Pak. j. sci. ind. res., vol. 40, nos. 1-4, January - April 1997

ANTIFUNGAL ACTIVITY OF THE PLANT *TRACHYSPERMUM AMMI*(L).

YAZDANA M. RIZKI, KANIZ FATIMA AND YASMEEN BADAR

PCSIR Laboratories Complex, Off University Road, Karachi-75280, Pakistan

(Received May 2, 1995; revised February 22, 1997)

Antifungal activity of the plant *T. ammi* (L.) Sprague, was carried out against fungi, *A. niger, A. flavus, F. solani, A. alternata* and *Helminthosporium* sp, as test organisms. Different parts of the plant were extracted in various solvents and zone of inhibition method was used to detect the fungi-toxic activity. Among all, particularly ethanol (95%) extract of the seeds was moderately effective against test fungi: benzene and pet ether extracts also showed some activity and root extracts in water, alcohol and acetone showed stimulating activity.

Key words: Fungi toxicity, *Trachyspermum ammi* (L), Antifungal activity.

Introduction

Trachyspermum ammi (Linn); Hindustani: Ajowan, sprague (Syn, *Carum copticum*; Bent & Hook F.) belongs to the family umbelliferae and grows in tropical regions. It is used as a food spice in the Indian subcontinent. It is known to possess several medicinal properties. The seeds of *T. ammi* (L.) have long been used in diarrhoea, atonic, dyspepsia, cholera, colic flatulence and indigestion. The roots possess diuretic and carminative properties [1].

The seeds of *T. ammi* (L) have aromatic smell, pungent taste and yield 4-6% of colourless to brownish essential oil, which is a rich source of thymol (containing 35-55%). The *T. ammi* seed oil, both as such or dethymolised, is also employed as an antiseptic and aromatic carminative [2].

Though *T. ammi* has been used commonly as a drug in traditional medicine and also as an insecticide, its potential as a fungicide has not been examined. Consequently, the present investigation was undertaken to screen this plant for antifungal property.

Materials and Methods

Plant material and preparation of extracts. The fresh plant *T. ammi* (L) was collected from the suburb area of Karachi, dried in air at room temperature (28-30°C) for 4-5 days. Different parts of the plant i.e. roots, stems and twigs, leaves and seeds were macerated separately in a mortar. Each macerated plant material was packed into a percolator and percolated with 7 different organic solvents like 95% ethanol, water, acetone, ethylacetate, chloroform, pet-ether (boiling pt. range 60-80°C) and benzene. The extracts by these solvents were made successively. One hundred grammes of the macerated part was extracted in 500 ml. of each solvent. This process was carried for 5 days to get maximum amount of the extract. The solvent of each extract was then evaporated in a rotary evaporator under reduced pressure at ± 40°C and the residues thus obtained were stored in the refrigerator. The crushed seeds produced maximum yield of essential oil about 2-3% which was brownish yellow in alcoholic extract.

Preparation of samples for testing. The concentrated residues were suspended or dissolved in an emulsifier, polyethylene glycol 400 (PEG 400), which shows no inhibitory effect on fungal or bacterial growth [3].

The organic extracts were dissolved or suspended in this solvent. Aliquots of this solution/suspension (5% extract solution) were used to test for the antifungal activity.

Antifungal testing. The hole-plate diffusion method was used to test the plant extracts against fungi. Five species of fungi were used for testing fungi toxic activity and they were (i) *Aspergillus flavus* and (ii) *Aspergillus niger*; these fungi are saprophytes but in favourable conditions become animal and human pathogens,the diseases being collectively known as aspergilloses [4]. These fungi also cause spoilage of food material. (iii) *Alternaria alternata* and (iv) *Helminthosporium* sp. which cause black-rot of many cash fruits and vegetables. (v) *Fusarium solanii*, which attacks many crops e.g. cotton, rice and chillies.

