Mycopath (2014) 12(2): 83-86 **Research Article**

Fusarium solani as biocontrol agent against parthenium weed

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Abstract

Parthenium weed (*Parthenium hysterophorus* L.) is known as an environmental, medical and agricultural hazard around the globe. During 2012-14, a series of surveys were conducted in Haryana, India and a leaf spot disease was regularly reported on parthenium grass in different parts of Kurukshetra, India and its adjoining areas. Pathogen was isolated from the infected leaves on potato dextrose agar) medium. Parthenium leaves were inoculated and the pathogen showed similar symptoms as occurred in nature, proving pathogenicity and Koch's postulates. Species identification of the pathogen was confirmed by CABI International Mycology Institute and the pathogen was identified as *Fusarium solani* (Mart.) Sacc. Phytotoxicity of fungal cultural filtrates was also confirmed on parthenium leaves in laboratory conditions. Due to the virulent nature of the isolated pathogen, it may be selected for further studies to develop mycoherbicide for control of this devastating weed.

Keywords: Biocontrol, fungal culture filtrates, *Fusarium solani*, *Parthenium hysterophorus*, phytotoxicity.

Introduction

Parthenium weed is an annual herb of Asteraceae family, originating from tropical Americas and now a weed of global significance in many countries around the world (Shabbir *et al*., 2012). It was reported that the seeds of this weed come to India with grains imported from USA under the USA PL 480 scheme and spread alarmingly like a wild blaze to almost all the states in India and established as a naturalized weed. In India, the weed was first reported in Poona (Maharashtra) as stray plants on rubbish heaps by Prof. Paranjape in 1951. Later on, it was reported by Rao (1956) as a new record for the country but the earliest record of this species in India goes back to 1814 by William Roxburgh, 'the Father of Indian Botany', in his book Hortus Bbengalensis (Rouxburg, 1814; Rao, 1956). Ever since the weed became a menace around the globe.With increasing weed population in both urban and rural localities, the associated problems like crop production, animal husbandry, human health and biodiversity are growing dramatically. To the weed scientists, Parthenium has proved a challenge because conventional methods have failed to suppress its growth and prevent its unchecked spread throughout the world, and still efforts are being made to control this weed by all possible means. In this context, biological control with plant pathogens is an effective, safe, selective and practical means of weed management as they are easy to isolate, grow and manipulate. They are inherently less harmful than conventional pesticides, effective in very small quantities and often decompose very quickly. They are economically feasible, safe and non- pathogenic to non-target organisms. When used as a component of IPM programmes, mycoherbicides can greatly decrease the use of conventional herbicides (Aneja *et al*., 2013). Since 1979, considerable progress has been made towards practical use of plant pathogens as safe and selective agents of weed management (Charudattan and Walker, 1982; Aneja *et al.*, 2013). The biological control of this weed using fungal pathogens under the mycoherbicidal strategy has been suggested as one of the most efficient method, owing to its long lasting, less costly and eco-friendly nature (Kumar, 2009). The objective of the present study was to isolate and identify fungal pathogens naturally occurring on parthenium weed in northern India and to assess its potential for parthenium management.

Materials and Methods

Isolation and identification of the pathogens

Leaf surfaces of Parthenium were washed with distilled and sterilized water in order to remove epiphytic fungi and adherent soil particles**.** The infected leaves were cut into 1.0-1.5 cm fragments, surface sterilized with 70% ethanol for 1-2 minutes and then rinsed in sterile distilled water 3 to 4 times. These fragments were

transferred on to the potato dextrose agar (PDA) medium (potato: 200 g, agar: 20 g, dextrose: 20 g, distilled water: 1000 mL) supplemented with streptomycin sulphate. Petri plates were incubated at 25 °C. for 3 to 4 days (Aneja *et al*., 2014). After appearance of fungal growth on leaf surface, fungus was sub cultured and purified on PDA. Seven days later morphological characteristics of the mycelium and conidia of pathogens were observed and preliminarily identified as *F. solani* (Ellis, 1971; Ellis, 1976; Bilgrami, 1991). The identification of pathogen was later confirmed at CABI International Mycological Institute, UK with reference No. 503548.

Pathogenicity test

The pathogenicity was determined *in vitro* conditions. Healthy leaves of parthenium were used for inoculation. The leaves were washed with sterile distilled water and wiped with a cotton swab dipped in 70% alcohol. Some of the leaves before inoculation were injured on adaxial surface by pricking with a flamed needle. Mycelial discs taken from 5 days old colony were placed on injured and uninjured portions. The inoculated leaves were kept in sterilized moist chambers and incubated at 25 °C. Regular observations were made for the appearance of symptoms after 3 days of incubation (Aneja *et al*., 2000).

Potential of cell free culture filtrate of *F. solani* against parthenium

Richard's medium (Agarwal and Hasija, 1986) containing KNO_3 10 g, KH_2PO_4 5 g, $MgSO_4$ $7H_2O$ 2.8 g, sucrose 35 g, distilled water 1000 mL, pH-3.84 ± 1 was used. Ten mycelial bits (2.5 mm) separated from seven days old culture of the fungus grown on PDA medium at 25 ± 2 °C were transferred to 1000 mL Erlenmeyer flasks containing 500 mL Richard's medium. Inoculated flasks were incubated at 25 ± 2 °C for 7, 14 and 21 days. CFCF was aseptically obtained by filtering the metabolized growth medium through pre **w**eighed Whatman filter paper No. 1. The supernatant was filtered through 0.25 µm (Sartorius) filter paper under vacuum conditions (Walker and Templeton, 1978).

