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Antibacterial Activity of Neem (*Azadirachta indica*) Plant Extracts against Bacterial Wilt of Tomato Caused by *Ralstonia solanacearum*

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Abstract **– Bacterial wilt is a major disease in solanaceous crop plants caused by *Ralstonia solanacearum*. Management of bacterial wilt is very complicated as there are no effective curative chemicals. The present study was conducted to analyze the *in vitro* antibacterial activity of *Azadirachta indica* against *R.solanacearum*. Neem extract was evaluated their antimicrobial activity assayed by three different methods namely, agar dilution, agar well diffusion, and disk inhibition zone methods on Tryptone soya agar (TSA) medium against *R. solanacearum.* Antibacterial effect of the extracts was studied and compared with commercial antibiotic. The results revealed that the average zone of inhibitions were in the aqueous and methanolic extracts were showed against ten *R. solanacearum* isolates in the range of 15 mm to 20mm, 13 mm to 21mm and 12mm to 23mm inhibition zone of different three methods respectively mentioned previously. The means and standard error of triplicate tests were recorded. The methanolic extract was found to be more effective than aqueous extract against *R. solanacearum*. The minimum inhibitory concentration (MIC) was determined by two-fold micro broth dilution method for the tested *R. solanacearum*. The MIC of the neem methanolic extract and aqueous extracts were 256µg ml^{-1} and 1024µg ml^{-1} respectively. Phytochemical analysis of neem leaf extract showed presence of phenols tannins, flavonoids, alkaloids, coumarins, saponins, steroides, terpenes quinones, proteins and amino acids and cardiac glycosides using methanolic extraction of bioactive compounds are solvent dependent. The results revealed that neem extracts have produced inhibitory activity against *R. solanacearum* and the same could be used to extract antibacterial compounds. The potential of *Azadirachta indica* extracts was found much better than most of the antibiotics.**

Keywords **– Bacterial Wilt, Phytochemicals, Neem, *Ralstonia solanacearum*, Tomato.**

I. INTRODUCTION

Bacterial wilt of tomato (*Lycopersicon esculentum*) caused by *Ralstonia solanacearum* is devastating disease and major constraint in the production of many important crops such as tomato, brinjal, chilli, tobacco, potato and banana (Kelman *et al.,* 1994). The yield loss may vary between 10.8 and 90.6 percent depending on the environmental circumstances and the stage at which infection occurs (Ramkishun, 1987). 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. The natural plant products derived from plant species has the capacity to control diseases caused by different pathogens (Guleria and Tiku, 2009). The research study focused on plant derived natural bactericides and their possible applications in agriculture to control plant bacterial diseases has intensified as this approach has enormous 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 (Varma and Dubey, 1999; Gottlieb *et al.*, 2002).

The negative effects of pesticides are been expansion the alternative control of plant disease, which includes the biological control, the induction of resistance and the use of natural products with induction of resistance and/or with direct antimicrobial activities. Control of bacterial wilt in diseased 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). The plants establish in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced on the market are obtained from natural resources. The use of plant extracts and phytochemicals both with known antimicrobial characteristics is of great significance, in the past few years a number of investigations have been conducted worldwide to prove antimicrobial activities from medicinal plants (Alonso-Paz et al., 1995). Green plants represent a pool of effective chemotherapeutants and can supply valuable sources of natural pesticides (Mahajan and Das, 2003). Biopesticides has been suggested as an effective substitute for chemicals (Kapoor, 2001). Reports are available on the use of several plant byproducts, which posses antimicrobial properties, on several plant pathogenic bacteria and fungi (Kilani, 2006). The use of plant extracts which are natural sources of antimicrobial substances, regarded as safe and degraded by natural soil microbes; they do not pose any health residual or

ecological problems at any concentration which they are used (Yang *et al.*, 2010). However, there is no single mean that would totally eliminate the disease, provide a complete cure or fully protect host plants against infection. *In vitro* and *in vivo* investigations by some researchers have confirmed the antimicrobial potential of some plant species (El-Ariqi, 2005).

