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Antifungal Activity of Medicinal Plants Metabolites Against Collar Rot of Chickpea Caused by *Sclerotium rolfsii*

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***Abstract* — Under laboratory condition, the fungitoxicity of alcohol extract of medicinal plants against *Sclerotium rolfsii* significantly varied with concentration and time intervals. All plant extracts were inhibitory to the mycelial growth of *Sclerotium rolfsii*. As concentration of extracts decreased, the effectiveness of extracts found to be slower in inhibiting mycelial growth. Therefore, maximum growth inhibition of *S. rolfsii* was recorded at 1000 µg ml^{-1} concentration in all cases. The extracts at 250 µg ml^{-1} concentration were failed to inhibit the mycelial growth of the pathogens. At this concentration, Ashwagandha leaf, Bawchi seed and Kali haldi root extracts were found to inhibit the mycelial growth of *S. rolfsii*. Ashwagandha recorded maximum average per cent inhibition of mycelial growth of *S. rolfsii* i.e. 25.818, 20.48 and 18.878 per cent at 1000, 500 and 250 µg ml^{-1} concentration, respectively.**

***Keywords* – Medicinal Plant Extract, Ashwagandha, Chickpea, *Sclerotium rolfsii*, Collar Rot.**

I. INTRODUCTION

Collar rot of chickpea caused by *Sclerotium rolfsii* is an important soil borne and fast spreading fungal pathogen, which causes considerable damage to the plant stand. Seedling mortality in chickpea due to *S. rolfsii* has been reported to vary from 54.7 to 95.00 per cent (Shrivastava *et al.,* 1984). Under field conditions, *S. rolfsii* has been reported to cause 22 to 50 per cent reduction in yield of chickpea. The disease appears in the early stages of the crop growth i.e. up to six weeks from sowing. Drying plants scattered in the field whose foliage turns slightly yellow before death is an indication of the disease. After infection, seedlings become chlorotic. The joint portion of stem and root turns soft, slightly contracts and begins to decay. Whitish fungal growth with white to dark brown mustard grain like sclerotia are seen on the white infected parts of the plant. The fungus can overwinter as mycelium in infected tissues or plant debris or as sclerotia near soil surface or buried in soil which serve as a major source of primary infection by germinating in response to alcohols and other volatile compounds released from decomposing plant material (Punja, 1985). Sclerotia disseminate by cultural practices with infected soil and contaminated tools, infected seedlings, water, wind and possibly as concomitant contaminants along with seeds. The pathogen being soil-borne, polyphagous in nature and longer persistence in soil, due to which its control with chemicals alone seems to be ineffective and uneconomical.

In chemical strategy, pesticides are used with a broad range or spectrum of activity and controlling several pests. Increasing awareness of public concern regarding a continued use of agro-chemicals that are damaging to biotic and abiotic environment are driving the search of more environmentally safe methods that will contribute to the goal of sustainability in agriculture (Herman *et al*. 2004). There are a number of disadvantages in using such chemical pesticides because of their broad spectrum of activity and these pesticides may destroy non-target organisms such as beneficial insects and parasites of destructive pests (Haas *et al.* 2000). However phytopatho--gens are the major constraint in crop production. The control of soilborne diseases such as collar rot of chickpea (*Sclerotium rolfsii*) is not feasible because of un-availability of soil fungicides and much higher cost required for controlling the disease (Maurya *et al*. 2007). Hence, the present study was undertaken to evaluate the medicinal plant extracts against the mycelial growth of *S. rolfsii* under *in vitro* condition.

II. MATERIALS AND METHODS

Different parts of the ten medicinal plant species such as leaves of Kalmegh (*Andrographis paniculata*), Vatraj (*Argyreia speciosa*), Ashwagandha (*Withania somnifera*), Roots of Kali haldi (*Curcuma ceasea*), Jangli Haldi (*Curcuma aromatica*), Kali musli (*Carculigo orchioides*), Shatavari (*Asperagus racemosus*) and seeds of Bawchi (*Psorolea carylifolia*), Vanjeera (*Vernonia anthelmintica*), Jangli sem (*Canavalia gladiata*) were collected from medicinal garden, Indira Gandhi Agriculture University, Raipur (C.G.) and used in the present study.

