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Outbreak and Management of Serpentine Leaf Miner, *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae), on Potato (*Solanum tuberosum* L.) Crop in India

Priyank Hanuman Mhatre · Shivaji Hausrao Thube · Om Navik · E. P. Venkatasalam · Sanjeev Sharma · Jagadeesh Patil, et al. ***[full author details at the end of the article]***

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Abstract
Serpentine leaf miner, from the genus *Liriomyza*, is one of the most economically important pests responsible for severe yield reductions in numerous vegetables crops worldwide including potato. Potato plays a significant role in the Indian economy. Potato production is being affected by several existing and new biotic stresses; correct and timely identification of new stresses is the key for their effective management. The Nilgiri hills located in Southern India is one of the oldest potato-growing areas of the country and is a prime centre in south India for potato production and supply. During the summer and autumn seasons of the year 2020, a leaf miner outbreak was observed in the potato-growing areas of the Nilgiri hills of Southern India. Here, we surveyed the potato fields to determine the species, damage severity, distribution, and possible alternate weed hosts of leaf miner. Based on the morpho-molecular characterization of the adult fly, the pest was identified as *Liriomyza huidobrensis*. During the outbreak, the incidence of this pest was 90–100%, with a damage severity ranging from 20 to 100% on the potato crop. The larval population varied from 1.2 to 14.83 larvae leaflet^{-1}. It was also observed that the damage incidence and severity of *L. huidobrensis* infestation was influenced by the crop age and foliar injury increased with the advancement of crop age. In addition to potato, *L. huidobrensis* incidence was also recorded on six cultivated crops such as carrot, beetroot, garlic, beans, double beans, and broccoli. The incidence of the pest was also noticed on one cover crop, lupine and seven weed species (*Amaranthus* spp., *Bidens pilosa*, *Chenopodium* spp., *Solanum nigrum*, *Galinsoga parviflora*, *Hypochaeris glabra*, and *Sonchus oleraceus*) commonly observed in the Nilgiri hills. Field experiments for the management of *L. huidobrensis* with two potato cultivars (Kufri Swarna and Kufri Sahyadri) were also conducted to have an immediate option against this pest. These studies recommend the application of abamectin for the management of *L. huidobrensis*. The polyphagous nature of the pest coupled with increasing trade, transport, and changing climatic scenario possesses the risk of its spread in new geographical areas along with the possibility to attain the status of a major pest of potato and other crops in near future. Additionally, the Nilgiri hills are identified as one of the world's biodiversity hotspots. Therefore in future, the studies

should focus on the exploration and conservation of biological control agents and evaluation of safer chemical molecules to minimize the crop losses and to avert its further spread in new localities.

Keywords Alternate hosts · Identification · Leaf miner · *Liriomyza huidobrensis* · Potato

Introduction

In the past few years, leaf miner flies in the genus *Liriomyza* (Diptera: Agromyzidae) have increasingly become the most economically important pests of vegetable and floriculture crops around the globe (Andersen et al. 2002). The genus *Liriomyza* was first proposed in 1894 by Mik; till date more than 456 species were reported with wide geographical distribution; among them nearly 24 species are economically important (Spencer 1973; Alves et al. 2014; Weintraub et al. 2017). Of these, three polyphagous species, namely *Liriomyza huidobrensis* (Blanchard), *Liriomyza trifolli* (Burgess), and *Liriomyza sativae* (Blanchard), are of particular importance as crop pests and are reported to be highly invasive species with worldwide distribution (Reitz et al. 2013). *Liriomyza huidobrensis* is extremely polyphagous and has been reported on 365 host plant species belonging to 49 plant families around the world (Weintraub et al. 2017). *Liriomyza huidobrensis* has its origin from Central and South America and was first reported in Europe in 1987. Since then it expands geographical range considerably in Europe and especially the Mediterranean region (CABI 2021). At present, *L. huidobrensis* is widely distributed and colonized in five continents covering more than 40 countries, except Australia and Antarctica (Weintraub et al. 2017). *Liriomyza huidobrensis* is highly polyphagous and has been recorded feeding on fifteen plant families, although its most preferred hosts include beans, beet, potatoes, tomatoes, peas, spinach and cut flowers such as chrysanthemum, carnations and gypsophila (Spencer, 1990). Young plants are more susceptible to infestation and severe infestation results in plant death, and older plants show reduced growth rate due to lesser photosynthetic activity. Even a few leaf mines in the lettuce, spinach, or celery can make the crop unmarketable. Feeding damage inflicted by adult females, and the oviposition puncture holes can affect the appearance of cut flowers and floriculture plants (Trumble et al. 2021). In potato, it is responsible for direct as well as indirect damage to the crop. In case of direct damage, the adult flies feed and oviposit on the plant leaves; this creates a spotted appearance on leaves. After egg hatching, larvae start feeding on the leaves that results in the production of larger mines by destructing foliar mesophyll (Parrella 1987), thereby reducing the yield of the crop by more than 50%, if left untreated (Mujica and Cisneros 1997). However, indirectly it is reported to act as a vector for plant diseases, during feeding and egg-laying by adult flies, as recently, *L. huidobrensis* was known to disseminate the fungus *Alternaria solani* in the potato crop (Soares et al. 2019). In India, other than *L. huidobrensis*, four leaf miner species, namely *L. brassicae* (Nimbalkar and Borle 1970), *L. compositella* (Udayagiri 1987), *L. trifolii* (Lakshminarayana et al. 1992), and *L. sativae* (Firake et al. 2018) have been reported; however, *L. huidobrensis* was first reported from Uttar Pradesh state of India during 1994 (CABI/EPPO 2002). Apart from the new record of this species in Uttar Pradesh and Manipur (Singh and Singh 2013), no further details are yet available related to the pest status of *L. huidobrensis* in India.

Recently, an outbreak of a new insect pest that mines potato leaf was detected in the Nilgiri hills of Southern India. This newly arrived leaf-mining pest causes severe damage to the potato leaf, and in many places, severe crop losses were also noted during the summer and autumn seasons of 2020 (unpublished). Therefore, a survey was conducted on an emergency basis during the autumn season of 2020, throughout the potato-growing regions of Nilgiri hills following the outbreak of the leaf-mining pest. The leaf-mining fly was detected and identified as *L. huidobrensis*. This leaf-mining species is morphologically very close to other species, particularly *L. trifolii* and *L. sativae* (Firake et al. 2018; CABI 2021); therefore, morphology coupled with molecular validation is required for the correct identification of the pest at the species level.