The above species of fungi were grown on Czapek's Dox Agar. For the hole-plate diffusion method 50µl of spore suspension made in broth, was thoroughly mixed with 25ml of sterile melted Czapek's Dox Agar at 30 -35°C, poured into sterilized petri-plates and left to solidify. Then with the help of a 12mm diameter borer the agar was removed to make wells in five positions in the petridishes. Four wells were aseptically filled with 40µl. of each plant extract, whereas 20µl of a commercial fungicide "Tecto" (Merck Sharp & Dhome) used as a standard for comparison, was poured in the fifth hole. After maintaining at 4°C for 1 hr. the petridishes were incubated for 4 to 7 days at 28 - 30°C. Zones of inhibition were measured in cm.

TABLE 1. ANTIFUNGAL ACTIVITY OF EXTRACTS OF *T. AMMI* IN SOLVENT SYSTEM

No.	Name of test fungus	Plant parts used	Solvent System (Inhibition zone in cm.)							
			Acetone	Benzene	Chloroform	Ethylacetate	Ethyl Alcohol	Pet- ether	Water	Tecto
1.	*A. niger*	Roots	+	-	-	-	++	-	++	2.5
		Stems	-	-	-	-		-	-	2.4
		Leaves	-	-	-	-		-	-	2.5
		Seeds	-	1.0	-	-	1.	1.2	-	2.5
2.	*A. flavus*	Roots	+	-	-	-	++	-	+	2.5
		Stems	-	-	-	-	-	-	-	2.6
		Leaves	-	-	-	-	-	-	-	2.5
		Seeds	-	1.1	-	-	1.5	1.2	-	2.4
3.	*Alternaria alternata*	Roots	+	-	-	-	++	-	+	2.5
		Stems	-	-	-	-	-	-	-	2.6
		Leaves	-	-	-	-	-		-	2.6
		Seeds	-	1.5	-	-	1.8		+	2.7
4.	*Helminthosporium* sp.	Roots	+	-	-	-	+		+	2.4
		Stems	-	-	-	-	-	-	-	2.5
		Leaves	-	-	-	-	-	-	-	2.5
		Seeds	-	1.5	-	-	1.9	1.1		2.6
5.	*Fusarium solanii*	Roots	++	-	-	+	++	-	+	2.5
		Stems	-	-	-	-	-	-		2.6
		Leaves	-	-	-	-	-	-	-	2.7
		Seeds	-	1.5	-	-	2.0	1.5	+	2.6

- = No activity, + stimulating activity

Results and Discussion

In our previous investigations, about 106 plant extracts were screened for fungitoxic activity [5,6]. Among all those plant extracts which showed antifungal activity, the plant *T. ammi* (L) showed more promising results. Therefore, this plant was selected to study the fungitoxic activity in detail.

The test results of different parts of the plant, extracted in various solvents, against the fungi are presented in Table 1. The antifungal activity was determined by measuring the zone of inhibition in cm, and the values below 0.01 cm were considered negative. The results were compared with a commercial standard broad spectrum chemical fungicide "Tecto" (Merck Sharp and Dhome, Holland).

The seed extracts of *T. ammi* showed activity against all the five species of fungi, 95% ethanolic extract being the most active. Besides, petroleum ether and benzene extracts were found to possess antifungal activity. Extracts of various parts of this plant in acetone, chloroform, ethyl acetate or water did not show any activity.

Root extracts in 95% ethanol, acetone and water surprisingly showed stimulating action i.e. promoted the fungal growth. Organic extracts of stems and leaves were devoid of activity. In root extracts growth promoting substances are present in high quantities, hence counter acting the effects of the inhibitory substances present if any.

Among tested fungi, *F. solanii* has shown more sensitivity towards seed extracts specially in 95% ethanol, also in benzene and pet. ether.

T. ammi seeds yield an essential oil, which is a rich source of thymol (35-55%). The oil has a powerful antiseptic property and is used as a medicine for the treatment of nasal catarrh and skin disease [1]. This suggests that it has a strong antibacterial effect.

