EFFECTS ON PHENOLIC PROHIBITINS FROM GROUNDNUT ON FUNGI

P. T. KALAICHELVAN and
A. MAHADEVAN
C. A. S. in Botany, University of Madras, Madras 600 025, India.

DESPITE several thought-provoking reviews on the participation of phenol in disease resistance[1-3], their mode of action on parasitic microorganisms continues to remain an enigma. Groundnut is an important oil crop constantly attacked by a wide range of fungi[4].

Groundnut shells var. TMV 7 (50 g) were individually extracted in 250 ml of acetone, benzene and ethanol for 10 h at 80°C using the Soxhlet apparatus and were filtered through cheese cloth and evaporated to dryness at 40°C on a water bath. The residue was dissolved in water and used in bioassays. Cultures of the leaf spot fungi *Alternaria alternata* and *Curvularia spicata* were grown in Czapek's Dox agar in petri plates. The spores were harvested after 8 days in sterile distilled water and centrifuged. The spore concentration was adjusted to 5000 spores/ml and used for spore germination assay.

All the extracts completely suppressed the germination of spores up to 6 h but after 8 h, germ tube emerged except in benzene extract in which no spores germinated (table 1).

Groundnut shells (10 g) were extracted with ethanol and concentrated[5] to 1 ml. Aliquots of 0.1 ml were spotted on paper and separated chromatographically using the solvent system benzene: acetone: water 10: 7: 3 v/v upper phase[6]. They were anthranilic acid, caffeic acid, *p*-coumaric acid,

