Review Article

Integrating Entomopathogenic Nematodes in Managing Pests of Honeybees

Mahfouz M.M. Abd-Elgawad

Plant Pathology Department, National Research Centre, Elbehoth St, Dokki 12622, Giza, Egypt.

Abstract | Honeybees (*Apis mellifera* L.) are key pollinators of many economic crops. The significance of the beehive products is also reflected at several levels including economics of local/export markets, and effects of related activities on the society and environment. However, accumulating reports and figures have been recording serious trends of losses in honeybee populations in the recent past. Different pests/pathogens rank high among other factors responsible for such losses. They resulted in less-than-desirable production in quantity and quality of apiculture. This has spurred enthusiasm among stakeholders and the general public due to the threat these pests pose to global food security. Recently, integrated pest management (IPM) schemes are emerging as the best option for controlling them as chemical pesticides can adversely affect both honeybee individuals and hive products. Added to polluting environment by such excessive chemicals, pests have also been developing their resistance. In contrast, entomopathogenic nematode (EPN) products are developing mostly to control pests of crops and are limited to few niche markets. Hence, expanding their safe and effective biocontrol spectra to key pests of honeybees are highlighted herein. Recent findings hold promise for their inclusion as components in IPM plans for sustainable systems. Related key findings and conclusions are presented herein. They are substantially based on prophylactic measures to avoid the conditions that draw pests of *A. mellifera* or assist them to build their populations. Maintaining healthy and robust honey bee colonies can reflect the importance of such preventive means. Using EPNs against three major bee pests that cause significant damage to beehives and results in considerable losses worldwide is addressed. These included the small hive beetle (*Aethina tumida* Murray), the greater wax moth (*Galleria mellonella* L.), and the lesser wax moth (*Achroia grisella* Fabricius). Other beneficial nematodes bode well for controlling further pests of *A. mellifera*. So, additional experimentation is needed to extend nematode efficacy against more pests/pathogens of *A. mellifera*. Together, such realistic cohort studies that address IPM schemes in beehives will lead to a better grasping of their impacts on colony health and the effects of other linked factors. It is concluded that sophisticated techniques of nematode characterization and application as benign/reliable alternatives to unhealthy pesticides against these pests will broaden EPN uptake and further activate the market of organic products.

Received | October 20, 2024; **Accepted** | July 07, 2025; **Published** | September 02, 2025
***Correspondence** | Mahfouz M.M. Abd-Elgawad, Plant Pathology Department, National Research Centre, Elbehoth St, Dokki 12622, Giza, Egypt; **Email:** mahfouzian@email.com
Citation | Abd-Elgawad, M.M.M, 2025. Integrating entomopathogenic nematodes in managing pests of honeybees. *Pakistan Journal of Nematology*, 43(2): 122-133.
DOI | https://dx.doi.org/10.17582/journal.pjn/2025/43.2.122.143
Keywords | *Achroia grisella*, Advanced technologies, *Aethina tumida*, Emerging nematode application, *Galleria mellonella*, Honeybee pests, Integrated pest management, *Pheromermis*

Introduction

Honey bee (*Apis mellifera* L. Hymenoptera: Apidae) populations are key insect pollinators of many economically important crops; comprising vegetables, fruits, and nuts. These pollinators recorded an approximate annual value of $17±18 billion in USA (Calderone, 2012). Moreover, honeybees and related products represent productive activities suitable for small and medium enterprises, and contribute to solving the problem of unemployment. Thus, Khattabi (2022) stressed that apiculture sector has several social, economic, health, and environmental benefits. Nonetheless, this sector suffered from numerous pest management inadequacies which have accounted for less-than-desirable pollination service in the recent past. Furthermore, such inadequacies downgraded quantity and quality of bee hives-productions in numerous countries. For instance, honeybees have been suffering considerable annual colony losses in USA. American beekeepers alone lost over 50% of their colonies in 2018 (United States Department of Agriculture, USDA, 2019). Likewise, the current figures indicate that the number of bee hives has decreased in Egypt from about 1.40 million hives in year 2000 to about 818 thousand hives in year 2017; a decrease of about 42% during this period. Consequently, honey production decreased from about 8.21 thousand tons in 2000 to about 4.14 thousand tons in 2017 (El-Tatawy *et al*., 2019). Hence, supporting apiculture is direly required to maximize the above-mentioned benefits and relevant activities. This is especially timely as current economic stresses threaten people everywhere with mounting conflicts, plagues, and mass migrations. In contrast, local consumption of bee products and exportation of honeybees and related products by some countries can provide foreign exchange revenue to alleviate such stresses. Admittedly, within the repercussions of these stresses, there is a challenge on the part of both private and governmental sectors to boost the size and quality of apiculture worldwide.

Various insects and pathogens are key barriers to optimize the qualitative and quantitative yield of apiaries (USDA, 2019; Khattabi, 2022). Chemicals are being used against pests and pathogens to enhance yields of apiaries (Glenny *et al*., 2017; Kegley *et al*., 2024). On the contrary, such chemical pesticides often destroy beneficial insects, give rise to secondary pest outbreaks, and evidence ecological pollution and health hazards (Abd-Elgawad, 2020). Therefore, the related problems have spurred enthusiasm for exploiting a full useful spectrum of safe and effective biological insecticides against pests of honey bees and their hive contents. Among them, entomopathogenic nematodes (EPNs) rank high for managing pests of honey bees. They are defined as a group of nematodes (thread worms), that cause death to insects. They possess virtually all the attributes of an ideal biocontrol agent. They are extraordinarily lethal with most insects being killed within 36 hrs. Dozens of important insect pests are susceptible yet nontarget populations are undisturbed. Despite their high pathogenicity to insects, their lack of threat to the environment led the U.S. Environmental Protection Agency to exempt nematodes, including transgenic strains, from registration requirements; early almost all countries provide a similar exemption. This high degree of safety means that unlike chemicals, or even *Bacillus thuringiensis*, nematode applications do not require masks or other safety equipment; and re-entry time, residues, pollution, and pollinator toxicity are not issues. Their application via irrigation has further improved grower acceptance. In addition to their ideal attributes as bioinsecticides, EPNs are compatible with existing equipment and have the potential to recycle in host populations of these pests (Abd-Elgawad *et al*., 2017; Shehata *et al*., 2021). Consequently, such merits attract stakeholders to harness them for effective and long-term inhibition of pest populations (Koppenhöfer *et al*., 2020). Contrary to chemical insecticides, EPNs can support both honeybee pollination services and sales of hive products via methods that neither degrade the environment nor harm human health. Species in the genera *Heterorhabditis* and *Steinernema* (Family: Heterorhabditidae and Steinernematidae, respectively) of these insecticidal nematodes can complete their lifecycles within their host bodies (Figure 1). Having invaded the hosts, EPNs release mutualistic bacteria which back the nematodes to feed on their hosts, develop, reproduce, and finally exit the host cadaver to seek for new hosts. More details of this process are reported elsewhere (Abd-Elgawad, 2017). This review focuses on optimizing the use of EPNs against main pests of honeybees. It highlights their application as a component in integrated pest management (IPM) with other practical inputs. Since IPM is defined as a comprehensive and sustainable technique to manage pests, it merges various methods like biological, physical, chemical, and cultural controls to diminish health, economic, and ecological risks. It prioritizes prevention and uses the least toxic and safe methods to manage pests effectively. These approaches aim to boost biocontrol technology for suppressing key pests of honeybees. While it can protect honeybees as pollinators and improve their useful products, it prevents unacceptable levels of unhealthy chemicals. Consequently, EPNs can minimize the risk to natural resources and the environment, and diminish the development of pest resistance to these chemicals. The focus of this review is on the utilization and potential of entomopathogenic nematodes (EPNs) as biological control agents (BCAs) against key pests of honeybees. Thus, the article addresses enhancing honeybee production by developing improved and sustainable approaches for relevant pest management.

