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Original Article

DNA barcoding confirmed the occurrence of invasive vegetable leaf miner, *Liriomyza sativae* Blanchard (Diptera:Agromyzidae) in Northeast India

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ABSTRACT

The vegetable leaf miner, *Liriomyza sativae* (Diptera:Agromyzidae), is an invasive polyphagous species originally known to be found in America and now spread in many parts of Africa, Asia, and the Pacific region. During 2016, *L. sativae* was observed for the first time infesting tomato (*Solanum lycopersicum* L.) leaves in experimental farms of an institute at Umiam (Meghalaya state of northeastern India). Based on museum specimens, this species was reported from India on tomato during 1994. Nevertheless, no further information is hitherto available from India apart from just new record. Considering the pest status of *L. sativae* across the globe, it is crucial to understand its expansion range, severity, biological attributes, and seasonal incidence on tomato in India. Taxonomic identification of different species of *Liriomyza* leaf miners is very complex due to morphological resemblance, and consequently, species-level identification is often done incorrectly by mistaking one species for another. Therefore, we characterized *L. sativae* at the molecular level and developed species specific DNA barcodes by using mitochondrial cytochrome oxidase gene. Moreover, the information on the correct distribution, seasonal incidence, and basic biological attributes of different stages of *L. sativae* is reported and discussed.

Introduction

In recent years, leaf miners of the genus *Liriomyza* (Diptera:Agromyzidae) have increasingly become important pests of vegetable and flower crops around the world (Andersen et al 2002). The vegetable leaf miner, *Liriomyza sativae* is an invasive species originally known to be found in America and now spread in many parts of Africa, Asia and the Pacific region (CABI 2016; Lonsdale 2011). It has been recorded feeding on nine plant families, although its preferred hosts tend to be the Cucurbitaceae, Fabaceae, and Solanaceae families. *L. sativae* is considered as one of the most devastating invasive New World species and is also known to be a vector of plant pathogens including plant viruses (Zitter et al 1980). *L. sativae* has spread in many regions of the world (Spencer 1973; 1990); therefore, it has been listed as an A1 quarantine pest by EPPO (OEEP/EPPO 1984). *L. sativae* was not known in the oriental region until 1994; however, Martinez (1994) recorded this species for the first time in the India. This report was originally published in the French language; when the severe incidence of *L. sativae* was observed in the Kampur region (Igatpuri area of Nasik district) of the Maharashtra state of India (Martinez 1994). However, due to the alphabetic similarity in the locality name “Kampur” with the “Kanpur” city of the Uttar Pradesh (UP) state of India; the distribution of *L. sativae* has mistakenly been interpreted and the name of “Kanpur (UP)” has been used in all subsequent reports and publications (CABI 2016; EPPO 2017). Apart from just new record of this species, no further details are yet available related to correct distribution, host plants and pest status of *L. sativae* in India.

Recently, the infestation of *L. sativae* was observed in a tomato crop at experimental farms of Indian Council of Agricultural Research (ICAR) Research Complex for north eastern hill (NEH)

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Region, Umiam (Figure 1). Morphologically, this species is very close to other leaf miner species, particularly *Liriomyza trifolii* and *Liriomyza huidobrensis*. In fact, all the species of the genus *Liriomyza* are similar and often mistaken for each other on quick examination. The critical inspection of the leaf mines and all stages of their development is crucial for accurate identification (CABI 2016). Owing to the existence of significant morphological similarities in between *Liriomyza* spp., the reliable identification of individual infestations must be done by the specialists (Spencer 1973). Methods of species detection in *Liriomyza* at the molecular level are also available and are being used for identification of different species of *Liriomyza* (Scheffer et al 2006; Nakamura et al 2013). To establish the correct identity of our specimens, we developed species-specific DNA barcodes by using mitochondrial cytochrome oxidase gene.

India is the second largest producer of tomato in the world after China (Anonymous 2015). During 2013–2014, the area under tomato cultivation in India was 0.88 million hectares with the production of 18.74 million tons (Anonymous 2015). Due to distinct climatic conditions, the vegetable crops including tomato are widely grown throughout the year in Northeast India. The northeastern India shares international borders with many countries, including China, Bhutan, Myanmar, Bangladesh, and Nepal, and therefore, the exchange of agricultural commodities may facilitate the rapid spread of pest and diseases in between countries. Tomato fruit borer (*Helicoverpa armigera*), the green peach aphid (*Myzus persicae*), cutworm (*Agrotis ipsilon*), leaf hopper (*Amrasca bigutulla bigutulla*) and tobacco white fly (*Bemisia tabaci*) are the important pest of tomato in Northeast India (Azad Thakur et al 2012). Recently, South American tomato pinworm (*Tuta absoluta*) has been observed infesting tomato crop in Meghalaya state (Sankarganesh et al 2017). Considering the pest status of *L. sativae* across the globe, it is crucial to understand its correct distribution, spread, severity, biological attributes, and seasonal incidence on tomato.

