Antifungal Activity of Medicinal Plants Against Chickpea Wilt Pathogen *(Fusarium oxysporum* f.sp. *ciceri)*

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SUMMARY

Extract of different parts of ten medicinal plants were evaluated against chickpea wilt pathogen (*Fusarium oxysporum* f.sp.) *ciceri* with three concentrations (1000, 500 and 250 µg ml^{-1}) at five different time internals. The fungitoxicity of alcohol extract of medicinal plants against wilt causing pathogen significantly varied with concentration and time intervals. All plant extracts inhibited the mycelial growth of the fungus *in vitro*. As concentration of extracts decreased, the effectiveness of extracts were also decreased against wilt pathogen. The maximum growth inhibition was recorded at 1000 µg ml^{-1} concentration and the per cent inhibition was observed maximum in Bawchi at 8 th DAI (45.55%) and at 7 th DAI (38.17%) followed by Ashwagandha at 8 th DAI (37.84%) and at 7 th DAI (36.25%) . At 4 th , 5 th and 6 th DAI, the per cent inhibition in alcohol extract at 500µg ml^{-1} concentration increased with increased in time upto 8 th DAI. Maximum per cent inhibition was observed in Bawchi at 8 th DAI (44.44%) and at 7 th DAI (36.36%) followed by Ashwagandha at 8 th DAI (33.33%) and at 7 th DAI (32.38%) . Bawchi treatment showed significantly highest per cent inhibition at 8 th DAI over rest of the treatments. The extracts at 250 µg ml^{-1} concentration were failed to inhibit the mycelial growth of these pathogens. At 250 µg ml^{-1} concentration, only Ashwagandha. Bawchi and Kali Haldi were found to inhibit the mycelial growth of the pathogen to some extent.

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Among pulse crops grown in India, chickpea occupies the premier position in terms of area as well as production. Chickpea is grown over an area of 10.6 m ha in the world and productivity with 820 kg ha^{-1}. India accounts for about 5.77 m tonnes (66.62%) of world chickpea production and 68.77 per cent (7.29 m ha) area with productivity 792 kg ha^{-1} (USDA, 2004). During the past 10 years, India accounted for 60-70 per cent world chickpea production. One of the major constraints limiting agriculture production is the difficulty in managing diseases caused by pathogens specially of soil borne nature. This problem further compounds when the diseases is incited by more than one pathogen. "Chickpea wilt complex" is one of the best example of such diseases for which four pathogens accounts *viz. Sclerotium rolfsii* Sacc., *Rhizoctonia bataticola* (Taub.) Butler, *R. solani* Kuhn and *Fusarium oxysporum* f.sp. *ciceri* (Padwick) Snyder and Hansen. These pathogens cause significant loss in yield and primarily responsible for wide gap in the yield levels in farmers field as also reported earlier (Dahiya, 2003). Chickpea wilt complex pathogens belong to a group of ubiquitous and diverse plant pathogens that occur widely in India as a root pathogens on different crops and also could be isolated from different varieties of the same host species. Therefore, these pathogens differ in their cultural, morphological and pathogenic behaviour.

MATERIALS AND METHODS

In vitro studies on *Fusarium oxysporum* f.sp. *ciceri* (Padwick) Snyder and Hansen were conducted in the Department of Plant Pathology, Indira Gandhi Agricultural University, Raipur (C.G.).

Evaluation of medicinal plant extracts against wilt complex fungi:

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 plants garden, Indira Gandhi Agriculture University, Raipur (C.G.) and used in the present study.

In 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 materials were 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 evaporated 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 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±2ºC in BOD. The observations were made for *F. oxysporum* f.sp. *ciceri* observations were made at 4, 5, 6, 7 and 8th day of incubation. The inhibitory effect of plant parts 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).

RESULTS AND DISCUSSION

Per cent inhibition of Fusarium oxysporum f.sp. ciceri (Padwick) Snyder and Hansen:

1000 mmg «l^{-1} concentration:

