Indian Journal of Ecology
Vol. 53, Issue 1, January-March 2026: 107-114
Journal homepage: http:/www.indianecol.org
DOI: https://doi.org/10.55362/IJE/2026/4765

Manuscript Number: 4765

Unraveling Genetic Variability in Brinjal Shoot and Fruit Borer, *Leucinodes orbonalis* (Guenee) using Mitochondrial Cytochrome Oxidase I (COI)

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Received: September 30, 2025 Revision Submitted: January 21, 2026 Accepted: February 10, 2026

***Abstract*:** The present study was undertaken to provide insights regarding population genetic structure of *Leucinodes orbonalis* from diverse locations. Molecular characterization of *L. orbonalis* populations collected from seven locations was carried using mitochondrial cytochrome oxidase I (COI). Collected specimens were subjected to DNA extraction, PCR amplification and sequencing of the targeted gene. The samples of *L. orbonalis* were collected from three locations of Himachal Pradesh, two locations each from Punjab and Haryana. The pair-wise genetic distance of *L. orbonalis* populations collected from different regions of Himachal Pradesh, Punjab and Haryana ranged from 0.0000 to 0.0044. This suggests that there is not much variation in the population of *L. orbonalis* from different locations. The phylogenetic analysis revealed two distinct clades based on the COI gene of mitochondrial region of *L. orbonalis*. The population of *L. orbonalis* from Shimla (MW363534), Solan (MW344268), Una (MW363538), Karnal (MW363539), and Jalandhar (MW363537) were clustered together in clade I due to their close proximity, while population of *L. orbonalis* from Firozpur (MW363535) and Bhiwani (MW363536) were clubbed in clade II. The average nucleotide compositions were adenine (30.46 %), thymine (39.57 %), guanine (14.02 %) and cytosine (15.93 %).

***Keywords*:** *Leucinodes orbonalis,* Mitochondrial cytochrome oxidase I (COI), Population, Phylogenetic analysis, Molecular characterization.

1. Introduction

Eggplant (*Solanum melongena* L.) is the major and economically important vegetable crop with high nutritive value and cultivated in the hot-wet climatic conditions in most parts of Asian countries (Thapa, 2010). In India, there are approximately 2500 varieties of brinjal with diverse shapes, ranging from oval or egg-shaped to long or club shaped. The color of the fruits ranges from white, yellow, green and purple to nearly black.

One of the major factors which is responsible for low productivity of brinjal is a variety of insect pests which not only reduce the yield but quality also. Among these, the brinjal shoot and fruit borer, *Leucinodes orbonalis* (Lepidoptera: Pyralidae) has been reported to be the serious pest which reduces the crop yield up to 60-70 per cent and inflicts the colossal loss in production (Sharma et al., 2017) and *L. orbonalis* is the most noxious and ubiquitous pest among the different insect pests of brinjal (Singh et al., 2019).

Insecticide treatments against this pest are not providing adequate control and repeated use of existing insecticides has resulted in the development of resistant biotypes by means of genotypic variations in these insects (Alyokhin et al., 2008). In response to the stresses, the populations of *L. orbonalis* have changed to adapt the ill-effects by changing their eco- behavioral pattern, feeding physiology and reproduction, in addition to the changes in their molecular machineries. It is therefore necessary to explore the genotypic variations that occur within the population of a species to design and formulate effective insect pest management strategies (Padwal and Srivastava, 2020). To minimize the hazardous effects of chemical pesticides, world agro-analysts intend to shift their insect pest management strategies solely towards biological control, a method which encompasses the broad range of concepts from using the different cultural practices and introduction of living organisms to control the pest (Baker et al., 2020).

