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Antifungal activity of some botanicals extracts against *Fusarium oxysporum f. sp. pisi* a causal agent of wilt of pea (*Pisum sativum* L.)

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Abstract
Pea (*Pisum sativum* L.) is an important cash as well as pulse crop in India. Wilt diseases caused by *Fusarium oxysporum f. sp. pisi* has reported as one of serious threat to the crop production affects plant growth and yield. In the present study antifungal activity of four botanical extracts i.e., Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus globulus*), Lemongrass (*Cymbopogon citratus*) and Ginger (*Zingiber officinale*) were tested in *in-vitro* condition against wilt *F. oxysporum* isolated from diseased plants of pea. Three replicates of different concentrations of the botanical extract *viz* 5, 15 and 25 per cent were tested against the pathogenic fungus, respectively, using Poison food technique. Result showed that growth of fungal mycelia was inhibited with increase in concentration of botanical extract. Lemongrass extract (25%) inhibited the maximum (35.93%) growth of fungal mycelia followed by Neem extract (32.59) and Ginger extract (27.78%) with Eucalyptus extract showed minimum inhibition (25.19%).

Keywords: botanical extracts, *fusarium oxysporum f. sp. pisi*, poison food technique, growth inhibition

Introduction
Pea (*Pisum sativum* L.) is a legume crop and is native to South Europe and grown a garden or field crop throughout the temperate regions of the world and was originally cultivated in the Mediterranean countries. In India, pea is one of the most important *Rabi* pulse crop, in which the crop is grown on a field scale for its dry seeds and smaller scale for green peas. It is being highly rich in protein contents and is consumed as both i.e., pulse as well as vegetable food. It is also a good source of carbohydrates, vitamins A and C, calcium, phosphorus and has a small quantity of iron (Kripalini *et al.* 2018) [5].

India is the second largest producer of pea in the world. The crop occupies 459 thousand hectares in India and shares 21 per cent production of the world. The production of pea in Uttarakhand is 93.40 tonnes with 1.72 per share (Anon 2018) [1]. In this state people prefer pea in fresh state as well as in processed form like frozen, canned or dehydrated.

The climate of Uttarakhand is known for high humidity which favours several diseases of pea caused by fungi, bacteria and viruses. Among these diseases, wilt disease caused by *Fusarium oxysporum* f. sp. *pisi* is the most destructive disease of pea and occurs almost every year. Wilt is a soil-inhabiting fungus surviving from year to year in the soil as thick-walled, very hardy spores that can sit in the soil surviving all kinds of conditions for more than 10 years. When the plant is in its early growth stage, the fungus kills it outright but the attack at the later stage results in shrivelled grains and heavy yield losses. A lot of work has been done on the management of fusarium disease through plant extracts.

Verma and Dohroo (2003) [11] evaluated twelve different botanicals against *F. oxysporum* f. sp. *pisi* causing wilt of pea in Himachal Pradesh. Garlic clove extract was completely inhibitory to the wilt pathogen and the growth rate of the fungus was recorded to be zero at 24 h of incubation. The antifungal activity of ethanol extracts of medicinal plants was evaluated against *F. oxysporum* f. sp. *pisi* by modified disc technique by Sahani and Saxena (2009)[9] various plant extracts resulted in inhibition on the growth of mycelium however bark of *Euphorbia nerifolia* exhibited absolute toxicity against the test fungus (98.0%).

Minz *et al.*, (2012) [6] tested antifungal potential of aqueous extracts of forty plants of different families against *F. oxysporum* f. sp. *ciceri*, causal agent of wilting of chick pea. Among forty plant species tested aqueous extract of *Chenopodium ambrosioides* recorded significant antifungal activity against the test fungi.

Gupta *et al.*, (2015) [3] evaluated the antifungal activity of crude extracts of some plants against *F. oxysporum* f. sp. udum, a causal agent for wilt disease of pigeon pea and found that the crude extract of leaf of *Phyllanthus nurai* Linn and *Vitex negundo* Linn exhibited maximum toxicity against the test fungus.

The present study was undertaken with the objective to study "Antifungal activity of four botanical extracts in *in-vitro* condition against *F. oxysporum f. sp. pisi a causal agent of wilt disease in Pea*" at the School of Agriculture, Uttaranchal University, Dehradun, Uttarakhand from (September 2020 to April 2021).

