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ORIGINAL ARTICLE

Olive leaf gall midge (*Dasineura oleae* Angelini, Diptera, Cecidomyiidae): determination of olive tree infestation rates and quantification of parasitism by indigenous parasitoids

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Abstract
The olive leaf gall midge is a serious insect pest on olive trees in the Palestinian Territories. It induces galls on the leaves and other vegetative organs of olive trees causing reduction in the yield. The main objectives of the present study were to determine the infestation rate with *Dasineura oleae* on olive trees cultivated over variable altitudes in the Palestinian Territories, to identify the indigenous parasitoids of *D. oleae* and to determine the overall rates of parasitism by the identified parasitoids on *D. oleae*. Results indicated that the infestation rates with *D. oleae* varied significantly depending on the altitudes of sampling sites ranging from 12.1 to 52.7% at 635 and 229 m above sea level (a.s.l.), respectively. Two species of indigenous hymenopterous parasitoids on *D. oleae* were identified: *Platygaster oleae* Szelenyi (Platygastridae) and *Zeytinus hatayensis* Doğanlar (Eulophidae) (new record for the Palestinian Territories). The overall rates of parasitism by the identified parasitoids varied significantly with the altitude of the sampling sites from 12.6% (Assira El-Shamalieh, 635 m a.s.l.) to 55.5% (Aljarochia, 102 m a.s.l.). Partitioning of the overall rates of parasitism between the identified parasitoids indicated that *P. oleae* was found at all sampling sites (82 to 635 m a.s.l.) with proportions of 60.8–100%, whereas *Z. hatayensis* with proportions of 0–39.1% and was not found at altitudes above 492 m a.s.l. The overall results suggest the use of *P. oleae* and *Z. hatayensis* in biocontrol of *D. oleae* in the Palestinian Territories.

Keywords Parasitism · Biocontrol · Indigenous parasitoids · *Olea europaea* · *Platygaster oleae* · *Zeytinus hatayensis*

Introduction

Dasineura oleae Angelini (1831) has been reported as a native gall inducer on olive, *Olea europaea* L., in the whole Mediterranean area from the most western part in Portugal up to the eastern part (Syria, Lebanon, Jordan, Israel and the Palestinian Territories). The galls induced by *D. oleae* affect the distribution of nutrient elements in the infested leaf tissues and other vegetative organs of olive trees, especially in the stalks of flowers (inflorescence). Infestation of the stalks by *D. oleae* may additionally lead to yield loss. Galls induced in these tissues interrupt the nutrient flow to the growing fruits, and small-sized fruits are produced (Batta 2019).

Al-Tamimi (1997) showed that the olive leaf gall midge is widely distributed in Jordan in all economically olive planted orchards. At some sites, it causes serious damage by infesting up to 55–62% of leaves. Darvas et al. (2000) evaluated *D. oleae* as a serious pest in Syria and a causer of damages in other countries of the Mediterranean area. *D. oleae* has been generally accepted as a secondary pest of olive in Turkey and in the countries of Mediterranean region (Skuhravá and Skuhravý 1997). Al-Tamimi (1997), Hrncic (1998), Simoglou et al. (2012) and Boselli and Bariselli (2015) stated that *D. oleae* has been considered as an important pest of some olive orchards in Jordan, Montenegro, Greece and Italy, respectively. In Turkey, the olive leaf gall midge has been reported from Bursa, Denizli, Antalya, Hatay and the Aegean region (Trotter 1903; Alkan 1952a, b;

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İyriboz 1968; İren and Ahmet 1973; Skuhravý et al. 1980; Skuhravá et al. 2005). Doğanlar et al. (2011) mentioned that *D. oleae* and its parasitoids develop high population densities in the Hatay province (Turkey) by their works in the period from 2007 to 2010. In the Palestinian Territories, Batta (2019) reported the presence of *D. oleae* as a serious pest on olive trees grown at different sites and governorates of the country.

Larvae of *D. oleae* cause slight, indefinite, elongate swellings (galls) on olive leaves (Fig. 1a–c). Per gall, only one larva develops. The whole development from egg to adult takes place inside the gall. Galls of *D. oleae* may also occur on terminal parts of young shoots of olive trees if the density of gall midge population in the area is high (Coutin and Katlabi 1986; Avidov and Harpaz 1969; Al-Tamimi 1997; Darvas et al. 2000; Batta 2019). Usually, only one generation per year develops in many areas of olive production in several countries of Mediterranean region (Al-Tamimi 1997; Darvas et al. 2000; Doğanlar et al. 2011), but at favorable conditions a second generation may develop at certain sites as in the subcoastal regions of olive production in the eastern Mediterranean countries such as Syria and the Palestinian

