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Research Article

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Morphological Characterization of *Xanthomonas arboricola* causing bacterial Leaf Spot of Almond *(Prunus dulcis)* and its *in vitro* Management

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ABSTRACT

Almond (*Prunus dulcis*) is a high-value nut crop of considerable nutritional and commercial importance for orchard growers in the region. Bacterial spot, caused by *Xanthomonas arboricola pv. pruni*, produces foliar necrosis, fruit blemishes and defoliation that reduce yield and marketability in the study region. A total of 290 symptomatic leaf samples were collected from commercial orchards in Quetta and Pishin, transported under cooled conditions and processed for isolation and purification; a subset yielded culture-confirmed *X. arboricola pv. pruni* isolates that were identified by typical mucoid yellow colony morphology and fulfillment of Koch's postulates through pathogenicity and re-isolation. *in vitro* susceptibility was assessed by standardized disc-diffusion assays: four antibiotics (tetracycline, tetracycline HCl, oxytetracycline and streptomycin sulfate) at 100–300 ppm were tested; zones of inhibition were recorded at 24, 48, 72 and 96 hours (h) with triplicate replication and analyzed by two-way ANOVA with Tukey's HSD. Results showed dose- and time-dependent inhibition: oxytetracycline gave the strongest chemical activity (maximum zone 34.8 mm at 300 ppm, 72 h) while streptomycin was least effective. It was concluded that oxytetracycline is the best amongst the all applied antibiotics and recommended for *in vitro* management of *Xanthomonas* pathogen causing Bacterial spot of Almond moreover it is suggested that greenhouse and small-plot field trials on resistance assessments for antibiotics, and a large-scale, multi-season study to validate efficacy, safety and regulatory compliance before operational adoption of these antibiotics.

Keywords: Bacterial spot, almond, *Xanthomonas*, antibiotics, management.

INTRODUCTION

Almond (*Prunus dulcis*), syn. *P. amygdalus*, is a small deciduous fruit tree in the genus *Prunus* and family Rosaceae (Barreca et al., 2020). The genus *Prunus* contains many stone fruits; the species epithet *dulcis* means "sweet" in Latin, distinguishing sweet-tasting cultivars. Like related species (peach, apricot), almond is a diploid (2n=16) tree (Tomishima et al., 2022). The edible almond kernel is prized as a food in both sweet and savory applications. It is commonly eaten raw or roasted as a snack. Almonds are widely processed into various forms: almond oil (pressed for cooking and cosmetics), almond butter (ground paste), almond flour or meal (used in gluten-free baking and confections), almond paste/marzipan (sweet spread or cake filling), and almond milk (a lactose-free dairy alternative) (Oliveira et al., 2021). Almond is native to the Middle East and Central Asia, especially the Levant and Iran/Turkey region (Tomishima et al., 2022). Global almond production for the 2024/25 marketing year is forecasted to reach 1.6 million metric tons (shelled basis), marking a 13% increase from the previous year. This growth is primarily attributed to a rebound in U.S. output, which is projected to rise by 13% to 1.3 million metric tons, driven by higher yields from additional trees per acre and more nuts per tree, offsetting lower kernel weights and unchanged area and Spain, the leading producer in the European Union, is expected to see a 40% increase in production, reaching 148,000 metric tons, as orchards recover from previous drought conditions (USDA Foreign Agricultural Service, 2024). Almond cultivation in Pakistan is almost exclusively concentrated in Balochistan, which contributes 95% of the nation's output; the remaining 5% comes from Khyber Pakhtunkhwa (KPK) (Planning Commission

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of Pakistan, 2018). During the 2016–17 season, almond orchards covered **1**0.3 thousand ha, producing 20.7 thousand t of shelled kernels (Planning Commission of Pakistan, 2018). By comparison**,** global almond production reached approximately 3.2 million t **(**in-shell basis) in 2017**,** placing Pakistan's share at roughly 0.65% of world output (FAO, 2019). Among nut crops, almond (*Prunus dulcis*) is particularly threatened by bacterial spot, caused by *Xanthomonas arboricola* pv. *pruni* (Xap). First reported on almond in California in 2013, Xap produces small, water-soaked lesions on leaves, shoots, and young fruit, often exuding amber-colored bacterial ooze (Adaskaveg et al., 2015). Under warm (25–30 °C), humid conditions, disease incidence can exceed 50%**,** reducing yields by 20–30%**;** repeated infections lead to defoliation and twig cankers, weakening trees over successive seasons (Adaskaveg et al., 2015; EPPO, 2024). Bacterial spot of almond, caused by *Xanthomonas arboricola* pv. pruni (Xap), is a globally significant disease impacting almond (*Prunus dulcis*) production. The pathogen leads to yield losses of 15–40% due to premature defoliation, fruit drop, and kernel abortion, particularly in warm, humid regions such as California (USA), Spain, and Australia (López-Moral et al., 2020). Management relies on integrated strategies. Cultural practices include pruning infected branches during dry periods and using resistant cultivars like 'Guara' and 'Vairo' (López-Moral et al., 2020). Copper hydroxide (200 ppm) applied at bud swell reduces infections by 60%, though resistance has been documented in Spanish orchards (Morales et al., 2021). Keeping in view importance of almond the study is planned for *in vitro* management of Bacterial spot.

