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Effect of fungicides and plant extracts on radial growth and sclerotia formation of *Rhizoctonia solani* causing web blight of urdbean

Shalu Chandel, Sanjeev Kumar, Mayank Bishnoi and Balkishan Chaudhary

Abstract
Twelve individual and combination fungicides *viz*., Tebuconazole, Thiophanate methyl, Azoxystrobin, Fluopyram, Mancozeb, Propineb, Metalaxil + Mancozeb, Tebuconazole + Trifloxystrobin, Fluopyram + Tebuconazole, Thiophanate methyl + Mancozeb, Bascold + Pyrclostrobin, Azoxystrobin + Difenconazole and seven plant extracts *viz*., leaves of Tulsi, Stevia, Alovera and clove of Garlic, rhizome of Ginger, stem of Giloy, root of Ashwagandha were evaluated to find out the efficacy of various fungicides and plant extract against *Rhizoctonia solani* causing web blight of Urdbean under *in vitro* condition. Among the fungicides, Tebuconazole and Mancozeb was found best fungicide which almost completely inhibited the radial growth an sclerotia production of *R. solani.* Among the combination fungicides, Fluopyram + Tebuconazole fungicide completely inhibited the radial growth and sclerotia production of *R. solani*. Among plant extracts, Garlic clove extract @ 20 percent found best as it inhibited 77.7 growth of *R. solani*.

Keywords: Effect, plant extract, fungicides, *Rhizoctonia solani*, urdbean, web blight

Introduction
Urdbean or Blackgram (*Vigna mungo* L.) is one of the ancient pulse crop and extensively grown in India. It is a wholesome crop with a short growing season that is an excellent source of digestible protein (25-28%), carbohydrate (62-65%), fibre (3.5-4.5%), ash (4.5-5.5%), and oil (1-1.5%) on a dry basi. (Singh, 1982) [12]. The biggest producers of black gram in India during Kharif are the states of Madhya Pradesh (14.38 lakh ha), Uttar Pradesh (7.01 lakh ha), Rajasthan (4.56 lakh ha), Maharashtra (2.87 lakh ha). (Annonymous, 2021) [1]. Among the fungal diseases web blight of urd bean caused by *Rhizoctonia solani* Kuhn is a serious problem of Urdbean and causes yield losses up to 20-30% (Shailbala and Tripathi 2007) [13]. It is considered one of the important causes for stagnated productivity of the crop in the country (Dubey and Patel, 2001) [5]. The first symptoms appear as small circular brown spot on the leaves. These spot enlarge often show concentric banding and surrounded by irregular water soaked areas. The mycelium on infected leaves appear as spider web thus suggested the name web blight disease. (Dwivedi and Saxena 1974) [3]. Disease management is an essential part of integrated crop management strategies. Fungicides, botanicals, have been found to be useful in the management of urdbean diseases. Various systemic and non-systemic fungicides were evaluated against *Rhizoctonia solani* to manage the web blight disease and reduce crop losses (Khan *et al*. 1998) [8]. Various plant products like plant extracts were shown to exert biological activity *in vitro* and *in vivo* and are used as bio-fungicidal compounds (Pawar and Thaker 2006; Fawzi *et al*. 2009) [11, 6]. The objective of the performed research work was to investigate the antifungal activity of plant extracts and new generation fungicides *in vitro* on growth and sclerotia formation of *R. solani* under *in-vitro* condition.

Materials and Methods
The urdbean plants showing the typical symptoms of web blight were collected from the field of Jawaharlal Nehru Agriculture during the month of September. The lesions that showed the initial and distinct typical signs were identified for pathogen isolation. The selected leaves were washed with fresh and sterile water in order to remove the dust particles or extraneous soil and surface contaminants. Subsequently, young diseased tissues were cut into 2-3 mm pieces (containing 1/3 diseased and 2/3 healthy portions) with the help of sterilized blade.

