

## Bio-efficacy of alcoholic extracts of botanicals to mitigate pea root rot caused by *Fusarium solani* f.sp. *pisi*

### Abstract

The present investigation had been undertaken in the Department of Plant Pathology, College of Agriculture, Chaudhary Sarwan Kumar Himachal Pradesh Agriculture University, Palampur (India) during 2017-2019. Pea root rot caused by *Fusarium solani* f. sp. *pisi* has been observed as an alarming problem in pea growing regions of Himachal Pradesh and poses major constraints in pea cultivations. The alcoholic leaf extracts of four plants viz., *Eupatorium adenophorum*, *Eucalyptus* sp., *Vitex negundo* and *Ageratum conyzoides* were evaluated at different concentrations for their efficacy against *F. solani* f. sp. *pisi* under *in vitro* conditions. Among all extracts, *E. adenophorum* yielded maximum mycelial inhibition of 77.4 per cent followed by *Eucalyptus* sp. 74.9 per cent at 25 per cent test concentration. Thus, all test botanicals, *E. adenophorum* was found the most efficient against *F. solani* f. sp. *pisi*.

**Keywords:** *Fusarium solani*, Botanical, Pea

### 1. Introduction:

Pea (*Pisum sativum* L.) stands as a self-pollinating, esteemed cash crop predominantly cultivated in cooler climatic zones across the globe. Renowned for its dual utility as both a vegetable (green peas) and a pulse (dried peas), it boasts a rich nutritional profile abundant in protein, carbohydrates, and essential minerals (Choudhary, 1996). Furthermore, its nutritional composition encompasses flavonoids, carotenoids, and vitamin C, rendering it a potent source of antioxidants (Clark, 2007). The agricultural practice of intercropping pea with cereals not only serves to enhance soil fertility by harnessing atmospheric nitrogen through root nodule fixation but also underscores its versatility as a soil-enriching crop within organic farming paradigms.

Despite its agricultural prominence, pea cultivation confronts a litany of pathogenic challenges, foremost among them being root rot/wilt complex, Ascochyta blight, powdery mildew, bacterial blight, white rot, and rust. Notably, the emergence of *Fusarium solani* f. sp. *pisi*-induced root rot poses a grave threat to pea cultivation in regions of Himachal Pradesh. The susceptibility of local pea cultivars exacerbates the severity of the disease, manifesting primarily during the pre-flowering or flowering stages and precipitating a decline in crop vigour. Initial symptoms manifest as basal leaf yellowing, progressing to root maceration and necrosis, ultimately culminating in plant collapse. Such afflictions entail substantial economic repercussions, inflicting significant losses upon the agricultural community (Negi et al., 2008).

Compounding the challenge is the soil-borne nature of the disease, complicating its management within extant agricultural protocols. Chemicals are preferred seed dressers for controlling the disease incidence of pea root rot, but their harmful effect on soil ecology cannot be overlooked. Consequently, the exploration of botanical extracts, specifically alcoholic leaf extracts from various sources, emerges as a promising avenue for combating *Fusarium solani* f. sp. *pisi* under controlled *in vitro* conditions.

### 2. Materials and Methods

**Comment [D1]:** This description is not necessary in the summary. Authors should give us an introduction including context, objective, methods and major results.

**Comment [D2]:** Addict another keyword

## 2.1 Isolation of pathogen

Isolation procedures were conducted on diseased pea samples to ascertain the causative agents responsible for root rot disease, employing Potato Dextrose Agar (PDA) as the growth medium. The collected diseased samples were initially rinsed in tap water, air-dried, and sections were excised from the transition zone of root rot. These bits were subjected to surface sterilization using a 1.0% sodium hypochlorite solution for 15 seconds, followed by triple rinsing with sterilized water under aseptic conditions within a Laminar Air Flow chamber. Subsequently, the sterilized bits were desiccated using double-folded sterilized filter paper to eliminate residual moisture before being aseptically transferred onto PDA Petri plates. The inoculated plates were then incubated in a BOD (Biological Oxygen Demand) incubator for a duration of seven days at a constant temperature of  $26\pm 1^\circ\text{C}$ .

## 2.2 Evaluation of botanicals

Alcoholic extracts derived from four locally available botanical sources, namely *Vitex negundo*, *Eucalyptus* sp., *Ageratum conyzoides*, and *Eupatorium adenophorum*, were assessed for their efficacy against *Fusarium solani* f. sp. *psii* using the Poisoned Food Technique, with each treatment replicated three times. Leaves of each botanical species were harvested from the surroundings of the Palampur area and subjected to surface sterilization using a 1.0% sodium hypochlorite solution for a duration of 15 seconds, followed by gentle drying in shaded conditions. Subsequently, the dried leaves were finely powdered using a blender, and the resulting powder was carefully stored in clean paper bags at room temperature, adequately labelled for identification.