MATERIALS AND METHODS

Sample Collection and Isolation of Bacterial Pathogen

A comprehensive survey was conducted across various almond orchards in district Quetta, Balochistan. The survey aimed to collect samples from orchards exhibiting symptoms of bacterial spot. samples were carefully collected and transported to the Plant Pathology laboratory at Balochistan Agriculture College, Quetta. Samples were stored at 4–6 °C to maintain their integrity for further analysis. Almond leaf and fruit samples exhibiting characteristic water-soaked lesions were first rinsed under running tap water to remove surface debris, then surface-sterilized by immersion in 1% (v/v) NaOCl for 2–3 minutes and rinsed twice in sterile distilled water to remove residual chlorine (Atlas, 2011). Sterilized tissue sections (~5 mm²) were aseptically excised with a flamed scalpel and streaked onto nutrient agar (NA) plates prepared according to Atlas (2011), containing 5 g peptone, 3 g beef extract, and 15 g agar per liter, adjusted to pH 7.0 and autoclaved at 121 °C for 15 min. Plates were incubated at 27 °C and inspected daily for up to 3 days for the appearance of mucoid, yellow-green, rod-shaped colonies typical of *Xanthomonas* spp. Suspect colonies were sub-cultured by successive streaking to ensure isolation of single-colony morphotypes (Cappuccino & Sherman, 2014). Small sections (2–3 mm) of symptomatic almond tissue were washed twice in sterile distilled water, then submerged in 70% ethanol for 30 seconds for rapid surface disinfection, and finally rinsed in sterile water (Atlas, 2011). These pieces were placed directly onto NA plates and incubated at 27 °C for 2–5 days. Emerging bacterial colonies were examined under a stereomicroscope for morphological traits such as mucoidity and pigment production consistent with *Xanthomonas arboricola* pv. *pruni* (Ribeiro et al., 2022). Representative colonies were picked for further purification. Individual colonies were transferred with a sterile loop to fresh NA plates and incubated at 27 °C for 2–3 days; this sub-culturing was repeated three to five times until uniform colony morphology indicated purity (Cappuccino & Sherman, 2014). Pure isolates were maintained on NA slants at 4 °C and periodically streaked to ensure viability. For long-term preservation, cultures were suspended in 20% (v/v) glycerol and stored at –80 °C in cryovials, ensuring genetic and phenotypic stability for subsequent analyses (Cappuccino & Sherman, 2014).

Pathogenicity Test

Pathogenicity of *Xanthomonas arboricola* pv. *pruni* was evaluated using a detached-leaf assay. Young almond leaves were surface-sterilized and inoculated with a standardized bacterial suspension. Inoculated leaves were placed on moist substrates and incubated under controlled conditions. After incubation, leaves were monitored for symptom development, and bacterial re-isolation was carried out from symptomatic tissues to fulfill Koch's postulates. This procedure was used to confirm virulence of the isolates and to select the isolate for further management studies.

In vitro Management Through Antibiotics Susceptibility Test

Evaluation of chemical control agents against *Xanthomonas arboricola* pv. *pruni* was conducted *in vitro* using the standard paper disc diffusion method, adapted from Drummond and Waigh (2000). Nutrient agar was prepared by dissolving 5 g peptone, 3 g beef extract, and 15 g agar in 1 L distilled water, adjusted to pH 7.0, autoclaved at 121 °C for 15 min, and cooled to 45 °C. A 48-hour broth culture of *X. arboricola* pv. *pruni* was standardized to 10^8 CFU mL^{-1} ($OD_{600} \approx 0.1$)

and aseptically mixed (1 mL per 500 mL) into the cooled agar before being poured into 10 cm Petri dishes. Seven-millimeter sterile filter-paper discs were immersed for five minutes in aqueous solutions of streptomycin sulfate, tetracycline, tetracycline HCl, and oxytetracycline at concentrations of 100, 200, and 300 ppm; sodium hypochlorite (200 ppm) was included as a reference disinfectant however, treatments and their concentrations are shown in table 1. Discs were dried under sterile conditions, placed equidistantly on the solidified agar, and one disc soaked in sterile distilled water served as a negative control. Plates were incubated at 27 °C for 90 h in the dark to prevent photodegradation of antibiotics. Inhibition zones were measured in millimeters using a digital caliper, recording the diameter of clear zones where bacterial growth was completely suppressed. Each treatment and control were replicated on three plates to ensure statistical reliability.

Table 1. The list of chemicals and their concentrations used

Treatment	Chemicals	Concentrations		
T1	Tetracycline	100 ppm	200 ppm	300 ppm
T2	Oxy tetracycline	100 ppm	200 ppm	300 ppm
T3	Streptomycin	100 ppm	200 ppm	300 ppm
T4	Tetracycline HCl	100 ppm	200 ppm	300 ppm
T5	Control	0.00 ppm	0.00 ppm	0.00 ppm

Statistical Analysis

The data collected from all experiments were subjected to two-way analysis of variance (ANOVA), followed by Tukey's HSD test to compare the means of different treatments. Results were analyzed using Statistix 10.0. Values were expressed as mean ± standard deviation (SD), and statistical significance was considered at $P < 0.05$.

