

# **Invitro Evaluation of Botanicals and Biocontrol Agents against Pomegranate Bacterial Blight Pathogen**

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**ABSTRACT:** In India, pomegranate is regarded as a “vital cash crop”. Successful cultivation of pomegranate in recent years has met with different traumas such as pest and diseases. Among diseases bacterial blight caused by *Xanthomonas axonopodis* sp. *Punicae* is a major threat. In the present study the bacteria were isolated from infected pomegranate plant; fruit, leaves and stem and subjected to bio chemical analysis to identify *Xanthomonas*. Ethanol, methanol and aqueous extracts of four botanicals and three bio control agents were evaluated for their antibacterial activity against the harmful pathogen which was isolated from the infected pomegranate plant. Among these, the ethanol extracts of *Azadirachta indica* (Neem) and bio control agent *T. viride* showed significant inhibitory effect. These were applied to the infected plant and were tested.

**KEYWORDS:** Botanicals, pomegranate, biocontrol, bacterial blight.

## **I. INTRODUCTION**

Pomegranate (*Punicagranatum* L.) is an ancient fruit, belonging to the smallest botanical family Punicaceae. Pomegranate is a good source of carbohydrates and minerals such as calcium, iron and sulphur. It is rich in vitamin-C and citric acid is the most predominant organic acid in pomegranate [1]. The bark of the stem, root and rind of the fruit is used for slimming, control of dysentery, diarrhea and killing tapeworms [2].

In Karnataka, this crop has spread across different districts viz., Bijapur, Bellary, Bagalkot, Koppal, Chitradurga, Belgaum, Davangere, Tumkur, Bangalore and Gulbarga. Pomegranate, the commercial crop of the farmer turned as a bane after the outbreak of bacterial blight. Since 2002, the disease has reached the alarming stage and hampering the Indian economy vis-à-vis export of quality fruits. The disease accounted up to 70 – 100 per cent during 2006 in Karnataka and Maharashtra resulting in wipe out of pomegranate during the year 2007, the total output of pomegranate production in India was down by 60 per cent [3]. During 2008 – 09 the disease has reached its alarming stage bringing substantial damage to the crop and heavy loss to the farmers. However, the magnitude of the disease severity has raised several questions to researchers, farm managers, administrators, private firms and above all the farmers to safeguard the cultivation of this dollar earning crop.

Disease is characterized by the appearance of small, irregular water soaked, dark colored spots on leaves resulting in premature defoliation. Pathogen also infects stem and branches causing girdling and cracking symptoms. Spots on fruit are dark brown, irregular slightly raised with oily appearance, which split opens with ‘L’/‘Y’ shaped cracks at final stages. Under severe condition it destroys the entire orchard and causes heavy economic losses. There are large number of chemicals are available in the market as bactericides and their bioefficacy and suitability needs to be verified by in vitro and field studies, so as to incorporate the effective ones in the management package [4].

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## II. MATERIALS AND METHODS

### **Isolation and identification of bacteria from infected stem and fruit:**

Pomegranate fruits and stem showing typical symptoms of bacterial blight were collected from the field (farm in Chitradurga district) and the bacterium was isolated by dilution plate technique. Isolation of the pathogen is done within 48 hours after collection of samples on nutrient agar medium. Pure culture is established by repeated single colony transfer method. A reference culture was obtained from UAS, Dharwad.

The morphological characteristics such as cell shape and gram staining characters of the isolates were studied. The physiological and biochemical characters of the isolates were studied for hydrogen sulphide production, urease production and acid from different sugars viz., glucose, arabinose, lactose etc.

Koch's postulates were followed to prove pathogenic nature of *Xanthomonas* isolates. For pathogenicity test, inoculation has to be made by spraying bacterial suspension of 48 hours old culture onto the injured and uninjured stem and fruits of healthy plants. The characteristics symptoms were observed on pomegranate leaves after four days of inoculation as small water soaked lesions. After six days of inoculation it turned brown to black coloured lesions, which later developed into angular to irregular shaped spots along the margins, veins and veinlets of the leaf lamina. Reisolations were carried out from these lesions for each isolate and comparisons were made with original culture to confirm the identity of the pathogen. The reisolated culture resembled the original mother culture and thus pathogenicity test was confirmed [5].

