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Ethnobotanical and antimicrobial study of plants from Sariska National Park, Alwar as a potential source to control bacterial wilt disease of groundnut

Ramesh Chand Meena, Ashwani Kumar Verma*, Mohan Singh and Laxmi Meena*
Department of Botany, SPNKS Govt. P.G. College, Dausa, Rajasthan, India
*Department of Botany, Raj Rishi Govt. College, Alwar, Rajasthan, India
Corresponding Author E- Mail: rameshchandmeena1012@gmail.com

ABSTRACT
Bacterial wilt disease of groundnut caused by Ralstonia solanacearum is difficult to control due to seed and soil borne nature of the pathogen. In the present study, symptomatic groundnut seeds and plans were collected and bacterial pathogen Ralstonia solanacearum was isolated using standard methods. Isolated bacterial colonies were identified on the basis of morphological, cultural and biochemical characterizations. To evaluate the economical and eco-friendly disease management method a total of fifteen plants were selected from Sariska National Park, Alwar on the basis of their anti-bacterial properties and tested against the studied pathogen. Three plant species Ocimum basilicum, Ricinus communis and Lantana camara showed the maximum potential to control the R. solanacearum. Among three tested plants R. communis found significantly most effective to control wilt disease showing only 6.7% disease occurrence.
Keywords: *Antibacterial, Control, Wilt, Plant Extract, Ralstonia solanacearum.*

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INTRODUCTION

Ralstonia solanacearum formerly known as *Pseudomonas solanacearum* is the causal organism of bacterial wilt disease of various crops including groundnut [27]. In different regions bacterial wilt disease also named by brown rot disease of groundnut. Wilting of infected plants are caused by the blocking of vascular tissues which make by high molecular mass of exo-polysaccharide one (EPS1). *Ralstonia solanacearum* are able to produced the high molecular mass of polysaccharide one (EPS1) by their metabolic activity [7]. The pathogen infects various crops such as groundnut, tomato, potato, capsicum and eggplant in the tropical regions. For the control of bacterial wilt disease various plant extracts have been tested for their effective antibacterial properties [15]. Against the growth of *R. solanacearum*, extracts of some plants showed effective result in both in-vitro and field treatments [20]. The studied extracts of plant parts were applied on the infected groundnut crops to control the bacterial wilt disease [17].

In Rajasthan the incidence of *R. solanacearum* causing bacterial wilt disease of groundnut had been controlled by effective strategies. Effects of anti-bacterial activity of different plant extracts have been observed by *in vitro* methods in primary step. The losses of the production of groundnut crops have been noted due to harmful effect of bacterial wilt disease. The present study was aimed at investigating the potential roles of selected plants from Sariska National Park, Alwar to control the bacterial wilt disease of groundnut.

MATERIAL AND METHODS

Field survey and collection of the selected plant species from Sariska National Park:

A total of 15 plants were selected from Sariska National Park, Alwar to test the antibacterial efficacy against bacterial wilt pathogen of groundnut according to their known anti-bacterial properties. These plants were collected and identified with taxonomic keys. The selected plants were *Abutilon indicum, Aegle marmelos, Lantana camara, Capparis deciduas, Tribulus terrestris, Ocimum basilicum, Zyzyphus nummularia, Solanum virginianum, Ficus racemosa, Eucalyptus camaldulensis, Xanthium strumarium, Ricinus communis, Tephrosia purpurea, Cassia tora, Cordia dichotoma* collected from Sariska National Park to carry on ethno-botanical control studied on *R. solanacearum.*

Treatment with plant extracts on petriplate method:
A total of 15 cultured petriplate of growing *Ralstonia solanacearum* were prepared. A series of experiments were carried out by following petriplate method. All petriplate with bacterial culture were treated with leaf extract of selected plants which were collect from Sariska Nationa Park. Potential of different plant extract was observed and evaluated in the form of percent inhibition. The plants which have exposed the anti-bacterial effects on the growth of *R. solanacearum* were selected for the further studies.

