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Evaluation of crop residues and fungicides in inhibiting carpogenic germination of *Sclerotinia sclerotiorum*(Lib.) de Bary

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Sclerotinia sclerotiorum (Lib.) de Bary is a soilborne plant pathogen with a wide host range. The management of the pathogen is important to prevent the losses caused by it on vegetable, fruit and field crops. The main source of spread of infection is through carpogenic germination, thus a study was conducted to minimise the carpogenic germination by the use of cultural and chemical methods of disease management. An *in vitro* study was conducted to observe the effect of twenty crop residues and systemic and non systemic fungicides. Amongst the tested crop residues mustard crop residues were found to be most effective in inhibition of carpogenic germination. Rice crop residues delayed the carpogenic germination by thirty three days. Systemic and non systemic fungicides were tested for their efficacy in minimising the myceliogenic and carpogenic germination. Mancozeb 63% and Azoxistrobin11%+ Tebuconzole 18.3% SC were most effective in inhibiting mycelial growth. Carbendazim, Azoxystrobin and Propiconazole were equally effective in inhibiting myceliogenic germination while Nativo and Propiconazole were found most effective in inhibiting carpogenic germination at 60 µg ml^{-1} and thus can be successfully utilized for disease management.

Keywords: Carpogenic germination, crop residues, disease management, fungicides

INTRODUCTION

Crop residues often are a source of primary inoculums for many plant pathogens, but some of the crop residues of the non host crops might prevent the occurrence of diseases in plants. *Sclerotinia sclerotiorum* (Lib.) de Bary being a soil borne pathogen is highly influenced by the crop residues in the soil. The pathogen has wide host range and thus can cause serious losses in many economically important crops (Hegedus and Rimmer, 2005). The sclerotia produced by the pathogen are the resting structures and survives in soils for longer durations of about three years(Cosic *et al.* 2012).

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The germination of sclerotia occurs either by myceliogenic germination or carpogenic germination in which apothecial cups are formed and ascospores are released causing infection in plants and again produce sclerotia (Fig.1), adding inocula to soil and the cycle continues. The survival and longevity of sclerotia depends on several factors such as the type of soil and burial depth (Duncan *et al.*2006). The inoculum levels also depend on the number of sclerotia formed on the host crop as 250-500 sclerotia are produced in cabbage head infected with *Sclerotinia sclerotiorum* (Leiner and Winton, 2006). Crop residues might inhibit or delay the germination of sclerotia and can thus prevent the occurrence of disease. As crop residue management is also a serious issue of concern nowadays, utilising it by incorporating in soil for disease management might be a solution for the

multiple problems by one strategy. Only a handful of studies are there on the use of crop residue based amendments for suppressing the sclerotial germination. Sweet clover straw reduced the germination of sclerotia at the rate of application of 3 per cent and combined application of *Trichoderma harzianum* and wheat straw also suppressed the sclerotial germination (Huang *et al.*2002). Wheat straw mulch reduced the incidence of sclerotinia blight in groundnut (Fergusan and Shaw, 2001).

Establishment of the pathogen in soil makes it tedious to manage due to its wide host range and soil borne nature. Several fungicides have been studied for its management with different efficacies. Fungicides are mostly studied for their efficacy in inhibiting mycelial growth of the pathogen and only a few studies emphasised on minimising the carpogenic germination of the sclerotia (Huang *et al.*2002). Systemic and non systemic fungicides prevent the mycelial growth of the pathogen and thus can minimise the sclerotia formation. It was observed that fungicides, carbendazim, thiophenate methyl and phenylpyrrole completely inhibited the growth of the pathogen at all the concentrations tested *in vitro*. Mancozeb was found effective at higher concentration while, copper oxychloride was least effective as it did not cause substantial reduction in growth of the pathogen. An *in vitro* study revealed cent per cent inhibition of mycelial growth of *Sclerotinia sclerotiorum* by 50 ppm concentration of carbendazim and hexaconazole. 50ppm concentration of Propiconazole inhibited 96.39% mycelial growth. Non systemic fungicides like captan and mancozeb inhibited cent per cent mycelial growth at a higher concentration of 500 ppm, while copper oxychloride was ineffective at lower concentrations (Rakesh *et al*. 2016). This study was undertaken to evaluate the efficacy of crop residues and fungicides in inhibiting the carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* under *in vitro* conditions.

