Çankırı (Eldivan) Karaçam Ormanlarında Bulunan Çam Keseböceği [*Thaumetopoea pityocampa* (Den.&Schiff.) (Lepidoptera: Thaumetopoeidae)]'nin Yumurta Parazitoitlerinin Tespiti ile Etkinliği Üzerinde Araştırmalar

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Öz

Çankırı (Eldivan) Karaçam Ormanlarında bulunan Çam Keseböceği [*Thaumetopoea pityocampa* (Den.&Schiff.) (Lepidoptera: Thaumetopoeidae)'nin yumurta parazitoitlerinin tespiti ile etkinliğinin belirlenmesi amacıyla ele alınan bu çalışma, 2015-2016 yıllarında yürütülmüştür. *T. pityocampa*'nın yumurta koçanlarında bulun yumurtalar stereo-mikroskop altında sayılarak bunların durumları (parazitli- parazitsiz, parazitoit çıkan-çıkamayan, zararlı larvası çakan-çıkamayan) dikkate alınmak suretiyle yumurtaların açılma oranları (%), parazitlenme oranları (%) ile bir koçanda bulunan yumurta sayıları (adet) belirlenmiştir. Karaçam ibrelerinde 23 yumurta koçanında 4176 adet *T.pityocampa* yumurtasının sayım sonucuna göre, her koçanda ortalama 181.56 adet (28-211) yumurtanın bulunduğu; toplam yumurtalardan 3426 adedinden zararlı larvasının çıktığı (%82.04); larva gelişmesine karşın 294 adet yumurtanın açılmadığı (%7.04);parazitli 456 adet yumurtadan ise 382'sinin parazitoitli olduğu (%9.15), bunlardan 74'ünden parazitoit çıkışının olmadığı (%1.77) anlaşılmıştır. Buna göre parazitoitlerin, zararlı popülasyonu üzerinde ort.%10.92 oranında etkili olduğu belirlenmiştir. Aynı çalışmada, parazitli *T. pityocampa* yumurtalarında, çokluk sırasına göre, üç hymenopter yumurta parazitoit türü [*Ooencyrtus pityocampae* Mercet (Hym., Encyrtidae), *Anastatus bifasciatus* Geoffroy (Hym., Eupelmidae) ve *Baryscapus servadeii* Domenichini (Hym., Eulophidae]'nün çıktığı, Çankırı (Eldivan) karaçam orman alanında bulunduğu bu çalışma ile ilk kez belirlenmiştir.

Anahtar kelimeler: *Thaumetopoea pityocampa*, yumurta parazitoitleri, *Ooencyrtus pityocampae*, *Anastatus bifasciatus*, *Baryscapus servadeii*, Türkiye, Çankırı

Researches on Determination of the Egg Parasitoids and Efficiencies of the Pine Processionary Moth [*Thaumetopoea pityocampa* (Den.&Schiff.)] in Black Pine Forest in Çankırı (Eldivan)

Abstract

This study was carried out in order to determine the egg parasitoids the pine processionary moth [*Thaumetopoea pityocampa* (Den&Schiff) (Lepidoptera: Thaumetopoeidae)] and their efficiencies in the Anatolian Black Pine forests in Çankırı (Eldivan) between 2015 and 2016. The eggs within the egg batches of *T.pityocampa* were counted under stereomicroscope and status of each egg was noted as parasitized/non-parasitized, parasitoid emerged/not emerged, pest larvae hatched/not hatched and thus hatch ratio (%), parasitization ratio (%) and the average egg numbers for each batch were calculated. Results of the counts of 4176 *T.pityocampa* eggs within 23 egg-batches on Anatolian Black Pine needles show that egg-batches contain 181.56 (28-211) eggs, 3426 out of 4176 *T.pityocampa* eggs were hatched (82.04%) but 294 of the eggs were not hatched (7.04%), 382 egg parasitoids were emerged from 456 parasitized *T.pityocampa* eggs (9.15%) however there were not any emergence from 74 of the parasitized eggs (1.77%). Thus, the egg parasitoids were calculated to be efficient as much as 10.92% on *T.pityocampa* population. There were 3 hymenopteran egg parasitoids (*Ooencyrtus pityocampae* Mercet (Hym., Encyrtidae), *Anastatus bifasciatus* Geoffroy (Hym., Eupelmidae) and *Baryscapus servadeii* Domenichini (Hym., Eulophidae) respectively) were identified from the emerged parasitoids from the Anatolian Black Pine forest for the first time in Çankırı (Eldivan).

