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# FIRST REPORT OF *HAPLOTHRIPS TENUIPENNIS* BAGNALL (THYSANOPTERA, PHLAEOTHRIPIDAE) ON POTATO IN NORTH WESTERN HILLS OF INDIA

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**ABSTRACT: Thrips populations were sampled from potato in north western hills, central and plateau regions and their association with *Groundnut bud necrosis virus* (GBNV) was studied. We report the occurrence of *Haplothrips tenuipennis* Bagnall on potato for the first time from north western hills of India. The thrips was recorded from Shimla, Kufri and Fagu in Himachal Pradesh. The abundance of this species was higher in Shimla followed by Fagu and Kufri. Field collected populations of *H. tenuipennis* were tested for GBNV and 53, 35, 48% of them tested positive from Kufri, Fagu and Shimla locations, respectively. Also, 36.36 % potato samples from Shimla were found positive for GBNV followed by 25 % and 20% in Fagu and Kufri, respectively. The importance of the vector-virus complex under field conditions remains to be studied. Therefore, present reports only the occurrence of *H. tenuipennis* for the first time on potato in India.**

**KEYWORDS:** Thrips, potato, incidence, distribution, species, vector, GBNV

## INTRODUCTION

The diseases caused by thrips-transmitted tospoviruses (Genus Tospovirus, Family Bunyaviridae) are a major constraint for production of important vegetable, legume and ornamental crops in different parts of the world. *Potato stem necrosis disease* (PSND) caused by *Ground nut bud necrosis* (GBNV) is a serious problem on the early potato crop in parts of North-western/ central plains of India (Bhatnagar *et al*., 2014; 2017) and yield losses to the extent of 15 to 30% are reported (Khurana *et al*., 1989, 1997; Singh *et al*., 1997). In Madhya Pradesh and Rajasthan, up to 90% yield losses have been recorded in the early planted crops (Khurana *et al*., 1997). The disease is characterized by unusual symptom like stem and petiole necrosis, leaf spotting/deformation and stunting. It is transmissible through viruliferous thrips, particularly *Thrips palmi,* with typical stem/ petiole and foliar necrosis indicating systemic infection (Khurana *et al*., 1997). Most of the thrips species vectoring tospovirusers are polyphogous and have broad host range (Reddy, 1988). Besides GBNV, thrips also transmit a number of other tospoviruses such as *Groundnut yellow spot virus* (GYSV), *Watermelon bud necrosis virus* (WBNV) and *Tomato spotted wilt virus* (TSWV) (Reddy, 1988; Jain *et al*., 1998) in India. Few other thrips species namely, *Thrips hawaiensis, Megalurothrips distalis, Thrips palmi and Scirtothrips* sp. have been identified from early planted potato crops in India (Khurana *et al*., 2001; Bhatnagar and Thakur, 2008; Bhatnagar *et al*., 2011). Recently, Raigond *et al*. (2017) reported the incidence of thrips transmitted

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GBNV in potato and tomato in northwestern Himalaya. Therefore, field surveys were conducted for collection and identification of thrips infesting potato crops and their association with GBNV.

## MATERIALS AND METHODS

### Monitoring, collection and identification of *H. tennuipennis*

We collected a total 80 thrips and 45 potato leaf samples from standing potato crop in Shimla, Kufri, Fagu, Pune and Hassan during Summer and *Rabi* seasons in 2015 and 2016. The thrips samples were counted and collected by gently tapping the potato plant on to white sheet. The samples were preserved in 95% alcohol and were used for morphological and molecular identification. Morphological identification of thrips was facilitated by nodal institute, Zoological Survey of India, Kolkata using diagnostic keys. Further, individual thrips were used for confirming species identity by amplifying and sequencing mitochondrial *COI* gene. Single thrips sample was transferred to clean eppendorf tubes. Total genomic DNA was isolated from individual samples using DNA extraction kit (Qiagen DNeasy Blood & Tissue Kit) and was quantified using Nano drop (Thermoscientific, Leon-Rot, Germany). A portion of mitochondrial CO1 gene (700 bp) was amplified by Eppendorf thermal cycler (Hauppauge, NY) using the universal CO-I primers: LCO-1490; 5'-GCGTCAACAAATCATAAAGAT ATTGG-3' and Antisense, HCO-2198; 5'-TAAACTTCAGGGTGACCAAA AAATCA-3' (Hebert *et al*., 2003). The total reaction volume was 25 µl having 1.0 ul of each primer (10mM), 2.5 ul of 10x taq buffer, 2.5 ul of 2.0 mM dNTP, 1.0 ul of 2.5 mM $MgCl_2$ and 1.0 ul of 0.5-unit Taq DNA polymerase including 4 µl template DNA in 12 ul sterile distilled water (Sridhar *et al*., 2016). The amplified products were resolved on ethidium bromide and visualized in a gel documentation system.

