Research Paper :

Screening of *Sclerotium rolfsii* Sacc. isolates for tolerance and sensitiveness against commonly used fungicides

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SUMMARY

A study was carried out on tolerance and sensitiveness among 24 isolates of *Sclerotium rolfsii* collected from Latur, Solapur and Nanded districts of Maharashtra state. Twelve fungicides *viz*; benomyl, orthocide, mancozeb, pentachloronitrobenzene (PCNB), tebuconazole, hexaconazole, propiconazole, matalaxyl, thiram, difenoconazole, chlorothalonil, and copper oxychloride were screened against 24 isolates of *S. rolfsii* using poison food technique. Out of 24 isolates, seven isolates *viz.*, SR-03, SR-05, SR-7, SR-10, SR-14, SR-17, and SR-19 showed high levels of tolerance to all these 12 commonly used fungicides. Nine isolates SR-01, SR-02, SR-6, SR-8, SR-11, SR-13, SR-15, SR-18 and SR-24 were weekly tolerant. Remaining eight isolates were highly sensitive to all these fungicides. PCNB, benomyl, orthocide, difenoconazole and chlorothalonil were found to be the most effective in suppressing the growth of most of the tested isolates. The fungicides like thiram, and matalaxyl were the least effective where all the isolates showed tolerance. The isolates of *S. rolfsii* obtained from diseased stem of groundnut crop showed high tolerance to all these fungicides. The isolates, obtained from rhizospheric soil of diseased crop were found to be weekly tolerant and sensitive to these fungicides.

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Stem rot of sunflower cause by *Selerotium rolfsii* occurred 30 to 40 days after sowing. Stems are usually infected at or near the soil line. A brownish lesion develops at the base of the stem and eventually girdles the plant. Later, the entire plant withers and dies. White cottony mycelium and mustard-seed-type sclerotial bodies are conspicuous on the affected stem near soil level. About 10 to 15 per cent plants have been reported to be affected, amounting to 20 per cent loss in yield, if the sunflower crop is planted in July or August or in February or March. The fungal contamination of seeds and grains during storage and the metabolites produced by them are of considerable importance in reducing the seed germination and sprouting (Jain and Patel, 1961). Stem rot diseases cause severe seedling mortality resulting in "patchy" crop stand and ultimately reduce the yields. Management of this disease is very difficult and uneconomical with chemical fungicides alone because of resistance development to chemical fungicides. For the management of this disease, farmers are using different fungicides as seed treatments before sowing but farmers are not getting satisfactory results as the pathogen developed fungicide resistance. Intensification and monocropping of sunflower led to an increase in the incidence of stem rot. Management of this disease is difficult due to prolonged survival ability and wide host range of the pathogen. *S. rolfsii* is a devastating soil-borne plant pathogenic fungus with a wide host-range (Aycock, 1966, Punja, 1988), has prolific growth and ability to produce persistent sclerotia contributing in high degree of economic losses (Mahen *et al*., 1995). The fungus forms differentiated sclerotia and sterile mycelia like other sclerotium-producing fungi. Those characterized by small tan to dark-brown or black spherical sclerotia with internally differentiated rind, cortex, and medulla were placed in the form genus *Sclerotium* (Punja and Rahe, 1992). However, the teleomorphic state was discovered later (Punja, 1988), confirming that the fungus was a basidiomycete. *S. rolfsii* usually causes collar rot (Singh and Pavgi, 1965). Cultivation of resistant varieties is the ideal and feasible management of the disease and resistant sources against this disease had been identified in various countries (Sugha *et al*., 1991; Gurha and Dubey, 1982). Geographical variability among *S. rolfsii* populations was demonstrated by earlier workers (Harlton *et al*., 1995; Nalim *et al*., 1995; Okabe *et al*., 1998). Investigations on