Keywords: *Thaumetopoea pityocampa*, egg parasitoids, *Ooencyrtus pityocampae*, *Anastatus bifasciatus*, *Baryscapus servadeii*, Turkey, Çankırı

1. INTRODUCTION

The pine processionary moth [*Thaumetopoea pityocampa* (Den&Schiff.) (Lepidoptera: Thaumetopoeidae)] is the major pest of pine forests in geographical regions under the Mediterranean climate effect. *T.pityocampa* is common in an important part of the pine forests; mostly Turkish pine (*Pinus brutia* Ten.) and other pine species. The main host tree species covers over 1.5 million ha forests in the Mediterranean, Aegean and Marmara regions and also the coastal sides of the Black Sea region and south aspects of warmer slopes in Turkey (Avcı 2000).

Heavy infestations of *T.pityocampa* adversely affect diameter and length development of trees (Asan 1993; Kanat et al. 2005; Durkaya et al. 2009) and also the larvae may cause even tree deaths in case of not being controlled (Figure 1).

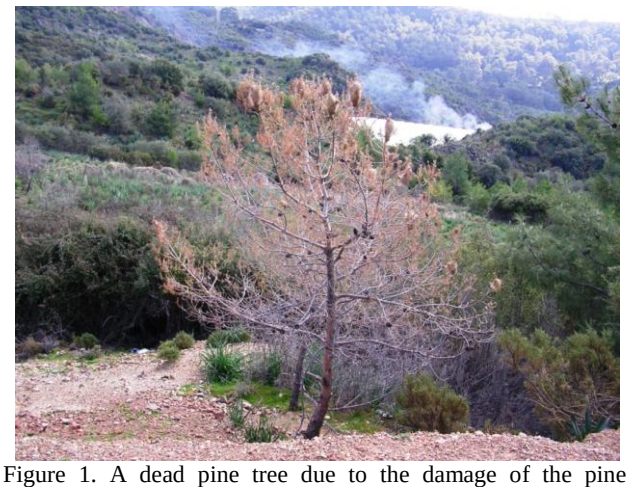

Figure 1. A dead pine tree due to the damage of the pine processionary moth [*Thaumetopoea pityocampa* (Den.&Schiff.)]

