Mysore J. Agric. Sci., 50 (3) : 514-520, 2016

Cultural, Physical Studies and Management of Tomato (*Solanum lycopersicum* Mill.) Root Rot Pathogen, *Rhizoctonia solani* Kuhn through Bio-Agents and Leaf Extract

EMAD ABD ATIA , Y. M. SOMASEKHARA AND C. GOVINDARAJU
Department of Plant Pathology, College of Agriculture, UAS, GKVK, Bengaluru-560 065

ABSTRACT

The cultural characters of *Rhizoctonia solani* were studied on the eleven different solid media, the maximum radial growth of *R. solani* was observed on Sabouraud's agar medium (80.50 mm) followed by Tomato extract agar (77.33 mm) and least colony diameter was observed in Richard's agar (49.50 mm). The favorable temperature and pH for the growth of *R. solani* was found with range from 25-30^{0}C and pH of 6 to 7. The efficacy of seventeen fungal and two bacterial bio-agents and nine leaf extracts were evaluated *in vitro* and under glass house condition against tomato root rot pathogen, *Rhizoctonia solani*. The per cent inhibition of fungal growth by *Trichoderma viride*-22-IIHR (88.96 %), *T. harzianum*-55-IIHR (88.34 %) showed maximum inhibition of *R. solani* in *in vitro* and reduced root rot incidence of tomato under glass house condition. Germination of tomato seeds increased in soil treated with *T. viride*-22-IIHR *and T. harzianum*-55-IIHR treated soil in glass house condition. The leaf extract of Tulsi, Simarouba, Lantana and Pongamia were showed maximum inhibition of *R. solani in vitro.*

THE widespread soil borne pathogen *R.solani* is responsible for serious damage to many economically important horticultural crops worldwide (Grosch *et al*., 2006). The different solid media had the most effect on the growth of fungal by effect on radial growth of mycelium, sclerotia production and colour of the colony. Veerendra Kumar (2004) studied the colony characters and sclerotia production of *Rhizoctonia* on different media. PDA showed mycelium having black colour with flat growth with uniform margin, but, a slightly raised growth with uneven margin was seen in Czapeck's agar and with the light black colour mycelium on Rose Bengal Agar.

Temperature plays an important role in the development offungal mycelia and spores which in turn influence the ability ofthe pathogen to incite infection in the host plant and the subsequents pread of the infection (Amborabé *et al*., 2005). Successful penetrations into host cells depend upon several fungal extra cellular enzymes which are affected by the rise or fall in temperatures (Freire, *et al.,* 1998: Webster and Weber 2007). Most fungi are able to grow in a wide pH range with an optimum between 5.5 and 8.0 (Datta *et al*., 2014). The pH of the medium of the fungal growth is important for the secretion of various fungal excreta necessary for the invasion of the host cell and the establishment of the disease.

Different methods have been used to control *R. solani,* being the most used cultural practices, solarization, chemical and biological control. This last method has been developed successfully during the last years. It is based on the reduction of inoculums or of pathogenic activity due to the natural presence of one or more organisms, through the management of the environment, the host or antagonists (Baker and Cook, 1974). The most common method for controlling these pathogens is the use of fungicides, but, the development of resistance in pathogenic fungi to common fungicides and increasing residual hazardous effects on human health and environmental pollution has given a thrust to search for new plant derivatives that can obstruct the fungal pathogenicity. Use of natural products for the management of fungal diseases in the plants is considered as a good alternate to synthetic fungicides, due to their less negative impact on the environment. Many higher plants and their constituents have been successful in plant disease control and proved to be safe and non phytotoxic; unlike chemical fungicides. Three weedy plants *viz., Lantana camara* and *Capparis decidua* has been used for

this purpose (Sharma & Kumar, 2009; Mangang *et al.*, 2012).

