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Inhibitory effect of plant oils and antibiotics against *Ralstonia solanacearum*

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ABSTRACT: Bacterial wilt of brinjal caused by *Ralstonia solanacearum*, considered as a destructive disease of brinjal crop. An experiment was conducted to test the effectiveness of plant oils and antibiotics against the growth of *Ralstonia solanacearum* under *in vitro* conditions in the Department of Plant Pathology, Odisha University of Agriculture and Technology, Bhubaneswar during 2021-2022. However eight different plant oils were tested. Among them, the maximum inhibition was observed in clove oil (15.47 mm) followed by linseed oil (14.53 mm). The antibiotics Streptocycline (12.57 mm) showed the considerably highest inhibition when tested against *R. solanacearum*, followed by Gentamycin (10.46mm), Ampicillin (9.12 mm), Cefuroxime (8.73 mm), and Chloramphenicol (8.66 mm),Levofloxacin (8.32 mm), Tetracycline (8.11 mm) and Ciprofloxacin showed (7.98 mm) were statistically at par.

Keywords: Antibiotics, inhibition, plant oils and *Ralstonia solanacearum*.

INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum* is considered to be one of the most destructive disease in the tropical, subtropical and temperate regions of the world and causing heavy economic losses (Bawari and Narendrappa 2019). Its broad host range includes more than 200 species in 50 families (Aliye *et al.,* 2008). One of the main challenges to growing brinjal in Odisha is the bacterial wilt disease. *R. solanacearum* is a gram-negative, rod-shaped, strictly aerobic bacterium with a single polar flagellum that measures 0.5-0.7 × 1.5-2.0 m in size. After 36 to 48 hours of growth at 28 °C, individual bacterial colonies are typically visible. In this strain, colonies were very fluidal and had a distinctive pink centre, whereas in other strains, fluidity and the pink centre were less obvious (Sambasivam and Girija 2006). Occasionally, colonies of the mutant or non-virulent type are uniformly rounded, smaller, and butyrous or dry. An experiment was carried out to find out which plant oils, bioagents, and antibacterial chemicals were most efficient at inhibiting the growth of *R. solanacearum* in *in vitro* conditions. Due to higher capacity of the *R. solanacearum* survival in diverse environmental conditions, high pathogenic variability and their existence with an extremely wide host range, the control of infection was a major challenge due to limited possibility for their chemical control, (Nguyen and Ranamukhaarachchi 2010)

MATERIALS AND METHODS

A. Isolation of R. solanacearum from bacterial wilt affected brinjal plant

The wilted brinjal plant samples were collected from the Khurda district of the Odisha. The collected plant samples were washed under tap water to remove the soil particle and air dried. The diseased plant stem bits were first surface-disinfected with 1% sodium hypochloride for one minute followed by repeated washing in sterile water for 50 seconds. Then the sterilized plant bits were transferred to nutrient agar plates and incubated at 28±2˚C for 48 hours. At the end of incubation period, both the virulent and avirulent

colonies of *R. solanacearum* were observed. The virulent colonies of *R. solanacearum* were milky, raised, irregular, fluidal colonies with pink color in the center (Fig. 1).

Fig. 1. Virulent colonies of *R. Solanacearum.*

B. In vitro study of different plant oils in inhibiting the growth of R. solanacearum

Eight different oils were collected from market were evaluated against the *R. solanacearum* under in vitro conditions were tested by well diffusion method (Kamal *et al*., 2008). 5.0 mm diameter wells were made in each agar plate with a sterilized cork borer. Essential oils were loaded into the wells in each petriplate separately and sterile distilled water served as control. Then the plates were placed for incubation at 30±20C for 24 hours. At the end of the incubation period, the zone of inhibition was measured and expressed in millimeters.

C. Evaluation of antibiotics against R. solanacearum

Fourteen antibiotics were tested (Table 2) to test their efficacy in inhibiting the growth of *R. solanacearum* by inhibition zone assay method. The virulent colonies of *R. solanacearum* was inoculated into 250 ml of nutrient broth. The inoculated flasks were incubated at 28^{o}C for 48 hours. Then the nutrient broth was poured onto the sterilized nutrient plates (Bawari and Narendrappa 2019). The antibiotic discs procured from HiMedia, Mumbai were used and the discs were placed aseptically on the petriplates. Then the plates were incubated at 28^{o}C for 48 hours and observed for the zone of inhibition zone around the antibiotic discs. The results obtained were analyzed statistically.

