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Efficacy of medicinal plant extracts against *Xanthomonas axonopodis* pv. punicae causing bacterial blight of Pomegranate

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ABSTRACT-
Pomegranate (*Punica granatum* L.) is economically importance fruit crop having high great therapeutic value. It is a good source of carbohydrates and minerals such as calcium, iron, sulphur, vitamin-C. The crop is infected by *Xanthomonas axonopodis* pv. punicae which caused 'bacterial blight' disease, which is responsible for deceased yield (nearly by 80 %) and quality of fruits affecting the marketing value of fruits. The disease symptoms can be initially found on stem part which gradually pervades to leaves and then to fruits. Management of this disease with the help chemicals and antibiotics is very difficult task and farmer suffers from economic losses. In the present study, efficacy of methanolic extracts of four medicinal plants were tested against *Xanthomonas axonopodis* pv. punicae *in vitro* by measuring the zone of inhibition by disc diffusion method. Out of four medicinal plant extracts *Withania somnifera* (Ashwagandha) showed maximum antibacterial activity and caused zone of growth inhibition of *Xanthomonas axonopodis* pv. Punicae at different concentration. *Withania somnifera* (Ashwagandha) leaves extract were found significantly superior in inhibiting the growth of *Xanthomonas axonopodis* pv. punicae with recorded average zone of inhibition (15.4 mm). The plant extracts showed antibacterial activity and caused inhibition of growth of *Xanthomonas axonopodis* pv. Punicae maximum inhibition of the test bacterium was showed by *Withania somnifera* (Ashwagandha) extracts among, *Justicia adhatoda* (Adulsa), *Ocimum sanctum* (Tulsi), and *Aloe barbadensis* (Aloe vera).
Keywords- Pomegranate, *Xanthomonas axonopodis*, Antibacterial activity, Medicinal Plants.

1. INTRODUCTION-

Pomegranate (*Punica granatum* L.) is a well known fruit crop of India. It is belongs to family Punicaceae and grown in tropical and sub tropical regions of the world. The fruit crop is native of Iran and also cultivated in the Himalayas in northern India, Spain, Morocco, Egypt, Iran, Afghanistan and Baluchistan since ancient times. It is widely cultivated in India and the drier part of southeast Asia, Malaya, Myanmar, China, Japan, USA (California), East Indies and tropical America. In India Pomegranate is commercially cultivated in Solapur, Sangli, Nasik, Ahmednagar, Buldhana, Beed, Aurangabad, Satara, Washim and districts of Maharashtra; Bijapur, Bellary, Koppal, Bagalkot, Belgaum districts of Karnataka and to a smaller extent in Gujarat, Andhra Pradesh and Telangana. (NHB-2018). Pomegranate cultivation accounts for the majority of state income and decrease in its production is major loss of income of state. Even though pomegranate has antibacterial and antiviral activity due to secondary metabolites present, still it gets affected by bacteria. In recent years the bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. punice is of the important

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disease of pomegranate causing heavy losses in yield and quality of fruits in pomegranate. The survey conducted in Pune and Sangli district of Maharashtra has reported that the farmers are using bleaching powder, Bordeaux mixture, di-ammonium phosphate, urea, farmyard manure to manage the disease without any success (Pawar and Kadam, 2012). So, different medicinal plants extracts *Withania somnifera* (Ashwagandha) extracts, *Justicia adhatoda* (Adulsa), *Ocimum sanctum* (Tulsi), and *Aloe barbadensis* (Aloe vera) are widely used for its antibacterial activity against causal organism. Therefore, the present studies were under taken and this will help to formulate the disease management strategies.

2. MATERIAL AND METHODS-

2.1 Collection of Plant Materials:

The medicinal plants samples for antibacterial activity testing were collected from Dhanwantari Medicinal Plant and Aromatic Nursery, Mahatma Phule Krishi Vidyapeeth, Rahuri. The following listed parts used in traditional medicines were collected in cotton bags by cutting it in to small pieces. *Withania somnifera* (Ashwagandha)- leaves, stem and root, *Justicia adhatoda* (Adulsa)- leaves and stem, *Ocimum sanctum* (Tulsi)- leaves, stem and root, *Aloe barbadensis* (Aloe vera)- leaves.

