Antifungal properties of leaf extract of *Ranunculus sceleratus* L.

S. B. Misra[1,2] and S. N. Dixit

Plant Pathological Laboratory, Gorakhpur University, Gorakhpur (U.P., India), 15 February 1978

Summary. Studies on various antifungal properties of the leaf extract of *Ranunculus sceleratus* L. showed that it was thermostable up to 100 °C, retained activity on autoclaving, and remained active up to 15 days at room temperature. It possessed quick fungicidal action, tolerance against heavy fungal inoculum, activity on broad pH range, broad fungicidal spectrum, non-phytotoxicity and non-systemic activity. The extract was lethal at 1:40 dilution and its volatile vapours were also fungitoxic.

Several plants have been reported to possess antifungal activity in their tissues[3-6]. Leaf extract of *R. sceleratus* L. has already been found by the authors[7-9] to exhibit fungitoxicity. In the present investigation, various antifungal properties of the expressed juice of the plant have been determined. The plant belonging to the Ranunculaceae is a herb found on sandy moist places near the water pools and rivers in Northern India. In Unani system of medicine, it is reputed to be a cure for plague and toothache. Besides, it has also been reported for its effectiveness against skin diseases[10].

Material and methods. Fresh leaves of the plant washed with 70% ethanol, repeatedly with fresh water and finally with sterilized water, were pulverised well, strained through 2 layers of muslin cloth and then filtered through Whatman No. 1 filter paper. The clear filtrate thus obtained was used for assay. *Alternaria tenuis* Nees, *Curvularia lunata* (Walker) Boedign, *Fusarium nivale* (Fries) Cesati and *Helminthosporium gramineum* Rab. ex Schlecht were used as test fungi. Determination of the antifungal properties of the extract was done by the poisoned food technique referred to as 'colony diameter measurement method', where each

Table 1. Antifungal properties of the leaf extract

Properties studied		Mycelial inhibition of the test fungi (%) At	Cl	Fn	Hg
Effect of temperature (°C)	40	100	100	100	100
	80	100	100	100	100
	100	100	100	100	100
	120	75.4	100	83.07	100
	300	66.6	75.4	82.5	75.8
Effect of autoclaving (At 15 lb pressure for 30 min)		100	100	100	100
Effect of storage (days)	5	100	100	100	100
	15	100	100	100	100
	30	68.4	65.5	42.4	42.8
	60	55.3	55.0	34.8	27.8
	120	28.5	12.9	15.6	21.3
Effect of exposure duration for killing fungi (min)	10	100	100	100	100
	5	100	100	100	100
	1	100	100	77.7	84.4
(sec)	45	79.6	82.7	68.2	71.1
	30	46.2	55.1	50.7	52.5
Effect of pH	2.5	100	100	100	100
	5.0	100	100	100	100
	5.5**	100	100	100	100
	7.0	100	100	100	100
	9.0	100	100	100	100
	11.0	0	10.9	8.3	0
Lethal dose (ml)	0.5	100	100	100	100
	0.25	64.0	83.0	81.2	86.6
	0.125	*	55.9	56.2	41.6
Activity of volatile vapours		100	100	100	100

At, *Alternaria tenuis;* Cl, *Curvularia lunata;* Fn, *Fusarium nivale;* Hg, *Helminthosporium gramineum;* Normal pH of the extract; *Growth accelerated.

Table 2. Fungitoxic spectrum of the extract

Test fungi	Inhibition at different doses (%) 1.0 ml	0.5 ml	0.25 ml
Absidia spinosa Lend.	100	100	100
Alternaria solani (Ellis & Mart) Jones and Grout	100	100	61.0
Aspergillus aculeatus Iizuka	100	143	*
A. flavipes Bainier and Sartory	100	100	30.0
A. flavus Link	100	68.0	*
A. fumigatus Fresenius	100	100	21.8
A. japonicus Saito	100	100	11.1
A. nidulans (Eidam) Winter	100	22.6	13.0
A. niger Van Tieghem	100	*	*
A. niveus Blochwitz	100	100	33.3
A. sydowi (Bainer & Sartory) Thomand Church.	100	48.5	57.1
A. terreus Thom	100	100	41.8
Cephalosporium sacchari Butler	100	100	100
Colletotrichum falcatum Went	100	100	47.5
Cunninghamella elegans Lendner	100	100	61.9
Monilia sitophila (Montagne) Sacc.	100	*	*
Mucor hiemalis Wehmer	100	100	44.0
Neocosmospora vasinfecta E.F. Smith	100	100	100
Periconia ignaria Booth	100	*	*
Pestalotia sp.	100	100	100
Phomopsis sp. Sacc.	100	100	100
Rhizopus nigricans Ehrenberg	100	100	57.8
Thielavia terricola (Gillman & Abbott)	100	100	41.7
Trichoderma viridis Pers. ex Fries	100	100	42.6
Verticillium terrestre (Link) Lindan Reinke and Berthold	100	100	100
Zygorhinchus heterogamous Vuillemin	100	100	100

*Growth stimulated.

treatment set contained 20 ml Czapek's agar medium, 1 ml extract, 0.8 ml antibiotic solution[6], a fungal disc (5 mm diameter, cut from the periphery of 7-day-old culture) inoculated in the centre. Control set was prepared similarly, except that equal amount of distilled water was added instead of the extract. Fungitoxicity of the volatile vapour of the extract was determined by Latham and Linn's method[11], and the effect of increased inoculum was observed in Czapek's liquid medium. Phytotoxicity and systemic activities of the extract at its lethal (1:40) and hyperlethal (1:20) concentrations were tested following Grewal[12] and Erwin et al.[13].

