POMEGRANATE BACTERIAL BLIGHT OF *Xanthomonas axonopodis* VMB13 -A SERIOUS THREAT TO POMEGRANATE PRODUCTION

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ABSTRACT

Pomegranate belongs to the family Punicaceae. It is an ancient vital fruit crop ofIndia. The fruits of pomegranate are known to possess therapeutic and pharmaceutical properties having high medicinal value. Amongst the diseases infecting pomegranate, the bacterial disease generally known as 'bacterial blight' caused byXanthomonas axonopodis pv. Punicae. Pomegranate Bacterial blight is amongst the basically devastating natural calamity inflicting huge losses to the crop productivity of pomegranate particularly in India during the last 24 years. Symptoms of this disease manifested as small,numerous, depressed, segregated, discoloured and usually water-soaked spots. Pomegranate 'the boon commercial fruit crop' to the farmer which turned as a big bane after the serious outbreak of bacterial blight. Many growers finding no alternatives to mitigate the disease effectively have uprooted their crop due to unbearable losses. Large scale infestation of Bacterial Blight Disease caused by Xanthomonas axonopodis pv. punicae has resulted inconsiderable damage to the crop.A study on isolation, morphological, biochemical features of this pathogen is of immense use in understanding the nature of the pathogen. The studies will further help in the management of the disease.The use of medicinal plant extracts is gradually becoming a method of choice in the management of different plant diseases as these are more ecofriendly and safe. There are several plant extracts used for control Xanthomonas axonopodis pv. punicae which helps the organic cultivation of pomegranate. Amongst various plant extracts screened, aqueous, methanol and ethanol leaf extracts of Azadirachta indica,Ricinus communisand Pongamia pinnata effectively retarded the growth of pomegranate bacterial blight pathogen under In Vitro condition. The present research work was initiated to find a suitable alternative to the synthetic antibiotics for the management of different plant diseases caused by bacteria. The present study was aimed to use three medicinal plantsViz. Azadirachta indica,Ricinus communis and Pongamia pinnataas antibacterial agent against Xanthomonas axonopodis pv. Punicae.Aqueous, ethanolic and methanolic extracts of these three plants were used for the present study as antibacterial agent against Xanthomonas axonopodis pv. Punicae. The antibacterial activity was tested by agar well diffusion assay. The maximum activity recorded in Azadirachta indica. Highest zone of inhibition (ZOI) was shown by aqueous extracts of all the tested medicinal plants while lowest zone of inhibition (ZOI) was shown by ethanolic extract of all the plants under study. The Plant extracts used exhibited best antibacterial activity having a potential to be used in the management of blight disease of Pomegranate as an alternative to chemical antibiotics.

Keywords : *Pomegranate, Bacterial blight, Xap., Medicinal Plants, Antibacterial activity*

Introduction

Pomegranate (Punica granatum L.) is a central fruit crop of subtropical and tropical regions of the world and is endorsed as a functional food and a nutraceutical source having health promoting benefits (Johanningsmeier, S. D. and Harris, G. K., 2011). Furthermore, the extended shelf life of pomegranate encourages giant demand in domestic and international markets. In the world, India is the largest producer of pomegranate having an annual production of 2,442 thousand tones grown in 209 thousand hectares (Anonymous, 2017).

The annual pomegranate fruit export is around 35,000 tonnes (Pal et al., 2014). Bacterial blight which was caused by Xanthomonas axonopodis pv. punicae (Xap) is a key constraint of pomegranate cultivation and the production (Hingorani, M. and Mehta, P., 1952). The major production limitation in pomegranate is bacterial blight disease which takes heavy toll of crop by reducing the market ability of fruits (Sharma et al., 2012a; Chand and Kishun,1991; Ravikumar et al., 2009).The causative agent of blight is Xanthomonas axonopodis pv. punicae. Bacterial blight typically affects above ground parts of

pomegranate like leaves, fruits and twigs(Ramesh, C. and Ram, K., 1991). The plant is susceptible to the blight durin gall stages of growth and resulting in huge economic loss.Bacterial blight mainly affects the above ground plant parts, specially leaves, fruits and twigs. Although the leaves show early water soaked lesions to late necrotic blighting,the fruits exhibit tisolated or coalesced water soaked lesions which is followed by necrosis with small cracks and splitting of the entire fruit. Stems display lesions around the nodes or injuries which forms cankers in the later stages.Suspected symptoms on floral parts have also been reported (Chand and Kishun, 1991; Rani et al., 2001).

