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Meticulous isolation and purification of causative agent: A decisive step in the study of Bacterial Blight Disease (BBD) of pomegranate

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ABSTRACT

Xanthomonas axonopodis PV *punicae* [Xap] is the causative agent of bacterial blight disease of pomegranate. The disease is severe and can cause upto 80 to 90% reduction in the yield of pomegranate. A variety of approaches have been tried for the control of the disease. The first step in such study is isolation of bacterial blight pathogen Xap from infected pomegranate fruits or leaves and further maintenance of pure culture. Xap develops colourless to faint yellow, highly mucoid colonies on Nutrient Glucose Agar [NGA] medium.. Many researchers get confused at this step with the colonies of Xap. In current research work also, correct isolation method of the pathogen was performed. Here, the suspected Xap pathogen was isolated from the infected plant parts by standard isolation procedure. The suspected pathogen was then streaked on NGA plate. The plate was incubated at 28^0C for 72 hr. After incubation, the critical point of study was the selection of Xap colonies. These colonies were selected as suspected Xap isolates. These isolates were purified by repeated subculturing and then preserved on sterile NGA slants. The confirmation of isolate as Xap, causative agent of Bacterial Blight Disease of pomegranate was done by applying Koch's postulates on healthy plants.

Key words*: Xanthomonas axonopodis PV punicae [Xap], NGA, Mucoid colonies*

Introduction

Pomegranate (*Punica granatum* L), belongs to family Lythraceae. It is an ancient fruit of tropical and subtropical countries of the world. It is commonly called as 'fruit of paradise' due to its multiple uses (Asgary *et al.,* 2014). According to National Horticulture Board of India, India is the largest producer of pomegranate in the world, about 36 per cent of the world's production and about 30 per cent of the international pomegranate trade (Ayyappan, 2015). Pomegranate plant is adversely affected by variety of diseases. These diseases decrease the yield of pomegranate considerably and cause economic loss. Among these various diseases, the highly hazardous is bacterial blight disease (Doddaraju *et al.,* 2019). This disease causes considerable pomegranate losses. The causative agent of the disease is *Xanthomonas axonopodis* PV. *Punicae [Xap]*. Various researchers are working throughout the world for control of the disease. Current research was also performed to develop an effective control method. The first step in this study was isolation of *Xap from* infected fruits and leaves. The infected materials were processed by routine microbiological technique. When the processed material was streaked on

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Nutrient Glucose Agar [NGA] plate, typical faint yellow, highly mucoid colonies were developed on the medium (Sharma *et al.*, 2017). These colonies were selected as suspected *Xap* colonies and processed for further characterisation. *Xap* produces colourless to faint yellow glue drop like colonies on NGA. The organisms were further purified by repeated subculturing on NGA plates. This initial step must be performed very critically to avoid further mistakes in methodology and interpretation.

Materials and Methods

Isolation and cultivation of organisms from diseased lesions of pomegranate

The infected pomegranate fruits and leaves were collected from pomegranate fields at Sangola, Solapur district. The infected tissues from diseased leaves and fruits were cut by using sterile surgical blade. The cut pieces were suspended in 70% ethanol solution for 30 seconds for surface sterilization. Then they were washed with sterile distilled water for 10-20 seconds twice or thrice to remove the traces of ethanol. These pieces were then transferred to sterile Petri plate containing 1 mL of sterile distilled water and gently crushed using forecep with sterile surgical blade. Due to crushing, oozing of bacterial cells occurred and the water became slightly turbid. This suspension was streaked aseptically on the surface of sterile nutrient glucose agar plate (Ashish and Arora, 2016). The streaked plates were then incubated at 28^0C for 72 hr and observed for development of suspected bacterial colonies with typical characters. The suspected colonies were again restreaked on sterile NGA plate for repeated subculturing for purification. Finally, pure *Xap* colonies were selected and preserved at 4^0C on sterile NGA slants for further work.

