

Potential of entomopathogens in biological control of the box-tree moth, *Cydalima perspectalis* (Lepidoptera: Crambidae)

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Abstract

The box-tree moth pyralid, *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae) is the most important insect pest of box trees. Although *C. perspectalis* is an invasive species, studies on the isolation, characterization and insecticidal activity of entomopathogens that cause disease in the natural populations of box tree moth are scarce. There are few studies on the entomopathogenic organisms that cause diseases in the natural populations of *C. perspectalis*. *C. perspectalis* populations can be controlled with entomopathogens such as *Bacillus thuringiensis*, baculoviruses, fungi and nematodes. *Bacillus thuringiensis* and baculoviruses are the most promising pathogens against *C. perspectalis*. Investigations to find the most effective entomopathogen against *C. perspectalis* should be continued. This also supports the need to find its specific entomopathogens causing diseases in its natural populations. The present paper compares data on potential entomopathogens found in the literature by discussing the current situation of the damage, distribution of *C. perspectalis* and the effects of the entomopathogens isolated from or tested against this pest insect.

Keywords: biological control, entomopathogens, box tree, *Cydalima perspectalis*

Introduction

The box tree, *Buxus sempervirens* L., is an evergreen shrub or small tree native to Europe, northwest Africa and South Asia. Box tree is one of the most valuable woods in Europe as its wood is very hard, dense, heavy, fine-grained and resistant to splitting and chipping (Piqué et al. 2021). It is also widely spread as an ornamental plant in Europe and artificially grown for different purposes in industry. Box tree plants have been threatened with extinction in recent years. Among many pests of box trees, the box-tree moth, *Cydalima perspectalis* (Walker, 1859) (Lepidoptera, Crambidae), is the most important pest with the most harmful effects on the trees. This species has two forms, white and melanic. In white ones, the outer edges of their forewings are covered with broad brown bands and white spots. In melanic ones, the forewings are wholly brown, with white spots on them. The insect has four biological stages, egg, caterpillar (larva), pupa and moth (Toper-Kaygin and Taşdelir 2019). Females lay eggs in groups on the underside of the leaves. After the larvae hatch from the eggs, they spread to the plant and feed in groups. The natural distribution area of *C. perspectalis* is the humid subtropical regions in the east of Asia; India, China, Japan and

Korea (Mally and Nuss 2010). In the last two decades, this pest has been recorded in Europe. *C. perspectalis* was first detected in southwestern Germany in 2007 (Krüger 2008). The great destruction of *C. perspectalis* in Germany in 2007 showed that this pest had come to this region before. The main entry route to this country is through the infested *Buxus* species brought from China to Europe for ornamental plantations (Krüger 2008, Muus et al. 2009).

C. perspectalis spread rapidly in Europe (Kenis et al. 2013, Nacambo et al. 2013, Götting and Herz 2016): after Germany (Krüger 2008), it was recorded in Switzerland (Billen 2007) and the Netherlands (Muus et al. 2009), and then in France (Feldtrauer et al. 2009), Austria (Rennwald and Rodeland 2009), Liechtenstein (Slamka 2010), Italy (Biondi 2010), Belgium (Casteels et al. 2011), Denmark (Hobern 2013) and Spain (Pérez-Otero et al. 2014). On the other hand, it expanded its spread to the east of Europe: Romania (Iamandei 2010, Székely et al. 2011), Hungary (Sáfián and Horváth 2011), the Czech Republic (Šumpich 2011), Slovenia (Seljak 2012), Slovakia (Pastoralis et al. 2013), Croatia (Matošević 2013), Greece (Strachinis et al. 2015), Bulgaria (Arnaudov and Raikov 2017), Montenegro (Hrnčić and Radonjić 2014), Bosnia and Herzegovina

(Ostojić et al. 2015), Serbia (Stojanović et al. 2015), Kosovo (Geci and Ibrahim 2018), and finally Turkey (Hizal et al. 2012) and Georgia 2015 (Matsiakh et al. 2016).