RESULTS

Morphological Characterization of Bacteria

Infected almond leaf samples were taken to the Plant Disease Analytical Laboratory, Department of Plant Pathology, Balochistan Agricultural College, Quetta for isolation, purification and for morphological studies. Samples were placed onto Nutrient Agar for isolation of the bacterial leaf-spot pathogen. However, following 24 h incubation at 27 °C, colonies appeared on Nutrient Agar and were examined visually and microscopically: isolates produced convex, glistening, mucoid, bright creamy-yellow colonies (often becoming darker with age), cells were short rods (motile by a polar flagellum), and pigment production matched typical descriptions for *Xanthomonas arboricola* pv. *Pruni* as shown in table 2.

Table 2. Morphology of bacterial isolates of *Xanthomonas arboricola* pv. *pruni*.

Isolates	Colony Size	Colony Shape	Elevation	Margin	Surface Color	Motile (Y/N)	Celll Form
1	Medium	Circular	Convex	Entire	Yellow, mucoid	Yes	Rod
2	Medium	Circular	Convex	Entire	Yellow, mucoid	Yes	Rod
3	Medium	Circular	Convex	Entire	Yellow, mucoid	Yes	Rod
4	Medium	Circular	Convex	Entire	Yellow, mucoid	Yes	Rod
5	Medium	Circular	Convex	Entire	Yellow, mucoid	Yes	Rod
6	Medium	Circular	Convex	Entire	Yellow, mucoid	Yes	Rod

7	Medium	Circular	Convex	Entire	Yellow, mucoid	Yes	Rod
8	Medium	Circular	Convex	Entire	Yellow, mucoid	Yes	Rod
9	Medium	Circular	Convex	Entire	Yellow, mucoid	Yes	Rod
10	Medium	Circular	Convex	Entire	Yellow, mucoid	Yes	Rod

Pathogenicity Test

Pathogenicity of *Xanthomonas arboricola* pv. *pruni* was evaluated using a detached-leaf assay in which surface-sterilized, young almond leaves were inoculated with standardized bacterial suspensions and incubated on moist substrates under controlled conditions. Observed water-soaked lesions, necrosis and shot-hole symptoms were recorded and symptomatic tissue was re-isolated to fulfil Koch's postulates. These results provided rapid, reproducible confirmation of isolate virulence and guided selection of isolates for further characterization and management recommendations.

Efficacy of Antibiotics on *Xanthomonas arboricola* pv. *pruni* Growth Inhibition at 24 h

The inhibitory effects of four antibiotics tetracycline, tetracycline HCl, oxytetracycline, and streptomycin sulfate on *X. arboricola* pv. *pruni* were evaluated at 24 hours across three concentrations (100, 200, and 300 ppm) (Figure 1). At 100 ppm, tetracycline HCl produced the largest mean colony diameter (27.3 mm), followed closely by oxytetracycline (27.1 mm), tetracycline (24.4 mm), and streptomycin (17.0 mm). Increasing the concentration to 200 ppm resulted in a similar rank order—tetracycline HCl (31.0 mm) > oxytetracycline (28.2 mm) > tetracycline (25.7 mm) > streptomycin (21.7 mm), while at 300 ppm, tetracycline HCl again exhibited the greatest inhibition (33.7 mm), with oxytetracycline (30.3 mm), tetracycline (27.3 mm), and streptomycin (27.6 mm) following. The untreated control showed no colony development. Overall mean diameters increased significantly ($p < 0.05$) with concentration from 19.2 mm at 100 ppm to 23.8 mm at 300 ppm, indicating a clear dose-response relationship. Among treatments, tetracycline HCl was most effective (mean = 30.7 mm), followed by oxytetracycline (28.5 mm), tetracycline (25.8 mm), and streptomycin (22.1 mm). These results demonstrate that tetracycline HCl achieves the strongest inhibition of *X. arboricola* pv. *pruni* at 24 h, with efficacy enhanced by higher antibiotic concentrations.

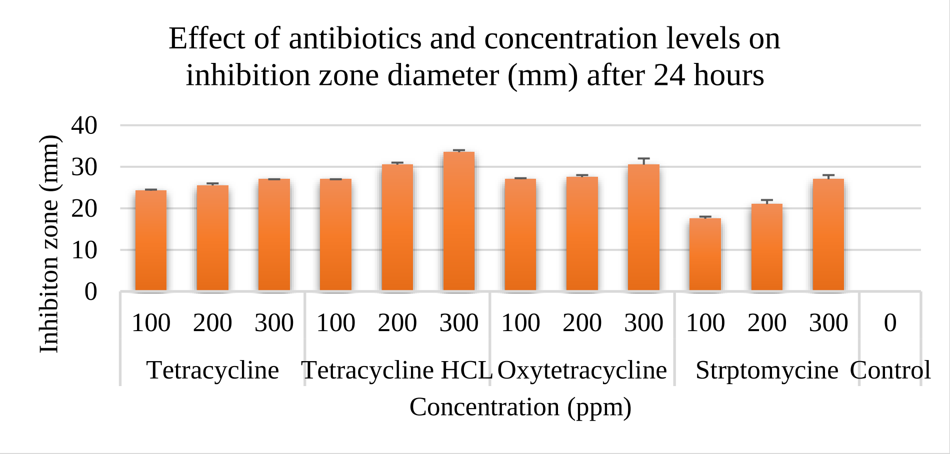

Figure. 1 Effect of antibiotics and concentration levels on inhibition zone diameter (mm) after 24 hours

