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Studies on various histopathological parameters to evaluate the biological control potential of *Alternaria macrospora* MKP1 against *Parthenium* weed

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ABSTRACT

Parthenium hysterophorus (Asteraceae) is known as one of the most troublesome weeds, causing severe environmental, economic, human and animal health problems in India and around the world. During a series of surveys for natural enemies of *P. hysterophorus*, a leaf blight pathogen was isolated from the affected parts of the *Parthenium* following the standard isolation techniques using potato dextrose agar (PDA) media. Koch's postulates were performed and found satisfactory for the isolate and proved to be pathogenic to this weed. On the basis of cultural, morphological and molecular characteristics, the pathogen was identified as *Alternaria macrospora* MKP1. The pathogen was studied for various histopathological parameters and the results showed that this pathogen has a good potential to control *Parthenium* weed.

Introduction

Parthenium hysterophorus L. (Asteraceace: Heliantheae), commonly known as parthenium, white top, congress grass, feverfew or carrot weed, is one of the worst weeds, threatening natural ecosystems and agro ecosystems in over 30 countries worldwide (Adkins and Shabbir, 2014). Parthenium has proved a challenge, because the conventional means of its control have failed due to their innate drawbacks. (Aggarwal et al. 2014). A great many chemical pesticides because of potential human health risks, environmental pollution, effects on non-target organisms and the development of pest resistance, have been or being phased out (Kaur et al. 2014). Efforts are still being made to control this weed by all possible means. In this context, biological control with plant

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pathogens is an effective, safe, selective and practical means of weed management. The concept of mycoherbicides using indigenous fungal pathogens provides a viable option at this juncture (Kaur et al. 2014). The mycoherbicidal potential of the pathogen is known to influence by environmental factors. To overcome these constraints, toxic metabolite and extracellular enzymes produced by the pathogen have also tried (Pandey et al. 2003). Pathogenic fungi occupy a relatively unexplored area in microorganism enzymes and Phytotoxins with different potentialities. They have not received extensive testing for their use as synergists of biocontrol agents. Thus, there is a need to study the weed pathogens, phytotoxins and enzymes produced by them and their integration with biocontrol agents for a holistic approach for an integrated weed management.

Materials and Methods

Isolation of the pathogen

The fungus *A. macrospora* MKP1 was isolated from the infected leaf portion of the parthenium weed (Aggarwal et al. 2014).

Morphological identification

The morphological characteristics of the mycelium, conidia and perithecia of fungal pathogen were studied using Lacto phenol cotton blue mount and preliminarily identification was done with the help of standard literature (Ellis 1976, 1971).

Molecular identification

Fungal pathogen was molecularly characterized by using the commercial service provided by Macrogen Inc., Advancing through Genomics, Korea (Kaur and Aggarwal, 2015)

In vitro Pathogenicity test

Healthy leaves of congress grass 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 of 8 mm were taken from 5 days old colony of isolated pathogen and placed on injured and uninjured portions. Then covered with sterile moist cotton. The inoculated leaves were kept in sterilized moist chambers and incubated at 25±2°C. Observations for the appearance of symptoms were made after 3 days of incubation (Aneja et al. 2000).

Optimization of cultural conditions

Optimization of cultural conditions was done using 'one variable at a time' approach. To determine the effect of various parameters on growth and conidial concentration, both solid and liquid media were used.

In solid media

Fifteen ml of a given media was poured into each sterile Petri plates and allowed to solidify. The plates with solidified medium were kept in an inverted position for 24 hrs to remove the thin film of water from the surface. Mycelial discs of 8mm diameter of the pathogen cut from the periphery of seven days old, actively growing colonies were placed in the centre of each plate and were incubated at 25±1°C for 7 days. Three replicates were taken for each medium, temperature, pH and relative humidity. Fungal growth was determined by calculating the area of radial growth for each colony (Abbas et al. 1995). Conidial concentration was determined by using haemocytometer (Tuite, 1969).

In broth

25 ml of broth was taken in each 100ml Erlenmeyer flask and sterilized at 121°C for 15 minutes. Flasks were inoculated each with an inoculum disc of 8mm diameter and incubated at 25°C for 7 days. Three replicates were run at each broth. For measuring dry mycelial weight, mycelial mats were harvested on pre-weighed Whatman filter paper No. 1, dried at 40°C to constant weight. Dry weight of the mycelium was then calculated. Conidial concentrations at different broth were measured using hemocytometer.

Effect of media

To see the effect of different media on the growth and sporulation of isolated pathogen, eight media include: Potato Sucrose Agar (PSA), Potato Dextrose Agar (PDA), Potato Dextrose Yeast Agar (PDAY), Parthenium Dextrose Agar (PeDA), Czapek's Dox Agar (CDA), Nutrient Agar (NA), Malt Extract Agar (MEA) and Sabouraud dextrose agar (SDA) were used and broth of the same was used for liquid medium.

Effect of temperature

The effect of temperature was studied in both solid and liquid media at different temperature conditions i.e., 5, 15, 25, 35 and 45°C.

Effect of pH

The effect of pH was optimized by preparing the solid and liquid medium with the different pH range from acid to alkali i.e. 3.5, 4.5, 5.5, 6.5 and 7.5.

Effect of Relative humidity

Moist chambers of different relative humidities were prepared using standard aqueous inorganic solutions. Different solutions used were: Na_2HPO_3 (93% R.H.); KCl (85% R.H.); NaCl (75% R.H.); $Ca(NO_3)_2$ (50% R.H.); and pure water (for 100% R.H.) (Aneja, 2003). Plates of potato sucrose agar medium were prepared by pouring 15-20 ml medium in to Petri plates. Saturated solutions of different relative humidity were poured aseptically into the lid of the PDAY plates. Plates were incubated at 25°C for 2 days to allow the agar medium to equalize with desired relative humidity. After 2 days, agar plates were inoculated with the disc of the fungus and moist chambers were incubated for 5-7 days at 25°C.

Histopathological studies

Conidia germination (In vitro)

For germination studies, conidia were harvested from 7 days old cultures grown on PSA. Germination and hyphal development of fungi was studied in cavity slides containing 1% glucose solution. Petri plates containing the cavity slides were incubated at room temperature and chlamydospores and conidia germinating in each slide were examined for 12h at every 30 min. interval. Germination was defined when the hypha produced by a chlamydospore and conidium to about half of its diameter (TeBeest et al. 1978).

Conidia germination (In vivo)

Surface sterilized seeds of congress grass were kept in moist chambers for 24-28 hrs at room temperature, and allowed to germinate. Germinating seeds were planted in plastic pots containing sand soil mixture. Plants were inoculated 15 days after the germination of seeds. 7-10 days old culture of the pathogen grown on PSA medium at 25 ±2°C was used for inoculation. Conidia were collected by scrubbing the fungal growth and collecting into a flask containing sterilized distilled water (plus .02% tween 80 as a surfactant) under aseptic conditions. Conidial concentrated was adjusted to 6×10^4 conidia/ml using hemocytometer. Plants were inoculated with conidial suspension until runoff with an automizer. After inoculation, leaves were collected at interval of 0,6,12,18,24 hrs and 02,03,04,05,06,07,08,09,10 days to see the germination of conidia on the leaves (Rathaiah, 1977; TeBeest et al. 1978; Evans and Fleureau, 1993).

Extracellular enzyme production

For detection of extracellular enzyme production, isolate from *Parthenium hysterophorus* was screened qualitatively for celluloytic, ligninolytic (laccase, lignin peroxidase and manganese peroxidase), pectinolytic and amylolytic enzyme production.

Qualitative method

Screening of fungal extracellular enzymes was done by qualitative method *i.e.* agar plate method. The functional role of extracellular enzymes by fungal pathogens was assessed by growing them on PDA for 6-7 days, incubated at 25^{o}C and placing 5 mm mycelial plugs on the solid media. After incubation, at room temperature, the zone of enzyme activity surrounding the fungal colony was measured as described by Patil et al. (2015). Procedure followed for the qualitative estimation of amylolytic, Proteolytic, Cellulolytic and Ligninolytic activity is given below. Amylase activity was assessed by growing the fungi on Glucose yeast extract peptone agar (GYP) medium with 0.2% soluble starch at pH 6.0. After incubation, the plates were flooded with 1% iodine in 2% potassium iodide (Sunitha et al. 2013). A qualitative determination of cellulolytic activity, Yeast Extract Peptone Agar medium containing 0.5% Carboxy-methylcellulose (CMC) was used. After 3-5 days of fungal colony growth, the plates were flooded with 0.2% aqueous Congo red solution and destained with 1M NaCl for 15 minutes. Appearance of yellow areas around the fungal colony in an otherwise red medium indicated cellulose activity (Sunitha et al. 2013). Pectinolytic activity was determined by growing the fungi in pectin agar medium (Pectin -5g, yeast extract-1g, agar- 15g pH 5.0 in 1L distilled water). After the incubation period, the plates were flooded with 1% aqueous solution of hexadecyl trimethyl ammonoium. A clear zone formed around the fungal colony indicated pectinolytic activity (Sunitha et al. 2013). For Ligninolytic activity one cm diameter plug cut from the growing edge of PDA cultures of selected isolates, was centrally inoculated on the surface of azure B agar. The medium containing (g/L) glucose-0.2%, KH_2P0_4-1, yeast Extract- 0.01, diammonium tartarate-0.5, $CuS0_4.5H_2O$-0.001, $MgSO_4 \cdot 7H_2O$-0.5, $FeSO_4$- 0.001, $CaCl_2 \cdot 2H_2O$-0.01, $MnSO_4.H_2O$-0.001and agar agar-20; was supplemented with 0.01 % w/v Azure B. The plates were incubated at 30 °C for 7 day. The uninoculated plate served as a control. The production of lignin peroxidase and Mn peroxidase was recorded as clearance of blue coloured medium. Laccase activity was also assessed by growing the fungi on solid medium with guaiacol as indicator. 0.01% guaiacol was added to the solid medium (PDA) and incubated at 25ºC. Guaiacol positive reaction was indicated by the formation of a reddish brown halo.

Phytotoxin Production

Preparation of Cell free culture filtrate (CFCF)

Richard's medium (Agarwal and Hasija 1986) containing KNO_3-10 g, KH_2PO_4-5 g, $MgSO_4$. $7H_2O$.-2.8g, Sucrose-35g, $FeCl_3$-Trace, distilled water-1000ml; pH-3.84 ± 1 was used for the fermentation. Ten mycelial bits (2.5mm) separated from seven days old culture of the fungus grown on PDA medium at 25 ± 2°C were transferred to 1000ml Erlenmeyer flasks containing 500 ml medium. Inoculated flasks were incubated at 25 ± 2°C in B.O.D incubator (Remi, India) for 7, 14, 21, 28 days. CFCF was aseptically obtained by filtering the metabolized growth medium through pre-weighed Whatman fillter 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 different 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 hrs (Sharma et al. 2004). 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 minutes, then washed thoroughly with distilled water. The surface sterilized seeds were placed on the moistened filer paper kept in the sterile petridish. Ten ml of cultural filtrate of 7, 14 and 21 days was poured into the moisture chamber. Suitable control was maintained with sterile distilled water. Plates were incubated at 26 ± 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.