Bio-control of soil-borne plant pathogens affecting agricultural plants can be controlled by the use of species of *Trichoderma, Aspergillus, Trichothecium* and *Epicoccum* in India. There are some antagonistic bacteria like *Bacillus subtilis*, *Enterobacter aerogenes, Pseudomonas fluorescence, Streptomyces spp.* and *Actinomycetes* in disease control. Chemical pesticides have already been proven to cause adverse environmental effects and result in health hazards to human as well as other organisms including beneficial natural enemies. So there is need to develop safer and environmentally feasible control alternatives. Biological control, i.e., the use of biological processes to lower inoculums density of the pathogen in order to reduce the disease producing activities thereby reducing crop loss, is a potential non hazardous alternative (Chet,1990) The objective of present study studied cultural, physiological studies for the growth of the pathogen and management of *R. solani* by using plant extract and bioagents.

Material and Methods

The various temperature (10,15, 20, 25, 30, 35 and 40°C), pH (4, 5, 6,7, 8, 9and 10) and media (Yeast extract, starch agar, malt extract agar, carrot extract, Richard's agar, Oat meal agar, Mathurs agar, PDA, Czapek agar dox media, Sabouraud's agar and host extract) were studied against tomato root rot pathogen *R. solani*. *In vitro* evaluation was carried out with 19 bio-agents against *R. solani* through dual culture technique. Nineteen bio-agents, seventeen fungal and two bacterial bio-agents were taken. Both bio-agents and test fungus were cultured on potato dextrose agar in order to get fresh and active growth of fungus.

Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile petri plates and allowed to solidify. For evaluation of fungal biocontrol agents, mycelial discs of test fungus were inoculated at one end of the petri plate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist, the bacterium was streaked one day earlier at one end of the petri plate at the middle of the petri plate and the test fungus placed at the other end. The plates were incubated at 27±1°C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

Affective of Six bio-agents viz., *T. viride*-16-IIHR, *T. viride*-22-IIHR, *T. viride*-GKVK1, *T. harzianum*-41-IIHR, *T. harzianum*-55-IIHR and *T. harzianum*-58-IIHR against *R. solani* were evaluated under glass house condition. The antagonist grow in potato broth and make talc based product 20 g of talc powder (10^8cfu / g) was mixed in *R. solani* infested soil. Each of the bio-agent was replicated three times, tomato seeds were sown and the observations on per cent of seed germination and per cent seedlings stands were recorded.

Nine locally available leaf extracts (Calotripis, Lantana, Lemon Grass, Neem, Simaroba, Tulsi, Nagadhale, Pongamia, Subabul) were evaluated against *R. solani* by following the procedure given by Gerard Ezhilan *et al.* (1994) with slight modification. Fresh plant leaves are washed with tap water and sterilized water. It was then processed with sterile distilled water at the rate of 1 ml g-1 of tissues (1:1 v / w) with the pestle and mortar and filtered through fine cloth. This also formed the standard leaf extract solution (100 %). The extract of different plant leaves are incorporated and sterilized at 1.1 kg cm^2 for 20 minutes.

Results and Discussion

Fungus showed slight difference in their growth on different solid media (Table I). Among different solid media, the maximum radial growth of *R. solani* was measured on Sabouraud's agar with mean colony diameter of 80.50 mm followed by Tomato extract agar (77.33 mm) and least mean colony diameter of 49.50 mm was observed in Richard's agar the optimum growth of the fungus was observed in Yeast extract agar (65.50), Carrot extract agar (64.17), Czapek dox agar (62.00) and Oat meal agar (60.50). Monga and Sheo Raj (1994), reported same result in their study

TABLE I

Growth of R. solani *on different culture media*

Media	Mycelium growth (mm)
Carrot extract agar	64.17
Richard's agar	49.50
Yeast extract agar	65.50
Oat meal agar	60.50
Mathur's medium	57.00
Malt extract agar	52.00
Czapek dox agar	62.00
Potato dextrose Agar	51.50
Starch agar	59.83
Tomato extract agar	77.33
Sabouraud's agar	80.50
Mean	61.80
CD @ 5 %	2.55
S.Em±	0.86
CV%	2.42

on root rot of cotton which caused by *R. solani* that the best medium for pathogen growth it was Sabouraud's agar and cotton (host) extract agar.

