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Original Research Article

Efficiency of *Ocimum sanctum* L. leaf extracts against bacterial wilt of tomato caused by *Ralstonia solanacearum* in tomato

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A B S T R A C T

Bacterial wilt caused by *Ralstonia solanacearum* is a major constraint for production of tomatoes (*Solanum lycopersicon*) affecting large varieties of solanaceous plants worldwide. Control of bacterial wilt is very difficult as there are no effective curative chemicals. Plants are considered as one of the most important source of medicine and drugs and have been used for treating different ailments in humans from the beginning of the civilization. *Ocimum sanctum* (Tulsi or Holy Basil) belongs to family *Labiaceae* and is known to be an important medicinal plant from earliest 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 *O. sanctum* against ten highly virulent isolates of *R. solanacearum*. The *O. sanctum* leaves were collected, dried and extracted using ethanol, methanol, hexane and ethyl acetate. 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 leaf extracts was more in the methanol extract (18mm) than in the ethanol, ethyl acetate and hexane respectively. All the solvent extracts of *O. sanctum* were active against *R. solanacearum*. 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 *O. sanctum* methanolic extract, ethanolic and aqueous extracts was 1024µg ml^{-1} while it was 2048µg ml^{-1} for the ethyl acetate, hexane extracts. The activities of the different solvent extracts are remarkable when compared with the water extracts. Hence, solvent extracts will enhance the efficacy of *O. sanctum* in the activity of *R. solanacearum* infections.

Introduction

Tomato (*Lycopersican esculentum* L.) is a widely consumed, tropical vegetable crop which is an excellent source of vitamin A, vitamin C, iron and phosphorus. In tropical Asia it is a significant cash crop from small farmers (Villareal, 1979). It tops the list of

industrial crops because of its outstanding processing quantities. It is a short duration and high yielding crop, and hence economically important. Its area of cultivation is rising day by day. In India, over 0.498 million hectares is under cultivation with 16.50 million tones production and 17.50 t/ha productivity (2010). The major restraint to tomato production in India is bacterial wilt caused by *R. solanacearum* (Yabuuchi *et al*., 1995). *Ralstonia solanacearum* is a highly varied, complex species which has a large host range of more than 200 species in 50 families (Aliye *et al.,* 2008). It also causes diseases in other economically important crops such as potato, eggplant, chilly and non *solanaceous* crops such as banana and groundnut (Anuratha *et al.,* 1990) proving to be a major constraint in the production of many important vegetables, fruit, and cash crops. The yield loss may vary between 10.8 and 90.6 percent depending on the environmental conditions and the stage at which infection occurs (Kishun, 1987). Bacterial Wilt poses a constant threat to tomato in Karnataka, Madhya Pradesh, Maharashtra and West Bengal in India. Infested soil and water act as primary sources of inoculum. 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). Bacterial wilt is among the most difficult diseases to control (Kucharek, 1998). Although crop rotation with non host crops may suppress soil borne populations of the pathogen (Ahmed *et al.*, 2000), the pathogen survive in the environment in association with weed hosts, which impairs the effect of crop rotation.

Many factors have been reported to be accountable for this with diseases and pests representing major constraints to production. Several authors have reported the contributions of many types of diseases affecting tomato production; however the effect of bacterium wilt has been reported to be the most damaging (Taiwo *et al.,* 2007). *R. solanacearum* affects the plants at their vegetative as well as their reproductive stages (Buddenhagen and Kelman, 1964; Hayward, 1991). The use of chemicals has not been effective in the control of bacterial wilt of tomatoes because the pathogen causing the disease *R. solanacearum* is a soil borne pathogen and is systemic in its action. High pathogen inconsistency, high survival rate in diverse environments and its extremely wide host range renders it difficult to control the disease. Chemical treatments are short lived and less effective. Therefore, the stratagem of biocontrol has evolved itself as a promising approach to create long lasting effect and facilitating sustainable agriculture (Nagorska *et al.,* 2007).

