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First Record, Distribution and Occurrence of A Protistan Entomopathogen, *Adelina mesnili* Perez (Coccidia: Adeleidae) in the Indian Meal Moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) Populations in Türkiye

Protistan Entomopatojen Adelina mesnili Perez'in (Coccidia: Adeleidae) Türkiye'deki Kuru Meyve Güvesi Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) Popülasyonlarında İlk Kaydı, Dağılımı ve Enfeksiyon Oranları

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ABSTRACT

Objective: *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) originating from South America is one of the important insect pests that damages storage products and is found on every continent. There is a new interest in using enthomopathogens for microbial control of *P. interpunctella* as well as other stored product pests. Coccidia as a group of protistan entomopathogens are host specific and their pathogenic effects on the hosts are more pronounced. Although this pathogenic effect results in increased host mortality or higher susceptibility to insecticides, the suppressive potential of coccidia in natural populations has not been adequately studied. In this study, characterization, distribution and occurrence of a coccidian entomopathogen was aim to show its natural suppressing potential in *P. interpunctella* populations.

Methods: During the three years (from 2019 to 2021), a total of 3.432 *P. interpunctella* samples (2.047 dead and 413 living larvae, 932 adults and 40 pupae) were collected from fourteen populations. After macroscopic examination, suspected samples were dissected in Ringer's solution and then prepared wet smears including host fat body were examined for presence of coccidian pathogens under a light microscope at a magnification of 400-1000X. The oocysts of the coccidian were measured and photographed using a microscope with a digital camera and soft imaging system.

Results: The pathogen was observed in the fat bodies of the larvae, pupae and adults. Oocysts measured as 29.52±3.32 (25.27-35.08) µm in diameter and they include 8 sporocysts. Sporocysts measured as 9.11±0.61 (8.90-9.85) µm. Forty-five of 3.432 *P. interpunctella* larvae, pupae and adults were found to be infected. Coccidian infections have also reached to the levels that can be considered high in some populations, as significant as 29.2%. The infection was observed in the three (21.4%) of the examined fourteen populations.

Conclusion: The coccidian entomopathogen presented in this study is the first Adeleid coccidian record from *P. interpunctella* populations in Türkiye. The detection of *Adelina mesnili* Perez (Coccidia: Adeleidae) in at least three populations and the infection rate reaching 29.2 percent, confirms that this pathogen has a considerable effect *P. interpunctella* populations that cannot be underestimated. Our results confirm that the coccidian pathogen is very effective in the larval stage.

Keywords: *Plodia interpunctella*, *Adelina mesnili,* stored product pest, distribution, biological control

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Türkiye PARAZİTOLOJİ Dergisi

ÖZ

Amaç: Güney Amerika kökenli *Plodia interpunctella* (Hübner) (Lepidoptera Pyralidae) her kıtada bulunan ve depolama ürünlerine zarar veren önemli zararlılardan biridir. *P. interpunctella* ve diğer depolanmış ürün zararlılarının mikrobiyal kontrolü için entomopatojenlerin kullanımına yönelik bir ilgi vardır. Bir protistan entomopatojen grubu olarak Coccidia, konakçıya özgü olup, konaklar üzerindeki patojenik etkileri daha belirgindir. Bu patojenik etki, artan konakçı ölümü veya insektisitlere karşı daha yüksek duyarlılık ile sonuçlanmasına rağmen, doğal popülasyonlarda coccidianların baskılayıcı potansiyeli yeterince araştırılmamıştır. Bu çalışmada, bir coccidian entomopatojenin karakterizasyonun yapılması, dağılımı ve enfeksiyon oranlarının belirlenerek *P. interpunctella* popülasyonlarındaki doğal baskılama potansiyelinin ortaya konulması amaçlanmıştır.