There are many studies on *T.pityocampa* mainly focusing on the biology, damage, hosts, and also mechanical and chemical control of this pest in Turkey (Acatay 1953; Besçeli 1969; Tosun 1975; Çanakçıoğlu and Selmi 1988; Çanakçıoğlu and Mol 1998). However, there are few detailed studies on the structure of egg batches, egg-laying behavior of the insect (Acatay 1953; Özkazanç 1987). Mechanical and chemical control measures against the pine processionary moth were generally effective in the short term but the damage of the pest continued on the long run. On the other hand, there are certain studies on the application of the preferred control measure, the biological control against *T.pityocampa*, due to continuous effect against many insect pests, since this control measure was effectively used against certain other pests in Turkey. There are many detailed studies on *T.pityocampa* in Bulgaria, Greece, Israel, Italy, France, and Portuguese especially. With these studies, the structure of the egg batches, larval hatching and parasitization ratios (Bellin et al. 1990; Schmidt 1990; Tsankov et al. 1996), egg depositing behavior (Tiberi 1983), egg parasitoids and efficiency ratios (Tiberi 1983; Tiberi 1984; Tiberi 1990; Tsankov et al. 1995; Tsankov et al. 1996; Schmidt et al. 1997; Schmidt et al. 1997; Tsankov et al. 1998; Tsankov et al. 1999), phenology and behavior of parasitoids (Battisti 1989; Tsankov 1990), mass production of parasitoids in laboratory conditions (Battisti et al. 1990; Masutti et al. 1992; Masutti et al. 1993) and the effects of host plants on parasitization (Tiberi 1984; Tiberi and Roversi 1987) were deeply researched. In addition, Doğanlar et al. (2002) report that the egg parasitoids emerged from the egg batches of *T.wilkinsoni* Tams were identified as *Ooencyrtus pityocampae* Mercet. (Hymenoptera: Encyrtidae), *Barycapus servadeii* Domenichini (Hymenoptera: Eulophidae) and *Anastatus bifasciatus* Geoffr. (Hymenoptera: Eupelmidae) and also their efficiency rates were 66.77%, 29.41%, and 1.23% respectively. Nasr et al. (2013) report that the adult parasitoid emergences from the collected egg batches were continued from May 1999 to April 2000 in Lebanon; parasitoid emerged eggs were smaller exit holes but *T.pityocampa* larvae hatched eggs were bigger holes; and the identification of parasitoids showed that three hymenopteran parasitoids were *Baryscapus servadeii*, *Ooencyrtus pityocampae* and *Anastatus bifasciatus* and also *B. servadeii* was the most common parasitoid followed by *O. pityocampae.*

Our literature search showed that there were not any researches on this subject in Çankırı forests. In our field observations in the Black Pine (*Pinus nigra*) forest in Eldivan (Çankırı), we observed that pine branches that have been infested with *T.pityocampa* were inserted onto isles surrounded with water (Figure 2) and help biological control efforts by enabling parasitoids to fly back to the forest area. Also, we observed that certain eggs in the batches had parasitoid emergence holes (Figure 3). Thus, we carried out this research in Eldivan (Çankırı) black pine forest in 2015 and 2016 in order to determine the egg parasitoids and the efficiency ratios parasitoids of *T.pityocampa* which is a serious insect pest in Turkish pine forests and expensive to control.

Figure 2. Insertion of Black pine branches that have been infested by *Thaumetopoea pityocampa* (Den.&Schiff.) onto the island surrounded by water in Eldivan (Çankırı)

Figure 3 Parasitoid emerged eggs of *Thaumetopoea pityocampa* (Den.&Schiff.) egg batch

2. MATERIALS AND METHODS

The main materials of this study which was carried out in order to determine the egg parasitoids of the pine processionary moth [*Thaumetopea pityocampa* (Den.&Schiff.)] and their efficiencies in Çankırı (Eldivan) were the black pine trees that have been infested with the pine processionary moth (*T.pityocampa*) and egg batches which were composed of the eggs of the pest. Stereomicroscope, various size nylon bags, ice box, PENS, brush, branch cutting scissors, ethyl alcohol (70%), open and capped glass tubes were utilized as auxiliary materials.

Studies were carried out as two steps: in the field and in the laboratory. Field studies were carried out in August and September periodically in Eldivan county black pine forest (1450 m) of Çankırı Forest District. Egg batches were sampled at four sides of the infested black pine trees. Egg batches were removed with an adjustable branch cutting scissor tool. The collected egg batches were inserted into nylon bags and stored in an ice box until laboratory.

In the laboratory, each egg batch inserted into a glass tube and the mouthparts of the tubes were sealed with a piece of moisturized cotton in order to prevent egg batches from drying. All glass tubes that contain an egg batch were stored at 25±1°C temperature, 60±5% relative humidity and 16:8 light: dark conditions. The glass tubes were checked daily and each cotton piece was moisturized to prevent egg batches from drying. Parasitoids emerged were kept until death, then removed from glass tubes and stored in ethyl alcohol for identification. This process was continued until the parasitoid emergence had completed. All evaluations were carried out after the parasitoids emerged.

In order to determine the parasitoid efficiency (infestation ratio), eggs in each egg batch were inspected under stereomicroscope after removing the scales on the egg batches with a fine needle without damaging the host eggs (Figure 4).

