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Incidence, Level of Damage and Identification of Insect Pests of Fruits and Leaves of *Ziziphus* Tree Species in Ethiopia

Tigabu R. Alle [1,2,*], Abdella Gure [3], Miriam F. Karlsson [4] and Samora M. Andrew [2,5]

[1] Sirinka Agricultural Research Centre, Woldia P.O. Box 74, Ethiopia
[2] Regional Research School in Forest Sciences, Sokoine University of Agriculture, Morogoro P.O. Box 3009, Tanzania; andrew.samora@udsm.ac.tz
[3] Wondo Genet College of Forestry and Natural Resources, Hawassa University, Shashamane P.O. Box 128, Ethiopia; abdellag66@gmail.com
[4] Department of Ecology, Swedish University of Agricultural Science, P.O. Box 7070, SE-750 07 Uppsala, Sweden; miriam.karlsson@slu.se
[5] Ecosystems & Conservation Group, College of Natural and Applied Sciences, University of Dar es Salaam, Dar es Salaam P.O. Box 35060, Tanzania
* Correspondence: redtiges1980@gmail.com; Tel.: +251-912347365; Fax: +251-333300084

Abstract: The *Ziziphus* tree species offer valuable socio-economic and ecological benefits but experience significant damage from insect pests. In Ethiopia, there is limited knowledge of the insects attacking *Ziziphus* fruits, and a study aimed to identify these pests, assess their impact and understand how different land use types (LUTs) affect them was conducted. Sampling involved collecting fifty fruits and ten leaves from each of ten randomly chosen *Ziziphus* trees per LUT within each agroecological zone from August to December in 2022 and 2023. Samples were visually assessed for incidence and infestation levels, and the five morphotypes were identified using molecular techniques through phylogenetic analysis. Fruit pest incidence varied during the season, yet a positive correlation (r = 0.84) was observed among the months and years when assessment took place. Most fruits showed low to medium infestation levels (5%–50%), while severe infestations (>75%) were predominant in the lowland agroecological zone. The insects that had caused the damage were identified as *Carpomya incompleta* Becker, 1903; *Drosophila hydei* Sturtevant, 1921; *D. simulans* Sturtevant, 1919 and *Zaprionus indianus* Gupta, 1970. Fruits showed higher incidence and infestation levels than leaves, indicating significant yield and income losses. Thus, implementing effective management strategies is vital to minimize these losses and achieve sustainable production in Ethiopia.

Keywords: land use; farmland; home garden; roadside; pest severity; yield losses

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1. Introduction

The genus *Ziziphus* (Mill.) (Paliureae, Rhamnaceae) comprises more than 135 plant species [1]. Ethiopia hosts four *Ziziphus* species, including *Ziziphus spina-christi* (L.) Desf, *Z. mucronata* Willd., *Z. mauritiana* Lam. and *Z. abyssinica* Hochst [2,3]. The *Ziziphus* tree species are tropical resources that offer substantial economic, environmental and social benefits to rural communities inhabiting arid and semi-arid regions [4,5]. The trees are steadily gaining attention among local communities owing to their resilience to harsh climatic conditions and consistent fruiting even amidst drought and climate change [6,7]. However, despite manifold benefits and growing community interest, *Ziziphus* production encounters setbacks attributable to various biotic and abiotic factors. Among these, the most significant biotic hindrances arise from insect pests, which commonly afflict *Ziziphus* leaves, flowers and fruits [8,9]. The presence of insect pests is a substantial risk factor diminishing both the quantity and quality of *Ziziphus* fruits and leaves [10], resulting in significant losses in production [11–13]. The species of insect pests and the incidence and intensity of these pests are subject to fluctuations in climatic conditions, seasonal changes, agricultural practices, ecological factors and the species of the *Ziziphus* tree [14].

Fruit flies (Diptera: Tephritidae), fruit borers (Lepidoptera: Carposinidae), termites (Termitidae: Isoptera), stone weevils (Coleoptera: Curculionidae), bark-eating caterpillars (Cossidae: Lepidoptera), scale insects (Hemiptera: Coccoidea) and mites (Acari: Tetranychidae) are among the common pests of *Ziziphus*, each inflicting varying levels of infestation [15–17]. Tephrididae fruit flies have previously been reported as the primary menace among the plethora of insect pests affecting *Ziziphus* species, posing severe threats to fruit growth, yield and quality, particularly affecting mature fruits [18,19]. Fruit fly infestations can result in significant reductions in fruit yield, reported as 13%–20% [20], 36%–40% [15] and, in extreme cases, up to 80%–100% [14]. Additionally, lepidopteran fruit borers cause substantial losses, with potential impacts of up to 70% [21]. Moreover, factors such as fruit maturity stage, seasonal and weather conditions and human activities including industrial operations and residential encroachments contribute to the variation in severity of insect pest infestations [12,19].

Assessing and evaluating the incidence and infestation levels of insect pests impacting the quality and quantity of *Ziziphus* fruits and leaves across diverse land uses and seasons is essential for determining variations and pinpointing critical infestation periods throughout the year [22]. Moreover, identifying the insect pests is crucial for determining the economically important pests and devising improved integrated management strategies. Morphological and molecular identification techniques serve as appropriate methods for identifying specific types of pests. Morphological analysis entails investigating the morphological characters of different body parts, which is crucial for easily identifying the taxa [23,24]. Molecular species identification, utilising genetic markers, represents a valuable addition to complement traditional morphological methods [25,26]; this approach, often achieved through mitochondrial DNA barcoding, involves the use of short DNA sequences for species identification [27,28].

The production and utilisation of *Ziziphus* fruits for food and other purposes is becoming increasingly common in Ethiopia mainly among the rural communities in some parts of Ethiopia. It is more valued as a food, feed and medicinal plant in the northern parts of Ethiopia than in other parts of the country, being particularly valued in some parts of the Tigray and Amhara regions where frequent drought can cause food shortages. It is known that *Ziziphus* fruits are not only used for domestic consumption by rural communities but also sold in the markets as a supplementary source of cash income for households.

Despite its increasing significance as an important tree species of multiple uses, and given the fact that *Ziziphus* fruit production is challenged by biotic and abiotic agents, particularly by insect pests, pertinent information regarding the species of insect pests affecting *Ziziphus* fruits and their incidence and infestation levels in Ethiopia is notably lacking. There is also a dearth of knowledge concerning the correlation between *Ziziphus* fruit insect pest incidence and other underlying factors such as agroecology and seasonal variations in the country. The current study was therefore conducted to document the species of insect pests associated with *Ziziphus* fruit damage and to assess their incidence and infestation levels in Ethiopia. The study hypothesized that there are relationships between Ziziphus fruit insect pest incidence and different land use types.

2. Materials and Methods

2.1. Study Sites

The study was conducted in three distinct land use types (LUTs), namely, farmland (FL), home garden (HG) and roadside (RS), in lowland and midland agroecological zones in the Bosset district of the East Shewa Zone of the Oromia regional state and the Bati district of the Oromia Special Zone of the Amhara regional state, respectively, in Ethiopia (Figure 1). These two zones within the Oromia regional state and the Amhara regional state were deliberately chosen due to the presence of *Ziziphus* species trees. Geog raphically, Bosset and Bati are situated at 8°34′59″ N and 39°28′59″ E and at 11°11′59″ N and 40°1′59″ E, respectively.

Bosset's elevation ranges from 1400 to 2500 m above sea level, while Bati's elevation varies from 1800 to 2200 m above sea level. The agroecological zones in the two districts also differ slightly in temperature, with temperatures fluctuating between 26 °C and 34 °C in Bosset and between 23 °C and 30 °C in Bati. Regarding precipitation, Bosset experiences abundant rainfall from June to August and a dry season from September to February, followed by a short rainy season from March to May. Similarly, Bati receives elevated rainfall between June and August, a dry spell stretching from September to February and a short rainy season between March and April. Bosset receives a mean minimum and maximum annual rainfall of 600 and 900 mm, respectively, while Bati receives 550–700 mm [29].

