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Research Article

## Control of *Ralstonia solanacearum* Infection in Tomato, Brinjal and Capsicum by antibiotic sensitivity test

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**Abstract:** *Ralstonia solanacearum* is one of the important dreaded soil's borne bacterial phytopathogen which causes enormous losses in the crop plants in tropical, subtropical and temperate region of the world. In India, the disease is highly prevalent and active throughout the year where the soil is acidic in the Eastern Plateau and Hill Region. Once the disease is established in the field, it cannot be controlled by chemical means. Keeping these in view, screening of potential antibiotics for the management of *R. solanacearum* was done.

In the present study, three strains of *R. solanacearum* were isolated from Brinjal, Tomato and Capsicum. Against these three strains of *R. solanacearum*, four antibiotics were screened through food poison techniques viz. Kasugamycin, Streptomycin, Ceftriaxone and Gentamicin. The different strains of *R. solanacearum* and antibiotic sensitivity showed varied response. Among the screened antibiotics, Gentamicin showed strong antibacterial efficacy which inhibited 100% Colony-forming Unit (cfu) at very low concentration (1 ppm) followed by Ceftriaxone which inhibited >50% cfu at 1 ppm against all three strains. However, Streptomycin also showed antibacterial efficacy and inhibited >50% cfu at 3 ppm, but Kasugamycin was found less antibacterial as compared to other tested antibiotics and inhibited >50% cfu at 4 ppm.

**Keywords:** Bacterial wilt, Antibiotics, *Ralstonia solanacearum*, antibacterial.

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### 1. Introduction

Bacterial wilt is a major disease in solanaceous crop caused by *Ralstonia solanacearum*<sup>1</sup>. The disease affects a wide range of economically important crops such as Tomato, Potato, Eggplant, Chilli and non-solanaceous crop such as Banana and Groundnut in India<sup>2</sup>. The causative agent is the bacterial pathogen *Ralstonia solanacearum*, which was formerly known as *Pseudomonas solanacearum* and is the causative agent of bacterial wilt of solanaceous crop<sup>3</sup>. It mainly attacks Potato, Tomato, Geranium, Eggplant, Capsicum and some solanaceous weeds like *Solanum nigrum* and *Solanum dulcamara*<sup>4,5</sup>.

It is an important soil borne, devastating bacterial phytopathogens, which are aerobic in nature, non-spore former, Gram-negative and is motile by a polar flagella tuft. This bacterium normally invades through wounds and colonizes the cortex and vascular

parenchyma and enters the xylem vessel and spreads up into the stems and leaves. The races of *Ralstonia solanacearum* identified by pathogenicity test on wide host range<sup>6</sup>. The species of this bacterium were classified into five races on the basis of different host range<sup>7</sup> and six biovars, according to the ability to oxidize disaccharides and three sugar alcohols<sup>8,9</sup>. During the following year, five biovars (based on carbon utilization pattern) and five pathogenic races were identified. Race1 occurs in the lowland tropics and warm temperate lands<sup>10</sup>. It colonizes the xylem, causing bacterial wilt in a very wide range of host plants<sup>11</sup>. The disease is called southern blight, *R. solanaceous* wilt and other common names where it occurs. The virulent and avirulent isolates of this bacterium were identified by Kelman's Tetrazolium chloride (TZC) agar test. In this test, virulent isolates produce pink or light red colour colonies with

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characteristic red colour center with whitish margin and avirulent produce off white margin<sup>8</sup>.

