

Eurasia Specialized Veterinary Publication

International Journal of Veterinary Research and Allied Science

2021, Volume 1, Issue 1, Page No: 1-8 Copyright CC BY-NC-SA 4.0 Available online at: www.esvpub.com/

Exploring the Role of Nanotechnology in Enhancing Apiculture Practices

Hossam F. Abou-Shaara^{1*}, Martin Staron², Dana Staroňová²

¹Department of Plant Protection, Faculty of Agriculture, Damanhour University, Damanhour, 22516, Egypt.

²Research Institute for Animal Production Nitra, Institute of Apiculture Liptovský Hrádok, Slovakia.

*E-mail \bowtie hossam.farag@agr.dmu.edu.eg

ABSTRACT

Nanotechnology, which involves the use of materials smaller than 100 nanometers to perform specific tasks, has gained attention in agriculture, industry, and medicine. As this field has advanced, various techniques for preparing nanoparticles have been developed. While nanotechnology has mainly focused on creating for managing plant diseases and pests, its applications in beekeeping are limited. Most research in this field has examined the use of bee products as nanoparticles for medical purposes. This article reviews the applications of nanotechnology in beekeeping, covering areas such as tools, instrumental insemination, nutrition, pollination, swarming, pest and disease control, and bee products. It also addresses the potential risks posed by nanoparticles to honeybees. This review aims to highlight emerging trends in beekeeping and to encourage further research on nanotechnology's role in improving bee management practices.

Keywords: Honey bees, Colonies, Pests, Diseases, Nanoparticles

Received: 10 February 2021 Revised: 28 May 2021 Accepted: 29 May 2021

How to Cite This Article: Abou-Shaara HF, Staron M, Staroňová D. Exploring the Role of Nanotechnology in Enhancing Apiculture Practices. Int J Vet Res Allied Sci. 2021;1(1):1-8.

Introduction

Nanotechnology refers to the use of materials or particles smaller than 100 nanometers to carry out specific tasks [1-4]. Nearly all substances, such as essential oils, and pesticides can be transformed into nanoparticles. Various techniques are employed for nanoparticle preparation, including gas condensation, chemical vapor deposition, and Sol-Gel methods [5-7], with nanoencapsulation achievable through several approaches [8]. Heavy metals like Ag, Ni, Fe, and Al are often used in nanomaterial production [6]. Once nanoparticles are created, their properties—such as chemical composition, shape, and size—must be analyzed, with tools like Gas Chromatography-Mass Spectrometry (GC-MS), X-ray Photoelectron Spectroscopy (XPS), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and UV–Vis Spectrophotometry playing crucial roles [9, 10]. While nanotechnology has been widely applied in agriculture, particularly for controlling plant diseases and pests [11], its use in beekeeping remains relatively underexplored. Beekeeping, however, offers significant economic and environmental value, and nanotechnology holds potential for various applications in the field. Current research on nanotechnology in beekeeping primarily focuses on the medical and nutritional properties of products by bees, such as propolis [12-15] and venom of bees [16]. This article outlines the possible advancements in beekeeping through nanotechnology, encouraging further research to enhance the industry.

Materials and Methods

This study is based on a comprehensive review of existing literature concerning the application of nanotechnology in beekeeping. All relevant aspects of beekeeping were taken into account during the manuscript's development

Abou-Shaara et al.,

and revision. The selected articles were organized into distinct categories: honey bee pests, feeding, beekeeping tools, pollination, swarming, instrumental insemination, honey bee diseases, and honey products of bees. These categories encompass the major areas of beekeeping. Each section also highlights suggestions for future research. A results section was included to summarize the reviewed studies, followed by a discussion on the potential risks associated with nanomaterials for honey bees. The discussion specifically addressed the use of heavy metals in nanomaterial preparation. Finally, a conclusion was provided, summarizing the findings from the reviewed studies. It should be noted that there is limited research on this area.

