DEVELOPMENT OF RESISTANCE TO PESTICIDE IN BACTERIAL BLIGHT OF BRINJAL PATHOGEN RALSTONIA SOLANACEARUM: A CHALLENGE IN DISEASE MANAGEMENT

Pushpa Patil*, Mohan Dalvi and Bharat Salvi
Regional Fruit Research Station, Vengurle - 416 516, Dist. Sindhudurg (M.S.), India.

Abstract
Development of resistance to pesticide in bacterial plant pathogens is one of the great concern for the control of bacterial diseases. Pesticidal resistance in plant pathogenic bacteria is known to occur in Israel, Lebanon, Newzealand, USA, Argentina, Brazil, Taiwan, Tongo and India, which posses serious threat in bacterial disease management. Existence and severity of bacterial blight of brinjal, season after season, in southern and western Maharashtra is a serious concern. India in spite of pesticidal sprays speculated the development of pesticidal resistance in the causal bacterium Ralstonia solanacearum. Bacteria in this region have been observed to develop resistant to streptomycin, Terramycin, Copper-oxychloride, Dithiocarbamate and Copper-oxychloride + Streptomycin with various mutation frequency. These pesticide resistant mutants were vigorous in growth and Pathogenicity like the native Ralstonia solanacearum population and therefore posses a challenge in the management of bacterial blight of brinjal

Key words: Ralstonia solanacearum, bacterial blight, pesticide resistance, brinjal.

Introduction
Bacterial blight of brinjal caused by Ralstonia solanacearum is an important disease of brinjal in Konkan region as well as in western parts of Maharashtra, India. The disease caused its first epidemic in 1992 in this region causing losses to the tune of Rs. 120 crores (Borkar, 1997). Dithiocarbamate are very popular for last six decades with brinjal as well as tomato growers all over the world and have been used successfully for the control of Ralstonia solanacearum (Harrison and Lockhart, 1963). Being a bacterial disease application of Dithiocarbamates, Copper fungicides and antibiotics viz Streptomycin, Streptocycline are in use for its control. However, in spite of repeated spray of these pesticides the disease prevalence is a serious concern among the brinjal growers. The reason seems to be that the pathogen might have developed pesticidal resistance to these fungicides and bactericides.

Streptomycin resistance in plant pathogenic bacteria particularly Erwinia amylovora infecting apple and pear has been observed in Israel, Labanon, Newzealand and U.S.A., in Pseudomonas cichorii infecting celery in U.S.A. (Patricia, 2001); Pseudomonas syringae infecting apple and pear, ornamental and landscape trees in New Zealand and U.S.A. (Young, 1997 and Hung & Burr, 1999) and in Xanthomonas campestris infecting tomato and pepper in Argentine, Bazil, Taiwan, Tongo and U.S.A. (Minsavaje et al., 1990; Stall and Thayer, 1962) and in Xanthomonas campestris infecting grapevine in India (Chand et al., 1991 and Borkar, 1997). Thus development of resistance in plant pathogenic bacteria against pesticide is emerging as an important challenge in plant disease management. Ready information about the pesticide resistance in the concern pathogen may be useful in determining strategies for control of the disease. The present investigation about pesticide resistance in Ralstonia solanacearum was studied and presented here.

Materials and Methods
The causal bacterium for bacterial blight of brinjal was isolated from infected leaf tissue on nutrient agar medium (NAM), purified, tested for pathogenicity and identified as Ralstonia solanacearum. Effect of various
pesticides i.e. fungicides and bactericides and their combination on *Ralstonia solanacearum* was studied by using poison food technique. The concentration of the bacterium (10^7 cfu) was plated on Nutrient Agar (NA) medium containing various concentrations of the pesticide. Formation of resistant mutant and mutation frequency to the concerned pesticide was calculated. Cross resistant pattern of concern mutant to other chemicals were also studied by following the same technique. Cultural and pathogenicity characteristic of the pesticide resistant mutant were studied. Pesticide resistant mutant were also isolated directly from infected tomato plants on nutrient agar medium (NAM) containing pesticides.

**Results and Discussion**

Bactericides *viz.* Streptomycin, Streptocycline, Terramycin and Bactericin-100, fungicides *viz.* Copperoxychloride, Dithiocarbamate, Bordeaux mixture and their combination *viz.* copperoxychloride+ Streptomycin/ Streptocycline and Bordeaux mixture + Streptocycline at higher concentration completely inhibited the growth of *Ralstonia solanacearum* whereas the concentrations lower than minimal inhibitory concentration (MIC) were infective and induce resistant mutant in *Ralstonia solanacearum* population particularly against the bactericide Streptomycin sulphate and Terramycin. The bacterium also formed resistant mutant against fungicide Copperoxychloride, Dithane M-45 and Dithane Z-78 and against combination of fungicide and bactericide (Copperoxychloride + streptomycin). The mutation frequency of *Ralstonia solanacearum* against the bactericide streptomycin and terramycin was 2 × 10^{-4} and 3.5 × 10^{-4}, respectively; against fungicide copperoxychloride, Dithane M-45 and Dithane Z-78 was 1.1 × 10^{-4}, 8 × 10^{-4} and 2.6 × 10^{-4}, respectively and against combination of copperoxychloride + streptomycin was 5 × 10^{-5} (table 1).