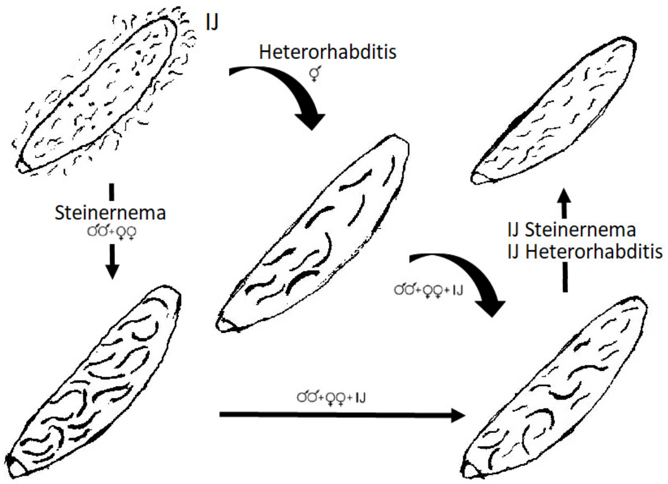

Figure 1: *Differences in developmental stages between species of Steinernema and Heterorhabditis reproducing on honeybee pests.*

The small hive beetle (Aethina tumida Murray, Coleoptera: Nitidulidae)

This pest is a predator and scavenger of honey bees as its larvae develop via feeding on honey, pollen, and *A. mellifera* brood. Its larvae usually damage the frames, ferment honey, and set adequate conditions for additional pests to develop. They can affect overall colony health, hive products, and finally all sorts of beneficial productivity. Although the pest is native to sub-Saharan Africa, it is an invasive species, and is currently found in Australia, America, Europe and Asia (Benuszak *et al*., 2019). Infesting a colony of honeybees usually starts when the *A. tumida* adults fly to a proper hive, mate, and lay eggs in clusters inside the wax frames. Then, the hatched larvae feed and develop. Its development speed is linked to food availability and temperature (Neumann *et al*., 2016). The developed larvae (called wandering larvae) drop beneath the hive to search for an adequate location to pupate. In the soil, often beneath the hive, these larvae develop into pupae and emerge as adults after 13-74 days depending on moisture content of soil and ambient temperature (Neumann *et al*., 2013). Usually, the beetle can multiply quickly when comb honey, bee brood, or/and pollen are available. Other factors can grant the beetles their current potential as a global threat to apiculture. These include long-distance flying efficiency, high overwintering capacity in honeybee clusters, and possessing host shifts to other bee species (Neumann and Elzen, 2004).

Current measures for *A. tumida* control comprise maintaining robust *A. mellifera* colonies, monitoring hive components to assure hygienic behavior in both breeding *A. mellifera* and their larvae/adults, discarding impaired frames, baiting *A. tumida* traps, and chemical applications in/around infested hives (Sanchez *et al*., 2021). These latter include treating soil with pyrethroids to kill all *A. tumida* stages. Benuszak *et al*. (2019) reviewed and detailed their usage. At least an area of 2 m radius around the hives should be chemically treated to a depth of at least 20 cm in USA. Also, Gard Star® (40% EC permethrin) at rate of 0.05% active ingredient (AI) is applied as soil drench. It is used to kill or inhibit new emerging beetles from entering nearby colonies via treating the soil underneath colonies that has severe larval infestation. A 1% solution of tetramethrin and cypermethrin was amply sprayed to drench the soil soon after soil ploughing to assure a good exposure of *A. tumida* in Italy (Granato *et al*., 2017). Soil drenching with permethrin solution (7.8 liters of 0.05% solution for 2 m radius and 0.2 m depth, but 19.5 liter for 0.5 m depth) showed high efficacy against *A. tumida* larvae (Smith *et al*. 2008). Because such pyrethroids are toxic to bees, their hives must not be sprayed (Hood, 2011; Benuszak *et al*., 2019). Moreover, exposure of non-target species such as wild bees should be averted. Additionally, soil treatment should occur after sunset, and related settings should be considered. Generally, large-scale *A. tumida*-chemical control should not be frequently applied as these insecticides are mostly effective against harmful *A. tumida* and useful honey bees simultaneously; both belong to the Class Insecta. Furthermore, they may adversely affect products of beehives in addition to bringing the above-mentioned complications.

Therefore, such chemicals to protect bees against *A. tumida* fall under the scope of the Biocidal Products Regulation set by the European Union (EU) under No 528/2012 (Benuszak *et al*., 2019). Consequently, these products should only be made available on the market and used provided proper authorization.

