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Antibacterial activity of commercially available chemicals against *Xanthomonas axonopodis* pv. punicae causing Bacterial blight of pomegranate under in vivo conditions

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Key words: Bacterial blight, *Xanthomonas axonopodis* pv. punicae, *In vivo,* Disease incidence, Disease reduction over control.

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Abstract

Xanthomonas axonopodis pv. punicae, causal agent of bacterial blight disease; has severely affected the pomegranate cultivation in many parts of Punjab, Pakistan. Extensive agricultural practices along with large amount of bactericides are required to control this disease. A field trail was conducted at orchards of Horticulture Research Institute, Faisalabad to study the in vivo efficacy of 5 chemicals in combination with cultural practices against the pathogen. Six treatments consisting of chemicals viz., Streptomycin sulphate, Bismerthiazol, Copper hydroxide, Copper oxychloride and Bordeaux mixture were sprayed with four replications and control plants were untreated. All the tested chemicals were found significantly effective ($P < 0.05$) compared to the control treatment in managing the disease. However, Streptomycin sulphate was proved to be most effective in reducing the disease incidence with least number of infected leaves (5.3%), lowest number of twigs infected (1.83%) and minimum fruit infection (12%).While disease reduction over control was calculated as 87, 85 and 88% in leaves, twigs and fruits respectively on application of streptomycin sulphate.

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Introduction

Punica granatum L. (pomegranate) is an ancient fruit, regarded as the "Fruit of Paradise". It is a native of Iran, and spread to the Mediterranean countries. Though it is a minor subtropical crop but its cultivation is increasing very rapidly. In Pakistan, Baluchistan is the major producer of pomegranates, followed by KPK and Punjab. It is being cultivated on nine thousand hectares with forty thousand tons' production. It is a good source of carbohydrates, vitamin-C and minerals (Malhotra *et al.*, 1983). Pomegranate is known to have several pharmaceutical and therapeutic values i.e., curing inflammation, mildly laxative, control of dysentery, diarrhea and killing tape worms. Plant residues are used in dying and tanning industries.

There are several production constraints but pests and diseases cause severe yield losses. Among diseases bacterial blight caused by *Xanthomonas axonopodis* pv. punicae is a major threat (Vauterin *et al.*, 1995; Kumar, 2007; Petersen *et al.*, 2010). In most areas it has reached the alarming stage and is assumed epidemic form.

Initial symptoms includes appearance of small, irregular water soaked, dark colored spots on leaves which on later stages enlarge, coalesce and led to defoliation (Kumar, 2007; Sharma *et al.*, 2017).

Girdling and cracking symptoms could be observed upon infection of stem and branches. Whereas spots on fruit split opens with 'L'/'Y' shaped cracks at final stages. If favorable conditions prevail, this disease may result in heavy economic losses and/or complete destruction of the orchard.

There is a little information available on management of *Xanthomonas axonopodis* pv. punicae in Pakistan. There is a need to evaluate the bio-efficacy of available bactericides against the disease along with incorporation of cultural practices. Keeping all these aspects in view, present study was conducted with the objective of *In-vivo* evaluation of bactericides against *X. axonopodis* pv punicae.

Material and methods

Site and plant selection

A field trail was conducted at orchards of Horticulture Research Institute, Faisalabad (31.4504° N, 73.1350° E) during Rabi (summers) season of 2016-17. Variety grown was Sanduri. Plants of the same age were selected for the experimental evaluation of chemicals.

Cultural practices

Diseased branches and leaves were pruned and burnt to lower the primary inoculum levels.

Preparation of Chemicals

Six treatments consisting of chemicals viz., Bordeaux mixture@ 4:4:50, Copper oxychloride 50 WP @3g/L of water, Kocide 3000 52.4 WG (copper hydroxide) @ 2.5 g/L of water, Flare 72 SP (Streptomycin sulphate)@ 1 g/L of water, Thrill 20% WP (Bismerthiazol) 2 g/L of water were prepared with three replications and control plants were untreated.

Application of chemicals

Chemicals were evaluated in combination with cultural practices for their efficacy against bacterial blight in pomegranate under in vivo conditions. First spray was given at the disease onset in April and treatments were continued at an interval of 15 days or if rainfall occurs. Spraying was done using manually operated high volume (knapsack) sprayer.