Materials and methods

The experiment was conducted at the Division of Crop Protection, ICAR Research Complex for NEH Region, Umiam, Meghalaya (India) from 2016 to 2017. Infested plant samples were collected from the institute farm and further reared in the laboratory. Adults were examined in the insect biosystematics laboratory of the institute and were identified with the help of taxonomic keys and available literature (IPPC 2014; OEEP/EPPO 2005). After identification at the species level, infested plant samples were also collected from the tomato fields in nearby localities in the Ri-Bhoi district of the Meghalaya state. Infested samples were brought to the laboratory and reared till adult emergence. Adults were examined for species-level identification with taxonomic keys. For confirmation of identification at the molecular level, the genomic DNA was extracted from the abdomen of two adults by using the DNeasy® Blood and Tissue Kit (Qiagen, Valencia, USA) as described in Behere et al (2015). Additionally, two adult specimens have been deposited as voucher specimens in Insect Museum of Crop Protection Division, ICAR Research Complex for NEH Region, Umiam, India. For amplification of the standard DNA barcoding region of mitochondrial cytochrome oxidase I gene, we used the procedure used for rose sawfly as described in Firake et al (2013). The Sanger sequencing of successful polymerase chain reaction (PCR) amplicons was carried out commercially by sending post PCR product (40 μL post PCR). Both the samples were sequenced from both the ends (5′ and 3′) for accuracy purpose. The DNA sequences were analyzed using Pregap4 and Gap4 programs within the Staden molecular biology analysis software (Staden et al 1998). Nucleotide sequences were aligned using the sequence alignment program Clustal X (Thompson et al 1997) and were checked manually. Sequence identity was determined by BLASTN search (Altschul et al 1997) against the nucleotide collection in the NCBI GenBank.

Seasonal incidence of *L. sativae* was studied in the experimental farm at Umiam, Meghalaya (India). Numbers of live mines per five random leaves were recorded at weekly basis from 10 plants grown in the field. The basic biological parameters of this pest were also studied under laboratory conditions (23±2°C temperature and 73±2% relative humidity); where infested branches of tomato plants were collected from the field and kept inside the cages (size: 45 × 45 × 45cm). Newly emerged adults were exposed to potted tomato plants (25–30 cm tall, i.e. during preflowering stage) in rearing cages. The 50% honey solution was provided as an adult food. Observations on oviposition period, fecundity, incubation period, larval period, pupal period, and adult longevity were recorded regularly.

Results and discussion

Morphological characters of the leaf miner specimens infesting tomato plants were found matching the characters of *L. sativae*. The species level identity of leaf miner was also confirmed at the molecular level. The standard insect DNA barcoding primers viz., LCO and HCO (Folmer et al 1994) used in this study successfully amplified the targeted fragment of cytochrome oxidase I gene.

Figure 1. Severely infested tomato leaves showing loose and irregularly scrolled mines of *L. sativae*.

Figure 2. Typical damage of *L. sativae* on tomato leaves.

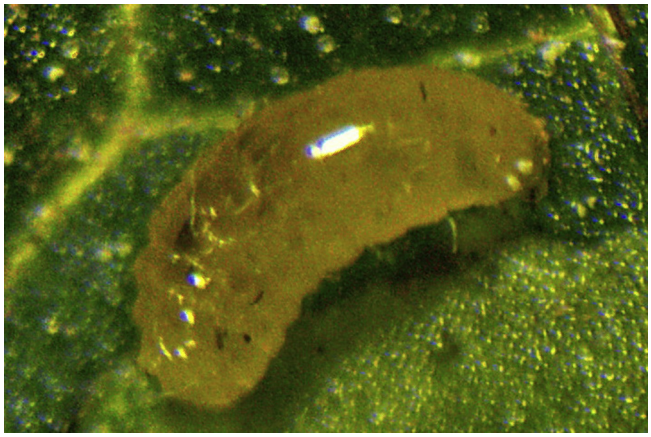

Figure 3. Yellow colored mature maggot of *L. sativae*.