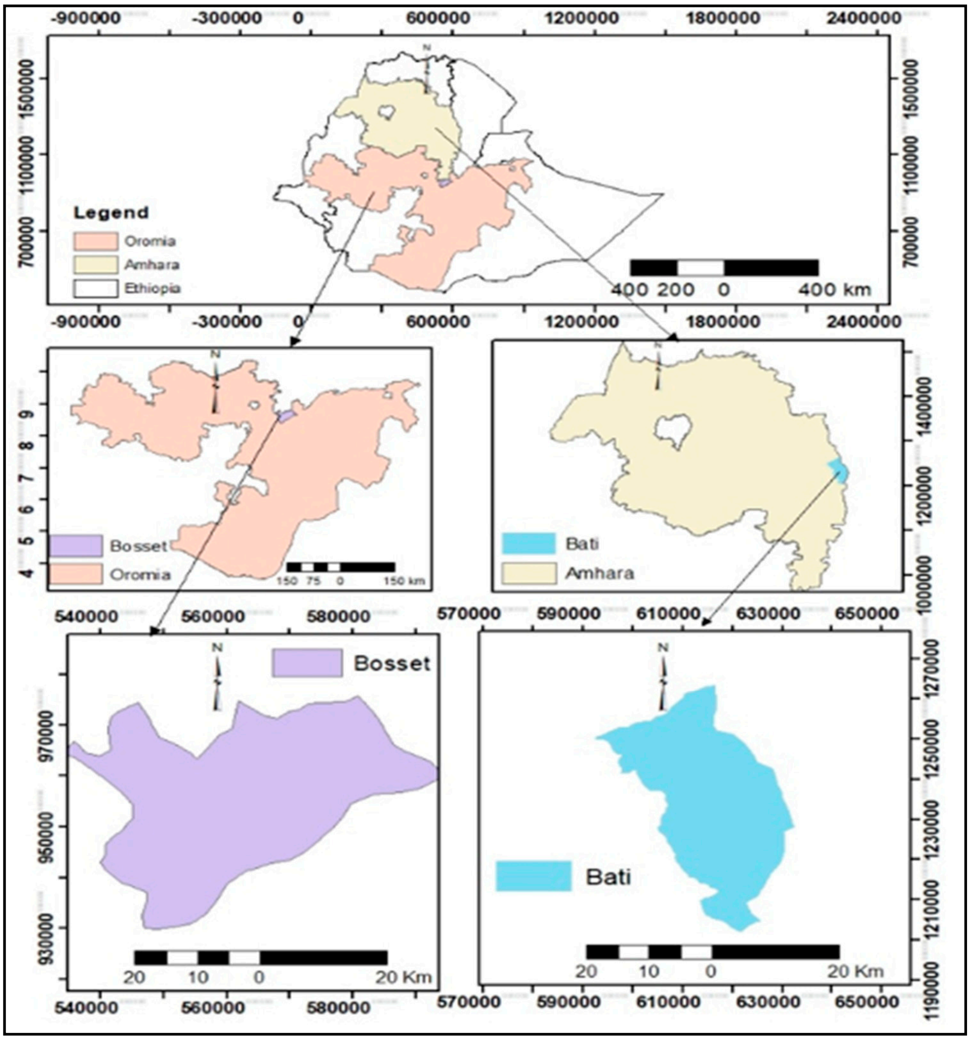

Figure 1. Location map of the study districts in Ethiopia.

2.2. Sampling and Data Collection

A three-stage stratified purposive sampling technique was employed to identify and select LUTs where *Ziziphus* species abundantly grow as well as areas where fruit collection and utilisation are actively practiced. As the initial stage, a reconnaissance survey was conducted in the North Omo Zone and Konso Zone of the Southern Nations, Nationalities and Peoples regional state, the East Shewa Zone of the Oromia regional state and the Oromia Special Administrative Zone and the South Wollo Zone of the Amhara regional state together with natural resource experts for the respective zones to identify suitable field sites for the study. Subsequently, two zones, namely, the East Shewa zone of the Oromia regional state and the Oromia Special Zone of the Amhara regional state, were selected based on the availability of *Ziziphus* trees and the culture of collection and utilization of *Ziziphus* fruits. Due to the difficulties in distinguishing *Ziziphus* spp. from each other, we have grouped them under the name *Ziziphus* spp. In the second stage, two suitable districts, one in each of the selected zones (the Bosset district on the East Shewa Zone and the Bati district of the Oromia Special Administrative Zone) were selected. In the third stage, three LUTs, namely, FL, HG and RS, were identified within each of the selected districts.

After the selection of suitable LUTs, a total of 30 trees from each of the districts (10 from each LUT) were randomly selected following FAO guidelines [30] and evaluated for incidence [31] and level of infestation [32]. Accordingly, approximately 50 ± 15 fruits and 10 ± 5 leaves per tree were collected randomly from ten trees from each of the LUTs in Bosset during the period from August to November and Bati during October–December in 2022 and 2023. Those months were selected for fruit collection because fruit maturation occurs in those months at the two study sites. Fruit collection involved shaking trees and beating the branches with long sticks, after which the fruits that fell to the ground were collected in paper bags that were properly labelled with the district name, land use type, tree number and date of collection. The fruits of each tree were transported from the field to the base station and separately sorted according to level of infestation into different categories based on criteria established by [32], which categorise infestation levels as very low ($\leq$5%), low (6%–10%), medium (11%–20%), severe (21%–50%) or very severe (>50%).

Similarly, leaf samples were collected from each of the ten trees from three randomly selected branches, one each from the bottom, middle and top of the crown, at a rate of 10 leaves per branch for a total of 30 leaves per tree; the leaves were placed in a paper bag labelled as described above in the case of fruit collection. The leaf samples were transported to the base camp for further evaluation. At the base camp, after mixing the 30 leaves obtained from the three levels of the crown of a sampled tree, 10 leaves per tree were randomly selected for evaluation of the incidence and level of infestation by insect pests on a scale from 0 to 5 through the visual scoring method described by [31]. According to this method, the different levels of leaf infestation are 0 = nil, no infestation; 1 = low (less than 5% of leaf area affected); 2 = medium (5%–20% of leaf area affected); 3 = severe (20%–50% of leaf area affected); 4 = very severe (50%–80% of leaf area affected); and 5 = extreme damage (80%–100% of leaves affected), complete or near-complete defoliation. The incidence of insect pests was calculated as a percentage of the total fruits and leaves inspected.

2.3. Insect Rearing and Identifications

To document and characterize insect pests affecting *Ziziphus* fruits in the study area, 15 fruits exhibiting the signs of insect damage were randomly selected from each LUT and incubated in insect-rearing cages at the forest entomology and pathology laboratory of Ethiopian Forestry Development (EFD) in Addis Ababa, Ethiopia, for 14–21 days. The insect-rearing cages were monitored daily to follow up on the development of the emerging insects, followed by adult counts every seven days. Matured insects were transferred to a killing jar containing a cotton swab immersed in 97% ethanol and transferred, using forceps, to clean vials (20 mL) for preservation. Insect specimens were stored at the EFD laboratory for DNA extraction until all necessary adults were collected from all study sites. Proper labelling comprising the site, agroecological zone and LUT from which the fruits were collected and the date of collection was ensured for both the rearing cages and the insect preservation vials.

Insects were identified based on morphological features including wings, legs, head, body colours, antennae, thorax and abdominal size and associated structures following available catalogues and identification keys [23,24]. Examination of insect structures was carried out using an EMZ-5 binocular 7×–45× zoom stereo microscope, and photographs were taken using a digital camera and sorted into morphotypes for documentation. Subsequently, based on similarity in morphological features, the insect specimens were categorized into ten different groups assumed to be different species. For the identification of the insects, besides the morphological features, molecular data were also employed. Molecular species identification, utilising genetic markers, represents a valuable addition to complement traditional morphological methods [23,33]. This approach, often achieved through mitochondrial DNA barcoding, involves the use of short DNA sequences for species identification [27,28]. Accordingly, the morphological categorization was followed by the sequencing of a genetic marker, Cytochrome c oxidase subunit 1 (COI), for molecular identification [34]. Polymerase chain reaction (PCR) and PCR-based typing methods serve as superior markers, making them ideal tools for species diagnosis [35,36].