Alcohol extract of different medicinal plants part at 1000 μg ml^{-1} was increased per cent inhibition of *F. oxysporum* f.sp. *ciceri* with time upto 6th DAI and then decreased except Kali Haldi, Bawchi and Ashwagandha upto 8th DAI (Table 1). At this concentration, the per cent inhibition was observed maximum in Bawchi at 8th DAI (45.55%) and at 7th DAI (38.17%) followed by Ashwagandha at 8th DAI (37.84%) and at 7th DAI (36.25%). At 4th, 5th and 6th DAI, it was maximum in Ashwagandha (21.27, 26.65 and 33.39%, respectively). Minimum per cent inhibition was observed in Shatavari at 8th DAI (3.70%) followed by Jangli Haldi at 4th DAI (4.52%). At 5th and 6th DAI, the per cent inhibition was minimum in Shatavari (6.25 and 11.66%, respectively), while at 7th DAI, it was minimum in Vatraj. At 8th DAI, Vanjeera showed no fungitoxic effect against *F. oxysporum* f.sp. *ciceri*. Bawchi treatment at 8th DAI showed significantly highest per cent inhibition over the rest of medicinal plants part treatments. The average per cent inhibition was observed maximum in Bawchi (32.414%) followed by Ashwagandha (31.080%) which were at par with each other and statistically superior over the rest of the treatments, whereas it was minimum in Shatavari (7.096%).

500 mmg «l^{-1} concentration:

The per cent inhibition in alcohol extract at 500 μg ml^{-1} concentration increased (Table 1) with increased in time upto 6th DAI and then decreased except in Bawchi, Ashwagandha and Kali Haldi upto 8th DAI. Maximum per cent inhibition of *F. oxysporum* f. s. *ciceri* was observed in Bawchi at 8th DAI (44.44%) and at 7th DAI (36.36%) followed by Ashwagandha at 8th DAI (33.33%) and at 7th DAI (32.38%). Bawchi treatment showed significantly highest per cent inhibition at 8th DAI over rest of the treatments. At all time interval, Bawchi showed maximum per cent inhibition except Ashwagandha (16.27%) at 4th DAI. Minimum per cent inhibition was observed in Shatavari at all time interval except Jangli Sem (6.25%) at 5th DAI. At 8th DAI, Vatraj, Jangli Haldi, Shatavari, Vanjeera and Kali Musli showed no fungitoxic effect against *F. oxysporum* f.sp. *ciceri*. The average per cent inhibition was significantly highest in Bawchi (33.152%) than other treatments. Besides Bawchi, Ashwagandha (26.148%) and Kali Haldi (19.25%) showed good fungitoxicity against *F. oxysporum* f. sp. *ciceri*, while Shatavari (3.336%), Kali Musli (5.546%), Vanjeera (5.808%), Vatraj (6.082%), Jangli Haldi (7.126%), Jangli Sem (6.050%) and Kalmegh (8.67%) showed negligible fungitoxicity (Table 1).

250 mmg «l^{-1} concentration:

Alcohol extract of different medicinal plants part at 250 mmg ml^{-1} increased per cent inhibition of *F. oxysporum* f.sp. *ciceri* (Table 1) with time upto 6th DAI and then decreased except Kali Musli upto 5th DAI and Kali Haldi, Ashwagandha and Bawchi upto 8th DAI. Shatavari treatment did not show fungitoxic effect against *F. oxysporum* f. sp. *ciceri*. Bawchi treatment showed maximum per cent inhibition (37.78%) at 8th DAI over rest of the treatments. At all time intervals, maximum per

Table 1: Per cent growth inhibition of *Fusarium oxysporum* f.sp.*ciceri* by different medicinal plants extract at different concentrations and different time intervals

