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Research Article

Impact of Plant Extracts and Chemicals against Bacterial Blight of Pomegranate

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Abstract

Bacterial blight of pomegranate caused by *Xanthomonas axanopodis* pv. *puniceae*, is devastating disease causing huge losses to the production especially in pakistan last few years. It is caused by a bacterium *Xanthomonas axanopodis* pv. *puniceae* which damage the crop severely in favorable environment conditions. Current research was conducted to evaluate four chemicals; Streptomycin, Copper oxy-chloride, kasugmicine, Oxy-tetracycline and four plant extracts; *Citrullus colocynths, Calotropis gigantea, Accica nelotica*, and *Moringa oleifera* against *Xanthomonas axanopodis* pv. *puniceae*.. In Laboratory conditions result showed that maximum inhibition zone of bacterial growth was expressed by streptomycin sulphate (38mm) followed by copper oxycholoride (38mm), kasugmicine (34mmm), and oxy-tetracycline (24mm) respectively at 3% concentration after 72 hours' time interval. In plant extracts, maximum inhibition was expressed by *Moringa olifera* (27.6mm) followed by *Accica nelotica* (26.3mm), *Citrullus colocynths* (16.6mm), and *Calotropis gigantea* (19.3mm) at 25%, 35% and 45% concentration. In laboratory conditions significant results shown by chemical (copper oxycholoride) and plant extract (*Moringa olifera*) Copper oxycholoride were applied in greenhouse. The copper oxychloride shows lowest disease severity (23%) while *Moringa olifera* 36% as compared to positive and negative control.

Keywords: Pomegranate; Chemicals; Plant extracts; Bacteria; Inhibition zone; *Xanthomonas axanopodis* pv. *puniceae;* Greenhouse.

Introduction

Pomegranate (*Punica granatum* l., *puniceae*) is highly nutritional and an important horticultural fruit crop which is grown on tropical and subtropical region (Kumar *et al.*, 2021). It is used for the treatment of various diseases such as atherosclerosis, breast cancer, skin cancer etc. (Amir *et al.*, 2019). High-quality pomegranate fruits are grown in dry climate (38 °C) (Viuda-Martos *et al.*, 2010). Pakistan ranked at 10th number with 54.3,000 tons production, cultivated on 13.4000 hectares area (Ali *et al.*, 2020). The disease

bacterial blight of pomegranate is caused by *Xanthomonas axanopodis* pv. *punicae* was reported first time 1992 in Pakistan, it causes 60–80% losses in favorable environmental conditions. The bacterium *Xanthomonas axanopodis* pv. *punicae* is gram-negativ and non-spore forming, with light yellow, round, convex, and smooth colonies. This is airborne, soil borne and seed born pathogen which enter in host through wounds or natural openings and it disseminate through rain, air, irrigation water, plant debris, insect vectors and equipment (Sharma *et al.*, 2017; Chowdappa *et al.*, 2018). The symptoms produced by this disease are water soaked lesion, asymmetrical to rounded black spots on leaves, dark brown to black necrotic area with yellow halo, large dark areas and silver bacterial ooze (Singh *et al.*, 2020). In the early stages, (*XAP*) affects stem, leaves and fruits and causes small leaf spots later on defoliation occurs, in severe attack L- or Y-shaped cracks are produced on fruit and cankerous lesion on the stem (Ippikoppa *et al.*, 2017). There are various method used for the management of bacterial blight of pomegranate i.e. biological, cultural and chemical control. Injudicious use of chemical affects the soil biodiversity and ecosystem and develops chemical resistance in pathogens also harmful effects for animals and plants (Brown *et al.*, 1990). In biological control which is safe and environment friendly, various strains of the Bacillus and Streptomyces have antimicrobial effect and attract the global interest for sustainability and disease management (Madhiazhagan *et al.*, 2002). Current research was designed for the management of bacteria blight of pomegranate through chemicals and plant extract and their combination which may be helpful for sustainable agriculture.

Methodology

Sample collection isolation and purification

Disease sample of Qandhari, sawa, sidhura were collected from pomegranate orchard in different areas of Ali Pur, Shujabad, Multan and Mailsi in 2022-23. Samples were sterilized with 1% sodium hypochlorite, drying on blotter paper and inoculate on prepared nutrient agar media in petri plates. Petri plates were labeled and placed in the incubator at 28-30 ◦ C temperature for 48-72 hours' time interval for identification and purification.