2.2 Plants Storage:

After collection of the plant material namely leaves, stem, root, and fruits parts were cleaned (running tap water for 10 min and then with distilled water for 5 min), shade dried (10 to 15 days) and powdered by using mixer grinder. The powdered plant material was stored at 4^{o}C until further use.

2.3 Preparation of Plant Extract:

The preparation of medicinal plants extracts were carried out by using Soxhlet apparatus by passing 30-40 cycles of methanol as solvent. The excess solvent present in the extract were allowed to evaporate by incubating for 5 days at 40^{o}C in hot air oven. The remaining weight of extract was measured.

2.4 Purification and Maintenance of Bacterial Culture:

The suspected bacterial colonies were picked up with the help of sterilized inoculation loop and streaked onto the surface of NSA medium. The inoculated plates were incubated at 25^{o}C to 28^{o}C for 3 days. Observations were made for the development of well separated typical, bright yellow, mucoid colonies, such pure colonies were further streaked onto the agar slants containing the NSA medium and incubated at at 25^{o}C to 28^{o}C for 3 days, and then cultures were stored in the refrigerator at 4^{0}C, which served as a stock culture for further studies. The five bacterial cultures obtained for isolation from the diseased samples of all the regions were designated as different isolates from *Xap*I to *Xap*V and were maintained in the same way for further use.

2.5 *In vitro* Efficacy of Different Medicinal Plants against *Xanthomonas axonopodis* pv. punicae by Paper Disc Diffusion Method:

Sensitivity of the isolate collected from Nevasa (*Xap*I), Shrirampur (*Xap*II), Sangamner (*Xap*III), Shirdi (*Xap*IV), Akole (*Xap*V), was tested by paper disc diffusion method. Derived concentration of the medicinal plants were freshly prepared in methanol. The bacterium *Xanthomonas axonopodis* pv. *punicae* was multiplied by inoculating the loopful culture in

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250 ml conical flask containing 100ml of nutrient broth medium. The inoculated flasks were incubated at 28°C for 72 hours. The 20 ml bacterial suspension was added to molten cooled 1000ml nutrient agar medium at temperature 45°C. The seeded medium was thoroughly mixed and poured into the sterilized petriplates and plates were allowed to solidify. The bactericides solutions were prepared at different concentrations as mentioned above. Similarly the aqueous extracts of leaves, stem, root, and fruits of *Withania somnifera Justicia adhatoda, Ocimum sanctum*, and *Aloe barbadensis* at different concentrations i.e. 5%, 10%, 15%. was prepared. The filter paper (Whatman No. 42) discs of 5 mm in diameter were prepared using punching machine. The discs were soaked in the respective solution for 5 minutes and transferred onto the surface of the seeded medium in petriplates. The plates were incubated at 25°C to 27°C for 72 hours and observed for the production of inhibition zone around the filter paper discs. The results obtained were analyzed statistically. Paper disc soaked in methanol served as control.

3. RESULTS AND DISCUSSION-

3.1 Collection of Diseased Samples:

In the present study diseased samples were collected during December 2018 to January 2021, from different regions of Ahmednagar district of Maharashtra. Infected part of the leaves along with some healthy portion was selected for isolation. (Plate 3.1)

Table 3.1 Samples collected from different locations of Ahmednagar district for isolation of *Xanthomonas axonopodis* pv. punicae and given code no. to isolates.