Medicinal plant	Plant part used	Concen-tration ($\mu g\ ml^{-1}$)	Per cent inhibition at different time intervals (DAI)					Average
			4^{th}	5^{th}	6^{th}	7^{th}	8^{th}	
1	2	3	4	5	6	7	8	9
Kalmegh	Leaf	1000	7.13 (15.46)	14.28 (24.51)	25.29 (30.15)	13.90 (21.89)	9.29 (17.71)	13.978 (21.94)
		500	7.06 (15.01)	8.86 (18.20)	12.48 (20.95)	8.15 (16.58)	6.80 (14.98)	8.670 (17.14)
		250	0 (0.00)	3.18 (10.09)	6.02 (13.93)	4.44 (12.15)	3.89 (11.33)	3.520 (9.50)
Vatraj	Leaf	1000	7.08 (15.20)	8.50 (16.86)	12.27 (20.48)	7.32 (15.59)	6.02 (14.17)	8.232 (16.46)
		500	6.68 (14.97)	8.17 (16.56)	11.20 (19.54)	4.36 (11.63)	0 (0.00)	6.082 (12.54)
		250	0 (0.00)	6.27 (14.27)	8.84 (17.27)	0 (0.00)	0 (0.00)	3.022 (6.31)
Kali Haldi	Root	1000	14.29 (22.19)	18.17 (25.22)	23.34 (28.89)	26.98 (31.29)	29.97 (33.17)	22.550 (28.15)
		500	11.90 (20.14)	16.67 (24.10)	18.96 (25.80)	22.24 (28.11)	26.48 (30.96)	19.250 (25.82)
		250	11.06 (19.42)	16.08 (23.53)	18.84 (25.68)	21.33 (27.45)	24.44 (29.61)	18.350 (25.14)
Jangli Haldi	Root	1000	4.52 (12.18)	8 .32 (16.75)	15.39 (23.07)	8.74 (17.18)	7.05 (15.36)	8.804 (16.91)
		500	4.46 (11.79)	9.66 (18.08)	15.79 (23.42)	5.78 (13.87)	0 (0.00)	7.126 (13.43)
		250	4.44 (12.11)	11.10 (19.36)	4.31 (11.80)	0 (0.00)	0 (0.00)	3.970 (8.65)
Bawchi	Seed	1000	18.82 (25.68)	27.01 (31.23)	32.52 (34.77)	38.17 (38.16)	45.55 (42.44)	32.414 (34.45)
		500	16.04 (23.56)	26.95 (31.26)	31.97 (34.43)	36.36 (37.08)	44.44 (41.80)	33.152 (33.63)
		250	14.28 (22.20)	26.15 (30.74)	31.94 (34.41)	36.60 (37.22)	37.78 (37.92)	29.350 (32.50)
Shatavari	Root	1000	4.67 (12.36)	6.25 (14.23)	11.66 (19.84)	9.20 (17.64)	3.70 (11.08)	7.096 (15.03)
		500	3.41 (10.60)	6.43 (14.22)	6.84 (15.05)	0 (0.00)	0 (0.00)	3.336 (7.97)
		250	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Jangli Sem	Seed	1000	6.36 (14.59)	9.65 (18.06)	12.50 (20.70)	9.10 (17.55)	5.69 (13.76)	8.660 (16.93)
		500	4.32 (11.99)	6.25 (14.24)	9.07 (17.52)	6.17 (14.29)	4.44 (12.17)	6.050 (14.04)
		250	0 (0.00)	4.61 (11.81)	5.13 (13.02)	2.85 (7.94)	2.36 (5.17)	2.990 (7.58)
Vanjeera	Seed	1000	6.52 (14.60)	10.96 (19.30)	17.27 (24.50)	11.52 (19.84)	0 (0.00)	9.250 (15.65)
		500	4.65 (12.46)	9.26 (17.64)	9.29 (17.73)	5.54 (11.17)	0 (0.00)	5.808 (11.80)
		250	2.39 (7.22)	5.00 (12.90)	9.07 (17.51)	5.11 (12.91)	0 (0.00)	4.314 (10.11)
Ashwagandha	Leaf	1000	21.27 (27.44)	26.65 (33.14)	33.39 (35.29)	36.25 (37.00)	37.84 (37.96)	31.080 (34.17)
		500	16.27 (23.63)	21.66 (27.71)	27.10 (31.36)	32.38 (34.66)	33.33 (35.25)	26.148 (30.52)
		250	14.27 (22.16)	19.38 (26.11)	22.20 (28.04)	25.75 (30.49)	28.89 (32.51)	22.098 (27.86)
Kali Musli	Root	1000	6.82 (15.08)	12.32 (20.53)	18.46 (25.39)	12.86 (21.02)	8.89 (17.26)	11.874 (19.86)
		500	5.37 (14.06)	9.10 (17.28)	13.26 (21.35)	0 (0.00)	0 (0.00)	5.546 (10.54)
		250	4.03 (9.35)	5.72 (13.79)	0 (0.00)	0 (0.00)	0 (0.00)	1.950 (4.63)

Figures in parenthesis are Arcsine transformed values; Average of three replications; DAI- Days After Inoculation

Source	S.E. ±	C.D. (P=0.05)
Treatment	0.3506	0.97
Concentration	0.1920	0.53
Treatment x Concentration	0.6073	1.69
Time interval	0.2479	0.69
Treatment x Time interval	0.7840	2.18
Concentration x Time interval	0.4294	1.19
Treatment x Concentration x Time interval	1.3579	3.78

cent inhibition was observed in Bawchi. At 8^{th} DAI, Bawchi, Ashwagandha and Kali Haldi showed fungitoxicity, while Vatraj, Jangli Haldi, Shatavari, Vanjeera and Kali Musli showed no fungitoxic effect. The average per cent inhibition was observed statistically superior in Bawchi (29.350%) followed by Ashwagandha (22.098%) and Kali haldi (18.35%), while it was minimum in Kali Musli (1.950%) followed by Vatraj (3.022%), Kalmegh (3.52%), Jangli Haldi (3.97%) and Vanjeera (4.314%). 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 (Table 1).

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