These pieces were surface sterilized in 1% sodium hypochlorite solution for 1–2 min and then washed thrice with sterile distilled water, to remove any traces of sodium hypochlorite. The pieces were then placed on pre-sterilized blotting paper to remove excess moisture under aseptic conditions in the inoculation chamber. The surface sterilized diseased pieces were then aseptically transferred on sterilized Potato Dextrose Agar (PDA) slants and poured PDA medium on Petri plates. Similarly, sclerotia of test pathogen were also used for isolation. Before isolation, the surface of sclerotia was sterilized by dipping in 1% sodium hypochlorite solution for 1-2 minutes and thereafter thoroughly washed thrice in sterile distilled water, to remove any traces of sodium hypochlorite solution. Then, these sclerotia were transferred aseptically on sterilized Potato Dextrose Agar (PDA) slants and poured PDA medium on Petri plates. Inoculated tubes and Petri plates were incubated in Biological Oxygen Demand (B.O.D.) incubator at 30±1°C. After 48 hrs of incubation, mycelial growth developed at margin was transferred to culture tubes containing PDA for further studies.

Effect of fungicides on radial growth and sclerotia formation of *R. solani*

In order to find out a suitable fungicide for management *R. solani* of urdbean. six fungicide namely Tebuconazole (1ml), Thiophanate-methyl (1gm), Azoxystrobin (1ml),, Fluoypyram (1ml), Mancozeb (2.5g), Propineb (2g) and six combination fungicides, namely Metalaxil + Mancozeb (2.5g/l), Tebuconazole + Trifloxystrobin (1g/l), Fluopyaram + Tebuconazole (1g/l), Thiophanate methyl + Mancozeb (2.5g/l), Bascold +Pyrclostrobin (1ml/l), Azoxystrobin + Difenconazole (1ml/l) along with control were evaluated against *Rhizoctonia solani in- vitro* by following the poisoned food technique. PDA poisoned with each fungicide quantity was poured into three sterilized Petriplates @ 20 ml/plate and allowed to solidify. Plates containing PDA without fungicide served as check. After solidification each Petriplate was inoculated with 5 mm mycelial disc aseptically. Plates were incubated at 30 ± 1^0 C and observation on radial growth of test fungus will be recorded after 144hours.Recorded data on radial growth was converted into percent growth inhibition by using following formula of Vincent (1947) [16].

Effect of botanicals on radial growth and sclerotia formation *Rhizoctonia solani* under *in- vitro* condition

Seven botanicals *viz. Allium sativum, Zingiber officinalis, Aloe barbadensis, Ocimum tenuiflorum, Tinospora cordifolia, Withania somnifera, Stevia rebaudiana* were tested for their antifungal activity against *R. Solani*. Extracts of plant parts such as leaf, rhizome, root, Stem and clove etc. were prepared by the standard method used, Fresh plant parts were washed with tap water followed by sterile distilled water, processed with sterile distilled water @1ml g-1 of plant tissue (1:1v/w) with pestle and mortar and filtered through a double layered cheese cloth. The filtrate so obtained formed the standard plant extract solution, The plant extract so prepared were screened *in vitro* against *R. Solani* sp. urdbean using poisoned food technique, Stock solution 10, 15 and 20 ml were mixed respectively with 90, 85 and 80 ml of sterilized molten Potato Dextrose Agar (PDA) media to obtained 10, 15 and 20 percent concentration of plant extract. The mixed medium was thoroughly shaken to ensure uniform mixing of extract. 20 ml of poisoned PDA was poured into sterile Petri plates. Three replications were maintained for each concentration. After solidification of poisoned media, the Plates were inoculated with mycelium disc (5 mm diameter) of vigorously growing pure culture colony of *R. Solani* urdbean, The control Petri plates in three replications were maintained using only sterile water without any plant extract but with mycelium disc (5 mm) for comparison. Plates were incubated at 30 ± 1 ºC and observation on radial growth after 144 hours. Recorded data on radial growth was converted into percent growth inhibition of incubation by using formula given by Vincent (1947) [16].

