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Research article

Expected potential hemipteran vectors of *Xylella fastidiosa* bacterium in olive and vineyard groves of the Egyptian northwestern coast

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ABSTRACT

Xylella fastidiosa was recently added to the list of threatening pathogens affecting more than 300 plant hosts. Hemipteran hoppers that feed on xylem have been documented as potential transmitters. Hemipteran hoppers, known vectors for plant pathogens via xylem feeding, pose significant risks to agriculture. Despite their role in transmitting diseases, comprehensive data on their species diversity, distribution, and seasonal patterns, particularly in critical agricultural zones, remain sparse. Hence, the current study was carried out at 14 sites (eight olive farms and six vineyards) during the 2021/2022 season to develop a comprehensive checklist of hopper species present on the Egyptian Northwestern Coast, including their seasonal and location distribution, to serve as a real roadmap supporting control strategies if the pathogen breaches Egyptian borders. Utilizing 560 yellow sticky traps, we collected data seasonally, resulting in the identification of 21 hopper species belonging to 14 genera within four families. Olive orchards harbored a higher number of hoppers compared to vineyards, with *Empoasca decipiens* being the most dominant species. Our findings provide a foundational checklist and highlight the importance of continued monitoring and detailed studies to support proactive control strategies against potential X. fastidiosa outbreaks. We used 560 yellow sticky traps at 10 traps per site (80 traps for olive orchards and 60 traps for the vineyard per season) throughout the study period. Traps were installed at two levels to catch hopper species harboring tree canopies and ground vegetation. Each trap was replaced every 7 days, and the collected trap sheets were sent to the laboratory for segregation and identification. The data revealed 21 hopper species belonging to 14 genera and 4 families, with cicadellid species being the most represented (14 species). Olive orchards harbored a higher number of hoppers than vineyards. *Empoasca decipiens* exhibited the highest dominance among the remaining species. Although summer sampling yielded the highest number of hopper species and trapped specimens, seasonal variation in the distribution pattern exhibited nonsignificant differences ($F = 1.7$ and $P = 0.173$). Ras El-Hekma had the highest species representation (21 species), whereas El-Negala had the highest species richness. The lowest species representation at the Barrani location had the highest abundance of caught specimens. Although there were fluctuations in the trapped specimens among the examined locations, statistical analysis revealed no significant differences ($F = 0.67$, $P = 0.58$). Cluster analysis revealed distinct groupings with different degrees of similarity for both seasonal and location distributions. The

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impact of trap height on the hopper capture pattern showed a biased tendency towards low traps. Diversity indices showed no significant differences between the examined locations. Although our results offer a foundation for potential control strategies against *X. fastidiosa*, further detailed studies are required to fill the knowledge gap regarding its suspected vectors. Such research will guide management strategies that can be applied in cases where this infectious bacterium crosses Egyptian borders.

1. Introduction

The gram-negative bacterium *Xylella fastidiosa* (Xanthomonadales: Xanthomonadaceae) has recently been added to the list of pathogens threatening over 300 plant hosts, including olives, vineyards, citrus, peaches, and plums, among others [[1,2\]](#page-7-0). The estimated losses of peach groves due to *X*. *fastidiosa* exceeded one million trees [\[3,4](#page-7-0)]. Ringenberg et al. [[5](#page-7-0)] monitored the ability of different *X*. *fastidiosa* subspecies to infect cultivated and wild plants. *X*. *fastidiosa* has been included as a quarantine pathogen in Europe in the last two decades of the 20th century, and most countries in the Mediterranean basin have been placed at potential risk due to the pandemic nature of this bacterium, similar to what happened in Italy [\[3\]](#page-7-0). Through its two life forms (motile and sticky), *X*. *fastidiosa* can clog the xylem vessels of infected hosts and impair sap flow, leading to characteristic symptoms [\[6\]](#page-7-0). Hemipteran piercing-sucking species of the families Cicadellidae (sharpshooter leafhoppers) and Aphrophoridae (spittlebugs) are potential vectors. Additionally, mechanical transmission from infected to sound hosts serves as another tool for the incidence of infection [\[5,6\]](#page-7-0). Among the surveyed *X*. *fastidiosa* vectors, only a few played crucial roles in transmission [\[7](#page-8-0)–9].

