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Antifungal Activities of Essential Oils and Crude Extracts of Some Aromatic Plants against *Fusarium* Rot of *Trichosanthes Dioica*

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Abstract - This experiment was conducted for assessing the antifungal activities of essential oils and crude extracts of some aromatic plants against *Fusarium* rot of *Trichosanthes dioica* was at the Central Department of Botany. Pathogenicity test was taken for the confirmation of disease by transferring the inoculum from pure culture. For the control of the above fungus essential oils and extracts of five aromatic plants *Zanthoxylum armatum, Mentha arvensis, Amomum subulatum, Valeriana jatamansi* and *Cymbopogon flexuosus* were used. Each oil and extracts were diluted in different concentration in 80% acetone and in distilled water respectively .The value of minimum inhibitory concentration and percentage of mycelia growth inhibition of oil and extracts were obtained as, oil of *C. flexuosus* showed the higest fungitoxicity (100%) at the 5.0 and 50 µlml^{-1} concentration followed by Z.*armatum, M. arvensis, A. subulatum* and *V. jatamansi* were found to be 10 and 100 µlml^{-1} respectively also the higest percentage of mycelial growth inhibitors were found to be *C. flexuosus* followed by *A. subulatum, Z. armatum, M. arvensis* and *V. jatamansi*.

Keywords : *inoculum, minimum inhibitory concentra-tion, mycelial growth, pathogenicity.*

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Keywords : inoculum, minimum inhibitory concentration, mycelial growth, pathogenicity.

I. Introduction

Pointed gourd (***Trichosanthes dioica*** Roxb) is a tropical vegetable crop, commonly found in Terai region of Nepal.

a) Post Harvest Damage

The fruits of this plant suffer a lot of damage from fungal disease during summer season. The disease forms a luxuriant wooly mycelium on the affected fruit which appears as if wrapped in absorbent cotton. The tissue in the interior of the fruit becomes watery, soft and the decaying matter emits a bad odour. The species of *Fusarium* are the commonest and most widely distributed in soil and on organic substances, which cause fruit rot on different plants. The rot of pointed gourd is carried out by *Fusarium solani* (Mart.) Sacc, Synder & Hansen.

The aim of the present study was to isolate the pathogen from infected pointed gourd and test its pathogenicity for studing the antifungal activities of oils and extracts of different aromatic plants against the *F. solani*.

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II. Methodology

The research was carried out with the following steps:

a) Collection of Host and Isolation of Pathogen

The infected pointed gourd was collected from local market of Kathmandu valley. A piece of fungal colony was transferred aseptically on a petri plate containing PDA media and was incubated in inverted position in an incubator at 25±2°C for one week. After one week, the mycelial growth was observed under a compound microscope.

b) Identification and Pathogenicity Test

After studying and observing the characteristics features of the pathogen, it was identified as *F. solani*. The characteristics features of the fungus were identified with the help of standard literature (Booth, 1977). For pathogenicity test, the inoculum from the pure culture of *F. solani* was transferred to the healthy fruit. When incubated at 25±2°C for 7 days, the characteristics symptoms were produced, which were found to be similar with the symptoms on fruits previously collected.

c) Maintenance of the Pure Culture

The pure culture of *F. solani* was preserved by sub-culturing in several slants and plates containing PDA media.

d) Preparation of one week old culture

The fungus from pure culture was inoculated into PDA media containing petriplates, and incubated. After seven days the inoculum disc was taken from the culture for further experiment.

e) Measurement of Spore

Measurement of spore was done by the help of ocular micrometer and stage micrometer. Ocular micrometer was calibrated by the help of stage micrometer.

Calculation of calibration factor applying the formula by:

$$\text{One ocular division} = \frac{\text{No. of division on stage micrometer}}{\text{No. of division of ocular micrometer}} \times 10\mu m$$

Collection and extraction of essential oils and extracts of aromatic plants

Plant samples of *V. jatamansi* , *A. subulatum, Z. armatum, M. arvensis* and *C. flexuosus* each 50 gm.were collected out of these *V. jatamansi* and *A. subulatum* were hydro- distilled for 6-8 hours in Clevenger's apparatus in 500 ml water for extraction of oiland rest were taken from HPPCL (Herb Production and Processing Company Limited). Similarly the crude extract of aromatic plants were made by the help of mortar with pistal.