India ranks second in potato production with a production of 48.53 million tonnes from 2.15 million ha area (FAOSTAT 2018). The Nilgiri hills located in Southern India are one of the oldest potato-growing areas in the country (Manorama et al. 2013). In the Nilgiri hills, the potato crop can be grown around the year because of its better geographical location receiving rainfall evenly and prevailing cooler temperatures, throughout the year. In the Nilgiri hills, the potato is being grown in more than 4500 ha area and is a prime centre in the south India for potato production and supply (Ravichandran et al. 2007). Due to the congenial climatic conditions, potato production in Nilgiri hills is being threatened regularly by several biotic stresses (Ravichandran et al. 2007). Among these, potato late blight (Mhatre et al. 2020a) and potato cyst nematode (Mhatre et al. 2021) are the regular problems, whereas, the potato tuber moth is considered as an emerging threat to the potato cultivation in this area (Mhatre et al. 2020b). Considering the economic importance and pest status of *L. huidobrensis* throughout the world, it is critical to understand its exact distribution, spread severity, biological attributes, and seasonal incidence on potato. Further, there was no report on the occurrence of *L. huidobrensis* infesting potato crop from Southern India. Understanding the exact identity of pest species, their population, spread, and alternate hosts are the crucial factors for formulating efficient management strategies for any pests. Therefore, here we report the morphological characteristics supported with molecular characterization by amplifying the mitochondrial cytochrome c oxidase I gene of *L. huidobrensis* for species-specific identification. We also assessed the pest incidence, damage severity, infestation level, and alternate hosts of *L. huidobrensis*, which provides the baseline information in understanding the possible cause of pest outbreak and designing the proper management strategies. In addition to this, to have an immediate option for the management of this pest, on an emergency basis, a field trial was also conducted using locally available insecticides for the management of this pest in the Nilgiri hills of India.

Materials and Methods

Field Survey and Sample Collection

During the autumn season (August to November) of 2020, a survey was conducted, and infested plant samples were collected from twelve potato-growing locations of the Nilgiri hills (Table 1). Informations like GPS coordinates (latitude, longitude, and elevation), crop age, potato cultivar, and cropping pattern were collected to understand the ecology/biology of the pest. In total, 60 fields (five fields per location) were assessed in this study; each location was approximately having an area of 0.4 to 0.5

Table 1 Survey of leaf miner, *Liriomyza huidobrensis* outbreak in potato-growing areas of Nilgiri hills

Location name	Latitude	Longitude	Elevation (metre above mean sea level)	Potato cultivar	Crop age (days)
Osahatti 1	11°22.126′	76°40.148′	2018	Kufri Jyoti	80
Osahatti 2	11°22.103′	76°40.344′	2027	Kufri Jyoti	80
Osahatti 3	11°22.199′	76°40.456"	2015	Kufri Jyoti	60
Appukodu 1	11°21.607′	76°39.606′	2080	Kufri Jyoti	50
Appukodu 2	11°21.573′	76°39.668′	2080	Kufri Himalini	50
Kallakorai	11°21.862′	76°39.956′	2006	Kufri Jyoti	35
IISWC Research farm	11°23.830′	76°39.975′	2218	Kufri Jyoti	80
Theetukal	11°23.849′	76° 39.774′	2210	Kufri Jyoti	65
Basavkal	11° 23.622′	76° 39.083′	2130	Kufri Jyoti	45
Kenthorai	11°26.752′	76°44.427′	2006	Kufri Girdhari	90
New Colony, Kenthorai	11°26.030′	76°44.369′	2142	Kufri Jyoti	70
New Colony, Kenthorai	11°26.030′	76°44.369′	2142	Kufri Himalini	75

ha. The sampling with live larvae of the leaf miner was carried out as per the methodology given by Alves et al. (2014). The leaf samples with active mines (upper, middle, and lower leaves) were collected from thirty individual plants from each location (six plants from each field). All the samples were placed in plastic bags with proper aeration and brought to the laboratory. The leaflets were further dissected and we recorded the presence of larvae leaflets^{-1}. The adult flies that emerged from the infested potato leaves collected from different locations were captured and preserved separately for morphological and molecular characterization in 70% and absolute alcohol, respectively.

Assessment of Pest Incidence and Damage Severity

A total of 60 potato fields were surveyed for the occurrence of leaf miners in the potato crop. Each sampled field was divided into three plots of approximately 10m^2 area and the observations on the presence of active leaf mines were recorded from the plants in middle rows. The upper, middle, and lower leaves of each plant were inspected for the damage incidence and damage severity. The pest damage incidence was estimated based on the presence of active mines with larvae and expressed in the percentage of infested plants. However, the damage severity of the pest infestation was calculated based on the visual observations and expressed in the percentage of leaf area damage as very low (0–20%), low (20–40%), moderate (40–60%), high (60–80%), and severe (80–100%) (Lopez et al. 2010).

Morpho-molecular Identification

Morphological identification of the pest emerged from the infested potato leaves was confirmed based on key diagnostic characters given by LucidKey (2021), Malipatil (2007), and Maharjan et al. (2014). Adult specimens from three locations (Osahatti 1, Apokodu 2, and Kenthorai) identified using morphological criteria were further subjected to molecular characterization using the mitochondrial cytochrome c oxidase I (*mtCOI*) gene. DNA was extracted from the adult individual using the QiagenDNeasy Blood and Tissue Kit®, following the manufacturer's protocol. PCR reactions were performed to amplify partial *mtCOI* gene sequences with 50 µL reaction volume comprising of 3.0 µL of genomic DNA (50 ng/µL), 2.0 µL each of forward (LCO1490 5′-GGTCAACAAATCATAAAGATATTGG-3′) and reverse (HCO2198 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) primers (Folmer et al. 1994) (10 pmol/mL), 2.0 µL of 10 mM dNTPs, 5.0 µL of 10X PCR buffer containing $MgCl_2$, 2.0 µL of DyNAzyme II DNA Polymerase (2 U/µL) and 34.0 µL of molecular biology grade water (Thermo Fisher Scientific, USA). Thermocycling (Bio-Rad T100) consisted of an initial denaturation at 94° C for 3 min, followed by 30 cycles of denaturation at 94° C for 20 s, annealing at 50° C for 30 s, extension at 72° C for 30 s, and final extension at 72° C for 5 min (Thube et al. 2018). The amplified product was evaluated with electrophoresis using 1.0% agarose gel (Life Technologies, CA, USA). PCR purification (Geneaid Biotech Ltd., Taiwan) was done, and purified products were Sanger sequenced (Agrigenome Pvt. Ltd., "SmartCity Kochi", Infopark Road, Kakkanad, Kerala, India).

