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# *In vitro* evaluation of antibacterial chemicals against *Ralstonia solanacearum* causing bacterial wilt of potato

**Ritu Kumari, RK Ranjan and Swati Kumari**

**Abstract**
Potato (*Solanum tuberosum* L.) is one of the important food crops. *Ralstonia solanacearum* causing bacterial wilt of potato, considered as destructive disease of potato crops. It has an extensive host range of ~ 450 crop species across 54 families. In the present research work six antibacterial chemicals (3 antibiotics & 3 bactericides) were evaluated against *R. solanacearum.* Among the antibiotics Chloramphenicol showed highest antibacterial activity at 100ppm and 250ppm (15.96 and 19.60 mm inhibition zone respectively) whereas at 500ppm Streptocycline produced highest inhibition zone (28.03 mm). Among the bactericides Azoxystrobin showed highest antibacterial activity at all the three concentrations (0.1%, 0.2% and 0.3%) produced inhibition zone 10.10mm, 11mm and 12.26 mm respectively.

**Keywords:** *Ralstonia solanacearum*, chemicals, potato

## Introduction
Potato (*Solanum tuberosum* L.) is herbaceous tuber crop belonging to the family solanaceae. It is cultivated and recognized as popular vegetable throughout the entire tropical and subtropical region of the world (Hayward, 1991) [5]. In India, a major loss has been estimated due to late blight (10-70%), bacterial wilt (30-70%), black scurf (10-20%) diseases in potato (Kadian *et al*., 2007) [7]. Bacterial wilt of potato limits potato production worldwide in Asia, Africa and Central and South America, where it causes severe crop losses (Hayward, 1994; Elphinstone, 2005) [6, 1] and causes catastrophic yield loss. *R. Solanacearum* has extensive host range of ~ 450 crop species across 54 families (Wicker *et al*., 2007) [19]. The presence of disease in six continents out of seven is not an unusual fact because of its host range and diversity among the isolates (Fegan and Prior, 2005) [2]. In India, a study showed 10 to 100% incidence of bacterial wilt during the summer season (Kishun, 1985) [8]. Shekhawat *et al*., (2000) [13] reported 20-100% losses in chilli, 2-95% in tomato (Singh *et al*., 2010) [14] and 25-100% in brinjal due to bacterial wilt. Nearly 37% potato loss due to brown rot disease in potato in Mukteshwar region of Uttarakhand reported by Verma and shekhawat (1991) [3], upto 13.8-55% in Kumaun hills (Paharia, 1963) [11], 0.33 to 40% in Maharashtra Paharia, 1963 [11], 20-25% at Hyderabad (Nath *et al*., 1958) [10] and 75% in Karnataka (Gadewar *et al*., 1991) [3]. *R. Solanacearum* causes wilt diseases in plants by invading through xylem vessels. Its ability to persist in soil and infesting plant parts adds to the difficulty in elimination of inocula (Genin and Denny, 2012). Pathogenicity of *R. solanacearum* is dependent upon its densities and after reaching to certain level in plant it activates pathogenicity genes which in low densities would not be activated (Schell, 2000) [13]. Complications in controlling *R. solanacearum* are being faced because of the complex pathogenicity mechanism possessed by bacterium, its ability to grow endophytically, perpetuation in soil, dispersion with water, and its association with weeds (Wang and Lin, 2005) [18]. The most commonly used chemical treatment has been fumigation of contaminated soil/portion of the farm with methyl bromide. This is very expensive and tedious exercise and cannot be used on large areas. Hence, is the need to study antibacterial agent other than solvent extract to repress the growth of this bacterium. With an aim to develop effective antibacterial agent without any residual effect, the present study was conducted to analyse the *in-vitro* antibacterial potential of certain antibiotics and chemicals against *Ralstonia solanacearum.*

## Materials and Methods

### *In vitro* evaluation of antibacterial chemicals against *R. Solanacearum*

Antibiotics *viz*, streptomycin, streptocycline, chloramphenicol (Table-1) and bactericides *viz* copper oxy chloride, azoxystrobin, carbendazim+mancozeb (Table- 2) were evaluated at different concentrations to test their efficacy in inhibiting the growth of *R. solanacearum* by the inhibition zone assay method.