Yöntemler: Üç yıl boyunca (2019-2021), on dört popülasyondan toplam 3,432 *P. interpunctella* örneği (2,047 ölü, 413 canlı larva, 932 ergin ve 40 pupa) toplanmıştır. Makroskopik incelemeden sonra, şüpheli numuneler Ringer solüsyonunda disekte edilmiş ve daha sonra konak yağ dokusunu içeren preperatlar hazırlanarak Coccidian patojenlerin varlığı açısından ışık mikroskobunda 400-1000X büyütmede incelenmiştir.

Bulgular: Patojen larva, pupa ve erginlerin yağ dokusunda gözlenmiştir. 29,52±3,32 (25,27-35,08) µm çapında ölçülen ookistler, 8 sporokist içermektedir. Sporokistler 9,11±0,61 (8,90-9,85) µm olarak ölçülmüştür. İncelenen 3,432 *P. interpunctella* larva, pupa ve ergininin 45 tanesinin enfekte olduğu tespit edilmiştir. Coccidian enfeksiyonlarının bazı popülasyonlarda %29,2 gibi önemli sayılabilecek seviyelere ulaştığı tespit edilmiştir. Enfeksiyon, incelenen on dört popülasyonun üçünde (%21,4) gözlenmiştir.

Sonuç: Bu çalışmada sunulan coccidian entomopatojen, Türkiye'deki *P. interpunctella* popülasyonlarından elde edilen ilk Adeleid Coccidian kaydıdır. *Adelina mesnili* Perez'in (Coccidia: Adeleidae) en az üç popülasyonda saptanması ve enfeksiyon oranının %29,2'ye ulaşması, bu patojenin *P. interpunctella* popülasyonlarında küçümsenemeyecek kadar önemli bir etkiye sahip olduğunu doğrulamaktadır.

Anahtar Kelimeler: *Plodia interpunctella*, *Adelina mesnili,* depo zararlısı, dağılış, biyolojik mücadele

INTRODUCTION

Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae) originating from South America is one of the important insect pests that damages storage products and is found on every continent. It is a harmful species in a wide range of products such as dried figs, dried apricots, hazelnuts, raisins, oil seeds, cereals, flour and products, cocoa and spices. *P. interpunctella* larvae feed both inside and on the surface of the food. The infestation of *P. interpunctella* causes direct crop loss and economic cost.

There is a new interest in using enthomopathogens for microbial control of *P. interpunctella* as well as other stored product pests (1,2). Among the entomopathogens, protistan entomopathogens are often prevalent and persistent in natural populations of pest insects. Coccidia as a group of protistan entomopathogens are all intracellular parasites and multiply extensively in the host insect. Most species are host specific. Coccidia carry out extensive and more multiplication cycles in the insect host, so the pathogenic effects on the host are more pronounced (3). Although this pathogenic effect results in increased host mortality or higher susceptibility to insecticides, the suppressive potential of coccidia in natural populations has not been adequately studied. In this study, characterization, distribution and occurrence of a coccidian entomopathogen were studied to show its natural suppressing potential in *P. interpunctella* populations.

METHODS

Insect Samples

During the three years (from 2019 to 2021), a total of 3.312 *P. interpunctella* samples (2.032 dead and 413 living larvae, 830 adults and 37 pupae) were collected from warehouses, shops and houses in the fourteen provinces (Ankara, Aydın, Bolu, Denizli, Gaziantep, Isparta, İstanbul, İzmir, Kastamonu, Malatya, Ordu, Samsun, Siirt and Trabzon), widely dispersed geographically in Türkiye (Table 1).

