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Evaluation of Medicinal Plants Metabolites Against Root Rot of Chickpea Caused by *Rhizoctonia solani*

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Abstract **– The fungitoxicity of alcohol extract of medicinal plants against root rot of chickpea caused by *Rhizoctonia solani* significantly varied with concentration and time intervals. All plant extracts were inhibitory to the mycelial growth of *R. solani*. As concentration of extracts decreased, the effectiveness of extracts found to be slower in inhibiting mycelial growth. Therefore, maximum growth inhibition of *R. solani* 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 *R. solani*. At this concentration, Ashwagandha leaf, Bawchi seed and Kali haldi root extracts were found to inhibit the mycelial growth of this pathogen. The average per cent inhibition of mycelial growth of *R. solani* was found to be maximum in Bawchi at 1000 µg ml^{-1} (39.336%), 500 µg ml^{-1} (37.472%) and 250 µg ml^{-1} (34.482%) concentration followed by Ashwagandha treatment.**

Keywords **– Medicinal plant Extract, Ashwagandha, Bawchi, Chickpea, Root rot, *Rhizoctonia solani*.**

I. INTRODUCTION

The root rot (*Rhizoctonia solani*) is a major constraint in the chickpea production as it is emerging as a potential threat to chickpea cultivation in semi-arid regions due to moisture stress and high temperatures during the flowering to pod filling stage (Sharma *et al*., 2010). The annual yield loss due to this disease alone is 10-20 per cent (Vishwadhar and Chaudhary, 2001). The root rot disease generally appears around flowering and podding time. The disease may also appear at seedling stage, however, the susceptibility of the plant increases with age. The disease generally appears when day temperature is more than 30 ^{0}C and soil moisture content of 60 per cent. Drooping of petioles and leaflets is confined to those at the very top of the plant. Sometimes when rest of the plant is dry, the top most leaves are chlorotic. The leaves and stems of affected plants are usually straw colored. The lower portion of the tap root usually remains in the soil when plants are uprooted. The tap root is dark and is devoid of most of its lateral and finer roots. Dark sclerotial bodies can be seen on the roots or inside the wood.

For the management of *R. solani*, number of chemical fungicides were used but frequent and indiscriminant use of synthetic fungicides posed a serious threat to the environment. Due to the aforementioned problems, the people are now moving towards the use of natural plant extracts as fungicides. Plants contain hundreds or thousands of metabolites. Many herbal plants are the gift of nature to human beings as they have some medicinal property and can be used to control various infections and diseases (Khalil *et al*., 2007). Therefore, the present study was undertaken to test whether the medicinal plant extracts are efficient in reducing the mycelial growth of chickpea wilt complex fungi 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$\pm$2^{0}C in BOD. The observations were made at 36, 48, 60, 72 and 84 hours of incubation for the *R. solani*. 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 root rot of chickpea caused by *Rhizoctonia solani* with three concentration (1000, 500 and 250 µg ml^{-1}) at five different time interval. The fungitoxicity of alcohol extract of medicinal plants against *R. solani* significantly varied with concentration and time intervals (Table 1). The result revealed that all plant extracts inhibited the mycelial growth of *R. solani*. As concentration of extracts decreased, the effectiveness of extracts were also decreased against *R. solani*. The maximum growth inhibition of this fungus 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 *R. solani*. At 250 µg ml^{-1} concentration, only Ashwagandha, Bawchi and Kali haldi were found to inhibit the mycelial growth of *R. solani*.

Per cent Inhibition of Rhizoctonia solani Kuhn

1000 µg 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, Vatraj and Kali musli), upto 72h in 3 (Jangli haldi, Shatavari and Vanjeera) treatment and then decreased except Bawchi, Ashwagandha, Kali haldi and Jangli sem at 84 h. At this concentration statistically significant per cent inhibition was observed in Bawchi at 84h (58.89%) followed by Ashwagandha at 84h (48.89%) and Bawchi at 72h (47.51%), while it was mini--mum in Kali musli at 84h (4.44%). Significantly highest per cent inhibition was recorded in Bawchi treatment at 84h over the other treatments. All the treatments showed good fungitoxic effect against *R. solani* except Kali musli, which showed minimum per cent inhibition at all time interval. Bawchi treatment showed best fungitoxicity effect against *R. solani* and observed maximum per cent inhibition at all time interval except 36 h, at 36h it was observed in Ashwagandha (15.88%) treatment. The ave--rage per cent inhibition was found statistically higher in Bawchi (39.336%) (Plate 1) followed by Ashwagandha (36.406%). Besides Bawchi and Ashwagandha, Shatavari (28.632%), Kali haldi (25.096%), Vanjeera (24.894%) and Jangli sem (22.766%) were showed good fungitoxicity against *R. solani*.