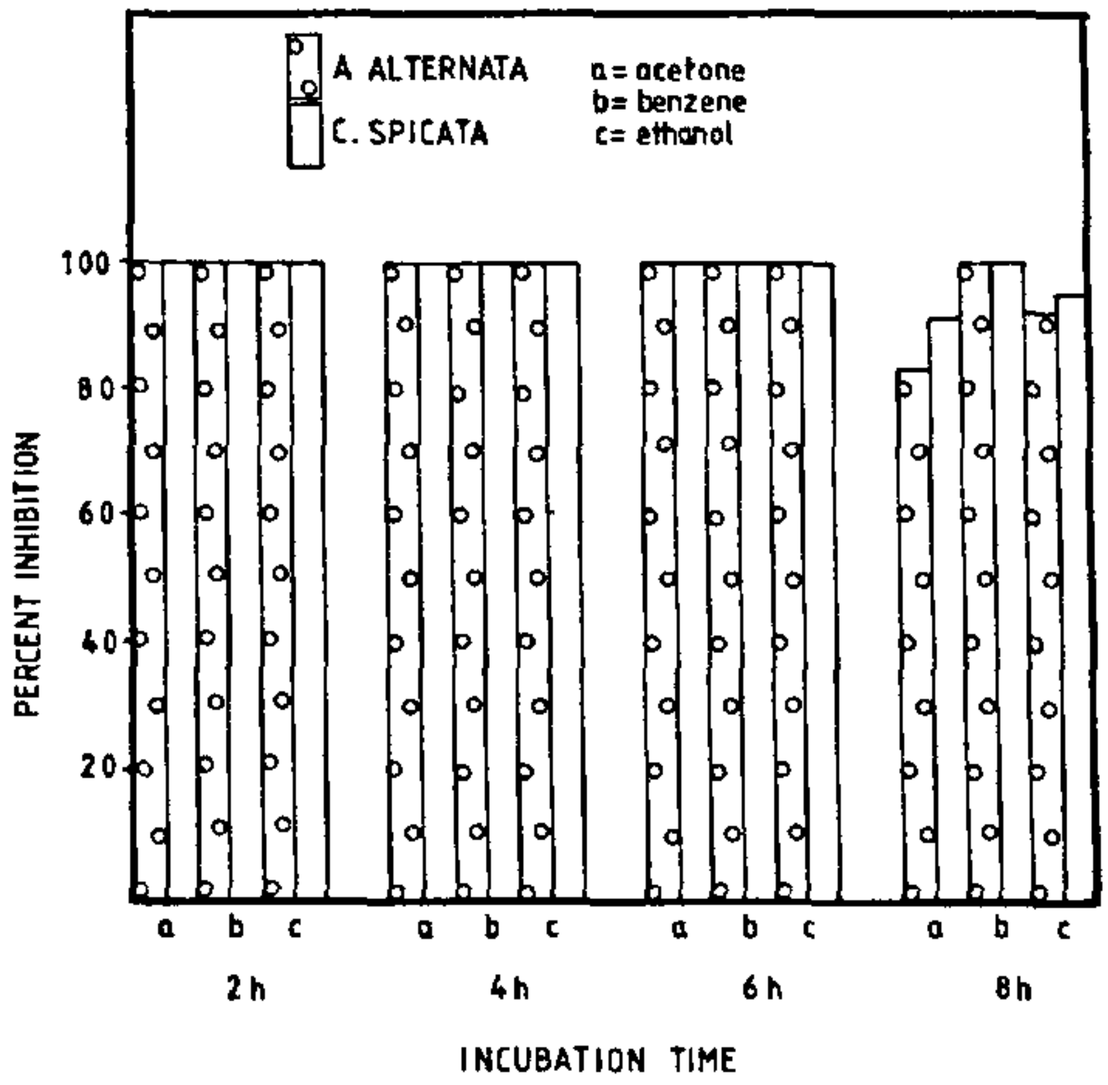

Figure 1. Effects of shell extract on the spore germination.

Table 1 *Effect of shell extract on the spore germinaion of fungi*

		Incubation at			
		2 h	4 h	6 h	8 h
		Per cent inhibition			
A. alternata	a	100	100	100	83
	b	100	100	100	100
	c	100	100	100	92
C. spicata	a	100	100	100	91
	b	100	100	100	100
	c	100	100	100	95

a: acetone; b: benzene; c: ethanol.

Table 2 *Effect of prohibitins on the germ tube elongation of fungi at the end of 6 h*

		Concn. mM					
		0.32	0.64	1.2	1.9	2.5	3.2
		Per cent inhibition*					
A. alternata	a	0	29	29	100	100	100
	b	0	29	57	100	100	100
	c	43	43	57	100	100	100
	d	0	43	71	100	100	100
C. spicata	a	20	52	100	100	100	100
	b	40	72	100	100	100	100
	c	72	96	100	100	100	100
	d	32	60	100	100	100	100

a: anthranilic acid; b: gentisic acid; c: salicylic acid; d: vanillic acid; *Percent inhibition was calculated with the control.

