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Efficacy of different phytoextracts against *Erysiphe polygoni* DC causing powdery mildew of fenugreek

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Abstract

Fenugreek (*Trigonella foenum graecum* L.) is one of the most important spices crop grown in India. Powdery mildew caused by *Erysiphe polygoni* DC is one of the major constraints in the production of fenugreek. In order to find out the efficacy of various phyto extract against *Erysiphe polygoni* experiment was carried out under *in vitro* condition. The relative efficacy of seven different phytoextracts were tested in different concentrations and different time of interval 24, 48 and 72 hours, respectively. Out of seven phytoextracts tested, two phytoextracts showed more than 50% inhibition at all concentrations on different time interval. Garlic and neem leaf extracts found best in spore germination inhibition at all the concentrations on different time interval. The significantly highest spore germination inhibition (81.82%) was recorded at 20 per cent concentration of garlic followed by 20 per cent concentration of neem (79.47%) on 72 hrs after the treatment.

Keywords: Fenugreek, powdery mildew, phyto extract, *erysiphe polygoni*, *in vitro*

Introduction

Fenugreek (*Trigonella foenum graecum* L.) is cultivated throughout India and belongs to the family *Leguminosae* (Balodi and Rao, 1991) [5]. Fenugreek has been cultivated in most of states of India. In India, the annual production of fenugreek is 2.40 lakh tones from an area of 1.81 lakh ha has spread all over the country (Anon., 2019) [1]. It is grown as an extensive scale in Rajasthan, Madhya pradesh, Gujarat, Uttar Pradesh, Maharashtra and Panjab. Rajasthan produces the lion's share of India's production, accounting for over 80% of the nation's total fenugreek yield (Kumawat and Shekhawat, 2015) [8]. This crop annual production is 12666 MT from an area of 6529 ha in Gujarat (Anon., 2019) [2]. Fenugreek is suffering from diseases like powdery mildew [*Erysiphe polygoni* DC.; *Leveillula Taurica* (Lev.)], downy mildew (*Peronospora trigonellae*), are serious diseases resulting 15 to 50 per cent seed yield losses (Kumawat and Shekhawat, 2015) [8]. It is a routine practice for farmers to spray fungicides onward from 45 days age to maturity of the crop to save seed yield from the epidemic of disease. Powdery mildew is a foliar pathogen and is very difficult to control. The efficacy of fungicides on spore germination considered as most suitable strategy for management. But the pesticides residues present in seed are main concern for human health and at national and international level. To overcome these problems, it is necessary to eco-friendly manage the disease by using phytoextracts under *in vitro* condition which may provide new information on its management of disease in the crop.

2. Materials and Methods

Various criteria are used to determine the effect of different physiological agents on germination, of which the most frequently used as the percentage of spores that produce germ tubes. Water is the prime factor in any germinating medium and for many spores is the only substance necessary to start germination. Its imbibition causes the first visible symptom of germination, the swelling of the spore, often to more than twice became its original size. Thus, absorption of water by spores resembles the imbibition activity of lyophilic colloids. This phenomenon, however, is dependent upon some vital mechanism of the spore, for dead spores do not swell and absorption varies with the viability of the spore. (David, 1950) [6].

Locally available plant species were tested for their efficacy against *Erysiphe polygoni*. Fresh leaves, cloves or rhizomes of respective plant species as shown in Table - 3.6 were first washed with tap water and then with sterilized water. Each sample was then homogenized in sterilized distilled water at the rate of 1 ml/g of tissues (1:1 V/W) with a pestle and mortar and filtered through fine muslin cloth. The filtrate was centrifuged at 5000 rpm for 20 minutes and

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The supernatant was filtered with sterilized sintered funnel (pore size 1-2 microns), which formed the standard plant extract solution (100%). The clarified extracts were stored in refrigerator at 4°C till used (Ansari, 1995) [4]. To prevent bacterial contamination, a pinch of streptomycin was added to each stalk solution.

Different phytoextracts were tested for their effect on spore germination inhibition of causal organism of powdery mildew by using spore germination inhibition technique. Powdery mildew disease infected leaves of fenugreek were collected from field. Spores were collected by gently rubbed sterilized brush on spotted part of leaves. So, spores were collected and added in sterilized distilled water. Muslin cloth bag was used for remove extra plant cells from spore suspension.

One drop of each phyto extract suspension and spore suspension was placed on a glass cavity slide, so concentration was obtained as per require for evaluation. The slides were then placed in petri plates lined with moistened coarse filter paper to provide sufficient humidity for germination of conidia for 24 hours at room temperature (25 °C±1). After 24 hours, 48 hours and 72 hours intervals observation of spore germination inhibition was taken by using light microscope at 40x objective lens.

