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# *In vitro* Efficacy Bioagents against Bacterial Leaf Spot of Chilli caused by *Xanthomonas axonopodis* pv. *vesicatoria*

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**Keywords**

*Xanthomonas axonopodis*, Bioagents, Completely Randomised Design, *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Acetobacter*, *Azospirrilum*, *Trichoderma konigii*

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**A B S T R A C T**

The *in vitro* study was conducted to evaluate efficacy of bioagents against *Xanthomonas axonopodis* pv. *vesicatoria* causing bacterial leaf spot of chilli. An investigation consisting nine treatments with three replications in Completely Randomized Design was carried out to evaluate *in vitro* effect of antagonistic microorganism against of *Xanthomonas axonopodis* pv. *vesicatoria***.** The bioagents *viz. Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Acetobacter*, *Azospirrilum*, *Trichoderma konigii*, were tested against *Xanthomonas axonopodis* pv. *vesicatoria*. The causal organism of bacterial leaf spot of chilli in *in vitro* by inhibition zone method All the treatments significantly inhibited bacterial growth of *Xanthomonas axonopodis* pv. *vesicatoria* over control.

## Introduction

Chilli (*Capsicum annum* L.) also known as red pepper is the member of family Solanaceae. Several sources concordantly put the origin of chilli in Bolivia or Brazil. Chilli originally from South America is referred as chillies, chilli, hot pepper, bell pepper, red pepper, pod pepper, cayenne pepper, paprika, pimento and *capsicum* in different parts of the world. Two species of chilli are under cultivation, the *Capsicum annum* L. is small in size, more pungent types, whereas, the *Capsicum frutescence* L. is somewhat larger, mild to moderately pungent types and referred as 'Dhobli Mirchi' and is used mostly as green vegetable.

India is the largest producer, consumer and exporter of chillies in the world. The important states growing chilli are Andhra Pradesh, Maharashtra, Orissa, West Bengal, Karnataka, Rajasthan and Tamil Nadu. As per the latest statistics, India produced 2955 thousand million tonnes of green chillies from an area of 292 thousand hectares in the year 2017-18 and produced 1304.38 thousand million tonnes of green chillies from an area of 794.12 thousand hectares in the year 2017-18 (Anonymous, 2018). Andhra Pradesh is the leading both in area and production contributing 25% area and 46% of production (Anonymous, 2018).

In Maharashtra State, the chilli is grown on area of 30.99 thousand hectares with annual production of 359.77 thousand tonnes and productivity of 2.08 (MT/ha) in years 2016-17. (Horticulture statistics at glance, 2017).Among diseases, bacterial leaf spot of chilli, caused by *Xanthomonas axonopodis* pv. *vesicatoria* is one of the most important and was first observed in 1914 in South Africa. Bacterial leaf spots on the fruits have been shown to account for up to 52 per cent causes weight loss in infected fruits (Jones *et al.*, 1986).

The disease is considered to be a major constraint to chilli production all over the world (Blancard, 1997). It attacks every part of the chilli plant. Infection on leaves causes defoliation, resulting in reduced marketable fruit weight for both staked and unstaked tomatoes (Dougherty, 1978; Pohronezny and Volin, 1983), and increase exposure of fruits to sun scald. But the main economic effect of the disease is the reduction in fruit weight and quality. Bacterial spots on the fruits have been shown to account for up to 52 per cent weight loss in infected fruits (Jones *et al.*, 1986).

There are large numbers of chemicals/ antibiotics available in the market as bactericides and their efficacy and stability needs to be verified in *in vitro* studies so as to incorporate the effective ones in the management packages. The effectiveness of bio-control depends on the choice of efficient species or isolates. The bio-control agents may act on the pathogen through antibiosis, competition for nutrients, parasitism of pathogen, disease suppression due to prevention of colonization of the pathogen and induction of resistance in plants. Under artificial inoculation conditions, application of bioagents *viz.*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Bacillus subtilis*, *Trichoderma harzianum*, *Trichoderma viride* and *Streptomyces griseoviridis* isolates were antagonistic to tomato bacterial spot disease. Application of bioagents as soil or seedling treatments was more effective than their application as foliar treatment. By considering this disease, efficient bioagents need to be explored to fit into the disease management (Narayanasamy, 2011).