For the first time *C. perspectalis* was recorded from İstanbul, Turkey, in 2011 (Hizal et al. 2012), and then from Düzce (Öztürk et al. 2016), Artvin (Göktürk 2017, Akıncı and Kurdoğlu 2018, 2019) and Bartın (Toper-Kaygın and Taşdeler 2019). Its spread continued rapidly in the Western Black Sea Region, and damages were recorded in Zonguldak, Karabük and Kastamonu (Toper-Kaygın and Taşdeler 2019). Recently, the pest has been reported in Hatay, the Mediterranean region of Turkey, whose distribution in this region has not been determined before (Ak et al. 2021). This recent record of the pest shows that it continues its distribution from North to South of Turkey. The data obtained in a recent study conducted in Artvin region related to the damage level of *C. perspectalis* in box tree forests confirms that *C. perspectalis* is the most dangerous defoliator of box tree stands in Turkey. Akıncı and Kurdoğlu (2019) found that most of the naturally growing box trees (63.4%) in Artvin region were damaged. The study observed that 25% of the naturally growing box trees that lost their leaves could not tolerate the loss. If the rapid spread of *C. perspectalis* continues and its population is not suppressed, the box tree habitats in Turkey will probably disappear completely (Ak et al. 2021). Turkey has extremely favourable conditions for the spread of *C. perspectalis*, especially since the average temperatures in the western and southern regions are above the current average and also the box trees, which are the hosts of the pest, are widely used in parks and gardens (Toper-Kaygın and Taşdeler 2019). It is predicted that *C. perspectalis* will rapidly increase its spread and damage in Turkish box tree forests and landscape areas (Toper-Kaygın and Taşdeler 2019). The recommended control methods against *C. perspectalis* in Europe are insecticides, biopesticides such as *Bacillus thuringiensis* (Bt), parasitoids and the removal of larvae manually or by spraying water on infested trees. However, although *B. thuringiensis* application is effective as a temporary solution to protect high-value natural stands of box trees, it cannot be applied in larger forests (Nacambo et al. 2013, Wan et al. 2014).

Currently, natural enemies of *C. perspectalis* are birds and polyphagous parasitoids, which show low predation and parasitism, possibly due to the high levels of toxic alkaloids retained by its larvae. Within the scope of chemical control, insecticides such as pyrethroids and neonicotinoids can control box tree moths (Korycinska and Eyre 2011). For et al. (2016) tested different chemical insecticides against *C. perspectalis* in Romania, including deltamethrin, lambda-cyhalothrin, thiamethoxam, thiacloprid, and imidacloprid + deltamethrin, but it was noteworthy that thiacloprid and lambda-cyhalothrin were found to be effective. Stan and Mitrea (2019) have tested thiacloprid, cypermethrin + chlorpyrifos, lamb-

da-cyhalothrin and tau-fluvalinate against *C. perspectalis* and achieved 98.70% mortality with these chemicals. After the application, it was observed that the damage of *C. perspectalis* decreased by 64% compared to the control group.

The damage of *C. perspectalis* on cultivated box trees can be prevented by chemical insecticides (Fora et al. 2016). However, their use is often undesirable and even limited in many places. Contrastly, chemical insecticides against *C. perspectalis* seem to continue in the near future. Applying chemical insecticides can cause serious adverse effects on organisms in the natural ecosystem. They seriously affect the environment, non-target arthropods, humans and other beneficial organisms (Desneux et al. 2007).

On the other hand, the targeted harmful insects can gain resistance to chemicals over time. Due to the wide application of broad-spectrum insecticides, *C. perspectalis* has already developed some resistance in China (Zhang et al. 2007). Therefore, new environmentally friendly pest control methods are of great need to protect natural *Buxus* forests. In recent years, alternative methods have been investigated against the use of chemicals in many countries (Li et al. 2004, Kawazu et al. 2007, Zimmermann and Wühler 2010, Bourdon et al. 2021, Oso et al. 2021). The development of novel biopesticides targeting this pest might help to protect box tree forests. The most remarkable and influential of these alternative methods is the use of entomopathogens. Entomopathogens are known as microorganisms infecting and killing insects.

Entomopathogens contribute to the natural regulation of insect populations (Sağlam et al. 2021). They naturally infect many insect species that are vectors of human diseases or pests of crops and forest trees. The insecticidal effect of entomopathogens to be used against pests varies between species and even between isolates of the same entomopathogenic species in different geographic regions. For this reason, scientists spend a great effort to find entomopathogens belonging to their biological richness within their geographic regions. Entomopathogens can spread spontaneously within the population of the pest by infecting them from individual to individual, or they can reach new generations of the pest (Yaman 2021). An entomopathogen in an insect that has died due to the disease within the current generation can reach individuals in the next generation differently and cause a disease and continue its existence in the population for years.