Observations were recorded and per cent inhibition of spore germination worked out as per the formula given below Vincent (1947) [10].

$$I = \frac{C - T}{C} \times 100$$

Where, I = Per cent inhibition

C = Number of germinated spores in control.

T = Number of germinated spores in treatment

3. Results and Discussion

Efficacy of phytoextracts on spore germination inhibition of *E. polygoni*

The efficacy of phytoextracts on inhibiting the spore germination of fungus under *in vitro* condition was determined by using slide spore germination inhibition technique. The data on cumulative spore germination inhibition are presented in Table 1 and fig 1.

It was evident from the Table 1 that all the phytoextracts were found effective in spore germination inhibition against the test pathogen. Among all seven phytoextracts tested the maximum mean inhibition was observed in *Allium sativum* L., *Azadirachta indica* A. Juss., and *Curcuma longa* L. with 65.07, 61.06 and 60.12 per cent mean spore germination inhibition, respectively on 24 hrs after treatment. Minimum effective phytoextract was *Lantana camara* L. with 29.32 per cent mean spore germination.

There was positive correlation in between concentration of phytoextract and spore germination inhibition. All phytoextracts within the concentration were significantly differ from each other, except *Lantana camara* L. with 33.61 and 36.92 per cent at 15 and 20 per cent concentration, respectively and *Allium cepa* L. with 36.67 and 38.02 per cent at 5 and 10 per cent concentration, respectively and statistically at par with their concentration.

Within the phytoextracts, the maximum concentration at 20 per cent *Allium sativum* L. (74.71%) and *Azadirachta indica* A. Juss. (72.18%) was found at par. The next effective

phytoextracts were *Curcuma longa* L. and *Allium sativum* L. at 20 and 15 per cent concentration with 73.62 and 67.48 per cent spore germination inhibition, respectively and found statistically at par with each other. But the least effective phytoextract was *Lantana camara* L. and *Ocimum sanctum* L. at 5 per cent concentration with 19.19 and 24.39 per cent spore germination, respectively and remained statistically at par with each other.

The interaction effect of phytoextracts and concentrations was found statistically non-significant with each other.

At 48 hrs, the maximum mean spore inhibition was found in *Allium sativum* L., *Azadirachta indica* A. Juss., and *Curcuma longa* L. with 68.68, 64.43 and 62.80 per cent mean spore germination inhibition, respectively. Minimum effective phytoextract was *Lantana camara* L. with 32.70 per cent mean spore germination.

Among the concentration, *Lantana camara* L. was found at par with their 37.40 and 40.02 per cent spore germination inhibition at 15 and 20 per cent, respectively. Then *Allium cepa* L. was found at par with 39.63 and 41.63 per cent at 5 and 10 per cent, respectively. All other remaining phytoextracts within the concentration were significantly differ from each other.

But the least effective phytoextract was *Lantana camara* L. at 5 per cent concentration with 22.17 per cent spore germination. Within the concentration of each phytoextract result found non-significant.

The interaction effect in between the concentrations and phytoextracts were showed that statistically non-significant.

The result of all six phytoextracts at 72 hrs has indicated that the maximum mean inhibition of was observed in *Allium sativum* L. with 72.12 per cent followed by *Azadirachta indica* A. Juss. With 68.23 per cent. And the next effective phytoextract was *Curcuma longa* L. with mean spore germination inhibition of 66.39 per cent. But the least effective phytoextracts were *Allium cepa* L., *Zingiber officinale* Rosc. And *Lantana camara* L. with 50.49, 50.22 and 36.66 per cent mean spore germination inhibition, respectively.

Within the concentration, *Allium cepa* L. was found at par with their 43.49 and 44.55 per cent spore germination inhibition at 5 and 10 per cent, respectively. All other remaining phytoextracts within the concentration were significantly differ from each other.

The interaction effect among the concentration and different phytoextract has revealed that statistically non-significant with each other.

The antifungal properties of naturally occurring substances in plant species showed that neem leaf extract was excellent source as spore germination inhibition of *E. cichoracearum*. Similarly, the neem based botanical of 2 per cent neem leaf extract found better for the control of powdery mildew of mustard in Gujarat (Anon., 1996) [3].

Maurya *et al.* (2004) [9] have reported more than 80 per cent spore germination inhibition of *E. pisi* causing powdery mildew in pea with neem and moth. In addition, they showed that *Allium sativum*, *A. cepa*, and *Zingiber officinale* are also effective in reducing disease and conidial germination. The same results were also reported by Dhaliwal *et al.* (2002) [7]. They indicated that complete inhibition of conidial germination of *uncinula necatar* causing powdery mildew of grapevine with phytoextract of garlic (*Allium sativum*).

