

THE ROLE OF TOXIN PRODUCED FROM *FUSARIUM OXYSPORUM* VAR. *LATHYRI* IN PRODUCTION OF WILT SYNDROME OF *LATHYRUS SATIVUS* L.

BY

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The present investigation has been undertaken in order to study the role of fungal toxin in disease development. In present investigation, a toxin was isolated from *F. oxysporum* var *lathyri* which causes wilt disease of *Lathyrus sativus*. The effect of the fungal toxin on healthy plant was shown by the production of characteristic disease symptoms when plants were treated with different concentration of toxin solution (1 : 50, 1 : 100, 1 : 500, 1 : 1000, 1 : 1500 and 1 : 2000). Severe wilting and maximum effect was noticed at 1 : 50 concentration. The toxin was found to be a Nivalenol diacetate compound.

INTRODUCTION

The role of microbial toxin in plant pathogenesis is well known. The toxin production capabilities of several formal specials of *Fusarium* are also shown by several researchers. The toxic nature of fusaric acid from *F. heterosporium* has been first recognised by Gaumann *et al* (1952) and Later they reported its occurrence from *F. oxysporum* f. sp. *Vasinfectum*, *F. oxysporum* f. sp. *Udum*. In 1966, Narayanan *et al* have shown that fusaric acid production from *F. vasinfectum* causing wilt of cotton is highest when the organism grows in extracts from host. Mall (1973) has detected fusaric acid *in vitro* and *in vivo* from the isolates of *F. oxysporum* f. *Tuberosii* causing potato wilt. According to him fusaric acid is selective in its production and action and its phytotoxic role in depend on its specific production. Prasad and Chowdhury (1974) have shown *in vitro* production of fusaric acid from *F. oxysporum* f. sp. *Udum* which causes wilt of *Cajanus cajan*. Szathamary *et al* (1976) have identified mycotoxins from species of *Fusarium* and *Stachybotrys* using TIC, CIC, gas chromatography and Mass spectrometry. Siegfried and Langerfeld (1978) have demonstrated toxin production from several species of *Fusarium*. Ghosal *et al* (1978) have also shown the toxin production from *Fusarium oxysporum* causing fusarial wilt of safflower. In the present experiment, an attempt has been made to show the production of toxins in culture filtrate of *F. oxysporum* f. *lathyri* and its role on wilting of *Lathyrus sativus*.

## MATERIALS AND METHODS

In order to study the toxin production, the test fungus was grown in Fries Medium. 50 ml of medium was taken in each Erlenmeyer flask (250 ml), plugged and sterilized at 15 lbs pressure for 15 minutes. Finally the flasks were inoculated with one uniform disc of inocula per flask (5 mm agar disc with actively growing mycelium of *F. oxysporum* var *lathyri* were cut out from 7 days old culture) and incubated for 21 days at 25°C. Then mycelial mat and culture filtrate were separated. Mycelial mats were washed with distilled water, dried at 60°C for 24 hours and then was kept in desiccator for 24 hours. After then it was powdered in a Willey mill and passed through 60 screen mesh.

The powdered mycelial grains were extracted with 95% ethanol. After proper grinding, the mixed solutions was centrifuged at 5000 r.p.m. for 15 minutes. Then the supernatant was decanted off and stored for chromatography for the detection of toxin.

The culture filtrate after harvesting was centrifuged at 20,000g for 30 minutes and the pellet was discarded. The supernatant liquid was concentrated at 80°C in water bath to approximately one fifth of its original volume. Then the preparation was treated with eight volumes of 95% ethanol, and the preparation was allowed to precipitate at 4°C overnight. The precipitate at 4°C overnight. The precipitate was then collected by centrifugation at 8,000g for 10 minutes. The pellet was dissolved in distilled water and passed through a 20.0 cm x 1.5 cm column of Dowex 50w-x8(20-50, U. S. mesh H<sup>+</sup> form) and then a 20.0 cm x 1.5 cm column of Dowex 50w-x8(20-50, U. S. mesh, formate form) to remove charged compounds and partially purified toxin was thus obtained. The toxin was further purified by passing it through a column of Sephadex G-25 (medium).

The toxin extracts were spotted on Silica gel G plate for thin layer chromatography using a mixture of chloroform : methanol : water (4.0 : 1.5 : 0.5) as a solvent. After running the solvent up to demarkation the plate was completely dried and spot was developed by spraying with concentrated sulphuric acids.

### Biological Test of Toxin on Seedlings

10 days seedlings was harvested intact from the earthen ware pots. The roots of seedling were washed thoroughly with continuous tap water and transferred to the conical flask containing different graded of toxin solutions (1 : 50 ; 1 : 100, 1 : 200, 1 : 500, 1 : 1000, 1 : 1500, 1 : 2000). After seven days, the change was noticed and the seedlings were weighed. Then the seedlings were dried at hot air oven at 60°C for 24 hours and after that stored in desiccator for 2-3 hours and again weighed.

## RESULTS AND DISCUSSIONS

The results obtained during the experimental period are recorded in Table 1.

The experimental data clearly indicate that root growth of the plant is heavily affected by toxins. Dry weight of *Lathyrus* root is decreased when 1 : 50, dilution of toxin concentration is applied. The dry weight of roots gradually increased towards normal when the dilutions are increased. It further reveals that the protein content of *Lathyrus* plant is also decreased when 1 : 50 dilution is applied. Again the protein content gradually increase when the dilution is gradually increased.

It has been found that when normal plants are placed in several dilutions of culture filtrate toxins, typical wilt symptoms appear in the plants. The effect of toxin is active up to 1 : 2,000 dilution.

TABLE 1. Data\* (mean) showing the effect of different concentrations of toxin on the root growth and protein content of *lythyrus* plant

| Conc. of Toxins | Root growth Dry wt. (mg) | Protein (%)      |
|-----------------|--------------------------|------------------|
| 1 : 50          | 25.25 $\pm$ 1.20         | 76.25 $\pm$ 1.26 |
| 1 : 100         | 29.25 $\pm$ 1.24         | 80.00 $\pm$ 1.40 |
| 1 : 200         | 35.00 $\pm$ 1.12         | 85.00 $\pm$ 1.40 |
| 1 : 500         | 38.00 $\pm$ 1.26         | 88.75 $\pm$ 1.20 |
| 1 : 1000        | 42.50 $\pm$ 1.20         | 81.25 $\pm$ 1.40 |
| 1 : 1500        | 54.00 $\pm$ 1.20         | 88.75 $\pm$ 1.26 |
| 1 : 2000        | 64.50 $\pm$ 1.40         | 96.25 $\pm$ 1.36 |
| Distilled water | 97.00 $\pm$ 1.60         | 97.50 $\pm$ 1.40 |

\*Data are expressed as average of five separate determinations  $\pm$  S. E. M.

The toxin has been purified and run through thin layer chromatography. It shows a purple brown coloured spot and becomes churred spot and fluoresces under long wave UV light. The  $r_f$  value of the spot is 0.91 and it is assumed to be Nivanlenol diacetate compound.

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