



Status of Bacterial Entomopathogens Used for Microbial Control of Arthropod Pests in Iran and Turkey

Rasoul Marzban ^{1*} , Gozde Busra Eroglu ² 

¹ Biological Control Research Department, Iranian Research Institute of Plant Protection, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran

² Department of Molecular Biology and Genetics, Faculty of Science, Erzurum Technical University, Erzurum, Turkey

Article History

Received : 22 Apr, 2023
Revised : 20 May, 2023
Accepted : 15 Jun, 2023

Keywords

Microbial biopesticides,
Entomopathogens,
Bacillus thuringiensis,
Biological control

Abstract

Bacterial entomopathogens, especially *Bacillus thuringiensis* (*Bt*), are the most popular entomopathogen in bacteria worldwide, thanks to their toxin genes and high virulence. In this context, to develop an alternative to chemical pesticides used to control agricultural and forest pest insects, *Bt* has been isolated from soils and many insects, and products in the form of formulations have been prepared. It is also known that *Bt*-based biopesticides are generally more specific and harmless to other organisms. The results demonstrate that countries have different conditions for releasing these organic products to the market and it is often difficult for scientists to obtain a license. This study discusses the current status of bacterial entomopathogens study in Iran and Turkey.



Corresponding Author:

Rasoul Marzban, Biological Control Research Department, Iranian Research Institute of Plant Protection, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran, r.marzban@areeo.ac.ir

Cite this article as:

Marzban, R., & Eroglu G., B. (2023). Status of Bacterial Entomopathogens Used for Microbial Control of Arthropod Pests in Iran and Turkey. *Natural Products and Biotechnology*, 3(1), 23-35. <https://doi.org/10.58465/natprobiotech.2023.04>

1. INTRODUCTION

Agricultural and forest pest insects cause economic losses of millions of dollars every year. Chemical pesticides are the leading strategies used to control these pests because of their short duration of action and ease of use. However, the broad spectrum of action of chemical pesticides destroys non-target organisms such as beneficial insects and natural enemies of destructive pests. These products are toxic to both the environment and humans and cause resistance development in target organisms over time (Karimi *et al.*, 2019; Khan *et al.*, 2023).

Another strategy involves the use of formulations of natural pathogen origin to protect agricultural crops from invaders such as pests, fungi, and weeds. These products are effective for the target organism and provide safe use for other living creatures and nature (Egbuna *et al.*, 2020). In recent years, more than 150 isolates of *Bacillus thuringiensis* (*Bt*) isolated from cultivated soils, were tested on different groups of insect pests after purification. In addition, some research has been carried out to determine the efficacy of those native isolates to select the ideal isolate (Marzban and Salehi, 2006). The diversity of the isolates *cry* and *cyt* genes have been studied (Salehi *et al.*, 2007; Salehi *et al.*, 2008; Seifi Nejad *et al.*, 2008; Nazarian Amirani *et al.*, 2009; Chalajour *et al.*, 2013; Rashki *et al.*, 2021). Moazami started to study on production and formulation of insect bacterial pathogens, especially *B. thuringiensis israelensis* H14 several decades ago. These studies are continuing and work is already well done on mass production and formulation of *Bt* in the Iranian research institute of plant protection and Agricultural biotechnology research institute (Marzban, 2012; Khorramvatan *et al.*, 2013; Khorramvatan *et al.*, 2014; Marzban *et al.*, 2014; Saberi *et al.*, 2014; Salehi *et al.*, 2015; Marzban *et al.*, 2016). With the increasing interest in organic agriculture in recent years, the

decrease in the use of chemical pesticides and the development of *Bt*-based microbial products instead is a tremendous development for the pesticide market.

Since the 1960s, biopesticides have been tested to control pests on wood trees and fruit gardens in Iran. The first biopesticide based on *B. thuringiensis* was successfully used against Gypsy moth in wood trees (Reardon *et al.*, 1994). During the past three decades, many research activities have been conducted on entomopathogens and antagonistic agents, such as fungi, bacteria, viruses, and nematodes. Furthermore, numerous efforts have been performed to obtain some biopesticide products supported by the Iranian Government and private companies at a commercial level (Karimi *et al.*, 2019).