Pathogenicity

Pathogenicity test was performed in greenhouse on three month pomegranate plants through syringe and spraying method. Relative humidity was maintained by humidifier to increase the chances of disease. Plant sprayed with distilled water was kept as a control.

Preparation of plants extracts

Four different extracts of *Acacia nilotica* (Keeker), *Calotropis procera* (Ak Plant) and *Moringa oleifera* (Moringa Plant) and fruit of *Citrullus colocynthis* (Bitter apple) were evaluated to check their antimicrobial properties. The leaves of these different plants were washed with tap water and dried in shade for two weeks. In case of *C. colocynthis*, the fruits were cut into half and shade dried for 3 weeks. The dried leaves and fruits of plants were grinded into fine powder. The equal weights of powder and distilled water were mixed and placed in incubator shaker at 150 rpm for 24 hours. Then, filtrate of aqueous extracts was obtained through muslin cloth and Whatman filter paper 44 after 6 hours. Three concentrations 25, 35 and 45% were prepared by adding 25, 35 and 45 mL of stock plant extracts in 75, 65, and 55 mL of distilled water, respectively.

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In vitro evaluations of plants extracts

The efficacy of different plant extracts (*Calotropis gigantean, Moringa oleifera, Acacia nelotica, Citrullus colocynths)* was checked at 25%, 35%, and 45% concentration against *Xanthomonas axanopodis* pv. *puniceae* through inhibition zone technique. A well was made in the center of petri plate with the help of cork borer and the extracts were poured in well with the help of 1000 µl pipette, allowed to settle down and absorbed by the media. Then, the plates were wrapped with paraffin tape, incubated at 27±1^{0}C for 24-72 hours and after 72 hours inhibition zone were measured in mm with the help of measuring tape.

Table 1. *In-vitro* evaluation of different botanicals extracts and its concentration.

Sr. No	C. Name	Botanical name	Plant parts	Active compound	Concentrations
1	Akk	*Calotropis gigantea*	Leaves	Rutin	25% ,35%,45%
2	Moringa	*Moringa oleifera*	Leaves	peptides	25% ,35%,45%
3	Keekar	*Acacia nelotica*	Leaves	Alkaloids	25% ,35%,45%
4	bitter apple	*Citrullus colocynths*	Fruit	linalool	25% ,35%,45%

In-vitro valuation of chemicals against *X. axanopodis* pv. *puniceae*

The efficacy of different chemicals (Kasugmicine, Copper oxychloride Streptomycin sulphate and Oxy-tetracycline) was checked at 1%, 2%, 3% concentration against *Xanthomonas axanopodis* pv. *puniceae* through inhibition zone technique. A well was made in the center of petri plate with the help of cork borer and chemicals were poured in well with the help of 1000 µl pipette, allowed to settle down, plates were wrapped with parafilm tape, incubated at 27±1^{0}C for 24-72 hours and after 72 hours inhibition zone were measured with the help of measuring tape (Raghuwanshi *et al.*, 2013).

Table 2. *In-vitro* evaluation of different chemicals and its concentration.

Chemicals	Concentration
Kasugmicine	1%, 2%, 3%
Copper oxychloride	1%, 2%, 3%
Streptomycin sulphate	1%, 2%, 3%
Oxy-tetracycline	1%, 2%, 3%

Evolution of chemicals and plants extracts against bacterial blight of pomegranate in green house conditions

Three months healthy (Qandhari, Sawa and Sidhura) plants were brought from a nursery, placed in green house at the department of plant pathology, Bahauddin zakariya university Multan. The experiment was done under (CRD). The plants were inoculated with 5 µl (10^{7}cfu) bacterial suspension of *Xanthomonas axanopodis* pv. *puniceae*, into the axial surface of leave and foliar application by spraying method, plants were covered with plastic sheet to maintain humidity. After 8 days of inoculation, 50 ml foliar spray of 45% of *M. oleifera* and 3% copper oxychloride were applied; inoculation was also done through syringe method. In control sawa variety was inoculated only with *Xanthomonas axanopodis* pv. *puniceae* for comparison. All the plants were irrigated on regular basis and after 7 days interval, disease severity was measured.

$$\% \text{ Severity} = \frac{\text{Number of infected leaves} \times \text{Grade}}{\text{Total Number of leaves} \times \text{Max. Grade}} \times 100$$

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Table 3. Disease rating scale.