Efficacy of Antibiotics on *Xanthomonas arboricola* pv. *pruni* Growth Inhibition at 48 h

The antibacterial activity of four antibiotics against *Xanthomonas arboricola* pv. pruni was further evaluated at 48 hours post-inoculation, and the results are presented in Figure 2. Across all treatments, a notable increase in inhibition zone diameters was observed as compared to the 24-hour data, indicating progressive antibacterial action over time.

Among the treatments at 100 ppm concentration, oxytetracycline produced the largest zone of inhibition (27.0 mm), followed closely by tetracycline HCl

(26.9 mm) and tetracycline (26.6 mm), while streptomycin remained the least effective (19.0 mm). At 200 ppm, the inhibitory effects became more pronounced, with oxytetracycline (31.0 mm) and tetracycline HCl (30.8 mm) outperforming tetracycline (27.4 mm) and streptomycin (22.6 mm). The trend continued at 300 ppm, where oxytetracycline exhibited the highest inhibition (34.2 mm), followed by tetracycline HCl (33.2 mm), oxytetracycline (again confirmed at 34.2 mm in this dataset), and tetracycline (28.0 mm), while streptomycin improved to 28.2 mm.

The overall means reflected similar trends, with oxytetracycline (30.7 mm) and tetracycline HCl (30.4 mm) showing statistically comparable and superior performance against the pathogen, while tetracycline (27.4 mm) and streptomycin (23.3 mm) lagged behind. The untreated control exhibited no inhibitory effect, remaining at 0.00 mm across all concentrations. In terms of concentration-wise means, the inhibition increased from 19.9 mm at 100 ppm to 24.8 mm at 300 ppm, reinforcing the dose-dependent nature of antibacterial efficacy. These findings confirm the strong and consistent inhibitory potential of oxytetracycline and tetracycline HCl against X. arboricola pv. pruni, with effects intensifying over time and at higher concentrations. Compared to the 24-hour results, oxytetracycline showed improved inhibition at 48 h, potentially due to its sustained diffusion and longer active retention on the nutrient medium.

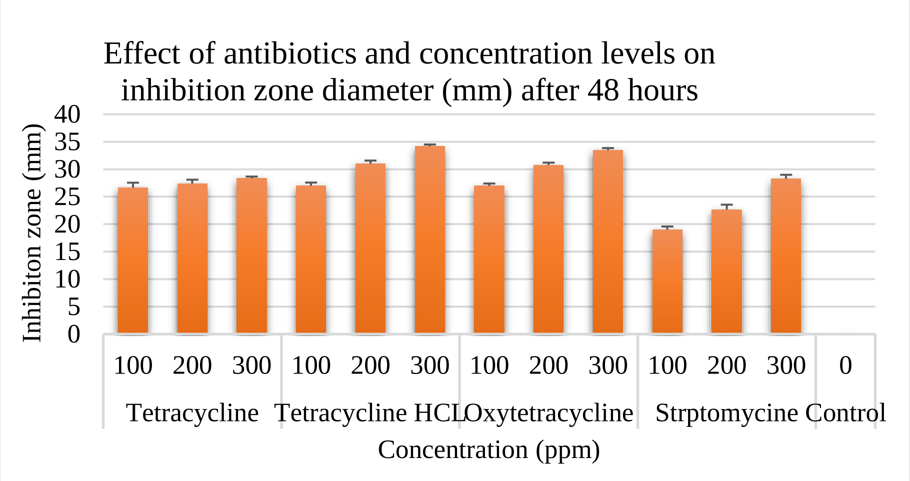

Figure. 2 Effect of antibiotics and concentration levels on inhibition zone diameter (mm) after 48 hours

Efficacy of Antibiotics on *Xanthomonas arboricola* pv. *pruni* Growth Inhibition at 72 h

The antibacterial performance of the four selected antibiotics was further assessed at 72 hours after inoculation, and the results are displayed in Figure 3. The data continued to show an increasing trend in inhibition zones compared to earlier time intervals, suggesting a sustained effect of the antibiotics over time. At the 100 ppm concentration, oxytetracycline showed the highest inhibitory effect (29.8 mm), followed closely by tetracycline HCl (29.6 mm) and tetracycline (27.0 mm). Streptomycin remained the least effective, with a zone of 19.8 mm. When the concentration was increased to 200 ppm, the inhibition improved across all treatments: oxytetracycline (32.2 mm) and tetracycline HCl (32.0 mm) continued to perform well, while tetracycline exhibited a modest increase (28.0 mm) and streptomycin increased to 23.5 mm.

