Biological Control Potential of Parthenium Hysterophorus Against Fusarium Solani – A Cause of Fusarium Wilt in Potato

Shazia Shafique, Sobiya Shafique

Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan

Abstract

Fusarium wilt, caused by *Fusarium solani,* is potential disease of potato in Pakistan. Recent studies have shown that fungal plant pathogens can be controlled by plant products as the plant materials are biodegradable, display structural diversity and complexity. Presently the pathogenic potential and biological control of *Fusarium solani* was studied by inoculating potato plants with three strains of *F. solani,* to screen the most virulent isolate among *F. solani* FCBP-016, *F. solani* FCBP-434 and *F. solani* FCBP-470. It was found through pathogenicity test that *F. solani* FCBP-434 was the most pathogenic among three *F. solani* strains with variation in genetic level. This disparity in genetic constitution might be a cause of its high pathogenicity. Further, antifungal bioassays were conducted to confirm mycotoxic potential of different plant parts i.e., root, shoot and leaf of *P. hysterophorus* against *F. solani* FCBP-434 using 1, 2, 3, and 4% concentrations of the aqueous, methanol and n-hexane extracts. Bioassays revealed that among the three solvents of *P. hysterophorus*, the growth of *F. solani* FCBP-434 was greatly inhibited at 1 & 2% conc. of aqueous and methanol extracts of leaf and stem while in case of n-hexane extract 3 & 4% conc. were proved more effective. Among root extracts, the higher concentrations of aqueous and n-hexane exhibited more promising results by causing reduction of 85 & 74%, respectively, whereas in methanol extract again lower conc. were more inhibitory.

Keywords: Fusarium wilt, Potato, Biocontrol, *P. hysterophorus,* Aqueous and organic solvents.

1. Introduction

Potato (*Solanum tuberosum*) is graded at third level among food after wheat and rice and ranks fifth in Pakistan for its total production [1]. Diseases are the most important factor for its low per acre production. Fusarium wilt is a fungal disease which can be caused by *Fusarium* species, particularly *F. solani*. Plant extracts and essential oils show antifungal activity against a wide range of fungi [2]. Therefore, the development of biopesticides has been focused as a viable pest control strategy in recent years. Among these, *Parthenium hysterophorus* is important. The allelopathic and antifungal potential of *P. hysterophorus* is due to release of phytotoxic substances such as caffeic, ferulic, vanillic, chlorogenic, *p*- coumaric and parthenin, *p*-hydroxybenzoic acids, ambrosin and coronopilin [3]. The antifungal activities of root and shoot extracts of two Asteraceous plant species viz. *P. hysterophorus* L and *Ageratum conyzoides* were determined against *Macrophomina phaseolina* (Tassi) Goid., the cause of charcoal rot disease of sunflower (*Helianthus annus* L.). A

measured reduction in *M. phaseolina* biomass was evaluated due to aqueous extracts of different concentrations [4]. On the basis of efficacy of plant extracts, present study has been designed to find out the effective plant product for the management of *F. solani* by using *P. hysterophorus* and molecular characterization of *F. solani.*

2. Methodology

Pure cultures of *Fusarium solani* FCBP-016, *F. solani* FCBP-434 and *F. solani* FCBP-470 were taken from FCBP, Institute of Agricultural sciences, University of the Punjab, Lahore. They were maintained and subcultured on Malt extract agar medium at 4 °C monthly.

For pathogenecity test the protocol of Grogan et al. [5] was adopted. Pathogenicity test was performed by inoculating the conidial suspension @ 10-15 mL/plant containing 2 x 10^5 conidia mL^{-1} on 1-month-old potted potato plants and soil with three selected isolates of the fungus. Disease severity was calculated with the help of following formula.

$$\text{Disease severity} = \frac{\text{Affected area of a plant}}{\text{Total area of the plant}} \text{ x } 100$$

Screening of the most pathogenic isolate was carried out on the basis of pathogenicity test. The most pathogenic species was isolated and subjected to further biocontrol assays.

The genomic DNA of three different isolates of *F. solani* was extracted by CTAB method [6] with some modifications.