## Materials and Methods

### *In vitro* evaluation of bioagents

An investigation consisting of nine treatments with three replications in Completely Randomized Design was carried out to evaluate *in vitro* effect of antagonistic microorganism against of *Xanthomonas axonopodis* pv. *vesicatoria*. The bioagents *viz. Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Acetobacter*, *Azospirrilum*, *Trichoderma konigii*, were tested against *Xanthomonas axonopodis* pv. *vesicatoria*. The causal organism of bacterial leaf spot of chilli in *in vitro* by inhibition zone method and result of growth of pathogen, their inhibition and inhibition per cent over control are presented in Table 1.

A heavy suspension (3 days old) of *X. axonopodis* pv. *vesicatoria* multiplied in nutrient broth (20ml) was mixed with nutrient agar medium (1000 ml) contained in Erleyenmayer`s flask. Fifteen to twenty min. medium were poured into the sterilized petriplates and allowed to solidify. A loopful culture of each of antagonistic (bacterium) organisum was placed in the centre of petriplates containing the seeded medium. In case of fungal antagonists, mycelial discs of 5 mm (diameter) size taken from actively growing culture were placed in the centre of the plates. The inoculated plates were then incubated at 30˚C for 72 hour. Observation were recorded for the zone of inhibition produced by antagonistics microrganisms around the growth of the pathogen. Observation on radial of test pathogen and percent inhibition over control will be calculated. By the formula of Vincent *et al.,* (1927).

$$I = \frac{C - T}{C} \times 100$$

where, T = Growth of the pathogen treatment
C = Growth of the pathogen in control

## Results and Discussion

### *In vitro* efficacy of bioagents

Among the eight bioagents tested, *Bacillus subtillis* and *Psuedomonas fluroscence* were found effective in inhibiting the growth of pathogen with an inhibition zone of 12.00 mm and 11.67 mm diameter followed by *Acetobacter* with 9 mm inhibition zone.

**Table.1** *In vitro* effect of antagonistic microorganism against *Xanthomonas axonopodis* pv. *Vesicatoria*

| Tr no. | Treatments | Mean zone of inhibition* (mm) |
|---|---|---|
| $T_1$ | *Trichoderma viride* | 1.00 (5.74)* |
| $T_2$ | *Trichoderma harzianum* | 2.33 (8.79) |
| $T_3$ | *Trichoderma hamatum* | 2.00 (8.13) |
| $T_4$ | *Bacillus subtilis* | 12.33 (20.56) |
| $T_5$ | *Acetobacter* spp. | 9.00 (17.46) |
| $T_6$ | *Azospirillum* spp. | 5.67 (13.77) |
| $T_7$ | *Trichoderma koningii* | 1.33 (6.63) |
| $T_8$ | *Pseudomonas fluroscence* | 11.67 (19.97) |
| $T_9$ | Control | 90.00 (0.00) |
| | **S.E. ±** | 0.46 |
| | **C.D. at (P=0.001)** | 1.37 |

