IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
Volume 9 | Issue 1 | January, 2014 | 113-116

RESEARCH ARTICLE

Phytotoxins of *Ascochyta cypericola* causing leaf blight of *Cyperus rotundus* L.

■ P.K. RATNA KUMAR AND A. NARAYANA RAO

SUMMARY

Phtotoxin/s production by *Ascochyta cypericola* causing leaf blight of *Cyperus rotundus* was studied by growing the fungus in varied media and conditions. The culture filtrates, their ethyl acetate extracts exhibited toxicity. The extracts on TLC resolved into an array of components of which one was purified and identified as –p-hydroxy phenyl propionate by IR, protonNMR and ^{13}CNMR spectroscopy. Even the release of the toxin by spores during germination is reported.

Key Words : *Ascochyta cypericola*, *Cyperus rotundus,* Phytotoxins

How to cite this article : Ratna Kumar, P.K. and Narayana Rao, A. (2014). Phytotoxins of *Ascochyta cypericola* causing leaf blight of *Cyperus rotundus* L. *Internat. J. Plant Sci.*, **9** (1): 113-116.

Article chronicle : Received : 18.09.2013; **Revised :** 13.10.2013; **Accepted :** 30.10.2013

Plant pathogens have many desirable characteristics that make them ideal candidates as biocontrol agents for weeds (Zettler and Freeman, 1972; Freeman *et al.* 1974). Since 1970 there has been a dramatic increase in research efforts directed toward the incorporation of plant pathogens into biological control programmes for both aquatic and terrestrial weeds (Charudattan, 1978; Freeman and Charudattan, 1981; Templeton, 1982).

Upadhyay *et al.* (1991) collected leaves showing severe blight disease symptoms on *Cyperus rotundus* in Banaras, India and have isolated a new pathogen named *Ascochyta cypericola,* and claimed it as a potential mycoherbicide in the control of the noxious weed. The fungus induced both sunken lesions on the stem, sheath and involucre, and reddish brown striations on the leaves of infected plants suggesting the interaction of one or more phytotoxins. A phytotoxic biphenyl ether named cyperine was isolated by Stierle *et al.* (1991) from culture filtrates of *A.cypericola*. Hence, attempts were made to study the phytotoxin production other than cyperine by *A.cypericola* isolated from *C.rotundus* infesting the local fields around Visakhapatnam during a survey undertaken in search of pests of the noxious weed.

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
P.K. RATNA KUMAR, Department of Botany, Andhra University, VISAKHAPATNAM (A.P.) INDIA

Address of the Co-authors:
A. NARAYANA RAO, Department of Botany, Andhra University, VISAKHAPATNAM (A.P.) INDIA

MATERIAL AND METHODS

Always freshly isolated cultures of *A.cypericola* were used. One-litre Erlenmeyer flasks each with 300ml liquid medium sterilized in an autoclave and inoculated with small mycelial agar blocks maintained at 30±1^{0} C were employed throughout the study. Initially MIDC, Potato dextrose, Czapek-Dox, Richard's and Sabouraud's media were employed. The flasks were maintained as still and shake cultures. After 10,15,20,25and 30 days of incubation the cultures were filtered through muslin doth and 2ml aliquots of the culture filtrates were bioassayed using ragi (*Eleucina coracana*) seedlings. The remaining 1.0 l each of the culture filtrates was extracted with ethyl acetate using a separatory funnel. The ethyl acetate extracts were then concentrated by evaporating ethyl acetate at 60^{0}C in an evaporator. The dark brown residues were taken in GD and their dilutions were bioassayed using ragi seedlings and also by leaf punch method (NarayanaRao, 1971).

The residues of ethyl acetate extracts from 1.0 l each of

culture filtrates obtained from the above media inoculated with the fungus were then subjected to silica gel TLC using benzene : ethyl acetate (7:3) as the solvent. One of the developed plates was exposed to iodine vapours, and the reacted zones were marked. The corresponding zones from unexposed plates were scrapped and each zone was eluated with 3.0ml methanol. The eluates were centrifuged, dried at 30 ± 1^0C and the residues were taken in 2.0ml of sterilized GD and then bioassayed with ragi (*Eleucina coracana*) seedlings and leaf punch method.

Then, ethyl acetate extract from 12 l of culture filtrate obtained from cultures grown in potato dextrose liquid medium for 15days as shake cultures was evaporated to dryness. The dry residue was dissolved in about 3.0ml ethyl acetate and subjected to silica gel column chromatography. On the column of 20 cm length the extract was placed and was eluated with benzene: ethyl acetate (7:3) solvent. Sixty-five fractions each of 20ml were collected and dried at room temperature of 30 ± 1^0 C, and taken in 10ml methanol. UV spectra of these 65 were taken, and those that have similar spectra were pooled. Of these, one was a major one. It was crystallized from methanol and was purified by further TLC. The UV (Fig. 1) and IR (Fig. 2) spectra of it were taken. In order to further confirm the structure the proton NMR (Fig. 3) spectra were taken in CDCL3/TMS. Still for further confirmation of the structure it was subjected to ^{13}CNMR (Fig. 4) spectroscopy. . And then its phytotoxic activity was determined by bioassay with ragi (*Eleucina coracana*) seedlings and leaf punch method.

