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### COMPARATIVE EFFICACY OF TEN COMMERCIAL FUNGICIDES FOR THE CONTROL OF *RHIZOCTONIA SOLANI*, THE CAUSE OF BLACK SCURF DISEASE OF POTATO

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#### ABSTRACT

Black scurf disease of potato caused by *Rhizoctonia solani* is one of the major problems causing considerable yield losses throughout the world. The control of *R. solani* is mainly based on the application of fungicides. Therefore, in the present study, ten fungicides belonging to different groups were evaluated for their *in vitro* effectiveness against *R. solani*. Highly significant inhibitory effects of fungicides were recorded on mycelial growth, sclerotial production, and germination of sclerotia of *R. solani*. In the *in vitro* test, the sensitivity of *R. solani* was found to vary greatly with the fungicides and their concentrations. The mycelial growth of *R. solani* was found to be the most sensitive to Antracol, Benlate, Captan, Dithane M-45, and Ridomil while the least sensitive to Polyram Combi. An intermediate sensitivity of the fungus was noticed to Daconil, Brassicol, Bavistin, and Vitavax. Sclerotial production by the fungus was the most sensitive to Ridomil and Benlate. At 50 ppm concentration, Benlate and Ridomil completely inhibited the sclerotial production. Dithane M-45 and Captan reduced the production of sclerotia greater than Daconil, Brassicol, and Bavistin. The least effective fungicide in reducing sclerotial production was Polyram Combi. Similarly, the most effective fungicide in reducing sclerotial germination was Benlate. The fungicides, Ridomil and Dithane M-45 showed an intermediate effect on the germination of sclerotia while Daconil and Captan proved to be the least effective fungicides in sclerotial germination of *R. solani*.

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#### INTRODUCTION

Potato (*Solanum tuberosum* L.) occupies a prominent position among vegetable crops grown all over the world. Besides being used as a vegetable crop, it has also been esteemed as one of the members of staple food crops. Due to its high productivity coupled with better quality of starch, it has been appreciated by the growers and relished by the consumers. Incidentally, potato has

come to rescue of mankind during war and peace time. Potato has played a remarkable role in human diet as a supplement of wheat and rice, and it has been found to produce more food per unit area than the cereals. Potato comprises of 80% water, 2% proteins and 18% carbohydrates. As food, it is one of the cheapest sources of carbohydrates and furnishes appreciable amounts of vitamins B1 and C as well as some minerals. Besides, it

yields a variety of commercial products such as starch, alcohol, dextrose, glucose and lactose and lactic acid. Potato having high nutritive value, contributes more protein and iron than other vegetables in the average diet and is also a useful source of thiamine, niacin and several other nutrients including fiber. Almost 86% area under potato cultivation and production of Pakistan is achieved from Punjab. Okara, Sahiwal, Kasur, Sialkot, Sheikhupura, Jhang, Narowal, Pakpattan, Gujranwala, Toba Tek Singh, Khanewal and Lahore are the major potato growing districts of Punjab. The rest of the share is from Khyber Pukhtunkhwa (9%), Baluchistan (4.5%) and Sindh (0.5%).

Pakistan is self-sufficient in potatoes for household consumption and relies for more than 99% on locally produced seed potatoes. Presently, it is estimated that the total annual domestic production amounts to around 2.02 million metric tons of which 280000 metric tons are used as seed and 1.7 million metric tons are available for consumption after post-harvest losses. It accounts to 11 kg per capita per annum with a population of roughly 150 million. The present per hectare yield is awfully low against 25-40 tons obtained in potato growing countries. This is due to various agronomic and environmental factors as well as due to the attack of various pests and diseases. Black scurf is one of the serious diseases of potato and causes considerable losses to the crop.

The disease is caused by the fungus *Rhizoctonia solani* which was originally described by Julius Kühn from potato in 1858 (Kühn, 1858). *R. solani* is a soil-borne Basidiomycete, ubiquitous in distribution and has complex biology. Because of the lack of conidia and the scarcity of the sexual spores, *R. solani* exists as vegetative hyphae and sclerotia in nature. The fungus is dispersed mainly via sclerotia, contaminated plant material or soil spread by wind, water or during agricultural practices. The fungus can survive long periods as saprophyte in the soil. Nutrients leaking from actively growing plant cells or decaying plant material attract the fungus. Nutrient usage by *R. solani* differs since it has been reported to be suppressed in saprophytic systems rich in organic nutrients. However, *R. solani* seems to have an ability to utilize other carbon sources, such as cellulose, that are only rarely used by other micro-organisms which makes it efficient competitor when resources are limited (Deacon, 1996).

