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RESEARCH ARTICLE

Eco friendly management of *Ralstonia solanacearum* causing rhizome wilt of ginger with bioagents, botanicals and neem based commercial formulations

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ABSTRACT

Rhizome wilt has been an important threat to the cultivation of ginger. To manage the disease, an *in vitro* evaluation of antagonistic microorganisms, botanicals and commercial neem based formulations were tested against *Ralstonia solanacearum,* an incitant of rhizome wilt. The investigation was carried out to evaluate commercially available plant based pesticides and biological control agents which are relatively safe, economical and non-hazardous and can be used successfully for the management of bacterial diseases in plants. The results of the experiment indicated that Soapnut + Meswak at 20 per cent showed highest inhibition of the bacterium with 1.41 cm inhibition, followed by the combined effect of cow urine + cow dung + lime (fermented for 48 – 72 hours) at 20 per cent which showed an inhibition of 1.25 cm and both the results were on par with each other and were found significantly superior over other treatments. Among the biocontrol agents, *Pseudomonas fluorescens* resulted in maximum inhibition of *Ralstonia solanacearum* and among the neem based commercial formulation, achook has shown significantly superior efficacy at all the concentrations with greater efficacy (1.31 cm) at 30 per cent concentration. Whereas, other products were less and moderately effective among interactions.

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INTRODUCTION

India is considered as a *"magical land of spices"*. No other country in the world has such a diverse variety of spice crops as India. Indian spices are known for their excellent aroma, flavour and pungency not easily matched by any other country. India has been a leading spice-producing, consuming and exporting country of the world. Ginger (*Zingiber officinale* Rosc.) is one of the important spice crops of India. Commercially the dry rhizome is derived from *Zingiber officinale* Rosc. It is an herbaceous perennial, but grown as an annual. It is the member of the family Zingiberaceae. Rhizome wilt has been an important threat to the cultivation of ginger since, it was reported in 1907 by Butler from Surat area in Gujarat. The disease is usually caused by a fungus, bacterium and plant parasitic nematode, *Meloidogyne* spp. The wilts caused by fungus and bacterium ultimately lead to rhizome wilt which is reported to be caused by *Fusarium oxysporum* f. sp. *zingiberi* Trugello and bacteria, *Ralstonia solanacearum* (Smith) Yabuuchi *et al*., in early stage of the crop. *Pythium* spp. is also noticed.

MATERIAL AND METHODS

***In vitro* evaluation of botanicals/organic products on the growth of *R. solanacearum* :**

Fresh plant materials were collected and washed first in tap water and then in distilled water; 100g of fresh sample was chopped and macerated in a surface sterilized pestle and mortar by adding 100 ml of sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth, filtrate thus used as a stock solution. To study the mechanism of plant extracts and organics, inhibition zone assay method was followed.

A heavy suspension (72 hours old) of *Ralstonia solonacearum*, multiplied in nutrient broth (20 ml) was mixed with molten (50°C) nutrient agar medium (1000 ml) contained in an Erleyenmayer's flask, the bacterial suspension was then seeded to the lukewarm nutrient agar medium (1000 ml). The seeded medium was poured into the sterilized Petriplates and plates were allowed to solidify.

Five, ten and twenty per cent each of plant extracts was prepared by mixing 5,10, and 20 ml of stock solution with 95,90 and 85 ml of sterilized distilled water, respectively.

The filter paper discs (Whatman No .44) measuring 5 mm in diameter were soaked in the respective concentrations for 5 min and transferred onto the surface of seeded medium in the Petriplates. The inoculated plates were kept in refrigerator at 5°C for 4 hours to allow the diffusion of chemicals into the medium. The plates were then incubated at 30°C for 72 hours. The observations were taken for the production of inhibition zone around the filter paper discs. The results obtained were analyzed statistically.

At the end of incubation period, observations were recorded for the production of inhibition zone representing the efficacy of plant extracts in inhibiting the growth of pathogen. The inhibition zone in each plate was measured in terms of millimeter in diameter and data obtained were analyzed statistically.

In vitro* evaluation of antagonistic micro organisms on the growth of *Ralstonia solanacearum :

Six biocontrol agents *viz.*, *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma koningii*, *Trichoderma virescense*, *Pseudomonas fluorescens*, and *Bacillus subtilis* were evaluated for their efficacy against the growth of *Ralstonia solanacearum* by inhibition zone assay method. The cultures of these bio control agents were obtained from Department of Plant Pathology, UAS Dharwad, and Institute of Organic farming UAS, Dharwad.

A heavy suspension of *Ralstonia solanacearum* multiplied in nutrient broth (20 ml) was mixed with lukewarm nutrient agar medium (1000 ml) contained in "Erleynemayers" flask. The inoculated flasks were incubated at 30°C for 72 hours. The bacterial suspension was then seeded to the lukewarm nutrient agar medium (1000 ml). The seeded medium was poured into the sterilized Petriplates and plates were allowed to solidify.

