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Short Communication

Characterization of *Rhizoctonia solani* causing Fruit rot of Strawberry (*Fragaria* x *ananassa* Duch.) in Wayanad and *in vitro* Evaluation of Fungicides, Organic preparations and Bioagents for its Management

Amrutha P. and Reshmy Vijayaraghavan
Department of Plant Pathology, College of Horticulture, Kerala Agricultural University, Vellanikara, Thrissur, India.
Corresponding author Email: amruthapvijayan8@gmail.com

ABSTRACT

Strawberry, one of the most delicate, sweet and refreshing temperate fruit has grabbed the minds of several farmers and consumers all over the world. Several fungal diseases affect it. As part of the study, surveys were carried out in major strawberry growing parts of Kerala *viz*., Wayanad, Idukki, Malappuram and Thrissur. However, rotten fruits with dark and hard encrustations were collected only from Wayanad district during 2015-16. Pathogen was isolated by following the standard protocol and Koch's postulates were proved. Upon culturing, the fungal isolate produced white mycelia turning brown on maturation with rapid growth. The hyphae were branched at right angles and did not produce spores. The pathogen was identified as *Rhizoctonia solani* based on cultural and morphological characters. *In vitro* evaluation was carried out with 9 fungicides and carbendazim 12% + mancozeb 63%, cymoxanil 8% + mancozeb 64%, propineb and Bordeaux mixture at all concentrations showed cent per cent inhibition. Copper hydroxide and difenoconazole inhibited the pathogen from 54.44 to 75 per cent and 58.88 to 70.55 per cent at 0.1, 0.15 and 0.2 and 0.05, 0.1 and 0.15, respectively. Copper oxychloride recorded less than 45 per cent inhibition, whereas carbendazim and potassium phosphonate were found to be least effective. Comparing the efficacy of organic preparations against *Rhizoctonia,* calphomil recorded highest inhibition of 55.33 to 63.88 per cent at different concentrations. Panchagavya and baking powder + vegetable oil mixture could inhibit the mycelial growth only by 23.33 to 25.50 per cent and 22.22 to 26 per cent, respectively. Whereas, neem oil was found to be least effective. Biocontrol agents were evaluated against the pathogen and *Trichoderma asperellum* could restrict growth of the pathogen by 66.67 per cent and *Pseudomonas fluorescens* by 33.33 per cent.

Keywords: Bioagents, Fungicides, Bioagents, *Rhizoctonia solani* and Strawberry

Strawberry (*Fragaria* x *ananassa* Duch.), a delicious fruit, is a hybrid species of *Fragaria* which is cultivated all over the world and is valued for its flavour, aroma and colour. Strawberry is an excellent table fruit and has a great demand in processing industry such as preserves, juices, jam and ice cream. United States is the largest producer of strawberry followed by China and Spain. Panchgami-Mahabaleshwar in Maharashtra accounts for 85 per cent of India's production. Kerala has become an important producer of strawberry as it is being cultivated in high range areas likeMunnar, Vattavada and Kanthalloor of Idukki district and several other places in Wayanad. But, the crop is seriously inflicted by several diseases caused by *Colletotrichum* spp., *Pestalotia* sp., *Gnomonia comari*, *Botrytis cinerea*, *Rhizoctonia solani*, *Alternaria alternata, Rhizopus nigricans* and *Mucor* spp. among which, *R. solani* causing hard rot causes significant yield losses. Dodge and Stevens (1924) first reported rotting of strawberry fruits in Florida incited by *R. solani* that lead to half the loss of total plants. Sharma and Bhardwaj (2001), De Los Santos *et al.* (2003) and Timudo-Torrevilla *et al*. (2005) has also investigated on strawberry fruit rot caused by *R. solani*. As the crop shares a major part of the fruit industry, there is an emerging trend

for the cultivation and processing of the fruits. Thus the present investigation was taken up to study the loss caused by the disease in specific locations of Kerala, symptomatology and characterization of the associated pathogen, managementof the pathogen usingf ungicides, organic preparations and selected biocontrol agents *in vitro*.