Results and Discussion

Beekeeping tools

Beekeeping requires a variety of essential tools, including beehives, hive tools, smokers, pollen and propolis traps, bee venom collectors, and traps for pests. These tools are typically made from materials such as plastic, metal, wood, or fibers. Nanotechnology, which has numerous industrial applications, can enhance the performance of these tools by improving their durability, strength, and resistance to extreme temperatures. For instance, wall coatings with silica-based nano-composite emulsions have shown improved solvent resistance and performance. Similarly, nanotechnology can be applied to enhance beekeeping equipment. Beehives, for example, can be coated with materials that protect them from environmental factors like sun and rain. Insulated hives have demonstrated better performance under high temperatures compared to uninsulated ones. It is anticipated that beehives treated with nanoparticle-based insulation will outperform those using conventional materials. Nanotechnology can also improve the strength and adaptability of other beekeeping tools, making them better suited for varying environmental conditions. This is especially important as climate change and rising temperatures pose significant challenges to beekeeping. Additionally, beehives could be treated with hygienic materials developed through nanotechnology to protect the health of honey bees. For example, hives coated with nano-silver have been found to protect against diseases, while uncoated hives showed higher bacterial growth and greater disease symptoms. More research is needed to enhance beekeeping tools and promote the growth and sustainability of the beekeeping industry [17-22].

Feeding

Honey bees primarily rely on nectar and pollen for nourishment. However, beekeepers often supplement their colonies with artificial feed when natural flowering plants are scarce or absent. Artificial feeding is typically categorized into two types: protein feeding and sugar feeding. Sugar feeding, which is vital for the survival of colonies, particularly in winter, can be made from various ingredients such as honey, sugar, and water. Protein feeding is based on the use of pollen or its substitutes. Inadequate nutrition can lead to health problems for honey bees, making them more vulnerable to diseases. Research suggests that food formulated as nanoparticles may have distinct properties compared to its conventional form, potentially improving the health and digestive functions of honey bees. Therefore, further research is suggested to explore the use of nanotechnology in creating nano-foods for honey bees, especially those that could be combined with specific medications to combat gut or hemolymph pathogens [23-30].

Pollination

Pollination is crucial for the reproduction of many plants, and honey bees play a significant role in pollinating a wide range of crops. Chemicals, such as pheromones and attractants, are sometimes used to direct foraging activity toward particular plants. When these chemicals are prepared as nanoparticles, their properties can be enhanced. For instance, studies have shown that chitosan nano-conjugated pheromones can influence reproductive behavior in fish. Further research is encouraged to examine the potential of nanoparticles in regulating foraging behavior and enhancing pollination efficiency [30-35].

Swarming

Swarming plays a crucial role in the reproduction of bee colonies. However, natural swarming is often problematic for beekeepers, as it can lead to weakened colonies, especially when the mother queen is lost in the process. To manage swarming, beekeepers employ various methods, including swarm lures, with pheromones being particularly effective in attracting swarms. When swarm lures are developed as nanoparticles, they may offer more

efficient attraction capabilities. Moreover, the effectiveness of these nanoparticle-based lures could last longer due to their altered properties compared to traditional lure formulations [36-42].

Instrumental insemination

In honey bee colonies, queen mating occurs naturally in the air within specific areas called drone congregation areas (DCAs). However, instrumental insemination is a critical technique for controlling queen mating. This process involves collecting semen from drones, narcotizing the virgin queens, and then inseminating the queens using specialized tools. Nanotechnology can improve the physical and hygienic properties of the tools used in instrumental insemination. By using biocompatible magnetic nanoparticles, molecular-based targeting can aid in the selection of healthy sperm from samples like boar semen. Nanopurification methods can offer non-invasive techniques for sperm selection based on epigenetics. A similar approach could be applied to the purification of drones' semen, allowing for the selection of the most viable sperm and ensuring the genetic quality of insemination (genetic paternity purification) [43-50].

Honey bee pests

Various pests, including hornets, moths, and beetles, can threaten beehives by feeding on bees, wax, and stored food. These pests are distributed across different regions, and their impact on colonies varies by location. Hornets, particularly those from the Vespa genus, attack adult bees in flight and invade colonies to feed on honey and bees. The recent invasion of *Vespa velutina* hornets in some European areas has raised concerns. Small hive beetles are highly destructive to bee colonies and are found in several countries across Africa, Europe, America, and Asia. Wax moths also pose a significant threat to bee colonies, damaging wax combs inside or outside hives. Nanotechnology can play a role in developing attractants for these pests, which can be used in various trap designs to capture them. Additionally, herbal extracts and essential oils have shown effectiveness in controlling wax moths and small hive beetles. When formulated as nanoparticles, these substances could enhance their pest-control efficacy. Further research is needed to explore these potential applications [51-55].

