Yaman *et al.*
Egyptian Journal of Biological Pest Control (2022) 32:82
https://doi.org/10.1186/s41938-022-00583-x

Egyptian Journal of Biological Pest Control

RESEARCH Open Access

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

Mustafa Yaman[1*], Tuğba Sağlam[1] and Ömer Ertürk[2]

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

*Correspondence: muyaman@hotmail.com

[1] Department of Biology, Faculty of Arts and Science, Bolu Abant İzzet Baysal University, 14030 Bolu, Turkey
Full list of author information is available at the end of the article

properties for a biological control agent. The results indicate that *M. dispora* can be an important natural suppressing protistan entomopathogen in *P. interpunctella* populations.

Keywords: *Plodia interpunctella*, Stored product pest, *Mattesia dispora*, Virulence, Distribution, Natural suppressor

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, Siirt, and Trabzon), widely dispersed geographically in Turkey.

Macroscopic–microscopic examination and characterization of protist pathogens

Macroscopic examination of a field-collected insect sample may offer some clues as to disease-causing entomopathogens in its natural populations. Therefore, the samples were firstly taken for macroscopic examination. After macroscopic examination, living and dead samples suspected with a symptoms of disease were separated individually for microscopic examinations. All samples were dissected in Ringer's solution and then prepared wet smears including host fat body, malpighian tubules, gut epithelium, and hemolymph were examined for the presence of protistan entomopathogens under a light microscope at a magnification of 400–1000×. When infection was found, the slides were air-dried and fixed with methanol, then stained with a freshly prepared 5% solution of Giemsa stain. They were then washed in running tap water, air-dried, and examined under a microscope (Yaman 2020). The oocysts of the protist pathogens detected by the light microscopy were measured and photographed using a microscope with a digital camera and soft imaging system. A part of the infected specimens was used for preparing samples for transmission electron microscopy (TEM) using previously reported techniques.

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 $10–14\times10^6$ oocysts/ml concentrations and diluted to 1.6×10^6 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 ± 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 900–1100 nm. The oocyst wall was quite thick, measuring 600 to 800 nm. Each oocyst contained 8 sporozoit (Figs. 3 and 4).

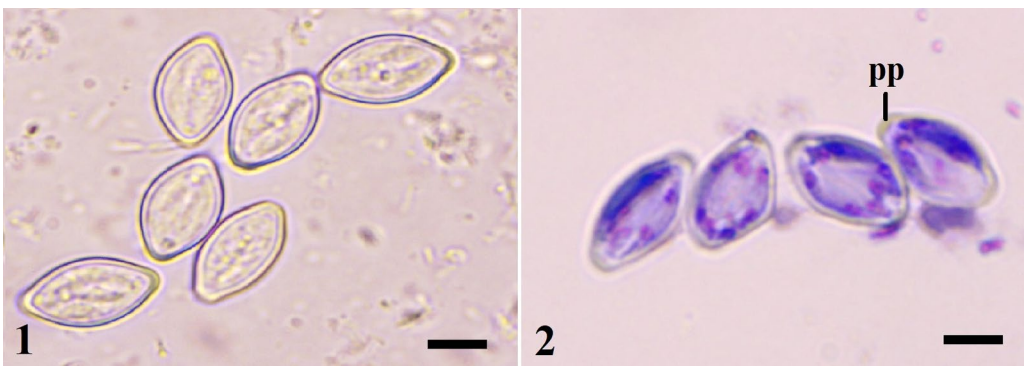
During the study, 3432 samples of *P. interpunctella* samples including larvae, adults, and pupae were dissected and searched for neogregarine infection in the 14 localities of Turkey from the years 2019–2021. 2047 dead and 413 living larvae, 932 adults, and 40 pupae were examined for the presence of 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%.