In ragi (*Eleucina coracana*) seedling bioassay, the seeds of ragi were thoroughly washed under running tap water and then with sterilized GD. The washed seeds were placed on sterilized Whatman No.1 paper discs in Petri plates. Sterilized GD was then added to moison the discs. The seedlings that have shown small plumule and radicle were placed in 100ml beakers containing 2ml of test solutions. The controls received sterilized GD. In each beaker 5 seedlings were placed. The beakers were then closed with Alfoil and incubated at 30 ± 1^0C in a glass chamber for five days. Then the shoot and the longest root were measured.

In leaf punch method, the upper surface of the middle portion of the first intact mature leaf from the top of *C.rotundus* plants was punched1.0cm apart with a fine glass capillary tube in order to hold the drops placed on punched areas. About 5 or 6 0.01ml drops of the test solution were placed on the punched area with glass capillary sterilized GD drops were placed as controls.

RESULTS AND DISCUSSION

The root growth was markedly inhibited than the shoot growth of ragi seedlings by the culture filtrates and their dilutions of the different media. The per cent inhibition of root growth was considerable by culture filtrates of 15day old cultures. Of the ethyl acetate extracts from culture filtrates, the extract of potato dextrose culture was more toxic followed by Czapek-Dox, Sabouraud's, MIDC and Richard's. The extracts from culture filtrates obtained from 15day old culture were more toxic than the other since root growth was inhibited markedly by all the dilutions. Since the culture filtrates and ethyl acetate extracts might be containing toxic components, the TLC technique was used to resolve the toxic components present in the culture filtrates of the fungus. TLC has resolved the crude ethyl acetate extracts into six distinct bands or zones. Of the bands, the one at 0.75 Rf inhibited the root growth of ragi seedlings totally when compared to the other minor ones and also produced large necrotic spots on leaves in the leaf punch method. This component was obtained in considerable quantity by silica gel column chromatography. Further, TLC of this component yielded pure compound, which was crystallized. The UV spectrum in methanol of the compound is given in Fig. 1. The IR spectrum taken showed the presence of hydroxyl (34000^{-1} cm) and ether bridge (116^{-1} cm), and a carbonyl group at (1706^{-1}cm) and aromatic ring (1586^{-1} cm) indicating the presence of a hydroxyl, a C = O and ether. Based on the above data it is proposed that the compound may be containing aromatic hydroxyl group and an ether bridge together with a carbonyl fraction. In order to further confirm the structure of the compound, the proton NMR spectra of the compound was taken in $CDCl_3$ / TMS. The ^{1}HNMR spectra showed the presence of a methyl group at δ 1.0($CH_{3)}$;(3<u>HS)</u>; methylenes (δ 1.3 – 1.8) and a peak counting for 2H at d 4.2 (t, 2H; o-CH_2 protons) and δ 7.8 – 7-6 (aromatic protons, 4H) indicating that the compound may be an ester and based on the above data the structure of the compound is proposed as in Fig. 5. The structure of the compound is further confirmed by ^{13}CNMR spectroscopy.

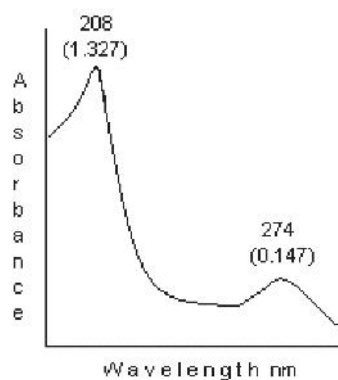

Fig. 1 : UV Spectrum in methanol of p-hydroxy phenyl propionate

The ^{13}CNMR spectra showed the presence of 4 protons at

δ 13.7 (-<u>C</u> H_3)

δ 19.2(impurity)

δ 30.64(-<u>C</u> H_2)

δ 65.57(-o-<u>C</u>H_2) and

δ 185.9(-C-O; carbonyl carbon).