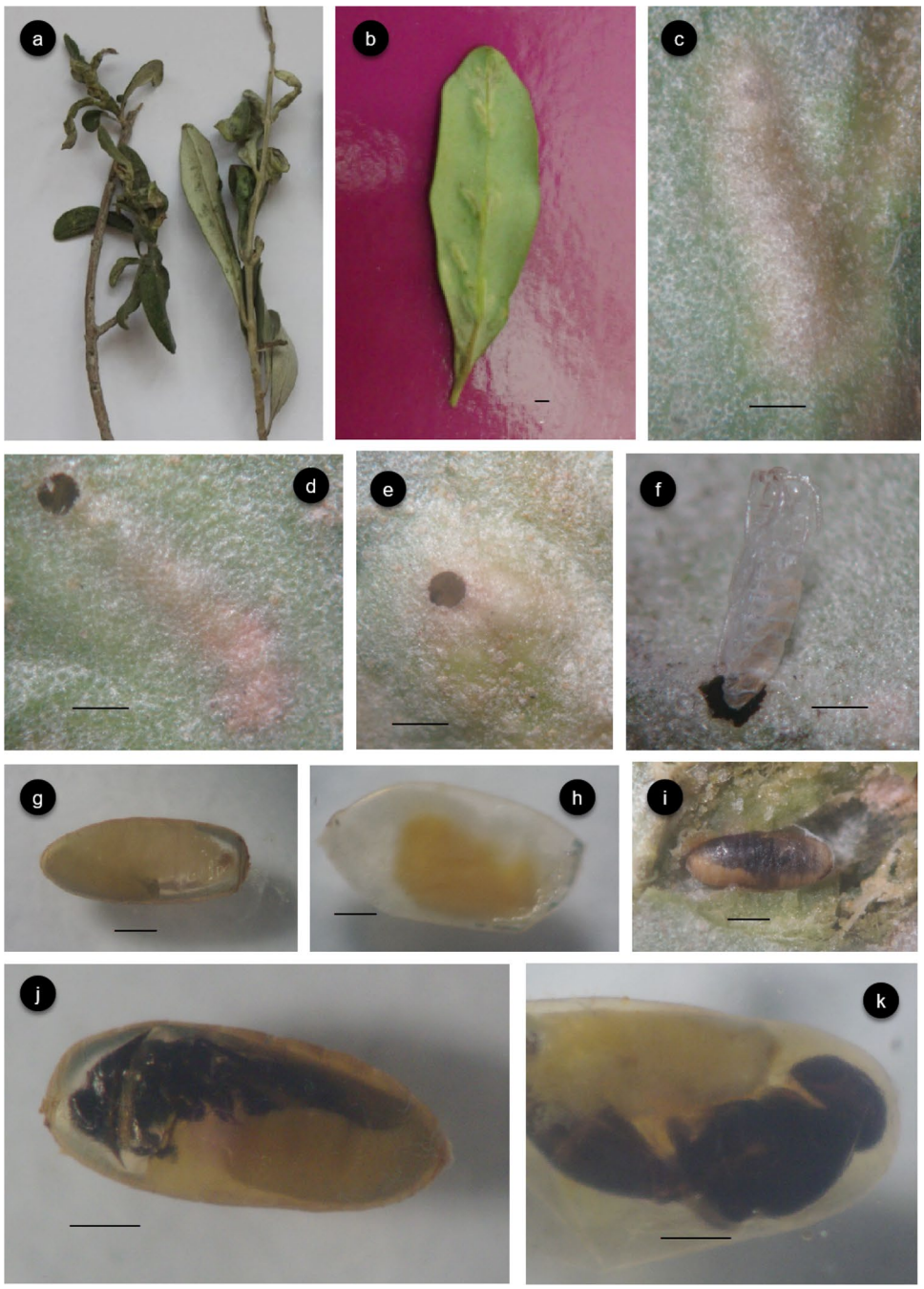

Fig. 1 Damage caused by *Dasineura oleae* on olive trees and mode of parasitism by indigenous parasitoids, **a** damage caused by *D. oleae* on leaves and shoots of olive trees, **b** infestation with *D. oleae* on olive leaves appeared as indefinite and elongate galls on the lower leaf surface, **c**: enlarged view of *D. oleae* gall on the lower surface, **d–e** circular exit holes for emergence of the adult parasitoids from *D. oleae* galls, **f** emergence of *D. oleae* adults after completing the life cycle in form of irregular exit hole with puparium left near the hole after emergence, **g–h** unparasitized pupae of *D. oleae*, **i–k** parasitized pupae of *D. oleae* with different developmental stages of the parasitoid living inside the pupae. Scale bars = 100 µm (in the above illustrations)

Territories (Baidaq et al. 2015; Ramadhane et al. 2017; Batta 2019). The adult flies of *D. oleae* are usually present in the field from March to the beginning of May for the first generation and from October to the beginning of November for the second generation (Batta 2019).

Only little work has been conducted on mortality factors of *D. oleae*. Doğanlar (1992) mentioned *D. oleae* on leaves and flowers of *O. europaea* as new host for *Quadrastichus sajoi* (Szelenyi) from Syria. Al-Tamimi (1997) stated that climatic conditions such as temperature and relative humidity, in addition to the natural enemies, especially the parasitoids, were given as the main mortality factors of *D. oleae* in Amman district, Jordan. Doğanlar et al. (2009) reared and described *Quadrastichus dasineurae* Doğanlar, LaSalle, Sertkaya and O. Doğanlar (Hymenoptera, Eulophidae) as larval parasitoid of *D. oleae* in Hatay province, Turkey. Doğanlar (2011) reared some parasitoids from galls of *D. oleae* and *Lasioptera oleicola* Skuhravá on leaves and shoots of *O. europaea* as larval and pupal parasitoids of *D. oleae* in Hatay province, Turkey.

In the Palestinian Territories, olive trees are cultivated in areas with variable altitudes ranging from 82 m above sea level (a.s.l.) in the subcoastal areas to 635 m a.s.l. in the hilly areas. The infestation rates with *D. oleae* in relation to the altitudes of the areas planted with olive trees have not been studied in this country. Similarly, parasitism on *D. oleae* has not been examined in this country. Therefore, the objectives of the present study were: (1) to determine the infestation rate with *D. oleae* on the leaves of olive trees cultivated at variable altitudes, (2) to identify the species of indigenous parasitoids on the larvae of *D. oleae*, (3) to describe the species of indigenous parasitoids by providing with the necessary illustrations, (4) to determine the overall rates of parasitism on *D. oleae* by the identified indigenous parasitoids in the sampling sites with variable altitudes, (5) to partition the overall rates of parasitism on *D. oleae* between the identified species of parasitoids in the sampling sites and check the relationship between the rate of parasitism and the altitude of the sampling sites.

