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A preliminary study of the biodiversity and bioecological parameters related to aphid's species in an organic citrus orchard in Northeastern Tunisia

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Abstract

The Aphididae family contains many polyphagous species. Four species were identified in an organic Citrus orchard in Northeastern Tunisia in the current study. These species are *Aphis gossypii*, *A. spiraecola, Macrosiphum euphorbiae*, and *Toxoptera aurantii*. The most important and abundant species was *A. gossypii* followed respectively by *A. spiraecola*, *M. euphorbiae*, and *T. aurantii*. Monitoring species dynamic population showed that aphids began to occur in February and reached then high levels from March to May. Various structure and beta diversity indexes were calculated. The obtained results demonstrated that there was a high dominance of a few or one species. The evenness index was high and had a significant positive correlation with the Berger-Parker index. The Equitability index was low. The richness index had a strong positive correlation with Margalef's richness index and the number of species. However, Menhinick's diversity index had a weak positive relationship with the Specific richness.

Keywords: Aphids, Citrus, biodiversity, structure indexes, beta diversity indexes

1. Introduction

Biodiversity is one of the elemental properties of nature and a source of immense potential for economical use. It is fundamental for agricultural activities, livestock, forestry, fishing, and industry ^[1]. Biological diversity or biodiversity are expressions interesting in the variability of life on the earth or to the property of living systems to be distinct. It includes plants, animals, microorganisms, ecosystems, and ecological processes during a functional unit ^[2]. It is considered a sign of the well-being of the ecosystem ^[3]. Biological diversity may be a central theme of ecological theory and has been the topic of many discussions. Researchers have currently developed an outsized number of parameters for measuring biodiversity as an indicator of the state of ecological systems, with practical applicability for conservation, management, and environmental monitoring ^[4]. Whittaker ^[5] has defined three levels of Biodveristy indexes: the alpha diversity indexes, which is the within-habit or intracommunity diversity, the Beta or between-habitat diversity that describes the complementarity among and between biological habitats and the Gamma diversity that is the diversity of the whole landscape.

Insects form a particular diversified class of great importance in ecosystems ^[6]. They are helpful in evaluating various environmental disturbances ^[7, 8]. Aphids are a group of insects extremely widespread in the world. They are reported in the tropics and subtropical, temperate regions and steppes ^[9]. Aphids belong to the order of Homoptera, sub-order of Sternorryncha, and super-family of Aphidoidea ^[10]. More than 250 species of the Aphidoidea superfamily are crop pests ^[11]. *Citrus* aphids are serious pests causing damage directly by sap-feeding resulting in leaf deformation, underdeveloped shoots, and honey residues causing sooty mold, or indirectly by virus transmission ^[12]. Many studies have been carried out in Tunisia to identify different aphids' species on other crops, thus on *Citrus*. Those studies have mentioned that almost all the identified species of *Citrus* belong to the family of Aphididae and the subfamily of Aphidinae. They are mainly *Aphis gossypii, Aphis spiraecola, Toxoptera aurantii,* and *Myzus persicae* ^[13, 14, 15].

This work aims to assess different beta diversity indexes of aphids' species identified in an organic *Citrus* orchard in Northeastern Tunisia and to study the different relationships between them through correlation analysis.

2. Materials and Methods

2.1 Experimental site

The study was carried out in an organic *Citrus* orchard located in the region of Mograne within the Higher School of Agriculture of Mograne in the governorate of Zaghouan (36° 25'46.05"N 10 ° 05'37.00"E, elevation 146 m) in Northeastern Tunisia (Figure 1) from January to June 2021. The orchard

has an area of approximately 66330 m^2 (6.63 ha), it is divided into 10 plots (micro-plots) of 6630 m^2 containing 10 lines; each line contains 20 trees. There are multiple varieties in the different plots like Thomson's navel, Valencia late, Maltese, clementine, mandarins, and pomelo with a spacing of 3m x 3m.



