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Antibacterial Activity of *Curcuma longa* (Turmeric) Plant Extracts Against Bacterial Wilt of Tomato Caused by *Ralstonia solanacearum*

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Abstract: ***Bacterial wilt caused by Ralstonia solanacearum is a major constraint for production of tomatoes (Solanum lycopersicon). It affects large varieties of solanaceous plants worldwide. Control of bacterial wilt is very tricky as there are no effective curative chemicals. Plants are considered as one of the most important source of medicine and drugs and they have been used for treating different ailments in humans worldwide from the beginning of the civilization. Turmeric (Curcuma longa) belongs to Family Zingiberaceae and Curcuma longa is known to be an important medicinal plant from initial period in India. 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 turmeric plant against ten highly virulent isolates of R. solanacearum. The antibacterial activity of the extracts was assayed by agar well diffusion method on Tryptone Soya agar. The results revealed that the average zone of inhibition of the rhizome extract was ranging at 20-26mm against R. solanacearum. Various concentrations of the extracts were prepared by dissolving extracts in DMSO. The means and standard error of triplicate tests were recorded. The minimum inhibitory concentration (MIC) was determined by two-fold micro broth dilution method for the tested pathogens. The MIC of the turmeric extract was 2-20µg ml^{-1}. The activities of the solvent extract are remarkable when compared with the water extracts. Hence, solvent extract will enhance the efficacy of turmeric in the activity of R. solanacearum infections.***

Keywords: *Ralstonia solanacearum*, plant extracts, tomato, *Curcuma longa*, minimum inhibitory concentration, bacterial wilt.

1. Introduction

Ralstonia solanacearum causes bacterial wilt, a soilborn vascular disease that is arguably one of the most economically important bacterial diseases in the world. It attacks over 450 plant species including ornamentals such as geranium, and limits the production of such economically important crops as tomatoes, tobacco, potatoes and bananas (Kelman *et al.*, 1994). (Kisun, 1987) reported that the yield loss may vary between 10.8 and 90.6 percent depending on the environmental circumstances and the stage at which infection occurs. Bacterial Wilt poses a constant threat to tomato in Karnataka, Madhya Pradesh, Marathwada region of Maharashtra and West Bengal in India. The pathogen infects susceptible plants in roots, usually through wounds (Pradhanang *et al.*, 2005) and colonizes within the xylem preventing the water movement into upper portion of the plant tissue (Kelman, 1998).

Control of bacterial wilt in infested soils is very difficult. It is generally considered that crop rotation with a non host crop is of minimal value because of the wide range of crop and weed hosts of the pathogen (Hayward, 1991). At present no conventional bactericides are known to provide effective control of this soil borne pathogen. Management of disease using bactericides causes environment pollution and the bactericide residues are harmful to human health. Public awareness about residual effects of pesticides in food and environment and development of pesticide resistance in plant pathogen population has challenged the plant pathologists to search for non-toxic bactericides for substituting the recommended chemicals. The intensive and indiscriminate use of pesticides in agriculture has caused many problems to the environment such as water, soil, animals and food contagion; poisoning of farmers; elimination of non-target organisms and selection of phytopathogens, pest and weed insensitive to certain active ingredients (Stangarlin *et al.*, 1999). Soil treatments with traditional general-purpose fumigants such as methyl bromide did not provide satisfactory control of the disease (Chellemi *et al*., 1997). Due to the aforementioned considerations, there is a continuous search to develop new management strategies to reduce the dependence on the synthetic agrochemicals. Among the safe methods, botanicals and biocontrol agents are attracting much attention.

The natural plant products derived from plant species has the capacity to control diseases caused by viruses, bacteria and fungal pathogens. Research focused on plant-derived natural bactericides and their possible applications in agriculture to control plant bacterial diseases has intensified as this approach has huge potential to inspire and influence modern agro-chemical research. Many reports revealed that, plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Gottlieb *et al*., 2002).

India has a rich history of using plants for medicinal purposes. Turmeric (*Curcuma longa* L.) is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases (Eigner *et al.*, 1999). *Curcuma longa*, botanically related to ginger (*Zingiberaceae* family), is a perennial plant having a short stem with large oblong leaves and bears ovate, pyriform or oblong rhizomes, which are often branched and brownish-yellow in colour. Turmeric is used as a food additive (spice), preservative and coloring agent in Asian countries, including China and South East Asia (Khattak *et al.*, 2005). It is also considered

as auspicious and is a part of religious rituals. Although as a dye it is used similarly to saffron, the culinary uses of the two spices should not be confused and should never replace saffron in food dishes. Its use dates back nearly 4000 years, to the Vedic culture in India where it was used as a culinary spice and had some religious significance. The name derives from the Latin terra merita "meritorious earth" referring to the colour of ground turmeric which resembles a mineral pigment. Turmeric has been used for 4,000 years to treat a variety of ailments (Bhowmik *et al.*, 2009).

