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Full Length Research Paper

Analysis of medicinal weeds associated with phytoplasma by morphological characters

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Phytoplasmas have been documented in more than 100 weeds plant species, including dicots and monocots. The wide host range of phytoplasmas is an important parameter for the epidemiology of its diseases. Overlapping plant hosts and vectors also give ample opportunities for them to interact and exchange genetic information. Some of them share host plants, though genetic exchange might also occur between these mollicutes. Phytoplasmas have been detected in most organs of infected plants, where they colonize the sieve tubes of the phloem. Infestations of floral tissue by them have been observed, but it is believed that seed transmission is generally not possible because the sieve tubes lack a direct connection to the seed. However, they can still occur in the seed. Suspected phytoplasma symptoms were observed in some weeds such as *Datura stramonium* L., *Achyranthes aspera* L., *Ranunculus scleratus* L. and *Ageratum conyzoides* L., found in Gorakhpur and its adjoining areas in Eastern U.P. India. The aim of this work is to predict phytoplasma infected plant and differentiate it from other causal organism like: bacteria, virus, fungi, etc. Phytoplasma are plant pathogenic mollicutes, originating from bacteria, which cause severe loss of diversity of many plants, but we selected some medicinal plants which are used in a direct way as a form of leaf, stem, flower, etc. These weeds are highly affected by phytoplasma, thereby making them lose their diversity; however, these weeds are present in moderate amount in this region. The major symptoms considered are witches' brooms, little and yellow leaf, phyllody disease, little leaf necrosis and witches' brooms, and abnormal proliferation of twinges, which at some point in time cause the death of complete plants. All weeds plants are confirmed on the basis of nested PCR and Phylogenetic analysis. These weeds are highly used medicinally in direct and indirect ways. These are severally affected by phytoplasma infection. Identification of the diagnosis of phytoplasma remains a challenge because of its rapid lethal course and lack of consistency to particular clinical signs and symptoms. Moreover, many difficulties occur because its concentration in host plants highly fluctuates over all seasons. These plants are firstly identified on the basis of peculiar symptoms of phytoplasma, after which they are confirmed by electron microscopy, and a particular group of phytoplasma is then identified by PCR, gene sequencing, and blast analysis.

Key words: Phytoplasma, dicots, monocots, phloem.

INTRODUCTION

Phytoplasma are specialized bacteria that are obligate parasites of plant phloem tissue and transmitted through insects (vectors). They were first discovered in 1967 and were named mycoplasma-like organisms or MLOs (Doi et al., 1967). They cannot be cultured *in vitro* in cell-free media. They are characterized by lack of a cell wall, a pleiomorphic or filamentous shape, normally with a diameter less than 1 micrometer, and having very small genomes. Plants infected by phytoplasmas exhibit variety of symptoms that suggest profound disturbances in the normal plant physiology. They are most prevalent in

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these regions of the Eastern U.P. *Achyranthes aspera* L., *Datura stramonium* L., *Ranunculus scleratus* L. and *Ageratum conyzoides* L., are important weeds growing in eastern U.P. *Datura* are cultural weeds, *Achyranthes* are medicinally important, *Ranunculus* are used as plants extracts for biological experiments and *Ageratum* are used in plants biodiversity, and are also used as medicinal weeds. These weeds are phytoplasma and they cause diseases in several weeds which result in serious threat as a source of alternative natural host for the spread of phytoplasma pathogen to other economically important plants, thereby creating a chance of causing severe losses. Detection of phytoplasma associated with diseases of weed crops is very important to check the possibility of further spread of their diseases to other commercial crops; hence this study was planned to identify the phytoplasma associated with many weeds in and around Gorakhpur because there is no authentic definite symptoms in the affected plant.

MATERIALS AND METHODS

Survey for phytoplasma diseases on weeds

From the different geographical regions of Gorakhpur and its adjoining areas, symptoms such as little leaf, whiting and yellowing, witches' brooms, stunting, phyllody, veriscence, etc., affecting plants' samples were observed. In other different regions like University campus Gorakhpur, fields and road side of Gorakhpur and its adjoining areas, symptoms such as little leaf, whiting and yellowing, witches' brooms, stunting, phyllody, veriscence, etc., showing suspected *A. aspera, D. stramonium, A. conyzoides* and *R. scleratus* infected samples were identified on the basis of morphological changes on the healthy plants (Figure 1).

Fluorescence microscopy, scanning electron microscopy and transmission electron microscopy

In this study, samples of branch and root tissues were taken for fluorescent DAPI staining. It was observed that branch, root, flowers and stem segments obtained from plants with and without symptoms for scanning electron microscopy (SEM) and transmission electron microscopy (TEM), showed pleiomorphic shape bodies when the plants were put in phloem sieve tubes, but the healthy plants did not show any bodies. Thus, this clearly confirms the occurrence of phytoplasma infection.

