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Review article (Derleme makale)

The usage possibilities of entomopathogenic fungi in the control of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)

Entomopatojen fungusların Batı çiçek thripsi, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) ile mücadelede kullanım olanakları

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Abstract

The western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), one of the main pests of agricultural areas, causing occurrence of significant economic losses if no control measure is taken. In recent years, the use of entomopathogenic fungi (EPF), an alternative method of control, has come into the prominence. EPF have a different place among insect pathogens in terms of direct infection of hosts through the integument. The most common fungal pathogens of *F. occidentalis* are *Verticillium lecanii*, *Beauveria bassiana*, *Entomophtholares* spp. and *Metarhizium anisopliae*. In Turkey, only *V. lecanii* and *B. bassiana* are commercially licensed against *F. occidentalis*. In this review, the usage possibilities of these two fungi, as well as the use of other species in controlling the pest will be discussed.

Keywords: *Frankliniella occidentalis*, entomopathogenic fungus, control, Turkey

Öz

Tarım alanlarının ana zararlılardan biri olan Batı çiçek thripsi, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) mücadele edilmediği takdirde önemli ekonomik kayıplara neden olmaktadır. Bu zararlı ile mücadalede son yıllarda alternatif bir mücadele yöntemi olan entomopatojen fungusların kullanımı ön plana çıkmıştır. Entomopatojen funguslar, konukçularını integümentten doğrudan geçerek enfekte etmesi bakımından böcek patojenleri arasında farklı bir yere sahiptirler. *F. occidentalis*'in en yaygın olarak görülen fungal patojenleri; *Verticillium lecanii*, *Beauveria bassiana*, *Entomophtholares* spp. ve *Metarhizium anisopliae*'dır. Ülkemizde sadece *V. lecanii* ve *B. bassiana F. occidentalis*'e ticari olarak ruhsatlıdır. Bu derlemede, gerek bu iki fungus gerekse diğer türlerin zararlı ile mücadelede kullanım olanakları tartışılacaktır.

Anahtar sözcükler: *Frankliniella occidentalis*, entomopatojen fungus, mücadele, Türkiye

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Introduction

Many factors, both abiotic and biotic, affects crop production. One of the most important biotic factors is *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) that is a polyphag species and attacks to many vegetable and ornamental plants both indoor and outdoor conditions worldwide. The pest is native to the Southwestern United States, but has spread to other parts of the world, including Europe, Australia (Kirk & Terry, 2003). It was the first time detected in Turkey (Gazipaşa, Antalya) on greenhouse grown cut-flowers and vegetables in 1993 (Tunç & Göçmen, 1994), and then spread rapidly and soon became one of the major pests of greenhouse crops leading to serious economic losses in the Mediterranean and Aegean Regions of Turkey. This insect damages the plant in several ways; (1) The major damage is caused by the adult ovipositing in the plant tissue, (2) The plant is also injured by feeding, which leaves holes and areas of silvery discoloration when the plant reacts to the insect's saliva, (3) larvae (first and second instars) feed heavily upon foliage and new fruit just beginning to develop from the flower, and (4) Perhaps, the most important damage by the pest, it serves as a vector for some important plant viruses, such as Tomato Spotted Wilt Virus (TSWV), which results in a significant damage in tobacco, pepper and tomato plants (Sakimura, 1962; Pappu et al., 2000). It is also the vector for Impatiens Necrotic Spot Virus (INSV) that is an economically important pathogen in a broad host range of ornamental plants (DeAngelis et al., 1993; DeAngelis et al., 1994; Goldbach & Peters, 1994; Sakurai et al., 2004). The most effective way to protect plants from these viruses is to prevent their contamination to plants by controlling the pest (Robb & Parrella, 1995). However, the control of this pest is very difficult because of being its eggs in plant tissue, pre-pupal and pupal stages in the soil or plant litter on the ground, as well as nymphs and adults inside the flowers or flower buds. This greatly reduces the chance of success with this species (Robb & Parella, 1995). It also has the potential to develop extremely fast resistance to synthetic neonicotinoid insecticides (Minakuchi et al., 2013).

In Antalya, the control of this pest relies on repetitive applications of synthetic insecticides with higher doses than their recommended label rates, but the desired results can not be obtained. The excessive use of synthetic insecticides is accompanied by high level of insect resistance along with the residual problem on crops. Resistance to most available chemical insecticides has been observed (Dağlı & Tunç, 2007; Gao et al., 2012). Due to all these problems and the damage potential of this pest to crop plants, the microbial control of this thrips by the microorganisms, which can be an alternative control method for the pest, is on the agenda.