The Egyptian Northwestern Coast (ENC) has a mountainous topography with a natural inclination northward toward the Mediterranean Sea. The prevailing environmental conditions in the ENC are harsh (low precipitation rate, soil salinity, sandstorms, and hot summer seasons). Because rainfall is the sole source of fresh water, many water harvesting techniques (dikes, reservoirs, and cisterns) have been adopted by local communities to collect adequate amounts of water for irrigation and drinking [\[10](#page-8-0)]. During the rainy winter season, many indigenous flora thrive and act as suitable refuges (shelters) for certain herbivores [\[11](#page-8-0)]. Certain noxious species, especially piercing-sucking species, may find ways to attack planted crops [[12,13\]](#page-8-0), coinciding with the dry conditions during summer, and may constitute a veritable menace through their potential to act as pathogen transmitters. Olive, *Oleae uropaea* L. (Lamiales: Oleaceae), grape, *Vitis vinifera* L. (Vitales: Vitaceae) orchards are among the main agricultural profiles in this area because of their low water requirements and resilience to the prevailing conditions [\[13](#page-8-0)–15]. However, a combination of local weather, environmental conditions, natural plant cover, and the presence of olive and vineyard groves could constitute an ideal environment for the spread of *X*. *fastidiosa* infection, especially where its vector(s) are available [\[5,](#page-7-0)[8](#page-8-0)]. The shortage of available data regarding xylem feeding hoppers in both olive and vineyard orchards in the ENC region was the main motivation for the current study. The objective was to develop a comprehensive checklist of hopper species present in this coastal area, including seasonal, location, and plantation distribution, to serve as a real road map supporting control strategies when the pathogen crosses Egyptian borders.

2. Materials and methods

2.1. Study area

The Egyptian Northwestern Coast falls under the Matrouh Governorate, ~450 km west of Cairo. This coastal area extends from El-Hammam in the east to El-Salloum in the west, adjacent to Libyan borders. The main rain-fed cultivation of olives and vineyard orchards extends from Ras El-Hekma City, ~100 km west of El-Hammam City, to El-Salloum. Accordingly, observational sites dedicated to the practical implementation of the current study were selected to cover these rainfed areas (Table 1 and [Fig. 1](#page-2-0)).

Table (1)

Coordinates of the experimental sites.

U: trap hanged in plant canopy, D: traps hanged in the ground cover.

2.2. Site selection and trap distribution design

The proposed design for the sticky trap installation was based on the methodology adopted in Ref. [[5](#page-7-0)]. Fourteen sites were selected (eight olive groves and six vineyards) during the 2021/2022 season. The selection criteria were based on the commonly cultivated varieties in the ENC, including Shamlaly and Picual olive varieties [[16\]](#page-8-0), Barrani and Banat for the vineyard [\[17](#page-8-0)], as well as tree age (approximately 15-year-old olive trees except for one site in Barrani with approximately 5-year-old trees, and approximately 10-year-old in the case of vineyard trees). These sites received common agricultural practices in the coastal area (rainfed irrigation, slight fertilizer addition before the rainy season, and irregular winter pruning). In total, 560 yellow sticky traps $(22 \times 30 \text{ cm})$ were individually fixed on wooden stands to prepare for seasonal distributions at the observation sites. Ten traps were installed at each site, *i*.*e*., 80 traps for olive sites and 60 traps for the vineyard/season. In the case of olive sites, five traps were placed 40 cm above the ground with a 20 m separating distance between each neighboring trap to catch hopper species harbored by the ground vegetation. The other five traps were hanged 1.5–2 m height to catch the hopper species that harbored the tree canopies. In the Barrani olive location with 5-year-old trees, ten traps were hung at a height of 40 cm. As the height of the vines never exceeds 1.5 m, traps approximately 50 cm high were installed. Notably, the Barrani variety is the most common under such rain-fed conditions, whereas the Banat variety has limited cultivation. Yellow sticky traps were installed seasonally, and traps were replaced every 7 days. Following the installation period, the trap sheets were individually delivered to the laboratory in clear plastic bags to remove the caught specimens from the sticky material (see [Table 2](#page-3-0)).