The use of botanicals or non chemical methods however, has a long history in the control of diseases (Secoy and Smith (1983); Calvet *et al.,* (2001) and Pradhanang *et al.,* (2003). Crude extracts from ten different plants were therefore investigated for their antimicrobial activities against *R. solanacearum*. India is a land of biodiversity in terms of plant species. Various plants have been mentioned in Ayurveda, an ancient Indian Sanskrit literature, for their therapeutic advantages (Kaushik, 1988). Plant extracts has been used in the treatment of microbial diseases from ancient time in traditional medical systems. Ability of using most of the medicinal plants for the treatments for various plant diseases may lie in the antioxidant and antimicrobial effect of phytochemicals (Akinmoladun *et al.,* 2007). *Tulasi* (Holy Basil) is a traditional plant considered sacred by the Hindus. This religion links the plant with the Goddess

figure as described in the *Puranas*. Hindus regard it as an earthly manifestation of goddess Vrindavani, who is dear to Lord Vishnu. The name "*Tulasi*" in Sanskrit means "the incomparable one." The *Shyama Tulasi* or Krishna Tulsi (*Ocimum sanctum* L. syn. *Ocimum tenuiflorum*) possesses great medicinal value as mentioned in *Charak Samhita*, an ancient Indian literature. It is a most common household plant in India and grows wild in tropics. Native to India, it is a short lived perennial herb or small shrub of Mint family Labiatae (*Lamiaceae*). It has small leaves with a strong smell and purple flowers. The foliage is green or purple, strongly scented. Oil extracted from leaves of this plant possesses important insecticidal properties (Nanasombat and Lohasupthawee, 2005).

Some of the phytochemicals of medicinal importance present in *Ocimum sanctum* have already been identified (Deshpande *et al.*, 1997). *Ocimum sanctum* as an insecticide, nematicide, fungicide and antimicrobial compound also has been reported (Mishra *et al.*, 2011). However, reports on the antibacterial activity of *O. sanctum* extracts against soil borne pathogens are limited. As such, extract of phytochemicals from *O. sanctum* plant possess useful pharmacological applications. Various insectides and pesticides used for the management of crops pose a risk to human health and cause various side effects. As healthy agricultural crops are very important to produce safe food, it seems necessary to study the prevention of tomatoes infection from these diseases without using chemical agents.

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 need 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 *Ocimum sanctum* plant extracts on the *in vitro* growth and development of *R. solanacearum*.

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 24–48h. 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). *R. solanacearum* were also isolated from a freshly wilted tomato plant by streaking a loopful of flowing ooze containing the bacteria onto sterile TZC agar plates (Danks and Barker, 2000). The suspected colonies were subjected to different colony characteristics, biochemical, physiological, hypersensitive and pathogenicity tests for confirmation of the identity of the pathogen (Vanitha *et al*., 2009; Narasimha Murthy *et al*., 2012).

The identification of the ten selected strains based on pathogenicity was further confirmed by molecular methods based on 16S rRNA sequencing for *R. solanacearum*. NCBI BLAST search was performed and the top hit sequences were multiple aligned and

phylogenetic tree was constructed using CLUSTAL X2 2.1 (Windows version) software by Neighbor Joining (NJ) analysis with 1000 bootstrap replications based on the algorithm (Waterman, 1986). The sequences were deposited to NCBI database.

Collection of Suitable Plant Material

Ocimum sanctum was collected in the month of June of 2013 from semiarid, unshaded land of Jnanabharathi campus, Bangalore University, Bangalore, Karnataka, India (Figure 1). Leaves suitable for extraction were plucked and were washed under running tap water followed by sterilized distilled water. Leaves were air-dried, powdered and were subjected to the following extraction protocols.