Phylogenetic Relationships

The sequence of the *mtCOI* gene of the specimens was used to study the phylogenetic relationships between the related species of *Liriomyza*, and *Helicoverpa armigera* was used as an outgroup. The obtained sequences were aligned using BioEdit (Biological sequence alignment editor—Tom Hall, http://www.mbio.ncsu.edu/BioEdit/bioedit.html), and BLAST was performed using NCBI (http://www.ncbi.nlm.nih.gov/) as well as the BOLD database (http://www.boldsystems.org/). Sequence similarity was analysed with the available sequences, and sequences were deposited in NCBI. The accession numbers of the respective sequences of *Liriomyza* spp. were obtained from the GenBank using BLAST tool of the NCBI, and the accessions are cited in the phylogenetic tree. All alignments with other relevant sequences were produced by ClustalW parameters in MEGA 7.0. The Phylogenetic analysis of sequence data was done using the neighbour-joining method using MEGA 7.0 (Kumar et al. 2016). The branch support was estimated by bootstrap analysis (1000 replicates) using the same parameters as the original search. Pairwise distances were computed using MEGA 7.0. Codon positions included were 1^{st}+ 2^{nd}+3^{rd}+ Noncoding. All positions containing gaps and missing data were eliminated.

Identification of Alternate Hosts

During the survey, it was noticed that the surrounding flora of the potato crop was also infected with leaf miners. Hence, based on the presence of the leaf mines on crop and weed hosts near the vicinity of the potato, a random sampling was carried out from all the locations and the leaf samples with active mines were collected from all the infested alternate hosts. The samples were placed in plastic bags with proper aeration, brought to the laboratory and observed till the emergence of adult flies. The adult leaf miners were identified by morphological characteristics and molecular sequence.

Field Experiment

Based on the pest outbreak noticed during the summer and autumn seasons of the year 2020, two field experiments were conducted with locally available insecticide molecules during the autumn season (August to November) of 2020 on an emergency basis for the management of this new pest problem of this area. The experiments were conducted at the research farm of ICAR-Central Potato Research Institute, Regional Station, Udhagamandalam, Tamil Nadu, India, on the potato crop under rainfed conditions (average rainfall of 1300–1400 mm year^{-1}). The first experiment was conducted using the recently released late blight and potato cyst nematode-resistant cultivar Kufri Sahyadri (Joseph et al. 2019), whereas, the second experiment was conducted using a popular cultivar of this region Kufri Swarna (Mhatre et al. 2020a). These cultivars are resistant to potato cyst nematode (PCN) and thus help minimize the experimental errors caused by PCN damage. For these trials, well-sprouted, medium-sized seed potato tubers were planted in a 9 m^2 plot with the spacing of 60 cm (row to row) × 20 cm (plant to plant); there were five rows of 15 tubers in each plot. In experimental plots, the nutritional (NPK-90:135:90 kg ha^{-1}; farmyard manure-15 t ha^{-1}) and cultural practices (weeding, hoeing, earthing up, haulm killing, etc.) were followed as per the recommendations (Ravichandran et al. 2007).

The experiments were organized in a randomized block design with seven treatments and three replicate plots for each trial. The treatments were T_1, deltamethrin; T_2, cypermethrin; T_3, abamectine; T_4, dinotefuran; T_5, spinetoram; T_6, fenpyroximate; T_7, novaluron; and T_8, untreated control. The detailed information about insecticides evaluated is provided in Table 2. The spray volume for each treatment was 500 L ha^{-1}. The untreated control plots were sprayed with only water without insecticides. The first spray of insecticide was applied at approximately 10% incidence of *L. huidobrensis* to the potato branches. Four sprays of each insecticide were applied at 7 day interval based on the pest damage incidence on the potato foliage.

The observations were terminated when the damage incidence reached 100% in the untreated plants; at this time, the damage incidence and severity of the pest infestation were calculated. The damage incidence was recorded based on the percentage of no. of damaged leaves per plant (no. of damaged leaves ×100/total no. of leaves), and damage severity was calculated based on the visual observations of total area damage per plot (very low (0–20%), low (20–40%), moderate (40–60%), high (60–80%), and severe (80–100%)) (Lopez et al. 2010). At the time of harvesting, the tuber yield was recorded and expressed in tonnes ha^{-1}.

Statistical Analysis

Percent data on damage incidence by *L. huidobrensis* was normalized using arcsine transformation, and numerical data on larvae leaflet^{-1} was square-root transformed before analysis. The analysis was undertaken on the transformed data, and un-transformed data were presented in mean ± SE. The ANOVA was performed on larvae leaflet^{-1}, percentage of damage incidence in survey samples, percentage infection of the leaflet by *L. huidobrensis*, and yield of the potato crop under field experiments. A significant mean comparison was conducted using Tukey post hoc test ($P \leq 0.05$). All the statistical data analyses were performed using SPSS version 21.0 (IBM Corp 2012).

Table 2 Insecticides evaluated against *Liriomyza huidobrensis* infesting potato during autumn season (August to November)

Active ingredients	Sub-group or exemplifying active ingredient	IRAC MoA number*	Rate (g (a.i.)/ha)**
Deltamethrin 2.8 % EC	Pyrethroids, Pyrethrins	3A	14.00
Cypermethrin 25 % EC	Pyrethroids, Pyrethrins	3A	62.50
Abamectin 1.9 % EC	Avermectins	6	9.50
Spinetoram 11.7 % EC	Spinosyns	5	58.50
Dinotefuran 20 % SG	Neonicotinoids	4A	100.00
Fenpyroximate 5% EC	METI acaricides and insecticides	21A	25.00
Novaluron 5.25 % EC	Benzoylureas	15	26.25

**IRAC-MOA*, Insecticide Resistance Action Committee–Mode of Action, http://www.irac-online.org/modes-of-action/

***a.i.*, active ingredient

Results

Damage

In the survey, it was observed that the leaf miner was present in all the surveyed areas. Among the surveyed locations, no significant differences were observed in the damage incidence of *L. huidobrensis*, as the damage incidence ranged from 90 to 100% (F = 1.26, df = 11, 44, P = 0.28). However, the damage intensity among the surveyed location ranged from very low to severe (Table 3). Moreover, increased foliar injury was observed with the advancement in the crop age. The damage intensity was high (60–80%) to severe (80–100%) at a crop age of and above 70 days. There was no significant difference observed among the potato cultivars in terms of pest damage incidence (F = 0.44, df = 2, 57, P = 0.65), whereas significant differences were observed in the number of *L. huidobrensis* larvae leaflet^{-1} across the locations (F = 52.28, df = 11, 348, $P < 0.0001$). Several larvae (on an average of 1.2 to 14.83 larvae leaflet^{-1}) and their mines were observed on potato leaves, and the severely infected fields were seen with reduced and blackened leaf foliage (Fig. 1; Table 3).