Azadirachta indica is well known in India and its neighboring countries. It is popularly known as Indian Neem (margosa tree) or Indian lilac. It is an evergreen tree, cultivated in various parts of Indian subcontinent. Every part of the tree has been used as traditional medicine for household remedy against various human ailments. Neem has been extensively used in Ayurveda, Unani and homoeopathic medicine and has become a cynosure of modern medicine. The tree is still regarded as 'village dispensary' in India. Chemical investigation on the products of the neem tree extensively undertaken in the middle of the isolation of nimbin, the first bitter compound isolated from neem oil, more than 135 compounds have been isolated from different parts of neem (Ganguli, 2002). Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex (Subapriya and Nagini, 2005).

Recently the use of some natural products has emerged to inhibit plant pathogens. Antimicrobials of plant origin are efficient in the treatment of plant diseases with no side effects that are often associated with synthetic ones. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally variable with more than 140 compounds isolated from different parts of the tree (Subapriya and Nagini, 2005). Quercetin and β-sitosterol, were the first polyphenolic flavonoids purified from neem fresh leaves and were known to have antibacterial and antifungal properties (Mahmoud *et al.*, 2011). The problems encountered with the use of chemical microbialcides include environmental degradation, resistance problem in target organism, etc. Their frequent use changes the ecological conditions which demand the need for developing ecofriendly herbal antimicrobial agents. In such a situation, natural plant products, which proved to be a correct choice, replace some of the chemicals in order to control plant diseases. Many focus on determining the antimicrobial activity of plant extracts found in folk medicine, essential oils or isolated compounds such as alkaloids, flavonoids, sesquiterpene, lactones, diterpenes, triterpenes or napthoquiones, among others. Some of these compounds were isolated or obtained by bio-guided isolation after previously detecting antimicrobial activity on the parts of the plant. Hence, the present study is conducted on *in vitro* evaluation of *Azadirachta indica* different solvent extracts against *R. solanacearum* causing bacterial wilt in tomato plants.

II. Material and Methods

A. Isolation and identification of R. solanacearum

The collected suspected tomato 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 were placed on Kelman's TZC (2, 3, 5 Triphenyl tetrazolium chloride) medium (Kelman, 1954). Isolation from rhizosphere soil samples were done by dilution plate technique on modified semi selective medium, South Africa (SMSA) agar medium (Elphinstone *et al.*, 1996). The plates were incubated at 28 ± 2 ^{o}C for 24–48 h. 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.

B. Plant extracts

Fresh healthy leaf of Neem leaf and floral parts were collected from Bangalore University, Karnataka. Neem aqueous extract was prepared by mixing 15.0 g of dry powder of neem leaves with 100 ml of sterile distilled water in a round bottom flask with occasional shaking. The extract was then filtered through a muslin cloth for coarse residue and finally through Whatman No. 1 filters paper and kept in an airtight amber coloured container at 4 °C until further use. In Methanolic extract, Air-dried 10g of dry powder of neem leaves powder was mixed with 100ml methanol, the mixtures were placed at room temperature for 24h on shaker with 150 rpm. The solution was filtered through Whatman filter no.1 and thus obtained was concentrated by complete evaporation of solvent at room temperature to yield the pure extract. Stock solution of methanolic crude extracts was prepared by mixing well the appropriate amounts of dried extracts and appropriate solvent. The solution was stored at 4 °C after collecting in sterilized screw cap tubes until further use (Pankaj *et al.*, 2011).

Fig.1. Neen plant and leaves.

C. Inoculum Preparation

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).

