Biological Control of Bulb Mites

Subjects: Entomology | Biotechnology & Applied Microbiology

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Mites of the genus *Rhizoglyphus* (Acari: Acaridae) are serious pests of plants belonging to the orders Liliales and Asparagales such as onions, garlic, lilies, and tulips. Their control by synthetic pesticides is becoming problematic as a result of resistance development in these mites and environmental and health issues. New pest control methods thus need to be developed. Efforts to develop biological control programs for bulb mites have taken place in a number of countries. Several biocontrol agents have been tested against *Rhizoglyphus* spp. under laboratory and some also under field conditions. The most promising results have been obtained with acaropathogenic/entomopathogenic fungi and predatory mites as described below. Other possible prospective control agents attacking mites are viruses, bacteria, and protista, but except for some bacteria their efficacy against bulb mites has not been investigated yet.

Rhizoglyphus | biological control | natural enemies | predatory mites | entomopathogenic fungi | Metarhizium | nematodes | food web | multitrophic interactions | holistic approach | Liliales | Asparagales

1. Bacteria

The soil bacterium *Bacillus thuringiensis* Berliner has been proven to be effective against some mite pests [1][2]. To the researchers' knowledge, there are only two reports on the efficacy of this biocontrol agent or its toxins against bulb mites. The study by Carter et al. [3], did not find any significant effect of *B. thuringiensis* Cry3Aa and Cry3Bb1 coleopteran-active delta-endotoxins on *Rhizoglyphus robini* (Claparède). On the other hand, a recent (2020) Chinese patent [4] claims genetically modified *B. thuringiensis* to be highly efficient for *Rhizoglyphus* spp. control with the example of *Rhizoglyphus echinopus* (Fumouze and Robin) where its population was reduced by 93.2%.

Nermuť et al. [5] investigated whether metabolites of nematode symbiotic bacteria of the genus *Xenorhabdus* sp. or *Photorhabdus* sp. could be suitable for *R. robini* control. Mortality of mites treated by culture supernatants of these bacteria varied considerably among *Xenorhabdus* species and strains. The most effective were strains of *X. doucetiae*, *X. bovienii*, *X. griffiniae* and an unidentified *Xenorhabdus* sp., causing mortality between 10% to almost 30%. Despite the low mortality, some bacterial strains had a repellent effect on mites [5].

2. Acaropathogenic and Entomopathogenic Fungi

Acaropathogenic (APFs) and entomopathogenic fungi (EPFs) are common in nature, have a cosmopolitan distribution and cause natural epizootics in populations of insects, mites or other arthropods [6][7]. Fungal pathogens are a permanent component of mite natural habitats [8]. The advantage of EPFs, in contrast to other biocontrol agents, is that most of them are able to persist in soils for months or even years, in the absence of arthropod hosts [9][10][11]. A recent (2022) study by Konopická et al. [12] evaluated species richness and density of these fungi in soil samples collected in onion and garlic fields. EPFs *Beauveria* spp., *Cordyceps* spp., *Lecanicillium* spp., *Metarhizium* spp. and *Purpureocillium* spp. were isolated. The highest density was observed in the genus *Metarhizium* in which the average density of colony forming units (CFU) per 1 mL of soil sample reached 1.47×10^4 while the lowest density was observed in the genus *Beauveria*. Interestingly, soils in the Czech Republic contained about ten times higher number of EPFs compared to Israel [12].

Besides Acari-specific pathogens such as *Hirsutella thompsonii* (Fisher) and *Neozygites* spp. (Entomophthorales), 'nonspecialist' mitosporic fungi (Hyphomycetes) such as *Beauveria bassiana* (Bals.-Criv.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorokin, *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha and Spatafora (formerly *Isaria fumosorosea*), *Cordyceps farinosa* (Holmsk.) Kepler, B. Shrestha and Spatafora (formerly *Isaria farinosa*), and *Lecanicillium lecanii* (Zimm.) Zare and W. Gams have potential to control some mite species [13][14][15][16].

