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In vitro Evaluation of Phytoextracts against *Xanthomonas axonopodis* pv. *Punieae*

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ABSTRACT

Pomegranate "the boon commercial fruit crop" to the farmer turned as a big bane after the severe outbreak of bacterial blight. Many growers finding no options to mitigate the disease effectively have uprooted their crop owing to unbearable losses. In recent years, there has been a major thrust on residue free organic pomegranate production. Taking the task into consideration, efficient botanicals and bio-agents need to be explored to fit into the management schedule. Keeping all these aspects in view, the present study was undertaken to explore the potential of aqueous and different solvent extracts of medicinal plant against Xanthomonas axonopodis pv. punicae. In the experiment of in vitro evaluation of phytoextracts of medicinal plants against Xanthomonas axonopodis pv. punicae solvent extract of Curcuma amada (Ambihalad), Syzygium aromaticum (Clove), Dhatura strumarium (Dhatura), Curcuma longa (Turmeric), Allium sativum (Garlic), Eucalyptus globules (Nilgiri) were found effective against Xanthomonas axonopodis pv. punicae at all concentration. Out of six botanicals two botanicals had shown maximum zone of inhibition effectivity against Xanthomonas axonopodis pv. Punicae. Moreover it is also indicated that inhibition zone of bacterium increased with increase in concentration.

Key wards: *Phytoextract, inhibition, Xanthomonas*

INTRODUCTION

Ever since the farmers of Maharashtra and Karnataka who ever cultivating pomegranate, they were always on profitable side from Rs. 60,000 to Rs. 1,00,000 per ha, but since 2002, the growers are in dire straits due to the severe outbreak of bacterial blight caused by *Xanthomonas axonopodis* pv. *punicae*. Maharashtra and Andhra Pradesh resulting in huge yield losses both in terms of quality and quantity.

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The disease continued to damage the crop (for subsequent years up-till now), although farmers have adopted all possible and available protection measures, the disease could not be mitigated effectively due to rapid build up of inoculums and wide spread of the disease. Pomegranate "the boon commercial fruit crop to the farmer turned as a big bane after the severe outbreak of bacterial blight. Many growers finding no options to mitigate the disease effectively have uprooted their crop owing to unbearable losses.

Losses due to pests and diseases are very high. Although, 25 to 30 per cent of total cost of production is being spent on plant protection especially pesticides. Among the diseases infecting pomegranate, the bacterial disease popularly known as 'bacterial blight' caused by *Xanthomonas axonopodis* pv. *punicae* is the main disease (Hingorani and Singh, 1959). The bacterial blight is also known as nodal blight or black spot, oily spot or *Telya* in Maharashtra (Jadhav *et al.*, 2009). Chand and Kishun (1991) noticed the epidemics of bacterial blight of pomegranate causing 60 to 80 per cent losses at Indian Institute of Horticultural Research (IIHR) experimental plots.

In recent years, there has been a major thrust on residue free organic pomegranate production. Taking the task into consideration, efficient botanicals and bio-agents need to be explored to fit into the management schedule. Keeping all these aspects in view, the present study was undertaken to explore the potential of aqueous and different solvent extracts of medicinal plant against *Xanthomonas axonopodis pv. punicae.*

MATERIALS AND METHODS

Isolation

The infected leaves, small twigs and fruits of pomegranate collected from bacterial blight disease affected orchards from PGI field MPKV, Rahuri.

In vitro evaluation of botanicals against *Xanthomonas axonopodis* pv. *Punicae*

In order to study the efficacy of different phytoextracts (botanicals) under *in vitro* conditions, different concentrations of botanicals were prepared. Following plants were selected for obtaining leaf extracts at 10, 20 and 30 % against *Xanthomonas axonopodis* pv. *punicae.*

