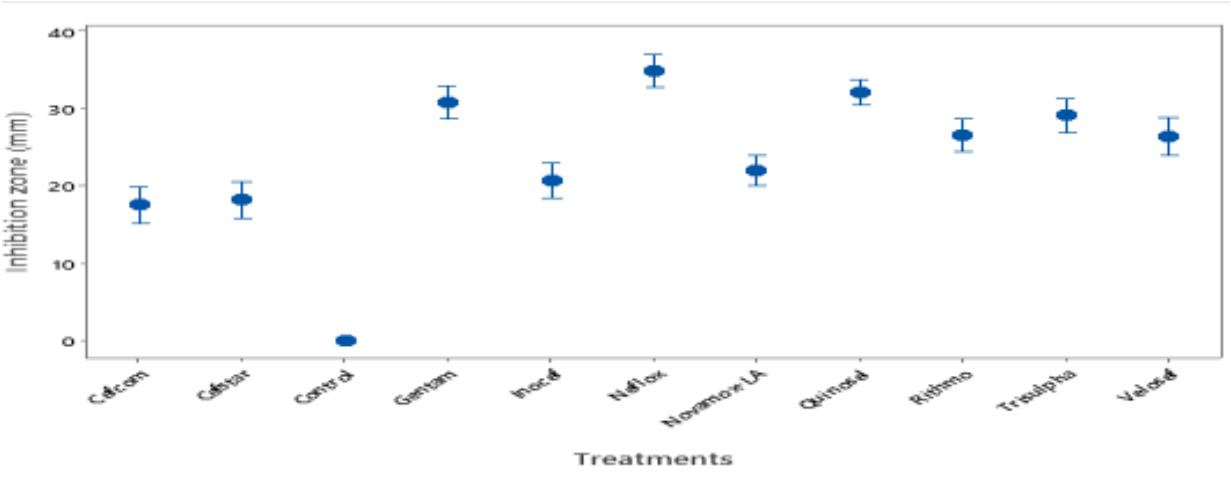

Figure 1. Evaluation of different antibiotics against *X. campestris* pv. *vesicatoria* under lab conditions.

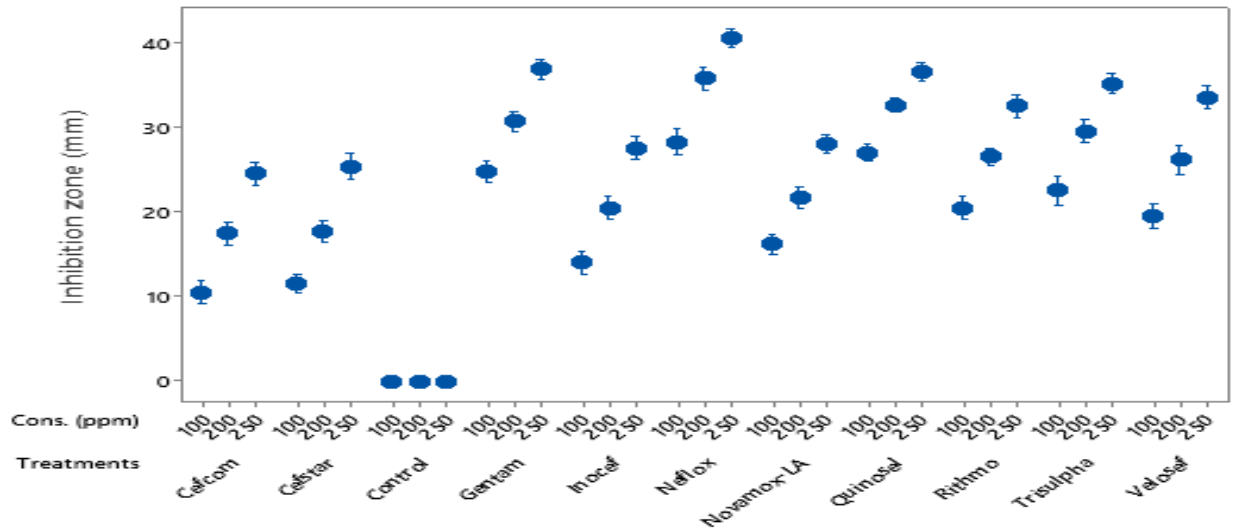

Figure 2. Impact of interaction between treatments and concentrations on development of inhibition zone against *X. campestris* pv. *vesicatoria* under lab conditions.