This study aims to review and discuss the possibility of using entomopathogenic organisms for the biological control of *C. perspectalis*. For this aim, several publications concerning entomopathogenic studies on *C. perspectalis* are discussed in detail, and entomopathogens from different groups, such as viruses, bacteria, fungi and nematodes, tested against or isolated from *C. perspectalis* are listed.

Materials and methods

A detailed literature review concerning *C. perspectalis* has been carried out. The literature review was conducted using Web of Science, Scopus and Google Academic, and the literature sources were grouped under three headings: damage and distribution of *C. perspectalis*, control methods, and entomopathogenic studies for its biological control. Entomopathogenic studies are discussed under four titles based on pathogen type. As a result of the literature review, an evaluation was made about the place of entomopathogens among the means of the biological control of *C. perspectalis*.

Viral entomopathogens

According to the literature, there is no specific virus record of *C. perspectalis* isolated so far from its natural populations. However, the fact that they infect mainly lepidopteran pests increases the possibility of having a baculovirus from *C. perspectalis* populations. *C. perspectalis* is invasive, especially in the last 15 years and has not attracted much attention in the previous years. In the last decade, only three entomopathogenic viruses originating from other insect hosts have been tested on this pest, and promising results were obtained (Table 1). In the first study, Rose et al. (2013) investigated the efficacy of *Anagrapha falcifera* nucleopolyhedrovirus (AnfaNPV), which was found to infect the larvae of pickleworm, *Diaphania nitidalis* (Lepidoptera: Rambidae), on *C. perspectalis* in order to find an effective control agent for biological control. Two AnfaNPV isolates termed Dn10 and BI-235 were tested against neonate *C. perspectalis* larvae in a seven-day bioassay experiment using the box tree leaf disks. At the end of the experiment, they determined median lethal concentrations (LC₅₀) as 7.8×10^5 and 2.3×10^6 OBs/ml for the isolates Dn10 and BI-235, respectively. As a result of this study, the isolate BI-235 of AnfaNPV was found as significantly virulent to neonate *C. perspectalis* larvae with high infec-

tion rates in a fat body, epidermis and tracheal matrix of the host.

In a second bioassay experimental study, Oberemok et al. (2017) found a significant effect of *Lymantria dispar* nucleopolyhedrovirus (LdMNPV) on the viability of *C. perspectalis* as a potential control agent for the pest. They also showed that the mortality of LdMNPV-infected box tree moth could be increased topically using antisense DNA fragments from the RING domain of the LdMNPV inhibitor of the apoptosis gene.

Gninenko et al. (2018) tested two foreign commercial viruses, *Neodiprion sertifer* nucleopolyhedrovirus (neovir) and *Lymantria dispar* nucleopolyhedrovirus (pinkvir), on *C. perspectalis* larvae under laboratory conditions. After 15 days of bioassay experiments, *Neodiprion sertifer* nuclear polyhedrosis virus (neovir) showed 94% larval mortality and *Lymantria dispar* nuclear polyhedrosis virus (pinkvir) 90% larval mortality. They offered that both foreign viruses can ensure high mortality rates on *C. perspectalis* caterpillars, which enables their applications in box tree protection.

Bacterial entomopathogens

The literature data shows a list containing the entomopathogenic bacteria of *C. perspectalis* populations in Table 1. Studies have been conducted mainly in the form of trials of commercial preparations or bacterial pathogens from different insect hosts. Li et al. (2004) tried the commercial preparation of *B. thuringiensis* var. *kurstaki* (Berliner 1915) against *C. perspectalis* in China, but they could not get a remarkable result. Burjanadze et al. (2019) tested the 1% concentrations of two commercial *B. thuringiensis* var. *kurstaki* preparations, Lepidocid CKM and DiPel® against L₂-L₅ instars larvae of *C. perspectalis* in the laboratory bioassay and field trial. After 12 days-experiment, they found 97.5% mortality with DiPel® and 84.5% mortality with Lepidocid CK-M in the laboratory bioassay and 60.6% mortality with Lepidocid CK-M, 88.6% mortality with DiPel® in a field trial. The results of this study indicate

