
Non-host Specific Phytotoxin of *Phomopsis Vexans*

SITANSU PAN, SK. J. ISLAM AND SHRESTHA DUTTA

Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur,
Nadia 741252, West Bengal

Phomopsis vexans, the causal organism of leaf blight and fruit rot of brinjal, produced non-host specific phytotoxin(s) in culture medium. The toxin(s) produced showed a positive correlation with the virulence of the pathogen to a limited extent. The symptoms produced on groundnut leaves following dipping of the seedlings in different concentrations of toxin(s) preparation were not identical to the natural one. The results suggested the presence of toxic principle(s) in the culture filtrate of the pathogen and its effect(s) to be non-host specific. The toxic principle(s) was thought to act as secondary determinant of disease.

Key words : Toxin(s), *Phomopsis vexans*, brinjal, leaf blight, fruit rot

INTRODUCTION

Toxic metabolites are commonly produced by plant pathogenic fungi (Rudolph, 1976). Some of these toxic metabolites are specific to only certain genotypes of higher plants or hosts (Nishimura and Kohmoto, 1983 ; Scheffer and Livingston, 1984). However, none of the non-host specific toxins have been critically evaluated for their possible roles in plant diseases. But several of the host specific toxins have undergone extensive analyses (Yoder, 1980; Sen, 1988). Non-host specific toxin(s), however, have been reviewed by Wheeler and Luke (1963) and Mitchell (1984). In the present experiment attempts have been made to study the role of metabolic byproducts of *Phomopsis vexans* (Sacc. & Syd.) Harter in pathogenesis and disease development.

MATERIALS AND METHODS

Three isolates of the pathogen viz., 25F, 17L and 10S with varied levels of virulence (Islam and Pan, 1990) were grown for 15 days at $28 \pm 1^\circ\text{C}$ in 250 ml Erlenmeyer flasks each containing 50 ml Richard's synthetic broth medium. The fungal mats were harvested by filtering through folds of Whatman No.1 filter paper. The filtrates were centrifuged separately for each isolate at 2000 g for 15 min in a model PR-2 International Refrigerated Centrifuge. The decant was collected and finally passed through a sterilized sintered glass filter (IG-5) to ensure that all fungal spores and mycelial fragments, if any, have been completely removed.

Partial purification of toxin(s)

Each cultural filtrate was extracted three times with ethyl acetate : water (1:1, v/v) and the organic layer was discarded. The pH of the aqueous layer was adjusted to 3.0 by adding required amount of 0.1 N HCl and re-extracted thrice with ethyl acetate : water (1:1, v/v) and the pH of the aqueous layer was readjusted to 6.8 with the help of 0.1 N NaOH and used as partially purified toxin in various investigations.

Biossay of toxin(s)

Assay of the toxin filtrate was first tried on sprouted eggplant seeds (cv. Pusa Purple Cluster). The root and shoot primordia did not emerge separately from the germinated seeds and the emerged sprout differentiated into root and shoot. The effect of toxin(s) on elongation of root and plumule thereby could not be studied on eggplant seeds. Toxin(s) preparation was finally assayed on sprouted rice seeds (cv. IR-36) adopting the technique of Samaddar and Scheffer (1968) with modification (Pan and Sen, 1986). The toxin(s) preparation was diluted as N, N/2, N/4 and N/8 (N = undiluted concentration) using sterile distilled water. Five ml of each dilution was pipetted for each dilution separately. Ten uniform, just sprouted rice seeds were placed on folded sterilised filter paper in each sterilised Petriplate. Suitable controls were maintained using similar dilutions of the uninoculated medium. The observations on radicle and plumule elongation was recorded after 72 h incubation at $28 \pm 1^\circ\text{C}$.

Translocability of the toxin(s) preparation was tested on 15 days old eggplant seedlings (cv. Pusa Purple Cluster). The seedlings exhibited general wilting of the leaves without manifestation of characteristic symptoms. The subsequent studies were then made on 15 days old groundnut seedlings (cv. JL-20) as described earlier (Pan and Sen, 1986).

RESULTS AND DISCUSSION

It appeared from the results (Table 1) that the roots were more sensitive to the toxic effect of culture filtrate compared to shoots. In culture filtrate the root and shoot development expressed as length in mm was always lower than that of corresponding development in uninoculated control. The nutritional support provided by the uninoculated control medium, particularly in higher dilutions (N/4 and N/8) contributed to growth and vigour of the radicle and plumule of the germinated rice seeds. The results also showed that the medium used inhibited shoot and root growth significantly at normal dilution. At lower dilutions the growth of root and shoot was stimulated. In culture filtrates, treated similarly, response was similar but the degree of inhibition was much higher showing thereby that some inhibitory substance(s) was secreted in the medium by the pathogen. At the highest dilution root growth was same as in water while in diluted uninoculated medium it was almost double. The results further showed that among the three isolates the toxin preparation of the most virulent isolate, 25F, was more inhibitory to root and shoot development than other two. When the germinated rice seeds were kept in the toxin(s) preparation for longer period (72-96 h) the features like development of root tip necrosis, formation of necrotic spots on the root surface in all isolates and coiling of roots in some cases were noted. Coiling of roots was probably due to interference of the toxic principle with normal physiological processes of the germinated rice seeds.

