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Evaluation of *Carica papaya* Leaf Extracts for their Efficacy on Control of Bacterial Wilt of Tomato caused by *Ralstonia solanacearum*

K. Narasimha Murthy[1], K. Soumya[3*], C. Srinivas[2] and S.R. Niranjana[1]

[1]Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore –570 006, Karnataka, India
[2]Department of Microbiology and Biotechnology, Jnanabharathi Campus, Bangalore University, Bangalore- 560 056, India
[3]Department of Microbiology, Field Marshal K. M. Cariappa College, A Constituent College of Mangalore University, Madikeri – 571201, Karnataka, India

**Corresponding author*

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A B S T R A C T

Management of bacterial wilt is very difficult as there are no efficient curative chemicals. *Carica papaya* leaf extract was evaluated their antimicrobial activity against *Ralstonia solanacearum.* The zone of inhibitions showed against ten *R. solanacearum* at range of 5.96mm to 15mm of different solvent extracts like aqueous, ethanol, ethyl acetate, hexane, and chloroform. The MIC of methanol at 512 µg/ml, ethanol at 2048 µg/ml, ethyl acetate at 1024 µg/ml, hexane at 1024 µg/ml, chloroform at 1024 µg/ml, aqueous at 2048 µg/ml and streptomycin at <8 µg/ml. The seed treatment with *C. papaya* leaf extracts increased the seed germination and vigor index (1218.61) when compared to control (1152.69). Under greenhouse conditions plants treatments with *C. papaya* extracts were increased plant growth and decreased wilt incidence about 42.29-52.14%. In field study the reduction of wilt by *C. papaya* leaf extracts at 100mg/ml concentration. *C. papaya* leaf extracts increased the yield by 15.08% (1.3t/ha) and decreased the wilt incidence by 52.14%.

Introduction

Plant diseases caused by different fungal and bacterial pathogens are the major constraints of tomato production (Jones *et al.*, 1991). Bacterial wilt caused by *Ralstonia solanacearum* is a destructive disease in the production of tomatoes (Ji *et al.*, 2005). This *R. solanacearum* belongs to the Betaproteobacteria, is accountable for bacterial wilt on more than 200 plant species from 50 botanical families, including impartment crops such as tomato, potato, pepper, eggplant, banana, and tobacco (Aliye *et al.*, 2008). The direct yields losses of tomato vary between by *R. solanacearum*

vary widely 0 to 91% (Elphinstone, 2005) and 10.8 to 90.6% depending on the environmental conditions (Kishun, 1987). Bacterial Wilt poses a continuous danger to tomato in Karnataka, Kerala, Maharashtra, Odisha, Jharkhand, Goa, West Bengal, Himachal Pradesh, Jammu and Kashmir, Uttarakhand and Northeastern states in India (Singh *et al.,* 2016). *R. solanacearum* inhabits the vascular tissue of its host plants. The *R. solanacearum* in general invades host roots from primary sources of inoculum through soil, wounds or natural openings at the site of secondary roots emerge (Hayward 1991; Pradhanang *et al.,* 2005). *R. solanacearum* colonizes in the root cortex and vascular tissues and finally enters the xylem vessels and spreads areal parts of the host. After the pathogen colonized the xylem, a large number of bacterial cells and blocking the water movement into upper parts of the plant. Affected plants suffer chlorosis, stunting, wilting, and usually die rapidly.

Bacterial wilt disease is most difficult to control and the effectiveness of present strategies for control of this disease is inadequate. No conventional bactericides are known to provide successful management of this *R. solanacearum* pathogen (Ahmed *et al.,* 2000; Williamson *et al.,* 2002). Management in chemical pesticides is usually considered as the most efficient and fastest approach for phytopathogens control however, there is no effective chemical product is available for control of bacterial wilt. *In vitro* and *in vivo* investigations by some investigators have established the potential antimicrobials from some plant species (El-Ariqi, 2005). In a challenge to change this situation, some alternative techniques of control have been adopted. Within this situation is the usage of plant extracts which are natural sources of antimicrobial compounds, regarded as environmental safe and biodegradation by natural soil microorganisms; there is no any health residual or environmental problems at any type of concentration of plant extracts used but effective against plant pathogens (Shivpuri *et al.,* 1997; Yang *et al.,* 2010). Usage of the majority medicinal plants for the management for various plant diseases in the activity of antimicrobial effect of phytochemical components (Akinmoladun *et al.,* 2007). Recent investigations the use of plant extracts have innovative move toward to management of phytopathogenic diseases. Plant extracts are regarded as constituents in pest management programmes (Belabid *et al.,* 2010). Compared to the synthetic drugs, antimicrobials of plant source are not associated with many side effects and have massive potential against many infectious pathogens (Barbour *et al.,* 2004). The objective of this work was to evaluate the effect of papaya leaf extracts for controlling wilt disease of tomato caused by *R. solanacearum* under *in vitro* and *in vivo* conditions.