Materials and Methods

Isolation and purification of pathogen

The plant showing typical characteristic symptoms of wilt disease of pea were collected from the research farm and brought to the laboratory. The isolation of the fungus was made by tissue isolation technique on potato dextrose agar (PDA) and incubated at 25±2 ºC. The resulting fungal culture was purified in aseptic condition by hypha tip method.

Preparation of Plant extracts

Fresh leaves of Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus globulus*), Lemongrass (*Cymbopogon citratus*) and Rhizome of Ginger (*Zingiber officinale*) were collected, washed with distilled water and kept for shade dry under room temperature for 15-20 days. After drying grind as fine powder by electric grinder pestle & mortar. Soak the powders in distilled water at 1:10 ratio for 24 hrs. Filter the soaked materials through double layered muslin cloth/ bacterial filter, followed by Whatman No.1 filter paper. Store the extracts in 4 °C in pre-sterilized flasks and tested against the pathogen at 5, 15, and 25 per cent concentrations.

Table1: List of the plants and part used for extract preparation

Treatment	Plants	Scientific name	Plant parts
T_1	Neem	*Azadirecta indica*	Leaves
T_2	Eucalyptus	*Eucalyptus spp.*	Leaves
T_3	Ginger	*Zingiber officinale*	Rhizome
T_4	Lemongrass	*Cymbopogon citratus*	Leaves

In-vitro Evaluation of Treatments

Poisoned food technique was used to check the efficacy of treatments. Purified plate of 7-10 days old culture taken and 8mm pieces of culture was cut out with the help of Cork borer. Media was poisoned with treatments at different concentrations of plant extracts (5%, 15%, and 25%) respectively and placed inside incubator at 25±2 ºC. Completely Randomized Design was followed with three replications of each treatment and plate having no treatments served as control. Growth was checked after 3, 6 and 9 days intervals.

Zone of Inhibition calculated by using formula (Vincent, 1947):

I=[C-T)/C] X 100

Where, I = Per cent growth inhibition,

C = Growth in control plates,

T = Treated plates growth

Experimental Results

Efficacy of different botanical extract on radial mycelia growth and per cent inhibition of *Fusarium oxysporum* f. sp. *pisi*

Radial mycelia growth

Data given in Table 2 show that the effectiveness of the extracts increased with the increase in time of DAI and concentration of the extracts. After 3 DAI, at 5 per cent concentration eucalyptus showed minimum radical growth diameter (23.00 mm) as compared to 30.00 mm in control. At 15 and 25 per cent concentration, lemon grass proved better as compared to other treatments with 20.00 and 15.67 mm diameter respectively which was significantly lower as compared to other treatments. The trend remained almost same after 6 and 9 DAI with lemon grass extract proving better treatment as compared to other treatments. After 6 DAI, the radical diameter was 52.00 mm in case of lemon extract which decreased to 45.00 and 41.67 mm at 15 and 25 per cent concentration respectively. After 9 DAI also lemon grass proved best with 67.33, 60.00 and 57.67 mm radical diameter as compared 90.00 mm in control at 5, 15 and 25 per cent concentration respectively. Lemon grass was followed by neem in effectiveness at 3, 6 and 9 DAI at all the concentrations. Among all the treatments ginger proved least effective throughout the experimentation.

Table 2: Effect of Botanical extract against *Fusarium oxysporum* f. spp. *Pisi* (Mycelia Growth Colony Diameter in mm)*

Treatment	3 DAI			6 DAI			9DAI		
	Radical growth (mm)			Radical growth (mm)			Radical growth (mm)		
	5%	15%	25%	5%	15%	25%	5%	15%	25%
T1	24.00	22.33	20.00	53.33	49.00	45.67	72.67	65.00	60.67
T2	23.00	22.33	21.33	55.67	52.67	46.00	76.00	73.33	67.33
T3	24.00	23.33	23.00	55.67	53.33	49.00	79.33	70.00	65.00
T4	23.33	20.00	15.67	52.00	45.00	41.67	67.33	60.00	57.67
T5(control)	30.00	30.00	30.00	60.00	60.00	60.00	90.00	90.00	90.00
C.D. at 5%	0.02	0.13	0.07	0.14	0.17	0.28	0.32	0.17	0.26
SE(m)	0.15	0.82	0.42	0.89	1.09	1.73	2.00	1.08	1.61

*= Mean Of Three Replications.