Morpho-molecular Identification

Based on the morphological observation of the adult flies such as coloration, the pattern on the body, head frons, hind margin of eye, abdominal tergite, antenna, mesonotum, scutellum, femora, etc., the insect was identified as *Liriomyza huidobrensis*, belonging to family Agromyzidae of the insect order Diptera. The adult flies were small in size (body length: 1779.27±35.77 µm; thorax width: 541.64±0.87 µm) and quite distinctive with a shiny black body with two yellow spots on their side and one on its back. The head was with yellow frons, the hind margin of the eye was black, the second tergite of the abdomen was divided, the enlarged third antennal segment was noticed, mesonotum was black, matt with bright yellow scutellum, and yellow femora with brownish striations were also observed (Fig. 2). In addition to this, larger mines typically caused

Table 3 Damage caused by *Liriomyza huidobrensis* in potato-growing areas of Nilgiri hills

Location name	Damage incidence (%) (mean ± SE)*	Damage severity**	Larvae leaflet^{-1} (mean ± SE)*
Osahatti 1	100±0.0^{a}	High	14.40±0.96^{a}
Osahatti 2	100±0.0^{a}	Severe	7.50±0.83^{b}
Osahatti 3	100±0.0^{a}	Moderate	14.83±1.01^{a}
Appukodu 1	100±0.0^{a}	Low	6.07±0.92^{bc}
Appukodu 2	100±0.0^{a}	Very low	3.93±0.60^{cd}
Kallakorai	90±6.32^{a}	Very low	3.00±0.44^{d}
IISWC Research farm	100±0.0^{a}	Severe	2.00±0.17^{d}
Theetukal	100±0.0^{a}	Very low	1.20±0.21^{d}
Basavkal	90±10.0^{a}	Very low	1.50±0.24^{d}
Kenthorai	100±0.0^{a}	High	2.27±0.20^{d}
New Colony, Kenthorai	100±0.0^{a}	High	8.42±0.73^{ab}
New Colony, Kenthorai	100±0.0^{a}	Severe	3.90±0.63^{cd}

*Means followed by the same letters in the column are not significantly different (ANOVA, Tukey's (HSD) test, P <0.05)

**Damage severity: very low (0–20%), low (20–40%), moderate (40–60%), high (60–80%), and severe (80–100%).

by the *L. huidobrensis* were also observed on the potato leaves (Fig. 1d). The molecular characterization of *L. huidobrensis* was confirmed based on the *mtCOI* gene, which yielded a single fragment of approximately 688 base pairs (accession no. MW467885 and MW467884) and 698 base pairs (accession no. MW467886).

Phylogenetic Relationships

The comparison of obtained sequences (MW467885, MW467884, and MW467886) revealed 100% sequence similarity corresponding to *L. huidobrensis* isolates from South Korea (KC136091), Zimbabwe (KU244272), Sri Lanka (KX373669), and Zambia (KX373670), 99.70% with Australia (KR476572) and 99.56% with China (EU219615) and USA (FJ435888). The phylogenetic relationships between 11 species of *Liriomyza* are presented in Fig. 3. All the *L. huidobrensis* isolates formed a monophyletic assemblage in this consensus tree except for two isolates (the USA and Australia). *Liriomyza langi* was found nearer to *L. huidobrensis.* The species *viz.*, *L. baptisiae*, *L. chinensis*, *L. cicerina*, and *L. chenopodii* did not group with any other species, whereas *L. sativae* and *L. trifoliearum*, *L. philadelphivora* and *L. fricki* formed separate monophyletic groups. *Helicoverpa armigera* was kept as an outgroup. Bootstrap support in this clade was ranging from 57 to 100.

Identification of Alternate Hosts

Other than on the potato crop, the presence of pests (adult fly or mines or larvae) was also noticed on other important and commonly cultivated crops of this region such as carrot, beetroot, garlic, beans, double beans, and broccoli. In addition to this, a cover

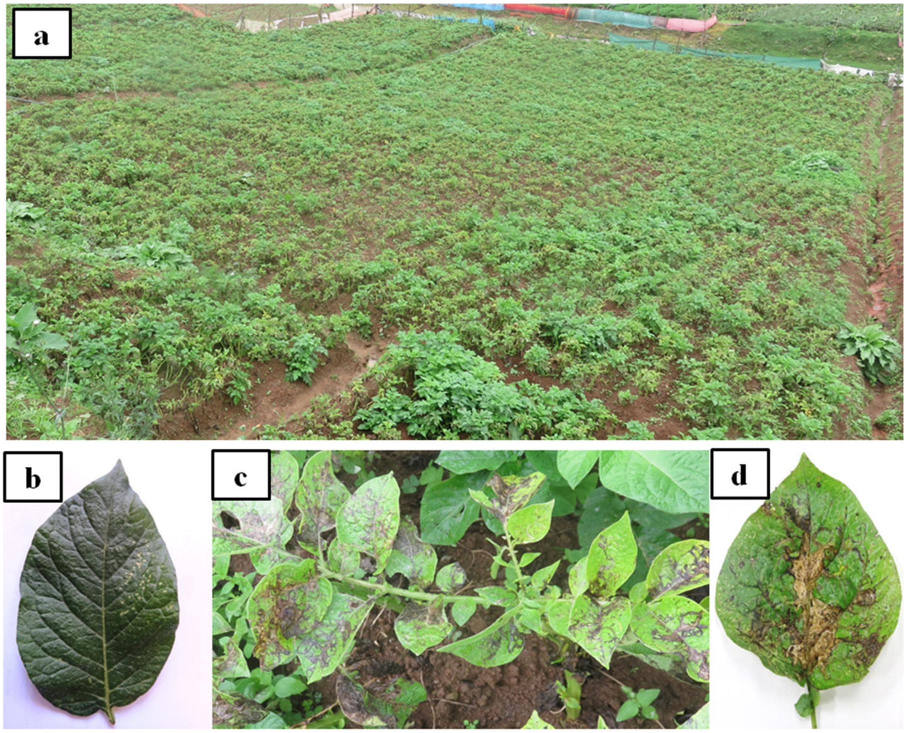

Fig. 1 Leaf miner attack in potato crop at Nilgiris: (**a**) field infestation at 75-day-old crop, (**b**) initial white spotting on leaf by leaf miner fly, (**c**) infected plant, (**d**) typical pattern of leaf mining by *Liriomyza huidobrensis*

crop (*Lupinus* spp.), and some weed species (*Amaranthus* spp., *Bidens pilosa*, *Chenopodium* spp., *Solanum nigrum*, *Galinsoga parviflora*, *Hypochaeris glabra*, and *Sonchus oleraceus*) near the vicinity of the potato crop were also found infected with leaf miner fly (Fig. 4).