Fig 1: Geographical localization of the experimental site

2.2 Sampling method

Aphids were monitored weekly from January 13th until June 16^{th,} 2021. From each micro-plot, ten trees were chosen randomly thus making a total number of 100 sampled trees per week from the orchard. From each tree, four twigs of 30 cm in length were sampled weekly and randomly from the five quadrants of each tree. All samples were taken to the laboratory of Entomology of the Higher School of Agriculture of Mograne for further examination and identification. The

second sampling way consisted of beating branches of the same chosen *Citrus* trees on a white tissue. The fallen specimens were aspirated using a simply manufactured aspirator and conserved in small vials containing a solution of 70% alcohol for further identification.

2.3 Bio-ecological parameters

Two structure indexes and nine beta diversity indexes were used for the study (Table 1)

Table 1: Structure and diversity indexes we	ere used for the study
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Index	Formula					
Structure indexes						
The number of Occurrence	$NOI = \left(\frac{C}{D}\right) * 100$					
Index or frequency ^[16, 17]	Where C is the number of individuals of each species in the sample and D is the number of individuals of all species in the sample.					
The specific density of	Dm = n/A0					
species ^[15]	Where n is the number of individuals of each species and A0 is the surface					
	Diversity indexes					
Specific Richness ^[5, 18]	Rs = The number of species or taxa, and it is the simplest and most commonly applied index used to represent diversity.					
Margalef's richness index [19]	$MR = (S-1)/\ln(n)$					
	Where S is the specific richness and n is the total number of individuals					
Menhinick's richness index	$DMn = S/\sqrt{n}$					
[20]	Where S is the specific richness and n is the total number of individuals					
Simpson's/Gini-Simpson's	$GSDI = \sum pi^2$					
diversity index ^[21]	Where pi is the proportion of the species i obtained by dividing the number of individuals of the targeted species					
	(ni) by the total number of individuals of the species that have been found (n).					
Simpson's dominance index	$SDI = 1{GSDI}$					
[21]	Where GSDI is the Simpson's/Gini-Simpson's diversity index					
Danaan Dankan'a daminanaa	d = Nmax/N					
index ^[22]	Where Nmax is the number of individuals of the most abundant species and N is the number of individuals of all					
mdex	the species that have been found.					
Shannon Weaver's diversity	$H' = -\sum pi * Ln(pi)$					
index ^[23]	Where pi is the proportion of the species i obtained by dividing the number of individuals of the targeted species (ni) by the total number of individuals of the species that have been found (n).					
	I = Hs/S					
Equitability index ^[24, 25]	Where $Hs = -\Sigma pi ln(pi)$ with pi the proportion of the species i obtained by dividing the number of individuals of the targeted species (ni) by the total number of individuals of the species that have been found (n); and S the observed number of species.					

Evenness index [20]	
Where H is the observed diversity and S is the observed number of species	

2.4 Statistical analysis

The different indexes have been measured by the statistical software PAST[®] version 3.24 (2019): Paleontological Statistics software package for education and data analysis ^[27]. A one-way ANOVA followed by a Student Newman and Keuls (SNK) Post Hoc test was used to compare the cumulative means of aphid species during the study period. Pearson correlation was performed on the different calculated indexes. The ANOVA, Post Hoc test and the correlation analysis were done with IBM SPSS (Statistical Package for the Social Sciences) version 23 Edition 32 bits.

3. Results

3.1 Aphid's fauna found in the citrus orchard

The aphid fauna found on citrus has been identified according to several identification keys ^[28, 29, 30, 31]. The identification led to four species (Table 2); are the cotton aphid *Aphis gossypii* Glover (1877) (Figure 2. A), the green citrus aphid *Aphis spiraecola* Patch (1914) (Figure 2. B), the black orange aphid *Toxoptera aurantii* Boyer de Fonscolomb (1841) (Figure 2. C), the green and pink potato aphid *Macrosiphum euphorbiae* Thomas (1878) (Figure 2. D).

Order	Sub-order	Family	Sub-family	Genus	Species
				Macrosiphum	euphorbiae
Hemiptera	Sternorryncha	Aphididae	Aphidinae	Aphis	gossypii
					spiraecola
				Toxoptera	aurantii

Table 2: Different species of aphids identified in the Citrus orchard



gossypii, B: A. spiraecola, C: T. aurantia, D: M. euphorbiae)

3.2 Structure indexes

Monitoring populations' dynamics

Monitoring of the total population of each species has revealed that the emergence of the majority of aphid's species took place from late February reaching high levels from March to May (Figure 3).