Sr. No.	Location	Name of Taluka	Code no.
1.	Nevasa	Nevasa	*Xap*I
2.	Shrirampur	Shrirampur	*Xap*II
3.	Sangamner	Sangamner	*Xap*III
4.	Shirdi	Rahata	*Xap*IV
5.	Akole	Akole	*Xax*V

3.2 Isolation of *X. axonopodis* from Diseased Samples:

A total of five isolates of *X. axonopodis* were isolated from infected leaves collected from different localities of Ahmednagar. The causal organism was isolated from the infected leafs showing typical symptoms of bacterial blight. The isolates were purified by streak plate method on NSA medium at 25°C to 28°C. Repeated isolation from the infected plant parts yielded well separated, typical, yellow, mucoid, colonies of bacterium on medium after 72 hours of incubation at 28°C to 30°C (Plate 3.1). Colonies were purified by streaking the isolated colony on same media used for isolation and pure colonies obtained were further streaked on to the NSA slants and kept for incubation at 28°C to 30°C for 72 hours. Cultures so obtained were stored in the refrigerator at 5°C, which served as a stock culture for further studies. Present findings corroborates with the findings of Raghuwanshi *et al.,* (2013) who reported the isolates showing typical characters of *X. axonopodis* pv. *punicae* with yellow mucoid shining colonies were obtained on NSA medium.

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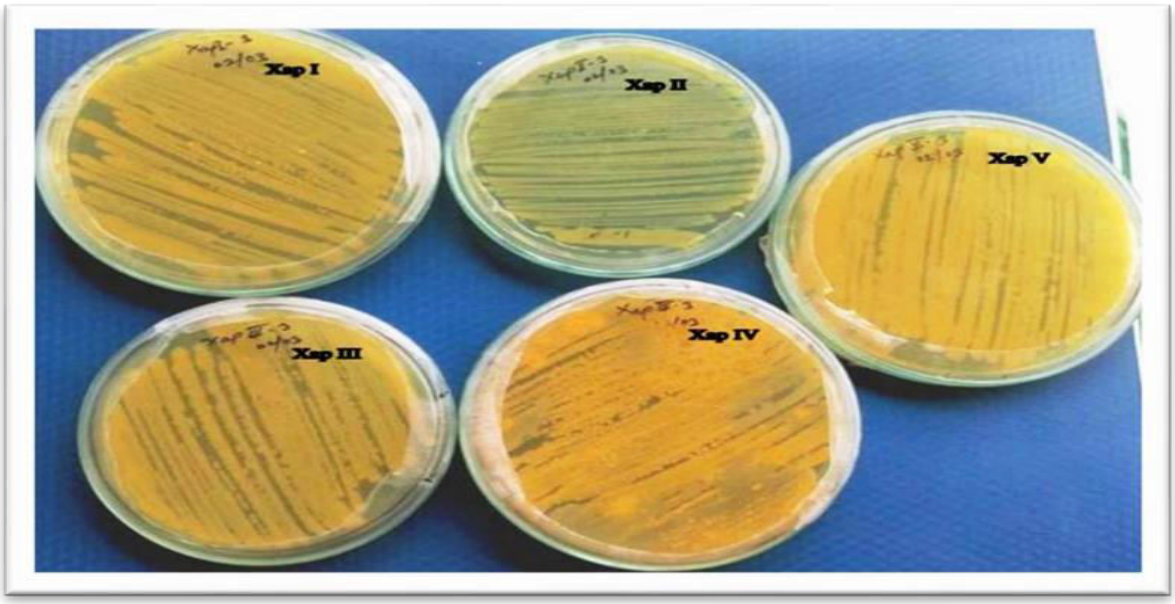

Plate 3.1 Isolated plates of *X. axonopodis* pv. *punicae* from various locations on NSA medium.

3.3 Efficacy of Different Medicinal Plants Extracts against *Xanthomonas axonopodis* pv. punica*e*:

In order to assess the *in vitro* efficacy of different medicinal plants extracts against *Xap*I to *Xap*V an experiment was conducted and the evaluation was made by paper disc diffusion method. (Plate 3.2).

