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***Orijinal araştırma* (*Original article*)**

Egg parasitoids of *Thaumetopoea pityocampa* (Den & Schiff.) (Lepidoptera: Thaumetopoeidae) and natural effectiveness in Terzioğlu Campus (Çanakkale)

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Terzioğlu Kampüsü'ndeki (Çanakkale) *Thaumetopoea pityocampa* (Den & Schiff.) (Lepidoptera: Thaumetopoeidae)'nın yumurta parazitoitleri ve doğal etkinliği

Öz: Çalışma Çanakkale Onsekiz Mart Üniversitesi Terzioğlu kampüsündeki doğal orman alanında 2018'de yapılmıştır. Çalışmanın amacı kızılçamlarda *Thaumetopoea pityocampa*'nın (Den & Schiff.) yumurta parazitoitlerinin doğal etkinliklerinin belirlenmesidir. Yumurtalar eylül ve ekimde toplanarak cam tüplere aktarılmış ve 24±1Cº sıcaklık ve 60-70% orantılı nem koşullarındaki iklim dolabına yerleştirilmiştir. Yumurtalar günlük olarak kontrol edilmiş ve çıkış yapan erginler ergin ömrünü incelemek için yeni cam tüplere aktarılmıştır. Toplam yumurta paketi sayısı 72 iken her bir pakette ortalama 250,27 yumurta bulunmuş ve toplam 18.018 yumurtada ortalama parazitleme oranı %18.16 (%1.13-%97.32) olarak belirlenmiştir. Çıkış yapan türlerin oranı 26% *Anastatus bifasciatus,* 32% *Baryscapus servadeii* ve 42% *Ooencrytus pityocampae'dir*. 24±1Cº sıcaklık ve %60-70 orantılı nemde ergin ömürleri *O. pityocampae*, *B. servadeii* ve *A. bifasciatus* için sırasıyla 18.77, 8.46 ve 8.85 gün olarak belirlenmiştir.

Anahtar Kelimeler: *Thaumetopoea pityocampa, Ooencrytus pityocampae, Baryscapus servadeii, Anastatus bifasciatus*

Abstract: The study was conducted in the natural forest area in Terzioğlu campus of Çanakkale Onsekiz Mart University in 2018. The purpose of the study was to determine the natural effectiveness of the egg parasitoids of *Thaumetopoea pityocampa* (Den & Schiff.) on red pines. Eggs collected in September and October were transferred into glass tubes, then they were placed in a climate chamber with 24±1Cº temperature and 60-70% r.h. Eggs were checked daily and emerged adults were transferred to new glass tubes to examine adult longevity. Total number of egg batches was 72 with a mean number of 250.27 eggs on each batch, and parasitism rate of eggs was 18.16% (1.13%-97.32%) from a total of 18018 eggs. Ratio of the species were 26% *Anastatus bifasciatus* Geoffroy, 32% *Baryscapus servadeii* Geoffroy and 42% *Ooencrytus pityocampae* Mercet. In 24±1Cº tempreature and 60-70% r.h., adult longevities of *O. pityocampae, B. servadeii* and *A. bifasciatus* were determined as 18.77, 8.46 and 8.85 days, respectively.

Keywords: *Thaumetopoea pityocampa, Ooencrytus pityocampae, Baryscapus servadeii, Anastatus bifasciatus*

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Introduction

Pine processionary *Thaumetopoea pityocampa* (Den. & Schiff., 1775) (Lepidoptera: Thaumetopoidae), which is common in Mediterranean countries, is a pest of pinales, especially calabrian pine (*Pinus brutia* Ten.) (Mendel, 1990). In Turkey, the pest is important in Mediterranean, Aegean and Marmara regions and other forest areas with Mediterranean climate (Çanakçıoğlu & Mol, 1998). However, in recent years a new species was determined as *T. wilkinsoni* Tams starting from south and spreading to other forests of Turkey and the studies have recorded that *T. pityocampa* is common in Northern Aegean and Thrace, while *T. wilkinsoni* is common in Mediterranean, Aegean and Blacksea regions (İpekdal et al., 2015; Keleş et al., 2018).