Figure 4 *Thaumetopoea pityocampa* (Den.&Schiff.) egg batches

While inspecting, all eggs were recorded into 3 categories: parasitoid emerged eggs (smaller, irregular emergence holes with parasitoid wastes), larvae hatched eggs (relatively bigger, regular hatching holes and transparent eggs), non-hatched or non-emerged eggs (Figure 5). Parasitoid emergence ratio (%) was calculated by dividing the parasitoid emerged eggs by total eggs in 23 egg batches, and non-emerged parasitoid ratio (%) was calculated by dividing the infested but non-emerged *T.pityocampa* eggs by total eggs in 23 egg batches. Parasitoid efficiency (%) on the insect pest was calculated as adding up these two ratios. Also, the dominant parasitoid species from 23 egg batches was determined.

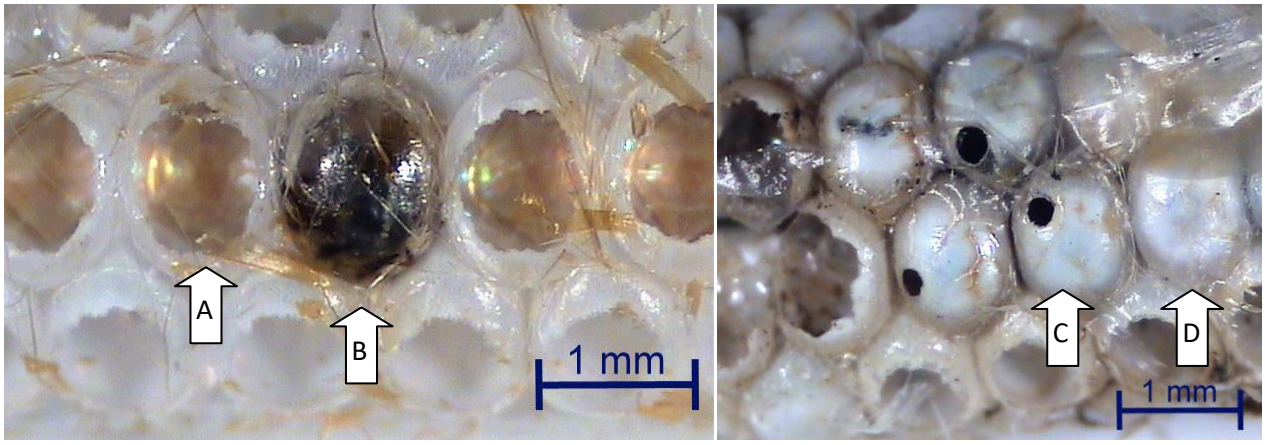

Figure 5. *Thaumetopoea pityocampa* (Den.&Schiff.) larvae emerged eggs (A), an egg with parasitoid (B), parasitoid emerged egg (C) and non-hatched/emerged egg (D)

The egg parasitoids of *T.pityocampa* were identified by Prof. Dr. Mustafa AVCI (Süleyman Demirel University, Faculty of Forestry, Forest Entomology and Protection Division).

All data collected and calculated were organized as figures and tables and relationships were discussed.

3. RESULTS AND DISCUSSION

The results of this study which was carried out in order to determine the egg parasitoids of the pine processionary moth [*Thaumetopoea pityocampa* (Den.&Schiff.)] their efficiency ratios in Eldivan Black Pine (*Pinus nigra*) forest in Çankırı Forest District show that three egg parasitoid species, *Ooencyrtus pityocampae* Mercet (Hym., Encyrtidae), *Anastatus bifasciatus* Geoffroy (Hym., Eupelmidae) and *Baryscapus servadeii* Domenichini (Hym., Eulophidae) have been identified. The dominant parasitoid species was found to be *O.pityocampae* (Figure 6A) followed by *A.bifasciatus* (Figure 6B) and *B.servadeii* (Figure 6C) with regard to abundance. Status of the egg batches in glass tubes in the laboratory is given in Table 1 according to the inspection conducted on November 1st, 2016 under stereo-microscope.