It was reported that avirulent type could be easily differentiated by the pigmentation from the virulent types<sup>12</sup>. In India, a study showed 10 to 100% incidence of bacterial wilt in summer<sup>13</sup>. The soil born vascular pathogen is widely distributed in tropical, subtropical climates and effects usually broad range of crops including monocots and dicot plants<sup>8, 14</sup>. Infected soil and surface water, including irrigation water are the primary sources of the inoculum. The pathogen infects roots of susceptible plants, usually through wounds<sup>15</sup>. Colonization by the bacterium within the xylem prevents water movement into the upper portion of plant tissues<sup>12</sup>. As the disease develops, all leaves may wilt quickly and desiccate although they remain green<sup>16</sup>. This bacterium was characterized into biovars on the basis of oxidation of carbohydrate. Biovars of *R. solanacearum* were differentiated according to their ability to oxidize disaccharides and hexose alcohol<sup>8</sup>. Aqueous extracts of some plants having medicinal properties did not have inhibitory effects on *Ralstonia solanacearum*<sup>17</sup>. The most commonly used chemical treatment has been fumigation of contaminated soil/portion of the farm with methyl bromide<sup>18</sup>. This is very expensive and tedious exercise and cannot be used on large areas. The other product used at field level is sodium hypochlorite. It is appropriate for spot treatment of the holes left behind after roguing of the wilting plants and for general field sanitation<sup>19</sup>. Its use is expensive and tedious.

Hence, is the need to study antibacterial agent other than solvent extract to repress the growth of this bacterium. In the present study, four antibiotics have been taken, namely Kasugamycin, Ambistryn, Ceftriaxone and Gentamicin. With an aim to develop effective antibacterial agent without any residual effect, the present study was conducted to analyze the *in-vitro* antibacterial potential of certain antibiotics against three different isolates of *Ralstonia solanacearum*. The antibacterial activities of antibiotics were assayed by using Food Poison techniques. Four above antibiotics were used against three isolates of *Ralstonia solanacearum*.

These compounds, which include aminoglycosides, tetracyclines and macrolides, interfere with essential steps of protein synthesis. Most antibiotics interact with ribosomal RNA<sup>20</sup>. The ribosome is the target of many important antibiotics, inhibiting the protein synthesis of the bacterium and thereby ceasing its growth and propagation.

## 2. Materials and Methods

### 2.1 Field survey, Identification and Sample collection

A field survey was conducted to determine the prevalence of bacterial wilt in solanaceous crops such as Brinjal, Tomato and Capsicum, growing in different

areas of Ranchi, Jharkhand, India. The typical symptoms of bacterial wilted plants were observed. Infected leaves of the plants by *Ralstonia solanacearum* turned yellow and remain wilted after a time. The area between leaf veins died and becomes brown. Usually, the main stem of the affected plants remained upright even though all the leaves may become wilted and dead. Stem streaming methods were a valuable diagnostic tool for quick detection of bacterial wilt in the field<sup>21</sup>. A thin thread of ooze was seen when an infected stem cuts across and cut ends held together for a few seconds in test tubes containing sterilized distilled water. Slimy sticky ooze forms tan-white to brown beads. Internal symptoms were also obtained, a dark brown to black streaking in the vascular tissues was revealed. All three strains were isolated from wilted plants by streaking a loop full flowing ooze containing bacteria onto sterile TTC media. Turbid bacterial suspensions were obtained when those stems were cut and placed in test tubes filled with sterilized distilled water. Bacterial colonies were obtained after incubation of 24-36 hrs. Red colonies were isolated.



Fig. 1(a). Wilt in Capsicum.



Fig. 1(b). Wilt in Tomato.



Fig. 1(c). Wilt in Brinjal.



Fig. 2. Flowing ooze containing bacteria.

## 2.2 Purification

For further purification bacterium were inoculated in nutrient agar media by streak plate technique. In these media, two types of isolates were revealed. On the plates of Brinjal and Capsicum, isolates were whitish in color. Tomato isolates were golden yellow in color. For further use, bacterial isolate were stored in King's B media. Finally, a pure culture of all these three isolates was obtained.



Fig. 3. Bacterial isolates in TTC medium.

## 2.3 Antibiotic sensitivity test

An antibiotic sensitivity test of Food Poison Technique was performed. Three isolates of the bacterium from Tomato, Brinjal and Capsicum were selected to test the antibacterial activity of four drugs, namely Kasugamycin, Ambistryn, Ceftriaxone, and

Gentamicin, whose chemical structures are shown in Fig. 4a-4d.

