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Bio-efficacy of Alcoholic Extracts of Botanicals to Mitigate Pea Root Rot Caused by *Fusarium solani* f.sp. *pisi*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

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, among all test botanicals, *E. adenophorum* was found the most efficient against *F. solani* f. sp. *pisi*. These findings suggest that *E. adenophorum* extracts could be used as eco-friendly alternatives to control *Fusarium solani* f. sp. *pisi*.

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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 [1]. Furthermore, its nutritional composition encompasses flavonoids, carotenoids, and vitamin C, rendering it a potent source of antioxidants [2]. 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 [3].

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 [4]. 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 [5].

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 [6].

2. MATERIALS AND METHODS

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±1°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. *pisi* 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 [7]. 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±1°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 [8].

$$\mathrm{I}\,(\%) = \frac{\mathrm{C} - \mathrm{T}}{\mathrm{C}} \mathrm{x}\, 100$$

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.

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 Fig. 2.

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

Botanicals	*Fusarium solani* f. sp*. pisi*									
	Mycelial growth (mm) at different concentrations (%)					Mycelial inhibition (%) at different concentrations (%)				
	5	10	15	20	25	5	10	15	20	25
Vitex negundo	46.3	43.6	39.0	36.0	31.0	48.6	51.6	56.6	60.0	65.6
Eupatorium adenophorum	50.3	33.3	28.3	21.6	20.3	44.1	63.0	68.6	76.0	77.4
Eucalyptus sp.	47.3	40.0	33.3	23.6	22.6	47.4	55.6	63.0	73.8	74.9
Ageratum conyzoides	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	-	-	-	-	-