On the contrary, EPN proved safety and efficacy in controlling *A. tumida* infecting beehives in USA (Shapiro-Ilan *et al*., 2010; Sanchez *et al*., 2021) and elsewhere (Cabanillas and Elzen, 2006; Cuthbertson *et al*., 2012; Benuszak *et al*., 2019). Efficacy of EPNs against *A. tumida* populations was so high that they caused 76-100% mortality (Cabanillas and Elzen, 2006; Ellis *et al*., 2010; Cuthbertson *et al*., 2013). In England, The Department for Environment, Food and Rural Affairs (DEFRA) found that the commercial EPN species, *Steinernema kraussei* and *S. carpocapsae* at a rate of 10,000 infective juveniles (IJs)/ml water could induce 100% mortality of *A. tumida*-pupating larvae in sand in laboratory bioassays. Contrary to *S. kraussei*, *S. carpocapsae* could still prove efficacy when the dose rate for either species was decreased to 25%. Further analysis for the timing of application found that EPNs, especially *S. carpocapsae*, were still able to invade and kill pupating larvae that had been in sand for 3 weeks (Cuthbertson *et al*., 2013; DEFRA, 2013). On the other, *S. kraussei* was reliable in suppressing *A. tumida* populations in three different soil types. These types represent soils found in the south of England. However, the EPN efficacy was reduced at temperature < 10°C (Benuszak *et al*., 2019). The latter authors speculated that more tests on EPN efficacy against *A. tumida* under field conditions will offer a greater grasping of how EPNs may be utilized in a contingency response too. Obviously, including EPNs as components in IPM schemes are the best strategies to avoid using excessive chemicals for *A. tumida* control (Fulton *et al*., 2019).

Sanchez *et al*. (2021) reviewed six EPN species that have shown promise in laboratory bioassays for controlling *A. tumida*. These are *Heterorhabditis indica* Poinar, Karunakar, and David; *Heterorhabditis bacteriophora* Poinar; *Steinernema carpocapsae* Weiser; *Steinernema feltiae* Filipjev; *Steinernema kraussei* Steiner; and *Steinernema riobrave* Cabanillas, Poinar, and Raulston. After entering *A. tumida* wandering larval host, the EPN-IJs release their symbiotic bacteria that live within the EPN into host's hemocoel (Abd-Elgawad, 2024a). Each of these six EPN species has a different mutualistic bacterium species that is in charge of killing the infected insect and creating a suitable setting for EPN multiplication (Boemare *et al*., 1993). Several EPN generations are produced within the host cadaver ahead of IJs leaving of the cadaver to search for a new host (Figure 1).

Having documented the EPN effectiveness against *A. tumida* in several continents (Ellis *et al*., 2010; Shapiro-Ilan *et al*., 2010; Hill *et al*., 2016; Benuszak *et al*., 2019; Sanchez *et al*., 2021), they should provide significant biocontrol options for IPM programs. To perfect their uses, however, biotic/abiotic factors of the related settings as well as the best EPN-host matching should be considered. According to the different settings/scenarios, various outlooks may be valid. That is because related biotic/abiotic factors must also be advantageous in the existing scenario. For biotic factors, changes in EPN levels may occur directly/indirectly by impacting EPN hosts and/or natural enemies (Campos-Herrera *et al*., 2019; Toth *et al*., 2022). Likewise, abiotic factors as soil moisture/texture (Campos-Herrera *et al*., 2013), mulching/adding organic matter (Abd-Elgawad *et al*., 2024), salinity (Nielsen *et al*., 2011), and pH (Campos-Herrera *et al*., 2019) could alter EPN population densities in fields that have beehives. This is especially significant as many commercial beekeepers transfer their beehive pallets to fields/orchards planted with vegetables, fruits, and nuts for the pollination service too. Neumann *et al*. (2013, 2016) confirmed that soil structure and condition have also shown to impact *A. tumida* pupation success. However, the related tests mostly used sterilized soil that killed all biota from the soil in order to limit confounding variables within the tests (Ellis *et al*., 2004; Neumann *et al*., 2013, 2016). In contrast, sterilizing the soil offers unrealistic conditions as it eliminates natural biotic competition in the soil and probably changes the physical/chemical properties. Therefore, Sanchez *et al*. (2021) examined the impact of six EPN species on *A. tumida* wandering larvae in natural and sterilized soil in three Alabama soil types at typical low moisture levels. They found that pupation success of different larval population densities was similar at the three soil types; Kalmia loamy sand (KLS), Benndale fine sandy loam (BFSL), and Decatur silt loam (DSL). Among the six EPN species, *S. carpocapsae* attained the best efficacy across all tested EPN population densities in both autoclaved and natural soil. Both *S. riobrave* and *H. indica* fulfilled the next highest efficacies, but they were significantly less effective than *S. carpocapsae*. While *S. carpocapsae* parasitized 87% *A. tumida* wandering larvae across all population densities tested, it colonized 94% of *A. tumida* in the KLS soil, 80% in the BFSL soil, and 47% in the DSL soil. So, the authors concluded that *S. carpocapsae* is a promising BCA to be implemented into IPM system on *A. tumida*. They stressed that *S. carpocapsae* may benefit beekeepers in IPM system on a local, state, and national level; boost the *A. mellifera* bee colony health, and subsequent pollination rates in locations where *A. tumida* exist.

Also, there are possible variations in the degree of susceptibility regarding different *A. tumida*-life stages to the tested EPN species/strains. As with other hosts, the EPN biocontrol impact on *A. tumida* generally depends on the used EPN species or strain, the IJ dose, and the insect-life stage (Abd-Elgawad *et al*., 2017). Basically, the engaged species of the two EPN genera (*Steinernema* and *Heterorhabditis*) naturally live in soil and require an insect host to reproduce, but they have different hunting styles and each species have different environmental and host preferences (Koppenhöfer *et al*., 2020). *Steinernema* spp. often hunt their hosts using ambush (sit and wait) foraging but *Heterorhabditis* spp. hunt using cruising (seek and attack) foraging. Also, both *H. indica* and *S. riobrave* enter the body of the *A. tumida* larva or adult by entering into an orifice (i.e., the mouth, anus, or spiracles). Additionally, *H. indica* has the benefit over *S. riobrave* as it can also enter an *A. tumida* adult via the intersegmental members of the beetle cuticle (Askary and Abd-Elgawad, 2021). Nevertheless, both *H. indica* and *S. riobrave* are completely harmless to honeybees, humans, pets, and plants. Furthermore, estimate of EPN fitness/quality reared in *A. tumida* in terms of measuring nematode infectivity (power to invade), virulence (power to kill), viability (percentage of emerged living infective juveniles), and reproductive capacity (yield per insect) (Shehata *et al*., 2019) could be used to boost the *A. tumida* biocontrol.