Table 1 shows that a total of 4176 eggs of *T.pityocampa* within 23 egg batches were inspected and the average of the eggs in batches was 181.56 (28-211) eggs. Also, 3426 *T.pityocampa* larvae hatched out of 4176 eggs (82.04%). However, 294 out of 4176 eggs were not hatched (7.04%). A total of 382 egg parasitoids emerged out of 456 parasitized eggs (9.15%). However, there were not any emergences from 74 out of 456 parasitized eggs (1.77%). These data show that the egg parasitoids were effective as high as 10.92% of the *T.pityocampa* egg population.

Figure 6 Egg parasitoids of the pine processionary moth: *Ooencyrtus pityocampae* Mercet (Hymenoptera: Encrytidae) adult (A), *Anastatus bifasciatus* Geoffroy (Hymenoptera: Eupelmidae) adult (B) and *Baryscapus servadeii* (Domenichini) (Hymenoptera: Eulophidae) adult (C)

Table 1 Status of *Thaumetopoea pityocampa* (Den.&Schiff.) eggs in batches that have been collected from the pine forest in Çankırı (Eldivan) according to the inspection under stereomicroscope

No of the Egg Batches	Eggs in the Batch	Parasitoid Emerged Eggs	Parasitized Eggs but no Emergence	Non-hatched eggs with *T.pityocampa* Larva	*T.pityocampa* Larva Hatched Eggs	Parasitization Ratio in Eggs (%)
1	203	25	5	13	160	14.78
2	198	19	3	29	147	11.11
3	205	9	3	23	170	5.85
4	211	0	0	0	211	0.00
5	204	21	2	11	170	11.27
6	181	0	0	5	176	0.00
7	163	0	0	0	163	0.00
8	194	2	0	7	185	1.03
9	207	7	0	5	195	3.38
10	199	41	17	27	114	29.15
11	235	21	3	23	188	10.21
12	161	4	1	4	152	3.11
13	157	22	2	8	125	15.29
14	208	26	3	19	160	13.94
15	176	0	0	1	175	0.00
16	202	0	0	0	202	0.00
17	148	67	2	0	79	46.62
18	218	46	2	4	166	22.02
19	128	9	3	9	107	9.38
20	182	8	0	32	142	4.40
21	205	34	9	22	140	20.98
22	28	13	5	5	5	64.29
23	163	8	14	47	94	13.50
Total	4176	382	74	294	3426	
Mean	181.56	16.61	3.22	12.78	148.95	
Ratio (%)		9.15	1.77	7.04	82.04	10.92

The literature regarding the parasitoids of *T.pityocampa* eggs are summarized below. Can and Özçankaya (2003) state that *O.pityocampae*, the most abundant egg parasitoid in their study, is a well-known parasitoid of *T.pityocampa.* Researchers also state that this parasitoid had developed an ability to find host eggs and it has two generations. While the first generation of the parasitoid lasts to 4 weeks, the second generation lasts up to 11 months. A small portion of the parasitoid adults fly between May and August, however, a large portion of the parasitoid adults fly when host eggs are available. Halperin (1990) and Bellin et al. (1990) state that adult *O.pityocampae* females lay about 27 (8-47) eggs on average. Researchers also state that *O.pityocampae* has diapause in each flight period; the first diapause (short period) starts after the air temperature cools down in fall and continues until April when it is suitable for insect development. Then the longer diapause continues until the fall season. Same researchers also report that the parasitization ratio of *O.pityocampae* as 3-6%. Ayar (2007) identified *O.pityocampae* adults in Isparta (Turkey) from the egg batches of an another pine processionary moth, *Thaumetopoea wilkinsoni* (Lep., Thaumetopoeidae) in spring. After some efforts for mass production of this parasitoid in the laboratory on *Bombyx mori* L. (Lep., Bombycidea),

732 out of 1000 *B.mori* eggs were parasitized by *O.pityocampae* and 698 parasitoid adults emerged. Thus the researcher concluded that *O.pityocampae* could be suitable for mass production. Tunca et al. (2015) report that *O.pityocampae*, the major egg parasitoid of *T.pityocampae*, has been reared more than 9 generations on a new laboratory host: *Philosamia ricini* (Donovan) (Lepidoptera: Saturniidae).