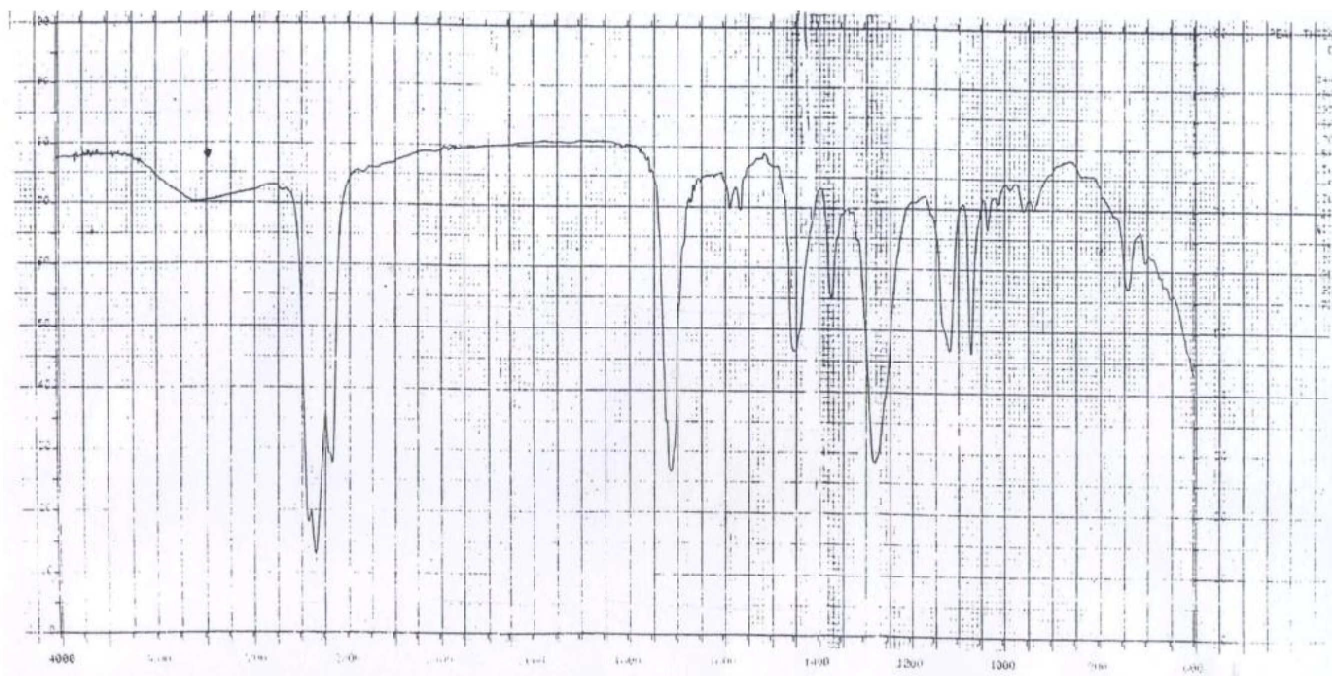

Fig. 2 : IR spectrum of p-hydroxy phenyl propionate

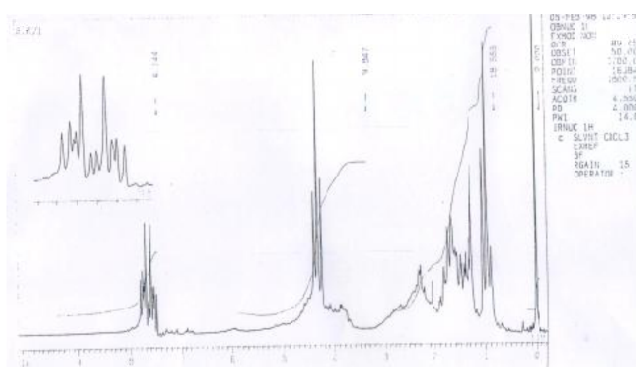

Fig. 3: ¹HNMR of p-hydroxy phenyl propionate

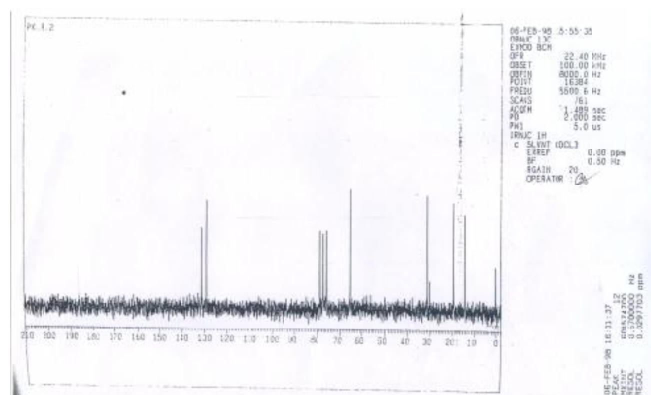

Fig. 4 : ¹³CNMR of p-hydroxy phenyl propionate

Barring few impurity peaks, the data are in accordance with proposed structure of the compound. Hence, the name of the compound present is –p-hydroxy phenyl propionate.