Materials and Methods

Plant material preparation

Fresh leaves of *C. papaya* were collected from Bangalore, Karnataka and the collected dust free leaves were allowed to dry under shade at room temperature. These dried leaves were mechanically powdered and stored in an airtight container and these powdered materials were used for further analysis.

Preparation of leaves extracts of *Carica papaya*

Aqueous extraction

Ten grams of air dried *C. papaya* leaves powder was extracted in 500ml of distilled water with slow heat and it was filtered through muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was

collected and filtered through Whatman filter No.1. The extract was autoclaved at 121°C with 15 lbs pressure and stored at 4 °C until further use.

Solvent extraction

Ten grams of air dried *C. papaya* powder was extracted with 100ml of solvents like methanol, ethanol, ethyl acetate, hexane and chloroform kept on a rotary shaker for 150 rpm for 24 h at room temperature. Subsequently, it was filtered through Whatman filter No.1 and centrifuged at 5000 rpm for 15 min. The supernatant was collected and solvent was evaporated to make the final volume one fifth of the original volume and final concentration is 100mg/ml. It was stored at 4°C in airtight bottles for further studies (Pankaj and Purshotam, 2011).

Isolation and identification of *R. solanacearum*

The wilted tomato and soil samples were collected from the field survey brought to the laboratory. Collected rhizosphere soil and plant materials were plated onto 2, 3, 5 Triphenyl tetrazolium chloride (TZC) medium (Kelman, 1954) and incubated at 28 ± 2 ^{o}C for 24–48 h. Characterizations of isolated pathogens were carried out by subjected to various biochemical, biovar, physiological, hypersensitive and pathogenicity tests (Narasimha Murthy *et al.*, 2012). The molecular identification based on 16S rRNA sequencing for *R. solanacearum* and phylogenetic tree was constructed (Waterman, 1986) and the sequences were deposited to NCBI database.

Preparation of bacterial inoculum

Inoculum of *R. solanacearum* was prepared by growing cells of the bacterium on CPG broth (1 g Casamino acids, 10 g peptone, 5 g glucose in one liter distilled water) for 48 hours at 28 °C and 150 rpm on rotary shaker (Kleman, 1954). The bacterium cells were centrifuged at 12,000 rpm for 10 min at 4°C. The pellet was mixed with distilled water and bacterial suspensions were adjusted to 0.45 at A610 nm using UV– visible spectrophotometer to obtain the concentration approximately1×10^{8} colony forming unit (CFU/ml) (Ran *et al.*, 2005).

Antibacterial activity of extracts against *R. solanacearum*

Extracts of *C. papaya* antagonistic against *R. solanacearum* by agar well diffusion method (Shrisha *et al.*, 2011). Petriplates containing 20 ml of tryptone soya agar medium, seeded with 100 µl *R. solanacearum* inoculum, the media was allowed to solidify and wells were prepared in plates with the help of a sterilized cork borer. 100 µl of the extracts were introduced into the wells and plates were kept at 2–3 h for to allow the diffusion of extracts and incubated at 28 ± 2 °C for 24–48 h. The pure solvents in equal volume served as negative control and Streptomycin antibiotic disc (30 µg) was used as positive control. After incubation the diameter of the zone of inhibition was measured in mm. The experiments were conducted in triplicate under aseptic conditions.

Detection of minimum inhibitory concentration (MIC)

The micro plate dilution method was used to determine the MIC values for *C. papaya* leaves extracts with antibacterial activity. This test was performed in sterile 96–well microtitre plates. For the evaluation of the active plant extract, diluting the various concentrations ranging from 8µg/ml to 4096 µg/ml were prepared and final concentration of *R. solanacearum* was 1×10^{8}cfu/ml. The wells were filled with 50 µl of respective

solvent and 100 µl of the *C. papaya* extracts were added to the wells by serial two fold dilution and streptomycin antibiotic was used as positive control. The plates were incubated at 28 ± 2 °C for 24 h, after incubation the MIC was determined as the lowest concentration of plant extracts that exhibited no visible growth of the *R. solanacearum* in the wells by visual reading when compared with the control (Mazzanti *et al.,* 2000).

Effect of *C. papaya* leaf extracts on tomato seed germination and seedling vigor index

The effect of *C. papaya* leaf extracts on seed germination and vigor index of tomato seedlings were evaluated under laboratory conditions. The germination tests for fresh *R. solanacearum* inoculum and *C. papaya* leaf extracts were carried out according to the paper towel method (ISTA, 2005). The vigor index was calculated by using the formula VI = (mean root length + mean shoot length) × Germination percentage (Abdul Baki and Anderson, 1973). The experiment was conducted with four replicates of hundred seeds each and the entire experiment was repeated thrice.

