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# TOXIN PRODUCTION BY *CURVULARIA LUNATA* VAR. *AERIA* CAUSING LEAF BLIGHT IN FRENCH BEAN (*PHASEOLUS VULGARIS*)[1]

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## ABSTRACT

*Curvularia lunata*, highly pathogenic to bean leaves produced thermostable toxic principle in synthetic media which induced symptoms similar to the disease. Toxin production was maximal after 12 days of incubation in Fries modified No. 3 medium at 30°C. Cultural conditions greatly influenced toxin production. Sucrose served as the best carbon source and ammonium sulphate and ammonium tartarate as the best nitrogen source for toxin production. The findings also confirmed that the toxin produced by *Curvularia lunata* is non specific.

## INTRODUCTION

Visible symptoms of leaf blight of french bean (*Phaseolus vulgaris*) are caused not only by *Curvularia lunata* var. *aeria* but also by the toxin produced by it. The first visible symptoms could be observed 4 to 5 days after inoculation as slight chlorotic and faint brown spots. After 7 days, dark lesions had appeared and 8 days after inoculation extensive chlorosis and necrosis occurred in large areas of the leaves. This rapid rate of symptom development led us to the hypothesis that a toxin is involved in symptom development. In this paper, we report our results on the optimum condtions required for the production of the toxin.

## MATERIAL AND METHODS

*C. lunata* var. *aebria* was isolated from diseased leaves of local fields. The fungus was grown on a shallow layer of Fries modified 3 medium (Pringle and Scheffer, 1963) containing 30 g sucrose, 5 g ammonium tratarate, 1 g $NH_4NO_3$, 1 g $KH_2PO_4$, 0.5 g $MgSO_4$. $7H_2O$, 0.1 g NaCl, 0.13 g $CaCl_2$. $2H_2O$, 1 g Difco yeast extract and distilled water to 1000 ml. Aliquots of 50 ml of basal medium were dispensed in 500 ml Erlenmeyer flasks, sterilized, inoculated and kept for 16 days at 30 oC. After incubation of 2, 4, 6, 8 10, 12 14 and 16 days the mycelial mats were harvested and washed several times with distilled water so as to remove the salts and colloidal particles associated with the myceliim. Filter papers containing mycelial mats were dried at 70°C in an electric oven for 3 days to obtain constant weight and reweighed. Culture filtrate was obtained, final pH was noted and used for toxin assays after centrifugation at 5000 ×g.

Before testing for toxin content the culture medium was filter through

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0.22u bacterial filter. Toxin assay was done by suspending host cuttings. Plants were grown in soil at room temperature. Petioles were excised from the stem under water and the cuttings immediately placed in serial dilutions of the toxic preparations under continuous cool white flourescent light at room temperature. The highest dilution at which necrosis of the leaf blade was observed after 48 hours was recorded as the end point; the reciprocal of this end point was taken arbitrarily as the number of activity units of the toxin per ml of the original solution e.g 1/10-10 units $ml^{-1}$.

Uninoculated Fries modified 3 medium was used as control. Each value represents average of 3 replicates with 3 plants each. The influence of environmental factors (temperature, pH, carbohydrate concentration, and nitrogen concentration) on toxin production was tested in a like manner.

## OBSERVATIONS

### I. *Time course of Toxin Production:*

The results in Fig. 1 revealed that the culture filtrate of *C. lunata* contained toxic principle, which was first detected after 4 days at a dilution of 1/4. The toxin production rose from this value to 1000 units $ml^{-1}$ in 12 days and later on remained constant. Mycelial dry weight followed a similar trend but it gradually decreased after reaching a maximum in 14 days. Whereas the amount of toxin remained constant up to the end of the incubation period and the toxin titre remained stable for at least 30 days of incubation.

### II. *Effect of temperature on toxin production:*

Toxin production was maximum at 30 °C (Fig. 2). The pathogen failed to grow at and below 10°C and above 40°C temperature. The toxin in the culture filtrate was heat stable (121 °C for 15 minutes at 15 psi).

