Characterization, distribution, and virulence of protistan entomopathogen, *Mattesia dispora* (Sporozoa, Gregarina) in the Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae) populations in Turkey

Mustafa Yaman1,*, Tuğba Sağlam1 and Ömer Ertürk2

**Abstract**

**Background:** Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) is the dominant pest on the stored products throughout the world. As an alternative to chemical insecticides, entomopathogens can be natural suppressors for pest populations. For this reason, the study of entomopathogens existing in the natural population of a pest contributes to the decision-making process of controlling that pest. In the present study, characterization, distribution, and virulence of protistan entomopathogen, the Turkish strain of *Mattesia dispora* in the Indian meal moth, *Plodia interpunctella* populations were presented.

**Results:** During the microscopic observations, a protistan entomopathogen was found in the populations of *P. interpunctella* in Turkey. It was identified as the Turkish strain of *Mattesia dispora*, a neogregarine pathogen. Typical fresh navicular oocysts of the pathogen were 13.28 ± 0.41 (13.1–14.41) μm in length and 7.72 ± 0.51 (6.6–8.54) μm in width (*n* = 50). Oocysts stained with Giemsa measured 12.32 ± 0.78 (10.88–13.24) μm in length and 7.01 ± 0.26 (6.5–7.43) μm in width. Polar plugs were recognizable clearly by light and electron microscopy, measuring 900 to 1100 nm. The oocyst wall was quite thick, measuring 600 to 800 nm. Each oocyst contained 8 sporozoites. 2.047 dead and 413 living larvae, 932 adults, and 40 pupae, collected from 14 different locations from 2019 to 2021 were examined for the presence of the protistan entomopathogen. In total, 225 of 3,432 *P. interpunctella* adult and larvae were found to be infected with this pathogen. Total infection occurred as 5.2 for *M. dispora*. Infection rates by *M. dispora* were 4.8% for dead larvae, 14.8% for living larvae, and 2.1% for adults. On the other hand, *M. dispora* infections reached 33% in some populations. *M. dispora* infections were observed in the seven (50%) of the examined populations. Furthermore, the Turkish strain of *M. dispora* had a high pathogenic effect against the second/third instar larvae of *P. interpunctella*. The average mortality rate was 98.33%.

**Conclusions:** Little is known about neogregarine infections as a natural suppressing factor in pest populations. The Turkish strain of *M. dispora* is very common and widespread in the populations of *P. interpunctella*. Furthermore, it has very high virulence on the *P. interpunctella* larvae. Such a widespread infection and very high virulence are desirable.
Entomopathogens that cause disease in pests are natural organisms (Baki et al. 2021; Yaman et al. 2021). During the three years (2019–2021), a total of 3312 insect samples (2032 dead and 413 living larvae; 830 adults and 37 pupae) were collected from warehouses, shops, and houses in the 14 provinces (Ankara, Aydın, Bolu, Denizli, Gaziantep, Isparta, İstanbul, İzmir, Kastamonu, Malatya, Ordu, Samsun, Siırt, and Trabzon), widely dispersed geographically in Turkey.

Background
The Indian meal moth, Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae), is the dominant pest on the stored products throughout the world. Synthetic insecticides applied to stored products or fumigation are used to reduce losses of stored products (Freitas et al. 2020). However, it has been realized that these insecticides are not quite innocent over the years. Because they pose a great risk to both nature and beneficial insects as well as humans. Therefore, there is an urgent need to develop an alternative safe pest control strategy with less harmful effects on humans and the environment (Kumar et al. 2012), which is possible with entomopathogenic organisms (Baki et al. 2021; Yaman et al. 2021). Entomopathogens that cause disease in pests are natural suppressors of pest populations. For this reason, the study of entomopathogens existing in the natural population of a pest contributes to talking the decision-making process to control that pest.