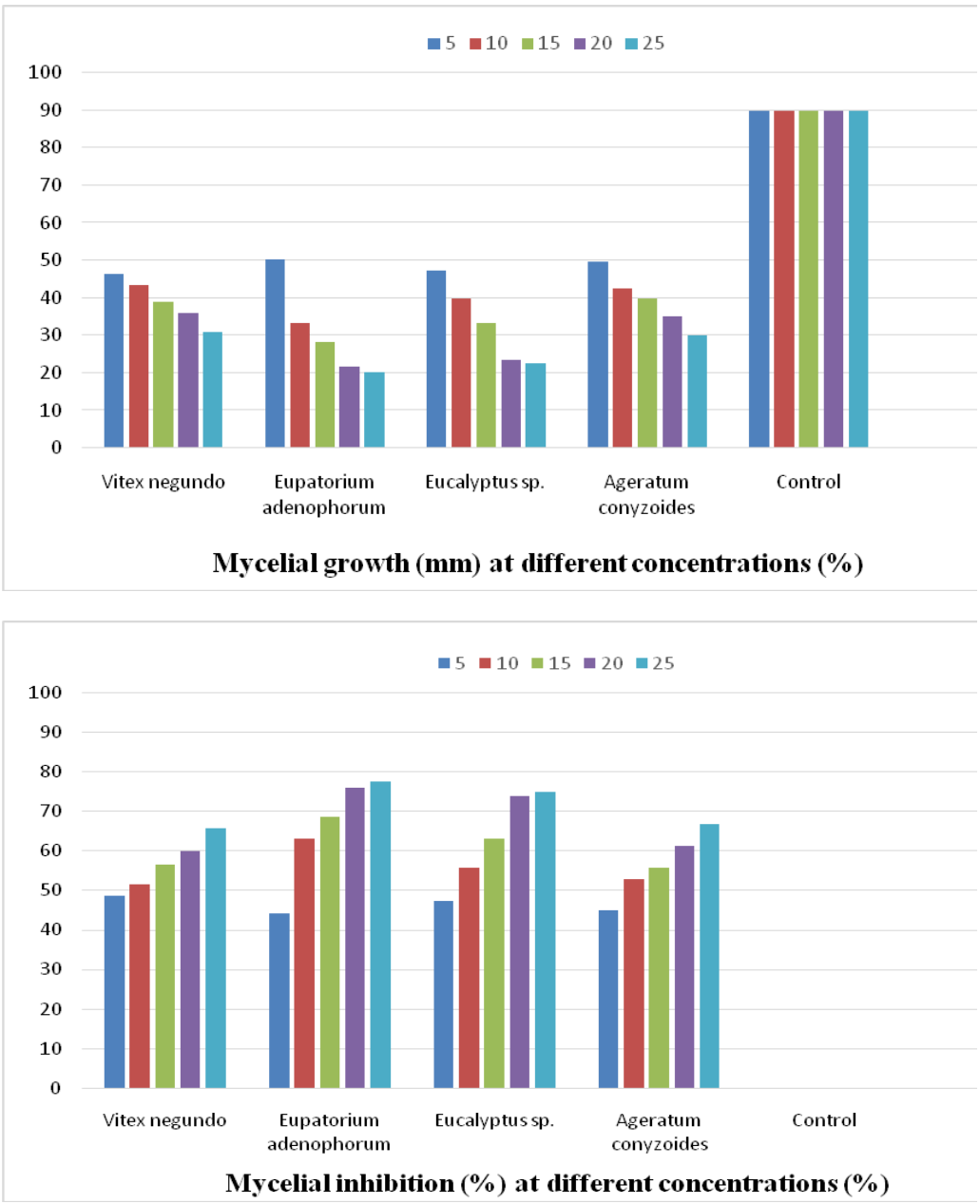

Fig. 1. Mycelial growth (mm) and mycelial inhibition (%) with alcoholic extracts of botanicals against *Fusarium solani* f. sp. *pisi*.

The results of these findings shows the effectiveness of the extracts, the concentration-dependent response, and the relative performance of each botanical. It is evident that all botanical extracts showed antifungal properties against *Fusarium solani* f. sp. *pisi*, as indicated by the reduction in mycelial growth compared to the control (untreated). This reaffirms the potential of natural plant extracts as alternatives to synthetic fungicides in managing fungal pathogens. There was a noticeable trend of increasing antifungal activity with higher concentrations of the extracts. This concentration-dependent response suggests a dose-response relationship, where higher concentrations of the extracts led to greater inhibition of fungal growth. This aligns with the principle that higher concentrations of bioactive compounds present in the extracts can exert stronger inhibitory effects on the pathogen. So, among the four botanicals tested, *Eupatorium adenophorum* emerged as the most effective, demonstrating the highest mycelial inhibition of 77.4% at the 25% concentration. This suggests

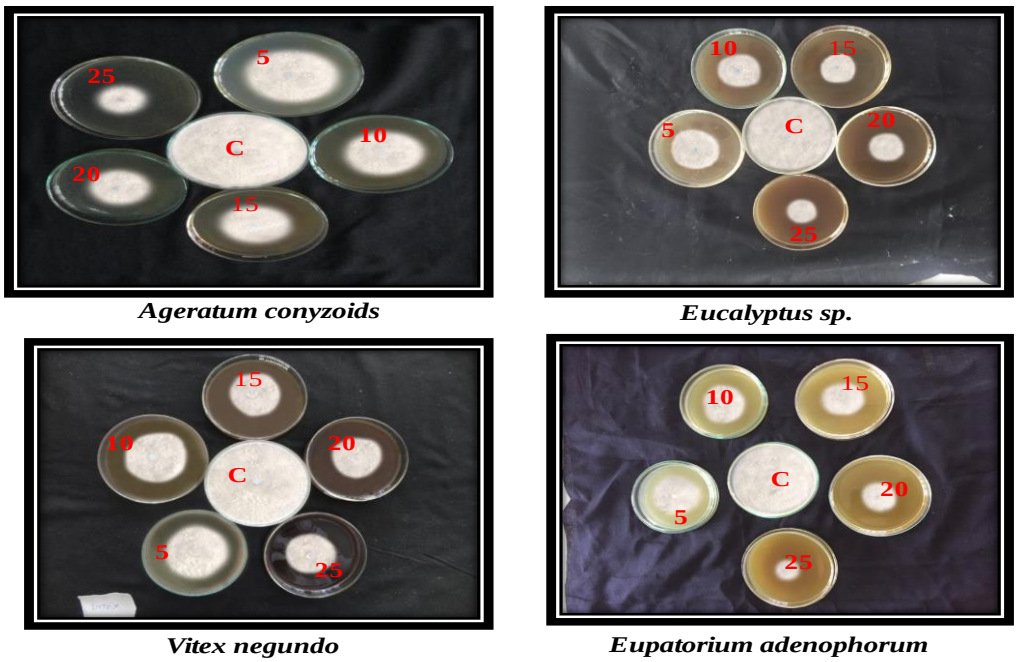

Fig. 2. Mycelial inhibition of *Fusarium solani* f. sp. *pisi* with alcoholic extracts of botanicals

that *E. adenophorum* possesses potent antifungal compounds that are particularly effective against *Fusarium solani* f. sp. *pisi.* 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 [9,10]. Hence, the chopped raw material of *E. adenophorum* can be used as soil amendment against the disease. Bhattarai and Shrestha [11] also revealed that the extracts of *E. adenophorum* (50 and 10% concentration) were found highly effective against *F. oxysporum, F. moniliforme* and *Aspergillus niger*. These results highlight the potential of these botanical extracts as natural antifungal agents for managing *Fusarium solani* f. sp. *pisi*. However, further studies are required to elucidate the specific bioactive compounds responsible for the observed antifungal activity and to assess their efficacy under field conditions.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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