Different researchers and scientists across the world are using molecular techniques like DNA barcoding for precise identification (Zhang et al., 2010; Shashank et al., 2015; Murali et al., 2017; Palraju et al., 2018). Amplified fragment length polymorphism-polymerase chain reaction (AFLP-

PCR) analysis was done to characterize 29 natural populations of *L. orbonalis* from different brinjal growing regions of India. Data analysis helps to determine the extent of inter- and intra- population variability amongst the different populations (Saini et al., 2006). Zhang et al. (2010) suggested a simple resampling approach for estimating several key sampling sizes for a DNA barcoding project.

Chakravarthy (2015) suggested that DNA barcoding is a major tool in fast and authentic identification of insects belonging to different orders and families. Murali et al. (2017) studied the molecular characterization of 21 population of *L. orbonalis* collected from Karnataka, Andhra Pradesh, New Delhi and Uttar Pradesh. They amplified CO I gene, the sequencing resulted in 600-636 bp and amino acid composition of nucleotide, which varied from 195-206 in different populations. Certain amino acids were either present or absent in different populations indicating their diversity.

Species level identification is dependent on different diagnostic characters. Morphology based identification is a hectic task due to time consumption. So, there is an unmet need to introduce the quick, economical and undisputed approaches to analyze the shoot and fruit borer taxonomically. This research was aimed to characterize populations of brinjal shoot and fruit borer, *L. orbonalis* for their genetic diversity from different location of Himachal Pradesh, Punjab and Haryana using universal primer (LCO1490/HCO2198) as a tool of the molecular marker.

2. MATERIAL AND METHODS

2.1. Survey and Collection of Insect Samples

Brinjal shoot and fruit borer (infested fruit and shoot) were collected from different locations of Himachal Pradesh, Punjab and Haryana (Table 1).

2.2. Maintenance of Population of *L. orbonalis* Collected from Different Locations

Larvae of *L. orbonalis* were collected in vials containing 90 per cent ethanol from each location. Collected specimens were brought to Department of Entomology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan. After washing with alcohol larvae were transferred to clean glass vials with the help of forceps and were preserved in 90 per cent ethanol for the DNA analysis and stored in -20ºC. For DNA analysis, the larvae were used after the removal of the intestine.

2.3. Study on Genetic Variability

The protocols used to study genetic diversity of *L. orbonalis* from different locations were discussed under the following subheadings: a.) DNA extraction; b.) Preparation and running of Agarose gel; c.) PCR amplification; d.) Visualization of PCR product; e.) DNA Sequencing and analysis.

a. DNA extraction

Total genomic DNA of *L. orbonalis* was extracted from larvae was followed by using the HiPurA® Insect DNA Purification Kit. The DNA purification procedure using the miniprep spin column comprising of three steps *viz.,* adsorption of DNA to the membrane, removal of residual contaminants and elution of pure genomic DNA. The eluate contained pure genomic DNA was stored for further use at -20°C in the deep freezer.

b. Preparation and running of agarose gel

For gel preparation of 0.8% concentration, 1.6g of

Table 1. Details of locations surveyed for sampling

States	District	Place of collection	Agro- climatic zones	Latitude	Longitude	Altitude (mamsl)
Himachal Pradesh	Shimla	Rohru	High hill	31° 12' 9.68" N	77° 45' 17.42" E	1554
	Solan	Nauni	Mid hill	31° 51' 44.75" N	77° 10' 9.15" E	1275
	Una	Rampur	Low hill	31° 27' 53.75" N	76° 16' 8.90" E	393
Punjab	Jalandhar	Phagwara	Central plain zone	31° 13' 26.4720" N	75° 46' 14.8728" E	234
	Firozpur	Dindsha	Western plain zone	30° 55' 24.3588" N	74° 36' 36.7704" E	182
Haryana	Karnal	Brass	Eastern zone	29° 41' 8.2644" N	76° 59' 25.9692" E	240
	Bhiwani	Chang	Western zone	28° 47' 56.5656" N	76° 8' 0.6504" E	225

