Himachal Journal of Agricultural Research 33 (2):218-222, 2007

Antifungal activity of some plant extracts against *Sclerotium rolfsii* Sacc.

Meena Devi and S. K. Sugha
Department of Plant Pathology
CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062, India.

Abstract

Antifungal activity of aqueous and ethanolic extract and dried powder of *Achyranthes aspera L.*, *Artemisia tridentata* Nutt., *Eucalyptus longifolia* Link., *Cedrus deodara L.*, *Lantana camara* L., *Ranunculus muricatus L.*, and *Swertia angustifolia* Buch-Ham. was evaluated against *Sclerotium rolfsii* Sacc. through mycelial growth inhibition and control of pre- and post-emergence damping-off of chili seedlings. Aqueous extract of fresh *R. muricatus* plants and dried powder of *A. tridentata* and ethanolic extracts of fresh plants of *C. deodara* ,*E, longifolia* and *R.muricatus* and dried powder of *A. tridentata C. deodara, E. longifolia*, *R. muricatus* and *S. angustifolia* caused complete inhibition of mycelial growth of pathogen. Aqueous and ethanolic extracts of fresh and powder of other test plants also reduced the mycelial growth of the pathogen significantly over check-1 (medium devoied of plant extract). Soil amendment with partially crushed fresh plants of *A. aspera* and *A. tridentata* and that of dried powder of *L. camara* was found effective in suppressing pre- and post-emergence damping-off of seedlings, respectively, whereas soil drenching with extracts of test plants was comparatively less effective.

Keywords: Antifungal activity, botanicals, *Sclerotium rolfsii*, damping-off.

Introduction

Sclerotium rolfsii Sacc., a soil borne pathogen infects more than 500 plant species (Aycock, 1966; Sarma *et al.*, 2002) and causes severe losses in many crops. Control of pathogen is difficult due to its prolific saprophytic growth habitat and production of large number of sclerotia which persist in soil for several years (Punja, 1985). Control measures effective for a particular crop or area may not be adaptable else where and repeated application of fungicides resulted in varying success with residual problem of pesticides in soil. Some compounds of higher plant origin have proved successful as natural fungitoxicants because of their nonphytotoxicity and biodegradable nature (Shivpuri *et al.*, 1997). Hence it was thought worthwhile to generate information on antifungal activity of some plants growing in Himachal Pradesh against the pathogen, the subject matter of present study.

Material and Methods

Isolation of pathogen

The pathogen was isolated on PDA medium from diseased chili seedlings and pure culture was obtained through hyphal tip method. For pot culture studies, the pathogen was mass multiplied on oat grains. One hundred gram oat grains were soaked overnight in 250 ml conical flask in water, sterilized at 1.03 Kg/cm^2, inoculated with pathogen aseptically and incubated at 22±1^{0}C for 14-days.

Preparation of extracts

Aqueous and ethanolic extracts of fresh plants were prepared by taking one gram plant material per ml of water/ethanol. The plant materials were thoroughly washed separately with tap water, crushed in a sterilized pestle mortar and filtrated through double layered muslin cloth. Filtrate were collected in beakers and stored in refrigerator (4°C) for subsequent studies. Plant materials were oven dried at 45°C for 4-days to a constant weight and pulverized to obtain dry powder. For preparation of aqueous/ethanolic extracts of dried plant material (100g fresh material gave 25g on drying) 0.25g was used per ml of sterilized water/ethanol. Ethanol was completely evaporated in a water bath at 40 ± 2 °C and concentrate was diluted with sterilized distilled water.

Evaluation of extracts

Aqueous/ ethanolic extracts of fresh plants and their dried powders were evaluated through poisoned food technique (Vincent, 1947). Extracts were mixed with PDA in 1:2 ratio in conical flask and autoclaved. Autoclaved poisoned food was poured in sterilized petriplates, cooled, inoculated with 5 mm disc of

7 days old culture and incubated at 25 ± 1°C. Medium devoid of extracts and other poisoned with captan (0.2%) served as check-I and II, respectively. The experiment was repeated thrice with three replicates/treatment. Data on mycelial growth were recorded after 48 hr of incubation when petriplates devoid of extracts were fully covered with mycelial growth of pathogen and per cent inhibition in each treatment was calculated.