Statistical analysis

The experimental results were in triplicate each time and expressed as mean ± SD and results were statistically evaluated using SPSS software version 16 using Dunnett's t test. *$p<0.05$ compared to negative control.

Identification of CFCF

Organic solvent extraction

Phytotoxins were extracted from the broth of 21 days old culture of selected pathogens grown as stationary culture (28 ± 2°C) on Richard's broth. The CFCF was obtained as described earlier and concentrated to 1/50 folds of the original volume. Solvents used were Diethyl ether, chloroform, ethyl acetate and butanol.

Assessment of the biological activity

All layers were subjected to in vacuo dessication at 40°C in a rotatary vacuum evaporator to remove the solvent and to obtain the residues. Residues were named as Fraction A (Butanol). Fraction B (Chloroform). Fraction C (Diethyl ether), Fraction D (Ethyl acetate). The test residues were prepared as stocks using distilled water and were tested for their phytotoxic activity using detached leaf bioassay (Strobel, 1973; Karr et al. 1974).

Identification of the active phytotoxin

It was done from the solvent extract of fungal culture filtrate by using thin layer chromatography (TLC).

Thin layer chromatography (TLC)

TLC Silica gel 60 F254 plates (Cat. No.1.05554.0007) of Merck, Germany and pre-coated silica slides were used for performing TLC. The TLC was performed at room temperature using properly equilibrated chromatography glass chamber.

Equilibration of chromatographic chamber

One centimetre height of solvent system was taken in a clean glass chamber. The chamber was covered with air tight lid and allowed to saturate with solvent vapours. The inner wall of the chamber was lined with a piece of filter paper, the lower edge of which was dipped in the solvent present in the chamber, to ensure the even distribution of solvent vapours throughout the chamber. Thin layer chromatography of phytotoxic fraction was performed on silica gel (0.25 mm) with ethyl acetate: Petroleum ether (0:100, 1:99 and 5: 95), chloroform: methanol (0:100, 1:99 and 5: 95), ethyl acetate: di ethyl ether (0:100, 1:99 and 5: 95) solvent systems. The plates were allowed to run for about 10-15 minutes. Spots were visualized by staining with iodine vaporization (Iodine adsorbed on silica) (Singh et al. 2010).

Application of samples

Cell free culture filtrate was applied on the TLC plate, using a capillary, as small dots at about 0.5cm above the lower edge of the TLC plates. Proper precautions were taken to avoid uneven spreading of the test sample and the plates were air dried for five minutes.

Detection of spots

The TLC was performed at room temperature using properly equilibrated chromatographic glass chamber. The various components present in the crude extract appeared in the form of bands on the plates. The developed plates were visualized in Iodine chamber as well as in UV light.

Host range studies

Test plants were selected on the centrifugal phylogenetic relationship with the target weed (Wapshere, 1974) and on the basis of their economic importance (Mitchell, 1988). Host specificity of the selected fungal pathogens was tested against 11 plant species belonging to the family Solanaceae, Brassicaceae (Cruciferae), Liliaceae, Poaceae and Fabaceae. The host plants tested for their host range are Tomato (*Lycopersicon esculentum*), Lobia (*Phaseolus lunatus*), Wheat (*Triticum aestivum*), Mung (*Vigna radiata*), Jowar (*Sorghum vulgare*), Rice (*Oryza sativa*), Onion (*Allium cepa*), Garlic (*A. sativum*), Mustard (*Brassica campestris*), Potato (*Solanum tuberosum*) and Sunflower (*Helianthus annuus*). Plants were grown in plastic pots (5 seeds/pot) and were inoculated at ages ranging from 1 to 3 weeks post germination. For inoculum production, pathogen was inoculated on PDA medium for 8 days, growth was scraped and put into a flask containing sterilized distilled water. Aqueous conidial suspension of the pathogen was amended with surfactant and standardized at $6x10^4$ conidia/ml with a hemocytometer. Plants were injured with sterilized needle and inoculated by spraying the leaf and stem surfaces to run off with the conidial suspension. Controls were sprayed with sterilized distilled water plus surfactant only. Plants were monitored daily for 2 weeks for studying the appearance of symptoms.

Evaluation of Biocontrol Potential

Preparation of inoculum

The inoculum of the selected pathogens was prepared by growing fungus culture on the Potato sucrose agar media. Since these media were found to be the best for growth and sporulation for these fungi. The microbial mass (conidia + mycelium) was harvested by flooding the culture growing on above media. This liquid microbial mass was transferred into an Erlenmeyer flask and stirred on a magnetic stirrer for 20-25 minutes. Inoculum concentration was adjusted to 6×10^4 conidia + mycelium/ml using haemocytometer.

Pathogenicity test

Seeds of congress grass were grown in 24 plastic plots of 2 X 2.5" size with 5 seeds/pot. Two sets of pots were prepared (12 pots each). One set without inoculum was kept as control, while on

another experimental set, inoculum was sprayed twice onto the leaves surfaces using an automizer. First inoculation was made seven days after planting when most plants were at the cotyledon to first leaf stage. Second inoculation was carried out 15 days after the first spray. Spraying was done on two types of plants, i.e. pricked (with sterilized needle) and unpricked which were again kept under two different conditions: (i) uncovered; and (ii) covered with polythene bags to maintain relative humidity. An equal number of controls were also kept so that plants in both sets received all possible combinations of inoculated Vs uninoculated, covered Vs uncovered. The inoculum was applied to the plants within 2 hrs of sunset to avoid drying and to allow for a natural dew period shortly thereafter. Observations were made after four days intervals for the development of disease i.e. onset of symptoms and percent area covered by the disease till the death of the plants. Plants were observed weekly for the development of disease symptoms. Date of appearance of infection was recorded from the onset of typical symptoms. Plants were monitored daily for 4 weeks for studying the symptoms and disease was calculated as percent number infection.

Effect of Adjuvants on biocontrol efficiency of pathogens

Four adjuvants, tween 80, tween 20, glycerol and triton X 100 were selected for the study. The conidial/mycelial suspensions for spraying on experimental plants were prepared in sterile distilled water as follows, inoculum + 0.5% Tween 80, inoculum + 0.02%, Triton X 100, inoculum + 0.5% tween 20 and inoculum + 0.05% glycerol (Daigle and Connick, 2002). Control plants were sprayed with sterile distilled water + surfactant. Plants were observed weekly for the development of disease symptoms and disease was calculated as percent number infection.

Results and Discussion

During surveys, it was observed that the congress grass population was found affected by a leaf blight disease at different parts of Kurukshetra district of Haryana. Microscopic study and molecular analysis (ITS rDNA sequence analysis) of the infected part revealed that the pathogen was *A. macrospora* strain MKP1 (Figure 1 A, B).

Pathogenicity test

Typical disease symptoms were produced on both injured and uninjured leaves in *in-vitro* and the inoculated pathogen was re-isolated and found similar to the original isolate in cultural characteristics thus confirming the pathogenicity of pathogens to *P. hysterophorus* and completing the Koch's postulates (Figure 1 C, D).

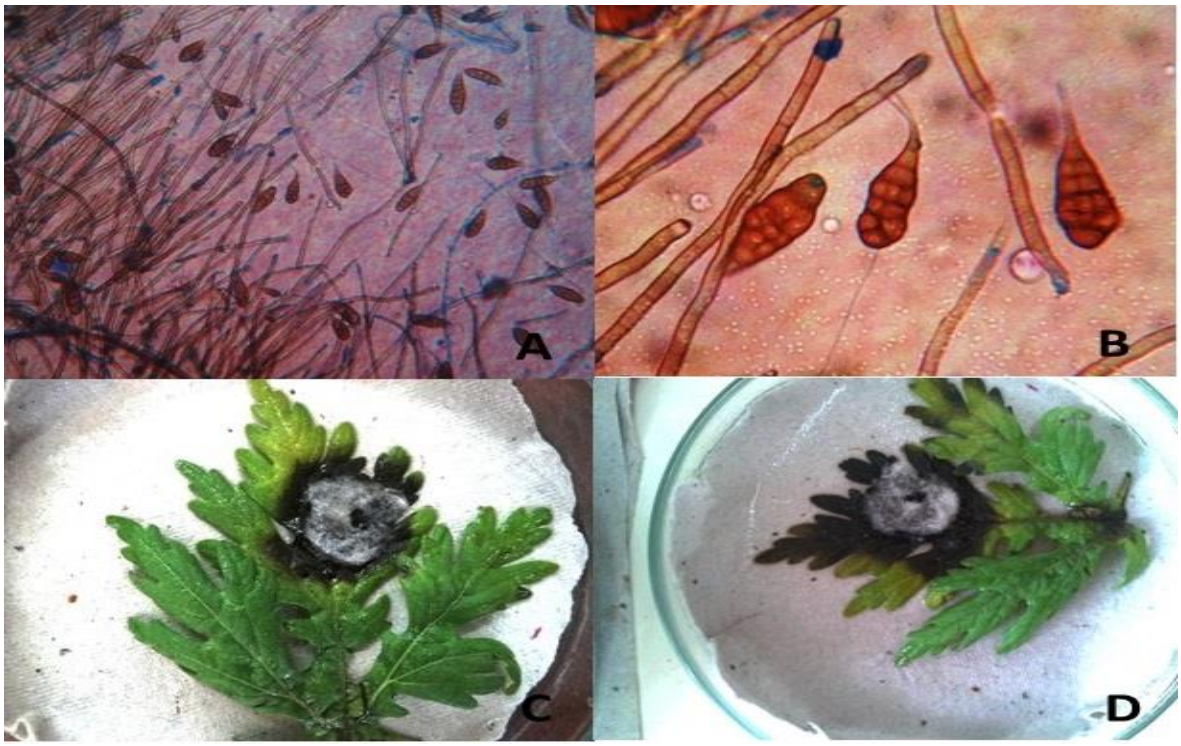

Figure 1. *Alternaria macrospora* MKP1 (A) Abundant conidia with conidiophores at 10x; (B) Conidia with conidiophores at 40x; (C) *In vitro* pathogenicity after 1 day; (D) *in vitro* pathogenicity after 3 days.