The degree of EPN-host matching should be the optimum based on sophisticated technology too. In this vein, characterizing EPN biogeography for augmentation and conservation biocontrol may call for fine-scale taxonomic resolution (e.g., high throughput genome sequencing, HTS), as exceedingly related species can show divergent phenotypes for key properties (Dritsoulas *et al*., 2021). Differentiation of these EPN traits such as insect host specificity and habitat adaptation via HTS should be exploited for the best EPN strain-*A. tumida* host matching.

Notwithstanding the utility of such advanced tools to characterize key EPN traits, practical transformational technologies that lead to their significant marketing are still lacking. Some related EPN products are sold on market, though their sell scale is either local, small, or has not been officially registered. Thus, a globally standard procedure for governmental approval especially for such non-registered products is suggested. Enlightening beekeepers/stakeholders with the efficacy of EPN usage against pests of *A. mellifera* and their merits to secure safe foods should be a priority for attracting end-users. The related labels for *A. tumida* biocontrol instruct to apply the nematodes about three times a year for the best results usually during early spring and early fall. Otherwise, adequate times are selected to comply with optimum temperature for EPN biocontrol activity. Also, it is better to continue using EPNs every couple of weeks until *A. tumida* is under control in the treated bee yards. Although the EPN dose depends on the severity of *A. tumida* problem, the recommended dose is approximately 5 million IJs per 10 hives (Anonymous, 2024). Delivering EPNs into the infested soil and near enough the *A. tumida* larvae or/and adults are paramount.

Three different methods of EPNs can generally be applied (Abd-Elgawad, 2025). Augmentation or inundative biocontrol method is done within a short time. So, relevant biotic/abiotic actors should be optimized to get the best results. It is the most used EPN biocontrol technique, but stakeholders should earnestly promote the other two techniques, i.e., classical and conservation biocontrol methods. More surveys are required in settings where the EPNs will have to develop the target merit (trait) for classical biocontrol (Salama and Abd-Elgawad, 2001). For conservation biocontrol, perennial systems are more advantageous for this method due to relative soil stability. Perennials in Florida citrus groves could implement definite scenario to safely and effectively control the related pests by EPNs before the spontaneous introduction of the citrus greening disease. However, combating this disease required new agricultural practices that led to suppressing native and augmented EPNs (Campos-Herrera *et al*., 2013).

Eventually, EPNs should be used as an integral part of IPM plans to treat infestations by *A. tumida*. Other IPM components should include maintenance of strong *A. mellifera* colonies and monitoring hygienic behavior for optimized reproduction of *A. mellifera*. Examples are getting rid of damaged frames, using *A. tumida* traps, and treating with chemicals in/around infested hives (Anonymous, 2024). Because pyrethroids are used to kill all *A. tumida* stages in soil (Benuszak *et al*., 2019), further studies on pyrethroid concentrations that may be compatible with EPNs and *A. mellifera* in the treated bee yards are needed. Optimistically, another investigation (Aioub *et al*., 2021) suggested that combining EPNs with specific pyrethroid concentrations can be a practical strategy for managing a different pest, *Pieris rapae* L. (Lepidoptera: Pieridae). If so, using similar technique in bee yards could pave the way to applying novel control method against *A. tumida*.

The greater (Galleria mellonella L.) and lesser (Achroia grisella Fabricius) wax moths (Lepidoptera: Pyralidae)

Wax moths are globally the most damaging pests of honeybee wax combs especially in both storage and weak bee colonies (El-Gohary *et al*., 2018). Tunnels made in the wax combs mixed with white silk secretions, larval feces, and different pest stages are the main symptoms of the wax moths-infestations made in these combs during feeding on them. The larvae can feed also on pollen, cast larval skins, and honey. Additionally, moving stages of wax moths can transmit the pathogens that seriously aggravate *A. mellifera* colony health. Generally, *G. mellonella* is more pervasive and damaging pest of bee wax combs than the lesser wax moth. If both moth species are found together, *G. mellonella* larvae are so dominated that they force *A. grisella* to feed on the hive floor (Egelie *et al*., 2022).

The most key aspect of managing both species of wax moths as well as other pests is to keep up with healthy and strong honey bee colonies (Kwadha *et al*., 2017; Egelie *et al*., 2022; Kegley *et al*., 2024). Obviously, chemical insecticides can effectively be used as control measure of wax moths too. Currently, two toxic chemicals, Paradichlorobenzene and Phestoxin are used (Said *et al*., 2019). So, they can protect all combs in storage but not combs having honey for human consumption. Simply, the odor of these chemicals is often absorbed by honey, and though the bees do not object to this odor, such honey is unfit for human consumption. Said *et al*. (2019) stressed that wax moths could develop resistance to these insecticides. Moreover, toxic residues within the wax are frequently found due to the use of these insecticides. As stored wax combs are the main target of *A. grisella*, there are several ways to limit the related damage of these combs. Either extreme cold or heat will kill all life stages of *A. grisella* even eggs (Egelie *et al*., 2022). However, freezing at 20°F for 24-48 hours is a better option because heating may melt and spoil the wax combs. After freezing, the treated combs should be stored in airtight plastic bags to avoid re-infestation. Fumigation is also possible but more expensive, less desirable treatment in stored hive materials. Moreover, fumigation may not be used on honey combs that the beekeeper needs to sell/consume. Additionally, fumigants may be very dangerous for the user.

Thus, safe and reliable methods are still being explored for controlling both species of wax moths. These included natural materials; e.g., plant essential oils (EOs) (Said *et al*., 2019) and other biopesticides based on living microbes and/or their bioactive compounds (Abd-Elgawad, 2023; Han *et al*., 2023). Of five tested natural essential oils, rosemary, *Salvia rosmarinus* Spenn (Lamiales: Lamiaceae) oil treatment showed the best effect against *G. mellonella* larvae. The major components of its essential oil were glycerol triacetien (39.22%), 1.8-cineole (16.82%), l-menthole (10.64%), a-camphor (8.71%), Pinene (7.16%), d-limonene (7.04%) (Said *et al*., 2019). Other components of essential oil proved effectiveness against additional parasites and should be tested against pests of honeybees (Abd-Elgawad and Omer, 1995). Nonetheless, many inherent factors oppose using EOs as biopesticides due to their natural lipophilicity and high volatility. In addition, two major impediments of using EOs are manufacturing constraints and production costs. To overcome these restrictions, Gupta *et al*. (2023) suggested incorporating some sophisticated techniques such as the EOs-nanoencapsulation, RNA-Seq in biopesticide development, and bioinformatics. Such sophisticated tools have the potential to be generally used against other pests to advance sustainable agricultural systems (Abd-Elgawad, 2024b). They can be used to control pests of crops that are pollinated by bees. Recently, honeybees were reported to transfer harmful pesticide residues from the pollinated orchards to the beehives (Ostiguy *et al*., 2019; Kegley *et al*., 2024). Therefore, a decrease in application of chemical pesticides on bee-attractive crops especially during pollination time would need to occur.