Table 1. List of medicinal plants used for extract preparation

Sr. No.	Common Name	Botanical Name	Plant Part Used
1	Garlic	*Allium sativum* Linn.	Bulb
2	Nilgiri	*Eucalyptus globules* Labill.	Leaf
3	Sankhapushpi	*Evolvulus aslinoides* Linn.	Leaf
4	Karanj	*Pongamia pinnata* Pierre.	Leaf
5	Mentha	*Mentha spicata* Linn.	Leaf
6	Ekdandi	*Tridex procumbence* Linn.	Leaf
7	Aloevera	*Aloe vera* Linn.	Leaf
8	Tulsi	*Ocimum sanctum O.Gratissium* Linn.	Leaf
9	Erend	*Ricinus communis* Linn.	Leaf
10	Ashwaganda	*Withania somnifera* Kaul.	Leaf
11	Parijatk	*Nyctanthes arbor-tristis* Linn.	Leaf
12	Periwrinkle	*Catharanthus roseus* Linn.	Leaf
13	Ritha	*Sapindus mokorossi* Gaerth	Leaf
14	Adulasa	*Adhatoda vasica* Nees.	Leaf
15	Onion	*Allium cepa* Linn.	Bulb
16	Ambihalad	*Curcuma amada* Linn.	Rhizome
17	Custard Apple	*Annona squamosa* Linn.	Leaf
18	Ruei	*Calotropis gigantean* Linn.	Leaf
19	Papaya	*Carica papaya* Linn.	Leaf
20	Turmeric	*Curcuma longa* Linn.	Rhizome
21	Lemongrass	*Cymbopogan flexuosus*	Leaf
22	Methi and Ranmethi	*Melilotus parviflora* Linn.	Leaf
23	Bel	*Aegle marmelos* Roxb.	Leaf
24	Pimpal	*Ficus religiosa* Linn.	Leaf
25	Behada	*Terminalia belerica* Roxb.	Seed
26	Clove	*Syzygium aromaticum* Linn.	Flower
27	Dhatura	*Datura stromarium*	Leaf

		Linn.	
28	Babhool	*Acacia arebica* Willd.	Leaf
29	Sarpagada	*Rauwolfia sarpentina* Benth.	Leaf
30	Ladies Finger	*Abelmoschus esculantus* Linn.	Leaf
31	Apta	*Bauhinia recemosa* Lamk.	Leaf
32	Ranhalad	*Curcuma aromatic* Sulisb.	Rhizome
33	Kala Dhotra	*Datura metel* Linn.	Leaf
34	Wood Apple	*Feronia elephantum*	Leaf
35	Mango	*Mangifera indica* Linn.	Leaf
36	Drumstick	*Moringa oleifera*	Leaf
37	Bartondi	*Morinda citrifolia* Linn.	Leaf
38	Sabja	*Ocimum basilicum* Linn.	Seed
39	Isabgol	*Plantago ovate* Forsk.	Leaf
40	Guava	*Psidium guajava* Linn.	Leaf
41	Tamarind	*Tamrindus indica* Linn.	Leaf
42	Tagar	*Tabernaemontana divaricata*	Leaf
43	Carry Leaves	*Murraya koenigii* Linn.	Leaf
44	Ranmethi	*Melilotus parviflora* Linn.	Leaf

The fresh plant parts of above test plants were collected and washed thoroughly in distilled water. The respective plant parts (leaves, root, rhizome, rind, cloves, etc.) were macerated in appropriated volume (1:1w/v) of water individually in a mortar pestle. This macerated material was passed though double fold muslin cloth and the filtrate was filtered through Whatman Filter paper No. 1. The filtrate was centrifuged at 10000 rpm for 20 minute and the supernatant obtained was filter sterilized through G4 filter and collected in sterile screw-cap glass vials for further use. Antibacterial activity of plant extracts was evaluated using Agar diffusion method. Wells of 5mm diameter were made in solidified NAS media with the help of sterilized cork borer and loaded with filter sterilized solvent extracts of required concentration i.e. 10, 20, 30 per cent of botanicals. The another plate with similar quantity of methanol served as a respective check. Each treatment was replicated thrice. The plates were incubated at 28 ± 2^0C for 48 h. The inhibition zone around the well was measured with a millimeter scale.

RESULTS AND DISCUSSION

In vitro evaluation of botanicals against *Xanthomonas axonopodis* pv. *punicae*

Sensitivity of *Xanthomonas axonopodis* pv. *punicae* to botanicals at 10 per cent concentration

Data pertaining to sensitivity of *Xanthomonas axonopodis* pv. *punicae* to botanicals at low concentration i.e. 10 per cent. Results revealed that inhibition of growth of bacterium differed significantly due to different botanical extract.

