Indian Journal of Weed Science **46**(2): 155–160, 2014

Mycobiota associated with *Parthenium hysterophorus* isolated from North India

N.K. Aggarwal*, M. Kaur, V. Kumar and A. Saini
Department of Microbiology, Kurukshetra University, Kurukshetra 136 119

Received: 23 March 2014; Revised: 26 April 2014

ABSTRACT

Parthenium hysterophorus, one of the "worst weeds", is an erect and much branched annual or ephermeral herb causing colossal loss in terms of economic, environmental, animal and human health hazards. A survey on occurrence of the natural enemies of *P. hysterophorus* L. was conducted in Kurukshetra and its adjoining areas, Haryana, India. The *Parthenium* population at different places during different seasons were found to have various diseases. The native pathogens of *Parthenium hysterophorus* were studied and compared on the basis of pathogenicity by Koch's postulates. A total of twenty six pathogenic fungi, P1-P26 were isolated from different diseased *Parthenium* plants. All the isolates were preliminarily identified on the basis of cultural and morphological characteristics and it was observed that all of them belongs to the fungi imperfecti except isolates P19, P20 which belongs to Ascomycota. On the basis of pathogenicity, the isolates P2, P5, P7, P9, P12, P17 and P23 were selected and the effect of different media on the growth and sporulation of selected pathogens was tested. This study will help to develop mycoherbicides by using these fungal pathogens in combination or single.

Key words: Ascomycota, Ephermeral, Fungi imperfecti, Mycoherbicide, *Parthenium hysterophorus*

White top (*Parthenium hysterophorus* L.) is an annual herb of Asteraceae family, originating from tropical Americas and now a weed of global significance in many countries around the world. It was reported that the seeds of this weed came to India with grains imported from U.S.A. under the USA PL 480 scheme and spread alarmingly like a wild blaze to almost all the states in India and established as a naturalized weed. In India, the weed was first reported in Poona (Maharashtra) by Prof. Paranjape in 1955, as stray plants on rubbish heaps and was reported by Rao in 1956, as a new record for the country but the earliest record of this species in India goes back to 1814 by William Roxburgh, 'the father of Indian Botany', in his book *Hortus Bengalensis* (Rao 1956, Rouxburg 1814). It was estimated to spread in 35 million hactare of land in India representing wasteland including pastures, cropland and forestland (Sushilkumar and Varshney 2010).

With the ever increasing population of the weed in both urban and rural localities, the associated problems like crop production, animal husbandry, human health and biodiversity are also phenomenally growing day by day. To the weed scientist, *Parthenium* has proved a challenge because conventional methods have failed to suppress its growth and prevent its unchecked spread throughout the world, and still efforts are being made to control this weed by all possible means. In this context, biological control with plant pathogens is an effective, safe, selective and practical means of weed management. Since 1979, considerable progress has been made towards practical use of plant pathogens as safe and selective agents of weed management (Charudattan and Walker 1982, Aneja *et al.* 2013, Kour 2014). The biological control of this weed using fungal pathogens under the mycoherbicidal strategy has been suggested as one of the most efficient method, owing to its long lasting, less costly and eco-friendly nature. The objective of the present study was to search for fungal pathogens naturally occurring on *Parthenium* weed in Northern India that could be used for reducing *Parthenium* population to economic levels.

***Corresponding author:** mani7yu@gmail.com

MATERIALS AND METHODS

Isolation of the pathogens

Surveys were conducted during 2013-14 in Kurukshetra and adjoining area to search pathogens associated with *Parthenium*. Leaf surfaces were washed with distilled and sterilized water in order to remove epiphytic fungi and adherent soil particles**.** The infected portions of the leaves were cut into 1.0-1.5 cm fragments, surface sterilized with 70% ethanol for 1-2 minutes and then rinsed in sterile distilled water 3 to 4 times. These fragments were transferred on to the potato dextrose agar (PDA) medium (potato: 200g,

agar-agar: 20 g, dextrose: 20 g, distilled water: 1000 ml) Petriplates supplemented with streptomycin sulphate and were incubated at 25 °C (Aneja *et al*. 2014)

Identification

5 mm diameter discs from 7 day old PDA cultures, taken from the advancing mycelial margins with a cork borer, were placed at the centre of PDA and maintained at 25±2 under continuous darkness. Seven days later, the colony appearance and their diameters on PDA were determined. The morphological characteristics of the mycelium, conidia and perithecia of pathogens were observed and preliminarily identified by consulting monograms (Ellis 1971, 1976, Bilgrami 1991).