The maximum averages of the different recorded species have been observed in April when spring shoots reached their peaks. The survey showed that *A. gossypii* is the most abundant species throughout the study period. However, no significant difference (p < 0.05) was observed between the maximum averages of *A. spiraecola*, *T. aurantii*, and *M. aurantii* (Figure 4).



Fig 3: Monitoring populations' dynamics of the different aphid species in the Citrus orchard



*Means with different alphabetic letters (a, b) are statically different (p <= 0.05) according to the Student Newman and Keul (SNK) PostHoc test. * Bars represent the standard error

Fig 4: Cumulative mean values of aphids' species throughout the study period in the Citrus orchard

Occurrence index or Frequency

The distribution of frequency of the various species identified in the citrus orchard is represented in Figure 5. *A. gossypii* and *M. euphorbiae* were the most abundant species (62% and 27% respectively) followed by *A. spireacola* and *T. aurantii* (9% and 2% respectively).



Fig 5: Distribution of the frequency of the different species of aphids in the *Citrus* orchard

Specific density

The results of the specific density of the different species revealed low values. The maximum value slightly exceeds 0.15 recorded by the most abundant and frequent species *A. gossypii* (Figure 6). That means that the number of individuals is very low compared to the total area of the orchard. That may be explained by the presence of aphids in some micro plots and their absence in others due to their preference towards some varieties of *Citrus* trees.



Fig 6: The specific density of the different species of aphids in the *Citrus* orchard

3.3 Diversity indexes

Nine diversity indexes have been studied (Table 3). The different obtained indexes showed high values of dominance indexes with 0.81 and 0.85 for Simpson Dominance index and Berger Parker dominance index respectively. This is due to the high dominance and abundance of *A. gossypii* compared to the other species. Consequently, the other registered diversity indexes are very low with 0.14 for Simpson diversity and 0.24 for Shannon diversity. In addition, the equitability showed a low value of about 0.26 (less than 0.6) indicating thus a disturbing environment. For richness indexes, Margalef's and Menhinick's richness indexes did not exceed 1 (respectively 0.16 and 0.18) and species richness was about 4.

Table 3: Values of the diversity indexes in the organic Citrus orchard

Diversity index	value
Specific richness (Rs)	4
Simpson Dominance (SDI)	0,81
Simpson diversity (GSDI)	0,14
Shannon index (H')	0,24
Evenness index (E)	0,78
Margalef's richness index (MR)	0,16
Menhinick's richness index (DMn)	0,18
Equitability index (J)	0,26
Berger Parker dominance index (d)	0,85

Diversity indexes evaluation in the organic citrus orchard

Monitoring Simpson Dominance and Equitability indexes revealed that dominance reached its maximum (SDI=1) almost during all the study period. Moreover, Berger Parker's dominance index was always high.

The index reached its maximum (d=1) almost during all the study periods indicating that the proportion of the abundance of one species in the field is high. The maximum-recorded levels of Simpson dominance and Berger Parker dominance indexes have been associated with maximum levels of Evenness (Figure 7).



Fig 7: Monitoring Dominance, Evenness, and Equitability indexes in the Citrus orchard

As a consequence of the high level of dominance observed in the Citrus orchard, the diversity was very low or absent throughout the study period. The Simpson diversity index (GSDI) ranged between 0.3 and 0.65 recorded on February 03rd and June 02nd, 2021, respectively. The Shannon index (H') ranged from 0.5 to 1.1 recorded on the same dates (Figure 8).



Fig 8: Monitoring Simpson diversity and Shannon indexes in the Citrus orchard

Regarding richness indexes, they were relatively low. Menhinick's richness index (DMn) ranged from 0.23 to 0.67 recorded between January 13th and June 09th, 2021, respectively. Margalef's richness index (MR) ranged from 0.35 to 0.5 registered between February 03rd and May 26th, 2021, respectively (Figure 9).