The fungus has a wide host range and can cause various diseases on important crop plants of the world as well as

ornamental plants and forest trees (Ogoshi, 1996). Disease symptoms include leaf blights, leaf spots, damping-off, rots on roots, shoots and fruits, canker lesions on sprouts and stolons, and sclerotial diseases (Hide, 1981; Jeger and Velvis, 1995; Jeger et al., 1996). However, some *R. solani* strains form symbiotic mycorrhizal relationships with orchid plants (Carling et al., 1999; Chang and Chou, 2007).

The infection initiates when mycelia or hyphae from a germinating sclerotium start to grow towards a suitable host as a result of attracting chemical exudates (Keijer, 1996). After the first contact, loose and still unattached hypha starts to grow over the plant and within a few hours, the hypha flattens and directional growth over the epidermal cells is initiated. Before actual active penetration of the host, T-shaped hyphal branches form thick infection cushions that attach strongly to the host epidermis (Keijer, 1996; Mantecón, 2008). Topological signaling, namely identification of the appropriate host by its surface structure, seems to be important for establishment of the infection. The fungus enters the plant actively by finding a weak spot on the surface where it can break down the protecting layer (Weinhold and Sinclair, 1996). Passive entry by the fungus into the plant is rare and limited to leaf pathogenic isolates (Weinhold and Sinclair, 1996) and it is not the usual infection mechanism for soil-borne *R. solani* (Keijer, 1996). Swollen hyphal tips on infection cushions concurrently form infection pegs that penetrate the cuticle and epidermal cell walls into the host epidermal tissue and outer layer of the cortex (Demirci and Döken, 1998). Penetration is established by using hydrostatic pressure, even though degrading enzymes such as cutinases (Baker and Bateman, 1978), pectinases (Bertagnoli et al., 1996; Jayasinghe et al., 2004) and xylanases (Peltonen, 1995), are most probably also involved in infection and penetration. When inside the host, the fungus starts to grow inter- and intra-cellularly degrading the tissue, which can be seen as necrotic lesions on epidermal tissue of shoots, roots and stolons or as damping-off of the young seedlings (Demirci and Döken, 1998).

The pathogenic fungus can be managed by developing resistant varieties. However, there are no resistant varieties available therefore; the objective of the present study was to assess the comparative effectiveness of some fungicides for the management of the disease.

## MATERIAL AND METHODS

### The Pathogen

The pathogen, *Rhizoctonia solani*, was isolated from potato tubers affected with black scurf disease. For isolation of the pathogen, sclerotia bearing tubers were surface disinfested by dipping in 0.1% mercuric chloride solution for one minute and then washed three times in sterilized water. The tubers were placed in sterilized moist chamber and were incubated at 28°C for 24 hours. The hyphal tips of the fungal colonies growing from the sclerotia were transferred to agar slants of Waksman agar medium. The pathogenicity of *R. solani* isolated from potato tubers affected by black scurf disease was tested using soil and seed infestation methods.

### Sterilization of soil and tubers

The soil used in the experiments was sterilized by autoclaving twice at 121°C for 40 minutes. The sterilized soil was used for pot experiment. The healthy potato tubers were disinfested by cleaning their surface with cotton lint pre-soaked in methylated spirits.

### *In vitro* effect of fungicides against *Rhizoctonia solani*

Ten fungicides viz. Antracol, Benlate, Brassicol, Bavistin, Captan, Daconil, Dithane M-45, Polyram Combi, Ridomil and Vitavax were collected from the market and evaluated for their comparative toxicity against mycelial growth, sclerotial production and sclerotial germination of *R. solani*.

### Effect of fungicides on mycelial growth and sclerotial production of *R. solani*

Sensitivity of *R. solani* mycelium to different fungicides at three concentrations (10, 20, and 50 ppm) was studied using modified technique of Borum and Sinclair (1968). Fungicidal concentrations were obtained by adding requisite amount of stock solution to autoclaved (121°C for 15 minutes) Waksman agar medium cooled at about 45°C. The sterilized medium without fungicide served as control. Three plates (9 cm diameter) were poured with the medium for each treatment. After solidification of the medium, agar plugs (4 mm diameter) containing *R. solani* mycelium were cut from the culture plates using a sterilized cork borer and were placed in the center of each plate. The plates were incubated at 30°C. The mean diameter of mycelium was recorded eight days after inoculation. Percent inhibition of *R. solani* colonies in each treatment was calculated. The same experiment was performed to see the effect of fungicides on the number of sclerotia. The plates were incubated and sclerotia produced by *R. solani* were

counted after 15 days in each dilution.