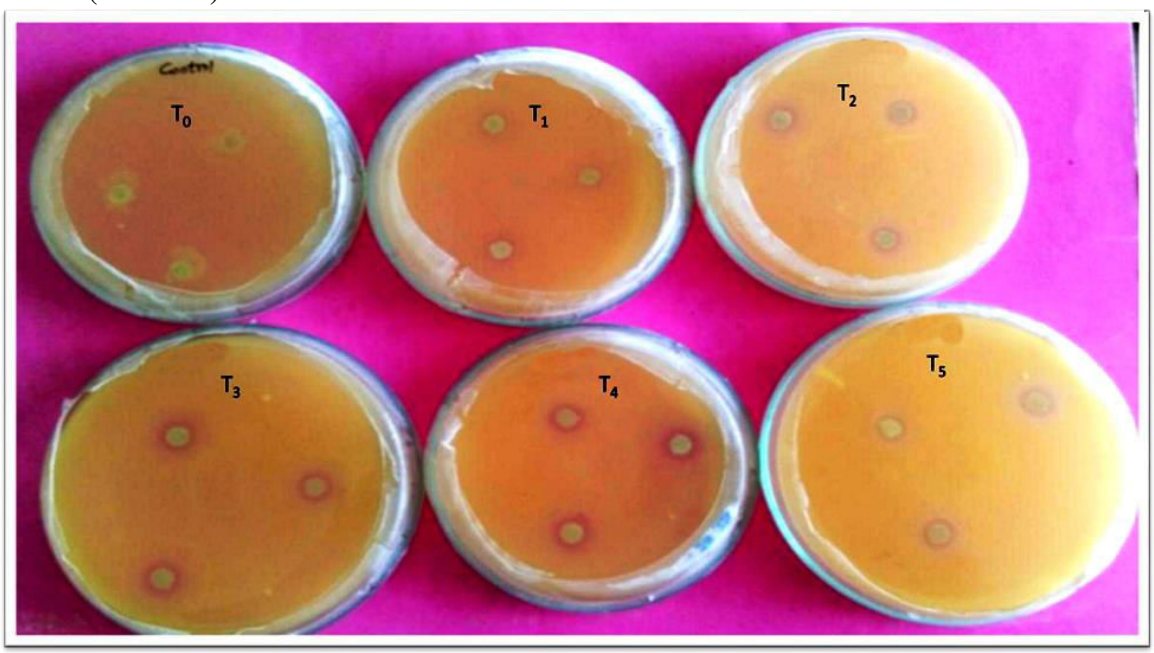

Plate 3.2 Bacterial plates (*Xanthomonas axonopodis* pv. *punicae*) showing inhibitory activity of leaves extract of *Withania somnifera* at different concentrations. T_0 : Control, T_1 *Xap*I : 5%, 10% and 15%, T_2 *Xap*II : 5%, 10% and 15%, T_3 *Xap*III : 5%, 10% and 15%, T_4 *Xap*IV : 5%, 10% and 15%,
T_5 *Xap*V : 5%, 10% and 15%.

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The data presented in table 3.3, revealed the significant differences in zone of inhibition among the different treatments at different concentrations.

Table 3.3: Antibacterial activity of medicinal plants extracts on growth of *Xanthomonas axonopodis* pv. punicae.