Table 1: *In vitro* study of different plant oils inhibiting the growth of *R. Solanacearum.*

Treatments	Common Name	Scientific name	Plant part used
1	Neem	*Azadirachta indica*	Seeds
2	Karanj	*Pongamia pinnata*	Seeds
3	Eucalyptus	*Eucalyptus occidentalis*	leaves
4	Clove	*Syzygiumaromaticum*	Seeds
5	Mustard	*Brassica rapa*	Seeds
6	Sesame	*Sesamumindicum*	Seeds
7	Coconut	*Cocas nucifera*	Seeds
8	Castor	*Ricinus communis*	Seeds
9	Linseed	*Linumusitatissimum*	Seeds
	Control		

Table 2: List of antibiotics used against the *R. solanacearum.*

Sr. No.	Antibiotics	Dose(mcg/disc)
1.	Levofloxacin	5
2.	Tetracycline	30
3.	Amikacin	10
4.	Cefuroxime	10
5.	Erythromycin	30
6.	Rifampicin	30
7.	Chloramhenicol	5
8.	Cetriaxone	30
9.	Ciprofloxacin	30
10.	Streptocycline	30
11.	Gentamycin	30
12.	Ampicillin	15
13.	Cephotaxime	5
14.	Cefixime	5
15.	Control	

RESULTS AND DISCUSSION

A. Isolation of the R. solanacearum

Isolation of *R. solanacearum* was done from brinjal plant showing characteristics wilt symptoms. Infected plants showed milky bacterial streaming from the cut ends of stem hence were ooze test positive. The virulent colonies of *R. solanacearum* were very small, round, white, slightly fluidal, translucent, slightly raised surface were observed. The well separated colonies were picked up and purified by single colony isolation technique and then suspended in sterile distilled water and stored in plastic vials as stock for future use. The similar findings were also observed by (Chaudhry and Rashid 2011; Sagar *et al*., 2014).

B. Effect of different plant oils on growth of Ralstonia solanacaerum

The efficacy of eight different plant oils against the growth of *R. solanacearum* at 100% concentration (Table 3) were observed. Clove oil (*Syzygium aromaticum*) was found to be most effective in inhibiting the growth of *Ralstonia solanacearum* (15.47mm) followed by linseed oil with 14.53 mm inhibition zone was statistically at par with Karanj (*Pongamia pinnata*) oil (13.64 mm) and Mustard oil (11.41 mm). Neem oil showed zone of inhibition (7.37 mm), followed by Coconut oil showed (3.71 mm), Sesamum oil (2.44 mm) and Castor oil showed inhibition zone of (1.63 mm) were statistically at par. No inhibition zone was observed in control.

Table 3: Effect of different plant oils on growth of *Ralstonia solanacaerum*.

Sr. No.	Essential oils	Scientific name	Inhibition zone (mm)
1.	Mustard oil	*Brassica nigra*	11.41 (3.44)*
2.	linseed oil	*Linum usitatissimum*	14.53 (4.00)
3.	Coconut oil	*Cocos nucifera*	3.71 (2.05)
4.	Sesamum	*Sesamum indicum*	2.44 (1.71)
5.	Neem	*Azadirachta indica*	7.37 (2.80)
6.	Castor	*Ricinus communis*	1.63 (1.45)
7.	Clove oil	*Syzygium aromaticum*	15.47 (4.79)
8.	Karanj oil	*Pongamia pinnata*	13.64 (3.76)
9.	control	water	0.00 (0.70)
	SE(m)		0.026
	C.D.		0.078