2.3. Insect segregation

Segregation of caught insects from the sticky material was performed through successive exposure of trap sheets to a series of chemical solvents to remove the sticky material from the insect's body and preserve its morphological features [\[18](#page-8-0)]. Each trap sheet was soaked for 1 h. Then, the insect specimens were filtered and washed in ethylene glycol solution for 15–30 min. Thereafter, intact insect specimens were washed in xylene solution for 30 min and then left to dry on absorbent paper. The removed specimens of each sticky trap were inventoried by date, location, trap location, and installation height and then stored in 70 % ethanol vials as a preparatory step for separating hopper species from the other insects [[18\]](#page-8-0). Finally, the hopper specimens were point-mounted for scientific identification [\[19](#page-8-0)–21]. The voucher hopper specimens were deposited in the insect collection at the Plant Protection Department, Desert Research Center, Egypt.

2.4. Species composition, biodiversity measures, and data analysis

Following scientific identification, the distribution patterns of hopper species, families, and individuals at the plantation, seasonal, and location levels were estimated. The estimated mean values of caught individuals for both seasonal and location distribution trials were subjected to an ANOVA test to determine the significance level based on the F and *P* values. Diversity indices (species richness "Margalef index," diversity "Shannon index," evenness, and abundance) among observational sites were also calculated, and average values were compared statistically. Jaccard's coefficient of similarity for the caught hopper species and a hierarchical clustering dendrogram for both seasonal and location distributions were calculated using the unweighted pair group (UPGMA). Version 4.03 of the PAST program was used to calculate the indices [[22\]](#page-8-0).

3. Results

The final output of the current study provides a checklist of hemipteran hopper species (both plant and leafhoppers), families, and individuals harboring olives and vineyard orchards in the ENC. As shown in Table (2), caught hoppers represented 21 species belonging to 14 genera in 4 families (Cicadellidae, Delphaeidae, Tettigometridae and Psyllidae). Cicadellid species had the highest representation among the 14 species, whereas the Tettigometridae family showed the lowest contribution with only one species. In terms of the average captured specimens/plantation, olive orchards harbored a higher number of hoppers (66.52 \pm 31.98) with 58.38 relative abundance % than the grapevine ones (41.62 %). The highest numerical abundance of *Empoasca decipiens* individuals in

Fig. 1. The location of the experimental sites in the study area.

Table (2)

Checklist of hopper species and families and the number of specimens captured from the Egyptian Northwestern coast.

grapevine (415) and olive (617) sites categorized its dominance (43.13 %) among the remaining species. *Thamnotettix* species 3 and 4 had the lowest dominance percentages (0.17 %).

Seasonal sampling of hoppers is presented in Tables (3). In this approach, traps hung during the summer and spring caught the highest number of hemipteran hopper species (21 and 18 species, respectively), compared with the lowest number of species in

 $^{\mathrm{a}}$: Repeated species were recorded once.

autumn. Out of the total captured specimens (2393 individuals), summer sampling represented approximately half of the total catch $(\sim 46.84$ % abundance and 53.38 ± 26.11 mean specimens caught), whereas the lowest individuals were counted during winter (5.64 % abundance and 6.43 ± 2.48 mean specimens caught). Such variations in hopper distribution patterns exhibited non-significant differences among the observational seasons ($F = 1.7$ and $P = 0.173$). Cicadellid specimens comprised 941 individuals caught during the summer sampling trial ([Table 3](#page-3-0)), followed by spring catches. Tettigometrid counts made the lowest contribution throughout the study.