**Table 1:** Different antibiotics used in evaluation against *Ralstonia solanacearum*

| S. No. | Antibiotics | Concentration (ppm) |
|---|---|---|
| 1 | Streptomycin | 100, 250, 500 |
| 2 | Streptocycline | 100, 250, 500 |
| 3 | Chloramphenicol | 100, 250, 500 |

**Table 2:** Different bactericides used in evaluation against *Ralstonia solanacearum*

| S. No. | Bactericides | Concentration (%) |
|---|---|---|
| 1. | Copper oxy chloride | 0.1, 0.2, 0.3 |
| 2. | Azoxystrobin | 0.1, 0.2, 0.3 |
| 3. | Carbendazim+Mancozeb | 0.1, 0.2, 0.3 |

In this technique melted CPG medium was poured in sterile petri plates and allowed to solidify. Afterwards, one loop of freshly grown culture of virulent isolates (SP-3) of *R. solanacearum* was evenly spread onto the medium with a sterilized spreader. The solutions of the desired concentrations of the test antibiotics and antibacterial fungicides were prepared separately. 5.0 mm diameter well was made in each agar plate using sterilized cork borer. 50 µl of chemicals were loaded into the wells in petri plates separately. The well which was loaded with sterile distilled water was used as control. The experiment was performed in triplicate under aseptic conditions. Then the plates were incubated at 28 $^0$C and observed at 24 h after incubation for the production of inhibition zone. Inhibition zone was measured with the help of a scale in mm.

## Result and Discussion

### *In vitro* evaluation of antibacterial chemicals against *R. Solanacearum*

Six antibacterial compounds comprising of three antibiotics and three bactericides were tested for their efficacy in inhibiting the growth of *R. solanacearum* under *in vitro* condition by "inhibition zone technique".

### *In vitro* evaluation of antibiotics against *R. solanacearum*

Among three bacterial antibiotics tested with different concentration, the maximum inhibition zone was recorded in chloramphenicol at two concentrations 100 ppm and 250 ppm (15.96 mm and 19.60 mm) respectively. However, at 500 ppm, streptocycline showed greater efficacy and produced maximum inhibition zone (28.03 mm) followed by chloramphenicol (23.96 mm) (Plate 1). The minimum inhibition zone was recorded in streptomycin (0 mm, 8.43 mm and 9.30 mm) at 100, 250 and 500 ppm respectively. The results were presented in table- 3 and fig.-1

**Table 3:** Effect of antibiotics at different concentration on *R. solanacearum* under *in vitro* conditions

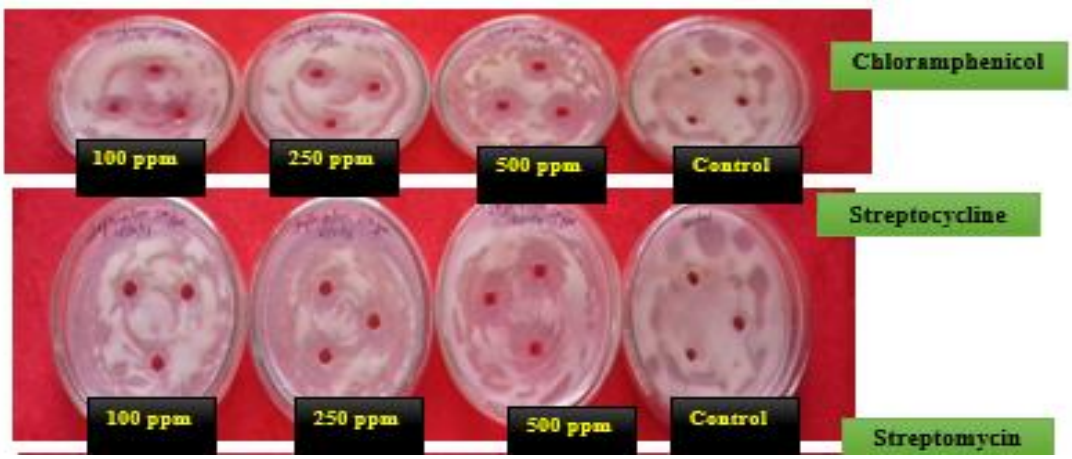
| S. No. | Antibiotics | Inhibition zone in mm | | |
|---|---|---|---|---|
| | | 100 ppm | 250 ppm | 500 ppm |
| 1. | Streptomycin | 0 | 8.43 | 9.30 |
| 2. | Streptocycline | 12.76 | 19.10 | 28.03 |
| 3. | Chloramphenicol | 15.96 | 19.60 | 23.96 |
| 4. | Control | 0 | | |
| Factors | | CD at 5% | S.Em± | |
| Antibiotic (A) | | 0.73 | 0.24 | |
| Concentration (B) | | 0.63 | 0.21 | |
| Interaction (A×B) | | 1.26 | 0.43 | |

