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Comparative Evaluation of Phytotoxicity of *Alternaria macrospora* Isolates, Potential Biocontrol Agent against Parthenium Weed

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ABSTRACT

Parthenium hysterophorus L. an exotic, pernicious weed is considered as one of the most troublesome weeds for agricultural sector by virtue of its high ecological amplitude and adaptability. Microbes and their by products is now proved to be a worthy alternative to toxic chemicals used for weed management. We determined the relative herbicidal potential of phytotoxin of four *Alternaria macrospora* isolates recovered from diseased leaves of parthenium by employing detached leaf, shoot cut and seed germination bioassay. There was a considerable diversity amongst various isolates. The isolate MKP2 from Kurukshetra showed the maximum Damage to the plant, whereas, isolate MKP4 also from Kurukshetra showed the lowest damage to the parthenium leaves. The wide range of phytotoxicity among *Alternaria macrospora* support the view that strain breeding for biological control of *P. hysterophorus* L. is warranted.

Key words: *Parthenium hysterophorus*, *Alternaria macrospora*, phytotoxicity, mycoherbicide

INTRODUCTION

Parthenium hysterophorus L. is a notorious weed creating problems for agriculture and public health. Conventional means of its control have failed due to their innate drawbacks. The concept of mycoherbicides using indigenous fungal pathogens provides a viable option at this juncture (Kaur *et al*., 2014). Biological, technological and commercial perspectives of this concept is now well documented in various publications. Mycoherbicidal potential of the pathogen is known to influence by environmental factors. To overcome these constraints, toxic metabolite produced by the pathogen have also tried (Pandey *et al*., 2003). Phytotoxins have not received extensive testing for their use as synergists of biocontrol agents. Thus, there is a need to study the weed pathogens, phytotoxins produced by them and their integration with biocontrol agents for a holistic approach for an integrated weed management.

During the survey it was observed that the isolates of *Alternaria macrospora* infected the weed and caused leaf spot and blight symptoms. Therefore, the present investigation was under taken with the *in vitro* evaluation of herbicidal potential of the pathogens against parthenium by detached leaf's shoot cut and seed germination bioassay.

MATERIALS AND METHODS

Isolation of pathogen: The isolates of *A. macrospora* were isolated from the infected leaf portion of the parthenium weed. The leaf was cut into small portions and sterilized in 70% ethanol then washed in sterile distilled water for four to five times. Leaf portions were then placed on PDA

medium plates supplemented with streptomycin sulphate. These were then incubated at ±25°C for 7 days. Isolated fungi were aseptically transferred to PDA plates and the pure cultures were incubated at above conditions. The pure culture was maintained on PDA slants (Aggarwal *et al*., 2014).

Morphological identification: Lacto phenol cotton blue mount was used to study the morphological characteristics of the mycelium, conidia and perithecia of fungal strain and preliminarily identification was done with the help of standard literature (Ellis, 1971, 1976; Bilgrami *et al*., 1991).

Molecular identification: Fungal pathogen was molecular characterized by using the commercial service provided by Macrogen Inc., Advancing through Genomics, Korea. Fungus genomic DNA samples were extracted using a InstaGene^{tm} Matrix (BIO-RAD.) The primers ITS1 primer (5-TCCGTAGGTGAACCTGCGG-3) and ITS5 (5-GGAAGTAAAAGTCGTAACAAGG-3) and ITS4 primer (5-TCCTCCGCTTATTGATATGC-3) were used for the PCR. The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30 μL reaction mixture by using a EF-Taq (SolGent, Korea) as follows: activation of Taq polymerase at 95°C for 2 min, 35 cycles of 95°C for 1 min, 55 and 72°C for 1 min each were performed, finishing with a 10 min step at 72°C. The amplification products were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). The purified PCR products of approximately 600 bp were sequenced by using 2 primers as described. Sequencing reaction was performed using a PRISM BigDye Terminator v3.1 Cycle sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95°C for 5 min, followed by 5 min on ice and then analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA) (Siddiquee *et al*., 2010; Satou *et al*., 2001).

Pathogenicity test: The pathogenicity was determined in *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. Mycelial discs taken from 5 days old colony were placed on leaves. 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 (Aggarwal *et al*., 2014).