Materials and methods

Collection of samples from different sampling sites of olive production areas

Samples of leaves and branches of infested olive trees with *D. oleae* were collected from different sampling sites spreading in olive production areas of the Palestinian Territories. Sampling sites have the following altitudes and coordinates: Kadouri in Tulkarm governorate (82 m a.s.l., coordinates: 32°18′48.3″N 35°01′19.9″E); Aljarochia in Tulkarm governorate (102 m a.s.l., coordinates: 32°20′48.0″N 35°02′50.5″E); Wadi Kana in Sulfeet governorate (229 m a.s.l., coordinates: 32°09′28.7″N35°06′38.5″E); Seilet El-Dahr in Jenin governorate (492 m a.s.l., coordinates: 32°18′52.8″N 35°11′21.4″E) and Assira El-Shamalieh in Nablus governorate (635 m a.s.l., coordinates: 32°14′59.9″N 35°16′05.6″E). Four plots with five olive trees each were randomly chosen in each sampling site (1000 m^2 area planted with olive trees). Then, randomly chosen samples of leaves and branches were collected from chosen plots within each site during the study that took place from November 15 (2018) to February 15 (2019) (the number of leaves collected per plot is indicated in Tables 1 and 2). The average annual temperature in the sampling sites was 14.8 °C (6–39 °C), and the average annual relative humidity was 58.9% (32–100%) (PMA 2018). The purpose of collecting the samples was to determine the infestation rates with *D. oleae* on olive trees and to determine the overall rates of parasitism caused by the indigenous parasitoids on *D. oleae* galls in the sampling sites.

Determination of infestation rates of olive trees with *D. oleae* in the different sampling sites

To determine the infestation rates with *D. oleae*, randomly chosen samples of olive leaves were collected from chosen plots in the sampling sites of olive production areas (four plots with five olive trees each per sampling site of 1000 m^2 area planted with olive trees as indicated in the previous section: collection of samples from different sampling sites). The following method was used to determine the rate of infestation: (1) counting the total number of leaves examined in each plot per sampling site, (2) counting the number of infested leaves with *D. oleae* in each plot per sampling site. The infested leaves should have the characteristic galls of *D. oleae* that could be seen on the lower leaf surface (Fig. 1b–c), (3) calculating the percentage of infestation with *D. oleae* on the leaves of each plot in each sampling site, and (4) calculating the average percentage of infestation with *D. oleae* per sampling site in each governorate. All counts for determining the infestation rate were repeated twice at different times during the period of the study for confirmation.

Rearing of indigenous parasitoids from *D. oleae* galls in the collected samples

For promoting the emergence of adult parasitoids from galls of *D. oleae*, the collected samples of infested leaves were incubated in closed transparent plastic bags (dimensions: 35 × 25 cm). Incubation of samples was done in an incubator under the following conditions: temperature of 20 ± 2 °C and illumination of 16 h per day. For each plot in each sampling site, emerged adult parasitoids were collected using an aspirator then preserved in screw-caped vials with 70%

Table 1 Rate of infestation with *Dasineura oleae* on olive trees and overall rate of parasitism on *D. oleae* at different sampling sites of olive production areas of the Palestinian Territories during the period from November 15, 2018, to February 15, 2019. Four plots were randomly taken in each sampling site (1000 m^2 area planted with olive trees), and each plot contained five neighboring olive trees within the site

Sampling site/governorate of olive production: coordinates and altitude of the site, in addition to the olive cultivar	Plots of olive trees taken in each sampling site (five trees/plot)	Total number of olive leaves randomly sampled/plot	Number of infested leaves with galls of *D. oleae*/plot	% of infestation with *D. oleae*/plot*	Total number of galls of *D. oleae* counted on infested leaves/plot	Number of galls of *D. oleae* parasitized on infested leaves/plot	% of overall rates of parasitism on *D. oleae* by all species of parasitoids/plot*
Kadouri/Tulkarm governorate (32°18′48.3″N 35°01′19.9″E) **82** m (269.0 feet) above sea level (a.s.l.); cultivar: Nabali	1	402	199	49.50	1025	582	56.78
	2	291	150	51.55	592	296	50.00
	3	525	211	40.19	905	401	44.30
	4	388	236	60.82	684	398	58.19
Average% per sampling site (s.s.)				50.5 ± 3.6 **cd**			52.3 ± 2.8 **D**
Aljarochia/Tulkarm governorate (32°20′48.0″N 35°02′50.5″E) **102** m (334.6 feet) a.s.l.; cultivar: Nabali-Mohassan	1	415	156	37.59	543	321	59.12
	2	208	140	67.31	880	428	48.64
	3	386	137	35.49	802	475	59.23
	4	540	173	32.04	740	406	54.86
Average% per s.s.				43.1 ± 7.0 **c**			55.5 ± 2.2 **D**
Wadi Kana/Sulfeet governorate (32°09′28.7″N 35°06′38.5″E) **229** m (751.3 feet) a.s.l.; cultivar: Nabali-Mohassan	1	590	284	48.13	1562	403	25.8
	2	225	126	56.00	880	274	31.1
	3	214	108	50.47	502	205	40.8
	4	302	170	56.29	1156	425	36.8
Average % per s.s.				52.7 ± 1.8 **d**			33.6 ± 2.3 **C**
Seilet El-Dahr/Jenin governorate (32°18′52.8″N 35°11′21.4″E) **492** m (1614.2 feet) a.s.l.; olive cultivar Nabali	1	513	121	23.59	203	56	27.59
	2	326	80	24.54	652	172	26.38
	3	411	110	26.76	908	216	23.79
	4	395	78	19.75	282	69	24.47
Average % per s.s.				23.7 ± 1.2 **b**			25.6 ± 0.8 **B**