Grades	Disease severity (%)	Response
0	0-0	Immune
1	0-5	Highly resistant
2	6-10	Resistant
3	11-25	Moderately resistant
4	26-50	Moderately susceptible
5	51-75	Susceptible
6	78-100	Highly susceptible

Results and Discussion

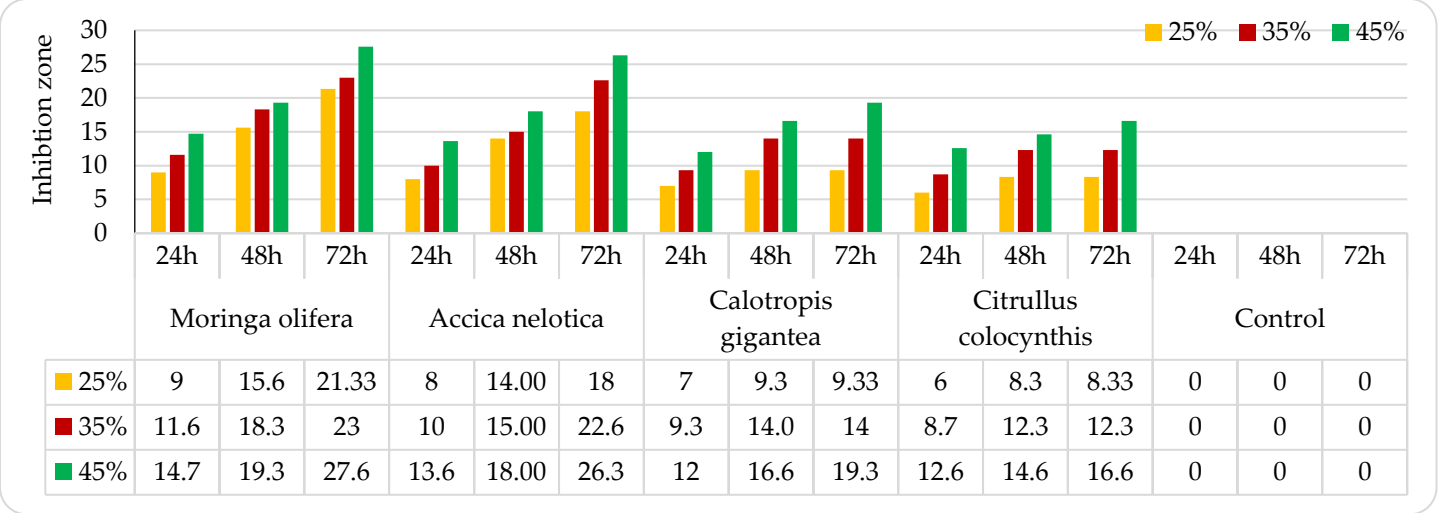
Pathogenicity test

Pathogenicity test was performed to fulfill Koch pastulates, the results showed that after the inoculation of *Xanthomonas axanopodis* pv. *puniceae* into the healthy plants, water soaked lesion, shiny appearance and dark brown spots on leaves were appeared after 15 days. Bacterium was re-isolated on nutrient agar media from artificially infected pomegranate leaves, purified which showed same biochemical and physical characters as of the original isolate (Figure 1).

In-vitro evaluation of plant extracts against bacterial blight of pomegranate

Four different plant extracts were evaluated in laboratory to check their efficacy against *Xanthomonas axanopodis* pv. *puniceae*. The results depicted that *M. oleifera* give significant antimicrobial activity (27.6 mm) at 45% concentration followed by *A. nilotica* (26.3mm) *C. gigantea* (19.3mm) and *C. colocynthis* (16.6mm) against colony growth of *Xanthomonas axanopodis* pv. *puniceae* after 72 hours' time interval (Figure 2).