The interaction between treatments and durations (T×D) revealed that the minimum inhibition zone was observed in Cefcom (13.50 mm, 13.55 mm, 13.66 mm), Inocef (14.16 mm, 14.50 mm, 15.00 mm), Cefstar (14.27 mm, 14.38 mm, 14.05 mm), Novamox-LA (14.61 mm, 15.22 mm, 15.83 mm), Rithmo (15.44 mm, 15.88 mm, 16.72 mm), Velocef (18.72 mm, 18.50 mm, 19.11 mm), Trisulpha (22.38 mm, 23.66 mm, 24.72 mm), Quinocel (24.11 mm, 24.22 mm, 24.61 mm), Gentam (24.27 mm, 27.61 mm, 30.33 mm), and Neflox (28.94 mm, 29.11 mm, 29.16 mm) after 24 hours, 48 hours, and 72 hours, respectively, as compared to the control (Table 2 and Figure 3).

Evaluation of different chemicals against *X. campestris* pv. *vesicatoria* under lab conditions

Under laboratory conditions, the results among the chemicals indicated that the maximum inhibition zone was expressed by Score (34.83 mm), Milvet (32.03 mm), Fossil (30.75 mm), Cabrio (29.05 mm), Evcin (26.50 mm), Electus super (26.38 mm), Topsin-M (21.94 mm), Forum Top (20.66 mm), Excel (18.16 mm), and Copper hydroxide (17.50 mm) when compared to the control (Table 3 and Figure 4).

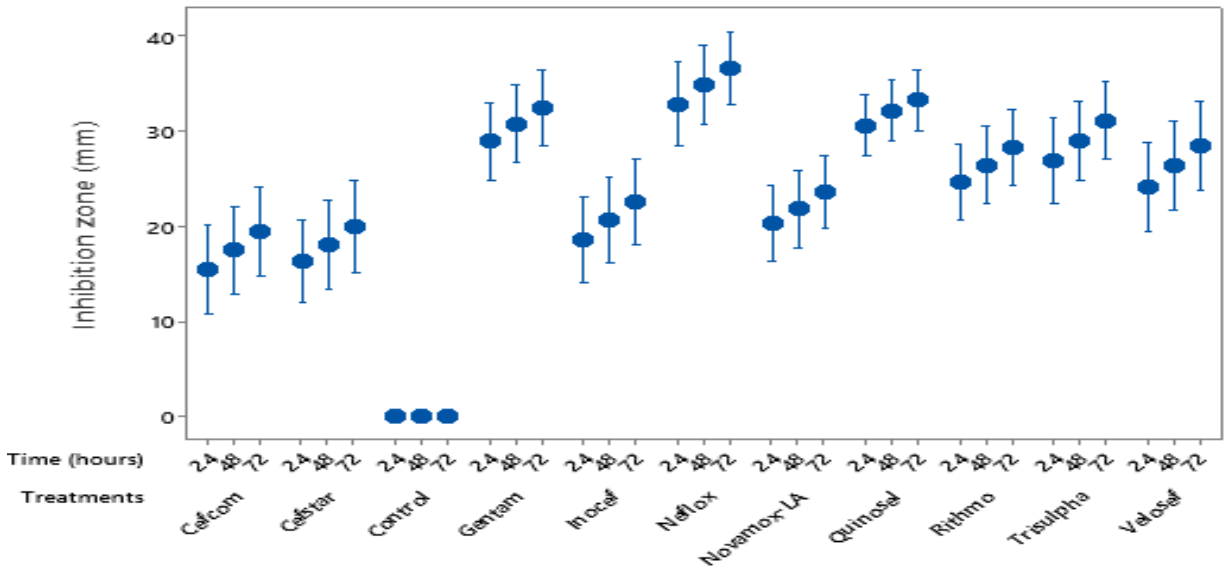

Figure 3. Impact of interaction between treatments and durations on the development of inhibition zone towards *X. campestris* pv. *vesicatoria* under lab conditions.