Han *et al.* (2023) developed a novel *Bacillus thuringiensis* (Bt) strain (designated BiotGm) that demonstrated efficacy against *G. mellonella* larvae with a LC_{50} value lower than 2 µg/g. However, BiotGm showed low toxicity levels with a LC_{50} = 20598.78 µg/ml for *A. mellifera* larvae but no adverse impact at concentration = 100 µg/ml for *A. mellifera* adults. The authors designed also an entrapment approach comprising a lure for *G. mellonella* larvae, BiotGm, and a trapping device that blocks bees from reaching the lure. Their method reduced the *G. mellonella* larval populations in laboratory and field trials. Although these results bode well for using Bt-based biocontrol of *G. mellonella* in beehives, Han *et al.* (2023) indicated the need for further optimization of their method. That is because the strain BiotGm may be seriously impacted by various ecological factors, including its formulation type and application technique as well as contaminants, temperature, ultraviolet radiation, pH, and rainfall. Hence, further field evaluations on its practicality are expected. On the other hand, because many EPN species have wide host ranges, some of them proved their ability to perfectly control *G. mellonella*. In this regard, *H. bacteriophora* could cause full (100%) mortality of *G. mellonella* larvae in natural bee hives. Moreover, *H. bacteriophora* did not affect larvae or adults of honeybees (Taha and Abdelmegeed, 2016). However, to avoid the sensitivity of EPNs as biologicals, they are much preferred to be used as an integral component in IPM programs. Consequently, compelling preventive measures via maintaining healthy bee colonies are the best components for IPM regarding either of the two wax moth species. Strictly speaking, if the beehives appear to be under stress, components for IPM may comprise manual removal of pests, freezing/heat treatment, or burning equipment. Other options are moth traps, insect zappers, and carbon dioxide fumigation rooms. Chemical control may be needed as a last resort (Kwadha *et al.*, 2017; Jack and Ellis, 2018; Kegley *et al.*, 2024).

Other pests and pathogens

Having recorded the favorable biocontrol potential of EPNs against the aforementioned pests, beneficial nematodes should be earnestly tried against further *A. mellifera*-pests and pathogens (Table 1). For instance, other entomogenous nematode species of the genus *Pheromermis* Poinar, Lane & Thomas (Mermithida: Mermithidae) showed promise in controlling *Vespa* hornets attacking beehives (Abou-Shaara and staron, 2019). *Vespa orientalis* is a main pest in several countries like Egypt. Using pathogens to control *Vespa* spp. has not been documented previously, due to the social nature of the hornets. Yet, isolating BCAs from hornet nests to develop novel control methods should be addressed via proper means such as the traps in the bee yards (Mazeed and Abd-Al Fattah, 2019) within IPM programs. Also, *Varroa destructor* Anderson and Trueman is the most damaging pest of honey bee colonies worldwide (Jack and Ellis, 2021). Pathogen-biopesticide cross correlations including *V. destructor* may indeed play a role in colony health, but are beyond the scope of this article. Also, long-term investigations are required to assess the persistence of these BCAs in the apiary settings and their potential progressive impacts on both target and non-target organisms over time (Dutka *et al.*, 2015).

Conclusions and Recommendations

The above-mentioned efficacies of biopesticides against pests of honeybees are promising, but further research aspects are warranted to perfect the application protocols for these and novel BCAs. These include examining the optimal timing, dosage, and combinations of IPM components to secure maximum effectiveness across different scenarios and climatic conditions. The most key aspect of these components in managing relevant pests/pathogens is to safeguard honeybee individuals in order to keep healthy and strong colonies. Robust beehives enable

Table 1: *Targeted pests of honeybees and their control using beneficial nematodes.*

Targeted pest species	Used nematodes	Efficacy	References
Aethina tumida	Steinernema carpocapsae	parasitized 87% wandering larvae of all tested populations	Sanchez *et al.*, 2021
Galleria mellonella	*Heterorhabditis bacteriophora*	Twice usage of high dose could induce full mortality of larvae and adults	Taha and Abd-elmegeed, 2016
Achroia grisella	Steinernema carpocapsae	Caused 100% larval mortality	San-Blas *et al.*, 2019
Vespa spp.	*Pheromermis* spp.	Adverse efficacy on the social wasps and produced sexuals	Abou-Shaara and Staron, 2019

worker bees to kill and/or evict pest larvae as well as seal areas where pests may lay eggs. Furthermore, although other pests and pathogens can inflict substantial damage to honeybee colonies, information is scanty about their interaction with beneficial nematodes. Therefore, more studies are direly needed to explore interactions of such pests/pathogens with beneficial nematodes such as *Pheromermis* spp. in the near future. Integrating EPNs into IPM strategies could significantly boost the sustainability of apiary management schemes, minimizing reliance on synthetic pesticides and alleviating their linked ecological risks. Accordingly, EPNs can contribute to the increasing evidence base that backs a paradigm shift for more environmentally sound pest management solutions in apiculture. Eventually, EPN applications into IPM plans against pests of *A. mellifera* can not only mitigate the issues of using unhealthy pesticides but may also widen EPN uptake and further stimulate the market of organic products. Although prevention is better than cure is a priority in controlling honey bee pests, the safe and effective use of nematodes offers promising hope. Future research directions should broaden EPN biocontrol spectra to other pest groups such as those infecting honeybee. Other facets for more uptakes of EPNs imply optimizing related EPN delivery/application, persistence, and integrating sophisticated technologies to reform their new directions. Therefore, while recent and above-mentioned EPN techniques against pests of honeybees should be adopted by beekeepers, researchers should work to provide possibly refined IPM strategies.

Acknowledgement

This research was supported by In-House project No. 13050112 entitled "Pesticide alternatives against soil-borne pathogens and pests attacking economically significant export crops." The author acknowledges the support in part of this study by the US-Egypt Project cycle 22 (No. 47725) entitled "Setting and assessing integrated pest management tactics to optimize livestock and honey bee production." This article is derived from the Subject Data funded in part by NAS and USAID, and that any opinions, findings, conclusions, or recommendations expressed in it are those of the authors alone and do not necessarily reflect the views of USAID or NAS.

