mobilizing lipids by non-specific esterases at the time of labour.

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ISOLATION OF TENUAZONIC ACID, A PHYTOTOXIN FROM *ALTERNARIA CRASSA* (SACC.) RANDS CAUSING LEAF BLIGHT AND FRUIT ROT OF *DATURA STRAMONIUM* MILL

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LEAF blight and fruit rot disease of *Datura stramonium* caused by *Alternaria crassa* was observed in the experimental fields of this Institute. The symptomatology of the disease indicated that the pathogen might elaborate a toxic metabolite during pathogenesis. Tenuazonic acid produced by *A. alternata*[1], *A. longipes*[2], *A. kikuchiana* and *A. mali*[3] and *Pyricularia oryzae*[4] has been shown to play an important role during pathogenesis. The present studies report the isolation of tenuazonic acid from *A. crassa* infecting *D. stramonium*, its phytotoxic effect on the host plant and the role in disease syndrome.

A pathogenic strain of *A. crassa* isolated from the leaves of *D. stramonium* was employed for these studies. The pathogen was grown in several 500 ml Erlenmeyer flasks filled with a nutrient liquid medium (g/l: glucose, 40; $(NH_4)_2HPO_4$, 2; KH_2PO_4, 11; $MgSO_4\ 7H_2O$, 0.5; KCl, 0.5 and yeast extract, 1.0). The inoculated flasks were incubated for 20 days at 25 $\pm$ 2°C. The culture was filtered through Whatman No. 1 filter paper and the filtrate was used to isolate the toxic metabolite following the method of Janardhanan and Husain[5].

Tenuazonic acid isolated from the culture filtrate of *A. crassa* was identified by TC on silica gel G using chloroform : methanol (90 : 10 v/v) solvent system. The compound was identified by UV and IR spectral analyses.

Toxicity of tenuazonic acid was tested on germinating seeds of *D. stramonium* at 10 μg to 100 μg/ml concentration. Toxicity was also tested on the leaves of *D. stramonium*. A drop of 200 μg/ml toxin was placed on the leaves followed by a fine needle prick. In both the experiments sterilized distilled water was used as control.

Results indicated that *A. crassa* produced significant amount of a toxic metabolite, tenuazonic acid, *in vitro*. TLC analysis of the toxin and the reference compound (tenuazonic acid) showed identical R_f value (0.4) and characteristic colour reaction when sprayed with methanolic $FeCl_3$. The compound had a UV absorption maxima at 220, 277 nm in acidic and 240, 280 nm in alkaline phase. IR spectrum revealed characteristic peaks at 3200, 3040, 1620, 1430 cm^{-1}. Thus the TLC combined with UV and IR spectral analysis confirmed the identity of the compound as tenuazonic acid.

The tenuazonic acid isolated from *A. crassa* induced typical necrosis on *D. stramonium* leaves 24 hr after application. The symptom of the toxicity consisted of necrotic spots surrounded by chlorotic halo which enlarged further resulting in death and defoliation. The toxic symptoms elaborated were almost similar to natural infection. Tenuazonic acid also induced complete inhibition of root and shoot elongation of germinating seeds of *D. stramonium* at 100 μg/ml concentration.

The present studies reveal that tenuazonic acid produced by *A. crassa* has a significant role during the leaf blight and fruit rot disease of *D. stramonium*. The

results also support the earlier findings of the role of tenuazonic acid in *Alternaria* blight of *D. innoxia* caused by *A. alternata*[5] and brown spot of tobacco caused by *A. longipes*[2].

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ACID-TEMPERATURE-SHOCK TREATMENT AS A METHOD FOR INDUCING RESTING SPORE GERMINATION IN SOME TROPICAL SYNCHYTRIA

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RESTING spores in the genus *Synchytrium* De Bary et Woronin are important structures in perpetuating the species. Germination of resting spores has been observed only in 42 of the total more than 200 species[1,2]. The presence of thick exosporium, lack of knowledge concerning the physiology and relatively a difficult task in germinating the resting spores are some of the factors for not determining the germination in the majority of the species. Initial difficulties were also observed in germinating resting spores of some tropical species of *Synchytrium*. Hence methods were evaluated for inducing resting spore germination and the results are presented in this communication.

Five species of tropical Synchytria *viz.*, *S. lagenariae* Mhatre and Mundkur, *S. trichosanthidis* Mhatre and Mundkur, *S. sesamicola* Lacy, *S. brassicae* Singh and Pavgi and *S. akshaiberi* Lingappa, were included in the present study. The dry infected material containing abundant resting spores of the species was soaked in tap water for 24–48 hr until the tissue became soft. The softened material was treated as follows:

(a) a dip in 0.5% con. HCl, H_2SO_4, NaOH or KOH (acid or alkali treatment)
(b) exposure to moist heat at 40–45°C for 1–2 hr (temperature-shock treatment)
(c) exposure of acid treated material to moist heat at 40–45°C for 1–2 hr (acid-temperature-shock treatment).

The treated material was washed under tap water for 30 min. The resting spores were extracted by crushing the material in a pestle and mortar. The host material was separated from the resting spores by repeated filtration through 2–3 layered coarse cloth and spores suspended in clean water were collected by sedimentation. The resting spores were sown for germination on filter papers in moist petri dishes or fixed on microslides by alternate wetting and drying method and incubated[3,4] at 30–35°C.

Among the different acid and alkalies tested, dilute hydrochloric acid was found to be satisfactory in corroding the thick exosporium. The alkalies on the other hand tended to cause damage to the resting spores, thereby making them nongerminable, although results with hydrochloric acid did not yield a very high per cent of germination (60–65%). A very high percentage of germination was secured when HCl-treated resting spores were subjected to moist heat at 40–45°C for 1–2 hr. The germination percentage ranged from 85–90%. This temperature shock-triggered germination could be correlated with the environmental conditions prior to the initiation of the disease. The incidence of *Synchytrium* gall disease of many crops appear in the gangetic plains of north India usually after the onset of monsoon showers in July. The soil (before the rains) is heated to 40–50°C because of the high summer heat prevalent in May–June. When monsoon showers are received the pre-heated soil emits steam or moist heat, which provides the necessary trigger for germination of resting spores. The resting spores present in the soil are known to resist high summer heat without endangering their viability[5]. This principle of temperature-shock treatment proceeded by a dip in acidulated water proved very effective in inducing resting spore germination of dormant resting spores. The technique appears to work well for other tropical species of *Synchytrium*.

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