Microbial control is defined as the process of removing the harmful effect of an organism causing damage in agriculture and forestry using microorganisms or microorganism's products (Demirbağ et al., 2008). Microbial control is made with pathogenic microorganisms that can infect insects. Entomopathogenic fungi (EPF) have an important place in this control method because they can infect more insect orders than other microorganisms (Deacon, 1983; Demirbağ et al., 2008).

In recent years, some studies have been conducted to determine the efficacy of EPF in controlling various thrips species including *F. occidentalis* (Ansari et al., 2008; Dura et al., 2012; Uçak et al., 2014; Mousavi et al., 2017). In a study by Shiberu et al. (2013), two indigenous isolates of EPF *Beauveria bassiana* (Balsamo) Vuillemin (PPRC-56) and *Metarhizium anisopliae* (Metschn.) Sorokin (PPRC-6) (Deuteromycota: Hyphomycetes) were evaluated for controlling onion thrips (*Thrips tabaci* L.) under field condition in Guder, Toke Kutaye district of Ethiopia. Both fungi achieved >75% mortalities at the 7^{th} day of application. In another study by Arthurs et al. (2013), commercial strains of *B. bassiana* (GHA), *M. brunneum* (F52) and *Isaria fumosorosea* Wize (Hypocreales: Cordycipitaceae) (Apopka 97) were tested for control of chilli thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae), an invasive pest of ornamental and vegetable plants in the Caribbean and southeastern United States. In laboratory assays, all three EPF were effective against the pest, and *M. brunneum* F52 reduced populations by 84-93%, *B.*

bassiana GHA by 81-94% and *I. fumosorosea* PFR-97 by 62-66%. More recently, Kivett et al. (2016) evaluated three commercial preparations of EPF, *B. bassiana* [BotaniGard®], *I. fumosoroseus* [NoFly™], and *M. anisopliae* [Met52®]) against the larvae and adults of *F. occidentalis* at two label rates (maximum and minimum) in the laboratory. Results indicated that (a) by 120 h of incubation, adult western flower thrips were more susceptible than larvae to maximum rates of unexpired *B. bassiana* and *I. fumosoroseus*; (b) unexpired products of *B. bassiana* and *I. fumosoroseus* had higher percentages of adult western flower thrips mortality than expired products at the maximum label rate. When all these studies are examined, it can be concluded that EPF may be a good alternative for the control of thrips. Moreover, this method has advantages such as being environment-friendly and not creating the problem of resistance.

Entomopathogenic Fungi and Their General Features

Entomopogenic fungi are the most common disease-causing organisms in insects (especially insect orders: Lepidoptera, Hemiptera, Hymenoptera, Coleoptera and Diptera). Apart from a few taxa, fungi have a different place among insect pathogens in terms of infecting hosts directly through the integument. For this reason, they are a microbial agent group that has come to the forefront in the microbial control of insects having piercing/sucking mouth parts, where the pathogens that need to be taken orally are ineffective.

Beauveria bassiana, *Metarhizium anisopliae* and *Verticillium lecanii* (Zimm.) (Deuteromycota: Hyphomycetes) are fungi species spread all over the world and widely used for biocontrol of insect pests such as thrips, aphids, whiteflies and weevils in greenhouse crops (de Faria & Wraight, 2007; Khan et al., 2012; Skinner et al., 2012). However, some fungus species have special host types. For example, *Hirsutella thompsonii* Fisher lives together with mites (Acarina), as well as aquatic fungi *Coelomomyces* spp. and *Culicinomyces* spp. with mosquitoes (Diptera: Culicidae). In some cases, a single fungal genus (for example, *Entomophthora*) has both narrow and broad host distribution. Within about 150 species of *Entomophthora*, *E. sphaerosperma* Fresenius has a relatively broad host range; *E. aphidis* Hoffman and some other species are specific to aphids whereas *E. acaricida* (Petch) is mite-specific (Demirbağ et al., 2008). Currently, there are eight commercial products based on five EPF registered in the European Union (EU) (Table 1).

