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TOXIN PRODUCTION BY *ALTERNARIA ALTERNATA* PATHOGENIC TO BRINJAL (*SOLANUM MELONGENA* L.)

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SOLANUM MELONGENA L. was found to develop a severe leaf spot disease during the rainy season of 1986. The pathogen was identified as *Alternaria alternata* (Fr.) Keissler based on the characteristics given by Ellis[1]. As the toxins produced by this pathogen play an important role in disease development[2,3], an attempt was made to test the toxigenic potential of *A. alternata* pathogenic to brinjal.

The fungus was cultured on Czapek-Dox broth for 30 days as stationary culture at room temperature (28±4°C). The toxicity of the culture filtrate was tested on seed germination and root elongation of tomato at different dilutions (1:0, 1:2, 1:4, 1:8, 1:10, 1:20, 1:40, 1:60, 1:80 and 1:100 of culture filtrate and distilled water). The petri dishes containing the seeds treated with the culture filtrates were incubated for 96 h and the germination percentage and root length were recorded. The detached leaves of brinjal and tomato were transferred to test tubes containing culture filtrate and symptoms were observed after 24 h. Chloroform extracts of the culture filtrates were employed for testing the antibacterial activity against *Bacillus megaterium* and *B. subtilis* using the seeded plate method[4].

The culture filtrates of the fungus grown on yeast-extract-sucrose broth for 30 days were screened for toxins[5–7]. The developing solvent system employed for TLC analysis was toluene-ethyl acetate-90% formic acid (5:4:1)[8]. On the basis of R_f values and fluorescence of spots, selected toxins were cochromatographed with authentic samples of *Alternaria* toxins and confirmatory tests were made[5,9]. UV spectral characteristics of the toxins were determined employing ethanol or water as carrier solvents[10].

The reduction in seed germination and root length over control was as high as 79% and 87% respectively. At lower dilutions only, the toxic principle was inhibitory to tomato, but at higher dilutions it stimulated the seed germination and root elongation. Epinasty with inward rolling of the leaf lamina with necrotic areas were the symptoms observed on detached leaves treated with the culture filtrate. Only wilting was noticed at higher dilutions. The solvent extractable metabolites produced by *A. alternata* were inhibitory to the test bacteria. The inhibition zones recorded in plates seeded with *B. megaterium* and *B. subtilis* were 0.88 and 1.47 cm^2 respectively.

TLC analysis of the solvent extracts revealed the existence of three phytotoxic compounds: (i) A brown coloured elongated spot with an R_f value extending from 0.25 to 0.36, (ii) and (iii) Compounds with blue fluorescence having R_f values of 0.39 and 0.56. Tests with ethanolic ferric chloride, *p*-anisaldehyde and UV spectral characteristics (peaks at 239 and 279 nm in water and 217 and 277 nm in ethanol) confirm the identity of the brown coloured compound as tenuazonic acid. TLC characteristics of the other two compounds did not coincide with those of alternariol and alternariol monomethyl ether. Besides phytotoxicity[11], the mycotoxic nature of tenuazonic acid is also well established in recent years[12]. Among *Alternaria* toxins, only tenuazonic acid is listed in the Rigistry of toxic effects of chemical substances[13]. The natural occurrence of tenuazonic acid in blast diseased rice plants[14] and tomato paste[9] was also reported.

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INHIBITION OF SPINACH MOSAIC VIRUS BY EXTRACTS OF SOME MEDICINAL PLANTS

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It was found earlier[1–7] that the extracts of different parts from a number of higher plants have antiviral properties. However, nothing is known about the antiviral properties of *Aconitum heterophyllum* Wall., *Anagallis arvensis* L., *Azadirachta indica* A. Juss., *Catharanthus roseus* (Linn.) G. Don, *Digitalis purpurea* L., *Glycyrrhiza glabra* L., *Hyoscyamus niger* L., *Ocimum sanctum* L., *Rauvolfia serpentina* Benth. ex Kurz. and *Withania somnifera* Dun. Hence, an attempt was made to determine the inhibitory effects of extracts of these plants on spinach mosaic virus[8] (SMV).

The culture of SMV was maintained on *Nicotiana glutinosa* L. by periodic inoculation inside the insect-proof glass-house. The crude suspension of the virus was prepared by macerating 5 g leaves of *N. glutinosa*, infected with SMV 8–10 days earlier, with 5 ml distilled water and squeezing through two layers of muslin cloth. The sap thus obtained was centrifuged at 10,000 rpm for 10 min. The pellet was discarded and the supernatant was taken as the standard virus solution. A standard solution of extracts of different parts of the plants was prepared by macerating 5 g of plant material with 5 ml of distilled water and squeezing later through two layers of muslin cloth. The extract thus obtained was centrifuged at 10,000 rpm for 10 min. The standard extracts of SMV and plant-parts were mixed in the ratio of 1:0.5 and 1:1. After 30 min the plants of *Chenopodium amaranticolor* Coste & Reyn., an indicator host for SMV, were inoculated separately with the mixtures of virus and plant extracts. In each case, five plants having six leaves of approximately the same leaf area were inoculated. Plants inoculated with distilled water in the ratio of 1:0.5 and 1:1 served as control. The number of local lesions evoked after 5–7 days of inoculation was counted. The percentage of inhibition was calculated as: $[(A-B)/A] \times 100$ (where A is the number of lesions in plants inoculated with virus solution diluted in distilled water and B the number of lesions in plants inoculated with virus solution treated with plant extract).

The results presented in table 1 indicate that extracts of all medicinal plants inhibited the spinach mosaic virus to a varying degree. The inhibition effect of plant extracts was directly correlated with increase in the concentration. Highest inhibition of SMV was achieved in the extracts of leaves of *O. sanctum* L., followed by the roots of *G. glabra* L., leaves of *A. arvensis* L., roots of *A. heterophyllum* Wall., leaves of *A. indica* A. Juss., leaves of *C. roseus* (Linn.) G. Don and roots of *R. serpentina* Benth. ex Kurz.

Fukushi[9] demonstrated that digitalin, a glycoside, destroyed the virulence of tobacco mosaic virus (TMV), while Kalichave *et al*[10] reported that quinine reduced the multiplication of TMV. Others[11,12] reported that tannin present in the plant extract was responsible for virus inhibition. It is likely that extracts of plant-parts tested might contain certain compounds which bring about inhibitory effects. This aspect needs further investigation.

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