Preparation of cell free culture filtrate: 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, $FeCl_3$-trace, distilled water 1000 mL, pH-3.84±1 was used for the fermentation. 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 medium. Inoculated flasks were incubated at 25±2°C in B.O.D incubator (Remi, India) for 21 days. The CFCF was aseptically obtained by filtering the metabolized growth medium through pre-weighed Whatman filter paper No. 1. The supernatant was filtered through the filter paper 0.25 μm (Sartorius), under in vacuum conditions (Walker and Templeton, 1978).

Detached leaf bioassay: Parthenium leaves detached from the plant were surface sterilized with 0.2% NaOCl and were incubated in a sterilized moist chambers having 21 days old 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).

Shoot cut bioassay: Shoots (15-20 cm in length) were taken and tip of the shoots were sterilized by washing with tap water and with 0.1% NaOCl solution for 3 min and immediately washed in sterilized distilled water to remove any trace of the chemical. The shoots were dipped in 100% concentration of 21 days old CFCF filtrates of different isolates. Suitable control was maintained with the help of sterile water. Flasks were incubated at 25±2°C in B.O.D. incubator. The effect of toxic metabolites was observed on the shoots after 24, 48 and 72 h (Singh *et al*., 2010).

Seed germination bioassay: The toxicity of CFCF against parthenium seeds was also tested by Seed Germination Bioassay. Seeds of parthenium were surface sterilized in 0.01% NaOCl solution for 15 min, then washed thoroughly with distilled water. The surface sterilized parthenium seeds were placed on the moistened filter paper kept in the sterile petridish. Ten milliliter of 21 days old cultural filtrate was poured into the moisture chamber. Suitable control was maintained with the help of sterile water. Plates were incubated at 25±2°C in B.O.D. incubator. Seed germination percent was recorded after seven days (Singh and Pandey, 2001; Thapar *et al*., 2002). The experiment was carried out in triplicate, respectively.

RESULTS

A congress grass population was found affected by various leaf spot and leaf blight diseases at different parts of Kurukshetra. The spots on PDA yielded four different colonies of fungal pathogens and microscopic study revealed that the pathogens belong to the genus *Alternaria*. Molecular analysis of the ITS1-5.8S-ITS2 rDNA region was carried out to confirm the species identity of all these pathogens. Fungal pathogens were molecular characterized by using the commercial service provided by Macrogen Inc., Advancing through Genomics, Korea. The results of the molecular Identification (ITS rDNA sequence analysis) showed that isolates are the different strains of *Alternaria macrospora* (*A. macrospora* strain MKP1, *A. macrospora* strain MKP2, *A. macrospora* strain MKP3, *A. macrospora* strain MKP4). When all the four strains were tested for pathogenicity on both injured and uninjured leaves the pathogens were re-isolated and found to be similar to the original isolates, thus confirming the pathogenicity of all the fungal isolates to parthenium and proving of Koch's postulates. A survey of available literature reveals that this species of *Alternaria* has reported for the first time on *P. hysterophorus* from India.

Detached leaf bioassay: Detached leaf bioassay was performed by treating parthenium leaves with 21 day's old metabolized broth for different time period (24, 48 and 72 h). Four distinct phytotoxic reactions were observed for all the four isolates tested against parthenium leaves (Fig. 1a-e). Disease reactions varied with the isolates, which ranged from chlorosis, necrosis and finally death of the parthenium leaves (highly aggressive). As depicted in Fig. 2 *A. macrospora* MKP2 exhibited maximum disease incidence followed by *A. macrospora* MKP1, *A. macrospora* MKP3 and least infectivity was recorded in case of *A. macrospora* MKP4. In general effect was less pronounced after 12 h and gradually enhanced till 72 h. Therefore, significant variation existed between all the isolates tested in the present investigation. The 21 days old cultural filtrate of *A. macrospora* MKP2 at 100% concentration exhibited maximum phytotoxicity, has immense potential to be develop as mycoherbicide agent against *P. hysterophorus*.

