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Survey on the Infestation of Parthenium weed and Occurrence of Insect and Mycobiota on the Weed in India

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Abstract

The noxious weed, *Parthenium hysterophorus L.* (Asteraceae: Heliantheae), commonly known as Parthenium, is considered as one of the most troublesome weeds for agricultural sector by virtue of its high ecological amplitude and adaptability. The study was carried out to see the status of Parthenium infestation and the occurrence of the natural enemies of *P. hysterophorus* in Haryana, India. In the extensive surveys made in different districts of Haryana, *Parthenium hysterophorus* was found as the most dominant weed infesting all over the areas in wastelands, roadsides, railway tracks, noncultivated area, grasslands and several agricultural crops. The Parthenium population at different places during different seasons were found to have various fungal diseases and leaf-feeding insect. The native pathogens of *Parthenium hysterophorus* were studied and compared on the basis of pathogenicity by Koch's postulates.

Introduction

Parthenium is a weed of global Significance. It is regarded as one of the worst weeds, because of its invasiveness, potential for spread, and economic and environmental impacts. *Parthenium hysterophorus* L. (Asteraceace: Heliantheae), commonly known as parthenium, white top, congress grass, feverfew or carrot weed, is one of the most aggressive invasive weeds, threatening natural ecosystems and agro ecosystems in over 30 countries worldwide (Adkins and Shabbir, 2014). Earlier, Parthenium was considered a problem in waste and vacant land but reports stared to appear about its infestation in field crops after 1970 (Narasimhan *et al*., 1977). Parthenium infestation in crops increased from 0.50 million hactares during 1980 to 14.25 million hactares in 2010 (Sushilkumar and Varshney, 2010). Grain yield reductions due to infestation of Parthenium up to 40% in agricultural crops, like rice, wheat, maize, pigeonpea,

blackgram, sorghum etc. are known (Khosla & Sobti 1979). Parsons and Cuthbertson (1992) reported that Parthenium caused a substantial yield loss in sunflower and sorghum in central Queensland, Australia. The adverse impacts of Parthenium weed on environment and agriculture have also been reviewed by Kassa (2016) in context to Ethopia. 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. (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). 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. Conventional means of its control have failed due to their innate drawbacks. The concept of integrated pest management using indigenous fungal pathogens and insects 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. This study was carried out to determine the status of Parthenium infestation in various agricultural crops and association of weed pathogens and insects with the weed.

Materials and Methods

Survey

Surveys were conducted in between 2012-2014, to see the percent occurrence and percent infestation of congress grass weed in various agriculturally important crops.

The percent occurrence of weed was calculated by applying the formula:

$$\% \text{ occurrence of weed} = \frac{\text{No. of fields having congress grass}}{\text{Total no. of fields surveyed}} \times 100$$

The percent infestation of a crop by the congress grass was calculated by the quadrate method, in ecological studies (Cox, 1990). The quadrates of 50 x 50 cm^2 were used for the sampling purpose.

$$\% \text{ infestation of weed} = \frac{\text{Total no. of quadrates in which Parthenium weed occurred}}{\text{Total number of quadrates used}} \times 100$$

Collection of diseased samples

Surveys were conducted to search naturally occurring fungal pathogens on *Parthenium hysterophorus* weed in different districts of Haryana in the year 2012-2013.

Infected plants were collected in sterilized polythene bags and brought to the laboratory for the study of symptoms, isolation, identification and pathogenicity tests of the pathogen/s involved.

Study of symptoms and sporulation of the pathogens

Diseased leaves and other infected plant parts were selected and observed with the aid of magnifying glass and a dissecting microscope. Leaves showing characteristic symptoms were taken and placed in moist chambers (a petri plate containing wet filter papers) and incubated at 25^{O}C for 7 days for sporulation of the pathogen/s involved. These were checked for the appearance of fruiting bodies and spores on leaf surface. Tissue sections were taken with fruiting bodies and one or two sections were placed on slide for microscopic observation. Add one drop of water before covering with cover slip and observed mount (wet mount) for presence of fruiting bodies and spores (Trigiano *et al.,* 2004).

Isolation of pathogenic fungal pathogens

Two methods were used for the isolation and identification of fungal pathogens from the infected leaves.

From sporulating structures in moist chambers

Infected leaves were surface sterilized with 70% alcohol for 30-160 seconds and washed with sterile distilled water and incubated in moist chamber at 25±2^{o}C for 2-3 days to allow the sporulation of the pathogen(s) involved. Pathogen/s were examined and isolated from the sporulating structures by streak plate method.

Direct isolation from the infected plant tissue

Infected portions of host tissues were surface sterilized with 70% alcohol and then washed with sterilized distilled water, 4-5 times and placed on Potato Dextrose Agar (PDA) media supplemented with streptomycin (10mg/l) and were incubated at 25±2^{o}C for 3-5 days for growth of the pathogen involved.

Purification and Storage

The fungus growing from leaf fragments were purified either by streaking or serial dilution method. The cultures were purified by single sporing and maintained on PDA slants at 4^{o}C (Bohra *et al.,* 2005). A spore suspension was prepared by transferring the fungal growth in the agar slant to sterile water kept in a sterilized test tube followed by vigorous shaking for a few minutes in order to disperse the spores from the spore-bearing structures. A serial dilution of spores was prepared by transferring serially 1 ml aliquots to a series of tubes containing 9 ml of sterile water. Aliquots (1 ml) of spore suspension at optimal dilutions mixed with melted media (at about 45°C) were transferred to sterile Petri dishes and the mixture was spread by tilting the dishes gently

in different directions for uniformly covering the entire surface of the bottom plate. The inoculated plates were incubated at temperatures that favour growth and spore germination. The plates were examined under the microscope at regular intervals and the locations of germinating spores were marked using a glass marking pencil. The marked germinating spores along with a small amount of medium were individually transferred to agar slants for development of colonies from the germinating spores (Ko *et al.,* 2001).

