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Original Research Article

In vitro efficacy of fungicides against the growth of foot-rot pathogen (*Sclerotium rolfsii sacc*.) of Brinjal

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A B S T R A C T

Nine fungicides, viz., Bavistin, Brassicol, Captan, Dithane M-45, DM-145, Fytolan, Manzate, Parasan and Sulfex were tested against *Sclerotium rolfsii* in vitro by food poising method. All the fungicides have showed adverse effect on the growth of *Sclerotium rolfsii*. Amongst the tested fungicides, Brassicol was found to be significantly effective against inhibition of growth, but it proved to be fungistatic in action, even in higher doses i.e., up to 3.0% concentration. Next to Brassicol, Manzate has been found to be the best as it gave 100% inhibition of growth at 0.1% concentration. Its o.1% concentration was found to be fungicidal in action. After Manzate, the Parasan and DM-145 have been found to be the next effective fungicides against *Sclerotium rolfsii*. The 0.25% concentration of these two fungicides cause 100% inhibition of growth and the same concentration (i.e., 0.25%) of these fungicides proved to be fungicidal. Dithane M-45 and Captan were also found to be toxic which cause 100% inhibition in growth at 0.5% and 2.0% concentration respectively. These two fungicides were also recorded as fungicidal in action at the same concentrations (i.e., 0.5 and 2.0%) . Sulfex, Fytolan and Bavistin have proved to be poor in this respect and did not show cent percent inhibition even in the higher dose i.e., 3.0% concentration.

K e y w o r d s

Sclerotium rolfsii, Fungicides, Brinjal, Mycelial growth

Introduction

The annual loss to world crops as a result of disease has been estimated at about 30,000 million dollars, a major loss of this due to fungal pathogen. Different methods were adopted for plant disease control. But in the recent past, considerable work has been done in the field of disease control through chemicals. The chemicals used for control of fungal diseases are mainly fungicides and antibiotics. Now a days, fungicides are known to be the most effective method of the fungal pathogen and disease control.

The bordeax mixture, discovered by Millardet (1885) had the greatest impact in plant pathology and it is still used against the powdery mildew of grapes in Europe. The Bordeax mixture was followed by lime

sulphur, formalin, Copper carbonate dust which has been used against plant diseases up to 1913. organic fungicides – Chloronil, Thiram, Diclone have been developed in the mid – 1930 onwards. Captan, the most effective fungicides, was developed only two decades ago. Many groups of chemical compounds are now available for plant disease control. These include heterocyclic nitrogenous compounds, quinines, phenols and antibiotics.

There is an increasing interest in chemotherapy of plants, the systematic fungicides are of different interest. In April 1966, a significant development in the field of fungicides took place when Von Schmeling and Kulka (1966) published a paper on systemic fungicidal activity of 1, 4 – oxathiin derivatives. To the end of 1968, only eight fungicides, besides antibiotics have been known but now about 40 systamic fungicides have been produced by different companies (Vyas, 1983).

There are enormous literature available on fungicides used for plant disease control (Punja et.al., 1982; Padule et.al., 1983; Gisi et.al., 1985; Mahaling and Anahosur, 1998; Mueller et. al., 2002; Bradley et.al., 2006; Gondal et.al. 2012; Maheshwari and Hare, 2013; Aujla et.al., 2013; Hoque et.al., 2014; Amrutha et.al., 2014). Khan et.al., 1995; Alam et.al., 2004; Sharma, 2006; Kopacki and Wagner, 2006; Yaqub and Shahzad, 2006; Nisa et.al., 2011; Sahi et.al., 2012; Jalander and Gachande, 2013; Parveen et.al., 2013; Nath et.al., 2013; Mahamadi and Rajaei, 2013; Rahman et.al., 2013 have studied the efficacy of different systemic and non systemic fungicides against various plant pathogens like *Fusariun*, *Alternaria*, *Phytophthora*, *Sclerotium* etc.

On the basis of above information in the present investigation, nine fungicides were evaluated for their efficacy against *Sclerotium rolfsii* in vitro. The aim of the present study was to compare the effect of some fungicides on *Sclerotium rolfsii* mycelial growth in vitro and identify the concentration of fungicide having fungicidal properties.

Materials and Methods

Isolation of Pathogen

The pure cultures of *Sclerotium rolfsii* was isolated from infected foot region of *solanum melongena* plant on potato dextrose agar medium. (Chaurasia et.al., 2014 a). The stock cultures were maintained by periodic transfer and stored in the refrigerator at 4° C for further studies. (Chaurasia, 2000; Chaurasia et.al., 2013).