Effect of *C. papaya* extracts on bacterial wilt incidence in tomato under greenhouse conditions

This experiment was performed in a greenhouse conditions, with the climatic conditions were maintained an average relative humidity of 80%, in darkness and 30 to 26±2 °C temperature regime (Neelu Singh *et al.,* 2012). Pots were filled with sterilized potting soil (soil, sand and coconut pith compost) and 50 ml of sterile water was added to each pot. The soil from each pot was then infested by adding 10ml of the *R. solanacearum* inoculum solution at 1×10^8 CFU/ml to obtain a final estimated population of 2.5×10^5 CFU/g of dry soil. Twenty days old tomato seedlings were transplanted five per pot and each plant was watered daily with 30 ml of sterile distilled water. The *R. solanacearum* infested pots were applied by soil drenching with 50 ml of *C. papaya* extracts concentration at 100mg/ml and controls received the same amount of sterile water. The wilt susceptible tomato cultivar Arka Meghali was used to assess the wilt incidence. For each treatment, the experiments have been repeated three times. After 30 days of transplanting, wilted tomato plants were sampled for isolation of *R. solanacearum* on modified TZC agar medium. Presumptive colonies of *R. solanacearum* were confirmed by biochemical and molecular characteristics (Deberdt *et al.,* 2012; Narasimha Murthy and Srinivas 2012). The plants including the roots were harvested from the pots and fresh weight, dry weight, mean shoot length, mean root length and disease incidence were measured to determine the effects of *C. papaya* extracts on plant growth. Treated plants were counted and uprooted separately and their weights recorded to measure growth promotion, compared with the untreated control (Lim and Kim 1997). Wilt incidence was recorded using the formula

$$\text{Percent wilt incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Effect of *C. papaya* extracts on bacterial wilt incidence in tomato under field conditions

The field trials were conducted at the farmer's plot near Chintamani, Karnataka, India during growing seasons. The individual field plots area was 25 m^2 containing fourteen rows with 100–120 seedlings per row and distance between rows were 50 cm. The field was maintained based on the tomato growing conditions (Narasimha Murthy *et al.,* 2016).

The treatment of leaf extracts was carried out like in greenhouse experiments. Wilt symptoms was recorded 7 days after pathogen inoculation. Disease incidence was calculated as described the earlier. Three plots were used as replications for each treatment as well as for the untreated control treatment. Field trials were repeated twice. The number of wilted plants in each treatment including the untreated control was continuously recorded up to 90 days after challenge inoculation and plant height, fresh weight, fruits per plants were calculated. At the time of harvest, ten plants from each replication were harvested to evaluate the total yield of each treatment as tons per hectare (t/ha).

Results and Discussion

Isolation and identification of *R. solanacearum*

Pink centers with white fluid colonies were selected and 50 isolates of *R. solanacearum* were isolated and identified (Figure 1). Microscopic studies the *R. solanacearum* was Gram negative, rod shaped and characterization of different physiological and biochemical tests. The molecular identification of *R. solanacearum* was confirmed by 16S rRNA gene sequencing (Narasimha Murthy *et al.*, 2012).

Antibacterial activity against *R. solanacearum*

Antibacterial activity of *C. papaya* extracts against ten highly virulent *R. solanacearum* was conducted. According to the results, *C. papaya* extracts showed the antibacterial activity against *R. solanacearum* isolates (Figure 2). Aqueous and solvent extracts were showed the zone of inhibition range of 9.57 to 11.82mm, 10.27 to 15.34mm, 6.78 to 11.33mm, 6.43 to 10.63mm, 7.33 to 11.17mm, 6.43 to 9.57mm, and 15 to 20mm of different solvent extracts that is aqueous, ethanol, ethyl acetate, hexane, chloroform and streptomycin respectively (Table 1).

Minimum Inhibitory Concentration

Minimum inhibitory concentrations of different *C. papaya* solvent extracts were demonstrated against *R. solanacearum.* The minimum inhibited extracts of Methanol at 512 µg/ml, Ethanol at 2048 µg/ml, Ethyl acetate at 1024 µg/ml, Hexane at 1024 µg/ml, Chloroform at 1024 µg/ml, Aqueous at 2048 µg/ml and Streptomycin at <8µg/ml (Table 2).

Effect of *Carica papaya* extract on tomato seed germination and seedling vigor index

Carica papaya extract treated seeds were increased germination and seedling vigor index as compared to control and decrease the germination with *R. solanacearum* inoculation. The extracts showed extensively higher mean root length, mean shoot length and vigor index with compared to control (Figure 3A; Table 3).