To evaluate fungitoxicity of partially crushed fresh test plants and their dried powders against the test pathogen, garden soil was steam sterilized for 2 consecutive days and made sick by mixing 1kg inoculum per 20 kg of sterilized soil. Partially crushed fresh test plants and their equivalent dried powder were mixed with sick soil in the ratio of 1:4 w/w before filling in pots.

Aqueous extracts of test plants were also evaluated for their fungitoxicity through soil drench method in pot culture. Pots were drenched @ 100 ml extract of test plants individually immediately, 24 and 48 hrs before sowing seeds of 'Kullu local'chili. Pots filled with sick soil and sterilized soil served as controls. Twenty seeds per pot and three pots per treatment were maintained. Seeds treated with captan @ 0.25% and untreated seeds in sick soil served as controls. Data on seed germination as well as pre- and post-emergence damping-off of seedlings were recorded from 7th day of sowing till one month.

Results and Discussion

Aqueous extracts of fresh plants of all the test plants significantly reduced the mycelial growth of the pathogen over check (Table 1). Aqueous extract of *R. muricatus* caused complete inhibition of mycelial growth of pathogen and differed significantly from rest of the test plants. *E. longifolia* and *C. deodara* were at par in reducing the mycelial growth. Aqueous extracts of *A. aspera* and *S. angustifolia* were comparatively less effective and those of *A. tridentata* and *L. camara* caused 46.6% and 57% inhibition of mycelial growth of the pathogen, respectively. Similarly aqueous extracts of dried powder of *A. tridentata* caused complete suppression of mycelial growth and those of *C. deodora, E. longifolia, R. muricatus* and *S. angustifolia* resulted in 83.7-95.2 per cent inhibition of mycelial growth of the pathogen. The aqueous extracts of dried powder of other plants caused 21.4 to 83.7% inhibition of mycelial growth of pathogen. Statistically, extract of dried powder of *C. deodara, R. muricatus and S. angustifolia* were at par in suppressing the mycelial growth of the pathogen.

Ethanolic extracts of fresh and dried powder of all the test plants caused significantly more inhibition of mycelial growth than the aqueous extracts. Ethanolic extract of fresh plants of *C. deodara, E. longifolia* and *R. muricatus* and dried powders of *A. tridentata, C. deodara, E. longifolia, R. muricatus* and *S. angustifolia* caused complete inhibition of mycelial growth of *S. rolfsii*. Ethanol than water seems to be a better solvent for studying the antifungal activity of plants. Antifungal activity of *E. longifolia* against *S. rolfsii* is in agreement with Singh and Dwivedi (1990). Antifungal activity of *A. tridentata* and *R. muricatus* and *L. camara* is documented against other pathogens but not against *S. rolfsii* (Bishit and Khulbe, 1995; Mishra and Tewari, 1990).

Soil amendment with partially crushed fresh plants of *A. aspera* followed by *A. tridentata* was found effective against *S. rolfsii* and supported very good seed germination and their effect was superior to soil amendment with fungicide (Table 2). *L. camara* followed by *A. tridentata* gave good control of post-emergence damping-off of seedlings. Whereas, soil amendment with other test plants was comparatively less effective, least being *E. longifolia*. Soil amendment with dried powder of *A. tridentata* and *L. camara* followed by *C. deodara* and *S. angustifolia* were effective over other test plants in providing protection against pre-emergence damping-off whereas, soil amendment with dried powder of *R. muricatus* followed by *A. aspera* were least effective against the pathogen. Of the various test plants, soil amendment with dried powder of *S. angustifolia* and *A. tridentata* provided protection against post-emergence damping-off. Effectiveness of dried powder of *L.camara* was superior over captan against post-emergence damping-off.