Identification of fungal pathogens

Morphology of various fungal isolates was studied by preparing lactophenol cotton blue mounts from moist chambers/plate cultures as well as from the infected host tissue sections. Morphological characteristics of fungal pathogens were studied at different stages for the identification of the pathogens. The isolates were identified by consulting the literature and various monographs (Ellis 1971, 1976).

Pathogenicity test (in vitro) and proving of Koch's postulates

Pathogenicity tests of fungal isolates were determined on healthy detached leaves. Leaves were washed thoroughly in running tap water and surface sterilized with 70% alcohol. Placing 8mm mycelial discs of various pathogens from 7 days old fungal cultures on healthy parthenium leaves under aseptic conditions made artificial inoculations. Inoculated leaves were kept in moist chambers and incubated at 25±2^{o}C. Observations for the appearance of symptoms were made 3-4 days after incubation/inoculation. Re-isolation of the pathogen was done from inoculated leaves by the methodology described earlier and the isolated pathogen was compared with the original isolate (Aneja and Singh, 1989; Aneja *et al.*, 2000).

Isolation frequency of pathogenic fungi from congress grass

The isolation frequency of pathogenic fungi was calculated in different season i.e. summer (March to June), rainy (July to October) and winter (November to February). The diseased specimens were collected and frequencies were recorded on the basis of their presence which were categorised as HF (high frequency, 70-100%), MF (moderate frequency, 30-70%), LF (moderate frequency, 1-30%) and A (Absent, when no disease was found in these seasons).

Selection of virulent pathogens

Selection of virulent pathogens was done on the basis of three parameters i.e. frequency, disease incidence and pathogenicity. Disease incidence was recorded as percentage of plants infected in a plant population with the help of following formula:

$$\% \text{ Disease incidence} = \frac{\text{No. of plants infected}}{\text{Total no. of plants counted}} \times 100$$

Insects associated with parthenium

Survey were conducted for naturally occurring/feeding insects (larvae and adults) on parthenium in Haryana. The adults were collected in glass jars and brought to the laboratory. The insects were also preserved in formalin solution for their identification

RESULTS AND DISCUSSION

Surveys

During the extensive surveys conducted in the various districts of Haryana, during 2012-2014 in different seasons, infestation of parthenium was recorded along the road sides, railway tracks, in strips along village linking roads, pastures, gardens, orchards, vacant plots in urban areas as well as agricultural crops. Vacant plots in urban Estates were having the most luxuriant growth of the weed. In fact, there was no plot visualized that was not occupied completely by parthenium. Different agricultural important crops were surveyed to see the percent occurrence and percent infestation of parthenium in various fields (Table 1). Of the total agricultural fields visited, parthenium was recorded in three cereals viz. rice (*Oryza sativa*), wheat (*Triticum aestivum*) and jowar (*Sorghum vulgare*). However, it was not recorded in maize (*Zea mays*) the other commonly grown cereal in the state. The vegetables infested by parthenium include lady's fingers (*Abelmoschus esculentus*), onion (*Allium cepa*), garlic (*A. sativum*), kakari (*Cucurbita maxima*), potato (*Solanum tuberosum)*, methe (*Trigonella foenum graecum*), Pea (*Pisum sativum*) and bitter gourd (*Momordica charantia*). Parthenium was found in three oil yielding crops viz. sunflower (*Helianthus annuus*), mustard (*Brassica compestris*) and taramira (*Eruca sativa*). Horsegram (*Cicer arietinum*), and barseem (*Trifolium alexandrium*) were the only pulses and forage crops, respectively, infested by this weed. Sugarcane crop (*Saccharum officinarum*) and turmeric (*Curcuma longa*) were the commonly grown sugar yielding crop and spice respectively, infested by parthenium. There was no infestation of parthenium in *Tagetes erecta* and *Memordica charantia,* this could be due to the allelopathic effects of these plants. Our study revealed that the infestation of *Saccharum officinarum*, *Cicer arietinum*, *Brassica compestris* and *Sorghum vulgare* by parthenium was much higher than that recorded in the other crops.

Kohli and Rani (1994) reported *P. hysterophorus* as a serious problem in cotton, groundnuts, potatoes, sorghum, okra, brinjal, chickpea and sesame. Parthenium is also proving to be problematic in a range of orchard crops, including cashew, coconut, guava, mango and papaya (Tripathi *et al*., 1991; Mahadevappa, 1997). Similar infestations of sugarcane and sunflower plantations have been noted in Australia (Parsons and Cuthbertson, 1992; Navie *et al.,* 1996), whilst in Brazil and Kenya, the principal crop affected is coffee (Njoroge, 1989; Kissmann and Groth, 1992). In Ethiopia, parthenium weed was observed to grow in sorghum, cotton, finger millet (*Eleusine coracana*), haricot bean (*Phaseolus vulgaris*), vegetables (potato, tomato, onion, carrot) and fruit orchards (citrus, mango, papaya and banana) (Taye, 2002). Shabbir (2006); Shabbir *et al.* (2011) reported the parthenium weed from wheat, rice, sugarcane, sorghum, maize, squash, gourd and water melon.

Table 1 Infestation of parthenium in various agricultural crops (2012-2014).