Cluster analysis revealed distinct seasonal groupings with varying degrees of similarity (Fig. 2). The hopper samples captured during summer and spring were separated into clear clusters with approximately 85 % similarity. Winter samples showed weak similarity to the spring–summer cluster (approximately 0.65 %). The weakest similarity (approximately 0.55 %) was detected between autumn samples and the remaining samples.

The species composition and abundance percentages among the examined locations were also investigated (Table 4a). All captured species were found at Ras El-Hekma (21 species). Both Barrani and El-Negala (20 species each) were ranked second at both locations, whereas Matrouh had the lowest species representation. The species richness over the observational sites at each location were noted. The two observational sites in El Negala had the highest species richness (10 %), whereas the five observational sites in Barrani had the lowest contribution (4 %). Despite having the lowest species representation, Barrani had the highest abundance of caught specimens (36.69 % and 219.5 \pm 185.44 mean catches) compared to the other locations (Table 4a). Table (4b) presents the distribution patterns of captured hoppers. *E*. *decipiens* had the largest number of individuals caught across all inventory locations. Statistical analysis revealed non-significant differences ($F = 0.67$, $P = 0.58$), despite the fluctuations in trapped specimens among the examined locations.

The distribution patterns of the captured hoppers were categorized into different groups using a hierarchical clustering approach [\(Fig. 3](#page-5-0)). El-Negala and Ras El-Hekma showed distinct clusters with approximately 95 % species similarity. Another cluster was observed between the Barrani and El Negala-Ras El Hekma clusters with approximately 93 % calculated similarity. Finally, the hopper community in Matrouh showed weak similarity with its detected counterparts, with 84 % similarity.

The effect of trap height on the hopper capture pattern was also estimated ([Table 5\)](#page-6-0). In the case of olive sites with two hanging trap levels, the general trend for hopper catch was in favor of lower hanging traps than those suspended at higher elevations. For example, the low-hanged traps at the Barrani olive site "S2D″ captured more species and individuals (18 species and 166 individuals) than those suspended at the higher elevation (S2U site). A similar trend was observed for the remaining olive cultivation sites. Although 14 species were collected at the "S5″ olive site (Zagarat 1, El-Negala), the caught individuals showed a biased towards the low traps (137 caught specimens). The only exception was the Ras El-Hekma olive site (S11), where one more species and more specimens were detected in the high-hang traps. In the case of vineyard sites with only one trap installation level, hopper capture was highest at S9 site (El-Bess, Ras El-Hekma), with 19 species and 193 caught specimens. The lowest number of caught species was detected at S14 site (Alam El-Harsh, Barrani) (11 species), whereas S13 site (El-Bess, Ras El-Hekma) witnessed the lowest number of trapped specimens (73 individuals). The diversity indices are listed in Table (5). Statistically, the calculated diversity indices (species diversity, richness, evenness, and abundance) showed no significant differences between the examined sites.

4. Discussion

The current inventory offers a checklist of hopper species that may act as *Xylella fastidiosa* transmitters along the northwestern Egyptian region of the Mediterranean coast. Proactive detection of *X*. *fastidiosa* vectors before the pathogen crosses country borders is an urgent issue, especially if the bacterium is detected near our environment [[5](#page-7-0)]. The detection of *Xylella* bacteria in Palestine-occupied lands [\[23](#page-8-0)], which are adjacent to Egyptian borders, was the main motivation for conducting the present study.