### **Evaluation of efficacy of plant extracts against bacterial pathogen**

Fresh leaves and bulb of different medicinal plants (Neem, Tulsi, Ginger, Garlic) are collected and washed, shade dried and then powdered using the blender and stored in air tight bottles[6]. 10 g of powder is added to 100 ml of solvent (methanol, ethanol, water) in a conical flask and plugged with cotton wool. After 42 hours the supernatant is collected and the solvent evaporated to make the crude extract and stored at 4° C [7].

### **Antibacterial activity testing**

Antibacterial activity of aqueous extract, solvent extracts was determined by cup diffusion / agar well diffusion method on nutrient agar medium. Cups/ wells are made in nutrient agar plate using cork borer (5 mm) and inoculum of bacteria is spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then aqueous extract, solvents extracts are placed in the wells made in inoculated plates. Similarly each plate carried a blank with solvent only in the center to serve as a control [7].

### **Determination of the efficacy of biocontrol agents against the bacterial pathogen**

Cultures of *Pseudomonas fluorescens* (NCIM 2174), *Bacillus subtilis*(NCIM 2548) and *Trichoderma viride*(NCIM 1060) are obtained from NCIM, Pune and used as biocontrol agents. Effect of bio control agents was studied by well diffusion method [8] and dual culture plate method [6].

## III. RESULTS AND DISCUSSION

Pomegranate fruits showing typical symptoms of bacterial blight are collected from the field and the bacterium was isolated by dilution plate technique. By gram staining technique the bacteria was identified to be gram negative rod shaped bacteria. Then the Hi media standard test kit for the identification of gram negative bacteria was used for further identification of bacteria.

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**Table 1: Physiological tests result**

Sl. no	Test	Test result	
		Isolate 1	Reference culture
1	Citrate	+ve	+ve
2	Lysine	-ve	-ve
3	Ornithine	-ve	-ve
4	Urease	-ve	-ve
5	Phenyl alanine	-ve	-ve
6	Nitrate reduction	-ve	-ve
7	H <sub>2</sub> S	+ve	+ve
8	Glucose	+ve	+ve
9	Adonitol	+ve	+ve
10	Lactose	-ve	-ve
11	Arabinose	+ve	-ve
12	Sorbitol	+ve	-ve

In accordance with Bergey’s manual chart and literature review[5], we confirmed Bacteria was Xanthomonas and this was also compared with reference culture result (table 1).

### Evaluation of efficacy of plant extracts against bacterial pathogen:

Extracts of fresh leaves and bulb of different medicinal plants (Botanicals) were checked for the antimicrobial activity against the pathogen (table2).

**Table 2 Efficacy of botanicals against reference and isolate1 culture**

Sl. no	Concentration (%)	Inhibition zone in mm ReferenceIsolate				Inhibition zone in mm Isolate1			
		Neem	Tulsi	Ginger	Garlic	Neem	Tulsi	Ginger	Garlic
1	control	0	0	0	0	0	0	0	0
2	10	2	1	3	0	2	1	2	1
3	20	8	2	3	8	5	1	3	1
4	30	10	2.5	4	10	8	2	3	1
5	40	12	5	5	11	10	3	4	2
6	50	14	8	5	11	10	3	4	4

The results of zone of inhibition study revealed that the leaves and bulb of 4 different plants are effective only with the ethanol extracts. No significant inhibition zones were found for methanol and aqueous extracts in four different samples. The Neem ethanol extracts are found effective. The maximum inhibition was shown by Neem ethanol extract at 50% concentration for reference culture. 40and50% showed 10mm zone of inhibition against pathogen isolate1. It is also found effective in field application.