Sr. No.	Crop	No. of fields visited	No. of fields having weed	Occurrence of weed %	Infestation %
1.	*Saccharum officinarum*	50	33	66%	64.15%
2.	*Eruca sativa*	30	17	56.67%	43.35%
3.	*Brassica compestris*	50	43	86%	87%
4.	*Helianthus annuus*	30	19	63.3%	44.9%
5.	*Oryza sativa*	40	7	17.5%	5%
6.	*Triticum aestivum*	50	39	78%	52.34%
7.	*Sorgham vulgare*	40	35	87.5%	74.23%
8.	*Trifolium alexandrium*	100	30	30%	54.61%
9.	*Cicer arietinum*	40	31	77.5%	71.25%
10.	*Pisum sativum*	50	5	10%	31.47%
11.	*Solanum tuberosum*	50	2	4%	49.79%
12.	*Allium cepa*	38	8	21%	30.4%
13.	*Allium sativum*	40	3	8%	28.35%
14.	*Zea mays*	45	0	0	0
15	*Tagetes erecta*	25	0	0	0
16.	*Cucurbita maxima*	30	17	56.67%	52.45%
17.	*Memordica charantia*	32	0	0	0
18.	*Abelmoschus esculentus*	50	2	4%	35%
19.	*Trigonella foenum-graecum*	30	4	14%	52.45%
20.	*Curcuma longa*	25	2	8%	13.45%

Congress grass when surveyed for its distribution in different districts of Haryana state has been found to be a very aggressive weed in various crops. Heavy infestation of congress grass was also observed along the road side, empty areas (Figure 2) and cultivated fields (Figure 1). In the months of October-November, numerous plants of congress grass were in an advanced state of decline with dead leaves.

Table 2: Characteristics and pathogenicity of fungal pathogens isolated from parthenium weed.

Isolates	Symptoms	Preliminary identification	Colony characteristics	Pathogenicity
P1	Leaf spot	*Pestalotia* sp.	Yellowish white with black sclerotia	+
P2	Leaf spot	*Alternaria* sp.	Green with white margin	+++
P3	Leaf spot	*Alternaria* sp.	Grey with green margin	++
P4	Leaf spot	*Alternaria* sp.	Light grey	++
P5	Leaf blight	*Torula* sp.	White green	++
P6	Leaf spot	*Fusarium* sp.	Whitish yellow	++
P7	Leaf blight	*Fusarium* sp.	Reddish white	++
P8	Leaf spot	*Fusarium* sp.	White orange	+
P9	Leaf blight	*Fusarium solani*	Pale green	++
P10	Leaf spot	*Cladosporium* sp.	Grey	+
P11	Leaf spot	*Cladosporium* sp.	purple-brown	+
P12	Leaf spot	*Alternaria alternata* agg.	Grey-green	++
P13	Leaf spot	*Acremonium* sp.	White	-
P14	anthracnose	*Colletotrichum* sp.	White to brown	+
P15	anthracnose	*Colletotrichum* sp.	Dark brow with black sclerotia	++
P16	Leaf spot	*Nigrospora* sp.	Grey to black	-
P17	Leaf blight	*Trichoconiellia padwickii*	Brown to grey with orange exudates	++
P18	Leaf spot	*Cladosporium* sp.	Grey green	+
P19	Leaf spot	*Cercospora**	-	-
P20	Leaf spot	*Chaetomium* sp.	Olive green	-
P21	Leaf spot	*Chaetomium* sp.	Olive to dark green	-
P22	Leaf spot, tip drying	*Dreschlera* sp.	Grey	++
P23	Leaf spot	*Curvularia* sp.	Grey	++
P24	Leaf spot	*Cuvularia* sp.	Green to black	++
P25	Leaf spot	*Epicoccum* sp.	Orange to brown	+
P26	Leaf spot	*Scedosporium* sp.	Orange white	+
P27	Leaf Spot	*Alternaria* sp.	Grey	+++
P28	Leaf blight	*Alternaria* sp.	Grey with green margins	+++
P29	Leaf Spot	*Alternaria* sp.	Dark grey	+++
P30	Leaf Spot	*Alternaria* sp.	Grey green	+++
P31	Leaf Spot	*Alternaria* sp.	Dark grey with white margins	+++