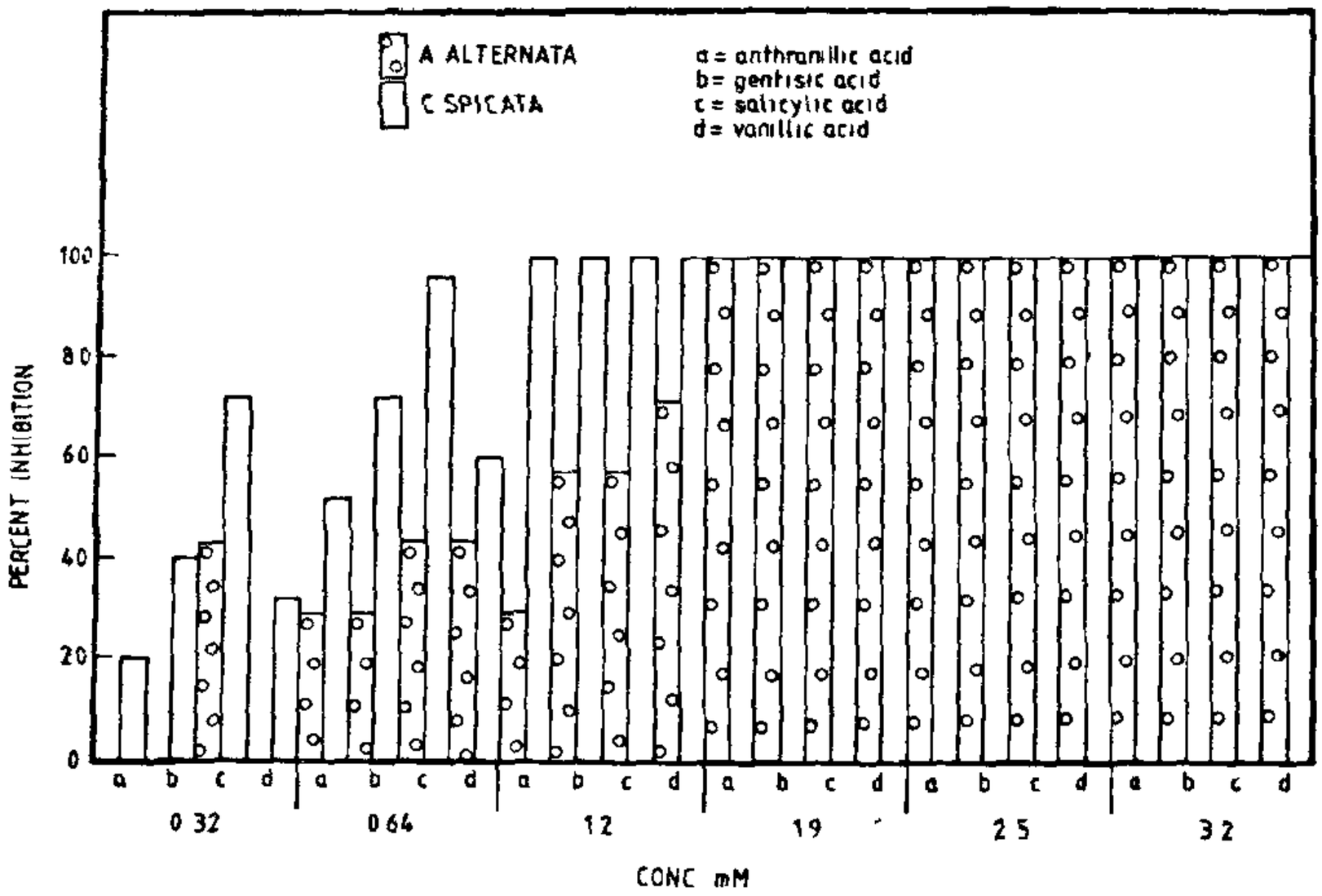

Figure 2. Effect of shell extract on the germ tube elongation of fungi.

gentistic acid, isovanillic acid, resorcinol, salicylic acid, scopoletin and vanillic acid. One g shell yielded anthranilic acid (0.90 μg), gentisic acid (2.63 μg), salicylic acid (13.75 μg), scopoletin (1.84 μg), vanillic acid (15.8 μg) and others in trace amounts. The spore germination with a few phenolics was assayed (table 2) and at lower concentrations of phenolics, germination was not affected but germ tube elongation was profoundly affected. Anthranilic, salicylic and vanillic acids at 1.9 mM caused 100% inhibition of *A. alternata*. All the phenols inhibited the spore germination of *C. spicata* at 1.2 mM (figure 2).

Mycelial segments (2 mm) were cut by a sterile corkborer from the periphery of 7-day-old cultures of the fungi grown over cellophane paper. The bits were suspended in 5 ml of solution containing test substances. Oxygen consumption was recorded in an oxygen electrode at 26°C. Corresponding controls were maintained.

Gentisic acid at 0.5 and 1.0 mM reduced the oxygen uptake by *A. alternata*. But at 2 mM, it completely reduced the oxygen uptake up to 10 min and even by 20 min it was only 0.1 mg/l (figure 1a). Salicylic acid (0.5 mM) reduced the oxygen uptake by 50% in 20 min. At 1.0 mM it reduced the uptake of oxygen by 67% while 2.0 mM concentration inhibited the respiration by 83% (figure 1b). Gentisic acid at 0.5 mM increased the respiration of *C. spicata*. But at 1.0 mM it reduced respiration by 18%. At 2 mM it caused complete inhibition in 5 min and 45% at the end of 20 min (figure 1c). Salicylic acid 0.5 mM inhibited respiration by 45%. At 1 mM and 2 mM it completely inhibited respiration up to 15 min. At 20 min there was little respiration (figure 1d). The precise step in respiration that is inhibited by the prohibitins is still not clear. The heart wood prohibitins of *Phellinus ignianius* uncoupled respirations[7]. They also inhibited phosphate uptake in yeast[8].

The authors thank UGC, New Delhi for financial assistance.

