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PARTHENIUM PHYLLODY IN EASTERN UTTAR PRADESH INDIA: EPIDEMIOLOGY AND HOST RANGE OF PHYTOPLASMA WITHIN IMPORTANT CROPS CULTIVATED IN EASTERN UTTAR PRADESH, INDIA

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ABSTRACT

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 roadside in Jaunpur, Azamgarh, Gorakhpur, and Varanasi districts of Uttar Pradesh, India, during summer 2010. To test whether *Parthenium* plants harbor phytoplasma, which may also infect important agricultural crop weeds and cultivated plants in Eastern U.P. India, phyllody symptoms were collected and assessed by polymerase chain reaction (PCR).

Keywords: *Parthenium* Phyllody Phytoplasma, 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. These is the 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 the competition and allelopathic effect on different crops (Navie *et al.*, 1996). *Parthenium* poses a health hazard to humans (Kologi *et al.*, 1997) and animals (Chippendale and Panneta 1994). *Parthenium* is also known to cause asthma, bronchitis, dermatitis, and hay fever in man livestock. *Parthenium* phyllody is a significant 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 yield reduction of 40% in crops (Khosla and Sobti, 1979) and a 90% reduction in forage production in grasslands (Nath 1988) were reported. The natural occurrence of phyllody was noticed ~ 10-15% of *Parthenium hysterophorus* growing wildly along the roadside 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, is recommended as an integrated *Parthenium* weed

management (PAG 2000).

More than 700 plant diseases are associated with phytoplasmas. Phytoplasmas are transmitted by insect vector of order Hemiptera, mainly by leafhopper species (Family Cicadellidae). Furthermore, three other families of fulgorids (Cixidae Delphacidae, Derbidae, Faltidae) are confirmed as phytoplasma vectors as well as psyllid species. A survey for natural enemies was carried out in Mexico by the Commonwealth Institute of Biological Control (now CABI Bioscience, Ascot, UK). Eight insect species and two rust fungi were introduced and released in Australia after preliminary screening in Mexico and final evaluation in quarantine in Australia (Evans 1997). Microorganisms associated with *Parthenium* were also studied in India, and such efforts are still in progress 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 the 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 aim to determine the host range of the pathogen with 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 evaluated as a percent of *Parthenium* plants with a disease symptom over the whole plants in a 4m×4m plots (16m²). Five counts were taken per field, and 3-5 fields were assessed at random at an 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 diseased *Parthenium* plants, analyzed 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 plants were air-dried and then stored at 4°C. Suspended insect vector, *Tylorlygus apicalis*, was also collected from different *Parthenium*-infested areas. The insects were killed by ethanol and air-dried or preserved in 70% ethanol for later examination. Accordingly, 0.5-1 g of the leaf containing midribs and floral parts consisting of phloem were grinded 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 ml of water and then subjected to electrophoresis in 1% agarose gel using 0.5x TBE as running buffer by adding ethidium bromide (1/50 ml) and then visualized 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 ml volume of reaction mixture containing 1 ml of the nucleic acid sample, 5 ml of