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variability within the population in a geographical region are important because these also document the changes occurring in the population. Pentachloronitrobenzene (PCNB) has been used with moderate success for many years for the control of stem rot (southern blight) of peanut (*Arachis hypogaea* L.), incited by *S. rolfsii* Sacc. (Brenneman *et al.*, 1991 and Harrison, 1961). However, there are numerous reports of inconsistent control of stem rot with fungicides (Brenneman *et al.*, 1991, Csinos, 1984, Csinos, 1985, Csinos, 1987, Edlich and Lyr, 1992, Hagan *et al.*, 1991). Smith *et al.* (1986) reported that PCNB provided less than satisfactory control of peanut stem rot in Texas and suggested that tolerance to PCNB may be partially responsible. In Georgia, control of stem rot with PCNB has been erratic also (Smith *et al.*, 1986). Considerable variation among isolates of *S. rolfsii* with respect to their sensitivity to PCNB has been reported (Anilkumar *et al.*, 1984, Brenneman *et al.*, 1991, Edlich and Lyr, 1992). Georgopoulos (1964) developed a strain of *S. rolfsii* that was tolerant to PCNB. Objectives of the present study were to 1.Understand tolerance and sensitivity of the *S. rolfsii* isolates to different fungicides 2. Find out most suitable fungicide for the management of stem rot disease of sunflower.

MATERIALS AND METHODS

Isolation and maintenance of S. rolfsii isolates:

Twenty four isolates of *S. rolfsii* were isolated from infected sunflower plants and rhizospheric soil samples. The diseased sunflower plants and soil samples were collected from sunflower growing areas of Latur, Nanded, and Solapur districts of Maharashtra state. Field survey was conducted during *Kharif* 2008 and 2009. Infected sunflower plants and soil samples were brought to the laboratory in separate polythene bags.

Isolation from infected plants:

As soon as the infected sunflower plants were brought to laboratory a small bit of 5 mm diameter was carefully excised from infected plant parts, surface sterilized with 0.1% $HgCl_2$ and transferred onto Potato dextrose agar (PDA) in Petri dishes. These plates were incubated for 5 days at 28^0C in BOD. After 5 days of incubation, pure culture of *S. rolfsii* was obtained by hyphal tip culture.

Isolation from soil:

One hundred mg soil samples was finely powdered and stirred in one ml sterile distilled water and serial dilutions were prepared and from 10^3 dilutions, 100 µl was spread in Petri dishes containing on Potato dextrose agar medium in three replicates. The plates were incubated at 28^0C for 4 days in BOD, and typical colonies of *S. rolfsii* were isolated and sub-cultured on PDA medium. Twenty four isolates of *S. rolfsii* were obtained from infected sunflower plants and rhizospheric soil samples. These isolates were maintained on PDA slants at 4^0C for further studies.

Fungicides:

Twelve fungicides *viz.*, Benomyl, Orthocide, Hexaconazole, Copper oxychloride, Mancozeb, Tebuconazole, Chlorothanil, Pentachloronitro- benzene, Thiram, Propiconazole, Matalaxyl and Difenoconazole were tested under *in vitro* conditions.

Screening against fungicides:

The sensitivity of *S. rolfsii* isolates to twelve fungicides at the rate of recommended doses was studied using poison food technique. A weighed quantity of each fungicide was amended in Potato extrose agar medium after autoclaving. Twenty ml of amended and non-amended medium was poured into 90 mm diameter Petri dishes. After solidification, the agar medium in the culture plates was seeded with 5 mm culture dises of three days old cultureof *S. rolfsii*. For each isolate and fungicide, five replications were maintained. The inoculated Petri dishes were incubated at 28^0C and radial colony growth (mm) of *S. rolfsii* was recorded after 72 hours of incubation. Isolates were grouped as tolerant "T" with radial growth of the fungus more than 50 mm, weakly tolerant "WT" with radial growth of the fungus between 30 to 50 mm and sensitive "S" with growth less than 30 mm.

The percentage growth inhibition of *S. rolfsii* was obtained by using the formula:

$$\text{Percentage growth inhibition} = \frac{A - B}{A} \times 100$$

where, A= Area covered by *S. rolfsii* in control (mm)

B= Area covered by *S. rolfsii* in different fungicide treatments (mm)

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Isolation of S. rolfsii:

Twenty four isolates of *S. rolfsii* were obtained from diseased sunflower plants and soil samples. The details of all these isolates are given in Table 1.