Mites and honey bee diseases

Mites, such as Varroa destructor, attack honey bees by feeding on their hemolymph at both immature and mature stages, while Tropilaelaps mites target only the immature stages. Honeybees are also susceptible to a range of bacterial, viral, fungal, and protozoan diseases, including Nosema. Previous studies on plant diseases have demonstrated that nanomaterials can be more effective than conventional treatments. For mites and diseases affecting honey bees, several control methods and materials, such as herbal extracts, propolis, and essential oils, have been explored. The effectiveness of these substances can be improved when formulated as nanoparticles. For instance, adding nanosilver (25 ppm) to bee feed has been shown to reduce the number of Nosema spores in laboratory conditions. Further research is required to develop safe, effective materials to control mites and honey bee diseases. Additionally, novel diagnostic techniques, such as a label-free colorimetric nanodiagnostic method, can be used to detect specific pathogens, such as Melissococcus plutonius, which causes European Foulbrood (EFB). This method, based on unmodified gold nanoparticles, offers a rapid and precise way to detect EFB and could be adapted for diagnosing other diseases like American Foulbrood (AFB), which presents a significant challenge to honey bee colonies [56-61].

Honey bee products

Beekeepers produce a range of valuable products from bee colonies, including royal jelly, honey, pollen, bee venom, beeswax, and propolis. These products serve as a significant source of income for many beekeepers and are utilized for both human consumption and medicinal purposes. In addition, beeswax is particularly useful in various industrial applications. The commercial value of these products of bees can be increased by improving their effectiveness. Nanotechnology offers the potential to enhance the properties of these products. For instance, nano-formulated propolis has shown promise as a treatment for cancer, with its antimicrobial properties exceeding those of traditional Chinese propolis. Propolis-loaded nano-in-microparticles have also demonstrated improved anti-cancer activity. Furthermore, chitosan nanoparticles carrying bee venom have proven effective against amebiasis, and melittin-loaded nano-liposomes have shown the ability to inhibit the growth of hepatocellular carcinoma (HCC) cells. More research is needed to explore the potential of bee products in nanoparticle form,

particularly in their ability to combat human and animal diseases, which could also boost their commercial appeal [62-66].

There is a wide variety of nanoparticles available for use in agriculture, and as their application increases, there is concern about the potential environmental pollution caused by these materials, particularly heavy metals in their production. Several studies have highlighted the negative effects of nanoparticles on honey bees. For instance, nanosilver at a concentration of 25 ppm added to bee feed was found to reduce the lifespan of worker bees in lab conditions. Similarly, high concentrations of ZnO nanoparticles were shown to lower the feeding rate of bees, potentially causing metabolic disruptions, such as reduced brain protein levels, decreased survival rates, and increased activities of AChE and GST, indicating a significant impact on the bees' nervous systems. Other studies revealed that the toxic effects of nanoparticles, including TiO2, ZnO-TiO2, and Ag-TiO2, on *Apis mellifera* increased with higher concentrations and prolonged exposure. Furthermore, cerium (IV) oxide nanoparticles led to sublethal changes in bees after chronic oral exposure, and sublethal concentrations of CdO or PbO nanoparticles in sugar syrup were found to negatively affect the histological and cellular structure of bee workers' midgut cells [67-73].

On a more positive note, certain studies have indicated that some nanoparticles pose no significant threat to honey bees. For example, silver nanoparticles, when used in beekeeping tools, did not show any harmful effects in honey or combs, suggesting that nanomaterials can be safely used in beekeeping. Additionally, nanoemulsions of hexanal and nanosized carbon black or titanium dioxide did not cause mortality or adverse chronic effects on bees' survival, feeding, or enzymatic activity. Similarly, ZnO nanoparticles at certain concentrations showed no negative impacts on bee survival or enzymatic functions, such as glutathione S-transferase and acetylcholinesterase [72-76].

The presence of nanoparticle residues in the products of bees due to direct applications on plants remains underexplored. However, honey bees are often used as bio-indicators for environmental pollution, so they, along with their products, could serve to monitor contamination levels from nanoparticles. Further research, both in laboratory and field settings, is needed to evaluate the potential risks of nanoparticles on bee behavior, physiology, colony productivity, and the impact on bee diseases and pests. While the risks associated with nanoparticles may be lower than those of traditional pesticides, a thorough assessment of their effects on honey bees is necessary before their widespread use in sustainable agriculture [76-80].

