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ANTIFUNGAL ACTIVITIES OF THE OILS OF *NIGELLA SATIVA* SEEDS

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ABSTRACT

Antifungal activities of the oils of *N. saliva* seeds were tested against twenty fungi including pathogenic and industrial strains. All the oils were found to have significant activities against the fungi, but the volatile oil showed stronger and wider range of antifungal activities. MIC values of the volatile oil was also determined against three pathogenic fungi and lowest MIC was found against *Aspergillus fumigants.*

Introduction

Nigella saliva lion (Fain: Ranunculaceac) is a pretty annual herbs, locally known as 'Kalajira'. This plant is widely distributed throughout India and Bangladesh and extensively cultivated in many parts of this sub-continent for its seeds (Kinikar and Basu 1984, Chopra et al 1982). It has been said by Prophet Mohammed (SM) that seeds of this plant can be used for the treatment of all diseases except death (Sahih-Al-Bukhari). The seeds of this plant is appetiser, stimulant, stomachie, anthelmentic, carminative, Purgative, diuretic and also used in tertian and puerperal fevers, paralysis, eczema, pityriasis, *eye*-scores, skineruption, snake-bite and scorpion sting (Kirtikar and Basu 1984, Chopra *et al* 1982). It was reported that seeds have marked emmenagogue action in dysmenorrhoea in 10-20 grain doses and abortifacients property in larger doses (Chopra *et al* 1982, Kirtikar & Basu 1984). Alcoholic extracts showed a lowering of blood pressure (Zawahry 1963). Hypotensive and antispasmodic glycosides and hypertensive alkaloid were isolated from this plant (Zawahry 1963, Zawahry and Kararra 1964). Antimicrobial properties of this species were also reported (Toppozada *et al* 1965, Rao *et al* 1978). In this paper the anti-fungal activities of *N. sativa* against wide ranges fungi were discussed.

Materials and Methods

Extraction of the oils:

The oils (NE-O), yield 25% was obtained from the seeds by cold expression in an oil mill. The oil was subjected to steam distillation and the distillate was removed by

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extraction with hexane. Evaporation of hexane gave the volatile oil (NE-S), yield 04%. The oil left after distillation was separated from water and designated as NE-R, 75% yield.

Fungi: *Aspergillus flavus, Aspergillus fumigatus, Aspergillus* fumigates (26934), *Aspergillus niger. Candida crusii, Cryptococcus neoformans, Humicola, Pichia membranaefaciens, Rhizopus arrhizus, Rhizopus oligosporous. Rhizopus* oryzae, *Saccharomyces cerevisiae, Saccharomyces cereviseae* var *ellipsoideus, Saccharomyces occidentails, Selecotium* Sp., *Trichoderma* Sp.(s), *Trichoderma* Sp.(w), *Trichophyton mentagrophytes. Wine yeast* (337) and *Unidentified* Sp. (Y-12).

Culture media:

Potato dextrose agar (DIFCO) medium was used for testing activities and to deter-mine MICs of the samples against the fungi.

Preparation of test materials discs:

10 mcl of each oils (NE-0, NE-S, NE-R) were absorbed on sterile matericel filter pa-per discs.

Antibiotics:

Standard griseofulvin 250 mcg/disc was used to compare the activities of the test materials in this experiment.

Procedure for testing antifungal activity and MIC determination:

The *in vitro* susceptibility *of* microorganisms to antimicrobial agents were measured by disc diffusion method (Bauer et al., 1966). The test was done in triplicate plates for each strain. The autoclaved agar medium cooled to 40°C was inoculated with the respective strains in an aseptic conditions, mixed thoroughly and poured into sterile petridishes. After solidification, the test materials and antibiotic discs were placed on the inoculated plates with a sterile Corcep. The plates were kept in refrigerator for few minutes to diffuse the materials in the medium and then incubated at room temperature for 48 hours. The antifungal activities was determined by measuring the diameter of zone of inhibition ex-pressed in mm. For the determination of MIC, the test material discs containing 0.25, 0.50, 0.75, 1.00, 1.50, 2.50, 5.00 mcl of the oil (NE-S) were placed on agar plates previously inoculated with the respective strains and then incubated.

Results and Discussion

The oils extracted from the seeds of *N. saliva* were screened for antifungal activities against twenty fungi of which five were pathogenic and rests were industrial strains. The activities were compared with standard Griseofulvin (250 mcg/disc) and the results are presented in the Table 1.

The volatile oil (NE-S) was found to have much higher degree and wider spectrum of activities than the other oils (i.e. NE-0 or NE-R). This oil in 10 mcl/disc showed very strong inhibition (40 mm) against five and moderate (20-39 mm) inhibition against eight fungi. It gave mild inhibition (20 mm) against six fungi and no inhibition against *Stlerotium sp.* On the other hand Griseofulvin showed activities against only two fungi (Sac*charomyces cerevisiae var ellepsoideus* and *S. occidentalis)* out of twenty fungi tested. The oil (NE-C) showed slightly wider range of activities than the fixed oils (NE-R) remained after steam distillation. This difference might be due to the presence of the volatile oil in the former.

Table 1: Antifungal activity of *Nigella sativa* seeds extracts (NE-S, NE-O, NE-R) against fungi.

Name of fungi	NE-S	NE-O	NE-R	Griseofulvin standard
	10 mcl/disc			25 mcg/disc
	Diameter of zone of inhibition in mm			
Aspergillus flavus	53.0	11.0	7.0	–
Aspergillus fumigatus	38.6	11.6	6.6	–
Aspergillus fumigatus (26934)	35.3	7.0	–	–
Aspergillus niger	16.0	6.0	–	–
Candida crusii	11.3	–	–	–
Cryptococcus neoformans	31.3	7.0	–	–
Humicola	41.0	9.3	7.0	–
Pichia membranaefaciens	25.0	–	–	–
Rhizopus arrhizus	9.3	–	–	–
Rhizopus oligosporous	8.6	–	–	–
Rhizopos oryzae	10.0	–	–	–
Saccharomyees cerevisiae	26.6	6.5	6.0	–
Saccharomyces cerevisiae var *ellepsoideus*	18.6	10.3	7.0	6.0
Saccharomyces occidentalis	59.3	14.6	10.3	7.0
Sclerotium Sp.	–	–	–	–
Trichoderma (s) Sp.	21.0	10.1	10.3	-
Trichoderma (w) Sp.	21.0	6.1	–	–
Trichophyton mentagrophytes	37.6	7.8	–	–
Wine yeast (337)	73.6	14.6	15.0	7.0
Unidentified Sp. (Y-12)	37.6	–	–	–

– = No inhibition

Table 2: MIC values of the extract NE-S against fungi.

Name of microorganisms	MIC values in mcVdisc
Aspergillus fTanis	> 0.50
Aspergillusfumigatus	< 0.50
Aspergillus niger	< 0.75

The volatile oil, the most active part of the seed was tested for MIC values against three pathogenic fungi and minimum value (< 0.50 mc/fdisc) was recorded against Aspergillus fumigatus (Table-2).

This represent the first record on the details antifungal properties of the oils of *N.* saliva. Recently activities of the alcoholic extract of the seeds of this species against only one fungus *(Candida albicans)* was published (Shah *et al* 1988). However, a good number of investigations were carried out on the antibacterial activities of the oils (Toppozada *et al,* 1965; Rao *et al* 1978).

The above results indicated the great potentiality of the volatile oils (NE-S) of *N.* sa*tiva* for using tropically against fungal diseases. However a thorough clinical trials are *re*quired before drawing any final conclusion of the effectiveness on the diseases caused by fungi.

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