This experiment was conducted to study the effect of temperature on growth and production of sclerotia at different temperature and results are presented in the Table II. The maximum mycelial growth of *R. solani* was observed at 30^{0}C, the growth of the fungus in this treatment was 52.83 mm, this was followed by 25^{0}C, the growth of the fungus in this temperature was 49 mm. The least fungal growth of the fungus (9.17 mm) was observed at 10^{0}C. The fungal growth in 40^{0}C was 38.66 mm, respectively. The fungus grew well at 30^{0}C (Table II).

The maximum mycelial growth of *R. solani* was obtained at pH of 6, the dry matter weight of the fungus in this treatment was 116.67 mg this was followed by the pH 7, the dry mater weight of the fungus in this pH was 103.33 mg and in pH 8 it was 100.00 mg. The least dry weight of the fungus (60.00 mg) was observed in pH 10. The dry weight of the fungus in pH 4, 5 and 9 was 70.00, 76.67 and 70.00 mg, respectively. The fungus grows well at the pH 6 (Table III). The maximum mycelial growth of all isolates was found at

TABLE II

Effect of different temperature on growth of R. solani

Temperature	Mycelium growth (mm)
10 ºC	9.17
15 ºC	13.00
20 ºC	13.50
25 ºC	49.00
30 ºC	52.83
35 ºC	42.67
40 ºC	38.67
Mean	31.26
CD @ 5 %	2.56
S.Em±	0.83
CV%	4.60

TABLE III

Effect of different level of pH on growth of tomato root rot pathogen, R. solani

pH level	Mycelial dry weight (mg)
4	70.00
5	76.67
6	116.67
7	103.33
8	100.00
9	70.00
10	60.00
Mean	85.24
CD @ 5 %	5.02
S.Em±	1.63
CV%	3.31

30°C and the optimum pH for maximum radial growth was 6 (Goswami *et al*., 2011). The best growth of *R. solani* was at pH 6.0 and temperature 30°C for all treatments (Datta *et al*., 2014).

The maximum inhibition of mycelial growth (88.96 %) was observed in *T. harzianum*-55-IIHR,

which was followed by *T. viride*-22-IIHR (88.34 %), whereas, *T. viride*-14-IIHR recorded least (47.54 %). *T. viride*-NBAIR, *P. fluorescens*, *T. harzianum*-NBAIR, *T. harzianum*-58-IIHR and *T. harzianum*-20-IIHR also were effective against *R. solani*, per cent inhibition was recorded (87.12, 87.12, 86.50, 85.28 and 82.82%). *T. viride*-GKVK2 (79.75 %), *T. viride*-GKVK1 (78.83%), *T. viride*-52-IIHR (77.91 %), *T. harzianum*-2-IIHR (74.85%), *T. viride*-16-IIHR (72.39%) and *T. harzianum*-41-IIHR (70.55 %) found effective. The results, thus obtained are presented in Table IV.

Upadhyay and Rai (1983) observed the coiling of hyphae in *R. solani* by *T.virens.* The interaction between *T. harzianum* and *R. solani* was studied by Benhamou and Chet (1993) and reported that coiling of *T. harzianum* hyphae around *R. solani* was an early event preceding hyphal damage; the contact between the two fungi was mediated by a fine extra cellular matrix originating from cells of *R. solani*. Of the five fungal bio-control agents tested, *in vitro* against *R. solani*, *T. harzianum* was the most effective in causing significant suppression (60 %) of both growth and sclerotia formation and followed by *T. virens*