Agarose powder (SeaKem® LE Agarose) was mixed with 200ml of 1X TAE (Tris-acetate-EDTA) buffer Solution in 250ml volumetric flask and the solution was heated until it was completely dissolved. For the visualization of nucleic acids, 2 µl Ethidium Bromide was added to the solution after the solution get cooled to 50-55°C. The solution was poured into the casting tray with the comb for solidification. After the gel gets solidified the comb was removed immediately. During the electrophoresis the gel was kept in the plastic tray, the wells were toward the negative electrode and the gel was submerged under 1X TAE buffer. For the detection of DNA in the sample, 2 µl of isolated DNA mixed with 2 µl 6X loading dye (Bromophenol Blue) was placed into the well carefully. Alongside the DNA samples, a DNA ladder was also placed in the well and the gel was run at 90 volts for 45 min to confirm the extraction. DNA was visualized by staining with ethidium bromide when ample migration has done (Corley, 2004).

c. PCR amplification

The PCR amplification of DNA samples was carried out in a thermocycler (ProFlex$^{\text{TM}}$ Base) using 20 µl reaction volume. The reaction mixture contained PCR pre-mix which is composed of Taq Buffer, dNTPs, $MgCl_2$, Taq DNA Polymerase along with the pair of primers, distilled water and genomic DNA.

Primers: The PCR amplification of mitochondrial region (COX1) was done with the universal primer for arthropods LCO1490/HCO2198 (Table 2).

PCR amplification*:* The reaction mixture was added to PCR tubes and the thermal cycler was programmed for 35 cycles with one cycle of initial denaturation at 94°C for 3 minutes whereas denaturation, annealing and elongation were repeated 36 times for 1 minute each at 94°C, 52°C and 72°C respectively. A final extension at 72°C for 30 minutes was carried out.

d. Visualization of PCR product

PCR product 2 µl from each tube was mixed with 2µl loading dye separately on a parafilm sheet and then loaded on 1.5 percent agarose gel (submerged in 1X TAE buffer and stained with 2 µl ethidium bromide for the detection of DNA) along with 2 µl DNA ladder (Genei 3kb DNA ladder) to determine the size of the amplified products. Then the gel was run at 90 volts for 60 minutes. Later the image analysis and photography were done under UV light by using gel documentation system (BIO-RAD, Model- Universal Hood II) and Image Lab™ Software.

e. DNA Sequencing and analysis

PCR product 10 µl from each sample was sent to Eurofins Genomic India Pvt. Ltd., Bangalore, Karnataka for the sequencing. The Sanger dideoxy method was used for sequencing of *L. orbonalis* samples. The sequences were subjected to BLAST (Basic Local Alignment Search Tool) in search of the homology of the partial sequences and were compared with similar sequence from NCBI database in order to find their phylogenetic relationship. The phylogenetic tree was constructed based on the CO1 sequence of *L. orbonalis* with Neighbor-Joining (NJ) method by using Mega X software (Saitou and Nei, 1987).

f. Genetic divergence: The genetic divergence was shown in terms of the number of base substitutions per site from between sequences. Analyses were conducted using the Maximum Composite likelihood model (Tamura et al., 2004) and evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).

Submission of sequence: The aligned nucleotide sequence in FASTA format was submitted to NCBI with all the necessary details. BankIt was used for the submission of nucleotide sequences to NCBI (Wheeler et al., 2001; Benson et al., 2003), from where accession numbers for all the sequences were received.

3. RESULTS AND DISCUSSION

3.1. Genetic Variability

The universal COI primer sequence was successfully used to amplify the COX1 region from all the DNA isolates. The amplified PCR product of DNA isolates (Figure 1).