Growth on different media

All the culture media tested for the growth of fungal pathogens supported the growth of test pathogen to various degrees. *A. macrospora* MKP1 showed excellent growth on PSA. PSA had the highest mycelial growth after five days. The mycelia of pathogens increased till the end of the experiment on PSA. The growth was good on PDAY, PeDA, PDA, MEA and CDA and lowest on SDA and NA (Table 1). Difference in surface and reverse coloration of fungal colonies was distinct on all the growth media. Similar results were observed in liquid media. Maximum dry weight of *A. macrospora* MKP1 (0.34 gm) was recovered at Potato Sucrose broth after five days.

Sporulation on different media

Sporulation of the tested pathogen was best on PSA followed by PDAY and PDA. The pathogens sporulate well on PeDA, MEA, CDA and SDA media. Poor sporulation was observed on NA (Table 1). If we consider both the parameters i.e., growth and sporulation which are the prerequisites of any mycoherbicide, for inoculum preparation, all the pathogens should be grown on PSA medium. Contrarily, in liquid media sporulation was nil in all tested broth.

Table 1. Experimental conditions for optimization of growth and sporulation in *A. macrospora* MKP1.

Parameters	*Alternaria macrospora* MKP1							
Medium[1]	PDA	PDAY	SDA	PeDA	MEA	PSA	CDA	NA
Colony diameter (cm)	6.72*±0.21**	6.45±0.18	5.24±0.03	6.59±0.45	5.68±0.08	6.17±0.28	5.02±0.28	3.11±0.17
Sporulation (x10⁴/ml)	15.72 ±0.01	17.95±0.18	10.24±0.13	16.59 ±0.45	15.68±0.08	19.43±0.05	7.02±0.12	5.11±0.17
Broth[1]	PDB	PDBY	SDB	PeDB	MEB	PSB	CDB	NB
Dry mycelial wt.(gm)	0.21±0.01	0.37±0.15	0.13±0.15	0.36±0.15	0.18±0.00	0.43±0.15	0.14±0.15	0.09±0.15
Temperature[2]	5°C	15°C	25°C		35°C		45°C	
Colony diameter (cm)	3.11±0.03	4.07±0.02	6.90±0.01		3.07±0.02		0.00±0.00	
Sporulation (x10⁴/ml)	1.11*±0.01	1.27±0.01	19.43±0.05		10.14±0.05		0.00±0.00	
Dry mycelial wt.(gm)	0.11±0.01	0.27±0.15	0.44±0.15		0.14±0.15		0.01±0.00	
pH[3]	3.5	4.5	5.5		6.5		7.5	
Colony diameter (cm)	4.56±0.53	5.71±1.17	6.63±0.44		6.81±1.23		6.54±0.87	
Sporulation (x10⁴/ml)	6.56±0.53	9.71±1.17	9.63±0.44		20.81±1.23		17.54±0.87	
Dry mycelial wt.(gm)	0.31±0.53	0.36±0.15	0.35±0.15		0.46±0.15		0.31±0.53	
Relative humidity[4]	H_2O	Na_2HPO_4	KCl		$Ca(NO_3)_2$		NaCl	
Colony diameter (cm)	4.28±0.53	6.65±1.17	3.50±0.44		2.86±1.23		1.94±0.87	
Sporulation (x10⁴/ml)	19.62±0.65	26.23±1.26	12.12±0.61		11.24±1.19		7.36±1.23	

Optimized conditions	pH	Media/ Broth	Relative humidity	Temperature
	6.5	PSA/PSB	95% (Na_2HPO_4)	25 °C

*Values, including diameter of the disc (8mm), are means of three replicates; **-Standard deviation; Conditions: 1 Temperature-25 OC, pH-7; 2Media- PSA and PSB, pH-7; 3Media- PSA and PSB, Temperature-25 OC; 4 Temperature-25 OC, pH- 6.5; Media- PSA

Effect of Incubation temperature, pH and relative humidity on the growth / sporulation of selected fungal pathogens

Effect of Incubation temperature on growth and sporulation In liquid medium

Maximum dry weight (0.44gm) of *A. macrospora* MKP1 was recovered at temperature 25°C after five days. The optimum temperature for the growth and sporulation of *A. macrospora* MKP1 was 25°C, above and below this temperature the growth declines. The isolate showed maximum biomass production at 25°C therefore this temperature can be used for incubation of this organism (Table 1).

Effect of Incubation temperature on growth and sporulation On solid medium

The variation in the diameter of the colonies was studied at temperatures 5°C, 15°C, 25°C, 35°C, 45°C for 5 days of incubation. It is concluded from the results that the temperature range 15°C to 25°C is good for the growth of this pathogen. All the temperature regimes tested supported the growth of the fungus except temp. 45°C. It was observed that at 25°C the fungi attained maximum growth after 5 days of incubation. However, the growth of the fungus was started to decline at 35°C and almost stops at 45°C, as these temperatures did not favour the growth of the fungus (Table 1). The selected pathogen showed best sporulation at 25°C on PSA medium. There was no sporulation at 45°C (Table 1). In broth culture, sporulation was nil for all tested incubation temperatures. Thus potential limiting factor for this pathogen is the inability to produce conidia in broth at all tested temperature. At a particular temperature, development of an organism was reliant on the rates of biochemical or enzymatic reactions which depend on the availability of substrates, enzymes. Similar results for the effect of temperature on growth and sporulation of *Alternaria*, has been studied by several workers. Ghorbani et al. (2000) showed that optimum temperature for disease development and consequent dry weight reduction by *A. alternata* on *Amaranthus retroflexus* plants was 20 to 30°C. Optimum temperature for most fungi lies between 24 and 30°C (Madan and Thind, 1998). Neergaar (1945), obseverd that the optimum temperature for the growth of *Alternaria alternata* was 25°C. However Kamal (1950), from India reported 25°C as optimum temperature for growth of *Alternaria alternata*. Better growth and sporulation of *A. tenuis*, was observed by Tandon (1961) at 26°C. Verma (1963), observed the optimum temperature of 25°C was essential for the growth and sporulation of *A. tenuis*.

Effect of pH on growth and sporulation

Best sporulation of the *Alternaria macrospora* MKP1 was observed at pH 6.5 in agar media whereas in broth culture, sporulation was nil at all tested pH ranges. Best growth of the *Alternaria macrospora* MKP1 was observed at pH 6.5 in both agar media and broth conditions. From the results it is concluded that the pH is also an important factor that determines the growth and sporulation of *A. macrospora* MKP1. Our results showed that this *Alternaria* pathogen have a wide range of pH for their growth and sporulation and the several workers have investigated the role of pH on growth and sporulation of *Alternaria* fungi with similar findings. Verma (1963) observed that optimum pH 6.60 was essential for the growth and sporulation of *A. tenuis*. Saad and Hagedorn (1970) were of the view that minimum, optimum and maximum pH for the growth and sporulation of *Alternaria alternata*, were 4.40, 6.50 and 7.60 respectively. Xu et al. (1984) while studying pH

requirements of two isolates, *A. alternata* noted its growth at pH range of 3.0-7.10 with optimum of 6.50. Chettananavar et al. (1987) obtained maximum growth of *A. alternata* at pH 6.50. Phylogenic relationship of *Alternaria macrospora* MKP1 with related genera are presented in Figure 2.

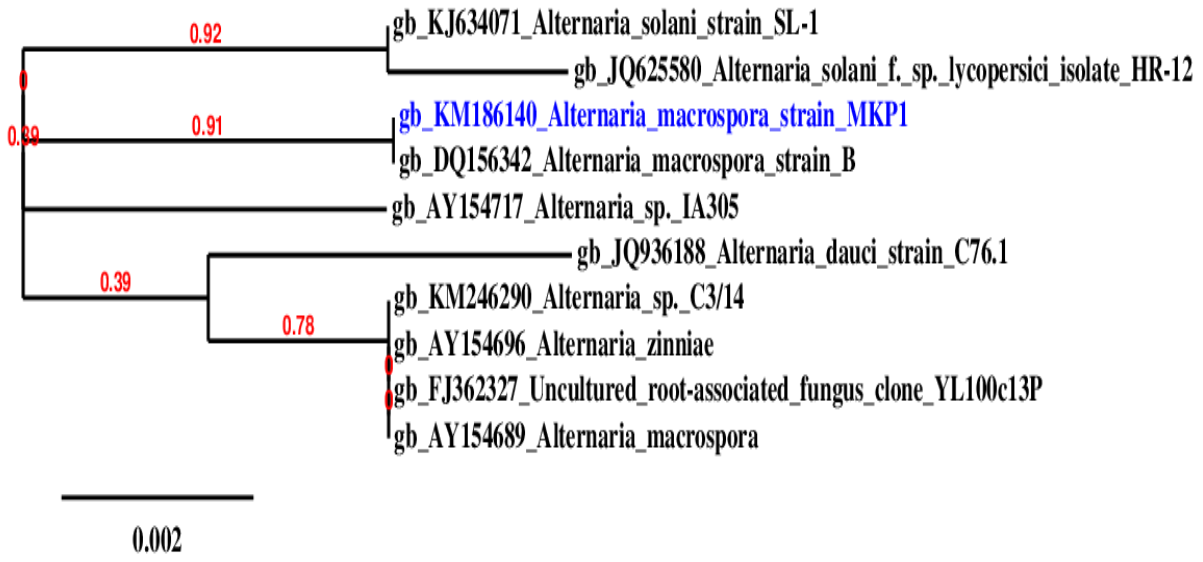

Figure 2. Phylogenic relationship of Alternaria macrospora MKP1 with related genera.

Effect of relative humidity on growth and sporulation

Results revealed that the growth and sporulation were affected a lot with the change of relative humidity. The isolate was capable to grow and sporulate at different levels of relative humidity. Maximum growth and sporulation of the pathogen was recorded when the relative humidity was between 93-100% because when pure water or disodium hydrogen phosphite (Na_2HPO_4) were placed in a petri dish the atmosphere becomes saturated with water vapours providing 100%, 93%, R.H. respectively (Table 1). Our results confirmed the previous findings that temperature and relative humidity are important cultural parameters determining factor for growth and sporulation of fungal pathogen (Aneja and Kaushal 1998; Aneja et al. 2000).

Histopathological studies (progression of disease development)

In vitro conidia germination

In vitro spore germination was studied in 1% w/v glucose solution and it was observed that the spores of *A. macrospora* MKP1 started germination after 20-30 minutes. After 4-5 hours the conidia produced three to four germinating tubes and after 12 hours all the conidia were germinated with a

number of secondary mycelium (characterized by the presence of septation in mycelium) and production of secondary conidiophores (Figure 3).