D. Antagonistic activity against R. solanacearum

The antagonistic activity of neem extracts were evaluated using three different methods namely, agar dilution, agar well diffusion and disc inhibition zone. Antagonistic activities against *R. solanacearum* of aqueous and methanolic extracts from neem powder was determined by agar dilution method, 15 ml of Tryptic Soy Agar (TSA) medium containing either the neem extracts were added to each of the Petri plates (9cm diameter). Then the isolates of *R. solanacearum* were inoculated on the agar surface. After incubation at 28 ± 2 °C for 24-48h, the antimicrobial activity was measured as diameter of the inhibition zone. The solvent (Methanol), in which extract was prepared, solvent was 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 and the antagonistic activity of the extract evaluated was expressed in terms of the average diameter of zone of inhibition in mm.

Antagonistic activity against *R. solanacearum* of aqueous and solvent extracts from neem powder was determined by standard agar well diffusion assay (Perez *et al.,* 1990). Petri dish containing 20 ml of TSA medium was seeded with 100µl inoculum of *R. solanacearum* (1×10^8 CFU ml^{-1}) and Media was allowed to solidify. Wells of 5 mm diameter were cut into solidified agar media with the help of sterilized cork borer. Aliquot 100µl of each neem extract was added in the respective well and petri plates were left undisturbed to allow diffusion of the sample for 2-3 h and incubated at 30°C for 24-48h (Narasimha Murthy *et al.,* 2012). Antagonistic activity against *R. solanacearum* of aqueous and solvent extracts from neem powder was determined by disc inhibition zone method, the TSA medium was inoculated with freshly prepared cells of *R. solanacearum* to yield a lawn of growth. After solidification of the agar, a number of sterilized discs were dipped into the solvent (negative control) or extract solutions and placed on the plates. After incubation at 28 ± 2 °C for 24-48h, the activity was measured as diameter of the inhibition zone formed around the disk. The experiment was performed in triplicate under aseptic conditions.

E. Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentrations (MIC) values were determined for extracts producing an inhibition zone of *R. solanacearum* that was found to be sensitive to the most active extract revealed by the previous screening tests (NCCLS, 1997). A stock solution of 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 $1x\ 10^8$ CFU ml^{-1} in each well. Micro titer plates were then kept at 28 ± 2 ^oC for 24h incubation. Following incubation, the MIC values are recorded as the lowest concentration of the extract that completely inhibited bacterial growth, giving a clear well.

F. Qualitative Method of Phytochemicals

Phytochemical tests were done to find the presence of the active chemical constituents from neem powder extracts such as phenols tannins, flavonoids, alkaloids, saponins, steroides, coumarins, quinones, proteins and aminoacids, terpenes and cardiac glycosides according to the methods described by Raman (2006).

III. RESULTS

A. Isolation and identification of R. solanacearum

After incubation pink centers with white fluid colonies were selected, a total of 50 isolates of *R. solanacearum* were isolated and identified. Microscopic studies revealed that bacterial isolates were Gram negative, rod shaped and it was confirmed by standard biochemical tests (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, KF924747 and KF924748 respectively..

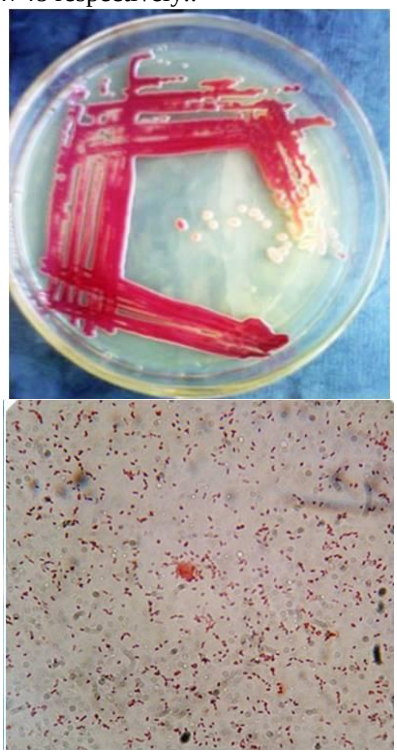

Fig.2. Colonies of *Ralstonia solanacearum* from infected tomato fields and Microscopic view of *R. solanacearum*