Data recording

Data was recorded on incidence and severity of disease on leaves, branches and fruits before and after spray. The disease severity was recorded by using 0-5 scale where 0= no disease, 1= <1%, 2= 1-10%, 3= 11-20%, 4=21-50% and 5= >50% disease. Percent disease reduction over control was measured by using the following formula.

$$\text{Disease incidence } \{(\text{DI})\ \%\} = \frac{\text{Number of sample with BLB symptoms}}{\text{Total number of samples}} \times 100$$

$$\text{Diseases reduction over control } (\%) = \frac{\text{DI in control} - \text{DI in treatment}}{\text{DI in control}} \times 100$$

Data analysis

There were six treatments and each treatment had

five plants. All he treatments were replicated thrice in Randomized Completely Block Design (RCBD). Readings were the mean of five plants and standard error was calculated. Data was subjected to analysis of variance (ANOVA) followed by Tukey's HSD (honest significant difference) using SPSS v 15.0 for windows.

Results

Antibacterial potential of five chemicals was assessed by a semi-scheduled spray and disease incidence (%) and severity was recorded at fortnightly interval. Disease reduction over control (%) was calculated for the last reading during data collection. First reading of disease incidence of bacterial blight of pomegranate was recorded 24 hours before first spray on appearance of pinhead sized infection spots. Initial disease incidence on leaves ranged 7.6-8.3%, did not significantly differ among the treatments compared whereas final reading taken after 6th spray showed a remarkable reduction in disease incidence as compared to control i.e., 63.7% (Fig. 1).

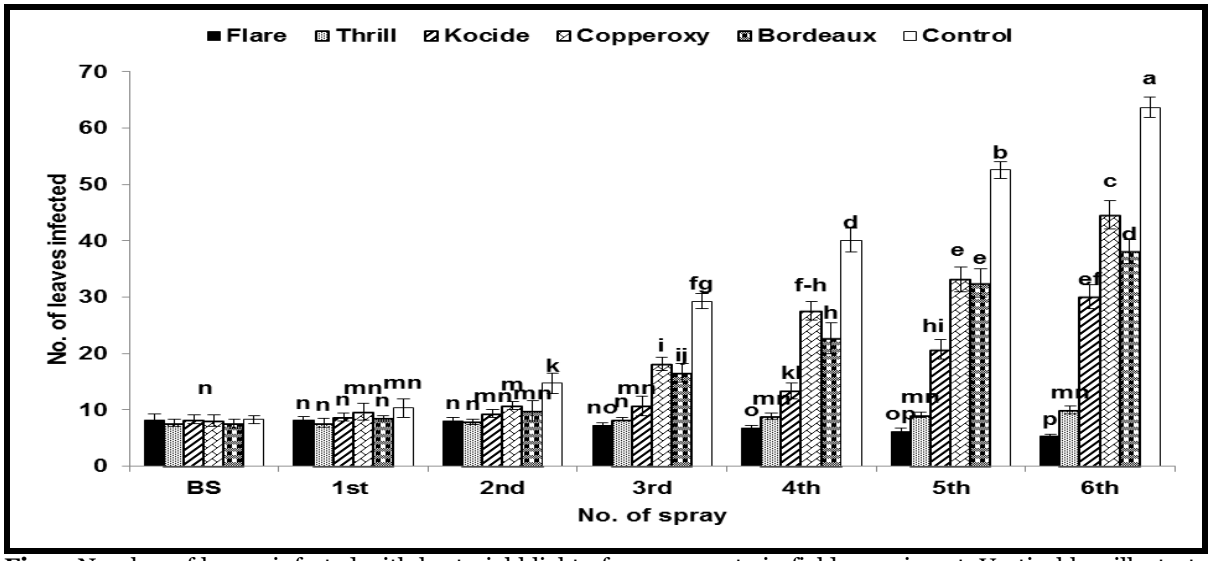

Fig. 1. Number of leaves infected with bacterial blight of pomegranate in field experiment. Vertical bar illustrate standard error of means. Values with different letters on top of bar show significant difference (P≤0.05) as determined by Tukey's HSD method. BS= Before spray.