Final 598bp sequences were obtained after trimming the ambiguous 5′ and 3′ ends of the sequences. Sequences of both the specimens of *L. sativae* were found to be similar and no single nucleotide polymorphisms were detected. A BLASTN search of 598bp sequence as a query in NCBI showed 99–100% homology with the *L. sativae* sequences deposited in NCBI GenBank (Accession numbers; KF962572, KR476573, KF962577, KC136060, EU219613 etc.) including complete mitochondrial genome of *L. sativae* (HQ333260). Since there was no variation among two sequences, a representative sequence of *L. sativae* has been deposited to the NCBI with accession number MF079257.

Vegetable leaf miner, *L. sativae* is the most destructive, invasive species in many countries, including neighboring China and Bangladesh (Amin et al 2014). In a recent report, relatively low levels of genetic diversity but moderate population genetic

Figure 4. The puparium of *L. sativae*, initially yellow in appearance and later turned into the brown color.

Figure 5. Dorsal view of adult of *L. sativae*.

structure was observed in *L. sativae* in China (Tang et al 2016). Also, genetic distance was not found correlated to the geographic distance, coupled with high levels of gene flow, suggesting a possible anthropogenic influence on the spread of *L. sativae* in China (Tang et al 2016). Considering the migration pattern, this species might have been migrated from adjacent areas of China or Bangladesh into the Meghalaya. Due to significant similarities in morphological characters of *Liriomyza* species; DNA barcode could only be the most reliable method of species detection in *Liriomyza* species complex (Scheffer et al 2006; Nakamura et al 2013)

Infestation of *L. sativae* has been observed in tomato fields at Umiam, Umroi Nongrah, Nongthymmai, and Mawnohsynrum villages of Meghalaya state and the infestation ranged from 2.1% to 42.5% infested leaves per plant. Severely infested plants were weakened or even totally destroyed. Infested leaves were found

Figure 6. Lateral view of adult of *L. sativae*.

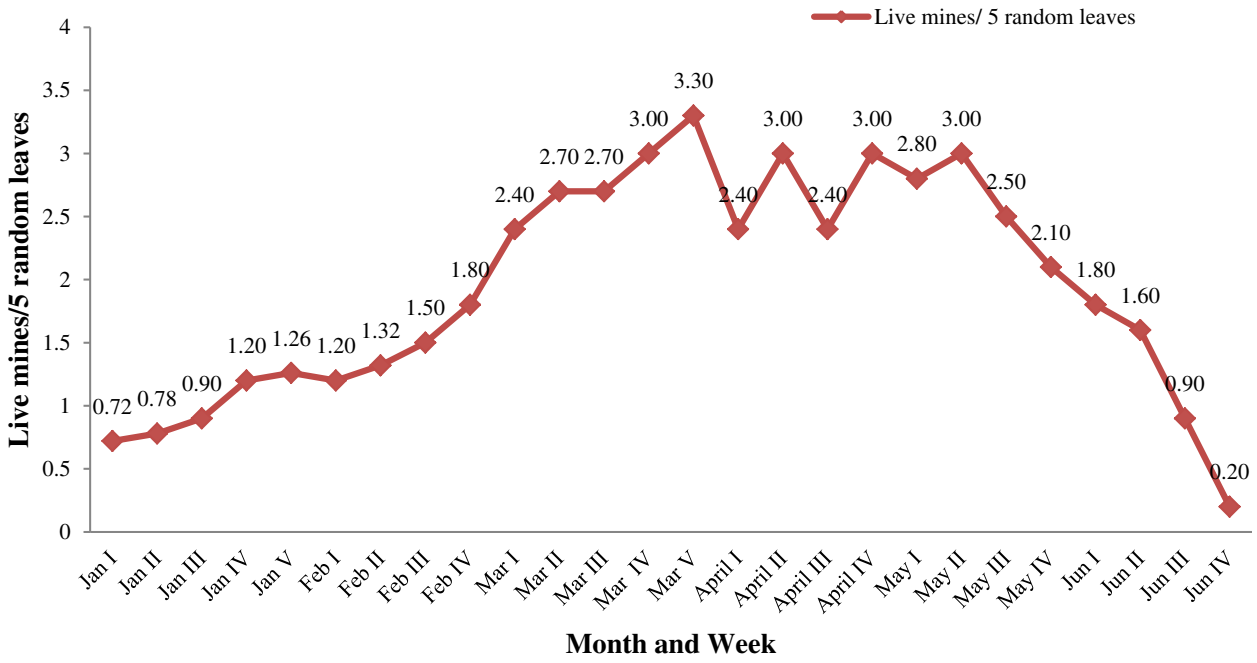

Figure 7. Seasonal incidence of *L. sativae* on tomato at Umiam during main season (from January to June, 2017).

more susceptible to wind damage. Frequent punctures on the leaves during egg laying by the females caused mottled appearance on foliage (Figure 2). After hatching, tiny maggots started feeding on the mesophyll portion of the leaves and caused irregular scrolled and loose mines on the foliage and young tender stem. The fecal deposits were also obvious in the mines. Leaf miner infested plants usually become weak since the photosynthetic ability of the plants reduces due to destruction of chlorophyll-containing cells during damage (Figure 2).

