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In vitro evaluation of different plant oils on growth of *Ralstonia solanacearum* causing wilt disease in *Capsicum annum*

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Abstract

Ralstonia solanacearum (Smith) Yabuuchi *et al.* is considered one of the most destructive plant pathogens in tropical, subtropical and temperate regions worldwide. It is a highly diverse and adaptive bacterium that differs in host range, geographical distribution, pathogenicity, epidemiological interactions and physiological properties. Bacterial wilt in capsicum caused by *Ralstonia solanacearum* is a global problem. It is difficult to control the bacterial wilt caused by *Ralstonia solanacearum* as it survives for longer period in soil, moves along with water, and has wide host range. Hence, in the present research study, the effect of different plant oils were evaluated against the pathogen *in vitro* by inhibition zone technique to find out the effective plant oil for use in management strategy of the disease. The experimental results revealed that, among the plant oils tested at 100% concentration, clove oil (*Syzygium aromaticum*) gives the best inhibitory effect to *Ralstonia solanacearum* with 33.99mm inhibition followed by karanj oil (*Pongamia pinnata*) with 15.66mm while the least inhibition is shown in case of sesamum oil (*Sesamum indicum*) with only 8.44mm inhibition zone against the test pathogen.

Keywords: *Ralstonia solanacearum*, capsicum, bacterial wilt, plant oils, management

Introduction

Capsicum (*Capsicum annum* L. var. *grossum* Sendt) is one of the most popular and highly profitable annual herbaceous vegetable crop belonging to the family solanaceae. China is the world's largest producer of bell peppers, followed by Mexico, Turkey, Indonesia. In India, capsicum is grown under 46 (000Ha) area and production was 327(000MT). The major capsicum growing states are Karnataka, Himachal Pradesh, Madhya Pradesh, Haryana, Maharashtra, Jammu and Kashmir, Jharkhand, Uttarakhand, Odisha, Telangana ^[1] [NHB, 2017]. Odisha produces 6.79(000MT) of capsicum from an area of 0.46(000Ha) in 2016-17. Bell peppers contain 94% water, 5% carbohydrates, and negligible fat (0.17g) and protein (0.86g). They are rich sources of vitamin C, containing 97% of the Daily Value (DV) in a 100 gram reference amount. Bacterial wilt caused by *Ralstonia solanacearum* [Smith ^[2], 1896; Yabuuchi *et al.* ^[3], 1995] is highly challenging and one of the most destructive diseases of solanaceous crops worldwide ^[4] [Hayward, 2005]. Upto 100% yield loss has been reported from wilt prone areas of the world ^[5] [Wang *et al.*, 1997]. Bacterial wilt of Capsicum is caused predominantly by biovars 1 and 3 of *R. solanacearum*. Since they belong to race 1, these biovars have a wide host range that guarantees long-term survival of the pathogen in soil in the absence of the main susceptible crop ^[6] [Champoiseau and Momol, 2008]. It is difficult to control the bacterial wilt caused by *Ralstonia solanacearum* as it survives for longer period in soil, moves along with water, and has wide host range ^[7] [Wang and Lin, 2005]. Owing to its no toxic residual effect, high target specificity and eco- friendly nature of the plant oils, different plant oils were tested for their efficacy in suppressing the growth of the pathogen *Ralstonia solanacearum* in laboratory condition by inhibition zone technique in this research study.

Materials and Methods

The capsicum plants showing wilting symptoms were collected from research field in Instructional farm, OUAT, Bhubaneswar. Isolation of the causal agents from diseased plants was performed by ooze test. The pathogenicity test was conducted by means of Koch's Postulate method on healthy capsicum seedlings of variety KSP-1070 (F1 hybrid) collected from local authorized seed dealer. The test was conducted under pot culture conditions in the Department of Plant Pathology, OUAT. The pathogen was re isolated from the inoculated plants and the isolate is further maintained for conducting *in vitro* evaluation of plant oils against the pathogen.