Observations. As is clear from table 1, the extract retained activity up to 100 °C, beyond which it became gradually ineffective; retained activity on autoclaving; remained completely active up to 15 days when stored at room temperature; killed the test fungi within 5 min; possessed maximum fungitoxicity between pH levels 5.0–9.0. The extract was fungicidal against all the test fungi and its lethal dose was found to be 0.5 ml i.e., 1:40 dilution. Volatile vapours emitted from the extract were also fungicidal (table 1). It possessed a wide range of fungitoxicity, inhibiting all the 26 fungi tested at 1 ml dose, 19 at 0.5 ml dose and 7 at 0.25 ml dose (table 2).

The extract was found to inhibit heavy fungal inoculum (10 discs each of 5 mm diameter). The extract exhibited no phytotoxicity during seed germination, root application and foliar spray studies. However, it did not prove systemic by either of the applications through roots and shoots.

Discussion. Disease resistance in plants has been attributed to the various chemicals present in their tissues. Strong fungicidal action exhibited by *R. sceleratus* in the present investigation may be responsible for its disease-free occurrence. It may be marked that Bhakuni et al.[14] have reported the plant to possess no fungitoxicity, while on the contrary Nene et al.[15] have found it to be active. It may be noted that Bhakuni et al.[14] missed the activity on account of their using dried material, as the plant has been found to loose activity on drying[16]. Loss of fungitoxicity due to dehydration has already been recorded by some workers with several other plants too[4,13]. The extract, on account of its thermostability, quick fungicidal action, activity at broad pH range, tolerance against heavy fungal inoculum, broad fungitoxic spectrum and no phytotoxicity during in vitro trials, proves to be of great significance for in vivo studies. It is hoped that the extract and its active principle(s) will prove to be of great therapeutic value for plants against different diseases.

1 Present address: Department of Botany, S.D. Degree College, Math-Iar, Deoria (India).
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3 K. Gilliver, Ann. appl. Biol. *34*, 136 (1947).
4 A.A. Abdullaeva, Dokl. Akad. Nauk. Uzbek. *1*, 43 (1959).
5 P.N. Thapliyal and Y.L. Nene, J. Sci. ind. Res. *26*, 289 (1967).
6 S. Gupta and A.B. Banerjee, Indian J. exp. Biol. *8*, 148 (1970).
7 S.B. Misra, R.R. Misra and S.N. Dixit, Nat. Acad. Sci. India *1974*, 76.
8 S.B. Misra and S.N. Dixit, Symp. Physiol. Micro-org. p. 221. 1976.
9 S.B. Misra and S.N. Dixit, Geobiosynthesis *4*, 29 (1977).
10 K.P. Trivedi, Dhanwantari *39*, 549 (1965).
11 A.J. Latham and M.B. Linn, Pl. Dis. Reptr *49*, 398 (1965).
12 J.S. Grewal, Personal communication 1972.
13 D.C. Erwin, J.J. Sims, D.E. Brown and J.R. Childrens, Phytopathology *61*, 964 (1971).
14 D.S. Bhakuni, M.L. Dhar, M.M. Dhar, B.N. Dhawan and B.N. Mehrotra, Indian J. exp. Biol. *7*, 250 (1969).
15 Y.L. Nene, P.N. Thapliyal and K. Kumar, Labdev. J. Sci. Technol. *6B*, 226 (1968).
16 S.B. Misra, Thesis, Gorakhpur University, India 1975.
17 E.M. Osborn, Br. J. exp. Path. *29*, 227 (1943).

Water compartments in the myelinated nerve. III. Pulsed NMR results[1]

V. Vasilescu, Eva Katona, V. Simplăceanu and D. Demco

Department of Biophysics, Faculty of Medicine, and Central Institute of Physics, IFTAR, Bucharest (Romania), 14 October 1977

Summary. 3 experimentally distinct transverse relaxation components of the water in frog sciatic nerve are obtained by Carr-Purcell-Meiboom-Gill technique. The relative weights of these components: ≈29%; ≈50%; ≈21% fit well with water compartments in this tissue as revealed by previous methods.

The state of water in tissues was, and still continues to be, the aim of many studies, due to its fundamental interest, both theoretical and practical. NMR techniques are widely used and a great deal of experimental data already accumulated, particularly on muscle water and to a somewhat lesser extent on water in nerve and other tissues[2]. However, the problem is far from being solved, the role and properties of tissue water still remaining a matter of considerable dispute[3]. Here we present results on the proton magnetic resonance (PMR) behaviour of the myelinated nerve, revealing the existence of 3 experimentally distinct types of water, with relative slow exchange processes between the various components.

Materials and method. The measurements were made on frog *(Rana temporaria)* sciatic nerves immediately after they were dissected or after keeping them for 30 min in normal Ringer solution. The total water content of the nerves was obtained by heat drying to constant weight at 105 °C. Their 'NMR-visible' water content was determined by calibration of the detector system, using samples of Cu^{2+}-doped water. The transverse relaxation behaviour of water protons in nerve and its variation with temperature and radio-frequency pulse separation, was observed using a Carr-Purcell-Meiboom-Gill sequence. The measurements were made on a Bruker BKR-322s spectrometer operating in this study at 45 MHz. The sequence pulse spacing (τ) was varied between 0.4 and 4 msec and the amplitudes of 256–1024 echoes were recorded. In each experiment, 8–64 transverse relaxation decays were averaged in a BNC-12 minicomputer. The temperature of the samples was gradually lowered from 300 to 268 °K.

Results. The figure shows a typical multiexponential trans-