



Entomopathogens as biological control of the mediterranean flour moth, *Ephestia kuehniella* (Lepidoptera: Pyralidae)

Akdeniz un güvesi *Ephestia kuehniella* (Lepidoptera: Pyralidae)'nın biyolojik kontrolünde entomopatojenler

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Abstract

Mediterranean flour moth *Ephestia kuehniella* (Zeller, Lepidoptera: Pyralidae) is one of the most important insect pests that invade the stored grain of many grains around the world. Chemical control is a very preferred method in the fight of this pest. However, due to the economic, social and environmental damages of chemicals, the interest in biological control, which is an alternative method, is gradually increasing. Entomopathogens have very important in biological control and that cause desired infections in pests. Entomopathogens include many species such as viruses, bacteria, protists, fungi and nematodes. In recent years, entomopathogenic nematodes; *Steinernema feltiae*, *Steinernema carpocapsae*, *Steinernema riobrave*, *Heterorhabditis bacteriophora*, *Heterorhabditis* sp., *Steinernema* sp., and *Xenorhabdus nematophila*, entomopathogenic fungi; *Paecilomyces fumosoroseus* (= *Isaria fumosorosea*), *Beuveria bassiana* and *Metarhizium anisopliae*, entomopathogenic bacteria; *Bacillus thuringiensis* subsp. *kurstaki*, *Photorhabdus temperata* and *Bacillus subtilis*, entomopathogenic protists; *Vairimorpha ephestiae*, *Leidyana ephestiae*, *Mattesia dispersa* and *Mattesia oryzaephili*, and virus; Nuclear Polyhedrosis Virus (NPV) were reported from *E. kuehniella*. In this review, it is aimed to evaluate the recent status of these entomopathogenic organisms found or tested for *E. kuehniella*.

Özet

Akdeniz un güvesi *Ephestia kuehniella* (Zeller, Lepidoptera: Pyralidae), dünyada birçok depolanmış tahıl istila eden en önemli böcek zararlılarından biridir. Zararlıyla mücadelede kimyasal mücadele çok tercih edilen bir yöntemdir. Bununla birlikte, kimyasalların ekonomik, sosyal ve çevresel zararlarından dolayı, alternatif bir yöntem olan biyolojik kontrole olan ilgi giderek artmaktadır. Entomopatojenler biyolojik kontrolde çok önemlidir ve zararlılarda istenen enfeksiyonlara neden olurlar. Entomopatojenlerin virüsler, bakteriler, protistler, mantarlar ve nematodlar gibi birçok türü vardır. Son yıllarda, entomopatojenik nematodlar; *Steinernema feltiae*, *Steinernema carpocapsae*, *Steinernema riobrave*, *Heterorhabditis bacteriophora*, *Heterorhabditis* sp., *Steinernema* sp. ve *Xenorhabdus nematophila*, entomopatojenik mantarlar; *Paecilomyces fumosoroseus* (= *Isaria fumosorosea*), *Beuveria bassiana*, *Metarhizium anisopliae*, entomopatojenik bakteriler; *Bacillus thuringiensis* subsp. *kurstaki*, *Photorhabdus temperata*, *Bacillus thuringiensis* ve *Bacillus subtilis*, entomopatojenik protistler; *Vairimorpha ephestiae*, *Leidyana ephestiae*, *Mattesia dispersa* ve *Mattesia oryzaephili* ve son olarak Nükleer Polihedrozis Virüsü (NPV) *E. kuehniella*'da bulunmuş yada ona karşı test edilmiştir. Bu derlemede, *E. kuehniella* popülasyonlarında bulunan veya test edilen bu entomopatojenik organizmaların değerlendirilmesi amaçlanmıştır.

INTRODUCTION

Lepidoptera, known as stored product pests, feeds on foods like grain. They increase the temperature and humidity in the grain during feeding, and also contaminate the products with the remains of feces and body parts (Ramos-Rodríguez et al. 2006, Barbosa Negrisoni et al. 2013). The European flour moth (*Ephestia kuehniella* (Zeller)) is known as a common warehouse pest in mills, feed mills and food. The adults usually live 2-3 weeks. Females lay about 150-200 (or even more) eggs on crops. The larvae emerging from the eggs quickly feed and cause the damage. The pest raises 5-6 annual

generations. *E. kuehniella* larvae of this trust both cause direct damage through nutrition and their presence on food also reduces product quality (Jallouli et al. 2013). *E. kuehniella* is known as first degree in flour and second degree harmful in cereals (Paula Pereira et al. 2002). *E. kuehniella* larvae not only cause direct damage by feeding, but also decrease the product quality with their presence in the product and the net they weave (Jallouli et al. 2013).

