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ANTIFUNGAL ACTIVITY OF PLANT EXTRACT OF *TRACHYSPERMUM AMMI*, ON ENDOPHYTIC FUNGI ISOLATED FROM *SCHLEICHERA OLEOSA* (KUSUM)

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ABSTRACT

Endophytic fungi colonize living plant tissues without producing any apparent disease symptoms or obvious negative effects infect they are responsible for producing many secondary metabolites. *Schleichera oleosa* (Kusum) is well known tree of medicinal importance in India. All parts of *Kusum* are used in Indian Traditional Healing. Various part of kusum tree are either favorite pick of traditional vaids for indigenous therapies and medicine or timber, food, animal feed, and oil. In spite of these properties, kusum is not free from microbial diseases. Many fungal and bacterial pathogens were reported to infect it. In the present study, endophytic fungi isolated from the leaves of *Schleichera oleosa* (Kusum). In all total eight fungi were isolated out of which antifungal activity of the organic solvent extracts(ethanol/ methanol) and hot water extract of *Trachyspermum ammi* at different concentrations i.e. 25%,50%,75%,100% was tested against; test fungi *Fusarium sp.*. The growth of the test fungi was inhibited by all the concentrations of extract prepared in hot water and methanol with maximum inhibition by 100% concentration, whereas higher concentrations of ethanol showed the inhibitory effect.

KEYWORDS: Entophytic fungi, *Fusarium*, Kusum and Inhibitory effect.

INTRODUCTION

The term endophyte include all organism that during a variable period of their life colonies the living internal tissues of their hosts. The word endophyte consist of two words, “endo” which means within and “phyte” which means plant. Endophytic fungi colonize living plant

tissues without producing any apparent disease symptoms or obvious negative effects infect they are responsible for producing many secondary metabolites. An Endophytic Fungi is an endosymbiont, in general they do not have any pathogenic effects on its host plant. They grow within their plant hosts without causing apparent disease symptoms (Petrini, O., 1991) and growth in this habitat involves continual metabolic interaction between fungus and host. *Schleichera oleosa* (Kusum) is well known tree of medicinal importance in India. All parts of *Kusum* are used in Indian Traditional Healing (Palanuvej C, Vipunngeun N., 2008). Various part of kusum tree are either favorite pick of traditional vaids for indigenous therapies and medicine or timber, food, animal feed, and oil.

Ajwain has been commonly used in traditional medicine systems for a variety of medicinal and pharmacological aspects (Lateef M, Iqbal Z, Akhtar MS, Jabbar A, Khan MN, Gilani AH., 2006). In Traditional Persian Medicine (TPM), Ajwain was well known from thousands of years. Persian practitioners usually used seeds of Ajwain as the most useful part of the herb (Avicenna. Al Qanun Fil Tibb [Hameed HA trans]. 1st ed., 1998).

MATERIAL AND METHOD

EXTRACT PREPARATION: Take the seeds of *Trachyspermum ammi* and wash it with distill water then dry it with Wattman filter paper and rest it for air dry then dry it at 40^0C for 24hr. After drying crush it into powder form then filter and collect it into air tight bottle.

PREPARATION OF SOLUTION (ETHANOL/METHANOL/HOT WATER)

Aqueous extract

500gm seeds of *Trachyspermum Ammi* were kept for maceration with 1000 ml of distilled water for 7 days. The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath. The extract was dried and used.

Alcoholic extract

500g of dried seeds of *Trachyspermum Ammi* was extracted exhaustively for 72hours in a bottle with ethanol/methanol.

Endophytic Fungi Isolated from *Schleichera oleosa* (Kusum)

Fungal endophytes were isolated from the *Schleichera oleosa* (Kusum). The plants samples were collected from the diverse region of Korba, Bilaspur, Katghora. The samples was collected in sterile plastic bags, sealed and carefully brought to the laboratory. In the

laboratory, the leaves of *Schleichera oleosa* (Kusum) surfaces were sterilized by soaking them in 1:5 dilutions of NaOCl (Sodium Hypochlorite solution) for 15 minutes. This will do to remove all microbial epiphytes. After that rinsed it in sterile distilled water and dipped in 70% ethanol for 10 minutes.