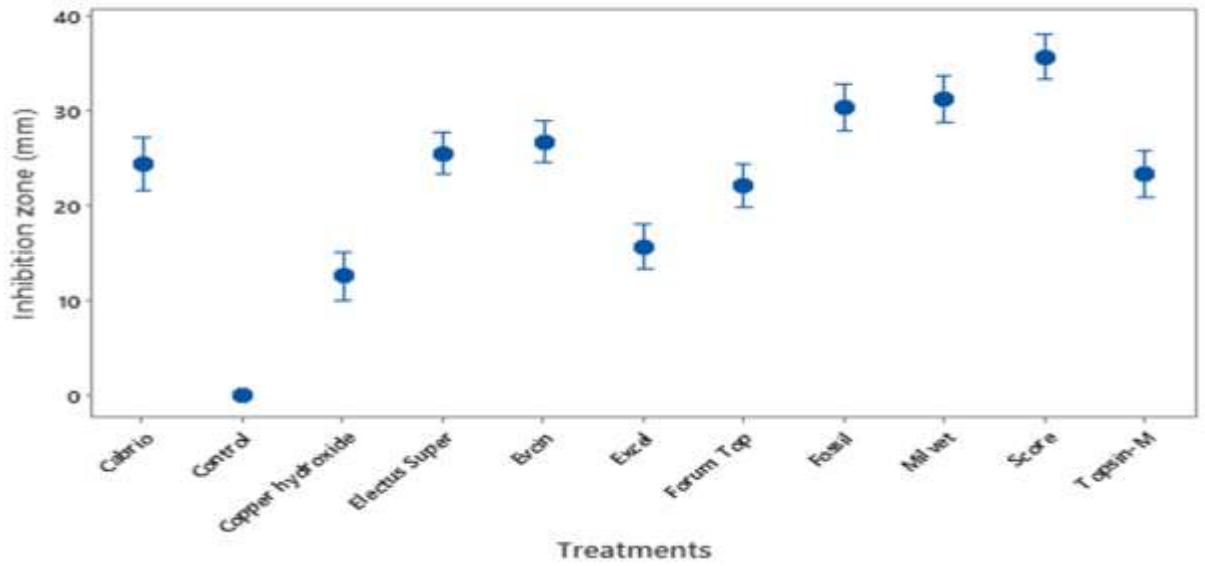

Figure 4. Effect of different chemicals on the inhibition zone of *X. campestris* pv. *vesicatoria* under lab conditions.

Table 3. Impact of different chemicals on inhibition zone of *X. campestris* pv. *vesicatoria* under lab conditions.

Treatments	Active ingredients (%)	Inhibition zone (mm)
Score	Difenoconazole (24.51%)	34.83a
Milvet	Sulfers	32.03b
Fossil	Difenoconazole + Azoxystrobin (29%)	30.75c
Cabrio	Pyrachlostrobin+ Metiram (60%)	29.05d
Evcin	Cosavet (80%)	26.50e
Electus super	Difenoconazole+ Azoxystrobin (30%)	26.38e
Topsin-M	Thiophenate-methyl (70%)	21.94f
Forum Top	BAS (53%)	20.66g
Excel	Azoxystrobin (80%)	18.16h
Copper hydroxide	Coside (52.4%)	17.50i
Control	Distil water	0.00j