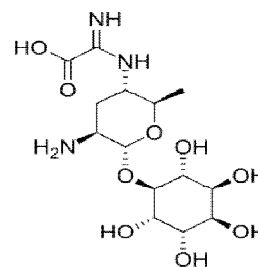


Fig. 4(a). Structure of Kasugamycin.

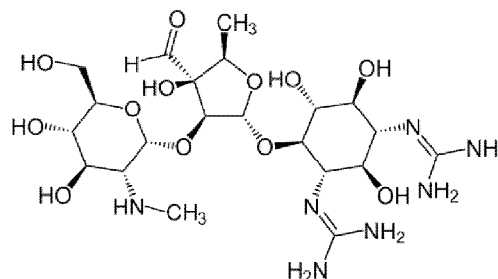


Fig. 4(b). Structure of Streptomycin.

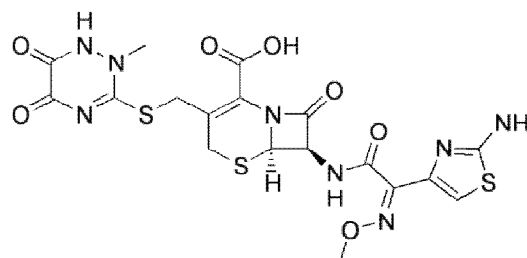


Fig. 4(c). Structure of Ceftriaxone.

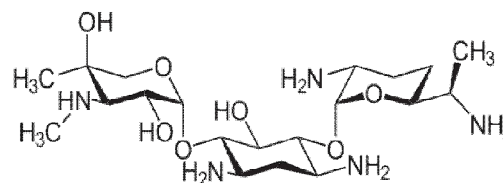


Fig. 4(d). Structure of Gentamicin.

Three replicates of each of the three isolates were tested against these drugs and four sets of experiment were done. For each set 45 Petri plates and 18 test tubes were taken, which were sterilized in hot air oven (160°C for 1 hour). 1 litre Nutrient Agar was prepared and sterilized in the autoclave (121°C at 15 psi for 20 min.) for each set of experiment. A stock solution of antibiotics was prepared. Before pouring different concentration of antibiotics (1 ppm, 2 ppm, 3 ppm, 4 ppm) was added into the sterilized media (1 litre medium was divided into 5 flasks containing 100ml each, before sterilization). Bacterial suspension of all three isolates was prepared by serial dilution up to  $10^{-6}$

and was inoculated by the spread plate method. After inoculation, these plates were incubated for 24 hours. Percentage inhibition was calculated.

### 3. Results and Discussion

The different strains of *R. solanacearum* and antibiotic sensitivity showed varied response. Among the screened antibiotics, Gentamicin showed strong antibacterial efficacy which inhibited 100% colony forming unit (cfu) at very low concentration (1 ppm) followed by Ceftriaxone which inhibited >50% cfu at 1 ppm against all three strains. However, Streptomycin also showed antibacterial efficacy and inhibited >50% cfu at 3 ppm, but Kasugamycin was found less antibacterial as compared to other tested antibiotics and inhibited >50% cfu at 4 ppm (Table-1).

The reason for bacterial growth inhibition, according to Barbera *et al.*, (2006)<sup>22</sup> is due to the fact that Kasugamycin (Ksg) binds within the messenger RNA channel of the 30S subunit. Ksg is also produced by the bacterium *Streptomyces kasugaensis* and has been used clinically in the treatment of *Pseudomonas aeruginosa* infections<sup>22</sup>. The drug Ambistryn contains streptomycin. It is aminoglycosides and are synthesized by different species of the genus *Streptomyces* whereas, Gentamicin comes from *Micromonospora purpurea*. Aminoglycosides bind to the 30S ribosomal subunits