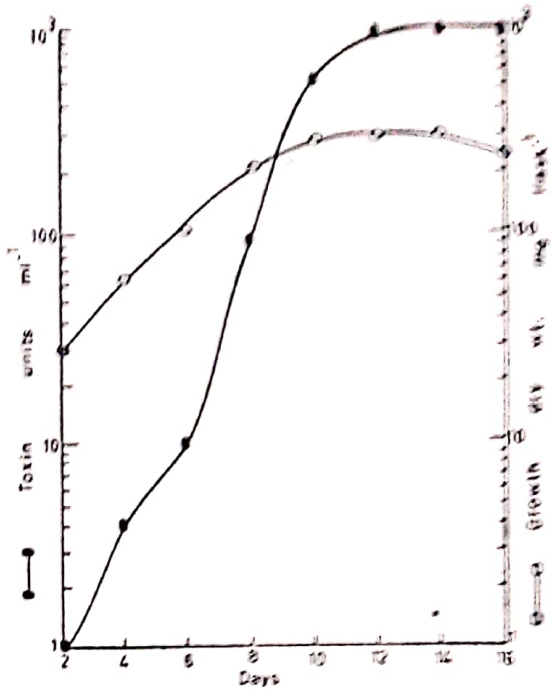


Fig. 1 : Time course curve of toxin production by *C. lunata*

### III. *Effect of pH on toxin production :*

Toxin production was maximum at pH 5.0. There was a sharp decline in the toxicity of culture filtrate above and below the optimum pH (Fig. 3).

### IV. *Effect of sugars :*

When various carbohydrates were substituted at equal sources content basis in Fries modified 3 medium, sucrose induced maximum toxin production which was followed by maltose, glucose, fructose starch, arabinose and glacatose. Raffinose was a very poor source. However, growth was maximum in arabinose followed by maltose sucrose, glucose, galactose starch, fructose and raffinose (Table I).

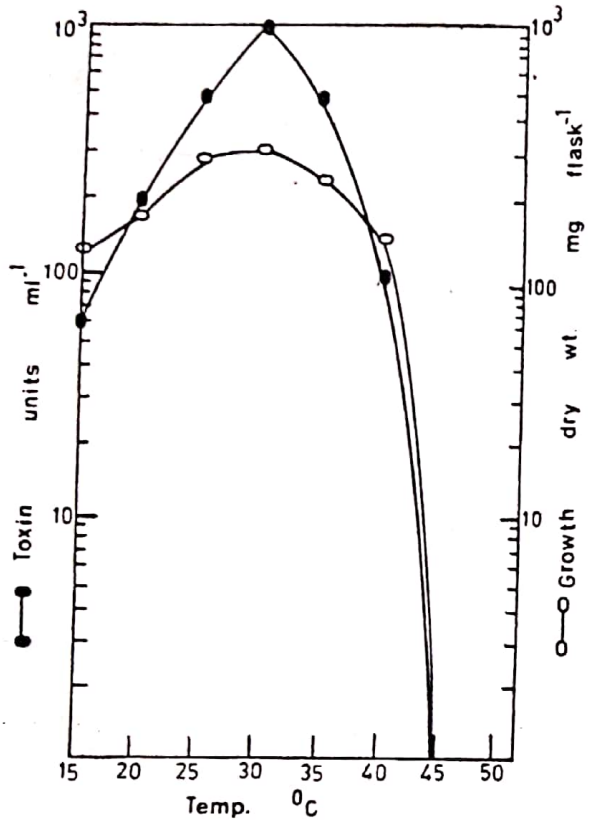


Fig. 2. Effect of temperature on toxin production and growth of *C. lunata*

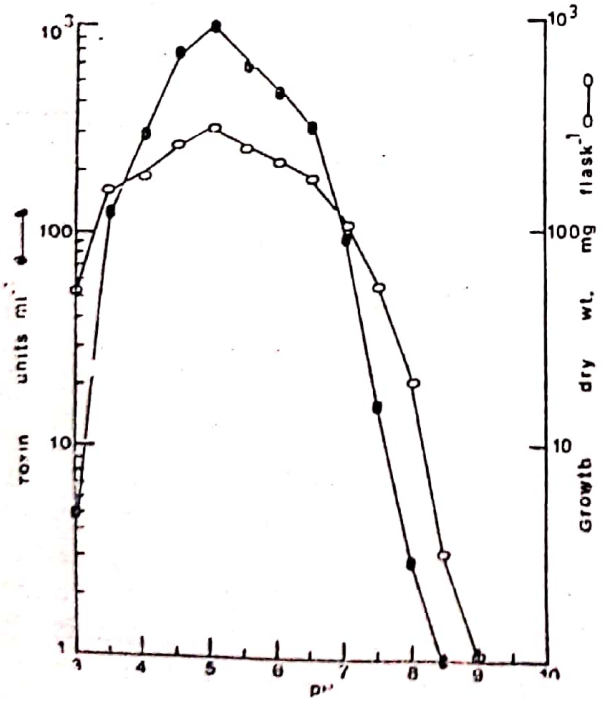


Fig. 3. Effect of pH on toxin production and of *C. lunata*.

