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Distribution and Occurrence of *Vairimorpha plodiae* (Opisthokonta: Microspora) in the Indian Meal Moth, *Plodia interpunctella* (Lepidoptera: Pyralidae) Populations: An Extensive Field Study

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Abstract. The Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae) is one of the most important stored product pests. Fumigation plays a significant role in the management of insect pests in stored-products. However, the use of fumigants is problematic because of their effects on the environment and high costs. Entomopathogenic organisms are environmentally friendly control agents and suppress pest populations under natural conditions. In this study, distribution and occurrence of a microsporidian pathogen, *Vairimorpha plodiae* (Opisthokonta: Microspora) in the populations of *P. interpunctella* from 12 localities representing Turkey between 2019 and 2020 are presented for the first time by confirming its effectiveness on natural populations. The presence of the microsporidian pathogen was found in 11 of 12 (91.7%) populations. In total, 863 of 3,044 samples were infected by the pathogen. Infection mean was 28.4% for all populations. Our results showed that *V. plodiae* infection reached to a considerably high prevalence (88.77%) in *P. interpunctella* populations and varied from 5.1 to 88.7% between the populations. In addition, microsporidia infections have been identified throughout Turkey. We found that *V. plodiae* can infect all life stages of *P. interpunctella.* Totally, 623 (28.5%) of 2187 larvae, 14 (37.8%) of 37 pupae, 226 (27%) of 820 adults were found to be infected by the pathogen. There were considerable differences between the dead and living larvae. The microsporidian infection was found in 26 (11.6%) of 225 living larvae, whereas it was found in 595 (30.5%) of 1,952 dead larvae. These results confirm that the microsporidia pathogen has a high spreading potential in *P. interpunctella* populations and can be a natural biological suppression factor on pest populations.

Keywords: *Plodia interpunctella*, *Vairimorpha plodiae*, biological control, stored product pest, entomopathogen.

INTRODUCTION

Although there are several beneficial roles of insects in research (as a model species) (Takov *et al.* 2020), medical and agricultural fields (Demirözer *et al.* 2020), several insects cause damage to plants growing and stored products in agriculture (Rees 2004; Kuyulu and Genç 2020; İnal and Kandemir 2020). The Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae) is a major pest of stored products (McGaughey 1985). Damage to stored products by *P. interpunctella* is a major problem for food manufacturers, retailers, and consumers (Campos and Phillips 2010). Fumigation plays a significant role in the management of insect pests in the stored-products. However, the use of

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fumigants is problematic because of their effects on the environment and high costs. Due to the harmful effects of chemicals on humans as well as non-target organisms (Arıkan and Turan 2020), different alternative control methods are sought today. Entomopathogenic organisms are environmentally friendly control agents and suppress pest populations under natural conditions. Therefore, the most reliable and promising agents against stored product pests are ecologically safely control agents (Adarkwah and Schöller 2012; Freitas *et al.* 2020), especially natural pathogenic microorganisms (Dales 1994).

The natural enemies of *P. interpunctella* have been investigated in more detail. There are numerous papers on natural enemies and pathogens of *P. interpunctella*, such as viruses, bacteria, protists, fungi, nematodes, and parasitoids (Adarkwah and Schöller 2012; Batta 2016; Cowan 1986; Knell *et al.* 1996; Malone 1984a; Mbata and Shapiro-Ilan 2005; Yaman *et al.* 2016). Microsporidia are important regulatory entomopathogenic microorganisms on insect populations due to their common and high infection in insects (Yaman *et al.* 2019, Yaman 2020). There are some records regarding the presence of microsporidian pathogens in *P. interpunctella* (Kellen and Lindegren 1971; Kellen and Lindegren 1973; Malone 1984a, 1984b; Yaman *et al.* 2016) and several studies on its natural enemies (Adarkwah and Schöller 2012; Batta 2016; Cowan 1986; Dales 1974; Knell *et al.* 1996, Malone 1984a; Mbata and Shapiro-Ilan 2005; Yaman *et al.* 2016). However, none of them includes the distribution and occurrence of the enemies in the natural populations of *P. interpunctella* in an extensive field study. Understanding the forces affecting the insect population provides valuable insight into disease dynamics and helps to manage insect populations. In this study, distribution and occurrence of *Vairimorpha plodiae* (Opisthokonta: Microspora) in the populations of *P. interpunctella* from 12 localities representing all Turkey between the years 2019–2020 is given for the first time by confirming its effectiveness on natural populations.