Then from the surface of sterilized leaves, segments are cut aseptically with sterile scalpel in a laminar-air-flow which is approximately 2mm X 2mm. The outer tissues of the sample were cut so as to expose the interior surface to PDA plates and incubated at 28^0C for the appearance of the fungal growth. After 7 to 10 days of incubation, mycelial growth appeared on the plates. Then the fungal colonies were aseptically transferred onto new PDA plates for obtaining pure culture. The identification of fungi was done using the culture characteristics and microscopic characteristics of fungal culture such as shape, color, pattern and arrangement of the mycelium, conidial arrangement, types of spore etc (Chowdhary P.N., 2000).

RESULTS AND DISCUSSION

The growth of the test fungi i.e. *Fusarium sp*. was inhibited by all the concentrations of extract prepared in hot water and methanol with maximum inhibition by 100% concentration is 11 mm zone and 8 mm zone respectively whereas only higher concentrations (75%, 100%) of ethanol showed inhibitory effect. 100% concentration of ethanol extract showed a zone of 5mm while diameter. The result shows that Hot water extract of *Trachyspermum ammi* is giving better antifungal activity**.**

Microscopic Examination

Fig. 1: ***Curvularia sp.***

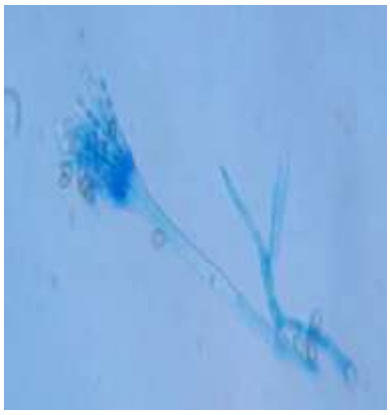


Fig. 2: ***Penicillium sp***

Fig 3: ***Mucor sp.***