Effect of *C. papaya* extracts on bacterial wilt incidence in tomato under greenhouse conditions

The reduction in disease incidence on tomato treated with *C. papaya* extracts at 100mg/ml concentrations in a growth chamber. The leaf extract treatment increased growth promotion as compared to the control. The treatment increased fresh weight, dry weight, shoots length, root length and reduced the wilt incidence in leaf extract treated seedlings. The disease incidence was decreased around 42.29-52.14% in plants treated with leaf extracts by soil drench method (Figure 3B; Table 4). The activity of *C. papaya* leaf extracts may be essential in the potential phytochemical compounds and leaf extract percentage, the period of pretreatment

determine efficiency for wilt control, as revealed in our research.

Effect of *C. papaya* extracts on bacterial wilt incidence in tomato under field conditions

The efficacy of *C. papaya* leaf extracts were revealed in the tomato fruit yield produced tabulated in Table 5. The control plot was yielded an average of 7.32 t/ha and *R. solanacearum* treated plot was yielded an average of 1.28 to 1.69 t/ha. Seedlings treated with leaf extract alone plot yielded an average of 8.62t/ha. As compared to the control plot, *C. papaya* leaf extract increased the tomato yield by 15.08% (1.3t/ha). Seedlings combined with *R. solanacearum* and leaf extract produces yielded an average of 5.95t/ha. As compared to pathogen treated plot (RS71.28 t/ha), leaf extract treated plot (8.62 t/ha) was increased yield by 85.15% (4.26t/ha). Tomato seedlings treated with leaf extract infected plot reduced the wilt incidence by 49.68% under field conditions as compared to pathogen treated plot (84.54% from RS2 infected plot). The *C. papaya* leaf extracts were found to be active in the management of bacterial wilt of tomato as chemical replacement.

Plants are the cheaper and safer preference sources of antimicrobials (Doughari *et al.*, 2007). The aqueous and solvent extracts investigated phytochemical screening from leaf extracts *C. papaya* was used to study the presence of alkaloids, flavonoids, terpenoids, glycosides, saponins, steroids, phenols, tannins, proteins, anthocyanins, anthocyanins and coumarins. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against phytopathogens. The antibacterial activity of plant extracts on *R. solanacearum* has been studied earlier (Larkin *et al.*, 2007). However, all the phytoconstituents were more in the solvent extraction than the aqueous as indicated by the intensity of the different confirmatory colors. This result can be attested to the work of Sikanda *et al.,* (2013) who also studied like finding and stated the effect of these phytochemical as a good antimicrobial agent on different test pathogens. In the present study, the leaf extracts of *C. papaya* was prepared using aqueous and solvent extraction method. Peter *et al.,* (2014) studied the leaf and root extracts of *C. papaya*, this research indicated that papaya leaves have potential natural antibacterial compounds.

In the ethanol extracts demonstrated a higher activity compared than the other solvents and aqueous extracts in *C. papaya* leaf samples (Uwah *et al.,* 2013). Doughari *et al.,* (2007) stated that the antimicrobial effect of this plant might be due to the bioactive compounds such as the phytochemical constituent present in the plant. The result further showed that the dry sample was effective against both Gram positive and Gram-negative bacteria while the fresh sample was more effective against Gram-negative bacteria (Okunola *et al.,* 2012).

In the antibacterial activity assay, the zone of inhibition at different range from solvent aqueous extracts. Anibijuwon and Udeze (2009) deliberated that the leaf and root of *C. papaya* using water and organic solvents were highest activity against *P. aeruginosa* and our study showed similar results in antibacterial activity against *R. solanacearum.* Antibacterial activity against *R. solanacearum* was found in high from *C. papaya* powder extracts against the bacterial wilt pathogen, MICs of solvent extracts were methanol at 512 µg/ml, ethanol at 2048 µg/ml, ethyl acetate at 1024 µg/ml, hexane at 1024 µg/ml, chloroform at 1024 µg/ml, aqueous at 2048 µg/ml and streptomycin at <8 µg/ml.