In Vitro Testing of Medicinal Plants

Collected plant part sample of 10 medicinal plant species were brought to the laboratory, spread on paper sheets and dried at room temperature. The plant samples were powdered and sieved through 1 mm mesh. The powdered plant material dissolved in alcohol in 1:4 (w/v) ratio and kept for 24 hour and filtered through double layer muslin cloth. The extract was centrifuged at 5000 rpm for 10 minutes and the supernatant was used to assess the bioactivity against all the three pathogens. The supernatant was kept at room temperature till it evaporates completely. The residue was dissolved in alcohol in ratio of 1:1 (w/v)- 1000 µg ml^{-1}, 1:2 (w/v)- 500 µg ml^{-1} and 1:4 (w/v)- 250 µg ml^{-1} concentrations. Discs of 5 mm size

Whatman No. 1 filter paper were used for the assay after sterilizing at 1.02 kg/cm^2 for 20 minutes. The discs were dipped in the alcohol extracts (250, 500 and 1000 µg ml^{-1}) and dried to evaporate the solvent. Five discs (2 treated with medicinal extract, 2 control with sterilized water) were kept in each Petri plate as shown in Plate 13 containing Potato dextrose agar (PDA) medium and inoculated with a 5 mm fungal disc at the centre. For each treatment and concentration, three replications were maintained against each of the pathogen. All the plates were incubated at 25±2^{0}C in BOD. The observations were made at 36, 48, 60, 72 and 84 hours of incubation for the *S. rolfsii*. The inhibitory effect of plant metabolites was worked out by using the following formula (Gautam *et al.*, 2003).

$$\text{Per cent inhibition} = \frac{X - Y}{X} \times 100$$

Where,
X= Diameter of control disc.
Y= Diameter of treated disc.

The results were analysed with 3 factor by factorial-Complete Randomized Design (factorial- CRD).

III. Results and Discussion

Extract of different parts of ten medicinal plants were evaluated against collar rot of chickpea caused *Sclerotium rolfsii* with three concentration (1000, 500 and 250 µg ml^{-1}) at five different time interval. The fungitoxicity of alcohol extract of medicinal plants against wilt complex causing fungi significantly varied with concentration and time intervals (Table 1). The result revealed that all plant extracts inhibited the mycelial growth of *S. rolfsii*. As concentration of extracts decreased, the effectiveness of extracts were also decreased against *S. rolfsii*. The maximum growth inhibition of *S. rolfsii* was recorded at 1000 µg ml^{-1} concentration in all cases. The extracts at 250 µg ml^{-1} concentration were failed to inhibit the mycelial growth of *S. rolfsii*. At 250 µg ml^{-1} concentration, only Ashwagandha, Bawchi and Kali haldi were found to inhibit the mycelial growth.

Per cent Inhibition of Sclerotium rolfsii Sacc. 1000 µ g ml^{-1} Concentration:

The per cent inhibition in alcohol extract at 1000 µg ml^{-1} of different medicinal plants part increased (Table 1) with time upto 60h in 3 (Kalmegh, Jangli haldi and Kali musli), upto 72h in 4 (Vatraj, Shatavari, Jangli sem and Vanjeera) treatments and then decreased except Kali haldi, Bawchi and Ashwagandha treatment upto 84h. At this concentra--tion, the per cent inhibition was recorded maximum in Kali haldi at 84h (31.88%) followed by Ashwagandha at 84h (31.48%) treatment, which were at par with each other and significantly superior over rest of the medicinal treatment except Bawchi (26.67%) treatment at 84h. Minimum per cent inhibition was recorded in Jangli haldi treatment at all time interval except at 36 h. At 36h, minimum per cent inhibition was observed in Kali musli (5.36%) treatment. Ashwagandha treatment recorded maximum per cent inhibition during all observation except Kali haldi (31.88%) treatment at 84h. The average per cent inhibition was observed in Ashwagandha (25.818%) (Plate 1), which was at par with Kali haldi (24.154%) and these treatments found significantly superior over rest of the treatments.

500 µg ml^{-1} Concentration:

The per cent inhibition of *S. rolfsii* in alcohol extract at 500 µg ml^{-1} concentration was increased (Table 1) with increased time upto 48h in Kali musli, upto 60h in 4 (Kalmegh, Vatraj and Jangli haldi), upto 72h in 3 (Shatavari, Jangli sem and Vanjeera) treatments and then decreased except Kali haldi, Ashwagandha and Bawchi treatment upto 84h. The per cent inhibition of *S. rolfsii* was maximum in Ashwagandha at 84h (27.78%) followed by Bawchi at 84h (26.30%) and were significant superior over other treatments except Kali haldi at 84h (24.44%) and at 72h (24.41%). Minimum per cent inhibition was observed in Kali musli and Jangli haldi treatment.

