Research Journal of Agriculture and Biological Sciences, 6(6): 728-731, 2010

Herbicidal Activity of Mycelial and Cell Free Extracts of *Fusarium oxysporum* F.sp. Ciceris Against Rice Weed *Cyperus Iria* l

S. Karthick Raja Namasivayam and Aruna

Department of Biotechnology, Sathyabama University, Chennai 600119, Tamil Nadu, India

Abstract: The present study was undertaken to evaluate herbicidal activity of *Fusarium oxysporum f.sp*. Ciceris isolated from infected cowpea mycelial and cell free extracts against rice weed *Cyprus iria* L adopting leaf necrosis assay. Extracts were prepared from fungal mycelia and mycelia free filtrate derived from modified Fries media, sabouraud maltose yeast extract broth, sabouraud sucrose yeast extract broth,sabouraud dextrose broth and potato dextrose broth and extracted with different solvents. Chloroform and ethanol mycelial extract from modified fries media and Chloroform, methanol mycelia free filtrate extract from sabouraud sucrose yeast extract broth recorded maximum herbicidal activity. The extracts were induced necrotic lesions as fungal conidia after 24 hours on detached leaves. The effect of temperature on herbicidal activity of mycelial and mycelia free filtrate extracts of chloroform and ethanol from modified fries media and sabouraud sucrose yeast extract broth reveals the extracts could retained the herbicidal activity upto 90 ^{o}C and induced necrotic lesions on detached leaves.

Key words: *Fusarium oxysporum f.sp*. Ciceris, Cyprus *iria*, herbicidal activity

INTRODUCTION

Agriculture in India is one of the most important sectors of its economy. It is the means of livelihood of almost two thirds of the work force in the country and according to the economic data for the financial year 2006-07, agriculture accounts for 18% of India's GDP. About 43 % of India's geographical area is used for agricultural activity. Though the share of Indian agriculture in the GDP has steadily declined, it is still the single largest contributor to the GDP and plays a vital role in the overall socio-economic development of India. Crop losses from weeds orldwide average 10% annually. Herbicides application is almost essential in modern agriculture to maintain high productivity. Increased awareness of the environmental damage caused by continued use of conventional synthetic herbicides has aroused great interest in biodegradable, selective and environmentally friendly herbicides. The increasing incidence of herbicide resistance in weeds has stimulated research for herbicidal compounds with new or noval site and mode of action. The use of microbially-derived compounds in biological control of weeds may represent a promising alternative to the use of chemicals. Microbial metabolites have become the focus of attention of researchers searching for natural product alternatives to conventional herbicides. Microbial toxins are metabolites produced by plant pathogens (fungi, bacteria), which play a role in host pathogen interactions and in disease expression[1,2]. They are low molecular weight substances produced by ome pathogens which are capable of reproducing symptoms similar to that found in natural fections in plants[3] Members of the *Fusarium oxysporum* species complex (FOSC) are the most common phytopathogenic Fusaria. They cause vascular wilts of over 100 cultivated plant species, including tomato, potato, sugarc ane, bean, cowpea, date and oil palm, as well as cooking and dessert bananas. In the present study, mycelia and cell free extracts prepared from *Fusarium oxysporum* infected cowpea isolate were evaluated against herbicidal activity against rice weed *Cyprus iria* adopting leaf necrosis assay

MATERIALS AND METHODS

Fungal Strain: *F.oxysporum* f.sp.Ciceris was isolated from infected cowpea plants adopting standard condition and the isolated fungi was identified based on cultural and morphological characteristics.Pure culture was maintained on potato dextrose agar slant as monosporic culture

Inoculam Preparation: The fully grown fungal culture on potato dextrose agar (PDA) slants was flooded with sterile distilled water and the tween 80 (0.1%), scrapped with sterile glass rod and the suspension was filtered through cheese cloth to remove mycelia debris and filtrate containing spores was used as inocula.

