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In-vitro evaluation of some essential oils against a pathogen isolated from wilt of chickpea in Dehradun

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Abstract
The present study Antifungal activity of essential oils in *in-vitro* evaluation against *Fusarium oxysporum* causal agent of wilt of chickpea (*Cicer arietinum* L.) was conducted at the laboratory. Chickpea is most important crop grow in India wilt diseases is caused by *Fusarium oxysporum* was serious threat to crop production. Three essential oil clove, Linseed, Castor with three replication was conducted for each concentration at 0.5%, 1.0%, 1.5%. By using food poision food technique. Maximum average inhibition was reported with the treatment, castor oil (48.15%), clove oil (44.44%) and Linseed oil (34.44%). Thus, all essential oil against wilt of chickpea and inhibited over untreated control. Most effective in order best antifungal potential are castor oil, clove oil and Linseed oil, respectively. All essential oil have their antifungal potential to their developed as potent fungicides in organic farmin and integrated disease management.

Keywords: essential oil, wilt of chickpea (*Fusarium oxysporum*), poison food technique, inhibition

Introduction
Chickpea (*Cicer arietinum* L.) is known in India by different names: Bengal gram, gram and chana. Chickpea are of Desi and Kabuli type. Desi types have small seeds with angular, sharp edges and the seed coat can vary from black to cream or yellow. The flowers of Desi type are generally pink 80-90 per cent of the world's chickpea crop. The Kabuli types have large rounded seeds shaped like a ram's head with cream beige or white seed coats.

India is the largest producer of chickpea accounting for 64% of the global production. It provides a high quality protein (20-22%) for vegetarians' diet; is rich in fiber, minerals and β-carotene. It also fixes the atmospheric nitrogen (40 kg N/ha) thus reduces the need of nitrogenous fertilizers. The optimal conditions required for growth and development include 18-26 ºC night temperature, 21-29 ºC day temperature and annual rainfall of 560-660 mm.

Chickpea production is constrained by diseases and insect-pests. In general, soil borne diseases (*Fusarium* wilt, collar rot, dry root rot, etc.) are more prevalent in central and peninsular India. Among the insect-pests, wilt collar rot is the most severe yield reducer throughout India. *Fusarium* wilt disease of chickpea was prevalent at Eastern plateau of India i.e., Ranchi, Dumka, Darisai and Chatra and the disease incidence was varied from 38.7 to 59.2% (Kumar *et al*., 2012). Chickpea (*Cicer arietinum* L), was first observed in India by Butler (1918). The genus *Fusarium* had many soil borne species which were distributed all over world and known as plant pathogens since a long time ago (Moss and Smith, 1984). The chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* was reported to be widely distributed in near about 32 countries of the world and at national scenario; six fungal diseases have been reported to be important and causing considerable damage to the crop (Haware *et al*., 1986; Nene *et al*., 1996).

Therefore the present study was carried to find out "*In-vitro* evaluation of some essential oils against a pathogen isolated from wilt of chickpea in Dehradun."

Material and Methods
All *in vitro* experiments were planned and carried out at the laboratory of Plant Pathology Department, School of Agriculture, Dehradun.

Isolation and identification of the pathogen
Chickpea plant with typical wilt symptoms was collected from different location from Dehradun and brought to the laboratory. In tap water, the roots and stems of infected plants the roots and stem were split open, and the sterilized sharp blade curt into small bites

(2.5mm). such bites were then disinfected for a minute with an aqueous solution of sodium hypochlorite (0.1%) and then thoroughly washed into sterile distilled water thrice to eliminate traces of sodium hypochlorite, if any, and transferred aseptically to PDA medium in Petri plates and incubated in B.O.D incubator at 25±2 ºC for 5 days. Plate fungal growth was investigated and subcultured on PDA slants. By routine cultivation of culture. It had been distilled and stored for the further research on agar slants.

***In- vitro* evaluation of essential oil**

Three essential oils *viz*. clove, linseed and castor oil were analyzed to see the effect against the pathogen *in vitro*. Three concentration i.e. 0.5, 1.0, 1.5 percent were taken for the evaluation. Desired concentrations obtained by adding a sufficient amount of oil to PDA by Poison food technique in Petri plates. Every treatment retained three replications. Oil free PDA acted as control. Each plate was inoculated with 8 mm diameter mycelia disk taken from *Fusarium oxysporum* culture grown on PDA, which is nine days old. The observation were made when the fungus was reached on the control plate or attained at the bottom of the plate.

List of Essential oils	
Treatment	**Essential oils**
T1	Clove oil
T2	Linseed oil
T3	Castor oil
T4	Control

Experimental details

Design: CRD
Replication: Three
Treatments: Four

Radial mycelia growth observation colony diameter of the *Fusarium oxysporum* f. sp. *Cicer*. was recorded at 3, 6, 9-day intervals and continued until untreated control plates were completely covered with mycelia growth and calculation of per cent inhibition by applying the following formula (Vincent 1927)

$$I = [(C-T)/C] \times 100$$

Where,
I = % of growth inhibition
C = Diameter of fungal colony (mm) in control plates
T = Diameter of fungal colony (mm) in treated plates

Results and Discussion

Efficacy of essential oils against *fusarium* oxysporum

Radial mycelia growth

The inhibitory activity against radial mycelia growth of pathogen was observed at all concentrations of clove oil, linseed oil and castor oil. When we increase the concentration of essential oils then mycelia growth was to be less.

At 0.5% mycelia growth was recorded in the range of (59.67mm) linseed oil to (65.00mm) castor oil. The highest mycelia growth was reported with castor oil (65.00mm), clove oil (63.33mm) and less effective was found at linseed oil (59.67mm) mycelia growth of pathogen over- under fully grown untreated control.

