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In vitro assay of antibacterial activity of botanical extracts against *Ralstonia solanacearum*

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Abstract
Brinjal (*Solanum melongena*) is considered as the second most important vegetable crop in India. Bacterial wilt caused by *Ralstonia solanacearum* was the major constraint in brinjal. The antibacterial activity of 24 ethanol phytoextracts were investigated against the bacterial wilt pathogen *Ralstonia solanacearum* under *in vitro* by agar well diffusion method at 10% and 20% concentrations. Among all the 24 botanicals tested, *Allium sativum*, showed maximum inhibition zone of 10.62 mm followed by *Allium cepa* showed 10.32 mm respectively at 20% concentration. At 10% concentration, maximum inhibition zone was observed in *Ocimum sanctum* 3.93 mm followed by *Zingiber officinale* showed 3.29 mm respectively.

Keywords: Antibacterial activity, botanicals, inhibition and *Ralstonia solanacearum*

Introduction
The brinjal (*Solanum melongena* L.) belongs to the family solanaceae, is one of the major vegetable crop in India. Bacterial wilt caused by *Ralstonia solanacearum* E.F. Smith (Yabbuchi *et al*., 1995) [3] is a major disease and devastating the production of many economically important crops such as tomato, brinjal, chilli, tobacco, potato and banana (Kelman *et al*., 1994) [7]. This microorganism attacks over 450 plant species distributed in tropical, subtropical and warm temperate regions of the world (Hayward, 1991) [6]. In Odisha, the brinjal is grown in an area of 630.12 thousand ha with production of 8671.95 thousand metric tonnes with the productivity of about 13.76 MT/Ha. (Anon., 2019-20) Cultivation of brinjal facing different diseases problem among those bacterial wilt of brinjal *R. Solanacearum* (Yabuchi *et al*., 1995) [13] is the most harmful disease causes heavy loss up to 39-40 per cent in Odisha region. The bacterial wilt symptoms in brinjal are characterized by wilting of one branch followed by two or three branches followed by complete wilting of the plants. The naturally derived plant products have the capacity to control diseases in plants caused by phytopathogens like fungi, bacteria and phytoplasma (Guleria and Tiku, 2009) [5]. Plant metabolites and plant-based pesticides are appear to be one of the better alternatives because they have a minimal environmental impact and significantly lower risk to consumers than synthetic pesticides. (Gottlieb *et al*., 2002) [4]. Hence, the present study is conducted on *in vitro* evaluation of various plant extractss against *R. solanacearum* causing bacterial wilt in solanaceous and other crops.

Material and Methods
The leaf extracts of 24 plant species (Table 1) were screened for their antibacterial activity against the bacterial wilt pathogen, *R. solanacearum* under *in vitro.* The experiment was designed to evaluate the antibacterial activity of 24 ethanol phytoextracts against *R. solanacearum* by using agar well diffusion method (Mahajan *et al*, 1991) [8]

Isolation of Pathogen
Brinjal plants showing typical symptoms of vascular discolouration caused by *R. solanacearum* were collected and diagnosis of the disease was done by ooze test. The lower part of the infected stem was cut into small pieces aseptically, and surface sterilized in 70 percent alcohol then washed in three series of sterile water to remove traces of alcohol. The infected tissue pieces were then suspended in a test tube containing sterilized water for 10-15 minutes. The bacterial suspension was isolated on the surface of TZC medium with spreader. The inoculated plates were incubated at 30 ^{0}C for 48 hours.

The well-separated typical colonies of *R. solanacearum* were observed and picking up the highly virulent colonies were streaked separately on the surface of TZC medium contained in Petri dishes. Pure colonies of *R. solanacearum* were picked up with sterile inoculation loop and suspended in sterile distilled water and kept in sterile propylene culture tubes then stored at 4°C in refrigerator for further studies.

