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Antibacterial Activity of Potassium Phosphite against Bacterial Blight Causing *Xanthomonas axonopodis PV. Punicae* in Pomegranate

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Abstract
Pomegranate cultivation in many part of India has been severely affected by bacterial blight caused by *Xanthomonas axonopodis pv. punicae.* Many studies were carried out to find alternative and potent products over existing methods. In the present study Potassium Phosphite was evaluated for antibacterial activity against *Xanthomonas axonopodis* by well diffusion method in vitro. Different concentrations of Potassium Phosphite were prepared separately and checked against pathogen for 24 hours. Among these concentrations Potassium Phosphite at 5000 ppm showed maximum zone of inhibition of 26 mm whereas concentrations of 1000 ppm exhibited least zone of inhibition. All the concentrations show inhibitory effect on growth of *Xanthomonas axonopodis pv. punicae.* This study would be highly useful to control the bacterial blight of pomegranate with the compatibility of Potassium Phosphite with other compounds.

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1. Introduction

1.1 Pomegranate

Pomegranate (*Punica granatum*) *is* an ancient fruit crop of India and also regarded as the fruit of paradise. Pomegranate has high nutritional, medicinal value and health benefits (Julie Jurenka, 2008; Aallwyn *et al*., 2014). The fruit has a wide consumer market and high economical value for its attractive, juicy, sweet characteristics and is a good source of carbohydrates and minerals such as Calcium, Iron and Sulphur. India is one of the major producer of pomegranate in the world with average total production of 8 lakh tons per annum (Kumar et al., 2011). However, recent emergence of bacterial blight has adversely affected the pomegranate production. Bacterial blight of pomegranate caused by *Xanthomonas axonopodis pv. punicae*, has become a major hurdle in pomegranate farming in the states of Maharashtra, Karnataka and Andhra Pradesh of India. Control of this pathogen is a challenge in agriculture. The disease conquered in all the seasons with varying degree of severity. The pathogen can infect at any stage of growth in the life of plant. The damage is observed on fruits which develop black oily spots later become completely black, then splits and dries off. In advanced stage of infection, tissue necrosis occurs on leaves and twigs. In the case of fruits the disease develops into cracks and later the fruit becomes completely black and dries off (Sandhya D. and Lakshmi M., 2016).

1.2 Potassium Phosphite

Potassium Phosphite is widely used in the management of fungal diseases in agriculture, horticulture and natural environments (Daniel R.et al, 2005). Potassium phosphite has also been

widely used in agriculture for the many advantages that it offers, especially to increase phosphorus uptake by the plant compared to products based on traditional phosphate (Cohen and Coffey, 1986; Jackson, 2000). Potassium phosphite is also known as Potassium Phosphonate, Potassium salt of Phosphorous acid. Phosphite works by boosting the plant's own natural defenses (Burra et al., 2014; Eshraghi et al., 2011; Lim et al., 2013; Massoud et al., 2012). Phosphite is not toxic to people or animals and its toxicity has been compared to table salt. Potassium phosphonate is an agent that has a systemic effect against fungal diseases, in particular against downy mildew (Pereira et al., 2010).

1.3 Aim and Objective

To isolate pathogenic microorganism from affected fruit sample and to screen antibacterial activity of Potassium Phosphite against microorganisms causing bacterial blight of Pomegranate.

2. Material and Methods

2.1 Isolation and identification of pathogens

Pomegranate fruit samples (Bhagwa variety) infected with bacteria were collected from city-Miraj, Maharashtra and identified by the S P College of Agriculture, Chiplun, Maharashtra, India. The infected portion (1cm) was removed from the fruit, surface sterilized with mercuric chloride solution (0.1%) for one minute, washed three times with sterile saline. The infected lesion was squeezed gently with a sterile scalpel into a sterile test tube containing 3 ml of sterilized saline. The suspension was serially diluted and plated in sterilized petriplates containing solidified *Xanthomonas* agar medium. Later the suspension was inoculated into a sterile petriplate and incubated at 30 ° C for 24 hrs (Yenjeerappa S.T. 2009).