Aqueous Extraction

Air-dried powder of *O. sanctum* leaves (15 g) was boiled in 500 ml distilled water till one fourth of the extract, initially taken, was left behind after evaporation. The solution was then filtered using muslin cloth. Filtrate was centrifuged at 5000 rpm for 15 minutes. The supernatant was again filtered using Whatman filter no.1 under strict aseptic conditions and the filtrate was collected in fresh sterilized bottles and stored at 4°C until further use.

Organic Solvent Extraction

Air-dried powder (10g) was thoroughly mixed with 100ml organic solvent *viz*., ethanol, methanol, hexane or ethyl acetate. The mixtures were placed at room temperature for 24h on shaker with 150 rpm. Solution was then filtered through Whatman filter no.1. The filtrate thus obtained was concentrated by complete evaporation of solvent at room temperature to yield the pure extract. Stock solutions of various organic crude extracts were prepared by mixing well the appropriate amounts of dried extracts and suitable solvent to give rise a final concentration of 100 mg/ml. Each solution was stored at 4°C after collecting in sterilized screw cap tubes until further use (Pankaj and Purshotam, 2011).

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) (Kelman, 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* of all aqueous and organic extracts from dried leaves of *O. sanctum* plant were 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 leaves extract was added in the respective well and the plates were incubated at 28 ± 2°C for 24–48 h. Organic solvents, in which extracts were prepared, were used as negative control while Streptomycin antibiotic of one unit strength was used as positive control. 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 respective extract at the end of incubation period.

Determination of Minimum Inhibitory Concentration

Extracts producing an inhibition zone ≥15 mm in diameter were screened to determine 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 8µg/ml to 4096 µg/ml. 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^oC for 24h incubation. Following incubation, the MIC was calculated as the lowest concentration of the extract inhibiting the visible growth of bacterial strain.

Results and Discussion

Isolation and identification of *R. solanacearum*

A total of 50 *R. solanacearum* isolates were obtained from the wilted tomato plant samples collected from different locations during field survey. Virulent isolates grown on TZC medium were highly fluidal, white coloured with a light pink centre and round to irregular margin. Microscopic studies revealed that bacterial isolates were Gram negative, rod shaped, non spore forming, strictly aerobic bacteria 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, *R. solanacearum* strains were grouped into highly pathogenic, moderately pathogenic, weakly pathogenic and avirulent.

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 different *O. sanctum* extracts against ten *R. solanacearum* pathogens, were studied. Results of the study are shown in the Table 1. According to the results, all different types of extracts obtained from *O. sanctum* leaves showed the antagonistic activity against all tested *R. solanacearum* strains. Data indicated that the most active extract was methanol which had inhibitory activity against ten *R. solanacearum* in the range of 9 mm to 18 mm zone of inhibition, Ethanol extract was inhibited range of 6 mm to 15 mm, Ethyl acetate extract was inhibited range of 7 mm to 13 mm and Hexane extract was inhibited range of 5 mm to 12mm zone

of inhibition zone (Table 1).

Minimum inhibitory concentrations of different active extracts from leaves of *O. sanctum* had been demonstrated in Table 2–7, *R. solanacearum* were inhibited at 1024μg ml^{-1} by methanol extract, ethanol extract, aqueous extracts and antibiotic while *R. solanacearum* was inhibited at 2048μg ml^{-1} concentration of ethyl acetate and hexane extracts Table 2–7.

According to the results, all different types of extracts obtained from *Ocimum sanctum* leaves have shown antagonistic activity against all tested *R. solanacearum*. The antibacterial effect of crude medicinal plant extract of *Ocimum gratisimum*, *Brassica oleracae* and *Ipomoea batatas* on *Ralstonia solanacearum* are also reported (Wagura, 2011). The antibacterial activity of *Ralstonia* with plant extracts have been reported earlier (Lopez *et al.,* 2005; Larkin and Griffins, 2007).