On the other hand, the interactions between treatments and concentrations (T×C) showed that the minimum inhibition zone was expressed by Copper hydroxide (10.50 mm, 17.50 mm, 24.50 mm), Excel (11.50 mm, 17.66 mm, 25.33 mm), Forum Top (14.00 mm, 20.50 mm, 27.50 mm), Topsin-M (16.16 mm, 21.66 mm, 28.00 mm), Electus super (20.50 mm, 26.50 mm, 32.50 mm), Evcin (19.50 mm, 26.16 mm, 33.50 mm), Cabrio (22.50 mm, 29.50 mm, 35.16 mm), Fossil (27.00 mm, 32.61 mm, 36.50 mm), Milvet (24.77 mm, 30.66 mm, 36.83 mm), and Score (28.27 mm, 35.72 mm, 40.50 mm) at concentrations of 200 ppm, 400 ppm, and 600 ppm, respectively, as compared to the control (Table 3 and Figure 5). The interaction between treatments and durations revealed that the maximum inhibition zone was observed by Score (32.88 mm, 34.94 mm, 36.66 mm), Milvet (30.66 mm, 32.16 mm, 33.27 mm), Fossil (28.94 mm, 30.83 mm, 32.50 mm), Cabrio (27.00 mm, 29.00 mm, 31.16 mm), Evcin (24.16 mm, 26.50 mm, 28.50 mm), Electus super (24.66 mm, 26.50 mm, 28.33 mm), Topsin-M (20.33 mm, 21.83 mm, 23.66 mm), Forum Top (18.66 mm, 20.66 mm, 22.66 mm), Excel (16.33 mm, 18.16 mm, 20.00 mm), and Copper hydroxide (15.50 mm, 17.50 mm, 19.50 mm) after 24 hours, 48 hours, and 72 hours, respectively, as compared to the control (Table 3 and Figure 6).

Evaluation of chemicals and antibiotics against leaf spot of tomato under greenhouse conditions

Under controlled greenhouse conditions, the results showed that the least disease severity was observed

with Score + Neflox (7.50%), followed by Neflox (10.70%) and Score (13.46%), in comparison to the control (Table 4 and Figure 7). On the other hand, the interaction between treatments and concentrations (T×C) indicated that the maximum disease severity was observed with Score (6.00%, 10.50%, 15.61%), Neflox (9.00%, 13.50%, 17.88%), and Neflox + Score (3.00%, 7.33%, 12.00%) at 1%, 2%, and 2.5%, respectively, in comparison to the control (Table 4 and Figure 8). The interaction between treatments and days (T×D) revealed that the minimum disease severity was exhibited by Neflox + Score (6.00%, 7.33%, 9.16%), Neflox (9.11%, 10.66%, 12.33%), and Score (11.88%, 13.55%, 14.94%) after 5, 10, and 15 days, respectively, in comparison to the control (Table 4 and Figure 9).