The initial water-soaked lesions emerge only after 6 to 7 days of infection under favorable field conditions and further develop into late necrotic blighting (Sharma, 2017). Fruits display isolated water-soaked lesions then followed by necrosis having small cracks, leading to the splittingof the entire fruit. Acute disease outbreaks can cause 60% to 80% yield losses (Ramesh, C. and Ram, K., 1991). Xanthomonas axonopodis pv. punicae (Xap) is a gram negative, rod shaped bacterium which measures 0.4µm to 0.75µm × 1.0µm to 3.0µm with single polar flagellum (Sharma, K.et al., 2015).Xanthomonas axonopodis pv. punicae (Xap) produces smooth, circular, light yellow, glistening mucoid, butyrous and convex colonies with entire margins.

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To control the bacterial blight of pomegranate, there are various management practices which have been developed; the present research work was concentrated on eco-friendly management in promoting the green alternative for the management of bacterial blight of pomegranate and reducing the pesticide usage.

Materials and Methods

1. Collection of Diseased Plant Parts of Pomegranate Tree

Pomegranate plants were observed for presence of symptoms of bacterial blight disease. The infected leaves of plants were collected from pomegranate field located at Bhada, Latur:District, Maharashtra, India. Samples of infected leaves were collected from blight affected pomegranate plants. The sterile scissor used to cut the branches. Then the infected leaves were put in polythene bag and closed it securely. It was then brought to the laboratory and kept it in shade, cool place and preserved for further study (Divya, 2013).

2. Preliminary identification and isolation of the pathogen from affected parts of Pomegranate plant

Ooze test was performed as per description of Sharma et al., (2010) which is based on the microscopic observations for confirmation of presence of the pathogen in infected leaves. Pomegranate leaves which shows typical symptoms of oily spot (bacterial blight) were collected from the field. In this test, the infected leaves were washed with the sterile distilled water. Further, all the selected infected leaves were sterilized with 0.1% mercuric chloride ($HgCl_2$) for 10 minutes and followed by repeatedly washing with sterile distilled water and next blot dried. Then fresh young lesion containing leaf was selected. The small bits of infected tissue were cut down from affected portion of plants such as leaves and pericarp of the fruits with the help of a sharp sterile scalpel. Then it was placed in a drop of sterile saline taken on glass slide and observed from cut ends under high power objective of the compound microscope. Jet of bacterial cells started oozing out from the cut pieces of the tissue in water were observed under microscope called as ‘ooze’ (Schaad, 1992).

Further, suspension of bacteria prepared from infected tissues taken from affected leaves, twigs and fruits were used for

isolation of well separated bacterial colonies on suitable medium. Singh et al., (2015) stated the disease diagnosis depends on visual symptoms on plant parts and ooze test.

3. Isolation of bacteria exudes out from ooze

The leaf having the infected part was washed and further crushed in sterile distilled water. The extract obtained was then streak inoculated on the surface of a sterile Glucose Yeast Extract Calcium Carbonate Agar (GYECC Agar) (Yenjerappa, 2009). The plate was further incubated at 30^0C for 3 days. As per the literature, well isolated, gummy, mucous, yellow colored colony was selected for further study (Yenjerappa, 2009).

4. Colony characteristics of the pathogen

Colony characteristics Viz. size, shape, margin, elevation, consistency, opacity, color were studied on medium such as nutrient glucose agar (Yenjerappa, 2009).

5. Morphological characteristics of the pathogen

The morphological characteristics of the pathogen such as Gram nature, motility, spore, cell arrangements, cell shape, capsule staining were studied as per the standard procedures described by Schaad (1992).

6. Molecular identification of the bacterial pathogen

Molecular identification of the promising bacterial isolate was carried out by 16S rRNA were sequenced (Mondal et al., 2012) at National Center for Cell Sciences, University of Pune Campus, Pune, Maharashtra, India.

7. Phylogenetic analysis:

The generated sequences were analyzed at the National Center for Biotechnology Information Bethesda, MD. www.nbi.nlm.nih.gov/BLAST for closed homology using BLASTn algorithm. The related sequences for the isolates were downloaded from the NCBI database were aligned by using CLUSTAL X2 multiple sequence alignment tool, the Phylogenetic evolutionary history was inferred using the Neighbor Joining Method analysis (Tamura et al., 2004). Phylogenetic analyses were conducted in MEGA 4.0. Phylogenetic tree building was performed using MEGA 4.0 (Tamura et al., 2007).