Pest control methods are known as natural control, legal control, mechanical control, physical control, cultural control, biological control, chemical control and

integrated control. One of the most commonly used methods in Lepidopteran species (such as *E. kuehniella*) is fumigation. Fumigation is a chemical control method. But, chemicals have extremely serious negative effects on the environment and human health. The use of chemical compounds has negative effects on non-target organisms (Arikan and Turan 2020). Due to the side effects of chemicals, this control method should be limited. Therefore, in recent years, alternative methods have been investigated against the use of chemicals in many countries due to their negative effects on both human health and the ozone layer (Freitas et al. 2020). The most remarkable of these alternative methods is the method of struggle with the use of entomopathogens. The UK government had designed a project to control pests in stored foods. In this project, instead of organophosphate, pesticide and methyl bromide, use of entomopathogenic fungi was investigated. As a result of this project, it has been observed that some isolates obtained from *Beauveria bassiana* cause 100% death in some pest species within 7 days (Cox et al. 2002). Among the important hidden pests encountered in stored products, *E. kuehniella* is a very common pest (Stejskal et al. 2020). In this context, there are many publications and studies on entomopathogens detected from *E. kuehniella*. According to the available literature, entomopathogens

detected from *E. kuehniella* are viruses, bacteria, protists, fungi and nematodes.

Entomopathogens tested against or isolated from *E. kuehniella* for biological control

Viruses

Entomopathogenic viruses are significant agents of biological control due to features such as non-toxicity to vertebrates and eco-friendliness (Clem and Passarelli 2013). Among the viruses, baculoviruses have very important due to their entomopathogenicity (Hails 2001, Karabörklü et al. 2018). There are many baculovirus origin products that are commercially produced for this purpose (Clem and Passarelli 2013). According to literatures, a list including entomopathogenic viruses of *E. kuehniella* populations and are as agents for natural control of this is given in the Table 1.

Lynn et al. (2004) performed nucleopolyhedrovirus inoculation to *E. kuehniella* cultures. Most grafted *E. kuehniella* cultures have been found to be highly susceptible to nucleopolyhedrovirus. Researchers think that their results may be useful in biocontrol research.

Table 1. Entomopathogenic viruses found or tested for *E. kuehniella*.

Entomopathogen Group	Entomopathogen Species	References
Viruses	Nucleopolyhedrovirus (NPV)	Lynn and Ferkovich (2004), Yaman et al. (2015).

Yaman et al. (2015) isolated a Nucleopolyhedrovirus (NPV) from laboratory-supported larvae of *E. kuehniella*. Under the light microscope, a large number of polyhedral inclusion bodies (PIBs) were observed in hemolymph, trachea and midgut. In addition, some diamond shaped inclusion bodies, such as virion-free spindles, were also observed in this study. Researchers reported that Nucleopolyhedrovirus (NPV) was first time isolated and characterized from *E. kuehniella*.

Bacteria

Entomopathogenic bacteria cause mass death in insects. These organisms enter the host through digestion and produce toxins and other pathogenic factors that disrupt the midgut epithelium, thereby causing septicemia and death of the insect host. *Bacillus thuringiensis* (Bt) (Berliner) is common in soil, is a deadly pathogen of many pests and is the most widely used

entomopathogenic biological control agent (Jurat-Fuentes and Jackson 2012). There are currently more than 40 Bt products available for the control of insect pests, which make up 1% of the global pesticide market. Especially, these bacterial preparations are used against Lepidopteran species (Krieg et al. 1983, Jamoussi et al. 2009). According to this information and the literature data obtained, a list containing the entomopathogenic bacteria of *E. kuehniella* populations and agents for their natural control is given in Table 2.