### *In vitro* evaluation of bactericides against *R. solanacearum*

Among three antibacterial chemicals tested, azoxystrobin showed maximum inhibition zone (10.10mm, 11mm and 12.26mm) followed by carbendazim+mancozeb combination (9.73mm, 10.30mm and 10.86mm) at 0.1%, 0.2% and 0.3% respectively. The minimum inhibition zone was recorded in copper oxy chloride (8.76mm, 9.96mm and 10.20mm) at 0.1%, 0.2% and 0.3% respectively (Plate 1). Results are presented in table- 4 and fig.-2

**Table 4:** Effect of bactericides at different concentration on *R. solanacearum* under *in vitro* conditions

| S. No. | Chemicals | Inhibition zone in mm against *R. solanacearum* | | |
|---|---|---|---|---|
| | | 0.1% | 0.2% | 0.3% |
| 1. | Carbendazim + Mancozeb | 9.73 | 10.30 | 10.86 |
| 2. | Copper oxychloride | 8.76 | 9.96 | 10.20 |
| 3. | Azoxystrobin | 10.10 | 11 | 12.26 |
| 4. | Control | 0 | | |
| Factors | | CD at 5% | | S.Em± |
| Chemicals (A) | | 0.50 | | 0.17 |
| Concentration (B) | | 0.43 | | 0.14 |
| Interaction (A×B) | | 0.86 | | 0.29 |



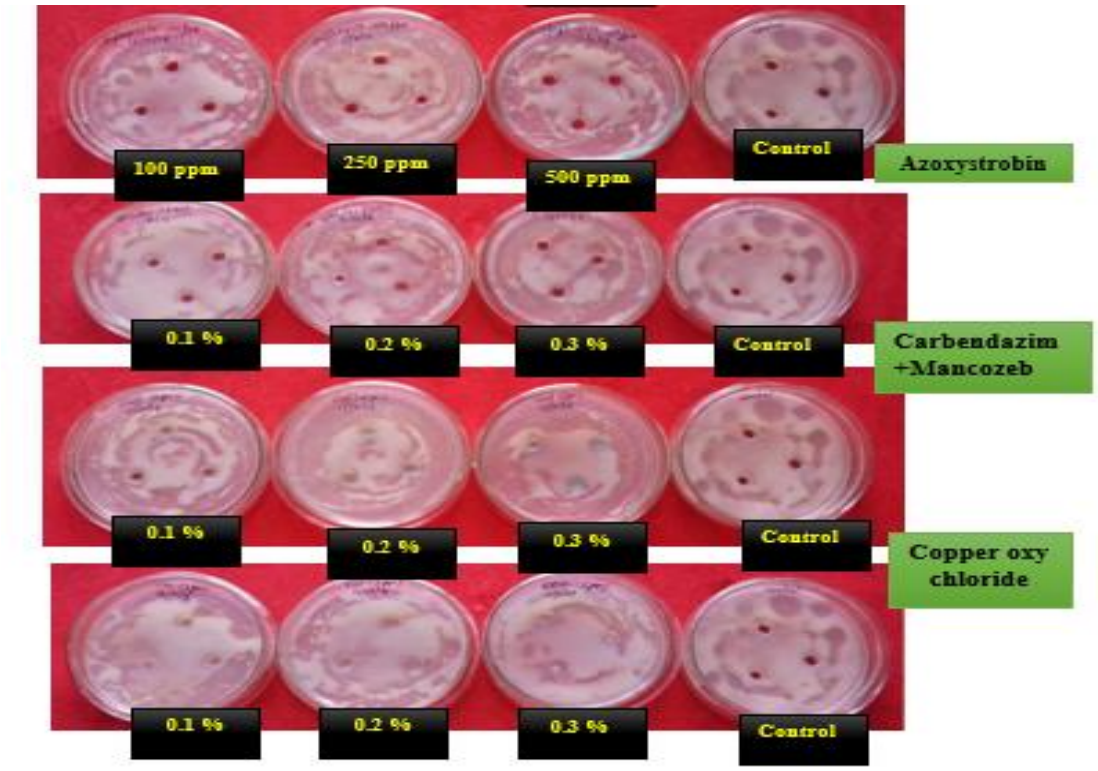



**Plate 1:** Inhibition zone produced by different antibacterial chemicals against *R. solanacearum* under *in vitro* conditions.