8. Collection of Medicinal Plant parts for Antibacterial Activity

The different medicinal plants used in the present study Viz. Azadirachta indica, Ricinus communis and Pongamia pinnata were collected from different parts of Bhada village. The collected plant parts were identified from the Department of Botany, Lal Bahadur Shastri Mahavidyalaya, Dharmabad, Nanded, Maharashtra. The medicinal plants used in this study are generally found in this geographical area and their medicinal importance is emphasized in the literature of utilization of medicinal plants in plant disease control. These plant parts were then further used to perform In vitro antibacterial activity against the isolated representative pathogen Viz. Xanthomonas axonopodis VMB13 of bacterial blight of pomegranate (Fatima et al., 2012).

9. Preparation of Medicinal Plant Extracts

9.1. Preparation of Aqueous Extract of Selected Medicinal Plant Parts

Twenty gram of thoroughly washed fresh plant parts Viz. root, stem, leaves and bark were macerated with 100ml sterile distilled water taken separately in a warring blender for 10 minutes. The crush of each plant was then filtered through muslin cloth having double layer and then centrifuged (Remi Centrifuge model R-8C DX+R-81A) at 4000 rpm for 30 minutes. The supernatant was then filtered just before subjecting it to antibacterial activity assay (Raghavendra et al., 2006).

9.2. Preparation of Solvent Extracts of Selected Medicinal Plants

Twenty gram of dried powder of each plant was filled in the thimble and extracted consecutively with the solvents Viz. aqueous, ethanol and methanol by using a Soxhlet extractor (Soxhlet Complete Borosil, Code 3840) for 48 hours. All these extracts were separately prepared in Soxhlet extractor. Further, all these extracts were concentrated using rotary flash evaporator (Superfit Rotary Vacuum Flash Unit PBU-6D) and preserved at 4^0C in airtight bottle for further use. Afterwards, all these extracts were subjected to antibacterial activity assay (Raghavendra et al., 2006).

10. Determination of Antibacterial Activity

The antibacterial activity of aqueous extracts and solvent extracts of all three plants under study were determined by agar well diffusion method (Cruickshank et al., 1975) on the sterile nutrient glucose agar medium. Cell density was adjusted to 10^6 - 10^7 CFU/ml on the basis when culture reaches 0.1 optical units at 600nm with spectrophotometer (Schaad, 1992). The inoculum containing 10^6-10^7 CFU/ml of 72 hours old culture of Xanthomonas axonopodis pv. punicae VMB13 was spread inoculated on medium with the help of sterile glass spreader. Wells were prepared in sterile nutrient glucose agar plate by using sterile cork borer (5mm). 100µl of aqueous extract and solvent extract was put in the wells prepared in the inoculated plates. 100µl of solvent was placed in a blank well made in sterile nutrient glucose agar plate. It was done to determine the antibacterial activity of solvent against the pathogen. All the plates were kept in refrigerator at 4^0C for 20 minutes for diffusion and incubated for 72 hours at 30^0C. The diameter of zone of growth inhibition around the wells was measured in millimeter (mm) (Raghavendra et al., 2006). All the experiments were performed in triplicates

Results and Discussion

Identification of the pathogen causing Bacterial Blight of Pomegranate

Confirmation of the presence of Pathogen by ooze test

Microscopic observation of the organism streaming from the edges of the cut tissues of infected leaf of pomegranate plant indicated the presence of pathogen *Xanthomonas axonopodis pv. Punicae VMB13*.Similar results of ooze test were demonstrated by Singh *et al*.,(2015).

Isolation of Bacterial Cells Exudes out from ooze

The bacterial cells which were obtained from ooze were isolated by inoculating on sterile glucose yeast extract calcium carbonate agar (GYCA) medium. The colonies appeared after 72 hours of incubation at 30^0C and were mucous, gummy and yellow colored. The well isolated colony was selected and re-streaked on glucose yeast extract calcium carbonate agar (GYCA) for further study. Similar colony characterizations were also reported by Digvijay *et al*., (2014) and Yenjerappa, (2009).