In Turkey, many entomopathogenic bacteria have been isolated from different insects for about 40 years, and their use as a biological control agent has been investigated. The first trial of *B. thuringiensis* product with Turkish isolates was studied by Çakmakçı *et al.*, (1985) with the TUBITAK-Tarmik-3 project. In this project, *B. thuringiensis* was isolated from died *Hyponomeuta malinellus* larvae collected from Kütahya, Niğde, and Ankara. The formulations of these strains and two commercial products (Dipel and Thuricide) were compared *in vitro* against the larvae of *Ephestia kuehniella*, *Galleria mellonella*, *Amorphogynia necessaria*, and *Malacosoma neustria*. The most effective local *Bt* strain was selected and field trials were conducted in Ankara. Studies in field trials were carried out on both *H. malinellus* and beneficial insects in the region. Özkazanç (1986) used different doses of Tarmik-3 *Bt* for the control of *Thaumetopoea pityocampa* and reported that the mortality rate was between 40-99% as a result of this study. Alten and Boşgelmez (1990) reported that 2 *Bt* (Iskenderun-310109 and Icel-330218) and 2 *Bacillus sphaericus* (Iskenderun-310111 and Icel-3302120) isolates of Turkish origin have high virulence for the control of 2nd and 3rd instar larvae of *Culiseta longiareolata*. Çakmakçı *et al.*, (1990) investigated the toxicity of *B. thuringiensis* subsp. *israelensis* (number 330211) isolated from the İçel region of Turkey to mammals on mice and guinea pigs. In this study, 2×10^8 bacterial stock suspensions per mL were given to the experimental animals by different means such as subcutaneous intraperitoneal, intravenous, oral, percutaneous, inhalation, and eye irritation test for each vaccination, and no signs of disease were observed in any of the animals as a result of the experiment. Sezen and Demirbağ (1999) were isolated a *B. thuringiensis* subsp. *kurstaki* from hazelnut pest, *Balaninus nucum* larvae, and adult in Trabzon. In insecticidal activity studies using this isolate, 100% mortality was detected on *Agriotes lineatus* at 1.8×10^9 cfu/mL concentration (Danışmazoğlu *et al.*, 2012). Besides, *Bt* isolate was applied to *Spodoptera littoralis* and *Agelastica alni* and 100% and 72.3% mortality were observed, respectively (Çakıcı *et al.*, 2014; Eski *et al.*, 2017). Sezen *et al.*, (2008) isolated *B. thuringiensis* ssp. *tenebrionis* (Xd3) from *Xyleborus dispar*. This isolate was applied to different hosts (*Agelastica alni*, *Amphimallon solstitiale*, and *Melolontha melolontha*) at a concentration of 1.8×10^9 cfu/mL, and 100% mortality was observed in all of them. Katı *et al.*, (2009) isolated two *B. thuringiensis* subsp. *morrisoni* from *Thaumetopoea pityocampa* in Turkey. As a result of virulence tests, Tp6 isolate caused 100% mortality in *Agelastica alni* and *Leptinotarsa decemlineata* larvae, while Tp14 isolate caused 100% mortality in *Malacosoma neustria* larvae. Yılmaz *et al.*, (2012) investigated the insecticidal activity of *Bt* SY49.1 strain in their study on *Ephestia kuehniella* and *Plodia interpunctella* and reported that spore-crystal mixtures caused more than 90% mortality in the larvae of the pests. Yıldız and Sezen (2017) isolated 13 isolates of *Serratia marcescens* (Eca1 and Eca3), *Serratia* sp. (Eca 11), *B. thuringiensis* (Eca2, Eca4, Eca6-10, Eca12 and Eca13), and *B. thuringiensis* (Eca5) from *Cadra cautella*. The insecticidal activities of these isolates were performed against three insect species from the Lepidoptera group that caused serious damage in warehouses. The highest insecticidal activity was 57% for Eca9 isolate in 3rd instar larvae of *C. cautella*, 100% for Eca9 isolate in 3rd instar larvae of *Plodia interpunctella*, and 100% for Eca10 and Eca3 isolate in 3rd instar larvae of *E. kuehniella*. Accordingly, they reported that Eca9, Eca3, and Eca10 isolates could

be valuable as potential biological control agents for the control of warehouse pests. Eski *et al.*, (2018) *Bt* (Se13 strain) isolated from *Spodoptera exigua*, at a concentration of 1.8×10^9 cfu/mL, caused 75% mortality 48 hours after the application and 100% mortality after 7 days in the 3rd instar larvae of *S. exigua*. Eski *et al.*, (2019) microencapsulated a local *Bt* Se13 isolate to make it environmentally stable. As a result of the efficacy studies of this product on *Spodoptera exigua*, the LC₅₀ value was determined as 1.6×10^4 cfu/mL⁻¹ and they reported that this biopesticide is promising for field applications of *S. exigua* control. Eski *et al.*, (2022), reported that *Bt* Xd3 against *Leptinotarsa decemlineata* showed 83% and 73% mortality in larvae and adults, respectively, within 10 days when applied at 10^9 cfu/mL⁻¹ concentration. Usta (2022), *Bt* from *Cydalima perspectalis* collected from Artvin province and reported that this bacterium showed an 85% mortality rate on the pest. Eski (2023) tested 13 local *Bt* strains isolated from soil samples for the control of *Tuta absoluta* and declared that *Bt*-B3 isolate is a promising biological warfare agent for integrated pest control of *T. absoluta* in Turkey.

1.1. Application of Biological Control Agents

In Iran, although the main approach to pest control in the country has been dominantly chemical control for years, biological pest control with about 90 years of practical background has occupied a meaningful part of pest control programs. It started with the classical approach in 1933 by introducing the Australian ladybird, *Rodolia* (= *Novius* = *Vedalia*) *cardinalis* to control an exotic pest, Australian scale, *Icerya purchasi*, on citrus. Afterward, other natural enemies such as *Cryptolaemus montrouzieri*, *Prospaltella berlesei* and *Phytoseiulus persimilis* were introduced to the country (Karimi and Kamali, 2021). The augmentation method was also practiced in 1946 by augmenting sun pest egg parasitoids and releasing them on infested wheat which lasted for 2 decades. From a conservation point of view, there is an outstanding example in the history of biological control. In 1972, sugarcane stem borer broke out and chemical control failed in suppressing the pest outraged population. The idea of banning chemical application made the natural enemy (*Platytenomus hylas*) escapes from chemical pressures and boosts its population which resulted in a sustainable pest control strategy (Karimi and Madadi, 2021). At present, about 100 private companies are involved in the biological control industry. Natural enemies such as *Trichogramma* sp., *Habrobracon hebetor*, *Chrysoperla carnea*, and *Cryptolaemus montrouzieri* are masse produced by private companies under close monitoring of Plant Protection Organization and released in selected farms based on protocols provided by the research section. Moreover, microbial biological control agents based on *B. thuringiensis*, *B. subtilis*, *Trichoderma* sp., are finding their role in sound crop protection. Since the 1970s, biopesticides have been tested to control pests on wood trees and fruit gardens in Iran. The first biopesticide based on *B. thuringiensis* was successfully used against gypsy moth in wood trees (Karimi *et al.*, 2019). During the past three decades, many research activities have been conducted on entomopathogens and antagonistic agents, such as fungi, bacteria, viruses, and nematodes. Furthermore, numerous efforts have been performed to obtain some biopesticide products supported by the government and private companies at the commercial level.