The damage of *T. pityocampa* is caused by intense feeding on leaves of pine trees by the larvae. In high larvae intensity, pine trees can be completely defoliated (Devkota & Schmitt, 1990). Especially in young forests, *T. pityocampa* damage may cause growth deficiency (Jacquet et al., 2012; Erkan, 2018). Tents, where the larvae live in winter months, is detrimental to aesthetics of parks and allergens from the larvae cause health problems in humans and other mammalians (Battisti et al., 2011; İpekdal & Çağlar, 2011). Also, damage from a few years of feeding causes the trees to weaken, which opens the way to secondary tree pests. In recent years, 40% of the control applications against pests in forest areas are against *T. pityocampa* (Anonymous, 2012).

Adult flight period of *T. pityocampa* starts in mid-July and continues until the end of September, which can change with region (Küçükosmanoğlu & Arslangündoğdu, 2002). Females of *T. pityocampa* lay their eggs by binding pine leaves together in a form similar to a corn cob, with egg numbers changing between 37-312 (Schmidt et al., 1999). Larvae emergence from these eggs were determined as 20-86% (Sarıkaya 2004) and parasitoid emergence were determined as 10-40% (Mirchey et al., 2004). In different regions of Turkey, 194.7 eggs per egg batch and larval emergence rate of 63.7% were determined by Keleş et al. (2018).

Control of *T. pityocampa* in forest areas of Terzioğlu campus of Çanakkale Onsekiz Mart University is important because of the student usage of these areas as recreational areas. This study was conducted with the purpose of determining the natural parasitism of *T. pityocampa* eggs in this area for better control of the pest.

Materials and methods

The study was conducted in the forest areas of Terzioğlu campus in Çanakkale Onsekiz Mart University in 2018.

Properties of the Experiment Area

Terzioğlu campus is built on a 3000 da area with 350 m elevation, including the radar hill. Most of the campus, which is starts at 200-250 m from Çanakkale Strait and rises at an angle of 0-30 º, is covered with forest. Forest is mainly consisting of Calabrian pine (*Pinus buritia*) and some shrubbery and heathland. In these shrubberies and heathlands, which are interwoven with university buildings, damage from *T. pityocampa* and larvae nests are apparent.

Materials of the study are pheromone traps of *T. pityocampa* and egg batches of *T. pityocampa* collected from trees, culture containers, glass tubes of differing sizes and parasitoids emerging from the egg batches.

Methods

Delta traps with "0,9 Mg (Z)-13-Hexadecen-11-Yn-1yl Acetate" are placed on Calabrian pines at 1,7 m height on 12.06.2018 at 100 m (Trap 1) and 175 m (Trap 2) elevations, to determine the adult flight of *T. pityocampa* and the suitable period to collect egg batches in Çanakkale Onsekiz Mart University Terzioğlu Campus. Traps were checked twice a week and number of adults in traps were recorded. Traps were cleaned for following counting and sampling was finished at the beginning of September.

Egg batches of *T. pityocampa* were collected every day around the area with the pheromone traps between 25.09.2018 and 22.10.2018. All directions and different heights on trees were checked to find egg batches. Determined egg batches were collected by cutting the branch with a pruning shear. Collected egg batches were transferred to the laboratory in plastic boxes and scales on the egg batches were cleaned off with a brush. The total number of eggs and the number of parasitized eggs were calculated after counting the eggs under a stereo binocular microscope (Olympus SZ51). Then, egg batches were placed into glass tubes (1x10 cm) and these tubes were transferred to a climate chamber with 24±1Cº temperature, 60-70% r.h. and 16:8 L:D photoperiod conditions.