Soil is considered to be a very favorable environment for EPFs application due to the high humidity conditions necessary for spore germination and host infection. On the other hand, soil-inhabiting bulb mites might have evolved at least to some extent resistance to EPFs. Indeed, a compound named hexyl rhizoglyphinate found in *R.* robini cuticle was shown to possess antifungal activity [17]. The role of other compounds, such as the monoterpenoids robinal [18] and isorobinal [19] in adaptation of bulb mites to live next to some acaro/entomopathogenic fungi in soil environments remains to be explored.

To the researchers' knowledge, only four studies have tested whether APFs or EPFs are effective in controlling bulb mites under laboratory or greenhouse conditions [14][15][20][21]. Sztejnberg et al. [21] reported no pathogenicity of an isolate of *Hirsutella kirchneri* (Rostrup) Minter, Brady and Hall, obtained from the cereal rust mite, *Abacarus hystrix* Nalepa (original accession number CMI 257456) against *R. robini*, despite several procedures attempted for mite infection (spraying, dipping, tipping fungal cultures over hosts).

Konopická et al. [14] evaluated the effecacy of 17 isolated and 3 reference strains of EPFs against *R. robini* females. Results revealed high variability in *R. robini* mortality among EPF species and strains. The highest efficacy against *R. robini* mites was found in the strain of *M. anisopliae* isolated from soil samples collected in the Czech Republic which caused mortality up to 99.3%, and strain of *Metarhizium indigoticum* (Kobayasi and Shimizu) Kepler, S.A. Rehner and Humber from Israel causing 98.3% mortality, four days from spray application. The concentration-response models indicated that the latter strain was more virulent than *M. anisopliae* strains. The median lethal concentration (LC_{50}) in *M. indigoticum* strain was estimated as 1.01×10^4. *Cordyceps fumosorosea* strains did not cause mortality higher than 40%. The lowest virulence was then found in *Beauveria* spp. strains causing mortality of mites between 5 and 25%.

Another recent (2020) study by Ment et al. [15] demonstrated high efficacy of *Metarhizium brunneum* Petch (isolate Mb7) against *R. robini*, which was susceptible to directly applied Mb7 conidia. Conidia of this fungus applied in vitro at concetration 1 × 10^7 caused 43% and 100% mortality of mites at three- and seven-days post inoculation, respectively and the estimated LT_{50} value was 4.3 days. Drench application in potted onion experiments also significantly reduced bulb mite populations compared to the untreated control.

The fungus *Metarhizium* spp. was also effective against *R. robini* in the study by Ko et al. [20]. In total, 11 isolates were selected for further study through a re-evaluation of the pathogenicity of the isolates. Conidial suspension with a concentration 1 × 10^7 conidia/mL of fungi *Metarhizium pinghaense* Q.T. Chen and H.L. Guo (isolate 3–1–2) and *M. anisopliae* (isolates 3–2–2 and 4–18–3) caused more than 80% mortality after 7 days. An isolate of *Metarhizium pemphigi* (Driver and R.J. Milner) Kepler, S.A. Rehner and Humber (isolate 1–1–1) and two isolates of *M. anisopliae* (isolates 4–3–2 and 4–31–2) showed mortality of 90% or more on the fifth day and 100% mortality after seven days.

3. Entomopathogenic Nematodes

Nematodes are not common parasites of mites but some mite-parasitic species are known. For example several allantonematid nematodes use mites as definitive hosts [13]. Usually the host is not killed but is slowly sterilised. Since these obligate parasites have not been raised on artificial media, their usefulness as biological control agents is limited. Mites can also serve as intermediate hosts of nematode parasites of vertebrates [13].