g) Determination of yield of essential oils

The volume of oil extracted was noted down. The yield of the essential oil was calculated in terms of % using the following formula.

$$\% \text{ of essential oil} = \frac{\text{Volume of essential oil}}{\text{Weight of sample}} \text{ X100}$$

h) Dilution of essential oil and crude extract

Essential oils were diluted into different concentration of 0.625, 1.25, 2.5, 5.0, 10.0, $\mu l ml^{-1}$ with 80 % acetone and each crude extracts was diluted to different concentrations, 0.625, 1.25, 2.5, 5.0, 10.0, 20, 30, 40, 50 & 100 $\mu l ml^{-1}$ with distilled water.

i) Assessment of toxicity of oil and extract against fungal pathogen

The toxicity of the oils was assessed by using the poisoned food technique 0.5ml of each concentration of each oil and extract was taken in presterilized cooled petriplate and 9.5 ml of PDA media was poured on that with gently swirling to mix the contents thoroughly. In control set the essential oil was replaced by equal volume of acetone (80%) and the control set of crude extract was replaced by equal volume of distilled water. The inoculum disc (4mm diameter) taken from the 7 days old culture of the pathogen, was placed aseptically at the centre of each plate and turned upside down in its position. The plates were then incubated at 25±2°C for 7 days. All the experiments were revised thrice. The percentage inhibition of mycelial growth of test fungus was calculated separately.

j) Calculation of percentage of mycelial growth inhibition

Fungitoxicity was assessed in terms of percentage inhibition of mycelial growth of test fungus.

$$\% \text{ inhibition of mycelial growth} = \frac{g_c - g_t}{g_c} \text{x100}$$

Where,

g_c = growth of mycelial colony after incubation in control set i.e. diameter of colony in control set - diameter of inoculum disc

g_t = growth of mycelial colony after incubation period in treatment set i.e. diameter of the colony in treatment set - diameter of inoculum disc.

k) Determination of minimum inhibitory concentration of essential oil and extract

The minimum inhibitory concentration (MIC) is the concentration of any substance in the external medium which just inhibits cell division of a normal cell.

MIC has been expressed as the minimum dose of the essential oil and extract required for complete (100%) inhibition of mycelial growth of the test fungus.

III. Result and Disscussion

a) Assessment of fungitoxicity of essential oils and extracts

Essential oils and extracts of different plant species showed different efficacies in the inhibition of the mycelial growth by measuring the colony size of culture plates.

Table 1 : Percentage of inhibition of *F.solani* mycelial growth using essential oils of *C. flexuosus*

S.N	Concent-ration of oil $\mu l ml^{-1}$	Inoculum size (mm)	Colony size (mm)			Mean colony size (mm)	Mycelial growth (mm)	% of inhibition of mycelial growth
			I	II	III			
1	0	4	42	42	42	42.00	38.00	0.00
2	0.62	4	19	18	19	18.67	14.67	61.40
3	1.25	4	12	11	12	11.67	7.67	79.82
4	2.50	4	8	7	8	7.67	3.67	90.35
5	5	4	4	4	4	4.00	0.00	100.00
6	10	4	4	4	4	4.00	0.00	100.00

Minimum inhibitory concentration (MIC) =5 $\mu l ml^{-1}$

Essential oil of *C. flexuosus* showed mycelial inhibition as 0%, 61.40%, 79.82%, 90.35%, 100% & 100% at 0, 0.625, 1.25, 2.5, 5.0, 10.0 $\mu l ml^{-1}$ oil concentration against *F.solani* respectively.

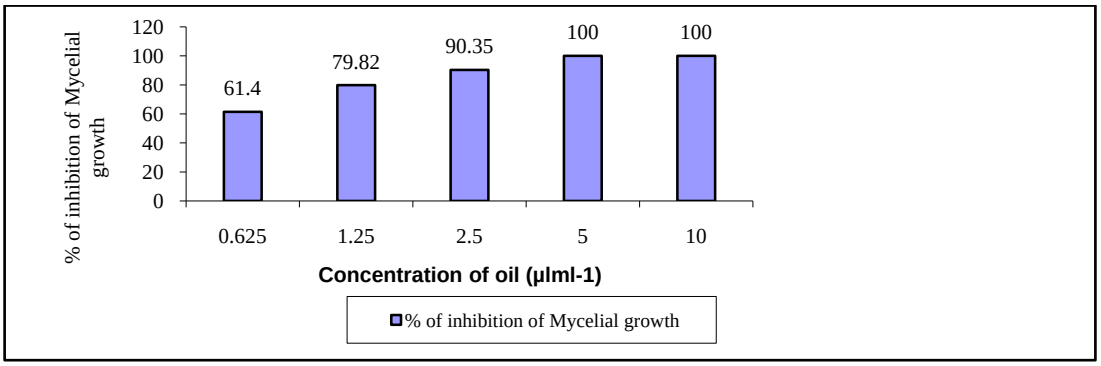

Figure 1 : Percentage of antifungal activity of *C. flexuosus* oil against *F. solani*