TABLE I

EFFECT OF DIFFERENT CARBOHYDRATES IN INDUCTION OF TOXIN PRODUCTION BY *CURVULARIA LUNATA* (AFTER 15 DAYS OF GROWTH)

| Carbon source | Growth mg flask$^{-1}$ | Toxin units ml$^{-1}$ |
|---|---|---|
| Glucose | 190 | 450 |
| Sucrose | 320 | 1000 |
| Fructose | 175 | 320 |
| Maltose | 340 | 600 |
| Arabinose | 365 | 150 |
| Raffinose | 150 | 10 |
| Galactose | 190 | 100 |
| Starch | 185 | 200 |

Effect of various concentrations of sucrose on toxin production was investigated (Table II). Toxin production increased with increase in amount of sucrose from 1 to 3% but further increase in the amount of sucrose up to 10% had no effect on toxin production. However, mycelial dry weight increased

TABLE II

EFFECT OF DIFFERENT CONCENTRATIONS OF SUCROSE ON TOXIN PRODUCTION BY *CURVULARIA LUNATA* (AFTER 15 DAYS OF GROWTH)

| Per cent sucrose concentration | Growth mg flask$^{-1}$ | Toxin units ml$^{-1}$ |
|---|---|---|
| 1 | 155 | 5 |
| 1.5 | 223 | 80 |
| 2 | 290 | 250 |
| 2.5 | 310 | 500 |
| 3 | 355 | 1000 |
| 5 | 365 | 1000 |
| 10 | 410 | 1000 |

progressively in media containing upto 10% sucrose.

V. *Effect of nitrogen source:*

Table III revealed that among the various nitrogen sources inorganic nitrogen sources were superior to organic nitrogen sources. Ammonium sulphate, ammonium tartarate, sodium nitrate, ammonium nitrate and ammonium oxalate markedly favoured toxin production by *C. lunata*.

TABLE III

EFFECT OF DIFFERENT NITROGEN SOURCES ON TOXIN PRODUCTION BY *CURVULARIA LUNATA* (AFTER 15 DAYS OF GROWTH)

| Nitrogen sources | Growth mg flask$^{-1}$ | Toxin units ml$^{-1}$ |
|---|---|---|
| Ammonium sulphate | 210 | 1000 |
| Ammonium nitrate | 258 | 750 |
| Sodium nitrate | 270 | 840 |
| Ammonium tartarate | 365 | 1000 |
| Ammonium chloride | 262 | 500 |
| Potassium nitrate | 255 | 250 |
| Asparagine | 405 | 35 |
| Glutamic acid | 285 | 10 |
| Glycine | 305 | 20 |
| Tryptophane | 362 | 60 |

VI. *Dilution end point:*

Fifteen day old culture filtrate was highly active up to 1.80 dilution but further dilution reduced the activity markedly and 1:1000 was the dilution end point (Table IV).

VII. *Host specificity:*

Table V shows the reaction of various plant species to fungal toxins. Red kidney bean (*Phaseolus vulgaris* L) was highly senstive to the toxin. Cowpea and broad bean were not infected by the pathogen but were extremely sensitive to the toxins showing total collapse and chlorosis within 48 hours. All other test plants were entirely or almost unaffected by the fungus and its toxin.

## DISCUSSION

It has been observed that *C. lunata* produced toxic substances; the toxin production rose to maximum on the 12th day in culture medium and remained stable for at least 30 days. Luke and Wheeler (1955) observed that maximum toxin production by *Helminthosporium victoriae* occurred between 13 to 21 days and then declined. Smedegard and Nelson (1969) reported maximum toxin production by *H. maydis* after 13 to 17 days in Fries medium. Nisa and Bisen (1982) found that *Coniella granati* produced maximum toxin after 25 days of incubation. Bisen *et al.* (1983) observed that maximum toxin production by three pathotypes of *Pseudomonas phaseolicola* on the 9th day. Onesirosan *et al.* (1975) reported maximum toxin production by *Corynespora cassiicola* between 12 to 30 days.