At 1.0% mycelia growth was recorded in the range of (63.67mm) Castor oil to (71.67mm) Linseed oil. The highest mycelia growth was reported with linseed oil (71.67mm), clove oil (64.67mm) and less effective was found at castor oil (63.67mm) mycelia growth of pathogen over- under fully grown untreated control.

At 1.5% mycelia growth was recorded in the range of (46.67mm) castor oil to (59.00mm) linseed oil. The highest mycelia growth was reported with linseed oil (59.00mm), clove oil (50mm) and less effective was found at castor oil (46.67mm) mycelia growth of pathogen over- under fully grown untreated control.

Table 1: Essention oils against Fusarium wilt of chick pea (Mycelia Growth Colony Diameter in mm)*

Treatment	**3 DAI**			**6 DAI**			**9DAI**		
	Radial growth (mm)			**Radial growth (mm)**			**Radial growth (mm)**		
Dose	0.5%	1%	1.5%	0.5%	1%	1.5%	0.5%	1%	1.5%
T1	26.00	24.67	20.67	44.33	41.00	32.00	63.33	64.67	50.00
T2	27.33	25.00	26.00	42.33	40.00	46.00	59.67	71.67	59.00
T3	24.33	27.33	25.67	41.00	42.67	40.00	65.00	63.67	46.67
T4(control)	30.00	30.00	30.00	60.00	60.00	60.00	90.00	90.00	90.00
C.D.	3.12	2.65	2.81	5.29	7.85	9.07	19.91	9.89	20.53
SE(m)	0.94	0.80	0.85	1.60	2.37	2.74	6.01	2.99	6.20

*= mean of radial growth, C.D = colony diameter mean, S.D (m) = Stander error mean

Mycelia inhibition

The result was examined that the essential oils were tested (@ 0.5, 1.0, 1.5% each) inhibited mycelia growth of *Fusarium oxysporum f. sp. Cicer arietinum l.* over untreated control. Further, the per cent mycelia inhibition of pathogen was increased with the increase in the concentration of the essential oil.

At 0.5% inhibition was recorded in the range of (27.78%) castor oil to (33.70%) linseed oil. The highest inhibition was reported with linseed oil (33.70%), clove oil (29.63%) and less effective was found at castor oil (27.78%) mycelia growth of pathogen over- under fully grown untreated control.

At 1.0% inhibition was recorded in the range of (20.37%) linseed oil to (29.26%) castor oil. The highest inhibition was reported with castor oil (29.26%), clove oil (28.15%) and less effective was found at linseed oil (20.37%) mycelia growth of pathogen over- under fully grown untreated control.

At 1.5% inhibition was recorded in the range of (34.44%) linseed oil to (48.15%) castor oil. The highest inhibition was reported with castor oil (48.15%),clove oil (44.44%) and less effective was found at linseed oil (34.44%) mycelia growth of pathogen over- under fully grown untreated control.

Table 2: Mycelial per cent inhibition of *Fusarium oxysporum*

Treatment	3 DAI			6 DAI			9DAI		
	inhibition%			inhibition%			inhibition%		
Dose	0.5%	1%	1.5%	0.5%	1%	1.5%	0.5%	1%	1.5%
T1	13.33	17.78	31.11	26.11	31.67	46.67	29.63	28.15	44.44
T2	8.89	16.67	13.33	29.45	33.33	23.33	33.70	20.37	34.44
T3	18.89	8.89	14.44	31.67	28.89	33.33	27.78	29.26	48.15
T4(control)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

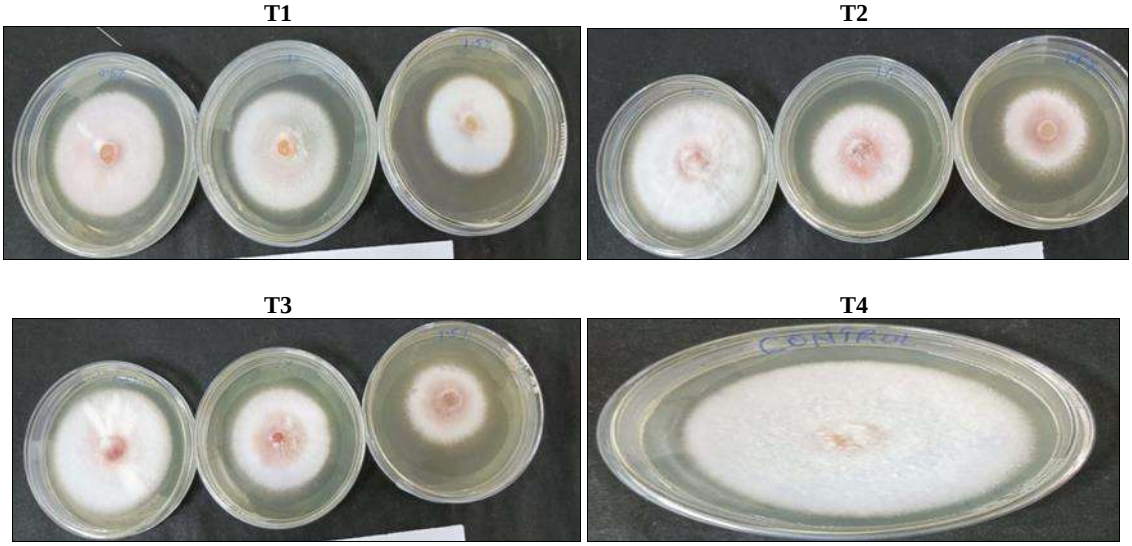

Fig 1: *In-vitro* efficacy of essential oils against *F. oxysproum*

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