Isolation and Characterization of Toxin from Alternaria helianthi Inciting Blight in Sunflower

S.P. Patole1*, K. Shankara1, R.S. Pradhan1 and S.B. Dhore2

1Central Integrated Pest Management Centre (CIPMC), Nagpur, Maharashtra, India
2Department of Botony, Hislop College, Nagpur, Maharashtra, India

*Corresponding author

A B S T R A C T

Alternaria helianthi the causal agent of sunflower leaf blight was isolated from a monoconidial culture derived from blight infected sunflower leaves of MSFH-8. Alternaria helianthi was first cultured on PDA and then transferred to Potato Dextrose broth. Toxin was purified from the cultural filtrate by treatment with organic solvents. The identity of the toxin was established by estimating carbohydrate and protein content. Based on the quantity of carbohydrate and protein the toxin was identified as glycoproteinaceous in nature. The toxin inhibited seed germination and affected root and shoot growth.

Keywords
Sunflower, Toxin, Alternaria helianthi, Leaf blight.

Introduction

Sunflower (Helianthus annuus L.) is a major oilseed crop in India and other countries like Australia, Africa, USA and Yugoslavia. The increase in area and production is concomitant with an increase in the incidence and severity of foliar diseases. Among the major foliar diseases, Alternaria leaf blight caused by Alternaria helianthi (Hansf.) Tubaki and Nishihara is an economically important disease and is reported to cause up to 80 per cent losses particularly in Kharif season (Balasubrahmanyam and Kolte, 1980; Amaresh 1997). Several toxins produced by microorganisms were reported to be responsible for the induction of diseases in plants. These microorganisms produce toxic metabolites in culture media and in plant tissues which are involved in the disease syndrome (Owens, 1969; Wood et al., 1972). Several species of Alternaria are known to produce different types of toxic metabolites (Otani et al., 1974: Bhaskaran and Kandaswamy 1978). Attempts were made to isolate the toxin produced by A. helianthi and study the effect of toxin on seed germination and seedling growth.

Materials and Methods

The pure culture of A. helianthi was isolated from a monoconidial culture derived from blight infected sunflower leaves of MSFH-8. Alternaria helianthi was first cultured on PDA and then transferred to Potato Dextrose broth.
broth. Potato Dextrose broth was dispensed at the rate of 200 ml per 500 ml conical flask. After sterilising them, one cm disc of the fungus from periphery of seven days old culture which was grown on Potato Dextrose Agar medium was inoculated and incubated at 27±1°C for 30 days. Mycelial mats were separated from the broth culture by filtration through cheese cloth and finally it was filtered by using Whatman No. 42 filter paper. Further, culture filtrate was centrifuged at 12000 rpm for 10 minutes to remove spores. The supernatant was reduced to 1/5th of its original volume by evaporating at 40°C in hot water bath. Gradually two volumes of acetone was added to the culture filtrate with constant stirring till the precipitation was completed and allowed to stand overnight at 4°C. The precipitate was separated by centrifugation at 20000 rpm for 10 min at 4°C and discarded. The acetone was removed from the supernatant liquid by hot water bath (40°C).

This solution was extracted three times with two parts of water saturated 1-butanal by centrifugation at 20000 rpm for 10 min at 4°C. The water phase was discarded and the butanol phase were combined and kept on hot water bath (40°C) till complete dryness.

This solution was extracted twice with 400 ml aliquots of diethyl ether and the ether phases were discarded by centrifugation at 20000 g for 10 min at 4°C. The water phase was taken to dryness by hot water bath. Completely moisture was removed and dried product was stored in clean and air dried bottle. The toxin was recovered as a brown powder.

Pure toxin of 3 mg was dissolved in 1.0 ml of sterilised distilled water to get 3000 ppm concentration. This solution was diluted with distilled water to get 2000, 1000, 500, 200 and 100 ppm of toxin concentrations. To assess the phytotoxicity of toxin, the sunflower seeds were surface sterilized with sodium hypochlorine (2.0% NaOCl) solution and washed in sterile distilled water. Effects of toxin on seed germination and seedling growth was studied by soaking sunflower seeds at different concentration of toxin viz., 100, 200, 500, 1000, 2000 and 3000 ppm for 1 hr and sterile distilled water served as control. One hundred seeds were placed on moist blotters in petri plates and incubated at 27±1°C for seven days.