In vitro efficacy of fungicides against *Sclerotium rolfsii*

The following nine fungicides were evalulated for their efficacy on mycilial growth of *Sclerotium rolfsii* by food poisoning technique (Schemitz, 1930; Chaurasia, 2014b):

1. **Bavistin**
   Methyl-2-banzimidazolecarbamate
2. **Brassicol**
   75% pentachloronitro-benzene
3. **Captan**
   N-trichloromethylmercapto-4-cyclohexene 1, 2-dicarboximide
4. **Dithane M-45**
   78% manganese ethylene bisdithiocarbamaate
5. **DM – 145**
   Mancozeb 75% and adjuvents 25%
6. **Fytolan**
   88% Copper as copper oxychloride
7. **Manzate**
   Mancozeb 75% WP.
8. **Parasan**
   Phenyl mercury acetate 1% Hg.

9. Sulfex
80% sulphar

The effect of above nine fungicides each with ten concentrations i.e., 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% were tried against *Sclerotium rolfsii*. Solutions of measured quantity of fungicides, prepared in sterile distilled water, then was added into sterile melted Potato dextrose agar to get the concentrations of 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% in the medium.

Approximately 20 ml of poisoned melted Potato dextrose agar medium was poured into each sterilized petridish. After solidification, each petridish was inoculated asceptically by placing the 8.0 mm diameter of inoculum in the center. The Potato dextrose medium without the addition of fungicide was considered as control. Each set was run in triplicates. The radial growth of pathogen, in each set was measured after 72 hours of incubation and the per cent inhibition over control was also calculated by the equation given by Vincent, 1927 and Chaurasia et.al., 2014 b.

$$I = \frac{(C - T)}{C} \times 100$$

Where,

I = Per cent of inhibition
C = Average Growth of *Sclerotium rolfsii* in control Petridishes.
T = Average Growth of *Sclerotium rolfsii* in each fungicide treated Petridishes

In order to study whether the effective concentration of fungicide was fungistatic or fungicideal in action to the pathogen, after 72 hours of incubation, the inoculum disc from those petridishes showing no growth has been transferred to fresh potato dextrose agar medium and the results were recorded after 72 hours of incubation.

Results and Discussion

The result obtained during the present investigation are presented in Table 1 and 2. From the results, it is clear that the radial growth of *Sclerotium rolfsii* was adversely affected by the fungicides. The effective fungicides, probably may act as antifungal agent and impacts its poisoning effect on metabolic process of pathogen, therefore, the growth of the pathogen might be adversely affected. Amongst the tested fungicides, Brassicol proved to be the most effective against *Sclerotium rolfsii* in which 100% inhibition in radial growth was recorded even in a very low concentration, i.e., 0.025%. Next to Brassicol, Manzate has been found to be significantly effective and 100% inhibition in radial growth was recorded above 0.05% i.e., at 0.1 per cent concentration. The results obtained with Brassicol are in correlated with the findings of Chauhan (1977) and Richhariya (1984), who has also reported significant response of Brassicol against *Sclerotium rolfsii*. Gould (1954) Cooper (1956), Aycock (1959), Harrison (1959) and many other investigators have also recommended the use of Brassicol against *Sclerotium rolfsii* on peanut and other ornamental and vegetable crops. Rahman et.al., (2013) found mancozeb (Manzate) as the most effective fungicide against *pestalotia palmarum*. After Manzate, the Parasan has been found to be the next effective fungicides against *Sclerotium rolfsii* in which 100% inhibition in radial growth was recorded at 0.25 per cent concentration. The lower concentration i.e., 0.025 per cent of this fungicide was so significantly found to effective and 72.56% inhibition in radial growth was recorded over control. DM-145 was also found to be toxic like Parasan at 0.25 per cent concentration but at lower concentration i.e., 0.025% of this fungicide have a less toxic

effect in comparision to Parasan. The 0.5% concentration of Dithane M-45 and 2.0% concentration of Captan were also found to be most toxic, where 100% inhibition in radial growth was recorded. Ohazurik (1996), Yaqub and Shahzad (2006) have also found some systemic fungicides including Dithane M-45 to be effective against *Sclerotium rolfsii*. Gupta et.al. (1983) and Mustika et.al. (1984) have also reported Dithane M-45 significantly reduced the conidial germination and growth of *Alternaria alterneta* and *Fusarium oxysporum* respectively. Alam et.al., (2000) reported the efficacy of fungicides on the inhibition of *Bipolaris sorokiniana* and found Dithane M-45 was the most effective fungicides. They stated that at 500 to 2500 ppm and 1/10 to 1/1000 ml concentrations were most effective after 5 to 30 minutes immersion. Alam et.al., (1999) reported the growth inhibition (in vitro) of chilli fruit rot pathogen *Alternaria tenuis* and found that Dithane M-45 proved to be the most effective against *Alternaria tenuis* when immersed for 5 to 30 minutes at 500 to 2500 ppm concentrations. Sulfex, Fytolan and Bavistin were also effective but the inhibition in the radial growth gradually increased with the increase in the concentrations and cent per cent (i.e., 100%) inhibition could not be recorded up to 3.0% concentration (plate 1).