3.2. Analysis of Nucleotide Sequence

To find out the per cent similarities, the nucleotide

Base sequence of COI primer

Primer	Sequence (5´ to 3´)
Forward	GGTCAACAAATCATAAAGATATTGG
Reverse	TAAACTTCAGGCTGACCAAAAAATCA

Table 2. Primers along with their nucleotide sequences and properties

Primer	Sequence (5´ to 3´)	Molecular weight (g/mol)	Length (Base pair)	Temperature (°C)	GC content (%)
Forward	GGTCAACAAATCATAAAGATATTGG	7722.1	25	56.4	32.0
Reverse	TAAACTTCAGGCTGACCAAAAAATCA	7940.2	26	58.5	34.6

sequences of *L. orbonalis* were aligned with the reference of NCBI (National Centre for Biotechnology Information) using BLAST tool (Basic Local Alignment Search Tool) which revealed the taxonomic identifications *viz.,* genus, species, sub-species and family and a comparative analysis of test sequence and reference sequence (NCBI database) were provided. The mtCOI sequences of *L. orbonalis* from all seven locations of the present study were submitted to the NCBI GenBank vide accession numbers (Table 3).

The populations of *L. orbonalis* have changed to adapt the ill-effects by changing their eco- behavioral pattern, feeding physiology and reproduction, in addition to the changes in their molecular machineries. It is therefore necessary to explore the genotypic variations that occur within the population of a species to design and formulate effective insect pest management strategies. DNA-based identification system, which is in the mitochondrial gene, cytochrome c oxidase subunit 1 (COI), can help in taxonomic classification of animal kingdom (Hebert et al., 2003). Chang et al. (2014) reported that *L. orbonalis* is the most detrimental South and Southeast Asian insect pest of eggplant. To help reduce the impact of this pest, population genetic diversity and structure of *L. orbonalis* were examined in eight populations from six countries using mitochondrial cytochrome c oxidase subunit I DNA sequences. Therefore, present investigation was undertaken on brinjal shoot and fruit borer, *L. orbonalis* for genetic diversity of different population.

For the genetic variability of *L. orbonalis,* the genomic DNA was collected from different locations of Himachal Pradesh, Punjab and Haryana and PCR analysis was performed using mitochondrial DNA specific primer. The universal base sequence of COI primers was successfully used for the amplification of COX1 region from all the DNA isolates and resultant amplicon of expected size around 600-700 bp was obtained and sequenced. Under analysis of nucleotide sequences, sequences of *L. orbonalis* were aligned with the reference of NCBI (National Centre for Biotechnology Information) using BLAST tool (Basic Local Alignment Search Tool) which revealed the taxonomic identifications. The accession number for all the samples were submitted.

3.3. Variation in Composition of Nucleotides

The present COI sequences of *L. orbonalis* from different regions of Himachal Pradesh, Punjab and Haryana showed significant variations in their nucleotide contents (Table 4). The average AT and GC content of all the specimen was 70.00% and 29.93%, respectively. The maximum AT content

Figure 1. Visualization of DNA samples on agarose gel electrophoresis (Where Ladder= 3Kb DNA ladder and DNA samples *viz.,* S11= Solan, S21= Una, S31= Shimla, H11= Bhiwani, H21= Karnal, P11= Jalandhar, P21=Firozpur

Table 3. GenBank accession no. of mtCOI sequences of the *Leucinodes orbonalis* collected from different locations

States	District	Place of collection	Submission ID	Gen Bank accession no.
Himachal Pradesh	Shimla	Rohru	SUB8692751	MW363534
	Solan	Nauni	SUB8691815	MW344268
	Una	Rampur	SUB8692807	MW363538
Punjab	Jalandhar	Phagwara	SUB8722720	MW363537
	Firozpur	Dindsha	SUB8722721	MW363535
Haryana	Karnal	Brass	SUB8722711	MW363539
	Bhiwani	Chang	SUB8722709	MW363536

(70.93%) was from the population of Haryana (Chang-Bhiwani) and the minimum AT content (69.45%) from the population of Himachal Pradesh (Rohru- Shimla). The highest GC content (30.53%) and lowest GC content (29.02%) was from Rohru- Shimla and Chang- Bhiwani, respectively. Overall, the average nucleotide compositions for *L. orbonalis* were found to be adenine (30.46%), thymine (39.57 %), guanine (14.02 %) and cytosine (15.93%), respectively.

