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Screening of Actinomycetes effects against Pathogen causing Bacterial Blight of Pomegranate

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ABSTRACT

Pomegranate is called 'fruit of paradise' due to its versatile uses. Its fruit, leaves, stem all have nutritional as well as medicinal uses. However, pomegranate yield is adversely affected by infection of plant by various plant pathogens. One of the major harmful diseases is bacterial blight of pomegranate [commonly known as 'telya disease'] caused by *Xanthomonas axonopodis* PV *punicae.* The pathogen affects the fruit, leaves as well as stem. A variety of approaches have been tried to control the disease including cultivation methods, use of botanicals, use of antibiotics, use of chemicals etc. However, no single therapy has been found to be a sure shot control method. The current research work has been designed to achieve the control of disease using biological agent. Here, specifically actinomycetes were isolated from healthy pomegranate cultivated soils. During this research, 24 different actinomycete cultures were isolated and purified. All these cultures were tested for antagonistic activity against bacterial blight pathogen by giant colony technique. The antagonistic activity was determined by calculating inhibition ratio. The inhibition ratio is the ratio of total length of inhibitory area to the total length of streak line. Some actinomycete isolates showed promising antagonistic activity against *Xanthomonas axonopodis.* Further work is in progress.

Key words*: Pomegranate, Xanthomonas axonopodis PV punicae, Actinomycetes.*

Introduction

Pomegranate occupies the important portion of world fruit trade. India is the world's largest producer of pomegranates (743.1 thousand tonnes per year). However, yield of pomegranate decreases considerably due to infection of plant by various plant pathogens. One of them is bacterial blight of pomegranate [commonly known as 'telya disease'] caused by *Xanthomonas axonopodis* PV *punicae* (*Xap*) (Jain and Desai, 2018). The pathogen affects the various plant parts like fruit, leaves and stem and finally causes death of plant. It considerably decreases the yield of pomegranate (Raghuwanshi *et al.*, 2013). A variety of control methods have been used for control of disease like proper cultivation methods, use of botanicals like neem and tulsi, use of antibiotics like streptocycline, use of chemicals etc..(Doddaraju *et al.*, 2019). However, these methods have been found useful only on lab scale. A new approach is the use of biological agent. In the current research work, actinomycetes were isolated from healthy pomegranate cultivated soils. After repeated subculturing and purification, 24 different actinomycete cultures were isolated. The antagonistic activity of these actinomycete cultures against bacterial blight pathogen was checked by giant colony technique. Here, inhibition ratio was calculated. The actino-

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mycete isolates showing promising antagonistic activity against *Xanthomonas axonopodis* were selected for further research work.

Materials and Methods

Soil samples were collected from healthy pomegranate cultivated fields.These soil samples were inoculated in Glycerol Aspergine broth for enrichment of actinomycetes (Kumar and Jadeja, 2016). The broth was incubated at 30 ^{0}C for 15 days. A loopful of enriched sample was streaked on Actinomycete Isolation Agar plates. The plates were incubated at 30^{0}C for 7 days. After incubation, typical actinomycete colonies were selected (Ragunathan, 2010). The actinomycete cultures were purified by subculturing. Thus, 24 cultures of actinomycetes were purified. These cultures were checked for their antagonistic activity against bacterial blight pathogen *Xap* by giant colony technique (Bavishi *et al.*, 2017).

Initially, the isolated actinomycete culture was individually streaked at the centre of sterile nutrient glucose agar plate in the form of a straight line. All the plates were incubated at 28 ^{0}C for 7 days till satisfactory growth was obtained. Then a loopful of suspension of pure culture of *Xap* was aseptically streaked on the same plate at right angles (perpendicular) to the growth of actinomycete. The plates were again incubated at 28 ^{0}C for 72 hrs. After incubation, the total length of *Xap* inhibition and the total length of *Xap* streak line was measured. The ratio of total length of microbial inhibition to total length of streak line was calculated. The cultures showing high inhibition ratio were selected for further work.