Results and Discussion

Effect of fungicides

Among the fungicides, Tebuconazole and Mancozeb was found best fungicide which almost completely inhibited the radial growth and microsclerotia production of *R. solani* (Table–1; Fig. 1). Thiophanate-methyl, Fluopyram and Azoxystrobin were second next in order of toxicity resulting, respectively 92.8, 91.3 and 88.3 percent inhibition of radial growth. Propineb was found least effective as it inhibited 66.2% growth of *R. solani*. No microsclerotia production was observed in Tebuconazole and Mancozeb, Thiophanate-methyl, Fluopyram and Azoxystrobin, while poor microsclerotia production was observed in Propineb. Hunjan *et al*. (2012) [7] also reported that tebuconazole has higher level of efficacy against *R. solani* of rice in laboratory condition. BcLalit. (2019) [2] reported the Thiophanate methyl showed 100 percent mycelia inhibition of *Rhizoctonia Bataticola* in blackgram, These results supports the present findings.

Among the combination fungicides, Fluopyram +Tebuconazole completely inhibited the radial growth and microsclerotia production of *R. solani* followed *by* Azoxystrobin + Difenconazole and Thiophanate methyl +Mancozeb. All the three fungicides were statistically at par with each other (Table 2; Fig 2). Metalaxyl + Mancozeb, Tebuconazole +Trifloxystrobin and, Bascold Pyrclostrobin were next in order of toxicity resulting, respectively 81.1, 79.2 and 72.9 percent inhibition of radial growth. The 48.10% growth of *R. solani* was recorded in control after 144 Hrs. of inoculation. No microsclerotia production was observed in Fluopyram+Tebuconazole, Azoxystrobin +Difenconazole and Thiophanate methyl + Mancozeb and Metalaxyl + Mancozeb, Tebuconazloe +Trifloxystrobin while poor sclerotia production was recorded in Bascold +Pyrclostrobin. Hunjan *et al.* (2012) [7] reported that fungicide *viz*., Trifloxystrobin + Tebuconazole showed higher level of efficacy against *R. solani* of rice in laboratory conditions. Sriraj *et al*. (2014) [14] also reported that Tebuconazole+ Trifloxystrobin effective against the pathogen in inhibiting the mycelia growth and sclerotial production at lower concentration. In the present study, Fluopyram +Tebuconazole and Tebuconazole+ Trifloxystrobin was found effective against the pathogen in inhibiting the mycelial growth and sclerotial production of *R. solani*.

Effect of botanicals

Data presented in Table-3, clearly revealed that garlic clove extract was most effective as they inhibited the growth of *R solani* by 77.7% followed by ginger extract (75.5%) at 20 per cent concentration. Tulsi leaf, Aloevera leaf and Ashwagandha leaf extract were also found very promising as

they produced 65.5, 62.2 and 58.8 percent inhibition, respectively, at 20 percent concentration. Minimum inhibition was recorded in leaf extract of *Stevia rebaudiana* (42.2%). The poor sclerotia formation was observed in garlic clove extract and ginger extract while fair to abundant sclerotia formation was observed in other treatment. At lower concentrations i.e. 10 and 15 percent growth inhibition due to Tulsi leaf, Aloevera leaf, Giloy leaf and Ashwagandha were less than 58 percent. Muzammil *et al*. (2014) [10] and Meena *et al*. (2014) [9] reported that *Allium sativum* inhibited radial growth of *R. bataticola* of sunflower. Dubey and Dwivedi (1991) [4] found fungi toxic properties of *A. sativum* against vegetative growth and microsclerotia viability of *R bataticola*. Tandel *et al*., (2010) [15] tried phytoextracts of eleven plant species against *R solani* of green gram and revealed that the onion bulb extract produced maximum inhibition (98.14%) followed by extract of acacia, ginger, neem, garlic and Karanj.

Table 1: Effect of single fungicides on radial growth and sclerotia formation *Rhizoctonia solani* under *in vitro* condition

Fungicide	Dose/liter	Percent growth inhibition after 144hrs*	Percent growth inhibition after 144hrs	Sclertia Formation after 15 days of inoculation
Thiophanate methyl	1 gm	4.90	92.8	-
Fluopyram	1 ml	5.93	91.3	-
Mancozeb	2.5 gm	0.26	99.2	-
Azoxysytrobin	1 ml	8.00	88.3	+
Propineb	2gm	23.23	66.2	++
Tebuconazole	1ml	0.13	99.8	-
Control		68.90	-	++++
CD		1.413		