### GBNV detection in thrips and potato plants

The total RNA was extracted from individual thrips and potato leaf using Axygen Mini Total RNA extraction kit (Axygen, USA) and stored at -20°C as per the standard protocol (Raigond, *et al*., 2017). Total RNA isolated was used for c-DNA synthesis by using random primers with Revert Aid c-DNA synthesis kit from Fermentas Life Sciences. The coat protein gene of GBNV was amplified in a thermo cycler using the primers (560 bp). The cDNA of 125 samples (80 thrips; 45 potato plant samples) were processed for GBNV detection. The total reaction volume of 20 µl contained 5 pmol of each primer, 2 µl of Taq buffer A, 2.0 mM of each dNTP and 1.0 unit of Taq DNA polymerase (Fermentas Life Sciences, UK) including 2 µl template cDNA. PCR was performed using an initial denaturation at 94°C for 5 min followed by 35 cycles at 94°C for 1 min, an annealing step at 62°C for 1 min, 72°C for 1 min and a final extension at 72°C for 10min. The amplified products were loaded on 1% agarose gel, stained with ethidium bromide and visualized in a gel documentation system.

The PCR amplicons were gel eluted using MinElute® gel extraction kit (Qiagen, Hilden, Germany) by following manufactures protocol. The eluted products were further used for cycle sequencing in a total volume of 20 µl mix containing 3 µl of reaction mixture, 2 µl of sequencing buffer (Big dye® Applied Biosystems, UK), 4 µl (0.8 pmol) of selected primers and 5 µl of eluted DNA (Baswaraj *et al*., 2017). The cycle sequenced product was purified, denatured at 95°C for 2 min. and sequenced in an ABI3500 genetic analyzer (Applied Biosystems). The sequences obtained

were aligned, assembled and BLAST analyzed for identification of thrips and GBNV.

## RESULTS AND DISCUSSION

Infestation of thrips in potato crops is reported from various parts of India. However, recently Raigond *et al*. (2017) reported that thrips and thrips-transmitted tospoviruses are emerging in India including north western hills. Hence, we monitored the thrips species in potato and their association with tospoviruses. Field collected thrips populations were morphologically identified as *Haplothrips tenuipennis* using key diagnostic characters as per Mound and Collins (2000) and Mound (2005). Morphological identification was further authenticated by PCR amplification and sequencing of the COI gene of the thrips (658 bp) (Fig.1). The BLAST analysis of the test sequences showed 98 % similarity with the reported sequences of NCBI repository (National Centre for Biotechnology Information). The results confirmed the identity of thrips species as *H. tenuipennis*. Therefore, the thrips recorded on potato was confirmed as *H. tenuipennis* by both morphological and molecular markers. This is the first record of *H. tenuipennis* on potato in north western hills (Himachal Pradesh). Previous records include Andaman Island, Assam, Madhya Pradesh, Maharashtra, Rajasthan, Tamil Nadu, Andhra Pradesh (Ramasubbarao and Thammiraju, 1994) and West Bengal (Tyagi and Kumar, 2016). Earlier, four species of thrips namely *T. hawaiensis, M. distalis, T. palmi and Scirtothrips* sp. have been reported to infest potato crops (Khurana *et al*., 2001).