Macroscopic-microscopic Examinations

Insect specimens suspected of having disease were subjected to macroscopic examination. The most common symptoms in larvae

Table 1. Sampling localities and dates

Sampling localities	Sampling date
Ankara	08.07.2021
Aydın	12.06.2019, 02.07.2019, 22.07.2019, 18.06.2020, 30.06.2020
Bolu	22.05.2019, 28.06.2019, 08.07.2019, 20.08.2019, 05.09.2019, 05.09.2019, 12.09.2019, 20.01.2020, 18.02.2020, 11.03.2020, 23.03.2020, 30.04.2020, 01.06.2020, 13.07.2020, 17.06.2021, 12.07.2021
Denizli	01.06.2019, 28.06.2019
Gaziantep	05.07.2019, 05.08.2019, 11.09.2019, 22.07.2020, 27.07.2020, 25.07.2021
Isparta	02.05.2019, 13.07.2019, 06.08.2020, 31.08.2020, 26.05.2021
İstanbul	20.12.2019, 16.03.2020, 02.04.2020
İzmir	12.06.2019
Kastamonu	08.12.2021
Malatya	13.06.2019, 21.06.2019, 12.09.2019, 16.07.2020, 20.08.2020
Ordu	18.06.2019, 21.06.2020
Samsun	10.06.2019, 10.07.2020, 28.07.2021
Siirt	28.06.2019
Trabzon	15.06.2019, 10.07.2020

were discolored death, slow movement, loss of appetite. After macroscopic examination, suspected samples were dissected in Ringer's solution and then prepared wet smears including host fat body were examined for presence of coccidian pathogens under a light microscope at a magnification of 400-1000X. When an infection was found, the slides were air-dried and fixed with methanol, then stained with freshly prepared 5% solution of Giemsa stain. They were then washed in running tap water, air-dried and examined under a microscope (4). The oocysts of the coccidian pathogen detected by the light microscopy were measured and photographed using a microscope with a digital camera and soft imaging system.

Statistical Analysis

A chi-square test was used to compare observed results. A p-value less than 0.05 was considered significant.

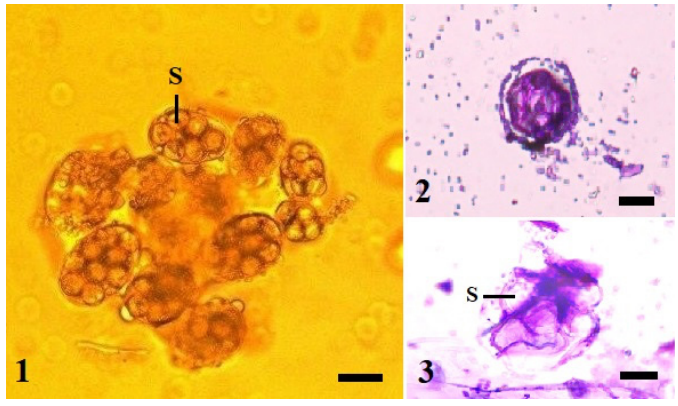
RESULTS

During the microscopic observations, a coccidian entomopathogen were found in the populations of *P. interpunctella* in Türkiye. The pathogen was observed in the fat bodies of the larvae, pupae and adults. Polysporocystic oocysts of the pathogen were the evidence of the infection (Figures 1-3). Oocysts measured as 29.52±3.32 (25.27-35.08) µm in diameter and they include 8 sporocysts. Sporocysts measured as 9.11±0.61 (8.90-9.85) µm.

During the study, 3.432 samples of *P. interpunctella* samples including larvae, adults and pupa were dissected and searched for the coccidian infections in the fourteen localities of Türkiye between the years 2019-2021. Totally, 2.047 dead and 413 living larvae, 932 adults and 40 pupae were examined for the presence of the coccidian pathogen, 45 of 3.432 *P. interpunctella* larvae, pupae and adults were found to be infected. Total infection occurred as 1.3% for the pathogen (Table 2). On the other hand, the average of coccidian infections for all populations was found as 0.2% for dead larvae, 0.2% for living larvae and 4.2% for adults. Coccidian infections have also reached to the levels that can be considered high in some populations, as significant as 29.2% (Tables 2, 3). The infection was observed in the three (21.4%) of the examined fourteen populations (Figure 4).