Table 1 (continued)

Sampling site/governorate of olive production: coordinates and altitude of the site, in addition to the olive cultivar	Plots of olive trees taken in each sampling site (five trees/plot)	Total number of olive leaves randomly sampled/plot	Number of infested leaves with galls of *D. oleae*/plot	% of infestation with *D. oleae*/plot*	Total number of galls of *D. oleae* counted on infested leaves/plot	Number of galls of *D. oleae* parasitized on infested leaves/plot	% of overall rates of parasitism on *D. oleae* by all species of parasitoids/plot*
Assira El-Shamalieh/Nablus governorate (32°14′59.9″N 35°16′05.6″E) **635** m (2083.3 feet) a.s.l.; olive cultivar Nabali	1	395	62	15.70	251	32	12.75
	2	350	46	13.14	622	81	13.02
	3	580	55	9.48	203	21	10.34
	4	412	42	10.19	292	42	14.38
Average % per s.s.				12.1 ± 1.2 **a**			12.6 ± 1.3 **A**
Overall average %/sampling site				**36.4**			**35.9**

Bold indicates the altitude of the sampling sites in the governorates and its importance in the spread of the pest and its parasitoids

*Means in each column followed by the same letter are not significantly different at $P \leq 0.05$ using ANOVA and Tukey HSD test for means separation

*Standard error of the means (SE) was calculated and added to the average % per sampling site as "Mean ± SE"

ethanol as a mounting medium for being identified and then described. The incubation period was extended until the emergence of all adult parasitoids from infested galls in the samples (collection time was elapsed from November 15, 2018, to February 15, 2019).

Description and identification of indigenous parasitoids reared from *D. oleae* galls

Specimens of males and females of the parasitoids preserved in screw-caped vials with 70% ethanol as a mounting medium were used for identification and description of the parasitoid species reared from *D. oleae* galls on the infested leaves. Adults of each parasitoid species were morphologically examined under the dissecting and compound microscopes. Afterward, preparations of microscopic slides for the different body parts of these adults were made to be examined during the identification. Representative pictures for the male and female adults of parasitoids and their body parts were taken. Finally, microscopic examination for identifying and describing of each parasitoid species was performed. Illustrations were also provided for the following characteristics: body color, female and male genitalia, the mesosoma, the gaster, the antennae and legs, in addition to the venation of the fore wings. These characteristics were used in the description of each parasitoid species.

Determination of overall rates of parasitism on *D. oleae* galls in the different sampling sites

The randomly chosen samples of infested leaves with *D. oleae* were taken from the different plots (four plots with five olive trees each per sampling site of 1000 m^2 area planted with olive trees as indicated in the previous section: collection of samples from different sampling sites). These samples were used to determine the overall rate of parasitism on *D. oleae* galls in the different sampling sites. The period of this study has extended from November 15, 2018, to February 15, 2019. The following method was used during the study: (1) counting the total number of *D. oleae* galls on the infested leaves in each plot and in each sampling site then, (2) counting the total number of parasitized galls of *D. oleae* on the sampled leaves in each plot and in each sampling site. The parasitized galls are characterized by having circular exit holes in their walls (Fig. 1d-e) that clearly indicate the emergence of the adult parasitoids, whereas the unparasitized galls of *D. oleae* have irregular holes (ruptures) in their walls. In addition, the pupal skins (puparia) are left behind when the adult flies emerge from pupae (Fig. 1f), (3) calculating the percentage of overall rate of parasitism on *D. oleae* galls on the infested leaves of each plot and each sampling site, and (4) calculating the average percentage of overall rate of parasitism per sampling site in each governorate for the different species of indigenous parasitoids on

Table 2 Partitioning of the overall rates of parasitism (indicated in Table 1) between two identified species of parasitoids on *Dasineura oleae* on olive trees at different sampling sites in the Palestinian Territories during the period from November 15, 2018, to February 15, 2019. Four plots were randomly taken in each sampling site (1000 m^2 area planted with olive trees), and each plot contained five neighboring olive trees within the site