Yoldaş (1997) states that *A.bifasciatus*, another egg parasitoid identified in our study, has a low potential as a biological control agent against *T.pityocampa*. Halperin (1990) reports that this egg parasitoid develops on Pentatomidae and Lepidoptera eggs in Israel and its parasitization ratio was lower than 0.1%.

Tiberi (1990) reports that the adults of *B.servadeii*, the other egg parasitoid species from our study, are generally seen with *O.pityocampae* adults between September and October and both parasitoids may lay eggs into same host egg. *B.servadeii* may produce with parthenogenesis and it could stay in diapause for two years and also a specialized parasitoid of the pine processionary moth. Halperin (1990) states that this parasitoid species is a common parasitoid species in the Mediterranean countries. The researcher also states that this parasitoid's abundance in Bulgaria was much higher than Spain and Israel and the parasitization ratio of *B.servadeii* on individual egg batches could be up to 37%; however, the general parasitization ratio was about 5.5%.

Masutti and Battisti (1990) state the importance of the parasitoids such as *Phryxe caudata*, *O.pityocampae*, and *B.servadeii* in control of *T.pityocampa* populations infesting various pine species such as *P.nigra* and *P.sylvestris* in Italy.

Schmidt et al. (1997) report that there were 175 (34-245) eggs on average in 25 *T.pityocampa* egg batches collected from the Atlas Mountains in Morocco. Researchers also report larval hatching ratio as 72.7% and non-hatched egg ratio as 27.3% and parasitoids' effect on eggs as 21.4% of which were parasitized by abundantly by *B.servadeii* followed by *O.pityocampae*. Nasr et al. (2013) reported that adult parasitoids emerged from collected egg batches between May of 1999 and April of 2000 in Lebanon and emergence holes of parasitoids on the eggs were smaller in diameters, however, *T.pityocampae* larvae hatched holes were much bigger. Researchers also state that they identified three egg parasitoid species (*B. servadeii*, *O. pityocampae* and *A.bifasciatus*) from collected *T.pityocampa* egg batches and the most abundant parasitoid species were *B.servadeii* followed by *O.pityocampae*.

Mirchev et al. (2015) report four egg parasitoids of *T.pityocampa* as *O.pityocampae*, *B.servadeii*, *A.bifasciatus* and *Trichogramma* sp. (Hym., Trichogrammatidae); *O.pityocampae* was the dominant egg parasitoid among identified egg parasitoids and the abundance of *B.servadeii* was about four times of the polyphagous species *A.bifasciatus*. The same researchers also state that *Trichogramma* sp. adults were rarely found.

O.pityocampae is the most common egg parasitoid in Italy and has two generations. The highest parasitization by *O.pityocampae* occurs in the second generation as up to 45.97% (Tiberi 1978; Tiberi 1983; Tiberi 1984).

Our literature search on the egg parasitoids of *T.pityocampa* in Turkey showed that there was not any previous study on this subject in Çankırı forests. Thus, it could be stated that the three hymenopteran egg parasitoid species (*O.pityocampae*, *A.bifasciatus*, and *B.servadeii*) are determined for the first time in Çankırı forest areas. Doğanlar et al. (2002) report that *O.pityocampae*, *B.servadeii*, and *A.bifasciatus* were identified in laboratory from the egg batches of *T.wilkinsoni* which were collected from Hassa and Kırıkhan counties of Hatay (Antakya) and Polateli (Kilis) pine sites and the natural efficiency ratios of the parasitoids were found to be 64.77%, 29.41% and 1.23% respectively. We think that these results are similar to our results in sense of the dominant parasitoid species and the parasitoid species identified.