Table 1. Entomopathogenic organisms found in or tested for *C. perspectalis*

	Entomopathogens	References
Viruses	<i>Anagrapha falcifera</i> nucleopolyhedrovirus (AnfaNPV) <i>Neodiprion sertifer</i> nucleopolyhedrosis (NsNPV) <i>Lymantria</i> nucleopolyhedrosis (pinkvir) <i>Lymantria dispar</i> nucleopolyhedrosis virus (LdMNPV)	Rose et al. 2013, Oberemok et al. 2017, Gninenko et al. 2018
Bacteria	<i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> <i>Bacillus thuringiensis</i> <i>Bacillus thuringiensis</i> subsp. <i>kenyae</i> <i>Bacillus cereus</i> , <i>Brevibacillus brevis</i> <i>Vibrio hollisae</i> <i>Bacillus</i> spp.	Li et al. 2004, Götting and Herz 2018, Harizanova et al. 2018, Burjanadze et al. 2019, Makarenko et al. 2019, Hulusjan et al. 2021, Tozlu et al. 2022, Usta 2022
Fungi	<i>Beauveria bassiana</i> <i>Isaria fumosorosea</i> <i>Metarhizium</i> spp. <i>Beauveria</i> spp.	Lee et al. 1997, Zamani et al. 2017, Harizanova et al. 2018, Burjanadze et al. 2019, Álvarez and Fernanda 2020, Zemek et al. 2020, Tozlu et al. 2022
Nematodes	<i>Steinernema carpocapsae</i> <i>Heterorhabditis bacteriophora</i>	Choo et al. 1991, Götting and Herz 2018

that infestation can be significantly reduced through two successive applications with these commercial *B. thuringiensis* var. *kurstaki* preparations. Makarenko et al. (2019) conducted a bioassay experiment against *C. perspectalis* using two bacterial pesticides, Lepidocide and Bitoxibacillin, combined with an insecticide, aktofit. They found that treating plants with biological preparations Lepidocide and Bitoxibacillin (100 ml/10 L of water) in combination with Aktofit is effective in protecting against *C. perspectalis*. These biological pesticides are recommended to protect against box tree moths in botanical gardens and arboreta.

Harizanova et al. (2018) investigated bacterial species composition and diversity in the gut of *C. perspectalis* caterpillars. They isolated five bacterial isolates and identified them at the species level as *Acinetobacter schindleri*, *Enterococcus casseliflavus*, *Klebsiella mobilis*, *Paenibacillus anaericanus* and *Paenibacillus popilliae*. As a result of the study, *P. popilliae* was the most prevalent pathogen (52.4%) as an agent affecting caterpillar survival in natural populations of pests. However, the insecticidal potential of these bacteria was not investigated on the pest. In another study using entomopathogens (Salioglu 2020), *Bacillus subtilis* and three types of bacteria obtained from different hosts, encoded as FD-1, FDP-8 and FD-63, were tested against *C. perspectalis* and mortality rates ranging from 60 to 85% were obtained. Recently, two bacterial studies on the biological control of *C. perspectalis* were carried out. In one of them, the insecticidal effects of *Bacillus thuringiensis* subsp. *kenyae*, *Bacillus cereus*, *Brevibacillus brevis* and *Vibrio hollisae* were determined on *C. perspectalis*. 100% mortality was achieved with *B. cereus*, *B. brevis* and *V. hollisae* on *C. perspectalis* larvae (Tozlu et al. 2022).

Bacterial entomopathogens in *C. perspectalis* populations were also monitored in some studies. *Bacillus* species were the most common pathogens. Harizanova et al. (2018) monitored bacterial entomopathogen flora in *C. perspectalis* populations and found an average contamination of 10.2% with *Bacillus anaericanus* and 52.4% with *Bacillus popilliae*. In a similar study, Hulujan et al. (2021) found that most larvae have been infected with *Bacillus* species. Moreover, *Bacillus thuringiensis* was isolated from *C. perspectalis* larvae in natural populations, and 85% mortality was obtained with this isolate in bioassay trials (Usta 2022).

Fungal entomopathogens

Fungal entomopathogens are important biological control agents and have been the subject of intensive research for over 100 years (Vega et al. 2012). There are commercially available fungal biopesticides in a safe alternative control against chemical use. Most insects are highly susceptible to fungi infections (Lovett and Leger 2017, Litwin et al. 2020, Baki et al. 2021). According to the literature, a list including the entomopathogenic fungi of *C. perspectalis* as agents for natural control of this pest is given in the Table.