The plant world is a rich storehouse of natural chemicals that can be exploited for use as pesticides. The total number of plant chemicals may exceed 4,000,000 and of these 10,000 are reported are found to be secondary metabolites whose major role in plants is defensive (Grayer and Harborne, 1994). Higher plants are much more important in the production of economically important organic compounds, pharmaceuticals and pesticides (Hostettman and Wolfender, 1997). Many species of higher plants have not been described, are much less surveyed for chemical or biologically active constituents and new sources of commercially valuable pesticides (Verma and Dubey, 1999). This is mainly due to lack of information on the screening/evaluation of diverse plants for their antibacterial activity.

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). Ji *et al.* (2007) has also reported the use of plant derived volatile compound thymol to suppress bacterial wilt of tomatoes as part of an integrated management package for the disease. The extracts investigated in this study are from plants that are locally available and environmentally friendly.

Ethanol, Ethyl acetate, Methanol, Hexane solvents and aqueous extracts of the leaf were subjected to a preliminary test of antimicrobial activities against phytopathogenic bacteria, *R. solanacearum.* It is clear from the present results that all the extracts exhibited pronounced activities against the tested bacteria. *In vitro* antibacterial activities against some pathogenic bacteria have been reported by Alam *et al.* (2008). The antibacterial activities of *Ocimum sanctum* leaf extract were promising. In *Ocimum sanctum* extracts, the growth of *R. solanacearum* maximum inhibited by Methanol, this tends to show that the active ingredients of these plant leaves are better extracted with the Methanol solvent than all other solvents. High activity of *Ocimum sanctum* extracts against *R. solanacearum* was achieved in Methanol followed by ethyl alcohol, Ethyl acetate and hexane. In general, the activities against the *R. solanacearum* used have shown good activity when compared with standard antibiotic.

Table.1 *In vitro* antagonistic activity of aqueous and organic extracts of *Ocimum sanctum* leaves against *R. solanacearum*

Type of Extract		Zone of Inhibition in mm									
		RS1	RS2	RS3	RS4	RS5	RS6	RS7	RS8	RS9	RS10
Organic Extract	Methanol	13.5±0.3	14.0±0.4	10.25±0.4	9.39±0.6	17.52±0.4	11.0±0.8	14.21±0.7	12.0±0.5	10.56±0.7	13.54±0.8
	Ethanol	7.9±0.2	9.56±0.5	11.34±0.5	14.92±0.7	9.65±0.5	12.0±0.6	10.56±0.5	8.65±0.6	6.78±0.3	13.0±0.4
	Ethyl Acetate	8.65±0.6	10.50±0.4	11.62±0.2	9.87±0.3	8.86±0.6	12.33±0.4	11.54±0.8	7.89±0.6	9.46±0.4	11.34±0.7
	Hexane	7.89±0.01	5.9. ±0.5	9.56±0.3	11.72±0.3	10.46±0.4	8.21±0.3	12.0±0.8	10.54±0.9	9.45±0.6	8.34±0.7
Aqueous Extract		12.45±0.1	11.21±0.6	10.33±0.2	12.86±0.6	9.21±0.5	10.5±0.4	12.46±0.9	10.25±0.7	9.76±0.4	9.89±0.3
	Streptomycin	24.65±1.2	27.33±1.6	23.56±1.9	26.5±1.3	24.17±1.7	22.54±1.1.	27.46±1.6	26.62±1.9	21.56±1.5	23.21±1.8
Controls	Methanol	4.21±0.4	5.72±0.7	3.0±0.03	7.5±0.2	5.32±0.1	4.21±0.5	3.92±0.2	4.56±0.3	3.66±0.1	5.33±0.4
	Ethanol	4.24±0.5	5.64±0.6	3.33±0.01	4.32±0.05	3.21±0.08	4.62±0.07	2.33±0.01	3.65±0.01	5.72±0.4	4.36±0.3
	Ethyl Acetate	2.3±0.03	4.54±0.2	5.76±0.06	3.56±0.1	4.96±0.08	3.54±0.1	2.89±0.02	5.65±02.	4.71±0.2	3.24±0.4
	Hexane	4.73±0.05	3.54±0.1	5.50±0.1	2.66±0.02	1.29±0.01	2.56±0.01	4.33±0.09	3.32±0.01	5.42±0.04	4.19±0.08

Values are presented as mean ± S.E. of triplicate experiments. + = Growth
*Mean of three values ± Standard Deviation.