500 µg ml^{-1} Concentration:

The per cent inhibition of *R. solani* in alcohol extract at 500 µg ml^{-1} concentration was increased (Table 1) with increased time upto 60h in 3 (Kalmegh, Vatraj and Kali musli), upto 72h in 3 (Jangli haldi, Vanjeera and Shata--vari) treatments and then decreased except Kali haldi, Bawchi, Jangli sem and Ashwagandha treatments upto 84h. At this concentration, maximum per cent inhibition was observed in Bawchi at 84h (55.55%) followed by Bawchi at 72h (47.29%) and Ashwagandha at 84h (42.48 %), minimum was observed in Vatraj at 48h (3.72%). Significantly highest per cent inhibition was recorded in Bawchi treatment at 84h over other treatments. Bawchi treatment recorded maximum per cent inhibition and found to be best at all time interval except at 36h, it was maximum in Ashwagandha (14.51%). The average per cent inhibition was found significantly higher in Bawchi (37.472%) (Plate 1) followed by Ashwagandha (33.378%). Besides Bawchi and Ashwagandha, Kali haldi (22.906%), Shatavari (21.228%), Jangli sem (20.186%) and Vanjeera (18.454%) were showed good fungitoxicity against *R. solani*, while Vatraj (2.934%), Kali musli (4.608%) and Kalmegh (14.964%) showed very less fungitoxicity. Vatraj treatment showed minimum per cent inhibition of *R. solani* at all time interval.

250 µg ml^{-1} Concentration:

Alcohol extract of different medicinal plants part at 250 µg ml^{-1} increased the per cent inhibition of *R. solani* (Table 1) with increased time upto 60h in 2 (Jangli haldi and Kali musli), upto 72h in 4 (Kalmegh, Shatavari, Jangli sem and Vanjeera) treatments and then decreased except Bawchi, Ashwagandha and Kali haldi, whereas Vatraj treatment showed no fungitoxic effect against *R. solani*. At this concentration, the per cent inhibition was maximum in Bawchi at 84h (51.11%) and at 72h (44.44%) followed by Ashwagandha at 84h (42.22%), minimum was observed in Kali musli at 36h (3.03%). Significantly highest per cent inhibition was recorded in Bawchi at 84h over the rest of the treatments. Bawchi treatment recorded maximum per cent inhibition at all time interval except at 36h, it was maximum in Ashwagandha (13.74%) treatment. Signifi--cant difference were in all observed in average per cent inhibition of *R. solani* in all treatments. The maximum average was recorded in Bawchi (34.482%) (Plate 1) followed by Ashwagandha (29.668%), Kali haldi (21.562 %) and Shatavari (17.386%), while it was minimum in Kali musli (3.768%) followed by Kalmegh (7.24%) and Jangli haldi (9.098%).

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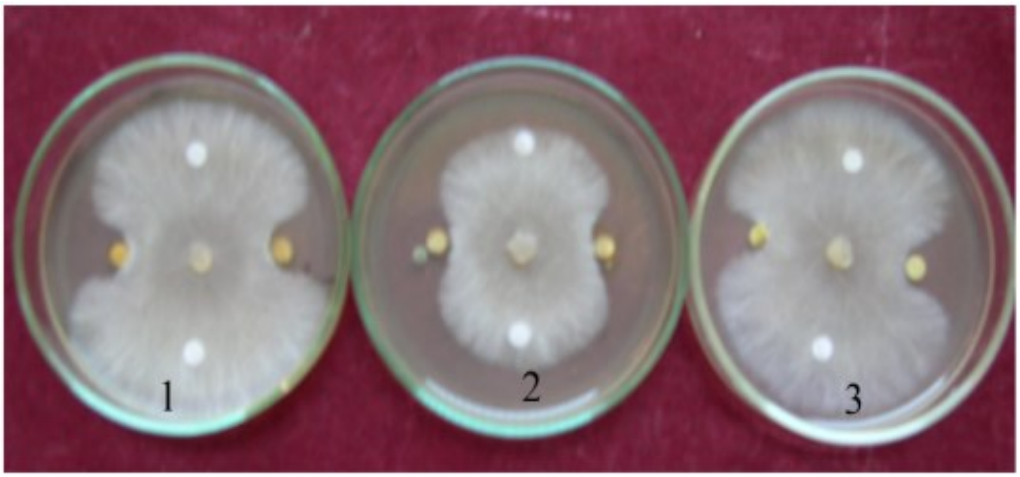
Table 1. Per cent growth inhibition of *Rhizoctonia solani* by of different medicinal plants extract at different concentration and different time interval.

