IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

DETECTION AND IDENTIFICATION OF *AGERATUM YELLOW VEIN VIRUS* INFECTING *PHYLLANTHUS NIRURI*: A MEDICINAL WEED IN EASTERN UTTAR PRADESH

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Abstract

During extensive survey of viral disease on medicinal plants of family Euphobriaceae in the summer season of 2006-07 at different districts of Eastern Uttar Pradesh (Gorakhpur, Deoria ,Kushinagar, Maharajganj), a variety of symptoms were recorded on *Phyllanthus niruri* plants. The infected plants showed mosaic and mottling. Yellow-green mosaic symptoms followed by yellow patches intermingled with normal green some time whole plant leaves become white and in advanced stage of infection defoliation take place. At several locations of survey areas on *P. niruri* where manifested with whitefly (*Bemisia tabaci*) were recorded. Infected plants samples were examined by electron microscopy and nested PCR by using CRv301 and CRc1152 primer pairs. The electron micrographs prepared with leaf dip method revealed the presence of geminate particles of size 20 nm in diameter. PCR products of the expected size ~870bp, were obtained from *P. niruri* samples. The results of the PCR investigation revealed that the P*hyllanthus niruri* plant showing yellow green mosaic and complete white leaves was positive for *Ageratum yellow vein virus*. The virus isolate was identified as *Ageratum yellow vein virus* of genus *Begomovirus* and family Geminiviridae. This is the first report of occurrence of *Ageratum yellow vein virus* on *P. niruri* from India on the basis of symptomatology, particle morphology and PCR analysis.

Index words: *Ageratum yellow vein virus,* molecular detection, symptoms, Geminivirdae and PCR

1.Introduction

Phyllanthus niruri L. (Stone breaker) belong family Euphorbiaceae is sparsely distributed throughout the tropical and subtropical countries of the world. This is an annual herb and widely spread in different areas of India (Gupta and Shukla, 2010). It is used in the Indian ayurvedic systems from the ancient times (Bagalkotkar 2006, Mohan et al.2015). *P. niruri* is a field weed and its genus *Phyllanthus* comprises of 600-700 species can be classified into 11 subgenus's with minor distinguishing features among them (Mao et al.2016).

P. niruri plant extract is used as a medicine and is recommended for bronchitis, anaemia, leprosy, asthma, urinar, inflammatory disorders, antifungal, antibacterial, hypoglycemic, hypotensive, analgesic and inhibitory effect on renal stone formation (Shilpa et al.2018, Satya et al.2012, Iqbal et al. 2007, Thyagarajan et al. 2002, Frietas et al.2002 ,Santos et al. 1995,Farouk et al. 1983, Jain et al.1967). The plant extract can also cure Hepatitis very effectively and it can be remedy for HIV-AIDS (Nyeem et al. 2017,Qian-Cutrone et al. 1996). Many alkaloids lignansniranthin, nirtetralin, and phyltetralin were isolated from *P. niruri* leaves which are a major component of many popular liver tonics (Chaube et al. 2015,Rastogi and Mehrotra, 1991).

So for six viruses, have been reported on *P. niruri, viz., Tomato leaf curl New Delhi virus* (Bargard et al. 2020*),*white fly transmitted *geminivirus* belonging to genus *Begomovirus* (Sivalingam and Verma, 2007), *Cucumber mosaic virus* (Kiranmai et al. 1998, *Croton yellow vein mosaic virus* (Devanna et al.2012), Yellow vein mosaic (Bhanu et al. 2015), *Ageratum yellow vein virus* (Liu et al. 2008, Srivastva and Shukla, 2016), *Cotton leaf curl virus* (Mann, 20018,Farooq et al. 2001, Whitefly transmitted Indian *begomovirus* (Saha et. al, 2012). But there is no report of any virus on survey area, hence in the present study an attempts has been made to identify the causal virus on this medicinal weed from Eastern Uttar Pradesh.