MATERIALS AND METHODS

Effect of various crop residues on carpogenic germination of sclerotia

An *in vitro* experiment was conducted in which sterilized soil collected from the nearby agricultural fields (40 g/plates) was placed in petri plates. Crop residues of twenty crops *viz*; rice, maize, sugarcane, pigeon pea, tomato, brinjal, cauliflower, turmeric, citrus, mustard, bean, broccoli, cowpea, bottle gourd, ridge gourd, okra, cucumber, moong bean, potato and wheat were evaluated for their effects on the carpogenic germination. The soil in the petri plates was mixed with 2% (w/w) of crop residues. Ten sclerotia were added in each petri plate and the soil moisture was adjusted to near field capacity (25% w/w, air-dried basis) by adding distilled water. Soil amended with distilled water was used as a control. Sclerotia of *S. sclerotiorum* were partially buried in the soil in each plate. Three replications were maintained for each treatment. The plates were incubated at 18±2°C. After 30 days, the sclerotia in each plate were observed for carpogenic germination (Fig. 2) and Percent Inhibition of Sclerotial germination (PISG) was calculated as follows:

PISG = (C-T)/C *100

where, C=Number of sclerotia germinated in control
T=Number of sclerotia germinated in treatment

Efficacy of fungicides against mycelial growth of S.sclerotiorum

Effect of the fungicides on mycelial growth of the pathogen was studied using Poison food technique. Seven systemic fungicides (azoxistrobin, propiconazole, carbendazim, tebuconzole, azoxistrobin11%+ tebuconzole 18.3%, mancozeb 63% +carbendazim 12% and tebuconazole 50%+ trifloxystrobin 25%) were used at four different concentrations of 5, 10, 15 and 20 µg $ml^{-1}$and five non systemic fungicides (mandipropamid, mancozeb, copper oxychloride, metiram and copper hydroxide) were tested at 50, 100, 150 and 200 µg ml^{-1} concentration (Fig. 4). Stock solution of 10,000 µg ml^{-1}concentration of each fungicide was prepared using sterilised distilled water in test tube. Required amount of solution was thoroughly mixed with PDA to get the desired concentration of fungicide and poured in petri plates. For each concentration three replications were maintained. The non toxicated PDA was used as check. After the solidification

of media, a 5mm disc of three days old culture of the test pathogen was cut with the help of cork borer and placed at the center of each petri plate. The petri plates were then incubated at 20±2^{0}C. After four days of incubation the radial growth was measured and per cent inhibition was calculated using the formula (Vincent, 1947)

Per cent inhibition=(X-Y)/X×100

Where,

X=Colony diameter in control
Y=Colony diameter in treated medium

Efficacy of fungicides against myceliogenic germination of sclerotia

The sclerotia were dipped in different concentrations of fungicides viz 60, 80 and 100µg ml^{-1}for 12 hr, then were dried with sterilised blotter papers and were then placed in petri plates with solidified PDA. Three replicates were maintained for each treatment. The plates were incubated at 20±2°C. The observations were taken after 4 days of incubation, the sclerotia in each plate were observed for myceliogenic germination and Percent Inhibition of Sclerotial germination (PISG) was calculated.

Efficacy of fungicides against carpogenic germination of sclerotia

For carpogenic germination, air-dried soil (40 g/ plates) was placed in petri plates. Ten sclerotia were added in petri plate and the soil moisture was adjusted to near field capacity (25% w/w, air-dried basis) and 10ml of different fungicide solution at three different concentrations viz 60, 80 and 100µg ml^{-1}was poured in the petri plate. Soil amended with distilled water was used as a control. Sclerotia of *S. sclerotiorum* were partially buried in the soil in each plate. There were three plates as replication for each treatment. The plates were incubated at 18±2°C. The observations were taken after 30 days of incubation, the sclerotia in each plate were observed for carpogenic germination and number of apothecia produced was noted down.

RESULTS AND DISCUSSION

Effect of crop residues on carpogenic germination

Mustard crop residues were the most effective in inhibiting sclerotial germination (Table 1). The results obtained in the study were in accordance with Shetty *et al*.(2000)who observed reduction in sclerotial viability and disease incidence of *S. minor* by incorporation of crop residues of broccoli. Rice crop residues delayed the carpogenic germination by thirty three days (Fig. 2). Minimum number of apothecial cups was formed in wheat crop residues. All the crop residues were effective in reducing the number of apothecial cups productions as compared to that of no residues. The results were in accordance with Ferguson and Shew (2001)who observed reduced disease incidence ofSclerotinia blight of groundnut by incorporation of wheat crop residue in soil. Similar results were observed by Huang *et al*.(2002) that is 68 per cent inhibition of carpogenic germination by incorporation of wheat crop residues and 90% inhibition by crop residues of yellow mustard. An inhibition of 62%was observed by maize crop residues (3%) and 30 per cent inhibition by beans.Hao *et al*. (2003) also observed reduced incidence of Lettuce drop disease, by crop rotation of lettuce with broccoli and the density of sclerotia was lowest in the plots where the rotation was followed. Silva *et al.* (2011) also stated that crop residues negatively affect the number of apothecia formed per sclerotium. There may be two probable reasons for the suppression of sclerotial germination by crucifer crops, the most expected one being, glucosinolates in crucifer crops breakdown, to produce volatile compound inhibiting the pathogen and the other one being volatile compound produced during the decomposition of crop residues, predispose the sclerotia to the attack by other soil microbes. The most probable reason for inhibition of carpogenic germination by crops residues other than crucifer crops may be the production of ammonia on decomposition of crop residues. The decomposition of crop residues might also increase the microbial populations which may inhibit the carpogenic germination.