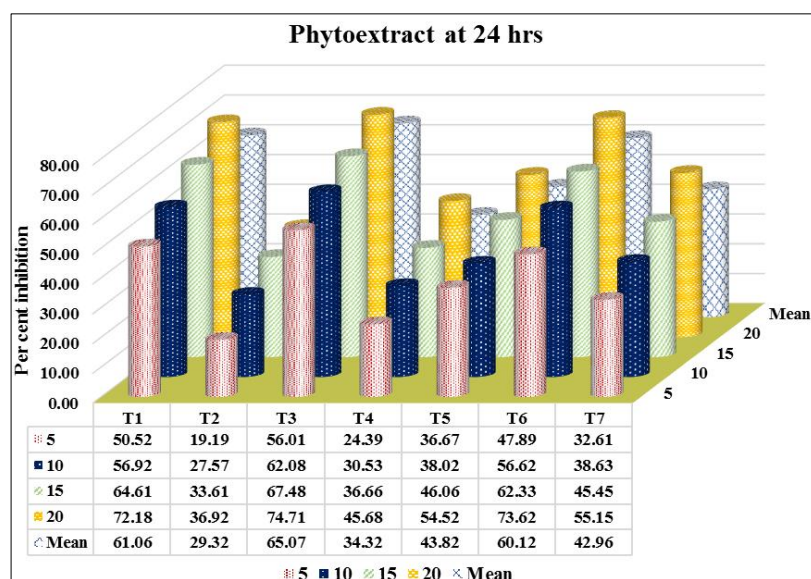
Table 1: Effect of phytoextracts on spore germination inhibition of *E. polygona* *in vitro*

S. No.	Phyto-extracts	Conc. (%)	Per cent spore germination inhibition after								
			24 hrs			48 hrs			72 hrs		
1.	<i>Azadirachta indica</i> A. Juss. (Neem leaf extract)	5	45.30 (50.52)*			47.00 (53.48)			49.22 (57.34)		
		10	48.98 (56.92)			51.08 (60.53)			52.80 (63.45)		
		15	53.49 (64.61)			55.80 (68.41)			58.48 (72.67)		
		20	58.17 (72.18)			60.19 (75.28)			63.06 (79.47)		
Mean			51.48 (61.06)			53.52 (64.43)			55.89 (68.23)		
2.	<i>Lantana camara</i> L. (Lantana)	5	25.98 (19.19)			28.09 (22.17)			30.68 (26.03)		
		10	31.68 (27.57)			33.94 (31.18)			35.73 (34.10)		
		15	35.43 (33.61)			37.70 (37.40)			40.01 (41.33)		
		20	37.42 (36.92)			39.25 (40.02)			42.24 (45.19)		
Mean			32.63 (29.32)			34.74 (32.70)			37.16 (36.66)		
3.	<i>Allium sativum</i> L. (Garlic)	5	48.45 (56.01)			50.17 (58.97)			52.44 (62.84)		
		10	51.99 (62.08)			54.14 (65.68)			55.92 (68.60)		
		15	55.23 (67.48)			57.59 (71.28)			60.14 (75.20)		
		20	59.81 (74.71)			62.58 (78.79)			64.77 (81.82)		
Mean			53.87 (65.07)			56.12 (68.68)			58.32 (72.12)		
4.	<i>Ocimum sanctum</i> L. (Tulsi)	5	29.60 (24.39)			31.55 (27.38)			32.17 (28.35)		
		10	33.54 (30.53)			35.77 (34.17)			36.72 (35.75)		
		15	37.27 (36.66)			39.50 (40.46)			40.01 (41.33)		
		20	42.52 (45.68)			44.13 (48.48)			45.13 (50.22)		
Mean			35.73 (34.32)			37.74 (37.62)			38.51 (38.91)		
5.	<i>Allium cepa</i> L. (Onion)	5	37.27 (36.67)			39.01 (39.63)			41.26 (43.49)		
		10	38.07 (38.02)			40.18 (41.63)			41.87 (44.55)		
		15	42.74 (46.06)			44.92 (49.86)			47.17 (53.79)		
		20	47.59 (54.52)			49.40 (57.65)			50.84 (60.12)		
Mean			41.42 (43.82)			43.38 (47.19)			45.28 (50.49)		
6.	<i>Curcuma longa</i> L. (Turmeric)	5	43.79 (47.89)			45.49 (50.86)			47.71 (54.72)		
		10	48.81 (56.62)			50.90 (60.23)			52.62 (63.14)		
		15	52.14 (62.33)			54.41 (66.13)			56.82 (70.06)		
		20	59.09 (73.62)			59.33 (73.99)			61.77 (77.63)		
Mean			50.96 (60.12)			52.53 (62.80)			54.73 (66.39)		
7.	<i>Zingiber officinale</i> Rosc. (Ginger)	5	34.82 (32.61)			36.62 (35.58)			38.91 (39.45)		
		10	38.43 (38.63)			40.54 (42.24)			42.22 (45.16)		
		15	42.39 (45.45)			44.57 (49.25)			46.82 (53.18)		
		20	47.96 (55.15)			49.75 (58.25)			52.56 (63.04)		
Mean			40.90 (42.96)			42.87 (46.33)			45.13 (50.22)		
			F	C	F*C	F	C	F*C	F	C	F*C
S.Em. ±			0.78	0.59	1.56	0.85	0.64	1.69	0.70	0.53	1.41
C.D. at 5%			2.20	1.67	NS	2.40	1.81	NS	1.99	1.51	NS
C.V. %			6.14			6.4			5.1		

