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Production of a Toxic Metabolite By *Alternaria helianthi* *in vitro* and *in vivo**

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Alternaria helianthi, the leaf spot pathogen of sunflower, produced toxic metabolite in culture, as well as in the infected leaf tissue. The toxin produced necrotic lesions on sunflower leaves. These spots, however, differed from the pathogen produced spots in that, these were not characterized by the yellow halo around. At 2000 ppm, the toxin inhibited sunflower seed germination by 9 per cent. The shoot and root growth was also inhibited when seeds were treated with the toxin at 1000 ppm. The toxin sprayed at 100 ppm resulted in an initial increase in respiration of leaf tissue, which decreased subsequently. The effect, in several respects, paralleled the effect of the pathogen on host respiration.

Many plant pathogenic microorganisms produce toxic metabolites in culture media and in plant tissues which are atleast in part involved in the disease syndrome (Wood *et al*., 1972). Several species of *Alternaria* are known to produce different types of toxic metabolites (Brian *et al*., 1949; Otani *et al*. 1974). The present paper reports on the production of a toxin by *Alternaria helianthi* (Hansf.) Tubaki and Nighihara, the leaf spot pathogen of sunflower (*Helianthus annuus* L.) and its role in the disease syndrome.

MATERIAL AND METHODS

A monoconidial culture of *A. helianthi* isolated from sunflower leaves of the culture EC 68413 was used in the study. Richard's medium, Czapek Dox medium and host leaf extract medium prepared as described elsewhere (Bhaskaran, 1976) were used for toxin production. The media were dispended at the rate of 50 ml. per 250 ml flask and autoclaved at 1 kg/cm² for 20 min. Flasks were incubated at 27°C for 15 days. The toxin was extracted from the culture filterate using peroxide free solvent ether (Purushothaman and Prasad, 1972). The final residue obtained was weighed and it was designated as crude toxin.

The crude toxin was dissolved in 2 ml of distilled methanol and chromatographed on Whatman No. 1 filler paper using a solvent system of n-butanol : acetic acid : water (4 : 1 : 1 v/v). The developed chromatograms were obser-

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ved under the ultra violet (U V) light directly or in the presence of ammonia vapour for the occurrence of fluorescent spots. Various detection reagents like two per cent alcoholic ferric chloride, alcoholic bromophenol blue, Folin Ciocalteu, aniline hydrogen pthalate, alkaline diazotized sulfanilic acid (DSA) and 0.1 per cent ninhydrin in acetone were used and the colour reactions of the spots and Rf values were recorded (Cheronis and Entrikin, 1963).

The fluorescent spot located was cut and eluted in 80 per cent methanol. The solution was evaporated to dryness and taken in spectral grade methanol. The adsorption spectrum of the compound was recorded in a Beckman DU spectrophoto meter. The toxin was isolated from the infected leaves by the procedure described by Purushothaman (1971).

The culture filtrate and different concentrations of the crude toxin were sprayed on 30 day old sunflower plants and 30 day old non-host plants like *Chrysanthemum indicum* L., *Tagetes erecta* L., *Zinnia elegans* L., *Acanthospermum hispidum* DC. and *Tridax procumbens* L., and kept in a moist chamber for 48 hr. Plants sprayed with uninoculated media served as check.

Effect of the toxin on respiration of sunflower leaves was studied by spraying one set of plants with 100 ppm. concentration of the toxin; another set of plants was sprayed with a conidial suspension of *A.helianthi*. The respiratory changes of toxin sprayed and pathogen inoculated sunflower leaves were studied using Warburg's respirometer (Umbreit *et al.*, 1964).

RESULTS AND DISCUSSION

In the preliminary studies, the culture filtrates obtained from the three media *viz.*, Czapek Dox, Richard's and host leaf extract, when sprayed on sunflower leaves, produced necrotic spots of 3 to 7 mm diameter after 72 hr indicating that the pathogen has elaborated some toxic metabolite(s) in the culture filtrate. Of the three media used, more quantity of the metabolite was produced by the pathogen in host leaf extract medium, followed by Czapek Dox and Richard's. The growth rate of the pathogen also followed a similar trend (Table I).