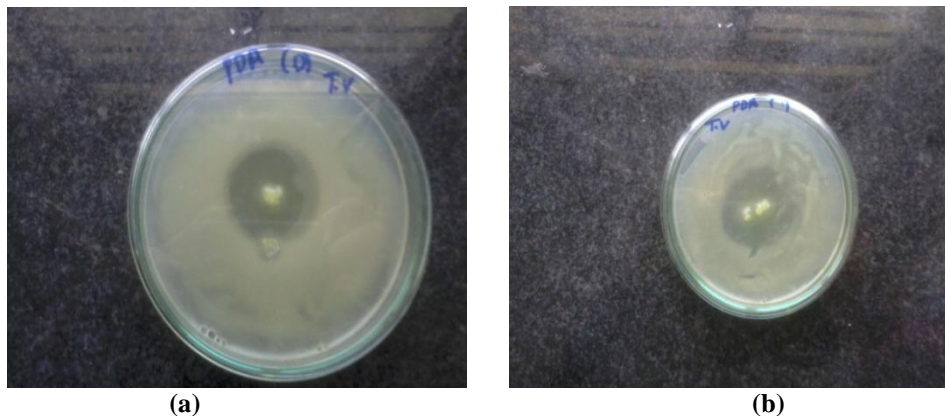
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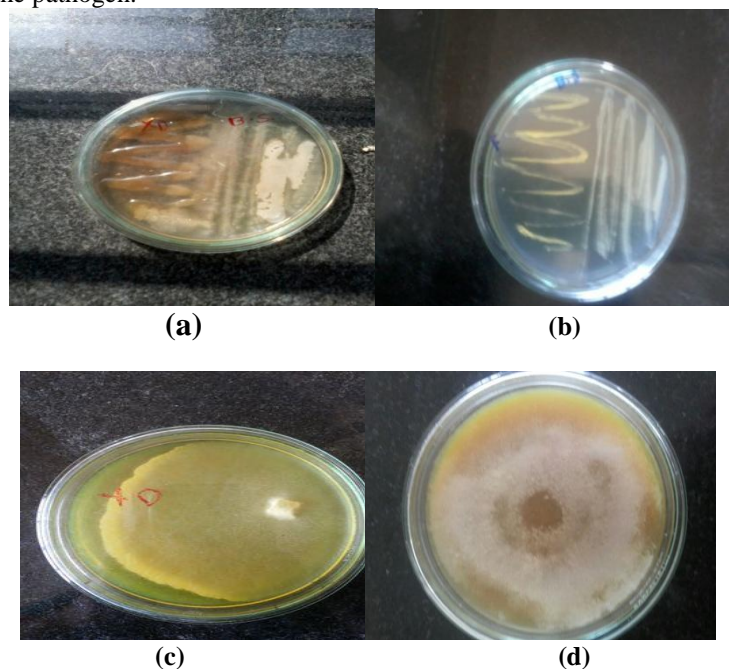
### Evaluation of efficacy of bio control agents against bacterial pathogen

In the light of present day constraints with the use of chemical pesticides in plant disease management, the biological control as an alternate option is gaining importance and awareness as the approach is ecofriendly and cost effective. Under biological control of plant diseases, various antagonistic organisms have been identified, which fight against the pathogens by different mechanisms viz., competition, lysis, antibiosis, siderophore production, etc. In this investigation *T. viride* showed maximum inhibition in both reference and Isolate 1 culture (Fig1), other biocontrol agents viz, *B. subtilis* and *P. fluorescens* were not effective against the pathogen in well diffusion method.



**Fig 1: In vitro evaluation of biocontrol agents by well diffusion method (a) Reference culture v/s *T. viride* (b) Isolate 1 culture v/s *T. viride***

Among the different antagonists tried as biocontrol agents in the dual culture method (Fig2), *Bacillus subtilis* and the fungal biocontrol agent *Trichoderma viridae* were found significantly superior over *Pseudomonas fluorescens* in inhibiting the growth of the pathogen.



**Fig 2: In vitro evaluation of biocontrol agents by Dual culture method (a) Reference culture v/s *B. subtilis* (b) Isolate 1 culture v/s *B. subtilis* (c) Reference culture v/s *T. viride* (d) Isolate 1 culture v/s *T. viride***

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Field application: Two plants in the field were used for the field application study for a month. Farmer sprayed the botanical extract of neem on the diseased plant once in week. It avoided spreading of disease. The spraying of the biocontrol agent *Trichoderma viride* with sterile water once in a week helped in fruit development.

## IV. CONCLUSION

In the present market, demand of Indian pomegranate fruit is decreasing because of using more chemical pesticides to control the disease, so with the present work we are stressing on using botanicals and biocontrol agents for the disease management and for yielding more crop.

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