Table 1 : Details of isolates of *S. rolfsii* Sacc. isolated from diseased sunflower plants and soil

Sr. No.	Accession no. of Isolates.	Locations	Type of sample for isolation
1.	NCPPS-SR-01	Ausaa	Soil
2.	NCPPS-SR-02	Nilanga	DS
3.	NCPPS-SR-03	Latur	DS
4.	NCPPS-SR-04	Udgir	Soil
5.	NCPPS-SR-05	Ahemadpur	DS
6.	NCPPS-SR-06	Solapur	Soil
7.	NCPPS-SR-07	Belati	DS
8.	NCPPS-SR-09	Sangela	DS
9.	NCPPS-SR-10	Kandhar	DS
10.	NCPPS-SR-11	Bhokar	DS
11.	NCPPS-SR-12	Loha	DS
12.	NCPPS-SR-13	Nanded	Soil
13.	NCPPS-SR-14	Savrkhed	Soil
14.	NCPPS-SR-15	Kevla	DS
15.	NCPPS-SR-16	Kushnoor	DS
16.	NCPPS-SR-17	Biloli	DS
17.	NCPPS-SR-18	Kotgil	DS
18.	NCPPS-SR-19	Karkheli	Soil
19.	NCPPS-SR-20	Shirur	DS
20.	NCPPS-SR-08	Wadhona	DS
21.	NCPPS-SR-21	Balsangi	DS
22.	NCPPS-SR-22	Hadolti	DS
23.	NCPPS-SR-23	Umrga	Soil
24.	NCPPS-SR-24	Jalkot	DS

DS: Diseased stem

Fungicides

The details of all fungicides used in present study are given in Table 2.

Screening against fungicides:

The findings in present investigation indicated that there was a significant variability among the isolates of *S. rolfsii* in this regard. Variability among the isolates of *S. rolfsii* was determined on the basis of their tolerance and sensitivity to different fungicides. On the basis of tolerance and sensitivity to different fungicides these isolates were categorized in to three groups *viz.*, tolerant, weakly tolerant and sensitive. The tolerant isolates were- SR-03, SR-05, SR-7, SR-10, SR-14, SR-17, and SR-19 (Plate 3). The weakly tolerant group included- SR-01, SR-02, SR-6, SR-8, SR-11, SR-13, SR-15, SR-18 and SR-24 (Plate 2). Remaining eight isolates *viz*. SR-04, SR-09, SR-12, SR-16, 20, SR-21, SR-22 and SR-23 were highly sensitive to all these fungicides (Plate 1). Among these fungicides benomyl, orthocide, copper oxychloride, pentachloronitrobenzene (PCNB), tebuconazole, hexaconazole and propiconazole were found to be effective in suppressing the growth of most of these tested isolates. Other fungicides like mancozeb, thiram, matalaxyl and difenoconazole were the least effective where few isolates showed tolerance (Table 3 and Fig. 1).

The substitutions of tebuconazole for chlorothalonil resulted in consistently higher yields and reduced incidence and/or severity of both foliar and stem rot diseases. There were strong correlations between the number of tebuconazole applications and stem rot incidence, pod yield and both grade and percentage damage of kernels. PCNB and carboxin have been used for many years in the USA to suppress stem rot. They only offer upto 50% control when used as either granules or liquid delivered via irrigation water. Tebuconazole provides good control than other fungicides. This fungicide and several other triazole types, sterol inhibiting fungicides have given greater than 80% control of stem rot at seasonal use rates of less than 1.0 kg/ha.

El-Tobshy *et al*. (1981) found that *Fusarium oxysporum* was very sensitive to fungicides during *in vitro* studies in which this fungus was equally inhibited by

Table 2 : Details of fungicides used in the present study

Trade name	Chemical name	Formulation	Manufacturer
Benlate	Benomyl	50 wp	R.B. Avari Entreprises Ltd.
Captan	Orthocide	50 wp	ICI (Pvt) Ltd.
Control (Total)	Hexaconazole	5% S.C.	Meghamani Industries limited
Cobox	Copper oxychloride	50 wp	Agricide (Pvt) Ltd.
Dithane M-45	Mancozeb	80 wp	Rohm and Hass Ltd.
Foilcur	Tebuconazole	25.9% EC	Bayer
Kavach	Chlorothanil	75% W.P.	Syngenta
PCNB	Pentachloronitro- benzene	100 wp	ICN Biomedicals
Thiram	Thiram	75% W.P.	Bayer
Tide	Propiconazole	25% EC	Meghamani Industries limited
Ridomil	Matalaxyl	68 wp	Novartis (Pvt) Ltd.
Score	Difenoconazole	25% EC	Syngenta