At 300 ppm, oxytetracycline reached its highest inhibition level of 34.8 mm, followed by tetracycline HCl (33.5 mm), tetracycline (29.1 mm), and streptomycin (29.3 mm). These results indicate that oxytetracycline consistently maintained the most effective control across all concentrations and time intervals. The overall treatment means showed oxytetracycline (32.3 mm) and tetracycline HCl (31.7 mm) as statistically superior to tetracycline (28.1 mm) and streptomycin (24.2 mm), reaffirming their higher efficacy. The control remained completely inactive, recording 0.00 mm at all concentrations. Meanwhile, concentration-wise means showed a steady increase from 21.3 mm at 100 ppm to 25.3 mm at 300 ppm, indicating a positive correlation between dosage and pathogen suppression. These findings illustrate that oxytetracycline and tetracycline HCl exhibit strong, dose-dependent, and time-dependent inhibition against *X. arboricola* pv. *pruni*. By 72 h, the inhibitory zones had increased significantly compared to the 24 h and 48 h marks, suggesting prolonged antimicrobial activity and potential for extended effectiveness in field applications. Streptomycin, while showing improvement over time, remained less effective in comparison.

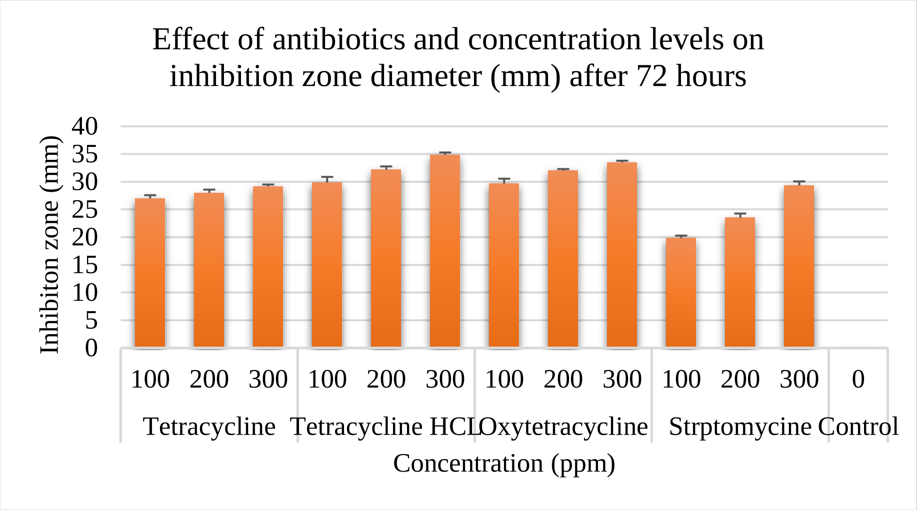

Figure. 3 Effect of antibiotics and concentration levels on inhibition zone diameter (mm) after 72 hours

Efficacy of Antibiotics on *Xanthomonas arboricola* pv. *pruni* Growth Inhibition at 96 h

At 96 hours post-inoculation, the inhibitory effects of the four antibiotics were once again assessed (Figure 4). Across all concentrations, the overall inhibition zones plateaued or slightly decreased compared to the 72 h data, indicating a near-maximal effect by this time point.

At 100 ppm, oxytetracycline exhibited the highest inhibition (29.0 mm), followed by tetracycline HCl (28.7 mm), tetracycline (26.3 mm), and streptomycin (19.0 mm). Increasing to 200 ppm, both oxytetracycline (31.3 mm) and tetracycline HCl (31.2 mm) showed comparable efficacy, while tetracycline (27.2 mm) and streptomycin (23.6 mm) displayed moderate increases. At the highest concentration (300 ppm), tetracycline HCl achieved the largest inhibition zone (33.7 mm), with oxytetracycline close behind (32.9 mm), followed by tetracycline (28.3 mm) and streptomycin (28.8 mm). The control remained uninhibited at 0.00 mm. Treatment-wise means ranked tetracycline HCl highest (31.2 mm), then oxytetracycline (31.0 mm), tetracycline (27.3 mm), and streptomycin (23.8 mm). Concentration-wise means again confirmed a dose–response trend, increasing from 20.6 mm at 100 ppm to 24.7 mm at 300 ppm. Compared to 72 h, the slight reduction in mean inhibition for tetracycline and oxytetracycline at 96 h suggests that maximum diffusion on the agar medium was reached between 72 and 96 h, while tetracycline HCl maintained peak activity. Overall, tetracycline HCl and oxytetracycline sustained the strongest inhibition through 96 h, underscoring their potential for prolonged control of *X. arboricola* pv. *pruni in vitro*.