Novelty Statement

This manuscript deals with the most important entomopathogenic (insecticidal) nematode species as natural bio-pesticides against key pests of honeybees. Updated techniques against *Aethina tumida* infestation in soil are suggested to pave the way of applying novel control technologies via IPM strategies.

Abbreviations

AI, Active ingredient; Bt, *Bacillus thuringiensis*; BCA, Biocontrol agent; DEFRA, Department for Environment, Food and Rural Affairs; EPNs, Entomopathogenic nematodes; EU, European Union; HTS, High throughput genome sequencing; IJs, Infective juveniles; EOs, Essential oils; IPM, Integrated pest management.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interests

The author has declared no conflict of interest.

References

Abd-Elgawad, M.M.M., 2017. Status of entomopathogenic nematodes in integrated pest management strategies in Egypt. In: Abd-Elgawad, M.M.M., Askary, T.H. and Coupland, J. (Eds). Biocontrol Agents: Entomopathogenic and Slug Parasitic Nematodes. CAB International, Wallingford, UK, pp. 473-501. https://www.cabi.org/animalscience/ebook/20173212295

Abd-Elgawad, M.M.M., 2020. Biological control agents in the integrated nematode management of pepper in Egypt. Egypt. J. Biol. Pest Contr., 30: 70. https://doi.org/10.1186/s41938-020-00273-6

Abd-Elgawad, M.M.M., 2023. Optimizing entomopathogenic nematode genetics and applications for the integrated management

Links Researchers

of horticultural pests. Horticulturae, 9(8): 865. https://doi.org/10.3390/horticulturae9080865

Abd-Elgawad, M.M.M., 2024a. Reproduction of entomopathogenic nematodes for use in pest control. In: Molinari, S. (Ed.). Methods in molecular biology: Plant-Nematode Interactions. Springer Nature, New York, USA, pp. 351–382. https://doi.org/10.1007/978-1-0716-3638-1_14

Abd-Elgawad, M.M.M., 2024b. Upgrading strategies for managing nematode pests on profitable crops. Plants, 13: 1558. https://doi.org/10.3390/plants13111558

Abd-Elgawad, M.M.M., 2025. Transformational technologies for more uptakes of entomopathogenic nematodes. Egypt. J. Biol. Pest. Contr., 35: 1. https://doi.org/10.1186/s41938-024-00838-9

Abd-Elgawad, M.M.M., Askary, T.H. and Coupland, J., 2017. Biocontrol agents: Entomopathogenic and slug parasitic nematodes. CAB International, Wallingford, UK, pp. 648. https://doi.org/10.1079/9781786390004.0000

Abd-Elgawad, M.M.M., Duncan, L.W., Hammam, M.M.A., Elborai, F. and Shehata, I.E., 2024. Effect of mulching manures and use of *Heterorhabditis bacteriophora* on strawberry fruit yield, *Temnorhynchus baal* and *Meloidogyne javanica* under field conditions. Arab J. Plant Prot., 42(3): 306-317. https://doi.org/10.22268/AJPP-001262

Abd-Elgawad, M.M. and Omer, E.A., 1995. Effect of essential oils of some medicinal plants on phytonematodes. Anz. Schädlingskd. Pflanzenschutz Umweltschutz, 68(4): 82-84.

Abou-Shaara, H.F. and Staron, M., 2019. Present and future perspectives of using biological control agents against pests of honey bees. Egypt. J. Biol. Pest Contr., 29: 24. https://doi.org/10.1186/s41938-019-0126-8

Aioub, A.A.A., El-Ashry, R.M., Hashem, A.S., Elesawy, A.E. and Elsobki, A.E.A., 2021. Compatibility of entomopathogenic nematodes with insecticides against the cabbage white butterfly, *Pieris rapae* L. (Lepidoptera: Pieridae). Egypt. J. Biol. Pest. Contr., 31: 153. https://doi.org/10.1186/s41938-021-00498-z

Anonymous, 2024. Beneficial Nematodes For Controlling Small Hive Beetles. Available at: https://www.funnybugbees.com/bee-pests-blog/beneficial-nematodes-for-controlling-small-hive-beetles (accessed 7 Oct 2024).

Askary, T.H. and Abd-Elgawad, M.M.M., 2021. Opportunities and challenges of entomopathogenic nematodes as biocontrol agents in their tripartite interactions. Egypt. J. Biol. Pest. Contr., 31: 42. https://doi.org/10.1186/s41938-021-00391-9

Benuszak, J., Brown, M., Chauzat, M.P., Duquesne, V., Franco, S., Gorlay-France, C., Kryger, P., Laurent, M., Mutinelli, F., Riviere, M.P. and Schafer, M., 2019. Guidance document for the management of the small hive beetle (*Aethina tumida*) infestation in soil. European Union Reference Laboratory for Honeybee Health (EURL). First version (April 2019).

Boemare, N., Akhurst, R.J. and Mourant, R.G., 1993. DNA relatedness between *Xenorhabdus* spp. (Enterobacteriaceae), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen. nov. Int. J. System. Evol. Microbiol., 43: 249-255. https://doi.org/10.1099/00207713-43-2-249

Cabanillas, H.E. and Elzen, P.J., 2006. Infectivity of entomopathogenic nematodes (Steinernematidae and Heterorhabdidae) against the small hive beetle *Aethina tumida* (Coleoptera: Nitidulidae). J. Apicult. Res., 45: 49–50. https://doi.org/10.1080/00218839.2006.11101314

Campos-Herrera, R., Pathak, E., El-Borai, F.E., Schumann, A., Abd-Elgawad, M.M.M. and Duncan, L.W., 2013. New citriculture system suppresses native and augmented entomopathogenic nematodes. Biol. Contr. 66: 183-194. https://doi.org/10.1016/j.biocontrol.2013.05.009

Calderone, N.W., 2012. Insect pollinated crops, insect pollinators and US agriculture: Trend analysis of aggregate data for the period 1992±2009. PLoS One 7(5): e37235. https://doi.org/10.1371/journal.pone.0037235

Campos-Herrera, R., Pathak, E., El-Borai, F.E., Schumann, A., Abd-Elgawad, M.M.M. and Duncan, L.W., 2013. New citriculture system suppresses native and augmented entomopathogenic nematodes. Biol. Contr., 66: 183-194. https://doi.org/10.1016/j.biocontrol.2013.05.009