Figure 1. Symptom of *Xanthomonas axanopodis* pv. *puniceae*

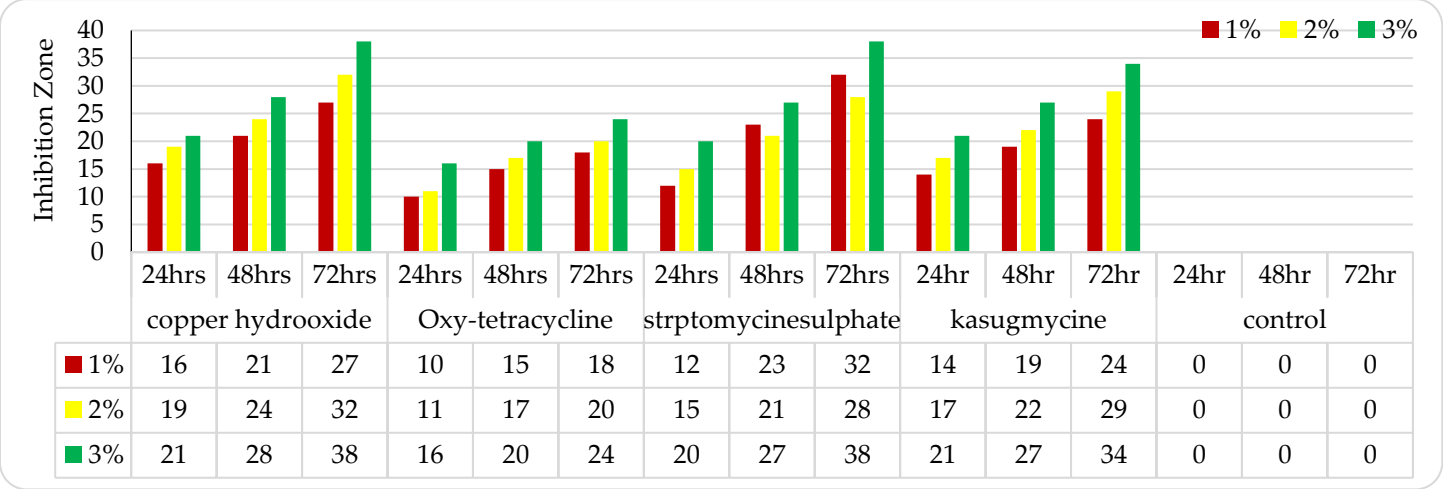


	Moringa olifera			Accica nelotica			Calotropis gigantea			Citrullus colocynthis			Control		
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
25%	9	15.6	21.33	8	14.00	18	7	9.3	9.33	6	8.3	8.33	0	0	0
35%	11.6	18.3	23	10	15.00	22.6	9.3	14.0	14	8.7	12.3	12.3	0	0	0
45%	14.7	19.3	27.6	13.6	18.00	26.3	12	16.6	19.3	12.6	14.6	16.6	0	0	0

Figure 2. *In-vitro* efficacy of botanicals against bacterial blight of pomegranate.

In-vitro evaluation of chemicals against bacterial blight of pomegranate

Four different chemicals were evaluated in laboratory conditions to check their efficacy against *Xanthomonas axanopodis* pv. *puniceae*. The results showed that copper-oxycholoride (38mm) and streptomycin sulphate (38mm) give maximum inhibition zone followed by kasugamycin (34mm) and oxy-tetracycline (24mm) against colony growth of *Xanthomonas axanopodis* pv. *puniceae* after 72 hours' time interval at 3% concentration (Figure 3).



	copper hydrooxide			Oxy-tetracycline			strptomycinesulphate			kasugmycine			control		
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hr	48hr	72hr	24hr	48hr	72hr
1%	16	21	27	10	15	18	12	23	32	14	19	24	0	0	0
2%	19	24	32	11	17	20	15	21	28	17	22	29	0	0	0
3%	21	28	38	16	20	24	20	27	38	21	27	34	0	0	0

Figure 3. *In-vitro* evaluation of chemicals against bacterial blight of pomegranate.

***In-vivo* experiment**

In laboratory conditions copper oxychloride and *M. oleifera* were more effective and showed significant results against *Xanthomonas axanopodis* pv. *puniceae* were selected for their evaluation under greenhouse condition. The results indicated that copper oxychloride decrease the bacterial activity by showing lower disease severity (20%) followed by negative control (25%), *M oleifera* (36%) and positive control (72%) respectively.