Collection and Preparation of plant extracts

The botanical species used in the present study were collected from the fields around college campus, O.U.A.T. Fresh extracts from the leaf, bulb (garlic and onion) rhizome (ginger) and fine powder turmeric were used for carrying out the experiment (Table 1). One g of the leaf, bulb and rhizome samples of all the tested plants were collected and washed in tap water and then rinsed in sterile distilled water. 100 g of fresh sample was chopped and macerated in a surface sterilized pestle and mortar by adding 100 ml of ethanol (1:1 w/v). The extract was filtered through Sterilized filter paper disc (Whatman no.1) measuring 10 mm diameter, the filtrate thus obtained was used as a stock solution. To study the antibacterial mechanism of plant extracts at two different concentrations *viz*., 10 per cent and 20 per cent were made by adding sterilized distilled water proportionately. The clear extract was used to test the antibacterial activity against *R. solanacearum* under *in vitro*.

Results and Discussion

The results of *in vitro* efficacy of 24 botanical extracts evaluated against *R. Solanacearum* by agar well diffusion method at 10% and 20% ethanol extract concentrations. Among all the botanical extracts showed the inhibitory effect at 20% concentration was found significantly more than the 10% concentration (Table 1).

At 20% ethanol extract concentration, the antibacterial activity of *A. sativum* was found significantly superior than rest of the plant extract with mean inhibition zone of (10.62 mm) followed by *Allium cepa* showed (10.32 mm). The inhibitory effect of *Ocimum sanctum* (9.64 mm), *Azadirachta indica* (9.17 mm) and *Zingiber officinale* (9.14 mm) were statically at par. The inhibitory effect of *Saraca asoca* (8.73 mm) followed by *Pongamia pinnata* (7.93 mm), *Piper betle* (7.66 mm) and *Lawsonia inermis* (7.58 mm) were also statically at par. *Curcuma longa* showed inhibitory effect of (6.51 mm) followed by *Cymbopogan flexiosus* (6.37 mm) followed by *Calotropis gigantean* showed inhibition zone of (5.69 mm) followed by *Tridax procumbens* (5.65 mm), *Carica papaya* showed (5.64 mm) and *Lantana camara* (5.53 mm) were at par. *Nerium indicum* (4.73 mm), *Aegle marmelos* (4.57 mm) and *Mentha arvensis* (4.37 mm) were at par. *Psidium guajava* showed inhibition zone of (3.73 mm) followed by *Catharanthus roseus* showed inhibition zone of (3.56 mm) were at par. A*loe barbadensis* showed inhibition zone of 1.47mm followed by *Eucalyptus citriodora* showed (1.33 mm). At 20% conc. *Rauwolfia serpentine* and *Nyctanathes arbortristis* (0.00 mm) did not show any inhibition against *R. Solanacearum*

At 10% conc of ehanol extract of botanicals, the antibacterial activity of *Ocimum sanctum* found to be effective and showed maximum inhibition zone of (3.93 mm) followed by *Allium sativum* showed inhibition zone of (3.29 mm) followed by *Allium cepa* showed (3.27 mm) and *Azadirachta indica* showed (3.19 mm) followed by *Saraca asoca* (2.85 mm) *Pongamia pinnata* showed (2.61mm) followed by *Zingiber officinale* showed (2.76 mm), *Cymbopogan flexiosus* (2.47mm) and *Piper betle* (2.08 mm) were at par. *Curcuma longa* showed inhibition zone of (1.95 mm) followed by *Lantana camara* (1.33 mm), and *Carica papaya* (1.28 mm) and *Nerium indicum* (1.13 mm) were at par. *Mentha arvensis* (0.93 mm) and *Tridax procumbens* showed inhibition zone of (0.91 mm). *Lawsonia inermis*

Whereas, *Nyctanathes arbortristis, Aegle marmelos, Psidium guajava, Catharanthus roseus, Eucalyptus citriodora, Aloe barbadensis, Rauwolfia serpentine* and *Calotropis gigantean* (0.00 mm) did not show any inhibition against *R. Solanacearum* at 10% conc of botanicals.

