International Research Journal of Plant and Crop Sciences (IRJPCS) ISSN 1711-3490, Vol. 6(3), pp. 196-200, December, 2021. Available online at www.advancedscholarsjournals.org © Advanced Scholars Journals

Full length Research paper

Parthenium Phyllody in Eastern Uttar Pradesh India: Epidemiology and Host Range of Phytoplasma within Important Crops Cultivated in Eastern Uttar Pradesh, India

Santosh Kumar Singh, Singh Raghuvanshi AK and Singh HV*

Department of Botany, T.D. College, Jaunpur.
*Department of Botany, S.G.R.P.G. College, Dobhi, Jaunpur

Accepted 22nd April, 2021

Parthenium hysterophorus **L. (family Asteraceae) is one of the 10 worst weeds in the world. This weed became the major invasive weed in both arable and grazing lands due to competitiveness and adaptability to different climates and soils. The natural occurrence of phyllody was noticed ~ 10-15% of *Parthenium hysterophorus* growing wildly along the road side in Jaunpur, Azamgarh, Gorakhpur and Varanasi districts of Uttar Pradesh, India during summer 2010. In order to test whether *Parthenium* plants harbor phytoplasma, which may also infect important agricultural crop weeds and cultivated plants in Eastern U.P. India showing phyllody symptoms were collected and assessed by polymerase chain reaction (PCR).**

Keywords : Parthemium Phyllody Phytotplasma, Nested PCR, 16Sr RNA

INTRODUCTION

Parthenium hysterophorus L is an annual herb of the Asteraceae family originating from Central America. It was introduced to tropical regions worldwide in the 1950s. This is 10 worst weeds in the world. It is herbaceous plant. In India, *Parthenium* is locally called “Congress grass”. It is believed that it entered India accidentally in the mid-1956 and is now considered as one of the most feared noxious weed species (Rao, 1956). Other than competition and allelopathic effect on different crops (Navie et al. 1996). Parthenium poses health hazard to humans (Kologi et al. 1997) and animals (Chippendable and Panneta 1994) like *Parthenium* is also known to cause asthma, bronchitis, dermatitis and hay-fever in man livestock. *Parthenium* phyllody is an important disease of *Parthenium hysterophorus* (Mathur, S.K. and V. Muniyappa). The weed became the major invasive weed in both arable and grazing lands due to competitiveness and adaptability to different climates and soils. In India, a yeild reduction of 40% in Agricultural crops (Khosla and Sobti, 1979) and 90% reduction in forage production in grass lands (Nath 1988) were reported. The natural occurrence of phyllody was noticed ~ 10-15% of *Parthenium hysterophorus* growing wildly along the road side in Jaunpur, Azamgarh, Gorakhpur and Varanasi districts of U.P., India during summer 2010.

The diseased plants are characterized by excessive branching, reduced plant height and leaf size as well as modification of the inflorescences into leaf-like structures that lead to sterility. Chemical control, though effective, is temporary and needs repeated application. Hence, the use of biocontrol agents including insects, pathogens and strong interfering smoother crops and plants are recommended as element of integrated *Parthenium* weed management (PAG 2000). More than 700 plant diseases are associated with phytoplasmas. Phytoplamas are transmitted by insect vector of order Hemiptera, mainly by leaf hopper species (Family Cicadellidae). Furthermore, within three other families of fulgorids (Cixidae Delphaciae, Derbidae, Faltidae) are confirmed as phytoplasma vectors as well as psyllid species. Survey for natural enemies were carried out in Mexico by the Common wealth Institute of

*Corresponding author: E-mail :singhsantosh589@gmail.com

Biological Control (now CABI Bioscience, Ascot, UK). Eight insect species and two rust fungi were introduced and released in Australian after preliminary screening in Mexico and final evaluation in quarantine in Australia (Evans 1997). Micro-oganisms associated with Parthenium were also studied in India and such efforts are still in porgress towards the development of indigenous pathogens as bioherbicides (Kumar 1998).