2.4. DNA Extraction

Following the morphological categorization of the insect specimens as described above, two insect specimens from each of the morphological groups were randomly selected for genomic DNA extraction. DNA was extracted from whole insect bodies [37] using the Quick-Start Protocol, and extraction of genomic DNA was conducted at the Holleta Agricultural Research Centre, National Biotechnology Institute, Holleta, Ethiopia. The DNeasy Blood and Tissue Kit (cat. nos. 69,504 and 69,506) was employed during the DNA extraction process. The DNA concentration was determined with an ultraviolet fluorescence spectrophotometer (Eppendorf, Germany), and the DNA was stored at −20 °C [38].

2.5. Polymerase Chain Reaction (PCR), DNA Sequencing and Phylogenetic Analyses

The amplification and sequencing of the COI region of DNA was conducted at the molecular biology laboratory of the International Livestock Research Institute (ILRI) in Nairobi, Kenya. Amplification of mitochondrial COI subunit 1 was carried out using the forward primer LCO1490 (5′-ggtcaacaaatcataaagatattgg-3′) and the reverse primer HC02198 (5′-taaacttcagggtgaccaaaaaatca-3′) with 2 U of Fast Start Taq DNA polymerase in a 20 µL reaction volume under standard conditions (L and H refer to light and heavy DNA strands, and the numbers (1490 and 2198) refer to the position of the 5′ nucleotide, while the 3′ end of each primer is on a second-position nucleotide) [39]. Four ladders (designated as molecular markers), each approximately 710 base pairs in size, confirmed the successful amplification of the COI target region. PCR was performed (200 µM each dNTP, 200 nM each primer, 2 mM $MgCl_2$) with 2 µL of the DNA extract as a template using the forward and reverse primers. The DNA fragments were amplified through 35 cycles with parameters of two minutes at 95 °C, one minute at 40 °C and one and one-half minutes at 72 °C, followed by a final extension step at 72 °C for seven minutes. PCR products were confirmed by gel electrophoresis using 1.5% *w*/*v* Hi-Res standard agarose/1X TAE gel (Cambridge Reagents, Thermo Scientific (Thermo Fisher Scientific, Waltham, MA, USA)), stained with the dye GelRed Nucleic Acid Gel Stain.

The amplified DNA fragments were sequenced by employing the Sanger dideoxy method [40]. Purified PCR product (25 ng/1 µL), primer (5 pmol/µL) and Big Dye Terminator v3.1 (0.5 µL) were brought to 10 µL in molecular H_2O and sequenced using the following protocol: 2 min at 96 °C (10 s at 96 °C, 10 s at 50 °C and 4 min at 60 °C) × 30 and a final hold at 72 °C for 4 min in an ABI 9700 thermocycler. Sequencing reactions were analysed on an Applied Biosystems 3730 DNA Analyzer. All sequences obtained from the genetic analyser were edited using BioEdit v. 7.7.1 bioinformatics software, and consensus sequences were obtained for each of the sequenced specimens [41]. After the consensus sequences were obtained, the DNA sequences were saved in FASTA format and were sent as query sequences to the National Centre for Biotechnology Information (NCBI) database to be verified through the Basic Local Alignment Search Tool (BLAST) [42]. Accordingly, after the BLAST search was performed in the NCBI database, matching sequences from the database providing possible species names with varying percentages of similarity to the query sequence along with additional descriptive information on the insect it originated from were obtained. Subsequently, the best sequence matches were saved and used as reference taxa for phylogenetic analysis and thus to confirm the identity of the insect species obtained from *Ziziphus* fruits collected during the current study.

For subsequent phylogenetic analysis, explorations of identical sequence arrangements were made using BLASTN, and the identities of the closest relatives of the sequences (similarity $\geq$ 99%) were retrieved from GenBank. The relationships of taxa were inferred using the neighbour-joining method [43]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was noted next to the branches [44]. The tree was drawn to scale, with branch lengths (next to the branches) in the same units as the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method [45] and presented in units of the number of base differences per site. The analysis encompassed 8, 12, 7, 9 and 13 nucleotide sequences for *Carpomya incompleta*, (Diptera: Tephritidae); the drosophilids *D. hydei* Sturtevant 1921, *Z. indianus* Gupta 1970, and *D. simulans* Sturtevant 1919 (all Diptera: Drosophilidae) and *Psyttalia concolor* Szepligeti 1910 (Braconidae) (Opiinae; Psyttalia), respectively. All positions containing gaps and missing data were eliminated using the complete deletion method. The final dataset comprised a total of 524, 450, 223, 597 and 532 positions for *C. incompleta*, *D. hydei*, *Z. indianus*, *D. simulans* and *P. concolor*, respectively. Supplementary Materials https://submit.ncbi.nlm.nih.gov/subs/sra/SUB14806558/metadata_SUB14806558 (accessed on 30 October 2024). Evolutionary analyses were conducted using Molecular Evolution Genetic Analysis (MEGA) MEGA11software version 11.0.10 [46].

2.6. Statistical Analysis

A one-way analysis of variance (ANOVA) was employed to examine variations in the percentage of incidence and infestation levels across the different LUTs, agroecological zones and fruit production years. Normality and homogeneity of the variances were checked using the Shapiro–Wilk test and Levene's test, respectively, prior to ANOVA [47] and considered significant at $p \geq 0.05$. Levene's test was used to calculate the homogeneity of variance of the data. The data exhibited normal distribution and homogeneity of variance the significance of mean differences was assessed through Tukey's Honest Significant Difference (Tukey's HSD) post hoc test. In testing statistical hypotheses, a significance level of $\alpha = 0.05$ was adopted. The Pearson correlation coefficient (r) was calculated to assess the relationships between insect pest incidences and agroecological zones (AEZ), LUTs, assessment months and fruit production years. Furthermore, linear regression analysis was employed to identify the factors contributing to the high incidence of insect pests on *Ziziphus* trees. To describe insect pest incidence percentage, a multiple linear regression model (MLRM) was used (Equation (1)). Regression analysis was tested against normality assumptions. The applicability of MLRM was tested by evaluating the linear relationships between the explanatory and explained variables.

$$\mathrm{Y} = \beta_0 + \beta_1 \mathrm{X}_1 + \beta_2 \mathrm{X}_2 + \cdots + \beta_p \mathrm{X}_p + \varepsilon \tag{1}$$

where β_0 is the y-intercept, y is the dependent variable, β_p is the slope coefficient for each explanatory variable, X_p are the explanatory variables and ε is the residual or model error term. The underlying hypothesis is that at least one explanatory variable has a significant effect on the incidence of insect pests on *Ziziphus* fruits.

Next, the multicollinearity of the explanatory variables was analysed. For the detection of multicollinearity between explanatory variables, the variance inflation factor (VIF) [48] was used (Equation (2)).

$$\mathrm{VIF} = \frac{1}{1 - R^2} \tag{2}$$

In the next step, the homoscedasticity distribution of regression residuals was analysed using Equation (3) [49]:

$$\mathrm{W} = nR^2 \tag{3}$$

where n is the number of observations and R^2 is the coefficient of determination of the auxiliary regression expressed by the following equation:

$$e^2 = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 + \beta_4 X_2 + \beta_5 X_1 \times X_2 \quad (4)$$

The applicability of MLRM was tested by evaluating the linear relationships between the explanatory and explained variables.

The critical value of the test is $X^2 = X^2\alpha, p$, where α is the level of significance and p is the number of variables in the auxiliary regression. The critical area is given by the following inequality:

$$nR^2 > X^2{}_{p-1,\,\alpha} \quad (5)$$

Finally, the Shapiro–Wilk test was used to check whether the residuals were normally distributed. All statistical analyses were performed using R free software version 4.3.2 [50].

