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## Soil amendment with compost as an alternative for management of *Ralstonia solanaceum* in capsicum annum

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### Abstract

Pepper bacterial wilt is caused by the bacterial pathogen, *Ralstonia solanacearum*. It is the most destructive disease of many solanaceous crops such as potatoes, tobacco, pepper, tomatoes, and eggplant and is a significant source of crop loss worldwide. Physical, cultural and chemical controls have been employed to combat this destructive disease. However, none of these strategies has been able to control the disease completely due to the broad host range and genetic diversity of the pathogen, its prolonged survival in the soil and survival on vegetation as a latent infection. Owing to co-management strategies, biological control is the best approach for human health and environmental friendly motivations. In the present study, compost made from leaf waste of medicinal plants such as *Azadirachta indica*, *Barleria prionites*, *Ficus benghalensis*, *Ficus religiosa*, and *Capparis decidua* was found effective in management and control of this pathogen. An improved germination was observed in Mundawar soil upto 95%, Thanagazi soil achieved an impressive 97% germination rate, and Behror soil yielded a remarkable 94% germination rate. Also, incidence of bacterial dropped from 13, 10 and 7 in normal soil to 7, 6, 11 percent in enriched soil from Mundawar, Thanagazi and Behror tehsils respectively.

**Key words:** *Ralstonia solanacearum*, compost, seed vigour index, soil

## Introduction

The bacterial wilt disease affecting solanaceous crops, originally identified as caused by *Bacillus solanacearum*, was first described by Smith in 1896 in the USA. The pathogen is now known as *Ralstonia solanacearum* (Smith, 1896) as reclassified by Yabuuchi et al. (1995), with several synonyms over time, including *Bacillus solanacearum* Smith, 1896, *Pseudomonas solanacearum* (Smith, 1896, 1914), and *Burkholderia solanacearum* (Smith, 1896; Yabuuchi and Arakawa, 1993). *Ralstonia solanacearum* has an unusually broad host range, infecting over 450 plant species across 54 plant families (Sharma et al., 2016a). Among its major hosts are economically significant crops like *Lycopersicon esculentum* (tomato), *Capsicum annum* (sweet and hot peppers), and *Solanum tuberosum* (potato) (Chandrashekara and Prasannakumar, 2010). Soilborne pathogens, including *Ralstonia solanacearum*, are major contributors to crop losses in many vital plants (Elphinstone et al., 2005). Ranked as the world's second most devastating phytopathogen (Mansfield et al., 2012), *Ralstonia spp.* are Gram-negative, oxidase- and catalase-positive, aerobic bacilli that thrive in soil and water environments (Lampropoulos et al., 2021). This bacterial wilt disease is one of the most destructive to several agriculturally significant crops such as eggplant, tomato, pepper, potato, and ginger, severely impacting crop yield and quality on a global scale (Cai et al., 2021). Due to its high destructive potential, *R. solanacearum* is one of the most intensively studied plant pathogens today.

Over 310 species of plants belonging to 42 plant families are infected by *Ralstonia* (Genin, 2010; Genin and Denny, 2011; Paudel et al., 2020). Most common use of capsicum includes as condiments, nutritional food, and many more beneficial uses in Indian, American, and Chinese culture for the treating acne, indigestion and inflammation of the joints (Meghvanshi et al., 2010). *R. solanacearum* is reported to affect different crops in various countries, causing an annual loss of \$848 million (Charkowski et al., 2020).

In addition to its high economic value, capsicum is one of the most common vegetables owing to the combination of the fruit's colour, flavour, and nutritional qualities (Materska and Peruka, 2005; Al-Snafi et al., 2015). It is one of the most challenging diseases, causing severe damage to pepper plants throughout the world, especially in the tropical and subtropical regions, and parts of the warm temperate regions (Du et al. 2017). The disease is known as “Green wilt” disease since the

leaves of the infested plant stay green when the plant begins to show wilt symptoms (Jiang *et al.* 2017).