C.D = Colony Diameter Mean

S.E (m) = Standard Error Mean

Mycelia inhibition

The data given in (Table 3 and Plate 1) show that maximum mycelia inhibition of fungus was recorded in case of lemon grass extract almost throughout the experimentation. The inhibition increased with the increase in period of DAI and concentration of the extracts. At 3 DAI though at 5 per cent concentration eucalyptus gave better results as compared to other treatments, lemon grass was significantly superior then other treatments till the termination of the experiment. The inhibition was 33.33 and 47.78 per cent at 15 and 25 per cent concentration at 3 DAI, 13.34, 25.00 and 30.55 per cent at 5, 15 and 25 per cent concentration at 6 DAI and 25.18, 33.33 and 35.93 per cent at 5, 15 and 25 per cent concentration at 9 DAI respectively in case of lemon grass. In this case Lemon grass followed Neem in effectiveness and minimum inhibition was recorded in case of ginger.

Table 3: Mycelia per cent inhibition of *Fusarium oxysporum* f. spp. *pisi*.

Table 3. Mycelia per cent inhibition of *Fusarium oxysporum* f. spp. *pisi*.									
Treatment	**3 DAI**			**6 DAI**			**9DAI**		
	inhibition%			**inhibition%**			**inhibition%**		
	5%	15%	25%	5%	15%	25%	5%	15%	25%
T1	20.00	25.56	33.33	11.11	18.34	23.89	19.26	27.78	32.59
T2	23.33	25.56	28.89	7.22	12.22	23.33	15.56	18.52	25.19
T3	20.00	22.22	23.33	7.22	11.11	18.33	11.85	22.22	27.78
T4	22.22	33.33	47.78	13.34	25.00	30.55	25.18	33.33	35.93
T5(control)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

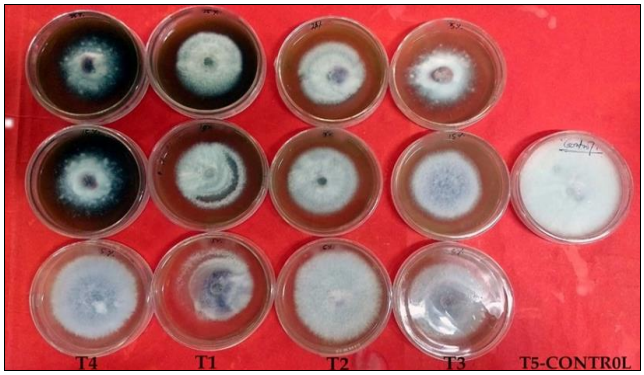

Plate 1: Inhibitory effect on radial growth of *Fusarium oxysporum f. spp. Pisi*

Discussion

The effects of neem (*A. indica*) and willow (*Salix babylonica*) aqueous extracts on fusarium wilt disease in tomato seedlings were investigated by Hanaa *et al*., (2011) [4]. Treatment of tomato plants with neem aqueous extract reduced the percentage of disease incidence to the level of 25.5 per cent after 6 weeks of infection.

Singh *et al.,* (2013) [10] evaluated the antifungal activity of aqueous extract of *Cymbopogon citrates* for controlling *Saprolegnia parasitica* (*in vitro*) isolated from endangered fish Tor putitora. The aqueous extract of *C. citrates* possessed high degree of antifungal activities against the test pathogen.

Ramaiah *et al.,* (2015) [7] conducted *in vitro* antifungal assay against *Fusarium oxysporum* f. sp. *lycopersici* using plant extracts of fifteen plants. Out of fifteen plants, *Azadirachta indica* inhibited 75 per cent growth of the *Fusarium oxysporum* f. sp. *lycopersici*.

Dwivedi and Sangeeta (2015) [2] analyzed the antifungal potentiality of aqueous extract of five medicinal plants against *F. oxysporum* f. sp. *ciceri* by using poisoned food technique. Among all medicinal plant extracts, *Tinospora cordifolia*, *C citratus* and *Moringa oleifera* showed the promising antifungal potentiality against *F. oxysporum* f. sp. *ciceri* with maximum inhibition of 100 per cent at 75 per cent concentration followed by *Z. officinale* and *Trachyspermum ammi* respectively.