Intensive surveys were conducted in different strawberry growing tracts of Kerala *viz.,* Wayanad, Malappuram, Idukki and Thrissur during December-January, March-April and July-August.During the surveyin Ambalavayal, Wayanad during December-January, rotten fruits showing similar symptoms were collected and percent incidencewas recorded (Wheeler, 1969).The pathogen was isolated from infected fruits as per the standard protocol on potato dextrose agar. The cultures thus obtained were maintained at 4°C for further studies. Pathogenicity tests were carried out by placing mycelial bits on fruits wounded with sterile needle and observed for development of symptoms. Symptoms observed during the survey were recorded and the symptoms shown during the artificial inoculation of pathogen for pathogenicity tests were noted down and compared.

Cultural and morphological characters *viz*., colony colour, pigmentations, colour of hyphae, branching pattern, presence of septation on hyphae or conidia were studied. The isolate were identified up to genus level and sent to National Centre for Fungal Taxonomy (NCFT), New Delhi for further confirmation.

Nine fungicides (Table 1) and four organic preparations (neem oil, panchagavya, Calphomil and baking powder + vegetable oil mixture) each at three different concentrations (recommended, lower and higher) were evaluated *in vitro* against *R. solani* by poison food technique (Zentmeyer, 1955). Media without the fungicide or organic formulation served as control. The plates were then incubated at room temperature (26-±1°C). Organic formulations were disinfected under UV light for one hour before mixing with PDA medium to avoid contamination. Tween 20a non-ionic surfactant, @ 0.2 per cent, was added to the mixture of PDA and neem oil to ensure perfect mixing of neem oil with the medium (Neves *et al*., 2001). Then required quantity of each formulation was mixed with the medium and plated. Observations were taken daily noting the growth rate of fungal pathogen until the cultures on control plates attained full growth. The per cent inhibition of mycelial growth in each treatment was recorded (Vincent, 1947).Efficacy of reference cultures of fungal and bacterial biocontrol agents from Kerala Agricultural University (KAU) *viz*., *Trichoderma asperellum* and *Pseudomonas fluorescens* were tested against fungal pathogen of strawberry following dual culture technique (Dennis and Webstar, 1971).

During the survey, rotten fruits with disease were collected during December-January from Ambalavayal, Wayanad. During pathogenicity testing symptoms developed as brownish sunken lesions two days after inoculation, which later turned water, soaked spreading to an area of 4cm^2. Under field conditions, rotten fruits collected from open fields of Ambalavayal were black and hard encrustations were formed on either side of the fruits, which ultimately led to fruit rot that was reported,by Dodge and Stevens (1924) and De Los Santos *et al.* (2003).

Mycelia of pathogen in culture appeared white,later turning light brown producing long thread like hyphae showing fast abundant growth, which attained 90mm within three days in Petri plate. Margin of colony was circular, smooth and hyphae were hyaline gradually turning brown and branching at 90° below the septa with distinct constrictions. Sporulation was absent and the hyphal length ranged from 121.23 to 150.98 µm with new hyphae arising at right angles (Fig. 1). Based on these cultural and morphological characteristics, the isolate was identified as *Rhizoctonia* spp. and for further confirmation, upto the species level, the isolate was send to NCFT, New Delhi where the cultures were identified as *Rhizoctonia solani* and thereafter maintained in the repository with ID. No. 8232.16. These observations were similar with that of Sneh *et al*. (1991), Nechet and Halfeld-Vieira (2007) and Lal and Kandhari (2009) in various crops like pigeon pea and rice.

