graminicola are equally resistant to the other three isolates, viz., Pattambi, Agartala and Jorhat. The stability of resistance in these varieties has been established.

3 August 1987; Revised 7 November 1987

1. Rao, Y. S., Jena, R. N. and Krishnaprasad, K. S., *Indian J. Nematol.*, 1977, **7**, 98.
2. Roy, A. K., *Indian J. Nematol.*, 1973, **3**, 72.
3. Rao, Y. S., In: *Rice research in India*, (ed.) S. Y. Padmanabhan, ICAR, New Delhi, 1985, p. 591.
4. Swain, B. N., Prasad, J. S. and Rao, Y. S., *IRR Newslett.*, 1986, **11**, 25.
5. Franklin, M. T. and Goodey, J. B., *J. Helminthol.*, 1949, **23**, 175.
6. Sahu, S. C. and Chawla, M. L., *IRR Newslett.*, 1986, **11**, 40.

A WILT TOXIN FROM *FUSARIUM OXYSPORUM* F. SP. *CUMINI* PATEL AND PRASAD

H. N. GOUR and SANJEEV AGRAWAL*
Department of Plant Physiology and Biochemistry, SKN College of Agriculture, Jobner 303 329, India.
** Present address: Department of Genetics, The University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030, USA.*

CUMIN cultivation has received a serious threat from *F. oxysporum* f. sp. *cumini*, a causative agent of wilt syndrome, which devastates the total standing crop. The toxins of *Fusarium* spp have been considered to be the active factors in causing wilt syndrome. The Botany School at Madras[1] contributed significant knowledge to wilt toxins using cotton isolate of *Fusarium*. Reports are scanty on the cumin wilt isolate producing such disease-inducing active factors, which produce wilt syndrome in isolated form without involving fungus and these fungal products could then be used in screening the varieties for disease resistance.

The present report is a part of the ongoing research project on cumin wilt toxins. The successful isolation of toxins of *F. oxysporum* f. sp. *cumini*, reproduction of wilt symptoms, some properties and host range of toxins and screening of some outstanding cumin lines for disease resistance are reported here.

The causal fungus from infected vascular track of cumin roots was isolated. One-year-old fungal culture of *F. oxysporum* f. sp. *cumini* Patel and Prasad was used in toxin extraction. Koch's postulates were proved under controlled conditions before the start of the experiments for toxin extraction. The fungus was identified and confirmed by IMI, Kew, UK (IMI No. 294847). Seven-day-old culture was inoculated in 250 ml conical flasks each containing 30 ml of Czapak-Dox medium (composition per litre: KH_2PO_4, 1 g; $NaNO_3$, 2 g; $MgSO_4.7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4.7H_2O$, 0.01 g; sucrose, 30 g, pH 7) and incubated at 25 ± 2°C for 21 days. Culture filtrates, pooled by centrifuging the medium containing fungal growth at 5,000 *g* for 20 min, were fractionated with different solvents using acetone (1:2), methanol (1:2), ethanol (1:2) or ammonium sulphate for partial purification of the active factors. Chilled solvents were added to each of the 50 ml of culture filtrates and kept at 4°C for 24 h. The precipitate thus collected was dissolved in double-distilled water and bioassayed using healthy fresh plants of highly susceptible variety (RS-1). Sterilized uninoculated Czapek-Dox medium and distilled water were used for the control experiments. The wilt symptoms were rated on 0–4 point scale where 0 = plants healthy; 1 = 0–25% wilt; 2 = 26–50% wilt, 3 = 51–75% wilt and 4 = severe wilt.

Some properties of toxins were studied. The host range of fungal metabolites was examined using plants of fenugreek (NLM Prabha), fennel (UF-32) and coriander (UD-41, UD-373, UD-374). A preliminary screening of twelve outstanding promising lines against toxin and the fungus was carried out. Symptoms were rated after 48 h of treatment.