The highest adenine (A) was observed from the Phagwara- Jalandhar specimen (31.60%) and lowest (30.06%) from Rampur- Una specimen. The highest percentage of thymine (T) with a value of 40.27 per cent was recorded from Chang-Bhiwani specimen and lowest 38.52 per cent was from Phagwara- Jalandhar specimen. The highest percentage of guanine (G) was 14.28% in the Nauni–Solan specimen, while the lowest was 13.27% in the Chang–Bhiwani specimen. In contrast, cytosine (C) content was highest in the Rohru–Shimla specimen (16.31%) and lowest in the Chang–Bhiwani specimen (15.75%).

The sequence region showed AT rich nucleotide composition typical of insect mitochondrial gene (Crozier and Crozier, 1993). The average AT and GC content of all the specimen was 70.00 and 29.93%, respectively. Overall, the average nucleotide compositions for *L. orbonalis* were adenine (30.46%), thymine (39.57 %), guanine (14.02 %) and cytosine (15.93%), respectively. The present observations on nucleotides composition of *L. orbonalis* slightly differed from the results of Shashank et al. (2015). They reported mean nucleotide compositions for *L. orbonalis* from different locations of India to be A (31.8 %), T (40.0 %), G (14.9 %) and C (13.3 %), respectively, which is in accordance with the results obtained in the present study.

3.4. Phylogenetic Analysis

The phylogenetic tree was constructed with Neighbour-Joining (NJ) method using Mega X programme (Saitou and Nei, 1987). This analysis recognized two distinct clades (Figure 2); the population of *L. orbonalis* from Shimla (MW363534), Solan (MW344268), Una (MW363538), Karnal (MW363539), and Jalandhar (MW363537) were clustered together in clade I due to their close proximity, while population of *L. orbonalis* from Firozpur (MW363535) and Bhiwani (MW363536) were clubbed in clade II. Second clade show some genetic difference in the phylogenetic analysis and molecular characters, which does not hold much significant importance in context to species evolution but their variability suggests the impact of geographical evolution of genetic behavior. However, both clades are of the same genera and species.

Under phylogenetic analysis of all the samples collected it was resulted that the pair-wise genetic distance of *L. orbonalis* populations collected from different regions of Himachal Pradesh, Punjab and Haryana ranged from 0 to 0.0044. This suggests that there is not much variation in the population of *L. orbonalis* from different locations. Shashank et al. (2015) also reported genetic distance amongst *L. orbonalis* population that ranged from 0.00 to 0.016 in different geographical localities of India and inferred that there was no significant molecular diversity. Murali et al. (2017) reported the pair-wise genetic distance of *L. orbonalis* populations collected from different regions of India which ranged from 0.00 to 0.051 and inferred that there was no significant molecular diversity within *L. orbonalis* of different geographical locations of India with respect to COI but there is difference in genetic distance among the populations.

The pair-wise genetic distance (Table 5) of *L. orbonalis* populations collected from different regions of Himachal

Table 4. Average nucleotide composition of COX-1 gene sequences among all the *Leucinodes orbonalis* specimens collected from different locations

DNA isolates	A	T(U)	G	C	AT	GC
Rohru- Shimla	30.33	39.12	14.22	16.31	69.45	30.53
Nauni- Solan	30.10	39.78	14.28	15.82	69.88	30.10
Rampur- Una	30.06	39.86	14.16	15.90	69.92	30.06
Phagwara- Jalandhar	31.60	38.52	13.85	16.01	70.12	29.86
Dindsha- Firozpur	30.54	39.56	14.06	15.82	70.10	29.88
Brass- Karnal	30.50	39.43	14.16	15.90	69.93	30.06
Chang- Bhiwani	30.66	40.27	13.27	15.75	70.93	29.02
Average	30.46	39.57	14.02	15.93	70.00	29.93

Pradesh, Punjab and Haryana ranged from 0 to 0.0044. This suggests that there is not much variation in the population of *L. orbonalis* from different locations.