3. Results

3.1. Factors Influencing Ziziphus Fruit and Leaves Insect Pest Incidences

The visual examination revealed insect pest infestations in both *Ziziphus* fruits and leaves and the damage was prevalent across all the study sites. Various symptoms of damage were observed on both fruits and leaves caused by various insect pests. Insect damage was evident across all agroecological zones and land uses and throughout the two-year assessment period. The timing of fruit collection (month) and production years were significant factors influencing the incidence of *Ziziphus* fruit insects, where variations were observed within months of each year (Table 1). Only the production year was found to influence leaf insect pest incidence percentage. Agroecological zone and land use did not influence leaf insect pest incidence (Table 1).

Table 1. Factors influencing the insect pest incidences of *Ziziphus* fruits in Ethiopia.

		Values							ANOVA	
Variables	**Coefficients (B)**	**S.E**	**t-Statistics**	**P**	**VIF**	**R**	**$R^2{}_{adj}$**	**RMSE**	**F-Value**	**P-Level**
Constant	95.8	2.43	39.4	0.000		0.298	0.096	10.1	11.6	0.000
AEZ	−2.3	2.25	−1.02	0.307	4.37					
LUT	0.26	0.66	0.40	0.690	4.37					
AY	−6.14	1.07	−5.71	0.000	1.00					
Constant	39.42	2.47	15.9	0.000		0.187	0.027	13.5		
AEZ	−1.81	2.83	−0.64	0.524	3.94				4.3	0.005
LUT	0.42	0.83	0.51	0.609	3.94					
AM	−0.21	0.06	−3.53	0.000	1.00					

AEZ = agroecological zone; LUT = land use type; AY = assessment year; AM = assessment month; B = slope coefficient; VIF = variance inflation factor; R = correlation coefficient; $R^2{}_{adj}$ = adjusted R squared; RMSE = root mean square error.

The incidence and infestation levels on *Ziziphus* fruits varied across assessment months in each fruit production year. The highest incidence overall occurred in November 2022, and November and December contained the highest incidence in 2023 (F = 7.69; df = 5; $p < 0.05$; Figure 2). The mean, standard deviation, minimum and maximum values were 83.8, 10.35, 77.8 and 88.7, respectively.

Insect pest incidence exhibited a significant correlation with the factors examined in this study. A significant positive correlation ($p < 0.05$) was observed between land use types and AEZ, as well as assessment months and production years over the two-year study period for both fruits and leaves (Table 2).

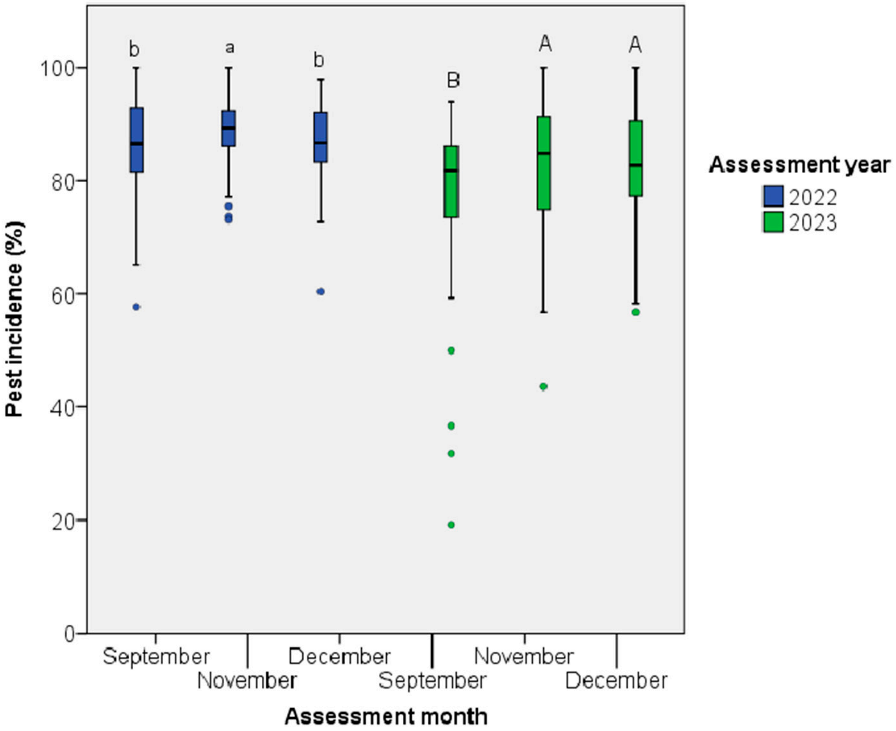

Figure 2. Mean ± SE incidence of insect pests on *Ziziphus* fruits across the assessment months during the 2022 and 2023 fruiting seasons in Ethiopia; a, b means marked with different letters to indicate statistically significant difference (means followed with the same letter within the same fruit production year are not significantly different at $p < 0.05$).

Table 2. Pearson correlation coefficients between determinants of insect pest incidence on *Ziziphus* fruits in Ethiopia.

Fruits				
Determinant Factors	**Insect Pest Incidence**	**AEZ**	**Land Use Types**	**Assessment Year**
AEZ	0.071			
Land use type	0.053	0.88 *		
Assessment year	0.289	0.001	0.00	
Assessment month	0.173	0.001	0.00	0.84 *
Leaves				
Determinant Factors	**Insect Pest Incidence**	**AEZ**	**Land Use Types**	**Assessment Year**
AEZ	0.02			
Land use type	0.01	0.86 *		
Assessment year	0.27	−0.02		
Assessment month	0.18	0.004	0.004	0.85 *

AEZ = agroecological zone; * = high significant association of insect incidence with the abiotic factors.

3.2. *Ziziphus Tree Fruits and Leaves Insect Pests Infestation Levels*

The average level of non-infested fruits per tree ranged from 6% to 13%. Most fruits had low to medium infestation levels, consistently observed over the study years. Significant variations emerged in severe- and very severe level infestation between the two assessment years, peaking in the 2023 fruiting season (Table 3). In contrast, insect pest occurrence on *Ziziphus* leaves was notably low, with the majority showing no signs of infestation, indicating overall leaf health. No significant differences were found among the three LUTs regarding leaf infestation levels (Table 3). However, despite the low infestation level, there was notable variation between the two fruiting years. Additionally, variations were observed between the two AEZs, with the lowland AEZ experiencing the highest leaf infestation levels. However, no instances of very severe pest infestation levels of leaves were recorded in either AEZ (Table 3).

Table 3. Incidence and infestation level of insect pests on *Ziziphus* fruits in three land use types in Ethiopia.