1 May 1987; Revised 12 August 1987

1. Mahadevan, A., *The biochemical aspects of plant disease resistance*, Today & Tomorrow's Printers & Publishers, New Delhi, 1982, p. 397.
2. Pan, S., Mukherjee, B., Ganguly, A., Mitra, S. R. and Bhattacharyya, A., *Z. Pflanzenkr. Pflanzenschutz*, 1985, **92,** 392.
3. Glazener, J. A., *Physiol. Plant Pathol.*, 1982, **20,** 11.
4. Jackson, C. R. and Bell, D. K., *Univ. Georgia Coll. Agric. Exp. Stn. Res. Bull.*, 1969, **56,** 137.
5. Ibrahim, R. K. and Towers, G. H. N., *Arch. Biochem. Biophys.*, 1960, **87,** 125.
6. Reddy, M. N. and Rao, A. S., *Phytopathol. Z.*, 1975, **83,** 103.
7. Lyr, H., *Naturwissenschaften*, 1960, **47,** 499.
8. Lyr, H., *Uber den wirkungsmechanisms Von Thujaplicinen and Pinosylvinmonomethylether in*

wirkungsmechanismen von Fingiziden und Antibiotika, Academie Verlag, Berlin, 1967, p. 27.

ENHANCED REPRODUCTIVE POTENTIAL OF *NEOCHETINA BRUCHI* HOSTACHE FED ON WATER HYACINTH PLANTS FROM POLLUTED WATER BODIES

KAISER JAMIL and K. N. JYOTHI

Biology Division, Regional Research Laboratory, Hyderabad 500 007, India.

CLOSE relations are known to exist between the phytophagous insects and their food plants. Only among female insects special nutritional requirements for reproduction are observed in addition to energy. The effects of various chemicals on plants may affect the insect species feeding on them. Sex ratio, reproduction and survival of the females varied in several species of insects with the food plant of their host[1–5]. We report here the enhanced reproductive activity of *Neochetina bruchi* fed on water hyacinth plants grown in polluted water, rich in several inorganic molecules.

Eichornia crassipes (Mart) solms, commonly known as waterhyacinth has become a serious concern as a weed pest in recent years. Several pathogens, mites, insects and other arthropods are found attacking this weed, of which two species of weevils, *Neochetina eichorniae* and *N. bruchi* are found as promising bio-control agents[6]. Hence, the above two species of Neochetina have been introduced in India and host specificity tests were carried out under quarantine conditions at the Commonwealth Institute of Biological Control and the National Centre for Biological Control, Bangalore. The above weevils collected from Bangalore are being successfully reared in the laboratory glass house. Adults of *N. bruchi* were selected for the present study. Water hyacinth plants were collected from two different polluted areas around Hyderabad. The above water bodies are heavily contaminated with industrial effluents from nearby industries like starch and brewery. The plants along with the polluted water were placed in 5 l beakers. Five pairs of freshly emerged adults of *N. bruchi* were introduced into each of these beakers. The insects released on plants collected from the laboratory pond were kept as control. The experiments were done in triplicates. The insects were examined for egg laying and hatching. The plants were replaced and the eggs and surviving females were counted daily. Their numbers and observations were recorded and the average mean fecundity rates determined.

For anatomical study, ovaries of the insects were collected from the polluted water bodies and the laboratory pond. Dissections were performed in the insect ringer solution. The material was stained with borax carmine, progressively dehydrated, cleared in cedarwood oil and fixed in canada balsam. Total amino acid and protein content of the leaves from the polluted water body were estimated and compared with that of the laboratory pond[7,8].

The effect of industrial pollutants on the fecundity of *N. bruchi* is shown in table 1. Females of *N. bruchi* laid tiny oval-round creamish eggs in the petioles of water hyacinth plants. The eggs hatched after 3–4 days into minute first instar larvae which tunnelled into the petiole for further development. The larval period lasted for a month inside the petiole. The 3rd instar larvae pierced out through the base of the petioles and pupated among the roots of water hyacinth. The life span of the adults was 3–4 months. The pre-oviposition period was 3–4 days.

Table 1 *Fecundity of N. Bruchi fed on water hyacinth plants from different bodies**

Place	Fecundity during			
	1st week	2nd week	3rd week	4th week
Laboratory pond (control)	285 ± 5.19	236 ± 8.83	128 ± 5.49	102 ± 2.86
Area 1 (Banjara hill)	286 ± 0.86	254 ± 4.10	243 ± 8.28	180 ± 6.73
Area 2	289 ± 5.92	286 ± 4.70	266 ± 4.67	212 ± 4.90

* Average of triplicate experiments: The number of pairs of insets studied was 5.