Campos-Herrera, R., Stuart, R.J., Pathak, E., El-Borai, F.E. and Duncan, L.W., 2019. Temporal patterns of entomopathogenic nematodes in Florida citrus orchards: Evidence of natural regulation by microorganisms and nematode competitors. Soil Biol. Biochem., 128:193-204. https://doi.org/10.1016/j.soilbio.2018.10.012

Cuthbertson, A.G.S., Mathers, J.J., Blackburn, L.F., Powell, M.E., Marris, G., Pietravalle, S., Brown, M.A. and Budge, G.E., 2012. Screening commercially available entomopathogenic biocontrol agents for the control of *Aethina tumida* (Coleoptera: Nitidulidae) in the UK. J. Insects, 3: 719–26. https://doi.org/10.3390/insects3030719

Cuthbertson, A.G.S., Wakefield, M.E., Powell, M.E., Marris, G., Anderson, H., Budge, G.E., Mathers, J.J., Blackburn, L.F. and Brown, M.A., 2013. The small hive beetle *Aethina tumida*: A review of its biology and control measures. J. Curr. Zool., 59: 644–653. https://doi.org/10.1093/czoolo/59.5.644

DEFRA., 2013. R&D project 'investigation into potential control measures for small hive beetle. PH0513'. The Food and Environment Research Agency, UK.

Dritsoulas, A., El-Borai, F.E., Shehata, I.E., Hammam, M.M., El-Ashry, R.M., Mohamed, M.M., Abd-Elgawad, M.M. and Duncan, L.W., 2021. Reclaimed desert habitats favor entomopathogenic nematode and microarthropod abundance compared to ancient farmlands in the Nile Basin. J. Nematol., 53: 1-13. https://doi.org/10.21307/jofnem-2021-047

Dutka, A., McNulty, A. and Williamson, S.M., 2015. A new threat to bees? Entomopathogenic nematodes used in biological pest control cause rapid mortality in *Bombus terrestris*. Peer J., 3: e1413. https://doi.org/10.7717/peerj.1413

Egelie, A.A., Mortensen, A.N., Barber, L., Sullivan, J. and Ellis, J.D., 2022. Lesser wax moth *Achroia grisella* Fabricius (Insecta: Lepidoptera: Pyralidae). UF/IFAS Extension Service, University of Florida, pub. No. EENY637/IN1108. https://edis.ifas.ufl.edu

El-Gohary, S.G., Yousif-Khalil, S.I., El-Maghraby, M.M.A. and Abd-Alla, Sh.M., 2018. Mass rearing of the greater wax moth, *Galleria mellonella* L. Zagazig J. Agric. Res., 45(2): 495-503. https://doi.org/10.21608/zjar.2018.49174

Ellis, J.D., Hepburn, H.R., Luckman, B. and Elzen, P.J., 2004. Effects of soil type, moisture, and density on pupation success of *Aethina tumida* (Coleoptera: Nitidulidae). J. Environ. Entomol., 33: 794-798. https://doi.org/10.1603/0046-225X-33.4.794

Ellis, J.D., Spiewok, S., Delaplane, K.S., Buchholz, S., Neumann, P. and Tedders, W. L., 2010. Susceptibility of *Aethina tumida* (Coleoptera: Nitidulidae) larvae and pupae to entomopathogenic nematodes. J. Econ. Entomol., 103: 1–9. https://doi.org/10.1603/EC08384

El-Tatawy, N., Shafeiy, M., Basuony, N. and El-Gendy, D., 2019. An economic study of honey production in Beheira governorate. J. Sustain. Agric. Sci., 45(4): 229-237.

Fulton, C.A., Hartz, K.E.H., Reeve, J.D. and Lydy, M.J., 2019. An examination of exposure routes of fluvalinate to larval and adult honey bees (*Apis mellifera*). J. Environ. Toxicol. Chemist., 38: 1356–1363. https://doi.org/10.1002/etc.4427

Glenny, W., Caviglin, I., Daughenbaugh, K.F., Radford, R., Kegley, S.E. and Flenniken, M.L., 2017. Honey bee (*Apis mellifera*) colony health and pathogen composition in migratory beekeeping operations involved in California almond pollination. PLoS One, 12(8): e0182814. https://doi.org/10.1371/journal.pone.0182814

Granato, A., Zecchin, B., Baratto, C., Duquesne, V., Negrisolo, E., Chauzat, M.P., Ribière-Chabert, M., Cattoli, G. and Mutinelli, F., 2017. Introduction of *Aethina tumida* (Coleoptera: Nitidulidae) in the regions of Calabria and Sicily (southern Italy). J. Apidol., 48: 194-203. https://doi.org/10.1007/s13592-016-0465-3

Gupta, I., Singh, R., Muthusamy, S., Sharma, M., Grewal, K., Singh, H.P. and Batish, D.R., 2023. Plant essential oils as biopesticides: Applications, mechanisms, innovations, and constraints. Plants, 12: 2916. https://doi.org/10.3390/plants12162916

Han, B., Zhang, L., Geng, L., Jia, H., Wang, J., Ke, L., Li, A., Gao, J., Wu, T., Lu, Y., Liu, F., Song, H., Wei, X., Ma, S., Zhan, H., Wu, Y., Liu, Y., Wang, Q., Diao, Q., Zhang, J., Dai, P., 2023. Greater wax moth control in apiaries can be improved by combining *Bacillus thuringiensis* and entrapments. Nat. Commun., 14: 7073. https://doi.org/10.1038/s41467-023-42946-4

Hill, E.S., Smythe, A.B. and Delaney, D.A., 2016.

Assessing the role of environmental conditions on efficacy rates of *Heterorhabditis indica* (Nematoda: Heterorhabditidae) for controlling *Aethina tumida* (Coleoptera: Nitidulidae) in honey bee (Hymenoptera: Apidae) colonies: A citizen science approach. J. Econ. Entomol., 109: 106112. https://doi.org/10.1093/jee/tov296

Hood, M., 2011. Handbook of small hive beetle. Clemson University, USA.

Jack, C.J. and Ellis, J.D., 2018. Wax Moth Control. U.S. Department of Agriculture, UF/IFAS Extension Service, University of Florida, pub. No. ENY121. https://doi.org/10.32473/edis-aa141-2018

Jack, C.J. and Ellis, J.D., 2021. Integrated pest management control of *Varroa destructor* (Acari: Varroidae), the most damaging pest of (*Apis mellifera* L. (Hymenoptera: Apidae)) colonies. J. Insect Sci., 21(5): 6. https://doi.org/10.1093/jisesa/ieab058

Kegley, S.E., Radford, R., Brown, T.J., Anderson, J., Cox, D., El-lis, S. and Marcy, G.W., 2024. Longitudinal analysis of honey bee colony health as a function of pesticide exposure. Agronomy, 14: 2505. https://doi.org/10.3390/agronomy14112505

Khattabi, A.M., 2022. Honey bee breeding (in Arabic). Media Support Centre Press, Ministry of Agriculture, Malawi, Egypt.

