

Original Research Article

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Survey and Collection of Bacterial Wilt (*Ralstonia solanacearum*) Isolates from Different Agro Climatic Zones of Karnataka

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ABSTRACT

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Bacterial wilt caused by *Ralstonia solanacearum* (E. F. Smith) is one of the devastating disease with global distribution affecting brinjal. The disease affects solanaceous vegetables in most states of India. Investigation on survey and collection of bacterial wilt (*Ralstonia solanacearum*) isolates from different agro climatic zones of Karnataka was undertaken. The roving survey was conducted during 2015-16 for assessing the bacterial wilt incidence in different agro climatic zones of Karnataka (India) viz., Bengaluru Rural, Mysuru, Dharwad, Hassan, Bidar, Kolar, Vijayapura, Tumakuru and Chikkaballapur and collection of disease samples was also done. The per cent disease incidence varied significantly in all places and maximum disease incidence in brinjal was recorded in Chikkaballapur (27.60%) followed by Dharwad (22.51%), while, the least incidence was observed in Mysuru (4.62%).

Introduction

Brinjal (*Solanum melongena* L.) is one of the important vegetable crop, belonging to the family Solanaceae. It features virtually in all the dishes of every household in India, irrespective of food preferences, income levels or social status. As a part of the most basic or sophisticated Indian meal, brinjal is used in the preparation of a number of sumptuous dishes. Further, in recent years brinjal is being exported in the form of products like bainganbhartha, chatni, pickles etc. to Middle East countries. Brinjal is the second most important vegetable growing in India after potato. In the world, brinjal is

grown in an area of about 1.86 million hectare, with production of 49.78 million tonnes with a productivity of 26.07 tonnes per hectare (Anon., 2014). In India, it is mainly grown in Odisha, Bihar, Karnataka, West Bengal, Andhra Pradesh, Telangana, Maharashtra and Uttar Pradesh.

The total area, production and productivity of brinjal crop in India is 0.922 million hectare, 13.5 million tonnes and 19.1 tonnes per hectare, respectively (Anon., 2014) and it contributes about 8.3 per cent of total Indian vegetable production. In Karnataka, it is grown in an area of 0.15 lakh hectare with a production of 4.02 lakh tonnes and

productivity of 25.4 tonnes per hectare (Anon., 2014). Successful cultivation of brinjal crop has been hindered due to attack of many pest and devastating diseases. Among diseases, bacterial wilt caused by *Ralstonia solanacearum* (Smith) is a major limiting factor. It has been the most ubiquitous and serious bacterial disease throughout the tropical, sub-tropical and temperate regions of the world (Hayward, 1991).

In India, this disease is of major concern and is serious in major parts of Karnataka, Kerala, Odisha, Maharashtra, Madhya Pradesh and West Bengal (Rao *et al.*, 1976) causing economic yield losses upto 86.14 per cent (Sabita *et al.*, 2000).

Materials and Methods

Survey

Roving survey was conducted to know the incidence of bacterial wilt of brinjal and collection of *R. solanacearum* isolates causing bacterial wilt from different agro climatic zones of Karnataka. The plants showing typical symptoms were collected from the fields representing different agro climatic areas. Total number of plants and number of wilt affected plants were counted from randomly selected rows in the field and per cent wilt incidence was calculated.

$$\text{Per cent disease incidence} = \frac{\text{Total number of plants wilted}}{\text{Total number of plants}} \times 100$$

Collection of sample

After recording the observation, the wilted plants showing typical symptoms of bacterial wilt were uprooted and collected. The collected samples were labelled and packed in polythene bags and kept at 4° C for the purpose of isolation of the causal organism.

Isolation of *Ralstonia solanacearum* from bacterial wilt infected plants

Brinjal plants showing typical symptoms of bacterial wilt caused by *Ralstonia solanacearum* collected from different agro-climatic zones of the state were used for the isolation of the causal organism. The preliminary diagnosis of the disease was done by checking the bacterial ooze from the discolored vascular tissues of the affected plants. The bacteria was isolated from the infected plants by extracting the ooze in sterile distilled water taken in small vials followed by dilution plating on Triphenyl Tetrazolium Chloride (TTC) agar medium.