During the recent decades, many herbal extracts have been extensively tested and a myriad of reports have been documented outlining the uses of plant extracts to control the animal and plant diseases (Opara *et al.*, 2010). A good number of reports outlined the antimicrobial effects of some medicinal plants for plant disease control. Some plant extracts were documented as effective inhibitors of phytopathogenic bacteria (Leksomboon *et al.*, 2000). Antimicrobial activities of several plant extracts against bacterial wilt of tomatoes were evaluated and quite satisfactory results were obtained. However, no attempts have been made to identify and characterize the antibacterial plant extracts to control the bacterial wilt pathogens of tomatoes. The use of plant extracts is found to be an efficient way of controlling plant diseases compared to synthetic chemicals as plant extracts have numerous advantages over it. So, there is an urgent necessitate to search for efficient, safe and eco-friendly alternative pesticides. Very little work has been done to investigate the use of natural plant products to control bacterial wilt. The study was therefore carried out to determine the effect of *Curcuma longa* plant extracts on the *in vitro* growth and development of *R. solanacearum* colonies. Hence, the present study is conducted on *in vitro* evaluation of turmeric (*Curcuma longa*) plant extracts against *R. solanacearum* causing bacterial wilt in tomato crops.

2. Materials and Methods

Isolation and identification of *R. solanacearum*:

Affected tomato plants showing typical symptoms of wilt were collected from different agro climatic zones of Karnataka. The collected plant materials were surface sterilized with 1% NaOCl solution for 1 to 2 min, followed by three repeated washings with distilled water and blot dried. Then the plant sections (0.5–1 cm) were placed on Kelman's TZC (2, 3, 5 Triphenyl tetrazolium chloride) medium (Kelman, 1954). The plates were incubated at 28 ± 2^{o}C for 24–48 h. Isolation from rhizosphere soil samples was done by dilution plate technique on modified semi selective medium, South Africa (SMSA) agar medium (Elphinstone *et al.*, 1996). The suspected colonies were subjected to different colony characteristics, biochemical, physiological, hypersensitive and pathogenicity tests for confirmation of the identity of the pathogen. The identification of the ten selected strains based on pathogenicity was further confirmed by molecular methods based on 16S rRNA sequencing for *R. solanacearum* (Narasimha Murthy *et al.*, 2012).

Preparation of plant extracts:

One gram of the *Curcuma longa* rhizome samples were thoroughly washed with tap water and then rinsed in sterile distilled water. The samples were ground in mortar and pestle with 10.0 ml sterile distilled water separately. The turmeric one gram of power was dissolved in 10.0 ml sterile distilled water. The content was filtered through Whatman's filter paper No.1 and the filtrate was used as 10% plant extract. The clear extract was used for testing its antibacterial activity against *R. solanacearum* under *in vitro*.

Preparation of bacterial inoculums:

Inoculum of the *R. solanacearum* was prepared by culturing it in Casamino acid Peptone Glucose (CPG) broth (1 g of Casamino acids, 10 g of peptone, 5g of glucose in 1000 ml of distilled water) (Kleman, 1954). Cultures were centrifuged at 12000 *g* for 10 min at 10°C. The pellet was resuspended in distilled water and was adjusted spectrophotometrically to 1×10^{8} CFU ml^{-1} (colony forming unit) (Ran *et al.*, 2005).

***In vitro* antagonistic activity against *R. solanacearum*:**

In vitro antagonistic activities against *R. solanacearum* from dried Turmeric plant was determined by standard agar well diffusion assay (Perez *et al.*, 1990). Petri dishes (size 9cm diameter) containing 20ml of cool Tryptic Soy Agar (TSA) (at 40°C) was seeded with 100µl inoculum of *R. solanacearum* (1×10^{8} CFU ml^{-1}). Media was allowed to solidify and then individual Petri dishes were marked for the bacteria inoculated. Wells of 5 mm diameter were cut into solidified agar media with the help of sterilized cork borer. Aliquot 100µl of each rhizome extract was added in the respective well and the plates were incubated at 28 ± 2°C for 24–48 h. The experiment was performed in triplicate under aseptic conditions. The antagonistic activity for each of the extract evaluated was expressed in terms of the average of the diameter of zone of inhibition (in mm) produced by the rhizome extract at the end of incubation period.