**Composition of the toxin produced by Alternaria helianthi**

**Carbohydrate**

One ml of the toxic filtrate of the fungus and partially purified fraction in diethyl ether and acetone extracts were kept in a boiling water bath with 0.5 ml of 2.5 N HCl for 3 h for hydrolysis and cooled to room temperature. The sample was neutralised with sodium carbonate until the effervescence ceased. The volume was made up to 25 ml and centrifuged at 10 000 rpm for 30 min. From that 1 ml of supernatant was taken and 4 ml of anthrone reagent was added and heated for 8 min in boiling water bath. The change in colour of the sample from green to dark green was read at 630 nm. The carbohydrate content in the samples was calculated using a glucose standard graph (Hedge and Hofreiter 1962).

**Protein**

The protein content of the toxin was estimated by the Lowry et al., (1951) method. Pipette out 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard into a series of test tubes. Pipette out 0.1ml and 0.2 ml of the sample extract in two other test tubes Make up the volume to 1ml in all the test tubes. A tube with 1ml of water serves as the blank. Add 5ml of reagent C to each tube including the blank. Mix well and allowed to stand for 10 min. Then add 0.5ml of reagent D, mix well and incubate at room temp in the dark for 30 min. Blue colour is developed. Take the readings at 660nm.
Table.1 Effect of purified toxin on seed germination and seedling growth of sunflower

| Sl. No. | Toxin concentrations (ppm) | Seed germination | Shoot growth | Root growth |
|---------|-----------------------------|------------------|--------------|-------------|
|         |                             | Per cent of seed germination | Per cent inhibition of seed germination over control | Shoot growth (mm) | Per cent inhibition of seedling growth over control | Root growth (mm) | Per cent inhibition of seedling growth over control |
| 1.      | 100                         | 100.00 (89.42)   | 0.00 (0.00)  | 11.90 (20.17) | 4.03 (11.19)  | 10.58 (18.98) | 0.66 (4.66)  |
| 2.      | 200                         | 96.33 (78.98)    | 3.66 (11.01) | 11.06 (19.43) | 11.02 (19.35) | 10.37 (18.78) | 2.66 (9.27)  |
| 3.      | 500                         | 85.68 (67.80)    | 14.32 (22.19)| 9.33 (17.78)  | 24.72 (29.80)| 8.23 (16.66) | 22.74 (28.43)|
| 4.      | 1000                        | 76.33 (60.89)    | 23.67 (29.10)| 7.16 (15.52)  | 41.91 (40.33)| 6.37 (15.20) | 35.48 (36.55)|
| 5.      | 2000                        | 57.32 (49.23)    | 42.65 (40.76)| 5.60 (13.68)  | 54.76 (47.74)| 4.91 (12.79) | 53.91 (47.24)|
| 6.      | 3000                        | 24.66 (29.77)    | 75.34 (60.22)| 2.53 (9.15)   | 79.57 (63.14)| 2.15 (8.43)  | 79.94 (63.40)|
| 7.      | Control                     | 100.00 (89.42)   | 0.00 (0.00)  | 12.40 (20.61) | 0.00 (0.00)  | 10.65 (19.05)| 0.00 (0.00)  |

** Figures in the parentheses indicate arcsine transformed values.
Draw a standard graph and calculate the amount of protein in the sample

Results and Discussion

The toxin was isolated from liquid culture grown on potato dextrose broth. The carbohydrate content of the toxin was 44.00 mg/100 mg, whereas the protein was 4.18 mg/100 mg. Since the toxin contained both carbohydrate and protein, it was glycoproteinaceous in nature. The glycoproteinaceous nature of the fungal toxin has also been reported to be produced by *Rhizoctonia solani* in rice (Vidhyasekaran et al., 1997) and *Corynespora cassiicola* in *Phyllanthus amarus* (Mathiyazhagan et al., 2005).

The present study is in agreement with work of Uma Maheswari and Sankaralingam (2010) who extracted toxin from *Alternaria alternata* and identified that the carbohydrate content of the toxin was greater, ranging from 1975 to 2950 μg/ml, whereas the protein was lower, ranging from 117 to 175 μg/ml. They identified the toxin as glycoprotein nature.

