EFFECTIVENESS OF PSEUDOMONAS FLUORESCENS AGAINST BACTERIAL CANKER XANTHOMONAS CAMPESTRISPV. CITRI IN ACID LIME CV. KAGZI LIME

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DOI: http://dx.doi.org/10.24327/IJRSR.2020.1105.5316

ABSTRACT
Investigations on antagonistic activity of selected Pseudomonas bacterial strain against citrus bacterial canker disease was carried out in the farmers field. Results of experiments revealed that bacterial strain Pseudomonas fluorescens was inhibitory on X. citri with various antagonistic activities. Pseudomonas fluorescens @ 20 gm / lit water was sprayed on acid lime for the management of citrus canker disease. The number of disease spots on leaf and fruits were recorded from 1.03 to 1.31 in the treatment Pseudomonas fluorescens @ 20 gm / lit and 1.07 to 1.23 in the control. In conclusion, the integration of Pseudomonas fluorescens was possible in IPM programme to manage citrus canker disease in acid lime.

INTRODUCTION
Pseudomonas fluorescens encompasses a group of common, nonpathogenic saprophytes that colonize soil, water and plant surface environments. It is a common gram negative, rod-shaped bacterium. As its name implies, it secretes a soluble greenish fluorescent pigment called fluorescein, particularly under conditions of low iron availability. Certain members of the P. fluorescens have been found potential agents for biocontrol which suppress plant diseases by protecting the seeds and roots from fungal infection. They are known to enhance plant growth and reduce severity of many fungal diseases (Hoffland et al. 1996, Wei et al. 1996). This effect is the result of the production of a number of secondary metabolites including antibiotics, siderophores and hydrogen cyanide (O’Sullivan & O’Gara 1992). Hass and Defago (2005) reviewed the mechanisms by which P. fluorescens control pathogenic microorganisms in detail. Competitive exclusion of pathogens as the result of rapid colonization of the rhizosphere by P. fluorescens may also be an important factor in disease control. The present review discusses the occurrence, distribution, growth requirements of P. fluorescens and diseases controlled by the bacterial antagonist in different agricultural and horticultural crops.

Acid Lime (Citrus aurantifolia Swingle), belongs to the family Rutaceae. Citrus is the third largest fruit crop grown in India after mango and banana. It is generally grown under both tropical and subtropical climatic conditions. Commercially sweet orange, mandarin and acid lime are grown in different agro climatic regions. Citrus fruits like oranges, limes, lemons, etc. have been under cultivation in India since time immemorial. Citrus trees have been derived from eastern Asia domestic species and are presently planted in many countries including Argentina, Australia, Brazil, China, Cuba, Egypt, India, Israel, Italy, Japan, Mexico, Morocco, South Africa, Spain, USA and Iran (Khodakaramian and Ghasemi, 2002). Citrus is a very important fruit tree and being grown in several Northern and Southern provinces (Khodakaramian and Ghasemi, 2002).
The area under Acid Lime in India is 255.20 thousand hectares with production of 2523.50 thousand MT and productivity of 9.9 MT (Annon., 2017). In Gujarat, Citrus is cultivated in 46279 hectares with production of 605613 MT and productivity of 13.09 MT/ha (Annon., 2017). In Mehsana district, the area, production and productivity is 12516 ha, 177852 metric tons and 14210 kg/ha, respectively (Annon., 2019).

Citrus bacterial canker is one of the most important and serious diseases of citrus around the world (Cubero and Graham, 2002; Gottwald et al., 1993; Graham, 2001; Graham et al., 1992; Khodakaramian and Ghasemi, 2002; Leite and Mohan, 1990; Leite et al., 1987; Bora and Bhagabati, 1996; Rodrigues et al., 1998; Schubert et al., 2001; Stall and Civerolo, 1991; Timmer et al., 2000; Vernière et al., 1998).

Almost all citrus cultivars are susceptible to this pathotype, but the degree of susceptibility to this disease is higher among some grape fruits, limes, lemons and some orange cultivars (Gottwald et al., 1993; Timmer et al., 2000; Vernière et al., 1998).

The symptoms of Asian citrus canker appear on leaves, fruits and twigs and include lumpy lesions that first appear on the upper side and later on the upper side of the leaves (Khodakaramian and Ghasemi, 2002; Bora and Bhagabati, 1996). Along with the spread of the lesions, their sides become lumpy which is called volcano shape lesion (Khodakaramian and Ghasemi, 2002; Bora and Bhagabati, 1996). Sever infection of citrus trees with pathotype results in leaf and fruit defoliation, twig dryness and finally complete decline of the tree (Khodakaramian and Ghasemi, 2002; Bora and Bhagabati, 1996).