TABLE I. Growth and toxin production by *A. helianthi* in different liquid media

Medium	Mycelial dry weight (mg)	Crude toxin (mg/100 ml medium)
Richard's	96	8.2
Czapek-Dox	108	8.8
Host leaf extract	240	13.1

When different concentrations of the crude toxin were sprayed on sunflower leaves, there was symptom expression at concentrations of 5 ppm and above. However, the symptoms were not identical to those incited by inoculation of the pathogen in that, the pathogen inoculated leaves had an yellow halo around the necrotic spot, whereas the toxin sprayed leaves produced necrotic spots alone. Indicating that in the leaf spot disease of sunflower, the toxin alone might be solely responsible for

symptom production. Other factors are persumably associated with.

The toxin did not produce any symptom on the leaves of non-host plants even at a concentration of 1000 ppm. When the effect of the toxin on sunflower seed germination was tested, it had no effect at concentrations of 200 ppm or below. But at concentration of 2,000 ppm, it inhibited seed germination by nine per cent (Table II). The

TABLE II. Effect of toxin treatment on the germination of sunflower seeds

Toxin concentration (ppm)	Seed germination (per cent)
2000	91 (3.00)
1000	94 (2.45)
500	99 (1.00)
200	100 (0.00)
100	100 (0.00)
Control	100 (0.00)
C.D. (P=0.05)	0.72

Data in parentheses are transformed values ($\sqrt{100-X}$ transformation)

TABLE III. Effect of toxin treatment (1000 ppm) on shoot and root length (cm) of sunflower seedlings

	Age of the seedlings (days)				
	3	4	5	6	7
Healthy shoot	*0.2	1.0	2.0	6.3	10.9
Toxin treated shoot	0.0	0.8	1.5	5.5	10.2
Healthy root	0.5	4.0	6.6	10.2	10.5
Toxin treated root	0.0	3.3	6.2	9.5	10.4

* Average of 50 seedlings in five replications

	C.D. (P=0.05)	
	Shoot	Root
Treatments	0.2	0.3
Age of seedlings	0.3	0.4
Interaction	NS	NS

shoot and root lengths were inhibited markedly, when the seeds were treated with a 1000 ppm solution of the toxic metabolite (Table III). Such stunting effect on the growth of the host plant has been reported for the toxin produced by *A. solani* also. Brian *et al.* (1959) stated that alternaric acid severely retarded the growth of radish, mustard and tomato seedlings.

Leaves of sunflower plants were sprayed with 100 ppm concentration of the toxin and its effect on the respiratory changes of the leaf was compared with that of pathogen inoculated leaves. Immediately after the treatment, there was an increase in the respiratory rate in toxin treated as well as in the pathogen inoculated leaves. In the necrotic lesion produced by the toxin and that produced by fungus inoculation, there was an initial increase in respiration (on 3rd day) when compared th the healthy leaves. But the resiratory rate decreased subsequently from 5th day onwards. Thus, the respiratory rate of the toxin treated and pathogen inoculated leaves followed the same pattern (Table IV). This indicates that the toxic metabolite of the pathogen may play a role in the deranged metabolism of the sunflower leaves infected by *A. helianthi*.

The toxin isolated from the culture filtrate and that from the infected sunflower leaves were separated on a chromatogram and both of them were found to have the same Rf value(0.268) and the colour reaction of the spots to various detection reagents were also identical. The compound gave an yellow fluorescence under U.V. light either in

TABLE IV. Effect of toxin (100 ppm) and pathogen on the respiratory activity* of sunflower leaf (Average of four estimations)

Sampling time	Healthy	Toxin treated		Pathogen inoculated		
		Necrotic	Apparently healthy	Necrotic	Halo	Apparently healthy
0 hr	2216	**	2280	**	**	2233
24 hr	2315	**	3632	**	**	3133
3rd day	2275	2855	3757	3612	**	3538
5th day	2308	1912	3648	3585	**	3680
8th day	2105	1633	3833	1833	4917	4283

* μl of O_2/hr/g dry weight of tissue

** Necrotic spot and halo not formed

the presence or absence or ammonia vapour. The colour of the spot on chromatogram was brick red with DSA, blue with Folin-Ciocalteu reagent and yellow with alcoholic bromophenolblue. It gave no colour reaction with alcoholic ferric chloride, aniline hydrogen pthalate and ninhydrin reagent. The U.V. absorption spectrum of the compound gave a peak between 260 and 265 nm wavelength. The reaction of the spot to various detection reagents and the U.V absorption spectrum reveal that the toxin isolated presumably be phenolic in nature.

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