Number of emerged parasitoids was counted daily to determine the number of different parasitoid species. The emerged adult parasitoids were differentiated by morphological properties and these data were used to calculate the ratio of different parasitiod species in the general population. Then, they were placed in glass tubes individually to determine their longevity. Thin lines of honey were placed into the tubes with a needle as food for parasitoid adults. Parasitoids were checked daily until death and the number of days were recorded for each individual. Longevity experiment was conducted with 50 repetitions for each species. Species of parasitoids were identified by Prof. Dr. Mustafa AVCI from Isparta University of Applied Sciences.

Results and discussion

At the end of the study, a total of 52 adults were collected from two traps (Figure 1, 2). Adults were captured one week after trap placement and an important portion of the adults were captured in August. Adult flight has ended at the beginning of October. According to Küçükosmanoğlu & Arslangündoğdu (2002), adult flight period starts in mid-July and lasts until the end of September, depending on the region.

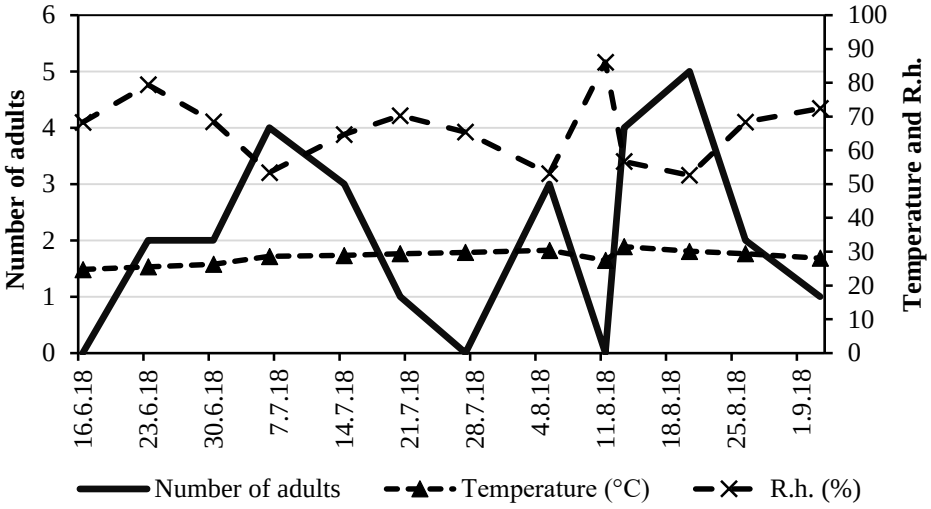

Figure 1. Number of *Thaumetopoea pityocampa* adults and climate data in Trap 1.

A total of 72 *T. pityocampa* egg batches were collected in samplings on Calabrian pines in two different areas. Mean number of eggs on each egg batch was found as 250.27. Şimşek et al. (2017) have reported that *T. pityocampa* has a mean number of 181.56 eggs on each batch and larval emergence rate from these eggs is 82.04% with a parasitism rate of 9.15%, on black pines. According to Keleş et al. (2018), mean number of eggs in each batch changes with elevation and mean number of eggs per batch was 194.7. The study by Doğanlar et al. (2002) in Hatay region has shown that, egg numbers per egg batch for *T. wilkinsoni* change between 153 and 181, depending on the region. Even though, numbers of eggs per egg batch were higher than other studies, it is apparent that egg numbers can change with region and elevation.