Fig. 1(a-e): Effect of 21 days old CFCF of *Alternaria macrospora* strains on parthenium leaves after 72 h incubation. Phytotoxic effect due to (a) *A. macrospora* MKP1, (b) *A. macrospora* MKP2, (c) *A. macrospora* MKP3, (d) *A. macrospora* MKP4 and (e) Control

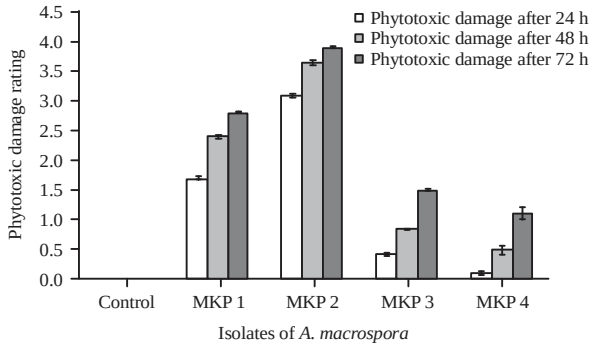

Fig. 2: Effect of 21 days old CFCF of four strains of *Alternaria macrospora* by detached leaf biossay

Shoot cut bioassay: As evident from Fig. 3, when shoots of test weed parthenium were immersed in 21 days old CFCF of the test fungal strains, phytotoxic damage occurred which was observed after 24, 48 and 72 h. The *A. macrospora* MKP2 exhibited maximum phytotoxic damage to the parthenium shoots followed by MKP1, MKP3 and MKP4. Phytotoxic effect was less after 24 h and mild at 48 h but was maximum at 72 h (Fig. 4). The shoots exhibited blackening of stem, drooping and curling of leaves, severe chlorosis and necrosis, finally leading to death of the toxin treated shoots.

Fig. 3(a-e): Effect of CFCF of *Alternaria macrospora* isolates on parthenium shoots after 72 h incubation. Phytotoxic effect due to (a) *A. macrospora* MKP1, (b) *A. macrospora* MKP2, (c), *A. macrospora* MKP3, (d) *A. macrospora* MKP4 and (e) Control

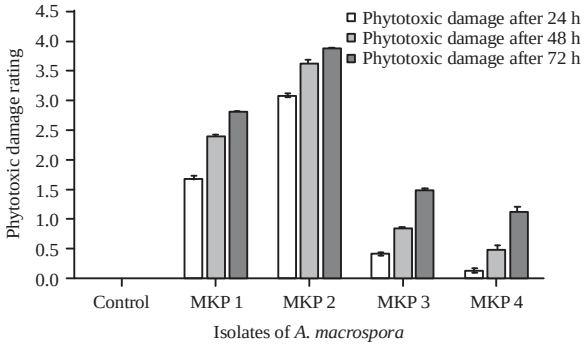

Fig. 4: Effect of 21 days old CFCF of four strains of *Alternaria macrospora* by shoot cut biossay

Seed germination bioassay: Percentage inhibition of seed germination is different according to different culture filtrates. Results of the parthenium seed germination by 21 days old cultural filtrate revealed that the cultural filtrate of *A. macrospora* MKP2 significantly decrease the germination of seeds as compare to the *A. macrospora* MKP1 and *A. macrospora* MKP3 cultural filtrate and least effect on inhibition of seed germination was recorded in case of isolate *A. macrospora* MKP4 (Fig. 5). In control (distilled water) negligible reduction occurred in germination and about 90% of seeds were germinated. 100, 77.7, 55.5, 44.4% germination