Biology of the *L. sativae* was studied under laboratory conditions on tomato plants. Adult flies started laying eggs just below the leaf surface after 2.33±0.33 days of release and the oviposition period was found to be 4.33±0.67 days. Average fecundity was recorded to be 43±8 eggs per female. The incubation period was found to be 2.67±0.33 days. Soon after hatching, tiny maggots started feeding by making typical (loose and irregular scrolled) mines on the leaves and completed the larval period in 4.33±0.67 days. Mature maggot looks yellow in color (Figure 3). Larvae of *L. sativae* pupariate usually on the foliage. Initially, the puparium was yellow in appearance and later turned into the brown color (Figure 4). Tiny adults started emerging mostly during the morning hours after completing the pupal period of 10.67±0.67 days. Adult flies (Figures 5 and 6) lived for 8.33±0.67 days. Little variations may be possible in biology of herbivore species on different host plants and locality. In previous reports, *L. sativae* was found to complete egg to the adult period in 15.9±0.04 days on melon plants (Araujo et al 2013) and 18.4 days on castor plants at 25°C (Parkman et al 1989). Also, incubation period and fecundity varies with temperature and host species (Harris and Tate 1933). In general, the development rate of the insect usually increases with the increase in temperature up to certain level (Firake and Khan 2013).

Infestation of *L. sativae* was started from the seedling stage of tomato plants in the nursery. Seasonal incidence of *L. sativae* was studied in tomato crop and the infestation was observed throughout the growing period of tomato, but the damage was higher during March and April months (Figure 7). Several reports indicated that the weather parameters, particularly temperature, have substantial effects on the biology and ultimately on pest infestation. About 20–24°C temperature is suitable for growth and development of *L. sativae* (Harris and Tate 1933; Spencer 1973; Parkman et al 1989).

The present study is the first report of the incidence and biology of *L. sativae* on tomato in the Northeast India. The invasion of this species might be from the adjacent areas of China or Bangladesh in the past or recently. This report also provides the correct distribution of *L. sativae* in India. Considering the pest status, timely monitoring would be required to understand the bionomics of this leaf miner species on tomato and other vegetable crops. Also, regular sampling would be essential in and nearby region to understand the species composition of leaf miners on tomato. Information generated in this study, including correct distribution and molecular characterization of *L. sativae* would be vital for future research on this species. Management practices available for other leaf miner species may be evaluated for management of *L. sativae* on tomato. Further exploration of host range and natural enemies of *L. sativae* would be another yet important area for future research.

Conflicts of interest

The authors declare that there is no conflicts of interest.

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References

Altschul SF, Madden TL, Shafer AA, Zhang J, Miller W, Lipman DJ. 1997. Gapped BLAST and PSIBLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25:3389–3402.

Amin S, Scheffer SJ, Lewis ML, Pasha MK, Bhuiya BA. 2014. DNA barcoding of the vegetable leafminer, *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) in Bangladesh. *DNA Barcodes* 2:29–33.

Andersen A, Nordhus E, Vu TT, Tran TTA, Ha QH, Hofsvang T. 2002. Polyphagous *Liriomyza* species (Diptera: Agromyzidae) in vegetables in Vietnam. *Tropical Agriculture* 79:241–246.

Anonymous. 2015. *Indian horticulture database. National horticulture board, ministry of agriculture*. Government of India. http://nhb.gov.in/area-pro/NHB_Database_2015.pdf [Date accessed: 06 August 2016].

Araujo EL, Nogueira CHF, Menezes Netto AC, Bezerra CES. 2013. Biological aspects of the leafminer, *Liriomyza sativae* (Diptera: Agromyzidae) on melon (*Cucumis melo* L.). *Ciencia Rural* 43:579–582.

Azad Thakur NS, Firake DM, Behere GT, Firake PD, Saikia K. 2012. Biodiversity of agriculturally important insects in North Eastern Himalaya: an overview. *Indian Journal of Hill Farming* 25:37–40.

Behere GT, Firake DM, Burange PS, Firake PD, Azad Thakur NS, Ngachan SV. 2015. Utility of ITS1 as a molecular tool for reliable identification of Henosepilachna pusillanima and H. septima (Coleoptera: Coccinellidae). *Journal of Genetics* 94: 19–23.