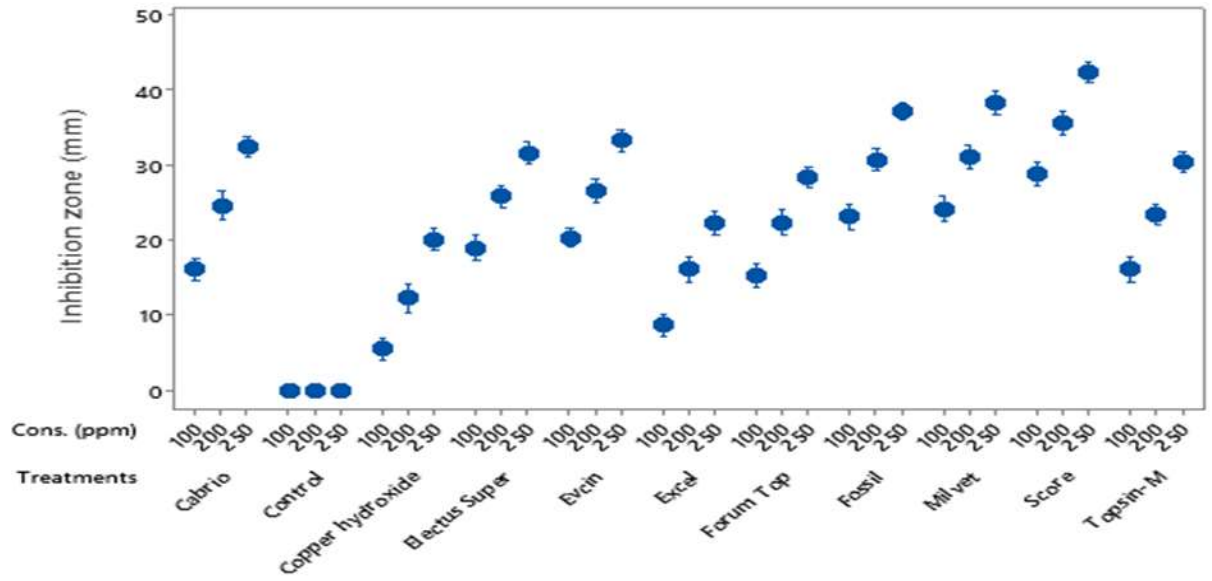

Figure 5. Impact of interaction between treatments and concentrations on development of inhibition zone for *X. campestris* pv. *vesicatoria* under lab conditions.

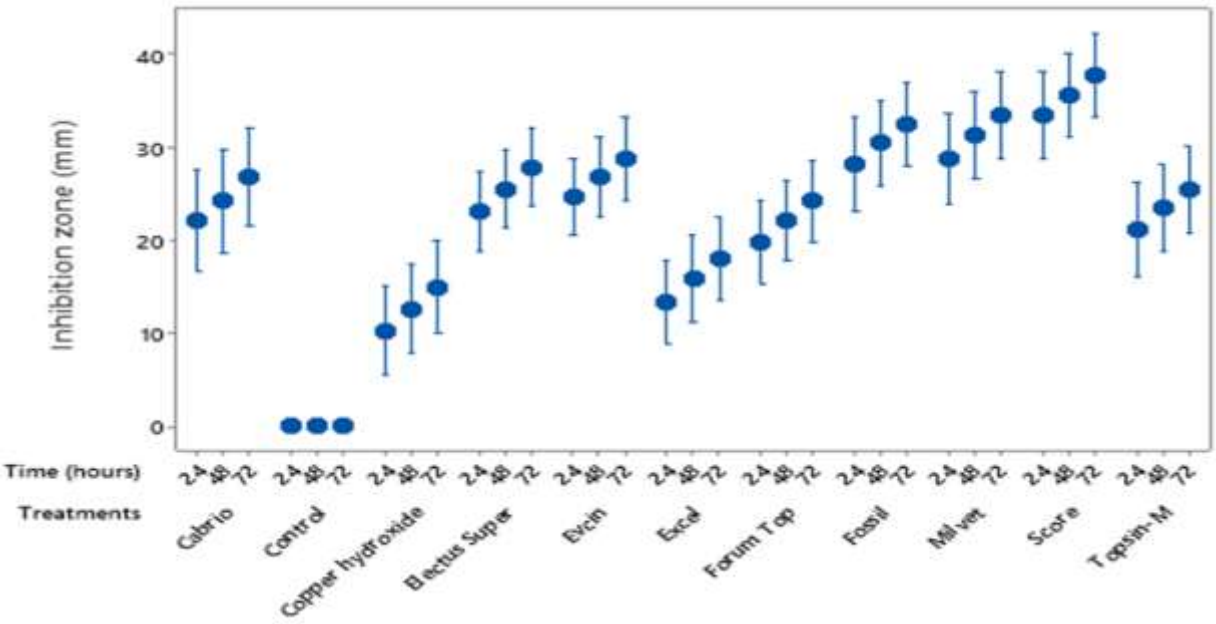

Figure 6. Impact of interaction between treatments and days on the development of inhibition zone of *X. campestris* pv. *vesicatoria* under lab conditions.