Determination of Minimum Inhibitory Concentration

Various concentrations of the extracts were prepared by dissolving extracts in Dimethyl sulfoxide (DMSO). The minimum inhibitory concentrations (MICs) by standard two-fold micro broth dilution methodology given by NCCLS (1997). A stock solution of each active extract was serially diluted in 96-wells micro titer plate with CPG broth to obtain a concentration ranging from 0.1µg/ml to 20µg/ml. Extracts were first diluted to the highest concentration (20 µg/ml) to be tested and then serial two fold dilution was made. A standardized inoculum for each bacterial strain was prepared so as to give inoculum size of approximately 1x 10^{8} CFUml^{-1} in each well. Micro titer plates were then kept at 28 ± 2^{o}C for 24h incubation. Following incubation, the MIC was calculated as the lowest concentration of the extract inhibiting the visible growth of bacterial strain.

3. Results

Isolation and identification of *R. solanacearum*:

After incubation pink centers with white fluid colonies were selected and a total of 50 strains of *R. solanacearum* were isolated and identified (Figure 1). Microscopic studies revealed that bacterial isolates were Gram negative, rod shaped and it was confirmed by standard biochemical tests. Pathogenicity was confirmed by the development of wilt symptoms on test plants after 7 days of inoculation followed by reisolation and identification of the causal organism from diseased plants. The inoculated plants lost turgidity, leaves started drooping and plants wilted suddenly. Based on the development of visible symptoms (Narasimha Murthy *et al*., 2012). The identification of the *R. solanacearum* isolates was confirmed by molecular analysis. The BLAST analysis of the sequences showed 98% to 99% identity to several isolates of *R. solanacearum* strains. Among 50 isolates, ten highly virulent strains were characterized and they were identified as *R. solanacearum* - RS1, RS2, RS3, RS4, RS5 RS6, RS7, RS8, RS9 and RS10 with Gen bank Accession numbers KF924739, KF924740, KF924741, KF924742, KF924743, KF924744, KF924745, KF924746, KF924747and KF924748 respectively.

In vitro* antagonistic activity against *R. solanacearum

Antibacterial activity of turmeric plant extracts against ten *R. solanacearum* pathogens, were studied. Results of the study are shown in the figure 2; the zone of inhibition was ranging at 20-26mm (figure 3). According to the results, all different types of extracts obtained from Turmeric plant rhizomes showed inhibition zone antagonistic activity against all tested *R. solanacearum* strains. The commercial bactericides, Streptocycline used as standard check showed inhibition zone measuring 22 mm diameter and water used as control check did not show any inhibition against *R. solanacearum*. Minimum inhibitory concentrations of different active extracts from rhizomes of Turmeric had been demonstrated in Table 1, *R. solanacearum* were inhibited at 2-20 µg ml^{-1} by DMSO extracts.

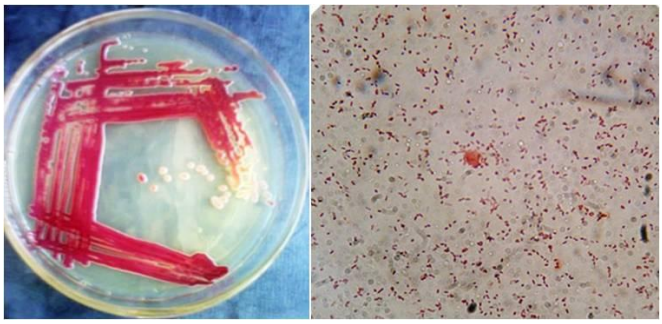

Figure 1: Colonies of *Ralstonia solanacearum* from infected tomato fields and Microscopic view of *R. solanacearum*

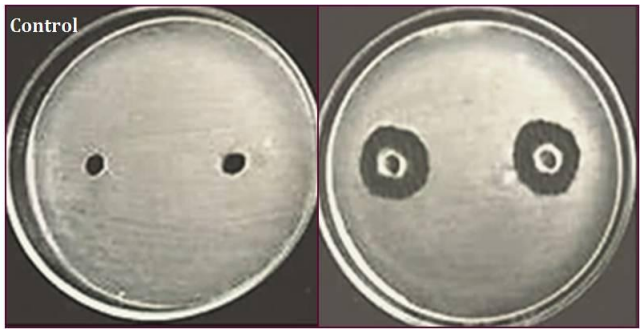

Figure 2: Antagonistic activity rhizome extracts of turmeric plant against *R. solanacearum*