Corresponding Author: S. Karthick Raja Namasivayam, Department of Biotechnology, Sathyabama University, Chennai 600119, Tamil Nadu, India
E-.mail: biologiask@gmail.com

Preparation of Mycelial and Cell Free Extracts: 500 ml. of Modified Fries broth media (MFM), potato dextrose broth (PDA), Sabouraud sucrose yeast extract broth((SSYB) sabouraud dextrose broth (SDB) and sabouraud maltose yeast extract broth (SMYB) was prepared and sterilized by autoclaving. 0.1 ml of the spore suspension was inoculated into respective media, incubated under shaking condition at 30 ^{o} C,100 rpm for ten days. After the incubation period , the media was filtered through cheese cloth and the collected mycelia and filtrate was extracted with methanol, chloroform, acetone, ethanol, butanol and distilled water (1:5 ratio) Solvent extracts were then concentrated using a flash evaporator, the final residues collected in screw cap vials and used for further study

Screening of Herbicidal Activity:

Sample Collection: Rice weed were collected from the rice field in an area around Porur, Chennai in sterile polythene bags, kept in ice box and brought to the laboratory

Herbicidal Activity (Leaf Necrosis Assay): Initially leaf necrosis assay was carried out with *F.oxysporum* fungal conidia. The expanded leaves of *Cyprus iria* cut into 6-9cm^2 , surface sterilized with ethanol and washed with sterile distilled water to remove ethanol from surface. The cut pieces were inoculated with 10^8 spores/ml of *F.oxysporum* condia by wounding them with sterile needle on the surface of the leaf and transferred to Petri plate containing moistened cotton ball and filter paper. Later plates were incubated at 25˚C for one week[2]. The leaf bioassay with respective extracts was performed as descried earlier with 10mg/ml final concentration. A daily observation was made for the development of necrotic lesions on the extracts inoculated leaves.

Effect of Temperature on Herbicidal Activity: The effect of temperature on herbicidal activity was studied.Chloroform and ethanol extract derived from modified fries media extracts with final concentration of 10mg/ml was heated at 40˚C, 50˚C, 60˚C, 70˚C and 80˚C for one hour. After the heat treatment, herbicidal assay was performed using leaf necrosis assay as discussed earlier.

RESULT AND DISCUSSION

Considering the increasing awareness of herbicide resistance and the restrictions of the use of chemical agrochemicals in agriculture against weeds novel compounds from microorganisms may provide new chemistries for weeds that may otherwise difficult to control. Boyetchko[4] submitted that microbially derived compounds may be pursued either as templates for new synthetic chemical herbicides or as pathogens applied directly to the target weed.In the present study, herbicidal activity of *Fusarium oxysporum* f.sp.Ciceris was evaluated against rice weed *Cyprus iria.* Among the different extracts derived from media, Chloroform and ethanol mycelial extracts derived from modified fries media recorded distinct herbicidal activity against *Cyprus iria* . First symptom appear within 24 hours as weak chlorotic marking which subsequently developed into well defined deep brown lesions. The diameter of necrotic area was 7.0mm^2(Table 1 & Figure1 } The fungal conidia also caused same effect and the diameter of necrotic lesions 7.2mm^2 (Figure 2)Herbicidal activity was not recorded in acetone,butanol and distilled water extracts of mycelia derived from remaining media and all the extracts of filtrate from all the media except chloroform: and methanol extracts of sabouraud sucrose yeast extract broth. Both the filtrate extracts induced viable necrotic lesions at 6.0mm^2 diameter (Table 2). Complete necrosis of leaflet was observed after 96 hours. The herbicidal activity of fungi and their metabolites on various weeds have been reported by many workers Zhang and Watson[8] isolated phytotoxin produced by *Exserohilum monoceras*, a potential bioherbicide for control of *echinochloa sp*. Chanudattan and Rao[5] isolated Bostrycin and 4-deoxybostricin from culture filtrate of *Alternaria eichokorniae* known to cause phytotoxic effect on water hyacinth. Phytotoxin produced by *Collectotrichum dematium* produces viable necrotic lesions on various weeds[7]. Physiological and ultrastutural effect of “fumonisin” a phytotoxin produced by *Fusarium monoliforme* was studied by Abbas *et al*[1]. Quayyum *et al*[6] isolated necrosis inducing host specific protein toxin (SGP) from spore germination fluid of *Alternaria panax*. Herbicidal activity was not recorded in mycelial and filtrate extracts derived from potato dextrose broth, sabouraud maltose yeast extract broth and sabouraud dextrose broth. Effect of temperature on herbicidal activity reveals no distinct effect Chloroform and ethanol mycelial extract from modified fries media heated at respective temperature induced necrotic lesions with surface area of 7.0, 7.0, 7.0 .6.7 and 6.7mm^2 respectively. Similar effect was observed in methanol extracts (Table 3) Surface area of necrotic lesions induced by chloroform mycelia cell free extract of sabouraud sucrose yeast extract broth was 6.0,6.0,6.0,5.8 and 5.8mm^2 and methanol extract induced necrotic lesion similar to chloroform extracts which clearly reveals the extracts retained its herbicidal activity under high temperature. The mimicking of pathogenic necrotic symptoms produced filtratev ced by chloroform and ethanol mycelial extract from modified