Fig 9: Monitoring richness indexes in the Citrus orchard

Correlation between diversity indexes

Relationships between diversity indexes have been studied using the Pearson correlation. There was a strong positive correlation between diversity indexes and richness indexes. For instance, that was observed between the Specific richness index (Rs) and Shannon index (H') (r= 0.761; p < 0.01), Margalef's richness index (MR) and Shannon index (H') (r=0.758; p < 0.01) and between Simpson diversity index (GSDI) and Margalef's richness index (MR) (r=0.729; p < 0.01) (Table 3). However, Menhinick's richness index (DMn) had a weak positive correlation with diversity indexes such as Simpson and Shannon diversity indexes (r=0.24 and r=0.23, respectively). Furthermore, Margalef's richness index (MR) had a strong positive correlation with specific richness (r=0.850; p < 0.01), however, Menhinick's richness index (DMn) had a weak correlation with specific richness (r=0.15). On the other hand, the Berger Parker dominance index (d) has a negative correlation value with Specific richness (Rs) (r=-0.09). However, it had a significant correlation with the Evenness index (E) (r=0.483; p < 0.05).

For the Evenness (E), it had a negative correlation with diversity indexes such as Specific richness (Rs) (r=-0.3), Simpson diversity index (GSDI) (r=-0.02), Shannon index (H') (r=-0.07), and Equitability index (J) (r=-0.055). Regarding Equitability (J), it had a strong significant correlation with Margalef's richness index (MR) (r=0.767; p < 0.01) and Simpson diversity index (GSDI) (r=0.975: p < 0.975). Moreover, this parameter had a significant negative correlation with Berger Parker dominance index (d) (r=-0.479: p < 0.05) and Simpson dominance index (SDI) (r=-0.610: p < 0.01).

	Specific	Number of	Simpson	Simpson	Shannon	Fanitability (F)	Berger Parker
	richness (Rs)	individuals (N)	dominance (SDI)	diversity (GSDI)	index (H')	Equitability (E)	index (d)
Evenness (E)	-0,30	-0,28	0,492*	-0,02	-0,07	-0,05461708	0,483*
Margalef's richness index (MR)	0,850**	0,14	-0,41	0,729**	0,758**	,767**	-0,28285726
Menhinick's richness index (DMn)	0,15	-,441*	-0,01	0,24	0,23	0,31670621	0,05523366
Equitability (E)	,711**	0,15	-0,610**	0,975**	0,970**		-0,479*
Berger Parker dominance index (d)	-0,09	0,06	0,982**	-0,527**	-0,513*	-,479*	
Simpson dominance index (SDI)	-0,23	-0,01		-0,641**	-0,631**	-,610**	0,982**
Simpson diversity index (GSDI)	0,711**	0,15	-,641**		0,996**	0,975**	-0,527**
Shannon index (H')	0,761**	0,21	-,631**	,996**		0,970**	-0,513*
Number of individuals (N)	0,458*		-0,01	0,15	0,21		

Table 4: Intra-correlation between various indexes using Pearson coefficient

* Correlation at 0.05 (2-tailed), ** Correlation at 0.01 (2-tailed)

4. Discussion

The study occurred on a Citrus orchard in the region of Mograne from the governorate of Zaghouane in Northeastern Tunisia. The field contains various trees of *Citrus spp*.

The species identified in the study were *Aphis spiraecola*, *Aphis gossypii*, *Toxoptera aurantii*, and *Macrosiphum euphorbiae*. These species were found on Citrus in Tunisia by other researchers ^[12, 14, 32, 33, 34]. Indeed, Ben Halima-Kamel and Ben Hammouda ^[14] found that the species *Aphis spiraecola* was the most abundant in the fields of *Citrus* spp during the second push that is the spring push, and that infestations depend on the development of young stems. Our results coincide with the results of Ben Halima-Kamel and Ben Hamouda ^[14]. Indeed, *Aphis gossypi* was the most

dominant species with less abundance of *Toxoptera aurantii* in the orchard. The same authors added that these species are the main species that cause significant damage in the Cap Bon region. *Aphis gossypii* and *Toxoptera aurantii* are species that havean important economic incidence in the Mediterranean region ^[35]. *Aphis sipraecola, Aphis gossypii*, and *Toxoptera aurantii* are vectors of Tristeza ^[36].