Fruits						
LUT	**Mean (±SE) Fruits Examined/Tree**	**Mean Infestation Level per Tree**				
		Very Low	**Low**	**Medium**	**Severe**	**Very Severe**
Farmland	58.0 ± 1.4	6.0 ± 0.4 ^{c}	20.8 ± 0.8	21 ± 0.9	9.0 ±0.7	5.3 ± 0.5
Home garden	56.9 ± 1.1	7.1 ± 0.5 ^{b}	19.9 ± 0.7	20.7 ± 0.9	8.6 ± 0.5	5.0 ± 0.5
Roadside	59.4 ± 1.2	8 ± 0.5 ^{a}	19.7 ± 0.7	21.7 ± 0.7	9.0 ± 0.6	5.1 ± 0.6
			AEZ			
Lowland	59.0 ± 0.9	9.8 ± 0.5	20.4 ± 0.5	21.6 ± 0.6	8.2 ± 0.4 ^{a}	5.9 ± 0.4 ^{b}
Midland	57.1 ± 0.8	9.0 ± 0.5	19.8 ± 0.5	20.6 ± 0.5	9.3 ± 0.4 ^{b}	4.2 ± 0.2 ^{a}
			Year			
2022	55.6 ± 0.6	7.1 ± 0.3 ^{b}	20.1 ± 0.4	20.8 ± 0.4	7.9 ± 0.3 ^{b}	4.9 ± 0.3
2023	60.6 ± 1	11.7 ± 0.6 ^{a}	20.2 ± 0.6	21.4 ± 0.6	9.8 ± 0.5 ^{a}	5.0 ± 0.3
Leaves						
LUT	**Mean (±SE) Number of Leaves Examined/Tree**	**Mean Infestation Level per Tree**				
		Nil	**Low**	**Medium**	**Severe**	
Farmland	52.6 ± 1.1	34.5 ± 1.1	13.1 ± 0.5	6.6 ± 0.4	4.5 ± 0.4	
Home garden	49.2 ± 1.1	32.7 ± 1.1	12.5 ± 0.4	6.5 ± 0.4	3.7 ± 0.4	
Roadside	53 ± 1.2	33.8 ± 1.2	13.4 ± 0.5	7.3 ± 0.5	4.4 ± 0.2	
			AEZ			
Lowland	51.1 ± 0.8	32.9 ± 0.8 ^{a}	12.8 ± 0.4	7.3 ± 0.3 ^{b}	4.2 ± 0.3	
Midland	52.7 ± 1.2	35.2 ± 1.1 ^{b}	13.6 ± 0.4	6 ± 0.4 ^{a}	4.1 ± 0.3	
			Year			
2022	50 ± 0.8	30.5 ± 0.6 ^{a}	14.2 ± 0.4 ^{b}	7.7 ± 0.4 ^{b}	4.1 ± 0.3	
2023	53.2 ± 1.1	36.9 ± 1.1 ^{b}	11.8 ± 0.4 ^{a}	6.0 ± 0.4 ^{a}	4.2 ± 0.2	

Means followed by a common letter in the same column for each factor (LUT, AEZ and year) are not significantly different at $p < 0.05$, Tukey's HSD test; means are followed by the standard error (SE); LUT: land use types; AEZ = Agroecological zones.

3.3. Morphological Characterization and Molecular Identification

In the present study, mature adults of insects that were captured from *Ziziphus* fruits incubated in insect-rearing cages in the laboratory were categorized into ten different groups based on similarities and differences in morphological characters of wings, legs, head, body colour, antennae, thorax and abdomen.

The BLAST search results with the DNA sequences obtained after sequencing the COI region of 20 insect specimens randomly selected from the ten morphologically separated groups revealed that, the insect groups collected from *Ziziphus* fruits in Ethiopia were related to six different insect species. Accordingly, based on the highest per cent similarity (≥99) of the query sequences with those of the GenBank sequences (Table 4), five of the 20 query sequences matched with *C. incompleta* and *C. vesuviana*, six with *D. hydei*, four with *Z. indianus*, three with *Simulium* spp., one with *D. simulans* and two with *P. concolor*. Phylogenetic analyses using the sequences obtained from our study and those of the highly matching GenBank sequences for each of the six taxa revealed that there seems to be more diversity of insects associated with *Ziziphus* fruits in Ethiopia than the results from the BLAST search mentioned above.

Table 4. Fruit fly isolates used in the insect identification study in Ethiopia.

Species Name	Isolate Number	Host	Origin	Collector	Percent Identity	Accession
C. incompleta	AHL2	*Z. jujuba*	Iraq	Tahir, H.M.	99.85	ON045003
C. incompleta	AHL1	*Z. jujuba*	Iraq	Tahir, H.M.	99.85	ON045002
C. incompleta	Italy 01	*Z. jujuba*	Italy	Zhang, Y.	99.71	NC_071720
C. vesuviana	I1	*Z. jujuba*	Spain	Garrido, J.I.	99.68	OK147923
C. vesuviana	China, Xinjiang 01	*Z. jujuba*	China	Zhang, Y.	95.31	MT121231
C. vesuviana	Iran 01	*Z. jujuba*	Iran	Zhang, Y.	95.31	NC071721
C. vesuviana	FUN12	*Z. jujuba*	China	Jing, L.	95.43	KU131576
C. vesuviana	ZFBO T01 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU5908887
C. vesuviana	ZFBO T04 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU8343087
C. vesuviana	ZFBO T17 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU1834357
C. vesuviana	ZFBA T06 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU6288145
C. vesuviana	ZFBA T18 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU1912175
D. hydei	CRX36794.1	Melon	Italy	Patrizia, T.	99.43	LN867077
D. hydei	DHYDE20161106	Berry	China	Qian, Z.Q.	99.14	MK659821
D. hydei	Africa	Berry	China	Wang, B.C.	98.85	DQ471603
D. hydei	CH55	Melon	Iran	Oshaghi, M.A.	99.7	OR077700
D. hydei	DQ37	Melon	New Zealand	Hodge, S.	99.55	KJ671602
D. hydei	Duke.Bio203L	Berry	USA	Spana, E.	99.39	MT807009
D. hydei	TEN104-102	Melon	Spain	Vilchez, R.I.	99.84	OK037195
D. hydei	QDE57910.1	Melon	South Africa	Liana, I.A.	99.53	MK251432
D. hydei	ABH5	Melon	Spain	Vilchez, R.I.	99.38	OK037196
D. hydei	15085-1641.58	Melon	Spain	Evans, A.L.	99.38	EU390734
D. hydei	AQ49	Berry	China	Wang, B.C.	93.97	DQ471601
D. hydei	ZFBO T03 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU3000047
D. hydei	ZFBO T02 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU2682351
D. hydei	ZFBO T09 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU3295235
D. hydei	ZFBO T14 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU7962979
D. hydei	ZFBA T08 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU7664841
D. hydei	ZFBA T12 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU7906397
D. simulans	UKG21278.1	Melon	China	Li, T.	99.86	MN046104
D. simulans	sm21	Peach	Brazil	Montooth, K.L.	99.86	KC244283
D. simulans	AU023	*Ziziphus*	Kenya	Ballard, J.W.	99.86	AY518674
D. simulans	Sc00	Melon	Seychelles	Ballard, J.W.	99.86	AF200844
D. simulans	DSR	Apple	Madagascar	Ballard, J.W.	99.86	AF200841
D. simulans	DSW	Apple	USA	Ballard, J.W.	99.86	AF200840
D. simulans	C167	Banana	Kenya	Ballard, J.W.	99.86	AF200839
D. simulans	KY215	Banana	Kenya	Ballard, J.W.	99.71	AY518672
D. simulans	KY007	Apple	USA	Ballard, J.W.	99.71	AY518670
D. simulans	SL3	Melon	Spain	Satta, Y.	99.71	M57911.1
D. simulans	simw501	Apple	Brazil	Montooth, K.L.		KC244284
D. simulans	ZFBO T13 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU2055377
Z. indianus	haplotype 6	Fig	Brazil	Mendonca, M.P.	98.96	KC994628
Z. indianus	Duke.Bio203L	Fig	USA	Mohamed, N.	99.1	MN448022
Z. indianus	haplotype 5	Fig	DRC	Mendonca, M.P.	98.81	KC994627
Z. indianus	ABR08559.1	Fig	Brazil	Amir, Y.	98.81	EF632369
Z. indianus	ABR08548.1	Fig	Brazil	Amir, Y.	98.66	EF632358
Z. indianus	ABR08551.1	Fig	Madagascar	Amir, Y.	98.51	EF632361
Z. indianus	ABR08549.1	Fig	Madeira	Amir, Y.	98.51	EF632359
Z. indianus	ZFBO T05 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU2155377
Z. indianus	ZFBO T10 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU2212817
Z. indianus	ZFBO T11 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU2280917
Z. indianus	ZFBA T19 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU2348287

