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Review / *Derleme*

Entomopathogens in control of urban pests

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Summary: Entomopathogens including viruses, bacteria, fungi, protozoa, rickettsia and nematodes are non-infective to vertebrates. There are lots of researches on their efficacy on urban pests. *Bacillus thuringiensis* var. *israelensis* (Bti) is effective in controlling mosquitos and black flies, *B. thuringiensis kurstaki* and *B. thuringiensis entomocidus* controls Lepidoptera caterpillars. *B. thuringiensis tenebrionis* controls some beetle species. Most of viruses kill immature stages of Lepidoptera and Hymenoptera. Baculoviruses are specific to only a few pests of Lepidoptera and mosquitoes. Fungi have wide spectrum of hosts and the ability to enter via cuticle. They can infect many insects (Dictyoptera, Orthoptera, Dermoptera, Culicidae, Muscidae, Simulidae, Tabanidae, Cimicidae, Vespidae, Formicidae, Lepidoptera, Termitidae) and ticks (Ixodidae and Argasidae). The most common entomopathogenic fungus species are *Beauveria bassiana* and *Metarhizium anisopliae*. Protozoa are pathogenic for insect and some ticks. *Nosema* is the most common genus. However there is necessity to evaluate the potential of Nosema-Ixodidae and Nosema-Insect interactions for tick and insect bio-control. Rickettsias are obligatory intracellular organisms and some species affect ticks. Nematode species in the families of Steinernematidae (*Steinernema* spp., *Neosteirnema* spp.), Heterorhabditidae (*Heterorhabditis* spp.) and Mermithidae are effective on insects and ticks. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) carry the pathogenic bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp.) in their intestines. These bacteria are able to kill the host within 24-48 hours. Some entomopathogens or microbial pathogens can be mass-produced, and suitable for commercial use. Current applications include control programs for agricultural, forest and urban pests. This review emphases on the potential of different entomopathogens for bio-control of urban pests and brief information about microbial control agents.

Key words: Entomopathogens, pest, microbial control, virus, bacterium, fungus, protozoa, rickettsia, nematode, insect, tick.

Kent zararlılarının kontrolünde entomopatojenler

Özet: Virüsleri, bakterileri, fungusları, protozoaları, riketsiyaları ve nematodları içeren entomopatojenler omurgalı canlılar için enfektif değildir. Kent zararlılarına entomopatojenlerin etkinlikleri konusunda birçok çalışma vardır. *Bacillus thuringiensis* var. *israelensis* (Bti) sivrisineklerin ve simuliidlerin mücadelesinde etkilidir. *B. thuringiensis kurstaki* ve *B. thuringiensis entomocidus* lepidopter zararlıları, *B. thuringiensis tenebrionis* ise bazı coleopter zararlıları kontrol eder. Virüslerin birçoğu lepidopter ve hymenopter larvalarını öldürür. Bakulovirüsler, lepidopter zararlıların ve sivrisineklerin sadece bir kaç tanesine özelleşmiştir. Funguslar, geniş bir konakçı aralığına sahiptir ve kütikuladan giriş yapabilirler. Birçok böceğe saldıran entomopatojen fungusların en yaygını *Beauveria bassiana* ve *Metarhizium anisopliae*'dir. Protozoalar böcekler ve bazı keneler için patojeniktir. *Nosema* protozoaların en yaygın cinsidir. Ancak protozoalar ile biyolojik böcek ve kene mücadelesi için, Nosema-Ixodidae ve Nosema-insekt etkileşim potansiyelinin değerlendirilmesine ihtiyaç vardır. Riketsiyalar, obligat intraselüler organizmalardır ve bazı türleri keneleri etkiler. Steinernematidae (*Steinernema* spp., *Neosteirnema* spp.), Heterorhabditidae (*Heterorhabditis* spp.) ve Mermithidae familyalarındaki nematod türleri böcekler ve keneler üzerinde etkilidir. Entomopatojenik nematodlar (Steinernematidae ve Heterorhabditidae) bağırsaklarında patojenik bakteriler (*Xenorhabdus* spp. ve *Photorhabdus* spp.) taşırlar. Bu bakteriler konakçısını 24-48 saat içerisinde öldürme yeteneğindedirler. Bazı entomopatojenler veya mikrobiyal patojenler kitle halinde üretilebilirler ve ticari kullanım için uygundurlar. Mevcut uygulamalar, tarımsal, orman ve kent zararlılarının mücadelelerini içermektedir. Bu derlemede, farklı entomopatojenlerin kent zararlıları ile biyolojik mücadeledeki potansiyelleri ve mikrobiyal mücadele ajanları hakkında kısa bilgi verilmiştir.