Table 1: Percent inhibition of ethanol extracts of botanicals used to test their antimicrobial properties against *Ralstonia solanacearum*

Treatments	Common name	Scientific name	10%	20%
1	Neem	*Azadirachta indica*	3.19 (2.05)	9.17 (3.19)
2	Onion	*Allium cepa*	3.27 (2.07)	10.32 (3.36)
3	Lemon grass	*Cymbopogan flexiosus*	2.47 (1.86)	6.37 (2.72)
4	Karanj	*Pongamia pinnata*	2.61 (1.90)	7.93 (2.99)
5	Indian snake root	*Rauwolfia serpentina*	0.00 (1.00)	0.00 (1.00)
6	Lantana	*Lantana camara*	1.33 (1.53)	5.53 (2.56)
7	Eucalyptus	*Eucalyptus citriodora*	0.00 (1.00)	1.33 (1.53)
8	Night jasmine	*Nyctanathes arbortristis*	0.00 (1.00)	0.00 (1.00)
9	Bael	*Aegle marmelos*	0.00 (1.00)	4.57 (2.36)
10	Turmeric	*Curcuma longa*	1.95 (1.72)	6.51 (2.74)
11	Indian mint	*Mentha arvensis*	0.93 (1.38)	4.37 (2.32)
12	Periwinkle	*Catharanthus roseus*	0.00 (1.00)	3.56 (2.14)
13	Aloe vera	*Aloe barbadensis*	0.00 (1.00)	1.47 (1.57)
14	Papaya	*Carica papaya*	1.28 (1.51)	5.64 (2.58)
15	Ashoka	*Saraca asoca*	2.85 (1.96)	8.73 (3.12)
16	Ginger	*Zingiber officinale*	2.76 (1.94)	9.14 (3.18)
17	Garlic	*Allium sativum*	3.29 (2.07)	10.62 (3.41)
18	Nerium	*Nerium indicum*	0.00 (1.00)	4.73 (2.39)
19	Calotropis	*Calotropis gigantea*	0.00 (1.00)	5.69 (2.59)
20	Betel vine	*Piper betle*	2.08 (1.76)	7.66 (2.94)
21	Tulsi	*Ocimum sanctum*	3.93 (2.22)	9.64 (3.26)
22	Henna	*Lawsonia inermis*	1.13 (1.46)	7.58 (2.93)
23	Coat buttons	*Tridax procumbens*	0.91 (1.38)	5.65 (2.58)

24	Guava	*Psidium guajava*	0.00 (1.00)	3.73 (2.17)
25	Control		0.00	0.00
	SE(m)±		0.03	0.02
	CD (0.05)		0.09	0.04

Gopalakrishnan *et al.* (2014) [3] observed that *in vitro* assessment of 23 botanicals against *R. solanacearum* tested, garlic extract showed a higher zone of inhibition of about 49 mm diameter at 10% concentration. Sinha K. (2016) [12] also demonstrated the aqueous extract of garlic (*Allium sativum*) at 100% concentration found to be very effective against *R. solanacearum.* (Bannihatti *et al*, 2016) [2] reported that solvent extracts of *A. sativum* (19.10 mm) recorded maximum inhibition zone followed by *A. cepa* (17.38mm) at 20% concentration.

(Singh and Jagtap, 2017) [14] also observed that acetone extracts of *Allium sativum* showed inhibition zone (12.3mm) followed by *A. cepa* (11.1mm) at 20% concentration Phytoextracts of *A. sativum*, *A. cepa*, *C. lunga*, *O. sanctum* and *Z. officinale* were showed antibacterial activity against *R. solanacerum*, demonstrated earlier by several workers (Murthy and Srinivasan, 2012; Owoseni and Sangoyami, 2014; Pankaj *et al.*, 2015) [9, 10, 11].