Biological control studies in Turkey started in the Ottoman period. In those years, mainly beneficial insects were procured from abroad and released into nature, and pest populations were controlled. The first example of this was provided in 1910 by bringing the predator *Rodolia cardinalis* from Chios to control the *Icerya purchasi* species, which is harmful in citrus orchards and some fruits (İslamoğlu, 2021). In 1965, Antalya Biological Control Research Station was established and applications were made here for many years in the form of reproduction and release of predator and parasitoid species (Erkiliç and Demirbaş, 2007).

The isolation and use of local microbial pathogens in biological control started in Turkey towards the end of the 1980s. So far, many local isolates of bacterial, fungal, nematode,

protozoan, and virus origin have been obtained by researchers. Most of these isolates were biotested under laboratory conditions, their host spectra were determined, and pot and field trials were completed. Among the microbial pathogens, studies on *Bt* isolates stand out in Turkey. Thanks to the toxin genes of *Bt* isolate, both its virulence and its host spectrum are relatively broad, this has made it possible to focus on studies in this field. However, although there are many local, national, and effective *Bt* isolates in the research laboratories of our country, commercialization of these products to the market has not been possible until now due to the high cost of the licensing process. For this reason, foreign biopesticide products supplied from abroad are used in Iran and Turkey. However, the high cost of foreign products has pushed agricultural producers to use lower-cost chemical products. However, it is known that the use of products of foreign isolate origin may have a negative effect on natural strains and the susceptibility of the host may vary according to foreign isolates. Therefore, local isolates suitable for growth as microbial control agents should be preferred for the identification of new species present in each geographical region.

1.2. Application of *B. thuringiensis* as Biopesticide

The reason why the use of microbial pesticides is still not widespread is the lack of social awareness; distrust of farmers (Mishra *et al.*, 2015); and lack of opportunities to support the use of microbial pesticides (Kumar and Singh, 2015). However, producers who think that biological control alone will not be sufficient for pest control should be given the necessary information and, if necessary, should be directed to integrated control methods. By supporting farmers with an affordable price policy and consistent success of microbial products, the use of products containing microbial agents can be expanded (Marrone, 2009; Marzban and Askari 2010; Regnault-Roger, 2012). *B. thuringiensis* has been used in Iran for three decades. It has been mostly used on agricultural and forestry pests. The product was produced by a private company in semi-solid culture in 1995. However, the product has not been registered due to high levels of microbial contamination (10-20%), beta-exotoxin presence, and unsuitable formulation. Later, the bacterium was produced using the liquid fermentation method in two private companies, Biorun Co. and Mehr Asia Biotechnology Company. The used serotypes of *B. thuringiensis* consist of *kurstaki*, *israelensis*, and *aizawai*. The current market for *Bt*-based products in Iran is approximately \$400,000 sharing only 1.0% of the total market. The most important *Bt* crop used in Iran is *B. thuringiensis* subsp. *kurstaki* and *B. thuringiensis* subsp. *israelensis* H14 and is commercialized for the control of many pests. The efficiency of biopesticides based on *B. thuringiensis* has been proved on pests of forestry, tropical crops, vegetables, industrial plants, orchards, and fruits in both glasshouse and field conditions. The target pests are gypsy moth, *Lymantria dispar*; garlic worm, *Dyspessa ulula*, indian meal moth, *Plodia interpunctella*, sugar beet armyworms, *Spodoptera littoralis*, *Spodoptera exigua*; cabbage butterfly, *Pieris brassicae*, *Plutella xylostella*; potato tuber moth, *Phthorimaea operculella*; grape berry moth, *Lobesia botrana*; european corn stem borer, *Ostrinia nubilalis*; colorado potato beetle, *Leptinotarsa decemlineata*; apple mine moth, *Yponomeuta malinellus*; rice stem borer, *Chilo suppressalis*; pea bollworm, *Helicoverpa virescens*; *Agrotis* spp.; oak white moth, *Leucoma wiltshire*; cotton bollworm, *Helicoverpa armigera*; mosquito, *Anopheles* sp. In recent years, more than 30 tons per year of *B. thuringiensis* products have been used for controlling lepidopteran pests in the forest, tropical crops, vegetables, industrial plants, orchards, and fruit orchards. In addition, some research has been carried out to explore native isolates to determine their efficacy and screen the ideal isolate for the control of coleopteran pests. (Saber, *et al.*, 2020; Saber, *et al.*, 2023)

B. thuringiensis israelensis H14 strain has high virulence in Diptera species (mosquito and housefly larvae) (De Barjac and Sutherland, 2012). Various studies have shown that *B. thuringiensis* H-14 is more effective against *Culex* and *Aedes* than *Anopheles* mosquitoes

(Moazami, 2002). *B. thuringiensis* H-14, which is known to be harmless to humans, pets, fish or plants, has been produced as a formulation for over ten years. There are 10 *Bt* based products registered and used between 1996-2021 in Turkey. Since all of these products are imported, they are quite expensive. This situation leads to the continuation of the intensive use of chemical pesticides in Turkey. For this reason, there is a need to increase the support for the introduction of local biopesticides produced in research laboratories of universities to the market with the support of companies to reduce the foreign dependency of the country and to expand the use of environmentally friendly products. Thus, it will contribute to both the economic and health security of Turkey.

1.3. Quality Control and Registration of Microbial Biopesticides

In order to commercialize biopesticide products so that they can compete with chemical pesticides, the required tests need to be carried out more easily. Otherwise, the development and commercialization of microbial biopesticides is a very difficult and time-consuming process. The Iran Agricultural Research, Education, and Extension Organization is improving and developing biopesticides for use in sustainable development. In 1994, the High Council for Development of the Application of Biologic Substances and Optimized Use of Fertilizer and Pesticides in Agriculture was established at the Ministry of Agriculture. The council's main objective is to reduce pesticide use by 30%. The government has also decreased subsidies for pesticides and is proceeding with a full omission of pesticides. The government provides credits and bank loans for the production of biopesticides. In addition, the production of biopesticides is exempted from a series of taxes and duties. One of the most important issues in turning *Bt*-based insecticides into a commercial product is that the product maintains its stability. While most manufacturers tested this with live spore counting in the 1980s, the correlation between spore count and toxicity later revealed that this test was unreliable. Thus, in 2010, the quality control scheme for biopesticides was revised by the Plant Protection Research Institute of Iran and the Plant Protection Organization (Marzban, 2004).

