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Short Communication

Selective isolation and screening of fungi with herbicidal potential and evaluation of herbicidal activity against *Vernonia* species

S. Karthick Raja Namasivayam*, A. Aruna and Shreya Hazra Choudhury

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

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In irrigated agriculture, weed control through chemical herbicides, creates various environmental and health hazards which lead to the search for an alternate method of weed management, which is an eco-friendly and effective means. Biological control of weeds using microorganism is now extensively studied to control various economic important weeds. In the present study, herbicidal activity of crude metabolites extracted from the fungal strains isolated from the soil was evaluated against *Vernonia* species. A total of 125 fungal soil isolates belonging to four fungal species such as *Alternaria alternata, Paecilomyces farinosus, Penicillium expansum,* and *Fusarium oxysporum* were isolated. The respective fungal isolates were cultivated in modified Fries media under standard condition. The mycelia and the filtrate were extracted with ethyl acetate and the concentrated extract was evaluated for the herbicidal activity adopting leaf necrosis assay. Among the different isolates, extract prepared from *A. alternata* and *P. farinosus* showed maximum herbicidal activity. The present study would suggest the possible use of the fungal extract as a bio-herbicidal agent after the effective formulation and mass production through standard conditions.

Key words: Herbicidal activity, fungi, extracts, *Vernonia species,* formulation.

INTRODUCTION

Weeds are an ever present and an increasingly significant constraint to agriculture production worldwide. The overall impact of weeds on crop production can be crudely calculated, with average loss varying from 10 to 20% (Charudattan and Rao, 1981). Current production levels can only be maintained through the regular and wholesale application of pesticides, particularly of chemical herbicides which account for almost 50% of agrochemical market (Woodburn, 1995). Chemical control has been internationally accepted but usage is restricted for the fact that effective weed herbicides can often affect many other plants, not to mention current public concern regarding environmental problems associated with chemical pesticide usage in general (Defago et al., 2001). Biocontrol agents (BCAs) have been recognized not only as a replacement for chemical pesticides, but also as a viable part of well designed, integrated weed management systems, utilizing living

*Corresponding author. E-mail: biologiask@gmail.com.

organisms or plant pathogens to control or reduce the population of undesirable weed species. Biological weed control is an approach utilizing living organism to control or reduce the population of an undesirable weed species (Watson, 1991). A bio-herbicide is a herbicide that is based on a living organism such as fungi, bacteria or protozoa. Bio-herbicides are employed to control weed species with a native pathogen applying them in massive doses in area infected with target weed flora. Bio-herbicides offer many advantages like high degree of specificity of target weed, no effect on non-target and beneficial plants or man; absence of weed resistance, development and absence of residue build-up in the environment (Karthick and Aruna, 2010).

Bio-herbicides include phytopathogenic microorganisms or microbial compounds useful for weed control. Collectively these organisms and/or their natural products are called bio-herbicides. Microorganisms produce secondary metabolites, which act on plants and other organisms which sometimes cause inhibition of growth, diseases and even death (Abbas et al., 2002). The initiative for using pathogens, phytotoxins from pathogens, and other microorganisms as biological weed-control agents began about three decades ago. Since then, numerous microbes have been screened for phytotoxic potential, and several dozen evaluated as bio-herbicides as reported by various researchers and summarized (Defago et al., 2001). Due to the interest in this area, many other weed pathogens and phytotoxins (from athogenic and nonpathogenic microorganisms) will be discovered that possess bio-herbicidal activity (Muller et al., 2009). Most bio-herbicides have been targeted toward agronomic weeds, but these agents may also be useful to control weeds in non-agronomic areas (recreational areas, forests, rights-of-way, lawns, gardens, etc.) where synthetic herbicides are not registered, or where their use is cost-prohibitive (Hussain et al., 1999). In the present study, herbicidal activity of metabolites extracted from fungi was evaluated against *Vernonia* species

MATERIALS AND METHODS

Isolation of fungi

The fungal isolates were isolated from local soil sample adopting soil dilution method (Clark, 1965). 25 g of soil sample was diluted in 225 ml of sterile distilled water. From this suspension, 10 ml was added to 990 ml of water and from this 1 ml was spread in Petri dishes in triplicates containing sabouraud dextrose agar (SDA) supplemented with chloramphenicol (100 mg/ml). The fungi were identified by colony morphology and microscopic staining with lactophenol cotton blue. The isolated fungi were maintained on sabouraud dextrose agar slant as monosporic culture.