There is a new interest in using entomopathogens for microbial control of P. interpunctella as well as other stored product pests (Yaman et al. 2021). Detection of different natural pathogens and parasites of P. interpunctella for controlling the population can be most successful in the biological control of this pest. Among the entomopathogens, protistan entomopathogens are often prevalent and persistent in natural populations of pest insects, studies on their use as potential microbial insecticides have generally been limited due to their high host specificity and difficulties with mass production. In addition, their suppressive potential in natural populations has not been adequately studied. Some entomopathogenic protists such as microsporidia, neogregarines, and coccidia are known to infect storage pest insects, however, entomopathogens naturally occurring in P. interpunctella populations have not been enough investigated. In this study, characterization, distribution, and virulence of protistan entomopathogen, M. dispora in P. interpunctella were studied to document the natural suppressing potential of this pathogen in P. interpunctella populations.

Methods
Insect samples
During the three years (2019–2021), a total of 3312 P. interpunctella samples (2032 dead and 413 living larvae; 830 adults and 37 pupae) were collected from warehouses, shops, and houses in the 14 provinces (Ankara, Aydın, Bolu, Denizli, Gaziantep, Isparta, İstanbul, İzmir, Kastamonu, Malatya, Ordu, Samsun, Siırt, and Trabzon), widely dispersed geographically in Turkey.

Bioassay tests for the potential of the isolated protistan entomopathogen
Virulence of the isolated protistan entomopathogen was tested against the second/third instar larvae of P. interpunctella. Oocyst of the pathogen was harvested from the infected larvae at the 10–14×10⁶ oocysts/ml concentrations and diluted to 1.6×10⁶ to obtain the required concentration for experimental treatments. Second/third instar larvae of P. interpunctella larvae were fed on nut tablets dipped into the neogregarine pathogen suspensions. Three replications (Experimental group 1, 2, and 3) of the experimental group and two replications (Control group 1 and 2) of the control were used. Each bioassay group was performed by 20 insect larvae under the same laboratory conditions. All tested groups were
kept at 24–28 °C and 35–45% RH and 18:6 photoperiod of laboratory conditions for 21 days. Observations were recorded daily and dead larvae were removed immediately. Experimental bioassays were repeated 3 times on different days and data was corrected, using Abbott’s formula (Abbott 1925).

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

**Results**
After macroscopic observations, disease-suspected specimens from the infected colonies were ailing obviously with symptoms such as slow movement, loss of appetite and color change, and in certain numbers dying although optimum living conditions are provided.

During the microscopic observations, a protistan entomopathogen was found in the populations of *P. interpunctella* in Turkey (Figs. 1 and 2). It was a neogregarine pathogen. The neogregarine pathogen was observed in only larvae and adults of *P. interpunctella*, not in the pupae. The infected tissue was the fat body and hemolymph of the host. All life cycle stages such as oocysts, micronuclear and macronuclear merozoites, and gamonts of the neogregarine pathogen were observed in the wet or stained smear preparations.

Typical fresh navicular oocysts of the pathogen were $13.28 \pm 0.41$ (13.1–14.41) μm in length and $7.72 \pm 0.51$ (6.6–8.54) μm in width ($n = 50$). Oocysts stained with Giemsa measured $12.32 \pm 0.78$ (10.88–13.24) μm in length and $7.01 \pm 0.26$ (6.5–7.43) μm in width. Polar plugs were recognizable clearly by light and electron microscopy, measuring 900–1100 nm. The oocyst wall was quite thick, measuring 600 to 800 nm. Each oocyst contained 8 sporozoit (Figs. 3 and 4).

During the study, 3432 samples of *P. interpunctella* samples including larvae, adults, and pupae were dissected and searched for neogregarine infection in the 14 localities of Turkey from the years 2019–2021. 2047 dead and 413 living larvae, 932 adults, and 40 pupae were examined for the presence of the neogregarine pathogen, 180 of 3432 *P. interpunctella* adults and larvae were found to be infected by this pathogen. Total infection occurred at 5.24% (Tables 1 and 2).

Neogregarine infection was observed in 7 of the examined 14 populations. The average of neogregarine infections for all populations was found as 4.8% in dead larvae, 14.8% in living larvae, and 2.1% in adults (Table 2). However, neogregarine infections had reached levels that can be considered high in some populations, as significant as 33%.