Eight different oils were collected from nearby homeopathic store for *in vitro* study against the pathogen. Test was done by paper disc method. Filter paper discs were first sterilized in autoclave at 10lbs for 15mins. Then the discs were dipped in different oils for 15 to 20 mins for soaking of sufficient oil. Bacterial suspension was spread on the nutrient agar plate, 3 paper discs were placed with four replications on each plate after immersing those discs in different oils separately. Filter paper discs dipped in sterile water served as check. Then those plates were placed for incubation at 30 ± 2 °C for 24 hours. After incubation inhibition zones were measured. The statistical data analysis was done in Completely Randomized Design (CRD) with 4 replications. The antibacterial activity was recorded as the width (mm) of clear zones of inhibition surrounding the diffusion disc after 24hr and the average zone of inhibition was calculated.

Table 1: List of plant oils used in *in vitro* assay study

Sl. No	Oils used	Scientific name	Place of collection
1.	Karanj	<i>Pongamia pinnata</i>	Local market
2.	Clove	<i>Syzygium aromaticum</i>	Local market
3.	Sesamum	<i>Sesamum indicum</i>	Local market
4.	Virgin coconut	<i>Cocos nucifera</i>	Local market
5.	Almond	<i>Prunus dulcis</i>	Local market
6.	Mustard	<i>Brassica nigra</i>	Local market
7.	Castor	<i>Ricinus communis</i>	Local market
8.	Neem	<i>Azadirachta indica</i>	Local market

Results and Discussion

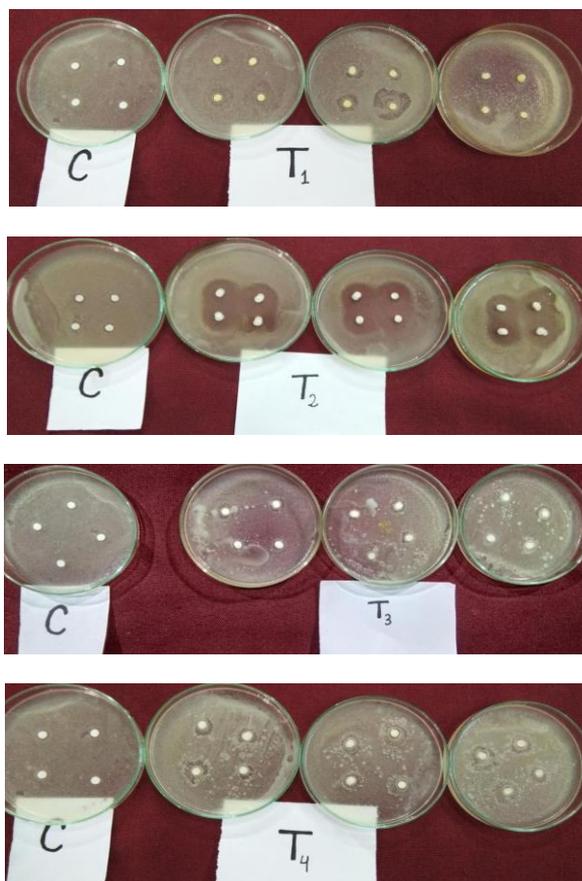
The effect of eight different plant oils at 100% concentration were studied on growth of *R. solanacearum* and formation of inhibition zone around the paper discs were measured at 24 hours after incubation with the help of a scale.