Conclusion

Nanotechnology shows great potential in advancing beekeeping practices, with various applications across the field. Further research is needed to explore its potential and identify the most effective formulations for different tasks. Additionally, the potential risks posed by nanoparticles to honey bees, whether as target or non-target organisms, warrant further investigation.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

- 1. Hagab RH, Kotp YH, Eissa D. Using nanotechnology for enhancing phosphorus fertilizer use efficiency of peanut bean grown in sandy soils. J Adv Pharm Educ Res. 2018;8(3):59-67.
- Mahmoud ZH, Falih MS, Khalaf OE, Farhan MA, Ali FK. Photosynthesis of AgBr doping TiO2 nanoparticles and degradation of reactive red 120 dye. J Adv Pharm Educ Res. 2018;8(4):51-5.
- Al-Ghamdi M, Aly MM, Sheshtawi RM. Antimicrobial activities of different novel chitosan-collagen nanocomposite films against some bacterial pathogens. Int J Pharm Phytopharmacol Res. 2020;10(1):114-21.

- Rashid FL, Hadi A, Al-Garah NH, Hashim A. Novel phase change materials, MgO nanoparticles, and waterbased nanofluids for thermal energy storage and biomedical applications. Int J Pharm Phytopharmacol Res. 2018;8(1):46-56.
- 5. El-Nour KMA, Eftaiha AA, Al-Warthan A, Ammar RA. Synthesis and applications of silver nanoparticles. Arab J Chem. 2010;3(3):135-40.
- 6. Rajput N. Methods of preparation of nanoparticles-a review. Int J Adv Eng Technol. 2015;7(6):1806-11.
- 7. Pacioni NL, Borsarelli CD, Rey V, Veglia AV. Synthetic routes for the preparation of silver nanoparticles: a mechanistic perspective. InSilver nanoparticle applications: in the fabrication and design of medical and biosensing devices 2015 Feb 21 (pp. 13-46). Cham: Springer International Publishing.
- Bilia AR, Guccione C, Isacchi B, Righeschi C, Firenzuoli F, Bergonzi MC. Essential oils loaded in nanosystems: a developing strategy for a successful therapeutic approach. Evid Based Complement Altern Med. 2014;2014:651593. doi:10.1155/2014/651593
- El-Deeb NM, El-Sherbiny IM, El-Aassara MR, Hafez EE. Novel trend in colon cancer therapy using silver nanoparticles synthesized by honey bee. J Nanomed Nanotechnol. 2015;6(2):265. doi:10.4172/2157-7439.1000265
- Abbassy MA, Abdel-Rasoul MA, Nassar AM, Soliman BS. Nematicidal activity of silver nanoparticles of botanical products against root-knot nematode, Meloidogyne incognita. Arch Phytopathol Plant Prot. 2017;50(17-18):909-26.
- 11. Saxena A, Jain A, Upadhyay P, Gauba PG. Applications of nanotechnology in Agriculture. J Nanosci Nanoeng Appl. 2018;8(1):20-7.
- 12. Kim DM, Lee GD, Aum SH, Kim HJ. Preparation of propolis nanofood and application to human cancer. Biol Pharm Bull. 2008;31(9):1704-10.