Preparation of the alcoholic extracts proceeded as follows:

**2.2.1 Alcoholic extract:** 100 grams of each powdered botanical sample were suspended in 100 millilitres of methanol within a conical flask sealed with a cotton plug. The suspension was placed on a rotary shaker set at a speed of 190-220 revolutions per minute (rpm) for 24 hours to facilitate extraction. Subsequently, the supernatant of each extract was collected and subjected to evaporation until the final volume was reduced to one fourth of the original volume. The resulting concentrated extracts were then stored in airtight bottles at a temperature of 4 degrees Celsius.

Five concentrations (5%, 10%, 15%, 20%, and 25%) of each botanical extract were prepared by diluting the stock solution using the Dilution Method. Double-strength solutions of these five concentrations were added to Potato Dextrose Agar (PDA) in Petri plates, with three replications per concentration, following the method described by Falck (1907). A control was established by adding medium mixed with sterilized distilled water in equal quantities to Petri plates.

Using sterilized cork borers and needles, all plates were inoculated with 5 mm mycelial discs of *Fusarium solani* f. sp. *solani*. The inoculated plates were then incubated at a temperature of  $26\pm 1^\circ\text{C}$  for a period of seven days or until the pathogen completely covered the control plates with mycelial growth.

Observations on the radial growth of the pathogen were recorded for each replication of the treatment and control plates. The percentage of mycelial inhibition was calculated using the formula described by McKinney (1923).

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

Where;

I (%) = Per cent mycelial inhibition

C = mycelial growth in control

T = mycelial growth in treatment

### 2.3 Statistical analysis

The data of experiment was pooled and subjected to appropriate statistical analysis. All the data were analysed in the computer using CPCS-1 and OPSTAT software. The significance of treatments was taken at 5 per cent level of significance.

## 3. RESULTS AND DISCUSSION

### 3.1 Identification of pathogen

Isolations were made from disease samples of root rot of pea collected from pea growing areas of the state. Based on morphological, cultural, and pathogenic characteristics, *Fusarium solani* f. sp. *pisi* was identified as the causal agent of pea root rot.

### 3.2 Evaluation of botanicals

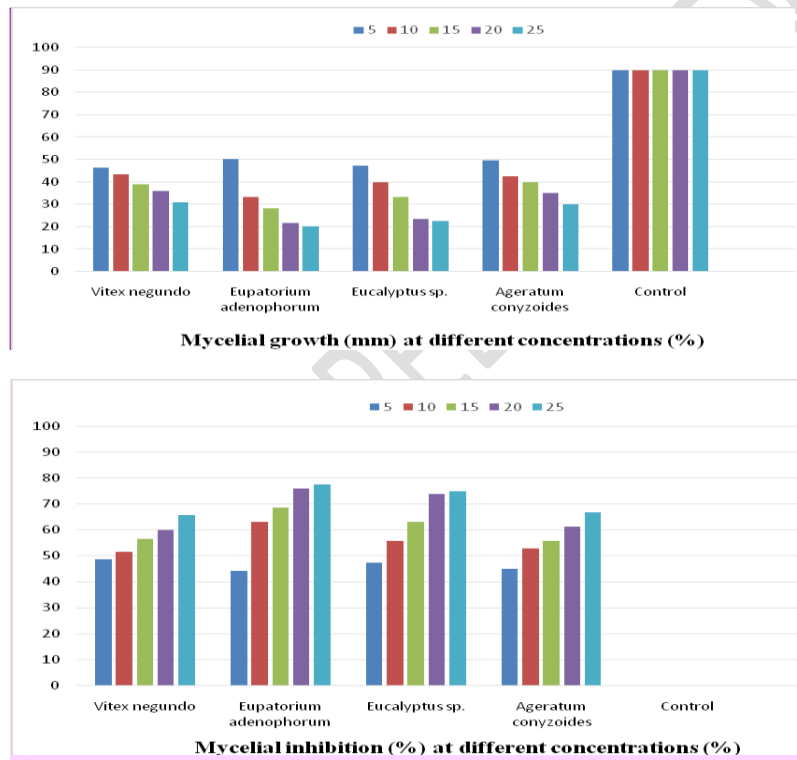
The alcoholic leaf extracts of four botanicals viz., *Eupatorium adenophorum*, *Eucalyptus* sp., *Vitex negundo* and *Ageratum conyzoides* were also evaluated at different concentrations i.e. 5, 10, 15, 20 and 25 per cent for their antifungal properties through Poisoned Food Technique against *F. solani* f. sp. *pisi* and data presented in the table 1.