RESULTS AND DISCUSSION

On the basis of morphological characteristics showing different types of symptoms, all samples were associated with phytoplasmas infection because all symptoms showed favorable conditions like: optimal temperature, humidity and light. However, all weeds were affected by phytoplasma which are medicinally important.

A. aspera contains free oleanolic acid in the roots which amount to 54%. It was observed from the roots that ecdysterone and oleanolic acid were isolated. In the unripe seeds, saponins, oleanolic acid, amino acids and hentriacontane (a long chained carbohydrate) were found. In the shoots, an aliphatic dihydroxyketone 36, 37-dihydroxyhenpentacontan-4-on and triacontanol could be found. Two long chain compounds, isolated from the shoots, were characterized as 27-cyclohexylheptacosan-7-ol and 16-hydroxy.26-methylheptacosan-2-On by chemical and spectral investigations (Misra et al., 1993). The petrol extract of the shoots produced a yellow semi-solid mass. From this mass, a pink coloured essential oil with a pleasant odour and an aliphatic alcohol (17-pentatriacontanol) were found. The whole plant was extracted with methanol. After the removal of the solvent, the residue was extracted successively with different solvents and with butanol by column chromatography. Ecdysterone, a phytoecdysone, was yielded and characterized by its colour and special chemical reactions with the following contents (g/kg): 0.25 (seeds), 0.09 (roots), 0.04 (stem, leaves). The pronounced insect moulting hormonal activity of this extract from the roots was observed due to the presence of ecdysterone (Banerji et al., 1970). In an investigation for alkaloids, only one indication was found in stems. *A. aspera* was assessed only by color reactions and not with modern techniques. Therefore this result can be neglected because it is in contradiction to the general characteristics of the family Amaranthaceae to which *A. aspera* belongs (Gariballa, 1983). *A. aspera* associated with phytoplasma was also studied by Raj et al. (2009a).

D. stramonium is used as medicine for treating asthma and gastrointestinal problems; it is also used for treating aches, abscesses, arthritis, boils, headaches, hemorroids, rattlesnake bites, sprains, swellings and tumors (Sandoval, 1998). It acts as a sedative in large doses and as a stimulant and deliriant in high ones. *D. stramonium* is an anodyne, antibiotic, antispasmodic and narcotic. Relieving the pains of rheumatism and sciatica when applied as an ointment, and easing the spasms of Parkinson's disease are unproven accounts of the effects of Jimson weed. Most parts of the plant are used for medicinal reasons. Eating the seeds rapidly gets the plant to the nervous system, but also increases the risk of lethal overdose. The leaves can be dried and smoked to relax the bronchiole muscles of the throat, and leaves are used also to line beds of those with insomnia. In an infusion, fresh leaves take to hot water, or a poultice using any variation of the recommended parts. *D. stramonium* contains active compounds such as hyoscine, as well as atropine, hyoscyamine, apohyoscine, and meteloidine. Thus, it is poisonous and hallucinogenic as well as acting as a pain killer (Duke, 1992).