Table 4. *Cont.*

Species Name	Isolate Number	Host	Origin	Collector	Percent Identity	Accession
P. concolor	PRJ076	*Ziziphus*	Morocco	Rugman-JP.F.	99.09	EU761024
P. concolor	TN0216	*Ziziphus*	Italy	Rugman-JP.F.	99.09	EU761022
P. concolor	TN0222	*Ziziphus*	USA	Rugman-JP.F.	99.09	EU761021
P. concolor	TN0227	*Ziziphus*	Morocco	Rugman JP.F.	98.94	EU761025
P. concolor	TN0223	*Ziziphus*	Italy	Rugman-JP.F.	98.94	EU761023
P. humilis	Ps29	*Ziziphus*	South Africa	Barbara, V.A.	95.61	MH841897
P. humilis	Ps24	*Ziziphus*	South Africa	Barbara, V.A.	95.61	MH841896
P. humilis	Ps25	*Ziziphus*	South Africa	Barbara, V.A.	95.61	MH841895
P. humilis	QTC30726.1	*Ziziphus*	Portugal	Powell, C.	95.61	MW279213
P. humilis	TN0220	*Ziziphus*	South Africa	Rugman-JP.F.	95.45	EU761031
P. humilis	TN0223	*Ziziphus*	Namibia	Rugman-JP.F.	95	EU761030
P. humilis	ZFBA T15 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU2497311
P. humilis	ZFBO T16 022	*Ziziphus*	Ethiopia	Tigabu, R.		QU2608411

The molecular identification unveiled the presence of four fruit fly species, comprising one Tephritidae species and three Drosophilidae species, as pests affecting *Ziziphus* fruits. The tephritid *C. incompleta* was observed, alongside several drosophilids: *D. hydei*; *Z. indianus*; and *D. simulans* Sturtevant 1919 (all Diptera: Drosophilidae). In addition, the Braconidae species, *P. concolor*, a parasitoid of *C. incompleta* and *Simulium* spp., (Diptera: Simuliidae) was identified during the current study. Given that *Simulium* spp. was found to be a non-insect pest of *Ziziphus*, it was excluded from further analysis. All insect pests were detected across the two AEZs and the three LUTs. *Carpomya incompleta* exhibited the highest occurrence across all three LUTs throughout the assessment months and in both AEZs (Table 5). The count of emergence holes, indicative of adult insect activity, ranged from one to four per fruit, averaging about three holes per fruit. Interestingly, during the rearing phase of the adults, two distinct types of insect pests were observed emerging from a single fruit in certain samples. The extent of damage inflicted by each species was not determined, but the cumulative damage caused by the aforementioned species was assessed.

Table 5. Species of insect pests recorded across LUTs, assessment months and AEZs in Ethiopia.

Type of Insect Pest	Percentage of Insect Pests Recorded Across the Different							
	Land Use Types			Assessment Months			AEZ	
	Farm Land	Home Garden	Roadsides	September	October	November	Low Land	Mid Land
C. incompleta	51.4	47.8	39.0	43.6	41.2	36.3	48.3	42.8
D. hydei	18.6	23.9	22.9	15.7	15.9	17.0	22.9	20.2
D. simulans	19.9	17.6	31.1	31.6	33.0	31.0	21.8	27.4
Z. indianus	6.3	8.8	4.6	6.7	7.4	10.8	5.6	6.0
P. concolor	3.7	1.9	2.5	2.4	2.5	5.0	1.3	3.7

3.4. Phylogenetic Characterization

Phylogenetic analyses using the neighbour-joining method for each taxon revealed further insights on whether the insects from our study were closely related to their respective matches from GenBank. Accordingly, five sequences (ZFBO T01 022, ZFBO T04 022, ZFBA T06 022, ZFBO T17 022 and ZFBA T18 022) that closely matched with *Carpomya* sp. However, though they have formed a distinct and strongly supported clade of their own with a bootstrap value of 100%, the insects in the current study were most closely related to *C. incompleta* and *C. vesuviana* from the *Carpomya* sp. retrieved from the NCBI. This clade was further divided into four branches that are also strongly supported by bootstrap values but negligible genetic distances. Similarly, those sequences from GenBank that were claimed to be from *C. incompleta* and *C. vesuviana* formed a clade of their own, which was further divided into distinct *C. incompleta* and *C. vesuviana* (Figure 3a).

In the phylogenetic analysis of six sequences, ZFBO T03 022, ZFBO T14 022, ZFBO T02 022, ZFBA T12 022, ZFBA T08 022 and ZFBO T09 022, they were found to be similar to those of *Drosophila* spp. Among the different *Drosophila* spp., retrieved from GenBank, the highest sequence similarity with *D. hydei* was observed with the two insects, i.e., ZFBO T03 022 and ZFBO T14 022, which showed a strongly supported clade, while the remaining four (ZFBO T02 022, ZFBA T12 022, ZFBA T08 022 and ZFBO T09 022) formed a separate and strongly supported clade on their own, suggesting that those insects represented by these sequences were not exactly identical to *D. hydei* (Figure 3b) but are among the *Drosophila* sp. Likewise, the phylogenetic analysis of the sequence (ZFBO T13 022) that matched with the *D. simulans* revealed that it formed a strongly supported but small clade with only one of the *D. simulans* sequences while the rest of the *D. simulans* sequences formed a separate clade (Figure 3c) which indicated that the species are among the *Drosophila* species. This indicates that the specimen from *Ziziphus* from Ethiopia is actually a *D. simulans* specimen.