In this study, a total of 15 thrips samples from Kufri, 20 from Fagu, and 25 from Shimla were processed to detect GBNV (560bp). PCR results revealed that thrips from these locations were found positive for GBNV (Fig 2). A maximum, 53.33 % thrips samples collected from Kufri were found positive for GBNV followed by 48 % and 35 % from Shimla and Fagu, respectively (Table 1). These results shows that *T. tenuipennis* may acquire tospovirus.

A total of 10, 12 and 11 potato leaf samples from Kufri, Fagu and Shimla were tested for GBNV. Results revealed that several potato samples collected from Shimla, Fagu and Kufri were positive for GBNV. Maximum, 36.36% potato samples from Shimla were

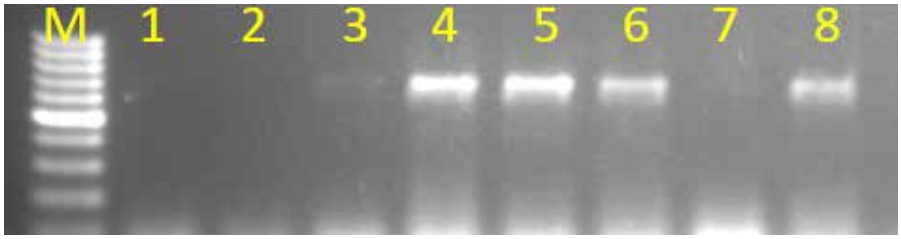


*Fig. 1. PCR amplification mtCOI gene of thrips from Shimla, Kufri, Fagu*


*Fig. 2. GBNV detection in individual thrip, +ve, WC, lane 1-2 kufri, 3-4 Fagu, 5-8 Shimla, 8-10 Pune and 11-12 Hassan.*

**Table 1. Field collected samples of *Haplothrips tenuipennis* and detection of GBNV**

| Location | Total sample processed | No. of positive samples | Percent positive samples |
|---|---|---|---|
| Kufri | 15 | 8 | 53.33 |
| Fagu | 20 | 7 | 35 |
| Shimla | 25 | 12 | 48 |

found positive for GBNV followed by 25% and 20% in Fagu and Kufri, respectively (Table 2). Results clearly indicate that the infection of GBNV was higher in the potato fields of Shimla hills.

It is evident from the previous reports that, early potato crop was more prone to thrips infestation and GBNV infection during September to October in which favourable high mean temperatures (30°C) exists (Kaushal *et al*., 2010). It is also reported that incidence of GBNV in potato was positively correlated with thrips population (Khurana *et al*., 2001). If temperature rises as described in IPCC, the thrips population may infest early potato crop during entire season and it may also infest seed and ware potato during main season. Earlier, the association of *T. palmi* with GBNV in potato was reported from Deesa, Gwalior and Chhindwara of Madhya Pradesh and Kota of Rajasthan (Jain *et al*., 2004; Akram *et al*., 2004; Bhatnagar *et al*., 2008) and in Pantnagar area of Uttarakhand (Pundhir *et al*., 2012). The peculiarity in tospovirus transmission by thrips is that only larvae can acquire the viruses while adults

**Table 2. Percentage infection of GBNV in potato**

| Location | Number of samples processed | Samples infected with GBNV | % Infection |
|---|---|---|---|
| Kufri | 10 | 2 | 20 |
| Fagu | 12 | 3 | 25 |
| Shimla | 11 | 4 | 36.36 |
| Pune | 8 | 0 | 0 |
| Hassan | 4 | 0 | 0 |

can transmit (Whitfield *et al*., 2005). Thrips may acquire but enable transmit virus due to midgut barrier, dissemination barrier, and salivary gland barrier (Ghosh *et al*., 2017).

## CONCLUSION

To conclude, this is the first report of *H. tenuipennis* on potato crop in north western hills of India. GBNV was found associated with 35 to 53.33% of the thrips samples, 20 to 36.36% of potato leaf samples collected from North-western hills. Systematic studies on acquisition, inoculation and transmission of GBNV under controlled conditions will help understand the importance of the thrips in field spread of the virus.

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