Table 3 : Tolerance and sensitivity of different isolates of *Sclerotium rolfsii* Sacc. screened against twelve fungicides

Fungicides	*Sclerotium rolfsii* Isolate numbers																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Benomyl	WT	S	T	S	T	WT	T	WT	S	T	WT	S	WT	T	WT	S	T	WT	T	S	T	S	S	WT
Chlorothanil	T	WT	T	S	T	WT	T	S	S	S	WT	S	WT	T	S	T	T	WT	T	S	S	T	S	T
Copper oxych	WT	WT	T	S	T	S	T	WT	S	S	S	S	WT	T	WT	S	T	WT	T	WT	S	S	S	S
Hexaconazole	WT	WT	T	S	T	WT	T	WT	S	S	WT	S	T	T	WT	S	T	WT	T	S	S	T	T	WT
Mancozeb	T	T	T	T	T	WT	T	T	T	S	WT	S	WT	T	T	S	T	WT	T	S	S	S	S	WT
Matalaxyl	WT	WT	T	S	T	WT	T	WT	S	S	WT	S	WT	T	WT	S	T	WT	T	WT	S	S	S	T
Orthocide	WT	S	T	S	T	S	T	WT	S	S	S	S	WT	T	T	S	T	WT	T	S	S	WT	S	T
PCNB	WT	WT	T	S	T	WT	T	WT	S	S	S	S	WT	T	WT	S	T	WT	T	S	S	S	S	WT
Propiconazole	WT	WT	T	S	T	WT	T	WT	S	S	WT	S	WT	T	WT	S	T	WT	T	S	S	WT	S	WT
Tebuconazole	WT	WT	T	S	T	WT	T	WT	S	S	WT	S	WT	T	WT	S	T	WT	T	S	S	S	S	S
Thiram	T	T	T	T	T	T	T	WT	S	S	T	S	WT	T	WT	S	T	WT	T	T	S	T	S	WT