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

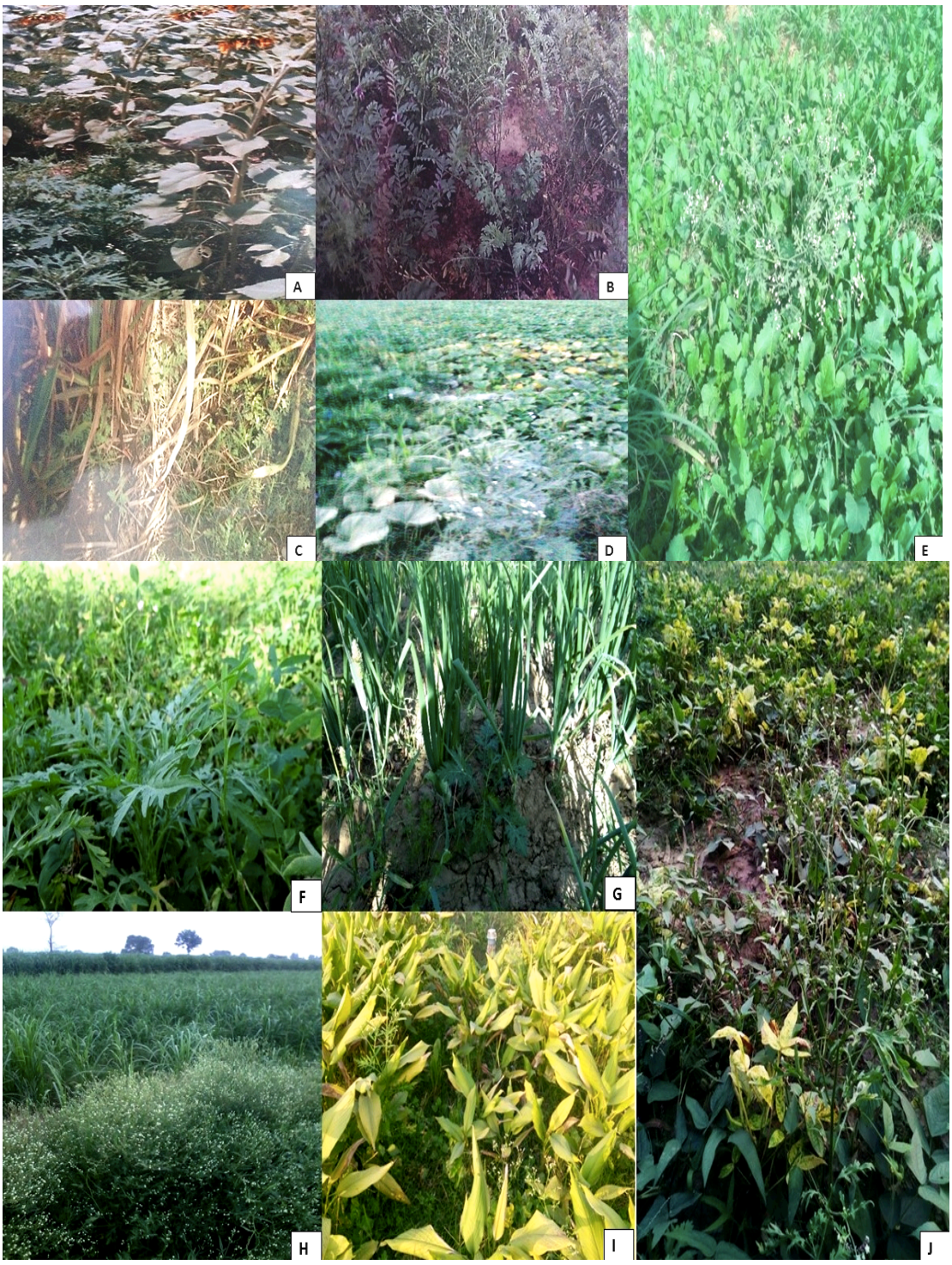


Figure 1 Infestation of *Parthenium hysterophorus* in various crops at Kurukshetra (A) *Helianthus annuus*; (B) *Cicer arietinum*; (C) *Saccharum officinarum;* (D)*Cucurbita maxima;* (E)*Brassica compestris*; (F) *Trifolium alexandrium*; (G) *Allium cepa*; (H) *Sorghum vulgare*; (I) *Curcuma longa*; (J) *Vigna radiata*.

Figure 2 Infestation of *Parthenium hysterophorus* in (A) Orchards; (B) House lawns; (C) Water canal; (D) Railway tracts; (E) Along roadsides; (F) Pastures; (G) School ground; (H) Bus stands.

Figure 3: (A) Larvae of *Zygogramma bicolorata* on axillary buds; (B) Defoliation of parthenium by pupae of *Zygogramma bicolorata* (C-D) Adult *Zygogramma* beetle (E) Complete defoliation of large population of parthenium weed by *Zygogramma* insect.

The data collected on the basis of information gathered from the farmers as well as on the basis of personal observation, it has been found that the invasion of this weed into agriculture system has been a major threat to agricultural productivity. The finding on infestation recorded by other workers and our findings make it clear that the infestation of congress grass is going to pose a serious threat to agricultural crops, plantation crops and vegetables in our country in the year to come. The need of the hour is to stop the spread and check of congress grass for boosting the productivity level of different crops.

Collection of infected leaves

Infected leaves with different types of symptoms were collected in sterilized polythene bags and brought to the laboratory for the study of symptoms, isolation, identification and pathogenicity tests of the fungal pathogen/s involved.

Leafs spots and necrosis were the common symptoms observed. Although all the stages of leaves showed infection, the mature leaves were more heavily affected. During survey conducted to search virulent fungal pathogens, congress grass population was found to be heavily infected by leaf spot diseases. The leaf spots disease of congress grass were reported from several places such as Kurukshetra, Shahbad, Pipli, Pehowa, Jyotisar, Ambala, Karnal, Panipat, Chandigarh, Sirsa, Yamunanagar, indicated the wide spread nature of the diseases in various districts of Haryana.