Effect of plant oils on growth of *R. solanacearum*

Sl. No.	Oils used	Scientific name	Treatments	Concentration (%)	Mean zone of inhibition(mm)
1.	Karanj	<i>Pongamia pinnata</i>	T ₁	100	15.66
2.	Clove	<i>Syzygium aromaticum</i>	T ₂	100	33.99
3.	Sesamum	<i>Sesamum indicum</i>	T ₃	100	8.44
4.	Virgin Coconut	<i>Cocos nucifera</i>	T ₄	100	13.11
5.	Almond	<i>Prunus dulcis</i>	T ₅	100	9.55
6.	Mustard	<i>Brassica nigra</i>	T ₆	100	14.66
7.	Castor	<i>Ricinus communis</i>	T ₇	100	11.88
8.	Neem	<i>Azadirachta indica</i>	T ₈	100	13.10
9.	Control		C	100	0.00
SE(m)				1.55	
C.D.				4.64	
C.V.				20.09	

The experimental results revealed that Clove (*Syzygium aromaticum*) oil which was significant over all other treatments has given the maximum inhibition (33.99mm) on the growth of *Ralstonia solanacearum* followed by Karanj (*Pongamia pinnata*) oil with 15.66mm inhibition zone. Mustard (*Brassica nigra*) and Virgin coconut (*Cocos nucifera*), Neem (*Azadirachta indica*) and Castor (*Ricinus communis*), Almond (*Prunus dulcis*) and Sesamum (*Sesamum indicum*) were statistically at par. Sesamum (*Sesamum indicum*) oil has given minimum inhibition of 8.44mm. In control, no inhibition zone was observed.

In an experiment carried out by Huang and Lakshman (2010) to study the inhibitory effects of clove oil against bacteria, 7 bacteria representing 7 genera of plant pathogenic bacteria were used among which *Ralstonia solanacearum* was one of the bacteria chosen to carry out the test. Through Agar diffusion technique followed by them, it was found that among the 7 representative bacteria tested, *Ralstonia solanacearum* was most sensitive against clove oil. Further greenhouse experiments conducted using clove oil as pre-plant soil fumigant showed significant control of wilt disease of Tomato and Geranium caused by *Ralstonia solanacearum* [8]. Hence, it can be observed that similar findings are also made during our study on effectiveness of plant oils against *Ralstonia solanacearum*. Our experiment proved that clove oil has potential inhibitory effects against *Ralstonia solanacearum*.

**Fig 1:** Different plant oils showing zone of inhibition to growth of *R. solanacearum* at 24 hours after incubation (T₁-T₄)