Aqueous extract was primed according to Bajwa et al. [7]. While the method of Alkhail [8] was followed for preparation of extract in methanol and n-hexane. The lower conc. of 1, 2, 3 & 4% of aqueous, n-hexane and methanol extracts of leaf, stem and root were prepared by adding appropriate quantity of sterilized distilled water. To make methanol and n-hexane control, 2 mL of methanol and n-hexane were dissolved in sterilized distilled water to make final volume 100 mL, in respective flasks.

Extract bioassays were carried out in liquid medium according to Bajwa et al. [9]. Their dry weight yield was determined after 24 h oven drying at 60 °C and percentage inhibition in biomass production was calculated as:

$$\text{Biomass reduction (\%)} = \frac{\text{Biomass in Control - Biomass in extract treatment}}{\text{Biomass in Control}} \text{ x } 100$$

All the data was subjected to analysis of variance (ANOVA) followed by Duncan's Multiple Range Test to delineate mean differences [10].

3. Results

3.1. Pathogenecity Test

The pathogenicity of three isolates of *F. solani* (FCBP-016, FCBP-434, FCBP-470) was assessed which enabled the reproduction of typical symptoms of the disease over the time-

scale after 15 days of incubation at 25 °C. Five levels of pathogenicity were detected on potato plants (Fig. 1). *Fusarium solani* FCBP-434 was proved to be the most pathogenic. Rapid results were obtained with only two primers i.e. A-02 and B-05 and the dendrogram generated from each primer and cluster of primers by MINITAB are presented Fig. 2. The RAPD data obtained with 13 primers was evaluated to analyze the genetic parity or disparity among different genotypes. A dendrogram was constructed on the basis of genetic distances by UPGMA method and two main groups of cluster were identified in the homology tree: F. solani FCBP-016 and F. solani FCBP-470 on one side and F. solani FCBP-434 on the other side (Fig. 3). F. solani FCBP-016 and F. solani FCBP-470 are 100% similar to each other but they show only 44.34% similarity with F. solani FCBP-434. The findings of pathogenicity test and molecular analysis showed that F. solani FCBP-434 have high pathogenic potential so it was subjected to further biocontrol assays.

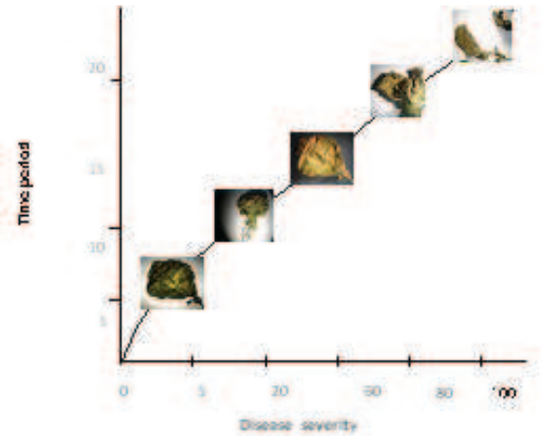

Fig 1. Periodic progression of Fusarium wilt of potato.

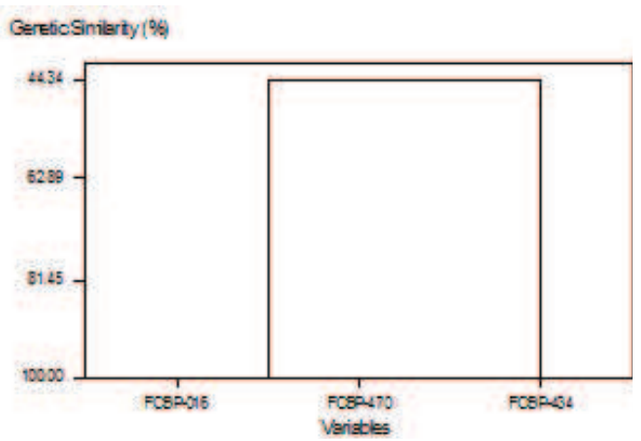

Fig 2. Homology tree constructed by UPGMA method for genetic similarity amongst genotypes of F. solani FCBP-016, 434 and 470.