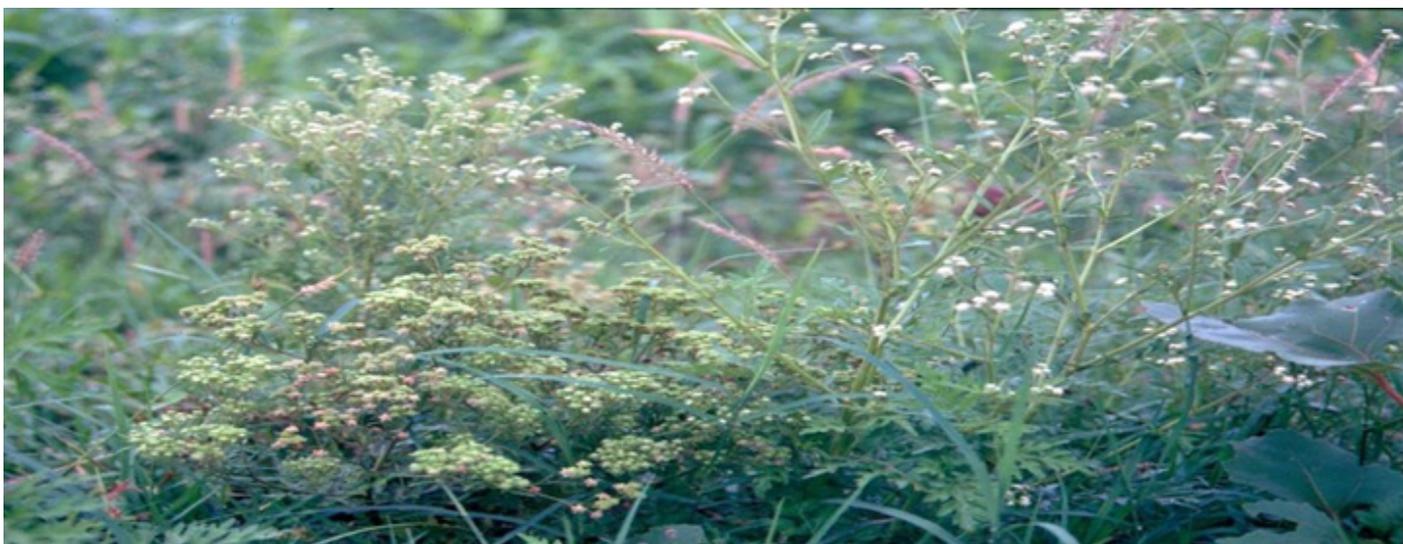
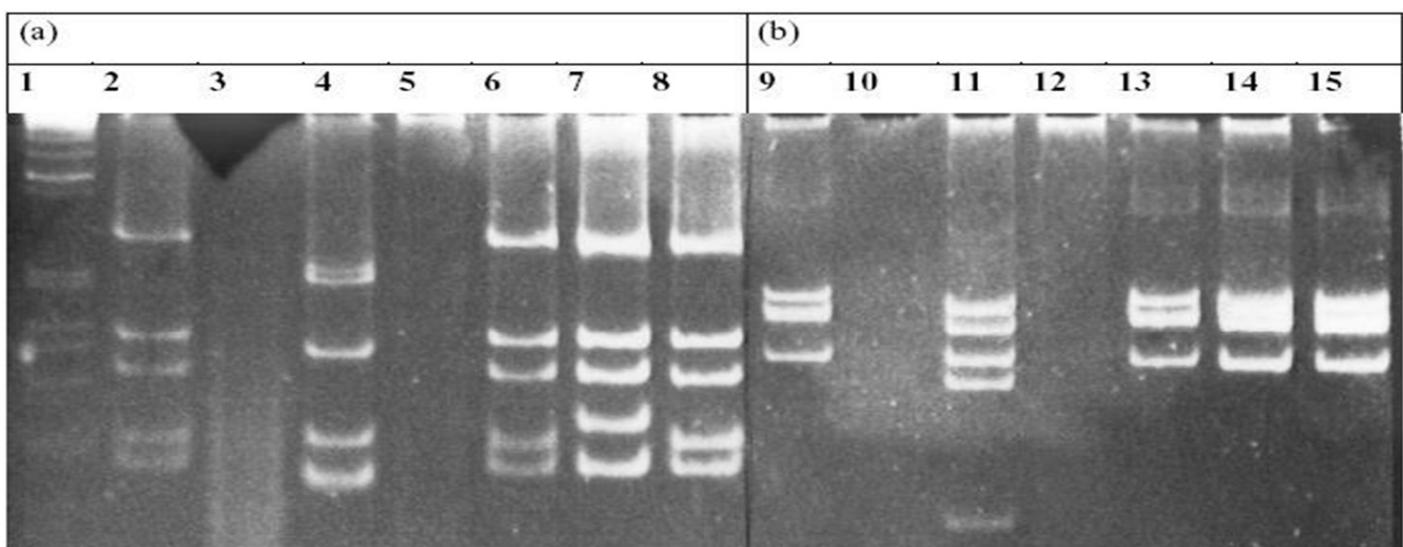


Figure. 1- Parthenium Phyllody From Eastern Uttar Pradesh (Gorakhpur, Jaunpur, Ajamgarh)