Morphological Characterizations of the pathogen

The bacterium was Gram negative, rod shaped,capsulated cells,non-spore forming which appeared singly, in chains and in pairs also. These results of morphological characterizations of the VMB13 isolate were in agreement with the reports described by Divya (2013).

Colony Characterizations of the pathogen

Colony diameter of the pathogen on GYCA mediumisas shown in (**Table 1**).

Table 1: Colony Characterizations of *Xanthomonas axonopodis VMB13* on GYCA medium incubated at 30^0C after 72 hours

Size	Shape	Margin	Elevation	Consistency	Opacity	Colour	Gram nature
2mm	Circular	Entire	Flat	Moist	Opaque	Bright Yellow	Gram negative

Molecular identification of Isolated Promising bacterial culture VMB13

Molecular identification of the promising bacterial isolate was carried out by 16S rRNA sequencing.

Phylogenic Analysis of VMB13

The phylogenetic tree was constructed by using Neighbour joining method by Kimura – 2 parameter with 1000 replicates in MEGA 4.0. According to the sequencing similarities anmultiple alignments, the present isolate was identified. The sequence obtained of the present isolate have been deposited in **DNA Databank of Japan (DDBJ)** and accession number obtained (**Figure I**).

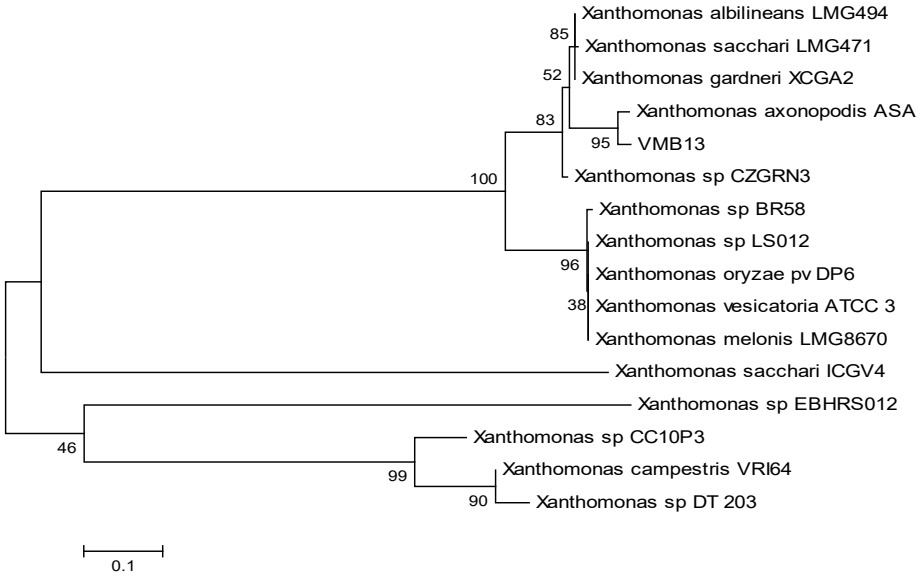


Figure I: Phylogenetic placement of VMB13 (Accession Number LC530853).

The gene sequences showing relationships among strain VMB13 and the closest type strain species of Xanthomonas. Numbers at nodes indicate percentage of bootstrap support based on a Neighbor-joining analysis of 1,000 resampled datasets. Bar 0.1 substitutions per nucleotide position.

Determination of antibacterial activity

In Vitro antibacterial potential of aqueous, ethanolic and methanolic extracts of three medicinal plants *Viz. Azadirachta indica, Ricinus communis and Pongamia pinnata*parts like leaves, bark, stem and root extracts against *Xanthomonas axonopodis* pv. *punicae VMB13* were determined by Agar well diffusion method on sterile nutrient glucose agar medium. Among the different medicinal plant extracts used for antibacterial activity, aqueous extract of allthe three medicinal plants under study were found very effective in inhibiting the maximum growth of the pathogen *VMB13*. Thediameter of zone of inhibition is depicted in **Table 2,Figure II**, **Figure III** and **Figure IV**.