On the other hand, the virulence of the protistan pathogen against *P. interpunctella* larvae was also determined. Bioassay tests showed that the protistan pathogen had a high pathogenic effect against the second/third instar larvae of *P. interpunctella* (Fig. 5). Two of the three experimental groups had a 100% mortality rate, while a 95% mortality rate was achieved in one experimental group. In contrast, one larval death was observed in only one of the control groups. The average mortality rate was determined as 98.33%.

**Discussion**
In this study, 14 sampling populations were included to represent the whole of Turkey, a neogregarine pathogen of *P. interpunctella* was detected for the first time. Morphological and ultrastructural results showed that the described neogregarine had the typical characteristics of members of the genus *Mattesia* (Family Lipotrophidae: order Neogregarinorida (Apicomplexa)). It closely resembles *Mattesia dispora*, first described from the larvae of the flour moth, *Ephestia kuehniella* by Naville (1930),

![Figs. 1–2 Ocysts of Mattesia dispora recorded from Plodia interpunctella in Turkey. Fresh 1 and Giemsa-stained oocysts 2 of neogregarine pathogen, pp, polar plug. Bars, 5 μm](image-url)
then recorded from different hosts including *P. interpunctella* (Yaman et al. 2021). *Mattesia* species discussed here was observed first in the larvae and adults of *P. interpunctella* and identified as a Turkish strain of *M. dispora*. Yaman et al. (2019) described this pathogen from the laboratory cultures *E. kuehniella* in Turkey. Both hosts are closely related insect pests of stored products and they often share the same habitat. On the other hand, Suzaki et al. (2006) identified a new gregarine parasite, *Lediyana* sp. of *P. interpunctella*, however, although a quite large number of samples was examined, this pathogen was not observed in any of the 14 populations in Turkey.

Neogregarines occur naturally in lepidopteran pests. Some are highly pathogenic. So, have been recognized as potential biocontrol agents against lepidopteran pests. However, the use of protistan pathogenic 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 (Dales 1994). Several studies on pathogens and parasites of stored-product pests, mainly have focused on the isolation and characterization of pathogenic microorganisms. A few of them were carried out on the protist pathogens of *P. interpunctella*. Until now, microsporidian pathogens, *Nosema plodiae* (Kellen and Lindegren 1973), *Vairimorpha plodiae* (Sagal et al. 2021), neogregarine pathogen, *Mattesia dispora* (Wendell and Dicke 1964), gregarine pathogen, *Lediyana* sp. (Suzaki et al. 2006) were studied as a microbial pathogen in *P. interpunctella*. There is only one study on the distribution, occurrence, and potential of a microsporidium, *Vairiomorpha plodiae* in *P. interpunctella* under natural conditions (Sagal et al. 2021). However,

### Table 1 Occurrence of *Mattesia dispora* in *Plodia interpunctella* populations

| Locality   | Examined sample | Infected sample | Infection rate (%) |
|------------|-----------------|-----------------|--------------------|
| Ankara     | 51              | 1               | 1.8                |
| Aydin      | 101             | –               | –                  |
| Bolu       | 1.115           | 20              | 1.8                |
| Denizli    | 9               | –               | –                  |
| Gaziantep  | 494             | 53              | 10.7               |
| Isparta    | 120             | –               | –                  |
| Istanbul   | 121             | –               | –                  |
| Izmir      | 45              | 1               | 2.2                |
| Kastamonu  | 120             | –               | –                  |
| Malatya    | 499             | 1               | 0.2                |
| Ordu       | 193             | 3               | 1.5                |
| Samsun     | 299             | 101             | 33.8               |
| Siirt      | 145             | –               | –                  |
| Trabzon    | 120             | –               | –                  |
| Total      | 3.432           | 180             | 5.24               |

### Table 2 Occurrence of *Mattesia dispora* in the different life stages of *Plodia interpunctella*

| Life stage          | Examined sample | Infected sample | Infection rate (%) |
|---------------------|-----------------|-----------------|--------------------|
| Larva (living)      | 413             | 61              | 14.8               |
| Larva (dead)        | 2.047           | 99              | 4.8                |
| Adult               | 932             | 20              | 2.1                |
| Pupae               | 40              | –               | –                  |
| Total               | 3.432           | 180             | 5.24               |

Figs. 3–4 Ultrastructure of mature oocysts of *Mattesia dispora*, TEM. Longitudinal 3 and cross 4 sections of oocyst including eight sporozoites. S sporozoites, OW oocyst wall, PP polar plug. Bars, 1 μm
there are no other studies on the distribution and potential of protistan entomopathogens in the natural populations of *P. interpunctella*. In this study, the presence, distribution, and virulence of *M. dispora* in 14 populations of *P. interpunctella* were investigated. *M. dispora* was detected in 7 (50%) of the 14 populations examined.