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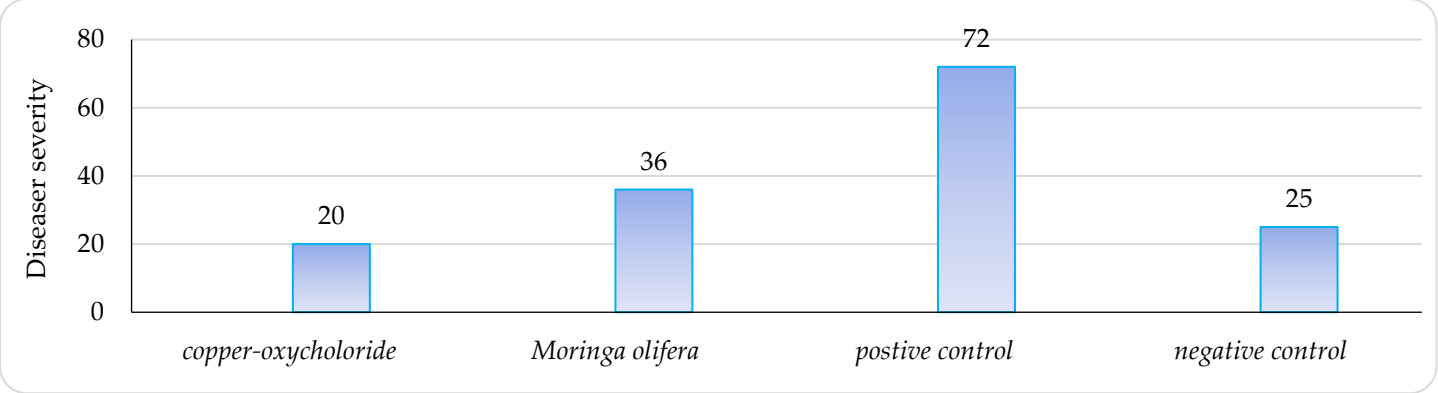

Figure 4. *In-vivo* evaluation of chemicals and botanicals extracts.

Discussion

Bacterial blight of pomegranate is an important disease which causes huge losses in favorable environmental conditions especially last few years and losses reached upto100% in severe conditions (Sharma *et al.*, 2017). The bacterium *Xanthomonas axonopodis* pv *punicae* is gram negative, single celled forming chains and rod-shaped with round endpoints (Jamadar *et al.*, 2011). The symptoms appear on all plant parts, leaf and fruit spots as well as cankers on stems, branches and trunks. The symptoms on the leaves are small, irregular, water-soaked lesions on the abaxial surface with a yellowish transparent appearance and leaf spots increase and turn black to dark brown. Fruits are most exposed part of the plant and symptoms on fruits start with water-soaked lesions that later turn to dark brown spots. Formation of cracks on necrotic fruit spots and splitting of fruits is prevalent. Likewise, Petersen *et al.*, (2010) observed early water soaked lesions to late necrotic blighting, the fruits show isolated or coalesced water soaked lesions followed by necrosis with small cracks and splitting of the entire fruit.

In current study in-vivo and in-vitro evaluation of four different plants extracts *Calotropis gigantean, Moringa oleifera, Acacia nelotica, Citrullus colocynths* and four chemicals copper-oxycholoride, streptomycin sulphate, kasugamycin and oxy-tetracycline was done against *Xanthomonas axanopodis* pv *puniceae* through inhibition zone technique. Among plant extracts *M. oleifera* give significant antibacterial activity (27.6 mm) at 45% concentration in laboratory whereas in chemicals copper-oxycholoride (38 mm) and streptomycin sulphate (38 mm) give maximum inhibition zone at 3% concentration after 72 hours intervals. In greenhouse studies, the results indicated that copper oxychloride decrease the bacterial activity by showing lower disease severity (20%) followed by *moringa olifera* (36%) and control (72%) respectively. It is concluded that application of chemicals against various disease is used in wide range because control through chemicals is easy, cheap and quick action but there are several constraints like potential harmful to non-target species, harmful for human and environment so their application should decrease by alternate pathways which is the application of plant extracts. Plant extracts are eco-friendly, bio-degradable, have medicinal value, potential to suppress specific plant diseases, either through directly or indirectly through anti-microbial action or resistance induction.

Conflict of Interest

The authors have not declared any conflict of interest.

Authors Contributions

All the authors contributed equally to the manuscript.

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