When results are evaluated together, we inspected 4176 eggs on 23 egg batches and we found that 456 eggs were parasitized with three identified hymenopter parasitoid species, *Ooencyrtus pityocampae* Mercet (Hym., Encyrtidae), *Anastatus bifasciatus* Geoffroy (Hym., Eupelmidae) and *Baryscapus servadeii* Domenichini (Hym., Eulophidae) with a total parasitization ratio of 10.92%. The most abundant and dominant of the egg parasitoids identified was *O.pityocampae* followed by *A.bifasciatus* and *B.servadeii*. These parasitoid species of *T.pityocampa* were determined in Eldivan (Çankırı) black pine forests for the first time with this study. With varying existence ratios in population and efficiency ratios, the determined egg parasitoid species and dominant egg parasitoids in other countries look similar to our findings.

4. CONCLUSION

Results of this study suggest that it should be the next thing to do to check if the natural enemies (*O. pityocampae*, *A. bifasciatus* and *B. servadeii*) of *T.pityocampa* determined in Eldivan forest area for the first time, exist in other regions where this insect pest exist. Current control measures against this pest should be carried out with method(s) to prevent natural enemies to be harmed. In order to achieve this goal, biopesticides that could be used when needed against this pest and their side effects, the best controlling time and also the most effective doses of the currently used insecticides and their side effects on parasitoids should be extensively researched. In the later studies, it would be most beneficial to research the bio-ecology, mass production, releasing techniques and alternative hosts of the egg parasitoid that has been found to be the most efficient one on the pest in this study. Understanding the negative effects of chemical control, the abundance of the parasitoids and predators in nature, increased social conscience of the environment have put forward other control methods such as Biological Control. Importation of natural enemies at international level have got easy and utilization of the modern technology for mass rearing of natural enemies, and financial support by governments from time to time could be mentioned as some of the reasons for speeding up biological control studies.

Today's policies and strategies of Ministry of Forestry and Water Management and the Ministry of Food, Agriculture, and Livestock suggest reducing chemical application while increasing biological control, biotechnical methods and integrated pest management (IPM) in Turkey. In many other countries such as U.S., there are intense governmental efforts for switching to control methods utilizing no chemicals or as low chemicals as possible. There are certain studies in the U.S. that about 75% of agricultural products have grown with Integrated Pest Management by the lead of the government (Anonymous 1995). Integrated Pest Management applications that the biological control methods turn out a bigger portion and no or minimized spraying applied are even on the top of the national product protection strategies of developing Asian countries. However, it is not possible to say that the governmental support for the biological control is satisfying. Nevertheless, it is satisfactory that the ministries previously mentioned gave attention to producing beneficial insects. We believe that achieving this goal is closely related to government support firstly and also being supported by researchers, producers and every part of the human population.

The phytophagous insects feed and complete their life cycles on their host plants. It is very important to know how and where the natural enemies, mainly predators, and parasitoids, complete their life cycles and the importance of winter shelters in their life cycles. In this sense, it should be the next thing to do to check if the natural enemies (*O. pityocampae*, *A. bifasciatus* and *B. servadeii*) of *T.pityocampa* determined in Eldivan Mountain forest area for the first time, exist in other regions where this insect pest exist. Also, susceptibilities of pine species against the pine processionary moth should be researched. Natural enemies should be taken into consideration in silvicultural applications and forest ecosystems should be arranged for the survival of natural enemies. Bio-pesticides should be preferred instead of wide spectrum pesticides and spot-spraying should be preferred if it is a necessity when activities of natural enemies are minimal level. Shelters for natural enemies should be prepared and certain other plants and trees producing honeydew, nectar, pollen etc. should be included in stand composition. While removing infested trees, natural enemies should be allowed to return back to forest area from the infested tree parts with egg batches. Alternative preys and hosts of the natural enemies should be protected and should be imported if necessary. Furthermore, bioecological studies should be carried out for controlling the pest and also the effects of the chemical compounds on the wildlife and beneficial insects should be investigated in detail since protection of the natural enemies in the forest that has been the source of the ecological balance.

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