Table 1. Registered entomopathogenic fungi for greenhouse crops in Europe (Gwynn, 2014)

Species	Isolate/Strain	Commercial name	Target pest
Beauveria bassiana (Balsamo) Vuillemin	ATCC 74040, GHA	Naturalis, Botanigard	Primarily whiteflies and thrips
Isaria fumosorosea Wize (formerly *Paecilomyces fumosoroseus* Wize)	Apopka 97, FE9901	PreFeRal Nofly	Primarily whiteflies
Lecanicillium muscarium Petch (formerly *Verticillium lecanii* Zimmerman)	Ve-6	Mycotal	Primarily whiteflies
Metarhizium brunneum Petch (formerly *Metarhizium anisopliae* Metschnikoff)	Bipesco 5, F52	Taerain Met52EC	Primarily whiteflies and thrips
Purpureocillium lilacinus (Thom) Samson (formerly *Paecilomyces lilacinus*)	251	BioAct	Root-knot nematodes

All these EPF have been reported to be effective, when sprayed in suspension, against thrips, aphids, whiteflies and weevils in greenhouse crops (de Faria & Wraight, 2007; Dura et al., 2012; Khan et al., 2012; Skinner et al., 2012).

A survey of literature indicates that EPF have some general features (Deacon, 1983; Demirbag et al., 2008; Kılıç & Yıldırım, 2008). These are;

(1) Generally, they can grow in mycological media such as potato dextrose agar or malt extract agar without the need for extra nutrients. However, *Entomophthora* spp. are exempted because they need a feed medium containing an animal material.

(2) EPF generally have an optimum growth temperature of 20-25°C and can not develop at 37°C or higher temperatures. As a result, except for some allergenic fungi, they do not show any serious problems affecting humans and other warm-blooded mammals.

(3) They develop by prolonging typical hyphae in solid substrates. In immature cultures, however, a few of them develop in the form of yeast-like budding cells called blastospores.

(4) EPF produce asexual spores on host or petri dishes under humid conditions and become an infection source in nature. These are basically different from bacterial endospores. Because fungal spores are produced in large quantities, they can be easily distributed by factors such as wind and rain.

(5) One of the most important features of EPF is that they have stable forms and saprophytic properties in adverse environmental conditions. For this reason, they can be isolated from soil and organic residues and their chances of being used in biological control are increasing.

The most important features of EPF used in the control of insect pests are the toxins produced by them. These toxic substances can cause different interactions on the host by suppressing the insect's immune system in a short time. The toxins and general activities of some toxin-producer fungi are given in Table 2.

Infection Mechanism of Entomopathogenic Fungi

The fungus spores that contact and stick to the insect body germinate and emit hyphae. They enter the cuticle, spread through the hemocoel and cause death slowly, with rapid proliferation of toxins, or proliferation of intense hyphae, disrupting the bodily functions of the host. The fungus then form spores, and spread them that will infect other insects, and thus fungal diseases can spread among insect populations. Sporulation, germination and infection usually require moist conditions. Figure 1 summarizes the development of fungus on the insect.

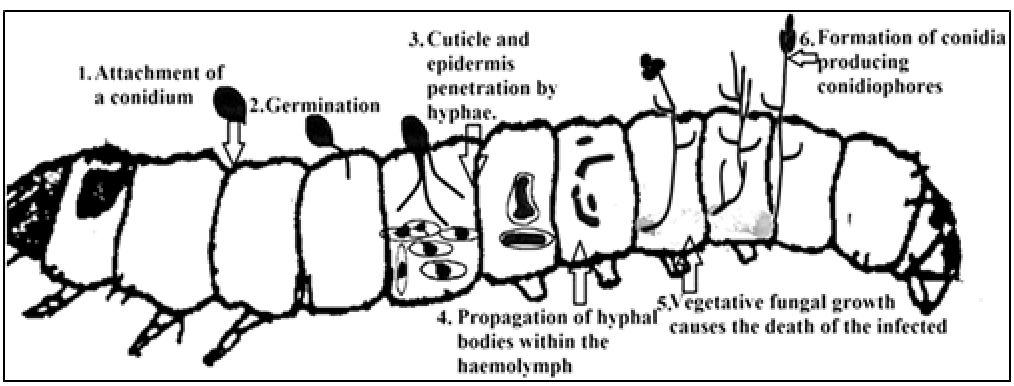

Figure 1. Mode of action of entomopathogenic fungi on insect larvae (Senthil-Nathan, 2015).