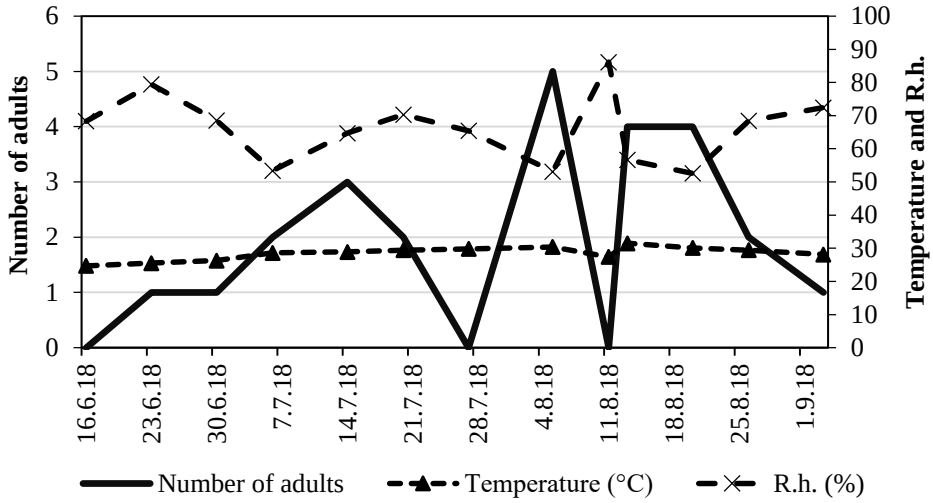

Figure 2. Number of *Thaumetopoea pityocampa* adults and climate data in Trap 2.

Most of the eggs batches placed in glass tubes were parasitized (94.44%), with parasitism rates changing between 1.13% and 97.32%, from a total of 72 egg batches. A total of 18.018 eggs were counted in these batches and 18.16% (3.273 eggs) of these eggs were parasitized (Table 1 and Figure 3). According to Schmidt et al. (1999), 47.2% to 79.7% of the eggs collected from Iber Peninsula have hatched and parasitism rates were between 11.3% and 31.7% with no parasitoid emergence from 44% of parasitized eggs. Can & Özcankaya (2003) has reported their findings as the highest parasitism was in İzmir with 19.8% and the lowest parasitism was in Muğla with 5.5%, in Aegean region. High number of parasitized eggs in our study shows that parasitoid presence is high in the study area.

Table 1. Number of *Thaumetopoea pityocampa* eggs, parasitism and emergence rates of parasitoids in laboratory conditions (24±1Cº, %60-70 r.h., 16:8 L:D) in 2018 (%)

# of egg batches	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
# of eggs	236	225	280	296	224	280	272	152	231	207	267	261	240	259	231
# of parasitized eggs	8	14	65	30	218	77	79	27	39	0	61	79	0	12	16
Parasitism rate (%)	3.39	6.22	23.21	10.1	97.32	27.50	29	17.76	16.88	0.00	22.85	30.27	0.00	4.63	4.63
# of emerged parasitoids	7	6	44	25	71	37	31	20	33	0	18	62	0	7	11
Parasitoid emergence rate (%)	87.50	42.86	67.69	83.33	32.57	48.05	39.24	74.07	84.62	0.00	29.51	78.48	0.00	58.33	68.75
# of egg batches	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
# of eggs	196	217	264	288	203	217	231	264	280	231	224	259	231	245	224
# of parasitized eggs	15	14	147	146	0	39	69	19	64	6	23	33	56	39	19
Parasitism rate (%)	6.93	7.65	55.68	50.69	0.00	17.97	29.87	7.2	22.86	2.6	10.27	12.74	24.24	15.92	60.61
# of emerged parasitoids	14	10	4	97	0	26	47	17	31	0	6	20	54	19	13
Parasitoid emergence rate (%)	93.33	71.43	2.72	66.44	0.00	66.67	68.12	89.47	48.44	0.00	26.09	60.61	96.43	48.72	68.42
# of egg batches	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
# of eggs	238	248	272	192	297	252	256	248	196	256	175	280	328	224	225
# of parasitized eggs	22	40	28	30	47	34	18	27	25	41	51	30	136	29	42
Parasitism rate (%)	9.24	16.13	1.29	15.63	15.82	13.5	7.03	10.89	12.76	16.02	29.14	10.71	41.46	13	18.7
# of emerged parasitoids	14	37	16	26	24	31	14	23	22	40	43	21	117	21	31
Parasitoid emergence rate (%)	63.64	92.50	57.14	86.67	51.06	91.18	77.78	85.19	88.00	97.56	84.31	70.00	86.03	72.41	73.81