Koppenhöfer, A.M., Shapiro-Ilan, D.I. and Hiltpold, I., 2020. Entomopathogenic nematodes in sustainable food production. Front. Sustain. Food Syst., 4: 125. https://doi.org/10.3389/fsufs.2020.00125

Kwadha, C.A., Ong'amo, G.O., Ndegwa, P.N., Raina, S.K. and Fombong, A.T., 2017. The biology and control of the greater wax moth, *Galleria mellonella*. Insects, 8: 61. https://doi.org/10.3390/insects8020061

Mazeed, A.R.A. and Abd-Al Fattah, Y.A., 2019. Effectiveness of baited traps for controlling oriental hornet *Vespa orientalis* (Hymenoptera: Vespidae) in Sohag governorate apiaries. Egypt. J. Plant Prot. Res. Inst., 2(2): 309-314.

Neumann, P. and Elzen, J.E., 2004. The biology of the small hive beetle (*Aethina tumida*, Coleoptera: Nitidulidae): gaps in our knowledge of an invasive species. J. Apidol., 35: 229-247. https://doi.org/10.1051/apido:2004010

Neumann, P., Evans, J.D., Pettis, J.S., Pirk, C.W.W., Schäfer, M.O., Tanner, G. and Ellis, J.D., 2013. Standard methods for small hive beetle research. J. Apicult. Res., 52: 1-32. https://doi.org/10.3896/IBRA.1.52.4.19

Neumann, P., Pettis, J.S. and Schäfer, M.O., 2016. Quo vadis *Aethina tumida*? Biology and control of small hive beetles. J. Apidol., 47: 427–466. https://doi.org/10.1007/s13592-016-0426-x

Nielsen, A.L., Spence, K.O., Nakatani, J. and Lewis, E.E., 2011. Effect of soil salinity on entomopathogenic nematode survival and behaviour. Nematology, 3: 859-867. https://doi.org/10.1163/138855411X562254

Ostiguy, N., Drummond, F.A., Aronstein, K., Eitzer, B., Ellis, J.D., Spivak, M. and Sheppard, W.S., 2019. Honey bee exposure to pesticides: A four-year nationwide study. Insects, 10: 13. https://doi.org/10.3390/insects10010013

Said, S.M., Hammam, M.A. and Abd-El Kader, S.K., 2019. Insecticidal activity against the greater wax moth (*Galleria mellonella* L.) and chemical composition of five plant essential oils. Menoufia J. Plant Prot., 4: 145-161. https://doi.org/10.21608/mjapam.2019.122986

Salama, H.S. and Abd-Elgawad, M.M.M., 2001. Isolation of heterorhabditid nematodes from palm tree planted areas and their implication in the red palm weevil control. Anz. Schadlingskunde J. Pest Sci., 74: 43-45. https://doi.org/10.1111/j.1493-0280.2001.01010.x

San-Blas, E., Campos-Herrera, R., Dolinski, C., Monteiro, C., Andaló, V., Garrigós Leite, L., Rodríguez, M.G., Morales-Montero, P., Sáenz-Aponte, A., Cedano, C., Carlos López-Nuñez, J., Del Valle, E., Doucet, M., Lax, P., Navarro, P.D., Báez, F., Llumiquinga, P., Ruiz-Vega, J., Guerra-Moreno, A. and Patricia Stock, S., 2019. Entomopathogenic nematology in Latin America: A brief history, current research and future prospects, J. Invert. Pathol., 165: 22-45. https://doi.org/10.1016/j.jip.2019.03.010

Sanchez, W., Shapiro, D., Williams, G. and Lawrence, K., 2021. Entomopathogenic nematode management of small hive beetles (*Aethina tumida*) in three native Alabama soils under low moisture conditions. J. Nematol., 53. https://doi.org/10.21307/jofnem-2021-063

Shapiro-Ilan, D., Morales-Ramos, J., Rojas, M. and Tedders, W., 2010. Effects of a novel ento-mopathogenic nematode-infected host formulation on cadaver integrity, nematode yield, and suppression of *Diaprepes abbreviatus* and *Aethina tumida*. J. Invertebr. Pathol., 103: 103-108. https://doi.org/10.1016/j.jip.2009.11.006

Shehata, I.E., Hammam, M.M.A. and Abd-Elgawad, M.M.M., 2021. Effects of inorganic fertilizers on virulence of the entomopathogenic nematode *Steinernema glaseri* and peanut germination under field conditions. Agronomy, 11: 945. https://doi.org/10.3390/agronomy11050945

Shehata, I.E., Hammam, M.M.A., El-Borai, F.E., Duncan, L.W. and Abd-Elgawad, M.M.M., 2019. Comparison of virulence, reproductive potential, and persistence among local *Heterorhabditis indica* populations for the control of *Temnorhynchus baal* (Reiche & Saulcy) (Coleoptera: Scarabaeidae) in Egypt. Egypt. J. Biol. Pest Cont., 29: 32. https://doi.org/10.1186/s41938-019-0137-5

Smith, M., Goodrum, L., Chinneck, N. and Stedman, M., 2008. The importance of hive health in apiculture from a veterinary perspective. UK Vet: Companion Animal, 13: 65-69. https://doi.org/10.1111/j.2044-3862.2008.tb00530.x

Taha, E.H. and Abdelmegeed, S.M., 2016. Effect of entomopathogenic nematodes, *Heterorhabditis bacteriophora* on *Galleria mellonella* in bee hives of *Apis mellifera*. J. Biol. Sci., 16: 197-201. https://doi.org/10.3923/jbs.2016.197.201

Toth, S., Toepfer, S., Szalai, M. and Kiss, J., 2022. Limited influence of abiotic and biotic factors on the efficacy of soil insecticides and entomopathogenic nematodes when managing the maize pest *Diabrotica v. virgifera* (Coleoptera: Chrysomelidae). Agronomy, 12: 2697. https://doi.org/10.3390/agronomy12112697

United States Department of Agriculture/National Agricultural Statistics Survey, 2019. Honey Bee Colonies (ISSN: 2470-993X): Washington, DC, USA. www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Bee_and_Honey.