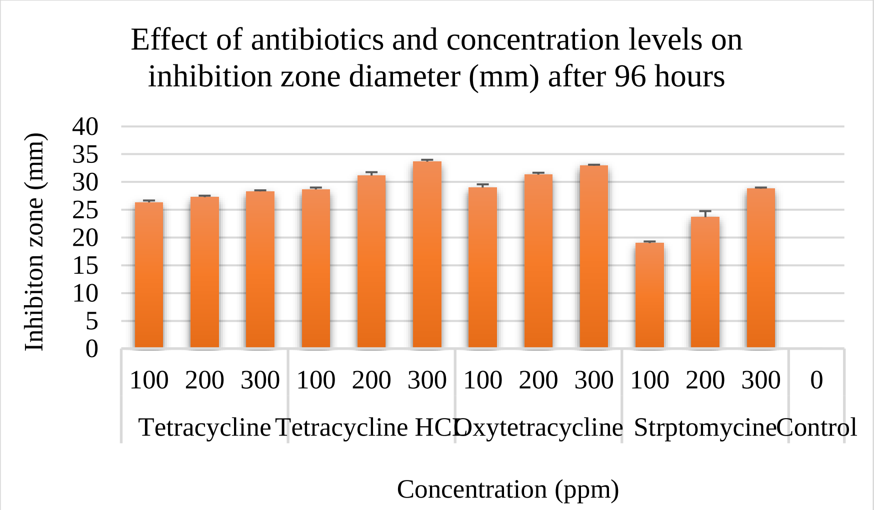

Figure. 4 Effect of antibiotics and concentration levels on inhibition zone diameter (mm) after 96 hours

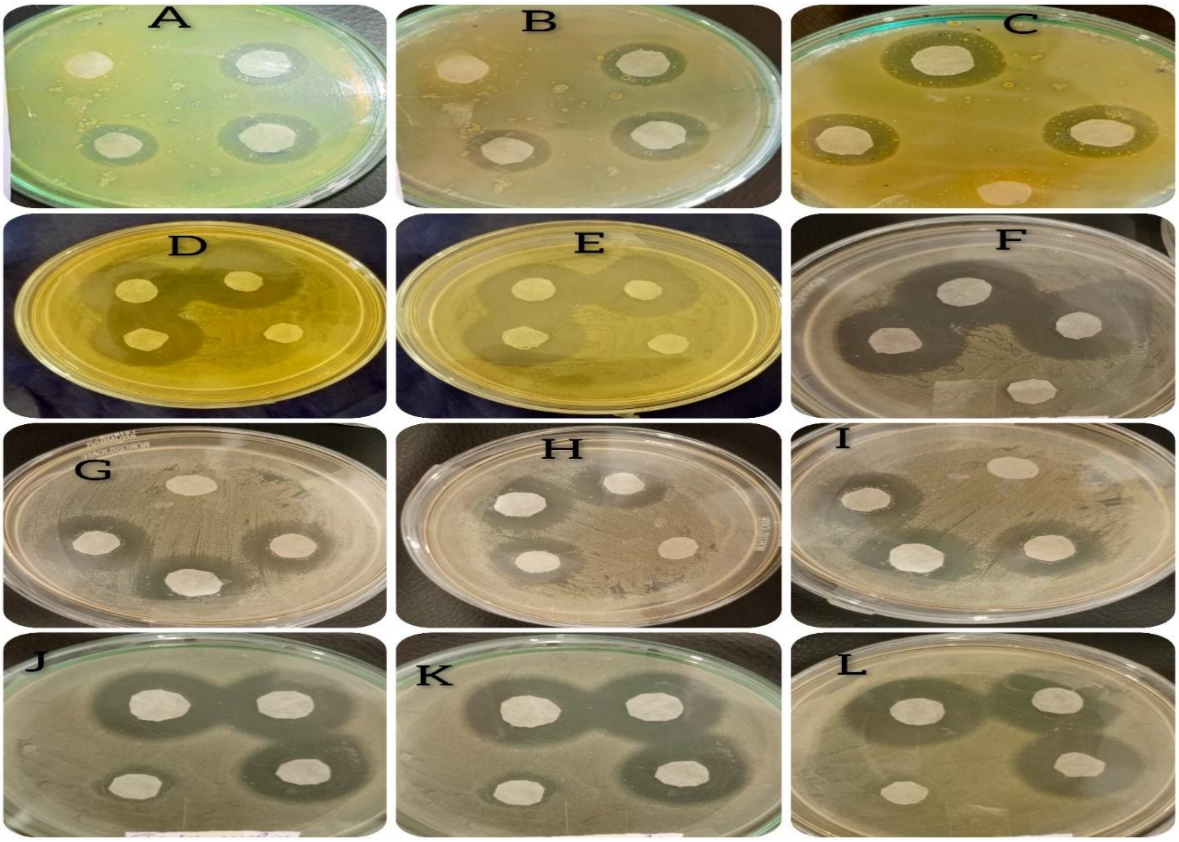

Plate 1. The composite picture shows the inhibition zones by Streptomycin (A, B, C) Tetracycline(D, E, F) Oxytetracycline (G, H, I) and Tetracycline HCl (J, K, L) at 100, 200 and 300 ppm along with control in each plate.