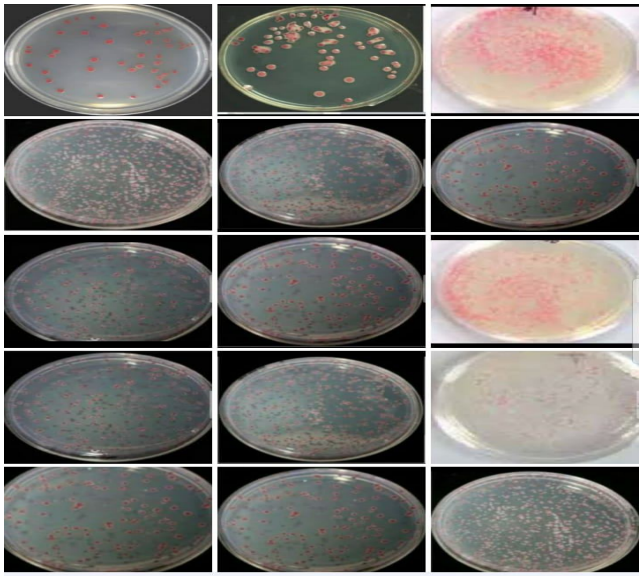

Figure -01: Potential of plant extracts against petriplate of *R. Solanacearum*

Table-01: Potential of different plant extracts against petriplate of *R. Solanacearum*

S.N.	Common name	Scientific name	Inhibition in percentage
1.	Kanghi	*Abutilon indicum*	27.9
2.	Bel	*Aegle marmelos*	21.3
3.	Lantana	*Lantana camara*	64.6
4.	Teti	*Capparis deciduas*	19.4
5.	Gokhru	*Tribulus terrestris*	17.6
6.	Basil	*Ocimum basilicum*	78.0
7.	Jhadi Ber	*Zyzyphus nummularia*	26.1
8.	Peeli Kateli	*Solanum virginianum*	32.8
9.	Gular	*Ficus racemosa*	29.0
10.	Safeda	*Eucalyptus camaldulensis*	24.6
11.	Bilwa	*Xanthium strumarium*	34.7
12.	Castor oil plant	*Ricinus communis*	98.2
13.	Sarphonk	*Tephrosia purpurea*	27.0
14.	Panwad	*Cassia tora*	28.3
15.	Lisoda	*Cordia dichotoma*	21.0

Infected groundnut seeds and plant parts were collected from groundnut growing farmer fields from different districts of Rajasthan. After that cut into small pieces and soaked in tap water and stand for one hour and then mixed again completely. In sterile container mixed the prepared content and twenty four hour old culture of *R. solanacearum.* Transferred the prepared the new combination of suspension into knapsack sprayer. In the small groundnut growing plots applied approximately one liter suspensions per plots. Irrigated plots were inoculated by applying of *R. solanacearum* suspension left for three days.
Treatment with plant extracts in groundnut growing plots: The investigation of wilted groundnut treatment was carried out in between June and July 2021. Selected plants exposed positive result *in vitro* experiments were used directly on the test plot treatments. The prepared suspensions of plant extracts were applied in the dilute form on groundnut growing plots.
Stem, leaf and flower parts of studied fifteen plant parts were collected from Sariska National Park. The process of harvesting was done in the hours of early morning and then packed in polythene bags. These bags were carried to the plots where the test was done. Plant parts were chopped into small pieces and soaked for 01 hour in water. Prepared mixtures were applied into the inoculated groundnut plots.

According to Ooshiro *et al* [19] each five liters of water solvent use 02 kg of extracts of harvested plant parts. Spreading the extracts applied plants turning from one side to cover the whole plant for treatment the wilt disease. The soil was aseptically mixed and allow to fumigation after the treatment by extracts of finally selected plants and left to four days. These tests were done by five replications and three treatments through randomized complete block design. Treatments were done by extracts of *O. basilicum*, *R. communis* and *L. camara* showed maximum inhibition of bacterial pathogen in petriplate method indicated by T1, T2 and T3 respectively along with negative (T4) and positive control (T5). The negative control (T4) done without apply of any extract and the positive control (T5) used with the selected extracts.

Above mentioned plots are planted by disease-free seeds of groundnut after seven days of treatment and noted the development of bacterial wilt disease symptoms for two months.