The distribution of hemipteran hopper species was observed at seasonal, location, and plantation scales. The prevailing environmental conditions (harsh climatic conditions, sandstorms, soil salinity, and sporadic precipitation rates) and anthropogenic activities (rainfed cultivation practices under the valley rehabilitation approach) [\[14](#page-8-0)] may be the main factors governing the overall representation of the hopper species and caught specimens. The characteristic arid conditions on the Egyptian northwestern coast

Figure (2). Cluster analysis (dendrogram) of hopper communities (seasonal catches).

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Table (4a)

Composition and abundance (%) of hopper species captured from the examined locations at the Egyptian Northwestern Coast.

Table (4b)

Distribution pattern of hopper species captured from the Egyptian Northwestern Coast.

Figure (3). Cluster analysis (dendrogram) of hopper communities (location catches).

(approximately 100 mm rainfall) [\[10](#page-8-0)] could be a crucial cause for the lower representation of hoppers compared to their counterparts in wet conditions. For instance, a monitoring study in the wet region of southern Brazil reported higher leafhopper diversity than in semi-arid areas [\[5\]](#page-7-0). Climate change's negative impacts could further deteriorate or fragment the feeding habitats (natural ground flora) of hoppers, thereby lowering their abundance [[9,24](#page-8-0)]. Agricultural practices, such as winter intra-plowing (among trees),

Table (5)

Impact of trap height on the diversity indices of hopper species captured from the Egyptian Northwestern Coast.

commonly aim to enhance soil water retention capacity but may affect hopper populations by destroying natural ground vegetation. In contrast, the flourishing natural ground vegetation beneath olive trees (where local farmers avoid plowing) provides a favorable habitat for hopper populations. Conversely, the limited growth pattern of grapevines, which hinders sunlight access to underlying flora during the vegetative growth season, and their deciduous nature during winter, render them less favorable habitats for hemipteran hoppers.

The high capability of cicadellid species to adapt to more than one feeding habitat (polyphagous behavior) $[25,26]$ $[25,26]$ may be a reasonable reason for their high abundance patterns at our observational sites. Among these, *E*. *decipiens* showed the highest representation. The wide host range of both the domestic and wild flora of this species [[27,28\]](#page-8-0), may synchronize its global distribution [[29\]](#page-8-0). Generally, the temporal diversity of floral species may govern hopper distribution, *i*.*e*., the greater the seasonal diversity of palatable hosts, the greater the dispersal pattern of hoppers. In this context, the coastal area under exploration harbors many floral species previously documented as favorable hopper habitats; including wild flora like malva (*Malva* sp., F.: Malvaceae), tamarix (*Tamarix* sp., F.: Tamaricaceae), Knotweed (*Polygonum* sp., F.: Polygonaceae), artemisia (*Artemisia* sp., F.: Asteraceae), thyme (*Thymus* sp., F.: Lamiaceae), deverra (*Deverra* sp., F.: Apiaceae), capparis (*Capparis* sp., F.: Capparaceae), saltbush atriplex (*Atriplex* sp., F.: Atripliceae), colocynth (*Citrullus* sp., F.: Cucurbitaceae), and acacia (*Acacia* sp., F.: Fabaceae) [\[30](#page-8-0)–33] and cultivated crops such as beans, wheat, barley, melons, and certain vegetables (cucumbers, tomatoes, and eggplants). The potential role of naturally developing vegetation in plant hopper colonization has been documented [\[34,35](#page-8-0)].

The distribution patterns of the surveyed hoppers were considered on both temporal and spatial scales. The dominant patterns of hopper species and their individuals caught in the summer and spring sampling trials were observed. Besides the growing nature of olive trees (evergreen) and grapevines (deciduous), the prevailing environmental conditions during the winter (harsh cold and rainy) and autumn (cold and sand storm) seasons [[12\]](#page-8-0) may play an effective role in adjusting the insect population [[36\]](#page-8-0). documented a higher abundance of cicadellid hoppers under warm and humid conditions in Egypt, compared to cooler and drier conditions where lower hopper populations were observed.