Isolation of fungal pathogens

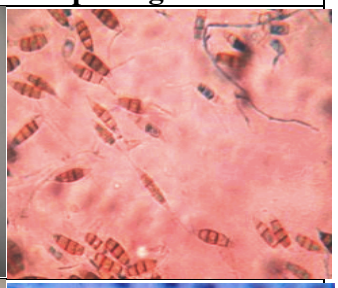
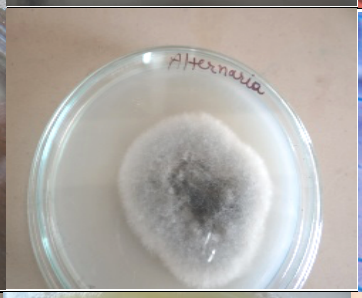
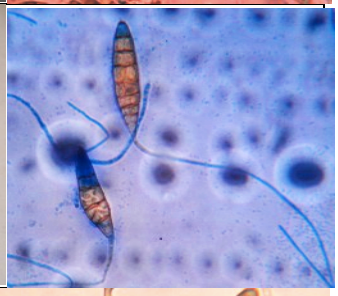
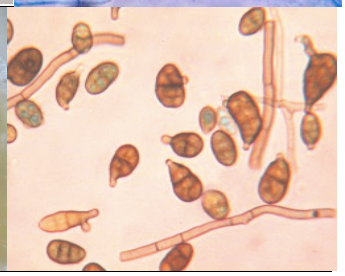
A total of thirty one fungal pathogens namely *Alternaria* sp. (nine different species), *Cladosporium* sp. (three different species), *Curvularia* sp. (two different species), *Colletotrichum* sp. (two different species), *Drechslera* sp., *Fusarium* sp. (four different species), *Chaetomium* sp. (two different species), *Acremonium* sp., *Trichoconiellia padwickii*, *Pestalotia* sp., *Epicoccum* sp., *Nigrospora* sp., *Scedosporium* sp. and *Torula* sp. were isolated from infected tissues on PDA and PDAY supplemented with or without streptomycin sulphate (10mg/l) (Table 2). On the basis of sporulating structures produced on the live parthenium leaves, one pathogens namely *Cercospora* sp., was identified**.** *Scedosporium*, *Torula* sp. and *Trichoconiellia* sp.has been reported first time on this weed during the survey (Plate 1). 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

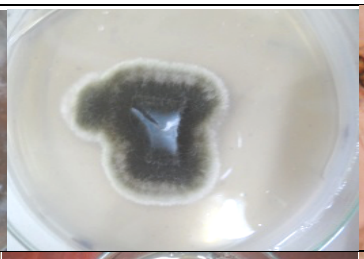
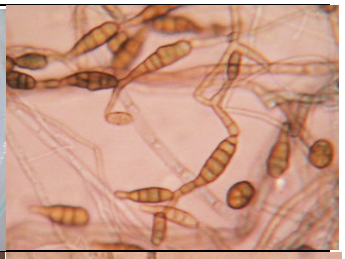
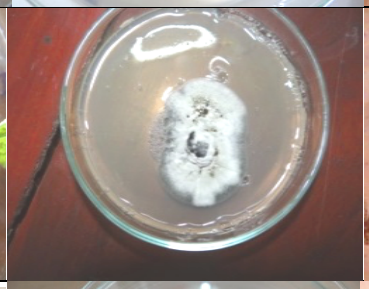
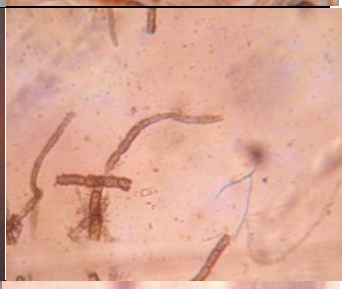
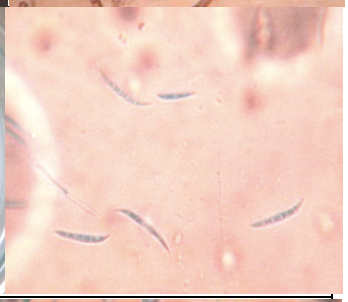
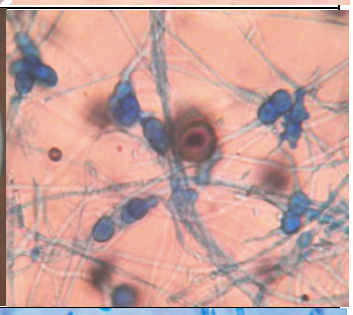
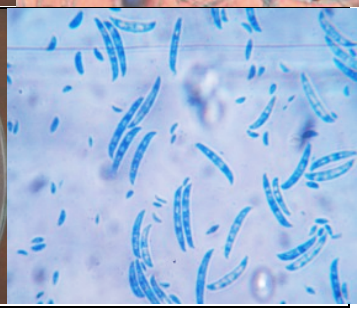
Selection of virulent pathogens