RESULTS AND DISCUSSION

In petriplate method, among fifteen plant extracts tested against bacterial pathogen maximum inhibition showed by plant extract of *Ocimum basilicum* (78%) followed by *Lantana camara* (64.6%).plant extract of *Tribulus terrestris* showed minimum control (17.6%) over studied bacterial pathogen. The results of effects on growth of *R. solanacearum* have been shown in Figure- 01 and Table- 01. Bacterial wilt disease is powerfully control by the farmers in groundnuts plotes to control of vegetative plant parts. In Rajasthan wilt control plant *R. communis* is easily available in the groundnut producing regions and can be used for wilt control. Other factors which have found in soils such as pH, organic matter content and microbial communities are suggested by many researchers for suppression of bacterial wilt disease in the soil (Elsas *et al.,* 2005). According to Sandra [21] the cropping of the effective plants are uses for bacterial wilt control to reduce the causing pathogens. These wilt controlling plants manage the loss of groundnuts production due to decrease by bacterial wilt disease. Among of 15 plant species, finally we have selected 03 plants (*Ocimum basilicum*, *Ricinus communis*, *Lantana camara*) which have maximum potential to control the *R. solanacearum*. This finding shows that use of these experimented plants have potential approach to control of bacterial wilt disease. It can be expected that these plants possess more antibacterial activity that is effective to control of *R. solanacearum* with economical and eco-friendly means. We applied the same treatment directly on groundnut crops in the fields to get effective results timely. Plots treated with *R. communis* had significantly low bacterial wilt incidence having been reduced by more than 90%. Treatment with the other two plants, *O. basilicum* and *L. camara* reduced the disease by 38% and 21% respectively.

Assessment of wilt disease occurrence: For the assessment of wilt disease, noted the symptoms of disease from 21 days to 90 days after planting. Each disease symptoms were observed on the crops. For the calculation of disease incidence noted the symptoms of bacterial wilt disease from all the plots. Symptoms of Bacterial wilt disease appearance of wilt symptoms are started after three weeks from planting. Highest wilting percentage in the earlier part of second month (July 2021) has been noted in the test of T1 (*O. basilicum*) which is found of seven plants wilted out of fifteen. Incidence of bacterial wilt disease has been occurred less than 10% on those plots which are treated with *R. communis*. While the incidences of bacterial wilt disease have been occurred approximately 62 and 79% by the treatment with *O. basilicum* and *L. camara* respectively which are not more significantly in the compare of untreated groundnut fields. Those plots which have treated with extract of *R. communis* shows minimum number of wilted plants in the second month (July 2021) with only one diseased plant. The negative control (T4) had Wilt symptoms are shows on a few plants which have treated by negative control in second months (July 2021) after the planting. Those plots which are treated by extract of *L. camara* shows more wilting plants in second month by (33.33%) with the found of five plants out of fifteen and noted in the third month by (53.33%) with the found of eight wilted plants out of fifteen growing plants. Under the negative control no any wilt occurrence observed possibly due to absence soil-borne pathogen of *R. solanacearum*. Some groundnut growing plots are showing in following images which are treated with the described experiments (T1, T2, T3, T4 and T5).

Fig.- 2 &3: Showing the wilted plots after four weeks which have treat with (T1) *O. basilicum* and (T2) *R. communis.*

Those plots which have treated by positive control (T5) have showed highest wilt incidence plant in the compare of other treated control except T3 during the first month (June 2021) second month (July 2021) after the planting. *O. basilicum* (T1), *L. camara* (T3) Positive control (T5) all had over Above than 60% wilt occurrence have been observed in the tested result of T1, T3 and T5 experiments. All those plots which treated with extract of *L. camara* have been recorded 100% wilting in the second month (July 2021). The extracts of *O. basilicum* and *L. camara* have effective control on the growth of *R. solanacearum* in the in-vitro experiment but not same control on the bacterial disease in the groundnut growing fields. Very little effect observed on control of the incidence of bacterial wilt disease by extracts of *O. basilicum* and *L. camara* so these plants have not shows effective results. Other factors could have affected the growth of *R. solanacearum* which have showed by the experimented observation. According to Weiss [26] the application process of releases plant materials are shows effective control management with the application of the temperature in the greenhouse as was found out. In the integrated disease management (IDM) test the process of biological control found as a normal. To reduce the bacterial wilt incidences, integrative research techniques have been created through plant species that can be intercropped with groundnut plant with helpful supportive microbes to defy soil pathogens and integration of some parts of the plant to reduce survival of *R. solanacearum* [19].