Table 2. Some toxins produced by entomopathogenic fungi (Boucias & Pendland, 1998)

Toxins	Toxin-producer fungus	General infection modes
Azoksibensonoid	*Entomophthora virulenta*	Various toxic effects
Sordisepin	*Cordyceps* spp.	Blocks RNA synthesis
Beauverisin	*Beauveria bassiana* *Isaria* sp. *Fusarium* sp.	Ionophor is soluble in lipid layers and increases membrane permeability for specific ions. In this way, it damages the cell organelles and their functions.
Bassionolid	*Beauveria bassiana*	Ionophore
Siklosporin A	*Beauveria bassiana* *Tolypocladium* sp. *Verticillum* sp. *Fusarium* sp.	Blocks a step in Ca ++ dependent signal transduction in vertabrate T cells. This causes immunosuppression. It can also suppress insect defense cells.
Oosporin	*Beauveria bassiana*	Red pigment, antimicrobial
Oksalik asit	*Beauveria brongniartii*	″
Destruksin	*Metarhizum anisapliae* *Aspergillus ochraceus*	It can affect Ca ++ channels in muscle membranes, immunosuppression and cytopathic.
Sitokhalasin	*Metarhizum anisapliae*	Blocks the elongation of the actin filament.
Swainsonin	*Metarhizum anisapliae*	Indolizidine alkaloid
Hirsutellin A	*Hirsutella thompsonii*	The ribosomal inhibitory protein (RIP) causes a specific cleavage of the rRNA and inhibits protein synthesis.
Efrapeptin	*Tolypacladium inflatum*	It inhibits mitochondrial ATPase activity.
Aflotoksin	*Aspergillus* spp.	It inhibits the reproductive mechanism in insects, carcinogenic in vertebrates.
Kojik asit	*Aspergillus flavus*	Antimicrobial
Restriktoksin	*Aspergillus* spp.	RIP-type toxin

Entomopathogenic Fungi Used in the Control of *Frankliniella occidentalis*

Entomopathogenic fungi are found in sub-divisions of Mastigomycotina, Zygomycotina, Ascomycotina and Deuteromycotina belonging to Eumycota division. The two most important groups of them are Entomophtholares (Zygomycotina) and Moniales (Deuteromycotina) (Yaman, 2012). Until today, 700 EPF belonging to a minimum of 90 genera have been identified. Among them, species such as *B. bassiana*, *M. anisopliae*, *I. fumosorosea* and *V. lecanii* are commercially produced and used in many countries for control of a large number of pests, including *F. occidentalis* (Rath, 2000).

Verticillum lecanii

Verticillum lecanii has been reported to be very effective on some Lepidoptera, Hymenoptera and Diptera species along with thrips, aphids, coccids and mites (Alavo et al., 2001). Soil moisture affects the permanence of *V. lecanii*, and it is the question of the loss of the viability of conidies in the soil when moisture is not at an adequate level (Storey & Gardner, 1988).

Verticillum lecanii, commercially available in Europe, has been used for many years in controlling thrips and other greenhouse pests (Ravensberg et al., 1990; Helyer et al., 1992). In Turkey, Uçak et al. (2014) investigated the effect of some biopesticides including *V. lecanii* on *F. occidentalis* by using dry film technique and leaf dipping technique in the laboratory. Their results indicated that *V. lecanii* could suppress *F. occidentalis* effectively.

Neozygites parvispora **(D.M. MacLeod & K.P. Carl) Remaud. & S. Keller**

This fungus, belonging to Entomophtholeres, is an obligate pathogen of thrips and widely seen in open areas. However, it is rarely encountered in greenhouse conditions. Vacante et al. (1994) reported that *N. parvispora* made up an epizootic in a population of *F. occidentalis* in a pepper crop in a plastic greenhouse in south-eastern Sicily (Italy). They monitored weekly the progress of the epizootic in the thrips population from December 1990 to April 1991 and indicated that infections of *N. parvispora* caused up to more than 60% mortality of the mobile developmental stages (adults, 1^{st}- and 2^{nd}-instar larvae) of the pest, reducing consistently both the insect population density and the proportion of flowers and leaves infested by thrips. They also reported that all mobile developmental stages of the thrips were susceptible to the infections of *N. parvispora*, and the incidence of mycosis in each developmental stage was positively correlated with its respective proportion in the thrips population. Montserrat et al. (1998) monitored weekly a greenhouse cucumber crop from May to August 1996 for thrips, *F. occidentalis*, and their natural enemies. They encountered an epizootic in the thrips population due to *N. parvispora* lasted from the seventh week after transplanting until the crop was terminated, reaching a mean of 6.46 infected thrips per leaf. They also reported that several species of generalist predators (Hemiptera: Miridae, and Anthocoridae) colonized the crop, and controlled the thrips population together with *N. parvispora*.