Native natural enemies may be more effective than introduced because of more adaptability and no necessity of quarantine measures. Since no attempt has been so far in Eastern U.P., this approach can be explored to manage *Parthenium*. Hence, the search for pathogens causing disease to *Parthenium* at various levels is an essential step for future implementation of biological control in an integrated *Parthenium* management system in Eastern U.P., India. The objectives of this study aims to determine the host range of the pathogen with agricultural crops cultivated plants via insect vectors found in Eastern U.P., India and to determine the incidence and distribution of phyllody disease in different infested areas and to detect phyllody disease causing agent through PCR and electron microscope.

MATERIALS AND METHOD

Field surveys were conducted in major *Parthenium* infested areas of Eastern Uttar Pradesh like that of Gorakhpur, Azamgarh, Jaunpur and Varanasi. The incidence of *Parthenium* phyllody diseases was assessed in cultivated lands, vacant lands in grasslands. Incidence was assessed as percent of *Parthenium* plants with a disease symptom over the total plants in a 4m×4m plots (16m²). Five counts were taken per field and 3-5 fields were assessed at random at interval of 2-3 km per location. Data on disease symptoms, habitat, rainfall, temperature and soil data were collected. Diseased plant samples were also collected, tagged and pressed for later examinations in the laboratory. *Parthenium* and cultivated plants showing phyllody symptoms were collected from locations heavily affected by the weed. Extraction of DNA from dried *Parthenium* plant and suspected insect vector was carried out using the phytoplasma enrichment procedure developed by Ahrens and Seemueller (1992). After DNA extraction phytoplasma specific DNA fragments were amplified by polymerase chain reaction, PCR, (*Parthenium*, peanut and sesame) or nested PCR (grass pea) respectively. The PCR products were further characterized by Restriction Fragment Length Polymorphism (RFLP) analysis. Amplified fragments were sequenced allowing species identification of the pathogens.

In order to characterize the potential risk of vector insects, planthoppers were captured from phyllody

In electron microscopy stem section of diseased plants were cut and immersed in 6% phosphate buffered (0.1 M; diseased *Parthenium* plants, analysed for phytoplasma infection, and classified by morphological and molecular methods. Furthermore, transmission studies with leafhoppers of the species *Orosius cellulosus* Lindberg of the family *Cicadellidae* were carried out.

The plansts were air dried and then stored at 4ºC. Suspended insect vector, Tyloryligus apicalis, was also collected from different Parthenium infested areas. The insects were killed by ethanol and air-dired or preserved in 70% ethanol for later examination. Accordingly, 0.5-1 g of leaf containing mid ribs and floral parts consisting phloem were grinned using mortar and pestle by placing them on ice. For the plant bug, 5-12 individuals were taken and then chopped in 2-ml eppendorf tube. After extraction, the nucleic acid pellet was re-suspended in 100 □l of water, and then subjected to electrophoresis in 1% agarose gel using 0.5x TBE as running buffer by adding ethidium bromide (□□l/50 ml) and then visualised by UV transilluminator for the presence of DNA. DNA was amplified by Polymerase Chain Reaction (PCR) using the phytoplasma primer pair P1 and P7 for all samples. The primers were derived from highly conserved ribosomal sequences and prime at the 5' end of the 16S rRNA gene and in the 5' region of the 23S RNA gene, respectively. They were reported to be universal for phytoplasma detection and amplify a DNA fragment of approximately 1800 bp in length that includes the complete 16S rRNA gene of about 1535 bp in size, the 16S/23S rDNA spacer region of about 250 bp in length, and approximately 50 bp from the 5' end of the 23S rRNA gene (Schneider et al., 1995).

The reaction were performed in 50 □l volume of reaction mixture containing 1 □l of the nucleic acid sample, 5 □l of Taq polymerage buffer with $MgCl_2$ (1x) (Stock solution: 10x: 100mM Tris-HCl, 50 mM KCl, 15 mM $MgCl_2$, pH 8.3) 4 □l of dNTP – mix (1.25 mM each for dATP, dCTP, dGTP and dTTP), 1 □l of each primer pair (Stock solution : 50 □M at the concentration of 50 pmol), 1u/□l Taq- DNA polymerase (stock solution: 5U/ □l), in a total volume of 50 □l water.