3.2. Biological control through leaf extract

The data on dry biomass production in early growth phase of 7-10 days after incubation (DAI) revealed that all the conc. significantly decreased the fungal biomass as compared to control (Fig. 4). Amongst all concentrations 1% concentration exhibited the most promising results as it caused about 85% reduction in fungal biomass while 62, 52 and 41% decline in fungal biomass was depicted by 2, 3 and 4% aqueous extract, respectively. The response of *F. solani* in terms of dry biomass production was variable when grown in different concentrations of methanol extract of *P. hysterophorus*. Amongst these 2% concentration was the most effective in suppressing the biomass production up to 76% and 3% concentration was also found to decline fungal biomass production up to 67% In dry fungal biomass production with an increase in concentration of n-hexane extract revealed the maximum antifungal stress by 4% concentration causing a decline of about 66% in the biomass production of *F. solani*. It was followed by 3, 2 & 1% concentration which revealed a significant reduction, in the range of 56, 50 and 44%, respectively.

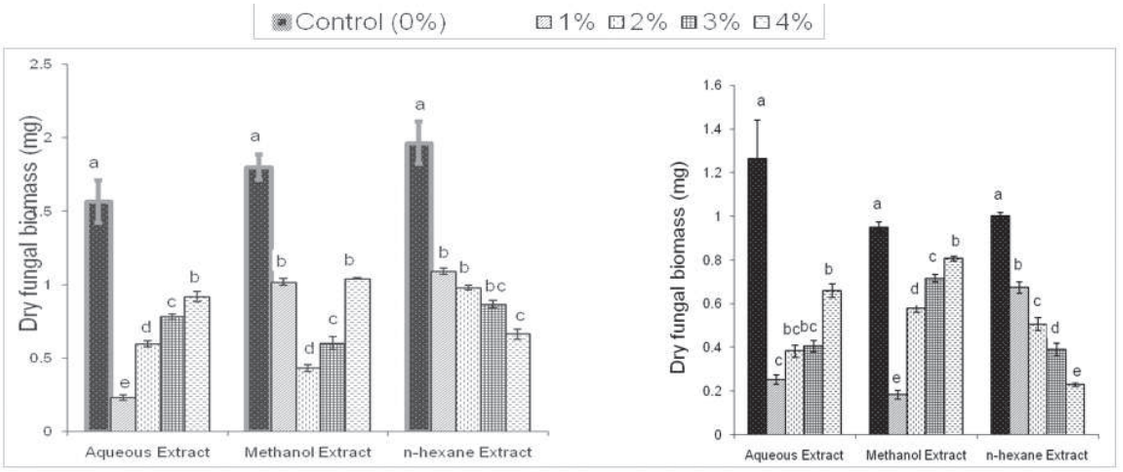

Fig 3. Effect of various conc.s of aqueous, methanol and n-hexane leaf extracts of P. hysterophorus on dry biomass production of F. solani FCBP-434.

Fig 4. Effect of various conc.s of aqueous, methanol and n-hexane stem extracts of P. hysterophorus on dry biomass production of F. solani FCBP-434.

3.3. Biological control through stem extract

All extract doses significantly inhibited the growth of the test pathogen in the same manner as depicted by aqueous leaf extract (Fig. 5). The greatest reduction in biomass production (70%) was observed in 1% extract. There was 48-70% reduction in fungal biomass production as noticed due to 1-4% concentrations of the extracts. All regimes of methanol extract of *P. hysterophorus* caused considerable inhibition in biomass production. The maximum inhibition in fungal growth was evidenced by 1% concentration which decreased biomass production upto 80%. While all the other concentrations (2-4%) caused about 15-38% reduction in biomass production, respectively. A variable response of dry biomass production in *F. solani* FCBP-434 was recorded to n-hexane extract of *P. hysterophorus* in different concentrations. *F. solani* FCBP-434 exhibited a significant reduction when exposed to different concentrations of extracts compared to control. The reduction in biomass ranged from 33 to 77%.