Soil drenching with test plants immediately before sowing resulted in 28.8 to 91.0 per cent pre-emergence and 4.6 to 63.7 per cent post-emergence damping-off, respectively. However,

Table 1. Effect of plant extracts on mycelial growth of *Sclerotium rolfsii*

Plant species	Aqueous extract				Ethanolic extract			
	A*		B**		A*		B**	
	1	2	1	2	1	2	1	2
Achyranthes aspera	839.7 (29.0)	822.6 (28.7)	19.6 (25.3)	21.4 (26.8)	162.1 (12.7)	35.1 (6.0)	84.7 (66.9)	94.9 (77.1)
Artemisia tridentata	558.9 (237.7)	0.0 (1.0)	46.6 (43.2)	100.0 (90.0)	40.6 (6.3)	0.0 (1.0)	96.7 (79.0)	100.0 (90.0)
Cedrus deodara	5.8 (2.6)	113.4 (10.4)	94.4 (85.8)	89.7 (71.2)	0.0 (1.0)	0.0 (1.0)	100.0 (90.0)	100.0 (90.0)
Eucalyptus longifolia	3.7 (2.1)	50.3 (7.2)	99.5 (86.0)	95.2 (87.4)	0.0 (1.0)	0.0 (1.0)	100.0 (90.0)	100.0 (90.0)
Lantana camara	455.1 (24.3)	395.2 (19.8)	57.0 (49.0)	62.0 (52.1)	69.7(8.3)	2.4 (1.8)	93.3 (75.1)	99.8 (87.5)
Ranunculus muricatus	0.0 (1.0)	114.7 (10.7)	100.0 (90.0)	86.8 (69.1)	0.0 (1.0)	0.0 (1.0)	100.0 (90.0)	100.0 (90.0)
Swertia angustifolia	778.6 (27.7)	169.7 (13.0)	26.5 (31.0)	83.7 (66.3)	3.1 (1.7)	0.0 (1.0)	99.7 (87.1)	100.0 (90.0)
Check I	1058.6 (32.6)	-	-	-	-	-	-	-
Check II	0.0 (1.0)	-	100.0 (90.00)	-	-	-	-	-
CD (P=0.05)	(2.1)	(2.6)	(14.1)	(8.5)	(1.6)	(1.1)	(2.7)	(1.5)

A=mycelial growth (mm^2); B=Per cent inhibition over check I; * Square root ** arc sine transformed values in parentheses
Check I = Medium devoid of any plant extract; Check II = Medium supplemented with 0.2% captan; 1= fresh plant, 2 = dried powder

Table 2. Effect of soil amendments on pre-and post-emergence damping-off of seedlings

Soil amendment	Damping-off (%)			
	1		2	
	A	B	A	B
Achyranthes aspera	0.0 (0.0)*	22.5 (27.9)	80.0 (61.5)	77.0 (61.5)
Artemisia tridentata	2.8 (6.8)	8.8 (17.0)	34.5 (35.9)	34.5 (35.9)
Cedrus deodara	23.0 (28.5)	55.8 (64.8)	41.8 (37.9)	56.3 (48.9)
Eucalyptus longifolia	56.2 (49.8)	65.5 (61.8)	56.2 (49.8)	77.3 (68.8)
Lantana camara	23.0 (28.5)	4.2 (8.4)	39.6 (38.6)	0.0 (0.0)
Ranunculus muricatus	51.5 (45.8)	52.1 (46.2)	91.3 (73.1)	100.0 (90.0)
Swertia angustifolia	48.4 (44.0)	23.8 (28.1)	45.8 (42.5)	20.6 (26.3)
Check I	34.5 (35.5)	69.6 (56.5)	-	-
Check II	30.0 (32.8)	3.9 (8.1)	-	-
CD (P=0.05)	(11.2)	(13.3)	(NS)	(16.0)

*Arc sine transformed values in parentheses; 1 partially crushed fresh plants;.2 dried plant powder; Check I : Sick soil devoid of any amendment; Check II : Captan (0.25%) treated seeds in sick soil; A= pre; B = post emergence damping-off

Table 3. Effect of pre-sowing soil drenching with plant extract on pre- and post-emergence damping-off of seedlings