A committee within the Ministry of Agriculture in Iran has the authority to register pesticides for use. All produced batches of *Bt* must be assayed for quality control. This quality control includes contamination with other microorganisms, β -exotoxin, suspensibility, potency, and viable spore count assay.

Turkey is in a very important position in terms of climate diversity, having large agroecological areas and growing many products of economic importance. In recent years, the number of producers engaged in organic farming has increased with government support. According to the revised directive published in the Official Gazette No. 27347 on 12.9.2009, under the Plant Protection and Quarantine Law No. 6968, registration procedures are carried out by the Ministry of Agriculture and the General Directorate of Rural Affairs Protection and Control. Since the criteria used in the inspection process of microbial pesticides are the same as the standards used for chemical pesticides, there are difficulties in the licensing process. For this reason, making regulations in the regulations of license and safety tests will make it easier for scientists to put their products on the market. Thus, by providing both lower-cost and more reliable products, the persuasion process of agricultural producers on the use of biopesticides will be facilitated. There are 16 registered microbial agent-based preparations in Iran (Table 1).

Natural Products and Biotechnology

Table 1. Microbial biopesticides registered in Iran (Iranian Plant Protection Organization)

Product name	Microorganism type	Active ingredient	Provider	Approval date	Formulation	Manufacturer/ Country	Target pest	Crop
CangMei		<i>Bacillus subtilis</i>	Giyah Parnian Atlas	2018	WP	Deqiang Biology /China	<i>Pyricularia oryzae</i>	Rice
				2022	WP		<i>Alternaria</i> spp.	Tomato
Pars Bacill		<i>Bacillus velezensis</i>	Royan Tisan Sabz	2019	SC	Royan Tisan Sabz/Iran	<i>Fusarium oxysporum</i>	Greenhouse tomato
Talaromin		<i>Talaromyces flavus</i>	Baharan Dasht Sahel	2018	P	Baharan Dasht Sahel/Iran	<i>Verticillium dahliae</i>	Potato
Trianium- P				2012	WP		<i>Fusarium oxysporum</i>	Greenhouse tomato
Trianium- G	Fungal/fungus like	<i>Trichoderma harzianum</i>	Giyah	2012	WP	Koppert /Netherlands	<i>Fusarium oxysporum</i>	Greenhouse cucumber
				2018	G		<i>Fusarium oxysporum</i>	Cantaloupe
Serenade		<i>Bacillus subtilis</i>	Bayer Parsian	2019	SC	Bayer/ Germany	<i>Botrytis cinerea</i>	Greenhouse strawberry
				2021	SC		<i>Fusarium oxysporum</i>	Greenhouse tomato
Rooein-1			Zist Fanavar Sabz	2020	P	Zist Fanavar Sabz/Iran	<i>Rhizoctonia solani</i>	Sugar beet
Polyversum		<i>Pythium oligandrum</i>	Bazargan Kala	2021	WP	Biopreparaty/ Czech Republic	<i>Pythium</i> spp.	Greenhouse cucumber
Naturalis-L		<i>Beauveria bassiana</i>	-	2001	SC	CBC/Italy	<i>Bemisia tabaci</i>	Cotton
Mycotal		<i>Lecanicillium muscarium</i>	Giyah	2012	WP	Koppert/ Netherlands	<i>Trialeurodes vaporariorum</i>	Greenhouse tomato
Biolep				2018	SC		<i>Lobesia botrana</i>	Grape
Biolep-p	Bacterial	<i>B. thuringiensis</i>	Biorun	2018	SC	Biorun/Iran	<i>Plutella xylostella</i>	Cabbage
				2018	WP		<i>Plutella xylostella</i>	Cabbage
Bactospeine			Valnet Bioscience	2019	WP	Valnet Bioscience/USA	<i>Helicoverpa armigera</i>	Cotton
				1968	WP		<i>Lymantria dispar</i>	Forrest trees
Bt			Giyah Parnian atlas	2022	WP	Biontech International/India	<i>Heliothis virescens</i>	Pea
MVP			Ecogen/USA	1975	WP	Ecogen/USA	<i>Leucoma wiltshire</i>	Forrest trees
Capsanem	Nematode	<i>Steinernema carpocapsae</i>	Giyah	2021	P	Koppert/ Netherlands	<i>Phthorimaea operculella</i>	Stored Potatoes

1.4. Registered Microbial Biopesticides

It is known that 74% of biopesticides, which have a market share of 5.61 billion USD in 2021, are bacteria-based (Thakore, 2006). Since the 1960s, biopesticides have been tested to control pests on tree trees and orchards in Iran. The first biopesticide based on *B. thuringiensis* was successfully used against the gypsy moth on woody trees. In the last three decades, many research activities have been conducted on entomopathogens and antagonist agents such as fungi, bacteria, viruses, and nematodes. In addition, many studies have been carried out to obtain some biopesticide products that are commercially supported by the Iranian government and private companies. *B. thuringiensis*-based products account for the largest share in the Iranian microbial biopesticide market. These products were first registered in Iran 50 years ago (under the trade names MVP® and Bactospin® on December 22, 1968 and May 9, 1975, respectively). Apart from *Bt*, other microbial products in the Iranian market are of fungal origin. On this subject, many researchers in Iran are working on microbial biocontrol agents against the problems of different plant pests (Moazami, 2002; Marzban and Tajbakhsh, 2004; Ranjy *et al.*, 2005; Keshavarzi, 2008; Seifi Nejad *et al.*, 2008; Nazarian Amirani *et al.*, 2009; Shojaaddini *et al.*, 2010; Marzban, 2012; Marzban *et al.*, 2014; Saberi *et al.*, 2014; Marzban *et al.*, 2016). According to the Turkish Statistical Institute, vegetable production in Turkey increased by 1.8 percent in 2021 compared to the previous year and reached 31.8 million tons. Rooted and tuberous vegetable production increased by 6.9 percent in vegetable subgroups. In this direction, Turkey's need for insecticides in agricultural production is also increasing. There are 29 registered microbial agent-based preparations (15 bacterial, 13 fungal, and 1 viral) in Turkey (Table 2).