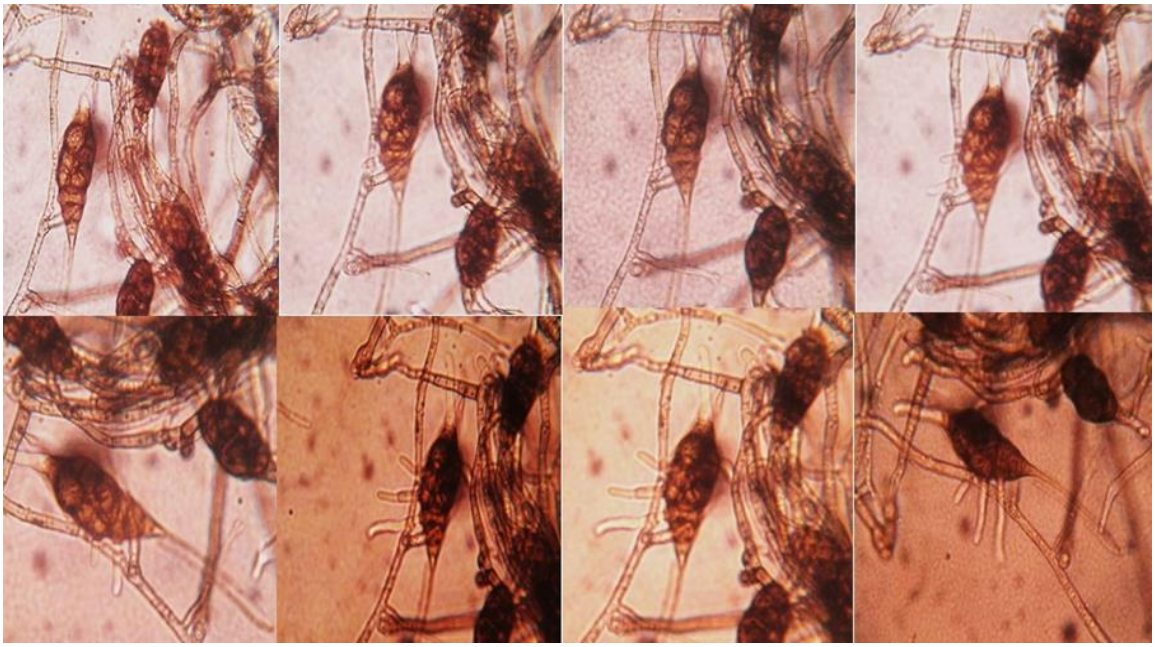

Figure 3. Different stages of spore germination of Alternaria macrospora MKP1 in glucose solution from single spore to germ tube development.

In vivo spore germination study

Leaves collected during 6, 12, 18, 24 hrs were taken in test tubes containing 5ml of sterilized distilled water and shaken well. 1-2 drops of the suspension from different test tubes were taken in different cavity slides to study the germination of conidia. It was observed that conidia of *A. macrospora* MKP1 started germinating 6-12 hrs after spraying of inoculum and after 18 hrs, 2-7 germ tubes started originating from different cells of each conidium. Conidia of the fungus can germinate either from one side or from both sides. conidium swells, forms a single germ tube and then divides and forms a second germ tube. Germ tube was separated from the conidium by a septum and a second germ tube develops opposite to the first germ tube. Germ tubes were aseptate and after 24 hrs they produced septate secondary mycelium and secondary conidia on congress grass plant leaves. Germination of conidia on leaves is a most desirable criterion for development of a biocontrol agent as a mycoherbicide to control congress grass. Results of *in vitro* and *in vivo* germination were different as the conidia germination takes more time on parthenium plants (*in vivo*) as compare to glucose solution (*in vitro*).

Penetration

Penetration of the host by the *Alternaria* isolates was observed through epidermis and stomata (mainly through epidermis) as evident from the stained sections of leaves taken 2-3 days after inoculation (Figure 4). The pathogens enter into the host tissue by formation of germ tubes and some intermediate swelling was observed in the germ tubes within the cortical cells from which infection hyphae, conidiophores and conidia aroused and penetrate to adjacent cells. Hyphae were visible clearly within the cortical cells. Plant tissue at the infected site got collapsed and extensive ramification of hyphae, conidia and conidiophores was observed with in the host's cortical cells after 72 hrs of inoculation.

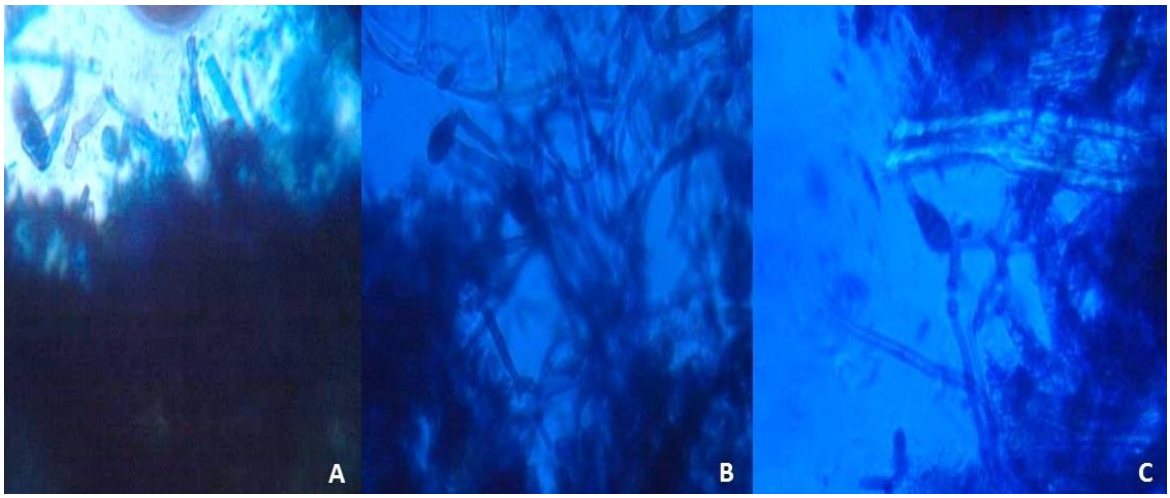

Figure 4. Conidia and conidiophores of Alternaria macrospora MKP1 on leaf surface after 72 hrs of inoculation.

Phtotoxin Production

Detached leaf bioassay

Detached leaf bioassay was performed by treating parthenium leaves with different day's old metabolized broth (Figure 5). Results indicated that 21 days old CFCF brought maximum phytotoxic damage followed by 14 and 7 days old metabolized medium (Figure 5). In general effect was less pronounced after 12 hrs and gradually enhanced till 72 hrs. Maximum damage was observed after 72 hrs with 100% CFCF of *Alternaria macrospora* MKP1. In general, toxin from 21 days old cultural filtrates caused severe chlorosis, necrosis and complete death of parthenium leaf (Figure 6).

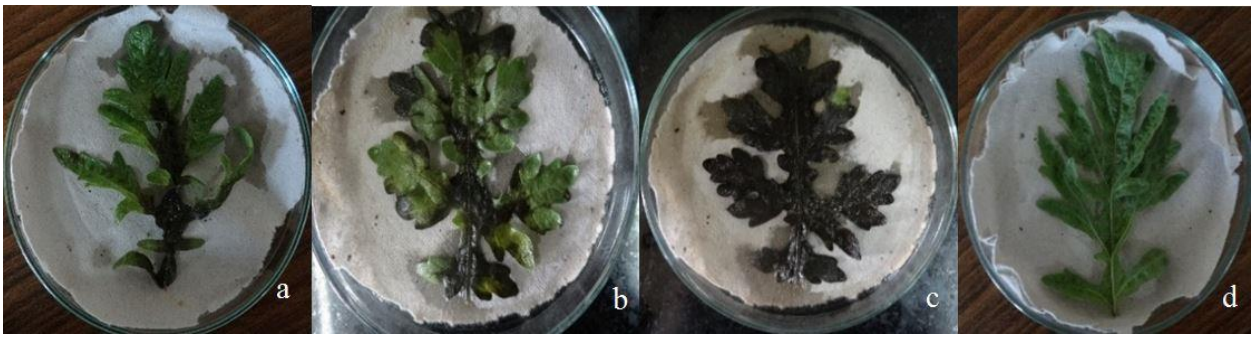

Figure 5. Effect of CFCF of Alternaria macrospora MKP1on parthenium leaf after 72 hrs incubation. Phytotoxic effect due to of (a) 7, (b) 14, (c) 21 days old fungal culture filtrates and control (d).

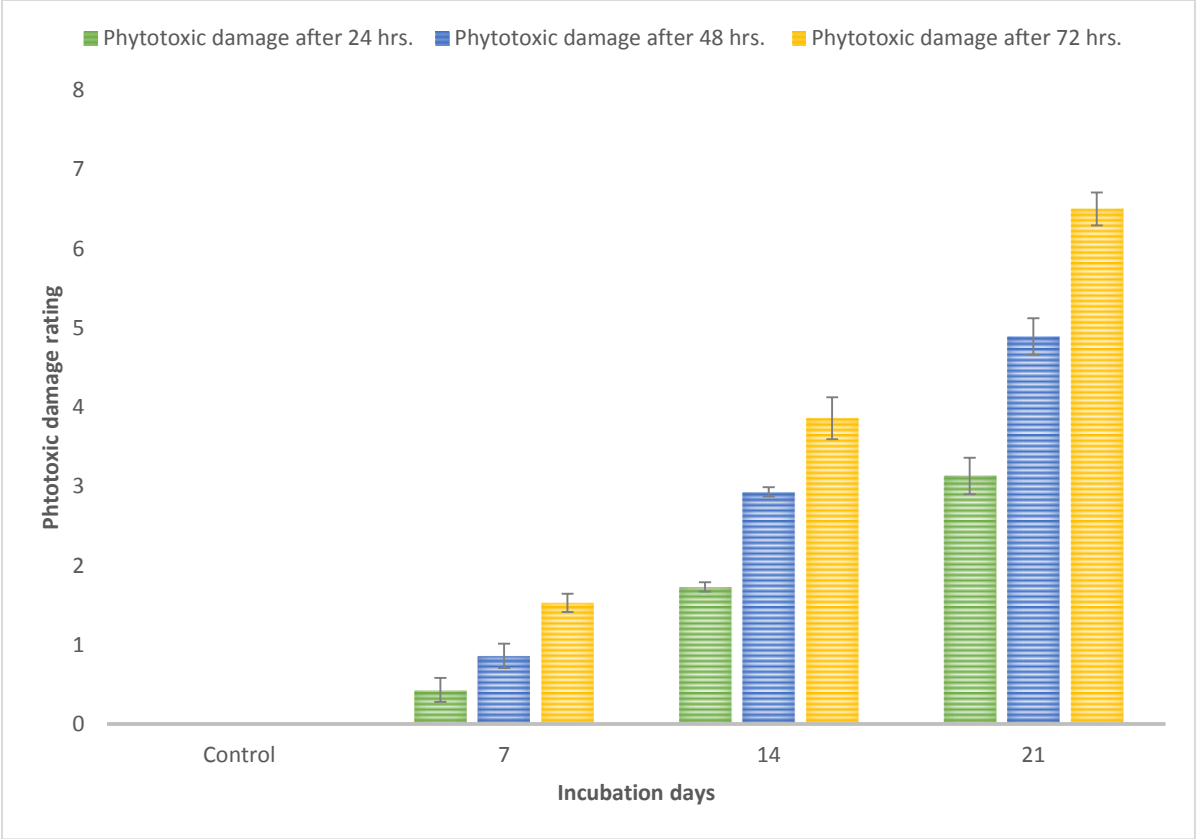

Figure 6. Damaging effect of different days old cell free culture of Alternaria macrospora MKP1 on detached leaves of parthenium weed. Vertical bars show standard errors of means of three replicates.