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

Type of Extracts		Zone of inhibition in mm									
		RS1	**RS2**	**RS3**	**RS4**	**RS5**	**RS6**	**RS7**	**RS8**	**RS9**	**RS10**
Solvent Extract	Methanol	12.66±0.5	15.34±0.2	11.52±0.7	10.27±0.3	13.45±0.5	12.33±0.6	13.73±0.4	13.79±0.2	12.43±0.5	11.25±0.4
	Ethanol	8.35±0.3	9.23±0.3	9.66±0.8	11.33±0.4	8.42±0.6	10.26±0.7	9.33±0.4	8.55±0.6	10.78±0.3	11.66±0.5
	Ethyl acetate	8.66±0.68	9.57±0.5	9.82±0.6	7.57±0.4	9.43±0.6	10.63±0.8	9.66±0.7	7.78±0.5	8.43±0.7	10.57±0.9
	Hexane	8.32±0.2	9.33 ±0.6	8.57±0.8	9.65±0.7	11.17±0.9	9.37±0.5	10.4±0.6	8.46±0.7	8.66±0.5	9.72±0.5
	Chloroform	9.57±0.5	8.43 ±0.7	9.32±0.5	8.57±0.4	9.57±0.5	8.12±0.8	9.37±0.8	8.28±0.5	9.57±0.7	8.32±0.8
Aqueous Extract		7.57±0.9	6.55±0.3	6.66±0.9	5.96±0.5	7.89±0.7	7.57±0.6	6.66±0.6	7.47±0.9	6.82±0.6	7.48±0.7
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
Solvent Control	Methanol	5.45±0.8	4.47±0.8	4.57±0.3	6.22±0.7	4.66±0.4	5.67±0.3	4.56±0.4	4.76±0.5	4.33±0.4	4.57±0.5
	Ethanol	4.56±0.3	5.66±0.4	4.21±0.2	4.56±0.1	3.45±0.2	4.66±0.2	3.45±0.1	3.43±0.1	5.57±0.3	4.33±0.5
	Ethyl acetate	2.33±0.05	3.66±0.3	4.66±0.1	3.57±0.1	4.78±0.2	4.33±0.1	3.31±0.2	5.66±0.2	4.57±0.3	4.12±0.2
	Hexane	3.89±0.1	4.32±0.2	5.67±0.2	2.66±0.1	2.37±0.09	2.21±0.02	3.97±0.2	4.21±0.2	4.57±0.2	4.45±0.1
	Chloroform	4.21±0.2	3.43±0.2	4.57±0.1	3.33±0.1	2.98±0.08	2.76±0.06	3.21±0.1	3.33±0.2	3.66±0.3	3.98±0.2

Values are presented as mean ± Standard errors of triplicate experiments. Mean of three values ± Standard Deviation. RS- *R. solanacearum*

Table.2 Minimum inhibitory concentrations of different extracts of *Carica papaya* against *R. solanacearum*

R. solanacearum	Extracts	Concentration (µg/ml)									
		4096	2048	1024	512	256	128	64	32	16	8
RS1	Ethanol	-	-	+	+	+	+	+	+	+	+
	Methanol	-	-	+	+	+	+	+	+	+	+
	Ethyl acetate	-	+	+	+	+	+	+	+	+	+
	Hexane	-	+	+	+	+	+	+	+	+	+
	Chloroform	-	-	-	+	+	+	+	+	+	+
	Aqueous	-	-	+	+	+	+	+	+	+	+
RS2	Ethanol	-	-	+	+	+	+	+	+	+	+
	Methanol	-	-	-	-	+	+	+	+	+	+
	Ethyl acetate	-	+	+	+	+	+	+	+	+	+
	Hexane	-	-	-	+	+	+	+	+	+	+
	Chloroform	-	-	+	+	+	+	+	+	+	+
	Aqueous	-	+	+	+	+	+	+	+	+	+
RS3	Ethanol	-	+	+	+	+	+	+	+	+	+
	Methanol	-	-	+	+	+	+	+	+	+	+
	Ethyl acetate	-	+	+	+	+	+	+	+	+	+
	Hexane	-	-	+	+	+	+	+	+	+	+
	Chloroform	-	-	-	+	+	+	+	+	+	+
	Aqueous	+	+	+	+	+	+	+	+	+	+
RS4	Ethanol	+	+	+	+	+	+	+	+	+	+
	Methanol	-	+	+	+	+	+	+	+	+	+
	Ethyl acetate	-	-	+	+	+	+	+	+	+	+
	Hexane	-	-	+	+	+	+	+	+	+	+
	Chloroform	-	-	+	+	+	+	+	+	+	+
	Aqueous	-	+	+	+	+	+	+	+	+	+
RS5	Ethanol	-	-	+	+	+	+	+	+	+	+
	Methanol	-	-	-	-	+	+	+	+	+	+
	Ethyl acetate	-	-	-	+	+	+	+	+	+	+
	Hexane	-	+	+	+	+	+	+	+	+	+
	Chloroform	-	-	-	+	+	+	+	+	+	+
	Aqueous	-	+	+	+	+	+	+	+	+	+
RS6	Ethanol	-	-	+	+	+	+	+	+	+	+
	Methanol	-	-	+	+	+	+	+	+	+	+
	Ethyl acetate	-	-	+	+	+	+	+	+	+	+
	Hexane	-	-	-	+	+	+	+	+	+	+
	Chloroform	-	-	-	+	+	+	+	+	+	+
	Aqueous	-	+	+	+	+	+	+	+	+	+
RS7	Ethanol	-	-	+	+	+	+	+	+	+	+
	Methanol	-	-	-	-	+	+	+	+	+	+
	Ethyl acetate	-	-	-	+	+	+	+	+	+	+
	Hexane	-	+	+	+	+	+	+	+	+	+
	Chloroform	-	+	+	+	+	+	+	+	+	+
	Aqueous	-	-	+	+	+	+	+	+	+	+