Entomophthora thripidium **(Wilding)**

This species can be found in greenhouse conditions and is effective in controlling thrips populations. It is generally active in spring and maintains its activity on the host until the end of the season. Thrips larvae and adults are susceptible to infections. *Entomophthora thripidium* infects only the abdominal organs of thrips (Malais & Revensberg, 2003). At room temperatures, the fungus completes its life cycle in about 4 days and does not have a resting spore stage. These preclude its commercial utility until these problems can be overome (Hubscher, 1983).

Metarhizium anisopliae

The disease caused by this fungus is sometimes called 'green lime disease' because of the green color of its spores. When its conidiophores contact an insect host, they germinate and the resulting hyphae enter the cuticle. Then, the fungus developing inside the body kills the insect host in a few days. This lethal effect is most likely due to the production of cyclic peptides (destruccines). The cadaver's cuticle is often red, and then the mildew turns green color as the ambient humidity is appropriate.

Metarhizium anisopliae has been reported to infect more than 200 insect species, including thrips (Cloyd, 1999; McNeil, 2005). Some previous studies in Turkey and other countries indicate that *M. anisopliae* can control the thrips well both indoor and outdoor conditions (Ekesi et al., 1998; Maniania et al., 2003; Ansari et al., 2008; Dura et al., 2012; Uçak et al., 2014). However, in a study, the effect of *M. anisopliae* and *V. lecanii* on pre-adult stages of *F. occidentalis* (especially 1st- and 2nd-instar larvae) was found to be lower than the adult stage due to molting process in immature stages (Vestergaard et al., 1995).

Beauveria bassiana

The fungus develops white color in Petri plate culture and produces specific dry and powdery conidia in the culture medium. *B. bassiana* can be used as a biological insecticide to control thrips, mites, whiteflies and many other arthropod pests (Erler et al., 2013, 2014; Erler & Ates, 2015; Topuz et al., 2016). After the spores stick onto the insect cuticle, they extend their hyphae into the insect's body and begin to multiply. Death of insects can take 3-5 days. An infected and dead insect forms a secondary spore source for subsequent infections (Long et al., 2000).

High humidity increases the activity of the conidia and the chance of future insect infections. Fungus spores easily die by solar radiation. However, they easily infect the arthropod hosts at cool and moderate temperatures (Goettel et al., 2000; Wraight and Ramos, 2002).

Studies carried out both in Turkey and in other countries have shown that *B. bassiana* can control thrips well (Gao et al., 2012; Uçak et al., 2014; Mousavi et al., 2017). For example, Mousavi et al. (2017) evaluated the efficacy of *B. bassiana* on *F. occidentalis* under the greenhouse conditions. Their results showed that the highest larval and adult mortality (95.5% and 98.4%, respectively) was achieved within seven days at 10^8 conidia /ml in comparison to the control.

Entomopathogenic fungi used on a commercial scale against thrips in Turkey

In Turkey, a limited number of EPF are licensed against important agricultural pests including thrips. In addition, all of these licensed preparations were isolated from abroad and made into commercial preparations. In Turkey, a limited number of studies on indigenous EPF are ongoing and there are no commercially licensed isolates against thrips (Kırışık & Erler, unpublished). Table 3 contains information on commercial products of exotic entonopathogenic fungi licensed against thrips.

Table 3. Specific information on commercial products of exotic entomopathogenic fungi licensed against thrips in Turkey

Commercial name	Content fungus	Recommended dose	Importer firm	Country of origin
Dopteril	*Beauveria bassiana* atcc. strain 74040	150 ml/100 l water	Boyut Dış Tic. A.Ş.	Italy
Nibortem	*Verticillium lecanii* strain Vl-1	25 ml/ha	Agrobest A.Ş.	India
Mycotal	*Verticillium lecanii* strain M	100 g/100 l water	Koppert Biologicals	England

Advantages and Disadvantages of Entomopathogenic Fungus Usage

There are some advantages and disadvantages to the practical use of EPF (Wan, 2003; Sevim et al., 2015). These are listed below:

Advantages

(1) In some cases, EPF have high host selectivity in terms of control with arthropod pests. Therefore, they do not affect populations of beneficial insects. However, some of them may have a very large host range.