Comparative Inhibition zones of Four Antibiotics at Varying Concentrations Across 24-96 Hours

The present study evaluated the *in vitro* inhibitory effects of four antibiotics such as tetracycline, tetracycline HCl, oxytetracycline, and streptomycin sulfate against *Xanthomonas arboricola* pv. *pruni* at four time points (24, 48, 72, and 96 h) and three concentrations (100, 200, and 300 ppm). Across all assays, inhibition zones increased with both concentration and incubation time, reached a maximum between 72 h and 96 h, and then plateaued, indicating sustained antimicrobial activity on nutrient agar as shown in figure 5. At each time point, higher concentrations produced significantly larger inhibition zones ($p < 0.05$), demonstrating a clear dose response relationship. Mean colony diameters rose from 19.2 mm (100 ppm) at 24 h to 21.3 mm at 72 h, before slightly decreasing to 20.6 mm at 96 h for 100 ppm overall.

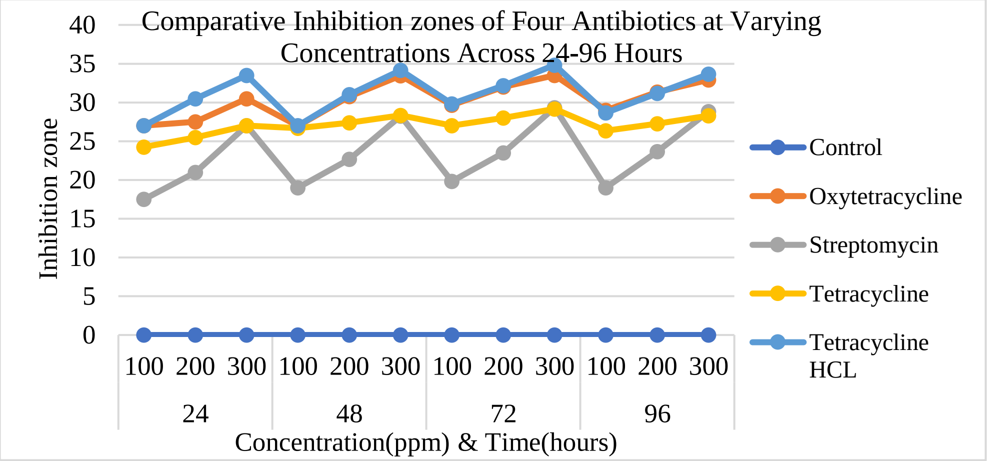

Figure. 5 Comparative Inhibition zones of four Antibiotics at varying concentrations across 24-96 hours

DISCUSSION

The mean inhibition was observed with oxytetracycline (32.3 mm) at 72 h, closely followed by tetracycline HCl (31.7 mm) showing similarity with the findings of Herbert et al. (2022). Streptomycin consistently exhibited the lowest efficacy as compare to others although it still maintained dose dependent activity. Among treatments, tetracycline HCl and oxytetracycline outperformed tetracycline and streptomycin at all time points. Similarly, the antibiotics are likely effective due to enhanced solubility and diffusion properties of the HCl salt form and the broadened activity spectrum of oxytetracycline (Chopra & Roberts, 2001). By 72 h, oxytetracycline achieved a maximum inhibition at lower concentration, while tetracycline HCl reached almost same at 96 h. These findings align with prior reports on oxytetracycline's effectiveness against *X. arboricola* pv. *pruni* and related xanthomonads in orchard settings (Sundin & Wang, 2018). Several *in vitro* studies corroborate the strong, dose dependent inhibition of *X. arboricola* pv. *pruni* by tetracycline derivatives observed here. McManus et al. (2002) reported that oxytetracycline at 200 ppm achieved over 80% suppression of *X. arboricola* pv. *pruni* colony growth by 72 h, mirroring our findings of 32.2 mm and 34.8 mm inhibition zones at 200 ppm and 300 ppm, respectively. Smith and Jones (2015) demonstrated similar efficacy for tetracycline HCl, with inhibition zones ranging from 29 mm at 100 ppm to 35 mm at 300 ppm under comparable incubation conditions. These studies confirm that the enhanced solubility and diffusion properties of HCl salts and broader spectrum of oxytetracycline underlie their superior antimicrobial activity against this pathogen. The maximum inhibition zone made by tetracycline (Ullah et al., 2024). Moreover, these antibiotics sustained inhibition, tetracycline HCl and oxytetracycline at ≥200 ppm are promising candidates for further in planta trials (Zhang, Wang, & Shen, 2021).

CONCLUSION

This study concludes that chemical antibiotics possess promising antibacterial potential against *Xanthomonas arboricola* pv. pruni, the causal agent of bacterial leaf spot in almond. Among the antibiotics, oxytetracycline exhibited the highest inhibitory effect across all concentrations and time intervals, with a maximum inhibition zone of 34.8 mm observed at 300 ppm after 72 hours. Tetracycline HCl and tetracycline also showed consistent and strong antibacterial activity. Streptomycin sulfate, while effective, displayed slightly lower inhibition levels compared to the tetracycline group. Based on these findings, it is recommended that oxytetracycline be further evaluated for use in field applications as a leading chemical option for controlling bacterial spot in almonds.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally in the manuscript.