Therefore, it is clear that Sulfex, Fytolan and Bavistin have poor inhibitory effect on the radial growth of pathogen as compared to other tested fungicides. This may be due to fact that the test pathogen might have developed some resistance towards these fungicides. Richhariya (1984) also reported poor response of Sulfex, Fytolan and Bavistin against tomato foot-rot pathogen *Sclerotium rolfsii*. *Sclerotium rolfsii* forms brownish sclerotia that can survive in soil for long period frequrently tolerating biological and chemical degradation due to the presence of melanin in the outer membrane (Cheit, 1975).

The results of fungistatic and fungicidal action of various fungicides are presented in Table 2. In order to find out whether the inhibition in radial growth was due to fungicidal or fungistatic action of fungicides, after 72 hours the inoculum disc was transferred in to fresh agar only in those cases of fugicides which showed the 100% inhibition over control. Thus Brassicol, Captan, Dithan M-45, DM-145, Manzate and Parasan were tested for their fangistatic or fungicidal in action.

In case of Brassicol only, in all the taken concentration, it was found that the growth of pathogen has started around the transferred inoculum in fresh agar medium. From this results, it is clear that Brassicol is fangistatic in action even in high dose of fungicide i.e. 3.0% concentration. In case of Manzate, the mycelium of inoculum disc did not survive even at very low concentration (i.e., at 0.1% concentration), therefore 0.1% of Manzate proved to be fungicidal in action and below this concentration Manzate was found to be fungistatic in action. Parasan and DM-145 have been found to be fungicidal in action at 0.25% concentration. Dithane M-45 and Captan have been found to be fungicidal in action at 0.5 and 2.0% concentration respectively and thus, fugus inoculum did not survive.

On the whole, it can be concluded that Brassicol was found to be significantly effective against *Sclerotium rolfsii*. The very low concentration (i.e., 0.025%) of Brassicol could cause 100% inhibition of growth but up to 3.0% concentration of it proved to be fungistatic in action. Next to Brassicol, Manzate has been found to be the best as it gave 100% inhibition of growth at 0.1% concentration its 0.1% concentration was found to be fungicidal.

Table.1 Effect of different concentrations of various fungicides on the growth of *Sclerotium rolfsii*

Fungicide	Radial Growth in mm*									
	Concentration (%)									
	0.025	0.05	0.1	0.25	0.5	1.0	1.5	2.0	2.5	3.0
Bavistin	80.2	78.5	74.3	66.0	52.4	37.0	33.2	30.6	28.5	26.1
	(2.19)	(4.26)	(9.39)	(19.51)	(36.09)	(54.87)	(59.51)	(62.68)	(65.24)	(68.17)
Brassicol	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0
	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)
Captan	78.3	68.5	52.1	28.5	10.0	5.8	3.0	00.0	00.0	00.0
	(4.51)	(16.46)	(36.46)	(65.24)	(87.80)	(92.92)	(96.34)	(100.00)	(100.00)	(100.00)
Dithane M-45	50.8	42.0	30.0	16.0	00.0	00.0	00.0	00.0	00.0	00.0
	(38.04)	(48.78)	(63.41)	(80.48)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)
DM-145	78.0	61.5	32.4	00.0	00.0	00.0	00.0	00.0	00.0	00.0
	(4.87)	(25.00)	(60.48)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)
Fytolan	80.0	78.5	74.3	62.0	42.1	27.0	20.3	16.5	12.2	8.0
	(2.43)	(4.26)	(9.39)	(24.39)	(48.65)	(67.07)	(75.24)	(79.87)	(85.12)	(90.24)
Manzate	68.5	52.2	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0
	(16.46)	(36.34)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)
Parasan	22.5	16.3	8.2	00.0	00.0	00.0	00.0	00.0	00.0	00.0
	(72.56)	(80.12)	(90.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)
Sulfex	45.5	34.2	26.2	22.0	19.5	15.0	11.0	8.5	7.3	6.2
	(44.51)	(58.29)	(68.04)	(73.17)	(76.21)	(81.70)	(86.58)	(89.63)	(91.09)	(92.43)
Control (No Fungicide)	82.0									
	(0.00)									