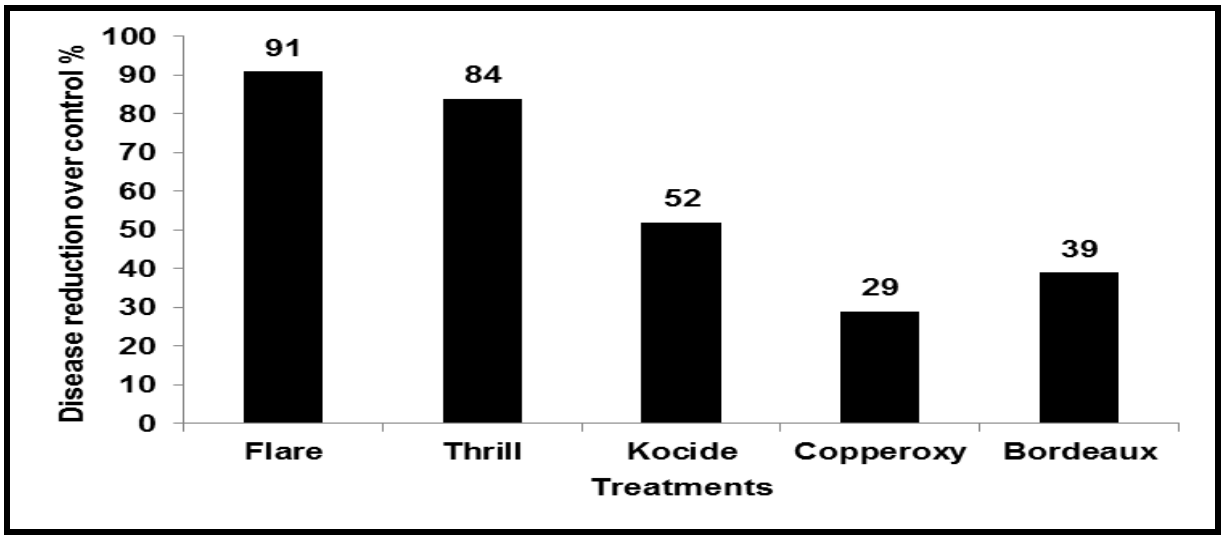

Fig. 2. Disease reduction (%) over control of *X. axonopodis* pv punicae using chemicals against infection development in pomegranate leaves.

Disease incidence in streptomycin sulfate was 5.3% followed by thrill (10%) and Kocide (30.1%). Highest disease incidence among treatments was exhibited by copper oxychloride i.e., 44.6%. Percent disease reduction over control was calculated and revealed that streptomycin sulfate significantly ($P < 0.05$) lowers the incidence of bacterial blight by 91.53% under in vivo conditions (Fig. 2) followed by Thrill (84.12%). Kocide, Copper oxychloride and Bordeaux mixture also reduced the disease incidence significantly i.e., 52.22%, 39.52% and 29.20% respectively.