3.4. Biological control through root extract

All the doses of aqueous root extracts significantly retarded growth of the test fungus pathogen (Fig. 6). The fungal biomass suppressed at lower concentration (1-2%) in a range of 14-45%. While higher concentrations (3-4%) proved the most effective as these caused a reduction up to 67-85%, respectively. In methanol treatment, the lowest concentration (1%) was the most effective in reducing and suppressing the target fungal pathogen up to 77% as compared to higher concentrations. The fungal biomass increased at higher concentrations (2-4%) but still a significant inhibition in biomass production was detected with reference to control.A similar kind of suppressive effect of various concentrations was recorded against target fungal pathogen as exhibited by n-hexane leaf and stem extract. All the concentrations significantly reduced the fungal biomass production. Amongst these 4% concentration was the most effective in suppressing the biomass production up to 74%.

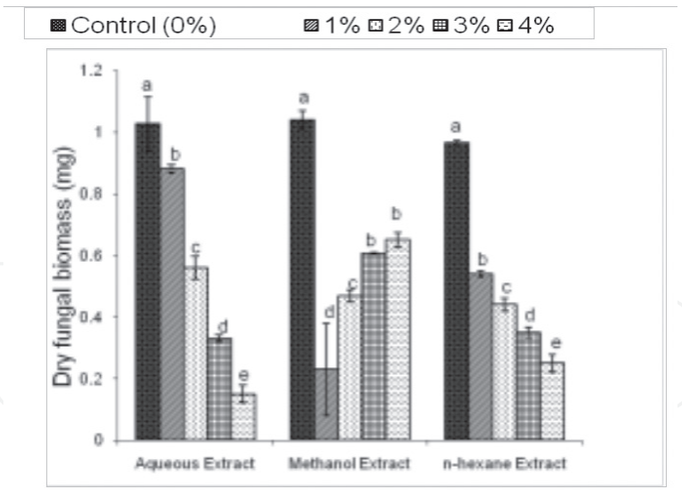

Fig 6. Effect of various conc.s of aqueous, methanol and n-hexane root extracts of P. hysterophorus on dry biomass production of F. solani FCBP-434.

4. Discussion

In the present study, pathogenicity test was carried out to assess the pathogenicity of three isolates of *Fusarium solani*. Among the isolates FCBP-434 proved to be more pathogenic and destructed the leaves on infected plants in 15 days after inoculation. Similar results were reported from various host plant - *F. oxysporum* combinations [11]. Molecular analysis of three isolates was also conducted. It was obvious from the results that *F. solani* FCBP-434 had genetic variability than other two isolates. In several studies RAPD fingerprinting technique has been employed to detect mutation, genetic relatedness and genetic variation within and between natural bacterial and human DNA and fungal populations [12, 13]. With the help of these two evidences about high pathogenic ability of *F. solani* FCBP-434, further its biological control assays were conducted.

It is obvious from the study that mycelial growth rate was significantly inhibited by antifungal compounds specifically at lower concentrations of aqueous leaf and stem extract. These results are supported from previous investigations in which leaf extracts of *Datura stramonium* have been shown to cause a decline in the development of rust pustules on wheat leaves [14]. Further increase in extract concentration exhibited significant difference in antimycotic activity as compared to 1% extract. In case of aqueous and organic root extract of *P. hysterophorus,* phytotoxins or antifungal compounds inhibited the biomass at higher concentration. The variation in antifungal activity of shoot and root extracts may be attributed to the different chemical nature of the compounds present in these parts [15]. Greater inhibition (42-76%) of growth of *F. solani* at 2-3% conc. of leaf methanolic extract was observed. While in case of stem and root methanolic extracts, lower conc (1-2%) were more effective as compared to higher conc. (3-4%). The pattern of gradually higher production of biomass in response to increasing conc. of aqueous extract was also similar to investigation of Bajwa et al. [16]. Comparative effectiveness of n-hexane extracts of selected test species revealed that higher concentrations were relatively more allelopathic thanlower concentrations. These results are supported by the work of Vir and Sharma [17] that employed 10% conc. of neem oil that exhibited 100% inhibition in *A. niger, D. rostrata* and *M. phaseolina.*

Thus it can be recommended that use of *P. hysterophorus* against *Fusarium solani* give better results as they are environmental safe alternatives and can be further exploited for formulating integrated disease management.