RS8	Ethanol	-	-	+	+	+	+	+	+	+	+
	Methanol	-	-	-	+	+	+	+	+	+	+
	Ethyl acetate	-	-	+	+	+	+	+	+	+	+
	Hexane	-	-	+	+	+	+	+	+	+	+
	Chloroform	-	-	+	+	+	+	+	+	+	+
	Aqueous	-	+	+	+	+	+	+	+	+	+
RS9	Ethanol	-	-	+	+	+	+	+	+	+	+
	Methanol	-	-	-	-	+	+	+	+	+	+
	Ethyl acetate	-	-	+	+	+	+	+	+	+	+
	Hexane	-	-	-	+	+	+	+	+	+	+
	Chloroform	-	-	-	+	+	+	+	+	+	+
	Aqueous	-	-	+	+	+	+	+	+	+	+
RS10	Ethanol	-	+	+	+	+	+	+	+	+	+
	Methanol	-	-	-	+	+	+	+	+	+	+
	Ethyl acetate	-	-	+	+	+	+	+	+	+	+
	Hexane	-	-	+	+	+	+	+	+	+	+
	Chloroform	-	+	+	+	+	+	+	+	+	+
	Aqueous	-	+	+	+	+	+	+	+	+	+
	Streptomycin	-	-	-	-	-	-	-	-	-	-

(-) No growth observed'; (+) Growth observed

Table.3 Effect of *Carica papaya* leaf extract on seed germination and seedling vigor of tomato under laboratory conditions

Treatments	Germination (%)	MRL (cm)	MSL (cm)	Fresh weight (g)	Dry weight (g)	VI
Control	92.66± 4.56	4.57±0.21	7.87±0.57	1.28±0.066	0.34±0.054	1152.69±18.66
RS1	34.0± 0.88	2.73±0.066	4.63±0.66	0.45±0.021	0.19± 0.012	250.24±5.86
RS2	33.63±0.57	2.76±0.074	5.07±0.33	0.43±0.14	0.2± 0.021	263.08±5.98
RS3	35.37±0.80	2.88±0.065	4.43±0.21	0.52±0.25	0.1± 0.032	258.04±4.76
RS4	33.66±0.66	2.78±0.074	5.86±0.56	0.48±0.21	0.1± 0.023	290.30±4.66
RS5	32.53±0.93	2.66±0.082	4.77±0.78	0.52±0.16	0.2± 0.036	239.53±5.57
RS6	35.87±0.57	2.67±0.054	4.66±0.66	0.50±0.21	0.1± 0.015	262.41±6.89
RS7	34.65±0.66	3.12±0.096	4.94±0.57	0.51±0.12	0.2± 0.034	279.27±5.48
RS8	36.67±0.87	2.89±0.091	5.88±0.68	0.47±0.16	0.1± 0.046	321.59±4.66
RS9	34.54±0.84	2.76±0.072	4.78±0.45	0.49±0.13	0.2± 0.033	260.43±6.57
RS10	33.33±0.67	2.83±0.066	4.96±0.43	0.48±0.15	0.2± 0.044	259.64±5.33
C. papaya	94.76± 4.36	6.18±0.57	8.68± 0.98	1.42± 0.066	0.48±0.066	1218.61±19.89

Values are presented as mean ± Standard Errors of triplicate experiments. Mean of three values ± Standard Deviation. MRL - Mean Root Length; MSL - Mean Shoot Length; VI - Vigor Index; RS- *R. solanacearum*

Table.4 Effect of *Carica papaya* leaf extract on bacterial wilt in tomato under greenhouse conditions