Table 2 : Percentage of inhibition of *F. solani* mycelial growth using extract of *C.flexuosus*

S.N	Concent-ration of extract µlml^{-1}	Inoculum size (mm)	Colony size (mm)			Mean colony size (mm)	Mycelial growth (mm)	% of inhibition of mycelial growth
			I	II	III			
1	0	4	55	55	55	55.00	51.00	0.00
2	0.62	4	50	50	51	50.33	46.33	9.15
3	1.25	4	23	23	22	22.67	18.67	63.40
4	2.50	4	18	18	18.5	18.17	14.17	72.22
5	5	4	10	10	9.5	9.83	5.83	88.56
6	10	4	9	9	9	9.00	5.00	90.20
7	20	4	7	7	6.5	6.83	2.83	94.44
8	30	4	6	5	5.5	5.50	1.50	97.06
9	40	4	5	5.5	5	5.17	1.17	97.71
10	50	4	4	4	4	4.00	0.00	100.00
11	100	4	4	4	4	4.00	0.00	100.00

Minimum inhibitory concentration (MIC) =50 µlml^{-1}

Extract of *C. flexuosus* showed mycelial inhibition as 0%, 9.15%, 63.40%, 72.22%, 88.56%, 90.20%, 94.44%, 97.06%, 97.71%, 100% & 100% at 0, 0.625, 1.25, 2.5, 5.0, 10.0, 20, 30, 40, 50, 100 µlml^{-1} oil concentration against *F. solani* respectively.

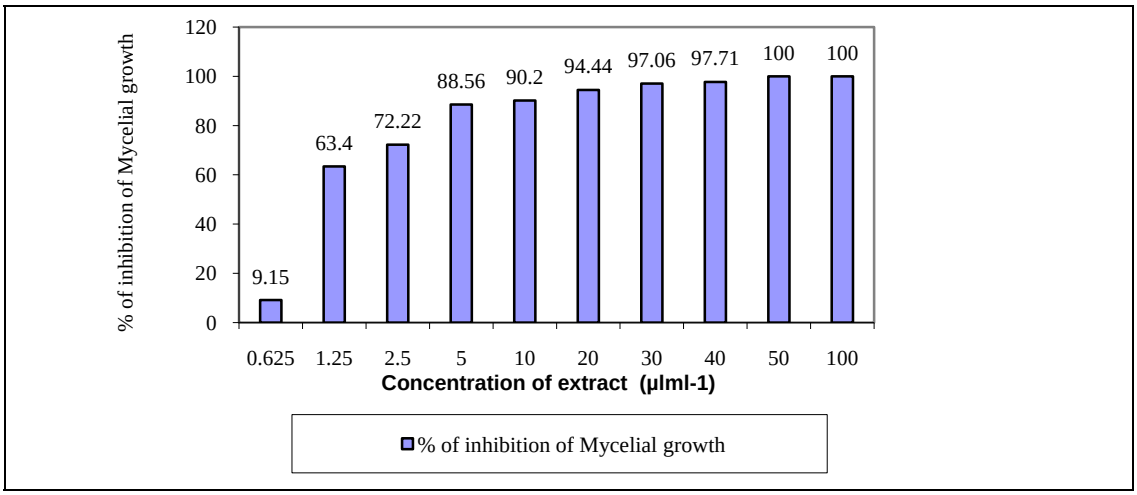

Figure 2 : Percentage of antifungal activity of *C. flexuosus* extract against *F. solani*

Minimum Inhibitory Concentration (MIC) value of Aromatic plants oils and extracts of *C. flexuosus* was 5.0 & 50.0 $\mu l ml^{-1}$ similarly 10.0 & 100.0 $\mu l ml^{-1}$ for *M. arvensis, V. jatamansi, A. subulatum* and *Z. armatum* respectively.