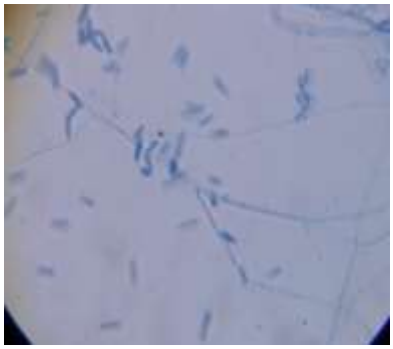


Fig 4: *Botrytis Cenerea*

Fig 5: *Fusarium*

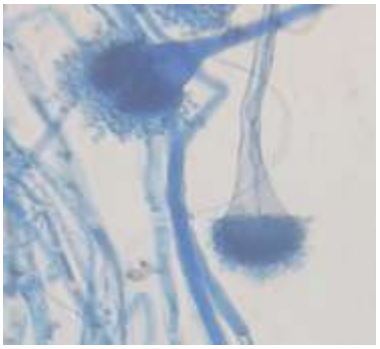


Fig 6: *Aspergillus Flavus*

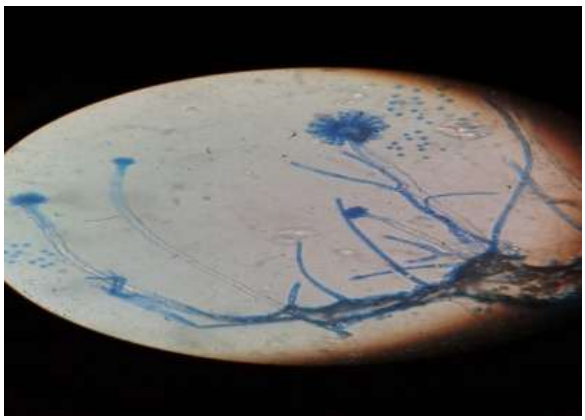


Fig 7: *Mucor sp.*

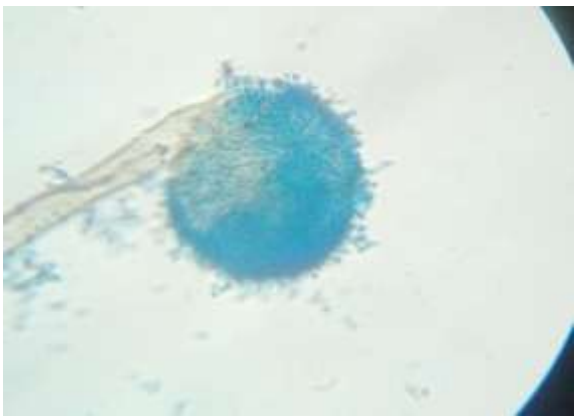


Fig 8: *Aspergillus niger*

OBSERVATIONS

Fig 9: Methanol 25%

Fig 10: Methanol 50%

Fig11: Methanol 75%

Fig12: Methanol 100%

Fig13: Ethanol 25%

Fig14: Ethanol 50%

Fig15: Ethanol 75%

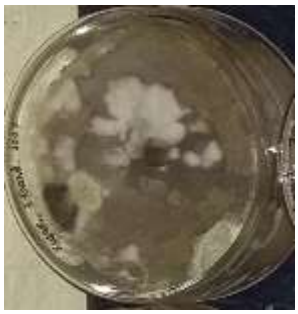


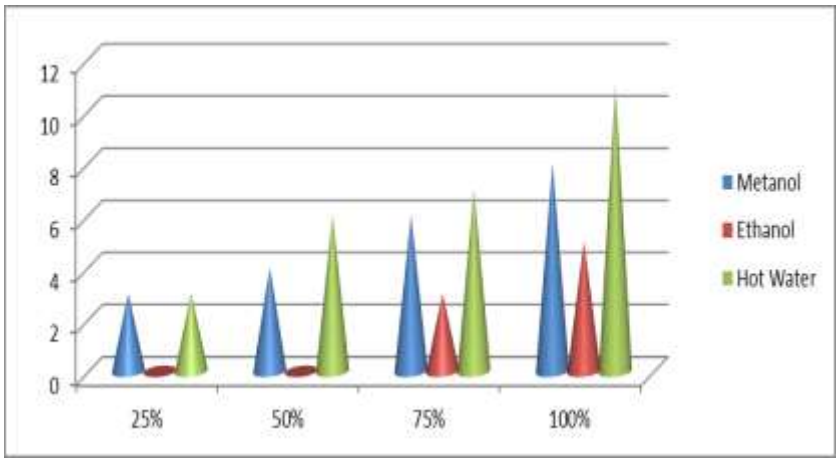
Fig16: Ethanol 100%




Fig17: Hot water 25% **Fig18: Hot water 50%** **Fig19: Hot water 75%** **Fig20: Hot water 100%**

Table 1: Anti fungal activity of Extract on *Fusarium sp*. at different concentration

ZONE OF INHIBITION				
S. NO.	**DIFFERENT CONCENTRATION**	**METHANOL**	**ETHANOL**	**HOT WATER**
1.	25 %	3mm	Nil	3mm
2.	50%	4mm	Nil	6mm
3.	75%	6mm	3mm	7mm
4.	100%	8mm	5mm	11mm



GRAPH 1: ZONE OF INHIBITION (mm)

CONCLUSION

On our study shows that *Schleichera oleosa* have good source of different kind of endophytic fungi. Our study indicates that seeds of *Trachyspermum ammi* can be used for meditational applications. Hot water, Ethanol and Methanol Extract of *Trachyspermum ammi* can be used to control the diseases in *Schleichera oleosa* which will be cost effective and eco- friendly.

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