Seed germination bioassay

Results of the Parthenium seed germination by different days old cultural filtrate revealed that the 21 days old cultural filtrate significantly decrease the germination of seeds as compare to the 7

and 14 days old cultural filtrate. Results after the treatment of the seeds of parthenium with cultural filtrate produced by *Alternaria macrospora* at 7, 14 and 21 days old metabolized broth are represented in Figure 5. In control (distilled water) negligible reduction occurred in germination and about 77.7% of seeds were germinated. 26.5, 45.63 and 100% germination inhibition occurred in the 7, 14 and 21 days old cultural filtrate respectively. Thus, the seeds imbibed the phytotoxin from 21 days old metabolized broth of *A. macrospora*, hampering the germination (Figure 7).

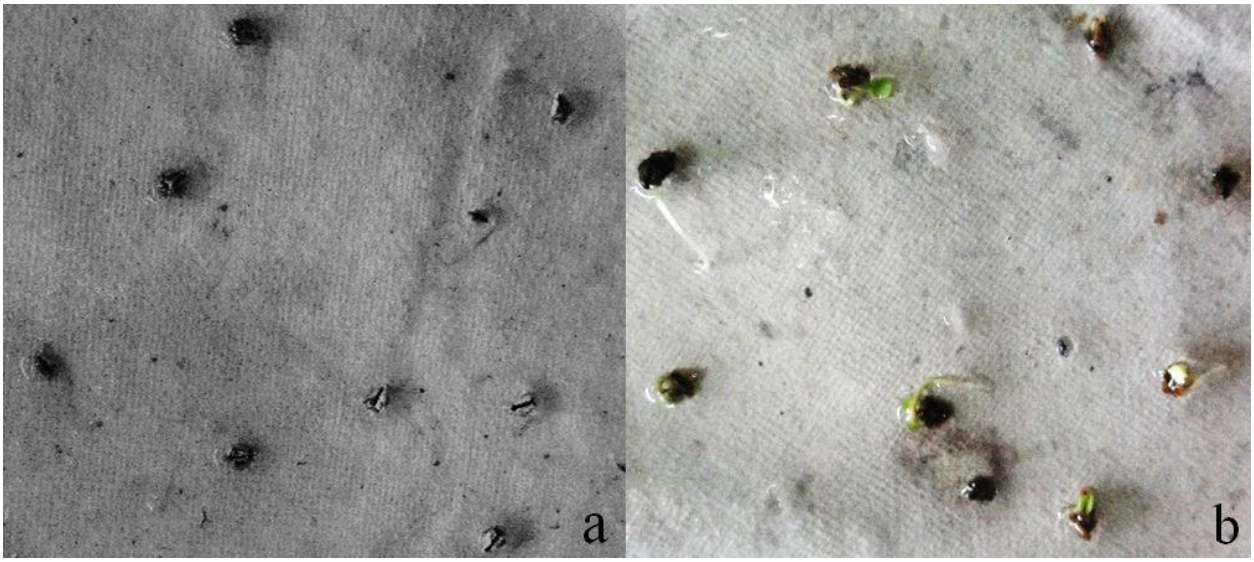

Figure 7. Phototoxic Activity of 21 days old cultural filtrate on Seed Germination of *parthenium* weed (a) 100% inhibition of seed germination by 21 days CFCF (b) 77 % inhibition in Control.

Identification of Cell Free Culture Filtrate (CFCF)

Solvent extraction CFCF of Alternaria macrospora MKP1

The cell free cultural filtrate of the *Alternaria macrospora* MKP1 isolates was extracted with different solvents and the following results were observed; a. Fraction A (Butanol fraction) was white in appearance. It produced least phytotoxic damage to parthenium leaves even after 72 hrs. b. Fraction B (Chloroform fraction) was yellow in appearance. It also failed to produce significant phytotoxicity on parthenium leaves at 12, 24 or 48 hrs. c. Fraction C (Diethyl ether fraction) was dark brown in appearance and produced remarkable chlorosis and necrosis on the leaves of test weed as early as 12 hrs. The Damage increased gradually and reached to its maximum after 48 hrs, ultimately leading to death of leaves after 72 hrs. d. Fraction D (Ethyl acetate fraction) was light brown in appearance and produced phytotoxic effects on parthenium leaves as severe chlorosis, necrosis after 72 hrs. The order of phytotoxicity of solvent extracted fractions was diethyl ether> ethyl acetate> chloroform > butanol. The phytotoxic moiety was present in diethyl ether extracted

fraction. Solvent extraction to recover the organic biological entities with novel activity has also been considered as one of the most effective method by many other workers (Pandey et al. 2002; Vikrant et al. 2006) who have employed similar solvents for extracting and isolating the phytotoxic compounds from the CFCF of other fungi. In several earlier reports on the isolation of tenuazonic acid from aqueous media, the metabolite was extracted into inorganic solvent (Benzene, Chloroform or ethyl acetate) prior to purification. Tenuazonic acid was extracted from CFCF of *A. alternate* (Figure 8).

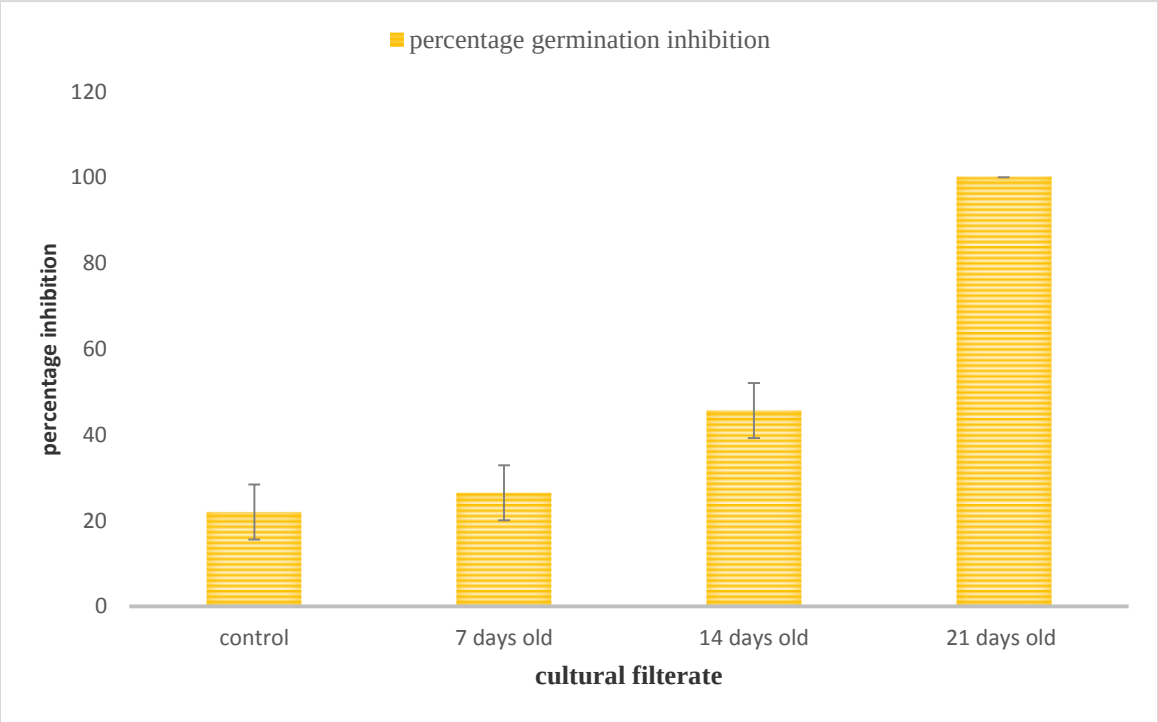

Figure 8. Effect of different days old CFCF of *A. macrospora* on Seed germination of *parthenium* weed. Vertical bars show standard errors of means of three replicates.

TLC slides analysis

Thin layer chromatography of cell free culture filtrate (F) was performed of fraction C (Diethyl ether) on silica gel with solvent system diethyl ether: ethyl acetate at different fractions i.e. 0: 100, 1: 99 and 5: 95. The slides were allowed to run for about 10-15 minutes. When slides were analyzed under iodine vapours, it showed fractions in the form of spots. TLC run in pure nonpolar solvent (0:100) and 5% polar solvent systems, slides did not show clear spots in the sample. 1% polar solvent system showed different sub fractions in the form of spots (Figure 9).