Loop full culture of the antagonistic organism was placed on the medium. In case of fungal antagonists, mycelial discs of 5 mm (dia) size taken from actively growing culture were placed in the centre of the plates. The inoculated plates were then incubated at 30°C for 72 hours. The observations for the production of inhibition zone around the antagonistic microorganisms was calculated and analyzed statistically.

***In vitro* evaluation of neem based commercial products on the growth of *R. solanacearum* :**

Four neem based commercial products *viz.*, achook, neem extra power, nimbicidin and neem oil (fighter) were evaluated under *in vitro* condition for their efficacy in inhibiting the growth of *R. solanacearum* at different concentrations 10, 20, 30 per cent by inhibition zone method.

RESULTS AND DISCUSSION

Botanicals next to bioagents are safe, ecofriendly and cost effective means of managing the crop diseases effectively. In the present study, among seven different plant extracts screened along with one organic product against *Ralstonia solanacearum*, none of the plant extracts was found to inhibit the growth of the pathogen completely. However, some of these plant extracts exhibited considerable amount of inhibition (Table 1).

In the light of present day constraints with the use of chemical pesticides in plant disease management, biological control is increasingly occupying the minds of scientists all over the world as they are ecofriendly and cost effective. In the recent years, the use of *Trichoderma* has gained more importance. These antagonistic microorganisms act on the pathogen by different mechanisms *viz.*, competition, lysis, antibiosis, siderophore production and hyperparsitism (Vidyasekaran, 1999).

***In vitro* evaluation of botanicals and organics on the growth of *R. solanacearum* :**

In vitro evaluation of botanicals was carried out with respect to inhibition zone produced due to inhibition of *R. solanacearum* at different concentrations.

Results indicated that soapnut + meswak at 20 per cent showed highest inhibition of 1.41 cm, followed by the combined effect of cow urine + cow dung + lime (fermented for 48 – 72 hours) at 20 per cent which showed an inhibition of 1.25 cm and both the results are at par with each other, which were found significantly superior over other treatments.

Interaction effect among the botanicals and concentration indicated the soapnut + meswak was found most effective at 20 per cent with a inhibition zone of 1.41 cm . There were no significant results obtained in garlic (0.86 cm),

tulsi (0.67cm), soapnut (0.75 cm) and meswak (0.73 cm) and all were on par with each other at concentration of 20 per cent, whereas, turmeric was found ineffective (Table 1).

In vitro evaluation of bioagents on the growth of *R. solanacerum* Mycelial disc method :

The antagonistic microorganisms *viz.*, *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma koningii* Oudem, *Trichoderma virens* Miller Gidden., *Pseudomonas fluorescens* Migula and *Bacillus subtilis* Cohn. were evaluated against *R. solanacerum* under *in vitro* condition by inhibition zone method as explained in the Material and Methods. Inhibition zone produced across the antagonistic microorganisms was recorded.

The results indicated that the antagonistic microorganism,

Table 1 : *In vitro* evaluation of botanicals/organics against the growth of *R. solanacearum*

Sr. No.	Name of the botanical /organic product	Botanical name	Parts used	Mean diameter of inhibition zone (cm) at different concentrations (%)		
				5	10	20
1.	Garlic	*Allium sativum*	Bulb	0.00 (1.00)*	0.66 (1.28)	0.86 (1.31)
2.	Turmeric	*Curcuma longa* L.	Rhizome	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
3.	Tulsi	*Ocimum sanctum* L. cv.Purple	Leaves	0.00 (1.00)	0.60 (1.26)	0.67 (1.29)
4.	Meswak /Toothbrush tree	*Salvadora persica*	Stem	0.60 (1.26)	0.66 (1.28)	0.73 (1.30)
5.	Soapnut/Sapindus	*Sapindus mukorossi*	Seeds + pulp	0.00 (1.00)	0.70 (1.30)	0.75 (1.32)
6.	Meswak (50%) + Soapnut (50%)	*Salvadora persica+ Sapindus mukorossi*	Stem+seeds+pulp	0.81 (1.34)	1.00 (1.41)	1.41 (1.55)
7.	Garlic (50%) + Turmeric (50%)	*Allium sativum+ Curcuma longa* L.	Bulb + rhizome	0.61 (1.26)	0.65 (1.28)	1.00 (1.41)
8.	Cowdung + Cowurine + lime (Fermented for 48-72 hours)		–	0.55 (1.24)	0.67 (1.29)	1.25 (1.50)
9.	Control			0.00	0.00	0.00

Factors	SEm±	CD at 1%
Botanicals/organics	0.0016	0.006
Concentration	0.0010	0.003
Interaction	0.0027	0.010

* Figures in the parenthesis are $\sqrt{x+1}$ transformed values

Table 2 : *In vitro* evaluation of bioagents against the growth of *R. solanacearum* (Mycelial disc method)