The emergence of the different species had been staggered over time. *Aphis gossypii* emerged first, which was around the beginning of February, followed by *Aphis spiraecola*. Moreover, according to Sellami *et al.* ^[15], the various specimens identified are divided into three groups according to their number of occurrences. Species with frequency fi $\geq 20\%$ are called frequent represented by *A*.

gossypii and A. spiraecola during this study. The second group of insects is within $20\% \leq fi \leq 10\%$ refers to moderately frequent species. The third group of insects contains rare species with fi <10%. That was the case of M. euphorbiae and T. aurantia with 9 and 2% respectively. These results coincide with a study in Japanmade by Komazaki ^[37] affirming that *A. gossypii* is a polyphagous species possessing thus primary hosts to settle down during winter. Blackman and Eastop ^[11] reported that A. gossypii is also parthenogenetic in the Mediterranean basin and A. spiraecola is parthenogenetic on Citrus spp. In addition, the two species had a parallel temporal evolution from the end of February until the end of the study. Indeed, a study on clementine gave the same results except that A. spiraecolawas more abundant than Aphis gossypii, this is explained by the fact that this species is influenced by the excessive production of proline that begins around the beginning of April and during June and September [38]. Our results confirm those of Marroquin et al. ^[39] who indicated that Aphis gossypii is more abundant on young shoots of Clementine than Aphis spiraecola with percentages of 53% and 32% respectively, Toxoptera aurantii stands at 11%. In Tunisia, Ben Halima-Kamel and Ben Hamouda ^[14] signaled that Aphis spiraecola and Aphis gossypii cause major damage to clementine trees with the sporadic presence of T. aurantii. A. spiraecola is the most abundant especially during summer.

According to Roger ^[16], diversity indexes represent the amount of information of a given sample. These indexes describe how individuals are distributed among various species. Diversity cannot be estimated by one index ^[40, 41]. So, nine diversity indexes have been used in our study: Simpson dominance, Simpson diversity, Shannon index, Evenness, Margalef's and Menhinick's richness indexes, Equitability, and Berger Parker dominance index.

Gorelick ^[42] affirms that the most used diversity indexes in ecological studies are Shannon and Simpson indexes. Our results showed that the mean value of the Shannon index was about 0.24 indicating a polluted environment ^[43, 44]. Indeed, the value of the Shannon index ranging between 1 and 2 describes a moderately polluted environment while values above 3 exhibit a stable ecosystem. Kanieski et al. [1] have shown that a value of 1.54 in an arboreal field indicates a low diversity. In addition, Dash [45] says that Simpson diversity values close to zero indicate that ecosystems are under stress conditions and low diversity. In our study, the mean value of the Simpson index was low (about 0.14). According to Brower and Zar^[46], this low diversity is due to a few numbers of species registered or to the abundance of few species so that the number of species identified in our study is low (Rs=4) and the dominance is high (SDI=0.81, d=0.85) (Table 3). Another study on Simpson diversity has demonstrated that the Simpson index reaches its maximum values when specific richness reaches a value from 10 to 12^[47]. In addition, the low diversity registered has been combined with a low mean value of equitability (J=0.26) (Table 3) which confirms the findings of Peet ^[48] affirming that diversification of species is higher in a community when there are more species and when the species are equally abundant.

The correlation between indexes has revealed that the Berger Parker index has a negative correlation with Specific richness (r=-0.09).That confirms the findings of Magurran ^[18] and Magurran and Phillip ^[49] who plotted the negative relationship between Species richness and Berger Parker index. In our study, the number of aphids' species was low (four species) and the mean value of Berger Parker dominance index was about 0.85 (Table 3). However, the Berger Parker index had a significant positive correlation with the Evenness index (r=0.483: p < 0.05). That explainsthese indexes' parallel temporal evolution (Table 4, Figure7).These results confirm the findings of Shah and Pandit ^[50] who revealed that the Berger Parker index depends totally on Evenness.