Screening of the major pathogens

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

Effect of different media on growth and sporulation

To see the effect of different media, on the growth and sporulation of fungal pathogens, seven media namely potato sucrose agar (PSA), potato dextrose agar (PDA), potato dextrose yeast agar (PDAY), *Parthenium* dextrose agar (PeDA), *Parthenium* dextrose yeast agar (PeDAY), maltose extract agar (MEA) and nutrient agar (NA) were used. Inoculated plates were incubated at 25 °C for ten days. Three replicates were run per medium per condition for all the test fungi. Fungal growth was determined by measuring the diameter of the colony at two places at right angle to each other and an average of the cross diameter was considered as growth of the fungus.

Conidial concentration of different pathogens on different media was measured by scraping the mycelial growth from the plate with distilled water and then homogenized on magnetic stirrer for 5 minutes, placed 1-2 drops of suspension on the hemocytometer slide and calculated the conidial concentration using microscope (Tuite 1969).

RESULTS AND DISCUSSION

During surveys conducted between 2013-14 in Kurukshetra and its adjoining area, over 47 diseased specimens were collected and examined for the fungal pathogens (Table 1). A total of twenty six fungal pathogens namely *Alternaria* sp. (four different species), *Cladosporium* sp. (three different species), *Curvularia* sp. (two different species), *Colletotrichum* sp. (two different species), *Drechslera* sp., *Fusarium* sp. (four different species), *Chaetomium* sp. (two different species), *Acremonium* sp., *Trichoconiellia padwickii*, *Pestalotia* sp., *Epicoccum* sp., *Nigrospora* sp., *Scedosporium* sp. and *Torula* sp. were identified. On the basis of sporulating structures produced on the live *Parthenium* leaves, one pathogens namely *Cercospora* sp., was identified.

Out of these twenty six pathogens, seven were selected on the basis of pathogenicity for evaluation of biocontrol potential and were tested for the growth and sporulation on different media. Species of *Alternaria, Fusarium* and *Curvularia* were already reported from *Parthenium hysterophorus* but *Torula* sp. and *Trichoconiellia padwickii* has been reported first time on this weed during the survey.

***Alternaria alternata* agg**

The symptoms appeared as small, central and marginal brown or black spots scattered on leaves. The spots became irregular in shape. When their size increased they turned brown to black in colour. Several such lesions may coalesce resulting in leaf drying. Culture grey green in colour with white margins and becoming black at maturity. Conidiophores light brown to golden brown, simple, branched, septate, straight or curved, smooth walled. Conidia light brown to olivaceous, borne long acropetal chains, ovoid or obclavate with a long or short beak, or ellipsoidal and without beak, smooth to echinulate, muriform with transverse and longitudinal septa. The beak, when present, is always smaller and lighter in colour than the conidial body (Fig. 1, A1-A4). Diseased specimens and culture have been deposited at CAB International Mycological Institute, UK with reference No. 502784 and IIBC, Ascot, UK.