Table 2. Microbial biopesticides registered in Turkey (Turkey Plant Protection Organization)

Product name	Microorganism type	Active ingredient	Provider	Approval date	Formulation type	Manufacturer/Country	Target pest	Crop
Agree 50 WG			Gennova	1996	WG	Certis/USA	<i>Helicoverpa armigera</i> , ...	Tomato
BIO-T Plus			Cansa	2009	Liquid	Biodalia Microbiological Technologies/Israel	<i>Lobesia botrana</i> , ...	Red pine, ...
Dacron			Safa	2012	Wettable powder	Jiangsu International Cooperation/China	<i>Cryptoblabes gnidiella</i> , <i>Helicoverpa armigera</i>	Orange, tomato
Delfin	Bacterial	<i>B. thuringiensis</i>	Agrikem	1996	WG	Certis/USA	<i>Tuta absoluta</i> , ...	Tomato, ...
Dipel			Sumitomo	2010	WG	Valent Biosciences/ABD	<i>Spodoptera littoralis</i> , ...	Tobacco, ...
Florbac			Sumitomo	2010	WG	Valent Biosciences/ABD	<i>Spodoptera littoralis</i> , ...	Tobacco, ...
Foray			Envirotek	2008	Liquid	Valent Biosciences/ABD	<i>Thaumetopoea pityocampa</i> , ...	Pine trees
IAB-BT			Nektar	2010	WG	Investigaciones Y Aplicaciones Bio Tecnologicas/Spain	<i>Helicoverpa armigera</i> , ...	Apple, ...
Rapax			Imes	2009	Liquid	CBC (Europe) S.r.l./Italy	<i>Helicoverpa armigera</i> , ...	Vineyard, tomato
Rebound			Hektaş	2005	Wettable powder	Shandong Lukang Biological Pesticides/China	<i>Tuta absoluta</i> , ...	Vineyard, ...

Natural Products and Biotechnology

Subtilex foliar		<i>Bacillus subtilis</i>	Bioglobal	2010	Wettable powder	Becker/ABD	<i>Botrytis cinerea</i>	Vineyard, tomato
Serenade			Bayer	2004	Liquid	Bayer/Germany	<i>Botrytis cinerea</i> ,...	Tomato, ...
Companion			Hekagro	2011	Liquid	Growth products/ABD	<i>Venturia inaequalis</i> ,...	Pear, cherry, ...
Biobac-WP			Atlantik	2008	Wettable powder	Biotech/Taiwan	<i>Sclerotinia sclerotiorum</i> , ...	Cherry, apricot, ...
AQ10		<i>Ampelomyces quisqualis</i>	Boyut	2010	WG	CBC (Europe) S.r.l./Italy	<i>Erysiphe necator</i> ,	Tomato
Bio Nematon	Fungal/fungus like	<i>Paecilomyces lilacinus</i>	Agrobest	2011	Liquid	T. Stanes and Company Limited/India	<i>Meloidogyne</i> spp.	Potatoes, ...
Bioact		<i>Paecilomyces lilacinus</i>	Bayer	2018	DC	Bayer/Germany	<i>Meloidogyne</i> spp.	Pepper, eggplant, ...
Blossom Protect		<i>Aureobasidium pullulans</i>	Nufarm	2012	WG	San Agrow Holding GmbH/Austria	<i>Erwinia amylovora</i>	Pear
Dopteril		<i>Beauveria bassiana</i>	Boyut	2009	Liquid	CBC (Europe) S.r.l./Italy	<i>Bemisia tabaci</i> , ...	Tomato, ...
Nostalgist		<i>Beauveria bassiana</i>	Agrobest	2012	Liquid	T. Stanes and Company Limited/India	<i>Melolontha melolontha</i> , ...	Cotton,...
Inferno		<i>Myrothecium verrucaria</i>	AMC-TR	2010	-	-	<i>Meloidogyne</i> spp.	Tomato
Nibortem		<i>Verticillium lecanii</i>	Agrobest	2012	Liquid	T. Stanes and Company Limited/India	<i>Frankliniella occidentalis</i> ,	Cucumber
Nogall		<i>Agrobacterium radiobacter</i>	Bioglobal	2005	Wettable powder	Becker Underwood/Australia	<i>Agrobacterium tumefaciens</i>	Cherry, peach
Remedier		<i>Trichoderma asperellum</i>	Tancan	2010	Wettable powder	Isagro S.P.A/Italy	<i>Alternaria</i> spp., ...	Strawberry, ...
Ruotshield			Hasel	2009	Granular	Biowork/ABD	<i>Rhizoctonia solani</i> ,...	Cucumber
T-22 Planter Box			Hasel	1999	Wettable powder	Biowork/ABD	<i>Botrytis cinerea</i> ,	Tomato, cotton
Trichoflow		<i>Trichoderma harzianum</i>	Enerji	2002	Wettable powder	McHort/New Zealand	<i>Fusarium</i> spp,...	Tomato
Priority		<i>Paecilomyces fumosoroseus</i>	Agrobest	2011	Liquid	T. Stanes and Company Limited/India	<i>Panonychus ulmi</i> , ...	Apple,...
Madex	Viral	<i>Cydia pomonella granulovirus</i>	Verim	2004	Liquid	Andermatt/Switzerland	<i>Grapholita funebrana</i>	Plum

Important studies on microbial control factors have been carried out in Turkey for the last 40 years. However, most of these studies remained in the literature and could not be commercialized. In this direction, producers of organic farming prefer imported products because they cannot reach ready-to-use domestic and national biological control agents. As a result, due to the expensiveness of these imported microbial pesticides, the orientation of the producer is generally towards the use of chemical pesticides.