The correlation between Equitability (J) and Evenness (E) was weak and negative (r=-0.055), indicating probably that these two indexes do not always refer to the same term as noticed in the literature. The existence of a few or one dominant species reduces the Equitability, regarding that, there was a significant negative correlation between Berger-Parker index and Equitability (r=-0.479: p < 0.05) and a positive significant correlation between Simpson dominance and Equitability (r=-0.610, p < 0.01). Moreover, the Equitability and Evenness had the same values when the dominance was low (Figure 7). In our study, the Evenness index has shown a weak and negative correlation with the Simpson index (r=-0.02). Shah and Padit ^[50] mentioned in their works that there was a significant negative correlation between these two indexes (r=-0.54; p < 0.05).However, Watling et al. [51] had found a positive correlation between them (r=0.54; p < 0.05). The evenness had shown also a negative correlation with the Shannon index (r=-0.07) which is in contrast with the findings of Shah and Padit [50] who found that there was a strong significant relationship (r=0.771; p < 0.01) between the two indexes. Our findings reject the confirmation that evenness is a component of the diversity in ecosystems ^[52].

Concerning the richness indexes, they have a highly significant (p < 0.01) correlation with Simpson and Shannon indexes, except for Menhinick's richness index that has a weak positive correlation with those indexes confirming then the findings of Ricotta^[53] and Liu *et al.*^[54] that the richness is a component of the diversity. Moreover, Ravera [55] affirmed that a reduced number of species is considered a factor of low diversity. Furthermore, Margalef's richness index has a highly positive correlation (p < 0.01) with the Specific richness index. That confirms the findings of Shah and Pandit^[50] affirming that Margalef's richness index depends on the number of species and it has no limit value so that it is sensitive to the sample's size. Kocatas [56] had demonstrated that this index is used to compare between sites. Kanieski et al. [1] confirmed also that Margalef's index depends on the sample's size. Therefore, Menhinick's richness index had a weak positive correlation that explains the affirmation of Kanieski et al. [1]. Indeed, Menhinick's richness does not consider the size of the sampledarea; it represents the real diversity even in a small area.

Schaaf *et al.* ^[57] found that values of 6.52 and 7.02 of the Margalef index reveal medium to high diversity and values of 1.1 and 1.7 of Menhinick indexes show a medium diversity. A value of 1.96 of Menhinick's index denotes high diversity Kanieski *et al.* ^[1].

5. Conclusion

Four Aphid species were identified in an organic *Citrus* orchard in northeastern Tunisia. These species are *Aphis spiraecola*, *Aphis gossypii*, *Toxoptera aurantii*, and *Macrosiphum euphorbiae*. *A. gossypii* was the most abundant species, followed by *M. euphorbiae*. *A. spireacola* and *T. aurantii* were rarely found on the orchard. Two structure indexes and nine beta diversity indexes were calculated.

Results showed that few or one species was dominant. Correlation between the different indexes showed that the evenness index had a significant positive correlation with the Berger-Parker index; the richness index had a strong positive correlation with Margalef's richness index and the number of species, and the Menhinick's diversity index had a weak positive relationship with the Specific richness. Other studies will be carried out on other locations and for longer periods.

Appendices Table 1: Descriptive statistics

	N	Moon	Std Dovistion	Std Ennon	td Error 95% Confidence Interval for Mean		Minimum	Morimum
	1N.	Mean	Stu. Deviation	Stu. Error	Lower Bound	Upper Bound	wiininium	Waxiniuni
A. gossypii	23	37.3039	46.26566	9.64706	17.2971	57.3107	.00	174.23
A. spiraecola	23	13.3361	15.58158	3.24898	6.5981	20.0741	.00	53.47
M. euphorbiae	23	2.7591	7.10253	1.48098	3122	5.8305	.00	31.87
T. aurantii	23	.3565	1.44841	.30201	2698	.9829	.00	6.88
Total	92	13.4389	28.37114	2.95790	7.5634	19.3144	.00	174.23

Appendices Table 2: Anova

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19659.377	3	6553.126	10.761	.000
Within Groups	53588.496	88	608.960		
Total	73247.873	91			

Student-Newman-Keuls ^a						
Granian	N	Subset for alpha = 0.05				
Species	IN	1	2			
T. aurantii	23	.3565				
M. euphorbiae	23	2.7591				
A. spiraecola	23	13.3361				
A. gossypii	23		37.3039			
Sig.		.181	1.000			

Appendices Table 3: PostHoc test

Means for groups in homogeneous subsets are displayed

a. Uses Harmonic Mean Sample Size = 23,000

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