According to studies, Rajasthan has poor soil microbial flora and very little organic matter in its soils (Sharma and Basotia 2018). Additionally, because there are no significant permanent streams in the area and little rainfall, the soils of the Alwar do not receive enough nitrogen replenishment through floods and other natural events. As a result, soil productivity in dry areas is also often low. A variety of pathogens affect pepper in this area including *R. solanaraceum* which is a soil-borne pathogen. To guarantee a productive and healthy pepper crop, both cultural practices and insecticides are used as the only option (Pitrat, 2012; Parisi *et al.*, 2020). In view of this, present study on investigating effect of soil amendments with organic composting for management of bacterial wilt of Capsicum was carried out.

## Methodology

### a. Isolation and identification of *R. solanacearum*

*Ralstonia solanacearum* was isolated from wilted capsicum plants grown in normal soil in the fields of Alwar, following the method of Kelman (1954). Briefly, wilted capsicum plants were brought to the laboratory, thoroughly washed under running tap water, and cut into small pieces. These pieces were surface-sterilized using 0.1% HgCl<sub>2</sub> for 1 minute, followed by three washes with double-distilled sterilized water (ddsH<sub>2</sub>O). The sterilized pieces were then placed in a beaker containing ddsH<sub>2</sub>O for 5 minutes, allowing milky white bacterial exudates to form. The resulting bacterial suspension was directly streaked onto Petri plates containing sterilized and solidified nutrient agar (NA) medium (composed of 10 g meat extract, 10 g peptone, 1.5 g NaCl, and 15 g agar, adjusted to 1000 ml). The plates were incubated at 28 ± 2 °C for 2 days. Subsequently, the isolate was re-streaked on triphenyl tetrazolium chloride (TTC or TZC) medium to obtain a single colony. The morphological characteristics of the isolates were then observed with the naked eye.

- a. **Preparation of compost:** This section of the study focused on compost and soil enrichment. Composting experiments were conducted at RR College in Alwar between September 2021 and February 2022. Plant remnants from medicinal plants such as *Azadirachta indica*, *Barleria prionites*, *Ficus benghalensis*, *Ficus religiosa*, and *Capparis decidua* were meticulously blended with organic matter to modify the initial carbon-to-nitrogen ratio. The mixture was layered in a compost pit, moistened, and turned every two weeks for aeration.

After 4-6 months, the compost matured into a nutrient-rich material, ready for soil amendment.

***b. Effect of compost on disease suppression:***

To study the effect of compost enriched soil on disease suppression visual observations for any symptom development on plant parts were performed. Also the growth of compost treated and plant pathogen treated plants will be compared for this purpose under following points:

***(i) Evaluation of pepper growth:***

The impact of compost on growth and incidence *R. solanaraceum* of pepper plants was observed in terms of plant height, number of branches, fruit weight, fruit yield per plant and vitamins in both seasons compared to the untreated plants.

***(ii) Effect on seed germination and seedlings growth***

Seeds of capsicum will be surface sterilized and sown in pots containing amended soil and nontreated naturally available soil. The observations will be made till the reproductive growth. Further the record of symptomatic seedlings, percent seed germination and root, shoot lengths will also be made. Seeds soaked in sterile distilled water were taken as negative control.

***(iii) Effect on germination percentage***

Effect of soil amendments on germination of seeds, number of ungerminated seeds, percent of healthy seedlings developed and total loss will be compared with seeds grown in non-treated naturally found soil in pot experiments.

**Results**

***a. Isolation and identification of *R. solanacearum****

100 sample of capsicum plants were grown in Mundawar, Thanagazi, Behror regions of Alwar district of Rajasthan. These capsicums were cultivated in normal soil. In Mundawar 13 bacterial infected plants were obtained out of 100 samples. Bacterial infection in samples taken from Thanagazi and Behror is 10 and 7 out of 100 sample. Bacterial infection percentage of Mundawar, Thanagazi, and Behror is 13%, 10% and 7% (Fig. 1).