- 13. Jingli YDW, Zhsgg F. Effect of Chinese propolis and nano-propolis on common pathogens in vitro. Chin Pharmacist. 2008;10:1167-9.
- 14. Rassu G, Cossu M, Langasco R, Carta A, Cavalli R, Giunchedi P, et al. Propolis as lipid bioactive nanocarrier for topical nasal drug delivery. Colloids Surf B Biointerfaces. 2015;136:908-17.
- 15. Elbaz NM, Khalil IA, Abd-Rabou AA, El- Sherbiny IM. Chitosan-based nano-in-microparticle carriers for enhanced oral delivery and anticancer activity of propolis. Int J Biol Macromol. 2016;92:254-69.
- 16. Mao J, Liu S, Ai M, Wang Z, Wang D, Li X, et al. A novel melittin nano-liposome exerted excellent antihepatocellular carcinoma efficacy with better biological safety. J Hematol Oncol. 2017;10:71.
- 17. Sahinler N, Gul A. The effects of propolis production methods and honeybee genotypes on propolis yield. Pak J Biol Sci. 2005;8(9):1212-4.
- 18. Dimou M, Thrasyvoulou A, Tsirakoglou V. Efficient use of pollen traps to determine the pollen flora used by honey bees. J Apic Res. 2006;45(1):42-6.
- 19. Fakhimzadeh K. A new device for venom collection and apicultural research. Am Bee J. 1990;130:785-7.
- 20. Brandeburgo MMA. A safe device for extracting venom from honey bees. Bee World. 1992;73(3):128-30.
- 21. Sanad RE, Mohanny KM. The efficacy of a new modified apparatus for collecting bee venom in relation to some biological aspects of honeybee colonies. J Am Sci. 2013;9(10):177-82.
- 22. Bacandritsos N, Papanastasiou I, Saitanis C, Roinioti E. Three non-toxic insect traps useful in trapping wasps enemies of honey bees. Bull Insectol. 2006;59(2):135-45.
- 23. Mizutani T, Arai K, Miyamoto M, Kimura Y. Application of silica-containing nano-composite emulsion to wall paint: a new environmentally safe paint of high performance. Prog Org Coat. 2006;55(3):276-83.
- 24. Abou-Shaara HF, Al-Ghamdi AA, Mohamed AA. Honey bee colonies performance enhance by newly modified beehives. J Apic Sci. 2013;57(2):45-57.
- Le Conte Y, Navajas M. Climate change: impact on honey bee populations and diseases. Rev Sci Tech Off Int Epizoot. 2008;27(2):499-510.
- Güneş ME, Borum AE, Özakin C, Girişgin AO, Aydin L. A new technic: efficacy of nano-silver coating of honey bee hives against some microorganisms. U Arı DU Bee J. 2012;12(1):23-30.
- 27. Brodschneider R, Crailsheim K. Nutrition and health in honey bees. Apidologie. 2010;41(3):278-94.
- 28. Huang Z. Pollen nutrition affects honey bee stress resistance. Terr Arthropod Rev. 2012;5(2):175-89.
- 29. Abou-Shaara HF. Effects of various sugar feeding choices on survival and tolerance of honey bee workers to low temperatures. J Entomol Acarol Res. 2017;49(1):6-12.