Occurrence of wilt disease: Observation results of wilt incidence are showed in image - 04 and table – 01 which are represented as average of diseased plants in all of treated five experiments. According to results of these above experimented, *R. communis* was found positively effective on the management of bacterial wilt disease of groundnut with controlling power of 93.3%. According to above experimented treatments it is verified that the extract of *R. communis* had powerful antibacterial capacity and successfully management on the disease of bacterial wilt of groundnut by controlling above than 90%. While those extract of other two plants *O. basilicum* and *L. camara* are did not controlled at the bacterial wilt disease and wilt incidences are showed above than 60%. Results of positive control are also not able to consider in reducing the wilt disease. Consumer friendly and environmentally awareness are effectively managed by the metabolities of phytopathogen approach [11].

Susceptible to restrict the isolates of *R. solanacearum* has been controlled by the use of *Casuarina equisetifolia* by branch puncture inoculation. Occasionally shows the symptoms of wilt disease in groundnut fields by the alternate host weed *Ageratum conyzoides* which distributed even in poor quality acidic soils in waste land. Symptoms of wilt disease are develops by the inoculation of disease isolates to groundnut. The environmental conditions that prevail performance of a cultivar is the end product of complex interactions between the genetic latent of the host can be emphasized. So in other hands we can say the disease management techniques are creat the additional knowledge to control the wilt pathogen. By the support of ICRISAT, AVRDC and ACIAR some collaborative programs are being progress in distribution of resistant germplasm. Due to presence of local pathogen situation and environmental conditions a few number of better breeding plans are evaluated. Maximum information be extracted from the trials conducted in the developing countries. Crop rotation trials were intended and executed by suppression capacity of *R. solanacearum* over a period of four years by its application. Groundnut production results are found more than 60% yield with occurrence minimum wilt incidence by the application of *Tagetes spp.* with non host crops. The interaction between plants and bacterial pathogen shows regularly.

The results of fungal extracts have showed deeply control on the bacterial disease in the in-vitro medium. Control management on the growth of *R. solanacearum* was not found similarly of all fungal metabolities. For the anti-bacterial activities generally fungal metabolites of *Trichoderma virens, Trichoderma koningi* and *Trichoderma harzianum* are used.

Khan, R.A.A. *et al* [13] reported by the research study that the results of different *Trichoderma spp*. which have used against *R. solanacearum* and it were observed by evaluation that the metabolites of *T. harzianum* were more effective than other applied strains. Various anti-bacterial compounds are create by the different fungal metabolites which are control the wilt disease in different level.

El-Hasan A. *et al* [6] reported that the effective response of various bioactive compounds of different isolates of *Trichoderma spp.* have different level control on growth of *R. solanacearum*. The causing of cell death was suggested by the effect of lysozyme to break down membrane and cell wall (Saito, H., et al. 2019). These activities can be normally observed in the analytical study of *R. solanacearum* in the micrograph of SEM. The fungal metabolites which have applied to infested soil with the presence of *Ralstonia solanacearum* can control the growth of bacterial population in soil which shows controlling response in the in-vitro experiment. The metabolites of *T. harzianum* were found effective than the other fungal metabolites in the results of in-vitro medium. So it is confirm that metabolites of *T. harzianum* have grater anti-bacterial capacity against the pathogen of *Ralstonia solanacearum*. The productions of ant-bacterial capacity are normally related with the fungal isolates.

According to Khan *et al* [13] an amendment with different application times was used in soil to the control of *R. solanacearum* in groundnut plants. Extra anti-bacterial compounds are released by the stability and compatibility of the experiments in the soil by the exhibited of longer application period. Low cost media was prepared by the extracts of fungal metabolities. The population of soil bacterial have reduced by the concentration of used extract and improved the growth of plants. Biocontrol agents of *Trichoderma* genus are normally used as fungal metabolities.