Table 4. Impact of different antibiotics and chemicals alone or in combination on disease severity of leaf spot of tomato under greenhouse conditions.

Treatments	Active ingredients (%)	Disease severity (%)
Score + Neflox	Difenoconazole (24.51%) + Florfenicol (23%)	7.50 d
Neflox	Florfenicol (23%)	10.70 c
Score	Difenoconazole (24.51%)	13.46 b
Control	Distil water	37.83 a
LSD	0.2554	

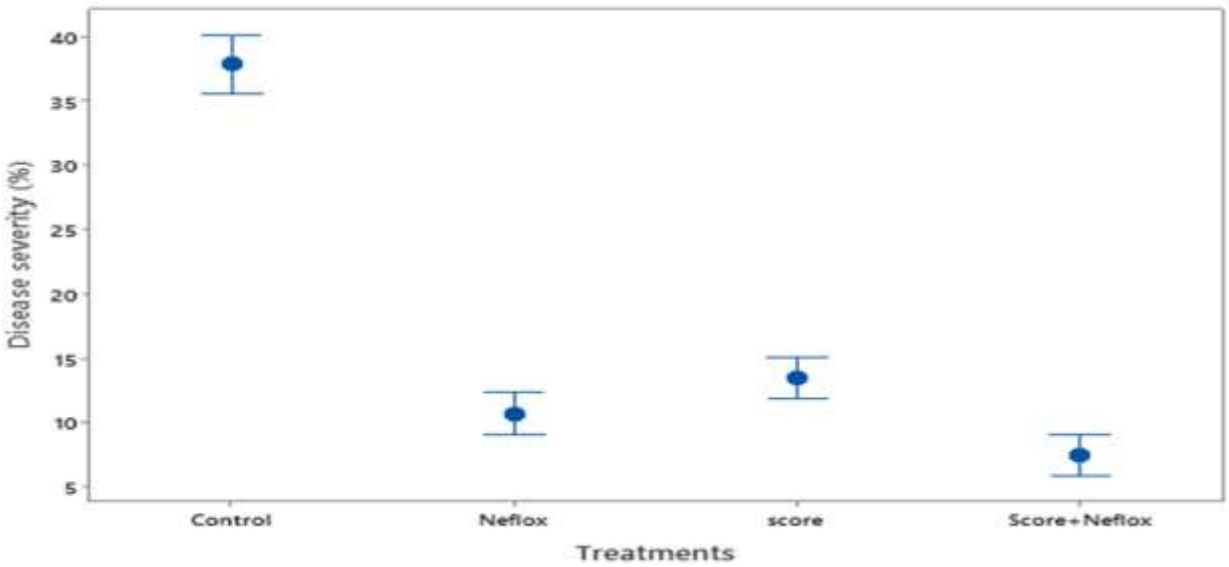

Figure 7. Evaluation of chemical and antibiotic alone or in combination against leaf spot of tomato under greenhouse conditions.