*Figures in the parenthesis indicate √x+0.5 transformation values

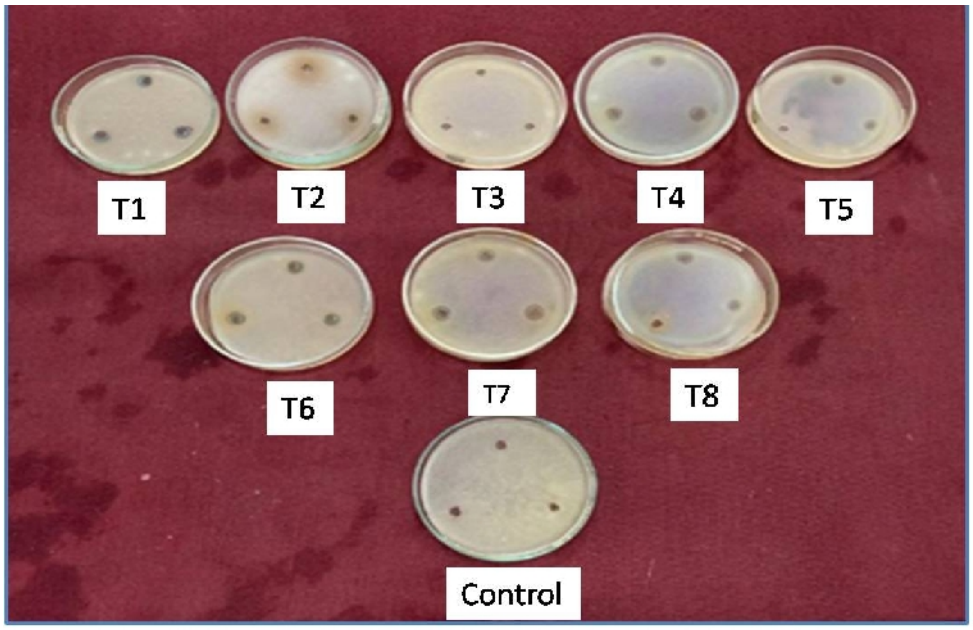

Fig. 2. *In vitro* efficacy of different plant oils against *Ralstonia solanacearum.*

C. In vitro evaluation of antibiotics against R. solanacearum

Fourteen antibacterial chemicals were tested by inhibition zone method to find out their effectiveness against the growth of *R. solanacearum* under *in vitro* condition and the results were presented in Table 4.

All the test antibiotics resulted in inhibiting the *R. solanacearum* growth. The maximum inhibition was observed in Streptocycline (12.57 mm) followed by Cefixime (12.45 mm). Gentamycin showed (10.46 mm) inhibition and Ampicillin (9.12 mm) followed by Cefuroxime (8.73 mm) were statistically at par.

Whereas, Chloramphenicol showed inhibition zone of (8.66 mm) followed by Levofloxacin (8.32 mm), Tetracycline (8.11 mm) and Ciprofloxacin showed (7.98 mm) were statistically at par. No zone of inhibition was noticed in control. Singh and Jagtap (2017) also found that Streptocycline at 400 ppm showed highest inhibition zone of 18.4 mm.

Table 4: *In vitro* evaluation of antibacterial chemicals against *R. Solanacearum.*

Sr. No.	Antibiotics	Zone of inhibition (mm)
1.	Levofloxacin	8.32 (2.96)*
2.	Tetracycline	8.11 (2.93)
3.	Amikacin	7.06 (2.74)
4.	Cefuroxime	8.73 (3.03)
5.	Erythromycin	3.37 (1.96)
6.	Rifampicin	5.41 (2.43)
7.	chloramphenicol	8.66 (3.02)
8.	Cetriaxone	4.54 (2.24)
9.	Ciprofloxacin	7.98 (2.91)
10.	Streptocycline	12.57 (3.61)
11.	Gentamycin	10.46 (3.31)
12.	Ampicillin	9.12 (3.10)
13.	Cephotaxime	4.89 (2.32)
14.	Cefixime	12.45 (3.59)
15.	Control	0.00 (0.70)
	S. Em±	0.67
	CD at (5%)	1.93

*Figures in the parenthesis indicate √x+0.5 transformation values

CONCLUSION

The effect of eight different plant oils on *R. solanacearum* revealed that maximum inhibition was observed in clove oil (15.47 mm) followed by linseed oil (14.53 mm). Huang and Lakshman, (2010) also observed that clove oil showed maximum inhibition against *R. solanacearum.* Kumari *et al.* (2021) also observed that Streptocycline exhibited highest inhibition zone of 28.03 mm at 500 ppm concentration. Singh and Jagtap (2017) also recorded that Streptocycline showed highest inhibition zoneof 18.4 mm and 21.7 mm at 400 and 500 ppm respectively (Murthy and Srinivas 2012; Raghu *et al.*, 2013 and Gupta and Razdan 2013).

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