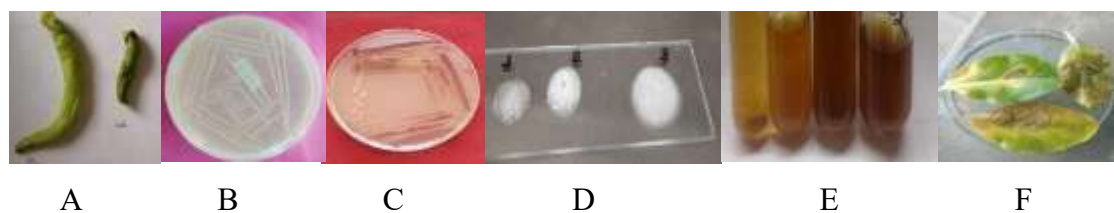


Fig 1: Biochemical tests for identification of *R. solanaraceum* isolated from *Capsicum annum* A. Healthy and Infected chilli; B. Streaked bacterial colonies on NA; C. colonies on TZC medium; D. catalase test; E- sugar utilization test; F. tobacco hypersensitivity test

**Table 1: Identification of *Ralstonia solanaraceum* isolated from capsicum**

| Sr. No. | Tests                          | Response      |
|---------|--------------------------------|---------------|
| 1.      | Gram staining                  | Gram negative |
| 2.      | KOH solubility                 | +             |
| 3.      | Acid production                | +             |
| 4.      | Starch hydrolysis              | +             |
| 5.      | Catalase test                  | +             |
| 6.      | Production of H <sub>2</sub> S | +             |
| 7.      | Motility test                  | +             |
| 8.      | Casein hydrolysis              | +             |

***b. Preparation of compost and compost extract:***

To study the effect of compost enriched soil on disease suppression visual observations for any symptom development on plant parts were performed. Also the growth of compost treated and plant pathogen treated plants will be compared for this purpose under following points:

***i. Evaluation of pepper growth and germination:***

The results of capsicum seed germination were examined in compost enriched and normal types of soil. The germination rate in Mundawar Soil and Behror Soil was approximately 81%, whereas in Thanagazi Soil, it was 74%. On the other hand, seeds planted in soil that had been enriched displayed superior germination rates. Mundawar Soil boasted a 95% germination rate, Thanagazi Soil achieved an impressive 97% germination rate, and Behror Soil yielded a remarkable 94% germination rate. Germination was observed in 81 seeds from Mundawar Soil and Behror Soil, and 74 seeds from Thanagazi Soil, out of 100 seeds in each case, when non-enriched soils were used. There were 16, 12, and 10 symptomatic Capsicum plants from the germinated seeds in Mundawar Soil, Thanagazi Soil, and Behror Soil, respectively. On the other hand, the use of enriched soil led to nearly perfect seed germination and fewer symptoms. The data presented in figures 3 and 4 demonstrate that the use of enriched soil effectively reduced overall plant loss. It is worth mentioning that soil derived from plant waste (compost) showed higher rates of symptom-free tomato seed germination when compared to normal soil (Fig. 2 and 3).