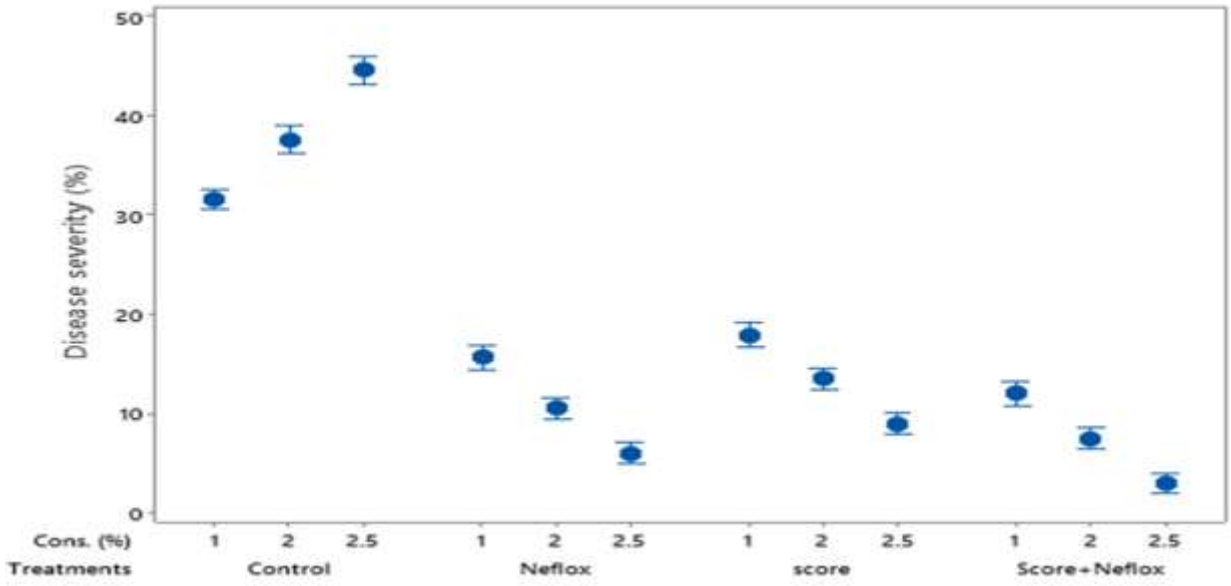

Figure 8. Impact of interaction between treatments and concentrations against leaf spot of tomato under greenhouse conditions.

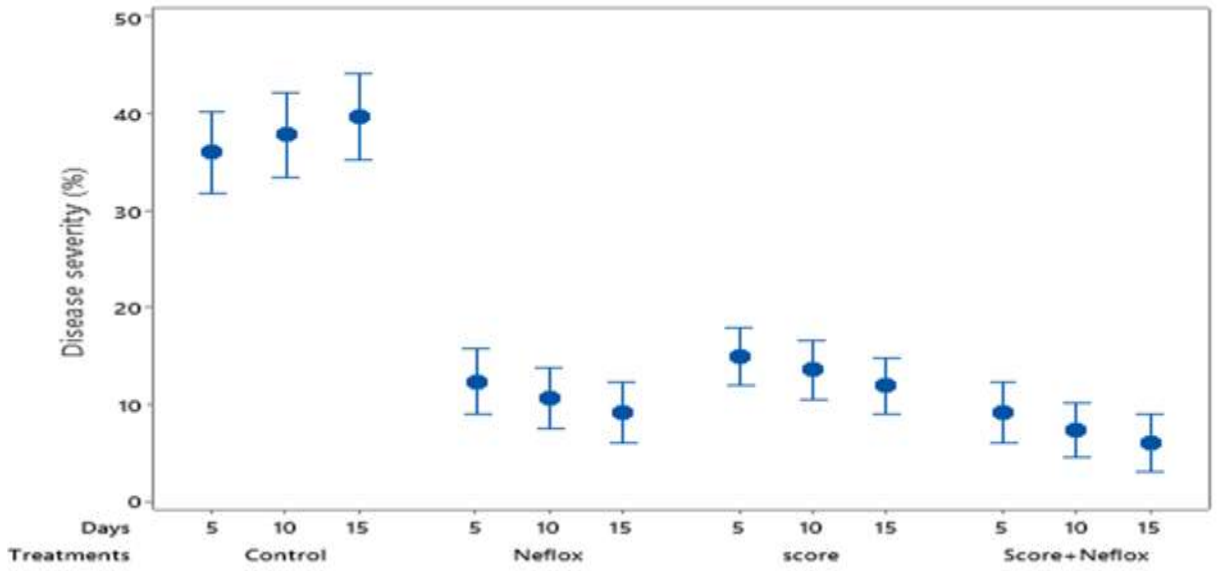

Figure 9. Impact of interaction between treatments and days on disease severity of bacterial leaf spot of tomato under greenhouse conditions.