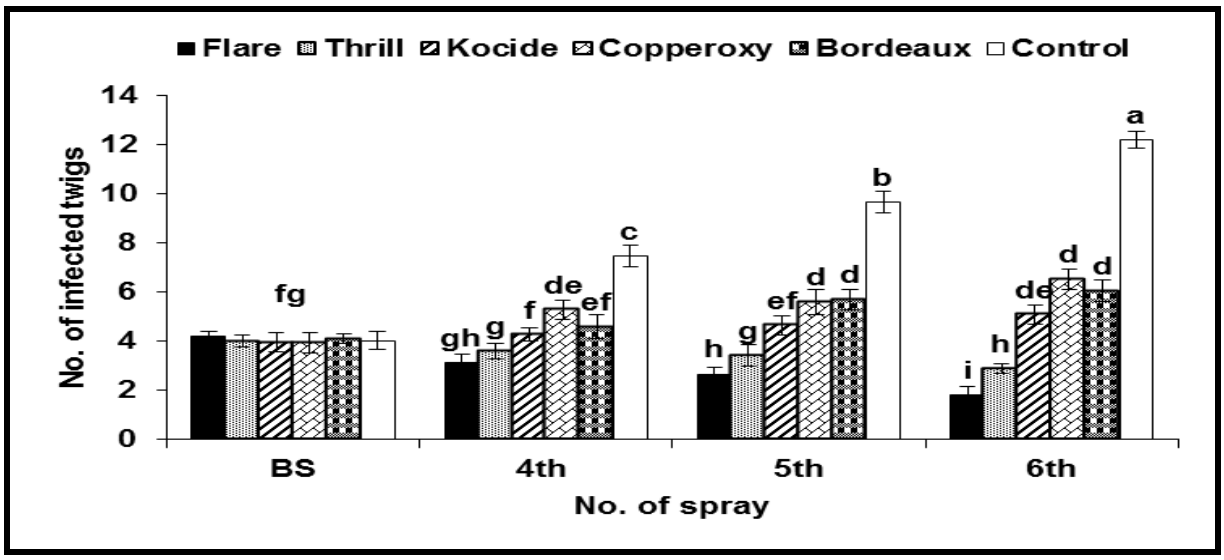

Fig. 3. Number of twigs and branches infected with bacterial blight of pomegranate in field experiment. Vertical bar illustrate standard error of means. Values with different letters on top of bar show significant difference ($P \leq 0.05$) as determined by Tukey's HSD method. BS= Before spray.

Data on number of twigs infected was recorded after 4rd, 5th and 6th spray and the highest incidence i.e., 12.23% was observed in untreated control(Fig. 3). The least incidence was observed in Flare treatment i.e., 1.83% followed by thrill 2.89%. Percent disease reduction over control was calculated and revealed that Flare significantly ($P < 0.05$) lowers the incidence of bacterial blight by 85.03%(Fig. 4).

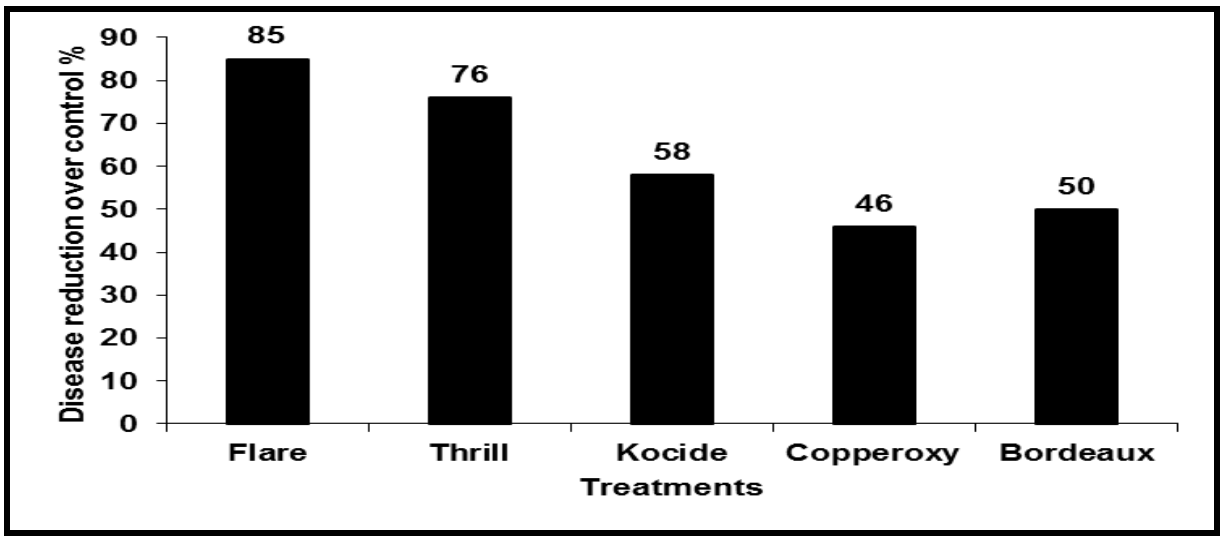

Fig. 4. Disease reduction (%) over control of *X. axonopodis* pv punicae using chemicals against infection development in pomegranate twigs and branches.

Significant reduction was also exhibited by Thrill, kocide, Bordeaux mixture and Copper oxychloride i.e., 76.36%, 58.29%, 50.36% and 46.52% respectively than the control treatment

Infected no of fruits were recorded at the time of harvest along with the total number of fruits per tree per treatment. Severely infected trees showed lower fruit onset and higher disease incidence on fruit.

The highest disease incidence on fruit i.e., 94.87% was observed in control treatment where no chemicals were applied (Fig. 5).