2.2 Preparation of Potassium Phosphite Solution

Potassium Phosphite powder was mixed in distilled water to get various concentrations- 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm**.** (1000 ppm = 1gm Potassium Phosphite/Liter of water)

2.3 Antibacterial Activity

The antibacterial activity of Potassium Phosphite was determined by agar well diffusion method (Perez et al., 1990). A sterile 5 mm borer was used to cut 4-5 wells at equidistance in the plate. 80 µl of the test compound was introduced into each well and allowed to diffuse properly by keeping the petri plates in refrigerator at 4°C for 2 hours followed by incubation at 37°C for 24 hours. The diameter of zone of inhibition (excluding well diameter) was taken as the measure of the antimicrobial activity of a particular extract. Each experiment was performed in triplicate.

3. Results

After incubation of the plates for 24 hrs at 30 °C results were observed and noted. It was observed that at concentration of 5000 ppm, the Potassium Phosphite exhibited significant antimicrobial activity against *Xanthomonas axonopodis pv. punicae* (Figure 2). From Table 1, it is observed that Potassium Phosphite showed zone of inhibition at all concentrations. It showed maximum activity at 5000 ppm and minimum at 1000 ppm. There was moderate activity at concentration 2000 and 3000 ppm. From Figure 1, it is clear that as per the concentration rise, there was increment in zone of inhibition.

Table 1: Potassium Phosphite Activity

Sr No	Concentration of Potassium Phosphite (ppm)	Zone of Inhibition (mm)
1	1000	10
2	2000	12
3	3000	14
4	4000	16
5	5000	26

Figure 1: Graphical Analysis of Pot Phosphite conc. Vs Zone of Inhibition

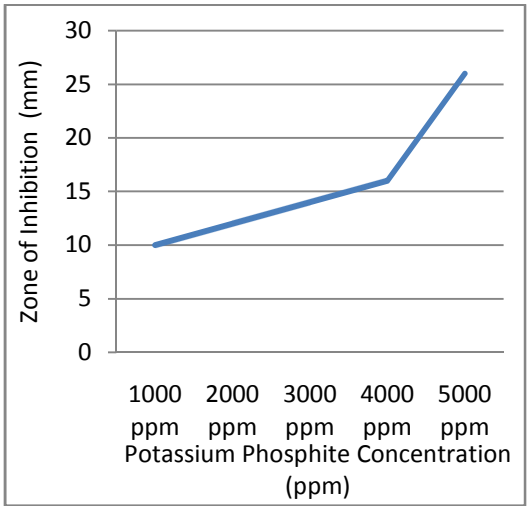
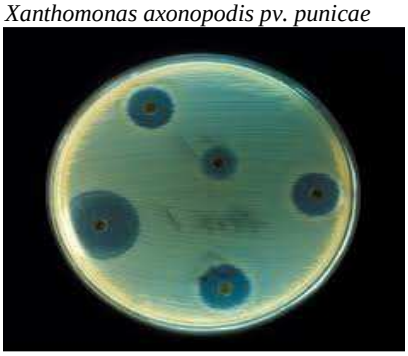


Figure 2: Potassium Phosphite Activity Against *Xanthomonas axonopodis pv. punicae*



4. Discussion

Bacterial Blight on Pomegranate caused by *Xanthomonas axonopodis pv. punicae*, is controlled by spraying various formulations of antibiotics and chemicals (Raghuwanshi et al., 2013; Lokesh et al., 2014). Major issue associated with these control measure is the decrease in plant immunity against further attack and other pathogens. Several recent laboratory studies show that application of phosphite compounds improves plant defence (Burra et al., 2014; Eshraghi et al., 2011; Lim et al., 2013; Massoud et al., 2012). This scenario demands an environmental friendly and effective method to control the pathogen. The present study screened antibacterial activity of Potassium Phosphite as a potent bactericide against *Xanthomonas axonopodis pv. punicae.* Phosphites are found to be much better than traditional phosphates. Potassium phosphite has significantly improved the agricultural credibility and acceptability of the new world.

Conclusion

Many bactericides in the market, efforts aim to treat diseases caused by *Xanthomonas axonopodis pv. punicae*. However, present study highlights activity of Potassium Phosphite against pathogenic isolates causing bacterial blight in pomegranate. Further study is necessary to understand the site of action of Potassium Phosphite at molecular level.

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