35 PCR cycles were conducted in automated Robocycler Temperature Cycler (Robocycler gradient 96). The following parameters were used: preheating at 95ºC for 5 min for the first cycle, denaturation at 95ºC for 30 seconds, annealing at 56ºC for 1 min and primer extension/polymerisation at 72ºC for 1 min and 30 seconds and the final polymerisation at 72ºC for 7 min. Control tubes without DNA template were used as negative control while the faba bean phyllody (FBP) and sunnhemp (Crotalaria juncea) phyllody (SUNHP) DNA templates were used as positive control. Aliquots of post reaction mixture (10 □l from each sample) were resolved in 1% agarose gel stained with ethidium bromide, and then visualised with UV illumination and the length of obtained DNA fragments was estimated

pH 6.8) glutaraldehyde for overnight at 4ºC on a shaker. The tissues were washed three times in phosphate buffer

(0.1 M; pH 6.8) each for 30 minutes. Tissues were then post fixed using 1% osmium tetroxide for 3 hrs and then washed. Dehydration was carried out by immersing the tissues in ethanol series. Infiltration was done by immersing in propylene oxide following the methods used in Sugarcane Research Station, Kuraghat, Gorakhpur. After infiltration, the tissues were embedded in complete Spurr's low viscosity medium (soft) at 4ºC overnight, and then transferred into gelatine capsule, and placed in an oven at 70ºC for 12-24h. Ultrathin sections were then cut with a glass knife in an ultramicrotome sections, stained with aqueous 4% uranyl acetate, counter stained with lead citrate and examined in transmission electron microscope.

RESULTS

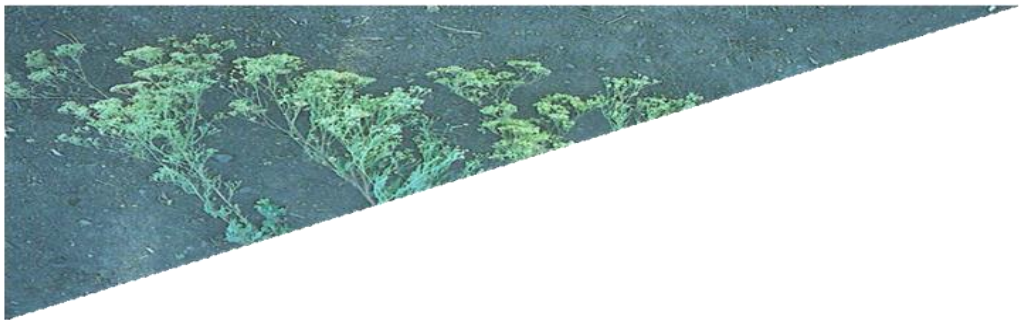
DNA fragments specific for phytoplasmas could be detected in *Parthenium hysterophorus* as well as in peanut (*Arachis hypogaea*), sesame (*Sesamum indicum*), and grass pea (*Lathyrus sativum*). After *Alul*-digestion of PCR-amplicons of Parthenium, seasame, peanut, and a *Vinca rosea* infected by faba bean phyllody (FBP positive control) showed identical restriction profiles, indicating a close relationship to FBP of the Peanut withches broom group.

Comparison of rDNA sequences of P1/P7 amplicons revealed that phytoplasmas detected in Parthenium plants were also present in sesame and peanut. Sequences identities of 1488 bp of the 16S rDNA sequence were above 99%, covering strains infecting sesame and peanut in other countries. Eastern Indian Parthenium, sesame, cynodon and peanut phytoplasma exhibited sequence similarities of 98% to phytoplasmas within the 16SrII species group (Peanut witches-broom group) including phytoplasmas originating from Eastern Indian, papaya, faba bean phyllody (FBP) (Scheinder B, et al., 1995), and the reference species *Candidatus* Phytoplasma aurantifolia, causing witches-broom disease of lime.

The planthopers collected from phyllody diseased Parthenium plants could be assigned to the genus *Hilda* of the family *Tettigometridae*. There were positive detections of phytoplasmas in almost every planthopper sample investigated. Because of the high similarity of the sequences from the 16S rDNA-gene, these phytoplasmas also belong to the phylogenetic clade 16Sr-II. Hence, members of *Tettigometridae* were described as potential vectors of phytoplasmas for the first time.