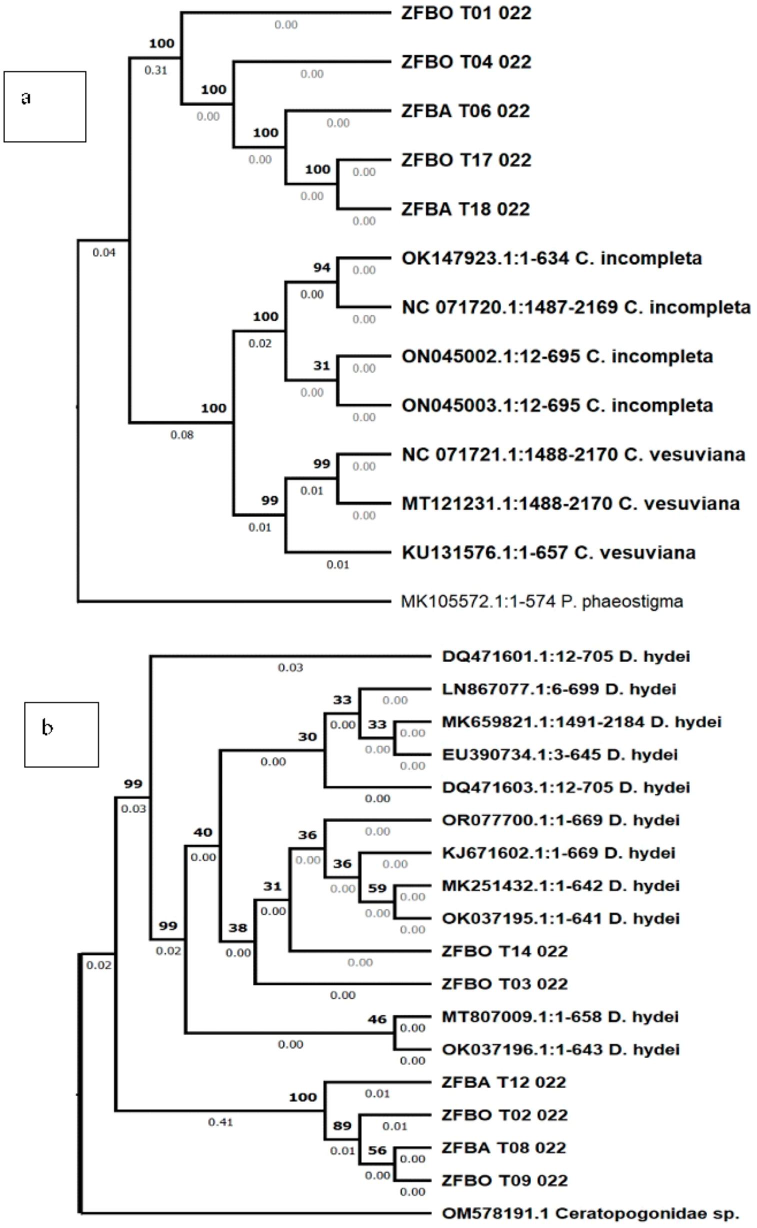

Figure 3. *Cont.*

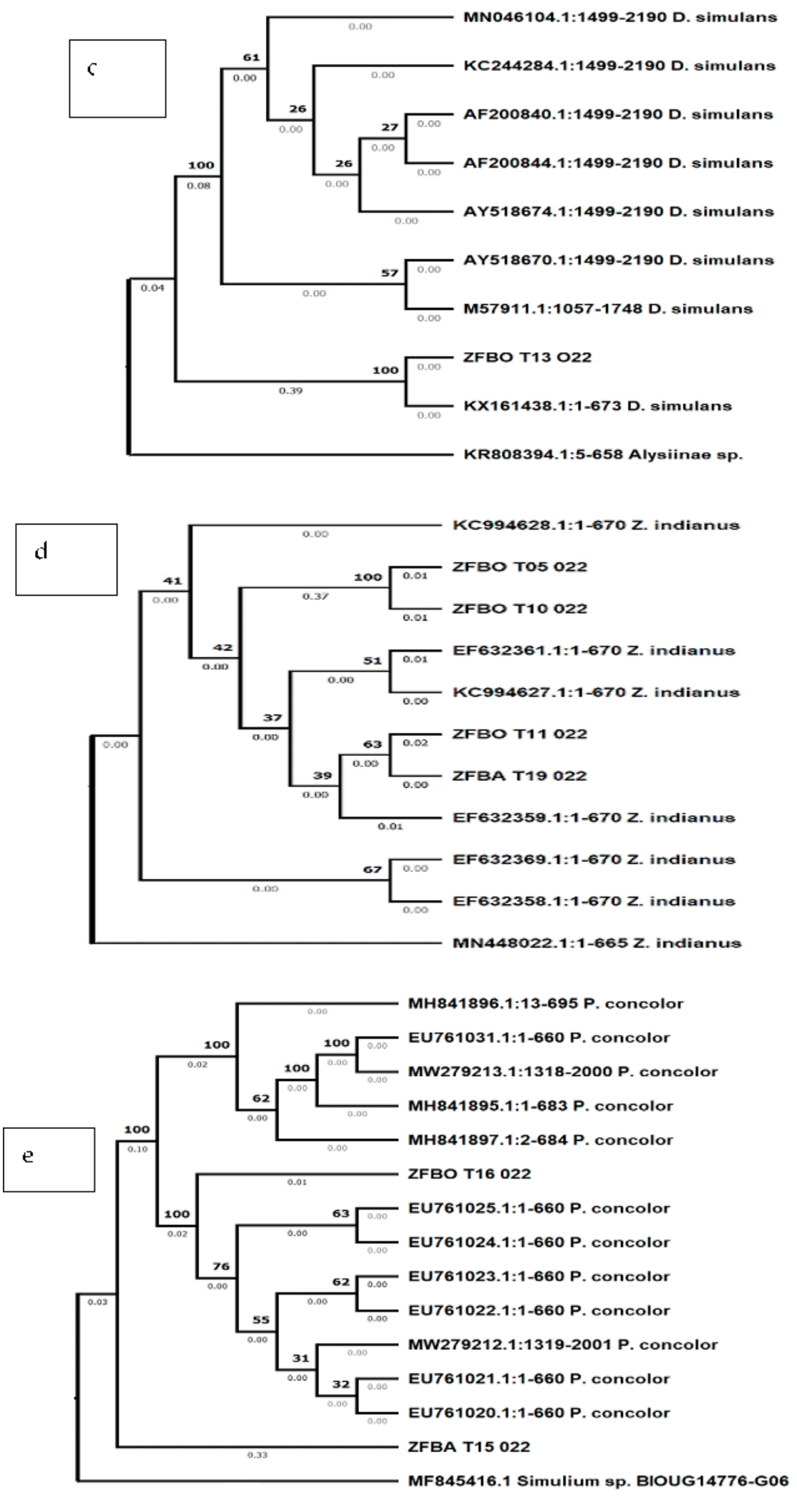

Figure 3. Phylogenetic tree drawn according to the neighbour-joining method based on barcode sequences from specimens of (**a**) *C. incompleta*, (**b**) *D. hydei*, (**c**) *D. simulans*, (**d**) *Z. indianus* and (**e**) *P. concolor* in Ethiopia.

Phylogenetic analysis of the sequences (ZFBO T05 022, ZFBO T10 022, ZFBO T11 022 and ZFBA T19 022) that matched *Zaprionus indianus* in the BLAST search result showed that they formed a clade with sequences of *Z. indianus* although the statistical support for the branches was not strong. However, two of the sequences (ZFBO T05 022 and ZFBO

T10 022) formed a strongly supported sub-branch within the clade, while the other two sequences (ZFBO T11 022 and ZFBA T19 022) formed another sub-branch with weaker bootstrap support (Figure 3d).

The phylogenetic analysis of the fifth taxon represented by sequences (ZFBA T15 022 and ZFBO T16 022) that showed the highest sequence similarity match with those of *P. concolor* based on the BLAST search revealed that only one of the two (ZFBO T16 022) formed a strongly supported clade with *P. concolor* sequences, while the second one formed a separate branch alone. Furthermore, the *P. concolor* sequences were also separated into two strongly supported clades (Figure 3e).

4. Discussion

The incidence of insect pests on *Ziziphus* fruits varied among the assessment months over two years. The percentage incidence of insect pests on *Ziziphus* fruits was above 77% across the AEZ and throughout the two fruit production years. The farmlands exhibited the highest percentage incidence during the 2022 fruiting year (89%), while the lowest incidence was observed in roadside areas (77%) during the 2023 fruit production year. Very severe infestations of >80% were observed in the lowland AEZ, likely attributable to the availability of a suitable environment, such as suitable temperature and humidity, for insect pest reproduction [15,51]. These variations in incidences and levels of infestations across different agroecological zones can also be associated with variations in temperature, humidity and rainfall, critical parameters for insect pest reproduction [52–55].

Insect pests contribute to yield reduction and post-harvest losses, consequently diminishing the quality and quantity of the fruit [56–58]. Such reductions in fruit quality can have adverse economic impacts on communities reliant on these fruits for their livelihood income [59]. Ensuring high fruit quality, such as size, shape, colour and freedom from defects and decay, is crucial for fetching higher prices [60,61]. Additionally, desirable qualities such as sweetness, softness, juiciness, thin skin and well-developed flavour render fruits more susceptible to insect pest attacks [57]. In the present study, the majority of fruits were classified under low, medium and severe infestation levels, with consistent patterns observed across production years and no significant variation among LUTs. The incidence and infestation levels on *Ziziphus* fruits exhibited variability across assessment months. In both assessment years, the highest percentage of incidence occurred during November and December, while the lowest was recorded in September. The various assessment months were positively correlated with the fruit production years, while no correlation was observed between the different LUTs, months of assessment and AEZs.

In contrast, the occurrence of insect pests on *Ziziphus* leaves was low, under 50%. No instances of very severe pest infestation levels were recorded, regardless of AEZ, LUT, production year, or assessment month. Despite the low percentage of incidence, significant variations were observed among the different LUTs, particularly on the roadside, which exhibited the highest percentage of infestation on *Ziziphus* leaves. Severe insect pest infestations on leaves could lead to complete defoliation, affecting growth and fruit production due to the cessation of photosynthesis [62,63]. Additionally, infestation levels were generally low over the two years, with the majority of leaves showing no signs of infestation, indicating their overall health. The percentage incidence of insect pests on *Ziziphus* leaves remained below 50% throughout all assessment months over the two production years. The highest percentage incidence was observed in September of the 2022 production year, while the lowest occurred in October of the 2023 production year, which could be associated with climate variability between the two production years [13]. The low percentage incidence and infestation levels might be due to the resistance of the leaves to insect pests [63,64]. Molecular identification unveiled the presence of four fruit fly species, comprising one Tephritidae species and three Drosophilidae species, as pests affecting *Ziziphus* fruits.