Table 1. Effect of different concentrations of toxin(s) produced by three isolates of *P. vexans* on the radicle and plumule growth of germinated rice seed (IR-36)

Isolates ^{ba}	Length ^b in mm in different dilutions							
	N ^c		N/2 ^c		N/4 ^c		N/8 ^c	
	R ^d	P ^e	R ^d	P ^e	R ^d	P ^e	R ^d	P ^e
25 F	9.5	5.4	33.5	14.6	48.6	28.4	55.3	34.3
17 L	10.0	8.0	41.1	17.6	51.1	30.8	56.5	35.4
10 S	10.3	8.3	41.7	19.8	52.8	32.5	58.9	36.2
Uninoculated control	21.8	7.9	47.2	21.0	72.3	42.5	96.7	52.0
Water control	53.4	24.5						

^a25F > 17 L > 10S isolates in relative degree of virulence

^bMean of hundred observations in each dilution

^cN, N/2, N/4 and N/8 represent folds of dilution over normal (N)

^dR = Radicle, ^eP= Plumule

Dipping of roots of groundnut seedlings in undiluted culture filtrate (N) produced spherical to irregular brown spots surrounded by a zone of yellow halo on the leaves irrespective of the isolate. The other symptoms produced included yellowing of the leaves and apical wilting of the seedlings. In higher dilutions the chlorotic spots were not visible but the seedlings showed generalised chlorosis, epinasty and shedding of leaves of varying magnitude. There was no development of symptoms when the culture filtrate was diluted 8 folds. A fair correlation was evident from the expressed symptoms and dilution of the toxin preparation, but the symptoms produced in these, however, were not identical to the natural one.

The inhibition of radicle and plumule growth technique has been used previously for assaying toxin(s) from several organisms (Oku and Nakanishi, 1963; Joffe, 1973). Present *in vitro* results showed that the pathogen produced non-host specific phytotoxin(s) which is possibly positively correlated with the virulence of the organisms to a limited extent. The results of *in vivo* experiments indicated that the toxin(s) were translocable and were accumulated in the plant tissue where it produced the visible symptoms.

Toxin(s) constitutes only a small part of the disease process. The observations of the present study speak for the presence of non-host specific toxic principles in the cultural filtrate of the isolates of *P. vexans* that possibly act as secondary determinants of disease development (Rudolph, 1976).

REFERENCES

- Islam, J. SK. and Pan, S. (1990). Variability among isolates of *Phomopsis vexans*. *Environ. and Ecol* 8: 315-319.
- Joffe, A. Z. (1973). *Fusarium* species of the sporotrichiella section and relations between their toxicity to plants and animals. *Z. Pflanz. Kran. Pflanzen.* 80 : 92-99.
- Mitchell, R. E. (1984). The relevance of non-host specific toxins in the expression of virulence by

- pathogens. *Ann. Rev. Phytopathology* **22** : 215-245.
- Nishimura, S. and Kohmoto, K. (1983). Host specific toxins and chemical structures from *Alternaria* species. *Ann. Rev. Phytopathol* **21** : 87-116.
- Oku, H. and Nakanishi, T. (1963). A toxic metabolite from *Ascochyta fabae* having antibiotic activity. *Phytopathology* **53** : 1321-1325.
- Pan, S. and Sen, C. (1986). Relative toxins production by parental and fungicide adapted isolates of *M. phaseolina*. *Indian J. Plant Pathol.* **4** : 57-62.
- Rudolph, K. (1976). Non-specific toxins. In R. Heitefuss, P. H. Williams (eds.) *Physiological Plant Pathology*. Vol. 4, New York, Springer Verlag, pp. 270 - 315.
- Samaddar, K. R. and Scheffer, R. P. (1968). Effect of the specific toxin in *Helminthosporium victoriae* on host cell membrane. *Plant Physiol.* **43** : 21-28.
- Scheffer, R. P. and Livingston, R. S. (1984). Host selective toxins and their role in plant disease. *Science, Washington* **223** : 17-21.
- Sen, C. (1988). Molecular basis of plant pathogenesis and its implications in disease management. In K. K. Janardhana and B. P. Singh (eds.) *Current Trends in Physiological Plant Pathology* (Dr. Akhtar Husain Festschrift). Lucknow, Association of Plant Pathologists of India, pp. 267-287.
- Wheeler, H. and Luke, H. H. (1963). Microbial toxins in plant disease. *Ann. Rev. Microbial.* **17** : 223-242.
- Yoder, O. C. (1980). Toxins in pathogenesis. *Ann. Rev. Phytopathology* **18** : 103-129.

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