***Alternaria* sp.**

The colony on PDA is green with white margins. The mycelium is branched and septate and the aerial hyphae are undeveloped. On living leaves of *Parthenium* symptoms are characterized as dark brown, irregular marginal spots. Conidiophores are solitary rarely in groups (1-3 in number), septate, light brown and straight to geniculate with 1-4 scars. Conidia mostly solitary, rarely in chains of 2-4, dark brown, smooth,

Table 1. Characteristic and percentage frequency of various fungal pathogens

Isolates	Symptoms	Preliminary identification	Colony characteristics	Pathogenicity	Frequency (%)
P1	Leaf spot	*Pestalotia* sp.	Yellowish white with black sclerotia	++	1.92
P2	Leaf spot	*Alternaria* sp.	Green with white margin	+++	9.62
P3	Leaf spot	*Alternaria* sp.	Grey with green margin	++	2.56
P4	Leaf spot	*Alternaria* sp.	Light grey	++	3.85
P5	Leaf blight	*Torula* sp.	White green	+++	5.13
P6	Leaf spot	*Fusarium* sp.	Whitish yellow	++	1.28
P7	Leaf blight	*Fusarium* sp.	Reddish white	+++	3.85
P8	Leaf spot	*Fusarium* sp.	White orange	+	6.41
P9	Leaf blight	*Fusarium solani*	Pale green	+++	5.13
P10	Leaf spot	*Cladosporium* sp.	Grey	+	5.77
P11	Leaf spot	*Cladosporium* sp.	purple-brown	+	4.49
P12	Leaf spot	*Alternaria alternata* agg.	Grey-green	+++	10.26
P13	Leaf spot	*Acremonium* sp.	White	+	1.92
P14	Anthracnose	*Colletotrichum* sp.	White to brown	+	4.49
P15	Anthracnose	*Colletotrichum* sp.	Dark brow with black sclerotia	++	5.77
P16	Leaf spot	*Nigrospora* sp.	Grey to black	+	3.85
P17	Leaf blight	*Trichoconiellia padwickii*	Brown to grey with orange exudates	+++	1.28
P18	Leaf spot	*Cladosporium* sp.	Grey green	+	1.92
P19	Leaf spot	*Cercospora**	-	-	-
P20	Leaf spot	*Chaetomium* sp.	Olive green	+	1.28
P21	Leaf spot	*Chaetomium* sp.	Olive to dark green	+	3.21
P22	Leaf spot, tip drying	*Dreschlera* sp.	Grey	++	2.56
P23	Leaf spot	*Curvularia* sp.	Grey	+++	4.79
P24	Leaf spot	*Cuvularia* sp.	Green to black	++	3.85
P25	Leaf spot	*Epicoccum* sp.	Orange to brown	+	3.85
P26	Leaf spot	*Scedosporium* sp.	Orange white	+	1.28

+ - low; ++ - moderate; +++ - high; * - Uncultured

with 3-5 transverse and 2-3 longitudinal septa, short beaked which sometimes swollen at the apex or ellipsoidal and without beak. Light brown to dark brown coloured chlamydospores present solitary or in chains (Fig. 1, B1-B4).

***Curvularia* sp.**

Symptoms appeared on leaves are brown to black leaf spots scattered all over the leaf. *Curvularia* sp. appears as shiny velvety-grey, fluffy growth on the colony surface with septate, dematia-ceous hyphae producing brown, geniculate conidiophores. Conidiophores arise in tufts of 4-6 from subcuticular stromata; erect, inflated at the base, dark brown, septate, nodulose with spiral conidial scars. Conidia olive brown, usually curved, ellipsoid 3 septate, rounded at the base, 2 central cells, larger and darker than the two nearly hyaline end cells (Fig. 1, C1-C4).

Fusarium solani

Symptoms are characterised as, dark brown, irregular, marginal and central spots. Culture pale to green in reverse; aerial culture pale. Hyphae are septate and hyaline. Conidiophores are simple (non-branched) or branched monophialides (phialides with a single opening). Macroconidia are moderately curved, stout, thick-walled, usually 3-5 septate, measure 4-6 x up to 65 µm long, and are borne on short conidiophores that soon form sporodochia. Microconidia are borne from long monophialides, are one to three-celled, 2-5 x 8-16 µm long, and occur in false heads only (in clusters of conidia at the tip of the phialide). Chlamydoconidia are present (sometimes profuse) and occur both singly and in pairs (Fig. 1, D1-D4). The identification of pathogen has been confirmed at CABI International Mycological Institute, UK with reference No. 503548.