On the other hand, entomopathogenic nematodes (EPNs) belonging to families Steinernematidae and Heterorhabditidae can be produced in large scale with some species commercially available and successfully applied against many pests [22][23][24][25]. The only study that has explored the potential of entomopathogenic nematodes to infect bulb mites, specifically *R. robini*, was published in 2019 [5]. In this study, the bulb mites were exposed to the infective juveniles of 20 strains of *Steinernema* and *Heterorhabditis* species applied at a dose of 300 infective juveniles per mite, and the invasion rate and mite mortality were assessed. The results showed that some EPNs, especially those with small body diameter, are able to invade and kill adult females of *R. robini*. The most promising species were *Steinernema huense* Phan, Mráček, Půža, Nermuť and Jarošová, *Heterorhabditis bacteriophora* Poinar and *Heterorhabditis amazonensis* Andaló, Nguyen and Moino, which caused mortality in *R. robini* up to 30%. The authors concluded that although some EPN species are able to invade and kill bulb mites, their efficacy is in general quite low and they do not seem to represent a viable option for bulb mite biocontrol as a standalone approach [5]. EPNs have, however, other important functions in soil as they can disseminate fungal spores [26], serve as prey for invertebrate predators including mites and springtails or as a host for nematode-trapping fungi, such as *Orbilia oligospora* (Fresen.) Baral and E. Weber, *Monacrosporium eudermatum* (Drechsler) Subram. and *Geniculifera paucispora* (R.C. Cooke) Rifai [27].

4. Predatory Mites

Biological control programs for bulb mites have focused on using predatory mites, mainly in the family Laelapidae (**Table 1**), which feed on soil-dwelling pests [28]. Studies prior to 1990 were limited to examination of predator behavior and their ability to feed and reproduce on a diet of bulb mites. Zedan [29] reported that protonymphs, deutonymphs, and adults of *Gaeolaelaps aculeifer* (Canestrini) feed and developed on all stages of *R. echinopus*. Reproductive potential of the predator was highest when it fed on adult prey, but fewer prey was consumed. Ragusa and Zedan [30] examined interactions between these two species collected from local populations in Italy, and found that both immature and adult *G. aculeifer* preferred to feed on immature rather than adult *R. echinopus*. In contrast to Zedan [29], reproductive potential was highest when predators fed on a diet of eggs and immatures of *R. echinopus*.

Table 1. List of biocontrol agents tested for control of bulb mites.

Group	Family	Species	References
Bacteria	Bacillaceae	*Bacillus thuringiensis* Berliner	[3][4]
	Morganellaceae *	*Xenorhabdus bovienii* Akhurst and Boemare	[5]
		Xenorhabdus budapestensis Lengyel et al.	
		Xenorhabdus cabanillasii Tailliez et al.	
		Xenorhabdus doucetiae Tailliez et al.	
		Xenorhabdus griffiniae Tailliez et al.	
		Xenorhabdus kozodoii Tailliez et al.	
		Xenorhabdus magdalenensis Tailliez et al.	
		Xenorhabdus nematophila (Poinar and Thomas) Thomas and Poinar	
		Xenorhabdus poinarii (Akhurst) Akhurst and Boemare	
		Xenorhabdus stockiae Tailliez et al.	
		Xenorhabdus sp.	
		Photorhabdus sp.	
Entomo-pathogenic fungi	Clavicipitaceae	*Metarhizium anisopliae* (Metsch.) Sorokin	[14][20]
		Metarhizium brunneum Petch	[15]
		Metarhizium indigoticum (Kobayasi and Shimizu) Kepler, S.A. Rehner and Humber	[14]