The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Maximum Composite Likelihood model (Tamura et al., 2004). This analysis involved seven nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 478 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).

Significant genetic variation among the populations was observed by Murali et al. (2021) and Kar et al. (2021). A wide range of genetic variation occurs in the genome of *L. orbonalis* collected from different geographical locations. Murali et al. (2021) investigated the genetic variability of *L. orbonalis* in the region of Tamil Nadu, South India and during their study, 60 RAPD ten-mer primers were used. Among all the primers used, 10 primers generated reproducible and scorable banding profile. Highest genetic diversity over 80 per cent genetic polymorphism was shown

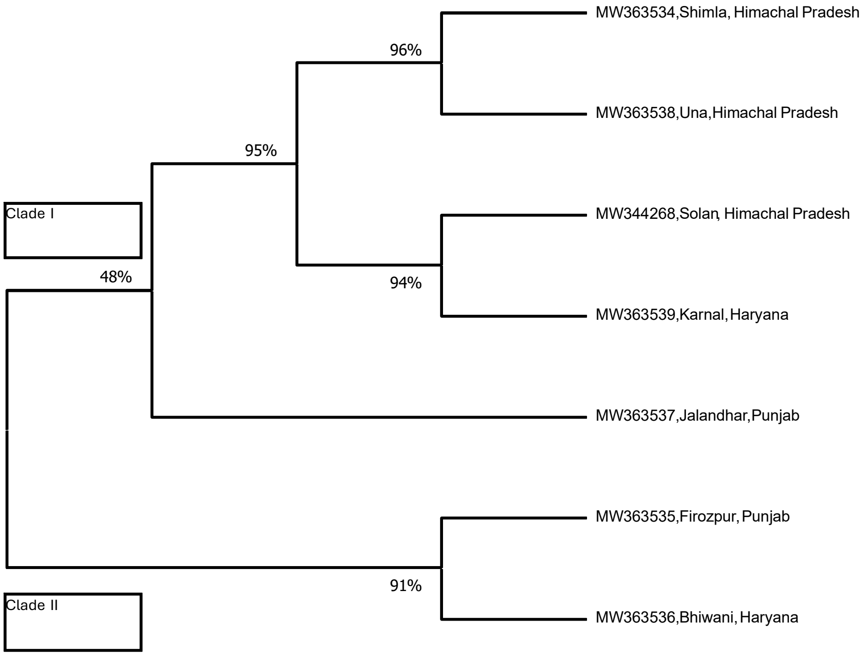

Figure 2. Phylogenetic tree of isolates of *Leucinodes orbonalis* collected from different locations of Himachal Pradesh, Punjab and Haryana

Table 5. Genetic divergence between COI sequences of nucleotide composition of COX-1 gene sequences among all the *Leucinodes orbonalis* specimens collected from different locations

Isolates	Locations	Isolates						
		1	2	3	4	5	6	7
1	Rohru- Shimla							
2	Nauni- Solan	0.0022						
3	Rampur- Una	0.0000	0.0022					
4	Phagwara- Jalandhar	0.0000	0.0000	0.0000				
5	Dindsha- Firozpur	0.0022	0.0044	0.0022	0.0000			
6	Brass- Karnal	0.0000	0.0000	0.0000	0.0000	0.0022		
7	Chang- Bhiwani	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	

by primers *viz.,* OPG 7, OPG 8, OPS 2 and OPS 7. Hence, significant genetic variation was observed among the population. Kar et al. (2021) also studied genetic variability among geographically confined populations of *L. orbonalis* from 30 locations of Odisha using twenty random RAPD markers. The PCR amplification resulted into 118 bands by 10 primers and 116 bands were found to be polymorphic out of which 13 were unique bands. Genetic similarity varies from 0.37 to 0.93, with a mean of 0.65 which indicated a significant genetic variability among the populations. COI is widely used for determining the genetic diversity of *L. orbonalis* among the populations. Kariyanna et al. (2022) observed genetic diversity of *L. orbonalis* populations from different geographic locations across the world and observed that no significant variability was found but difference in the amino acid composition was resulted.