***Fusarium* sp.**

The symptoms appeared as water soaked brown spots scattered on the leaf surface. These spots coalesced and formed larger brown spots. Culture whitish cream to deep rose red to burgundy in reverse with floccose texture; aerial culture white, becoming somewhat compressed by the formation of orange sporodochia due to the presence of conidial mass. Microconidia produced on polyphialidic conidiophores, usually looking like rabbit ears or X- shaped, straight, obovoid and 0-1 septate. Macroconidia scarce, sickle shaped, 3-5 septate. Chlamydospores present abundantly, in chains or cluster (Fig. 1, E1-E4).

***Torula* sp.**

Symptoms are characterized by the presence of black marginal necrotic spots on the leaves of *Parthenium*. Colonies are whitish green, effuse to

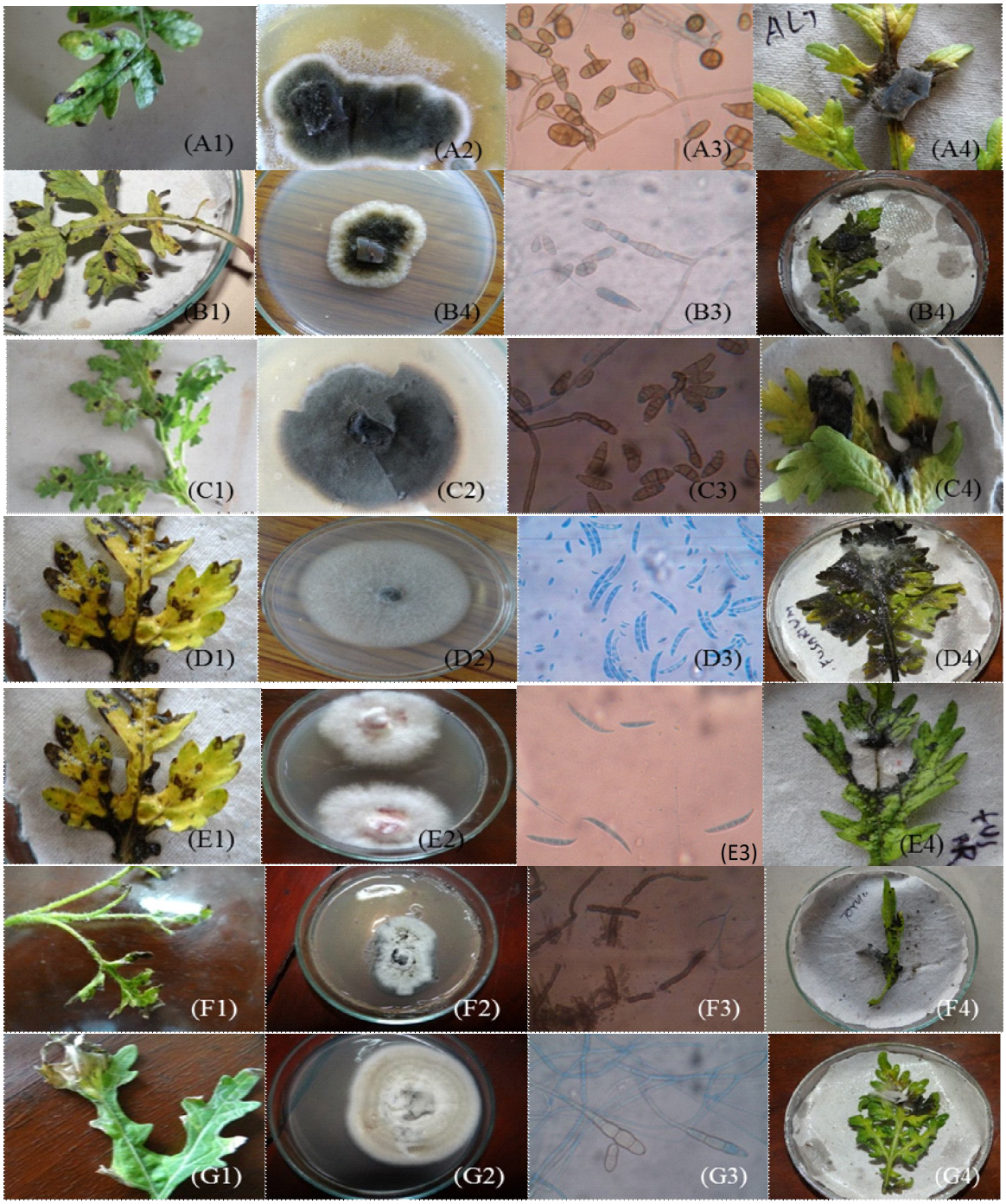

Figure 1. Symptoms, colony characteristic, microscopic view and pathogenicity test of fungal pathogen reported on *Parthenium* weed. *A. alternata* agg.(A1-A4); *Alternaria* sp.(B1-B4); *Curvularia* sp.(C1-C4); *Fusarium solani* (D1-D4); *Fusarium* sp. (E1-E4); *Torula* sp. (F1-F4); *Trichoconeilla padwickii* (G1-G4)

crusted-like with pronounced vertical streaking, becoming markedly thickened and friable in age. Conidia are dark brown phragmoconidia, typically roughened, borne in unbranched moniliform, acropetal chains. The wall of the distal conidial cell is characteristically thinner and lighter in pigmentation than the rest of the conidium (Fig. 1, F1-F4).

Trichoconeillia padwickii

