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Antifungal Activity of Medicinal Plants Metabolites Against Wilt of Chickpea Caused by *Fusarium oxysporum* f.sp. *ciceri*

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***Abstract* – Under *in vitro* condition, the fungitoxicity of alcohol extract of medicinal plants against wilt of chickpea caused by *Fusarium oxysporum* f.sp. *ciceri* significantly varied with concentration and time intervals. All plant extracts were inhibitory to the mycelial growth of *F. oxysporum* f.sp. *ciceri*. As concentration of extracts decreased, the effectiveness of extracts found to be slower in inhibiting mycelial growth. Therefore, 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 *F. oxysporum* f.sp. *ciceri*. At this concentration, Ashwagandha leaf, Bawchi seed and Kali haldi root extracts were found to inhibit the mycelial growth of *F. oxysporum* f.sp. *ciceri*. The average per cent inhibition of mycelial growth of *F. oxysporum* was found to be maximum in Bawchi at 1000 µg ml^{-1} (32.414%), 500 µg ml^{-1} (33.152%) and 250 µg ml^{-1} (29.35%) concentration followed by Ashwagandha treatment.**

***Keywords* – Medicinal plant Extract, Ashwagandha, Bawchi, Wilt, Chickpea, *Fusarium oxysporum* f.sp. *ciceri*.**

I. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the world's third most important pulse crop, after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.) (Vishwadhar and Gurha, 1998). Although, chickpea is predominantly consumed as a pulse, dry chickpea is also used in preparing a variety of snack foods, sweets and condiments and green fresh chickpeas are commonly consumed as a vegetable. *Fusarium* wilt caused by *Fusarium oxysporium* f.sp. *ciceri* Synd. and Hans. is one of the major soil / seed borne disease of chickpea (*C. arietinum* L.). When disease occurs at seedling stage, seedlings that die due to wilt disease can be confused with other diseases of wilt complex, if not examined carefully. Fusarium wilt infected seedlings collapse and lie flat on the ground retaining their dull green color. In case of adult plants, characteristic symptom is brown to black discoloration of xylem vessels. In susceptible plants hyphae are inter and intracellular in pith, xylem and cortex. The phytotoxin produced by the pathogen causes wilting and leaf burning. The roots of the wilting plants do not show any external rotting but when split open vertically, dark brown discoloration of internal xylem is seen (Nene *et al*., 1991). At national level the yield losses encountered due to wilt may vary between five to ten per cent. The pathogen is both seed and soil borne; facultative saprophyte and can survive in soil up to six years in the absence of susceptible host (Haware *et al*. 1986). For the management of this pathogen, 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. Hence, the present study was undertaken to test antifungal activity of medicinal plant metabolites against the chickpea wilt caused by *Fusarium oxysporium* f.sp. *ciceri* under *in vitro* condition.

II. MATERIALS AND METHODS

Different parts of the ten medicinal plant species such as leaves of Kalmegh (*Andrographis paniculata*), vatr-

-aj (*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 ± 2^0C in BOD. The observations for *F. oxysporum* f.sp. *ciceri* were made at 4, 5, 6, 7 and 8th day of incubation. 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 wilt of chickpea caused by *Fusarium oxysporum* f.sp. *ciceri* with three concentration (1000, 500 and 250 µg ml^{-1}) at five different time interval. The fungitoxicity of alcohol extract of medicinal plants against *F. oxysporum* f.sp. *ciceri* significantly varied with concentration and time intervals (Table 1). The result revealed that all plant extracts inhibited the mycelial growth of *F. oxysporum* f.sp. *ciceri*. As concentration of extracts decreased, the effectiveness of extracts were also decreased against *F. oxysporum* f.sp. *ciceri*. The maximum growth inhibition of *F. oxysporum* f.sp. *ciceri* 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 *F. oxysporum* f.sp. *ciceri*. At 250 µg ml^{-1} concentration, only Ashwagandha, Bawchi and Kali haldi were found to inhibit the mycelial growth of *F. oxysporum* f.sp. *ciceri*.

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

1000 µg ml^{-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%) (Plate 1) 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%) followed by Vatraj (9.25%).

500 µg ml^{-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 were 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 (Plate 1). 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.

250 µg ml^{-1} Concentration:

Alcohol extract of different medicinal plants part at 250 µg ml^{-1} was 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 not showed 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 interval, maximum per cent inhibition was observed in Bawchi. At 8th 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 (29.35%) (Plate 1) followed by Ashwagandha (22.098%) and Kali haldi (18.35%), while it was minimum in Kali musli (1.95%) 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.

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

Medicinal plant	Plant part used	Concen-tration ($\mu g\ ml^{-1}$)	Per cent inhibition at different time interval (DAI)					Average
			4th	5th	6th	7th	8th	
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)

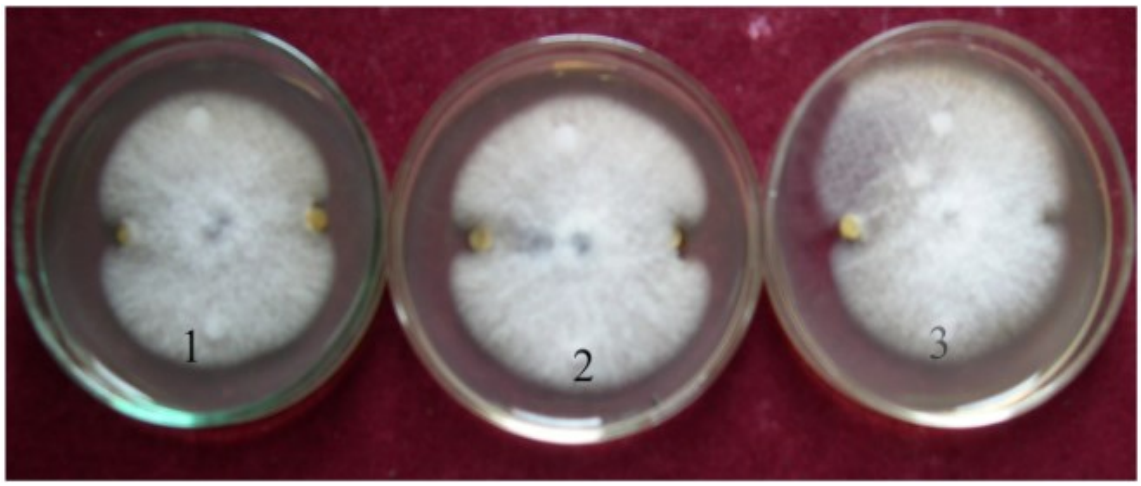
		250	11.06 (19.42)	16.08 (23.53)	18.84 (25.68)	21.33 (27.45)	24.44 (29.61)	18.350 (25.14)
Jangli Haldi	Root	1000	4.52 (12.18)	8.32 (16.75)	15.39 (23.07)	8.74 (17.18)	7.05 (15.36)	8.804 (16.91)
		500	4.46 (11.79)	9.66 (18.08)	15.79 (23.42)	5.78 (13.87)	0 (0.00)	7.126 (13.43)
		250	4.44 (12.11)	11.10 (19.36)	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)
1	**2**	**3**	4	5	6	7	8	9
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 replication.

DAI- Days After Inoculation

Source	**SEm ±**	**CD (5%)**
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



Fusarium oxysporum f.sp. *ciceri* (Bawchi at 6 DAI) (1. 1000 µg ml^{-1}, 2. 500 µg ml^{-1}, 3. 250 µg ml^{-1})

Plate 1. Effect of alcohol extract on medicinal plants on wilt complex fungi.