MATERIALS AND METHODS

Insect Samples

A total of 3044 *P. interpunctella* samples (820 adults, 1952 dead and 235 living larvae, and 37 pupae) were collected from different stored products such as nut, walnut, peanut, chestnut, dry fig, apricot, and flour in warehouses, shops, and houses in 12 provinces, widely dispersed geographically in Turkey during the two years (2019–2020). Since the sampling localities have different climatic and geographical characteristics, the biological periods of the pest may occur at different times. For this reason, sampling dates are given in detail in Table 1.

Microscopic Examination

Firstly, the samples were taken for macroscopic examination. After macroscopic examination, living and dead samples were separated individually. *P. interpunctella* larva, pupa, and adult 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 the microsporidian infection under a light microscope at a magnification of 400–1000X. When 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 (Yaman *et al.* 2019).

RESULTS AND DISCUSSION

During the study, 3044 *P. interpunctella* samples including larvae, pupae, and adults were dissected and searched for the microsporidian pathogen, *Vairimorpha plodiae* in the 12 localities of Turkey between the years 2019–2020. Microsporidium infection was confirmed by observation of the oval spores studied in detail by Yaman *et al.* (2016) as evidence of *V. plodiae* infection. The presence of the microsporidian pathogen was found in 11 (91.7%) of 12 populations (Figure 1). No infection was observed only in one population. Although sampling was done on different dates from different localities, no correlation was observed between sampling dates and infection rates. In total, 863 of the 3044 samples were infected by the pathogen. The infection mean was 28.4% for all populations. The results in this study showed that the *V. plodiae* infection reached to considerable high prevalence (88.77%) in *P. interpunctella* populations and varied from 5.1 to 88.7% between the populations (Table 2). The enzootic prevalence of microsporidian infection varies from occasional or continuous presence at low levels to wide levels that increase greatly (Solter *et al.* 2012). There are some reasons for that such as specific pathogen-host interactions, high host density, and the population dynamics of the host. On the other hand, larval population density and size of *P. interpunctella* may vary depending upon food resources and characteristics of the parental population. Furthermore, Gage (1995) mentioned that this species has a very cosmopolitan diet and populations vary considerably in nature from a few individuals to