Plant species	Damping-off (%)					
	Soil drenching before sowing					
	1		2		3	
	A	B	A	B	A	B
Achyranthes aspera	42.1 (40.3)*	29.6 (31.3)	47.8 (43.6)	10.0 (13.3)	72.6 (58.5)	10.0 (13.3)
Artemisia tridentata	64.0 (53.3)	31.3 (32.8)	61.0 (51.6)	34.7 (35.9)	95.0 (80.8)	0.00 (0.00)
Cedrus deodara	68.6 (57.0)	37.5 (37.5)	60.7 (51.1)	7.2 (11.1)	82.2 (66.3)	10.0 (13.3)
Eucalyptus longifolia	20.8 (25.5)	7.9 (16.8)	84.9 (67.2)	41.7 (40.1)	75.6 (60.6)	36.7 (37.2)
Lantana camara	38.8 (37.9)	4.6 (8.8)	40.6 (39.2)	83.3 (72.3)	81.6 (65.0)	33.3 (32.2)
Ranunculus muricatus	91.0 (72.7)	25.0 (22.5)	90.8 (72.6)	25.0 (22.5)	87.7 (70.1)	33.3 (27.4)
Swertia angustifolia	75.6 (60.6)	63.3 (52.7)	59.4 (50.5)	6.3 (10.4)	63.2 (53.0)	0.0 (0.0)
Check I	84.3 (65.5)	69.6 (56.5)	-	-	-	-
Check II	32.7 (34.6)	12.1 (20.3)	-	-	-	-
CD (P=0.05)	9.7	NS	NS	18.2	7.6	NS

*Arc sine transformed values in parentheses; 1, 2, 3, soil drenching at 0, 24 and 48 hr, respectively; Check I : Sick soil devoid of any drenching ; Check II : Sick soil drenched with captan @ 0.25%; A & B = pre- and post-emergence damping-off

soil drenching before 24 hr of sowing caused 40.6 to 90.8 per cent pre-emergence and 6.3 to 83.3 per cent post-emergence damping-off. Soil drenching with *S. angustifolia* caused minimum (6.2%) disease followed by *C. deodara* (7.2%) post-emergence damping- off. Whereas soil drenching 48 hr before sowing resulted in 63.2 to 95.0 per cent pre-emergence and 0.0-36.7 per cent post-emergence damping-off of seedling excepting *A. tridentata* and *S. angustifolia* which gave complete protection from post-emergence damping-off. Thus, soil drenching with test plants was comparatively less effective than soil amendment in providing protection against pre- and post-emergence damping-off due to *S. rolfsii.*

It was inferred that *Ranunculus, Lantana, Artemisia* and *Eucalyptus* possessed strong antifungal activity against *S. rolfsii* and could be exploited in one or other form for the management of this polyphagus pathogen.

References

Aycock R 1966. Stem rot and other diseases caused by *Sclerotium rolfsii*. N. Carol. Agric. Exp. Stn. Tech. Bull. pp 174, 202.

Bisht GS and Khulbe RD 1995. *In vitro* efficacy of leaf extracts of certain indigenous medicinal plants against brown leaf spot pathogen of rice. *Indian Phytopathology* **48**: 480-482.

Mishra M and Tewari SN 1990. Ethanolic extract toxicity of three botanicals against five fungal pathogens of rice. Nat. Acad. Sci. (Letters) **13**: 409-412.

Punja ZK, Smith VL, Campbell CL and Jenkins 1985. Sampling and extraction procedure to estimate numbers, spatial pattern, and temporal distribution of sclerotia of *Sclerotium rolfsii* in soil. Plant Disease **69** (6): 469-474.

Sarma BK, Singh DP, Singh HB, Singh Anand and Singh UP 2002. *Sclerotium rolfsii*- a threat to crop plants. Indian J. Plant Pathol. **20** (1&2): 1-14.

Shivpuri A, Sharma OP and Jhamaria SL 1997. Fungitoxic properties of plant extracts against pathogenic fungi. J. Mycol. Pl. Pathol. **27**: 29-31.

Singh RK and Dwivedi RS 1990. Effects of some higher plants and synergistic activity of their essential oils against *Sclerolium rolfsii* Sacc., foot rot pathogen of barley. Proc. Nat. Acad. Sci. India **60** (B): 39-42.

Vincent JH 1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature **15**: 850.

VIR SOFTECH