Table 2: In Vitro evaluation of antibacterial potential of selected medicinal plants against Xanthomonas axonopodis VMB13

Plants used	Diameter of Zone of growth inhibition (mm)														
	Aqueous					Ethanol					Methanol				
	C	L	B	S	R	C	L	B	S	R	C	L	B	S	R
Azadirachta indica	00	40	35	31	20	1.5	38	32	27	18	1.7	36	32	28	18
Ricinus communis	00	34	32	22	18	1.6	33	28	23	13	1.5	28	25	20	11
Pongamia pinnata	00	35	31	27	13	1.5	34	30	25	16	1.6	32	29	26	16

Note: Where, **C** –Control, **L** – Leaves, **B** – Bark, **S** – Stem, **R**- Root

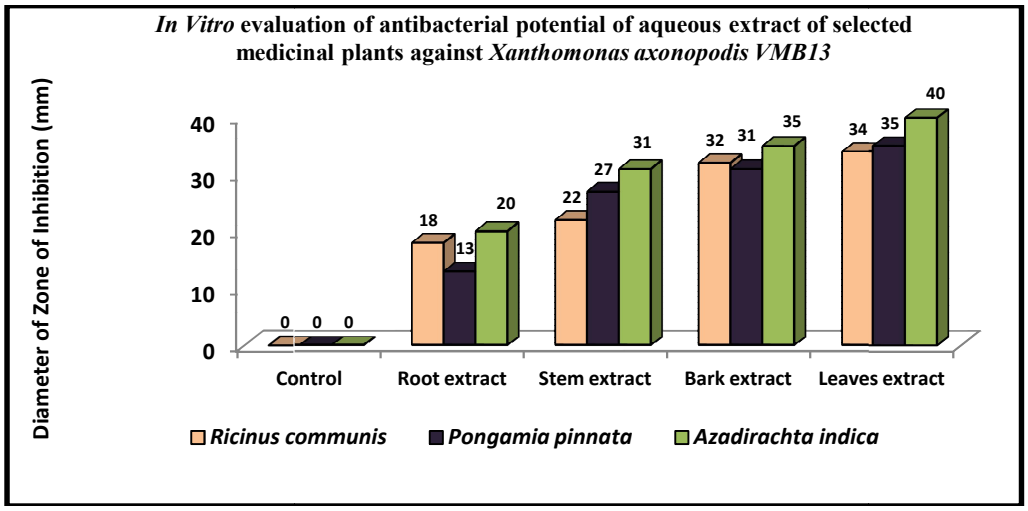


Figure II: In Vitro evaluation of Antibacterial Potential of Aqueous extract of selected medicinal plants against Xanthomonas axonopodis VMB13

It is found from the **Figure II** that the promising fungal isolate Xanthomonas axonopodis VMB13 shows the maximum zone of inhibition i.e. 40mm to aqueous leaves extract of Azadirachta indica followed by bark extract, stem extract and root extract which is 35mm, 31mm and 20mm respectively.Similarly, the promising fungal isolate VMB13 shows maximum zone of inhibition i.e. 35mm to aqueous leaves extract of Pongamia pinnata followed by bark extract, root extract and stem extract which is 31mm, 13mm and 27mm respectively.The promising fungal isolate VMB13 shows maximum zone of inhibition i.e. 34mm to aqueous leaves extract of Ricinus communis followed by bark extract, root extract and stem extract which is 32mm, 18mm and 22mm respectively.

Among the different plants used for antibacterial activity, aqueous extractsof *Azadirachta indica* plant, were showed significant antibacterial activityagainst the pathogen *Xanthomonas axonopodis VMB13*.

Murugan *et al*., (2012) reported that methanol and aqueous extracts of *Pongamia pinnata* Linn seed showed 10mm-13mm diameter of zone of growth inhibition against Xanthomonas oryzae pv. oryzae.