Forty four botanical *viz*., Garlic (*Alium sativum*), Guava (*Psidium guajava*), Nilgiri (*Eucalyptus globulus*), Mint (*Mentha spicata*), Ashwagandha (*Withania somnifera*), Adulsa (*Adathoda vesica*), Sarpagandha (*Rauwolfia serpentina*), periwinkle (*Catharanthus roseus),* Onion (*Allium cepa*), Aloe-vera (*Aloe* spp.), Tulsi (*Ocimum spp*.), Bel (*Aegle marmalose*), Dhatura (*Dhatura spp*), Turmeric (*Curcuma longa*), Sankhapushpi (*Evolvulusas linoides*), Karanj (*Pongamia pinnata),* Ekdandi (*Tridex procumbence),* Erend (*Ricinus communis),* Ambihalad (*Curcuma amada),* Custard Apple (*Annona squamosa*), Ruei (*Calotropis gigantean*), Papaya (*Carica papaya*), Lemongrass (*Cymbopogan flexuosus),* Methi (*Melilotus parviflora),* Pimpal (*Ficus religiosa),* Behada (*Terminalia belerica),* Babhool (*Acacia arebica),* Ladies Finger (*Abelmoschus esculantus*), Apta (*Bauhinia recemosa),* Ranhalad (*Curcuma aromatic),* Wood Apple (*Feronia elephantum),* Mango (*Mangifera indica),* Drumstick (*Moringa oleifera),* Bartondi (*Morinda citrifolia),* Sabja (*Ocimum basilicum),* Isabgol (*Plantago ovata),* Tamarind *(Tamrindus indica),* Tagar (*Tabernaemontana divaricata),* Carry Leaves (*Murraya koenigii),* Ranmethi (*Melilotus parviflora*).

Out of 44 only six botanicals *viz*, *Curcuma amada* (Ambihalad), *Syzygium aromaticum* (Clove), *Dhatura strumarium* (Dhatura), *Curcuma longa* (Turmeric), *Allium sativum* (Garlic), *Eucalyptus globules* (Nilgiri) were found effective against *Xanthomonas*

axonopodis pv. *punicae* at 10 per cent concentration. Among the six effective botanicals *Curcuma amada* (Ambihalad) exhibited maximum inhibition zone (15.13 mm) and per cent inhibition was 17.03 per cent over control recommended chemicals i.e. Bromopol and other botanicals also. It was followed by *Syzygium aromaticum* (Clove) (14.53 mm) and *Dhatura strumarium* (Dhatura), while the lowest inhibition zone was observed where Turmeric (9.8 mm) was used. Per cent inhibition over recommended chemicals (Bromopol) was less in all botanicals except Ambehalad.

These results are in conformity with reports of Mangamma and Sreeramulu (1991), Woughkaew *et a1*. (1997), Yenjerappa (2009) who reported the effectivity of extract of garlic (*Allium sativum*), Turmeric *(Curcuma longa)* against *Xanthomonas axonopodis* pv. *vasicatoria.*

Sensitivity of *Xanthomonas axonopodis* pv. *punicae* to botanicals at 20 per cent concentration

Out of 44 only six botanicals *viz*, *Curcuma amada* (Ambihalad), *Syzygium aromaticum* (Clove), *Dhatura strumarium* (Dhatura), *Curcuma longa* (Turmeric), *Alium sativum* (Garlic), *Eucalyptus globules* (Nilgiri) were found effective against *Xanthomonas axonopodis* pv. *punicae* at 20 per cent concentration. Among the six effective botanicals *Curcuma amada* (Ambihalad) exhibited maximum inhibition zone (18.13 mm) and per cent inhibition was 20.14 per cent more over recommended chemicals i.e. Bromopol and other botanicals also. It was followed by *Syzygium aromaticum* (Clove) (16.93mm) and *Dhatura strumarium* (Dhatura), while the lowest inhibition zone was observed where Turmeric (11. mm) was used. Percent inhibition over recommended chemicals (Bromopol) was less in all botanicals except Ambehalad.

These results are in conformity with reports of Sree and Sreeramula (2002), Alane and Swami (2015) and Dande *et al.*(2015), who reported the effectively of extract of garlic (*Allium sativum*) against *Xanthomonas axonopodis* pv. *punicae*, *Xanthomonas campestris* pv. *malvacearum* and *Xanthomonas axonopodis* pv. *vasicatoria.*

Sensitivity of *Xanthomonas axonopodis* pv. *punicae* to botanicals at 30 per cent concentration

Data pertaining to sensitivity of *Xanthomonas axonopodis* pv *punicae* to botanicals at high concentration i.e. 30 per cent, which revealed that inhibition of growth of bacterium differed significantly due to different solvent extract of botanicals.

Out of 44 only six botanicals *viz*, *Curcuma amada* (Ambihalad), *Syzygium aromaticum* (Clove), *Dhatura strumarium* (Dhatura), *Curcuma longa* (Turmeric), *Alium sativum* (Garlic), *Eucalyptus globules* (Nilgiri) were found effective against *Xanthomonas axonopodis* pv. *punicae* at 30 per cent concentration. Among the six effective botanicals *Curcuma amada* (Ambihalad) exhibited maximum inhibition zone (22.73 mm) and per cent inhibition was 25.25 per cent more over recommended chemicals i.e. Bromopol and other botanicals also. It was followed by *Syzygium aromaticum* (Clove) (20.5mm) and *Dhatura strumarium* (Dhatura), while the lowest inhibition zone was observed where turmeric (12.73 mm) was used Percent inhibition over recommended chemicals (Bromopol) was less in all botanicals except Ambehalad.