b) Comparative fungi toxicities of different essential oils

Table 3 : Comparative fungitoxicities of different essential oils in different concentrations

S.N	Concent-ration of oil $\mu l ml^{-1}$	% of Mycelial growth inhibition				
		Cymbopogon flexuosus	*Zanthoxylum armatum*	*Valeriana jatamansi*	*Mentha arvensis*	*Amomum subulatum*
1	0	0	0	0	0	0
2	0.625	61.4	58.77	48.61	40.35	22.76
3	1.25	79.82	71.93	70.37	53.51	35.77
4	2.5	90.35	87.77	78.7	80.26	45.53
5	5	100	97.37	85.9	92.98	97.56
6	10	100	100.00	100	100	100

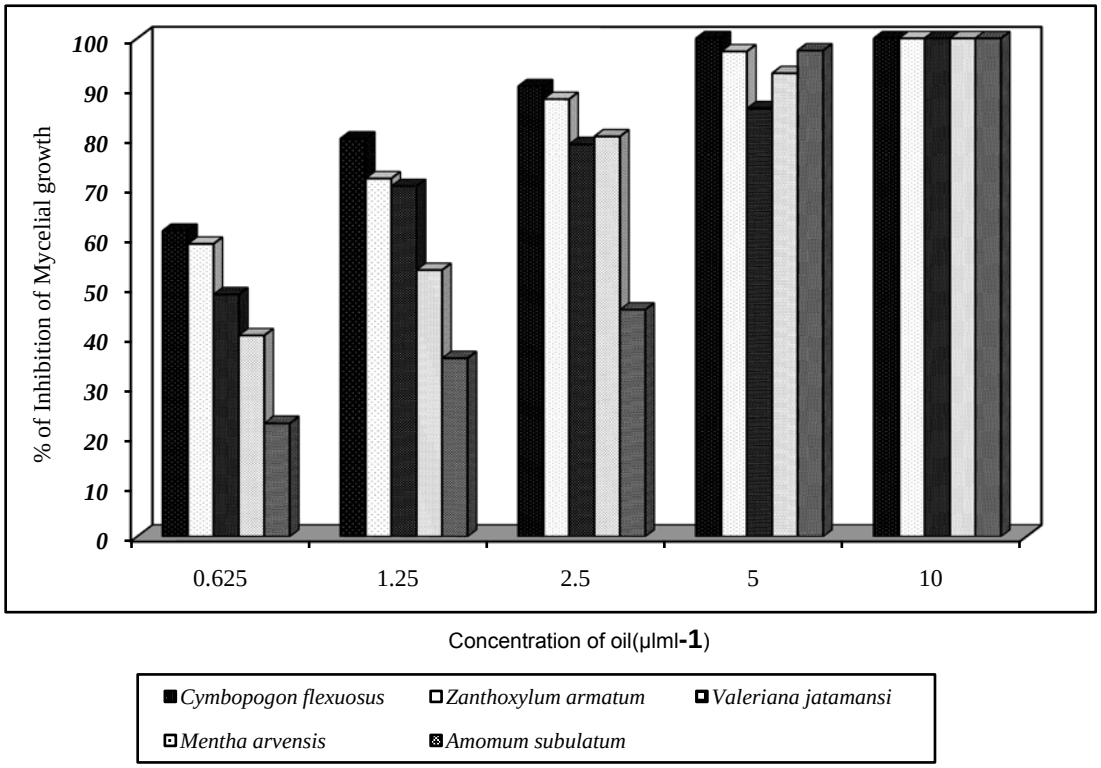

Figure 3 : Fungitoxicities of different essential oils in different concentrations

Table 4 : Comparative fungitoxicities of different extracts in different concentrations

S.N	Concent-ration of extract $\mu l ml^{-1}$	% of Mycelial growth inhibition				
		Amomum subulatum	*Zanthoxylu-m armatum*	*Valeriana jatamansi*	*Mentha arvensis*	*Cymbopogon flexuosus*
1	0	0	0	0	0	0
2	0.625	30.72	26.47	18.95	14.38	9.15
3	1.25	40.22	44.44	48.69	20.92	63.4

4	2.5	84.97	77.45	53.59	26.80.	72.22
5	5	87.91	80.64	58.17	59.48	88.56
6	10	89.87	86.27	62.75	68.28	90.2
7	20	91.83	90.20	77.78	73.53	94.44
8	30	94.77	90.52	84.97	77.78	97.06
9	40	96.73	96.08	87.58	83.33	97.71
10	50	98.37	99.02	98.37	90.52	100
11	100	100	100.00	100	100	100