and interfere with the protein synthesis. These antibiotics are acting on this bacterium due to its bactericidal activity against gram-negative pathogen. Streptomycin usefulness has decreased greatly due to widespread drug resistance<sup>23</sup>. Streptomycin showed inhibition up to 50% CFU at 3 ppm, but Gentamicin showed 100% CFU inhibition at 1 ppm. Ceftriaxone is a sterile, semisynthetic, broad spectrum cephalosporin antibiotic. The bactericidal activity of Ceftriaxone results from inhibition of cell wall synthesis. Cephalosporin also inhibits the transpeptidase reaction during peptidoglycan synthesis<sup>23</sup>. This has been statistically confirmed.

#### 3.1 Statistical Analysis

The above findings were statistically analyzed and the summary statistics for each of the selected data variables shows measures of central tendency, measures of variability, and measures of shape (Table-2). The standardized skewness, which is used to determine whether the sample came from a normal distribution. None of the values were found to be outside the range of -2 to +2 indicating that the values outside the expected range were found to be none. This clearly shows that the experiments followed a normal distribution as indicated by summary statistics of the three plant species selected.

Table 1. Antimicrobial efficacy of different antibiotics against three isolates of bacteria bacterial colonies ( $\times 10^{-6}$ ) % inhibition.

| Strains  | Conc. | % Inhibition |           |             |            |
|----------|-------|--------------|-----------|-------------|------------|
|          |       | Antibiotics  |           |             |            |
|          |       | Kasugamycin  | Ambistryn | Ceftriaxone | Gentamicin |
| Tomato   | 1ppm  | 30.96        | 39.63     | 80.79       | 43.57      |
|          | 2ppm  | 45.71        | 45        | 100         | 55.1       |
|          | 3ppm  | 52.39        | 61.27     | 100         | 100        |
|          | 4ppm  | 63.35        | 75.23     | 100         | 100        |
| Brinjal  | 1ppm  | 28.16        | 42.72     | 92.46       | 100        |
|          | 2ppm  | 36.3         | 86.36     | 100         | 100        |
|          | 3ppm  | 42           | 100       | 100         | 100        |
|          | 4ppm  | 56.73        | 100       | 100         | 100        |
| Capsicum | 1ppm  | 18.29        | 45.87     | 52.26       | 100        |
|          | 2ppm  | 33.48        | 68.01     | 70.4        | 100        |
|          | 3ppm  | 48.53        | 86.1      | 91.44       | 100        |
|          | 4ppm  | 56.29        | 99.38     | 94.4        | 100        |

Table 2. Summary statistics.

|                     | Kasugamycin |         |          | Ambistryn |         |          | Ceftriaxone |         |          | Gentamicin |         |          |
|---------------------|-------------|---------|----------|-----------|---------|----------|-------------|---------|----------|------------|---------|----------|
|                     | Tomato      | Brinjal | Capsicum | Tomato    | Brinjal | Capsicum | Tomato      | Brinjal | Capsicum | Tomato     | Brinjal | Capsicum |
| Standard Deviation  | 16.171      | 12.045  | 16.8231  | 9.605     | 27.139  | 23.2014  | 29.628      | 3.77    | 19.7217  | 16.171     | 0       | 0        |
| Coeff. of variation | 29.25%      | 29.52%  | 29.52%   | 10.09%    | 32.99%  | 32.99%   | 39.68%      | 3.84%   | 3.84%    | 29.25%     | 0%      | 0%       |
| Stand. Skewness     | 0.4158      | 0.6034  | -0.38782 | -1.633    | -1.3792 | -0.3672  | -0.1057     | -1.633  | -0.5385  | 0.4158     | 0       | 0        |