TABLE IV

In vitro evaluation of bio-agents against R. solani

Bio agents	Radial Growth of Trichoderma	Radial Growth of R. Solani	Per cent inhibition of R. solani by antagonist	Mode of parasitism
T.viride-GKVK1	72.33	11.50	78.83 (62.61) *	+++
T.viride-GKVK2	90.00	11.00	79.75 (63.26)	++
T.viride-GKVK3	90.00	17.67	67.48 (55.23)	++
T.viride-NBAIR	90.00	7.00	87.12 (68.96)	+++
T.harzianum-NBAIR	68.75	7.33	86.50 (68.45)	++
T.harzianum-2-IIHR	77.50	13.67	74.85 (59.90)	+++
T.viride-13-IIHR	62.75	27.00	50.30 (45.17)	+
T.viride-14-IIHR	50.50	28.50	47.54 (43.59)	-
T.viride-16-IIHR	90.00	15.00	72.39 (58.31)	+++
*T.harzianu*m-19-IIHR	53.75	20.50	62.27 (52.10)	-
T.harzianum-20-IIHR	90.00	9.33	82.82 (65.54)	++
T.viride-21-IIHR	77.50	16.50	69.63 (56.56)	+++
T.viride-22-IIHR	90.00	6.33	88.34 (43.59)	+++
T.harzianum-41-IIHR	90.00	16.00	70.55 (59.90)	+++
T.viride-52-IIHR	73.00	12.00	77.91 (45.17)	++
T. harzianum-55-IIHR	90.00	6.00	88.96 (65.54)	+++
T.harzianum-58-IIHR	90.00	8.00	85.28 (58.31)	+++
B. subtilis-NBAIR	-	26.83	50.61 (52.10)	-
P. flourecense-NBAIR	-	7.00	87.12 (68.96)	++
Control	-	54.33	-	
Mean	79.18	16.08	74.12	
CD @ 5%	2.93	1.24	1.43	
S.Em±	1.02	0.43	0.50	
CV%	2.23	4.67	1.50	

*The value in the parenthesis is arc sine transformed

(-) No parasitism; (+) Weak parasitism; (++) Medium parasitism; (+++) Strong parasitism.

(50 %). Further, *T. harzianum* against *R. solani* was found to effective was reported by Bunker and Mathur (2001). Under *in vitro* conditions, the bio-control agents, *T. viride* and *T. harzianum*, significantly inhibited the growth of *R. solani* as reported by Rehman *et al.* (2012). Several reports are available in use of *T. viride* in the management of *R. solani* (Choudhury *et al.,* 2003; Prasad, 2005; Paramasivan *et al.,* 2007; Rini and Sulochana, 2007).

T. viride-22-IIHR showed the maximum per cent of germination of tomato seeds, it was 83.33 per cent followed by *T. harzianum*-55-IIHR (70.00 %), *T. viride*-16-IIHR (68.33 %), *T. viride*-GKVK 1 (63.33 %) and *T. harzianum*-41-IIHR (53.33 %). *T. harzianum*-58-IIHR showed less effective against *R. solani* it was 45.00 per cent and the per cent seed germination in untreated control was 26.67 per cent

TABLE V

Evaluation of bio-agent on tomato root rot disease (R. solani) under glass house

	Per cent germination	Per cent seedlings stands after 21 days	Per cent incidence
T. viride-16-IIHR	68.33 (55.77) *	60.78 (51.23)	39.22 (38.77)
T. viride-22-IIHR	83.33 (66.14)	77.83 (62.11)	22.17 (27.89)
T. viride - GKVK1	63.33 (52.78)	59.17 (50.32)	40.83 (39.68)
T. harzianum-41-IIHR	53.33 (46.91)	50.78 (45.45)	49.22 (44.55)
T. harzianum-55-IIHR	70.00 (56.81)	64.05 (53.16)	35.95 (36.84)
T. harzianum 58-IIHR	45.00 (31.08)	42.00 (40.39)	58.00 (49.61)
Control	26.67 (42.13)	25.18 (30.12)	74.82 (59.88)
Mean	58.57	42.20	45.74
CD @ 5%	4.36	4.48	4.48
S.Em±	1.42	1.45	1.45
CV%	4.88	5.30	5.94