Field Experiment

In first experiment with cultivar Kufri Sahyadri, no significant differences ($F = 2.96$, $df = 7, 16$, $P = 0.457$) were observed in the damage incidence caused by *L. huidobrensis* before application of any treatment. However, significant differences were noticed after fourth insecticide application ($F = 38.07$, $df = 7, 16$, $P < 0.001$). The yield of Kufri Sahyadri was also significantly influenced by the application of different insecticides ($F = 5.09$, $df = 7, 16$, $P = 0.003$). Among the insecticides, abamectin was found effective in reducing 23% terminal damage incidence by *L. huidobrensis* and improved the yield of the crop by 24.44% (Figs. 5 and 6) over untreated control, whereas, the highest damage incidence (100%) and lowest tuber yield (30 t ha^{-1}) were noted in untreated control plots. The terminal disease severity was observed maximum, i.e., high in untreated control (60–80%), whereas it was found moderate (40–60%) in abamectin-treated plants.

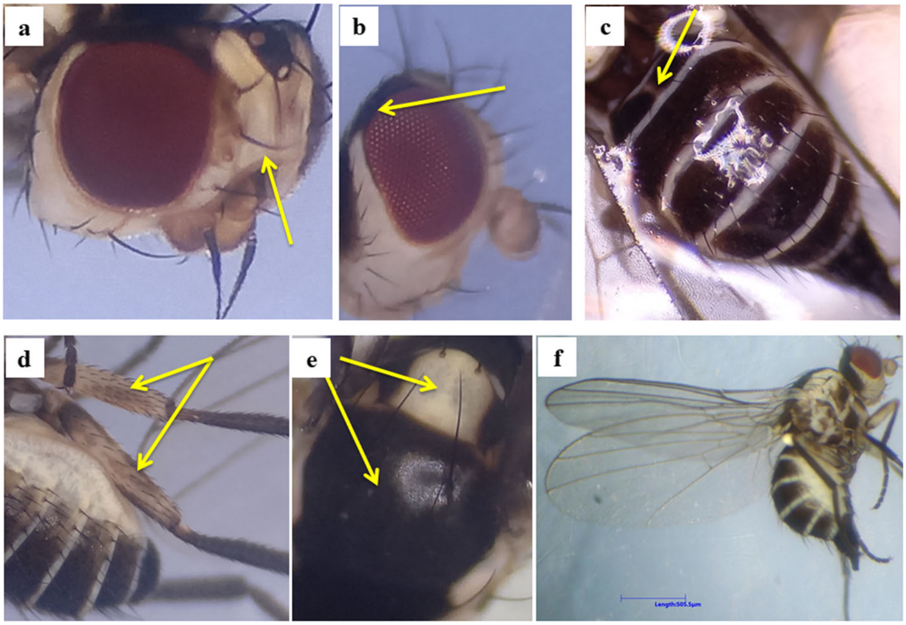

Fig. 2 Key diagnostic characters of *Liriomyza huidobrensis*: (**a**) yellow frons, (**b**) black hind margin of eye, (**c**) divided second visible tergite of abdomen, (**d**) femora yellow with brownish striations, (**e**) black and matt mesonotum with bright yellow scutellum, (**f**) adult fly

In second experiment with cultivar Kufri Swarna, there were no significant differences noticed among the initial damage incidences caused by *L. huidobrensis* (F =2.44,

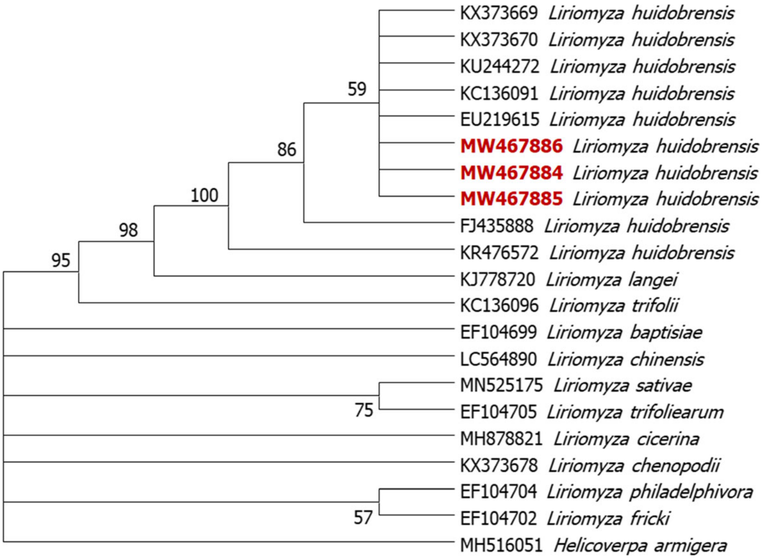

Fig. 3 Phylogenetic relationships of 11 species of *Liriomyza* spp. were inferred based on the analysis of mitochondrial cytochrome c oxidase subunit I (*mtCOI*) gene using the neighbour-joining method. *Helicoverpa armigera* was used as an outgroup. Numbers at the nodes represent bootstrap proportion for neighbour-joining (50% or more)