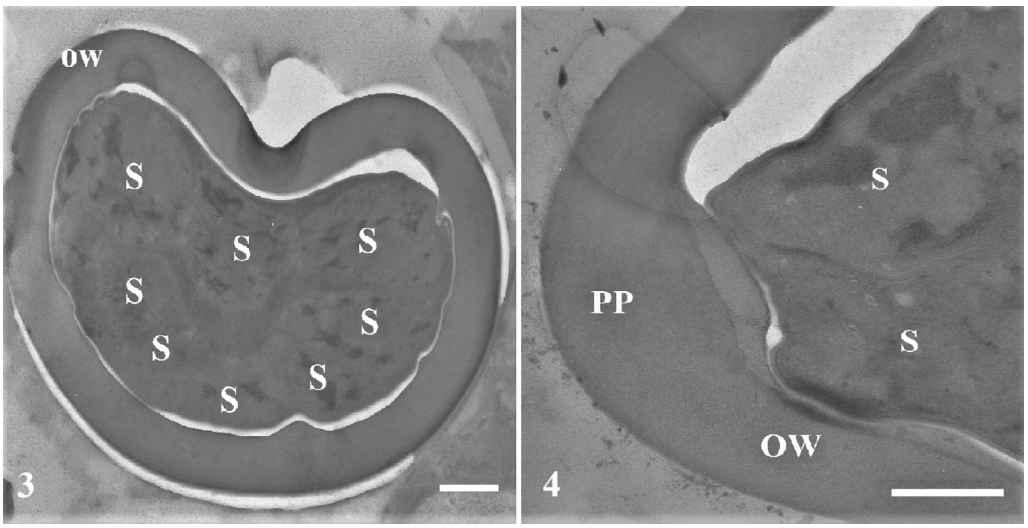
On the other hand, the virulence of the protistan pathogen against *P. interpunctella* larvae was also determined. Bioassay tests showed that the protistan pathogen had a high pathogenic effect against the second/third instar larvae of *P. interpunctella* (Fig. 5). Two of the three experimental groups 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



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

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

Locality	Examined sample	Infected sample	Infection rate (%)
Ankara	51	1	1.8
Aydın	101	–	–
Bolu	1.115	20	1.8
Denizli	9	–	–
Gaziantep	494	53	10.7
Isparta	120	–	–
İstanbul	121	–	–
İzmir	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

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* (Sağlam et al. 2021), neogregarine pathogen, *Mattesia dispora* (Wendell and Dicke 1964), gregarine pathogen, *Leidyana* 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 (Sağlam et al. 2021). However,

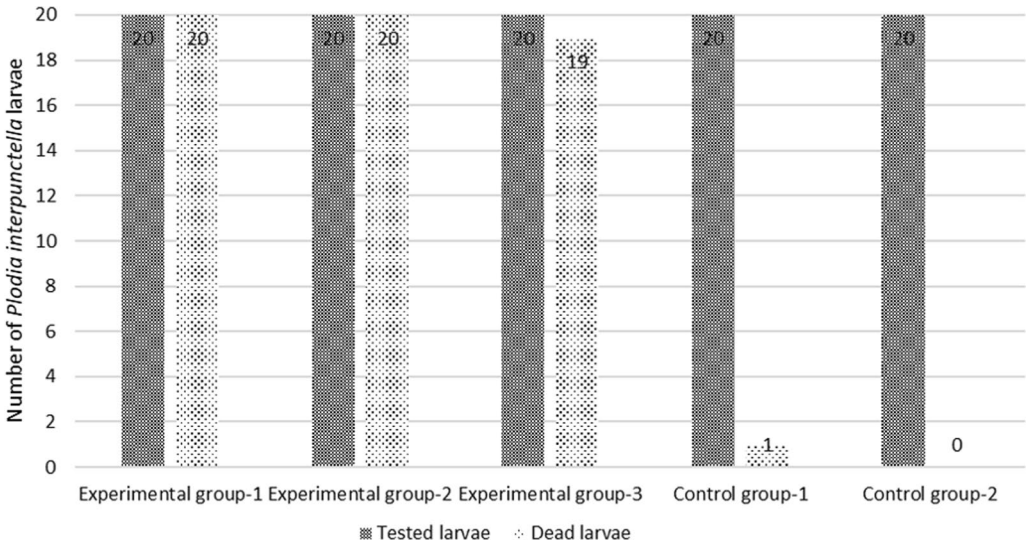


Fig. 5 Virulence of *Mattesia dispora* on the second/third instar larvae of *Plodia interpunctella*

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 host (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. oryzaephili* isolated from *Cryptolestes ferrugineus* and *M. dispora* obtained from *E. kuehniella* 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 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.

Acknowledgements
Not applicable.

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.

Funding
The study was financially supported with a research project by the Scientific and Technological Research Council of Turkey (Project no: 118O980).

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

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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

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

Received: 19 March 2022 Accepted: 26 June 2022
Published online: 30 June 2022

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