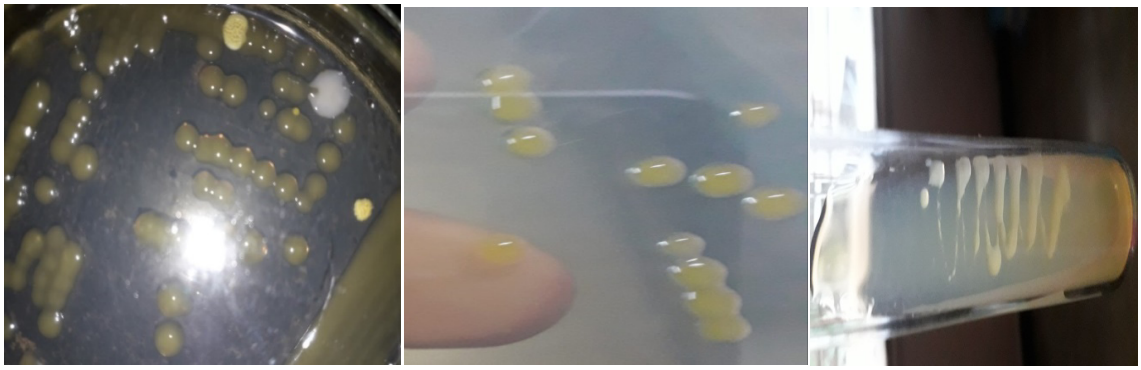
Koch's postulates studies

i) 0.1 mL of 10^8 CFU/mL suspension of Xap isolate was inoculated in immature fruits as well as leaves of healthy pomegranate plant & observed for development of bacterial blight of pomegranate. One plant was used as control and kept uninoculated.
ii) The infected tissue (oily spots) from fruits and leaves were subjected to isolation of causative agent on NGA medium. The development of typical bacterial blight diseased lesions indicates and confirms that the isolate is Xap causing pomegranate oily spot disease (Ashby, 2007).

Results and Discussion

Isolation and cultivation of organisms from diseased lesions of pomegranate

After incubation of NGA plates at 28^0C for 72 hr, various colonies were developed on NGA plate (Photoplate 1). Typical yellow, circular, entire, mucoid, high convex, glistening colonies were selected for purification. These colonies initially appeared like a minute glue drop. The colonies were initially colourless. Then the colour was slowly changed to cream colour to faint yellow. When these suspected bacterial colonies were restreaked on sterile NGA plate and incubated for 72 hours, pure culture of isolate was obtained (Photoplate 2). The colour of the colonies was slowly turned to brown. These isolates were further purified by repeated subculturing on Nutrient Glucose Agar plates (Photoplate 3). These purified cultures were used for Koch's postulates



Photoplate 1 **Photoplate 2** **Photoplate 3**

Mixed Colonies on NGAPure culture of Xap on NGA Growth of Xap on slant

studies.

Koch's postulates studies

It was found from the studies that the isolates were obtained from diseased lesions of pomegranate oily spots. When this isolate was injected in healthy pomegranate plants, it produced typical oily spot lesions on fruits and leaves. From these lesions again the isolate was grown on laboratory media i. e. NGA plate, confirming the isolate as causative agent of bacterial blight of pomegranate i. e. *Xanthomonas axonopodis* PV *punicae.*

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Conflict of interest

There is no any conflict of interest between the authors. Each author has a contribution in this research and publication work.

References

Asgary, S., Javanmard, S. and Zarfeshany, A. 2014. Potent health effects of pomegranate. *Advanced Biomedical Research*. 3(1): 100.https://doi.org/10.4103/2277-9175.129371

Ayyappan, S. I. 2015. Executive summery of vision 2015, (pp 1-15). *Vision 2050*.

Doddaraju, P., Kumar, P., Gunnaiah, R., Gowda, A.A., Lokesh, V., Pujer, P. and Anjunatha, G. 2019. Reliable and early diagnosis of bacterial blight in pomegranate caused by *Xanthomonas axonopodis*pv. *Punicae* using sensitive PCR techniques. *Scientific Reports*. 9 (1). https://doi.org/10.1038/s41598-019-46588-9

Sharma, J., Sharma, K. K., Kumar, A., Mondal, K. K., Thalor, S., Maity, A., Gharate, R., Chinchure, S. and Jadhav, V.T. 2017. Pomegranate bacterial blight: Symptomatology and rapid inoculation technique for xanthomonas axonopodis pv. punicae. *Journal of Plant Pathology*. 99(1): 109–119. https://doi.org/10.4454/jpp.v99i1.3825

Ashish and Arora, A. 2016. An overview of bacterial blight disease: A serious threat to pomegranate production. *International Journal of Agriculture, Environment and Biotechnology*. 9(4): 629. https://doi.org/10.5958/2230-732x.2016.00082.6

Ashby. 2007. Koch's postulated for plant pathogen. *British Mycological Society*. 1993–1994.