The bioassay with ragi (*Eleucina coracana*) seedlings showed that a concentration of 10 µg / ml of -p-hydroxy phenyl propionate inhibiting root growth to 57% and shoot growth 59%. The roots and shoots in test solution turned pale-yellow when compared to the control in water. The toxin produced typical necrotic spots on *C. rotundus* leaves at 0.1 ppm.

The release of the toxin by conidia during germination was also studied by extracting the germination fluid with ethyl acetate and subjecting it to silica gel TLC along with –p-hydroxy phenyl propionate as reference and by UV spectra of the zone at 0.75 Rf opposite to the reference clearly indicates that -p- hydroxy phenyl propionate is released by the spores during germination.

In the place of organisms, the strategy relies on substances the microbes produced, namely weed- damaging compounds known as phytotoxins (Strobel, 1991). In his paper "biological control of weeds", Strobel (1991), has given a list of phytotoxins under study for weed control. One of the toxins

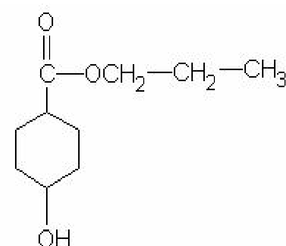

Fig. 5 : p-hydroxy phenyl propionate

is cyperine, a biphenyl ether isolated from culture filtrate of *A.cypericola* by Stierle *et al.* (1991). It is not selective but extremely toxic to purple nutsedge. In the present study, the culture filtrates and the ethyl acetate extracts from culture filtrates of *A.cypericola* when grown in different media and conditions showed toxicity to ragi (*Eleucina coracana*) seedlings. However, because of certain limitations no attempts were made to isolate and study cyperine, nonetheless, silica gel TLC resolved an array of toxic components differing in Rf and UV spectra. Further, by silica gel column chromatography a compound was isolated, purified and crystallized, which by IR, proton NMR and ^{13}CNMR spectroscopy revealed to be –p-hydroxy phenyl propionate. On silica gel TLC plates it has an Rf of 0.75 and UV absorption of 208 and 274.

This compound is produced by *A.cypericola* in all the media where the fungus was grown. The compound at 0.1 ppm inhibited root and shoot growth of ragi seedlings to an extent of @ 56%. At the same concentration it produced a necrotic spot on the leaves of *C.rotundus*. Since the toxin is assayed by abnormal test material *i.e.*, ragi (*Eleucina coracana*) seedlings show that the phytotoxic –p- hydroxy phenyl propionate is not selective in toxicity. Further, it is also shown that the compound is released by spores during germination. With regard to the other phytotoxic components as resolved by TLC need further study. In the words of Strobel (1991),"when they (weed killing fungi) produce such a toxin, they also apparently produce a family of compounds that are structurally related to it ". That the toxic components resolved by TLC, in this study are related or not has to be elucidated by further detailed analytical study.

Acknowledgements:

The authors were thankful to University Grants Commission, New Delhi, India for the finance support under UGC-SAP-CAS-I project in the Department of Botany, Centre of Advanced Studies, Andhra University, Visakhapatnam.

REFERENCES

Charudattan, R. (1978). Biological control projects in plant pathology - a directory. Misc.Pub, Plant Path. Department University of Florida, Gainesville, FL, 67p.

Freeman, T.E. and Charudattan, R. (1981). Biological control of weeds with plant pathogens. Prospectus 1980. Proc. V Int. Symp. Biol. Contr. Weeds. July 22-27 1980, Brisbane, Australia, Delfosse, E. S. (ed.). CSIRO, Melbourne, pp. 293-299.

Freeman, T.E., Zettler, F.W. and Charudattan, R. (1974). Phytopathogens as biocontrols for aquatic weeds. *PANS,* **20**: 181-184.

Narayana Rao, A. (1971). Studies on the toxins of *Pyricularia.* Ph.D Thesis, University of Madras. 103 pp.

Stierle, A. Upadhyay, R.K. and Strobel, G.A. (1991). Cyperine, A. phytotoxin produced by *Ascochyta cypericola.* A fungal pathogen of *Cyperus rotundus. Phytochem.,* **30** : 2191-2192.

Strobel, G.A. (1991). Biological control of weeds. *Scientific American,* **265** : 72-78.

Templeton, G.E. (1982). Status of weed control with plant pathogens. pp 29-44. In : *Biological control of weeds with plant pathogens*, R. charudattan and H.L. Walker. eds.Wiley New York, U.S.A.

Upadhyay, R.K., Kenfield, D., Strobel, G.A. and Hess, W.M. (1991). *Ascochyta Cypericola* sp. nov. causing leaf blight of purple nutsedge (*C.rotundus*). *Can. J. Bot.,* **69** : 797-802

Zettler, F.W. and Freeman, T.E. (1972). Use of plant pathogens as biocontrols of aquatic weeds. *Annu. Rev. Phytopath.,* **10** : 455-470.

9th Year
★★★★★ of Excellence ★★★★★