The toxin inhibited germination of sunflower seeds at all the six concentrations tested (Table 1). Per cent of seed germination ranged from 24.66 to 100 per cent. Toxin at 100 ppm concentration didn’t affect seed germination while toxin concentration of 200 ppm to 3000 ppm affected seed germination.

At 200 ppm the seed germination was 96.33 per cent while at 3000 ppm seed germination was 24.66 per cent. Minimum length of sunflower shoot (2.53 mm) and root length (2.15 mm) was observed at 3000 ppm. The present findings are in agreement with Islam and Maric (1980), Robeson and Strobel (1985), Amaresh and Nargund (2005), who reported that the toxin produced by *A. helianthi* inhibited seed germination and root elongation in sunflower.

References

Amaresh, Y. S., 1997. Studies on foliar diseases of sunflower (*Helianthus annuus* L.) with special reference to *Alternaria* leaf blight caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara. *M.Sc (Agri) Thesis*, Univ. Agric. Sci., Dharwad, 117 pp.

Amaresh, Y. S., and Nargund, V. B., 2005. Isolation of toxin from *Alternaria helianthi* causing blight in sunflower. *J. Mycol. Pl. Pathol.*, 35: 367-639

Balasubramanyam, N., and Kolte, S. J., 1980b. Effect of *Alternaria* blight on yield components, oil content and seed quality of sunflower. *Indian J. Agric. Sci.*, 50: 701-706.

Islam, U., and Maric, A., 1980. Contribution to the studies on biology, epidemiology and resistance of sunflower to *Alternaria helianthi*. *Zastita Bilja*, 35: 35-49.

Lowry, O. H., Rosebrough, N. J, Farr, A. L and Randall, R. J 1951. Total Protein Estimation by Lowry’s Method. *J. Biol. Chem.*, 193: 265.

Mathiyazhagan, S., Kavitha K, Chandrasekar G, Nakkeeran S, Manian K, Krishnamoorthy A. S, Sankaralingam A. and Fernando WGD. 2005. Toxin production by *Corynespora cassiicola* in *Phyllanthus amarus*, the stem blight pathogen. *Acta Phytopath. Entomol. Hungarica*, 40: 55–65.

Otani, U. S., Nishimura. And Kohmoto, K., 1974. Nature of specific susceptibility of *Alternaria kikll Cllianan* in Nijisseiki cultivar among Japanese pears. III Chemical and thermal protection against
effect of host specific toxin. *Ann. Phytopathol. Soc. Japan*, 40: 66-69.

Owens, L. D., 1969. Toxins in plant diseases. Structure and action. *Science*, 165: 18-25.

Robeson, D. J., and Strobel, G. A., 1985. Deoxyxradicin and 3-epideoxyxradicinol production by the sunflower pathogen *Alternaria helianthi*. *Ann. App. Biol.*, 107: 409-415.

Uma Maheswari, C., and Sankaralingam, A., 2010. Role of toxin produced by *Alternaria alternata* in leaf blight of watermelon and its degradation by biocontrol agents. *Arch. Phytopathol. Plant Protect*, 43: 41-50.

Vidhyasekaran, P., Ruby Ponmalar, T., Samiyappan, R., Velazhahan, R., Vimala, R., Ramanathan, A., Paranidharan, V. and Muthukrishnan, S., 1997. Host specific toxin produced by *R. solani* the rice sheath blight pathogen. *Phytopathology*, 87:1258–1263.

Wood, R. K. S., Ballio, A. and Graniti, A., 1972. *Phytotoxins in plant disease*. Academic Press London, 530 pp.

**How to cite this article:**

Patole, S.P., K. Shankara, R.S. Pradhan and Dhore, S.B. 2017. Isolation and Characterization of Toxin from *Alternaria helianthi* Inciting Blight in Sunflower. *Int.J.Curr.Microbiol.App.Sci.* 6(10): 2892-2896. doi: [https://doi.org/10.20546/ijcemas.2017.610.341](https://doi.org/10.20546/ijcemas.2017.610.341)