*Average of three replication

Table 2: Effect of combination fungicides on radial growth and sclerotia formation *Rhizoctonia solani* under *in vitro* condition

Combination Fungicide	Dose/liter	Percent growth inhibition after 144hrs*	Percent growth inhibition after 144hrs	Sclertia Formation after 15 days of inoculation
Fluopyram +Tebuconazole	1 ml	0.0	100.0	-
Tebuconazole +Trifloxystrobin	1 gm	10.0	79.2	+
Metalaxyl + mancozeb	2.5 gm	9.06	81.1	_
Azoxystrobin +Difenconazole	1 ml	0.16	99.6	_
Thiophinate methyl +mancozeb	2.5 gm	0.06	99.8	_
Bascold + Pyrclostrobin	1 ml	13.00	72.9	++
Control		48.33		++++
CD		1.397		

*Average three replication

Table 3: Effect of plant extracts on radial growth and microsclerotia formation of *R. Solani.*

S. No.	Name of Plant extracts	Local name	Parts used	Radial growth (mm) of target pathogen (5 DAI)*			Percent growth inhibition			No. of microsclerotia formed after 15 days
				10%	15%	20%	10%	15%	20%	
1	*Allium sativum*	Garlic	Clove	40.0	25.0	20.0	55.5	72.2	77.7	+
2	*Zingiber officinalis*	Ginger	Rhizome	55.0	32.0	22.0	38.8	75.5	75.5	+
3	*Aloe barbadensis*	Alovera	Leaf	75.0	80.0	34.0	16.6	12.5	62.2	++
4	*TinosporaCordifolia*	Giloy	stem	61.0	53.5	48.0	65.5	46.6	46.6	+++
5	*Ocimumtenuiflorum*	Tulsi	Leaf	53.3	41.0	31.0	40.7	54.4	65.5	++
6	*Withaniasomnifera*	Ashwagandha	root	55.0	51.0	37.0	38.8	58.8	58.8	++
7	*Stevia rebaudiana*	Stevia	Leaf	76.0	60.0	52.0	15.5	42.2	42.2	+++
8	Control	-	-	90.0	90.0	90.0	00.0	00.0	00.0	++++
	CD (0.05)			1.20	1.30	1.27				