- 30. Saffari AM, Kevan PG, Atkinson JL. A promising pollen substitute for honey bees. Am Bee J. 2004;144(3):230-1.
- DeGrandi-Hoffman G, Wardell G, Ahumada-Segura F, Rinderer T, Danka R, Pettis J. Comparisons of pollen substitute diets for honey bees: consumption rates by colonies and effects on brood and adult populations. J Apic Res. 2008;47(4):265-70.
- 32. Mattila HR, Otis GW. Influence of pollen diet in spring on development of honey bee (Hymenoptera: Apidae) colonies. J Econ Entomol. 2006;99(3):604-13.
- 33. DeGrandi-Hoffman G, Chen Y, Huang E, Huang MH. The effect of diet on protein concentration, hypopharyngeal gland development, and virus load in worker honey bees (Apis mellifera L.). J Insect Physiol. 2010;56(9):1184-91.
- 34. Gill RA. The value of honeybee pollination to society. InVI International Symposium on Pollination 288 1990 Aug 27 (pp. 62-68).
- 35. Pankiw T. Brood pheromone regulates foraging activity of honey bees (Hymenoptera: Apidae). J Econ Entomol. 2004;97(3):748-51.
- 36. Mott CM, Breed MD. Insulin modifies honeybee worker behavior. Insects. 2012;3(4):1084-92.
- Abou-Shaara HF. The foraging behavior of honey bees, Apis mellifera: a review. Vet Med. 2014;59(1):1-10.
- 38. Sharma R, Rather MA, Leela RV, Saha H, Purayil SB, Dar SA, et al. Preliminary observations on effect of nano-conjugated pheromones on Clarias batrachus (Linnaeus, 1758). Aquac Res. 2014;45(8):1415-20.
- Winston ML, Higo HA, Colley SJ, Pankiw T, Slessor KN. The role of queen mandibular pheromone and colony congestion in honey bee (Apis mellifera L.) reproductive swarming (Hymenoptera: Apidae). J Insect Behav. 1991;4:649-60.
- Schmidt JO. Attraction of reproductive honey bee swarms to artificial nests by Nasonov pheromone. J Chem Ecol. 1994;20:1053-6.
- 41. Moritz RF, Kryger P, Allsopp MH. Competition for royalty in bees. Nature. 1996;384(6609):522.
- 42. Neumann P, Moritz RF. Testing genetic variance hypotheses for the evolution of polyandry in the honeybee (Apis mellifera L.). Insect Soc. 2000;47:271-9.
- 43. Cobey SW. Comparison studies of instrumentally inseminated and naturally mated honey bee queens and factors affecting their performance. Apidologie. 2007;38(4):390-410.
- 44. Cobey SW, Tarpy DR, Woyke J. Standard methods for instrumental insemination of Apis mellifera queens. J Apic Res. 2013;52(4):1-18.
- 45. Durfey CL, Swistek SE, Liao SF, Crenshaw MA, Clemente HJ, Thirumalai RV, et al. Nanotechnology-based approach for safer enrichment of semen with best spermatozoa. J Anim Sci Biotechnol. 2019;10:14.
- 46. Štiavnická M, Abril-Parreño L, Nevoral J, Králíčková M, García-Álvarez O. Non-invasive approaches to epigenetic-based sperm selection. Med Sci Moni Int Med J Exp Clin Res. 2017;23:4677.
- 47. Villemant C, Barbet-Massin M, Perrard A, Muller F, Gargominy O, Jiguet F, et al. Predicting the invasion risk by the alien bee- hawking Yellow-legged hornet Vespa velutina nigrithorax across Europe and other continents with niche models. Biol Conserv. 2011;144(9):2142-50.
- Budge GE, Hodgetts J, Jones EP, Ostojá- Starzewski JC, Hall J, Tomkies V, et al. The invasion, provenance, and diversity of Vespa velutina Lepeletier (Hymenoptera: Vespidae) in Great Britain. PLoS One. 2017;12(9):e0185172.
- 49. Neumann P, Evans JD, Pettis JS, Pirk CW, Schäfer MO, Tanner G, et al. Standard methods for small hive beetle research. J Apic Res. 2013;52(4):1-32.
- Neumann P, Pettis JS, Schäfer MO. Quo vadis Aethina tumida? Biology and control of small hive beetles. Apidologie. 2016;47:427-66.
- 51. Ellis JD, Graham JR, Mortensen A. Standard methods for wax moth research. J Apic Res. 2013;52(1):1-7.
- 52. Rome Q, Perrard A, Muller F, Villemant C. Monitoring and control modalities of a honeybee predator, the yellow-legged hornet Vespa velutina nigrithorax (Hymenoptera: Vespidae). Aliens. 2011;31(31):7-15.
- 53. Demichelis S, Manino A, Minuto G, Mariotti M, Porporato M. Social wasp trapping in northwest Italy: comparison of different bait-traps and first detection of Vespa velutina. Bull Insectol. 2014;67(2):307-17.
- 54. Zaitoun ST. The effect of different Mediterranean plant extracts on the development of the great wax moth Galleria mellonella L. (Lepidoptera: Pyralidae) and their toxicity to worker honeybees Apis mellifera L.(Hymenoptera: Apidae) under laboratory conditions. J Food Agric Environ. 2007;5(2):289-94.