### Effect of fungicides on the germination of sclerotia of *R. solani*

The relative effect of five most effective fungicides on sclerotial germination was also studied. These five fungicides were relatively more effective to inhibit the mycelial growth and sclerotial production of *R. solani*. The sclerotia obtained from three weeks old culture were dipped in different fungicidal dilutions (10, 20 and 50 ppm) for 5 minutes. Water without fungicides served as control. Five treated sclerotia were placed in each plate. Three plates were kept under each treatment and incubated at 30°C. Germination of sclerotia was recorded after 24 hours of incubation.

### Statistical analysis

Completely randomized design was used in the experiments. Percent reductions in mycelial growth, sclerotial production and germination of sclerotia of *R. solani* were calculated over controls prior to statistical analysis (Iqbal and Mukhtar, 2020). All the data were subjected to Analysis of Variance (ANOVA) using GenStat package 2009, (12th edition) version 12.1.0.3278 (www.vsnl.co.uk). The differences among means were compared by Fisher's Protected Least Significant Difference Test at ( $P \leq 0.05$ ).

## RESULTS

### *In vitro* effect of fungicides on mycelial growth of *R. solani*

In the *in vitro* evaluation, the sensitivity of mycelial growth of *R. solani* to ten test fungicides varied considerably (Table 1). There was significant decrease in mycelial growth with the increase of concentration for each fungicide. Although none of the test fungicides completely inhibited the fungal growth at any of the three dosage rates yet the mycelial growth of *R. solani* was the most sensitive to Antracol, Benlate, Captan, Dithane M-45 and Ridomil while the least sensitive to Polyram Combi and with an intermediate sensitivity to Daconil, Brassicol, Bavistin and Vitavax. Antracol, Benlate, Captan, Dithane M-45 and Ridomil were statistically similar in reducing mycelial growth and there was no significant difference in colony diameter among the plates containing 10 ppm Antracol, Benlate, Captan, Dithane M-45 and Ridomil (Table 1). Similarly, the activity of Brassicol, Bavistin and Vitavax on fungus growth was same having no statistical significant difference (Table 1). The effectiveness of Daconil and Polyram Combi at 10 ppm was also statistically similar

and did not differ with each other in mycelial growth of *R. solani*. It is quite evident that with an increase in fungicide dosage rate, there was a significant difference in the effectiveness of all the fungicides. At 20 ppm dosage rate, Antracol, Benlate, Captan and Ridomil exhibited statistically a similar effect on the growth of fungus. The response of Vitavax, Dithane M-45 and

Bavistin on the fungus growth was intermediate and there was significant difference among their effectiveness while at 20 ppm dosage rate Polyram Combi, Daconil and Brassical were least effective. At 20 ppm dosage rate, Benlate and Captan were the most effective fungicides to inhibit the mycelium growth (Table 1).

Table 1. Effect of different fungicides on colony growth of *R. solani*.

Fungicide	Colony diameter (cm)			% decrease over control		
	10 ppm	20 ppm	50 ppm	10 ppm	20 ppm	50 ppm
Control	9.0	9.0	9.0	00.00 a	00.00 a	00.00 a
Antracol	6.3	4.2	3.6	30.00 d	53.33 g	60.00 e
Benlate	6.0	3.0	2.1	33.33 d	66.66 h	76.66 g
Brassicol	7.4	7.0	4.2	17.77 c	22.22 b	53.33 d
Bavistin	7.2	5.5	4.6	20.00 c	38.88 d	48.88 c
Captan	6.5	4.1	3.1	27.77 d	54.44 g	65.55 f
Daconil	8.2	6.6	4.4	8.88 b	26.66 c	51.11 cd
Dithane M-45	6.4	5.2	3.6	28.88 d	42.22 e	60.00 e
Polyram Combi	8.0	6.8	6.6	11.11 b	24.44 bc	26.66 b
Ridomil	6.5	4.3	3.2	27.77 d	52.22 g	64.44 f
Vitavax	7.1	4.8	3.8	21.10 c	46.66 f	57.77 e

Values are means of three replicates. Means sharing common letters do not differ significantly at 5%.

The colony growth of *R. solani* was most sensitive at 50 ppm concentration. Antracol, Benlate, Captan, Dithane M-45 and Ridomil showed the greatest effectiveness in reducing the growth of *R. solani* (Table 1). At 50 ppm dosage rate, the fungicide Benlate was the best to inhibit the fungus growth. It gave 76.66% decrease over control at 50 ppm (Table 1).