Anahtar sözcükler: Entomopatojenler, pest, mikrobiyal mücadele, virüs, bakteri, fungus, protozoa, riketsiya, nematod, böcek, kene.

Introduction

Pests are unwanted organisms, because their activities could harm people living in the same ecosystem. Urban pests can be generally characterized as organisms which affect human health, or which damage wooden support structures of buildings. The most common urban pests found in urban areas in the world are cockroaches (*Blattella germanica, Blatta orientalis,* *Periplaneta americana, Periplaneta australasiae*), ants (fire ants), termites, gray silverfish (*Lepisma saccharina*), fleas (*Ctenocephalides felis*), mosquitoes (*Aedes aegyti, Anopheles maculipennis, Culex pipiens*) houseflies (*Musca domestica*), stinging pests (*Vespula vulgaris, Vespula germanica*), carpenter bees (*Xylocopa* spp.), spiders (*Latrodectus mactans, Loxosceles reclusa, Chirocanthium mildei*), ticks (*Rhipicephalus sanguineus, Dermacentor variabilis, Amblyomma americanum, Hyaloma marginatum),* mites *(Sarcoptes scabiei),* bedbugs *(Cimex lectularius)*, and lice (*Pediculus capitus, Pediculus humanus*) (47). Most of these orders of insects and other arthropods include species that have medical importance, either because they bite, sting, suck blood, transmit parasites and pathogens, or because they induce allergies, delusional parasitoids or entomophobia (21-23, 47, 60, 61). The economic importance of the urban pests is high and its management costs lots of money. For example mosquito-borne diseases in Africa cost $12 billion annually (47). There are chemical and nonchemical methods of urban pest control. Microbial control is a nonchemical control and we can use entomopathogens for controlling of urban pests. In this review we focused on microbial control of some urban pests that are important in Turkey.

*Advantages and Disadvantages of Entomopathogens***:** Entomopathogens have some quite striking advantages, but unfortunately, pathogens also have serious disadvantages, particularly in the prospects of successful commercialization. *Advantages;* Host specificity, no toxic residues (human and environmental), unlikely or slow resistance development, compatibility with pesticides, ease of genetic modification. *Disadvantages;* High host specificity, problems of shelf-life and persistence in the field, unsightly insect corpses, and problems with development.

*Short information about Entomopathogens***:** Some researchers reported characteristic of entomopathogens (12, 42, 43). Below, we present some characteristic according to these authors;

*Characteristics of entomopathogens***:** They tend to be specific to certain species or groups of pests and kill them or reduce their reproduction, slow their growth, shorten their life. Effectiveness of entomopathogens may depend on environmental conditions or host abundance, usually relatively slow acting, may take several days to provide adequate control. They can cause rapid mortality, or epizootics under optimal environmental conditions, do not harm non-target organisms, such as beneficial insects, mammals, or plants.

*Fungi***:** Fungal hyphae penetrate the insect cuticle or "skin" and produce spores that multiply throughout the body. Fungi destroy the insect tissue or produce toxins, killing the insect host. Fungal hyphae penetrate outward through the softer parts of the insect and produce spores that are released into the environment to complete their life cycle. Insects that are attacked by fungi often retain their shape but usually become hardened, "mummy-like" and appear "fuzzy" from the fungal growth. Primary hosts of fungi include homopterous, dipteran, coleopteran larvae, Lepidoptera, trips, and mites, some entomo-pathogenic fungi are available in commercial formulations.