Several control strategies have been established for the management of *L. orbonalis.* But due to insecticidal resistance, various studies have reported on genetic diversity of *L. orbonalis* population of different locations so that a better understanding in the management strategies for these pests occurs. Thus, adoption of molecular marker method has been an effective tool in studying the genetic variability of *L. orbonalis* of different populations along with identifying and characterizing the insecticides resistance in populations of *L. orbonalis.*

CONCLUSION

This study revealed important insights into the genetic characterization of *Leucinodes orbonalis* populations across different regions. Molecular analysis showed the formation of two distinct clades: one comprising populations from Firozpur and Bhiwani with a similarity index of 91%, and the other including populations from Shimla, Solan, Una, Jalandhar, and Karnal with a similarity index of 95%. Pair-wise genetic distance among populations ranged from 0 to 0.0044, indicating low overall genetic divergence and a relatively homogeneous population structure. From a pest management perspective, this genetic uniformity suggests that control measures whether biological, chemical, or biotechnological—could be uniformly effective across regions without significant risk of localized resistance. Nonetheless, the slight divergence observed, particularly between the two clades, underlines the importance of ongoing regional monitoring to detect emerging variations. Integrating these molecular findings with ecological data, such as life fertility parameters, can contribute to the development of targeted, sustainable, and region-specific management strategies.

Acknowledgments

The authors are highly grateful to the Department of Entomology, College of Horticulture, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh, India) for conferring the laboratory facilities.

Authors' Contributions

Rakesh Daroch conceived and designed the experiments. Vanshdeep Dhanda performed the experiments. Vanshdeep Dhanda and Rakesh Daroch analyzed the data. Vanshdeep Dhanda and Chetna Mahajan wrote the paper and contributed to the critical review and editing of the manuscript. All authors read and approved of the final manuscript.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this research paper.

REFERENCES

Alyokhin, A., Baker, M., Mota-Sanchez, D., Dively, G., & Grafius, E. (2008). Colorado potato beetle resistance to insecticides. *American Journal of Potato Research, 85*, 395-313.

Baker, B.P., Green, T.A., & Loker, A.J. (2020). Biological control and integrated pest management in organic and conventional systems. *Journal of Biological Control*, *140,* 104095.

Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., &Wheeler, D.L. (2003). GenBank. *Nucleic Acids Research*, *31*, 23-27.

Chakravarthy, A.K. (2015). New Horizons in Insect Science: Towards Sustainable Pest Management. *Springer India,* 239-257.

Chang, J.C., Ponnath, D.W., & Ramasamy, S. (2014). Phylogeographical structure in mitochondrial DNA of eggplant fruit and shoot borer, *Leucinodes orbonalis* Guenee (Lepidoptera: Crambidae) in South and South-East Asia. *Mitochondrial DNA, 27*, 1-7.

Corley, R.B. (2004). A guide to methods in the biomedical sciences. 1st ed. Springer, New York ISBN: 0-387-22845-4.

Crozier RH and Crozier YC. 1993. The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. *Genetics, 113*, 97-17.

Hebert, P.D.N., Ratnasingham, S., & deWaard, J.R. (2003). Barcoding animal life: cytochrome c oxidase subunit 1 divergenences among closely related species. *Proceedings of the Royal Society of London. Series B: Biological Science, 270*, 596-599.

Kar, D., Ananya, K., Asit, R., Mahendra, G., Bibhudutta, P., & Budhadev, M. (2021). Genetic diversity of brinjal shoot and fruit borer (BSFB) populations of Odisha, India. *Iranian Journal of Science and Technology*, *Transaction A: Science*, *45*(1), 135-144.