4. CONCLUSION

Chemical pesticides, which are commonly used to control agricultural pests, pose significant health risks to living organisms. In particular, for natural enemies of insect pests using the same product over and over again for all pests both cause resistance in insects and

harm the environment. For this reason, it is necessary to increase the use of natural enemies and to introduce cheap, domestic, and organic alternative products to the market. *B. thuringiensis* species, which is one of the microbial control agents, has a high infectivity in insects, so there are many studies in the literature on this subject. However, since the licensing process of each country has different difficulties, scientists cannot offer the pathogens they have determined to be effective in the laboratory as a product to the markets. After 2000, Turkey and Iran registered at least one microbial pesticide per year, despite a delayed start. The most important reason for less development of microbial pesticides in Iran and Turkey is their expense and comparing their efficiency with chemical pesticides. As a result, producers should be informed about the use of microbial pesticides and product costs should be offered to producers at more affordable prices with government support.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research publishing ethics. The scientific and legal responsibility for manuscripts published in NatProBiotech belongs to the author(s).

Author Contribution Statement

Rasoul Marzban: Study conception and design, data collection, analysis and interpretation of results, draft manuscript preparation. **Gozde Busra Eroglu:** Data collection, analysis and interpretation of results, draft manuscript preparation.

Orcid

Rasoul Marzban  <https://orcid.org/0000-0001-5091-006X>

Gozde Busra Eroglu  <https://orcid.org/0000-0001-8988-1315>

5. REFERENCES

- Alten, B., & Boşgelmez, A. (1990). Effectiveness of several *Bacillus* isolates on *Culiseta longiareolata* (Macquart) (Diptera: Culicidae) larvae under natural conditions. *Doğa, Türk Zooloji Dergisi*, 14, 263-273.
- Çakıcı, F. Ö., Sevim, A., Demirbağ, Z., & Demir, İ. (2014). Investigating internal bacteria of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) larvae and some *Bacillus* strains as biocontrol agents. *Turkish Journal of Agriculture and Forestry*, 38, 99–110. <https://doi.org/10.3906/tar-1302-65>
- Çakmakçı, I., Ozer, N., Alten, B., Bor, N. M., Yorukan, S., Boşgelmez, A., & Gurkan, F. (1990). Safety Test On Mice with *B. thuringiensis* Isolate. *Journal of Islamic Academy of Sciences*, 3, 35-40.
- Çakmakçı, M. L., Boşgelmez, A., Soylu, O. Z., Bulut, H., & Gürkan, B. (1985). *B. thuringiensis*'in üretim olanakları ve Tarımda önemli zararlara neden olan bazı lepidopter türlerine karşı etkinliklerinin saptanması üzerine araştırmalar. *Tübitak TARMİK*, (3).
- Chalajour, H., Dolatabad, S. S., & Salehi, J. G. (2013). Genetic diversity among *B. thuringiensis* isolates from Iran based on random amplified polymorphic DNA (RAPD) marker. *Research on Crops*, 3, 945-949.
- Danışmazoğlu, M., Demir, I., Sevim, A., Demirbağ, Z., & Naçacıoğlu, R. (2012). An investigation on the bacterial flora of *Agriotes lineatus* (Coleoptera: Elateridae) and pathogenicity of the flora members. *Crop protection*, 40, 1-7. <https://doi.org/10.1016/j.cropro.2012.04.012>

- De Barjac, H., & Sutherland, D. J. (2012). Bacterial Control of Mosquitoes & Black Flies. Springer Dordrecht, 349 P. <https://doi.org/10.1007/978-94-011-5967-8>
- Egbuna, C., Sawicka, B., Tijjani, H., Kryeziu, T. L., Ifemeje, J. C., Skiba, D., & Lukong, C. B. (2020). *Biopesticides, safety issues and market trends. Natural remedies for pest, disease and weed control*. Academic Press, New York, pp 43-53. <https://doi.org/10.1016/B978-0-12-819304-4.00004-X>
- Erkılıç L., & Demirbaş, H. (2007). Biological control of citrus insect pests in Turkey. *CABI Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, 2 (056): 1-6. <https://doi.org/10.1079/PAVSNNR20072056>
- Eski, A., Demir, İ., Sezen, K., & Demirbağ, Z. (2017). A new biopesticide from a local *B. thuringiensis* var. *tenebrionis* (Xd3) against alder leaf beetle (Coleoptera: Chrysomelidae). *World Journal of Microbiology and Biotechnology*, 33, 1-9. <https://doi.org/10.1007/s11274-017-2263-0>
- Eski, A., Demir, I., Güllü, M., & Demirbağ, Z. (2018). Biodiversity and pathogenicity of bacteria associated with the gut microbiota of beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae). *Microbial pathogenesis*, 121, 350-358. <https://doi.org/10.1016/j.micpath.2018.05.012>
- Eski, A., Demirbağ, Z., & Demir, İ. (2019). Microencapsulation of an indigenous isolate of *B. thuringiensis* by spray drying. *Journal of microencapsulation*, 36, 1-9. <https://doi.org/10.1080/02652048.2019.1572238>
- Eski, A., Bayramoğlu, Z., Sönmez, E., Biryol, S., & Demir, I. (2022). Evaluation of the Effectiveness of Entomopathogens for the Control of Colorado Potato Beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Journal of Agricultural Science and Technology*, 24, 393-405.
- Eski, A. (2023). Susceptibility of different *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) populations to indigenous *B. thuringiensis* strains. *Turkish Journal of Entomology*, 47, 101-110. <https://doi.org/10.16970/entoted.1216414>
- İslamoğlu, M. (2021). *The place and importance of biological control in Turkey and some application examples*. Agricultural researches resource book, p. 159.
- Karimi, J., Dara, S. K., & Arthurs, S. (2019). Microbial insecticides in Iran: history, current status, challenges and perspective. *Journal of invertebrate pathology*, 165, 67-73. <https://doi.org/10.1016/j.jip.2018.02.016>
- Karimi, J., & Kamali, S. (2021). Overview: History of agricultural entomology and biological pest control in Iran. *Biol. Control Insect Mite Pests Iran. Prog. Biol. Control*, 18, 1–20. https://doi.org/10.1007/978-3-030-63990-7_1
- Karimi, J., & Madadi, H. (2021). *Biological control of insects and mite pests in Iran: A review from fundamental and applied aspects*. Springer, p. 621.
- Katı, H., Ince, I. A., Sezen, K., İsci, S., & Demirbağ, Z. (2009). Characterization of two *B. thuringiensis* ssp. *morrisoni* strains isolated from *Thaumatococcus panyocampa* (Lep., Thaumatocoeidae). *Biocontrol Science and Technology*, 19, 475-484. <https://doi.org/10.1080/09583150902836377>
- Keshavarzi, M. (2008). Isolation, Identification and Differentiation of local *B. thuringiensis* Strains. *Journal of Agricultural Science Technology*, 10, 493-499.