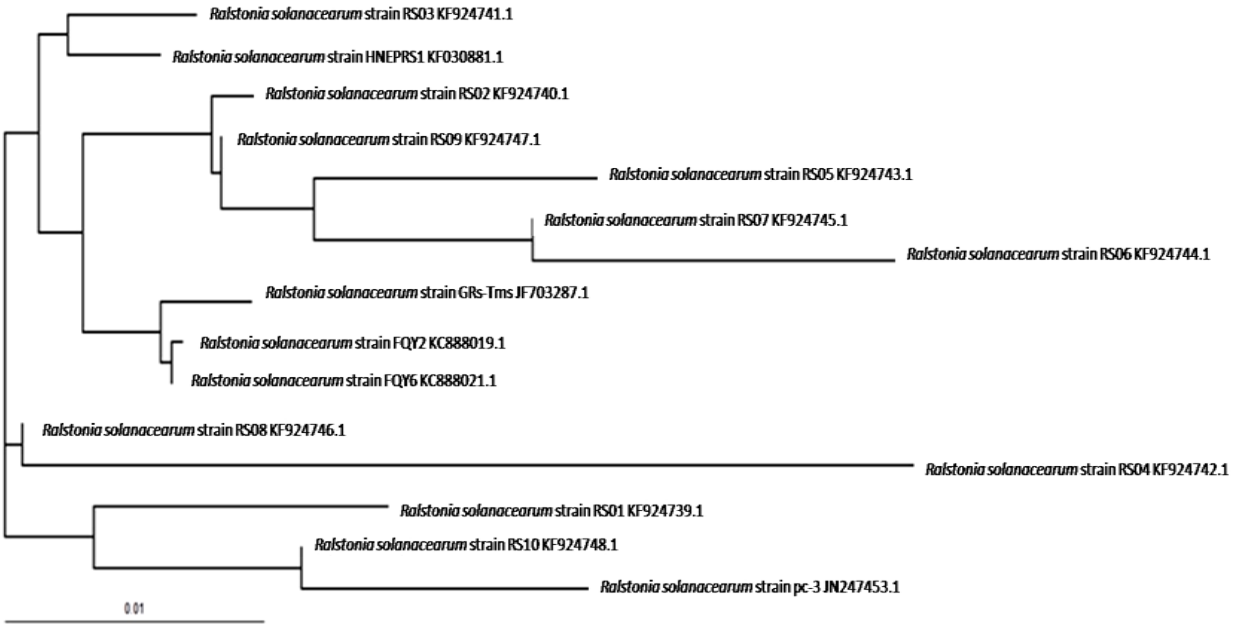

Fig.3. Phylogenetic relationships of *R. solanacearum* isolates inferred by Neighbor-Joining (NJ) bootstrap tree analysis of 16S rRNA sequences.

B. Antagonistic activity against R. solanacearum

Antibacterial activity of neem powder extracts against ten *R. solanacearum* pathogens, were studied. Results of the antagonistic activity were shown in the Table 1. According to the results, neem extracts showed the antagonistic activity against all tested *R. solanacearum* isolates. Aqueous and methanolic extracts were showed against ten *R. solanacearum* in the range of 15 mm to 20mm, 13 mm to 21mm and 12mm to 23mm inhibition zone of different methods, agar dilution, agar well diffusion, and disk inhibition zone respectively.

Table 1: Antagonistic activity of aqueous and methanolic extracts of neem powder against *R. solanacearum* by agar dilution, agar well diffusion and disk inhibition methods.