Herbicidal activity

The herbicidal activity was done adapting detached leaf necrosis assay. The *Vernonia* plants were collected from university playground. Fresh leaves were collected in sterile polythene bags, kept in ice box, and brought to the laboratory immediately. Initially leaf necrosis assay was carried with all the isolated fungal conidia. The expanded leaves were surface sterilised with ethanol and washed with sterile distilled water to remove ethanol from surface. The leaves were inoculated with 10^8 spores/ml of isolated fungal conidia by wounding them with sterile needle on the surface of leaf and transferred to Petri plate containing moistened cotton ball and filter paper. Later plates were incubated at 25°C for one week. The fungal isolates that showed necrotic lesions on detached leaves was selected for further study. Among the 125 isolates, four fungal isolates: *A. alternata SKA08 strain, P. farinosus SKP01 strain, P. expansum SKPC01* and *F. oxysporum SKF01 strain* fungal conidia recorded herbicidal activity by the formation of necrotic lesions.

Crude extraction of herbicidal metabolites

The fungal extracts were prepared from mycelia and filtrate collected from modified Fries media. 500 ml of modified Fries media (sucrose 10 g; casein hydrolysates 2 g; sodium nitrate 1.5 g; dipotassium hydrogen orthophosphate 1 g; potassium chloride 0.5g; magnesium sulphate 0.5 g; ferrous sulphate 0.01 g; distilled water 1l; pH 6.8) was prepared and sterilised by autoclaving. 0.1 ml of spore suspension derived from 10 days old slant culture of *A. alternata SKA08 strain, P. farinosus SKP01, P. expansum SKPC01 strain, F. oxysporum SKF01* strain was inoculated and the inoculated flasks were kept at 28°C on a rotatory shaker at 150 rpm for 21 days. The broth was filtered through three layers of cheese cloth, the mycelia and filtrate collected was extracted with double the volume of ethyl acetate. The concentrated extracts was dissolved in DMSO and used for herbicidal activity. Leaf necrosis assay was done with aliquots of 10, 20 and 30 µg/ml of the concentrated extract as described earlier. The inoculated leaves with respective extracts were observed for development of necrotic lesions.

Formulation of herbicidal metabolites

The extract which showed maximum herbicidal activity was formulated in minimal media as liquid formulation. The minimal media (solution A: sodium phosphate pentahydrate 1.28 g; potassium phosphate (dibasic) 0.3 g; sodium chloride 0.05 g; ammonium chloride 0.1 g; distilled water 100 ml; solution B: magnesium sulphate 1.232 g/5 ml; solution C: calcium chloride 0.555 g/5 ml; solution D: glucose 2 g/10 ml; add 0.2 ml of solution B + 10 µl of solution C and 10 ml of solution D to solution A) was prepared and transferred to screw caps vials, sterilized by autoclaving. 1 ml of *A. alternata* mycelia and filtrate extract was added. One set of vials were kept for autoclaving and other set without autoclaving. Similarly one set of vials kept at 4°C for 48 h other kept at room temperature. Herbicidal activity was performed with all these formulation as described earlier.

RESULTS AND DISCUSSION

Fungi have a worldwide distribution, and grow in a wide range of habitats, including deserts, hypersaline environments, the deep sea, on rocks, and in extremely low and high temperatures. Fungi are the major group of organisms known to produce a wide range of metabo-lites, which shows several physiological changes in plant species known as phytotoxins (Yoshida and Hiradate, 1999; Abbasi et al., 2010). Such activity is called phyto-

Table 1. Diameter (cm) of leaf necrotic lesions caused by fungal extracts and re-extraction from necrotic lesions.