*Figures in Parenthesis are arcsine transformed values

**Fig.1** Effect of bioagent against *Xanthomonas axonopodis* pv. *vesicatoria* in *In vitro*

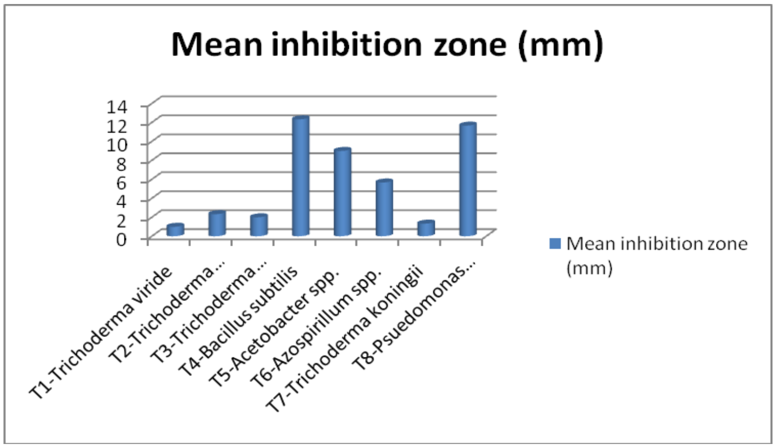
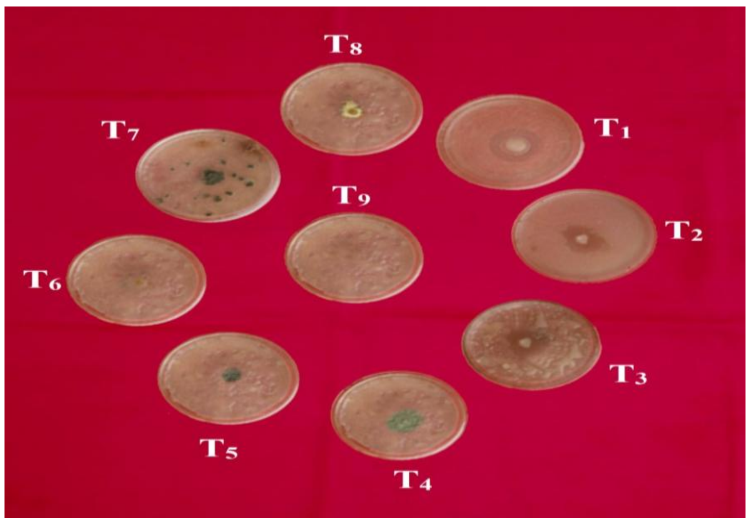



**Plate** *In vitro* effect of antagonistcs against *Xanthomonas axonopodis* pv. *vesicatoria*




The ecological consequences of overuse and misuse of chemicals/bactericides in plant disease management became a serious threat in inducing environmental contamination. Hence, the biological control as an alternate option is gaining importance and awareness among farming community as the approach is ecofriendly and cost effective.

Under biological control of plant diseases, various antagonistic organisms have been identified which suppress the pathogen by different mechanisms viz., antibiosis, hyperparasitism, competition, hypovirulence and by inducing systemic resistance by acting as elicitors.

Superior in inhibiting the growth of the pathogen with an inhibition zone of 12.33 and 11.67 mm diameter. The *Trichoderma harzianum* and *Trichoderma hamatum* inhibited growth of the pathogen to the extent of 2.33 and 2.00 mm per cent over control. These are followed by *Acetobacter* and *Azospirrillum* with an inhibition growth of pathogen (9.00mm and 5.67mm). The results are shown in Fig. 1.

Study conducted revealed that, among the eight bioagents tried *Bacillus substillis and Pseudomonas fluroscence* were found significantly over other bioagents. These findings are similar to the results reported by

Yenjerappa (2009) studied that among the different antagonists *Bacillus subtilis* and *Pseudomonas fluorescens* were found significantly superior over other antagonists in inhibiting the growth of the pathogen.

Laha *et al.*, (1992) stated that, fluorescent pigments produced by *Pseudomonas* were sequester Fe3 + and were considered as siderophores, which inhibits the large number of phytopathogenic bacteria and fungi, whereas biochemical studies by Valasubramanian *et al.*, (1994) showed that, efficient strains of *Pseudomonas fluorescens* produces an antibiotic phenazine-1-carboxylic acid (PCA), which hinders the growth of plant pathogenic bacteria.

Unnamalai and Gnanamanickam (1984) reported the inhibiting effect of *Pseudomonas fluorescens* on the growth of *Xanthomonas citri*.

Seleim *et al.*, (2011) evaluated bioagents viz. *Pseudomonas fluorescens*, *P. putida* and *Bacillus subtilis* against bacterial wilt *in vitro* and *in vivo*. Under greenhouse conditions, *Pseudomonas fluorescens* exhibited the highest disease reduction of tomato bacterial wilt disease followed by *P. putida* and then *B. subtilis*.

These findings are similar to the results reported by Apet *et al.*, (2018) studied efficacy of different bioagent viz., *T. hamatum, B. subtilis, P. fluorescence, T. virens, T. harzianum* and *T. viride* against *Xanthomonas axonopodis* pv. *punicae*. Maximum inhibition was observed due to the *P. fluorescence* (12.82 mm), while minimum with *T. virens* (2.38 mm).

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