- 55. Dekebo A, Seokmin H, Jung C. Attractiveness of the small hive beetle (Aethina tumida) to volatiles from honey bee (Apis mellifera) and beehive materials. J Apic. 2017;32(4):315-26.
- 56. Dietemann V, Nazzi F, Martin SJ, Anderson DL, Locke B, Delaplane KS, et al. Standard methods for varroa research. J Apic Res. 2013;52(1):1-54.
- 57. Anderson DL, Roberts JM. Standard methods for Tropilaelaps mites research. J Apic Res. 2013;52(4):1-6.
- Forsgren E, Budge GE, Charrière JD, Hornitzky MA. Standard methods for European foulbrood research. J Apic Res. 2013;52(1):1-4.
- 59. Fries I, Chauzat MP, Chen YP, Doublet V, Genersch E, Gisder S, et al. Standard methods for Nosema research. J Apic Res. 2013;52(1):1-28.
- 60. Ahmed AI. Chitosan and silver nanoparticles as control agents of some Faba bean spot diseases. J Plant Pathol Microbiol. 2017;8(9):2.
- 61. Fuselli SR, de la Rosa SB, Gende LB, Eguaras MJ, Fritz R. Antimicrobial activity of some Argentinean wild plant essential oils against Paenibacillus larvae, causal agent of American foulbrood (AFB). J Apic Res. 2006;45(1):2-7.
- 62. González MJ, Marioli JM. Antibacterial activity of water extracts and essential oils of various aromatic plants against Paenibacillus larvae, the causative agent of American Foulbrood. J Inv Pathol 2010;104(3):209-13.
- 63. Rosenkranz P, Aumeier P, Ziegelmann B. Biology and control of Varroa destructor. J Inv Pathol. 2010;103:S96-S119.
- 64. Abou-Shaara HF. Using safe materials to control Varroa mites with studying grooming behavior of honey bees and morphology of Varroa over winter. Ann Agric Sci. 2017;62(2):205-10.
- 65. Abou-Shaara HF. Calendar for the prevalence of honey bee diseases, with studying the role of some materials to control nosema. Korean J Appl Entomol. 2018;57(2):87-95.
- 66. Borsuk G, Paleolog J, Olszewski K, Strachecka A. Laboratory assessment of the effect of nanosilver on longevity, sugar syrup ingestion, and infection of honeybees with Nosema spp. Med Weter. 2013;69(12):730-2.
- Saleh M, Soliman H, El-Matbouli M, Sørum H, Fauske AK. A novel gold nanoparticles-based assay for rapid detection of Melissococcus plutonius, the causative agent of European foulbrood. Vet Rec. 2012;171(16):400.
- 68. Saber AES, Abdelwahab AK, El Amir AM, Nassar MI. Bee venom loaded chitosan nanoparticles as treatment for amoebiasis in mice. J Egypt Soc Parasitol. 2017;47(2):443-58.
- 69. Glavan G, Milivojević T, Božič J, Sepčić K, Drobne D. Feeding preference and sub-chronic effects of ZnO nanomaterials in honey bees (Apis mellifera carnica). Arch Environ Contam Toxicol. 2017;72:471-80.
- 70. Milivojević T, Glavan G, Božič J, Sepčić K, Mesarič T, Drobne D. Neurotoxic potential of ingested ZnO nanomaterials on bees. Chemosphere. 2015;120:547-54.
- Özkan Y, Irende İ, Akdeniz G, Kabakçi D, Sökmen M. Evaluation of the comparative acute toxic effects of TiO2, Ag-TiO2, and ZnO-TiO2 composite nanoparticles on honey Bee (Apis mellifera). J Int Environ Appl Sci. 2015;10(1):26-36.
- 72. Kos M, Kokalj AJ, Glavan G, Marolt G, Zidar P, Božič J, et al. Cerium (IV) oxide nanoparticles induce sublethal changes in honeybees after chronic exposure. Environ Sci Nano. 2017;4:2297-310.
- 73. Dabour K, Al Naggar Y, Masry S, Naiem E, Giesy JP. Cellular alterations in midgut cells of honey bee workers (Apis millefera L.) exposed to sublethal concentrations of CdO or PbO nanoparticles or their binary mixture. Sci Total Environ. 2019;651:1356-67.
- 74. Karthika S, Kumar NN, Gunasekaran K, Subramanian KS. Biosafety of nanoemulsion of hexanal to honey bees and natural enemies. Indian J Sci Technol. 2015;8(30):1-7. doi:10.17485/ijst/2015/v8i30/52668
- 75. Mohan C, Sridharan S, Subramanian KS, Natarajan N, Nakkeeran S. Effect of Nanoemulsion of Hexanal on honey bees (Hymenoptera; Apidae). J Entomol Zool Stud. 2017;5(3):1415-8.
- 76. Jemec A, Milivojević T, Drobne D, Sepčić K, Božič J, Glavan G. No chronic effects on biochemical biomarkers, feeding and survival of carnolian honeybees (Apis mellifera carnica) after exposure to nanosized carbon black and titanium dioxide. Acta Biol Slov. 2016;59(1):45-55.
- 77. Leita L, Muhlbachova G, Cesco S, Barbattini R, Mondini C. Investigation of the use of honey bees and honey bee products to assess heavy metals contamination. Environ Monit Assess. 1996;43:1-9.

- 78. Rashed MN, El-Haty MT, Mohamed SM. Bee honey as environmental indicator for pollution with heavy metals. Toxicol Environ Chem. 2009;91(3):389-403.
- 79. Roman A. Levels of copper, selenium, lead, and cadmium in forager bees. Polish J Environ Stud. 2010;19(3):663-9.
- 80. Oliveira CR, Domingues CE, de Melo NF, Roat TC, Malaspina O, Jones-Costa M, et al. Nanopesticide based on botanical insecticide pyrethrum and its potential effects on honeybees. Chemosphere. 2019;236:124282.