Symptoms on living leaves of *Parthenium*, are characterized as dark brown to black, irregular marginal spots. Colonies are whitish brown and black from reverse on PSA. Conidiophores are brown, straight to geniculate with 1-4 scars, up to 190 µm long, 5.7-9.5 µm thick. Conidia mostly solitary, rarely in chains of

Table 2. Colony diameter of different pathogens on different media

Pathogens	Colony diameter on different media (cm)						
	PDA	PDAY	PeDAY	PeDA	MEA	PSA	NA
Alternaria alternata agg.	6.72±0.21	7.95±0.18	6.84±0.03	6.59±0.45	7.68±0.08	6.97±0.28	6.11±0.17
Alternaria sp.	5.63±0.07	5.12±0.47	4.95±0.43	4.95±0.04	5.15±0.25	6.32±0.43	4.81±0.41
Curvularia sp.	5.85±0.25	6.55±0.32	5.4±0.42	5.29±0.67	5.31±0.41	6.39±0.35	5.25±0.62
Fusarium solani	5.88±0.08	6.95±0.22	6.39±0.34	6.45±0.51	6.87±0.63	7.15±0.55	4.95±0.16
Fusarium sp.	6.51±0.16	6.95±0.04	7.1±0.45	6.95±0.34	6.79±0.06	7.81±0.23	5.86±0.39
Torula sp.	4.35±0.51	4.53±0.31	4.14±0.58	4.05±0.53	4.36±0.24	4.79±0.71	4.20±0.11
Trichoconeilla padwickii	5.55±0.28	5.16±0.25	5.24±0.36	5.47±0.08	4.85±0.34	6.85±0.12	3.60±0.08

Table 3. Effect of different media on the sporulation of pathogens

Pathogens	Average spore count/ml ($x10^5$) on different media						
	PDA	PDAY	PeDAY	PeDA	MEA	PSA	NA
Alternaria alternata agg.	19.00±0.46	11.95±0.81	16.84±0.53	14.59±0.45	17.68±0.68	14.97±0.28	1.11±0.17
Fusarium solani	24.88±0.56	27.95±0.43	24.39±0.04	23.45±0.39	22.87±0.26	18.15±0.58	9.95±0.68
Fusarium sp.	16.51±0.16	18.95±0.04	10.1±0.44	12.95±0.61	16.79±0.38	15.81±0.22	8.86±0.33
Curvularia sp.	17.85±0.25	19.55±0.32	15.4±0.82	13.29±0.38	19.31±0.42	18.39±0.42	14.25±0.13
Trichoconeilla padwickii	5.55±0.27	7.16±0.25	4.24±0.16	4.47±0.23	6.85±0.34	6.85±0.21	2.60±0.02
Alternaria sp.	25.63±0.53	23.12±0.42	21.95±0.53	22.95±0.47	27.15±0.41	23.32±0.63	18.81±0.41