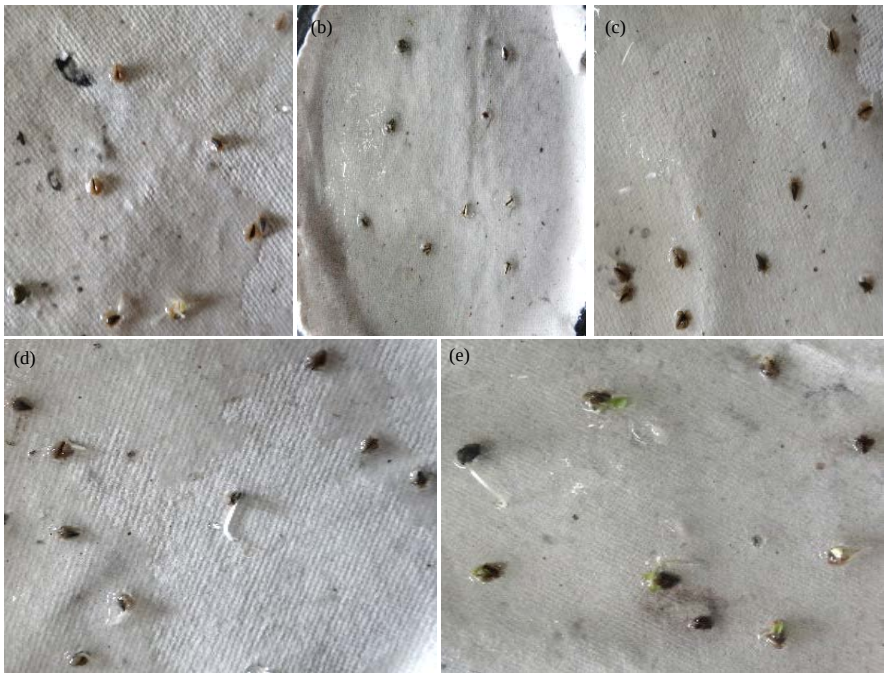

Fig. 5(a-e): Germination inhibition of parthenium seeds (a) 77.7% inhibition by cultural filterate of *A. macrospora* MKP1, (b)100% inhibition by cultural filterate of *A. macrospora* MKP2, (c) 55.5% inhibition by cultural filterate of *A. macrospora* MKP3, (d) 44.4% inhibition by cultural filterate of *A. macrospora* MKP4 and (e) 11.11% inhibition by control

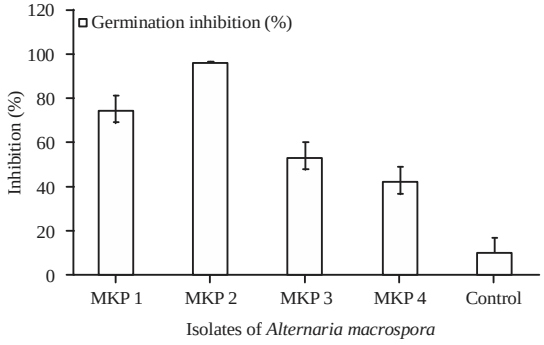

Fig. 6: Effect of 21 days old CFCF of *A. macrospora* strains on seed germination biossay

inhibition occurred in the MKP2, MKP1, MKP3, MKP4, respectively. Results after the treatment of the seeds of parthenium with cultural filtrate produced by the different isolates of *Alternaria macrospora* are represented in Fig. 6.

DISCUSSION

The biological control of weeds 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. Walker and Templeton (1978) suggested that the concept of the phytotoxic

metabolites of fungal pathogens is also an attractive proposal for biological control of weeds, so our work in this area aims for searching a potential pathogen with phytotoxic activity, which should be emerges as an effective mycoherbicide against this weed.

It is clear from the result that filtrate of *Alternaria macrospora* isolates inhibited seed germination and causes significant damage to the leaf and shoot of the parthenium which means that metabolites are discharged by the tested fungi in the media in which they were grown. Significant variation in phytotoxin and virulence of various pathogens at different levels viz., generic, species levels or even at intraspecific level have also recorded by many workers (Ansari and Agnihotri, 2000). Variation in virulence has been correlated with synthesis of different phytotoxins and of enzymes (Punja *et al*., 1985). Variations in phytotoxic damage with different days old metabolized broth on test weed have been reported by earlier workers (Pandey *et al*., 2005; Quereshi *et al*., 2006; Thapar *et al*., 2002), similar results on detached leaf bioassay have been obtained by Sharma *et al*. (2004). Inhibitory nature of the fungi was also recorded by different workers. Sinha and Prasad (1981) recorded inhibition of seed germination in mung due to *Alternaria alternata*, *Curvularia lunata* and *Macrophomina phaseolina*. this is due to toxins secreted in the media. The result of seed germination inhibition corroborate with the findings of Fulton *et al*. (1965), which strengthen the present data. These effects on seed germination may be due to inhibitory factor present in the fungal culture filtrate.

Thus, the main aim of this paper was to highlight the herbicidal efficacy of the phytotoxins of *Alternaria macrospora* isolates, as novel and lucrative source of potential herbicides for the management of weed, *Parthenium hysterophorus*. Therefore, it appears that the cultural filtrate used in this study must have some toxin or alike substance which needs further detailed investigation to isolate and identify the potential herbicidal constituents present in these fungal culture filtrates.

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REFERENCES

Agarwal, G.P. and S.K. Hasija, 1986. Microorganisms in the Laboratory-a Laboratory Guide of Mycology, Microbiology and Plant Pathology. Print House India, Lucknow, pp: 155.