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	12.50 (20.70)	16.95 (24.32)	27.71 (31.75)	22.58 (28.36)	14.44 (22.29)	18.836 (25.48)
		500	6.25 (14.47)	15.03 (22.75)	25.02 (30.00)	17.78 (24.93)	10.74 (19.09)	14.964 (22.25)
		250	4.92 (12.76)	5.87 (13.93)	7.13 (15.33)	10.00 (18.38)	8.28 (16.68)	7.240 (15.42)
Vatraj	Leaf	1000	11.51 (19.82)	17.35 (24.58)	24.29 (29.51)	20.71 (27.07)	18.96 (25.80)	18.564 (25.36)
		500	0 (0.00)	3.72 (9.09)	6.24 (14.38)	4.71 (12.52)	0 (0.00)	2.934 (7.20)
		250	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Kali Haldi	Root	1000	13.89 (21.89)	20.68 (26.72)	26.55 (30.99)	29.99 (33.20)	34.37 (35.87)	25.096 (29.73)
		500	13.04 (21.16)	18.88 (25.75)	22.21 (28.08)	26.66 (31.11)	33.74 (35.49)	22.906 (28.32)
		250	12.26 (20.47)	18.19 (25.09)	21.66 (27.73)	24.44 (29.62)	31.26 (33.98)	21.562 (27.38)
Jangli Haldi	Root	1000	11.54 (19.85)	14.44 (22.31)	19.23 (26.01)	23.20 (28.79)	18.89 (25.74)	17.460 (24.54)
		500	10.77 (19.16)	13.13 (21.24)	16.38 (23.82)	20.59 (26.98)	16.64 (24.09)	15.502 (23.06)
		250	3.58 (8.82)	10.34 (18.39)	13.60 (21.63)	9.09 (17.52)	8.88 (17.33)	9.098 (16.74)
Bawchi	Seed	1000	15.17 (22.92)	34.36 (35.55)	40.41 (39.65)	47.51 (43.57)	58.89 (50.12)	39.336 (38.36)
		500	13.13 (21.24)	30.98 (33.81)	40.41 (39.46)	47.29 (43.44)	55.55 (48.19)	37.472 (37.23)
		250	11.79 (20.03)	28.90 (32.38)	36.17 (36.97)	44.44 (41.80)	51.11 (45.64)	34.482 (35.36)
Shatavari	Root	1000	12.36 (20.58)	26.00 (30.62)	38.69 (38.45)	41.67 (39.12)	24.44 (29.62)	28.632 (31.68)
		500	8.69 (17.13)	18.33 (25.32)	22.20 (28.06)	32.59 (34.81)	24.33 (32.82)	21.228 (27.63)
		250	6.89 (15.15)	12.69 (20.80)	18.77 (25.59)	24.88 (29.92)	23.70 (29.11)	17.386 (24.11)
1	2	3	4	5	6	7	8	9
Jangli Sem	Seed	1000	10.77 (19.16)	19.37 (26.07)	23.92 (29.27)	28.66 (32.37)	31.11 (33.88)	22.766 (28.15)
		500	10.47 (18.86)	18.03 (24.90)	21.92 (27.89)	23.47 (28.93)	27.04 (31.32)	20.186 (26.38)
		250	6.44 (14.58)	12.37 (20.58)	16.91 (24.26)	23.70 (29.13)	17.82 (23.29)	15.508 (22.37)
Vanjeera	Seed	1000	12.16 (20.41)	18.18 (25.10)	30.92 (33.77)	36.66 (37.26)	26.55 (30.98)	24.894 (29.50)
		500	8.05 (16.46)	14.94 (22.64)	19.04 (25.87)	26.91 (31.24)	23.33 (28.87)	18.454 (25.01)

		250	6.25 (14.47)	11.11 (19.47)	14.88 (22.61)	21.70 (27.75)	13.33 (21.37)	13.454 (21.13)
Ashwagandha	Leaf	1000	15.88 (23.47)	30.80 (33.68)	39.68 (39.03)	46.78 (43.22)	48.89 (44.34)	36.406 (36.75)
		500	14.51 (22.38)	30.03 (33.20)	38.77 (38.51)	41.10 (39.87)	42.48 (40.66)	33.378 (34.92)
		250	13.74 (21.75)	23.09 (28.72)	28.92 (32.52)	40.37 (39.44)	42.22 (40.52)	29.668 (32.59)
Kali Musli	**Root**	1000	4.54 (12.22)	8.64 (17.05)	18.19 (25.19)	6.82 (18.47)	4.44 (12.11)	8.526 (17.01)
		500	4.54 (12.15)	7.91 (16.28)	10.59 (18.95)	0 (0.00)	0 (0.00)	4.608 (9.47)
		250	3.03 (5.85)	6.01 (13.95)	9.80 (18.08)	0 (0.00)	0 (0.00)	3.768 (7.58)

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

Source	**SEm ±**	**CD (5%)**
Treatment	**0.3228**	**0.89**
Concentration	**0.1768**	**0.49**
Treatment x Concentration	0.5591	1.55
Time interval	**0.2283**	**0.63**
Treatment x Time interval	0.7218	2.01
Concentration x Time interval	**0.3954**	**1.10**
Treatment x Concentration x Time interval	**1.2503**	**3.48**



Rhizoctonia solani (Bawchi at 60h)
(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.