Chemically oil of *T. ammi* seeds is composed of 50% phenols, mainly thymol and about 50% of terpenes [2]. Thymol crystallises easily from the oil, the remainder of the oil consists of p-cymene α-pinene, dipentene, γ-terpinene and carvacrol, the mixture being known as "thymene". Content of phenols were determined by NaOH aq. sol. as 45-75% soluble in 1 to 2.5 vol. and more of 80% alcohol [7-9].

Thymolysed oil has poor antifungal activity [10] but the dethymolysed oil, which is largely a waste product, reflected a wide range of antifungal activity indicating the possibility of its exploitation as a useful antitoxicant [9]. For the first time the toxicity of dethymolysed oil of *T. ammi* was reported against *Helminthosporium oryzae*. The dethymolysed oil has

shown strong fungicidal activity even in diluted form [11].

Essential oil isolated from leaves and seeds of *T. ammi* tested against the growth of *A. flavus* was found very toxic [12]. In our investigation only the seed oil was active against tested fungi. It may be due to more chemical contents present in alcoholic seed extract, (particularly phenols) than other parts of the plant.

It is also reported that essential oils from 15 medicinal plants were tested for antifungal activity against 16 species of human and plant fungi, with the oil from *T. ammi* completely inhibiting growth of all tested fungi [13].

The toxicity of the *T. ammi* seed oil persisted for at least 365 days at storage. The oil was thermostable (upto 100°C) and was more effective than the chemical fungicides, Agrosan G.M., Benlate, Ceresan, Bithane, M-45 and Thiovit, commonly used for control of plant disease [12,14].

Plant essential oils have been reported to be more active than some of the prevalent synthetic fungicides and thus stand good candidates as natural fungicides [13]. Besides, these oils are reported to be toxic to various human and plant pathogens [9]. Our data on the antifungal properties of *T. ammi* suggests that this plant should be examined further to evaluate its potential as a natural fungicide.

References

1. R.N. Chopra, I.C. Chopra, K.L. Manda and L.D. Kapur, *Indigenous Drugs of India*, (U.N. Dhur & Sens. Pvt. Ltd., 15 Bankin Chaterjee Street, Calcutta 1958), 2nd ed., pp.92-95.
2. B. Schimmel, Co. Lpz. 1903, Oct. 82, 1920, **3**, 14 (1928), J. Soc. Chem. Ind. London 604 (1918).
3. M. Ieven, D.A.V. Berghe, F. Mertens, A. Vlietinck and E. Lammens, Planta Medica, **36**, 311 (1979).
4. G.L. Chopra, *A Class Book of Fungi*, (S. Nagin & Co. Partap Road, Jullundur City, India 1947), 4th ed.
5. Y.M. Rizki, K. Fatima, A. Askari and S.I. Ahmed, Pak. j. sci. ind. res., **30**, 760 (1987).
6. Y.M. Rizki, E. Fatima, S.I. Ahmed and Y. Badar, Pak. j. sci. ind. res., **32**, 608 (1989).
7. Gildemeister and Noffmann, *Die Atherischen Ole* (1943) Vol. 111, 3rd ed., pp.496.
8. E. Guenther, *The Essential Oils* (D. Van Nostrand Co., Inc., New York, 1950) Vol. IV, pp. 551-52
9. A.K. Singh, A. Dikshit, M.I. Sharma and S.N. Dixit, Economic Botany, **34** (2), (1980).
10. S.S. Nigam and T.S.S. Rao, *Antimicrobial efficacy of some Indian essential oils* In: Kapoor L.D. and Ramkrishan ed. Advances In essential Oil Industry, (Today & Tomorrow's Printers & Publishers, New Delhi, 1977), pp 177-180.
11. A. Dikshit, A. Hussain, Fitoterapia, **55** (3), 171 (1984).
12. S.K. Dwivedi, N.K. Dubey, Mycopathologia, **121**(2), 101 (1993).
13. S.K. Sharma, V.P. Singh, Indian Drugs Pharm., **14**(1), 3 (1979).
14. S.C. Tripathi, S.P. Singh, S. Dube, J. Pathology, **116**(2), 113 (1986).