At 36 and 48h, the maximum inhibition was observed in plant extract of Ashwagandha (14.54 and 18.02%, respec--tively) followed by Kali haldi (12.33 and 17.24%, respectively), Bawchi (11.63 and 14.5%, respectively) and minimum inhibition in Kali musli (3.10 and 5.38%, respectively) followed by Jangli haldi (3.70 and 6.27%, respectively). At 60 and 72h, it was maximum in Kali haldi (23.98 and 24.41%, respectively), Bawchi (17.97 and 22.21%, respectively) and minimum in Kali musli (3.59%) and Jangli haldi (1.53%), respectively. At 84h, Kali musli and Jangli haldi was not showed fungitoxicity against *S. rolfsii*. The average per cent inhibition was higher in Ashwagandha (20.48%) (Plate 1) which was at par with the Kali haldi (20.48%) and Bawchi (18.606%). These treatments were statistically superior over the rest of the treatments.

250 µg ml^{-1} Concentration:

At this concentration, Vatraj, Jangli haldi and Kali musli did not show any fungitoxicity against *S. rolfsii*. The per cent inhibition by different medicinal plants part extract increased (Table 1) with time interval upto 60h in Kalmegh and Vanjeera, upto 72h in Shatavari and Jangli sem and then decreased except Ashwagandha, Bawchi and Kali haldi upto 84h. Maximum per cent inhibition was observed in Ashwagandha at 84h (24.26%) followed by Bawchi at 84h (23.26%) which were statistically superior over rest of the plant extract treatment except Kali haldi at 84h (18.89%). Minimum per cent inhibition was recorded in Shatavari (1.11%) followed by Kalmegh (3.12%) and Jangli sem at 36h (3.57%). Ashwagandha treatment recorded maximum per cent inhibition at all time interval except Bawchi treatment at 72h. At 36 and 72 h, minimum inhibition was observed in Kalmegh (3.12 and 4.80%, respectively), whereas at 48 and 60h, it was observed in Jangli sem (4.37 and 6.09%, respectively). The average per cent inhibition was maximum in Ashwagandha (18.878%) (Plate 1) followed by Bawchi (17.55%) which were at par with each other and statistically superior over rest of the treatments.

Similar findings were reported by Gautam *et al.* (2003), Kordali *et al.* (2003), Sharma and Bohra (2003) and Gautam and Chauhan (2004) in different plant extracts.

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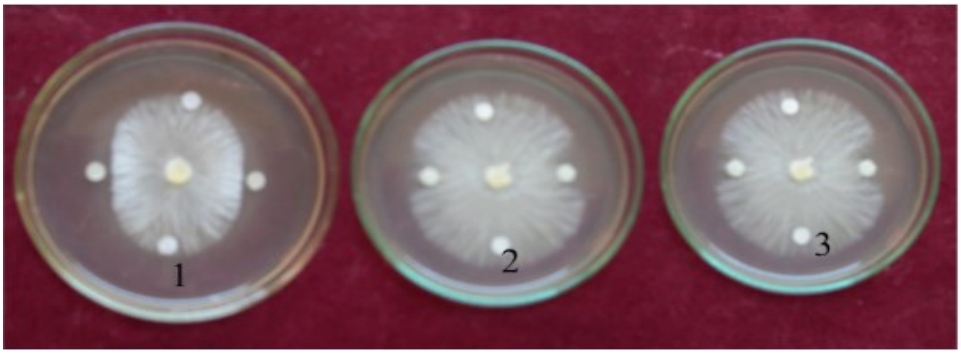
Table 1. Per cent growth inhibition of *Sclerotium rolfsii* by of different medicinal plants extract at different concentration and different time interval.