Sampling site/governorate of olive production: coordinates and altitude of the site, in addition to the olive cultivar	Samples taken from reared parasitoids on *D. oleae* in each plot & preserved in 70% ethanol	Total number of parasitoids taken from preserved specimens in the samples of each plot	Number of *Platygaster oleae* parasitoids counted in the samples of each plot	Number of *Zeytinus hatayensis* parasitoids counted in the samples of each plot	*% Platygaster oleae* parasitoid in the samples of each plot*	*% Zeytinus hatayensis* parasitoid in the samples of each plot*
Kadouri/Tulkarm governorate (32°18′48.3″N 35°01′19.9″E) **82** m (269.0 feet) above sea level (a.s.l.); cultivar: Nabali	1	128	110	18	85.94	14.06
	2	184	138	46	75.00	25.00
	3	150	109	41	72.67	27.34
	4	144	138	6	95.83	4.17
Average % per sampling site (s.s.)					82.4 ± 4.6 **c**	17.6 ± 4.5 **B**
Aljarochia/Tulkarm governorate (32°20′48.0″N 35°02′50.5″E) **102** m (334.6 feet) a.s.l.; cultivar: Nabali-Mohassan	1	102	68	34	66.67	33.33
	2	104	60	44	57.69	42.31
	3	142	90	52	63.38	36.62
	4	256	149	106	58.20	41.41
Average % per s.s.					61.6 ± 1.9 **a**	38.4 ± 1.8 **D**
Wadi Kana/Sulfeet governorate (32°09′28.7″N 35°06′38.5″E) **229** m (751.3 feet) a.s.l.; cultivar: Nabali-Mohassan	1	105	75	30	71.43	28.57
	2	115	65	50	56.52	43.48
	3	120	80	40	66.67	33.33
	4	85	71	14	83.53	16.47
Average % per s.s.					69.5 ± 4.9 **b**	30.5 ± 1.2 **C**
Seilet El-Dahr/Jenin governorate (32°18′52.8″N 35°11′21.4″E) **492** m (1614.2 feet) a.s.l.; olive cultivar Nabali	1	125	125	0	100	0
	2	115	115	0	100	0
	3	78	78	0	100	0
	4	46	46	0	100	0
Average % per s.s.					100 ± 0 **d**	0 ± 0 **A**
Assira El-Shamalieh/Nablus governorate (32°14′59.9″N 35°16′05.6″E) **635** m (2083.3 feet) a.s.l.; olive cultivar Nabali	1	69	69	0	100	0
	2	38	38	0	100	0
	3	52	52	0	100	0
	4	27	27	0	100	0
Average % per s.s.					100 ± 0 **d**	0 ± 0 **A**
Overall average %/sampling site					**82.7**	**17.3**

Bold indicates the altitude of the sampling sites in the governorates and its importance in the spread of the pest and its parasitoids

*Means in each column followed by the same letter are not significantly different at $P \leq 0.05$ using ANOVA and Tukey HSD test for means separation

*Standard error of the means (SE) was calculated and added to the average % per sampling site as "Mean ± SE"

D. oleae galls. All counts to determine the overall rates of parasitism were repeated twice at different times during the period of the study for confirmation.

Partitioning of the overall rates of parasitism between the identified species of indigenous parasitoids of *D. oleae* in the different sampling sites

After identification of each parasitoid species reared from galls of *D. oleae* in the different sampling sites of olive production areas, samples of preserved specimens of parasitoid adults in screw-caped vials with 70% ethanol were used during the study. The preserved specimens represent the parasitoids in the sampling sites (four plots with five olive trees each per sampling site of 1000 m^2 area planted with olive trees as indicated in the previous section: collection of samples from different sampling sites). The percentage of each parasitoid species in each plot and in each sampling site was determined according to the following method: (1) taking four samples of preserved specimens of parasitoid species representing four plots chosen per sampling site. Each sample contains the two species of parasitoids reared from *D. oleae* galls specific to the plots of sampling site, (2) counting the total number of preserved specimens of the two species of parasitoid adults in the plot, (3) counting the number of each species of parasitoid specimens in the plot according to the identified morphological characters of each species, (4) calculating the percentage of each parasitoid species in the sample in relation to the total number, (5) calculating the average percentage of each parasitoid species in the plots per sampling site and governorate. Collecting and preserving of parasitoid specimens elapsed from November 15, 2018, to February 15, 2019. All counts for determining the rate of parasitism by each parasitoid were repeated twice at different times during the period of the study for confirmation.

Data analyses

Statistical analysis was performed on the data obtained on the average % of infestation rates with *D. oleae* per plot in the sampling sites and governorate, and the average % of overall rate of parasitism by the two species of parasitoids on *D. oleae* galls per sampling site and governorate, in addition to the average % of each parasitoid species per plot in the sampling site and governorate. The analysis performed includes calculating of the standard error of the means (SE) in the different plots of the sampling sites and adding it to the means "Mean ± SE" as shown in Tables 1 and 2. Analysis of variance (ANOVA) and means' separation by Tukey HSD were conducted to check the presence of significant differences between the means in the different plots of sampling sites. Significance level was set at $P < 0.05$. All analyses were performed with the statistical software SAS® (SAS Institute, Cary, NC, USA).

Results

Rate of infestation of olive tree leaves with *D. oleae* versus altitude of sampling sites

The average % of infestation with *D. oleae* on the sampled leaves of olive trees in the different sampling sites representing the governorates of the Palestinian Territories varied from 12.1% in Assira El-Shamalieh (635 m a.s.l.) to 52.7% in Wadi Kana (229 m a.s.l.) (Table 1). At higher altitude such as Seilet El-Dahr (492 m a.s.l.) and Assira El-Shamalieh (635 m a.s.l.), the average % of infestation rate was lower, 23.7% and 12.1%, whereas at lower altitudes such as Kadouri (82 m a.s.l.), Aljarochia (102 m a.s.l.) and Wadi Kana (229 m a.s.l.), the average % of infestation rate was higher, 50.5%, 43.1% and 52.7% (Table 1). Significant differences (at $P \leq 0.05$) were obtained between the values of average % of infestation with *D. oleae* in the different sampling sites distributed over a range of altitudes from 82 to 635 m a.s.l. The overall average % of infestation with *D. oleae* in the different sampling sites of the Palestinian Territories was 36.4% (Table 1). These results indicate that the rate of infestation with *D. oleae* depends upon the altitude of the area in which olive trees are cultivated.