Nowadays, citrus trees are suffering from this disease in about 30 countries around the world. Citrus bacterial canker was first observed on lemon trees in Kerman province in 1989 and the causal agent was isolated and identified (Khodakaramian and Ghasemi, 2002). According to the previous studies some bacterial strains isolated from citrus trees in Southern provinces of Iran were capable of inducing typical canker symptoms on many citrus species including *Citrus aurantiifolia*, *C. ponceirus*, *C. grandis*, *C. sinensis*, *C. aurantium*, *C. jambhiri*, *C. limon*, *C. reticulata*, *C. medica* and *C. paradisi* (Khodakaramian and Ghasemi, 2002).

For controlling citrus bacterial canker disease different methods such as eradication of diseased trees, prevention of importation and distribution of infected plants, chemical methods, planting resistant varieties and the use of biological agents have been studied (Cubero and Graham, 2002; Gottwald et al., 1993; Graham, 2001; Graham et al., 1992; Khodakaramian and Ghasemi, 2002; Leite and Mohan, 1990; Leite et al., 1987; Bora and Bhagabati, 1996; Rodrigues et al., 1998; Schubert et al., 2001; Timmer et al., 2000; Vernière et al., 1998).

Recently, the use of bacterial and fungal antagonists has been applied to control several plant diseases including citrus canker (Heydari et al., 2005; Bora and Bhagabati, 1996; Shahriari et al., 2005). Considering all the above facts and with a view to have better management of canker and yield of fruits, a field experiments were conducted to find out the effectiveness of *P. fluorescens* on canker and yield of Acid Lime (*Citrus aurantiifolia* Swingle) cv. Kagzi Lime.

**MATERIAL AND METHODS**

The experiments were carried out by Krishi Vigyan Kendra, Mehsana, Gujarat in citrus canker affected orchards of different farmers during the year 2014-2016 to evaluate the antagonistic effect of bacterial isolates against causal agent of Bacterial Citrus Canker. Ten farmers selected from Sanganpur, Jagudan, Akhaj, Kahoda, Bhatasan villages citrus growing area of Mehsana district. The two treatment i.e. T1: spraying of streptomycin sulphate @ 1 gm + copper oxychloride @ 40 gm per 10 liter water and T2: *Pseudomonas fluorescens* @ 0.2 % (1 x 10^8 cfu ) 20 gm/10 liter water were applied in the month of June, August and December for the management of citrus canker. Spray of both the treatments applied on lime tree at first week of June, August and December in each year and observation recorded one month after spraying. Each treatment consists of 10 acid lime plants (kagzi lime variety) and replicated in 10 farmers field. Disease evaluation factor was the total number of lesions on 50 leaves as well as fruits appeared on trees one month after spray. Economics of the results was also calculated. The same experiments conducted for the year 2013-14, 2014-15 and 2015-16 as well as three year pooled results were recorded.

**RESULTS AND DISCUSSION**

The results of antagonistic activity of *Pseudomonas fluorescens* against bacterial citrus canker causal agent in field condition are shown in Table 1. Perusal of the data presented in Table 1 indicated that biological treatment i.e. *Pseudomonas fluorescens* treatment (T2) was found at par with chemical control treatment i.e. Streptomycin sulphate @ 1 gm + copper oxychloride @ 40 gm per 10 liter water (T1) in the sense number of spots on leaf during June, July and August month application in the year 2013-14, 2014-15 and 2015-16. The three years pooled data showed that average number of canker spots on leaf was 0.91 ± 0.05 in T1 and 1.03 ± 0.05 in T2 treatment (Table 1). The results obtained in three years experiments were more or less similar with finding of Khodakaramian and Ghasemi (2002) and Leite and Mohan (1990).

In case of bacterial canker spots on fruit in acid lime, results are presented in Table 2. These results indicated that biological treatment i.e. *Pseudomonas fluorescens* treatment (T2) was found at par with chemical control treatment (T1) in all three year experiment. The three years pooled data showed that number of canker spots on fruits was 1.23 ± 0.05 and 1.31 ± 0.03 in T1 and T2, respectively (Table 2). The results obtained in three years experiments were more or less similar with finding of Khodakaramian and Ghasemi (2002) and Leite and Mohan (1990).

Table 1 Effectiveness of Pseudomonas fluorescens against citrus bacterial canker in acid lime.