The formation of separate clades, supported by strong bootstrap values, among the fruit flies according to the sequences from specimens collected from *Ziziphus* fruits in

Ethiopia suggests that the insects may not be *C. incompleta* or *C. vesuviana* but may be a species that belongs to the same genus but not the same species. Furthermore, there seems to be some degree of genetic variation among the specimens collected from Ethiopia, which could only be verified with further analysis using additional molecular markers. In the present study, the tephritid *C. incompleta* was found to be the most dominant insect pest. *Carpomya incompleta* is a monophagous pest of *Ziziphus* spp., causing significant negative impacts and contributing to low yields and poor quality of fruit [65]. It has been recorded in several countries in Africa, Asia, the Middle East and Europe [66,67]. Additionally, another tephritid fruit fly, *C. vesuviana* Costa (Diptera: Tephritidae), which is also a monophagous pest of *Zizyphus* species, is a destructive pest contributing to low yield and poor quality of fruits [52,68,69]. Other tephritid fruit fly species, *Bactrocera zonata* and *B. dorsalis* (Diptera: Tephritidae), are common insects that frequently attack and severely damage *Ziziphus* fruits [63].

Alongside them, three drosophilids, namely, *D. hydei*, *Zaprionus indianus*, and *D. simulans*, were also found to be impactful insect pests of *Ziziphus* fruits in Ethiopia. The phylogenetic analysis of the sequences identified as *D. hydei* from BLAST search indicated that the specimen from *Ziziphus* fruits in Ethiopia was probably not a single species but two different species, one of them being *D. hydei*, since it formed a strongly supported branch along with *D. hydei* sequences, while the other group appears to be distinct from *D. hydei* and maybe another *Drosophila* sp. In this study, the Ethiopian isolate ZFBO T13 022 was which demonstrated intraspecific similarity with *D. simulans* isolate KX161438.1:1-673 retrieved from GenBank, supported by a robust bootstrap value of 100%, thereby indicating a complete resemblance of the Ethiopian isolate to *D. simulans*. *Drosophila hydei* and *D. simulans* were reported as impacting insect pests on many fruits such as bananas, apples, melons and berries (cherry, raspberry, blackberry, strawberry and blueberry) with wide distribution ranges caused due to the transportation of contaminated fruits and the changing climates [70,71]. Several Drosophilidae lay their eggs in decaying fruits and might be secondary pests, yet some species also lay eggs on fresh fruits, and their larvae feed on healthy fruit. *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) mainly infests healthy, unwounded thin-skinned fruits such as soft and stone fruits, cherries and apricots [72,73], and larval feeding and development on fruit flesh make fruits unmarketable [74].

The BLAST search result indicated that four sequences from the current study matched with those of *Z. indianus* from the GenBank database. Nevertheless, from the phylogenetic tree of the sequences from Ethiopia and those from GenBank, it could be said that the specimens from Ethiopia do not appear to be *Z. indianus* but may be a related species since the bootstrap support for the branches was low. Given the existence of several species under the genus *Zaprionus* and the fact that *Z. indianus*, also known as the fig fly, is a generalist that lives on a large number of host plants using their fruits for oviposition [75], the specimens from *Ziziphus* fruits in Ethiopia could be considered to belong to the genus *Zaprionus*. *Zaprionus indianus* was reported first from Tunisian olives [76] and, thereafter, was introduced to various countries across the globe and became pests of multiple fruits such as olive, berries, peach, apple and *Ziziphus* [77]. *Zaprionus indianus* mostly lays eggs in decaying fruit or fruit with injuries or mechanical damage, yet it can also oviposit in healthy, undamaged fruit such as figs, strawberries and guavas [78].

Psyttalia concolor is known to be a parasitoid of the tephritid pests and therefore has been extensively used in biological control programmes against these pests. It is also known to be a member of a complex of closely related species from Africa that are difficult to separate based on morphological features alone [79]. The grouping of one of our sequences with one group of *P. concolor* sequences might indicate that the species represented by the sequence is actually *P. concolor*, while the separation of the second sequence into a separate branch on its own might indicate that the specimen represents one of the closely related members of the *P. concolor* species complex which we could not name based only on the data we currently have. Furthermore, the finding of the insect specimens that are already in use for biological control against fruit fly pests is interesting for further research on biological control of the tephritid pests affecting *Zizyphus* fruits in Ethiopia. *Psyttalia concolor* native to the Mediterranean are used as biological control of arthropod pests and parasitize *C. incompleta* and the olive fruit fly (*Bactrocera oleae*) insects. They contribute a potential for agricultural pest controls [80].

Among the four fruit flies in the current study, *Z. indianus* showed the lowest record compared to *D. hydei*, *D. simulans* and *C. incompleta*. The variation in the distribution of insect pests across different LUTs, agroecological zones and fruit production years might be related to various abiotic factors such as temperature, rainfall and relative humidity, as well as biotic factors such as varietal resistance [16,73,81]. *Drosophila hydei* and *D. simulans* showed similar distributions among the three LUTs and the two AEZs. *Zaprionus indianus* showed the lowest record compared to *D. hydei*, *D. simulans* and *C. incompleta*. The variation might be related to various abiotic factors such as temperature, rainfall and relative humidity [16,81]. Various insect pests have been reported from different countries for their detrimental impacts on *Ziziphus* fruit production, attacking the fruits at various stages of maturity [63,71].

5. Conclusions

Ziziphus tree species, which serve various purposes for communities including consumption, market sale, firewood, construction material, farm tools and fencing, face challenges in growth and production in Ethiopia due to both biotic and abiotic factors. Among biotic constraints, insect pests present significant challenges to *Ziziphus* trees, affecting flower, fruit and foliage production. The present study provides valuable insights into the frequency and severity of insects infesting *Ziziphus* tree fruits and leaves, which have significantly hampered fruit production, resulting in considerable yield reductions. Across the study sites, various damage symptoms were observed on both fruits and leaves over the span of two years. These distinct symptoms observed consistently throughout the assessment period, were attributed to different species of fruit fly insects. Higher incidences of pests and more severe infestations were observed on fruits harvested from farmlands, whereas comparatively lower occurrences were recorded on fruits obtained from roadsides over the two fruit production years. Conversely, leaves exhibited notably lower percentages of incidence and infestation levels than fruits. In the lowland AEZ, higher percentages of incidence and infestation levels were observed. Analysis across different assessment months revealed peak percentages of incidence and infestation levels in November, with the lowest levels occurring in September across both LUTs and AEZs. These assessment months emerged as significant influences on the incidence of *Ziziphus* fruit insect pests. Moreover, this study identified the types of insect pests responsible for significant yield losses on *Ziziphus* fruits in Ethiopia. Both morphological (based on phenotypic characterizations) and molecular (based on DNA barcoding) techniques were employed to confirm the identification of specific insect pests. The study revealed that *C. incomplete*, *D. hyde*, *D. simulans* and *Z. indianus* were the fruit fly insect pests impacting *Ziziphus* fruits in Ethiopia. The isolates from Ethiopia formed separate clades among themselves and between the sequences retrieved from GenBank except for the Ethiopian isolate ZFBO T13 022, which demonstrated a complete resemblance to and belonged to the same clade as *D. simulans*.

Overall, fruits exhibited significantly higher percentages of incidence and infestation levels than leaves, indicating substantial yield losses due to fruit fly insect pests within the study areas. The major fruit fly insect pest impacting *Ziziphus* fruits was *C. incomplete*, while the least common was *Z. indianus*. Therefore, it is imperative to implement appropriate management strategies to mitigate significant yield losses and promote sustainable *Ziziphus* fruit production.

Supplementary Materials: The following supporting information can be downloaded at https://submit.ncbi.nlm.nih.gov/subs/sra/SUB14806558/metadata_SUB14806558 (accessed on 30 October 2024).

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Data Availability Statement: Data will be made available upon request.

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Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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