Name of fungi	Diameter of leaf necrotic lesions (cm)				
	Concentration (µg/ml)	Extraction		Reextraction	
		ME	FE	ME	FE
Alternaria alternata Spores	10	0.8	0.4	0.4	0.9
	20	1.0	1.0	0.9	1.0
	30	1.8*	1.5	1.5	1.1
		0.9			
Fusarium oxysporum Spores	10	1.0	0.5	0.6	0.5
	20	1.2	0.9	0.8	0.6
	30	1.5	0.8	0.9	1.2
		0.7			
Penicillium expansum Spores	10	0.5	0.0	0.0	1.0
	20	0.7	0.0	0.0	1.2
	30	1.7*	0.0	0.0	1.5
		0.6			
Paecilomyces farinosus Spores	10	1.0	0.2	0.4	0.7
	20	1.5	0.6	0.7	0.8
	30	1.8*	1.2	1.0	1.0
		0.9			

toxic activity. The metabolites of many fungi may have adverse or stimulatory effects on plants such as suppression of seed germination, mal formation and retardation of seedling growth reported that some fungal pathogens produce phytotoxins that effect seed germination and seedling growth and can be used to control various weeds (Hyang, 2008). In the present study, herbicidal activity of metabolites extracted from the fungal strain was evaluated against *Vernonia*. A total of 125 fungal strains were isolated from the soil. Among 125 isolates, herbicidal activity was recorded in *A. alternata SKA08* strain*, Paecilomyces farinosus SKP01, P. expansum SKPC01* strain and *F. oxysporum SKF01* strain spore treatment (Table 1). 0.9 cm of necrotic lesion was observed in *A. alternata* and *P. farinosus* followed by 0.7 cm in *F. oxysporum* and 0.6 cm in *P. expansum.* The extracts were prepared from all these 4 strains for the herbicidal activity. The mycelial and filtrate extract of all these strains showed necrotic lesions and concentration dependent variation on the development of necrotic lesions was observed. Maximum herbicidal activity was observed in *A. alternata* and *P. farinosus* mycelial extracts. The diameter of the necrotic lesion was found to be 1.8 cm at 30 µg/ml concentration. 0.8, 1.0 and 1.0, 1.5 cm of necrotic lesions was observed at 10 and 20 µg/ml mycelial extracts of *A. alternata* and *P. farinosus*. *F. oxysporum* also showed the herbicidal activity. The diameter of the necrotic lession was 1.0, 1.2 and 1.5 cm at 10, 20 and 30 µg/ml. *P. expansum* recorded necrotic lesions at the diameter of 0.5, 0.7 and 1.7 at respective concentrations. After re-extraction, all the tested strains mycelia and filtrate retained herbicidal activity. The diameter of the necrotic lesions was 0.9, 1.0, 1.1 in *A. alternata,* 0.5, 0.6. 1.2 in *F. oxysporum,* 1.0, 1.2, 1.5 in *P. expansum,* 0.7, 0.8, 1.0 cm in *P. farinosus* at respective concentration. The herbicidal activity of the extracts after reextraction from the necrotic lesions clearly reveals the virulence of the extracts. The filtrate extract also showed herbicidal activity. The filtrate extract of *A. alternata SKA08 strain* showed necrotic lesion at the diameter of 0.4, 0.9 and 1.5 cm at respective concentration. 0.4, 0.7 and 1.0 cm of necrotic lesion was observed in *P. farinosus SKP01 strain.* The diameter of the necrotic lesion in *F. oxysporum* extract was 0.6, 0.8, and 0.9 cm at respective concentration. But herbicidal activity was not recorded in *P. expansum* extracts at all concentrations. Similar effect was recorded in necrotic lesions after reextraction. No significant difference in the development of necrotic lesions was observed in re extraction treatment.

The liquid formulation of mycelia and filtrate extract of *A. alternata* in minimal media show distinct herbicidal activity. Autoclaved filtrate extract in minimal media showed maximum necrotic lesions of 1 cm diameter and mycelial autoclaved extract showed necrotic lesion of 0.6 cm diameter, but unsterilized refrigerated mycelial extract

caused maximum necrotic lesions. The diameter of necrotic lesion was 1.1 cm. Further study will be helpful to identify the mimicking of the pathogenic symptoms produced by fungal extracts of *A. alternata SKA08* strain on *Vernonia* species suggests a herbicidal role for the fungal extract in the necrotic lesions on the leaves of *Vernonia* species. Characterization of herbicidal compound in this fungal extract, mass production, formulation and herbicidal activity on other weeds (*in vitro* and field trial) will be carried out in future study.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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