DISCUSSION

Light microscopic observations of the fresh and Giemsa-stained preperations indicate that the coccidian pathogen found in *P. interpunctella* populations belongs to the genus *Adelina*. The recorded parasite has typical characters of the genus *Adelina* such as shape and size of oocysts (Figures 1-3), number (3-30) of sporocyst per oocyst and number of sporozoites per sporocyst (3). In the literature there is no coccidian record from *P. interpunctella* populations in Türkiye. The Coccidian entomopathogen presented in this study is the first *Adeleid* coccidian record from *P. interpunctella* populations in Türkiye. Number of sporocyst per oocyst and the host affinity is generally recognized as a valid taxonomic character to discriminate the pathogen at the species level. According to Yaman et al. (2), up to now fifteen species belonging to the genus *Adelina* have been described from host insects; their distinctive characteristics are



Figures 1-3. Adeleid polysporocystic oocysts of coccidian pathogen. (1) A group of oocysts in fat body, (2) Giemsa stained oocyst, (3) Giemsa stained sporocysts releasing from the damaged oocyst. Bars, 10 µm

Table 2. Occurrence of *Adelina mesnili* in *P. interpunctella* populations

Locality	Examined sample	Infected sample	Infection rate (%)
Ankara	51	-	-
Aydın	101	3	2.9
Bolu	1.115	-	-
Denizli	9	-	-
Gaziantep	494	-	-
Isparta	120	-	-
İstanbul	121	-	-
İzmir	45	-	-
Kastamonu	120	35	29.2
Malatya	499	-	-
Ordu	193	7	3.6
Samsun	299	-	-
Siirt	145	-	-
Trabzon	120	-	-
Total	**3.432**	**45**	**1.3**

shown in Table 4. The oocyst dimension and sporocyst number per oocyst is a good feature for comparison of the fifteen *Adelina* species from host insects. As seen in Table 4, our coccidian differs from thirteen *Adelina* species in oocyst size and similar with *Adelina mesnili* (8 sporocysts per oocysts). The number of sporocyst in an oocyst varies from 6 to 8 with 8 being the most common. Pérez (5) recorded the number of sporocysts in each of *A. mesnili* as generally 6 to 8, rarely 9 in the original description. The morphological features of the pathogen show similarities

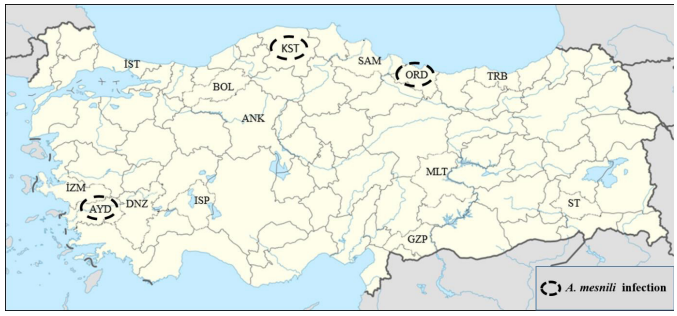


Figure 4. Distribution of *Adelina mesnili* infections in *Plodia interpunctella* populations in Türkiye

ANK: Ankara, AYD: Aydın, BOL: Bolu, DNZ: Denizli, GZP: Gaziantep, ISP: Isparta, İST: İstanbul, İZM: İzmir, KST: Kastamonu MLT: Malatya, ORD: Ordu, SAM: Samsun, ST: Siirt, TRB: Trabzon

with other species of the genus *Adelina* (Coccidia: Adeleidae) and especially resembles *A. mesnili*, described in lepidopteran hosts by Pérez (5) and observed in the artificial cultures of *P. interpunctella* and *Ephestia kühniella* by Steinhaus (6). Therefore, the coccidian pathogen was identified as the Turkish strain of *A. mesnili*. *A. mesnili* found in the present study was observed first in the larvae, pupae and adults of *P. interpunctella* in Türkiye.