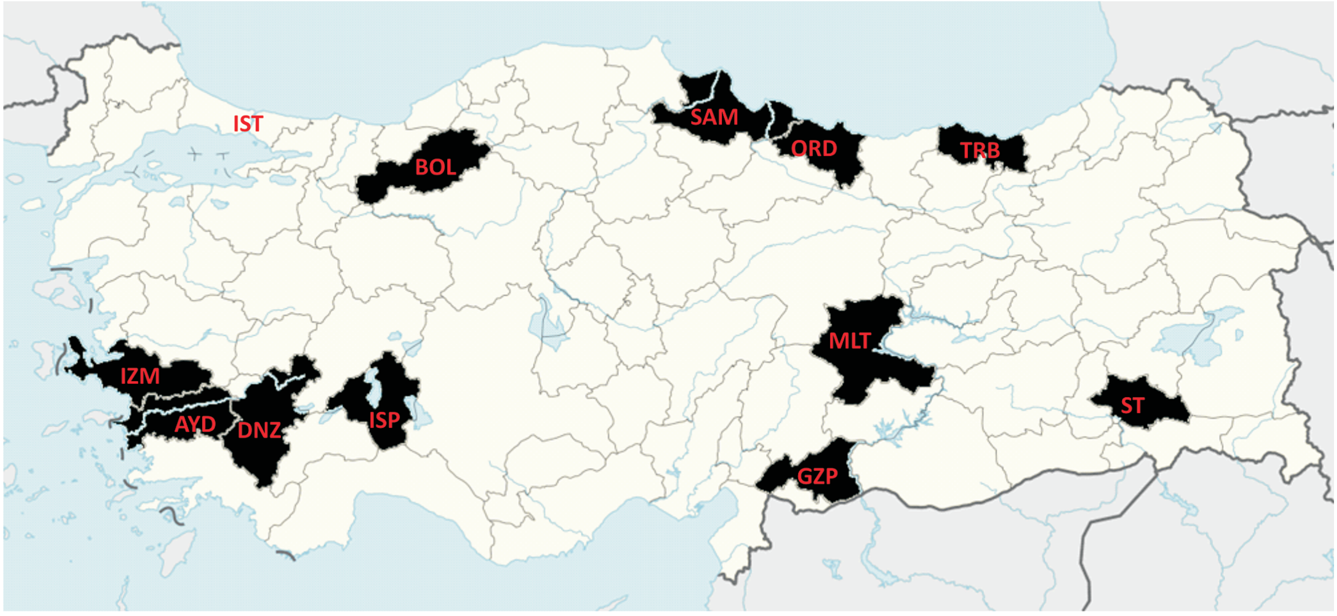

Fig. 1. The microsporidian pathogen, *Vairimorpha plodiae* infections in *Plodia interpunctella* populations in Turkey. Infected populations (note that the pathogen naturally disperses in all populations). (AYD: Aydın, **BOL:** Bolu, **DNZ:** Denizli, **GZP:** Gaziantep, **ISP:** Isparta, İST: İstanbul, İZM: İzmir, **MLT:** Malatya, **ORD:** Ordu, **SAM:** Samsun, **ST:** Siirt, **TRB:** Trabzon)

high densities developed over many generations. In addition, Sait *et al.* (1994) observed the dynamics of granulosis virus pathogen itself were highly unstable and there was little evidence for linked host-pathogen fluctuations. On the other hand, theoretical studies suggest that disease may play an important role in the population dynamics of insects (Sait *et al.* 1994). The variability in infection rates observed among populations in this study supports that the rate of infection in a population may depend on *P. interpunctella* population density and dynamics.The results of this study are also important as they support understanding how *V. plodiae* can help to regulate *P. interpunctella* populations under natural conditions and evaluate the potential of the microsporidian pathogen as a pest control agent.

During the study, we also determined the infection rates among the different life stages (larva, pupa, and adult) in different populations. It is showed that *V. plodiae* infects all life stages of *P. interpunctella.* Totally, 623 (28.5%) of 2187 larvae, 14 (37.8%) of 37 pupae, 226 (27%) of 820 adults were found to be infected by the pathogen (Table 3). The highest infection rate was observed in pupae (37.8%). When compared with the adult, larval and pupal infections, the pupal infection was higher than larval and adult infections and also total mean infection (Table 3). There is a statistically significant difference in the infection levels of pupae/living larvae and adults/living larvae of *P. interpunctella* (Pearson Chi-square, P: 0,000 < 0.05). Lower microsporidial virulence may allow the host to complete its life cycle and ensures transmission of the pathogen to the next host generation via the egg or embryo (Solter *et al.*, 2012). The prevalence of the microsporidian pathogen observed in all life stages of *P. interpunctella* confirms that the infection with the lower virulence of *V. plodiae* ensures transmission of the pathogen through all life stages and to the next host generation.

We also focused on the infection rates between dead and living larvae. We found considerable differences between the dead and the living larvae. The microsporidian infection was found in 26 (11.6%) of 225 living larvae, while it was found in 595 (30.5%) of 1952 dead larvae (Table 3). The dead larval infection was about three times more than the living larval infection. There is a statistically significant difference in the infection levels of living and dead larvae of *P. interpunctella* (Pearson Chi-square, P: 0,000 < 0.05). Microscopical observations also supported the differences between larval infections. The spore density of the microsporidian pathogen was higher in dead larvae. The higher infection levels observed in dead larvae show that the microsporidian pathogen is a main factor of the larval death.

Table 1. Sampling localities and dates for *Plodia interpunctella* populations

Sampling populations	Sampling Date
AYD	12.06.2019, 02.07.2019, 22.07.2019, 18.06.2020, 30.06.2020
BOL	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
DNZ	01.06.2019, 28.06.2019
GZP	05.07.2019, 05.08.2019, 11.09.2019, 22.07.2020, 27.07.2020,
ISP	02.05.2019, 13.07.2019, 06.08.2020, 31.08.2020
IST	20.12.2019, 16.03.2020, 02.04.2020
IZM	12.06.2019
MLT	13.06.2019, 21.06.2019, 12.09.2019, 16.07.2020, 20.08.2020
ORD	18.06.2019, 21.06.2020
SAM	10.06.2019, 10.07.2020
SRT	28.06.2019
TRB	15.06.2019, 10.07.2020