Treatments	Plant Height (cm)	MSL (cm)	MRL (cm)	MFW (g)	Dry Weight (g)	DI (%)
Control	27.47±1.57	17.54±0.78	9.12±0.78	10.23±0.66	1.96±0.048	0.00
RS1	16.66±0.66	7.45±0.57	5.56±0.54	4.89±0.33	0.63±0.033	79.94±2.56
RS2	17.12±0.57	8.56±0.66	4.45±0.33	4.45±0.21	0.58±0.052	81.14±4.66
RS3	16.78±0.89	7.54±0.43	5.78±0.42	5.37±0.32	0.67±0.066	84.48±3.57
RS4	17.23±0.76	8.76±0.66	4.84±0.57	4.61±0.15	0.82±0.048	78.89±4.48
RS5	16.63±0.54	9.23±0.57	5.63±0.66	4.78±0.12	0.91±0.082	81.76±3.89
RS6	16.78±0.66	8.54±0.89	6.45±0.23	5.32±0.48	0.59±0.067	86.43±5.66
RS7	16.54±0.57	7.89±0.76	5.62±0.48	5.46±0.33	0.61±0.033	87.33±4.84
RS8	16.89±0.65	8.67±0.57	6.58±0.32	4.84±0.12	0.65±0.067	88.78±6.57
RS9	16.65±0. 57	8.78±0.48	5.73±0.40	5.47±0.33	0.66±0.084	88.92±4.89
RS10	17.13±0.66	8.89±0.86	5.68±0.33	5.58±0.15	0.70±0.076	79.68±3.76
***C. papaya* extract**	30.89±3.47	15.34±1.33	11.23±0.89	12.54±1.12	2.28±0.066	0.00
RS + *C. papaya*	22.66±2.68	12.48±1.57	8.68±0.66	9.55±0.98	1.36±0.057	36.78±2.57

Values are presented as mean ± Standard Errors of triplicate experiments. Mean of three values ± Standard Deviation. MSL- Mean shoot length; MRL- Mean root length; MFW- Mean fresh weight; DI; Disease incidence of tomato plants treated by *Carica papaya* leaf extract and infested with *R. solanacearum* (RS)

Table.5 Effect of *C. papaya* extracts on tomato plant growth and fruits yield under field conditions

Treatments	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Fruits/ plant	Yield t/ha	Wilt Incidence (%)
Control	69.12±3.57	589.84±6.87	38.9±3.66	28.56±2.33	7.32±0.66	0.00
RS1	41.62±1.98	171.63±4.43	16.63±1.54	10.75±0.42	1.46±0.054	82.32±3.33
RS2	43.86±1.66	168.38±4.57	14.75±1.33	11.37±1.57	1.29±0.067	84.54±3.57
RS3	38.63±1.54	165.46±3.66	17.34±1.66	10.68±1.78	1.47±0.057	81.76±4.66
RS4	37.54±1.33	159.93±4.48	16.33±1.57	10.94±1.15	1.69±0.066	79.68±2.96
RS5	34.93±1.12	168.74±4.57	18.47±1.89	11.96±0.66	1.38±0.064	82.34±3.53
RS6	39.67±1.68	164.82±4.33	19.56±1.66	9.82±1.12	1.34±0.021	83.67±4.33
RS7	36.83±1.57	169.96±3.48	16.73±1.57	12.23±1.48	1.28±0.043	81.66±3.66
RS8	37.46±1.67	166.77±4.63	15.94±1.48	10.35±0.54	1.35±0.68	84.48±3.57
RS9	35.73±1.68	170.46±3.66	17.73±1.67	12.47±1.57	1.46±0.046	82.62±2.98
RS10	36.69±3.79	169.83±3.57	16.85±1.54	10.78±0.89	1.37±0.076	83.54±3.21
C. papaya	92.63±3.66	712.85±5.66	44.65±2.57	39.43±2.45	8.62±0.88	0.00
RS + *C. papaya*	66.58±2.89	433.44±4.68	36.43±1.66	28.64±1.89	5.95±0.24	34.86±1.57

Values are presented as mean ± Standard Errors of triplicate experiments. Mean of three values ± Standard Deviation

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

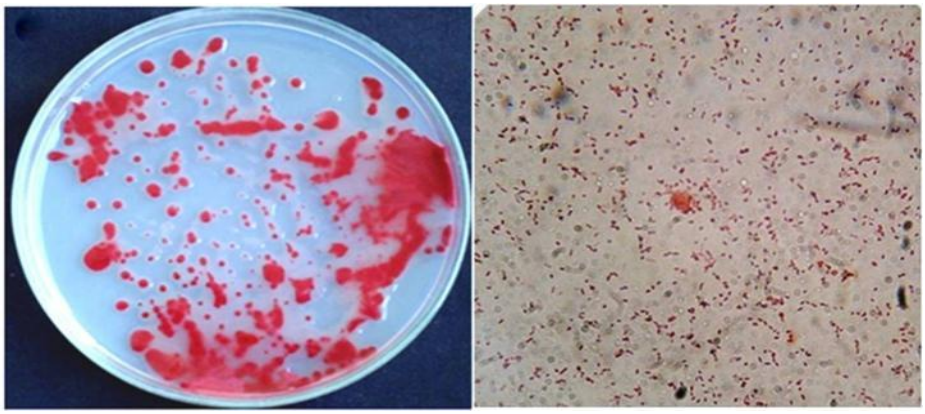


Fig.2 Zone of inhibition of *Carica papaya* leaf extracts against *Ralstonia solanacearum*