Parthenium leaves detached from the plant were surface sterilized with 0.2% NaOCl and were incubated in a sterilized moist chambers having cultural filtrate of pathogen at 25 ± 2 °C. The phytotoxic effect due to the application of toxin was observed after 24, 48 and 72 h (Sharma *et al*., 2004). Data were analyzed by applying Tukey's HSD test at 5% level of probability using computer software Statistics 8.1.

Results and Discussion

During the surveys in the various districts of Haryana in 2012-2014, infestation of parthenium was recorded in crops, uncultivated areas and roadsides. A leaf spot disease was observed on congress grass or parthenium in different parts of Kurukshetra. Symtoms of disease were observed as dark brown spots on leaves started from the margins, gradually spread towards the centre and become irregular shaped structures (Fig. 1 A). Young leaves showed less infection than mature leaves in the field indicated that young leaves were more resistant. Under severe conditions, the older diseased leaves were shed from the plant.

The pathogen was identified as *F. solani* (Fig. 1 B). The fungal culture was white to creamy from front and pale to green in reverse (Fig. 1B). Hyphae were septate and hyaline. Conidiophores were simple (non-branched) or branched monophialides (phialides with a single opening). Macroconidia were moderately curved, stout, thick-walled, usually 3-5 septate, measure 4-6 × 61-65 µm long, and on short conidiophores that soon form sporodochia. Microconidia were on long monophialides, and were one to three-celled, 2-5 × 8-16 µm long, and occur in false heads only, in clusters of conidia at the tip of the phialide as shown in Fig. 1C (Ellis, 1971). Chlamydospores were present (sometimes profuse) and occured both singly and in pairs. The identification of pathogen was confirmed at CABI International Mycological Institute, UK with reference No. 503548.

During *in-vitro* pathogenicity test, typical disease symptoms were produced on both injured and uninjured leaves and the inoculated pathogen was re-isolated and found similar to the original isolate in cultural characteristics thus confirming the pathogenicity of *F. solani* (Fig. 1D).

Detached leaf bioassay was performed by treating Parthenium leaves with different day's old metabolized broth (Fig. 2 A-D). Results indicated that 21 days old CFCF brought maximum phytotoxic damage followed by 14 and 7 days old metabolized medium (Fig. 3). In general effect was less pronounced after 12 hrs and gradually enhanced till 72 h. Maximum damage was observed after 72 h with 100% CFCF of *F. solani*. In general, toxin from 21 days old cultural filtrates caused severe chlorosis, necrosis and complete death of parthenium leaf.

Conclusions

The present study indicated that the *F. solani* seem to offer great potential for

development and exploitation as effective biocontrol agents against parthenium. Further work on its host specificity and evaluation as biocontrol agents is in progress in our lab, which may results in recognition the potential of *F. solani*. Further studies are required to isolate and identify the potential herbicidal constituents present in these fungal culture filtrates.

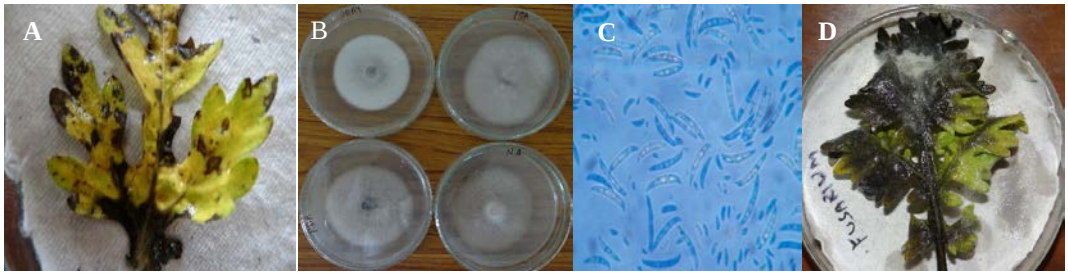

Fig. 1A-D: (A) Disease symptoms of the pathogen on parthenium leaf; (B) Colony of the pathogen after 7 days; (C) Macroconidia and microconidia of *Fusarium solani*, and (D) Infection on leave *in vitro* condition.

Fig. 2: Effect of cell free culture of *F. solani* on parthenium leaf after 72 hrs incubation. Phytotoxic effect due to of 7 (A), 14 (B) and 21 (C) days old fungal culture filtrates, and control (D).

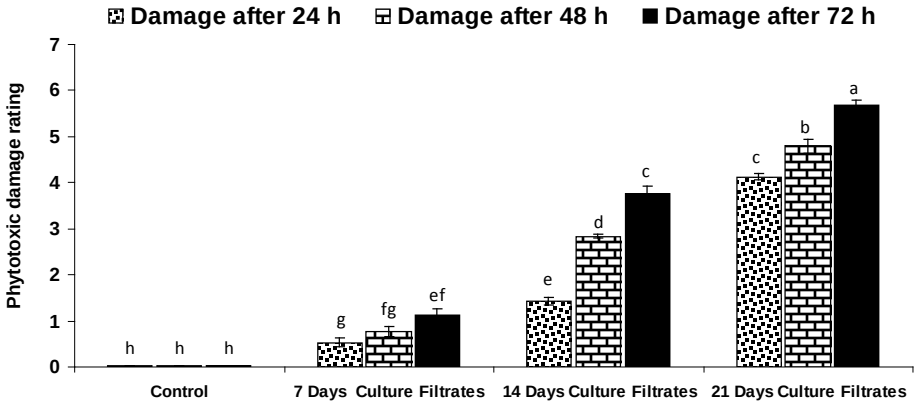

Fig. 3: Damaging effect of different days old cell free culture of *F. solani* on detached leaves of parthenium weed. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference ($P \leq 0.05$) as determined by Tukey's HSD test.

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