Table 1. Continued

# of egg batches	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
# of eggs	252	344	224	280	262	266	287	248	280	256	256	266	312	245	256
# of parasitized eggs	41	173	74	51	25	3	18	51	17	91	73	51	45	41	48
Parasitism rate (%)	16.27	50.29	33.04	18.21	9.54	1.13	6.27	20.56	6.07	35.55	28.52	19.17	14.42	16.73	18.75
# of emerged parasitoids	36	128	32	47	17	0	10	24	4	57	64	22	42	27	42
Parasitoid emergence rate (%)	87.80	73.99	43.24	92.16	68.00	0.00	55.56	47.06	23.53	62.64	87.67	43.14	93.33	65.85	87.50
# of egg batches	61	62	63	64	65	66	67	68	69	70	71	72	Mean	Total	
# of eggs	266	264	280	224	260	261	181	270	343	232	246	235	250.25	18.018	
# of parasitized eggs	0	61	68	72	23	19	78	19	9	33	69	4	45.45	3273	
Parasitism rate (%)	0.00	23.11	24.29	32.14	8.85	7.28	43.09	7.04	2.62	14.22	28.05	1.7	18.17		
# of emerged parasitoids	0	38	66	63	18	15	32	11	5	11	31	1	29.00	2054	
Parasitoid emergence rate (%)	0.00	62.30	97.06	87.50	78.26	78.95	41.03	57.89	55.56	33.33	44.93	25.00	62.76		

With these results in mind, in Terzioğlu Campus, which has the properties of a nature preservation area, 1/5 of the egg population of *T. pityocampa* is parasitized by egg parasitoids and larval emergence is prevented (Figure 3).

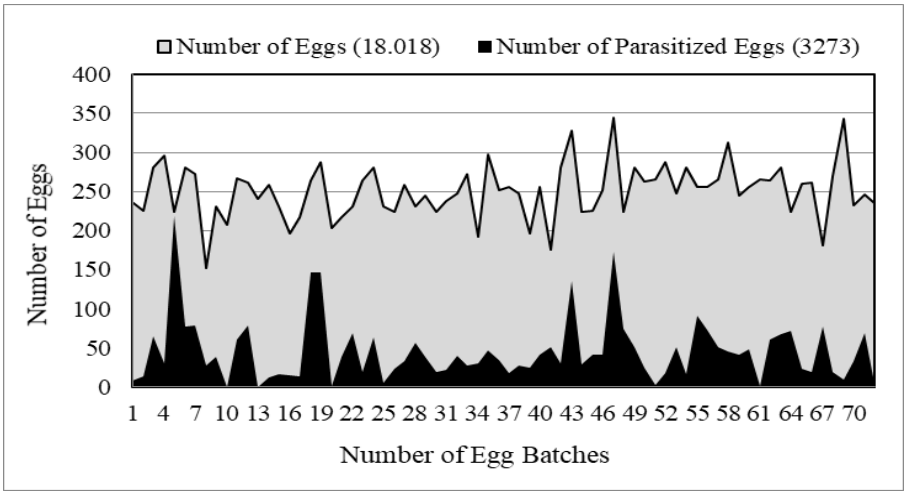

Figure 3. Number of parasitized eggs in egg batches of *Thaumetopoea pityocampa*

Parasitoid from *T. pityocampa* eggs were identified as *Anastatus bifasciatus* Geoffroy (Hymenoptera: Eupelmidae), *Baryscapus servadeii* Domenichini (Hymenoptera: Eupelmidae) and *Ooencrytus pityocampae* Mercet (Hymenoptera: Encrytidae). More than one parasitoid species were collected from same egg batches. Parasitism rates of these parasitoids were 42%, 32% and 26% for *O. pityocampae*, *B. servadeii* and *A. bifasciatus*, respectively in Terzioğlu Campus (Figure 4). *O. pityocampae* is considered as the most effective species by Tsankov et al. (1996) and was followed by *A. bifasciatus* and *B. servadeii* in Bulgaria. In Israel, the effectiveness of *O. pityocampae* and *B. servadeii* were found to be similar, while it was limited in *A. bifasciatus* (Halperin, 1990).