Sr. No.	Name of the Bio agent	Inhibition zone (cm)
1.	*Trichoderma harzianum*	0.85 (1.36)*
2.	*Trichoderma viride*	0.60 (1.26)
3.	*Trichoderma koningii*	0.75 (1.32)
4.	*Trichoderma virens*	0.00 (1.00)
5.	*Pseudomonas fluorescens*	1.85 (1.71)
6.	*Bacillus subtilis*	1.00 (1.41)
7.	Control	0.00
SEm±		0.011
CD at 1%		0.049

* Figures in the parenthesis are $\sqrt{x+1}$ transformed values

Table 3 : *In vitro* evaluation of bioagents against the growth of *R. solanacearum* (culture filtrate method)

Sr. No.	Name of the bio agent	Inhibition zone(cm)
1.	*Trichoderma harzianum*	0.60 (1.26)*
2.	*Trichoderma viride*	0.71 (1.31)
3.	*Trichoderma koningii*	0.60 (1.26)
4.	*Trichoderma virens*	0.80 (1.34)
5.	*Pseudomonas flourescens*	1.58 (1.60)
6.	*Bacillus subtilis*	1.00 (1.31)
7.	Control	0.00
SEm ±		0.0122
CD at 1%		0.0546

* Figures in the parenthesis are $\sqrt{x+1}$ transformed values

Table 4: *In vitro* evaluation of neem based commercial products on the growth of *Ralstonia solanacearum* by inhibition zone method

Sr. No.	Neem based products	Chemical content	Mean inhibition zone(cm) at different concentrations		
			10%	20%	30%
1.	Neem extra power	Azadirachtin 1000 ppm	0.61 (1.27)*	0.81 (1.34)	0.85 (1.36)
2.	Achook	Azadirachtin 0.15 %EC	0.60 (1.26)	0.85 (1.36)	1.31 (1.52)
3.	Nimbicidine	Azadirachtin 0.03% EC	0.00 (1.00)	0.61 (1.27)	0.75 (1.31)
4.	Fighter	Azadirachtin1500 ppm	0.00 (1.00)	0.66 (1.29)	0.93 (1.39)

Factors	SEm±	CD at 1%
Neem products	0.0055	0.021
concentration	0.0047	0.021
Interaction	0.0095	0.037

* Figures in the parenthesis are $\sqrt{x+1}$ transformed values

Pseudomonas fluorescens resulted in maximum inhibition of the *Ralstonia solanacearum* with an inhibition zone of 1.85 cm which was found significantly superior over other treatments followed by *Bacillus subtilis* (1.0 cm). Whereas, the fungal antagonists like *T.harzianum*, *T. viride*, *T. koningii* were moderately effective with slight inhibition zone produced around them and the effect of *T. virens* was zero without any inhibition (Table 2).

Culture filtrate method :

Same antagonistic microorganisms were further evaluated by culture filtrate method. The fungal antagonistic micro organisms were grown in potato dextrose broth. Then filtered through Watman Paper-44. This filtrate was taken for evaluation.

Among all the antagonistic microorganisms, *Pseudomonas fluorescens* resulted in maximum inhibition of 1.58 cm followed *Bacillus subtilis* (1.00 cm), whereas all the other fungal biocontrol agents were found least significant. The effect of *T. harzianum* and *T. virens* were least (Table 3).

Sivamani *et al*. (1987) examined the toxicity of *Pseudomonas fluorescens* towards bacterial plant pathogens of banana (*Ralstonia solanacearum*) and rice (*Xanthomonas campestris* pv. *oryzae*). They found that native strains of *Pseudomonas fluorescens* could be effective biocontrol agent against *Ralstonia solanacearum* and *Xanthomonas oryzae*.

***In vitro* evaluation of neem based commercial products on the growth of *R. solanacearum* :**

Four neem based commercial products *viz.*, achook, neem extra power, nimbicidin and neem oil were evaluated under *in vitro* condition for their efficacy in inhibiting the growth of *R. solanacearum* at different concentrations (10, 20, 30 %).

The results indicated that, among the four commercial products tested, achook has shown significantly superior efficacy at all the concentrations with greater efficacy (1.31 cm) at 30 per cent concentration. Whereas, other products were less and moderately effective among interactions. The efficacy goes increasing with increasing concentration. Nimbicidin and fighter did not show any inhibition at 10 per cent concentration (Table 4).

Prasad and Alankara Rao (1987) evaluated the antimicrobial effects of essential oils of five species of ocimum and all the samples showed antibacterial activity against gram positive and gram negative bacteria.

Meena (2007) evaluated the partially purified plant extracts of the growth of *Ralstonia* and *Xanthomonas* bacterium. They found that the Mahua flowers and Satyanashi were effective under *in vitro* disc diffusion technique at 1000 ppm con centration.

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