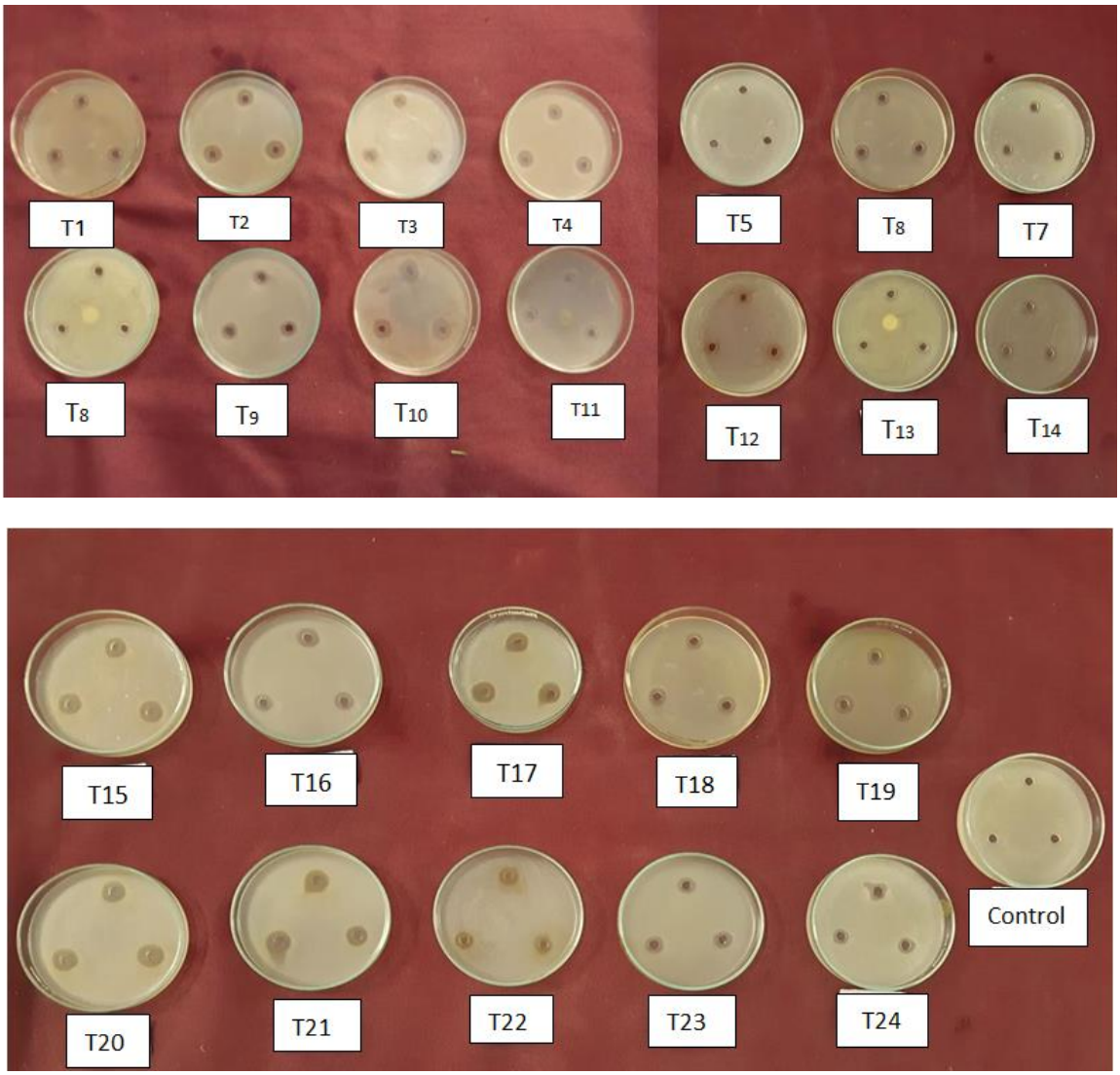

Fig 1: Percent inhibition of ethanol extracts of botanicals at 20% concentration used to test their antimicrobial properties against *Ralstonia solanacearum*

Conclusion

The present investigation revealed that many phytoextracts had inhibitory effect against *R. solanacearum. Allium sativum* exhibited maximum antibacterial activity effective against *R. solanacearum* with inhibition zone of 10.62 mm at 20% concentration. Botanicals are cheap commodities and also most economical and potential alternative to common agrochemicals and antibiotics. Hence their potential can be exploited in the management of bacterial wilt of solanaceous and other crops. Thus, further research is needed to study the antibacterial effect of different phytoextracts against *R. solanacearum*.

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