Description and identification of the parasitoids reared from *D. oleae* galls

Two species of indigenous hymenopterous parasitoids were reared from galls of *D. oleae* and then identified as *Platygaster oleae* Szelenyi (Platygastridae) and *Zeytinus hatayensis* Doganlar (Eulophidae) (new record for the Palestinian Territories). For this identification, morphological characters of males and females of each parasitoid species were described and then compared with the characters of the species described by the key given by Doğanlar (2011). The necessary illustrations for describing the above-mentioned characters are provided in Fig. 2.

***Platygaster oleae* Szelenyi** is an endoparasitoid in the larvae of *D. oleae* and develops inside it until the emergence of adult parasitoid. Morphological characters of the male and female adults that distinguish them from other parasitoid species are given in Fig. 2. The mean body length for the females = 1.12 ± 0.13 mm ($n = 50$ specimens) and for males = 0.90 ± 0.15 mm ($n = 50$ specimens).

***Zeytinus hatayensis* Doğanlar** is an endoparasitoid in the larvae of *D. oleae* and develops inside it until the emergence of adult parasitoid. Morphological characters of the male

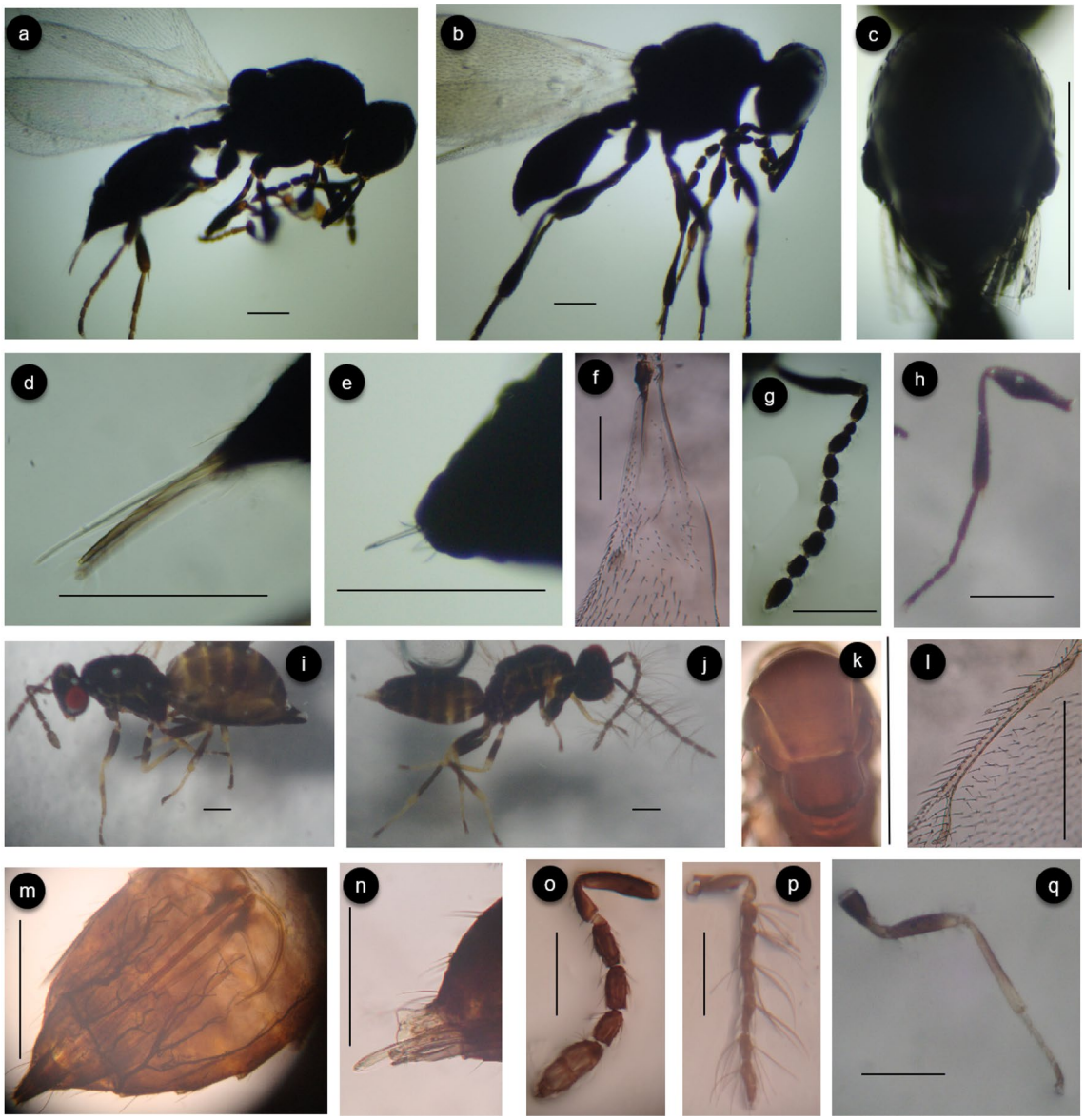

Fig. 2 Specific morphological characteristics of indigenous parasitoids of *Dasineura oleae* that distinguish them from other parasitoid species, **a**–**b** lateral views of female and male adults of *Platygaster oleae*, respectively, **c** dorsal view of the characteristic mesonotum and scutellum of *P. oleae*, **d**–**e** lateral views of ovipositor and aedeagus of female and male of *P. oleae*, **f** characteristic fore wing base of *P. oleae* adult, **g**: characteristic antenna of *P. oleae* adult, **h**: characteristic hind leg of *P. oleae* adult, **i**–**j** lateral views of female and male adults of *Zeytinus hatayensis*, respectively, **k** dorsal view of the characteristic mesonotum and scutellum of *Z. hatayensis*, **l** characteristic fore wing base of *Z. hatayensis*, **m**: ventral view of the gaster showing the ovipositor of *Z. hatayensis* female, **n**: lateral view of the gaster showing the aedeagus of *Z. hatayensis* male, **o**–**p** characteristic antennae of female and male of *Z. hatayensis*, respectively, **q** characteristic hind leg of *Z. hatayensis* adult. Scale bars = 100 µm (in the above illustrations)