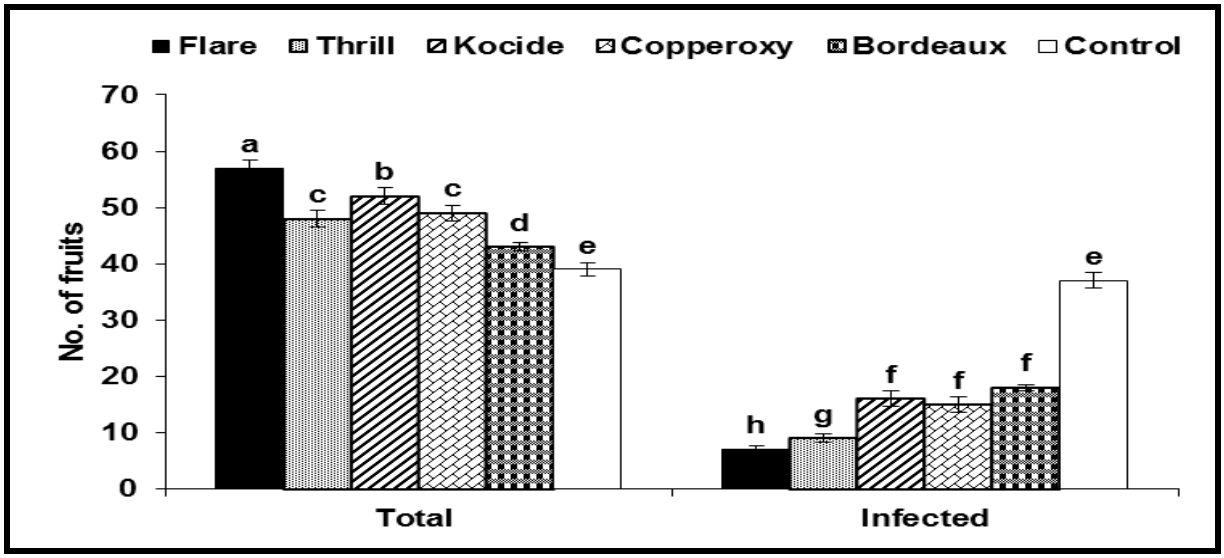

Fig. 5. Number of fruits infected with bacterial blight of pomegranate in field experiment. Vertical bar illustrate standard error of means. Values with different letters on top of bar show significant difference (P≤0.05) as determined by Tukey's HSD method.

This was followed by Bordeaux mixture, kocide and copper oxychloride i.e., 30.76%, 30.61%and 41.86%respectively. Thrill showed the disease incidence of 18.75% on fruit whereas the least incidence was observed in Flare treatment i.e., 12.28%. Percent disease reduction over control was calculated and revealed that Flare and thrill significantly lowers ($P < 0.05$) the incidence of bacterial blight by 87.71% and 81.25% (Fig. 6). Kocide, Copper oxychloride and Bordeaux mixture exhibited disease reduction over control treatment i.e., 69.38%, 69.23%, and 58.13% respectively.

Discussion

Pomegranate is grown on diverse range of climatic conditions. The wider range of climatic and environmental a condition in which pomegranate is grown indicates the nature and the diversity of the associated disease problems. Bacterial blight of pomegranate caused by *Xanthomon asaxonopodis* pv. *punicae* is known to occur in different pomegranate growing areas (Vauterin *et al.,* 1995; Icoz *et al.*, 2014). It is being considered as major disease of pomegranate crop which resulted in both qualitative and quantitative losses. In Pakistan, it was first observed in Chakwal and Islamabad during August 1988 (Akhtar and Bhatti, 1992).For the subsequent years, the disease continued to damage the crop,however its severe outbreak is observed in last few years. Faster inoculum build up rate and rapid spread under favorable environmental conditions are two main reasons that this disease could not be managed effectively.

The study was conducted during Rabi (spring) seasons of 2016-17 to evaluate the effectiveness of commercially available chemicals against bacterial blight of pomegranate. Overall all the tested chemicals were found effective when compared to untreated control. However, among the tested bactericidal chemicals, it was found that flare and thrill were statistically significantly effective in reducing the disease incidence at recommended doses. Disease reduction over control (%) was also measured significantly lowered in all chemical treatments than control where no chemical was applied. Percent disease reduction ranged between 29.20 to 91.53% on leaves, 46.52 to 85.03% on branches and 58.13 to 87.71% on fruits.