CABI. 2016. *Invasive species compendium. Data sheet of Liriomyza sativae (Vegetable leaf miner)*. http://www.cabi.org/isc/datasheet/30960 [Date accessed: 28 April 2017].

EPPO. 2017. *EPPO global database*. http://gd.eppo.int/taxon/LIRISA/distribution [Date accessed: 04 May 2017].

Firake DM, Behere GT, Firake PD, Rajkhoa DJ, Azad Thakur NS, Saini MS, Rahman Z, Ngachan SV. 2013. *Arge xanthogaster* (Hymenoptera: Argidae): a new threat to rose plants in Meghalaya, India. *Florida Entomologist* 96:1298–1304.

Firake DM, Khan MA. 2013. Do daily alternating temperatures affect the performance of egg parasitoids, *Trichogramma* spp. *Journal of Insect Science* 14: 1–14.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3:294–299.

Harris HM, Tate HD. 1933. A leaf miner attacking the cultivated onion. *Journal of Economic Entomology* 26:515–516.

IPPC. 2014. *Approval of international standards as ANNEX to ISPM 27:2006- diagnostic protocol for genus Liriomyza 2006-017*. https://www.google.co.in/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&cad=rja&uact=8&ved=0ahUKEwjlvujGudPTAhVIhbwKHfjMClgQFggrMAE&url=https%3A%2F%2Fwww.ippc.int%2Fstatic%2Fmedia%2Ffiles%2Fpublications%2Fen%2F2014%2F06%F24%2F2006-017_draftispm_genusliriomyza_2014-0612_agm.docx&usg=AFQjCNGYq0EVCKjR1L1IiVde6BDGxFiwJg&sig2=UrAx3TRBWqoSUXD5FXdWGw [Date accessed: 05 May 2017].

Lonsdale O. 2011. The Liriomyza (Agromyzidae: Schizophora: Diptera) of California. *Zootaxa* 2850:1–123.

Martinez M. 1994. A new pest menaces the Oriental Region: *Liriomyza sativae* Blanchard (Diptera, Agromyzidae). *Bulletin de la Societe Entomologique de France* 99 (4):356.

Nakamura S, Masuda T, Mochizuki A, Konishi K, Tokumaru S, Ueno K, Yamaguchi T. 2013. Primer design for identifying economically important *Liriomyza* species (Diptera: Agromyzidae) by multiplex PCR. *Molecular Ecology Resources*. https://doi.org/10.1111/1755 0998.12025.

OEEP/EPPO. 1984. Data sheets on quarantine organisms No. 131, Liriomyza trifolii. *Bulletin OEPP/EPPO Bulletin* 14:29–37.

OEEP/EPPO. 2005. Normes OEPP EPPO standards. *Bulletin OEPP/EPPO Bulletin* 35: 271–273.

Parkman P, Dusky JA, Waddill VH. 1989. Biological studies of *Liriomyza sativae* (Diptera: Agromyzidae) on castor bean. *Environmental Entomology* 18:768–772.

Sankarganesh E, Firake DM, Sharma B, Verma V, Behere GT. 2017. Invasion of the South American Tomato Pinworm, Tuta absoluta, in northeastern India: A New Challenge and Biosecurity Concerns. *Entomologia Generalis*. https://doi.org/10.1127/entomologia/2017/0489. In press.

Scheffer SJ, Lewis ML, Joshi RC. 2006. DNA barcoding applied to invasive leafminers (Diptera: Agromyzidae) in the Philippines. *Annals of the Entomological Society of America* 99:204–210.

Spencer KA. 1973, . *The agromyzidae of Venezuela. Revista de la facultad de Agronomia*, 7. Caracas: Universidad Central. pp. 5–107.

Spencer KA. 1990. *Host specialization in the world Agromyzidae (Diptera). Seris entomologica 45*. Dordrecht, Netherlands: Kluwer Academic Publishers. p. 440.

Staden R, Beal KF, Bonfield JK. 1998. The staden package. In: Misener S, editor. *Bioinformatics methods and protocol*. New York: Springer. pp. 115–130.

Tang XT, Ji Y, Chang YW, Shen Y. 2016. Population genetic structure and migration patterns of *Liriomyza sativae* in China: moderate subdivision and no Bridgehead effect revealed by microsatellites. *Bulletin of Entomological Research* 106 (1): 114–123.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24:4876–4882.

Zitter TA, Tsai JH, Harris KF. 1980. Flies. In: Harris KF, editor. *Vectors of plant pathogens*. New York: Academic Press. pp. 165–176.