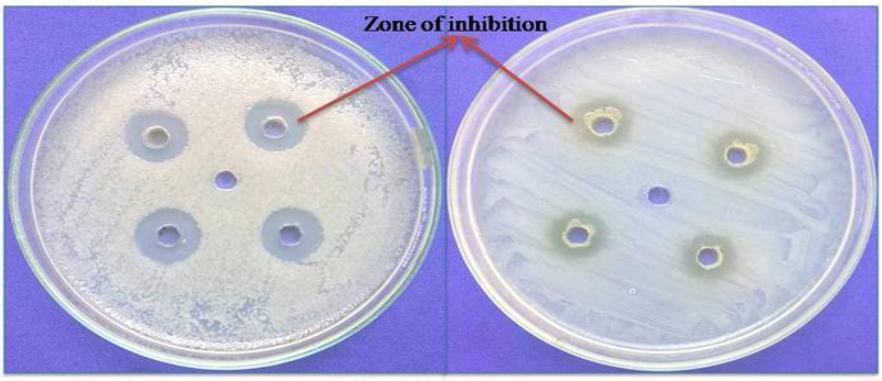
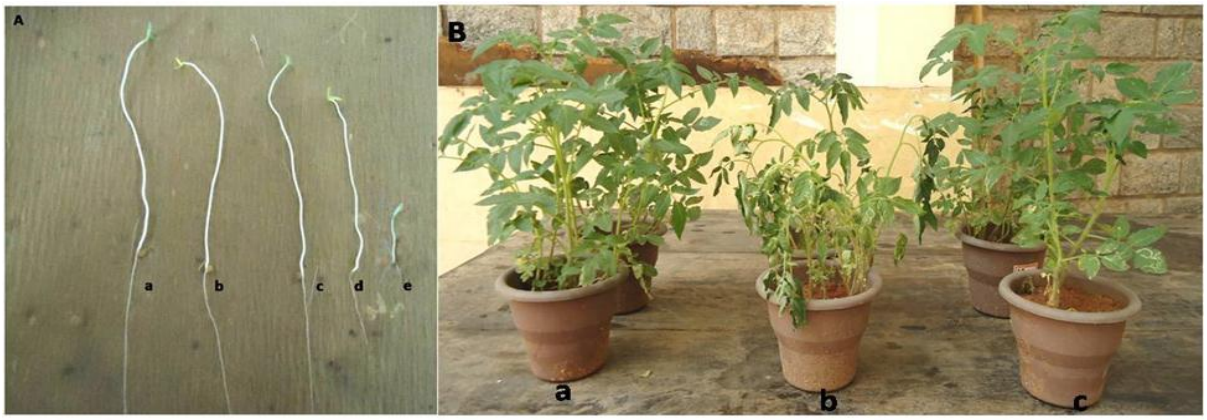


Fig.3A Effect of *Carica papaya* extract on tomato seed germination and seedling vigor index. Seed germination of tomato seedlings a and b- *C. papaya* leaf extract treatment, c and d- Controls and e- Pathogen treated seedlings **Fig.3B** Effect of *C. papaya* extracts on bacterial wilt incidence in tomato under greenhouse conditions. a -*C. papaya* extracts treated, b- *R. solanacearum* treated and c- *C. papaya* extracts and *R. solanacearum* treated



The results were evident the use of *C. papaya* leaf powder extracts has a potential to substitute the antibiotics to control the infection (Sumathi and Gowthami 2014). Thus, *C. papaya* could become promising natural antimicrobial agents with potential applications in agriculture for controlling the bacterial wilt of tomato. However, if plant extracts are to be used for control of plant pathogens in agriculture. The greenhouse and field trial experiments designated that tomato seedling with leaf extracts resulted in a significant decrease in bacterial wilt disease. These outcomes were similar to previous research on the part of plant extracts in the control of bacterial disease.

In our study revealed the antibacterial activity of solvent extracts of *C. papaya* against *R. solanacearum*, the causal agent of bacterial wilt. It may be concluded from this study that *C. papaya* leaf extracts were *in vitro* and *in vivo* against phytopathogens. The antibacterial activity of *C. papaya* extracts were found much better than the broad spectrum antibiotic. Plants extracts are originate to be an actual reservoir for the bioactive compounds and can offer valuable sources for the detection of natural pesticides (Akhtar *et al.,* 1997).

Further isolation and purification of the extracts are necessary to conclude the bioactive components responsible for their activity. It is important that research should continue to isolate and purify the bioactive components from *C. papaya* leaves responsible for the control of pathogens. The bioactive components in the extract of the *C. papaya* could be commercially exploited for the decrease of the wilt diseases in tomato plants. Although our results support the idea that *C. papaya* extracts are candidate for control of bacterial plant pathogens *in vitro* and *in vivo* conditions.

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