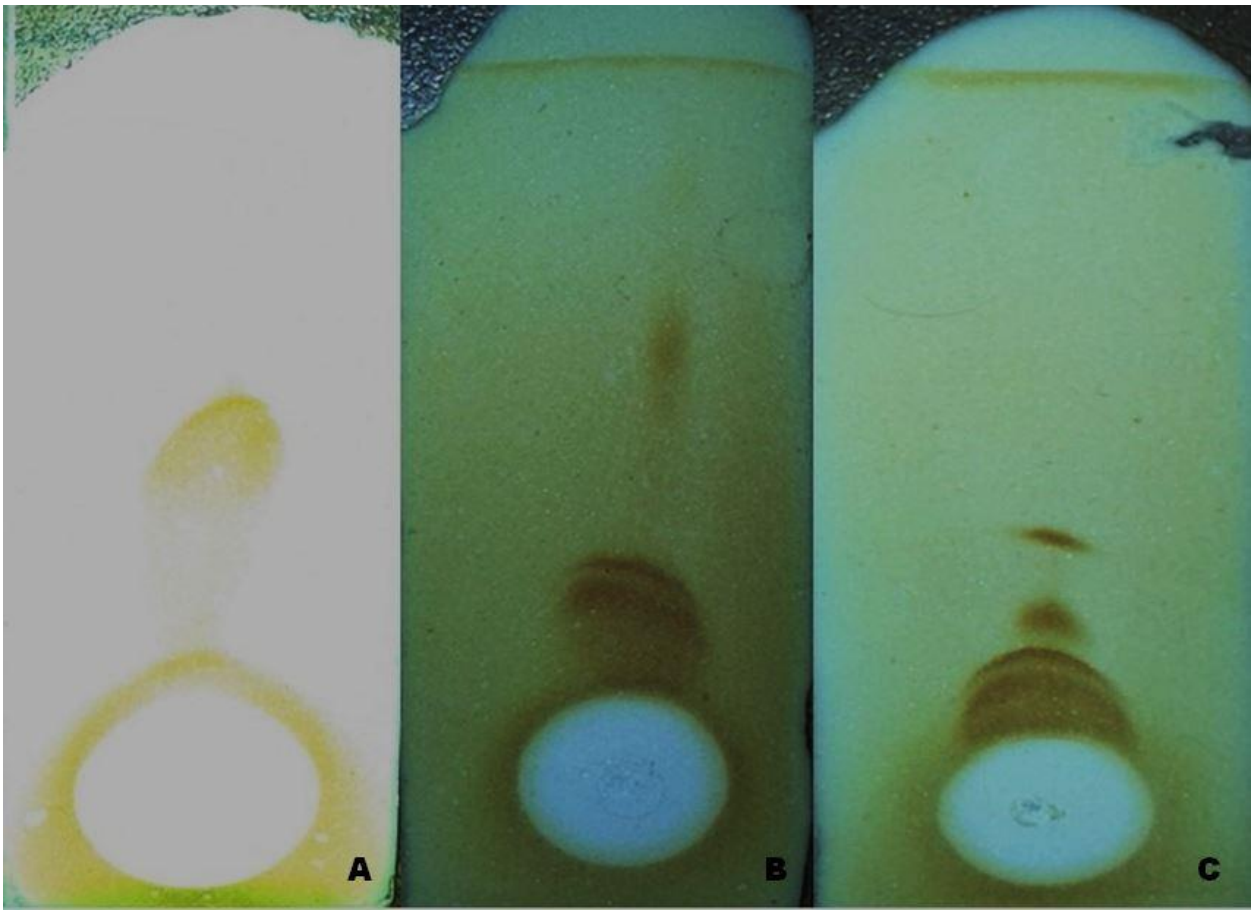

Figure 9. TLC plates in different solvent systems for A. macrospora MKP1 under iodine vapours (A) polar solvent; (B) 5% polar solvent; (C) 1% polar solvent.

Production of cell wall degrading enzymes

Results showed that the *Alternaria macrospora* MKP1 exhibited an ability to oxidize guaiacol, halo of intense brown colour was formed under and around the fungal colony (positive for guaiacol oxidation), indicating the presence of ligninolytic enzymes (Laccase) (Figure 10). Lignin peroxidase and manganese peroxidase assay revealed decolourization of the dye Azure-B by fungi which was positively correlated with production of lignin peroxidase and Mn dependent peroxidase. In solid plate screening the pathogen decolorized the Azure B dye and exhibited an ability of producing lignin peroxidase and Manganese peroxidase enzymes. The pathogen also showed the positive results for amylase production by halo zone formation on starch agar plates. The amylolytic potential of these pathogenic fungi may help them to degrade starch which is available in the host plant. On pectin agar plates *Alternaria macrospora* MKP1 showed clear zones on treatment with iodine-potassium iodide solution indicating the pectinolytic ability of this pathogen. The pectinolytic enzyme of this pathogenic fungi may help them to cause infection in the parthenium

plant. The elaboration of an array of cell wall splitting enzymes helps the pathogen for easy penetration of the host cell wall and subsequent colonization. Cellulose is a major structural constituent of the cell wall of host plants. Many phytopathogenic fungi produce cellulases in culture adaptively which hydrolyse cellulose and its derivatives (Muthulakshmi, 1990). The results obtained in the present study indicate that *Alternaria macrospora* MKP1 produced cellulase. Cellulolytic enzyme assay was performed to test the cellulolytic activity of *Alternaria macrospora* MKP1. Zone production by the tested fungi indicates the presence of cellulase enzyme (Figure 10).

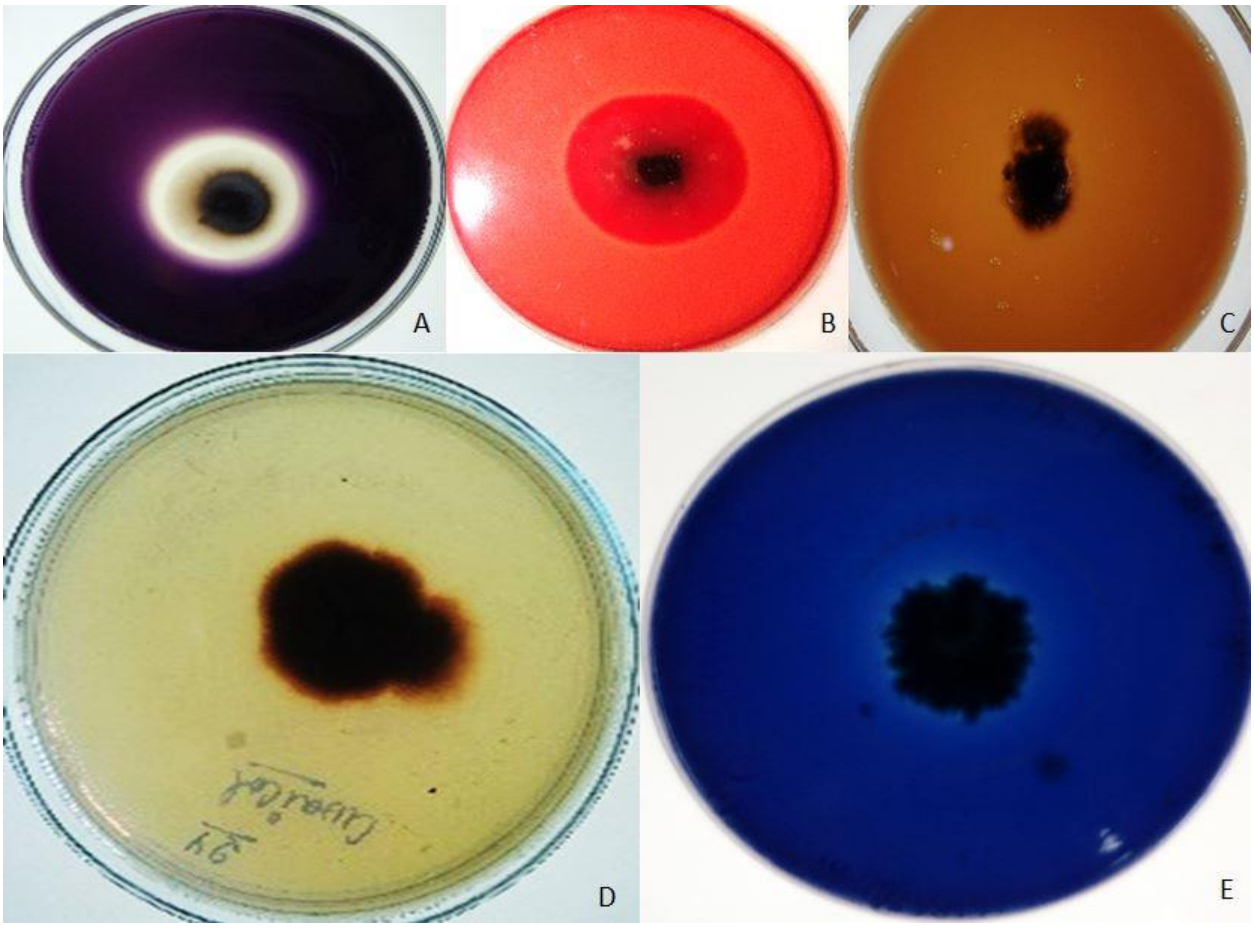

Figure 10. Evaluation of qualitative enzymatic activities of fungal pathogens on solid media by zone formation (A) Amylolytic activity on starch agar; (B) Cellulolytic activity on CMCase agar (C)Pectinolytic activity on pectin agar; (D) Laccase activity on guaiacol agar (E) Lignin peroxidase activity on azure B agar.

Several pathogenic ascomycetes and deutromycetes are known to produce lignin degrading enzymes. In our study the screened pathogen was able to produce all three ligninolytic enzyme which may contribute in the pathogenesis against Parthenium. A numbers of cell wall degrading enzymes have been shown to be produced by plant pathogens (Chenglin et al. 1996), which are known to facilitate cell wall penetration and tissue maceration in host plants. Since all the species of

Alternaria are intercellular in the host, the productions of these enzymes appears to facilitate the dissolution of host cell wall and middle lamella and help entry and establishment of the pathogen in the host and are possibly responsible for playing a vital role in pathogenesis through cell wall degradation and disintegration of tissues. Diseased *Parthenium hysterophorus* plants (covered and uncovered) in the experimental pots inoculated with *Alternaria macrospora* MKP1 (6x104conidia/ml) are presented in figures 11 and 12.

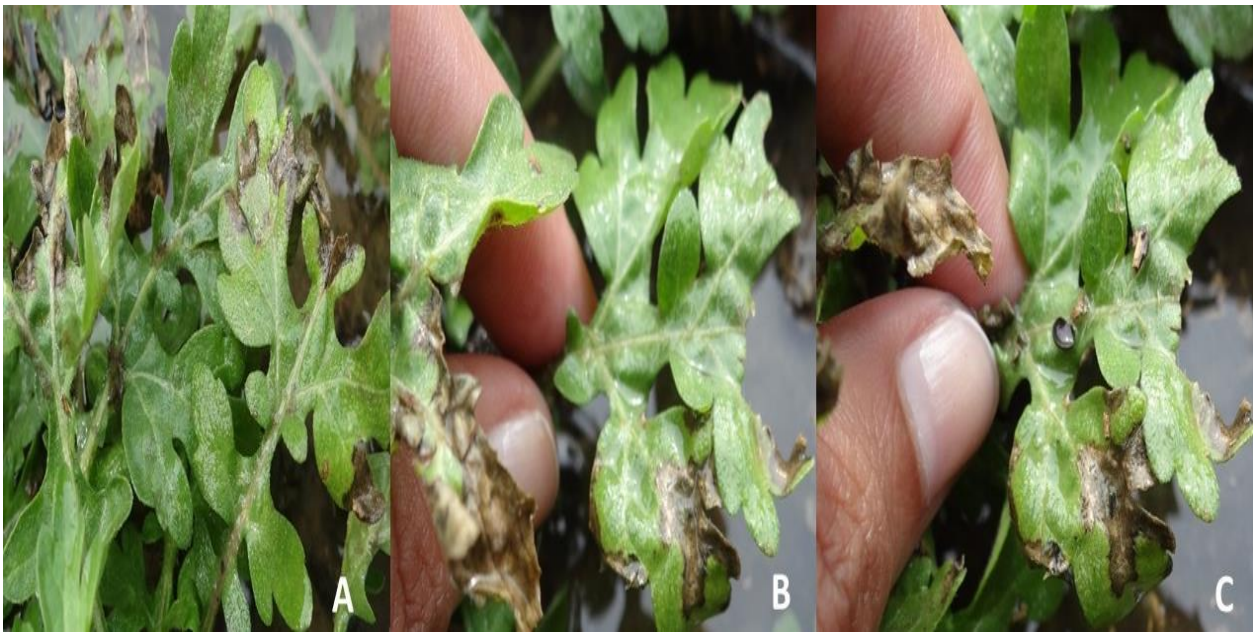

Figure 11. Diseased Parthenium hysterophorus plants (covered) in the experimental pots inoculated with Alternaria macrospora MKP1 (6 x 104conidia/ml).