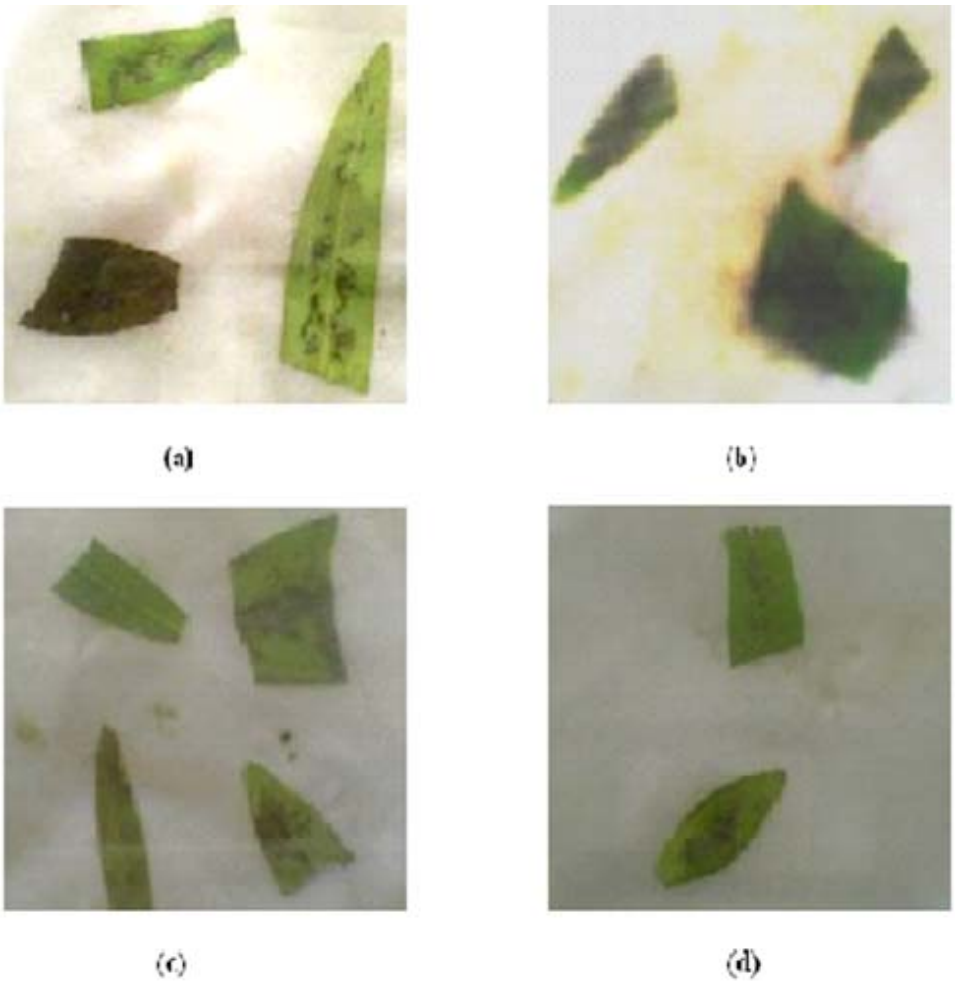

Fig. 1: Necrotic lesions produced by mycelia and cell free extracts of *Fusarium oxysporum* f.sp. ciceris against *Cyprus iria*
a) Necrotic lesions produced by mycelial chloroform extract
b) Necrotic lesions produced by mycelial ethyl acetate extract
c) Necrotic lesions produced by filtrate chloroform extract
d) Necrotic lesions produced by filtrate ethyl acetate extract

Table 1: Surface necrotic area of *Cyprus iria* with mycelial extracts of *Fusarium oxysporum* f.sp. Ciceris derived from various media