DISCUSSION

Bacterial leaf spot of tomato is the most devastating disease of tomatoes, caused by *Xanthomonas campestris* pv. *vesicatoria* (Kebede et al., 2014; Potnis et al., 2015). It is responsible for a 50% reduction in tomato crop yield, with disease incidence ranging from 22% to 50% (Kavitha and Umesha, 2007; Abrahamian et al., 2019). Various management strategies, such as the use of chemicals, biocontrol agents, antibiotics, and resistant cultivars, have been employed by scientists to manage bacterial leaf spot of tomatoes (Horvath et al., 2012; Aslam et al., 2017a,b, 2019; Aslam and Mukhtar, 2023a,b; Saeed et al., 2023; Shahbaz et al., 2023; Yaseen et al., 2023). Among all these strategies, resistant varieties are the most reliable way to manage this disease. However, when the disease appears in epidemic form, farmers have no option but to use chemicals due to their quick action and easy availability (Trueman et al., 2019; Iqbal and Mukhtar, 2020). That is why in the present study, chemicals and antibiotics were evaluated for their effectiveness against bacterial leaf spot of tomatoes. Under laboratory conditions, Score exhibited the maximum inhibition zone, and among antibiotics, Neflox showed the largest inhibition zone. The combination of Score and Neflox exhibited the lowest disease incidence (%) under greenhouse conditions. The results of the present research were supported by the findings of Itako et al. (2015), who evaluated different chemicals against *X. campestris* pv. *vesicatoria* and described that Score showed significant antibacterial potential against the pathogen. Similarly, Vallad et al. (2010) evaluated various antibiotics and found that Neflox and Gentam exhibited strong antibacterial potential against *X. campestris* pv. *vesicatoria*. The results of the present study were also consistent with the findings of Fayette et al. (2012), who evaluated different antibiotics alone or in combination under greenhouse conditions with other chemicals and copper bactericides at three locations in Florida. They reported that the severity of bacterial leaf spot of tomatoes was reduced by up to 37.5% by antibiotics compared to non-treated plants. Likewise, Graves and Alexander (2002), Griffin et al. (2017), and Obradovic et al. (2005) also assessed combinations of antibiotics and chemicals, and they reported that Score and Neflox exhibited the least disease severity under greenhouse conditions.

The primary component of Score is difenoconazole, which is used to treat numerous plant pathogens. Difenoconazole is a broad-spectrum fungicide that functions as a systemic fungicide. It inhibits the biological process that converts lanosterol into ergosterol, the end product of sterol production. This process is known as C-14 demethylation of lanosterol or 24-methylenedihydrolanosterol (Koller and Scheinpflug, 1987). Neflox is a quinolone/fluoroquinolone antibiotic. Neflox is bactericidal, and its mechanism of action relies on inhibiting bacterial DNA replication by binding to an enzyme called DNA gyrase, which is necessary for

unwinding the DNA double helix during replication. Notably, the drug exhibits 100 times higher affinity for bacterial DNA gyrase than for mammalian DNA.

CONCLUSION

In laboratory conditions, Score and Neflox exhibited the maximum inhibition of *Xanthomonas campestris* pv. *vesicatoria*. In greenhouse conditions, the combination of Score and Neflox resulted in the least disease severity compared to other treatments. Therefore, it can be concluded that Score and Neflox are highly recommended for farmers for the management of bacterial leaf spot in tomatoes.

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AUTHORS' CONTRIBUTION

SN wrote manuscript and conducted research; MA conceived idea and supervised research; MJA edited manuscript; MMJ analyzed data; MDG helped in making graph; MA helped in laboratory experiment; RWK helped in data analysis; MJM helped in data collection; MUA helped in reference correction; MW helped in data collection.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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