2.Materials and Methods

2(a). Survey and Symptomatology

Extensive surveys were conducted at the different districts (Gorakhpur, Deoria ,Kushinagar, Maharajganj) of Eastern Uttar Pradesh, to study the incidence of any virus infection on medicinally important weed plants of family Euphorbiaceae. During survey, yellow-green mosaic vein clearing, leaf curling symptoms were recorded from different districts of Eastern Uttar Pradesh. In advance stage of infection whole plant leaves become white and defoliation take place or plants complete dry. The leaves showed yellow patches intermingled with normal green. The infected plant samples were collected and examined for the etiology of the virus.

2(b). Electron Microscopy

Electron microscopy by leaf dip preparation was carried out as described by Brandes, (1964). Electron micrographs of well-separated *Phyllanthus* were taken at different magnification on plate film in JEM-1011. The negatives were magnified 5 times the original magnification and the measurements of the length of 100 particles (in nm) were taken in the prints.

2(c). DNA Extraction and PCR Amplification

The total DNA was extracted from 100 mg leaves tissue of infected as well as healthy leaf samples using the method described earlier by Dellaporta et al. (1983). The DNA pellet was suspended in 20 ml TE buffer. The quality and quantity of the genomic DNA was checked on 1% agarose gel and stored at -20^{o}C till further use. Total DNA was extracted from infected leaf samples and polymerase chain reaction (PCR) was performed using primers specific to amplify the coat protein gene of Indian isolates of *Tomato leaf curl virus* (*begomovirus*) viz., ATGKCSAAGCGWCCRGCAGA (CRv301) and TTWARAATGTAAWWKGAGCAG (CRc1152) were used (Reddy et al. 2005).

PCR reactions were carried out in a total of 50 µl volume containing 1 µl (20 ng) template DNA, 5.0 µl (10 X) PCR buffer, 1.0 µl (10 mM of each) dNTPs, 3 µl (25 mM) MgCl2, 1.0 µl (25 pmole) of each forward and reverse primers and 1.0 µl (3U) *Taq* DNA polymerase. Amplifications were performed in a Peltier thermal cycler following conditions: initial denaturation at 94°C for 5 min followed by a 30 cycles of denaturation at 94°C for 30 sec, primers annealing at 47°C for 30 sec and extension at 72°C for 40 sec. Later a final extension was given at 72°C for 5 min. The amplified products were electrophoresed with DNA 100bp marker in 1% agarose.

3.Results and Discussion

During extensive survey of viral disease on medicinal weed plants in the rainy season of 2007-2010 at different districts (Gorakhpur, Deoria, Kushinagar, Maharajganj) of Eastern Uttar Pradesh, a variety of symptoms were recorded on *Phyllanthus niruri* mosaic and mottling. Yellow-green mosaic, vein clearing leaf curling symptoms followed by yellow patches intermingled with normal green some time whole plant leaves become white and in advanced stage of infection complete defoliation take place. (Figure.1b-1e). At different locations of survey areas on *P. niruri* where manifested with whitefly (*Bemisia tabaci*) were recorded. The presence of virus was demonstrated by electron microscopy and electrophoresing the PCR products on agarose gels. The electron micrographs prepared with leaf dip method revealed the presence of geminate particles of size 20 nm in diameter (Figure 2a).

Fig. (1a) Healthy plant of *P.niruri* Fi.(1b)Infected plants: Virus symptoms of yellow mosaic,leaf yellowing yellowing of vein

Fig. (1c) Curling of leaves. Fig.(1d) Yellow vein mosaic complete yellowing/ defoliation of leaves

Fig.1(e) Yellowing of leaves in apical region.

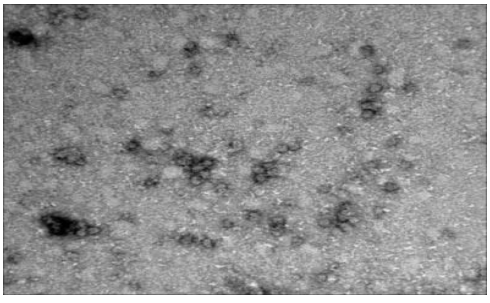

Fig. (2a) Electron micrograph showing geminate particles of size 20 nm in *P.niruri* plant with yellow vein mosaic disease.