F. oxysporum f. sp. *cumini* produced metabolites on Czapek-Dox medium which on partial purification with ethanol-induced disease symptoms of wilt resembling those produced by fungus itself. In field conditions the infected plants first showed changes in the colour of leaves from green to yellow, beginning from oldest leaves and extending upward to the younger leaves leading to wilting of the entire plant which ultimately dried up and could easily be pulled out of the soil. Toxin-treated plant cuttings indicated yellowing of older leaves within 12 h, the general yellowing after 24 h and the leaves dried completely after 48 h of treatment. Since the ethanol extracted toxin solutions caused severe wilt in cumin plant cuttings (rating 4), ethanol was the most suitable solvent for isolation of wilt toxins from culture filtrates. However, fractionation with acetone, methanol and ammonium sulphate was partly successful (table 1).

These toxins obtained in precipitate form by fractionation with ethanol were readily soluble in citrate

Table 1 *Partial purification of wilt toxins from culture filtrates of F. oxysporum cumin isolate with different solvents*

Solvent	Wilt index*
Ethanol	4.0
Acetone	2.5
Methanol	2.0
Ammonium sulphate	2.0
Sterilized uninoculated Czapek's medium	0 0
Distilled water	0.0

* Cuttings from plants were dipped in toxin preparations, unsterilized medium and distilled water for 48 h and symptoms were then recorded on a wilt index scale of 0–4.

buffer at pH 6 and toxic activity was enhanced on boiling.

Host range

Plants of crops like fenugreek (NLM Prabha), fennel (UF-32) and coriander (UD-41, UD-373, UD-374) were tested against fungus and its purified toxin sample, where both induced wilting symptoms in the three crops (the disease ratings ranged between 1 and 4). UD-41 variety of coriander showed resistance to toxin and fungus (table 2).

Screening of cumin varieties

Twelve breeders' varieties of cumin were screened against wilt fungus and its toxins. Table 3 shows the varietal reaction to toxin and the fungus. UC-199 and UC-19 lines showed considerable resistance, whereas all other ten lines were rated as susceptible to both the test organism and its toxins.

Table 2 *Host range of Fusarium oxysporum f. sp. cumini and toxins*

	Wilt index*	
Variety	*F. oxysporum***	Toxins
Fenugreek NLM Prabha	2	3
Fennel UF-32	2	4
Coriander UD-41	0	1
UD-373	1	2
UD-374	1	3

* Cuttings from plants were dipped in toxin preparations, unsterilized medium and distilled water for 48 h and symptoms were then recorded on a wilt index scale of 0–4; ** The symptoms by fungus appeared after 7-days of inoculations; Control plants dipped in sterilized medium and distilled water remained healthy during the treatments.

Table 3 *Screening of cumin varieties for resistance against wilt using isolated toxin*

	Wilt index*	
Variety	*F. oxysporum***	Toxins
UC-199	0	2
UC-198	1	2
UC-19	0	0
Local check	2	3
UC-11	3	3
GC-1	3	3
UC-89	2	3
UC-209	4	4
UC-208	3	4
RS-1	3	4
UC-24	3	4
MC-43-83	4	4

* Cuttings from plants were dipped in toxin preparations, unsterilized medium and distilled water for 48 h and symptoms were then recorded on a wilt index scale of 0–4; ** The symptoms by fungus appeared after 7 days of inoculations; Control plants dipped in sterilized medium and distilled water remained healthy during the treatments.

The present work with *F. oxysporum* f. sp. *cumini* fully supports the statement of Gaumann[2] that "every pathogenic organism is toxigenic". Fusarial toxins reported earlier were identified as fusaric acid, lycomarasmin, etc[3,4] but the nature of the present fusarial toxins is not known. Treatments with extracts from uninoculated Czapek-Dox medium had no effects, confirming that components of the autoclaved medium itself were not the active factors, but the active factors were the fungal products in the medium. These toxins could be easily separated by organic solvents especially ethanol and acetone. The grey precipitates formed were readily soluble in glass-distilled water, were heat stable and non-host-specific. These toxins could be used to screen the varieties of cumin for wilt resistance because, (i) the symptoms produced by the toxins were similar to those elicited by the fungus itself, and (ii) that the varietal reaction to the fungus and toxins was more or less similar in terms of wilt ratings as indicated in table 3.