Among the entomopathogenic fungi, *Beauveria bassiana* (Bals.-Criv.) Vuill. infects several species of insects and is used to control crop infestations by insects. Some studies tested this pathogen against *Cydalima perspectalis* (Álvarez and Fernanda 2020). Lee et al. (1997) found that *Cydalima (Diaphania) perspectalis* was not affected at 2.0×10^7 – 2.0×10^4 conidia/ml of *Beauveria bassiana*. Contrastly, Tozlu et al. (2022) tested *B. bassiana* on *C. perspectalis* larvae at the concentrations of 1×10^6 , 1×10^7 and 1×10^8 conidia/ml and found 100% mortality with 1×10^8 conidia/ml concentration under laboratory conditions.

In contrast to commercial preparations of viral and bacterial entomopathogens tested against *C. perspectalis*, two fungal entomopathogens were determined to cause natural infection in their natural populations. Zamani et al. (2017) observed *C. perspectalis* larvae infected with fungal mycelium in the natural populations. They isolated and identified that fungal pathogen as *Beauveria bassiana* species. *B. bassiana* is a common fungal entomopathogen isolated from several insect groups, especially Lepidoptera larvae in many countries. *B. bassiana* isolated from *C. perspectalis* in that study is the first report of the natural occurrence of a fungal pathogen on the box tree moth. They suggest that biocontrol of *C. perspectalis* by fungal pathogens can be used as an appropriate alternative or at least a supplement to chemical pesticides.

Harizanova et al. (2018) investigated microorganism species composition and diversity in the gut of *C. perspectalis* caterpillars. They isolated five fungal species and identified them at the genus level as *Metarhizium* sp., *Beauveria* sp., *Verticillium* sp., *Alternaria* sp. and *Mucor* sp., and found *Metarhizium* sp. and *Beauveria* sp. in high and similar prevalences of 29.6% and 28.6%, respectively. However, the insecticidal potential of these fungi was not tested on the pest. Similarly, Burjanadze et al. (2019) observed *C. perspectalis* adults infected with an entomopathogenic fungus identified as *B. bassiana* in the nature boxwood forest. This fungal pathogen was tested against L₂-L₅ instar larvae of *C. perspectalis* with the 1×10^8 concentration in laboratory and field experiments. They found 80% mortality in the laboratory bioassay and 60% in a field trial. According to the results, *C. perspectalis* infestation can be reduced through tree applications with *B. bassiana*. Zemek et al. (2020) tested the entomopathogenic fungus, *Isaria fumosorosea*, against *C. perspectalis* in laboratory experiments. For this, the last-instar larvae of the box tree moth were treated by the suspension of fungus conidia at concentrations ranging from 1×10^4 to 1×10^8 spores per 1 mL. They observed fungus infection mostly in pupae; however, the maximum mortality of 60% confirmed that *C. perspectalis* shows a very low susceptibility to *I. fumosorosea*. The most interesting results of the study were that several ungerminated fungal conidia were found on larval cuticles and that the hydroalcoholic extract of *B. sempervirens* leaves significantly inhibits the germination of *I. fu-*

mosorosea conidia and fungus growth. They suggested that the low virulence of the fungus might be due to the accumulation of host plant phytochemicals having antimicrobial activity in the larval cuticle of the pest.

Nematode entomopathogens

The literature data shows a list of the entomopathogenic nematodes for the natural control of *C. perspectalis* shown in Table 1.

Choo et al. (1991) tested *Steinernema carpocapsae* (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* identified from Korean forest soils (Rhabditida: Heterorhabditidae) against *C. perspectalis*. In the study, *C. perspectalis* larvae were exposed to *S. carpocapsae* and *H. bacteriophora*, and a high degree of mortality was observed in the larvae. Götting and Herz (2018) investigated the potential of Nemastar®, the commercial preparation of entomopathogenic nematode *Steinernema carpocapsae* on *C. perspectalis* larvae in both laboratory bioassays and field trials. *S. carpocapsae* was applied in different suspensions (10–200 EPN/100 µl, per larva) against the 2nd and 4th instar larvae, and high susceptibility was observed as 10–75% mortality for the 2nd instar larvae and 45–100% mortality for the 4th instar larvae. Contrastly, the desired result could not be obtained with the entomopathogenic nematode, *S. carpocapsae*, in field trials.