T : Tolerant WT : Weakly tolerant S : Sensitive

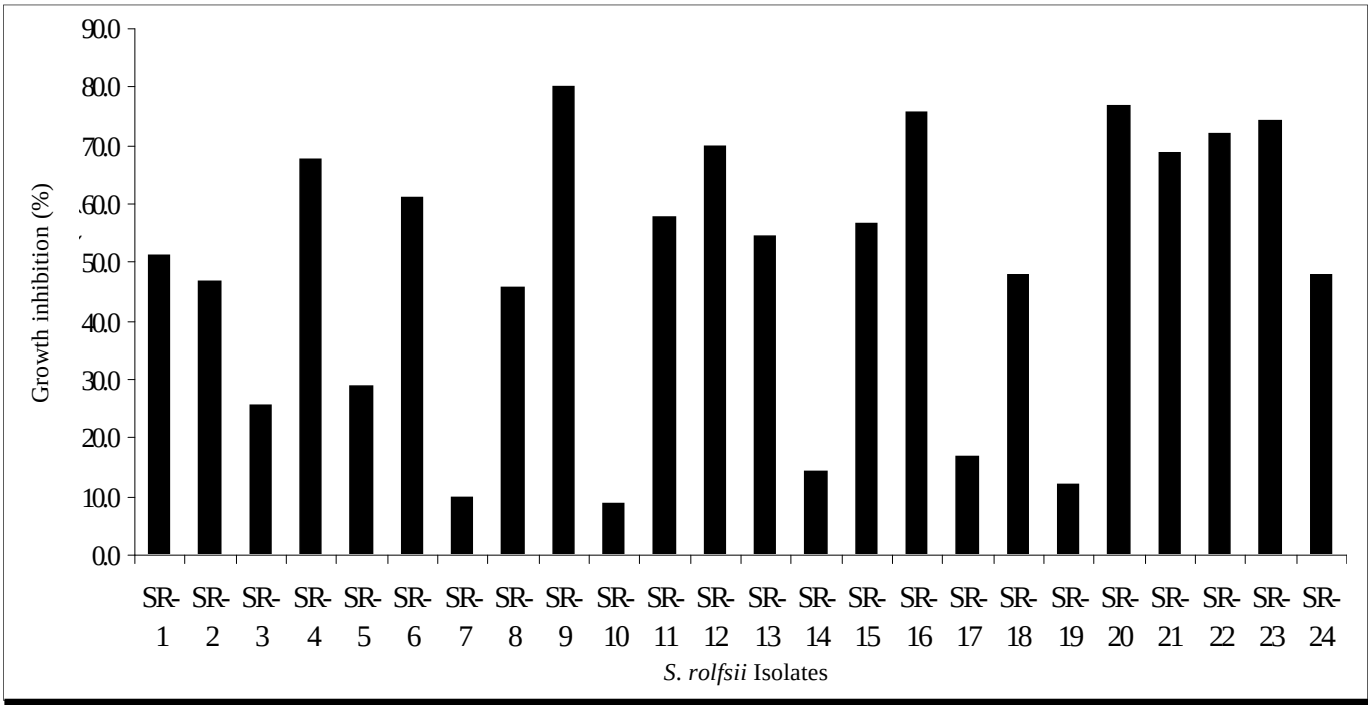

Fig. 1 : Percentage growth inhibition of *S. rolfsii* isolates by different fungicides

benomyl, thiabendazole and thiophenate methyl. Thus, the efficacy of benlate in the present study had been confirmed in the light of previous research. Similarly, benlate had been used against a wide spectrum of fungal diseases such as grey mold, powdery mildew and black spot of roses, scab and powdery mildew of apples, powdery mildew of cucurbits and strawberries (Scot *et al.*, 1979). Similarly, it was effective for the control of rice blast (Kamerwar-Row, 1976). The results obtained with benlate are in agreement with those of Bashir *et al*. (1985) who recommended it for the control of mungbean anthracnose (*Colletotrichum lindemuthianum*). The sensitivity of mycelia to benlate has also been reported for *Ascochyta lentis* (Ilyas *et al.*, 1992). Benlate is a systemic fungicide which is used against the fungal diseases as spray, soil drenching and seed treatment for air-borne, soil-borne and seed-borne diseases, respectively. Its systemic fungitioxicity in many plants had been reported by Erwin *et al*. (1969). The present results are also in accordance with the work of Johnson and Subramanyam (2000) who observed complete inhibition in radial growth of *S. rolfsii* with hexaconazole and propiconazole. Similar results were also reported by Isaiah *et al.* (2005).