7 August 1987; Revised 20 January 1988

1. Sadasivan, T. S. and Kalyansundaram, R., *Proc.*

Indian Acad. Sci., 1956, **43**, 271.
2. Gaumann, E., *Endeavour*, 1954, **13**, 198.
3. Gaumann, E., *Phytopathology*, 1957, **47**, 342.
4. Heinze, P. H. and Andrus, C. F., *Am. J. Bot.*, 1945, **32**, 62.

EFFECT OF COLCHICINE ON SEX EXPRESSION AND LEAF SHAPE IN MULBERRY

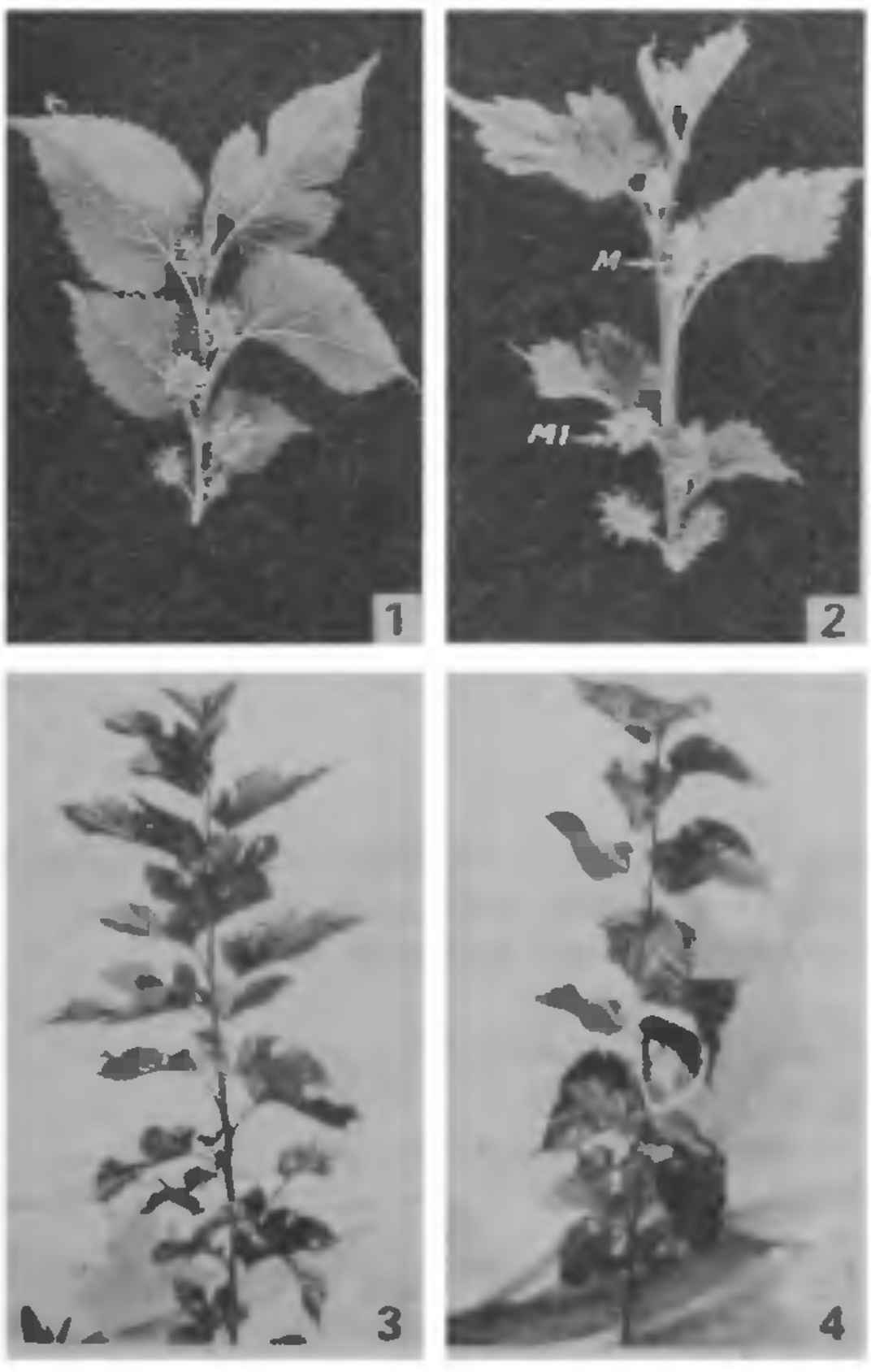
N. K. DWIVEDI, N. SURYANARAYANA, B. N. SUSHEELAMMA, A. K. SIKDAR and M. S. JOLLY