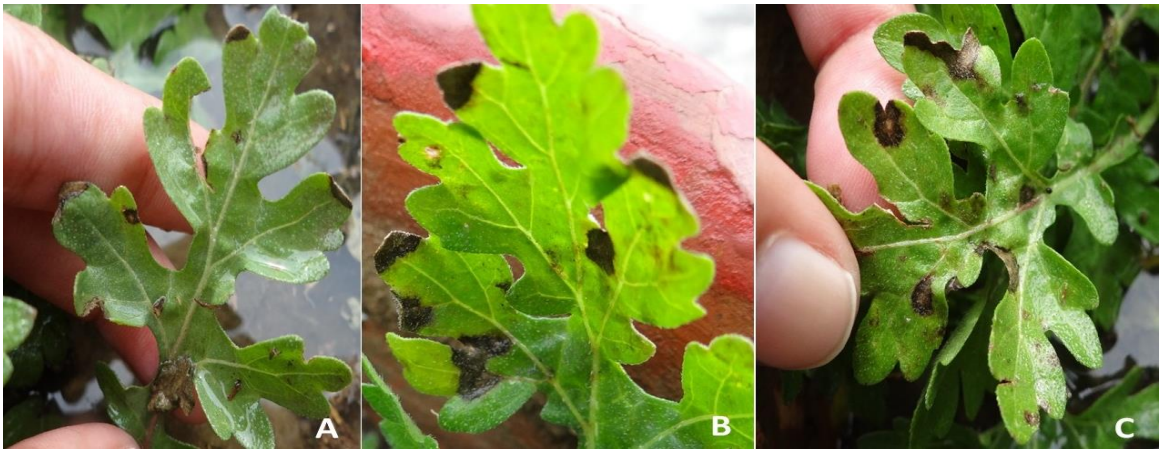

Figure 12. Diseased Parthenium hysterophorus plants (uncovered) in the experimental pots inoculated with Alternaria macrospora MKP1 (6x104 conidia/ml).

Host range studies

Host specificity of the *Alternaria macrospora* MKP1 was tested against 3 weeds viz. *Amaranthus viridis*, *Chenopodium album*, *Trianthema portulacastrum* and 11 crop plant species belonging to the family Solanaceae, Brassicaceae (Cruciferae), Liliaceae, Poaceae and Fabaceae. The host plants tested for their host range were Tomato (*Lycopersicon esculentum*), Lobia (*Phaseolus lunatus*), Wheat (*Triticum aestivum*), Mung (*Vigna radiata*), Jowar (*Sorghum vulgare*), Rice (*Oryza sativa*), Onion (*Allium cepa*), Garlic (*A. sativum*), Mustard (*Brassica campestris*), potato (*Solanum tuberosum*) and Sunflower (*Helianthus annuus*). All evidence of *Alternaria macrospora* MKP1 infection was limited to only weeds. These studies indicated that *Alternaria macrospora* MKP1 has a restricted host range. On the basis of host specificity test, which is a prerequisite requirement for the development of Mycoherbicide, we have selected *Alternaria macrospora* MKP1 and for the further studies as these pathogens were found to be host specific and fulfill one of the important criteria to be a promising mycoherbicide. Table 2 represents the results of host specificity test of *Alternaria* isolates against different weeds and crops.

Table 2. Response of various crop/weed species tested for susceptibility to selected *Alternaria macrospora* MKP1.

S. No.	Host	Family	Disease Reaction Due to *Alternaria macrospora* MKP1
		Weeds	
1.	*Amaranthus viridis*	Amaranthaceae	Sensitive
2.	*Chenopodium album*	Chenopodiaceae	Sensitive
3.	*Trianthema portulacastrum*	Aizoaceae	Sensitive
		Crops	
1.	*Solanum tuberosum*	Solanaceae	Resistant
2.	*Brassica campestris*	Brassicaceae	Resistant
3.	*Helianthus annuus*	Asteraceae	Resistant
4.	*Allium sativum*	Amaryllidaceae	Resistant
5.	*Oryzae sativa*	Poaceae	Resistant
6.	*Vigna radiate*	Fabaceae	Resistant
7.	*Sorghum vulgare*	Poaceae	Resistant
8.	*Triticum aestivum*	Poaceae	Resistant
9.	*Allium cepa*	Amaryllidaceae	Resistant
10.	*Phaseolus lunatus*	Fabaceae	Resistant
11.	*Lycopersicon esculentum*	Solanaceae	Resistant

Biocontrol potential of Alternaria macrospora MKP1

Typical disease symptoms due to *A. macrospora* MKP1 were observed on both pricked and unpricked leaves both in *in vitro* and *in vivo* conditions. The inoculated pathogen was re-isolated thus confirming the pathogenicity of the *A. macrospora* MKP1 to parthenium and usual Koch's postulates. The inoculum ($6x10^4$ conidia+mycelium/ml) of pathogen was artificially sprayed on the weed. The symptoms started on the leaves, after 3-4 days of inoculation, as a pin point dark brown spot on the margins as well as centre of the leaves, the characteristics of the infection was the presence of white dot at the centre of brown spots. In covered pots, per cent infection observed in uninjured and injured plants was 67.28±0.21 and 72.51±0.21 respectively and in uncovered pots, per cent infection in uninjured and injured plants was 61±0.21 and 65±0.21 respectively after 25 days post inoculation (Table 3). In spite of the infection, new leaves continue to emerge on a few plants and plants survived in a few cases. Data, when in inoculated and un-inoculated plants was statistically analysed, reveals that percent infection of parthenium leaves was highly significant in inoculated and un-inoculated plants in both covered and uncovered pots (The data was analyzed by one way ANOVA followed by Dunnett's t test compared to control at 5% significant level) (Table 4).

Table 3 Effect of foliar application of *Alternaria macrospora* MKP1 (percent infection) to *parthenium.*

S. No	Days of inoculation	Covered		Uncovered	
		Uninjured	Injured	Uninjured	Injured
1	5	5.20±0.23	7.23±0.21	2±0.23	5±0.21
2	10	9.31±0.21	11.26±0.40	7±0.34	9±0.23
3	15	29.81±0.34	33.92±0.31	21±0.24	25±0.41
4	20	53.13±0.43	61.19±0.23	43.12±0.21	51.32±0.51
5	25	67.28±0.23	72.51±0.21	61±0.19	65±0.25

Studies on the effect of different adjuvants on biocontrol efficacy of selected fungal biocontrol agents

Four adjuvants, glycerol, tween 80, tween 20 and triton x 100, were selected for the study.

Effect of adjuvant on Alternaria macrospora MKP1

Significant differences were evident among all the treatments in terms of pathogenicity of selected pathogen. The best of all the surfactants was Tween 80 followed by glycerol and Tween 20. The least effective surfactant was Triton X-100. Overall, the addition of surfactants resulted in increased pathogenicity of the fungus to *P. hysterophorus*. When tween 20 and glycerol was used as surfactant, highest per cent infection observed was 66% and 78% in covered pots, on the injured leaves, artificially inoculated with an inoculum of $6x10^4$ conidia/ml 25 days post inoculation (Table 4).

Table 4. Effect of foliar application of *Alternaria macrospora* MKP1 (percent infection) to parthenium, 25 days after inoculation.

Treatment	Percent infection on leaves[a]			
	Uncovered pots		Covered pots	
	Uninjured	injured	Uninjured	injured
Inoculated[b]	61.28[a]±3.06[c]	65.28±3.06	67.54±5.34	72.28±3.06
Control[d]	4.58±0.23	7.28±3.06	4.26±6.03	6.28±3.06

[a]Each value represents the average of 6 replicates, each replicate contained an average of 5 plants per pot. [b]Plants were sprayed to runoff with a suspension of $6x10^4$ conidia/ml and water. [c]± S.D. [d]Plants were sprayed the runoff with water only. The data was analyzed by one way ANOVA followed by Dunnett's t test compared to control at 5% significant level.

Spores applied in the conc. of $6x10^4$ conidia/ml with tween 80 produced 92 % percent infection in covered condition on injured leaves. Our results revealed that the addition of tween 80 significantly improved the effectiveness of the pathogen applied in $6x10^4$ conidia/ml as compared with the glycerol and tween 20 surfactant. Our results concluded that a total of 15-20% increase in biocontrol potential of selected fungal pathogens when applied with an adjuvant in the conc. of $6x10^4$ conidia/ml in pots (Table 4). Adjuvant formulation increased conidial germination and infection of target plants in the pots because an adjuvant reduced dew requirement by pathogen to cause infection on weeds. In every biocontrol programme, degree of Disease incidence and Disease Severity increased with increasing time period and gave maximum severity after 25 days. During the experiment, the disease severity increased between 15 and 25 days after inoculation of fungus + adjuvants and there was a decrease in chlorophyll content in diseased leaves as compared to healthy ones. During our study tween 80 was found to be a most suitable adjuvant for *Alternaria*

macrospora MKP1 compared to tween 20 and triton x 100, which can control congress grass upto 90%. There are several biological and environmental limitations which obstruct the efficacy of a biocontrol agent. In the present days, advances in adjuvants formulation and delivery system have been used to overcome some of these limitations and improve the efficacy of a biocontrol agent (Boyette et al. 1996). Formulation is recognized as a method to increase efficiency and efficacy of the biocontrol agent (Greaves et al. 1998; Evans and Reeder, 2001). For examples, oil emulsion formulation, may reduce dew requirements (Greaves, 1996; Shabana, 1997b; Greaves et al. 1998) and the number of spores required (Egley and Boyette, 1995). Sodium alginate granular formulation with several biocontrol agents has been successfully increased the biocontrol efficacy of the pathogen (Walker and Riley, 1982; Walker and Connick, 1983). A formulation containing corn oil, and Tween 20, 40, 60 and 80 and spores of *Alternaria helianthi*, a pathogen of cocklebur (*Xanthium strumarium*) reduced the dew period required for infection (Abbas and Egley, 1996). *Alternaria cassiae* mycoherbicide formulations were improved by studying the influence of pH, surfactants, and nutrients on germination (Daigle and Cotty, 1991). A formulation containing 0.01-1% Tween 80 significantly promoted spore germination. Effect of adjuvant on the biocontrol efficiency of *Alternaria macrospora* MKP1 biocontrol agent to *Parthenium hysterophorus* at 25 days after inoculation are presented in table 5.