In trasmission studies a successful acquisition of phytoplasmas by *Orasius cellulosus* was shown by means of positive detection of the pathogen in several leaf hoppers. Furthermore, detection of phytoplasmas in a single bait plant suggests that this species is suitable for tranmitting phytoplasmas. However, as the Parthenium plants used as baits developed no characteristic symptoms a successful transmission of phytoplasmas by Hilda sp. and *O. cellulosus* still has to be proven.

Parthenium plants with phyllody symptom were fixed, embedded and ultrathin stem sections of 200-300 nm were observed under transmission electron microscope. Phytoplasma-like bodies of different sizes and shapes were detected. Analysis of the electron microscope photographs in Sugarcane Research Station, Kuraghat, Gorakhpur also showed the pleomorphic, phytoplasma-like agent.



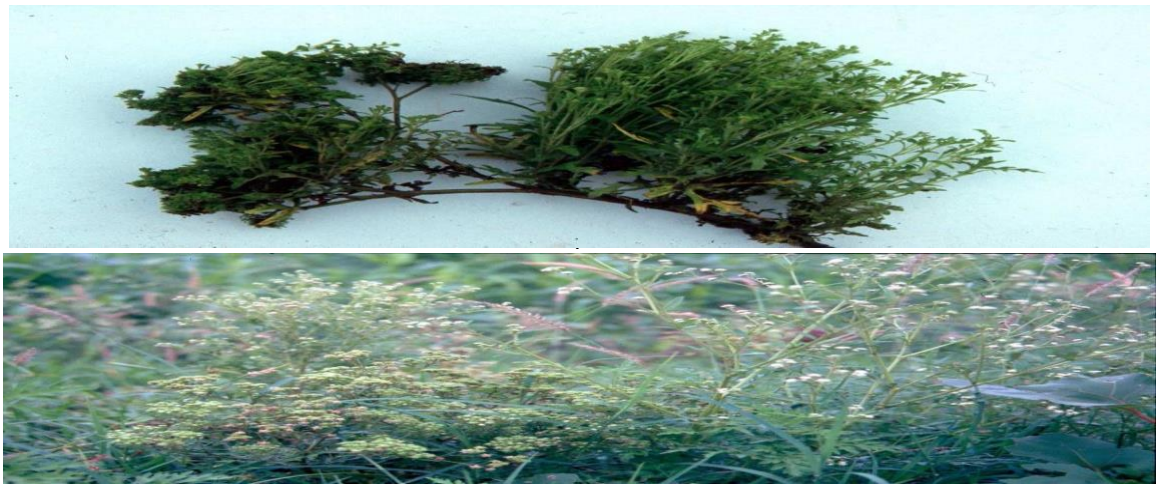


Figure. On Parthenium Phyllody From Eastern Uttar Pradesh (Gorakhpur, Jaunpur, Ajamgarh)
Lane Sample material Lane Sample material

1 DNA marker (1kb, Fermentas)

2 Phyllody diseased plant (Gorakhpur) 3 Phyllody diseased plant (Jaunpur)
4 Phyllody diseased plant (Ajamgarh) 5 Phyllody diseased plant (Varanasi)

Figure 2. PCR amplification of phytoplasma DNA from diseased parthenium and reference samples in faba bean (FBP) and sunnhemp (SUNHP) using the primers P1 and P7

Figure 3. Ultrathin stem sections of parthenium showing phytoplasmas of different sizes and shapes: a = x 20 000, b = x 25 000

DISCUSSION

Phytoplasma detected in Parthenium and crops of Eastern Uttar Pradesh, India, are closely related and potential vector insects are native in India. This suggests that Parthenium represents a pathogen reservoir for the phytoplasmas affecting agricultural crops in the Easter Uttar Pradesh. Since phytoplasma infections can lead to sterility of the infloresences, severe losses in yield of agricultural crops could be expected. Thus, control of Parthenium and putative vectors transmitting phyllody disease is important. In this study, the incidence of phyllody disease varied from 6-75% across different locations both during fallow and cropping season. Similary, Mathur and Muniyappa (1993) reported Bangalore, India. Seemueller et al. (1994) uses additional enzymes in PCR. The electron microscopy study by Phatak et al. (1975). The detection of phytoplasma in an insect also does not necessarily mean that the insect species can transmit the disease (Vega et al. 1993). Phyllody syndorme is reported in faba bean (Dereje and Tesfaye 1994). Mathur and Muniyappa (1993) carried out host range studies in India with 16 plant species belonging to Asteraceae, Fabaceae, Malvaceae, Pedaliaceae and Solananceae by inoculating the plants using leafhopper, *Orocius albicinctus*, as a vector.