Table.2 Minimum inhibitory concentration of active crude ethanol extracts of O*cimum sanctum* leaves against *R. solanacearum*

Type of Active Crude Extracts	*R. solana cearum*	Concentration of Extracts (in µg ml^{-1})										MIC (in µg ml^{-1})
		4096	2048	1024	512	256	128	64	32	16	8	
Ethanol	RS1	-	-	+	+	+	+	+	+	+	+	2048
	RS2	-	+	+	+	+	+	+	+	+	+	4096
	RS3	-	+	+	+	+	+	+	+	+	+	4096
	RS4	+	+	+	+	+	+	+	+	+	+	>4096
	RS5	-	-	+	+	+	+	+	+	+	+	2048
	RS6	-	-	-	+	+	+	+	+	+	+	1024
	RS7	-	-	-	+	+	+	+	+	+	+	1024
	RS8	-	-	+	+	+	+	+	+	+	+	2048
	RS9	-	-	+	+	+	+	+	+	+	+	2048
	RS10	-	+	+	+	+	+	+	+	+	+	4096

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

Table.3 Minimum inhibitory concentration of active crude Methanol extracts of O*cimum sanctum* leaves against *R. solanacearum*

Type of Active Crude Extracts	*R. solanacearum*	Concentration of Extracts (in µg ml^{-1})										MIC (in µg ml^{-1})
		4096	2048	1024	512	256	128	64	32	16	8	
Methanol	RS1	-	-	+	+	+	+	+	+	+	+	2048
	RS2	-	-	+	+	+	+	+	+	+	+	2048
	RS3	-	-	+	+	+	+	+	+	+	+	2048
	RS4	-	+	+	+	+	+	+	+	+	+	4096
	RS5	-	-	-	+	+	+	+	+	+	+	512
	RS6	-	-	+	+	+	+	+	+	+	+	2048
	RS7	-	-	-	+	+	+	+	+	+	+	1024
	RS8	-	-	+	+	+	+	+	+	+	+	2048
	RS9	-	-	-	+	+	+	+	+	+	+	1024
	RS10	-	-	+	+	+	+	+	+	+	+	2048

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

Table.4 Minimum inhibitory concentration of active crude Ethyl acetate extracts of O*cimum sanctum* leaves against *R. solanacearum*

Type of Active Crude Extracts	*R. solanacearum*	Concentration of Extracts (in µg ml^{-1})										MIC (in µg ml^{-1})
		4096	2048	1024	512	256	128	64	32	16	8	
Ethyl acetate	RS1	+	+	+	+	+	+	+	+	+	+	4096
	RS2	-	+	+	+	+	+	+	+	+	+	4096
	RS3	-	+	+	+	+	+	+	+	+	+	4096
	RS4	-	+	+	+	+	+	+	+	+	+	4096
	RS5	-	-	+	+	+	+	+	+	+	+	2048
	RS6	+	+	+	+	+	+	+	+	+	+	>4096
	RS7	-	-	+	+	+	+	+	+	+	+	2048
	RS8	+	+	+	+	+	+	+	+	+	+	>4096
	RS9	-	-	+	+	+	+	+	+	+	+	2048
	RS10	-	-	+	+	+	+	+	+	+	+	2048

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

Table.5 Minimum inhibitory concentration of active crude hexane extracts of *Ocimum sanctum* leaves against *R. solanacearum*