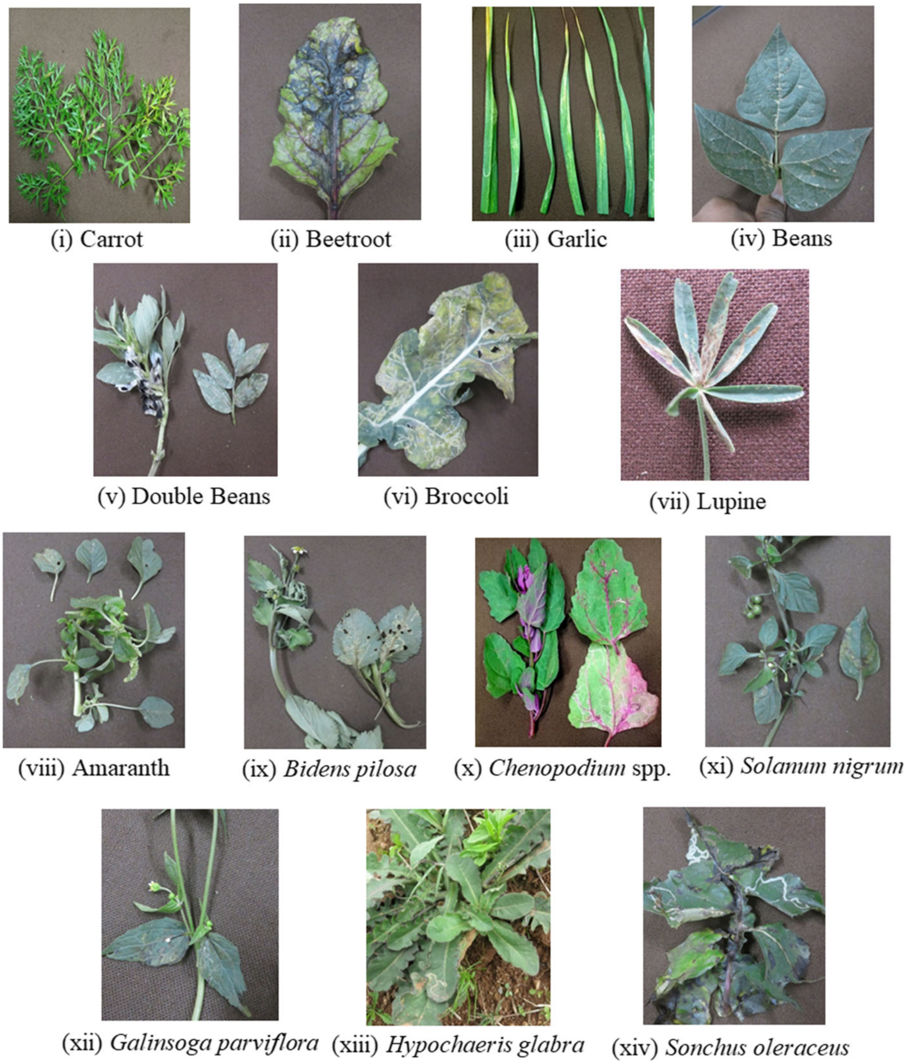

Fig. 4 Alternate hosts of *Liriomyza huidobrensis* showing leaf mining, observed near the vicinity of potato crop. Among these carrot, beetroot, garlic, beans, double beans, and broccoli are being cultivated as major crops in the Nilgiri hills. Lupine is being grown as cover crop, whereas amaranth, *Bidens pilosa*, *Chenopodium* spp., *Solanum nigrum*, *Galinsoga parviflora*, *Hypochaeris glabra*, and *Sonchus oleraceus* are the common weed flora noticed in this region

df = 7, 16, P = 0.067). However, significant differences were recorded after the fourth sprays of insecticides (F = 15.07, df = 7, 16, P < 0.001). The yield of cultivar Kufri Swarna was also significantly influenced by the application of different insecticides (F = 6.98, df = 7, 16, P = 0.001). Here also among the insecticides, abamectin was found effective in reducing 18% terminal damage incidence by *L. huidobrensis* and improved the yield of the crop by 20% (Figs. 5 and 7) over untreated control. The highest tuber yield was recorded in the application of abamectin (26.06 t ha^{-1}) followed by cypermethrin (24.32 t ha^{-1}) whereas the lowest tuber yield (21.78 t ha^{-1}) was noted

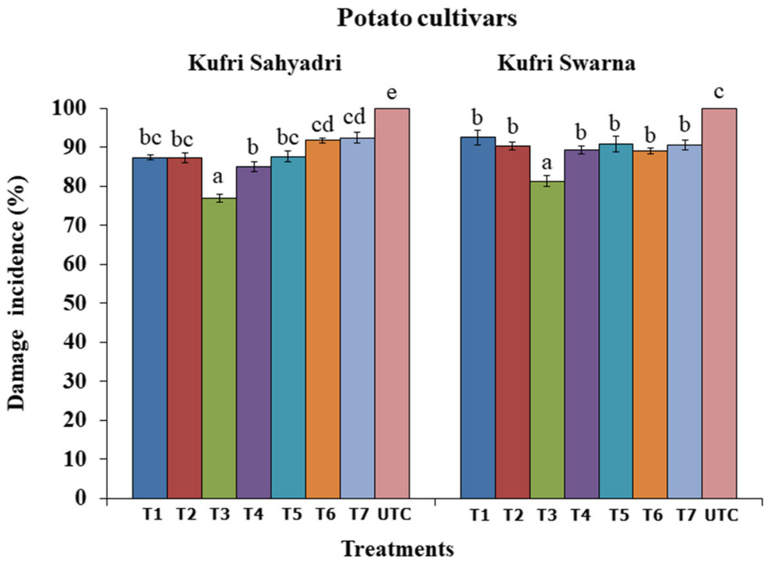

Fig. 5 Effect of insecticides on the terminal percent damage incidence of *Liriomyza huidobrensis* in Kufri Sahyadri and Kufri Swarna. Different lowercase letters indicate significant differences for different treatments ($P < 0.05$, Tukey's test). T1, deltamethrin; T2, cypermethrin; T3, abamectin; T4, spinetoram; T5, dinotefuran; T6, fenpyroximate; T7, novaluron; and T8, untreated control

in untreated control. The terminal disease severity was observed maximum i.e. severe in untreated control (80–100%) whereas it was found moderate (40–60%) in abamectin-treated plants and high (60–80%) in other treatments.