Mostafa et al. (2005) tested *B. thuringiensis* var. *kurstaki* against *E. kuehniella* fifth instar larvae. In the study, it was observed survival time shortened in the larvae fed with bacterial preparation.

Mahbubur Rahman et al. (2007) investigated the effects of *B. thuringiensis*-toxin formulation against the defense systems of *E. kuehniella* larvae. In the study was observed

that melanization significantly reduced in hemolymphes of larvae infected with *B. thuringiensis*-toxin. Researchers say that hemolymph melanization is associated with a high immune status.

Jamoussi et al. (2009) tested *Photorhabdus temperata* (Fischer-Le Saux et al.) and *B. thuringiensis* bacteria against *E. kuehniella* larvae. In this study, it was studied the first time effectiveness of the *P. temperata* vip3LB gene. Result of the study, it has been determined that recombinant bacteria expressing the vip3LB gene reduce or stop larval growth. Also, this study showed that vip3LB gene may increase the insecticidal activities of *B. thuringiensis* and *P. temperata*.

Ghribi et al. (2012) determined *E. kuehniella* larvicidal power of biosurfactant secreted by *Bacillus subtilis* (Ehrenberg). In the study, the biosurfactant tested against third instar larvae of *E. kuehniella*. Researchers found that

biosurfactants can be resistant to environmental stresses such as extreme pH, temperature and sunlight / UV radiation. According to researchers, thanks to these features of the biosurfactant secreted by *B. subtilis*, it can be used against *E. kuehniella* larvae.

BenFarhat et al. (2013) investigated the effect of *B. thuringiensis* and *Xenorhabdus nematophila* (Poinar and Thomas) cells on the growth of *E. kuehniella* larvae. In the study, mixture of *X. nematophila* and *B. thuringiensis* had negative effects on the growth of *E. kuehniella* larvae.

Jallouli et al. (2013) conducted research on the insecticidal activity of the entomopathogenic bacterium *P. temperata* against *E. kuehniella*. Larvae fed on with *P. temperata* culture did not appear adult form. In the treatment with a high concentration of *P. temperata* culture, *E. kuehniella* larvae was mortality rate 100%.

Table 2. Entomopathogenic bacteria found or tested for *E. kuehniella*.

Entomopathogen Group	Entomopathogen Species	References
Bacteria	<i>Bacillus thuringiensis</i>	Mostafa et al. (2005), Mahbubur Rahman et al. (2007), Jamoussi et al. (2009)
	<i>Bacillus subtilis</i>	Ghribi et al. (2012), BenFarhat et al. (2013)
	<i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i>	BenFarhat et al. (2013)
	<i>Xenorhabdus nematophila</i>	BenFarhat et al. (2013)
	<i>Photorhabdus temperata</i>	Jallouli et al. (2013)

Protists

Several groups of protists cause disease in insects and are of interest as agents for natural control of insect pests (Yaman 2020). These organisms are known as entomopathogenic protists. The most important entomopathogenic species belong to Microspora (Microsporidians), Sarcomastigophora (Flagellates and Rhizopods) and Apicomplexa (Gregarines and Coccidian). Microsporidian pathogens often cause the host (insect) to die (Yaman et al. 2019). Sometimes it reduces the nutritional abilities of the host by restricting its vital activities (Andreadis 1985, Didier 2005, Yaman 2010). For example, gregarine infection reduces the resistance of insects, negatively affects their development and reproduction. Thus, insects that are not resistant to adverse environmental conditions die more easily than healthy insects (Dales 1994, Lipa and Triggiani 1992, Valigurová and Koudela 2006). According to this information and the literature data obtained, a list containing the entomopathogenic protist (microsporidia and gregarine) of *E. kuehniella* populations and agents for their natural control is given in Table 3.

Weiser and Purrini (1985) documented the ultrastructure of the *Vairimorpha ephestiae* (Mattes) (Protozoa, Microsporidia) from *E. kuehniella* larvae. According to researchers, development of *V. ephestiae* are two types of sporogony; one ending with single binucleate spores, and a second with spores in octosporous pansporoblasts. These spores are uninucleate. Elongate oval spores of two sizes differ in number of nuclei. They have a long polar filament deposited in 14 coils in one layer beneath the spore wall.