Rawal and Adhikari (2016) [8] conducted study to determine the antimicrobial activity of dried ginger powder using paper disc diffusion assay against *F. oxysporum* f. sp. l*ycopersici* and found the potent antimicrobial activity of the ginger extract against the pathogen.

Zafar *et al.,* (2020) [12] four Plant Extracts applied and overall, all the Plant Extracts performed well while comparing to control. Different concentrations of plant extracts used with different days interval. *In-vitro* neem found to be most effective against mycelial growth followed by ginger and eucalyptus. In-vivo conditions four plant extracts used at different concentration of neem found to be most effective followed by garlic, ginger and Eucalyptus at all concentrations against Fusarium wilt disease.

In present study, it was shows that the all botanical extract against *Fusarium oxysporum* f. sp. *pisi* and inhibited its mycelia growth over untreated control. Botanical extract found most effective in order to T4-Lemongrass (*Cymbopogon citratus*), T1-Neem (*Azadirachta indica*), T4-Rhizome of Ginger (*Zingiber officinale*) and T2-Eucalyptus (*Eucalyptus globulus*).

Conclusion

In our present investigation, the results are quite encouraging because almost of the plant extracts showed antifungal efficacy against test fungus. However, the extracts of Lemongrass (*Cymbopogon citratus*), Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus globulus*), and Ginger (*Zingiber officinale*) can be used as an alternative drug for controlling diseases and Disease Management in pea crop.

References

1. Anonymous. Horticultural statistics at a glance. Horticulture Statistics Division, Department of Agriculture, Cooperation and Farmers' Welfare, Ministry of Agriculture and Farmers' Welfare, Government of India 2018.
2. Dwivedi SK, Sangeeta. Efficacy of some medicinal plant extract against *Fusarium oxysporum* f. sp. ciceri causing chickpea wilt, Asian Journal of Crop Science 2015;7(2):138.
3. Gupta AK, Chaudhary S, Samuel CO, Upadhyaya PP, Antifungal activity of some locally available plants against *Fusarium oxysporum* f. sp. udum, Int. J Curr. Microbiol. App. Sci 2015;4(11):695-699.
4. Hanaa RMF, Abdou ZA, Salama DA, Ibrahim MAR, Sror HAM. Effect of neem and willow aqueous extracts on fusarium wilt disease in tomato seedlings: induction of antioxidant defensive enzymes. Annals of Agricultural Sciences 2011;56(1):1-7.
5. Kripalini N, Biswas MK, Devi PS. Studies on Morphological, Cultural and Pathogenic Variability in Isolates of *Fusarium oxysporum* f. sp. pisi causing Wilt of Pea from Different Districts of Manipur, India, Int. J Curr. Microbiol. App. Sci 2018;7(11):2500-2506.
6. Minz Shweta, Samuel CO, Tripathi SC. The effect of plant extracts on the growth of wilt causing fungi *Fusarium oxysporum*, J Pharm. Biol. Sci 2012;4(1):13-16.
7. Ramaiah AK, Garampalli RKH. *In vitro* antifungal activity of some plant extracts against *Fusarium oxysporum* f. sp. lycopersici, Asian Journal of Plant Science and Research 2015;5(1):22-27.
8. Rawal P, Adhikari RS. Evaluation of antifungal activity of Zingiber officinale against Fusarium oxysporum f. sp. lycopersici, Advances in Applied Science Research 2016;7(2):5-9.

9. Sahani RK, Saxena AR. Fungitoxic properties of medicinal and aromatic plants against Fusarium oxysporum f. sp. pisi, Annals of Plant Protection Sciences 2009;17(1):146-148.
10. Singh R, Kumar A, Pandey NN, Gupta M, Kumar P, Haldar RS *et al. In vitro* evaluation of antifungal activity of aqueous extract of lemon grass (*Cymbopogon citrates*) against pathogenic fungi Saprolegnia parasitica isolated from endangered cold water fish Tor putitora, Journal of Ecophysiology and Occupational Health 2013;13(1/2):63.
11. Verma, S. and Dohroo, N.P., Evaluation of botanicals *in vitro* against Fusarium oxysporum f. sp. pisi causing wilt of pea, Plant Disease Research 2003;18(2):131-134.
12. Zafar W, Aslam MK, Atiq K, Arshad M. Management of fusarium wilt of pea through plant extracts and fungicides in relation to factors available on https://www.researchgate.net/publication/344804201,(2020).