**Table 1** *In vitro* evaluation of alcoholic extracts of botanicals against *Fusarium solani* f. sp. *pisi*

Botanicals	<i>Fusarium solani</i> f. sp. <i>pisi</i>									
	Mycelial growth (mm)					Mycelial inhibition (%)				
	at different concentrations					at different concentrations				
	5	10	15	20	25	5	10	15	20	25
<i>Vitex negundo</i>	46.3	43.6	39.0	36.0	31.0	48.6	51.6	56.6	60.0	65.6
<i>Eupatorium adenophorum</i>	50.3	33.3	28.3	21.6	20.3	44.1	63.0	68.6	76.0	77.4
<i>Eucalyptus</i> sp.	47.3	40.0	33.3	23.6	22.6	47.4	55.6	63.0	73.8	74.9
<i>Ageratum conyzoides</i>	49.6	42.6	40.0	35.0	30.0	44.9	52.7	55.6	61.1	66.7
Control (No treatment)	90.0	90.0	90.0	90.0	90.0	-	-	-	-	-

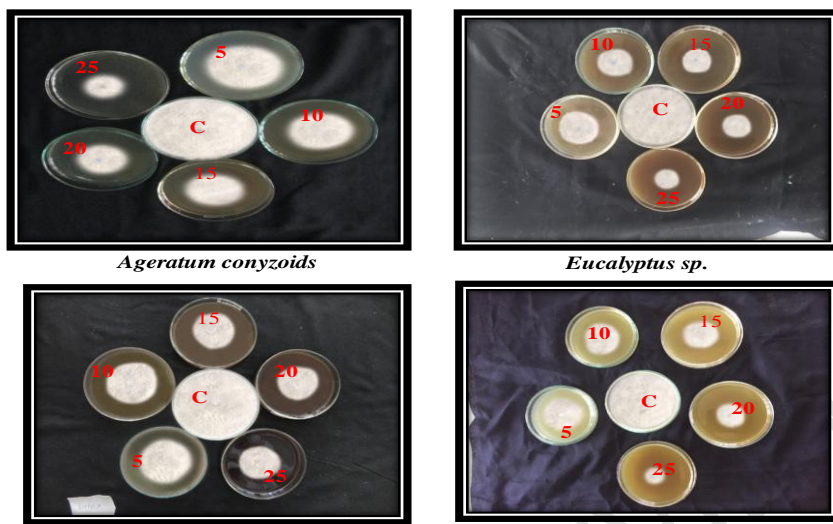
CD (p=0.05)	2.5	2.0	2.0	2.6	2.0	-	-	-	-	-
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The data in the table 1 revealed that alcoholic extracts of all the botanicals were effective against the pathogen to a varying extent. Antifungal activities of all phytoextracts increased significantly with the increase in the concentration from 5 to 25 per cent. The alcoholic plant extracts of all botanicals proved to be effective against the pathogen at 25 per cent concentration resulted in more than 60 per cent mycelial inhibition. Among all four, *E. adenophorum* was found best with mycelial inhibition of 77.4 per cent against *F. solani* f. sp. *pisi* followed by *Eucalyptus* sp. (74.9%), *A. conyzoides* (66.7%) and *V. negundo* (65.6%) at 25 per cent test concentration. The results obtained above have also been depicted in the bar diagram (Fig. 1) and plate 1.



**Fig.1** Mycelial growth (mm) and mycelial inhibition (%) with alcoholic extracts of botanicals against *Fusarium solani* f. sp. *pisi*.

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**Plate 1** Mycelial inhibition of *Fusarium solani* f. sp. *pisi* with alcoholic extracts of botanicals.

**Comment [D4]:** Figure???

The antifungal potential of aqueous and crude extracts of botanicals against *F. solani*, *F. oxysporum*, *R. solani* and *S. sclerotiorum* have also been reported by various workers in the recent past (Kumar and Tripathi 1991; and Devi and Paul 2005).

Among alcoholic extracts of all test botanicals, *E. adenophorum* was found best one against *F. solani* f. sp. *pisi*. Hence, the chopped raw material of *E. adenophorum* can be used as soil amendment against the disease.

**Comment [D5]:** Improve discussion

**4. Conclusion:** In conclusion, the study demonstrated the potent antifungal properties of alcoholic leaf extracts from *Eupatorium adenophorum*, *Eucalyptus* sp., *Ageratum conyzoides*, and *Vitex negundo* against *F. solani* f. sp. *pisi*. Increasing concentrations of the extracts correlated with higher efficacy, with *E. adenophorum* exhibiting the most significant inhibition of mycelial growth. These findings suggest the potential of *E. adenophorum* as a natural soil amendment for controlling fungal diseases in agriculture.

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