Mulberry Breeding and Genetics Section, Central Sericultural Research and Training Institute, Mysore 570 008, India.

MULBERRY was the first plant through which the sex in plants was demonstrated[1]. The regulation of sex expression[2] and leaf shape was attributed to genetic, environmental and chemical factors in angiosperms. Modification of sex expression in mulberry (*Morus* spp.) was reported by several workers using growth regulators, certain ionic chemicals and colchicine[3–7]. The present paper deals with the sex modification and leaf shape in a female cultivar of mulberry, popularly cultivated in the rainfed areas of Karnataka with lobed leaves viz. Mysore Local (*Morus indica* L.) by colchicine. This is part of the polyploidy breeding programme designed to improve the nutritive quality of leaves by inducing tetraploidy subsequently evolving triploids by hybridization with different desirable diploid strains.

The sprouting vegetative buds of female mulberry cultivar Mysore Local were treated with 0.35% aqueous solution of colchicine (Loba, India) prepared in 5% glycerine for 6 h for three consecutive days, using cotton plugs to keep the growing buds moist in November–December 1985. After the treatment, the cotton plugs were removed and the buds washed with distilled water. There were four replications for 6 buds for treatment. The control received only distilled water treatment. The pollen fertility was determined in 1% acetocarmine.

Flowers were induced after 10–15 days in treated as well as in control. The control buds produced 100% healthy female inflorescences (figure 1), while the treated buds produced male, mixed type and female inflorescences (figure 2). The frequency of production of male (21%) and mixed type (27.25%) of inflorescences was lower than that of healthy female (50.75%) inflorescences. Higher frequency of production of male (43.27%) and mixed type (25.08%) of inflorescences than that of suppressed (23.08%) and healthy female inflorescences (7.69%) was reported in cultivar Kanva-2 (*Morus alba* L.) which was treated with 0.4% aqueous solution of colchicine[5]. There were a few bisexual flowers (3.5%) in the mixed type of inflorescences along with male (56.33%) and female (40.17%) flowers on the same inflorescence. Pollen fertility was 89.33% and 85.67% in male and mixed type of inflorescences respectively. Female inflorescences of control buds dusted with pollen grains of male and mixed type of inflorescences showed 83.87% and 66.67% seed setting respectively. After three months of treatment a branch bearing entire leaves (figure 4) was isolated from treated batch whereas the branches grown from control as well as other treated buds bore only lobed leaves (figure 3). The branch with entire, dark green and smooth



Figures 1–4. **1.** Control branch bearing female inflorescences; **2.** Induced male and mixed inflorescences; **3.** Control branch bearing lobed leaves, and **4.** Branch developed from treated bud bearing unlobed leaves. (M, Male inflorescence; MI, Mixed inflorescence).