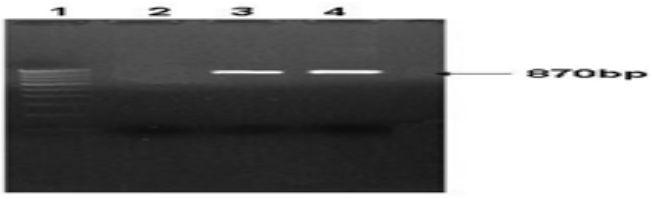

Fig.(2b).Amplification of coat protein gene of *Geminivirus* isolate using CRv301 and Crc1152 primer pair specific to coat protein gene of *Tomato leaf curl virus Begomovirus* (Lane 1: 100bp ladder; Lane 2: Healthy control; Lane 3& 4: Infected).

PCR carried out using coat protein specific primers to amplify the gene. PCR products of the expected size ~870bp, were obtained from *P. niruri diseased* samples but not band obtained from healthy tissues. The results of the PCR investigation revealed that the *Phyllanthus niruri* samples were positive for *Ageratum yellow vein virus*.

From earlier report the virus, *Ageratum yellow vein* virus (AYVV), has been identified as a *geminivirus* on the basis of host range, transmission virion morphology, serology and cytopathology (Tan and Wong, 1993, Wong et al. 1993). *Ageratum yellow vein virus* (AYVV) members of the Geminiviridae genus *Begomovirus* are transmitted by the whitefly (*Bemisia tabaci*) (Gennadius) to a wide range of vegetable and fibre crops worldwide in which they cause serious diseases (Briddon and Markham, 1995, Brown, 1994, Saunders and Stanley 1999).*Ageratum yellow vein virus* reported on *Ageratum conyzoides* (Haider, 1996, Tan and Wong, 1993). The single AYVV genomic component infects french bean and tomato suggests that *A. conyzoides* may act as a reservoir host for the pathogen (Tan et al. 1996). Although the virus was shown to have a rather limited host range, a feature typical of many dicotyledonous-infecting *geminivirus*, it could be transmitted by whiteflies to tomato (Tan et al. 1996). AYVV is a monopartite *geminivirus* resembling some isolates of *Tomato leaf curl china virus* (TYLCV) (Navot et al. 1991, Kheyr-Pour et al. 1991) and the closely related Australian isolate of TLCV (Dry et al.1993). AYVV associated with a new betasatellite infecting *Carica papaya* in China (Shen et al. 2014). AYVV causing infecting on *Nicotiana tabacum, N. glutinosa, Ageratum conyzoides, Oxalis corymbosa*, and *Phyllanthus urinaria* (Liu et al. 2008). AYVV were reported from Indonesia on *Ageratum conyzoides, Nicotiana benthamiana* (Kon et al. 2007a,). AYVV and *Ageratum yellow vein China* virus Infecting tomato, *Zinnia elegans* in Vietnam (Choi et al. 2019, Li et al.2013). Leaf curling and yellowing symptoms in *Phaseolus vulgaris* caused by *Ageratum yellow vein virus* from Japan (Tomitaka et al. 2020.). AYVV and *Papaya leaf curl Guangdong virus* infection in plants of *Euphorbia pulcherrima* was reported from China (Zhang et al. 2014). *A. conyzoides* has been identified as host of *Ageratum yellow vein virus.* AYVV-related *begomoviruses* are clearly emerging viruses and associated with tomato leaf curl disease in Java, Indonesia (Kon et al. 2007b).

1. Conclusion

On the basis of this study it was concluded that *Ageratum yellow vein virus* presence in the North Eastern Part of Uttar Pradesh from *P.niruri* plant. Hence, AYVV reported on *P. niruri* in the present study is the first report from India on the basis of symptomatology, particle morphology and PCR. The virus infected plant was identified as *Ageratum yellow vein virus* of genus *Begomovirus* and family Geminiviridae.

2. Acknowledgement:

Author highly thankful to head department of Botany , DDU Gorakhpur University, Gorakhpur for providing laboratory facility as well moral support during my research work.

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