Lane	Sample material	Lane	Sample material
1	DNA marker (1kb, Fermentas)	3	Phyllody diseased plant (Jaunpur)
2	Phyllody diseased plant (Gorakhpur)	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

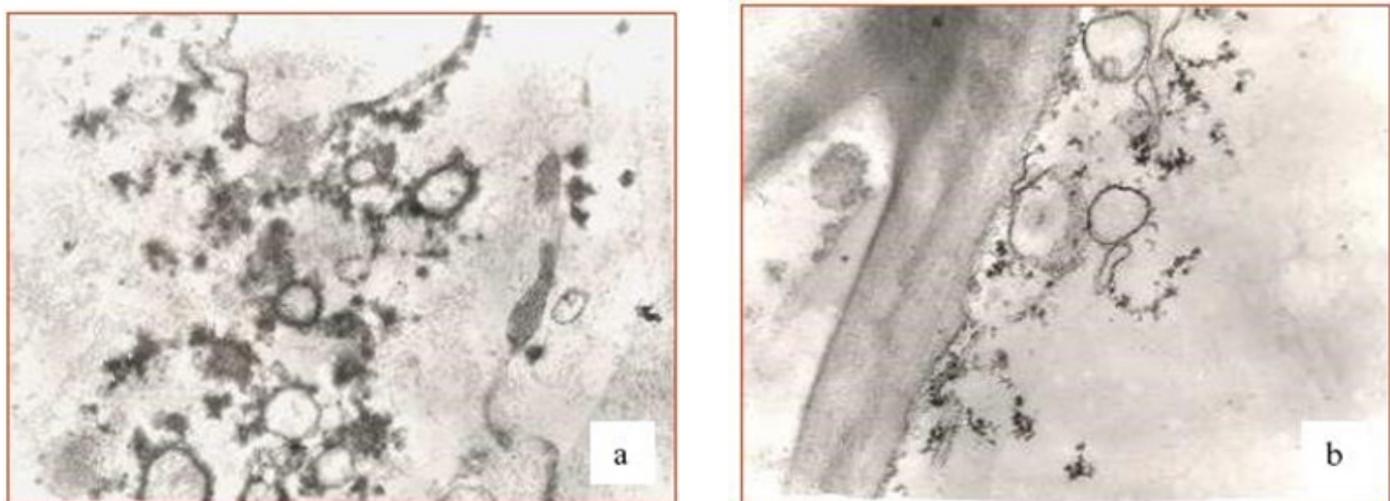


Figure 3. Ultrathin stem sections of parthenium showing phytoplasmas of different sizes and shapes: a = x 20 000, b = x 25 000

Taq polymerase buffer with MgCl₂ (1x) (Stock solution: 10x: 100mM Tris-HCl, 50 mM KCl, 15 mM MgCl₂, pH 8.3) 4ml of dNTP – mix (1.25 mM each for dATP, dCTP, dGTP and dTTP), 1 ml of each primer pair (Stock solution : 50 mM at the concentration of 50 pmol), 1u/ml TaqDNA polymerase (stock solution: 5U/ml), in a total volume of 50 ml water.

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

The electron microscopy stem section of diseased plants were cut and immersed in 6% phosphate-buffered (0.1 M; pH 6.8) glutaraldehyde for overnight at 4°C 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 the 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 4°C overnight and then transferred into a gelatine capsule and placed in an oven at 70°C for 12-24h. Ultrathin sections were then cut with a glass knife in an ultra-microtome section, stained with aqueous 4% uranyl acetate, counterstained with lead citrate, and examined in the 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 *AluI*-digestion of PCR-amplicons of *Parthenium*, sesame, 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 witches 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 planthoppers 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 transmission 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 transmitting 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.

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 Eastern Uttar Pradesh. Since phytoplasma infections can lead to sterility of the inflorescences, severe losses in yield of agricultural crops could be expected. Thus, control of *Parthenium* and putative vectors transmitting phyllody disease is essential. In this study, the incidence of phyllody disease varied from 6-75% across different locations both during the fallow and cropping season. Similarly, 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 syndrome 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 Solanaceae the plants using leafhopper, *Orocious albicinctus*, as a vector.

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