Medicinal plant	Plant part used	Concen-tration (µg ml^{-1})	Per cent inhibition at different time interval (h)					Average
			36	48	60	72	84	
1	2	3	4	5	6	7	8	9
Kalmegh	Leaf	1000	9.71 (17.94)	13.63 (21.66)	18.89 (25.76)	13.11 (21.17)	8.37 (16.82)	12.742 (20.67)
		500	7.78 (16.16)	12.29 (20.47)	16.29 (23.79)	11.07 (19.41)	7.98 (16.39)	1.082 (19.24)
		250	3.12 (10.18)	6.63 (14.69)	8.69 (17.14)	4.80 (10.37)	4.44 (9.84)	5.536 (12.44)
Vatraj	Leaf	1000	6.24 (14.41)	11.11 (19.67)	14.04 (21.96)	14.14 (22.05)	13.33 (21.37)	11.772 (19.90)
		500	6.20 (14.45)	10.25 (18.64)	13.97 (21.93)	13.25 (21.32)	11.11 (19.47)	10.956 (19.15)
		250	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Kali Haldi	Root	1000	13.63 (21.33)	18.19 (25.18)	26.92 (31.22)	30.15 (33.30)	31.88 (34.37)	24.154 (29.08)
		500	12.33 (20.55)	17.24 (24.52)	23.98 (29.28)	24.41 (29.60)	24.44 (29.62)	20.48 (26.71)
		250	8.64 (17.10)	11.87 (20.15)	14.26 (22.19)	16.28 (23.78)	18.89 (25.75)	13.988 (21.79)
Jangli Haldi	Root	1000	5.73 (13.85)	6.06 (14.24)	8.79 (17.21)	6.26 (14.47)	0 (0.00)	5.368 (11.95)
		500	3.70 (11.09)	6.27 (14.50)	8.78 (17.16)	1.53 (5.73)	0 (0.00)	4.056 (9.69)
		250	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Bawchi	Seed	1000	12.12 (20.36)	16.69 (24.09)	19.08 (25.88)	23.01 (28.66)	26.67 (31.09)	19.514 (26.02)
		500	11.63 (19.95)	14.55 (22.42)	17.97 (25.08)	22.21 (28.10)	26.30 (30.83)	18.606 (25.27)
		250	11.11 (19.44)	14.36 (22.25)	17.42 (24.66)	21.62 (27.68)	23.26 (28.82)	17.55 (24.57)
Shatavari	Root	1000	6.79 (15.05)	12.09 (20.33)	14.15 (22.09)	18.88 (25.73)	13.26 (21.32)	13.034 (20.90)
		500	6.67 (14.93)	8.14 (16.56)	9.43 (16.45)	13.01 (21.07)	7.41 (15.79)	8.932 (16.96)
		250	1.11 (3.51)	5.60 (13.37)	7.40 (15.78)	8.95 (17.31)	4.44 (9.94)	5.498 (11.98)
1	2	3	4	5	6	7	8	9
Jangli Sem	Seed	1000	8.58 (17.02)	9.88 (18.32)	15.13 (22.88)	15.95 (23.50)	9.52 (17.91)	11.812 (19.92)

		500	8.17 (16.59)	9.27 (17.72)	11.56 (19.78)	11.75 (20.02)	7.51 (15.89)	9.652 (18.00)
		250	3.57 (10.88)	4.37 (11.75)	6.09 (14.08)	7.18 (15.53)	0 (0.00)	4.242 (10.45)
Vanjeera	Seed	1000	11.23 (19.57)	14.76 (22.50)	17.06 (24.39)	19.99 (26.55)	16.31 (23.80)	15.87 (23.36)
		500	7.90 (16.32)	13.41 (21.45)	16.29 (23.79)	18.32 (25.33)	12.66 (20.83)	13.716 (21.36)
		250	6.98 (15.30)	9.43 (17.85)	16.04 (23.59)	15.79 (23.41)	8.89 (17.33)	11.42 (19.50)
Ashwagandha	Leaf	1000	15.64 (23.22)	24.11 (29.40)	28.05 (31.93)	29.81 (33.09)	31.48 (34.12)	25.818 (30.35)
		500	14.54 (22.41)	18.02 (25.10)	19.72 (26.34)	22.33 (28.18)	27.78 (31.79)	20.48 (26.76)
		250	12.12 (20.35)	16.97 (24.30)	19.74 (26.36)	21.30 (27.47)	24.26 (29.49)	18.878 (25.59)
Kali Musli	Root	1000	5.36 (13.38)	6.67 (14.93)	11.63 (19.93)	6.82 (15.01)	6.26 (14.48)	7.348 (15.55)
		500	3.10 (10.15)	5.38 (13.31)	3.59 (10.87)	2.98 (8.01)	0 (0.00)	3.01 (8.47)
		250	0 (0.00)	3.12 (8.22)	0 (0.00)	0 (0.00)	0 (0.00)	0.624 (1.64)

Figures in parenthesis are Arcsine transformed values; Average of three replication; h-hours

Source	SEm ±	CD (5%)
Treatment	0.3180	0.88
Concentration	0.1742	0.48
Treatment x Concentration	0.5508	1.53
Time interval	0.2248	0.62
Treatment x Time interval	0.7110	1.97
Concentration x Time interval	0.3894	1.08
Treatment x Concentration x Time interval	1.2315	3.42



Sclerotium rolfsii (Ashwagandha at 48h)
(1.1000 µg ml^{-1}, 2.500 µg ml^{-1} and 3. 250 µg ml^{-1})
Plate 1. Effect of alcohol extract on medicinal plants on wilt complex fungi.