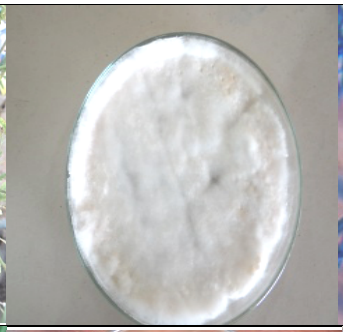
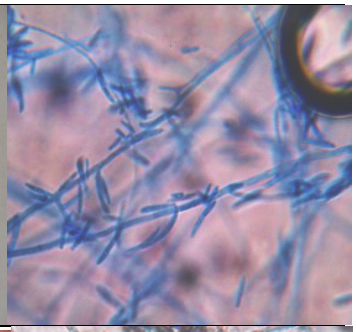
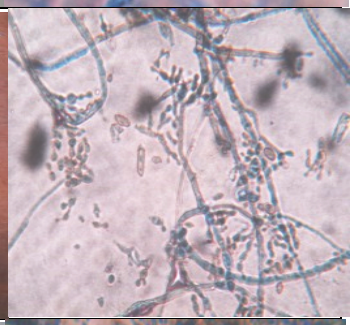
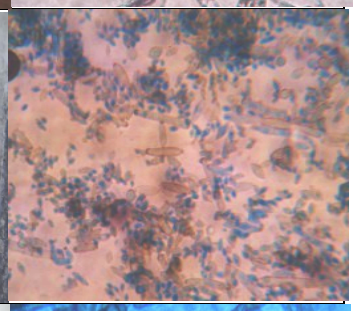
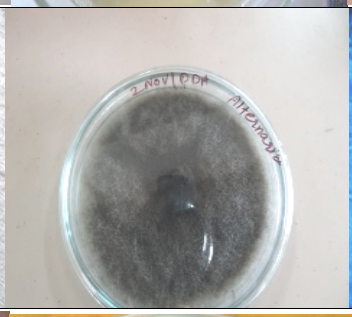
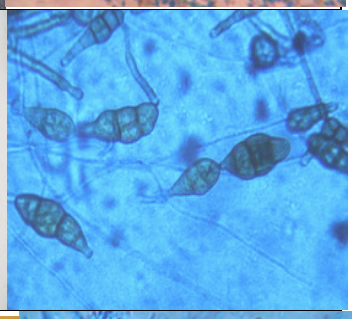
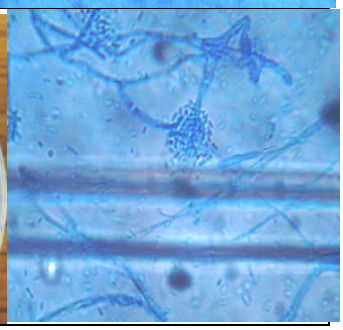
During surveys, total of thirty-one fungal pathogens were recorded on congress grass and their frequency in different season i.e. summer, rainy and winter. Six fungal pathogens namely *Alternaria* sp. (P27), *Alternaria* sp. (P28), *Alternaria* sp. (P29), *Alternaria* sp. (P30), *Alternaria* sp. (P31) and *Alternaria* sp. (P2) were selected on the basis of disease incidence, frequency and pathogenicity to see their biocontrol potential against congress grass, which were later on identified as *Alternaria macrospora* MKP1,

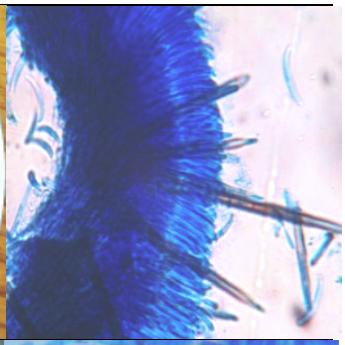
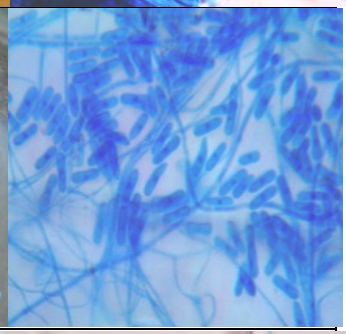
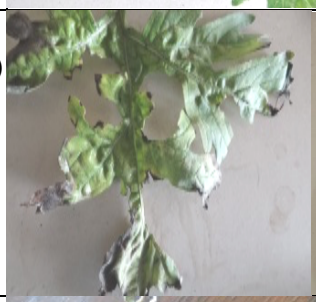
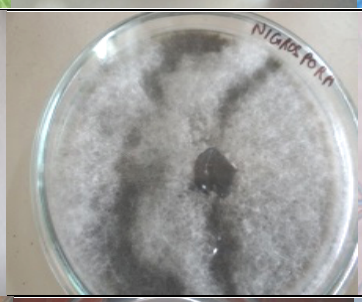
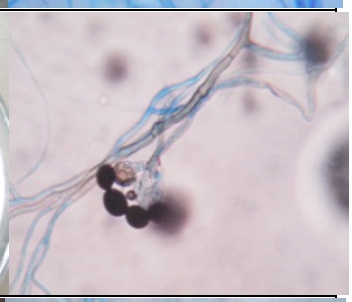
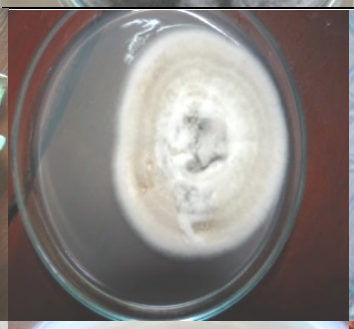
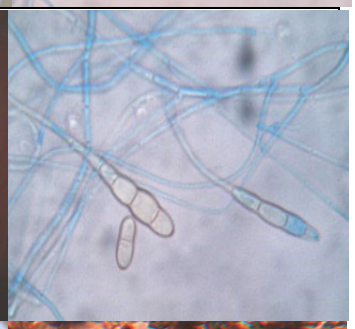
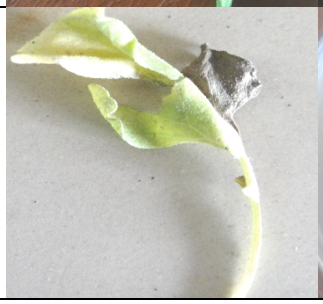
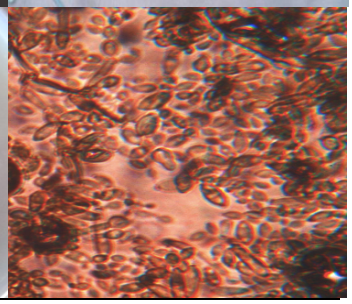
Alternaria macrospora MKP2, *Alternaria macrospora* MKP3, *Alternaria macrospora* MKP4, *Alternaria* sp. PMK1 and *Alternaria* sp. PMK2 (Table 3). Identification of the pathogens were confirmed from Commonwealth Agricultural Beaux International (CABI), (IMI) and sequenced from Macrogen Inc., Advancing through Genomics, Korea. Out of these selected pathogens the maximum disease incidence and pathogenicity were reported in P31 followed by P2 > P27> P28> P29> P30 (Table 3). During surveys, it was also observed that the disease symptoms produced by fungal pathogens were mainly confined to the older leaves near to the soil. Maximum fungal pathogens were found to be present on the lower portion (i.e. leaves with broader lamina and old ones) of the congress grass plant nearer to the soil. The availability of high humid conditions in the lower portions must be probable reason for this. Looking into the severity of the disease and damage caused to the congress grass weed in Northern India, the selected fungal pathogens seem to offer great potential and were selected for their evaluation as biocontrol agent for congress grass.

