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Factors Influencing Toxin Production by *Alternaria macrospora* Zimm.

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The pathogen *Alternaria macrospora* Zimm. produced toxic metabolite(s) in culture. Czapek's medium was found to be the best medium for toxin production. Maximum toxin production was noted after 16 days. Sucrose as carbon source, ammonium oxalate and urea as inorganic and organic nitrogen sources favoured maximum toxin production. Toxin production was maximum at pH 6 and 7 and at temperature of 27°C.

The importance of toxins on the causation of plant diseases is increasingly recognized. There are numerous reports available to demonstrate the production by pathogenic fungi and bacteria *in vitro* of substances that are toxic to plants. Sufficient evidence has accumulated to establish the role of toxin in the development of leaf spot diseases (Pringle and Scheffer, 1963; and Chandrasekharan Nair, 1972). *Alternaria macrospora* produced a toxin which was assayed by different techniques (Padmanaban and Narayanasamy, 1974). The influence of various factors affecting the toxin production by *Alternaria macrospora* Zimm. has been studied and the results are reported in this communication.

MATERIALS AND METHODS

The bacterial inhibition bioassay adopted by Kalyanasundaram (1954) was modified and followed to assay the activity of toxin produced by *Alternaria macrospora* grown under different conditions. Nutrient agar medium was melted, cooled and a heavy suspension of *Bacillus subtilis* Cohn. emund Prasmoviski was mixed with the medium. Twenty ml of the medium was poured in each Petri dish and was allowed to set. Then a well was dug out at the centre of the Petri dish using a 10 mm cork borer and 0.2 ml of the culture filtrate or uninoculated medium or sterile water was transferred to the well by means of a sterile pipette. Four replications were maintained. After 8 hours of incubation at room temperature the diameter of the inhibition zone developed around the well was measured. The results were expressed as the percentage mean area of inhibition zone in transformed value.

RESULTS AND DISCUSSION

Influence of carbon sources on toxin production

The fungus was grown in Czapek's medium amended with the addition of various carbon sources. Medium without any carbon source was

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kept as control. The culture filtrate was obtained after an incubation period of 16 days and toxin production was assayed by bacterial bioassay technique. Sucrose was the best carbon source in inducing maximum toxin production followed by galactose (Table I).

TABLE I. Effect of carbon sources on toxin production

Treatment	Toxin activity in mean area of inhibition zone in sq. mm. (Transformed value)
Sucrose	22.61
Fructose	9.88
Glucose	9.61
Galactose	16.13
Maltose	6.99
Lactose	7.69
Xylose	9.32
Sorbose	5.92
Arabinose	8.99
Starch	6.99
Dextrin	12.45
Glycogen	15.60
Control	0.71
C. D. (P=0.01)	1.42

Brain *et al.* (1951) have reported sucrose in the medium to be more suitable for producing high toxin titre in the case of *Alternaria solani*. Chandrasekharan Nair (1972) reported better toxin production by *Colletotrichum capsici* when sucrose was used as the carbon source in the medium.

Influence of nitrogen sources on toxin production

The fungus was grown in Czapek's medium incorporated with different inorganic and organic nitrogen sources. Medium without any nitrogen source was kept as control. The culture filtrate was obtained after an incubation period of 16 days and toxin production was assayed by bacterial bioassay technique.

Toxin production was maximum in ammonium oxalate followed by ammonium phosphate, and ammonium nitrate was significantly superior to other treatments (Table II

TABLE II. Effect of inorganic nitrogen sources on toxin production

Treatment	Mean area of inhibition zone in sq. mm. (Transformed value)
Ammonium nitrate	17.86
Ammonium sulphate	12.74
Ammonium chloride	12.46
Ammonium phosphate	18 60
Ammonium oxalate	19.34
Potassium nitrate	7.69
Sodium nitrite	6.54
Sodium nitrate	12.71
Control	0.71
C. D. (P=0.01)	1.59

Toxin production was better induced by urea followed by peptone and casein. Asparagin and beef extract were not suitable for toxin production (Table III). The favourable action of ammonium salts on toxin production by *Alternaria* spp. has been reported by many workers. Brian *et al.*, (1951) observed better toxin production by *Alternaria solani*, when ammonium nitrate was used as nitrogen source. Sathiabalan Samuel (1969) found ammonium oxalate, ammonium nitrate, peptone and yeast extract as the best sources of nitrogen for higher toxin

TABLE III. Effect of organic nitrogen sources on toxin production

Treatment	Mean area of inhibition zone in sq. mm. (Transformed value)
Peptone	18.35
Beef extract	11.08
Casein	12.74
Asparagin	11 64
Urea	20.30
Control	0.71
C. D. (P=0.01)	0.93

production. Arjunan (1970) also reported ammonium sulphate as the best source of nitrogen for the toxin production by *Helminthosporium turcicum*.

Influence of pH on toxin production

The pH of Czapek's medium was adjusted to 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 using Trombay pH meter. The test fungus was grown for a period of 16 days. The culture filtrate was assayed for toxin content. Production of toxin was favoured to the maximum extent by pH 6 and pH 7 (Table IV).

TABLE IV. Effect of different pH levels on the toxin production

Treatment	Mean area of inhibition zone in sq. mm. (Transformed value)
pH 2	0.71
pH 3	0.71
pH 4	22.71
pH 5	21.25
pH 6	27.42
pH 7	27.42
pH 8	22.71
pH 9	21.27
pH 10	19.33
pH 11	15.60
C. D. (P=0.01)	2.61

Sathiabalan Samuel (1969) observed that maximum toxin production by *Alternaria sesami* in the media with initial pH of 6.0 and 7.0. The toxin production was favoured at the same pH range in the case of *Pyricularia setariae* (Subramanian, 1969) and *Colletotrichum capsici* (Chandrasekharan Nair, 1972).

Influence of temperature on toxin production

The fungus was grown in Czapek's medium and incubated at different temperatures viz., 0, 5, 10, 15, 20, 27, 30 and 40°C. The culture filtrate was assayed for toxin content (Table V)

TABLE V. Effect of different temperature on toxin production

Treatment	Mean area of inhibition zone in sq. mm. (Transformed value)
0° C	0.71
5° C	0.71
10° C	6.29
15° C	8.99
20° C	20.54
27° C	20.79
30° C	9.02
40° C	0.71
C. D. (P=0.01)	1.58

Maximum amount of toxin was produced when the fungus was incubated at 27°C and 20°C. The influence of temperature on toxin production by *Colletotrichum capsici* (Syd.) Butl. & Bisby was distinctly revealed by the studies of Chandrasekharan Nair (1972) who observed maximum toxin production at 28—30°C.

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