ACKNOWLEDGEMENT

All the experimental work was done by Santosh Kumar Singh. Thanks are due to Dr. G.P. Rao, Scientist, Sugarcane Research Station, Kuraghat, Gorakhpur for facilities.

REFERENCES:

Ahrens U, E Seemuller (1992). Detection of DNA of plant pathogenic mycoplasma like organisms by a polymerase chain reaction that amplifies a sequence of the 16S rRNA gene. Phytopathology 82: 828-832.

Chippendale JF, FD Panneta (1994). The cost of parthenium weed to the Queensland cattle industry. Plant Protection Quarterly 9:73-76.

Dereje Gorfu, Tesfaye Beshir (1994). Faba bean diseases in Ethiopia. In : Tilaye A., G Bejiga, MC Saxena and MB Solh MB (eds) Cool-season food legumes of Ethiopia. Proceeding of the first national cool-season food legumes review conference, 16-20 December 1993, Addis Ababa, Ethiopia.

Evans HC (1997). *Parthenium hysterophorus*: A review of its weed status and the possibilities for biological control. Biocontrol News and Information 18: 89- 98.

Khosla SN, SW Sobti. (1979). Parthenium – a national hazard, its control and utitlity- review. Pesticides 13 : 121-127.

Kologi PD, SD Kologi, NP Kologi (1997). Dermatological hazards of parthenium in human beings. In : Mahadevappa M and VC Patil (eds) pp.18-19. Proceedings of the first international conference on parthenium managmenet, 6-8 October 1997, University of Agricultural Sciences, Dahrwad, India.

Kumar PS (1998). Biological suppression of parthenium with pathogens. In: Singh PS and SS Hussani (eds.). Biological Suppression of Plant Disease, Phytoparasitic Nematodes and Weeds. Bangalore, Karnataka, India, pp.192- 210.

Mathur SK, V Muniyappa (1993). Parthenium phyllody disease in India. In: Raychaudhuri SP and DS Teakle (eds.). Management of plant diseases caused by fastidious prokaryotes: Proceedings of the fourth Regional Workshop on Plant Mycoplasma, University of Queensland, Australia New Delhi, pp.21-34. May 2000).

Nath R (1988). *Parthenium hysterophorus* L.A general account. Agricultural Review 9:171-179.

Navie SC, RE McFadyen, FD Panetta, SW Adkins. 1996. The biology of Australian Weeds 27: *Parthenium hysterophorus* L. Plant Protection Quarterly 11: 76- 87.

PAG (Parthenium Action Group). 2000. Parthenium weed. Information document. (*http:/www.chris.tag.csiro.au/parthenium/information.html* :accessed 12

Phatak HC, T Lundsgaard, R Padma, R. Singh , VS Verma (1975). Mycoplasma- like bodies associated with phyllody of *Parthenium hysterophorus* L. phytopathology Zeitschriften 83: 10-13.

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

Schneider B, MT Cousin, S Klingkong, E Seemuller (1995). Taxonomic relatedness and phylogenetic positions of phytoplasmas associated with diseases of faba bean, sunnhemp, sesame, soybean, and eggplant. Journal of Plant Disease and Protection 102: 225-232.

Seemuller E, B Schneider, R Maurer, U Ahrens, X Daire, H Kison, KH Lorenz, G Firrao, L Avinent, BB Sears, E Stackebrandt (1994). Phylogenetic classification of phytopathogenic Mollicules by sequence analysis of 16S ribosomal DNA. International Journal of Systematic Bacteriology 44: 440- 446.

Vega FE, RE Davis, P Barbosa, EL Dally, AH Purcel, IM Lee (1993). Detection of plant pathogens in a non-vector species by the polymerase chain reaction. Phytopathology 83: 621-624.