*Bacteria***:** Bacteria need to be ingested to infect their hosts. The early insect larval instars are the most susceptible stages to the bacteria. Infected insects show a loss of appetite, sluggishness, discharge from the mouth and anus, discoloration, and liquefaction and putrefaction of the body tissues. *B. thuringiensis* is the most famous one in commercial formulations. Genes of *B. thuringiensis* encoding the toxins were inserted into some plants for controlling of insects.

*Viruses***:** Most insect viruses need to be ingested to successfully infect their host. Some spread from insect to insect during mating or egg laying. Viruses require living insects in which to grow. An infected insect appears sluggish, stops feeding; the cuticle will have a pale discoloration, and often hangs from its legs. The infected insect will die within one or two days after the symptoms appear. Granulovirusses (GVs and Cytoplasmic polyhedral viruses (CPVs) debilitate their hosts. The decomposing cadaver will burst, liberating the viral particles into the environment. Viruses usually attack the larval stage.

*Protozoans (Microsporidia)***:** Protozoans infecting a wide range of insects must be consumed to infect a host. Microsporidia slowly kill the host insect by reducing its reproduction or feeding activity. Infected insects appear sluggish and smaller than normal and may have difficulty molting, and are more susceptible to other mortality factors (other diseases or adverse weather).

*Nematodes***:** They enter the host through natural openings and soft parts between segments. Some of them are tiny (microscopic). Nematodes are effective against many soil or surface dwelling insect pests and some insects living in cryptic habitats. Most entomopathogenic nematodes kill their host within 24-48 hours. Insects killed by nematodes become brownish yellow to red or "milky" in color and the tissue turns to a gummy consistency. Some entomopathogenic nematodes are available in commercial formulations.

Microbial control of some important urban pests

*Cockroaches***:** Many researchers reported that entomopathogens kill cockroaches (53). The most promising of these pathogens are fungi, such as *Cordyceps blattae* (48), *Beauveria bassiana* (63) and *Paecilomyces fumosoroseus* and other fungi (52) being pathogenic for cockroaches. There is report of some strains of *B. thuringiensis* subsp. *kurstaki* caused up to 45% mortality of cockroaches when fed at high concentrations (*Blattaria orientalis, B. germanica* and

Periplanata americana). Various Microsporida and Haplosporida are pathogenic for cockroaches (48). Entomopathogenic nematodes are pathogens of cockroaches. They have been considered as effective in the field condition (3). Koehler *et al.* (26) showed that the time required killing 50% of the cockroaches exposed to *Steinernema carpocapsae* was related inversely to the moisture of their preferred habitats and cockroaches were the most susceptible.