	RS1	**RS2**	**RS3**	**RS4**	**RS5**	**RS6**	**RS7**	**RS8**	**RS9**	**RS10**
	Diameter of inhibition zone (mm) by agar dilution									
Aqueous extract	15.33±0.4	17.06±0.8	17.46±0.2	15.57±0.3	15.46±0.7	16.30±0.3	15.34±0.5	16.30±1.7	17.50±0.3	15.0±1.2
Methanolic extract	19.42±0.6	20.33±0.6	17.57±0.2	18.32±0.2	20.54±0.3	18.70±1.1	19.6 ± 0.5	17.5 ± 0.6	20.24±0.4	19.72±0.4
	Diameter of inhibition zone (mm) by agar well diffusion									
Aqueous extract	14.57±0.7	13.33±0.4	14.56±0.6	13.5±0.4	14.6±1.1	15.11±0.3	14.7±0.6	13.6±0.5	13.66±0.9	14.33±0.6
Methanolic extract	20.57±1.2	19.33±0.8	18.57±0.4	21.17±1.1	21.2±0.9	20.33±1.3	21.56±0.7	20.6±0.3	18.4±0.6	19.0± 0.5
	Diameter of inhibition zone (mm) by disk inhibition									
Aqueous extract	13.2±0.5	14.33±0.6	12.46±0.7	13.4±0.7	13.7±0.8	12.56±0.7	12.33±0.6	13.54±0.9	14.6±0.6	12.5±0.7
Methanolic extract	19.66± 1.2	20.7± 0.8	22.1 ± 0.6	21.3± 0.8	18.60±0.8	21.2±1.3	23.03±1.4	20.6±0.7	20.57±0.9	22.62±0.8
Methanol	5.2±0.2	4.66±0.4	4.21±0.3	5.33±0.3	4.52±0.4	3.43±0.2	4.56±0.3	3.6±0.7	4.33±0.4	4.57±0.5
Streptomycin	22.65±1.2	23.33±1.6	23.56±1.9	22.5±1.3	24.17±1.7	22.54±1.1	21.46±1.6	23.62±1.9	21.56±1.5	23.21±1.8

Data given are mean of three replicates ± standard error.

C. Minimum Inhibitory Concentration

Minimum inhibitory concentrations of neem powder extracts had been demonstrated, *R. solanacearum* were inhibited at 256µg ml^{-1} by methanol extract. It showed varying broad-spectrum antibacterial activity at different MIC against the *R. solanacearum* and aqueous extracts exhibited good antibacterial activity at the highest concentration 1024µg ml^{-1} and antibiotic while *R. solanacearum* was inhibited at 8µg ml^{-1} concentration (Table 2-4).

Table 2: Minimum inhibitory concentration of active methanol extracts of neem powder against *R.solanacearum.*

Type of Extract	*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 extracts	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 3: Minimum inhibitory concentration of aqueous extracts of neem powder against *R.solanacearum.*

Type of Extract	*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 4: 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

D. Phytochemical screening

The Phytochemical screening of methanolic neem powder extract showed the presence of Phenols Tannins, Flavonoids, Alkaloids, Saponins, Steroides, terpenes and Cardiac glycosides (Table 5).

Table 5: Phytochemical screening of neem powder extracts

Assay	Result
Phenols	+
Tannins	+
Flavonoids	+
Alkaloids	+
Saponins	+
Steroides	+
Terpenes	+
Glycosides	+
Mucilages	+
Anthraquinones	-
Coumarins,	+
Quinones,	+
Proteins and amino acids	+

+: Present; -: Absent.