Lord (2003) tested the mortality doses of *Mattesia oryzaephili* and *Mattesia dispora* against *E. kuehniella*. Doses of *M. oryzaephili* and *M. dispora* were given as a diet for *E. kuehniella*. At the end of the study, they determined the median lethal doses as 7.9×10^7 oocysts/g of diet for *M. oryzaephili* and 2.7×10^3 *M. dispora* on *E. kuehniella*.

Valigurová and Koudela (2005) described the structure of the *Leidyana ephestiae* from *E. kuehniella* larvae. The intestines of young and mature trophozoites of experimentally infected *E. kuehniella* larvae were examined by electron microscopy. Young trophozoites

were small, oval to ovoid, and possessed a simple, globular epimerite. Mature trophozoites have shown a large oval epimerite with many mitochondria and vesicles.

Valigurová and Koudela (2006) described the structure of the *M. dispersa*, from *E. kuehniella* larvae.

Table 3. Entomopathogenic protists found or tested for *E. kuehniella*.

Entomopathogen Group	Entomopathogen Species	References
Microsporidia	<i>Vairimorpha ephestiae</i>	Weiser and Purrini (1985)
	<i>Mattesia dispersa</i>	Lord (2003), Valigurová and Koudela (2006), Yaman et al. (2019)
Gregarines	<i>Mattesia oryzaephili</i>	Lord (2003)
	<i>Leidyana ephestiae</i>	Valigurová and Koudela (2005)

Fungi

Entomopathogenic fungi (EPF) form spore resistant to adverse environmental conditions, also have saprophytic life cycles. Entomopathogenic fungi do not have a toxic effect on mammals (Vega et al. 2012). They do not form resistant to insects. It infects the different stages of the host and the environment for a long time. With these features, they have an important place in biological control (Erkiliç and Uygun 1993). For this reason, there are many commercially available fungal preparations (Vega et al. 2012). And these preparations are successfully used to combat pests. However, it has disadvantages such as slow killing, high humidity, inability to be used with fungicides, expensive production and storage difficulty (Goettel et al. 2005, Sevim et al. 2015). According to literatures, a list including the entomopathogenic fungi of *E. kuehniella* as agents for natural control of this pest is given in the Table 4.

Willey et al. (2002) conducted a study on the use of entomopathogenic fungi for pest control in stored products. In the laboratory, researchers tested fungal samples against insects, moths (*E. kuehniella*) and mites. They found that *Beauveria bassiana* strains performed better than other entomopathogenic fungi against *E. kuehniella*. Researchers stated that *B. bassiana* may be an alternative biocontrol agent to pesticides.

Michalaki et al. (2007) investigated the insecticidal effect of entomopathogenic fungus *Paecilomyces fumosoroseus*. This EPF *Paecilomyces fumosoroseus*, was applied to the wheat in the storages in doses. As a result, researchers found that the mortality of *E. kuehniella* larvae did not exceed 56%.

Yaman et al. (2019) identified a neogregarine pathogen, *Mattesia dispersa* from *E. kuehniella* from Turkey for the first time. In addition, they also documented the occurrence of the pathogen in different life stages of *E. kuehniella*. The pathogen was determined cause different infection levels in the larvae, pupae and adults of *E. kuehniella*, 57.06, 85 and 3.17% respectively.

Abdalla et al. (2012) studied natural entomopathogenic agents against *E. kuehniella*. As a result of study, *E. kuehniella* larvae was found to sensitive to *B. bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii*.

Mahmoud Sabbour et al. (2012) tested *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea* against *E. kuehniella*. As a result of the study, it was observed that *E. kuehniella* was very sensitive to *M. anisopliae* and *B. bassiana*. In addition, *B. bassiana* has been found to be the most effective fungus species against *E. kuehniella*.

Faraji et al. (2013) tested entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* against the third instar larvae of *E. kuehniella*. The results showed that entomopathogenic fungi can be used as an alternative method to pest control in Insect Pest Management (IPM) programs.

Rahimi et al. (2013) tested the mixtures of pyriproxyfen and hexaflumuron, known as insect growth regulators with *B. bassiana* against *E. kuehniella*. The results of the study showed pyriproxyfen and hexaflumuron are selective and safe for non-target organisms. In addition, it has been found combination of pyriproxyfen and hexaflumuron with *B. bassiana* may be more effective against *E. kuehniella*.