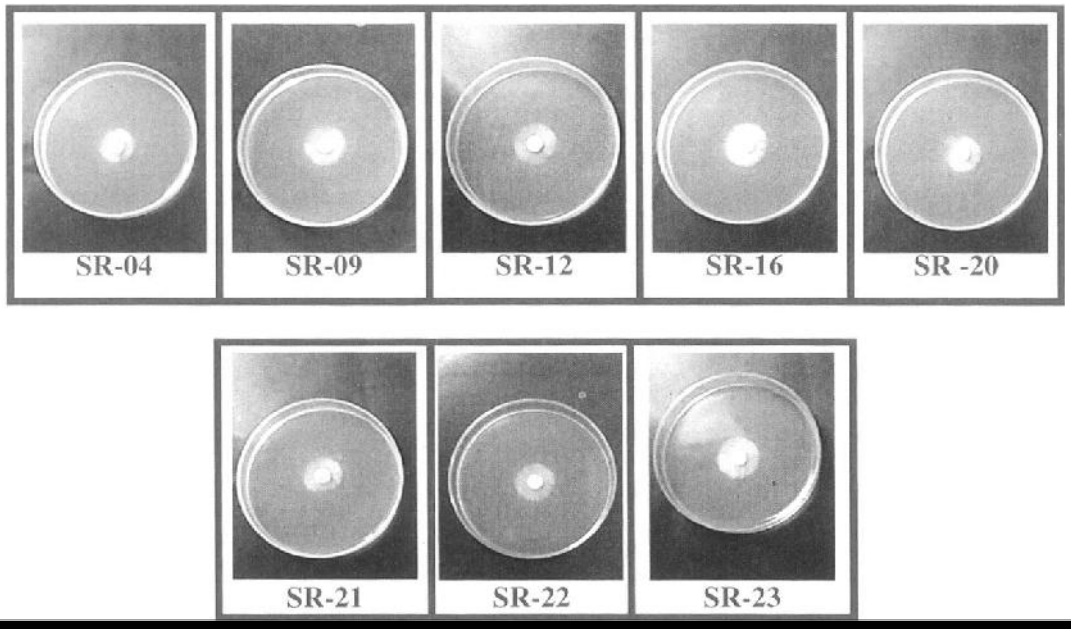

Plate 1 : Isolates of *S. rolfsii* sensitive to different fungicides

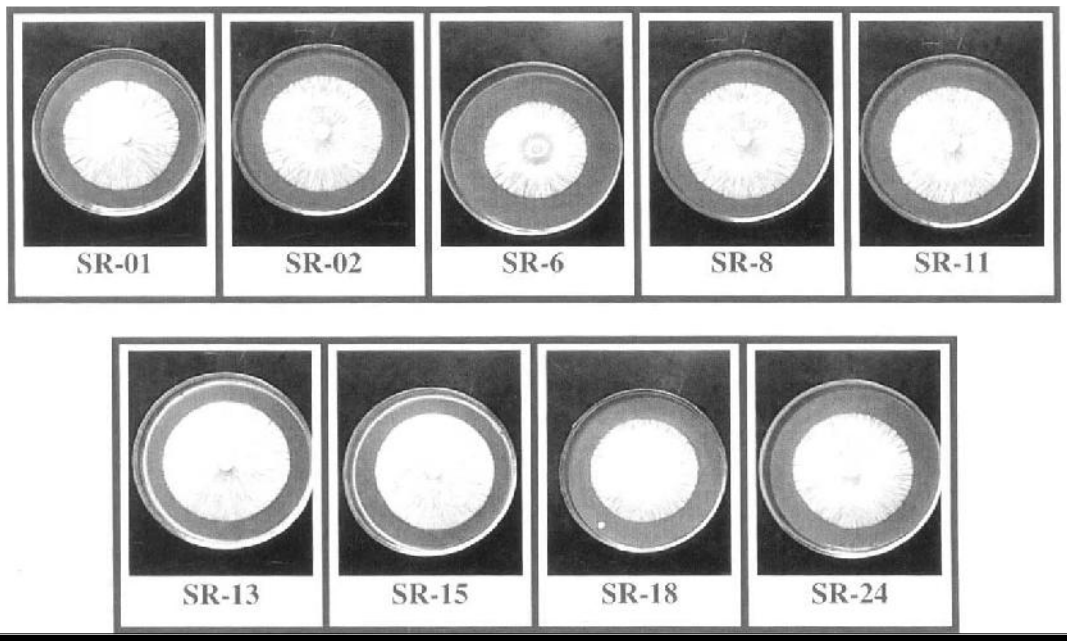

Plate 2 : Isolates of *S. rolfsii* weakly tolerant to different fungicides

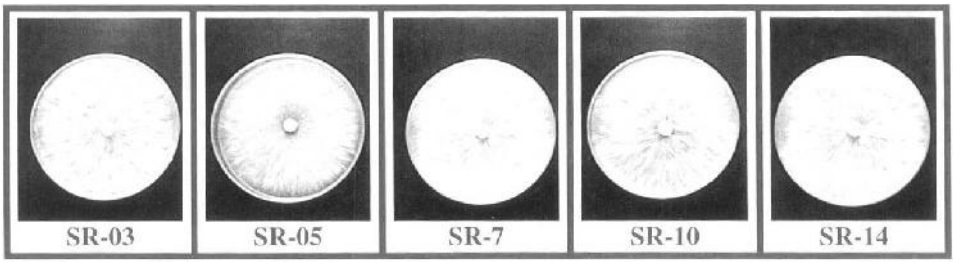

Plate 3 : Isolates of *S. rolfsii* tolerant to different fungicides

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