Type of Active Crude Extracts	*R. solanacearum*	Concentration of Extracts (in µg ml^{-1})										MIC (in µg ml^{-1})
		4096	2048	1024	512	256	128	64	32	16	8	
Hexane	RS1	-	+	+	+	+	+	+	+	+	+	4096
	RS2	-	-	+	+	+	+	+	+	+	+	2048
	RS3	-	-	+	+	+	+	+	+	+	+	2048
	RS4	-	+	+	+	+	+	+	+	+	+	4096
	RS5	+	+	+	+	+	+	+	+	+	+	>4096
	RS6	-	-	+	+	+	+	+	+	+	+	2048
	RS7	-	+	+	+	+	+	+	+	+	+	4096
	RS8	-	-	+	+	+	+	+	+	+	+	2048
	RS9	-	-	+	+	+	+	+	+	+	+	2048
	RS10	-	+	+	+	+	+	+	+	+	+	4096

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

Table.6 Minimum inhibitory concentration of Aqueous extracts of O*cimum sanctum* leaves against *R. solanacearum*

Type of Active Crude Extracts	*R. solanacearum*	Concentration of Extracts (in µg ml^{-1})										MIC (in µg ml^{-1})
		4096	2048	1024	512	256	128	64	32	16	8	
Aqueous	RS1	-	-	-	+	+	+	+	+	+	+	1024
	RS2	-	+	+	+	+	+	+	+	+	+	4096
	RS3	-	-	+	+	+	+	+	+	+	+	2048
	RS4	-	+	+	+	+	+	+	+	+	+	4096
	RS5	-	+	+	+	+	+	+	+	+	+	4096
	RS6	-	-	+	+	+	+	+	+	+	+	2048
	RS7	-	-	-	+	+	+	+	+	+	+	1024
	RS8	-	+	+	+	+	+	+	+	+	+	4096
	RS9	-	-	+	+	+	+	+	+	+	+	2048
	RS10	-	+	+	+	+	+	+	+	+	+	4096

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

Table.7 Minimum inhibitory concentration of antibiotic against *R. solanacearum*

Type of Active Crude Extracts	*R. solanacearum*	Concentration of Extracts (in µg ml^{-1})										MIC (in µg ml^{-1})
		4096	2048	1024	512	256	128	64	32	16	8	
Streptomycin	RS1	-	-	-	-	-	-	-	-	-	-	<8
	RS2	-	-	-	-	-	-	-	-	-	-	<8
	RS3	-	-	-	-	-	-	-	-	-	-	<8
	RS4	-	-	-	-	-	-	-	-	-	-	<8
	RS5	-	-	-	-	-	-	-	-	-	-	<8
	RS6	-	-	-	-	-	-	-	-	-	-	<8
	RS7	-	-	-	-	-	-	-	-	-	-	<8
	RS8	-	-	-	-	-	-	-	-	-	-	<8
	RS9	-	-	-	-	-	-	-	-	-	-	<8
	RS10	-	-	-	-	-	-	-	-	-	-	<8

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

High activity was found in extracts from leaves of *Ocimum sanctum* against *R. solanacearum.*

R. solanacearum were inhibited at minimum inhibitory concentration (MIC) of 1024µg ml^{-1} by methanol extract, ethanol extract and aqueous extracts while *R. solanacearum* was inhibited at MIC of 2048µg ml^{-1} concentration of ethyl acetate, hexane extracts. It was evident that the use of *Ocimum sanctum* solvent extracts has a potential to substitute the antibiotics for pathogen infection control. This kind of biological control 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.

As per the results of the present study, Ocimum extract has shown antimicrobial properties against *R. solanacearum*. The results of the present investigation are an important step towards isolation and characterization of the antibacterial agent against the *R. solanacearum* and its further evaluation for crop protection strategies. The solvent extracts will enhance the efficacy of phytochemicals in the control of *R. solanacearum* infections. Bacterial wilt of tomato caused by *R. solanacearum* is a systemic disease that cannot be efficiently control with foliar application of chemical pesticides. The traditional use of plants in the control of diseases dates to ancient times and presents no potential toxicity. The results from the present findings 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*. 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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