No statistical difference between both dead and living larvae was found. As seen in Table 3, the infection rates in the adults are higher than those in pupae. There is statistically significant difference in the infection levels of adult and pupa of *P. interpunctella* (Pearson chi-square, p=0.000 <0.05). Coccidians occur naturally in Lepidoptera. So, have been recognized as potential biocontrol agents against Lepidoptera. However, the use of pathogenic protist species as a control agent should be in the early stages of development. At the same time, extensive research is required to be used as a protective agent (7).

There have been several studies on pathogens and parasites of stored-product pests, mainly focused on isolation and characterization of pathogenic microorganisms. A few of them were carried out on the protistan entomopathogens of *P. inteerpunctella*. Until now, microsporidian pathogens, *Nosema plodiae* (8,9), *Vairimorpha plodia* (1,10-12), neogregarine pathogen, *Mattesia dispora* (13), gregarine pathogen, *Leidyana* sp. (14), have been studied as microbial pathogen in *P. interpunctella*. However, there is only one study on the distribution, occurrence

Table 3. Occurrence of *Adelina mesnili* in the different life stages of *Plodia interpunctella*

Life stage	Number of examined sample	Number of infected sample	Infection rate (%)
Larva (living)	413	1	0.2
Larva (dead)	2.047	4	0.2
Adult	932	39	4.2
Pupae	40	1	-
Total	**3.432**	**45**	**1.3**

Table 4. *Adelina* species that infect insects and their morphological features [improved from (15)]

Adelina species	Tissues infected	Num. sporocyst per oocyst	Oocyst diam. (µm)	Sporocyst diam. (µm)	Reference
A. akidium	Fat body	12-20	30-40	10	(16)
A. collembolae	Fat body	24	40	7.5-8	(17)
A. eryptocerci	Various	5-21	24-51	10-12	(18)
A. mesnili	Fat body	6-8	-	15	(5)
A. riouxi	-	8-18	30-40	7-10	(19)
A. sericesthis	Fat body	4-8	30-40	10.8-11.9	(20)
A. simplex	Gut	8-16	25-40	-	(21)
A. tenebrionis	Fat body	2-12	20-35	10-12	(22)
A. tenebrionis	Fat body	3-13	29.2-45	12.3-14	(15)
A. tipulae	Gut	4-10	35-40	-	(21)
A. transita	Various	6-20	30-40	10-11	(23)
A. grylli		4-22	-	-	(24)
A. triboli	Fat body	4-16	40	10-13	(25)
A. melolonthae	Fat body	4-12	35.62±4.04 (23.97-44.56)	11.70±0.42 (11.02-12.52)	(26)
A. mesnili	Fat body	6-8	29.52±3.32 (25.27-35.08)	9.11±0.61 (8.90-9.85)	In this study

and potential of microsporidian entomopathogen, *V. plodia* in *P. interpunctella* under natural conditions (1). There is no any study on distribution, occurrence and potential of microbial pathogens about *A. mesnili* (Coccidia: Adeleidae) in *P. interpunctella* under the natural conditions. In this study, characterization, distribution and occurrence of the coccidian entomopathogen of *P. interpunctella* from 14 localities representing all Türkiye between the years 2019-2021 is given in an extensive field study for the first time by confirming its first record from Türkiye and effectiveness on natural populations. *A. mesnili* was detected in three populations (21.4%).

CONCLUSION

Coccidians occurring naturally in insect pest populations are highly pathogenic for them, therefore they have been considered as potential natural suppressing factor in insect pest populations. The detection of *A. mesnili* in at least three populations and the infection rate reaching 29.2 percent, confirms that this pathogen has a considerable effect in *P. interpuctella* populations that cannot be underestimated.

* Ethics

Ethics Committee Approval: Ethics committee approval is not required as it is studied on harmful insects.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

* Authorship Contributions

Concept: M.Y., Design: M.Y., Data Collection or Processing: T.S., Ö.E., Analysis or Interpretation: M.Y., T.S., Ö.E., Literature Search: M.Y., T.S., Ö.E., Writing: M.Y., T.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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