Entomopathogenic Microorganisms in Animals Protection

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The control of ectoparasites requests the development of novel strategies and, among them, the use of entomopathogenic microorganisms appears as a promising tool to achieve an eco-friendly approach.

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1. Entomopathogenic Fungi

Several fungal species are well suited to control arthropods, being able to cause epizootic infection and most of them infect their host by direct penetration through the arthropod's tegument [1]. Most of the organisms are related to biological control of crop pests, but, more recently, have been applied to combat some livestock ectoparasites.

Entomopathogenic fungi (EPFs) have been identified by their growth onto insect cadavers and can be commercially produced to act as biopesticides. Species of *Beauveria, Metarhizium, Lecanicillium* and *Isaria* are relatively easy to mass produce [2]. One of the main concerns about their extensive employ would be related to their sensitivity to temperature as well as ultraviolet radiation [3] and to the presence of a suitable moisture degree, to allow the conidia to germinate [4]. On the other hand, EPFs seem to have a negligible risk of inducing resistance [5], despite their long-term persistence in the environment.

2. Entomopathogenic Bacteria

Among the entomopathogenic bacteria (EPBs), *Bacillus thuringiensis* showed the most relevant activity against arthropods. *B. thuringiensis* is a Gram-positive, rod-shaped, spore-forming bacterium, innocuous for humans, animals and plants. It can be isolated from different environments, such as soil, rhizosphere, phylloplane, freshwater, and grain dusts; furthermore, it can be found in invertebrates and insectivorous mammals [6]. Its entomopathogenic property is related to the production of highly biodegradable proteins. Its action to the insect pest relies on insecticidal toxin and an array of virulence factors [7]. *B. thuringiensis* produces, upon sporulation, insecticidal crystal inclusion formed by several proteins named Cry or Cyt proteins. These proteins have been proven to be toxic to insects belonging to the orders *Lepidoptera, Dipteran, Coleoptera, Hymenoptera, Homoptera, Orthoptera* and *Mallophage* [7]. *B. thuringiensis*, because of its known entomopathogenic activity, has been used worldwide for biological control against several agriculture pests for a long time.

Besides *B. thuringiensis*, *Lysinibacillus* (formely *Bacillus*) *sphaericus* is employed for preventing and controlling pests. Both agents are the only commercial entomopathogenic bacteria that are produced using mass production techniques and sold in sufficient commercial quantities.

3. Ticks

Ticks are large-bodied bloodsucking, nonpermanent parasitic Acari, feeding exclusively on vertebrates. They are divided into three families, among which Ixodidae (hard ticks) represent an important concern for mammalian health, although some of them also feed on birds, and can be carried between continents. Ticks exert a direct damage, feeding on their host. Saliva and/or mouthpart penetration can induce a toxic reaction in hosts, such as tick paralysis [8][9], or allergic state in human patients [10]. Heavy tick infestation can cause severe anemia, considering that an adult female tick can feed up to 2.0 mL of blood from the vertebrate host [11].

3.1. Fungi

The control of ticks by entomopathogenic fungi has been widely studied and, differently from insects, tick eggs are sensitive [12]. Tick species differ in their behavior, range of hosts and life cycle, so also, their sensitivity in comparison to a fungal species is not the same [13]. Furthermore, ticks are reported to be more tolerant to EPFs than other arthropods, so the amount of the inocula for tick control purposes should be larger. Different stages of ticks would exhibit differences in sensitivity versus EPFs. *R. sanguineus* engorged females and unfed other stages appeared more prone to fungal infection with *M. anisopliae* and *M. flavoviride* [14]. Nymphs were reported as less sensitive when compared with other stages [13][15]. A slight difference of sensitivity to *M. brunneum*, between adults and nymphs of *I. scapularis* was also reported [16][17] and larvae are considered the most susceptible stage to EPFs [18]. EPFs active against different tick species are summarized in Table 1.

Table 1. Entomopathogenic fungi (EPFs) active versus different tick species.