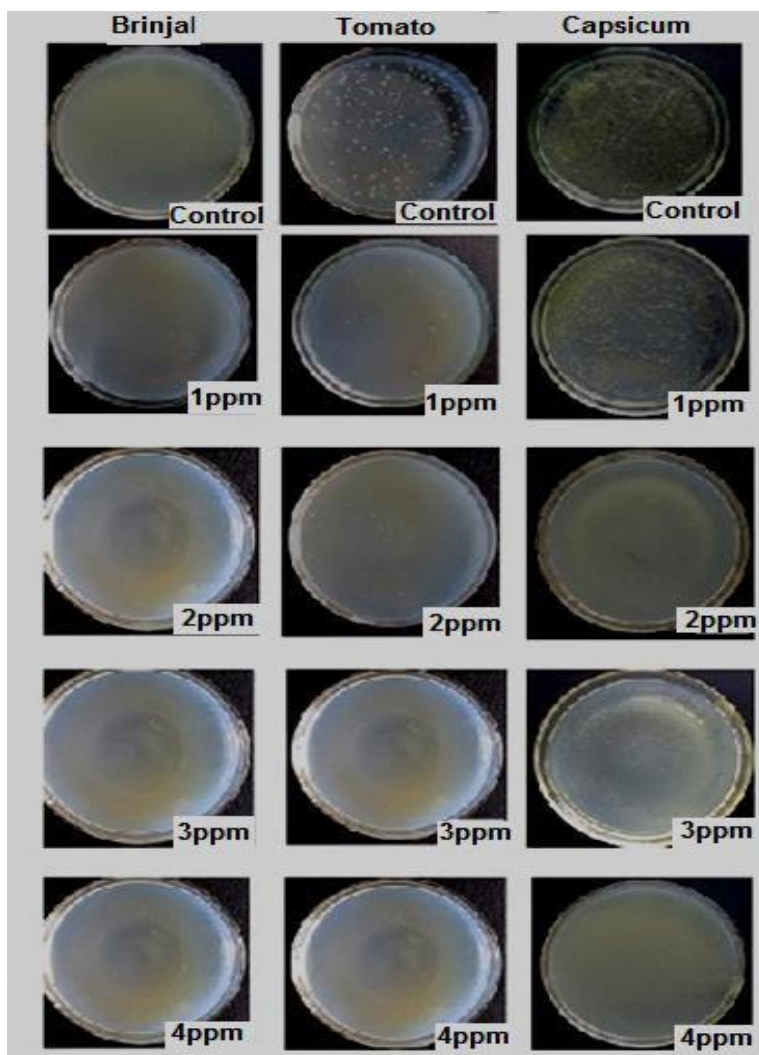


Fig. 5. Treatment of bacterial culture with Ceftriaxone.

### 3.2 ANOVA Result

The values obtained were subjected to ANOVA analysis (Table-3), which indicates that since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 4 variables at the 95.0% confidence level in all three experimental plants and the antimicrobial efficacy is found in all four antibiotics.

Table 3. ANOVA result.

| ANOVA<br>(Between<br>groups) | Tomato  |         | Brinjal |         | Capsicum |         |
|------------------------------|---------|---------|---------|---------|----------|---------|
|                              | F-Ratio | P-Value | F-Ratio | P-Value | F-Ratio  | P-Value |
|                              | 3.89    | 0.0373  | 13.51   | 0.0004  | 8.35     | 0.0029  |

### 4. Conclusion

After statistical analysis, it is evident that since all the three experimental plants showed 95.05% confidence level variables of antibiotics, the results are statistically significant. Among all the four antibiotics,

Gentamicin showed 100% efficacy at 1 ppm concentration, hence can be effectively used alone for the treatment of the bacterial wilt by *R. solanacearum*. Other antibiotics, used in the present study like Ceftriaxone and Ambistryn, showed 50% efficacy at 1 ppm and 3 ppm, respectively, while Kasugamycin showed less effective in controlling the disease.

Hence, it can be concluded that Gentamicin at the concentration of 1 ppm can be successfully used to curb the menace of *Ralstonia solanacearum* infection and can be a boon to the farmers and can be used under field management; while other antibiotics can be used in combination for effective management of the disease.

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