In vitro screening of fungicides against *R. solani* revealed that carbendazim 12% + mancozeb 63%, cymoxanil 8% + mancozeb 64%, propineb 70WP and Bordeaux mixture at all concentrations were 100 per cent effective (Fig. 2). According to Srinivas *et al*. (2013) and Raj *et al*. (2016), carbendazim 12% + mancozeb 63%showed cent per cent and propineb 96.27 per cent efficacy against *R. solani* of rice and chilli. However, copper hydroxide and difenoconazole

Table 1: *In vitro* evaluation of fungicides against *Rhizoctonia solani*

Fungicide	Conc (%)	Per cent Inhibition *Rhizoctonia solani*
Carbendazim 12% + Mancozeb 63%	0.15	100(10)[a]
	0.2	100(10)[a]
	0.25	100(10)[a]
Cymoxanil 8% + Mancozeb 64%	0.15	100(10)[a]
	0.2	100(10)[a]
	0.25	100(10)[a]
Copper hydroxide 77WP	0.1	54.4(7.45)[f]
	0.15	70.87(8.38)c
	0.2	75(8.7)b
Copper oxychloride 50WP	0.2	38.88(6.29)[i]
	0.25	41.66(6.51)[h]
	0.3	43.55(6.67)[g]
Propineb 70WP	0.25	100(10)[a]
	0.3	100(10)[a]
	0.35	100(10)[a]
Carbendazim 50WP	0.05	0(.7)
	0.1	0(.7)
	0.15	0(.7)
Difenconazole 25EC	0.05	58.55(7.69)[e]
	0.1	67.31(8.19)[d]
	0.15	70.44(8.4)[c]
Potassium phosphonate	0.25	0(.7)[j]
	0.3	0(.7)[j]
	0.35	0(.7)[j]
Bordeaux Mixture	0.5	100(10)[a]
	1	100(10)[a]
	1.5	100(10)[a]
CD (0.05)		0.031

*Mean of the three replications
In each column figure followed by same letter do not differ significantly according to DMRT.
"x+0.5 transformed values are given in parantheses

inhibited the pathogen from 50 to 70 per cent (Table 1). Apart from other fungicides, copper oxychloride recorded less than 45 per cent in inhibiting pathogen. Conversely, Srinivas *et al*. (2013) and Raj *et al*. (2016) recorded 70 to 100 per cent inhibition of *R.solani* of rice and chilli with copper oxychloride. Carbendazim and potassium phosphonate were found to be the least per cent effective. Nevertheless, Seema *et al*. (2010) pointed out higher efficacy of Carbendazim in inhibiting *R. solani* of tobacco.

Among all the organic preparations tested against *R. solani*,calphomil recorded the highest inhibition of 55.33 to 63.88 per cent at different concentrations

(Fig.2) while neem oil was found the least effective. However, panchagavya and baking powder + vegetable oil mixture could restrict the mycelial growth upto 26 per cent (Table 2). Several workers pointed out the antifungal activity of panchagavya against *R. solani* (Dogra, 2006; Ashlesha and Paul, 2014 and Jandaik and Sharma, 2016) in capsicum. Observations on the *in vitro* evaluation of *R. solani* with *Trichoderma asperellum* tested 66.67 per cent inhibition and *Pseudomonas fluorescens* exhibited only 33.33 per cent control (Fig. 2). In congruence with above findings, Amin and Razdan (2010), Seema and Devaki (2012) and Srinivas *et al*. (2013) noticed upto 63.52 and 71.4 per cent control over *R. solani*with *Trichoderma viride* infecting tomato, tobacco and rice.However, Mezeal (2014) noted a higher inhibition of 81.30 per cent with *P. fluorescens*against *R. solani* from tomato whereas, Tapwal *et al*. (2015) reported an inhibition of only 1.45 per cent and 5.10 per cent with *T. viride* and *T. harzianum* when tested against *R. solani*.

Table 2: *In vitro* evaluation of organic preparations against *R.solani*

Sl. No.	Formulation	Conc (%)	Per cent inhibition
1.	Calphomil	0.2	55.33(7.47)^{c}
		0.25	58.33(7.67)^{b}
		0.3	63.88(8.01)^{a}
2.	Neem oil	0.15	0
		0.2	0
		0.25	0
3.	Panchagavya	2.0	23.33(4.88)^{g}
		3.0	25.38(5.02)^{f}
		4.0	25.50(5.11)^{e}
4.	Baking powder + Vegetable oil	0.2	22.22(4.76)^{h}
		0.25	23.40(4.8)^{g}
		0.3	26.0(5.16)^{d}
CD(0.05)			0.019