Tick Species	EPFs	References
Amblyomma americanum	***Beauveria bassiana***	[19]
Amblyomma parvum	***Metarhizium anisopliae***	[20]
Amblyomma variegatum	***Beauveria bassiana***	[21]
Amblyomma variegatum	***Metarhizium anisopliae***	[21]
Amblyomma variegatum	***M. anisopliae + B. bassiana***	[22]
Boophilus microplus	***Beauveria bassiana***	[23]
Boophilus microplus	***Metarhizium anisopliae***	[24][25][23][26][27][28]
Boophilus sp.	***Fusarium sp.*** ***Metarhizium anisopliae***	[29]
Dermacentor albipictus	***Beauveria bassiana***	[30]
Dermacentor albipictus	***Metarhizium anisopliae***	[30]
Dermacentor albipictus	***Metarhizium brunneum***	[30]
Dermacentor marginatus	***Aspergillus fumigatus***	[31]
Dermacentor marginatus	***Trichothecium roseum***	[32]
Dermacentor reticulatus	***Isaria fumosorosea***	[33]
Dermacentor reticulatus	***Beauveria bassiana***	[33]
Dermacentor reticulatus	***Metarhizium anisopliae***	[33]
Dermacentor reticulatus	***Metarhizium robertsii***	[33]
Dermacentor sp.	***Beauveria bassiana***	[34]
Dermacentor variabilis	***Metarhizium anisopliae***	[16]
Dermacentor variabilis	***Beauveria bassiana***	[16]
Dermacentor variabilis	***Scopulariopsis brevicaulis***	[35]
Haemaphysalis longicornis	***Beauveria bassiana***	[36]
Haemaphysalis qinghaiensis	***Metarhizium anisopliae***	[37]
Haemaphysalis qinghaiensis	***Beauveria bassiana***	[37]
Hyalomma anatolicum	***Beauveria bassiana***	[38]
Hyalomma anatolicum	***Metarhizium anisopliae***	[38]
Hyalomma anatolicum	***Paecilomyces lilacinus***	[38]
Hyalomma lusitanicum	***Beauveria bassiana***	[39][40]
Hyalomma scupense	***Aspergillus fumigatus***	[31]

Tick Species	EPFs	References
Ixodes dammini	*Aspergillus ochraceus*	[29]
Ixodes dammini	*Metarhizium anisopliae*	[41]
Ixodes ricinus	*Conidiobolus coronatus*	[42]
Ixodes ricinus	*Aspergillus flavus*	[43]
Ixodes ricinus	*Aspergillus fumigatus*	[43]
Ixodes ricinus	*Aspergillus niger*	[34]
Ixodes ricinus	*Aspergillus parasiticus*	[34]
Ixodes ricinus	*Beauveria bassiana*	[29][33]
Ixodes ricinus	*Beauveria brognardi*	[42]
Ixodes ricinus	*Paecilomyces farinosus*	[42]
Ixodes ricinus	*Paecilomyces fumosoroseus*	[34][42]
Ixodes ricinus	*Penicillium insectivorum*	[43]
Ixodes ricinus	*Trichothecium roseum*	[32]
Ixodes ricinus	*Verticillium aranearum*	[42]
Ixodes ricinus	*Verticillium lecanii*	[34][42]
Ixodes ricinus	*Metarhizium anisopliae*	[33]
Ixodes ricinus	*Metarhizium robertsii*	[33]
Ixodes ricinus	*Isaria fumosorosea*	[33]
Ixodes scapularis	*Metarhizium brunneum*	[16][17][18][44]
Ixodes scapularis	*Metarhizium anisopliae*	[16]
Ixodes scapularis	*Beauveria bassiana*	[16]
Rhipicephalus annulatus	*Metarhizium brunneum*	[45]
Rhipicephalus appendiculatus	*Aspergillus sp.*	[46]
Rhipicephalus appendiculatus	*Fusarium sp.*	[46]
Rhipicephalus appendiculatus	*Metarhizium anisopliae*	[47][21][46]
Rhipicephalus appendiculatus	*Beauveria bassiana*	[47]
Rhipicephalus appendiculatus	*M. anisopliae + B. bassiana*	[21]
Rhipicephalus decoloratus	*Beauveria bassiana*	[48]
Rhipicephalus microplus	*Metarhizium robertsii*	[49][50][51][52]
Rhipicephalus microplus	*Beauveria bassiana*	[53][51][54][55][56][57][58][59]
Rhipicephalus microplus	*Metarhizium anisopliae*	[53][51][60][56][57][58][59]
Rhipicephalus microplus	*Paecilomyces lilacinus*	[53]
Rhipicephalus microplus	*Isaria fumosorosea*	[61]
Rhipicephalus microplus	*Isaria farinosa*	[61]
Rhipicephalus microplus	*Purpurocillium lilacinus*	[61]
Rhipicephalus sanguineus	*Aspergillus ochraceus*	[62]
Rhipicephalus sanguineus	*Fusarium sp.*	[63]
Rhipicephalus sanguineus	*Curvularia lunata*	[64]

Tick Species	EPFs	References
Rhipicephalus sanguineus	***Rhizopus thailandensis***	[64]
Rhipicephalus sanguineus	***Rhizopus arrhizus***	[64]
Rhipicephalus sanguineus	***Metarhizium anisopliae***	[13][14][15][16]
Rhipicephalus sanguineus	***Metarhizium flavoviride***	[14]
Rhipicephalus sanguineus	***Isaria fumosorosea***	[14]
Rhipicephalus sanguineus	***Beauveria bassiana***	[16]

3.2. Bacteria

Some bacterial species have been demonstrated to be pathogenic for ticks; thus, they are considered useful for biological control. Among EPBs, *B. thuringiensis* is the most studied agent with activity against ticks [65] and is largely employed in commercial insecticide formulations. The pathogenic action of *B. thuringiensis* normally occurs after the ingestion of spores by ticks, and the crystalline inclusions containing insecticidal δ-endotoxins specifically interact with receptors in the insect midgut epithelial cells [66].