*Mosquitoes***:** Mosquitoes are other urban pests that have microbial antagonists including fungus, protozoa, virus and bacteria. The discovery, in 1977, of the selective mosquito-pathogenic bacterium *B. thuringiensis* var. *israelensis* (Bti) curtailed widespread interest in the search for other suitable biological control agents (49), also Bti and *B. sphaericus* (Bs) are commonly applied into the water to control mosquito larvae (49). When a larva consumes the bacteria, protein crystals produced by the bacteria bind to and destroy the gut tissue of mosquito larva. The products remain effective for 24 to 48 hours. Bs products contain live bacteria and if conditions are favorable bacteria remain effective for more than 30 days (7). Also some fungi have been considered virulent and pathogenic for mosquitoes. The most important entomopathogenic fungi that infect mosquitoes belong to the genera *Lagenidium, Coelomomyces, Entomophthora, Culicinomyces, Beauveria,* and *Metarhizium* (49). *Coelomomyces* spp. is one of the most widely studied fungi that infects mosquitoes (27, 38, 49). *Leptolegnia* spp. are typically encountered in wild isolates. *Coelomomyces* spp. and *Leptolegnia* spp. are highly pathogenic, and they kill mosquito larvae within 72 hours post infection (49). *Entomophthora* spp. typically infects only adult mosquitoes (49). Also *B. bassiana* and *Metarhizium anisopliae* are able to control mosquitoes (49). The two types of viruses routinely found to be pathogenic to mosquitoes are defined as occluded or non-occluded (13). Occluded viruses belong to families (*Baculoviridae* and *Reoviridae*) and non-occluded belong to families (*Iridioviridae* and *Parvoviridae*) (13). Researchers reported that two types of viruses isolated from mosquitoes, but only one of them was found to be detrimental to the development of the mosquitoes larvae (5, 13). *Deltabaculo virus* dipteran-specific NPV is the only member of this family that is commonly associated with feral mosquito larvae. These viral particles infect the larval midgut epithelium resulting in a stunted appearance, delayed growth and death. *Cypoviruses* are also referred as cytoplasmic polyhedrosis viruses (CPV). Infections with CPVs are typically benign in natural conditions but can cause larval mortality only if the inoculum is very high. *Mosquito Iridescent Virus (MIV) is* cosmopolitan. *Mosquitodensovirus (MDV)* infections are usually subtle under natural conditions. Infected larvae will become lethargic, change their body color, have a contorted appearance, or appear whitish in color before expiring (5). Entomopathogenic nematode affect house flies rarely. Mermithidae are a family of elongated round-headed nematode worms. A large number of mermithid species have been described. One of them is *Romanomermis culicivorax* that has been mass-produced for large-scale evaluation. The potential of the mermithids for the control of ground-pool and rice-field mosquitoes could be rather high, wherever they can be recycled at operational levels after having been introduced (59).

*Housefly***:** *Musca domestica* (Diptera: Muscidae), commonly called the house fly. There are available numerous studies that document the attempted use of entomopathogenic fungi, bacteria, virus and nematodes against housefly under laboratory and field conditions. *Entomophthora muscae* is an important natural regulator of fly populations, but constraints imposed by production, storage stability, and low mortality rates have limited its use as an effective control agent (25, 51). *B. bassiana* has many advantages and has been developed for commercial fly control products (11, 30, 31). New improvements in genetic modification of *B. bassiana* could lead to new faster-acting bio-pesticide products that are competitive with conventional insecticides. Early research with exotoxin-producing strains of *B. thuringiensis* was promising, but the shift in emphasis to endotoxin-only strains with high activity against Lepidoptera limited discovery of fly-active strains (1, 36, 50). Surveys have suggested that strains with high levels of the Cry1B endotoxin are more virulent than other strains for muscoid flies (37). Recent successes with *B. thuringiensis* var. *israelensis* in poultry farms suggest that Bti warrants further study (56). House fly salivary gland hypertrophy virus (MdSGHV) has the appealing property of shutting down reproductive development in adult flies but attempts to develop infective baits have been hampered by the refractoriness of older flies to oral infection (15, 16, 32). Space sprays to treat flies directly may have more potential for delivering MdSGHV into fly populations (33, 34). Entomopathogenic nematodes such as *Steinernema* and *Heterorhabditis* spp. and their associated symbionts are virulent for fly larvae in certain substrates (41, 44, 55), but the harsh environments presented by poultry and swine manure are inimical to their survival (6, 17, 41, 45).

*Ticks***:** There are many pathogens of ticks. Some viruses cause damage to ticks' salivary gland and the synganglion. For example, *Alveonasus lahoransis, Boophlilus microplus* and *Ixodes persulcatus* are affected by some viruses and virus-like particles (40). Viruses do not play an important role in reducing tick populations, but virus and virus like organisms are too limited to determine their effect. Some rickettsias are parasites of ticks (4, 18). An infection with *Rickettsia prowazekii* was

Table. Entomopathogens: Some products of entomopathogens and their host range.
Tablo. Entomopatojenler: Bazı entomopatojen preparatları ve konakçı aralığı.