* After deducting the inoculum disc of 8.0 mm diameter

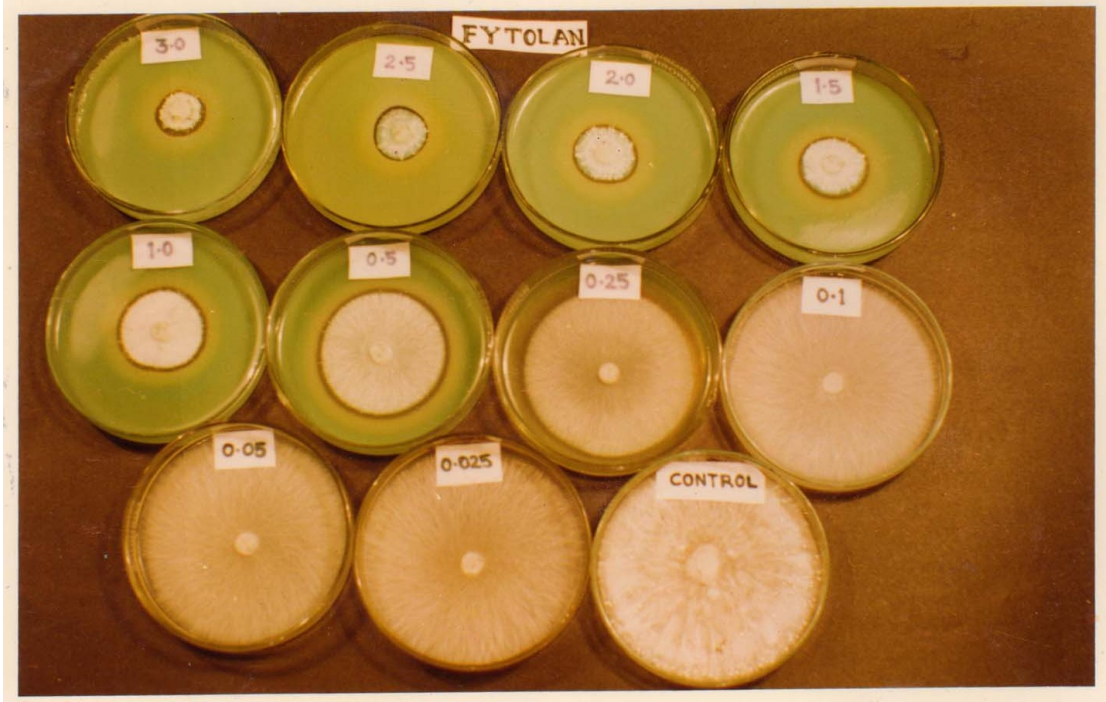
The data given in the brackets show per cent inhibition over control

Table.2 Fungicidal and fungistatic action of fungicides against *Sclerotium rolfsii*

Fungicide	Concentration (%)									
	0.025	0.05	0.1	0.25	0.5	1.0	1.5	2.0	2.5	3.0
Brassicol	+	+	+	+	+	+	+	+	+	+
Captan	+	+	+	+	+	+	+	-	-	-
Dithane M-45	+	+	+	+	-	-	-	-	-	-
DM-145	+	+	+	-	-	-	-	-	-	-
Manzate	+	+	-	-	-	-	-	-	-	-
Parasan	+	+	+	-	-	-	-	-	-	-

\+ Fungistatic - Fungicidal

Plate.1 Showing the effect of different concentrations of Fytolan on the growth of *Sclerotium rolfsii* (After 72 hours of inoculation)



After Manzate, the Parasan and DM-145 have been found to be the next effective fungicides against *Sclerotium rolfsii*. The 0.25% concentration of these two fungicides cause 100% inhibition of growth and the same concentration (i.e., 0.025%) of these fungicides proved to be fungicidal Dithane M-45 and Captan were also found to be toxic which cause 100% inhibition in radial growth at 0.5% and 2.0% concentration respectively. These two fungicides were also recorded as fungicidal in action at the same concentration. Sulfex, Fytolan and Bavistin have proved to be poor in this respect.

On the whole it can be concluded that amongst tested fungicides, only six fungicides were found to be more effective in which 100% inhibition was recorded. The relative degree of their effectiveness are as follows and the concentration in which 100% inhibition was recorded also given in brackets :

Brassicol (0.025%) > Manzate (0.1%) > Parasan (0.25%) >DM-145 (0.25%) > Dithane M-45 (0.5%) > Captan (2.0%)
Sulfex, Fytolan and Bavistin were proved to be poor and did not show cent per cent inhibition even in their higer dose i.e., 3.0% concentration.

All the above six fungicides in which 100% inhibition was recorded were tested for their fungicidal or fungistatic in action at various concentration. Manzate at 0.1%, Parasan at 0.25% and DM-145 at 0.25%, Dithane M-45 at 0.5% and Captan at 2.0% have been found to be fungicidal in action and showing toxic effect which killed the pathogen *Sclerotium rolfsii*. Only Brassicol was found to be fungistatic in action, in all the taken concentrations from 0.025% to 3.0%. In the light of present

investigation, fungicides like Brassicol, Manzate, Parasan, DM-145, Dithane M-45 and Captan may be of practical importance in control of foot-rot disease of brinjal incited by *Sclerotium rolfsii* which however requires field investigation.

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