Sabbour (2013) tested the different concentrations of *M. anisopliae* toxin, destruxin and nano-destruxin against *E. kuehniella* under laboratory and store conditions. Different concentrations of fungi toxin, destruxin and nano-destruxin were applied in the study. As a result of the study, it was determined the number of eggs decrease of *E. kuehniella*.

Shakarami et al. (2015) tested entomopathogenic fungus *B. bassiana* and three different botanical compounds against the third instar larvae of *E. kuehniella*. They conducted experiments under suitable laboratory conditions and suitable photoperiod. Results showed that all botanical compounds and entomopathogenic fungi exhibited larvicidal activity against *E. kuehniella*. In addition, it showed that *B. bassiana* and essential oil mixture had a synergistic effect on the mortality of *E. kuehniella* larvae.

Jarrahi and Safavi (2016) treated *E. kuehniella* larvae with *M. anisopliae*. Then, researchers examined the behavior performance in *Habrobracon hebetor*. In that study, they determined that it is appropriate to use *M. anisopliae* and *H. hebetor* together for biological control of *E. kuehniella*.

Athanassiou et al. (2017) tested the insecticidal activity of *M. anisopliae* against *E. kuehniella* larvae. In the study, the death of *E. kuehniella* larvae was observed between 41.1% and 93.3%. In addition, increase in temperature was found to be an important factor for *M. anisopliae* performance.

Seyedtalebi et al. (2017) tested five different *B. bassiana* isolates against *E. kuehniella*. Also, they evaluated the defense reactions of *E. kuehniella* against *B. bassiana*. In the results of working, researchers observed *E. kuehniella* was moderately sensitive to *B. bassiana*. The research provides an interesting perspective on the susceptibility of pests to fungal infections.

Table 4. Entomopathogenic fungi found or tested for *E. kuehniella*.

Entomopathogen Group	Entomopathogen Species	References
Fungi	<i>Beuveria bassiana</i>	Willey et al. (2002), Abdalla et al. (2012), Mahmoud Sabbour et al. (2012), Faraji et al. (2013), Rahimi et al. (2013), Shakarami et al. (2015), Seyedtalebi et al. (2017)
	<i>Metarhizium anisopliae</i>	Abdalla et al. (2012), Mahmoud Sabbour et al. (2012), Faraji et al. (2013), Sabbour (2013), Jarrahi and Safavi (2016), Athanassiou et al. (2017)
	<i>Paecilomyces fumosoroseus</i> (= <i>Isaria fumosorosea</i>)	Michalaki et al. (2007), Mahmoud Sabbour et al. (2012)
	<i>Verticillium lecanii</i>	Abdalla et al. (2012)

Nematode

Some entomopathogenic nematodes in the family Steinernematidae and Heterorhabditidae are used as effective biocontrol agents against many pests. These nematode types can also play an important role in Insect Pest Management (IPM) (Wouts 1991, Tulek et al. 2015). Because, they are considered non-toxic to humans, relatively specific to their target pests, and can be applied with standard pesticide equipment (Shapiro-Ilan et al. 2006). According to this information and the literature data obtained, a list containing the entomopathogenic nematodes of *E. kuehniella* and agents for its natural control is given in Table 5.

Ramos-Rodríguez et al. (2006) investigated the pathogenic effects of some species of the genus *Steinernema* against stored pests. Entomopathogenic nematodes *Steinernema riobrave* (Rhabditida: Steinernematidae), *S. carpocapsae* (Weiser) (Nematoda: Steinernematidae) and *S. feltiae* (Filipema) (Nematoda: Steinernematidae) were tested against different pest. As a result of the tests, it was found that *S. riobrave* has the best pathogenicity against *E. kuehniella*. According to the

results of the study, they reported that if tested more *S. riobrave*, the would be more reliable.

Athanassiou et al. (2008) tested the mortality effect of different strains of *Steinernema feltiae* against *E. kuehniella* larvae. Researchers have implemented *S. feltiae* doses against *E. kuehniella* larvae in different doses. As a result of the study, it was determined *S. feltiae* doses cause mortality between 52% and 100 %. Therefore, the researchers stated that *S. feltiae* could be a promising biological control agent against *E. kuehniella*.