Pathogen or Taxus	Products	Host range
Bacillus thuringiensis var. *kurstaki*	Bactur®, Bactospeine®, Bioworm®, Caterpillar Killer®, Dipel®, Futura®, Javelin®, SOK-Bt®, Thuricide®, Topside®, Tribactur®, Worthy Attack®	Lepidoptera larvae
Bacillus thuringiensis var. israelensis (*Bti*)	Aquabee®, Bactimos®, Gnatrol®, LarvX®, Mosquito Attack®, Skeetal®, Teknar®, Vectobac®	larvae of *Aedes* and *Psorophora* mosquitoes, black flies, and fungus gnats
Bacillus thuringiensis var. *aizawai*	Certan®	wax moth caterpillars, Lepidoptera larvae
Bacillus popilliae and *Bacillus lentimorbus*	Doom, Japidemic, ® Milky Spore Disease, Grub Attack®	larvae (grubs) of Japanese beetle
Bacillus sphaericus	Vectolex CG®, Vectolex WDG®	larvae of *Culex, Psorophora,* and *Culiseta* mosquitos, *Aedes* spp.
Beauveria bassiana	Botanigard®, Mycotrol®, Naturalis®	aphids, fungus gnats, mealy bugs, mites, thrips, whiteflies, mosquito species
Lagenidium giganteum	Laginex®	larvae of most pest mosquito species
Nosema locustae	NOLO Bait®, Grasshopper Attack®	European corn borer caterpillars, grasshoppers and Mormon crickets
Gypsy moth nuclear plyhedrosis (NPV)	Gypchek® virus	gypsy moth caterpillars, ticks
Steinernema feltiae *S. riobravis*, *S. carpocapsae* and other *Steinernema* species	Biosafe®, Ecomask®, Scanmask®, also sold generically (wholesale and retail), Vector®	larvae of a wide variety of soil-dwelling and boring insects
Heterorhabditis heliothidis	currently available on a wholesale basis for large scale operations	larvae of a wide variety of soil-dwelling and boring insects
Steinernema scapterisci	Nematac®S	late nymph and adult stages of mole crickets

fatal to ticks including *Dermacentor andersoni*, *D. marginatus, D. raticulatus* (9). Rickettsia like organisms infect females of *Rhipicephalus bursa* and they were found in its malphigian tubules and was found that they caused up to 50% mortalitiy of this tick (14). There are lots of bacteria infecting ticks (2, 19, 29). *Proteus mirabilis* might hold promise for biological control of adult ticks because it causes abnormalities or mortality in the next generation (8). Spores of *B. thuringiensis* affects tick *Ornithodoros serraticus* and cause up to 100% mortality. Some protozoa species like *Nosema* spp. are pathogenic to ticks (28, 58). But the major pathogens of ticks are fungi, because they have wide host range and are able to enter the host body via the cuticle (20). It was reported that fungal infection may cause death of up to 50% of *Ixodes* and other ticks (10, 24). However, representatives from only 6 genera out of the 57 major entomopathogenic fungi (Eumaycota, Deuteromycotina) are known to attack ticks (54). Entomopathogenic and entomoparasitic nematodes include species from families Mermithidae, Heterohabditidae and Steinernematidae that enter ticks via their cuticle and natural openings, and then kill hosts (35, 39, 62).

Potential use of Entomopathogens: Entomopathogenic fungi *B. bassiana*, *M. anisopliae*; nematodes from the families Heterorhabditidae and Steinernematidae: bacteria *B. thuringiensis* var. *israilensis*, *B. thuringiensis* var. *kurstaki*; virus NPV and GV groups are used increasingly in commercial formulation against urban pests. There is a brief summary in Table that includes some commercial microbial preparations against arthropod pests (46, 57).

In conclusion, microbial control agents offer effective alternatives for the control of many agricultural, forest and urban pests. Their greatest strength is their safety, as they are essentially nontoxic and nonpathogenic to human and environment. Although not every pest problem can be controlled by the use of a microbial control agent, these control agents can be used successfully to control many arthropod pests instead of toxic insecticides. Because most entomopathogens are effective against only a narrow range of pests and vulnerable to rapid inactivation in the environment, pest controls operators must properly identify target pests and plan the most effective application. Consequently, entomopathogens are likely to become increasingly important tools in pest management.

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