*The value in the parenthesis is arc sine transformed

(Table V). The maximum per cent stand of seedlings obtained in *T. viride*-22-IIHR (77.83 %), *T. harzianum*-55-IIHR (64.05 %), *T. viride*-16-IIHR (60.78 %), *T. viride*-GKVK1 (59.17 %), *T. harzianum*-41-IIHR (50.78 %), *T. harzianum*-58-IIHR (42.00 %) and in untreated control seedling stand was 25.18 per cent. The antagonist *T. viride*-22-IIHR and *T. harzianum*-55-IIHR reduced wilt incidence and obtained good seedling stands. Neha and Dawande (2010), has been found that the diseases caused by soil borne plant pathogen *R. solani* can be controlled by the antifungal activity of *Trichoderma* spp. and *P. fluorescens*. Five isolates of *P. fluorescens* along with both strains of *P. aureofaciens* significantly inhibited the growth of *R. solani* which caused cotton seedling damping-off disease as reported by Samavat *et al*. (2014). *T. harzianum*, *Epicoccum* sp. are effective bio-agents in controlling black scurf and dry rot of potato caused by *R. solani* and *Fusarium sambucinum* and could be considered as promising alternative to chemical products (El-Kot, 2008).

Tulsi leaf extract recorded maximum per cent inhibition of the fungus, the per cent inhibit was 29.69, 42.99 and 57.96 per cent at 10, 20 and 30 per cent, respectively followed by Simarouba leaf extract. In Simarouba leaf extract, the per cent inhibit was 28.66, 53.82 and 55.41 per cent at 10, 20 and 30 per cent, respectively. Lantana showed effective against fungus, the per cent inhibit was 43.42, 51.91 and 52.55 per cent at 10, 20 and 30 per cent, respectively. In Pongamia the per cent inhibit was 40.76, 42.04 and 52.87 per cent at 10, 20 and 30 per cent, respectively. Calotripis showed least per cent inhibition of the fungus. In this treatment recorded fungal growth up to 20.06, 28.24 and 37.26 per cent at 10, 20 and 30 per cent, respectively. However, the Tulsi, Simarouba, Lantana and Pongamia leaf extract found to be effective against tomato root rot pathogen, *R. solani* (Table VI) Shivapuri *et al*. (1997) studied the different plant extracts, among them, Pongamia leaf extracts was found to be effective against root rot pathogen, *R. solani*. The inhibition effect against *R. solani* may be due to production of fungistatic properties. Different Ocimum speices (Tulsi) can used as a potent natural antifungal agent against of *Rhizoctonia solani* as reported by Senthi *et al.* 2013.

TABLE VI

In vitro evaluation of leaf extract against R. solani

Plant extract	Common Name	Concentration (%) / Per cent inhibition over control			Mean
		10	20	30	
Calotropis gigantean	Calotripis	20.06 (26.61) *	28.24 (32.09)	37.26 (37.62)	28.52
Lantana camara	Lantana	43.42 (41.22)	51.91 (46.10)	52.55 (46.46)	49.29
Cymbopogon flexuosus	Lemon Grass	31.53 (34.16)	35.03 (36.29)	40.13 (39.31)	35.56
Azadirachta indica	Neem	39.58 (38.98)	40.13 (39.30)	43.63 (41.34)	41.11
Simarouba glauca	Simarouba	28.66 (32.37)	53.82 (47.19)	55.41 (48.11)	45.97
Ocimum tenuiflorum	Tulsi	29.69 (33.01)	42.99 (40.97)	57.96 (49.58)	43.55
Ruta graveolens	Nagadhale	33.44 (35.33)	37.58 (37.81)	44.27 (41.71)	38.43
Pongamia pinnata	Pongamia	40.76 (39.68)	42.04 (40.42)	52.87 (46.64)	45.22
Leucaena leucocephala	Subabul	41.08 (39.86)	43.31 (41.16)	46.82 (43.17)	43.74
Mean		34.25	41.67	47.88	41.27
CD @ 1%		Plant extract:0.91; Concentration:0.52: P×C:1.58			
S.Em±		Plant extract:0.17; Concentration:0.06: P×C:0.52			

*The value in the parenthesis is arc sine transformed

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(*Received :* October, 2015 *Accepted :* May, 2016)