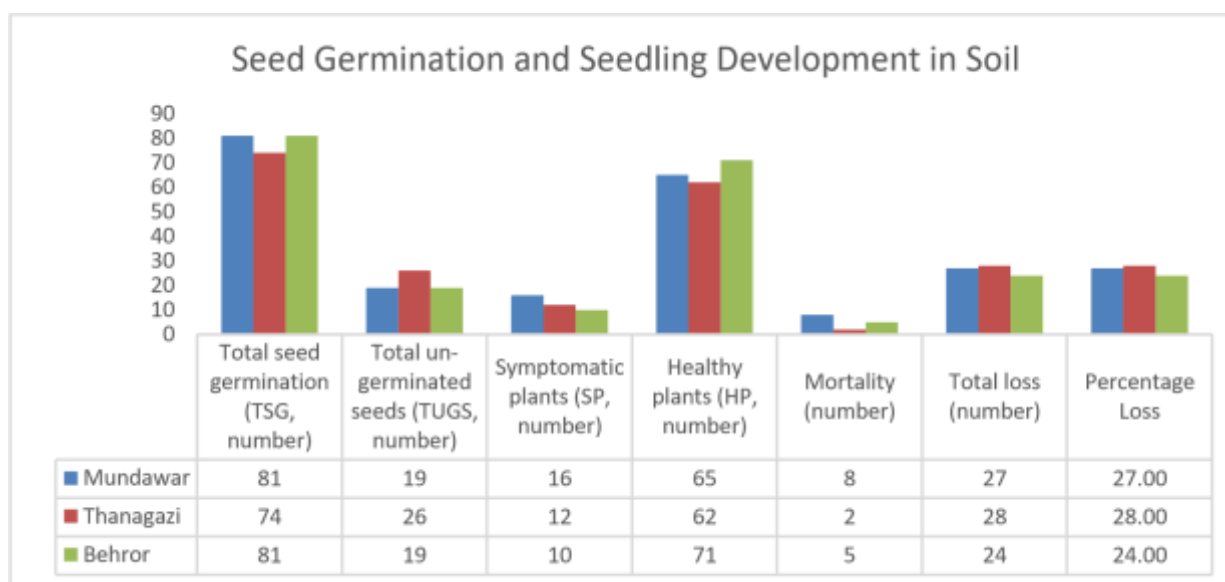


Fig 2: Evaluation of seed germination and capsicum seedling in normal soil of Alwar district.

| Seed Germination and Seedling Development in Enriched Soil |                           |                                    |                            |                             |                    |                     |                 |
|------------------------------------------------------------|---------------------------|------------------------------------|----------------------------|-----------------------------|--------------------|---------------------|-----------------|
| 6040200                                                    |                           |                                    |                            |                             |                    |                     |                 |
|                                                            | Total seed germination... | Total ungerminated seeds (TUGS,... | Symptomatic plants (SP,... | Healthy plants (HP, number) | Mortality (number) | Total loss (number) | Percentage Loss |
| Mundawar                                                   | 95                        | 5                                  | 1                          | 94                          | 0                  | 5                   | 5.00            |
| Thanagazi                                                  | 97                        | 3                                  | 0                          | 97                          | 0                  | 3                   | 3.00            |
| Behror                                                     | 94                        | 6                                  | 2                          | 92                          | 0                  | 6                   | 6.00            |
| 12010080                                                   |                           |                                    |                            |                             |                    |                     |                 |

Fig 3: Evaluation of capsicum seed germination and seedling health in compost enriched soil of Alwar district.

The length of Capsicum plants was assessed depending on the soil type it is grown. In Mundawar soil, the plants reached a length of 5.56 cm, while in Thanagazi soil and Behror soil, they measured 3.12 cm and 5.86 cm, respectively after 15 days of planting in normal soil. In enriched soil conditions, the length of capsicum plants showed variations: 6.47 cm in Mundawar enriched soil, 6.42 cm in Thanagazi enriched soil, and 6.1 cm in Behror enriched soil (Fig. 4).



Fig.4: A- Pot experiment and B- Effect of compost on capsicum plant grown in compost (top left) in comparison to grown in normal soil (top right) *ii. Effect of compost on disease suppression:*

Compost displayed a positive effect on the growth and yield of chili pepper (Fig. ). The root growth, the number of chilies per plot, and the size of the chili were significantly better in the compost treated soil (Enriched soil) than in normal soil. Data show that bioactive compost

improved chili growth by increasing shoot length, in plant fresh and weight as compared to that of the normal soil. Also, incidence of bacterial dropped from 13, 10 and 7 in normal soil to 7, 6, 11 percent in enriched soil from Mundawar, Thanagazi and Behror tehsils respectively.

**Table 3: Control of Incidence of Bacterial Pathogens in Enriched soil**

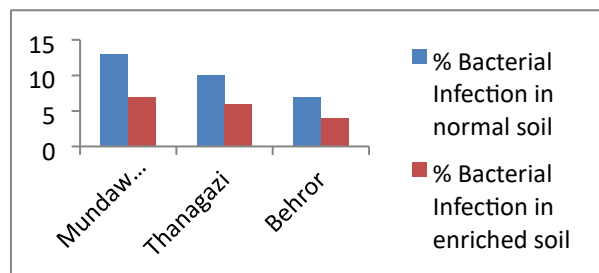


Fig 5: comparison of incidence of *R. solanaraceum* in normal and compost enriched soil