and female adults that distinguish them from other parasitoid species are given in Fig. 2. The mean body length for the females = 1.33 ± 0.15 mm (n = 50 specimens) and for males = 1.03 ± 0.17 mm (n = 50 specimens).

Evaluation of parasitism by the indigenous parasitoids on *D. oleae*

Females of the parasitoids *of P. oleae* and *Z. hatayensis* own a long, appendicular ovipositor for thrusting the walls of *D. oleae* galls and then depositing eggs inside the galls near the host larvae that live inside the galls. The hatched larvae of the parasitoid penetrate the larvae of *D. oleae* and feed inside it

possibly causing its death (Fig. 1i–k). Parasitized larvae of *D. oleae* are characterized by having partially or completely dark color (Fig. 1i–k) compared to the unparasitized larvae with yellow color (Fig. 1g–h). When the development of parasitoid larvae is completed, they pupate in the gall. Then, the adults emerge from hosting galls by chewing small circular exit holes in the wall of these galls (Fig. 1d–e). If the galls of *D. oleae* are not parasitized by either *P. oleae* or *Z. hatayensis*, the adult flies of *D. oleae* emerge from hosting gall. This is done by making, first, a rupture in the wall of these galls (irregular exit hole made by the mature pupae; Fig. 1f), and then, second, the adult flies emerge from exited mature pupae by leaving empty pupal skins (puparia) attaching to the leaves near the exit hole (Fig. 1f). This proves that *P. oleae* and *Z. hatayensis* are endoparasitoids of larvae and pupae of *D. oleae* on olive trees grown in the Palestinian Territories, and both species of parasitoids can be reared from galls of *D. oleae* on infested olive leaves.

Overall rate of parasitism by indigenous parasitoids reared from *D. oleae* on olive trees in the different sampling sites

The overall rate of parasitism caused by the different species of indigenous parasitoids on *D. oleae* galls was determined by calculating the average % of the overall rate of parasitism in randomly chosen samples of infested olive leaves with *D. oleae* in the different sampling sites of the Palestinian Territories (Table 1). The average % of the overall rate of parasitism varied from 12.6% in Assira El-Shamalieh (635 m a.s.l.) to 55.5% in Aljarochia (102 m a.s.l.) (Table 1). At higher altitudes such as Seilet El-Dahr (492 m a.s.l.) and Assira El-Shamalieh (635 m a.s.l.), the average % of overall rate parasitism was lower, 25.6% and 12.6%, respectively (Table 1). However, at lower altitudes such as Kadouri (82 m a.s.l.), Aljarochia (102 m a.s.l.) and Wadi Kana (229 m a.s.l.), the average % of overall rate parasitism was higher, 52.3%, 55.5% and 33.6%, respectively (Table 1). Significant differences (at $P \leq 0.05$) were obtained between the values of average % of overall rate parasitism in the different sampling sites. Therefore, the overall rate of parasitism is dependent on the altitude of the area in which olive trees are cultivated. The average % of overall rate of parasitism in the different sampling sites of the Palestinian Territories was 35.9% (Table 1).

Partitioning of the overall rate of parasitism between the identified species of indigenous parasitoids of *D. oleae* in the different sampling sites

After the identification and description of two parasitoid species from *D. oleae* (*P. oleae* and *Z. hatayensis*), the overall rate of parasitism determined in the above section was partitioned between these species of parasitoids as follows: For *P. oleae*, it varied from 61.6% in Aljarochia (102 m a.s.l.) to 100% in Seilet El-Dahr (492 m a.s.l.) and in Assira El-Shamalieh (635 m a.s.l.) (Table 2). For *Z. hatayensis*, it varied from 0% in Seilet El-Dahr (492 m a.s.l.) and in Assira El-Shamalieh (635 m a.s.l.) to 38.4% in Aljarochia (102 m a.s.l.) (Table 2). This would indicate that the average rate of partitioning the overall rate of parasitism depends upon the altitude of the area in which olive trees are cultivated. Significant differences (at $P \leq 0.05$) were obtained between the values of average % of partitioning the overall rate of parasitism in the different sampling sites. Also, *P. oleae* constituted the largest portion in the samples taken at all altitudes (ranged from 61.6% to 100% at 102 to 635 m a.s.l., respectively), whereas *Z. hatayensis* constituted the smallest portion in the samples taken at the same altitudes (ranged from 0 to 38.4% at 102 to 635 m a.s.l., respectively). Furthermore, *P. oleae* existed at all altitudes of sampling sites (82 to 635 m a.s.l.), whereas *Z. hatayensis* existed only at lower altitudes but not at higher ones exceeding 492 m a.s.l. The overall average rate of partitioning of parasitism on *D. oleae* by the two species of parasitoids in the different sampling sites was 82.7% for *P. oleae* and 17.3% for *Z. hatayensis* (Table 2).