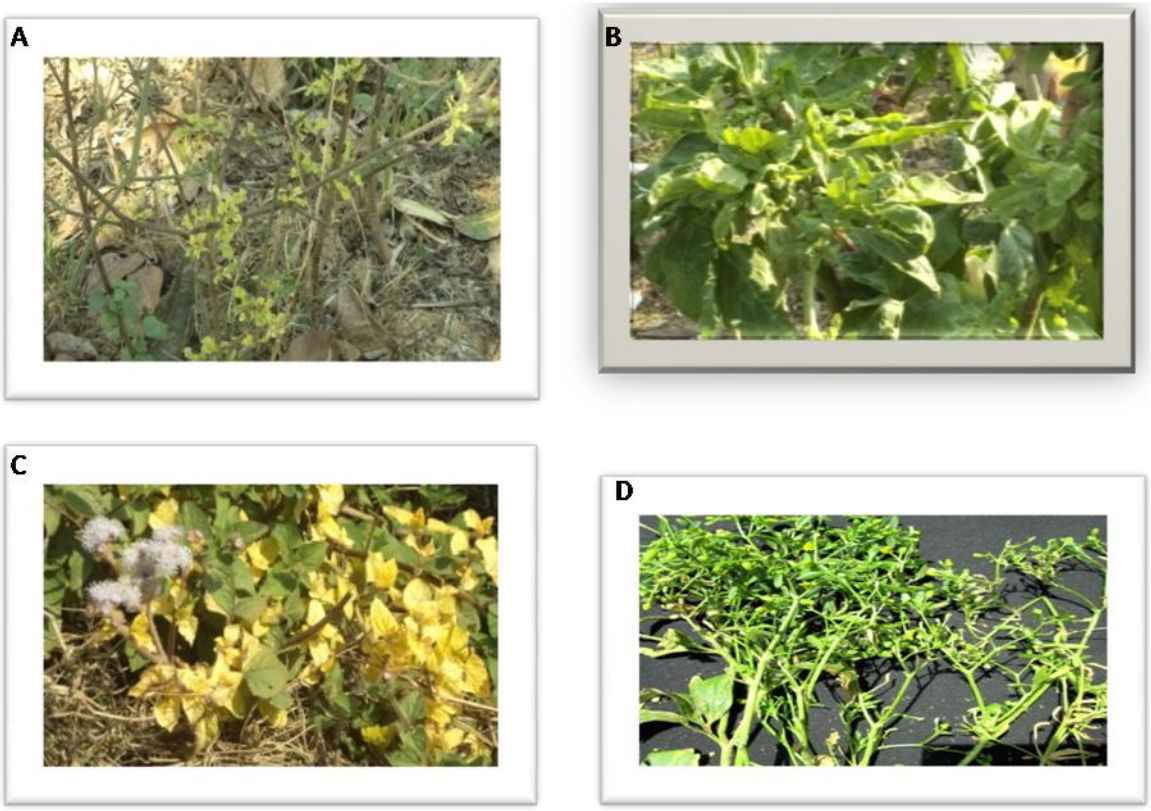

Figure 1. (A) Little leaf on *Achyranthes aspera* showing infected plants, (B) Witches' brooms symptoms on *Datura stramonium* showing infected plants, (C) Yellowing symptoms on *Ageratum conyzoides* showing infected plants, and (D) Phyllody symptoms on *Ranunculus scleratus.*

D. stramonium has been found to rapidly clear 2,4,6-trinitrotoluene (TNT) from munition waste sites, and transform it via nitroreduction. It might very well have similar properties as remediators of explosives (Lucero et al., 1999). However, phytoplasma associated with *D. stramonium* was reported by Raj et al. (2009b) and Singh et al. (2012).

A. conyzoides as a medicinal plant has limited uses due to its toxicity. It is used as an insecticide and nematicide with limited medicinal value but in some special cases, it can cause liver lesions and tumors. There was a mass poisoning incident in Ethiopia as a result of contamination of grain with *A. conyzoides*. However, the plant contains pyrrolizidine alkaloids: lycopsamine and echinatine. *A. conyzoide* is prone to becoming a rampant environmental weed when grown outside of its natural range; as such, it is an invasive weed in U.P. Nonetheless, its association with phytoplasma was first of all reported by Tiwari et al. (2012).

R. scleratus often forms colonies in wet depressions by reseeding itself. The preference is full to partial sunlight, wet conditions, and soil containing clay, clay-loam, or decaying organic material. This plant grows readily in shallow water, but tolerates occasional droughts that cause the surface water to evaporate. The foliage is rarely bothered by insects or diseases, though the foliage of 'cursed crowfoot' is more toxic than most *R. scleratus* (buttercups). In the past, beggars reportedly smeared the juices of the foliage on their faces and arms to create blisters that would solicit sympathy and money from passers-by. Thus, this study selected particular plants because of their aquatic nature which shows phytoplasma symptoms and loss of its biodiversity before the completed time.

Conclusions

On the basis of morphological character, showing different types of symptoms, and the literature survey, these four medicinal weeds were reported to be affected by phytoplasma infection seriously in this region. The genus of *A. aspera* weeds is also commonly called 'Chinchina', though the large genus of many species of flowering plants in the family Amaranthacae are native to warm, temperate, subtropical and tropical regions

throughout the world. The genus includes both annual and perennial herbaceous weeds. The yellow and little leaf symptoms were noticed on *A. aspera* plants growing in different places of Groakhpur, Eastern U.P., India during 2010-2012. *D. stramonium* is an annual invasive weed grown in India, and is used as a medicinal plant for therapeutic purposes. A disease characterized by little leaf was observed on plants of *D. stramonium* growing close to fields and road sides in Gorakhpur. The diseased plants exhibited proliferation of branches with shortened internodes and reduced-size leaves which give rise to the little leaf appearance. However, this is the first record of a 16SrVI phytoplasma affecting *D*. *stramonium*, which is suggested as a possible new '*Candidatus* phytoplasma' species based on sequence analysis. *A. conyzoides* phytoplasma is an isolate of 16SrI group (aster yellows), and is associated with little leaf disease. To the best of our knowledge, this is the first report of a '*Candidatus* phytoplasma asteris' associated with little leaf disease of *A. conyzoides* in Gorakhpur. *Candidatus* phytoplasma aster yellows were identified to be associated with *Ranunculus* phyllody disease which was first reported from Eastern Uttar Pradesh, India in the present study. Also, this is the first report from India on molecular detection and characterization of phytoplasma infection in *R. sceleratus*.

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