## Discussion

The substantial economic damage caused by *R. solanacearum* is largely due to its wide host range and extensive geographical spread in warm temperate regions around the world (Elphinstone, 2005). The pathogen contributes to significant yield losses, which can vary depending on the crop cultivar, soil type, climate, farming practices, and pathogen strain (Elphinstone, 2005; Tshifhiwa et al., 2024). Soil-borne diseases are among the most destructive plant diseases, accounting for an estimated 10-20% of annual crop yield losses (Savary et al., 2012; Yuliar et al., 2015). *R. solanacearum* is ranked as the second most destructive among the ten most harmful bacterial species affecting economically important crops (Mansfield et al., 2012). The pathogen has caused severe yield losses in various solanaceous crops, including up to 88% loss of tomatoes in Uganda and 70% loss of potatoes in India and other countries (Katafiire et al., 2005). Bacterial wilt has been reported to affect 50-100% of potatoes in Kenya (Muthoni et al., 2012). In Ethiopia, bacterial wilt incidence is nearly 100% in peppers, 63% in potatoes, and 55% in tomatoes (Assefa et al., 2015).

In this study, virulent colonies of *R. solanacearum* isolated from capsicum were developed on TZC agar medium. These colonies appeared as dull white or creamy, irregularly round fluidal colonies, opaque with a light pink or red center, while non-virulent colonies appeared dark red. These findings align with the observations of Dhital et al. (2001), Ahmed et al. (2013), and Chamedjeu et al. (2018), isolated *R. solanacearum* on TZC medium from infected potato plants. Symptoms observed on inoculated potato plants included the loss of turgidity in tender leaves, pale yellowing of lower leaves, drooping of infected leaves, flaccidity, and sudden wilting of the entire plant. These results are consistent with those reported by Shweta et al. (2018), who demonstrated the pathogenicity of *R. solanacearum* using a soil drenching method on potatoes. Gram staining results revealed that the test bacterium was gram-negative, rod-shaped, and retained reddish-pink coloration. Similar results have been reported by researchers such as Pawaskar et al. (2014), Singh et al. (2015), and Bannihatti (2015). As observed in the present study, the bacterium also formed slime threads when a 48-hour-old bacterial culture was mixed with 3% KOH solution, showing a positive reaction for the test (Table 1). This result corroborates the findings of Rahman et al. (2010), Chakravarty and Kalita (2011), and Singh et al. (2015), who reported *R. solanacearum* to be positive in the KOH solubility test.

It is well-recognized that compost use plays a crucial role in enhancing productivity, soil fertility, and sustainable agriculture. However, compost and compost extracts can alter the balance of soil microflora and suppress foliar diseases in some crops (Stone et al., 2003; Zaki et al., 2011). This study demonstrated that compost extracts could suppress the incidence of the bacterial wilt pathogen and control symptom development in capsicum. The observed suppressive activity of microbial-enriched compost extracts is likely biological in nature, as it has been suggested that the accumulation of soluble nutrients in compost enhances beneficial microorganisms that produce antimicrobial compounds (Ingham, 2005). Additionally, compost has been shown to control plant pathogens through single or multiple mechanisms, such as microbial antagonism by various mechanisms such as through antibiosis, parasitism, competition for nutrients/space, or induced plant resistance (Zhang et al., 1998; Litterick et al., 2004; Al-Mughrabi et al., 2008) or suppressive physicochemical properties (improved nutrient status of the plant, toxic compounds, or induced resistance) (Hoitink et al., 1997). Calcium also plays an essential role in plant defense against pathogens, as it is crucial for the structure of the middle lamellae of plant cells and in maintaining the selectivity of cell plasma membranes (Paula Júnior et al., 2009). However, disease reduction