*Average of 3 replication

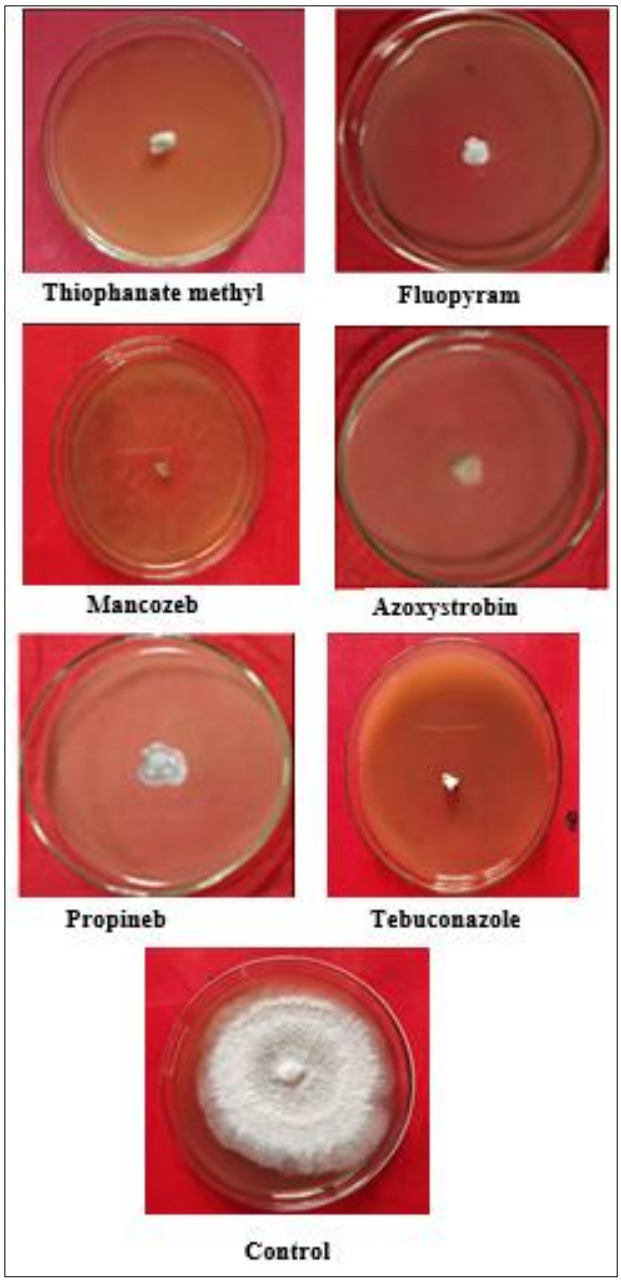

Fig 1: Effect of single fungicide on radial growth and sclerotia formation *Rhizoctonia solani*

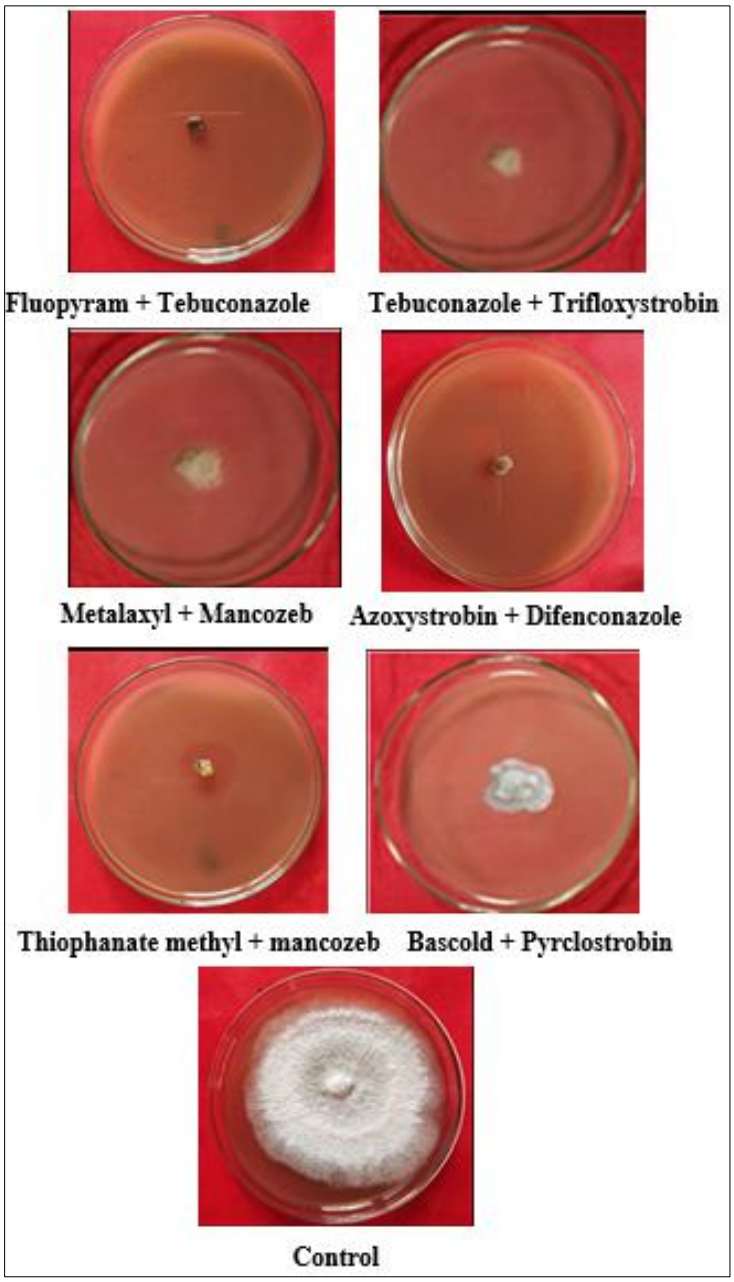

Fig 2: Effect of combination fungicide on radial growth and sclerotia formation *Rhizoctonia solani*

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