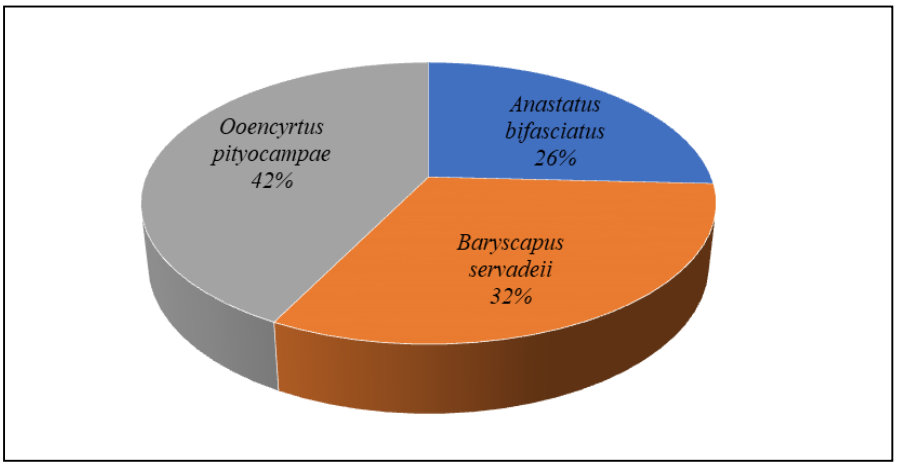

Figure 4. Distribution of parasitoids emerged from *Thaumetopoea pityocampa* eggs (%)

In Hatay region, eggs of *T. wilkinsoni* were parasitized at the rates of 64.77%, 26.41% and 1.23% by *O. pityocampae*, *B. servadeii* and *A. bifasciatus*, respectively (Doğanlar et al., 2002). According to other studies, *O. pityocampae* is the most effective egg parasitoid of *T. pityocampa* and parasitize the eggs of many Lepidopteran and Hemipteran species (Tiberi, 1990), and also can be mass produced on *Philosamia ricini* Danovan (Lepidoptera: Saturniidae) in laboratory conditions for biological control applications (Tunca et al., 2015). In addition, *O. pityocampae* is considered the most effective parasitoid species in Southern regions of Turkey and was followed by *B. servadeii* in effectiveness against *T. pityocampa* (Mirchey et al., 2015)

The emergence rate of parasitoids from 3.273 *T. pityocampa* eggs collected from September to December was 65.72% and parasitism related to egg batches is given at figure 5. Upon the examination of numbers of parasitoids emerged from the eggs, the number of adults from different species was divergent. Rate of *A. bifasciatus* emerged from the eggs collected between October and November was 98.65%. In December, January and February, adult emergence rate of *O. pityocampae* were 9.96%, 71.14% and 28.85%, while it was 1.6%, 14.23% and 81.15% for *B. servadeii*, respectively. According to this data most of *A. bifasciatus* adults have emerged soon after parasitism period, while majority of the other two species have emerged in January and February. These findings were evaluated as the rest of the unemerged parasitoids are overwintering and will emerge after winter, especially for *B. servadeii*. According to Bellin et al. (1990), parasitoids emerge in September and October, when parasitizations occur. But Tsankov et al. (1996) have reported even though parasitoid emergence occurs before winter, most of *B. servadeii* adults emerge in June, while *O. pityocampae* adults emerge in May. In Morocco, *O. pityocampae* completes adult emergence period before winter and 50% of *B. servadeii* adults emerge after overwintering (Schmidt et al., 1997). Tsankov et al.

(1996) also suggested that parasitoids overwinter in diapause and *O. pityocampae* is not a well-adapted parasitoid species for *T. pityocampa*.