Group	Family	Species	References
		Metarhizium pemphigi (Driver and R.J. Milner) Kepler, S.A. Rehner and Humber	[20]
		Metarhizium pinghaense Q.T. Chen and H.L. Guo	[20]
	Cordycipitaceae	*Beauveria bassiana* (Bals.-Criv.) Vuill.	[14]
		Beauveria brongniartii (Sacc.) Petch	[14]
		Cordyceps fumosorosea (Wize) Kepler, B. Shrestha and Spatafora	[14]
	Ophiocordycipitaceae	*Hirsutella kirchneri* (Rostrup) Minter, Brady and Hall	[21]
Entomo-pathogenic nematodes	Steinernematidae	*Steinernema carpocapsae* (Weiser)	[5]
		Steinernema huense Phan, Mráček, Půža, Nermuť and Jarošová	
		Steinernema surkhetense Khatri-Chhetri, Waeyenberge, Spiridonov, Manandhar and Moens	
		Steinernema sp.	
	Heterorhabditidae	*Heterorhabditis amazonensis* Andaló, Nguyen and Moino	[5]
		Heterorhabditis bacteriophora Poinar	
		Heterorhabditis beicherriana Li, Liu, Nermuť, Půža and Mráček	
		Heterorhabditis floridensis Nguyen, Gozel, Koppenhöfer and Adams	
		Heterorhabditis indica Poinar et al.	
		Heterorhabditis taysearae Shamseldean	
		Heterorhabditis sp.	
Predatory mites	Ascidae	*Protogamasellus minutus* Nasr	[31]
	Blattisociidae	*Lasioseius* sp.	[32]
		Lasioseius africanus Nasr	[33]
		Lasioseius allii Chant (mentioned as *Lasioseius bispinosus* Evans)	[34]

Group	Family	Species	References
[35]	Laelapidae	*Cosmolaelaps barbatus* Moreira, Klompen and Moraes	[28]
		Cosmolaelaps jaboticabalensis Moreira, Klompen and Moraes	[28]
		Gaeolaelaps aculeifer (Canestrini)	[29][30][34][35][36][37]
		Stratiolaelaps miles (Berlese) [34]	[34]
	Macrochelidae	*Macrocheles embersoni* Azevedo, Berto and Castilho	[38]
		Macrocheles muscaedomesticae (Scopoli)	[38]
		Macrocheles robustulus (Berlese)	[38]
	Parasitidae	*Parasitus fimetorum* (Berlese)	[34]
	Rhodacaridae [37]	*Protogamasellopsis zaheri* Abo-Shnaf, Castilho and Moraes (mentioned as *Protogamasellopsis posnaniensis* Wisniewski and Hirschmann)	[39]

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Other Mesostigmata have also been reported feeding on *R. echinopus* [28][32][39] and *R. robini* [31][33][34]. Wu et al. [32] reported that an unidentified species of *Lasioseius* (Blattisociidae) developed and reproduced by feeding on *R. echinopus*. Castilho et al. [39] demonstrated that *Protogamasellopsis zaheri* Abo-Shnaf, Castilho and Moraes (mentioned as *Protogamasellopsis posnaniensis* Wisniewski and Hirschmann) (Rhodacaridae) fed on *R. echinopus* and reproduced. Moreira and Moraes [28] observed that *Cosmolaelaps barbatus* Moreira, Klompen and Moraes and *Cosmolaelaps jaboticabalensis* Moreira, Klompen and Moraes (Laelapidae) oviposited when fed *R. echinopus*. Azevedo et al. [38] observed that *Macrocheles embersoni* Azevedo, Berto and Castilho, *Macrocheles muscaedomesticae* (Scopoli) and *Macrocheles robustulus* (Berlese) (Macrochelidae) were able to consume *R. echinopus*, but with a very low consumption compared to other prey. In the case of *R. echinopus*, Afifi et al. [31] observed the development and reproduction of *Protogamasellus minutus* Nasr (Ascidae) when fed with this prey. Also, Lesna et al. [34] reported that *Lasioseius allii* Chant (mentioned as *Lasioseius bispinosus* Evans) (Blattisociidae) and *Parasitus fimetorum* (Berlese) (Parasitidae) were successfully reared on *R. robini*, and the latter was able to suppress the prey when peat was used as substrate. Mowafi [33] observed the development and reproduction of *Lasioseius africanus* Nasr (Blattisociidae) feeding on *R. robini*. However, all of these studies were conducted in the laboratory and only in the case of *P. fimetorum* were small-scale population experiments conducted in closed flasks. Further studies are needed to explore the potential of these species as biocontrol agents in potted plant and field trials.

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