The numeric increase in hopper species in the Matrouh and El-Negala observational sites (10 % and 8.5 % richness, respectively) met the lowest number of caught specimens. This may be due to the expansion of anthropogenic activities (agriculture, tourism, and urbanization) that may play a major role in habitat deterioration or fragmentation, causing hopper population disruption [[37\]](#page-8-0). In contrast, the low population density, in addition to the relative distance of the Barrani location from the implemented human activities (especially touristic activity), could be the cause for the high number of hoppers (36.69 % abundance) by decreasing the likelihood of habitat deterioration.

The higher species representation and number of caught specimens in the low-hang traps than in the high-elevation ones could be attributed to the seasonal diversity of the palatable floral hosts in this coastal area $[26]$ $[26]$. The final findings of $[5]$ indicated that more hoppers were captured by low-hanging traps, which means a higher abundance in the natural ground vegetation. However, the distribution biased towards the elevated traps at the S11 olive site (Ras El-Hekma location) could be attributed to the full tillage practice of the ground vegetation beneath the olive trees (personal observation), which in turn may force the hoppers to migrate to the olive trees. Such an experiment could offer real insights into the potential of hopper species to migrate among different hosts [[8](#page-8-0)]. From a statistical point of view, the non-significant differences in the diversity indices among the observational sites could be attributed to the resemblance of the environmental and topographical conditions along this coastal area and may also indicate the slight impact of the implemented human activities on hopper population composition.

Xylella fastidiosa vectors are confined to four families in the order Hemiptera [[38\]](#page-8-0). Xylem-feeding sharpshooters (cicadellid species) were the only suspected vectors detected in this study. According to Ref. [\[39](#page-8-0)], all Cicadellidae species are assumed to play an effective potential in *X*. *fastidiosa* transmission. The source of potential threats in this coastal area may arise from the ability of these hemipteran species to migrate among both natural and cultivated (olive and vine) hosts [\[30](#page-8-0)] and, consequently, may act as *X*. *fastidiosa* transmitters if this bacterium crosses Egyptian borders [\[40](#page-8-0),[41](#page-8-0)]. The current study presents a comprehensive scientific list of *X*. *fastidiosa* potential vectors and their distribution along the northwestern Egyptian coast. As such, an area is considered an essential assemblage point for olive and vine cultivation, and many agricultural investments are based on their products, which constitute one of the main sources of income for local communities and investors. As a result of the expected rapid spread of the infection among olive trees or grapevines via these hemipteran species $[42]$ $[42]$, the socioeconomic impact will be catastrophic in cases where such infectious bacteria bridge borders. The triple relationship among *X*. *fastidiosa* pathogens, insect vectors, and plant hosts represents the core source of threat in terms of the incidence and spread of infection [\[43](#page-8-0)]. drew attention toward this concept as a "risk factor" with this approach. This means that the infection of grapevines by Pierce's disease could be conditioned by the proximity of the grapevines to the already infected hosts as well as the most abundant vectors [\[34,44](#page-8-0)].

5. Conclusion

The current study offers a comprehensive checklist of expected vectors of the infectious *Xylella fastidiosa* bacterium. However, more detailed studies should be conducted to fill the knowledge gap regarding suspected *X*. *fastidiosa* vectors in terms of, for example, vector density, vector dispersal capacity, vector population, and host-vector relationships. Such insights will guide management strategies and the efforts that will be exerted in case this infectious bacterium breaches Egyptian borders.

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Data availability statement

All data to support the conclusions have been provided in the manuscript.

CRediT authorship contribution statement

I. Ahmed Imam: Formal analysis, Data curation, Conceptualization. **I. Iman El-Sebaey:** Methodology. **Abdel Nasser A. Kobisi:** Methodology. **Manar A. Elagory:** Investigation. **Amany N. Mansour:** Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Ahmed I. Imam reports financial support was provided by Science and Technology Development Fund (STDF). 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 paper. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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