These results are in conformity with reports Iaram *et a1*. (2003), Satya and *et al*, (2005), Gargade and Kadam (2014), who reported the effectivity of extract of garlic (*Allium sativum*), Zimmu (*Allium cepa* + *Allium sativum*) and Dhatura (*Dhatura metel*) against *Xanthomonas axonopodis* pv. *punicae*, *Xanthomonas campestris* pv. *malvacearum* and *Xanthomonas axonopodis* pv. *oryzae.*

SUMMARY AND CONCLUSION

In the study of *in vitro* testing of botanical against *Xanthomonas axonopodis* pv. *punicae* it was observed that solvent extract of botanicals like *Curcuma amada* (Ambihalad), *Syzygium aromaticum* (Clove), *Dhatura strumarium* (Dhatura), *Curcuma longa*

(Turmeric), *Allium sativum* (Garlic), *Eucalyptus globules* (Nilgiri) were found be useful as a preventive measure to prevent the infection of bacterium. No any single botanical was found to be effective as a curative measures.

Inhibition of *Xanthomonas axonopodis* pv. *punicae* was directly proportionate with concentration, i.e. Ambihalad extract followed by Clove and Datura extracts were effective at 10 %, 20 % and 30 % concentration and shows maximum inhibition zone and inhibition percent as concentration increases.

REFERANCES

Alane, S.K. and Swami, C.S. 2016. Antibacterial activity of plant extracts against X*anthomonas axonopodis* pv. *punicae* causing bacterial blight of Pomegranate (*Punica granatum* L.). *Bioscience Discovery.* **7** : 70-73.

Chand, R. and Kishun, R. 1991. Studies on bacterial blight (*Xanthomonas campestris* pv. *punicae*) of pomegranate. *Indian Phytopath*, **44** : 370-371.

Dande, V , Sonawane, A, Goradiya S, Ghule, A, Mahajan M and Renake S,2015,A Study of a Unique Herbal Elixir as a Potential Green Pesticide for Integrated Pest Management of Pomegranate, *International J. Microbio. and Applied Sci.*, **4** : 388-398.

Gargade, V.A. and Kadam, D.G. 2014. Antibacterial activity of some herbal extracts and traditional biocontrol agents against *Xanthomonas axonopodis* pv *punicae*, *J. International Academic Res. for Multidisciplinary*, **2** , 140-152.

Hingorani, M.K. and Singh, N.J. 1959. *Xanthomonas punicae* sp. Nov. on pomegranate (*Punica granatum* L.). *Indian J. Agric. Sci*., **29** : 45-48.

Iqram, F., Khan, M.A. and Khan, S.M. 2003. *In vitro* evalution of plant extract and antagonistic fungi against *Xanthomonas compestris* pv. *citri* (Hasse) dye. *Pakistan J. Botany*, **35** : 967-970.

Jadhav, V.T. and Sharma, K K. 2009. Integrated management of diseases in pomegranate. Paper Presented In: *2nd Inter. Symp. Pomegranate and minor including Mediterranean Fruits*, Univ. Agric. Sci., Dharwad, June 23-27, pp. 48-52.

Mangamma, P. and Sreeramulu, V. 1991. Garlic extract inhibitory to the growth of *Xanthomonas campestris* pv. *vesicatoria*. *Indian Phytopath.*, **44** : 372-374.

Satya, V.K., Radhajeyalakshmi, R., Kavitha, K., Paranidharan, V., Bhaskaran, R., and Velazhahan, R. 2005. *In-vitro* antimicrobial activity of zimmu (*Allium sativum* L. and *Allium cepa* L.) leaf extract. Archives of Phytopathology and Plant Protection. **38** : 185-192.

Sree, D. S. and Sreeramulu, A. 2002. Inhibitory effect of plant extracts on the management of bacterial leaf spot pathogen (*Xanthomonas axonopodis* pv. *vesicatoria*) infecting *Capsicum frutescens* L. *J. Nat. Remedies*. **2** : 191-194.

Wongkaew, P.S., Hokawat, C. and Sinsiri, V.R. 1997. A study on antimicrobial effect of some medicinal plant extract against some plant pathogenic bacteria. *Kaen Kaset Khon Kaen Agric. J.* **25** : 25-29

Yenjerappa, S.T. 2009. Epidemiology and management of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Singh) Vuterrin *et al*., (1995), *Ph.D. Thesis*, Univ. Agri. Sci. Dharwad (India).