2, obclavte, rostrate, pale to golden brown, smooth to minutely verruculose, with 5-9 transverse and rarely seen longitudinal septa, body 72-106 × 17.1-26.6 µm, beak hyaline, filiform, septate, straight to geniculate sometimes swollen at the apex, often much longer than the body of the spore, 55-165 µm long and 1.9-3.8 µm thick. Diseased specimens and culture have been deposited at CABI, International Mycological Institute, UK (reference no. 502783)(Fig. 1, G1-G4).

Growth and sporulation on different media

Of the seven media tested for the growth and sporulation, *A. alternata* and *Curvularia* sp. showed excellent growth and sporulation on PDAY, its growth and sporulation was good on PDA, MEA, PSA, PeDA, PeDAY and poor on NA. *Alternaria* sp. showed best growth and sporulation on MEA media and was poor on NA. Growth of *Fusarium* sp. was best observed on PSA and it sporulate well on all the media but best on PDAY. Best growth and sporulation of *Fusarium solani* and *Trichoconeilla padwickii* were observed on PSA and PDAY respectively. The growth of *Torula* sp. was best on PSA but its spore count could not be performed because the conidia of this pathogen were indistinct.

Literature search revealed that a great deal of work has been done by the scientists to control this weed by fungal pathogens. Saxena and Kumar (2007) worked on the mycoherbicidal potential of *Alternaria alternata* ITCC (LC#508) in Northern India to control *Parthenium* weed, and reported 50% damage of plants *in vitro* detached leaf and whole plant bioassay at 96 hours post-treatment at a concentration of 1×10^6 spores/ml. *Sclerotium rolfsii* (teleomorph: *Athelia rolfsii*), incites a severe collar rot disease on *Parthenium* (Pandey *et al.* 1998, Shukla and Pandey 2006). Although, the pathogen is responsible for severe damage to weed, but the wide host range of the species creates doubt about its suitability as mycoherbicides. *Cladosporium* sp. (MCPL-461), a floral leaf pathogen of *Parthenium*, produces symptoms on the flowers, buds, and inflorescences, and causes sterility and reduces seed viability. The severity of pathogen to the reproductive organs led to serious damages of the *Parthenium* plants and may be used as a potential mycoherbicide against this weed (Kumar *et al.* 2009). Satyaprasad and Usharani (1981) reported powdery mildew causing *Oidium parthenii* on *Parthenium* at Hyderabad. The fungus appears as small, circular, white powdery spots on the surface of leaves, and spreads over the entire lamina on both the surfaces giving a powdery appearance to the plant. Severe infection leads to defoliation. Kauraw *et al.* (1997) reported *Fusarium pallidoroseum*, on *Parthenium* from Jabalpur. It was found to reduce seed germination, seedling vigour, height of plant, number of branches and number of flowers and reported as a potential biocontrol agent for *Parthenium* management.

But these pathogens suffered from one or the other disadvantages, so our work in this area aims for searching a potential pathogen which should be the host specific and emerges as an effective mycoherbicide against this weed.

A total of twenty nine fungal pathogens have been reported on *P. hysterophorus* weed from various parts of the globe. During our study on fungal pathogens, we have reported 26 pathogens on this weed. Out of these, *Torula* sp. and *Trichoconeilla padwickii* were reported first time on *Parthenium hysterophorus.* Looking into the severity of the disease and damage caused to the *Parthenium* weed during surveys in North India, the pathogens P2, P5, P7, P9, P12, P17, P23 seem to offer great potential for development and exploitation as effective biocontrol agents for checking *Parthenium* growth. Further work on its host specificity and evaluation as biocontrol agents is in progress in our lab, which may leave to recognize the potential of these pathogens.