5. References

[1] N.J. Malik. Potatoes in Pakistan. World MatPrinters, 8A. Saharwwardy Avenue, Islamabad. 1995, Pp **3**: 27.

[2] P. Masoko, J. Picard, J.N. Eloff. The antifungal activity of twenty-four southern African *Combretum* species (Combretaceae). *South. Afr. J. Bot.* 2007, **73**:173-183.

[3] B.B. Jarvis, N.B. Pena, M.M. Rao, R.S. Comezoglu, T.F. Comezoglu, N.B. Mandava. Allelopathic agents for *Parthenium hysterophorus* and *Baccharis megapotamica*. In *The Chemistry of Allelopathy, biochemical interactions among the plants, American Chemical Society.* 1985, pp: 149–59.

[4] R. Bajwa, S. Shafique, S. Shafique. Evaluation of antifungal activity of aqueous extracts of two Asteraceous plants species. *Mycopath,* 2007a, **5**(1): 29-33.

[5] R.G. Grogan, K.A. Kimble, I. Misaghi. A stem canker disease of tomato caused by *Alternata alternata* f. sp. Lycopersici. *Phytopathol*. 1975, 65: 880-886.

[6] M.A. Saghai-Maroof, K.M. Soliman, R.A. Jorgensen, R.W. Allard. Fungal DNA Isolation. Proceedings of the National Academy of Sciences. 1984, **81**: 8014-8018.

[7] R. Bajwa, S. Shafique, S. Shafique. Appraisal of antifungal activity of *Aloe vera. Mycopath.* 2007, **5** (1): 5-9.

[8] A.A. Alkhail. Antifungal activity of some extracts against some plant pathogenic fungi. *Pak. J. Biol. Sci.* 2005, **8**: 413–417.

[9] R. Bajwa, T. Anjum, S. Shafique, S. Shafique. Evaluation of antifungal activity of *Cicer arietinum* L. *Pak. J. Bot.* 2006, **38**: 175-184.

[10] R.G.D. Steel, J.H. Torrie. *Principiles and Procedures of Statistics.* McGraw Hill Book Co., Inc, New York, USA. 1980.

[11] G.H. Kim, Hur Jae-Seoun, W. Choi, Y.J. Koh. Fusarium Wilt of Winter Daphne (*Daphne odora* Thunb.) caused by *Fusarium oxysporum. Plant Pathol. J.* 2005, **21**: 102-105.

[12] A.P. Keinath, M.W. Farnham, T.A. Zitter. Morphological, pathological, genetic differentiation of *Didymella bryoniae* and *Phoma spp.* isolated from cucurbits. *Phytopathol.* 1995, **85**: 364-369.

[13] C. Jones, A. Kortenkamp. RAPD library finger printing of bacterial and human DNA: applications in mutation detection. *Teratogen. Carcinogen. Mutagen.* 2000, **20** (2): 49-63.

[14] I. Hassan, M.A. Nasir, M.R. Haque. Effect of different plant extract on brown rust and yield of wheat. *J. Agri. Res.* 1992, **30**: 127–31.

[15] S. Afsharypuor, A. Mostajeran, R. Mokhtary. Variation of Scopolamine an Atropine in different parts of *Datura metel* during development. *Planta Med.* 1995, **61**: 383-384.

[16] R. Bajwa, S. Shafique, T. Anjum, S. Shafique. Antifungal activity of allelopathic plant extracts IV: Growth response of *Alternaria alternata*, *Fusarium monili-frome* and *Drechslera hawaiiensis* to aqueous extract of *Parthenium hysterophorus* L. *Int. J. Agri. Bio.* 2004, **6**: 511-516.

[17] D. Vir, R.K. Sharma. Evaluation of neem oil for control of plant pathogens. *Asian. Farm. Chem.* 1985, **1** (7-8): 23-24.