- Khan, B. A., Nadeem, M. A., Nawaz, H., Amin, M. M., Abbasi, G. H., Nadeem, M., Ali, M., Ameen, M., Javaid, M. M., Maqbool, R., Ikram, M., & Ayub, M. A. (2023). *Pesticides: impacts on agriculture productivity, environment, and management strategies*. In *Emerging Contaminants and Plants: Interactions, Adaptations and Remediation Technologies* (pp. 109-134). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-031-22269-6_5
- Khorramvatan, S., Marzban, R., Ardjmand, M., Seifkordi, A., & Askary, H. (2013). The effect of polymers on the stability of microencapsulated formulations of *B. thuringiensis* subsp. *kurstaki* (Bt-KD2) after exposure to Ultra Violet Radiation. *Biocontrol Science and Technology*, 24, 462–472. <https://doi.org/10.1080/09583157.2013.871503>
- Khorramvatan, S., Marzban, R., Ardjmand, M., Seifkordi, A., & Askary, H. (2014). Preparation concentrated suspension of microencapsulated formulation of *B. thuringiensis* subsp. *kurstaki*. *Journal of BioControl in Plant Protection*, 2, 81-89.
- Kumar, S., & Singh, A. (2015). Biopesticides: Present Status and the Future Prospects. *Journal of Fertilizers & Pesticides*, 6(2). <https://doi.org/10.4172/jbfbp.1000e129>
- Marrone, P. G. (2009). *Barriers to adoption of biological control agents and biological pesticides*. In: Radeliffe, E. B., Hutchison, W. D., & Cancelado, R. E. (eds) *Integrated Pest Management*. Cambridge University Press, Cambridge, p. 163–178. <https://doi.org/10.1079/PAVSNNR20072051>
- Marzban, R. (2004). Biopesticides (microbial pesticides) registration and quality control. *Magazine of Zeitun*, 162, 16-22.
- Marzban, R., & Tajbakhsh, M. (2004). Comparison of several methods for detection and quantification of β -exotoxin in commercial *B. thuringiensis* products. *Applied Entomology and Phytopathology*, 71, 141-149.
- Marzban, R., & Salehi, J. G. (2006). *Distribution of B. thuringiensis in the Agricultural soils of Iran*. *Biotechnology, Agriculture and the Food Industry* (ISBN: 1-60021-040-6), Nova Science Publishers, Inc. (New York) USA, pp. 95-100.
- Marzban, R., & Askari, H. (2010). Microbial Pest Control Achievements, Challenges, and Vision. *Proceedings of the Congress on half a century of pesticide usage in Iran*. P. 9.
- Marzban, R. (2012). Investigation on the suitable isolate and medium for the reduction of *B. thuringiensis*. *Journal of Biopesticide*, 5, 144-147.
- Marzban, R., Saberi, F., & Shirazi, M. M. (2014). Separation of *B. thuringiensis* from fermentation broth using microfiltration: An optimization approach. *Research Journal of Biotechnology*, 9, 33-37.
- Marzban, R., Saberi, F., & Shirazi, M. M. (2016). Microfiltration and Ultrafiltration of *B. thuringiensis* Fermentation broth: Membrane performance and spore-crystal recovery approaches. *Brazilian Journal of Chemical Engineering*, 33, 783-791
- Mishra, J., Tewari, S., Singh, S., & Arora, N. K. (2015). *Biopesticides: Where we stand? Plant microbes symbiosis: Plant microbes symbiosis: applied facets*. New Delhi, Springer. pp. 37–75. https://doi.org/10.1007/978-81-322-2068-8_2
- Moazami, N. (2002) *Biopesticides production*. In: *Encyclopedia of life support systems, 3-encyclopedias of biological physiological and health sciences, biotechnology*. EOLSS Publishers Co.
- Nazarian Amirani, A., Jahangiri, R., Salehi, J. G., Seifinejad, A., Soheilvand, S., Bagheri, O., Keshavarzi, M., & Alamisaeid, K. H. (2009). Coleopteran-specific and putative novel cry genes