Conclusions

In biological control, pathogens such as bacteria, viruses, fungi and nematodes are very important. These pathogens generally do not harm beneficial insects and other non-target species, killing only pests (Rincón-Castro and Ibarra 2011). As a result of a detailed review of the articles on the use of entomopathogens in the biological control of *C. perspectalis*, it is seen that entomopathogenic studies that find more effective microbial control agents against *C. perspectalis* are very few compared to those of other pests. Furthermore, although *C. perspectalis* is an invasive species and causes severe damage to box tree forests, studies on the isolation, characterization and insecticidal activity of pathogens that cause disease in the natural populations of box tree moth are scarce. However, the following critical statements have been reached;

- The efficacy of only three baculoviruses has been investigated in the literature on *C. perspectalis* caterpillars. Baculoviruses have very narrow host ranges so that one virus infects one or a few host insect species, with one exception, *Autographa californica* multiple nucleopolyhedroviruses (AcMNPV) infecting many lepidopteran species. In contrast, no species-specific natural virus was recorded, and three foreign baculovirus preparations from different host species tested against *C. perspectalis* appear to affect its caterpillars significantly. This exciting situation makes us think that *C. perspectalis* populations can be controlled with
- viral entomopathogens such as baculoviruses. However, studies to find the most effective viral insecticide against the pest should continue investigating the effectiveness of different viral pathogens. This also supports the need to find the specific viral pathogens causing diseases in its natural populations.
- Studies on the use of entomopathogenic bacteria in the biological control of *C. perspectalis* have focused on determining the efficacy of commercial *Bacillus thuringiensis* var. *kurstaki* preparations on pest caterpillars. The effectiveness of *Bacillus thuringiensis* var. *kurstaki* preparations on the pest has been proven, and their use in field applications has been generally accepted. Use of *Bacillus thuringiensis* var. *kurstaki* preparations against the pest are recommended. However, there are cases when *Bacillus thuringiensis* var. *kurstaki* are insufficient. On the other hand, there is no study on the isolation, characterization and insecticidal activity of other bacterial entomopathogens (spore-forming or non-spore-forming bacteria) that cause disease in the natural populations of box tree moth. Only in one study gut flora of *C. perspectalis* was determined.
- By reference to the results of one study, it is expected that *C. perspectalis* can have a large range of fungal pathogens, including *Metarhizium* sp., *Beauveria* sp., *Verticillium* sp., *Alternaria* sp., *Mucor* sp. and *Isaria* sp. *C. perspectalis* infestation can be reduced through tree applications with *B. bassiana*. This pathogen can be a naturally suppressing factor in *C. perspectalis* populations even though considerably high mortalities have not been determined in experimental studies. Contrastly, *Isaria fumosorosea* is not very effective against *C. perspectalis*. Environmental factors such as ultraviolet light, temperature, and humidity can influence the effectiveness of fungal entomopathogens in the field. Furthermore, a very different view has been cited as the reason why fungal entomopathogens are less effective on *C. perspectalis*; low virulence of fungal entomopathogens might be due to the accumulation of host plant phytochemicals. This idea may be correct, as host plant phytochemicals can act as antimicrobials in the larval cuticle of the pest. Developing fungal entomopathogens as effective biological control agents requires knowledge of bioassay methods and production, formulation, and application methodologies. However, it is clear that the fungal pathogens of the pest still need to be studied in detail.
- The limited number of entomopathogenic nematode studies confirm that nematodes can exert a very adverse effect on *C. perspectalis*. *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* stand out as the most promising nematodes.
- Entomopathogens are mainly isolated from natural environments or diseased insects in natural pest populations. Genomic characteristics and different climatic and geographical factors can determine their insect-

ticidal potential on pest insects. This situation stimulates scientists to find entomopathogens in different geographical areas. On the other hand, there are a few studies on the entomopathogenic organisms that cause diseases in the natural populations of *C. perspectalis*. As a main result, studies to find the most effective entomopathogen against *C. perspectalis* should be continued. This also supports the need to find the specific entomopathogens causing diseases in its natural populations.

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