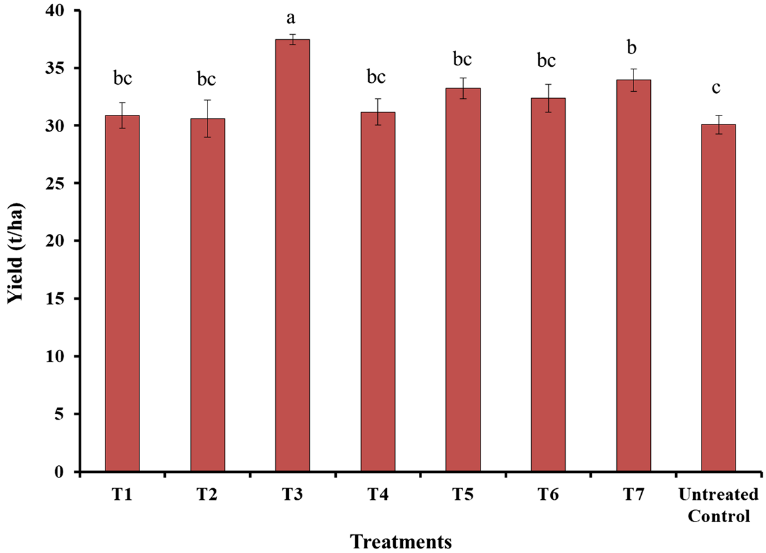

Fig. 6 Effect of insecticides on the tuber yield of Kufri Sahyadri. Different lowercase letters indicate significant differences for different treatments ($P < 0.05$, Tukey's test). T1, deltamethrin; T2, cypermethrin; T3, abamectin; T4, spinetoram; T5, dinotefuran; T6, fenpyroximate; T7, novaluron; and T8, untreated control

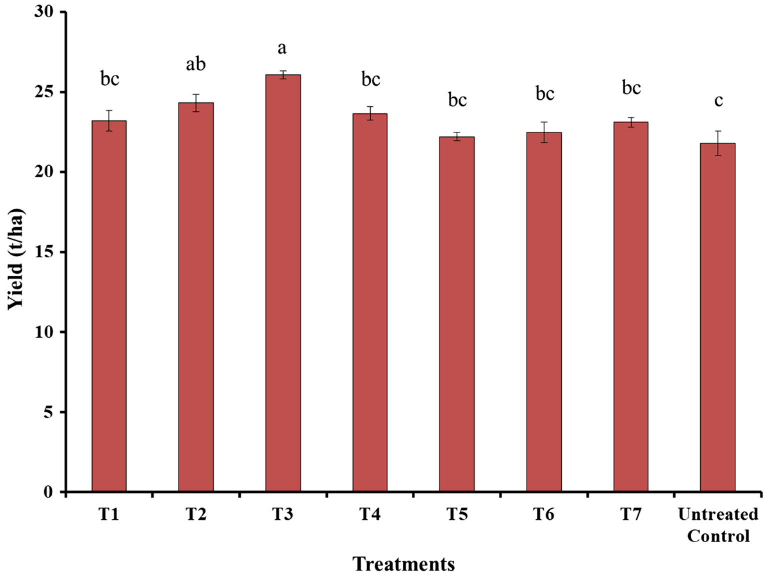

Fig. 7 Effect of insecticides on the yield of Kufri Swarna. Different lowercase letters indicate significant differences for different treatments ($P < 0.05$, Tukey's test). T1, deltamethrin; T2, cypermethrin; T3, abamectin; T4, spinetoram; T5, dinotefuran; T6, fenpyroximate; T7, novaluron; and T8, untreated control

Discussion

The Nilgiri hills of Southern India are one of the gifted places on the earth where farmers can grow potato and other temperate crops throughout the year, due to congenial climatic conditions. This place is known for the production of high-quality vegetable (Ravichandran et al. 2007; Santhi and Priya 2016). In the present study, an outbreak of a new pest, *L. huidobrensis*, was reported in the Nilgiri hills during summer and autumn seasons of 2020. In Nilgiri hills, to date only one species of *Liriomyza* (*L. trifolii*) infesting lettuce has been reported (Anjali et al. 2018); moreover, *L. huidobrensis* infesting potato is reported from Uttar Pradesh (CABI/EPPO 2002) and onion from north-eastern part (Manipur) (Singh and Singh 2013) of India. Hence, not much is known about the infestation and distribution of *L. huidobrensis* on potatoes in India. The outbreak of *L. huidobrensis* was noted in the summer and autumn seasons of 2020. Potato is considered a suitable host for the outbreak of this pest, and the results of our study are in agreement with Maharjan et al. (2014), who also reported the outbreak of *L. huidobrensis* from Miryang and Goryeong of the South Korean Peninsula in the potato crop during 2012. The reasons behind the outbreak and invasion of *L. huidobrensis* such as increasing trade of horticultural crops, indiscriminate use of pesticides, climate change scenario etc., in different parts of the world are well explained in a recent review by Weintraub et al. (2017). In Europe it was reported that, *L. huidobrensis* was regularly intercepted from horticulture imports within the region or in the new region (Baker et al. 2012). Indiscriminate use of pesticides against primary pests of potato was also suggested as one of the factors behind the outbreak of this pest, as the parasitoids against *L. huidobrensis* were eliminated, creating classic secondary pest outbreaks (Luck et al. 1977). However, in a study, it was clearly indicated that the species is expected to spread to the higher altitudes globally due to the climate change scenarios (Mujica 2016). The arrival of *L. huidobrensis* has been reported from Southeast Asia, particularly Malaysia, Indonesia, Philippines, and Vietnam mostly with the importation of flowers and infested potatoes (Hussain and Sivapragasam 2008; Rauf 1995; Shepard et al. 1996; Molitas-Colting et al. 2002; Andersen et al. 2008). Probably, the invasion and outbreak of *L. huidobrensis* in the Nilgiri hills are also attributed to the abovementioned factors but the exact cause of its entry into India is still not known.

It was observed that during the outbreak, the foliar injury by *L. huidobrensis* increases with the age of the potato crop. Similarly, Mujica and Kroschel (2013) also reported that the foliar injury increases with the age of the crop. Lopez et al. (2019) estimated the economic injury level (EIL) for *L. huidobrensis* (3.24 larvae leaflet^{-1} of tomato). In our study, it was found that in seven locations the larval count was above EIL (3.9–14.83 larvae leaflet^{-1}); this indicates the outbreak of the pest in many folds in the surveyed area. As discussed the reasons behind the outbreak could be increased trade, indiscriminate use of pesticides, and climate change scenario (Weintraub et al. 2017). In addition to this, in the Nilgiri hills, the availability of congenial climatic conditions and alternate host crops throughout the year are other probable reasons behind the outbreak. Therefore, it is predicted that *L. huidobrensis* may attain the status of a major and serious threat to the cultivation of potato as well as other crops of the Nilgiri hills, if left unaddressed.

In the present study, based on morphological characters and molecular analysis, the leaf miner fly was identified as *Liriomyza huidobrensis*. All the diagnostic characters are typically matched with the description of the *L. huidobrensis* given by LucidKey (2021), Malipatil (2007), and Maharjan et al. (2014). In addition to the morphological characters, *L. huidobrensis* is reported to cause larger mines in the spongy mesophyll of foliage and petioles (Parrella 1987); the types of mines evident in the present study were also typical to the mines produced by *L. huidobrensis* (Maharjan et al. 2014).