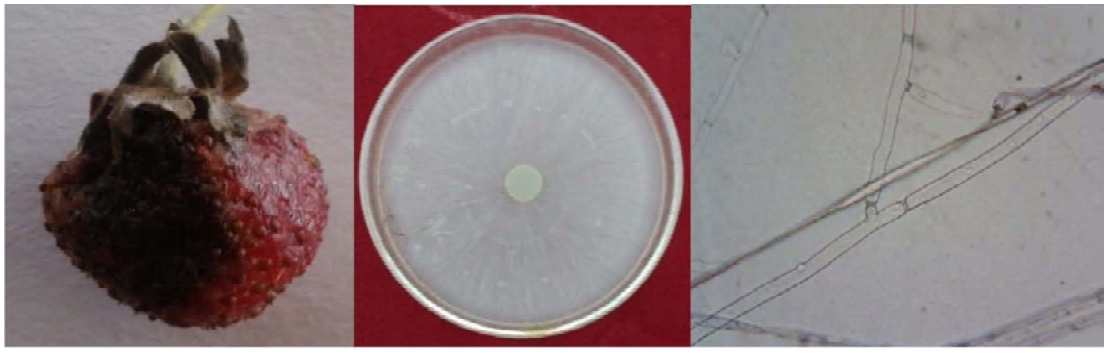
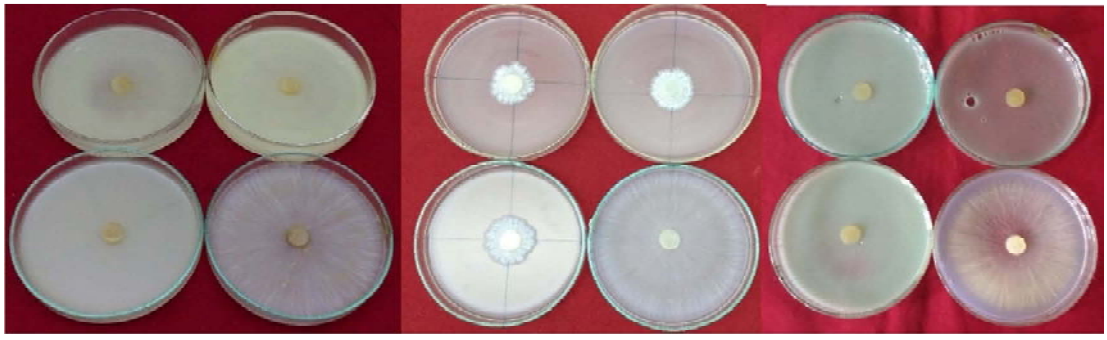
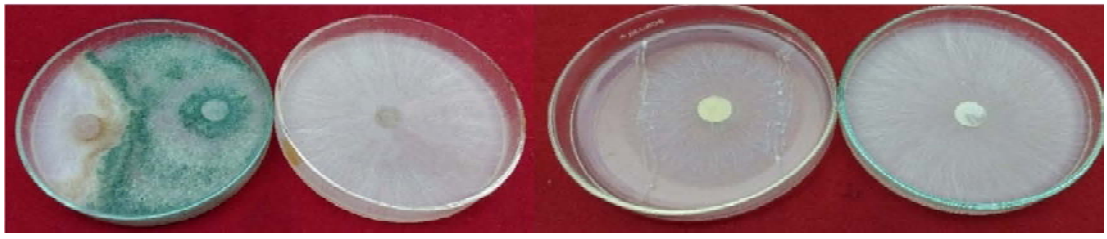


Fig. 1: (a) Rhizocotonia solani infected straw berry fruit, (b) R. solani on PDA,
(c) Hyphae of R. solani with right angle branching.



a) Cymoxanil 8%+ Mancozeb 64% b) Difenoconazole 25EC c) Carbendazim 12%+ Mancozeb 63%

d) Copper oxychloride 50WP e) Calphomil f) Neem oil



g) *Trichoderma asperellum* h) *Pseudomonas fluorescens*

Fig. 2: *In vitro* evaluation of fungicides and organic formulations against *Rhizoctonia solani*

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