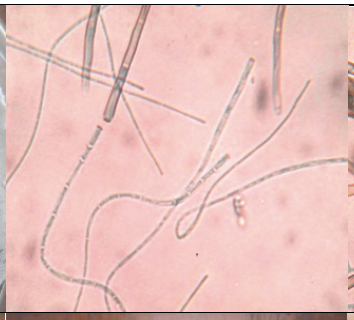
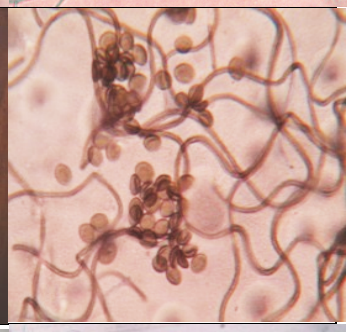
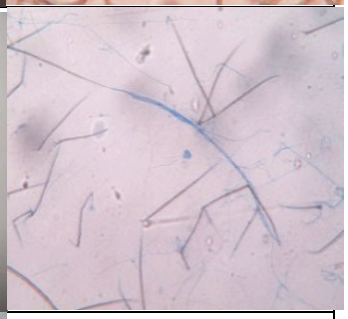
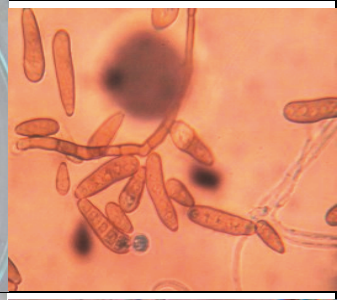
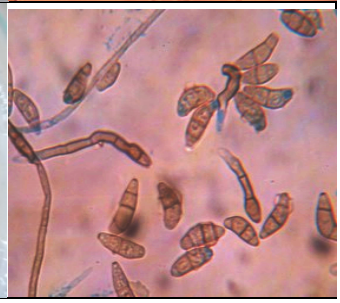
Plate 1: Symptoms, colony characteristics and morphological characteristics of fungal pathogens isolated from parthenium weed.

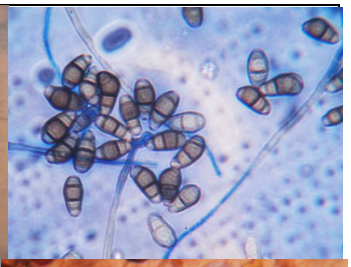
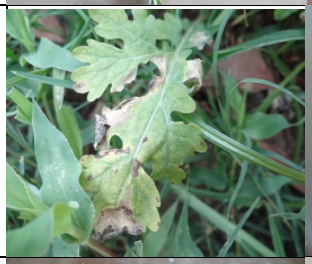
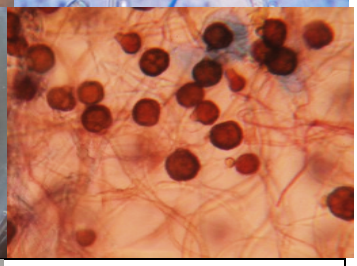
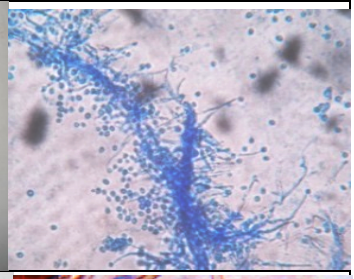
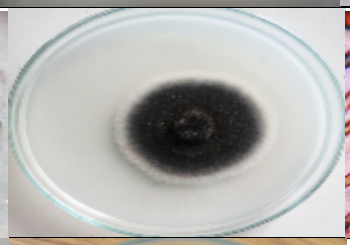
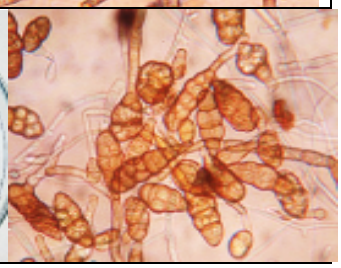
Isolates	Symptoms	Colony characteristics	Morphological view
P1 (*Pestalotia* sp.)			
P2 (*Alternaria* sp.)			
P3 (*Alternaria* sp.)			

P4 (*Alternaria* sp.)			
P5 (*Torula* sp.)			
P6 (*Fusarium* sp.)			
P7 (*Fusarium* sp.)			
P8 (*Fusarium* sp.)			

P9 (*Fusarium* sp.)			
P10 (*Cladosporium* sp.)			
P11 (*Cladosporium* sp.)			
P12 (*Alternaria* sp.)			
P13 (*Acremonium* sp.)			

P14 (*Colletotichu* sp.)			
P15 (*Colletotichum* sp.)			
P16 (*Nigrospora* sp.)			
P17 (*Trichoconeilla* sp.)			
P18 (*Cladosporium* sp.)			

P19 (*Cercospora* sp.)			
P20 (*Chaetomium* sp.)			
P21 (*Chaetomium* sp.)			
P22 (*Dreschelera* sp.)			
P23 (*Curvularia* sp.)			