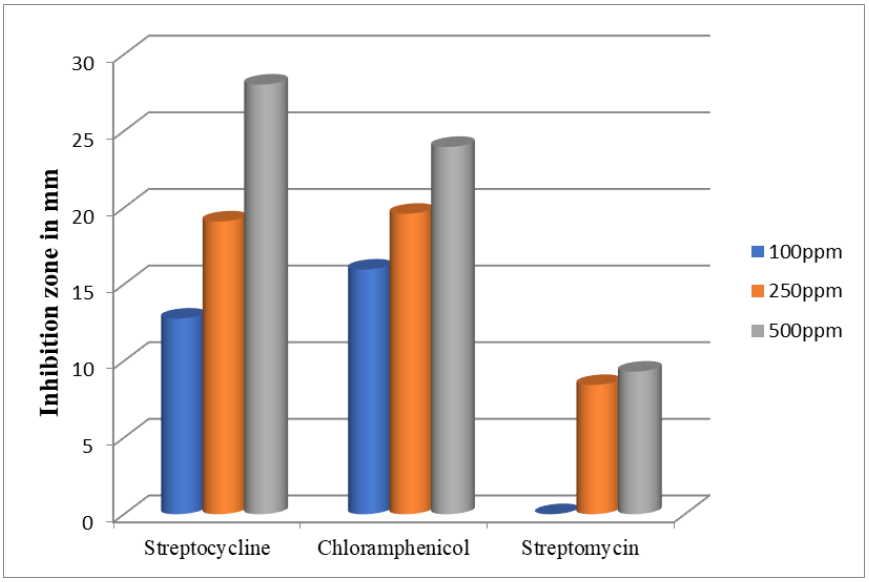



**Fig 1:** Evaluation of antibiotics at different concentration against *R. solanacearum* under *in vitro* conditions

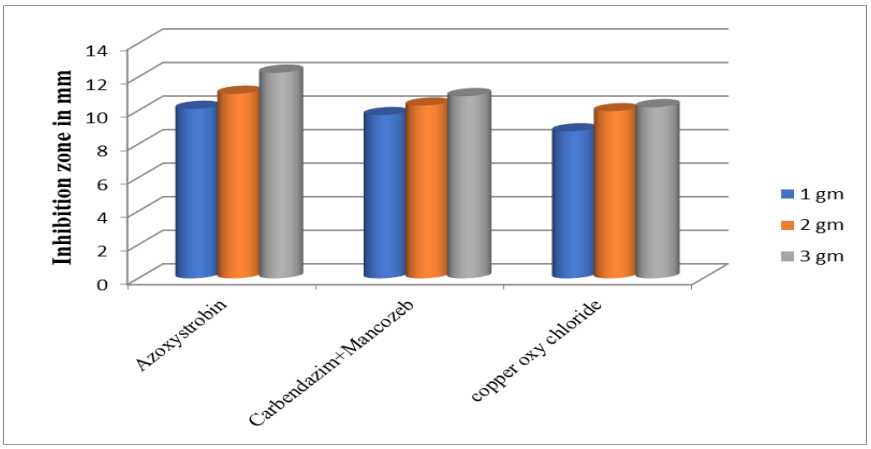



**Fig 2:** Evaluation of bactericides at different concentration against *R. solanacearum* under *in vitro* conditions

**Conclusion**

Six antibacterial chemicals (3 antibiotics & 3 bactericides) were evaluated against *R. solanacearum.* Among the antibiotics chloramphenicol showed highest antibacterial activity at 100 and 250 ppm (15.96 mm and 19.60 mm) whereas at 500 ppm streptocycline produced highest inhibition zone (28.03 mm). The statistical analysis also showed that at 100 ppm concentration chloramphenicol was found best; at 250 ppm concentration both antibiotics streptocycline and chloramphenicol were at par. However, at 500 ppm concentration streptocycline was found best. Among the bactericides azoxystrobin showed highest antibacterial activity at all the three concentrations (0.1%, 0.2% and 0.3%) produced inhibition zone 10.10mm, 11mm and 12.26 mm respectively. The statistical analysis also showed that azoxystrobin was found best at all the 3 concentration that is at 0.1%, 0.2% and 0.3%. However copper oxy chloride and carbendazim + mancozeb combination were at par at 0.2% and 0.3% concentration. Similar findings were made by Singh and Jagtap (2017) [15] who evaluated six antibiotics among which highest inhibition zone was recorded in the antibiotic streptocycline at 400 ppm (18.4 mm) and 500 ppm (21.7 mm). Singh and Jagtap (2017) [15] also evaluated 5 antibacterial fungicides in which copper oxy chloride produced inhibition zone 10.6 mm and 11.6 mm at 1500 ppm and 2000 ppm respectively and azoxystrobin produced inhibition zone 7.2 mm and 7.3 mm at 1500 ppm and 2000 ppm respectively.

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