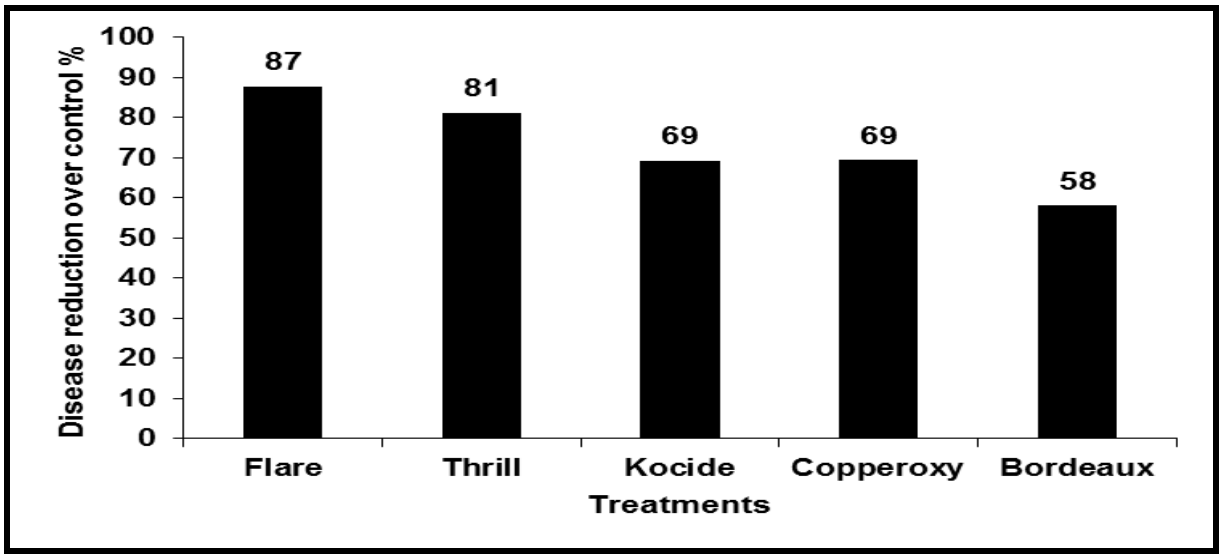

Fig. 6. Disease reduction (%) over control of *X. axonopodis* pv punicae using chemicals against infection development in pomegranate fruits.

Streptomycin sulphate (0.05%) or Streptocycline (0.05%) were found effective against bacterial leaf spot incidence on grape vine (Ravikumar*et al.*, 2002). Kishun and Sohi, (1984) reported that agrimycin-100 (0.1%) or Streptocycline @ 250 ppm managed *Xanthomonas campestris* pv. *campestris* associated with black rot of cabbage. Similarly Manjula *et al.,* (2002) reported that the sprays of Streptocycline or K cycline or bacterinol-100 effectively control bacterial blight of pomegranate. Streptomycin Sulfate is an aminoglycoside antibiotic with antibacterial property that inhibits peptide elongation and protein synthesis, by binding to the S12 protein of the bacterial 30S ribosomal subunit, consequently leading to bacterial cell death (PubChem CID:53384391). Few studies reported that Streptocycline (0.05%) combined with Copper oxychloride (0.2%) produce better results against the disease than upon individual application (Yenjerappa *et al.,* 2004; Lokesh *et al.*, 2014). Copper is used in various forms and classified as multisite compounds that act by disrupting cellular proteins (Mondal and Mani, 2012).

Bismerthiazol is widely used as a protectant bactericide for controlling leaf blight of rice caused by *X. oryzae* pv. oryzae. It also has curative efficacy and could inhibit growth of the bacteria (Liang *et al.*, 2015). Yu *et al.*, (2016) demonstrated that bismerthiazol was effective for controlling citrus canker in planta by inhibiting the growth of *X. citri* ssp. citri (Xcc) and triggering host defense responses.

This may involve the salicylic acid signaling pathway and the priming defense in citrus fruits by bismerthiazol, and it may serve as an effective alternative to copper bactericides for the control bacterial diseases.

Conclusion

Xanthomon axonopodis pv. punicae associated with bacterial blight of pomegranate pose a major threat to the crop. Chemicals were evaluated in combination with cultural practices for their efficacy against bacterial blight in pomegranate under in vivo conditions. It is concluded from present studies that pruning is pre-requisite for the management of bacterial blight and streptomycin sulfate and bismerthiazol effectively controlled *X. axonopodis* pv. *punicae* when applied at an interval of 15 days or if rainfall occur.

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