Worldwide, *L. huidobrensis* was found infesting 365 host plant species belonging to 49 plant families (Weintraub et al. 2017). In our study, the polyphagous nature of *L. huidobrensis* was noted; along with the potato crop, it was also found to infest six important cash crops, one cover crop, and seven weed species common to the Nilgiri hills. Moreover, *L. huidobrensis* can complete its life cycle in 17–65 days depending on the season, host plant, and temperature suitability (Lange et al. 1957; Parrella and Bethke 1984; Mujica and Cisneros 1997; Head et al. 2002). In the Nilgiri hills, the climatic conditions are congenial throughout the year for growing potato and other host crops of *L. huidobrensis*. Therefore, this pest can complete multiple generations and have the potential to become an emerging threat to the agriculture of this area. To avoid this, proper monitoring of the pest is a must, the population of *L. huidobrensis* adult fly can be monitored during the early crop season (30-40 days after planting) using yellow sticky traps (Mujica and Kroschel 2013), and the same was found effective in catching the adult fly populations in the present study (Supplementary Fig. 1). Therefore, as a preventive measure, the farmers of this area are advised to adopt proper weed management practices and destroy all unwanted plant material after the crop harvest; moreover, during the cropping season, the monitoring can be done using yellow sticky traps to adopt proper management practices.

The field experiments for the management of *L. huidobrensis* were planted with two potato cultivars, viz. Kufri Sahyadri and Kufri Swarna; both the cultivars are medium maturing but, Kufri Swarna is maturing in about 100 days during autumn season; however, Kufri Sahyadri takes more than 120 days for maturity. In these trials, the higher damage incidence of *L. huidobrensis* and lower tuber yield were recorded in Kufri Swarna compared to Kufri Sahyadri; this can be attributed to their maturity duration. In an earlier study, Mujica and Kroschel (2013) also recorded similar results of more damage and yield losses in early potato cultivars than late cultivars; they have reported 22–51% yield reductions by *L. huidobrensis* in different potato cultivars. In a study, Johnson et al. (1983) reported that *L. huidobrensis* can decrease photosynthesis by up to 62%. Further, this injury invites other external infections by microorganisms and results in a lower yield of the crop (Motteoni and Broadbent 1988).

In the present investigation, among the tested chemical insecticides, application of abamectin was found effective in reducing the damage incidence caused by *L. huidobrensis* in both the cultivars and it improved the crop yield significantly compared to untreated control. Abamectin is a translaminar insecticide belonging to a class of closely related macrocyclic lactones; it is a mixture of insecticidal avermectins either produced directly by the actinomycete bacterium, *Streptomyces avermitilis*, or generated through semisynthetic modifications (Fisher and Mrozik 1989). Abamectin is a neurotoxin that acts as a gamma-aminobutyric acid (GABA) agonist (Abalis et al. 1986). The affected insect becomes paralysed, stops feeding, and dies. In addition to larvicidal activity, it was also found to have repellent activity toward leaf miner adult flies (Parrella et al. 1988). Earlier studies reported the efficacy of different chemical insecticides including abamectin against leaf miners belonging to the genus *Liriomyza* (Parrella et al. 1988; van der Staay 1992; Mujica et al. 2001; Weintraub 2001; Fourouzan and Farrokh-Eslamlu 2017), and our results are in agreement with these studies. Therefore, for the management of the *L. huidobrensis*, the application of abamectin is recommended to the farmers of the Nilgiri hills of India. But the continuous application of any insecticide may lead to the development of insecticide resistance, which is one of the inherited abilities of *Liriomyza* spp., for becoming the major pests of the crops (Parrella and Keil 1984; Reitz et al. 2013). In addition to this, the Nilgiri hills are identified as one of the world's biodiversity hotspots; therefore, future studies should focus on understanding the biology of the pest, exploration and conservation of biological control agents, and integration of different management approaches including the use of safer insecticide molecules. This helps to avoid crop losses considering the safety of the flora and fauna of the Nilgiri hills, and further, it will also help contain the spread of *L. huidobrensis* to new geographical areas of the country.

Conclusion

Our study reports the severe outbreak of leaf miners on the potato crop in the Nilgiri hills of Southern India. Based on morphological and molecular characterization, the species of the pest was identified as *L. huidobrensis*, which is a new pest problem to this area. Our study identified fourteen alternate hosts (including common weed species) of the pest which emphasizes the importance of the cleaner cultivation in this area. In addition to this, the field trials conducted in two cultivars recommend the application of abamectin as an instant solution for the management of the pest outbreak. In the future, the studies should focus on bio-rational management of *L. huidobrensis* in the Nilgiri hills: the biodiversity hotspots of the world.

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Author Contribution PHM: conceptualization, methodology, investigation—conducting survey and field experiments, formal analysis, writing original draft, visualization. SHT: morphological identification and molecular validation, writing—review and editing. ON: morphological confirmation, formal analysis, writing—review and editing. VEP: supervision, review and editing. SS: supervision, review and editing. JP: formal analysis, writing—review and editing. SuS: formal analysis. DKL: writing original draft. SW: review and editing. TPP: molecular validation. MAS: methodology. MK: review and editing.

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Declarations

Conflict of Interest The authors declare no competing interests.

Ethics Approval No ethics have been violated in compiling this article.

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Affiliations

Priyank Hanuman Mhatre[1] · **Shivaji Hausrao Thube**[2] · **Om Navik**[3] · **E. P. Venkatasalam**[1] · **Sanjeev Sharma**[4] · **Jagadeesh Patil**[3] · **S. Subhash**[4] · **K. L. Divya**[1] · **Santosh Watpade**[5] · **Thava Prakash Pandian**[2] · **M. Abas Shah**[4] · **Manoj Kumar**[4]

✉ Priyank Hanuman Mhatre
priyank.iari@gmail.com

[1] ICAR-Central Potato Research Institute, Regional Station, Udhagamandalam, The Nilgiris, Tamil Nadu 643004, India

[2] ICAR-Central Plantation Crops Research Institute, Regional Station, Vittal, Dakshina Kannada District, Karnataka 574243, India

[3] ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, Karnataka 560024, India

[4] ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh 171001, India

[5] ICAR-Indian Agricultural Research Institute, Regional Station, Shimla, Himachal Pradesh 171001, India