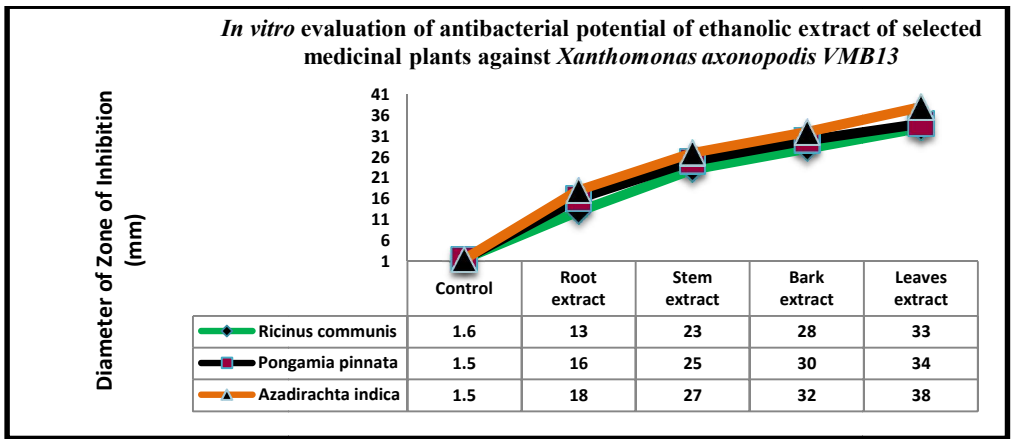


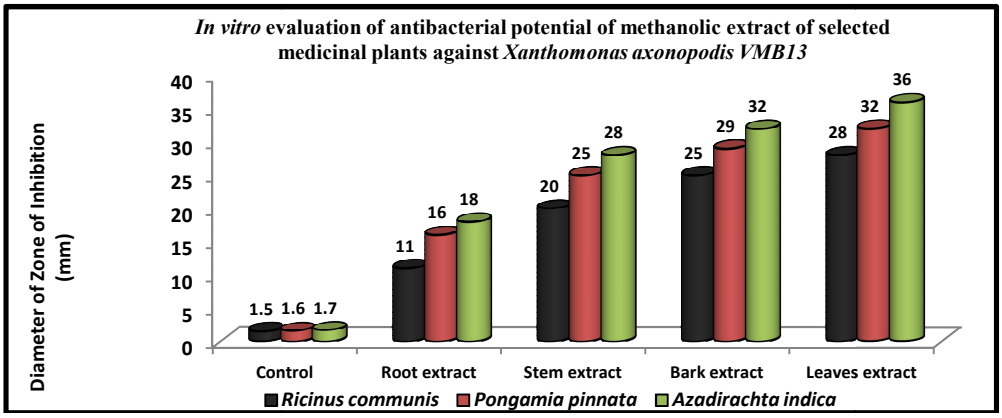
Figure III: In VitroEvaluation of Antibacterial potential of Ethanolic extract of selected medicinal plants against Xanthomonas axonopodis VMB13

It is found from the **Figure III** that the promising fungal isolate Xanthomonas axonopodis VMB13 shows the maximum zone of inhibition i.e. 38mm to ethanolic leaves extract of Azadirachta indica followed by bark extract, stem extract and root extract which is 32mm, 27mm and 18mm respectively.Similarly, the promising fungal isolate VMB13 shows maximum zone of inhibition i.e. 34mm to ethanolic leaves extract of Pongamia pinnata followed by bark extract, root extract and stem extract which is 30mm, 16mm and 25mm respectively. The promising fungal isolate VMB13 shows maximum zone of inhibition i.e. 33mm to ethanolic leaves extract of Ricinus communis followed by bark extract, root extract and stem extract which is 28mm, 13mm and 23mm respectively.

Among the different plants used for antibacterial activity, ethanolic extractsof *Azadirachta indica* plant, were found very effective in inhibiting the maximum growth of the pathogen *Xanthomonas axonopodis VMB13*.

Methanol seed extract of Acacia nilotica and aqueous, methanol and ethanol bark extracts of Acacia nilotica, showed significant antibacterial activity with diameter of zone of inhibition was ranged between 11.75mm to 27.90mm against phytopathogenic Xanthomonas pathovars Viz. Xanthomonas axonopodis pv. malvacearum associated with angular leaf spot of cotton Xanthomonas axonopodis pv. Phaseoli, common blight of bean and Xanthomonas campestris pv. vesicatoria associated with bacterial spot of tomato respectively (Raghvendra et al., 2006).

Figure IV: In VitroEvaluation of Antibacterial Potential of Methanolic extract of selected medicinal plants against Xanthomonas axonopodis VMB13



It is found from the **Figure IV** that the promising fungal isolate Xanthomonas axonopodis VMB13 shows the maximum zone of inhibition i.e. 36mm to methanolic leaves extract of Azadirachta indica followed by bark extract, stem extract and root extract which is 32mm, 28mm and 18mm respectively.Similarly, the promising fungal isolate VMB13 shows maximum zone of inhibition i.e. 32mm to methanolic leaves extract of Pongamia pinnata followed by bark extract, root extract and stem extract which is 29mm, 16mm and 25mm respectively. The promising fungal isolate VMB13 shows maximum zone of inhibition i.e. 28mm to methanolic leaves extract of Ricinus communis followed by bark extract, root extract and stem extract which is 25mm, 16mm and 20mm respectively.