Kariyanna, B., Bheemanna, M., Suprakash, P., Suraj, S., & Rupsanatan, M. (2022). Genetic variation and molecular tools for the management of brinjal shoot and fruit borer *Leucinodes* *orbonalis* Guenee (Lepidoptera: Crambidae) in Genetics methods and tools for managing crop pests, edited by AK Chakravarthy. *Springer Nature, Singapore*, 391-407.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K., (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution, 35*, 1547-1549.

Murali, S., Jalali, S.K., Shylesha, A.N., Swamy, T.M.S., Baskar, R., & Mruthunjayaswamy, P. (2017). Molecular characterization and their phylogenetic relationship based on mitochondrial cytochrome oxidase I of brinjal shoot and fruit borer, *Leucinodes orbonalis* (Guenee) (Lepidoptera:Pyralidae). *International Journal of Current Microbiology and Applied Science, 6*, 2527-2539.

Murali, P., Hilda, K., Ramakrishnan, M., Ganesh, A., Bhuvaragavan, S., & Janarthanan, S. (2021). Molecular genotypic diversity of populations of brinjal shoot and fruit borer, *Leucinodes orbonalis* and development of SCAR marker for pesticide resistance. *Molecular Biology Reports, 48,* 7787-7800.

Padwal Kanchan, G., & Srivastava, C.P. (2020). Phenotypic variability in *Leucinodes orbonalis* (Guenee) populations collected from different Agro-climatic zones of India. *Journal of Experimental Zoology India, 23*(1), 345-352.

Palraju, M., Paulchamy, R., & Janarthanan, S. (2018). Population genetic structure and molecular diversity of *Leucinodes orbonalis* based on mitochondrial COI gene sequences. *Mitochondrial DNA Part A*, *29*, 1231-1239.

Saini, T., Alamalakala, L., Parimi, S., Char, B., & Zehr, U. (2006). AFLP Variation within and among Indian populations of eggplant fruit and shoot borer (*Leucinodes orbonlis* Guenee). The 2006 ESA Annual Meeting.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*, 406-425.

Sharma, A., Rana, R.S., Sharma, K.C., Kumar, A., & Singh, S. (2017). Screening of brinjal cultivars resistance against brinjal shoot and fruit borer (*Leucinodes orbonalis* Guenee). *International Journal of Entomology Research, 2*(5), 69-75.

Shashank, P.R., Ojha, R., Venkatesan, T., Jalali, S.K., & Bhanu, K.R.M. (2015). Molecular characterization of brinjal shoot and fruit borer, *Leucinodes orbonalis* (Guenée) (Lepidoptera: Crambidae) based on mitochondrial marker cytochrome oxidase I and their phylogenetic relationship. *Indian Journal of Experimental Biology*, *52*, 51-55.

Singh, H., Kumar, A., Sharanappa., Sahu, R., & Khan, H.H. (2019). Efficacy of certain insecticides against Brinjal shoot and fruit borer [*Leucinodes orbonali*s (Guenee)] under field condition. *International Journal of Chemical Studies*, *7*(1), 2049-2051.

Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the Neighbor-Joining method. *Proceedings of the National Academy of Sciences USA*, *101,* 11030-11035.

Thapa, R.B. (2010). Integrated management of brinjal fruit and shoot borer, *Leucinodes orbonalis* Guen: an overview. *Journal of Institute of Agriculture and Animal Science, 30*, 1–16.

Wheeler, D.L., Church, D.M., Lash, A.E., Leipe, D.D., Madden, T.L., Pontius, J.U., Schuler, G.D., Schriml, L.M., Tatusova, T.A., Wagner, L., & Rapp, B.A. (2001). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, *29*, 11-16.

Zhang, A.B., He, L.J., Crozier, R.H., Muster, C., & Zhu, C.D. (2010). Estimating sample sizes for DNA barcoding. *Molecular Phylogenetics and Evolution, 54*, 1035-1039.