Table 1: Effect of crop residues on carpogenic germination of sclerotia

Crop Residues	% inhibition of sclerotial germination	Initiation of germination (in Days)	Number of stipes formed per plate	Number of Apothecial cups formed per plate
Rice	50.00	61.00	8.00	7.67
Maize	70.00	29.67	9.00	9.00
Sugarcane	33.33	29.67	16.33	15.00
Pigeon Pea	40.00	32.33	12.00	10.00
Tomato	30.00	30.00	14.00	12.67
Brinjal	46.66	30.33	13.00	13.00
Cauliflower	40.00	30.33	10.00	9.00
Turmeric	50.00	36.00	11.00	8.00
Citrus	30.00	31.67	14.00	10.00
Mustard	100.00	0.00	0.00	0.00
Bean	20.00	32.00	18.00	15.67
Broccoli	60.00	32.00	10.00	8.67
Cowpea	30.00	35.00	15.00	13.00
Bottle guard	30.00	31.67	12.67	11.00
Turai	30.00	30.00	14.00	11.67
Okra	20.00	31.33	17.67	17.00
Cucumber	30.00	30.00	15.00	13.33
Moong bean	26.66	32.00	14.00	12.00
Potato	53.33	31.00	12.67	11.33
Wheat	60.00	41.67	7.00	7.00
No residues	0.00	28.00	20.67	20.33
SEM±	0.14	0.89	0.20	0.24
CD at 5%	0.41	2.54	0.58	0.68

Table 2 : Effect of systemic fungicides on mycelial growth of *Sclerotinia sclerotiorum*

Systemic fungicides	Per cent Inhibition of radial growth*			
	5 µg ml^{-1}	10 µg ml^{-1}	15 µg ml^{-1}	20 µg ml^{-1}
Azoxistrobin11%+ Tebuconzole 18.3%SC	100.00	100.00	100.00	100.00
Mancozeb 63% +Carbendazim 12% WP	86.66	92.96	100.00	100.00
Tebuconzole 25.9% EC	91.85	91.33	100.00	100.00
Tebuconazole 50%+ Trifloxystrobin 25% WG	100.00	100.00	100.00	100.00
Carbendazim 50%WP	100.00	100.00	100.00	100.00
Azoxistrobin 23% SC	17.03	33.33	55.55	77.77
Propiconazole 25%EC	100.00	100.00	100.00	100.00
Check	0.00	0.00	0.00	0.00
	Fungicide (a)	Concentration (b)	Interaction (a×b)	
SEM±	0.51	0.36	0.10	
CD at 5%	0.14	0.10	0.28	

* Mean of three replications

Table 3: Effect of non systemic fungicides on mycelial growth of *Sclerotinia sclerotiorum*

Non Systemic fungicides	% Inhibition of radial growth*			
	50 µg ml^{-1}	100 µg ml^{-1}	150 µg ml^{-1}	200 µg ml^{-1}
Copper oxychloride50%WP	0.00	1.48	28.51	42.96
Metiram70%WG	12.96	29.25	52.96	74.44
Copper Hydroxide 53.8%DF	0.00	4.81	25.92	40.00
Mandipropamid 25% SC	0.00	0.00	31.48	48.14
Mancozeb75%WP	53.33	84.81	100.00	100.00
Check	0.00	0.00	0.00	0.00
	Fungicide(a)	Concentration(b)	Interaction (a×b)	
SEM±	0.48	0.39	0.97	
CD at 5%	1.38	1.13	2.77	