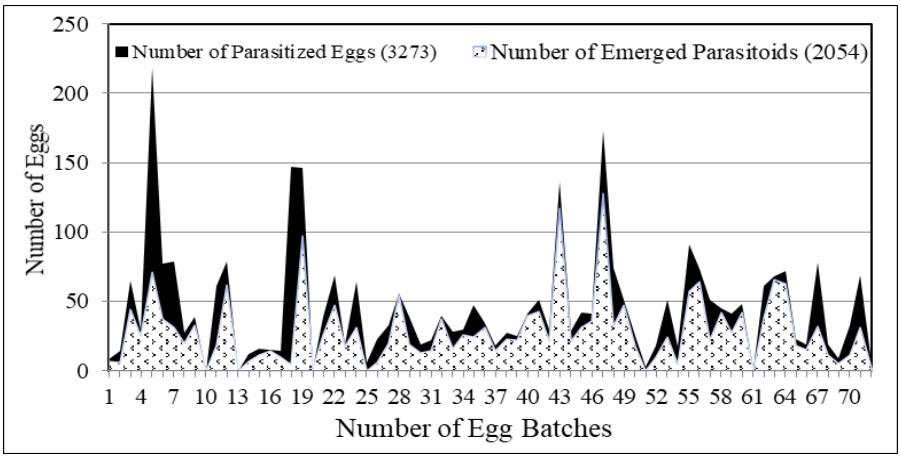

Figure 5. Numbers of parasitoids emerged from *Thaumetopoea pityocampa* eggs batches

In this study, adult longevity of *A. bifasciatus*, *B. servadeii* and *O. pityocampae* emerged from *T. pityocampa* eggs was determined as 8.85, 8.46 and 18.77 days in 24±1C° temperature and 60-70% r.h. conditions, respectively. Survival of parasitoids relies on long adult longevity, in addition to presence of alternative hosts. According to Halperin (1990), adult longevity of *O. pityocampae* in 19C° temperature is 90 days, while it is 30-35 days in 27-30C° temperatures. Kitt & Schmidt (1993) suggested that longer adult longevity makes it possible for the parasitoid to survive until the emergence of the next host. *O. pityocampae*, which has multiple generation per year and alternative hosts, diapauses a short period in autumn and a longer period in summer (Halperin, 1990; Bellin et al., 1990), and has two generations per year in Italy with a parasitism rate of 45.97% in the 2nd generation (Tiberi, 1990). We speculate that, parasitism rate and number of generations per year of *O. pityocampae* may increase with alternative hosts because of the wide range of ornamental plants and pest feeding on them.

Conclusion

Mean number of eggs on an egg batch collected from the pine trees in Çanakkale Onsekiz Mart University, Terzioğlu Campus was 250.25 from a total of 72 egg batches. From these egg batches, 68 of them (94.44%) were parasitized and 18.87% of the 18.018 eggs from these batches were also parasitized. From these parasitized eggs, rate of emerged adults was 42% *O. pityocampae*, 26% *A. bifasciatus* and 32% *B. servadeii*. Majority of the emerged adult parasitoids in October and November were *A. bifasciatus*, while it was *O. pityocampae* and *B. servadeii* in January and February.

The rate of 1/5 of parasitized eggs and 94.44% of parasitized egg batches shows that parasitoids are common in Terzioğlu Campus. Similar parasitism rates of the three species may be linked to the climate conditions, as well as to the alternative hosts living on the various ornamental plants in the campus. Hence, the fact of *A. bifasciatus* parasitizing the eggs of more than 30 species from Lepidoptera and Hemiptera orders (Battista et al.,1988; Halperin, 1990; Avcı, 2009; Stahl et al., 2018) and its usage against the pest *Halyomorpha halys* (Stahl et al., 2019) in different agricultural areas, supports this opinion. On the other hand, presence of alternative hosts for *O. pityocampae* and *B. servadeii* is thought to be helpful to increase the effectiveness of these species

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