IV. DISCUSSION

Natural products with pesticidal activity are being explored in order to make available the pesticides, which are eco-friendly, selective and can be locally produced, particularly for the farmers who cannot pay for expensive synthetic pesticides (Rhouma *et al.,* 2009). Excessive and random application of the chemicals has created several environmental and health hazards and also some phytopathogens have been developed resistance. The control of bacterial disease in plants is mainly achieved by the use of synthetic pesticides which can result in toxicity to animals and humans as well as accumulation in living systems (Ansari and Malik, 2008). In addition, plant pathogens commonly acquire resistance to chemical pesticides as a result of their repeated uses (McManus *et al.,* 2002). New molecules for the control of plant pathogens are mandatory. Recently, plant extracts have been tested against plant bacteria with a certain degree of success (Slusarenko *et al.,* 2008). The presence of antifungal components in higher plants has long been recognized as an important factor for disease resistance (Mahadevan, 1982). Such components being biodegradable and selective in their toxicity are considered valuable in controlling some plant diseases (Singh and Dwivedi, 1987). The antibacterial activity of *Ralstonia* with plant extracts have been reported earlier (Lopez *et al.,* 2005). However, the antibacterial activity of neem extract against *Ralstonia 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.* The use of neem 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 different crops (Jai Sunder *et al.,* 2011).

The use of naturally available chemicals from plants, which retards the reproduction of undesirable microorganisms, would be a more sensible and ecologically sound method for plant protection and will have an important 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). Several authors have linked the presence of these bioactive compounds to the antimicrobial properties of crude plant extracts (Reddy *et al.,* 2007). This activity of neem extracts, suggest that the active principles are soluble in water.

The result revealed that both the neem powder extracts have produced inhibitory activity against the *R. solanacearum* and the it was found much better than most of the antibiotic and the same may be useful against the *R. solanacearum.* In the present study the methanolic neem powder extract produced better antibacterial activity. This indicates that the active constituents of the leaf parts have more ability to dissolve in methanol than the water used in this study. According to the results, the antagonistic activity of neem leaf powder extracts against *R. solanacearum*. Aqueous and methanolic extracts were showed against ten *R. solanacearum* in the range of 15 mm to 20, 13 mm to 21 and 12mm to 23mm inhibition zone of different methods, agar dilution, agar well diffusion, and disk inhibition zone respectively.

Antibacterial activity against bacterial wilt pathogen was found in high from neem leaf powder extracts against the *R. solanacearum* were inhibited minimum inhibitory concentration (MIC) at 256µg ml^{-1} by methanol extracts and aqueous extract was inhibited MIC at 1024µg ml^{-1} concentration. It was evident that the use of neem leaf powder extracts has a potential to substitute the antibiotics to control the infection. This kind of biological control 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. Phytochemical screening from neem leaf extracts were used to study the presence of contained Phenols Tannins, Flavonoids, Alkaloids, Saponins, Steroides, Coumarins, Quinones, Proteins and aminoacids, Terpenes and Cardiac glycosides. 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 against *R. solanacearum* have been reported earlier (Larkin *et al.,* 2007). The aqueous plant extracts exhibited antibacterial property, the highest zone of inhibition was observed in leaf extract of *Musa paradisica* against *R. solanacearum* (Biswal, 2015). The finding of the result is encouraging and could be used as a source of antimicrobial compounds for reduce of bacterial wilt caused by *R. solanacearum.* It was evident that the use of neem leaf powder solvent extracts has a potential to substitute the antibiotics to control the *R. solanacearum*. This kind of biological come up to would be safe and environmental friendly.

V. CONCLUSION

This study revealed the antibacterial activity of methanolic extract of Neen against *R. solanacearum*, the causal agent of bacterial wilt in tomato. It may be concluded from this study that *Azadirachta indica* leaf extract has antimicrobial activity against plant pathogens. Simultaneously all the concentration of the extracts affected the bacteria however; the highest concentrations of the extracts had more pronounced effect in decreasing the growth of *R. solanacearum*. The antibacterial activity of neem extracts are found much better than the broad spectrum antibiotic. Further isolation and purification of the extracts are required to determine the bioactive components responsible for their activity. It is essential that research should continue to isolate and purify the active components of this natural herb and use in field conditions. The compounds in the extract of the plant could be commercially exploited for the reduction of the bacteria causing fatal diseases of plants. Although our results support the idea that neem extracts are candidate for control of plant diseases.

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