was accompanied by a gradual increase in peroxidase and polyphenol oxidase activity. In this study, soil amendment with compost treatments significantly controlled the incidence of the test pathogen and symptom development. Many researchers have reported that compost extracts increase peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase,  $\beta$ -1,3glucanase, and chitinase activity in okra, tomato, and onion plants when applied to foliage (Nwogbaga & Iwuagwu, 2015; Siddiqui et al., 2009). The induction of plant resistance by compost extract could be attributed to the presence of beneficial microorganisms responsible for inducing systemic resistance (Segarra et al., 2009b). Furthermore, the compost was rich in inorganic salts, organic carbon, and phenols, which can affect pathogen growth and phyllosphere microorganisms (Segarra et al., 2009a). Our results showed a general increase in plant growth characteristics (plant height, number of branches per plant, fruit yield per plant, and fruit freshness) due to the use of compost treatments as soil amendments compared to control plants grown in normal soil. The enhancement effect of compost may result from a higher concentration of plant nutrients such as N, P, K, and Mg, as well as root reinforcement induced by compost (Donn et al., 2014; Nadjjet et al., 2014). Rodriguez-Vila et al. (2016) confirmed that organic amendments improve soil properties by increasing organic matter, nutrient content, and microbial activity, thereby enhancing crop growth and yield.

According to Singh et al., (2017), the effectiveness of organic amendments against *R. solanacearum* in pot culture conditions, karanj cake (64.97%) had the greatest impact on average mortality reduction, followed by neem seed cake (43.95%), farm yard manure (37.36%), vermicompost (32.95%), poultry manure (32.24%), and sunflower seed cake (26.87%). Vermicompost had the highest average reduction in incidence (60.66%), followed by karanj cake (53.74%), neem seed cake (46.70%), and compost (41.33%). Bannihatti et al. (2019) evaluated ten organic amendments in pot culture as pre-sowing soil treatments against *R. solanacearum*. Similarly, in their evaluation of ten organic amendments used in soil contaminated with *R. solanacearum*, Kadam and Jagtap (2018) discovered that Karanj cake had the highest average mortality reduction (70.24%), followed by poultry manure (66.64%), neem seed cake (63.70%), groundnut cake (53.28%), goat manure (52.71%), FYM (45.64%). Neem extracts have produced inhibitory activity against *R. solanacearum*, according to research by Murthy et al. (2015), who also discovered that *Azadirachta indica* extracts have much greater antibacterial potential than the majority of

antibiotics. According to Zeeshan et al. (2016), under in vivo conditions, spent mushroom compost was effective in suppressing the bacterial wilt of tomatoes and improved agronomic traits like fresh plant weight, root length, plant height, dry plant weight, and the quantity of fruits and shoots per plant. The mushroom spent or cow manure has also been reported to be used composts with upto 100% control efficiencies against bacterial wilt of tomatoes (Ding, 2023). In the present study too, seed germination were examined in compost enriched and normal types of soil. The germination rate in Mundawar soil and Behror soil was approximately 81%, whereas in Thanagazi Soil, it was 74%. On the other hand, seeds planted in soil that had been enriched displayed superior germination rates. Mundawar Soil boasted a 95% germination rate, Thanagazi Soil achieved an impressive 97% germination rate, and Behror Soil yielded a remarkable 94% germination rate. Also, incidence of bacterial dropped from 13, 10 and 7 in normal soil to 7, 6, 11 percent in enriched soil from Mundawar, Thanagazi and Behror tehsils respectively. Thus it can be said that the use of compost as an alternative to suppress bacterial wilt has beneficially influenced capsicum productivity via improving the chemical, biological, and physical properties of soil, which influences plant health positively. Also, it is evident that compost may affect the incidence of *R. solanaraceum* and improves capsicum plant growth. **Conclusion**

Soil-borne plant pathogens cause significant damage to crops each year. Among these, *Ralstonia solanacearum* stands out as the second most destructive bacterial phytopathogen globally due to its highly damaging nature. Various control measures have been developed to manage this pathogen, but achieving efficient and eco-friendly management strategies that offer consistent and effective control remains a challenge. There is an ongoing need to develop more effective methods to combat this deadly disease in multiple crops under field conditions. In our study, the use of compost treatments as soil amendments led to a notable control in disease incidence and improvement in plant growth characteristics, including plant height, the number of branches per plant, fruit yield per plant, and fruit freshness, compared to control plants grown in untreated soil.

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