* Mean of three replications

Table 4: Effect of fungicides on myceliogenic germination of sclerotia

Fungicides	Per cent inhibition of sclerotial germination*		
	60 µg ml^{-1}	80 µg ml^{-1}	100 µg ml^{-1}
Copper oxychloride 50% WP	0.00	13.33	20.00
Metiram 70% WG	0.00	6.67	40.00
Copper Hydroxide 53.8% DF	0.00	20.00	26.67
Mandipropamid 25% SC	0.00	20.00	40.00
Mancozeb75%WP	20.00	20.00	60.00
Azoxistrobin11%+Tebuconzole18.3%SC	40.00	100.00	100.00
Mancozeb 63% +Carbendazim 12% WP	60.00	80.00	100.00
Tebuconzole 25.9% EC	73.34	100.00	100.00
Tebuconazole50%+Trifloxystrobin25% WG	80.00	100.00	100.00
Carbendazim 50%WP	100.00	100.00	100.00
Azoxistrobin 23% SC	100.00	100.00	100.00
Propiconazole 25%EC	100.00	100.00	100.00
Check	0.00	0.00	0.00
	Fungicide(a)	Concentration(b)	Interaction (a×b)
SEM±	1.24	0.59	2.16
CD at 5%	3.51	1.68	8.08

* Mean of three replications

Effect of fungicides on mycelial growth of Sclerotinia sclerotiorum

The efficacy of twelve fungicides was tested against mycelial growth of fungi using Poison food technique and thus different concentrations were used for systemic (5, 10, 15 and 20µg ml^{-1}) and non systemic fungicides (50, 100, 150 and 200µg ml^{-1}). Amongst all the tested systemic fungicides carbendazim (Fig. 3), propiconazole and azoxistrobin11%+ tebuconzole 18.3%SC was found to be highly effective in inhibiting mycelial growth with cent per cent inhibition at all tested concentrations while azoxistrobin 23% SC was least effective (Table2).

Effect of fungicides on myceliogenic germination of sclerotia

The results revealed that systemic fungicides, carbendazim 50%WP, azoxistrobin 23% SC and

Table 5 : Effect of fungicides on carpogenic germination of *Sclerotinia sclerotiorum*

Fungicides	Per cent inhibition of sclerotial germination* Concentrations		
	60 µg ml^{-1}	80 µg ml^{-1}	100 µg ml^{-1}
Copper oxychloride50%WP	40.00	60.00	70.00
Metiram70%WG	30.00	40.00	50.00
Copper Hydroxide 53.8%DF	50.0	70.00	80.00
Mandipropamid 25% SC	20.00	40.00	40.00
Mancozeb75%WP	53.33	60.00	70.00
Azoxistrobin11%+ Tebuconzole 18.3%SC	80.00	86.66	100.00
Mancozeb 63% +Carbendazim 12% WP	90.00	100.00	100.00
Tebuconzole 25.9% EC	86.66	100.00	100.00
Tebuconazole 50%+ Trifloxystrobin 25% WG	100.00	100.00	100.00
Carbendazim 50%WP	90.00	100.00	100.00
Azoxistrobin 23% SC	60.00	70.00	100.00
Propiconazole 25%EC	100.00	100.00	100.00
Check	0.00	0.00	0.00
	Fungicide(a)	Concentration(b)	Interaction (a×b)
SEM±	0.53	0.25	0.92
CD at 5%	0.15	0.72	0.26

* Mean of three replications

propiconazole 25% EC, showed cent per cent inhibition of myceliogenic germination even at lowest tested concentration (60 µg ml^{-1}). While amongst non systemic fungicides Mancozeb75%WP was found to be the most effective. The results indicated that with increase in concentration of fungicide the myceliogenic inhibition by all the fungicides also increased. Systemic fungicides were highly effective at all the tested concentrations where as non systemic fungicides were ineffective at low concentration but the efficacy increased with increase in concentration (Table 3).

Effect of fungicides on carpogenic germination

Twelve fungicides including seven systemic and five non systemic were tested at three different concentrations viz., 60, 80 and 100 µg ml^{-1} for their inhibitory effect on carpogenic germination of sclerotia. Propiconazole 25%EC and tebuconazole 50%+ trifloxystrobin 25% WG showed cent per cent inhibition of sclerotial germination at all tested concentrations while mandipropamid 25% SC was found to be least effective.

The low efficacy of fungicides against myceliogenic germination compared to that against carpogenic germination may be due to less duration of exposure of sclerotia to fungicides as in sclerotial dip method the sclerotia were dipped for 12 hours in fungicides whereas in case of carpogenic germination exposure of fungicides was for about thirty days. Also the difference in the efficacy of fungicides against carpogenic and myceliogenic germination may due to the difference in mobility of different fungicides in soil (Table 4).