#### ***In vitro* effect of fungicides on the production of sclerotia by *R. solani***

Like mycelial growth, the effect of ten fungicides on the production of sclerotia of *R. solani* also varied considerably (Table 2). At 10 ppm, the most effective fungicide in inhibiting the sclerotial formation by colonies of *R. solani* was Ridomil and it was statistically stronger in effectiveness to all other fungicides tested in the experiment. Benlate, Dithane M-45, Captan and Brassicol gave better reduction in the production of sclerotia and had significant difference among each other. On the other hand, the least effective fungicides were Polyram Combi and Bavistin having statistically the similar performance.

There was a significant decrease in sclerotial production with the increase of fungicide concentration. At 20 ppm concentration, sclerotial production was most sensitive

in Antracol, Benlate, Dithane M-45 and Ridomil and had a significant difference with each other. At the said concentration, Vitavax, Daconil, Captan and Brassicol had an intermediate effect on the sclerotial production of *R. solani* but these fungicides were more effective than Bavistin which had significantly greater reduction than Polyram Combi.

At 50 ppm, among all the fungicides, Antracol, Benlate, Ridomil, Dithane M-45 and Vitavax gave a remarkable reduction of sclerotia. Benlate and Ridomil checked the sclerotial production completely at 50 ppm. Both the fungicides statistically were non-significant with each other. Inhibition of sclerotia was also significantly greater in Dithane M-45 and Captan while Daconil, Brassicol and Bavistin showed an intermediate effect in reducing the production of sclerotia. Daconil and Brassicol had a significant difference with each other. Polyram Combi showed the least effect on the production of sclerotia of *R. solani* at 50 ppm (Table 2).

#### **Effect of fungicides on the germination of sclerotia of *R. solani***

The effectiveness of fungicides in inhibiting percent sclerotial germination is presented in Table 3. Among the 5 fungicides tested at 10 ppm, the most effective

fungicide in inhibiting the sclerotial germination was Benlate. The fungicides, Ridomil and Dithane M-45 showed an intermediate effect whereas Daconil and Captan were least effective. Although there was a general increase in the inhibition of germination of the sclerotia with an increase in the fungicides doses yet all the fungicides gave similar results in response to increase in doses. Out of five fungicides tested in the experiment at 20 ppm, Benlate and Ridomil proved to be the most effective fungicides in retarding germination of sclerotia and, both were statistically non significant with

each other (Table 3). The effect of Dithane M-45 treatment was statistically intermediate whereas Daconil proved to be the least effective. Thus an increase in the dosage rates of fungicides resulted in the increased inhibition of sclerotial germination. Among the five fungicides tested at 50 ppm, the most effective fungicides in retarding the sclerotial germination were Benlate and Ridomil. Dithane M-45 had an intermediate effect whereas Daconil and Captan were least effective and all were statistically non-significant with each other (Table 3).

Table 2. Effect of different fungicides on the production of sclerotia by *R. solani* per colony.

Fungicide	Mean sclerotial production			% decrease over control		
	10 ppm	20 ppm	50 ppm	10 ppm	20 ppm	50 ppm
Control	300	300	300	00.00 a	00.00 a	00.00 a
Antracol	160	20	2	46.66 c	93.33 g	99.33 i
Benlate	70	18	1	76.66 i	94.00 g	99.66 i
Brassiccol	130	80	55	56.66 f	73.33 d	81.66 d
Bavistin	175	105	62	41.66 b	65.00 c	79.33 c
Captan	125	75	40	58.33 g	75.00 e	86.66 f
Daconil	145	80	50	51.66 d	73.33 f	83.33 e
Dithane M-45	98	35	25	67.33 h	88.33 f	91.66 h
Polyram Combi	178	140	92	40.66 b	53.33 f	69.33 b
Ridomil	40	12	1	86.66 j	96.00 h	99.66 i
Vitavex	140	80	30	53.33 c	73.33 d	90.00 g

Values are means of three replicates. Means sharing common letters do not differ significantly at 5%.

Table 3. Effect of different fungicides on the germination of sclerotia by *R. solani*.

Fungicide	% germination			% decrease over control		
	10 ppm	20 ppm	50 ppm	10 ppm	20 ppm	50 ppm
Control	100	100	100	0.0 a	0.0 a	0.00 a
Benlate	45	30	16	55 d	70 d	84 c
Captan	65	46	25	35 b	54 b	75 b
Daconil	66	48	29	34 b	52 b	71 b
Dithane M-45	50	35	18	50 c	65 c	82 c
Ridomil	48	31	16	52 cd	69 cd	84 c

Values are means of three replicates. Means sharing common letters do not differ significantly at 5%.