Small pieces of discolored vascular tissue measuring 4-5 mm in length were cut from the discolored vascular tissues of the stem. The tissue was surface sterilized by immersing bits in 1 per cent sodium hypochlorite for 30 seconds. The disinfected tissue was washed in sterile water to remove traces of sodium hypochlorite, if any. The surface sterilized bits were then suspended in ten milliliter sterile distilled water taken in a test tube for ten minutes. After the water in the test tube became turbid due to oozing of the bacterial cells from the cut ends of the diseased tissue the bacterial suspension was serially diluted in nine milliliter sterile distilled water. One hundred microliter of the diluted bacterial suspension was poured onto the surface of solidified TriphenylTetrazolium Chloride agar (TTC) medium (Kelman, 1954) in sterilized Petri plates. The bacterial suspension was spread onto the surface of TTC medium with a sterilized spreader. The inoculated plates were incubated at 28°C for 48 hours.

At the end of the incubation period, the plates were observed for the development of well separated irregularly shaped, fluidal, dull white colonies with slight red center typical of virulent *R. solanacearum* colonies. The

concentration of the inoculum was adjusted to 0.3 Optical Density (OD) at 600nm wave length (approximately 1.0×10^8 cfu/ml) using spectrophotometer. The composition of the TTC agar medium used is as follows: 1.0 per cent peptone, 0.1 per cent Casein hydrolysate and 0.5 per cent Dextrose and 1.5 per cent agar in 1000 ml of distilled water. One ml of 1 per cent aqueous solution of triphenyltetrazolium chloride was added to 200 ml medium after sterilization and prior to pouring into the Petri plates.

Results and Discussion

Roving survey was conducted in different agro climatic zones of Karnataka for assessing the bacterial wilt incidence and collection of disease samples. The results of the survey were depicted in Table 1, Plate 1 and Figure 1. Based on the results of roving survey in major brinjal growing areas of Karnataka, it was evident that the incidence of *Ralstonia solanacearum* was highly prevalent across all brinjal growing areas. The per cent disease incidence varied significantly across all places and maximum disease incidence was recorded in Chikkaballapur (27.60%) followed by Dharwad (22.51%), while, the least incidence was observed in Mysuru (4.62%) followed by Hassan (6.37%) and Tumakuru (7.18%).

Wherever, wilted plant was observed, there it was strongly noted that the absolute association of *R. Solanacearum* with plant which was confirmed by ooze test. The high incidence of the disease in Karnataka region indicates that it is a recurrent problem in the brinjal growing areas.

This may be due to susceptibility of the cultivars or favourable environmental conditions like temperature and continuous rainfall experienced. The infected debris left in the field might serve as a major source of

infection, for causing epidemic throughout the season.

The results of present study was supported by Vanitha *et al.*, (2009), found the disease incidence in plants ranged from 9 to 39 per cent whereas, the incidence in seeds ranged from 4 to 18 per cent in major tomato growing districts of Karnataka state. Deepa *et al.*, (2003) confirmed the nine strains of *R. Solanacearum* belongs to Races 1 and 3 isolated from the bacterial wilt affected plants of brinjal, chilli and tomato in three different agro climatic zones of Kerala. Chandrashekara *et al.*, (2012) collected 57 isolates of *R. solanacearum* causing wilt on different host plants from different agro climatic zones of India. Narasimhamurthy and Srinivasa (2012) concluded that 85 isolates from different agro-climatic regions of Karnataka and other parts of India belong to biovar III.

Brinjal plants showing typical symptoms of bacterial wilt caused by *Ralstonia solanacearum* collected from different agro-climatic zones of the state were used for the isolation of the causal organism (Plate 2).

The bacteria was isolated from the different samples collected and designated as shown in Table 2. All the isolates showed the development of well separated irregularly shaped, fluidal, dull white colonies with slight red center typical of virulent *R. solanacearum* colonies (Plate 3). All the isolates collected showed gram negative reaction (Plate 4).

In conclusion the bacterial wilt of brinjal is a major disease occurring in almost all the major brinjal growing areas though the incidence varied. The isolates isolated all exhibited the typical characteristics of the *R. solanacearum*.

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