Athanassiou et al. (2010) tested *Heterorhabditis bacteriophora* Poinar (Nematoda: Heterorhabditidae), *S. carpocapsae* and *S. feltiae* against *E. kuehniella* larvae. They implemented these nematodes to *E. kuehniella* larvae in different doses. *S. feltiae* showed a mortality rates between 36.7% and 78.3% on *E. kuehniella* larvae. No death was observed in *S. carpocapsae* application. In the application of *H. bacteriophora*, very little mortality was observed. As a result of the study, it was determined the effectiveness levels of entomopathogenic nematodes increased depending on the dose.

Barbosa Negrisoni et al. (2013) tested some entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae against *E. kuehniella*. They found that most species and/or strains of entomopathogenic nematodes are hypersensitive to *E. kuehniella*. According to the researchers, entomopathogenic nematodes are shown as potential control agents for stored product pests.

Basheer et al. (2014) investigated the potential use of some entomopathogenic nematode isolates against *E. kuehniella* larvae. In this study, four nematode isolates were used. These isolates include *Heterorhabditis* sp. and *Steinernema* sp. species. Study results showed that *E. kuehniella* is very sensitive to nematode infections.

Table 5. Entomopathogenic nematodes found or tested for *E. kuehniella*.

Entomopathogen Group	Entomopathogen Species	References
Nematoda	<i>Steinernema</i> sp.	Barbosa Negrisoni et al. (2013), Basheer et al. (2014)
	<i>Heterorhabditis</i> sp.	Barbosa Negrisoni et al. (2013), Basheer et al. (2014)
	<i>Steinernema feltiae</i>	Ramos-Rodríguez et al. (2006), Athanassiou et al. (2008)
	<i>Steinernema carpocapsae</i>	Ramos-Rodríguez et al. (2006), Athanassiou et al. (2010)
	<i>Steinernema riobrave</i>	Ramos-Rodríguez et al. (2006)
	<i>Heterorhabditis bacteriophora</i>	Athanassiou et al. (2010)

CONCLUSION

The Mediterranean flour moth, *Ephestia kuehniella* (Zeller) (Lep., Pyralidae) is a major economic insect pest of stored products and cereals. Stored agricultural products and foods have an important place in nutrition and economy. The protection of these products is very important for manufacturers, consumers and exporters. Nevertheless, chemical methods have been used to protect these products. Chemical methods applied to both environmental and human health. There are serious adverse effects on. Therefore, biological control or microbial control are a good alternative to chemical control as it does not harm living organisms other than the target organism and is sensitive to the environment. For this purpose, microbial insecticides have significant potential to control insect pests. Many baculovirus origin products produced for this purpose and commercially available are introduced to the market (Clem and Passarelli 2013). In addition, today, there are many commercial preparations containing entomopathogenic fungi and the preparations are used in the fight against Lepidopteran pests (Goettel et al. 2005). Some species such as *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea* (= *Paecilomyces fumosoroseus*) are the most common commercially used in many countries to combat many pests (Rath 2000). Many commercial products and preparations containing *M. anisopliae* are already on the market or are under development (de Faria and Wraight 2007). Although there are many commercial preparations, they are not very useful in the control of storage pests since the storage conditions of fungi are not compatible with the applicable climatic

conditions (Doberski 1981). Also, *B. thuringiensis* and *B. thuringiensis* subsp. *kurstaki* are bacteria containing commercial preparations that are widely used against Lepidopteran. In addition, entomopathogenic nematodes (genera *Steinernema* and *Heterorhabditis*) are effective biological control agents used to control *E. kuehniella* (Shapiro-Ilan et al. 2002, 2009). Among the entomopathogens, *Bacillus thuringiensis* var. *kurstaki* and baculovirus specific to the pest seem to be most popular against *E. kuehniella* larvae. There is a new interest in using entomopathogens for biological control of *E. kuehniella* as well as other stored product pests. Detection of different natural pathogens and parasites of *E. kuehniella* and controlling the population will be most successful in combating with the pest. In this paper, a review of entomopathogenic organisms infecting *E. kuehniella* is presented to stimulate scientist to find possible biological control strategies to control *E. kuehniella* populations.

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