<table>
<thead>
<tr>
<th>Treat.</th>
<th>Year 2013-14</th>
<th>Year 2014-15</th>
<th>Year 2015-16</th>
<th>Three year pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June</td>
<td>July</td>
<td>August</td>
<td>Av. ± SD</td>
</tr>
<tr>
<td>T1</td>
<td>0.75</td>
<td>0.86</td>
<td>0.78</td>
<td>0.80 ± 0.06</td>
</tr>
<tr>
<td>T2</td>
<td>0.89</td>
<td>1.05</td>
<td>0.99</td>
<td>0.98 ± 0.08</td>
</tr>
</tbody>
</table>

Table 2 Effectiveness of Pseudomonas fluorescens against citrus bacterial canker in acid lime.

<table>
<thead>
<tr>
<th>Treat.</th>
<th>Year 2013-14</th>
<th>Year 2014-15</th>
<th>Year 2015-16</th>
<th>Three year pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June</td>
<td>July</td>
<td>August</td>
<td>Av. ± SD</td>
</tr>
<tr>
<td>T1</td>
<td>1.04</td>
<td>1.15</td>
<td>1.11</td>
<td>1.10 ± 0.06</td>
</tr>
<tr>
<td>T2</td>
<td>1.17</td>
<td>1.23</td>
<td>1.26</td>
<td>1.22 ± 0.05</td>
</tr>
</tbody>
</table>

Table 3 Effectiveness of Pseudomonas fluorescens against citrus bacterial canker in acid lime.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Yield (Kg/ha)</th>
<th>2013-14</th>
<th>2014-15</th>
<th>2015-16</th>
<th>Av. ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>14980</td>
<td>14205</td>
<td>14256</td>
<td>14480 ± 433.47</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>14760</td>
<td>13940</td>
<td>14105</td>
<td>14268 ± 433.71</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 Economics of Pseudomonas fluorescens against citrus canker in acid lime.

<table>
<thead>
<tr>
<th>Treat.</th>
<th>Gross return (in lacs/ha)</th>
<th>Cost of cultivation (in lacs/ha)</th>
<th>Net return (in lacs/ha)</th>
<th>BCR</th>
<th>Av. ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>2.21</td>
<td>4.26</td>
<td>3.44 ± 1.08</td>
<td>1.75</td>
<td>1.80</td>
</tr>
<tr>
<td>T2</td>
<td>2.24</td>
<td>4.18</td>
<td>3.41 ± 1.03</td>
<td>1.83</td>
<td>1.73</td>
</tr>
</tbody>
</table>

Results presented in Table 3 revealed that similar results was observed in the year 2013-14, 2014-15 and 2015-16 in acid lime productivity. The three year pooled data indicated that the average yield of acid lime was 14480 ± 433.47 kg/ha and 14268 ± 433.71 kg/ha in the treatment T1 and T2, respectively.

The data in Table 4 showed that Pseudomonas fluorescens treatment (T2) was found almost similar to chemical treatment (T1) with all the parameters of economics i.e. gross return, cost of cultivation, net return and cost benefit ratio during all the three years. The pooled results of three years (Table 4) revealed that the gross return (3.41 ± 1.03 lacs/ha), cost of cultivation (1.63 ± 0.26 lacs/ha) and net return (1.78 ± 1.18 lacs/ha) were recorded in biological treatment (T2), whereas in T1 treatment, gross return, cost of cultivation and net return were 3.44 ± 1.08, 1.65 ± 0.21 and 1.79 ± 1.14 Rs. lacs/ha, respectively. The three years average BCR was recorded as 2.12 ± 0.76 in T1 and 2.17 ± 0.85 in T2 biological treatment (Table 4).

CONCLUSION

The results obtained in farmer’s field condition indicated that the bacterial antagonists Pseudomonas fluorescens was capable of reducing the incidence of bacterial canker disease as compared to chemical control. Based on the results of this study, it can be concluded that biological control is potentially an effective method and may be used as an one of the important component of Integrated Pest Management (IPM) strategies for controlling and managing citrus bacterial canker disease which is considered to be one of the most damaging and destructive diseases of citrus growers around the world.

Acknowledgement:

Authors are very grateful to the ICAR, New Delhi for providing financial assistance for conducting on farm trial on citrus and thankful to host institution for providing facilities. We are also thankful to farmers for providing land and support to conducting experiments.

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How to cite this article:
DOI: http://dx.doi.org/10.24327/ijrsr.2020.1105.5316

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