The fact that *M. dispora* infects half of the populations studied and its infection rate reaches up to 33.8% in some populations (Table 1) indicates that *M. dispora* can be an important natural suppressing protistan entomopathogen in *P. interpunctella* populations in Turkey. In addition, *P. interpunctella* larvae and adults were found to be infected by the neogregarine pathogen. As shown in Table 1, *M. dispora* is very common in the populations of *P. interpunctella*. Such a widespread infection is a desirable property for a biological control agent (Pereira et al. 2002). Additionally, among gregarines only the neogregarines had a high pathogenic effect on their hosts by destroying the host’s fat body and exhausting energy sources. The results confirmed that *M. dispora* infections are desirable and significant natural suppressor factors in *P. interpunctella* populations.

In neogregarines, the members of the genus *Mattesia* are known as important pathogens of various insects with a significant pathogenic effect on their hosts (Valigurova and Koudela 2006). Therefore, their effects on several host insects have been investigated by several authors for microbial control. In an extensive study, the susceptibility of several insect pests of stored grain to 2 *Mattesia* species, *M. oryzae philii* isolated from *Crypto lestes ferrugineus* and *M. dispora* obtained from *E. kueh niella* were determined for microbial control (Lord 2003).

On the other hand, bioassay experiments showed that the Turkish strain of the neogregarine, *M. dispora* had a high virulence on the second/third instars’ larvae of *P. interpunctella* with a 98.33% mortality rate under the laboratory conditions. There was a statistically significant difference in the mortality levels of the experimental group and control group (Pearson Chi-square, *P*: 0.02 < 0.05). It is considerable high effect when compared other *Mattesia* spp. (Alfazairy et al. 2019).

Mass rearing of both insects’ *P. interpunctella* and *E. kuehniella* provides optimal conditions for reproduction and spread of the gregarine (Valigurova and Koudela 2006). The high infection of the Turkish strain of *M. dispora* in both laboratory and natural populations of *P. interpunctella* encouraged its mass production to be used in the biological control of *P. interpunctella*. There are some studies supporting this idea. Lord (2003) studied the alternative hosts that might be used for production of oocysts and revealed that *G. mellonella* larvae can serve as a medium for producing oocysts in larger quantities than the known small grain beetle hosts. Alfazairy et al. (2019) evaluated the potential of the Egyptian strain of the neogregarine, *Mattesia* sp., originally isolated from some stored grain insect pests in term of spore productivity, pathogenicity, and host range assays. According to those results, *P. interpunctella* could serve as a potential host for mass propagating the neogregarine pathogens. The present study confirms that *P. interpunctella* can serve as a medium for producing neogregarines in larger quantities and that *M. dispora* can be a natural suppressing factor of *P. interpunctella* population.
Conclusions
Neogregarines occur naturally in insect pest populations and are highly pathogenic for them, therefore they have been considered as potential control agents against insect pests. Little is known about neogregarine infections as natural suppressing factor in pest populations. The Turkish strain of *M. dispora* is very common and widespread in the populations of *P. interpunctella*. Furthermore, it had a very high virulence against the *P. interpunctella* larvae. *M. dispora* can be an important natural suppressing protistan entomopathogen against *P. interpunctella* populations.

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Author contributions
MY collected insects, identified the neogregarine pathogen and wrote the manuscript. TS collected and dissected insects. ÖE collected and dissected insects. All authors read and approved the final manuscript.

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Availability of data and materials
All datasets are presented in the main manuscript.

Declarations

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Not applicable.

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Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Department of Biology, Faculty of Arts and Science, Bolu Abant İzzet Baysal University, 14030 Bolu, Turkey. 2Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Ordu University, 52750 Ordu, Turkey.

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