4. Dermanyssus Gallinae

The genus *Dermanyssus* comprises hematophagous mite species, parasites of birds. The taxonomy of species within the genus was not clearly defined, until now [67]. *Dermanyssus gallinae* (poultry red mite) is very common in layer houses and is considered as the most damaging to laying hens worldwide [68]. Although birds are first choice hosts, *D. gallinae* feed on humans and other mammals, too [69], and can act as a vector for several pathogens of poultry [70], as well as zoonotic agents [71].

4.1. Fungi

Entomopathogenic fungi have been assayed to control the mite population. *B. bassiana*, *M. anisopliae, Trichoderma album*, and *P. fumosoroseus* are the most studied fungal species. The use of fungal entomopathogens to control arthropod pests as biological agents would be suggested considering their easy direct penetration through arthropod tegument, the lack of induction of host resistance, the ability to horizontally transmit from fungus-infected to uninfected arthropods, mostly in moist environments [72] and potential damage to flies, lice, and other pests [73][74]. *B. bassiana*, *P. fumosoroseus* and *M. anisopliae* were proven to kill several red mites, when administered in high doses, with a variability depending on the isolate [75][76][77][78], being able to cause high mortality within 5 days [76]. Different strains of *M. anisopliae* have been successfully applied to control the mites, under laboratory conditions, demonstrating differences in pathogenicity with a dose- and time-dependent effect [79].

4.2. Bacteria

The use of *B. thuringiensis* has been proposed as an alternative control method to chemical acaricides against *D. gallinae* in integrated management programs. It has been observed that *B. thuringiensis* var. *kurstaki* is able to damage the cuticle of *D. galllinae* and cause the loss of mobility of this mite in a period of 24 h [80]. Moreover, Torres and Hernandez [81] observed a moderate mortality of *D. gallinae* from day 2 of application (66%), which increased up to 78% at 7 days at a concentration of 35 mg/mL. Similarly, a previous study by Mullens et al. [82] on the fowl mite *Ornithonyssus sylviarum*, revealed that this mite was susceptible to *B. thuringiensis*, and the authors concluded that the entomopathogen had potential for the development of a control preparation for direct application to poultry.

5. *Psoroptes sp.*

Psoroptes mites are non-burrowing Acharina, responsible for ear and body mange of herbivores. *Psoroptes ovis* severely impacts on animal health. It induces an exudative dermatitis in beef cattle and sheep which, when not treated, can lead affected animals to lose condition and, sometimes, to death [83][84].

5.1. Fungi

In a comparative in vitro study with *Hirsutella thompsonii*, *M. anisopliae* was highly pathogenic and suitable for the control of *P. ovis* [85]. These features were furtherly corroborated by observing the efficiency of the mold in producing fatal infections, as well as the infectiveness of 5-day-old cadavers of mites [86]. The higher infectivity of *M. anisopliae* in comparison with *B. bassiana* was assessed in vivo, too [87]. The strong parasite killing of *M. anisopliae* seems to be related to its ability to induce the oxidative damage of mites [88].

5.2. Bacteria

The in vitro acaricidal effect of *B. thuringiensis* on *P. cuniculi* has been demonstrated. The bacterium can induce histological alterations of this mite, such as the presence of dilated intercellular spaces in the basal membrane, membrane detachment of the peritrophic matrix and morphological alterations in columnar cells of the intestine [89].

Similarly, the combined use of *B. thuringiensis* and ivermectin has been proposed by some authors to combat *Psoroptes* sp., in view of a potential synergistic or additive effect with the possibility of lowering the dose of ivermectin [89].

6. *Varroa destructor*

Varroa destructor is a parasite Mesostigmata mite, exerting a huge impact on beekeeping. It has become a global parasite, switching host onto Apis mellifera from Apis cerana. Varroasis is often a threat for colonies, when nearby colonies collapse [90].

V. destructor has been reported to be susceptible to the entomopathogenic fungi, M. anisopliae, B. bassiana, Verticillium lecanii, Hirsutella spp. [29][91], Hirsutella thompsonii [92][93], B. bassiana [94] and M. anisopliae [95][96]. Clonostachys rosea (formerly Gliocladium roseum) is an Ascomycete, belonging to Hypocreales, widely distributed in soil, and provided by an endophytic ability in tissues from several plants. The mold produces conidia and chlamydospores.

7. Zoonotic Potential of EPFs

Bacillus thuringiensis has been associated to different human infections; it has been cultured from marginal and apical periodontitis, wounds, corneal ulcera and gastrointestinal infections in humans [97]. Even though this EPB is not considered as a traditional zoonotic agent, its presence in different forms of human infections suggests that, at least in immunocompromised patients, it could represent a risk. *B. thuringiensis*, similarly to *B. cereus*, produces several virulence factors potentially acting against mammalian cells, such as hemolysins and enterotoxins [98].

For these reasons, the use of *B. thuringiensis* in pest control should be carried out with attention to avoid possible infections, mainly in operators.

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