P24 (*Curvularia* sp.)			
P25 (*Epicoccum* sp.)			
P26 (*Scedosporium* sp.)			
P27 (*Alternaria*)			
P28 (*Alternaria* sp.)			
P29 (*Alternaria* sp.)			

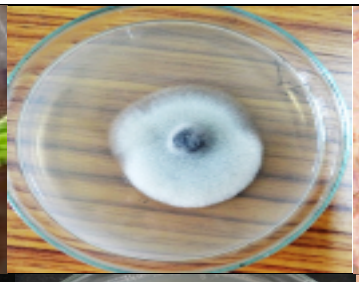
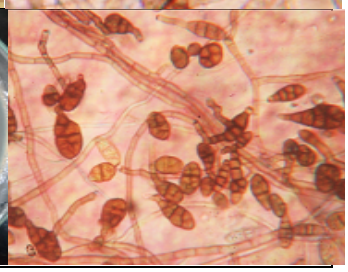
P30 (*Alternaria* sp.)			
P31 (*Alternaria* sp.)			

Table 3 Frequency, disease incidence and pathogenicity of fungal pathogens on congress grass in nature.

Sr No.	Fungal pathogen	% frequency of occurrence	Disease incidence (%)	Pathogenicity
1.	*Alternaria* sp. (P27)	29.7 %	74%	+++, severe symptoms
2	*Alternaria* sp. (P28)	24.5 %	73 %	+++, severe symptoms
3	*Alternaria* sp. (P29)	24 %	69 %	+++, severe symptoms
4	*Alternaria* sp. (P30)	25.5 %	67 %	+++, severe symptoms
5	*Alternaria* sp. (P31)	28.5%	80%	+++, severe symptoms
6	*Alternaria* sp. (P2)	26.5 %	76%	+++, severe symptoms

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.

Isolation frequency of pathogenic fungi from congress grass F

The perusal of data reveals that the frequency of *Alternaria macrospora* MKP1 *Alternaria macrospora* MKP2, *Alternaria macrospora* MKP3, *Alternaria macrospora* MKP4 was found to be high in winter season followed by rainy and summer season whereas *Alternaria* sp. PMK1 and *Alternaria* sp. PMK2 have highest frequency of occurrence in the rainy season followed by moderate in winter and lowest in summer (Table 4).

Table 4 Frequency of selected fungal pathogens on congress grass in different seasons of Northern India.

Sr No.	Fungal pathogen	Summer (March to June)	Rainy (July to October)	Winter (November to February)
1.	*Alternaria* sp. (P27)	LF	MF	HF
2	*Alternaria* sp. (P28)	LF	MF	HF
3	*Alternaria* sp. (P29)	LF	MF	HF
4	*Alternaria* sp. (P30)	LF	MF	HF
5	*Alternaria* sp. (P31)	LF	HF	MF
6	*Alternaria* sp. (P2)	LF	HF	MF

HF= 70-100%; MF= 30-70%; LF= 1-30%

Insects associated with parthenium weed

During the survey between 2012-2104, a leaf feeding beetle in rainy season a caterpillar in winter were found to be feeding on young as well as mature plants of parthenium. The beetles were identified as *Zygogramma bicolorata* (Figure 4.21) of coleoptera: Chrysomelida. The notable observation made about the *Zygogramma* beetle in nature were: in pastures, this beetle was found to be more active in rainy and spring season on mature and young parthenium plants, in response to feeding the larval stage of this beetle was more active than adults and the senescent leaves appear to be less preferred by larvae and adults, and fecundity was reduced drastically when reared only on mature leaves.

The female of *Zygogramma* beetle lays its yellow eggs on the lower surface of leaves either singly or in groups of 5-6. The eggs hatch in 3-4 days and the newly hatched larvae (Figure 3) migrate to the terminal and axillary buds. The older larvae defoliate the plant and were fully fed in 9-18 days and form pupae. The adult emerge from the pupae in 8-11 days. Singh (1977) reported that the females were capable of laying 317-3105 eggs in 79-167 days. This beetle could be used as a suitable biocontrol agent either alone or in integrated weed management against this weed.

CONCLUSION

Congress grass (*Parthenium hysterophorus* L.), is known for its notorious role as environmental, medical and agricultural hazards around the globe. It is a noxious weed due to competition for yields in various agricultural and vegetables crops such as mustard, wheat, sugarcane, sorghum, sunflower, gram, pea, potato and onion crops in northern India. Although various pre- and post-emergence chemical herbicides are available to control this weed but keeping in view the pollution hazards created by chemicals, the need of the hour is to intensify research on to control this weed either through biological agents or with an integrated approach using chemical plus biological agents. From the present study, it may be concluded that several potential pathogenic fungi and insect *Zygogramma* found in association with congress grass. Out of various fungal isolates, *Alternaria macrospora* MKP1, *Alternaria macrospora* MKP2 *Alternaria macrospora* MKP3 *Alternaria macrospora* MKP4 *Alternaria* sp. PMK1 and *Alternaria* sp. PMK2 were found to be highly aggressive towards congress grass and have a potential to control this terrestrial weed in association with the leaf feeding insect *Zygogramma bicolorata* as a part of intergerated pest management. Intensive work is still needed on the impact of the field environment and application technology on the efficacy of these pathogens as a mycoherbicide.

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Authors' contributions: *Manpreet Kaur (Research Scholar) performed the research experiment, wrote the manuscript and is the corresponding author of manuscript. Neeraj Kumar Aggarwal (Assistant Professor) and Vijay Kumar (Assistant Professor) have helped in designe the research work, data collection, modification and final correction in the manuscript.*

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