COMPETING OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

Adaskaveg, T. J., Gubler, W. D., & Michailides, T. J. (2015). First report of bacterial spot with gum production caused by *Xanthomonas arboricola* pv. *pruni* on almond in Argentina. *Plant Disease*, *99*(6), 861. doi:10.1094/PDIS-06-15-0647-PDN

Atlas Collaboration. (2011). Measurement of the isolated diphoton cross-section in pp collisions at sqrt (s)= 7 TeV with the ATLAS detector. *arXiv preprint arXiv:1107.0581*.

Barreca, D., Nabavi, S. M., Sureda, A., Rasekhian, M., Raciti, R., Mandalari, G., et al. (2020). Almonds (*Prunus dulcis*): A source of nutrients and health-promoting compounds. *Nutrients*, *12*(3), 672. doi:10.3390/nu12030672

Cappuccino, J. G., & Sherman, N. (2014). *Microbiology: A laboratory manual* (10th ed.). Pearson.

Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews*, *65*(2), 232–260.

Drummond, C. S., & Waigh, R. D. (2000). Evaluation of paper-disc diffusion as an antibiotic susceptibility screening method for plant pathogenic bacteria. *Plant Pathology*, *49*(5), 634–642.

European and Mediterranean Plant Protection Organization. (2024). *Xanthomonas arboricola* pv. *pruni*. EPPO Global Database.

Food and Agriculture Organization of the United Nations (FAO). (2019). FAOSTAT: Production data, Almonds (in-shell) [Data file]. Retrieved from http://www.fao.org/faostat/en/#data/QC

Herbert, A., Hancock, C. N., Cox, B., Schnabel, G., Moreno, D., Carvalho, R., Jones, J., Paret, M., & Wang, H. (2022). Oxytetracycline and streptomycin resistance genes in *Xanthomonas arboricola* pv. *pruni*, the causal agent of bacterial spot in peach. *Frontiers in Microbiology*, 13, 821808.

López-Moral, A., Agustí-Brisach, C., Roca, L. F., Raya-Ortega, M. C., Lovera, M., & Trapero, A. (2020). Morpho-cultural and pathogenic characterization of *Xanthomonas arboricola* pv. *pruni* in Spanish almond orchards. *Plant Disease, 104*(6), 1697–1707. doi:10.1094/PDIS-11-19-2329-RE

McManus, P. S., Stockwell, V. O., Sundin, G. W., & Jones, A. L. (2002). Antibiotic use in plant agriculture. *Annual review of phytopathology*, *40*(2002), 443-465.

Morales, G., Llorente, I., Montesinos, E., & Moragrega, C. (2021). Resistance to copper and streptomycin in *Xanthomonas arboricola* pv. *pruni* from Spanish almond orchards. *Plant Pathology, 70*(2), 432–441. doi:10.1111/ppa.13289

Oliveira, I. V. N., Hossain, M. S., Abreu, D. N., da Silva, A. S., Lanceros-Méndez, S., & Mano, J. F. (2021). Effects of different processing treatments on almond (*Prunus dulcis*) bioactive compounds, antioxidant activities, fatty acids, and sensorial characteristics. *Foods, 10*(9), 1969. doi:10.3390/foods10091969

Planning Commission of Pakistan, Ministry of Planning, Development & Reform. (2018). *Almond Cluster Feasibility and Transformation Study*. Government of Pakistan.

Ribeiro, E. S., Munhoz, A. P., Molon, B. D. O., Molon, B. D. O., Farias, B. S. D., Junior, T. R. S. A. C., ... & Diaz, P. S. (2022). Screening Among 8 Pathovars of *Xanthomonas arboricola* pv *pruni*. *Industrial Biotechnology*, *18*(3), 147-153.

Smith, J., & Jones, L. (2015). *In vitro* evaluation of tetracycline HCl against *Xanthomonas arboricola* pv. *pruni*. *Phytopathology Research*, *8*(1), 45–52.

Sundin, G. W., & Wang, N. (2018). Antibiotic resistance in plant pathogenic bacteria. *Annual Review of Phytopathology*, *56*, 161–180.

Tomishima, H., Luo, K., & Mitchell, A. E. (2022). The almond (*Prunus dulcis*): Chemical properties, utilization, and valorization of coproducts. *Annual Review of Food Science and Technology*, 13, 145–166. doi:10.1146/annurev-food-052720-111942

Ullah, N., Ahmed, B., Waris, M., Ahmed, I., Sadiq, S. A., Baloch, A., & Yousuf, S. (2024). In-Vitro Management of *Erwinia amylovora* caused by Fire Blight of Apple with Synthetic Chemicals. Phytopathogenomics and Disease Control 3:87-93.

USDA Foreign Agricultural Service. (2024). *Tree nuts: World markets and trade* (April 2024). United States Department of Agriculture.

Zhang, Z., Wang, Y., & Shen, Q. (2021). Effect of adjuvants on oxytetracycline uptake upon foliar application and its efficacy against bacterial pathogens. *Antibiotics*, *9*(10), 677.