Table 2. Occurrence of *V. plodiae* in *P.interpunctella* populations

Populations	Number of examined	Number of infected	Infection rate (%)
AYD	101	58	57.42
BOL	1114	389	35
DNZ	9	3	33.33
GZP	411	21	5.11
ISP	100	72	72
IST	121	0	0
IZM	45	15	33.33
MLT	499	48	9.61
ORD	192	15	7.81
SAM	187	166	88.77
SRT	145	69	47.58
TRB	120	7	5.8
Total	3044	863	28.4

On the other hand, the microsporidian pathogen, *V. plodiae* was found in 11 (91.7%) of 12 investigated localities, a considerable large area representing all Turkey (Figure 1). As shown in Figure 1, *V. plodiae* is very common and widespread in the populations of *P. interpunctella*. Such a widespread infection is a desirable property for a biological control agent (Pereira *et al.* 2002). The results confirmed that *V. plodiae* infections are desirable and significant natural suppressor factor in *P. interpunctella* populations.

There have been several methods of pest control for stored-product pests, but their effectiveness is limited, and alternatives are needed. However, there are few studies on natural entomopathogenic organisms suppressing *P. interpunctella* populations. Until now, microsporidian pathogens, *Nosema plodiae* (Kellen and Lindegren 1971, 1973), *Vairimorpha plodia* (Malone 1984a, Malone 1984b, Yaman *et al.* 2016), neogregarine pathogen, *Mattesia dispora* (Wendell and Dicke 1964), gregarine pathogen, *Leidyana* sp. (Suzaki *et al.* 2006), bacterial pathogen, *Bacillus thuringiensis* (Kantack 1959, Nwanze *et al.* 1975, Kinsinger and McGaughey 1976, McGaughey 1978), baculoviruses, nucleopolyhedrovirus (Hunter *et al.* 1973) and granulovirus (Wilson and Consigli 1985) and fungal pathogens, *Beauveria bassiana* (Adane *et al.* 1996), *Metarhizim anisoplia*, *Paecilomyces farinosus* and *Lecanicillium* (*Verticillium*) *lecanii* (Būda and Pečiulytė 2008), and nematodes, *Steinernema feltiae* (Oğuzoğlu and Özer 2007), *Steinernama riobrave* (Ramos-Rodríguez *et al.* 2007), *Heterorhabditis indica*, *Heterorhabditis marelatus* and *Heterorhabditis megidis* (Mbata and Shapiro-Ilan 2005) have been studied as potential control agents against *P. interpunctella*. However, there is no study on the distribution, occurrence and potential of microbial pathogen in *P. interpunctella* under natural conditions. In this study, distribution and occurrence of *Vairimorpha plodiae* (Opisthokonta: Microspora) in the populations of *P. interpunctella* from 12 localities representing all Turkey between the years 2019–2020 is given in an extensive field study for the first time by confirming its effectiveness on natural populations.

Microsporidian effects range from nearly benign to relatively virulent, with infections causing the death of the host. Microsporidia utilize insect tissues for their reproductive energy and development. They invade tissues to cause chronic infections in insects, and their effects are manifested in reduced fertility and pupal weight, decreased fertility, and shortened lifespan (Solter *et al.* 2012). The genus *Vairimorpha* in the phylum Microspora includes the most potential microbial insecticide candidate species. *V. necatrix* is a virulent and promising pathogen with high mortality in lepidopteran pests (Maddox *et al.* 1981; Down *et al.* 2004). Therefore, the results of this study on *Vairimorpha plodiae,* an important pathogen of the lepidopteran pest *P. interpunctella* are of great importance to put forth

Table 3. Occurrence of *V. plodiae* in the different life stages of *P. interpunctella*

Life stage	Number of examined sample	Number of infected sample	Infection rate (%)
Larva (Living)	235	28	12
Larva (Dead)	1952	595	30.5
Pupa	37	14	37.8
Adult	820	226	27.6
Total	3044	863	28.3

that the microsporidian pathogen, *V. plodiae* is very common and widespread and naturally occurs in *P. interpunctella* populations with the high infections in all life stages larvae, pupae, and adults.

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