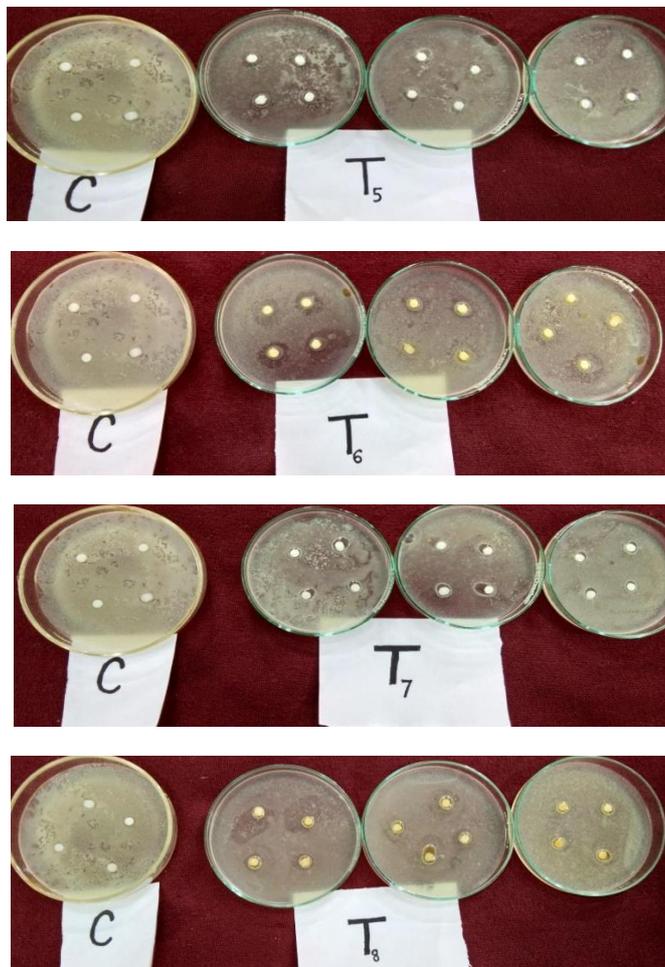
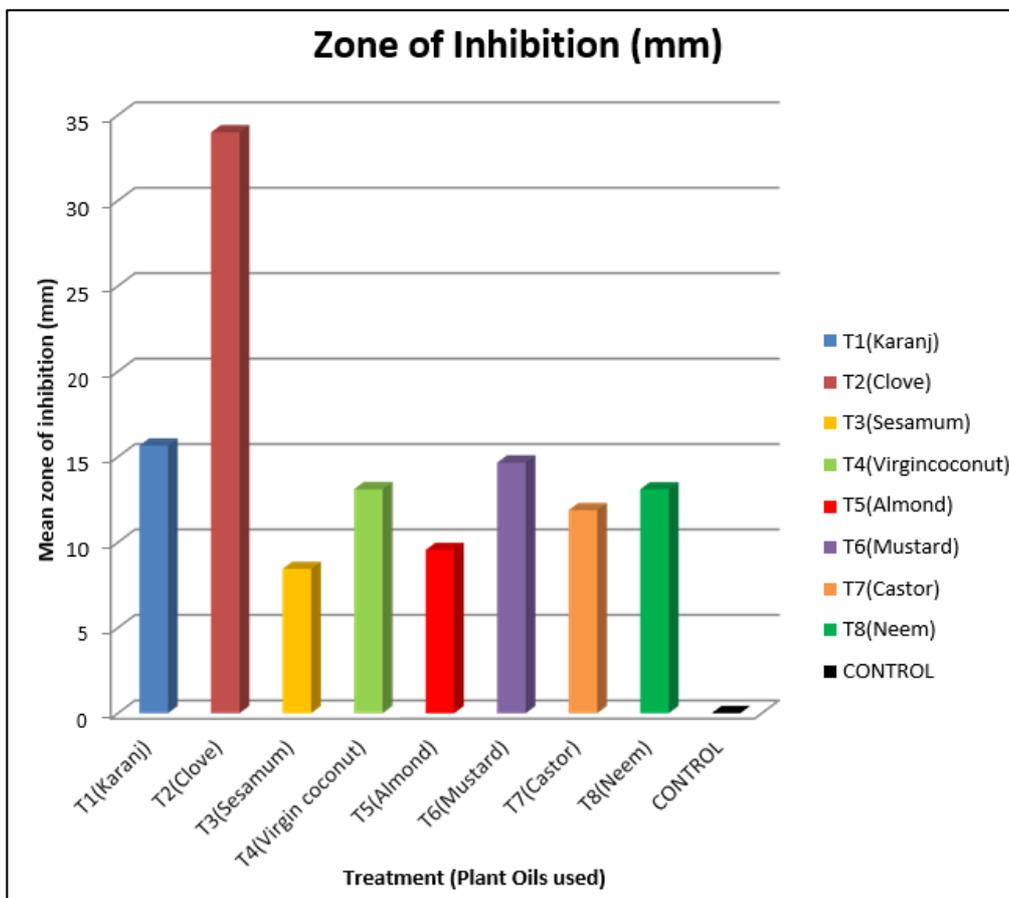


Fig 2: Different plant oils showing zone of inhibition to growth of *R. solanacearum* at 24 hours after incubation (T5-T8)



Zone of Inhibition

Conclusion

From the above experiment we can conclude that among the 8 plant oils tested against *Ralstonia solanacearum* at 100% concentration, clove oil (*Syzygium aromaticum*) gives the maximum antibacterial activity followed by karanj oil (*Pongamia pinnata*). This result indicates that we can go for further assessment through field trials to check the effectiveness of the plant oils in suppressing the growth of the bacteria so that bacterial wilt of capsicum can be effectively managed.

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