Discussion

To our best knowledge, this is the first study demonstrating the relationship between the rate of infestation with *D. oleae* on olive trees and the altitude of the area cultivated with this crop. However, certain authors reported the infestation rate with *D. oleae* but without specifying the altitudes of the infested areas. For example, Doganlar et al. (2011) and Perdikis et al. (2015) reported infestation rates of 8.0–78.2% and up to 89% on olive trees grown in Hatay province (Turkey) and in Crete (Greece) without specifying the altitudes of the sampling sites.

In the present study, we were able to prove that the infestation rates with *D. oleae* are dependent on the altitude of the sampling sites since we found that the infestation rates were more severe in the lower altitudes (82 to 229 m a.s.l.) compared to the higher altitudes (492 to 635 m a.s.l.). A possible explanation for this relationship could be that the lower altitudes are not far from the coast of Mediterranean sea, where temperatures and relative humidity are more favorable for the development of *D. oleae* compared to the higher altitudes where lower temperatures and less relative humidity are prevailing. Moreover, at the Palestinian conditions, *D. oleae* produces two generations per year in the subcoastal areas of olive production (lower altitudes) compared to only one generation per year in the hilly areas (higher altitudes) (Batta, 2019).

During the present study, we were able to identify two species of indigenous parasitoids on larvae and pupae of *D. oleae* on olive trees in the Palestinian Territories: *Platygaster oleae* and *Zeytinus hatayensis*. They are considered as the main indigenous parasitoids on *D. oleae* in the sampling sites because *P. oleae* possessed the greatest overall average rate of parasitism reaching at 82.7% and spread in all Palestinian sampling sites and governorates, whereas *Z. hatayensis* possessed the smallest overall average rate of parasitism reaching at 17.3% and spread in few Palestinian sampling sites and governorates. These characteristics would qualify *P. oleae* to be an excellent candidate for biocontrol of *D. oleae* on olive trees in the Palestinian Territories. In other countries such as Croatia, Jordan, Greece and Turkey, *P. oleae* has been reported to be a potential parasitoid on *D. oleae* and plays an important role in biocontrol of *D. oleae* (Szelenyi 1940; Al-Tamimi 1997; Fauna europaea 2010; Doganlar 2011).

The overall rate of parasitism on *D. oleae* is inversely proportional to the altitude of the area planted with olive trees; it was higher at the lower altitudes (82 to 229 m a.s.l.) when compared with the higher altitudes (492 to 635 m a.s.l.). Also, the development of the parasitoids at the lower altitudes was faster in comparison with the higher altitudes (Batta 2019). This could be attributed to the availability of favorable conditions, especially temperature and relative humidity in the areas with lower altitudes compared to the higher altitudes. Doganlar et al. (2011) reported that certain parasitoids of *D. oleae* such as *Z. hatayensis* do not exist and develop at higher altitudes in Hatay province (Turkey) because it needs certain levels of temperature and relative humidity for their development. However, high temperature accompanied with low relative humidity (> 35 °C and < 50% r.h.) causes high mortality in the emerged adults of *P. oleae* at the higher altitudes of olive orchards of Amman district (Jordan) (Al-Tamimi 1997). This would restrict the use of this parasitoid as biocontrol agents of *D. oleae* at the higher altitudes.

For partitioning of the overall rate of parasitism on *D. oleae* between the identified species of parasitoids, we were able to allocate certain level of parasitism rate for each one of these parasitoids within the overall rate of parasitism. This allocation is acceptable for assessing the efficacy of each parasitoid species in suppressing *D. oleae* population when used separately. However, the available literature for assessing the efficacy of each parasitoid species within the parasitoid complex on *D. oleae* infestation is extremely rare. This could be attributed to the practical difficulty in defining the characteristics of the hole from which each parasitoid species can make to exit from hosting gall. This is because each parasitoid species has its own characteristic exit hole. Doganlar et al. (2011) assessed the overall efficacy of 16 species of parasitoids from five families on *D. oleae* infestation in Hatay province, Turkey. They reported an overall rate of parasitism reaching at 65% but, they did not allocate certain level of parasitism rate for each one of these parasitoids within the overall rate of parasitism.

In conclusion, in the present study, we were able to determine the rates of infestation with *D. oleae* on olive trees in the Palestinian Territories and relate these rates to the different altitudes of the sampling sites. We were also able to identify two species of indigenous parasitoids on *D. oleae*: *Platygaster oleae* and *Zeytinus hatayensis* in the studied sampling sites. Other species of indigenous parasitoids may be identified when the sampling process is extended to other sampling sites or in other periods of sampling during the year. The diagnostic characters of these species, in addition to the necessary illustrations, were provided in this study. Furthermore, we determined the overall rates of parasitism on *D. oleae* by the identified species of parasitoids in the sampling sites, in addition to partitioning of these rates between the identified species of the parasitoids according to the sampling sites. As a result of this partitioning, *P. oleae* was found to be the dominant species of parasitoids with the largest portion of the overall rates of parasitism, whereas *Z. hatayensis* possessed the smallest portion of these rates. Moreover, the overall rates of parasitism and its partitioning between the parasitoid species were found to be related to the different altitudes in the sampling sites giving that *P. oleae* existed in all ranges of altitudes (82 to 635 m a.s.l.), whereas *Z. hatayensis* existed at low altitudes (82 to 229 m a.s.l.) but not at the altitudes of > 492 m a.s.l. The overall results of this study indicate the significant role of native parasitoids in regulation of *D. oleae* outbreaks and in the potential use of these parasitoids as biocontrol agents of this insect in the Palestinian Territories.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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