Among the different plants used for antibacterial activity, methanolic extracts of Azadirachta indica plant, were found very effective in inhibiting the maximum growth of the pathogen Xanthomonas axonopodis VMB13.

Alane and Swami (2016) reported in their study that acetone leaf extracts of Azadirachta indica (18mm), Ricinus communis (16mm), Metha spicata showed diameter of zone of inhibition 22mm, Murraya Koenigii (19mm), Allium sativum (20mm) and Tridax procumbens (17mm) against Xanthomonas axonopodis pv. punicae. Alcohol leaf extract of Azadirachta indica (14mm) inhibited growth of the pathogen followed by aqueous extract of Calotropis procera (10mm),Moringa oleifera (10mm) and Allium sativum (10mm).

Mahesh and Satish, (2008) reported methanol bark and leaf extract of Acacia nilotica exhibited 13mm and 15mm diameter of zone of inhibition against Xanthomonas axonopodis pv. malvacearum respectively.

Britto and Gracelin (2011a) also evaluated bio-efficacy of crude methanol leaves and flower extract of Datura metel Linn with diameter of zone of inhibition 25.30mm and 11.60mm respectively against Xanthomonas campestris.Ethanol seed extract of Azadirachta indica, ethanol leaf extract of Ricinus communis, ethanol leaf extract of Terminalia catappa, ethanol leaf extract of Adhatoda vasica, ethanol seed extract of Pongamia pinnata were found moderately effective in inhibiting the growth of pathogen where as remaining plant extracts showed least antibacterial activity against Xanthomonas axonopodis pv. punicae.

Conclusion

Many synthetic antibiotics are employed to control the various phytopathogens. The increased awareness of environmental problems with such chemical antibiotics has led to the exploration for non-conventional chemicals of having biological origin for the management of these plant diseases. Plant originated-antibacterial compounds can be one approach to plant disease management because of their eco-friendly nature.Laboratory screening of different plant extracts has given encouraging results which indicates their potential use in the management of disease caused by Xanthomonas axonopodis pv. Punicae. Several Plant extracts resulted in the antibacterial activity is having potential to use in the management of plant diseases as an alternative to the chemical antibiotics.

Acknowledgement

We would like to offer our sincere thanks to National Center for Cell Sciences, University of Pune Campus for the 16s rRNA sequencing of the isolate. Our sincere thanks to Principal, Lal Bahadur Shastri Mahavidyalaya, Department of Botany, Nanded for providing the Laboratory facilities.and seven star friend group