S.No.	Media	Surface necrotic area (mm)2					
		M	CH	A	BT	E	DW
1	Potato dextrose broth	0.0	0.0	0.0	0.0	0.0	0.0
2	Modified Fries media	0.0	7.0 ±0.1	0.0	0.0	7.0 ±0.4	0.0
3	Sabouraud dextrose broth	0.0	0.0	0.0	0.0	0.0	0.0
4	Sabouraud sucrose yeast extract broth	0.0	0.0	0.0	0.0	0.0	0.0
5	Sabouraud maltose yeast extract broth	0.0	0.0	0.0	0.0	0.0	0.0

Table 1: Surface necrotic area of *Cyprus iria* with mycelia free filtrate extracts of *Fusarium oxysporum* f.sp. Ciceris derived from various media

S.No.	Media	Surface necrotic area (mm)2					
		M	CH	A	BT	E	DW
1	Potato dextrose broth	0.0	0.0	0.0	0.0	0.0	0.0
2	Modified Fries media	0.0	0.0	0.0	0.0	0.0	0.0
3	Sabouraud dextrose broth	0.0	0.0	0.0	0.0	0.0	0.0
4	Sabouraud sucrose yeast extract broth	0.0	6.0±0.1	0.0	0.0	6.0 ±0.4	0.0
5	Sabouraud maltose yeast extract broth	0.0	0.0	0.0	0.0	0.0	0.0

Mean ± S.E M- methanol, CH- chloroform, A- Acetone, BT- Butanol, E- ethanol, DW- distilled water

Table 2: Effect of temperature on herbicidal activity of extracts

S.No.	Temperature (^{o}C)	Surface necrotic area (mm) ^{2}			
		CH Mycelia from MFM	M	CH Filtrate from SSYB	M
1	40	7.0 ±0.1.	7.0 ±0.1.	6.0±0.1.	6.0±0.1.
2	50	7.0 ±0.2.	7.0 ±0.3	6.0±0.3	6.0±0.2
3	60	7.0 ±0.3	7.0 ±0.1.	6.0±0.1	6.0±0.1
4	70	6.7±0.4	6.8±0.1	5.8±0.1	5.7±0.3
5	80	6.7±0.1	6.8±0.4	5.8±0.1	5.8±0.4

fries media on *Cyprus iria* suggest a herbicidal role for the extracts in *Cyprus iria* necrotic lesion. Identification of the compound responsible for the herbicidal activity in the extracts, mass production, formulation and herbicidal activity on other economic weeds (*In vitro* and field trial) will be carry out in future study.

REFERENCES

1. Abbas, H.K., S.O. Duke, W.T. Shier, 2001. Inhibition of ceramide synthesis in plants by phytotoxins. Advances in Microbial Toxin Research and its Biotechnological Exploitation, 211-229.
2. Amusa, N.A., 2005. Microbially produced phytotoxins and plant disease management African Journal of Biotechnology, 5: 405-414.
3. Ballio, A., F. Bossa, L. Camoni, D. Di Giorgio, M.C. Flamand, H. Maraite, G. Nitti, P. Pucci and A. Scaloni, 1996. Structure of fuscopeptins, phytotoxic metabolites of *Pseudomonas fuscovaginae*. *FEBS Lett.*, 381: 213-216.
4. Boyetchka, S.M., 1999. Innovative applications of microbial agents for biological weed control. In; Mukerji, K.G (ed.) Biotechnological appliocations in biocontrol of plant pathogens. New York Kluwer academic /Plenum publishers, USA, 73-97.
5. Charudattan, R., K.V. Rao, 1981. Bostrycin & 4-Deoxy bostrycin: Two non specific phytotoxins produced by *Alternaria eichorniae* Applied and environmental microbiology, 43: 846-849.
6. Quayyum, H.A., M. Gijzen and J.A. Traquair, 2002. Purification of a necrosis –Inducing host specific protein toxin from spore germination fluid of Alternaria panax APS Journal of phytopathology, 93(3): 323-328.
7. Yoshida, S., S. Hiradate, Y. Fujii and A. Shirata, 1999. Colletotrichum dematium produces phytotoxins in Anthracnose lesions of Mulberry leaves The American phytopathological Society, 90(3): 285-291.
8. Zhang, W. and A.K. Watson, 1997. Efficacy of *Exserohilum monoceras* for the control of *Echinochloa* species in rice (*Oryza sativa* L.). Weed Sci., 45: 144-150.