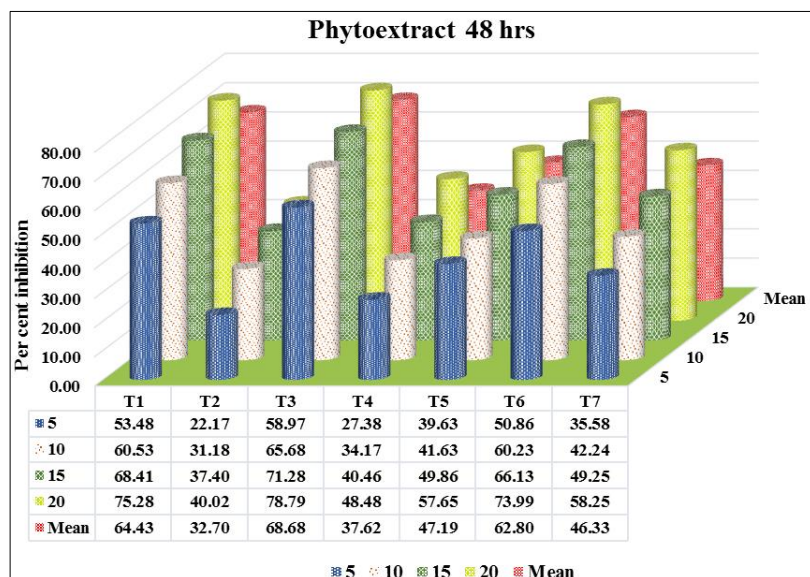
F= Phytoextract, C=Concentration, F*C=Interaction of phytoextract and concentration

@ =Mean of three replications

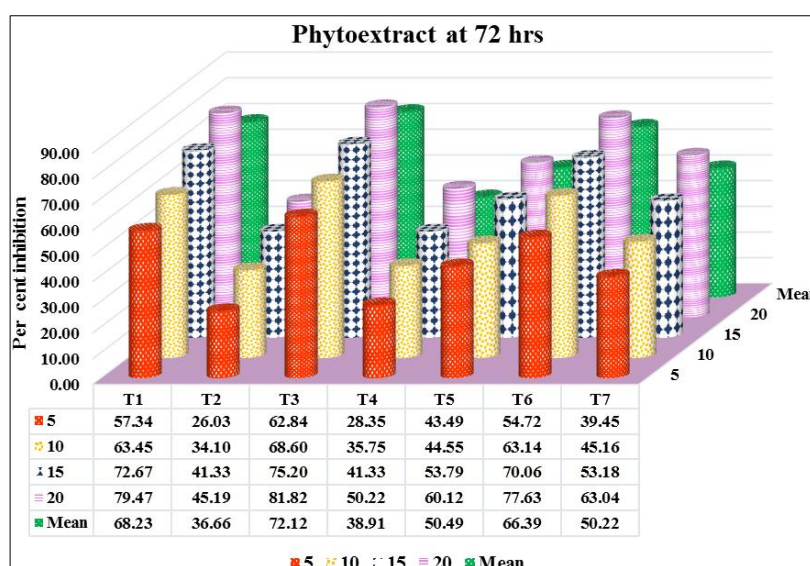
*Figures in parentheses indicate arc-sine re-transformed values.



(a)



(b)



(c)

Fig 1: Per cent spore germination inhibition of *E. polygoni* by different phytoextracts *in vitro*

4. Conclusion

In order to find out an effective and eco-friendly approach in controlling the powdery mildew disease of fenugreek under *in vitro* condition, different seven phytoextracts were tested, at 5, 10, 15 and 20 per cent concentration. The maximum mean spore germination inhibition was found in treatment of garlic with 65.07, 68.68 and 72.12 per cent followed by neem leaf extract with 61.06, 64.43 and 68.23 per cent at 24, 48 and 72 hrs, respectively but lantana was found as least effective as compared to other phytoextract treatments.

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