Results and Discussion

After initial incubation of nutrient glucose agar plate at 28 ^{0}C for 7 days, the actinomycete culture growth was observed at the centre of the plate in the form of straight line (Photoplate 1). After subsequent incu-

Table 1. Inhibition ratios of actinomycete cultures

Actino. Culture Number	Length of inhibition [cm] = X	Length of streak line [cm] =Y	Ratio of inhibition X/Y	Actino Culture Number	Length of inhibition [cm] = X	Length of streak line [cm] =Y	Ratio of inhibition X/Y
A1	3	3.5	0.86	A13	0.4	3.1	0.13
A2	2.7	3.0	0.90	A14	0.5	3.4	0.15
A3	0.5	3.4	0.15	A15	0.4	3.3	0.12
A4	2.9	3.3	0.88	A16	0.4	3.4	0.12
A5	0.4	3.4	0.18	A17	0.4	3.4	0.12
A6	2.8	3.5	0.80	A18	2.8	3.2	0.88
A7	3.0	3.5	0.86	A19	0.4	3.3	0.12
A8	3	3.5	0.86	A20	0.3	3.4	0.08
A9	3.2	3.5	0.91	A21	3.1	3.3	0.94
A10	3.0	3.4	0.88	A22	0.3	3.5	0.09
A11	2.8	3.3	0.85	A23	0.4	3.4	0.12
A12	0.5	3.5	0.14	A24	0.3	3.4	0.09

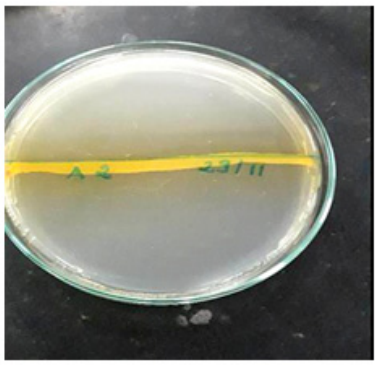

Fig. 1. Growth of actinomycete

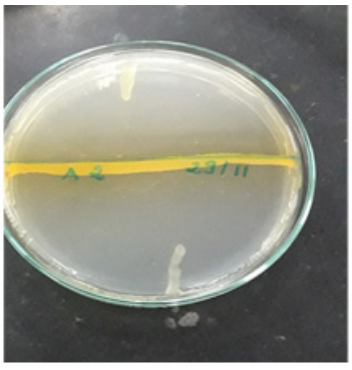

Fig. 2. Inhibition of SV

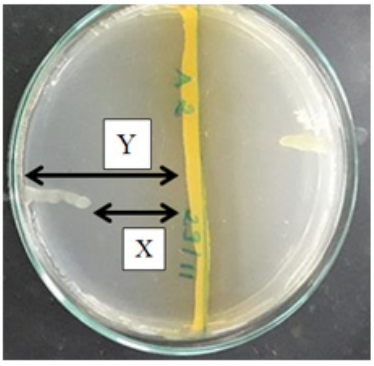

Fig. 3. Inhibition ratio

bation of plate at 28 ^{0}C for 72 hrs, the growth of *Xap* was observed, either on entire streak line or partially on streak line depending on the actinomycete culture (Photoplate 2).

After incubation, total length of *Xap* inhibition (X) and total length of *Xap* streak line (Y) was calculated (Fig. 3). The ratio of total length of microbial inhibition to total length of streak line (X/Y) was calculated as Inhibition Ratio. The inhibition ratio is directly proportional to the antagonistic activity of actinomycete against *Xap*. The inhibition ratios of all the 24 actinomycete cultures were calculated (Table 1).

The actinomycete cultures showing high inhibition (ratio marked as %) were selected for further research work. After identification of these cultures, these isolates were found to be the members of genus *Streptomyces* and *Nocardia*.

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Conflict of interest

There is no any conflict of interest among the authors.

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