(2) They do not have any adverse effects on humans and other vertebrates and also do not cause environmental pollution like synthetic pesticides commonly used.

(3) After application, they stay in the environment for a long time, resulting in a rapid death of the arthropod hosts.

(4) They do not cause problems such as pesticide resistance in controlling arthropod pests and thus provide a long-lasting control.

(5) They are suitable to be developed by biotechnological investigations.

Disadvantages

(1) The most serious disadvantage is that they are susceptible to UV radiation and low humidity and high temperature.

(2) They are adversely affected by fungicides.

(3) Synthetic pesticides generally kill arthropod pests in a short time whereas EPF require a longer period of time (sometimes 1-2 weeks).

(4) Production costs are generally higher than many synthetic pesticides.

Compability of Entomopathogenic Fungi with Natural Enemies

Use of EPF in greenhouse crops could have various direct and indirect effects on existing biological control systems based on arthropod natural enemies, potentially leading to both positive and negative outcomes for overall pest control. For example, *F. occidentalis* can be controlled effectively by phytoseiid predatory mites in sweet pepper, which provides pollen and nectar, but not in many ornamental plants that lack these supplemental food resources (Messelink et al., 2014). In such scenarios, the use of EPF has potential as a complementary measure in biological control programmes, as long as any potentially negative direct and indirect effects of these microorganisms are considered.

As for the direct effects of EPF on arthropod natural enemies, although isolate and species dependent, EPF generally have wider host ranges than other pathogens, such as bacteria and viruses and could potentially kill non-target arthropod natural enemies (Roy & Pell, 2000). However, killing effect of EPF is likely to vary significantly depending on the type of arthropod natural enemy. For example, greenhouse studies with predatory mirid bugs (Hemiptera: Miridae) showed that there were no negative effects of two commercial isolates of *B. bassiana* (GHA and ATCC 74040) on predator populations (Labbé et al., 2009; Hamdi et al., 2011). On the contrary, densities of predatory *Orius* (Hem.: Anthocoridae) species were significantly reduced due to infection by the GHA isolate of *B. bassiana* (Shipp et al., 2012), although in other studies there were no or only weak side effects on *Orius* spp. (Hamdi et al., 2011; Pourian et al., 2011). Predatory mites are generally not susceptible to commercial isolates of the entomopathogenic fungi *B. bassiana*, *M. brunneum* and *I. fumosorosae* both in laboratory and greenhouse trials (Ludwig & Oetting, 2001; Vergel et al., 2011; Wu et al., 2014). As for the side effects of EPF to parasitoid species, in the laboratory, leafminer and whitefly parasitoids can be highly susceptible (30–70%) to *B. bassiana* (Shipp et al., 2003), but in greenhouse trials only low levels of infection were observed in whitefly parasitoid populations (Labbé et al., 2009; Shipp et al., 2012). A recent study has shown that the mortality of different arthropods for the control of the western flower thrips ranged from 3 to 61% when combined with EPF in laboratory assays (Saito & Brownbridge, 2016). However, their results also indicate that compatibility and overall increased effects are observed when both biological control agents are applied. Although the majority of studies suggest that EPF are compatible with arthropod natural enemies, caution should be practiced with the application of EPF with broad host ranges.

Conclusion

Frankliniella occidentalis is a pest that causes economic losses in many crops both directly and indirectly, and its control must be made absolutely each growing period. However, when controlling this pest, human and environmental health is not often considered. In addition, the pest is capable to develop resistance to the insecticides easily due to its short life-cycle. For this reason, EPF that have not been reported to have resistance up to now can be used against the pest especially indoor crop production. They are currently being investigated not only for the control of thrips but also for many other important arthropod pests on various crops around the world, and some are commercially available. Biological control of *F. occidentalis* by EPF offers a sound alternative management strategy. However, as we have seen, there are very limited studies and information on the effect of EPF on *F. occidentalis* in our country. In conclusion, considering human and environmental health, further studies are needed in this regard.

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