References

1. **Anonymous (2017).** Horticultural statistics at a glance, National Horticulture Board, Govt. of India, 46.
2. **Divya, B. L. (2013).** Variability studies of Xanthomonas axonopodis pv. Punicae (Hingorani and Singh) vauterin et al. causing bacterial blight disease of pomegranate and it's management. Ph. D. thesis submitted to University of Agricultural Sciences, Bengaluru, Karnataka, India.
3. **Hingorani, M. and Mehta, P. (1952).** Bacterial leaf spot of pomegranate. Indian Phytopathol 5, 55–56 .
4. **Johanningsmeier, S. D. and Harris, G. K.(2011).** Pomegranate as a functional food and nutraceutical source. Annu. Rev. Food. Sci. T. **2**, 181–201.
5. **Pal, R., Babu, K. D., Singh, N., Maity, A. and Gaikwad, N.(2014).** Pomegranate Research in India–Status and future challenges. Progr. Hort. 46, 184–201.
6. **Ramesh, C. and Ram, K.(1991).** Studies on bacterial blight (Xanthomonas campestris pv. punicae) of pomegranate. Indian Phytopatholo. 44, 370–372.
7. **Ravikumar M.R., Shamarao J., Yenjereapp S.T., Patil H.B., (2009).** Epidemiology and management of bacterial blight of pomegranate caused by Xanthomonas axonopodis pv. punicae. Acta Horticulture 818: 291-296.
8. **Sharma, J. (2017).**Pomegranate bacterial blight: symptomatology and rapid inoculation technique for Xanthomonas axonopodis pv. punicae. J. Plant. Pathol. 99: 109–119.
9. **Sharma, K., Sharma, J. and Jadhav, V. (2015).** In Recent Advances in Diagonosis and Management of plant Disease:119–126 (Springer).
10. **Sharma K. K., Sharma J., and Jadhav V. T. (2010).** Status of bacterial blight of pomegranate in India. In: Fruit, vegetable, cereal science and biotechnology. 4 (2),Global Science Books publication, Japan:102–105.
11. **Sharma J.,and Jadhav V.T.(2012).** Network project on mitigating bacterial blight of pomegranate in Maharashtra, Karnataka and Andhra: Progress Report April 2011 to October 2012. National Research Centre on Pomegranate, Solapur, Maharashtra, India.
12. **Schaad N. W. (1992).** Laboratory Guide for Identification of Plant Pathogenic Bacteria. New Publishing House, New Delhi, India: 81-94.
13. **Chand R.,and Kishun R., (1991).** Studies on bacterial blight (Xanthomonas campestris pv. punicae) on Pomegranate. Indian Phytopathology. 44: 370-372.
14. **Singh N. V., Abburi V. L., Ramajayam D. Ravinder Kumar, Ram Chandra, Sharma K. K.,Sharma J. K., Dinesh Babu, Pal R. K., Mundewadikar D.M., Thangasamy Saminathan T.,Cantrell R., Nimmakayala P.,and Reddy U.K. (2015).** Genetic diversity and association mapping of bacterial blight and other horticulturally important traits with microsatellite markers in pomegranate from India. Mol Genet Genomics: 1-10.
15. **Yenjerappa S. T. (2009).** Epidemiology and management of bacterial blight of pomegranate caused by Xanthomonas axonopodis pv. punicae (Hingorani and Singh) vauterin et al. Th 9936 (accession no) submitted to University of Agricultural Sciences, Dharwad, Karnataka State, India.
16. **Mondal K., Rajenran T., Phaneendra C., Mani C., Sharma J., Shukla R., Pooja Verma G., Kumar R., Singh D., Kumar A., Saxena A. K.,and Jain R. (2012).** The reliable and rapid polymerase chain reaction (PCR) diagnosis for Xanthomonas axonopodis pv. punicae in pomegranate. African Journal of Microbiology Research. 6 (30):5950-5956.

17. **Tamura K., Nei M., and Kumar S. (2004).** Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA), 101:11030-11035.
18. **Raghavendra M. P., Satish S., and Raveesha K. A. (2006).** In vitro evaluation of anti-bacterial spectrum and photochemical analysis of Acacia nilotica. Journal of Agricultural Technology. 2(1):77-88.
19. **Fatima S., Baig M. R.,and Kadam V. B. (2012).** Antimicrobial activity of Acacia nilotica (L.) Del. plant extracts against Xanthomonas malvacearum bacteria. International Multidisciplinary Research Journal. 2(6):48-49.
20. **Tamura, K., Dudley, J., Nei M., Kumar S. (2007).** MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution . 24:1596–1599.
21. **Cruickshank R., Duguid J. P., Marmion B. R., and Swain R. H. A. (1975).** Medical Microbiology, 12th Ed., Living stone, London, New York, 812-825.
22. **Digvijay Bhardwaj S.,and Pankaj Kumar (2014).** Antibacterial activity of Juniperus communis L. and Vitex negundo against Xanthomonas axonopodis pv. punicae invitro. World Journal of Pharmaceutical Research. 3(10):1489-1500.
23. **Murugan A. M., Shanthi S., Arunachalam C., Sivakumar N., Elamathy S. andRajapandian K. (2012).** Study on cow urine and Pongamia pinnata Linn seed infarmyard: A natural, cost effective, eco-friendly remedy to bacterial leaf blight (BLB) of paddy. African Journal of Biotechnology. 11(40):9591-9598.
24. **Alane S. K. and Swami C. S.(2016).**Antibacterial activity of plant extracts againstXanthomonas axonopodis pv.punicae causing bacterial blight of pomegranate(Punica granatum L.). Bioscience Discovery. 7(1):70-73.
25. **Mahesh B. and Satish S. (2008).** Antimicrobial activity of some important medicinal plant against plant and human pathogens. World Journal of Agricultural Sciences.4 (S):839-843.
26. **Britto A. J. D. and Gracelin D. H. S. (2011).** Datura metel Linn. - A plant withpotential as antibacterial agent. International Journal of Applied Biology and Pharmaceutical Technology. 2(2):429-433.