The results indicated that with increase in fungicide concentrations, the efficacy of fungicides also increased and systemic fungicides were more effective compared to non systemic fungicides. The results were in agreement with Krishnamoorthy *et al*. (2017) who observed that Nativo (tebuconazole + trifloxystrobin) and carbendazim as equally effective against the pathogen. Rakesh *et al.*

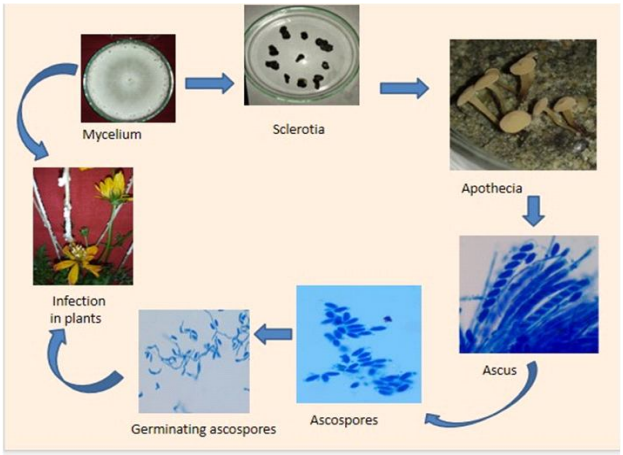

Fig.1: Disease cycle of *Sclerotinia sclerotiorum*

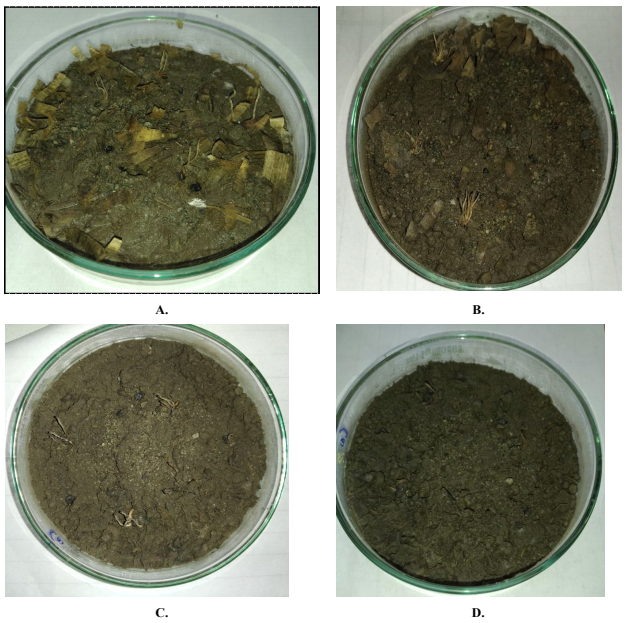

Fig. 2: Effect of crop residues on carpogenic germination, A.Maize, B. Broccoli, C. Turmeric and D. Wheat

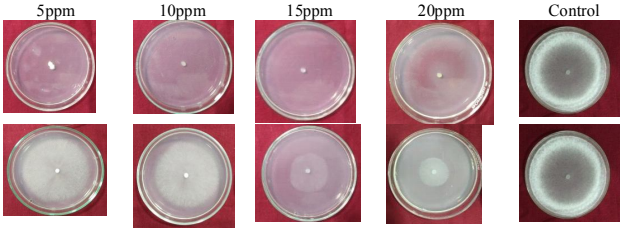

Fig. 3: Efficacy of systemic fungicides against mycelial growth; a.Carbendazim, b.Azoxistrobin

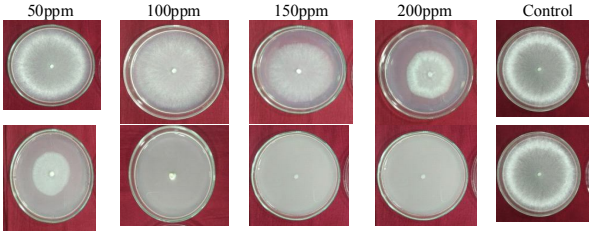

Fig. 4: Efficacy of non systemic fungicides against mycelial growth;a.Copper hydroxide b.Mancozeb

(2016) also observed that carbendazim was highly effective *in vitro* against *S. sclerotiorum* and completely inhibited mycelial growth. Propiconazole was effective in inhibiting mycelial growth. Also at low concentration, Copper oxychloride did not show any inhibitory effect on growth of mycelia but were effective at higher concentration. The results also aligned with Goswami *et al*. (2020) who observed complete inhibition of sclerotial germination on dipping in benomyl and carbendazim at 100 and 250 µg/ml respectively.

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DECLARATIONS

Conflict of Interest: Authors declare no conflict of interest.

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