## DISCUSSION

*R. solani*, the causal organism of black scurf disease of potato is widely prevalent in the soil and is responsible for poor quality and low yield of potato. The cheapest mean to control the disease is the cultivation of resistant varieties but unfortunately resistance is lacking in commercial potato cultivars. The present study indicated that the control of *R. solani* is possible through the use of suitable chemicals. These chemicals when

provided with suitable quantity and concentrations may exert an effective action to suppress the activity of the pathogen.

In the present study, the mycelial growth of *R. solani* was found to be the most sensitive to Antracol, Benlate, Captan, Dithane M-45 and Ridomil while the least sensitive to Polyram Combi and with an intermediate sensitivity to Daconil, Brassiccol, Bavistin and Vitavex (Table 1). Sclerotial production of the fungus was most

sensitive to Ridomil and Benlate. At 50 ppm concentration, Benlate and Ridomil checked the sclerotial production completely. Dithene M-45 and Captan reduced the production of sclerotia greater than Daconil, Brassicol and Bavistin, The least effective fungicide in reducing the sclerotial production was Polyram Combi (Table 2). In the *in vitro* test, the sensitivity of *R. solani* was found to vary greatly with the fungicides and their concentrations. Similarly, the most effective fungicide in reducing sclerotial germination was Benlate. The fungicides, Ridomil and Dithan M-45 showed an intermediate effect on the germination of sclerotia while Daconil and Captan proved to be the least effective fungicides in sclerotial germination of *R. solani*.

Many researchers have reported the effectiveness of different fungicides for the control of soil borne diseases caused by *R. solani* on different crops (Abdalla, 1975; Araki, 1985; Bruggen and Arneson, 1986; Kannaiyan and Prasad, 1979a, 1979b; Kataria and Grover, 1976; Kataria and Verma, 1989; Kesavan, 1984; Sundravadana et al., 2007). Hide and Read (1991) found that treatment of seeds with tolclofos-methyl reduced the severity of black scurf in America. Similarly, Özer and Bayraktar (2015) reported that fludioxonil gave good control of *Rhizoctonia* isolates in field experiments.

Benomyl has successfully controlled many diseases of different crops as leaf spot in sugar beet (Karaoglanidis et al., 2003), rice blast (Kamerwar, 1976), scab and powdery mildew of apples, cucurbits and strawberries (Scott et al., 1970). Marley and Gbenga (2004) found that benomyl reduced the mycelial growth of *Stenocarpella maydis in vitro*. It also inhibited the growth of *Fusarium oxysporum* (El-Tobshy et al., 1981). Mamza et al. (2010) reported that benomyl along with thiram and tricyclazole suppressed growth of *F. pallideroseum* isolated from castor. Khan and Khan (2006) found that both benomyl and carbendazim inhibited 100% mycelial growth of *M. phaseolina*. Carbendazim also inhibited the growth and sclerotial production of *M. phaseolina* (Suryawanshi et al., 2008). Similarly, seed dressing with fungicides enhanced seedling emergence and reduced mortality rate in legumes (Muthomi et al., 2007).

A number of mechanisms are involved in the suppression and inhibition of pathogens by fungicides. It was found from the present investigation that fungicides significantly caused reduction in growth and sclerotia of *R. solani*. Fungicides act by binding with  $\beta$ -tubulin polymers of pathogens which play a key role in nuclear

division and result in inhibition of polymerizing activity of microtubules. These also cause hindrance in different regulatory cellular activities including mitosis, meiosis and cell shape maintenance etc. (Nene and Thapliyal, 1979). Similarly, Carbendazim inactivates tubulin function of pathogen necessary for their maintenance and growth (Butlers et al., 1995). Variations in sensitivity have also been found among fungicides against different anastomosis groups (AGs) (Campion et al., 2003; Kataria et al., 1991; Muzhinji et al., 2018).

### CONCLUSIONS

It is concluded from the present studies that fungicides can be effectively used against black scurf disease of potato caused by *Rhizoctonia Solani*. As Benlate, Captan, Daconil, Dithane M-45 and Ridomil proved to be the effective against the fungus and hence recommended for the control of *R. Solani*. The findings of the studies are of great interest to plant pathologists, extension workers, farming community and the industry sector. As the fungus *Rhizoctonia* has been reported to possess a mixed reproductive mode of recombination including clonality and recombination therefore identification of insensitive isolates in some of the AGs should be done. It is therefore of paramount importance to institute monitoring programs to understand the population dynamics of *Rhizoctonia* populations exposed to fungicides to ensure continued effectiveness of fungicide control programs.

### AUTHOR'S CONTRIBUTION

The author designed the study, performed the experiments, collected and analyzed the data, wrote the manuscript and proofread the paper.

### CONFLICT OF INTEREST

The author declares no conflict of interest

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