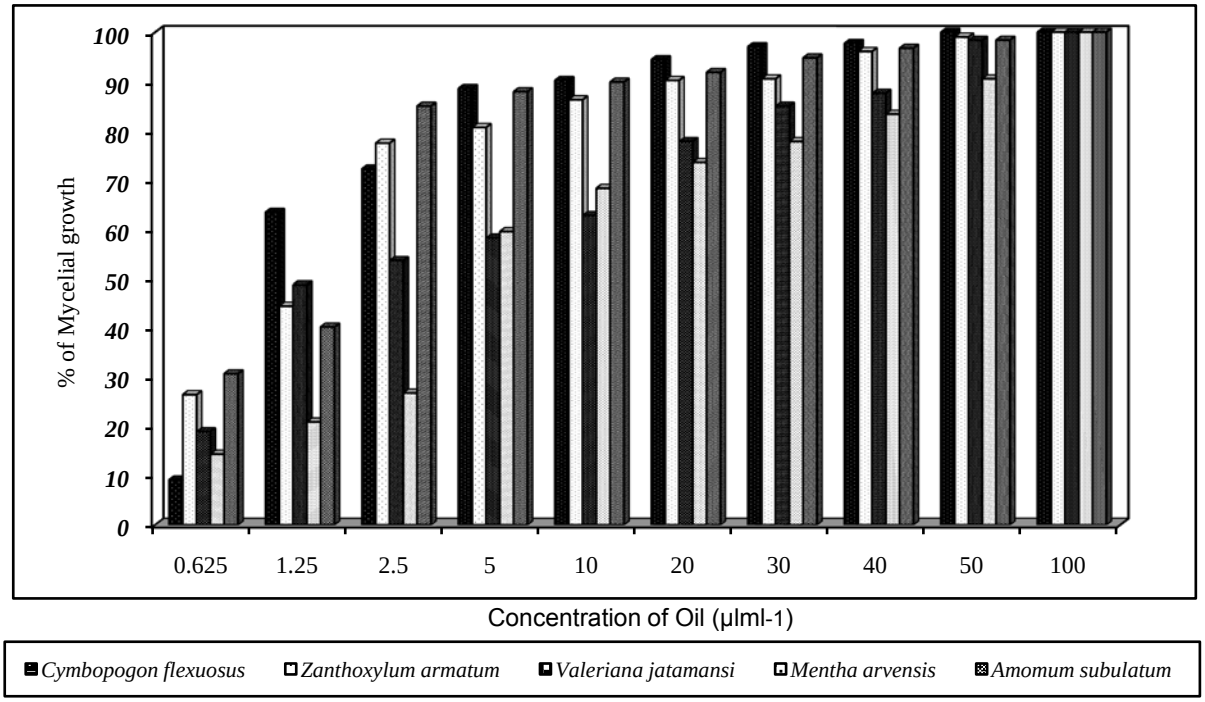

Figure 4 : Fungitoxicities of different extracts in different concentrations

Out of five test plants, essential oil and extract of *C. flexuosus* was found more effective in its fungitoxic properties and showed 100% inhibition of mycelial growth of the test fungus at MIC of 5.0μlml^{-1} and 50μlml^{-1} respectively. Similarly *Z. armatum, V. jatamansi, M. arvensis, A.subulatum* were found effective and showed 100% mycelial growth inhibition at 10μlml^{-1} and 100μlml^{-1} respectively. Also the percentage of mycelial growth inhibition were found to be different, according to their different concentration of each essential oil and extracts, the highest percentage of mycelial growth inhibition were found to be oil of *C. flexuosus, Z. armatum, M. arvensis, A. subulatum* and *V. jatamansi* respectively and similarly extracts of *C. flexuosus, Z. armatum, A. subulatum, V. jatamansi* and *M.* arvensis respectively. Thus comparative fungitoxicities of five different essential oils and extracts were observed against *F. solani*.

A perusal of literature showed that, similar experiments for the control of *F. solani* using essential oils and extracts has not yet been done in Nepal. So this is the first study to assess fungitoxicities of essential oils and extract against *F. solani*. This research work can be applicable for commercial scale of inhibitory activities.

IV. Acknowledgements

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