Aggarwal, N.K., M. Kaur, V. Kumar and A. Saini, 2014. Mycobiota associated with *Parthenium hysterophorus* isolated from North India. Indian J. Weed Sci., 46: 155-160.

Ansari, M.M. and S.K. Agnihotri, 2000. Morphological, physiological and pathological variations among *Sclerotium rolfsii* isolates of soybean. Indian Phytopathol., 53: 65-67.

Bilgrami, K.S., S. Jamaluddin and M.A. Rizwi, 1991. Fungi of India-List and References. Today and Tomorrow's Printers and Publishers, New Delhi, India, ISBN-13: 9781555282592, Pages: 798.

Ellis, M.B., 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.

Ellis, M.B., 1976. More Dematiaceous Hyphomycetes. Commonwealth Mycological Inc., England, USA., ISBN-13: 9780851983653, Pages: 507.

Fulton, N.D., K. Bollenba and G.E. Templeto, 1965. A metabolite from *Alternaria tenuis* that inhibits chlorophyll production. Phytopathology, 55: 49-51.

Kaur, M., N.K. Aggarwal, V. Kumar and R. Dhiman, 2014. Effects and management of *Parthenium hysterophorus*: A weed of global significance. Int. Scholarly Res. Notices. 10.1155/2014/368647.

Pandey, A.K., G.M. Shrivastava, A.K. Singh and S.K. Yenna, 2003. Herbicidal potential of secondary metabolites of *Sclerotium rolfsii* against Parthenium: A preliminary observation. J. Basic Applied Mycol., 2: 27-30.

Pandey, A.K., A.K. Singh, S. Quereshi and C. Pandey, 2005. Herbicidal activities of secondary metabolites of *Aspergillus* sp. against *Lantana camara*. J. Basic Applied Mycol., 4: 65-67.

Punja, Z.R., V.L. Smith, C.L. Campbell and S.F. Jenkins, 1985. Sampling and extraction procedure to estimate numbers, spatial pattern and temporal distribution of sclerotia of *Sclerotium rolfsii* in soil. Plant Dis., 69: 469-474.

Quereshi, S., A.K. Pandey, A.K. Singh and K.K. Yadav, 2006. Herbicidal potential of *Phoma* sp. FACC# 54. A preliminary evaluation. J. Basic Applied Mycol., 5: 60-61.

Satou, M., M. Ichinoe, F. Fukumoto, N. Tezuka and S. Horiuchi, 2001. *Fusarium* blight of kangaroo paw (*Anigozanthos* spp.) caused by *Fusarium chlamydosporum* and *Fusarium semitectum*. J. Phytopathol., 149: 203-206.

Sharma, P., S.R. Sharma and M. Sindhu, 2004. A detached leaf technique for evaluation of resistance in cabbage and cauliflower against three major pathogens. Indian Phytopathol., 57: 315-318.

Siddiquee, S., U.K.B. Yusuf and N.A.I.M. Zainudin, 2010. Morphological and molecular detection of *Fusarium chlamydosporum* from root endophytes of *Dendrobium crumenatum*. Afr. J. Biotechnol., 9: 4081-4090.

Singh, J. and A.K. Pandey, 2001. Incidence of storage mycoflora associated with some forest plants of ethnic values in M.P. Ind. J. Applied Pure Biol., 16: 43-45.

Singh, J., S. Quereshi, N. Banerjee and A.K. Pandey, 2010. Production and extraction of phytotoxins from *Colletotrichum dematium* FGCC# 20 effective against *Parthenium hysterophorus* L. Braz. Arch. Biol. Technol., 53: 669-678.

Sinha, M.K. and T. Prasad, 1981. Effect of fungal metabolites on seed germination, microbial association and seedling growth of Mung. Indian Phytopathol., 34: 515-517.

Thapar, R., A.K. Singh, A. Pandey and A.K. Pandey, 2002. Bioactivity of CFCF of *Curvularia lunata* in *Parthenium hysterophorus* L. J. Basic Applied Mycol., 1: 126-129.

Walker, H.L. and G.E. Templeton, 1978. *In vitro* production of a phytotoxic metabolite by *Colletotrichum gloeosporiodes* f. Sp. *Aeschynomene*. Plant Sci. Lett., 13: 91-96.