Culture filtrate retained its activity even after autoclaving at 121° C for 15 minutes which indicated its thermostable nature. Thermostability appears to be a basic characteristic of toxin (Luke and Wheeler, 1955; Das and Srivastave, 1969; Sundheim, 1972 Maity and Samaddar, 1977; Vijaya Kumar and Rao , 1979).

Naef-Roth (1972) noticed that lycomarasmin one of the toxins of wilt inducing species of *Fusarium*, when injected to the stem of tomat plant injures the leaves severely, whereas the stem remains undamaged. Ballio *et al.* (1972), Main and Pero (1972) and

TABLE IV

EFFECT ON BEAN LEAF OF A CULTURE FILTRATE (15 DAYS OLD) OF *CURVULARIA LUNATA*

| Concentration of culture filtrate | Phytotoxic damage rating* at time t (hours) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Culture filtrate | | | | Medium (Control) | | | |
| | 24 | 48 | 72 | 96 | 24 | 48 | 72 | 96 |
| Cell free culture filtrate | 5 | 5 | 5 | 5 | 0 | 0 | 1 | 1.5 |
| 1 : 1 | 5 | 5 | 5 | 5 | 0 | 0 | 0 | 0 |
| 1 : 10 | 5 | 5 | 5 | 5 | 0 | 0 | 0.5 | 0.5 |
| 1 : 20 | 4 | 5 | 5 | 5 | 0 | 0 | 0.5 | 1 |
| 1 : 40 | 4 | 5 | 5 | 5 | 0 | 0 | 0.5 | 0.5 |
| 1 : 80 | 3 | 4 | 5 | 5 | 0 | 0 | 0 | 1 |
| 1 : 160 | 2 | 3 | 4 | 5 | 0 | 0 | 0 | 0 |
| 1 : 320 | 2 | 2.5 | 3 | 5 | 0 | 0 | 0.5 | 1 |
| 1 : 640 | 1.5 | 2 | 3 | 4 | 0 | 0 | 0.5 | 0.5 |
| 1 : 750 | 1 | 1.5 | 2 | 3 | 0 | 0 | 0 | 0 |
| 1 : 1000 | 0.5 | 1 | 1.5 | 2 | 0 | 0 | 0 | 0 |

Cutting from 2 week old seedlings

* Rating scale 0=no symptoms to 5=complete chlorosis and collapse of plant cuttings were rated after exposure for indicated time. Each value represents the average of 3 replicates with 3 cuttings each.

TABLE V

RELATIONSHIP BETWEEN SUSCEPTIBILITY TO THE CULTURE FILTRATE OF *CURVULARIA LUNATA* AND DISEASE REACTION OF VARIOUS PLANTS (15 DAYS OLD CULTURE FILTRATE)

| Plant species | *Phytotoxic damage rating | | | |
|---|---|---|---|---|
| | Culture filtrate | | Medium (control) | |
| | 24 hours | 48 hours | 24 hours | 48 hours |
| Red Kidney | 3 | 4.5 | 0 | 0.5 |
| Cowpea | 2.5 | 4 | 0.5 | 1 |
| Broad bean | 1 | 1.5 | 0 | 0 |
| Tomato | 0 | 0.5 | 0 | 0.5 |
| Brinjal | 0 | 1 | 0 | 0.5 |
| Chillies | 0 | 1 | 0 | 1 |
| Pea | 0 | 0 | 0 | 0 |

*Cuttings from 2—3 week old seedlings

* Rating scale 0—no symptoms to 5— complete chlorosis and collapse of cuttings. Cuttings were rated after 24 and 48 hours of exposure.

Each value represents average of 3 replicates with 3 plants each.

Schlosser (1972) reported that the host tissues showed disease symptoms when the tissues were infected by the toxins produced by the pathogen. Bean leaves were affected by cell free culture filtrate obtained from 15 day old culture.

Our data further confirmed that the toxin produced by *C. lunata* is a nonsspecific one as it expressed symptoms on various other plants.

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