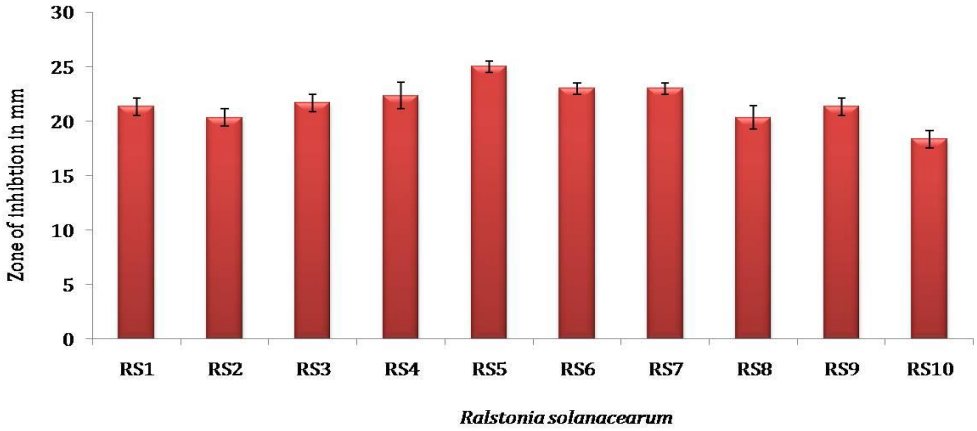

Figure 3: Zone of inhibition exhibited by rhizome extracts of turmeric plant against ten virulent strains of *R. solanacearum*

Table 1: Minimum inhibitory concentration of rhizome extracts of turmeric plant against *R. solanacearum*.

R. solanacearum	Concentration of Extracts (in µg ml^{-1})				MIC (in µg ml^{-1})
	20 µg ml^{-1}	2 µg ml^{-1}	1 µg ml^{-1}	0.1 µg ml^{-1}	
RS1	-	-	+	+	2
RS2	-	-	+	+	2
RS3	-	-	+	+	2
RS4	-	+	+	+	20
RS5	-	-	+	+	2
RS6	-	-	+	+	2
RS7	-	-	+	+	2
RS8	-	-	+	+	2
RS9	-	-	+	+	2
RS10	-	-	+	+	2

(-) represents 'No Growth Observed'; (+) represents 'Growth Observed

4. Discussion

Synthetic pesticides are nowadays widely used for the control of plant diseases all through the world because of their higher efficiency in controlling disease causing organisms. However, excessive and random application of these chemicals has created numerous environmental and health hazards and some phytopathogens have developed resistance (Rhouma *et al.,* 2009). Plants produce antimicrobial agents by secondary metabolism to protect themselves from pathogen attack, and therefore many plant species possess substantial antimicrobial activity (Macdonald, 2008).

According to the results, extracts obtained from turmeric plant rhizome shown to be with antagonistic activity against all tested *R. solanacearum*. The antibacterial effect of crude medicinal plant extract of *Curcuma longa*, *Brassica oleracae* and *Ipomoea batatas* on *Ralstonia solanacearum* were also reported (Wagura, 2011). The antibacterial activity of *Ralstonia* with plant extracts have been reported earlier (Lopez *et al.,* 2005; Larkin *et al.,* 2007). Though most of the botanicals have anti bacterial effect on most of the plant pathogenic bacteria and other microorganism (Chethana *et al.,* 2012), in the present study rhizome extracts of turmeric plant extracts antibacterial effect against *R. solanacearum*. This may be due to the variations in species, strains and biovars of phytopathogenic bacteria including *R. solanacearum*.
Biologically active plant derived pesticides are expected to play an increasingly significant role in crop protection strategies. Exploitation of naturally available chemicals from plants, which retards the reproduction of undesirable microorganisms, would be a more realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial pesticides for crop protection strategies, with special reference to the management of plant diseases (Gottlieb *et al.,* 2002). The extracts investigated in this study are from plants that are locally available and environmentally friendly.