Treatment	Name of Plant	Plant Part used	Concentration of Extract	Zone of inhibition (in mm)					Average Zone of inhibition (in mm)
				*Xap*I	*Xap*II	*Xap*III	*Xap*IV	*Xap*V	
T_0	Untreated Control (Methanol Solvent)		5%	0	0	0	0	0	0
			10%	0	0	0	0	0	0
			15%	0	0	0	0	0	0
T_1 to T_5	*Withania somnifera*	Leaves extract	5%	9	9	8	8	9	8.6
			10%	11	12	12	11	12	11.6
			15%	15	16	15	15	16	15.4
T_6 to T_{10}	*Withania somnifera*	Fruit extract	5%	0	0	0	0	0	0
			10%	8	8	8	8	8	8
			15%	10	9	9	10	10	9.6
T_{11} to T_{15}	*Withania somnifera*	Root extract	5%	10	9	9	10	10	9.6
			10%	12	11	12	12	12	11.8
			15%	15	15	16	15	15	15.2
T_{16} to T_{20}	*Ocimum sanctum*	Leaves extract	5%	0	0	0	0	0	0
			10%	7	8	7	8	8	7.6
			15%	9	10	10	9	9	9.4
T_{21} to T_{25}	*Ocimum sanctum*	Stem extract	5%	0	0	0	0	0	0
			10%	0	0	0	0	0	0
			15%	0	0	0	0	0	0
T_{26} to T_{30}	*Ocimum sanctum*	Root extract	5%	0	0	0	0	0	0
			10%	7	8	8	8	8	7.8
			15%	9	10	9	9	9	9.2
T_{31} to T_{35}	*Justicia adhatoda*	Leaves extract	5%	0	0	0	0	0	0
			10%	8	7	8	8	8	7.8
			15%	9	9	9	9	9	9
T_{36} to T_{40}	*Justicia adhatoda*	Stem extract	5%	0	0	0	0	0	0
			10%	0	0	0	0	0	0
			15%	0	0	0	0	0	0
T_{40} to T_{45}	*Aloe barbadensis*	Leaves extract	5%	0	0	0	0	0	0
			10%	8	8	8	8	8	8
			15%	10	10	9	9	9	9.4

From table, it becomes clear that, methanol extracts of medicinal plants were most effective in controlling the growth of *Xanthomonas axonopodis* pv. punicae. Among the different plants studied, methanolic leaves extract of *Withania somnifera* with average zone of inhibition (15.4 mm), methanol root extract of *Withania somnifera* with average zone of inhibition (15.2 mm), methanolic leaves extract of *Ocimum sanctum* with average zone of inhibition (9.4 mm), methanolic root extract of *Ocimum sanctum* with average zone of

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inhibition (9.2 mm), methanolic leaves extract of *Justicia adhatoda* with average zone of inhibition (9 mm) and methanolic leaves extract of *Aloe barbadensis* with average zone of inhibition (9.4 mm) caused inhibition at some extent.

4. DISCUSSION

This study reveals that, these plant extracts showed antibacterial activity and caused inhibition of growth of *Xanthomonas axonopodis* pv. punicae. The result presented in Table 3.3 revealed that leaves extract of *Withania somnifera* with average zone of inhibition (15.4 mm), root extract of *Withania somnifera* with average zone of inhibition (15.2 mm), leaves extract of *Ocimum sanctum* with average zone of inhibition (9.4 mm), root extract of *Ocimum sanctum* with average zone of inhibition (9.2 mm), leaves extract of *Justicia adhatoda* with average zone of inhibition (9 mm) and leaves extract of *Aloe barbadensis* with average zone of inhibition (9.4 mm). Similarly, the result presented in Table 3.3 and Plate 3.2 revealed that *Withania somnifera* leaves extract at 15% concentration was found most effective for controlling *Xanthomonas axonopodis* pv. punicae by forming average zone of inhibition (15.4 mm). *Withania somnifera* root extract was found second best effective plant extract which showed average zone of inhibition (15.2 mm) at 15% concentration followed by leaves extract of *Ocimum sanctum* with average zone of inhibition (9.4 mm), methanolic root extract of *Ocimum sanctum* with average zone of inhibition (9.2 mm), leaves extract of *Justicia adhatoda* with average zone of inhibition (9 mm) and leaves extract of *Aloe barbadensis* with average zone of inhibition (9.4 mm) showed. In present investigations the medicinal plants extracts observed effective in inhibition of growth of *Xanthomonas axonopodis* pv. punicae.

5. CONCLUSION

On the basis of the present in vitro study, it could be concluded that the maximum zones of inhibition were recorded in methanolic leaves extract of *Withania somnifera* as compare to other medicinal plants extracts. *Withania somnifera* leaves extract found significantly superior in inhibiting the growth of *Xanthomonas axonopodis* pv. punicae with average zone of inhibition (15.4 mm).

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