These pesticide resistant mutant were found pathogenic *i.e.* they induce water soaking susceptible reaction on brinjal and can be a threat in the control of the disease Fungicide of combination group *i.e.* Dithane Z-78 had effect on pigmentation of the bacterium. The bacterium might have lost some pigmentation when grown on Dithane Z-78 containing medium and formed albino colour colonies. The loss of pigment was irreversible *i.e.* transfer of albino mutant colonies on Dithane Z-78 free medium and medium containing other

<table>
<thead>
<tr>
<th>Tr. no.</th>
<th>Pesticide</th>
<th>Minimum inhibitory conc. (ppm)</th>
<th>Infective conc. (ppm)</th>
<th>Conc. at which R.S. mutant form (ppm)</th>
<th>Mutation frequency</th>
<th>Effect on bacterium</th>
<th>Pathogenicity of resistant mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Streptomycin sulphate</td>
<td>1000 ppm</td>
<td>500 ppm</td>
<td>100-500 ppm</td>
<td>2 × 10^{-4}</td>
<td>-</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>2</td>
<td>Streptocycline</td>
<td>25 ppm</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Terramycin</td>
<td>75 ppm</td>
<td>25 ppm</td>
<td>50-75 ppm</td>
<td>3.5 × 10^{-4}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Bactericin-100</td>
<td>50 ppm</td>
<td>25 ppm</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>COC</td>
<td>0.1%</td>
<td>0.05%</td>
<td>0.05-0.1%</td>
<td>1.1 × 10^{-4}</td>
<td>-</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>6</td>
<td>Dithane M-45</td>
<td>0.05%</td>
<td>0.025%</td>
<td>0.025-0.05 %</td>
<td>8 × 10^{-5}</td>
<td>-</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>7</td>
<td>Dithane Z-78</td>
<td>0.025%</td>
<td>0.01%</td>
<td>0.025-0.01%</td>
<td>2.6 × 10^{-4}</td>
<td>-</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>8</td>
<td>Bordeaux mixture</td>
<td>0.1%</td>
<td>0.05%</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>COC+ Streptocycline</td>
<td>0.025% + 50ppm</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Bordeaux mixture + Streptocycline</td>
<td>0.025% + 50ppm</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>COC + Streptocycline</td>
<td>0.2%+ 500ppm</td>
<td>0.025% + 75 ppm</td>
<td>0.05% + 75 ppm</td>
<td>5 × 10^{-4}</td>
<td>-</td>
<td>Pathogenic</td>
</tr>
</tbody>
</table>

**Table 1:** Development of resistance to pesticide in *Ralstonia solanacearum* of brinjal.

**Table 2:** Cross-resistance pattern of pesticide mutant of *Xanthomonas campestris* pv. *vesicatoria*.
chemicals did not change the albino colour mutant into yellow colonies. Loss of pigmentation in *Xanthomonas* and formation of albino strain was reported due to different phenol (Borkar, 1984). The present results indicate that besides phenol, Dithiocarbamates particularly Dithane Z-78 also induces albinism in *Ralstonia*. This may be the reason that why albino strains of *Xanthomonas* occur in nature (Durgapal, 1997).

Interestingly, Dithane M-45 resistant mutant when subjected to Dithane Z-78 treatment, the yellow pigmentation of the mutant was not changed indicating that only native virgin population of *Ralstonia solanacearum* can be changed by Dithane Z-78 for pigmentation character, but the population which already had survived during application of Dithane M-45 can not be changed for pigmentation character.

A general recommendation to combat any bacterial plant disease is the application of copper fungicide + Streptomycin. Therefore the reaction of Dithiocarbamate resistant mutant to the combination of Copperfungicide + streptomycin was studied. The result (table 2) indicated that Dithane M-45 resistant mutant has cross resistant for Dithane Z-78 and vice-versa, but did not have any cross resistant for copperoxychloride and streptomycin. Genetics of resistance to copper, Dithiocarbamate and Streptomycin in *Ralstonia solanacearum* was studied by Harrison and Lockhart (1963) and in *Xanthomonas campestris* pv. *malvacearum* by Nafde and Verma (1985). In several cases, where pesticide resistant mutant are known, these exhibit a slow growth and weak pathogenicity. (Borkar, 1997). However, the dithiocarbamate resistant mutant of *Ralstonia solanacearum*, due to their normal growth rate and pathogenicity posed the problem of rapid multiplication of pesticide resistant mutant in nature. The pathogen may develop resistance to certain formulation when these are used rigorously and repeatedly. Resistant mutant of *Ralstonia solanacearum* to Streptomycin, Copperoxychloride and Dithiocarbamate (the widely used pesticide in brinjal field) were also obtained directly from infected host on the medium containing this pesticide indicating that pesticide resistant mutant exists in natural infected host and may pose serious threat in recurrence of disease and therefore posses a challenge in the management of bacterial population.

**References**