Synthetic pesticides are nowadays widely used for the control of plant diseases throughout the world because of their higher effectiveness in controlling disease causing organisms. However, excessive and unsystematic application of these chemicals has created several environmental and health hazards and also some phytopathogens have been developed resistance (Rhouma *et al.,* 2009).Green plants have been shown to represents a reservoir of effective chematherapeutants and can provide valuable sources of natural pesticides (Dorman *et al.,* 2000). The use of *Curcuma longa* as a therapeutic agent for control of *Ralstonia solanacearum* will be of great use as the disease is highly prevalent and the biological control of the wilt will certainly enhance the quality of the plant products. Green plants are found to be an effective reservoir for the bioactive molecules and can provide valuable sources for the discovery of natural pesticides (Akhtar *et al.,* 1997). Therefore, in recent years medicinal plant extracts are intensively analyzed with an aim of isolating novel bioactive compounds.

In the present study, turmeric plant rhizome exhibited antibacterial activity against *R. solanacearum*, which is comparable to the commercial bactericide, streptocycline. The antibacterial effect of *Curcuma longa* has been shown against bacterial wilt pathogens. Turmeric extracts of the rhizome were subjected to a preliminary test of antimicrobial activities against phytopathogenic bacteria, *R. solanacearum.* It is clear from the present results that the extract exhibited marked activities against the tested bacteria. *In vitro* antibacterial activities against some pathogenic bacteria have been reported by Alam *et al.,* (2008). Though garlic extract and clove oil have shown to have high potential against several microorganisms (Jeyaseelan *et al.,* 2010) including *R. solanacearum* in the present study, the commercial exploitation in the management of bacterial wilt may be very expensive.

The extracts of turmeric plant rhizome were inefficient to inhibit the growth of the test bacterium as indicated by the results obtained in this study. This could be due to the fact that *Ralstonia* is a very difficult pathogen to inhibit and this may be a major contribution to its high occurrence and infectivity in soil, water, contaminated tools and infected seeds (Andersona *et al.,* 1999). The efficacy of solvent extracts of turmeric plant rhizome at various concentrations showed the potentials of their incorporation into effective management strategies of this important plant pathogen. These plants are commonly found in the environment and do not pose any threat to environmental safety as observed in many chemical pesticides (Sangoyomi *et al.,* 2011). The antibacterial activities of turmeric extracts were promising. In general, the activities against the *R. solanacearum* used have shown good activity when compared with standard antibiotic. High activity was found in extracts from rhizomes of *Curcuma longa* against the *R. solanacearum R. solanacearum* were inhibited minimum inhibitory concentration (MIC) ranging at 2-20µg ml^{-1}. This kind of biological come up to would be economical, safe, environmental friendly. These plants are also available in plenty and farmers can use it for control of wilt in the solanaceous crops. However, the chemical compounds are yet to be isolated from this plants which requires further detail study.

The antibacterial activity of *Ralstonia* with plant extracts have been reported earlier (Larkin et al., 2007; Walters *et al.,* 2009). However, the antibacterial activity of *Curcuma longa* extract against *R. solanacearum* is never reported earlier. The finding of the results is encouraging and could be used as a source of antimicrobial compounds for the control of bacterial wilt caused by *R. solanacearum.* It was evident that the use of *Curcuma longa* solvent extracts has a potential to substitute the antibiotics to control the infection. This kind of biological approach would be economical, safe, environmental friendly. These plants are also available in plenty and farmers can use it for control of wilt in the solanaceous crops. However, the chemical compounds are yet to be isolated from this plants which requires further detail study.

5. Conclusion

As per the results of the present study, turmeric extract has shown antimicrobial properties against *R. solanacearum.* The finding of the present investigation is an important step towards isolation and characterization of the antibacterial agent against the *R. solanacearum* and its further evaluation for crop protection strategies. Bacterial wilt of tomato caused by *R. solanacearum* is a systemic disease that cannot be efficiently control with foliar application of chemical pesticides. The use of plants in the control of diseases is as old as man and presents no potential toxicity. The results are very encouraging and the identification of the novel antibacterial compounds could be useful in the control of bacterial wilt infection in plant caused by *R. solanacearum.* The results are very encouraging and the identification of the novel antibacterial compounds could be useful in the control of bacterial wilt infection in plant caused by *Ralstonia solanacearum.* Pesticide companies may also use the findings as a baseline study for formulation of phyto based "green technology" for the management of bacterial wilt of tomatoes and other members of the *Solanaceae* family that are often infected by *R. solanacearum.*

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