- in Iranian native *B. thuringiensis* collection. *Journal of Invertebrate Pathology*, 102, 101–109. <https://doi.org/10.1016/j.jip.2009.07.009>
- Özkazanç, O. (1986). Studies on the effectiveness of Tarmik-3 (*B. thuringiensis*) on the control of pine processionary caterpillar and determination of its more effective dosages. *Forest Research Institute Technical Bulletin*, 14.
- Ranjy, H., Marzban, R., & Homaionifar, M. (2005). Comparative efficacy of chemical and biological insecticides for control of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), in potato fields. *Journal of Agricultural Sciences*, 3, 143-150.
- Rashki, M., Maleki, M., Torkzadeh-Mahani, M., Shakeri, S., & Nezhad, P. S. (2021). Isolation of Iranian *Bacillus thuringiensis* strains and characterization of lepidopteran-active cry genes. *Egyptian Journal of Biological Pest Control*, 31, 1-10. <https://doi.org/10.1186/s41938-021-00432-3>
- Reardon, R., Dubois, N., & McLane, W. (1994). *Bacillus thuringiensis* for managing gypsy moth: A review. US Department of Agriculture Forest Service, United States Department Agriculture. <https://doi.org/10.5962/bhl.title.141061>
- Regnault-Roger, C. (2012). *Trends for Commercialization of Biocontrol Agent (Biopesticide) Products*. In: Merillon, J.M. & Ramawat, K.G. (eds) *Plant Defence: Biological Control, Progress in Biological Control 12*. Springer Science + Business Media, Dordrecht. p 139–160. https://doi.org/10.1007/978-94-007-1933-0_6
- Saberi, F., Marzban, R., & Ardjmand, M. (2014). Optimization of *B. thuringiensis* production process in lab Fermenter. *Biological Control of Pests and Plant Diseases*, 3, 165-172. <https://doi.org/10.22059/JBIOC.2014.53960>
- Saberi, F., Marzban, R., & Ardjmand, M., Pajoum Shariati, F., & Tavakoli, O. (2020). Optimization of Culture Media to Enhance the Ability of Local *B. thuringiensis* var. tenebrionis. *Journal of the Saudi Society of Agricultural Sciences*, 19, 468-475. <https://doi.org/10.1016/j.jssas.2020.08.004>
- Saberi, F., Marzban, R., Ardjmand, M., Pajoum Shariati, F., & Tavakoli, O. (2023). Optimization of the Culture Medium, Fermentation Process and Effectiveness of Biopesticide from an Iranian *B. thuringiensis* var. tenebrionis (BN2). *Journal of Agricultural Science and Technology*, 25, 469-484. <http://dx.doi.org/10.52547/jast.25.2.469>
- Salehi, J. G., Pourjan Abad, A., Seifinejad, A., Marzban, R., Kariman, K., & Maleki, B. (2007). Distribution and diversity of Dipteran-species cry and cyt genes in native *B. thuringiensis* strains obtained from different ecosystems of Iran. *Journal of Industrial Microbiology and Biotechnology*, 35, 83-94. <https://doi.org/10.1007/s10295-007-0269-6>
- Salehi, J. G., Seifinejad, A., Saeedizadeh, A., Nazarian, A., Yousefloo, M., Soheilvand, S., Mousivand, M., Jahangiri, R., Yazdani, M., Amiri, M. R., & Akbari, S. (2008). Molecular detection of nematocidal crystalliferous *B. thuringiensis* strains of Iran and evaluation of their toxicity on free-living and plant parasitic nematodes, *Canadian Journal of Microbiology*, 54, 812–822. <https://doi.org/10.1139/W08-074>
- Salehi, J. G., Abbasalizadeh, S., Moradali, M. F., & Morsali, H. (2015). Development of a Cost Effective Bioprocess for Production of an Iranian Anti-Coleoptera *B. thuringiensis* Strain. *Journal of agricultural science and Technology*, 17, 1-14.

- Seifi Nejad, A., Salehi, J. G., Hosseinzadeh, A., & Abdmishani, C. (2008). Characterization of Lepidoptera-active cry and vip genes in Iranian *B. thuringiensis* strain collection, *Journal of Biological Control*, 44, 216–226. <https://doi.org/10.1016/j.biocontrol.2007.09.010>
- Sezen, K., & Demirbağ, Z. (1999). Isolation and insecticidal activity of some bacteria from the hazelnut beetle (*Balaninus nucum* L.). *Applied Entomology and Zoology*, 34(1), 85-89. <https://doi.org/10.1303/aez.34.85>
- Sezen, K., Kati, H., Nalcacioglu, R., Muratoglu, H., & Demirbağ, Z. (2008). Identification and pathogenicity of bacteria from European shot-hole borer, *Xyleborus dispar* Fabricius (Coleoptera: Scolytidae). *Annals of Microbiology*, 58, 173–179. <https://doi.org/10.1007/BF03175313>
- Shojaaddini, M., Moharramipour, S., Khodabandeh, M., & Talebi, A. A. (2010). Development of a Cost Effective Medium for Production of *B. thuringiensis* Bioinsecticide using food barley. *Journal of Plant Protection Research*, 50, 9-14. <http://dx.doi.org/10.2478/v10045-010-0002-8>
- Thakore, Y. (2006). The biopesticide market for global agricultural use. *Industrial Biotechnology*, 2, 192–208. <https://doi.org/10.1089/ind.2006.2.194>
- Usta, M. (2022). Local isolate of *B. thuringiensis* (Berliner, 1915) (Bacteria: Bacillaceae) from *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae: Spilomelinae) includes cry1, cry3 and cry4 genes and their insecticidal activities. *Turkish Journal of Entomology*, 46, 227-237. <https://doi.org/10.16970/entoted.1017243>
- Yıldız, İ., & Sezen, K. (2017). Microbial control using bacteria of the almond moth, *Cadra (Ephestia) cautella* Walker (Lepidoptera: Pyralidae). *Journal of Stored Products Research*, 74, 98-105. <https://doi.org/10.1016/j.jspr.2017.10.007>
- Yılmaz, S., Ayvaz, A., Akbulut, M., Azizoğlu, U., & Karabörklü, S. (2012). A novel *B. thuringiensis* strain and its pathogenicity against three important pest insects. *Journal of Stored Products Research*, 51, 33–40. <https://doi.org/10.1016/j.jspr.2012.06.004>