Application of the *Trichoderma spp*. to the soil is directly control the bacterial wilt disease [16]. A bacterial wilt disease in groundnut crops is easily evaluated and manages by the metabolities of *Trichoderma spp*. To manage the growth of *R. solanacearum* on groundnut crops by the potential of *Trichoderma* metabolites some effective methods are discussed. Many protection techniques are applied in recently times on the growth of *R. solanacearum*. The commercial preparation of *Trichoderma* metabolites are easily provide by this study in the market for the control on growth of *R. solanacearum* in groundnut crops. Study of this research is possibly help in control bacterial disease in other crops and may be useful for field of agriculture and research.

Grimault and Prior are suggested in [8] that tolerant plants which are not shows any symptoms after the infection of bacterial wilt disease can be apply diseased areas. Once transplanted, the plants are Infected plants are develops the immune capacity against to bacterial wilt disease for approximately more than 35 days and not seriously affected after once transplanted the wilt pathogen.

According to Dhital *et al* [4] and Kishore *et al* [14] reduced the wilt disease upto 80% by use of bleaching powder in the soils. According to Janse *et al* [14] promising results for disinfection of infested surface water shows by the hydrogen peroxide and peracetic acids. Develop a biological control method against the bacterial wilt disease by many strategies of wilt control [2, 3]. Nguyen and Ranamukhaarachchi are [18] suggested in 2010 that the biological management of the *R. solanacearum* via isolated bacterial strains from soil. According to Huang and Lakshman [10] the incidence of wilt disease can also reduced by the use of clove oil. The wilt disease is reduced above than 45% by the treatment of chitosan and wilt incidence decrease by above than 80% by the use of *Paenibacillus polymyxa* strain [1]. Wei *et al* reported in 2013 that for bacterial wilt of tomato the strain of *Ralstonia pickettii* used as an effective biocontrol agent. Elhalag *et al* [5] reported in 2018 that the use of bacteriophages are shows positive effect on bacterial wilt disease. Tu *et al* [23] reported in 2020 that various plant species which have different essential oils shows positive effect on casual agent of bacterial disease. He *et al* reported in 2014 that the use of calcium carbonate (CaCO3) in growing fields of groundnut crops help in reducing the incidence of *R. solanacearum* and it has been suggested for controlling of bacterial wilt pathogen by the used of calcium carbonate as a bio-control agent.

Anti-bacterial potential of *Ocimum balisicum, Ricinus communis* and *Lantana camara* are experimented for the control of bacterial wilt disease both in vitro and fields in groundnut crops in this study. The extract of *Geranium carolinianum* is also show the effective results for the control of *R. solanacearum* growth in the soil with solarisation process [19].

Above two treatments *R. communis* and negative control have reduced the wilt incidence by more than 70%. Whereas other three treatments positive control, *L. camara* and *O.bacillacum* have reduced the wilt incidences by less than 40%. Wilt incidence is reduced by treatment of *L. camara* and *O.bacillacum* found only 22% and 37% respectively. In the positive control found more wilt incidence due to apply the inoculation of casual agent *R. solanacearum* but not use any plant extract for the treatment of wilt disease. Wilt incidence is reduced by treatment of negative control by 72% due soil borne casual agent not survive in the presence of soil antiseptic methyle sodium. Treatment by the extract of *R. communis* has effectively reduced the wilt incidence by 93%. *L. camara* (T3) exhibited a very high Maximum wilt incidence is observed in those experimented plots which are treated with extract *L. camara* in the third month (August 2021) with 53.33% (Table- 02).

Table - 02: Average disease occurrence in the groundnut plots

Applied plant extract	Avarage occurrence	Incidence category
Oscimum basilicum	62.64%	A
Lantana camara	76.84%	A
Ricinus communis	7.05%	B
Applied negative treatment	27.98%	B
Applied positive treatment	67.78%	A

Among all of described experimented the treatment by *R. communis* showed effective control with reduced by 93% bacterial wilt disease of groundnut in tested plots. Other two plant extracts have not controlled the wilt disease with more effective. So it is confirmed that the extract of *R. communis* should be use before than planting of groundnut crops directly in the plots to control the bacterial wilt disease.

CONCLUSION

All the three studied plants tested for disease management in the field experiments, *R. communis* was found most potent to control the studied bacterial pathogens. The results of the study revealed the fact that bacterial wilt disease of groundnut can also be effectively managed with economical and eco-friendly means. Studied plants from Sariska National Park can be used to control bacterial pathogen *R. solanacearum* causing loss of the yield and quality of the groundnut crop.

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