Table 5. Effect of adjuvant on the biocontrol efficiency of *Alternaria macrospora* MKP1 biocontrol agent :- (percent infection) to *Parthenium hysterophorus,* 25 days after inoculation.

S. No.	Surfactant	Covered		Uncovered	
		Uninjured	Injured	Uninjured	Injured
1	Tween 80	89±0.21	92±0.33	74±0.26	87±0.41
2	Tween 20	61±0.32	66.26±0.13	57±0.23	61±0.43
3	Glycerol	72.81±0.21	78.92±0.43	51±0.44	69±0.13
4	Triton x 100	60.13±0.34	61.19±0.25	41.12±0.23	53.32±0.24
5	Control (distilled water)	65.28±0.21	69.51±0.34	61±0.29	65±0.23

Conclusion

Twenty-nine fungal pathogens have been reported on *P. hysterophorus* weed from various parts of the globe (Kaur et al. 2014). A literature search reveals that this is the first report of the

occurrence of *Alternaria macrospora* MKP1 causing leaf blight on parthenium weed from the world. Thus, the main aim of this paper was to highlight the various histopathological parameters to evaluate the biological control potential of *Alternaria macrospora* MKP1 against parthenium. Looking into the severity of the disease and damage caused to the parthenium weed, the pathogens seem to offer great potential for development and exploitation as effective biocontrol agents for checking parthenium growth. The cultural filtrate used in this study must have toxin or alike substance which needs further detailed investigation to identify the potential herbicidal constituents present in these fungal culture filtrates. The host specificity, excellent enzymatic activity, phytotoxins and pathogenesis of this isolate accentuates its potential in biocontrol of notorious weed parthenium.

Conflict of Interest

Authors declare no conflict of interest.

References

Abbas H.K, Duke S.O, Paul R.N, Riley R.T, Tanaka T. 1995. AAL-toxin, a potent natural herbicide which disrupts sphingolipid metabolism of plants. Pest Sci. 43: 181-187.

Abbas H.K, Egley G.H. 1996. Influence of unrefined corn oil and surface-active agents on the germination and infectivity of *Alternaria helianthi*. Biocontrol Sci Technol. 6: 531-538.

Adkins S, Shabbir A. 2014. Biology, ecology and management of the invasive parthenium weed (*Parthenium hysterophorus* L.). Pest Manage Sci. 70: 1023-1029.

Agarwal G.P, Hasija S.K. 1986. Microorganisms in the laboratory - a laboratory guide of mycology, microbiology and plant pathology. Print House (India) Lucknow, pp 155.

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

Aneja K.R. 2003. Experiments in Microbiology, Plant Pathology and Biotechnology. 4th ed. New Age International Publishers, New Delhi.

Aneja K.R, Kaushal S. 1998. Occurence of *Gibbago trianthemae* on horse purslane in India J Biol Con. 12: 157-159.

Aneja K.R, Khan S.A, Kaushal S. 2000. Management of horse purslane (*Trianthema portulacastrum* L.) with *Gibbago trianthemae* Simmons in India. In: Spencer NR (ed) Proceedings of the X International Symposium on Biological Control of Weeds. Montana State University, Bozeman, Montana, USA, pp 27-33.

Aneja K.R, Kumar V, Jiloha P, Kaur M, Sharma C, Surain P, Dhiman R, Aneja A. 2013. Potential bioherbicides: Indian perspectives. In: Salar RK, Gahlawat SK, Siwach P, Duhan JS (eds) Biotechnology: prospects and applications. Springer, India, pp 197-215.

Aneja K.R, Singh K. 1989. *Alternaria alternata* (Fr.) Keissler, a pathogen of water hyacinth with biocontrol potential. Int J Pest Manage. 35: 354-356.

Boyette C.D, Quimby P.C. Jr, Caesar A.J. 1996. Adjuvants, formulations, and spraying systems for improvement of mycoherbicides. Weed Tech. 10: 637-644.

Chenglin Y, William S.D, Carl S.C. 1996. Purification and characterization of a polygalacturanase produced by *Penicillium expansum* in apple fruit. Phytopathology. 86: 1160-1166.

Chettananavar S.N, Srikant K, Hedge R.K. 1987. Studies on leaf blight of wheat caused by *Alternaria alternata* (Fr.), Keissler. cultural and physiological studies. Mysore J Sci. 21: 313-317.

Daigle D.J, Connick W.J. 2002. Formulating Mycoberhicides. In: Osiewacz HD (ed) The Mycota X Industrial Applications. Springer-Verlag Berlin Heidelberg Southern Regional Research Center, New Orleans, Louisiana, USA.

Daigle O.J, Cotty P.J. 1991. Factors that influence germination and mycoherbicidal activity of *Alternaria cassiae*. Weed Technol. 5: 82-86.

Egley G.H, Boyette C.D. 1995. Water corn oil emulsion enhances conidia germination and mycoherbicidal activity of *Colletotrichum trunctatum*. Weed Sci. 43: 312-317.

Ellis M.B. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institue, England.

Ellis M.B. 1976. More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, England.

Evans H.C, Fleureau L. 1993. Studies on the rust, *Maravalia cryptostegiae,* a potential biological control agent of rubber-vine weed, *Cryptostegia grandiflora* (Asclepiadaceae: Periplocoideae), in Australia, II: Infection. Mycopathologia 124: 17-184.

Ghorbani R, Seel W, Litterick A, Leifert C. 2000. Evaluation of *Alternaria alternata* for biological control of *Amaranthus retroflexus*. Weed Sci. 48: 474-480.

Greaves M.P. 1996. Microbial herbicides-factors in development. In: Copping LG (ed) Crop Protection Agents from Nature. Royal Society of Chemistry, Cambridge, UK, pp 444-467.

Greaves M.P, Holloway P.J, Auld B.A. 1998. Formulation of microbial herbicides. In: Burges HD (ed) Formulation of Microbial Biopesticides. Dordrecht, The Netherlands, Kluwer, pp 203-233.

Kamal M. 1950. Leaf blight of *Pandanus* sp. Curr Sci. 19: 125-128.

Karr A.L, Karr D.B, Strobel G.A. 1974. Isolation and partial characterization of four host specific toxins of *Helminthosporium maydis* (race T.). Pl Physiol. 53: 250-257.

Kaur M, Aggarwal N.K. 2015. Biocontrol potential of four deadly strains of *Alternaria macrospora* isolated from parthenium weed. Plant Pathol J. 14: 72-78.

Kaur M, Aggarwal N.K, Dhiman R. 2016. Screening of Phytotoxicity of *Alternaria macrospora* MKP1 against *Parthenium hysterophorus* L. Arch. Phytopathology Plant Protect. 48: 890-897.

Kaur M, Aggarwal N.K, Kumar V, Dhiman R. 2014. Effects and Management of *Parthenium hysterophorus* a weed of global significances. Int Sch Res Notices. Doi.org/10.1155/2014/368647.

Madan M, Thind K.S. 1998. Physiology of fungi. A.P.H. Publishing Corporation, New Delhi, India.

Mitchell J.K. 1988. *Gibbago trianthemae*, a recently described hyphomycete with bioherbicide potential for control of horse purslane (*Trianthema portulacastrum*). Plant Dis. 72: 354-355.

Muthulakshmi P. 1990. Studies on fruit rot of chilli (*Capsicum annuum* L.) caused by *Alternaria tenuis* Nees. M.Sc. (Ag.). Thesis, Tamil Nadu Agric. Univ., Madurai, India, 139 p.

Neergaard P. 1945. Danish species of *Alternaria* and *Stemphylium.* London, Oxford Univ, Press, pp 129-1148.

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

Patil M.G, Pagare J, Patil S.N, Sidhu A.K. 2015. Extracellular Enzymatic Activities of Endophytic Fungi Isolated from Various Medicinal Plants. Int J Curr Microbiol Appl Sci. 4: 1035-1042.

Rathaiah Y. 1977. Stomatal tropism of *Cercospora beticola* in sugarbeet. Phytopathol. 67: 358-362.

Saad S, Hagedorn S.J. 1970. Growth and nutrition of *Alternaria* pathogenic to snap bean. Phytopathol. 60: 903-906.

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

Shabana Y.M. 1997b. Vegetable oil suspension emulsions for formulating the weed pathogen (*Alternaria eichhorniae*) to bypass dew. Z Pflanzenkr Pflanzenschutz. 104: 78-89.

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

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

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

Strobel G.A. 1973. The Helminthosporoside binding protein of sugarcane. J Biol Chem. 248: 1321-1328.

Sunitha V.H, Nirmala D, Srinivas C. 2013. Extracellular Enzymatic Activity of Endophytic Fungal Strains Isolated from Medicinal Plants. World J Agric Sci. 9: 1-9.

Tandon R.N. 1961. Physiological studies on some pathogenic fungi in U.P. Sci Res Committee, Monograph 34.

TeBeest D.O, Templeton G.E, Smith R.T. 1978. Temperature and moisture requirements for development of anthracnose on North jointvetch Phytopathol. 68: 389-93.

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

Tuite J. 1969. Plant Pathological Methods, Fungi and Bacteria. Burger Publishing company, MN.

Verma V.C. 1963. Effect of temperature and hydrogen ion concentration on three pathogenic fungi. Sydowia 25: 164-169.

Vikrant P, Verma K.K, Rajak R.C, Pandey A.K. 2006. Characterization of a phytotoxin from *Phoma herbarum* for management of *Parthenium hysterophorus* L. J Phytopathol. 154: 1-8.

Walker H.L, Connick W.J. Jr. 1983. Sodium alginate for production and formulation of mycoherbicides. Weed Sci. 31: 333-338.

Walker H.L, Riley J.A. 1982. Evaluation of *Alternaria cassia* for the biocontrol of sickle-pod (*Cassia obtusifolia*). Weed Sci. 30: 651-654.

Walker H.L, Templeton G.E. 1979. *In vitro* production of phytotoxic metabolites by *Colletotrichum gleosporoides* f sp *aeschynomene*. Plant Sci Lett. 13: 91-99.

Wapshere A.J. 1974. A strategy for evaluating the safety of organisms for biological weed control. Ann Appl Biol. 77: 201-211.

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