ACKNOWLEDGEMENT

The authors are thankful to the Director, CABI, International Mycological Institute, Egham, England for confirming the identification of the isolate. The author also thanks the University Grants Commission, New Delhi for providing financial assistance in the form of Maulana Azad National Fellowship for minority students.

REFERENCES

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

Aneja KR, Kumar V and Sharma C. 2014. Leaf-spot disease of *Trianthema portulacastrum* – a new record from world. *Journal of Innovative Biology* **1:** (2) 112-116.

Aneja KR, Kumar V, Jiloha P, Kaur M, Sharma C, Surain P, Dhiman R and Aneja A. 2013. Potential bioherbicides : Indian perspectives, pp. 197-215. In: *Biotechnology: Prospects and Applications*. (Eds. Salar RK, Gahlawat SK, Siwach P and Duhan J) . Springer, India.

Bilgrami KS, Jamaluddin S and Rizwi MA. 1991. *Fungi of India- List and References.* Today and Tomorrow's Printers and Publishers, New Delhi. 798.

Charudattan R and Walker HL. 1982. *Biological Control of Weeds with Plant Pathogens.* John Wiley, New York: 293p.

Ellis MB. 1971. *Dematiaceous Hyphomycetes.* Commonwealth Mycological Institute, England.

Ellis MB. 1976. More *Dematiaceous Hyphomycetes.* Commonwealth Mycological Institute, England.

Kaur M, Aggarwal NK, Kumar V and Dhiman R. 2014. Effects and Management of *Parthenium hysterophorus* : A Weed of Global Significances. *International Scholarly Research Notices.* DOI.org/10.1155/2014/368647

Kauraw LP, Chile A and Bhan VM. 1997. Evaluation of *Fusarium pallidoroseum* (Cooke) Sacc. for the biocontrol of *Parthenium hysterophorus* L., pp. 70-74. In: *Proceedings of the I*st *International Conference on Parthenium Management*, (Eds. Mahadevappa M and Patel VC) Dharwad, India.

Kumar A, Verma VC, Gond SK, Kumar V and Kharwar RN. 2009. Biocontrol potential of *Cladosporium* sp. (MCPL-461), against a noxious weed *Parthenium hysterophorus* L. *Journal of Environmental Biology* **300**(2): 307-312.

Pandey AK, Mishra J and Hasija SK. 1998. Effect of inoculum on mycoherbicidal potential of *Sclerotium rolfsii* against *Parthenium, Journal of Mycology and Plant Path*ology, **28**: 284-287.

Rao RS. 1956. *Parthenium*, a new record for India. *Journal of Bombay Natural History Society* **54**: 218-220.

Roxburg W. 1814. *Hortus Bengalensis or a Catalogue of the Plants Growing in the Honourable East India Company's Botanic Garden at Calcutta*. The Mission Press, Serampore. 62p.

Saxena S and Kumar M. 2007. Mycoherbicidal potential of *Alternaria alternata* ITCC4896 for the control of *Parthenium hysterophorus. Jornal of Plant Protection Research*, **47**: 213-218.

Satyaprasad K and Usharani P. 1981. Occurrence of powdery mildew on *Parthenium* caused by *Oidium parthenii. Current Science* **24**: 1081-1082.

Shukla R and Pandey AK. 2006. Maximization of production of oxalic acid from *Sclerotium rolfsii,* a mycoherbicidal agent against *Parthenium*. *Annals of Plant Protection Science*, **14**: 202-205.

Sushilkumr and Varshney Jay G. 2010. Parthenium infestation and its estimated cost management in *India*. *Indian Journal of Weed Science* **42**(1&2): 73-77.

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