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REVIEW

Nanoencapsulations of essential oils and microbial toxins as insecticides: Case studies for their further optimization

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Abstract

Indiscriminate use of excessive insecticides has mounted concerns over human health and environmental pollution issues. In contrast, nanoencapsulations of many insecticides can offer a broad range of reliable and mostly safe alternatives based on their nanoparticles (NPs). These NPs possess virtually all the attributes of ideal biocontrol agents due to their minute size which economizes usage to wide surface areas and boosts reactivity to increase effectiveness. Many of their formulations presented herein have proved to render both huge biocompatibility and supreme efficiency as insecticides. This review aimed to gather information relevant to expanding and advancing technological tools for optimizing application of insecticidal essential oils and microbial toxins with the targeted release and protective merits of encapsulation. It highlights their various applications to demonstrate their merits compared to other classical and commercial usages. The target pests included a variety of exemplifying insects of stored grains, farming, and disease-vectors. Yet, limited recorded data on their possible unfavorable impacts on humans and the environment might call for their attentive usage particularly in large-scale settings. Hence, the related modes of action of essential oils and microbial toxins are provided so that they could be used effectively and carefully. Their insecticidal merits lend further urgency to the need to optimize their role in pest management as related to challenges and opportunities which are discussed here to upgrade sustainable agricultural systems.

Keywords: biopesticide, modes of action, nanoformulations, risk assessment, sustainability

Introduction

The global burden of crop pests on key crops is being aggravated by continuous world population growth, climate change, and conflicts. Annual losses in food production exclusively caused by insect pests were recently recorded to reach 45% (Battu *et al.* 2023). The damage to crops is diffused not only worldwide but also to the whole plant, i.e., above- and below-ground organs. Ways to effectively control such crop pests are complicated by the dilemma of their interactions with many biotic and abiotic factors. Therefore, merging recent innovations such as ribonucleic acid (RNA)-sequencing in biopesticide development (Gupta *et al.* 2023), bioinformatics, and nanoencapsulation of certain insecticidal components (Yousef *et al.* 2023) can be incorporated into reliable pest management plans. Yet, some of these emerging innovations may be more reliable or acceptable than others, e.g., using genetic engineering for pest control is still a controversial approach since there are concerns about its safety (Afzal and Mukhtar 2024). Of special interest, nanotechnological research to upgrade pest/pathogen control measures has been growing exponentially (Elmer and White 2018). This is because nanopesticides can significantly reduce the amounts of traditional pesticide inputs which have globally reached as many as 4.6 million tons annually (Subhadarsini Pradhan *et al.* 2022). Unfortunately, more than 90% of them are lost to the air, run-off, and/or drift during their application which has brought about many health and ecological problems. Meanwhile, staggering figures have been recorded showing that the real amount of targeted pest uptake is only around 0.1% after loss of classical pesticides (Subhadarsini Pradhan *et al.* 2022). Admittedly, traditional usage of chemical pesticides leaves serious pollutants in the soil and water systems, in addition to accumulations of chemical residues in crops and food, which cause high risks to human health. Usually, their repeated usage can induce gradual resistance in the intended pests. In contrast, bio-active ingredients of nanopesticides can minimize such losses, improve their effectiveness and at least delay acquired resistance (Shen *et al.* 2023). Microbial pathogens that are also used as protective treatments against plant pests/pathogens can be incorporated into these techniques (Abd-Elgawad *et al.* 2010).

As insecticide-related nanotechnology is still developing, this review presents a brief overview of their classification and preparation. However, with the swift development of nanoinsecticides for sustainable agriculture, continuously updating state-of-the-art research in specific related disciplines is quite useful. Therefore, current challenges and opportunities for using nanoencapsulations of two bio-insecticidal groups believed to have safe and promising applications, i.e., essential oils (EOs) and microbial toxins are addressed. This review focuses on their economic and safe applications as well as modes of action for their effective usage by merging emerging and novel technologies.

A brief survey of classification and preparation of nanoinsecticides

Because nanomaterials represent three external dimensional particles, with at least one dimension at the scale of 1 to 100 nm (Sekhon 2014), there is a large variety of potential natural/engineered materials that can be defined as nanoparticles (NPs). Generally, they can be classified into various categories, e.g., according to their material-based source, shape, number of nano-scale dimensions, intended goal, and active ingredient (AI). The latter has two main divisions regarding insecticides, namely, biological and chemical AIs. Furthermore, nanoinsecticides addressed here have encapsulated product forms to make sure that the encapsulated AI can target the pest without being negatively affected by the related external surroundings. Nanoinsecticidal preparation techniques can also be divided into two major types based on dispersing and condensing reactions. The technique can be detected according to whether the NPs require a chemical reaction for condensing or are achieved directly from a macromolecule by physical dispersing. The first technique is a type of bottom-up approach, and the second is known as an up-bottom technique (Anandhi *et al.* 2020).

Nanoencapsulation of biological insecticides and their merits

Currently, NPs of single/composite nonmetals, metalloid/metallic oxides, carbon nanomaterials, and functionalized structures of liposomes, dendrimers, and quantum dots have begun to be used for plant protection measures (Elmer and White 2018). Among them, biological insecticides related to both EOs, and microbial toxins rank high due to their supposed eco-friendly and effective application as nanoinsecticides (Kala *et al.* 2020; Gupta *et al.* 2023). As nanoencapsulation aims at loading/coating the AI in nanometer sized capsules (Cano-Sarabia and Maspoch 2015), it can also be synthesized to provide several merits. These involve slow release to achieve a longer half-life period of efficacy, low dose/concentration of the encapsulated AI to economize and minimize its residuals, ensure safe handling, and enhanced bioactivities of AI due to targeted release and protective attributes (Sabry and Ragaei 2018). Various organic (e.g., synthetic or natural polymers), inorganic (e.g., elemental carbon and mesoporous silica) and organic-inorganic compounds have been utilized to encapsulate insecticides to meet specific deliveries and pests as well as their settings. These materials, known as nano-carriers, can have different forms and shapes too, e.g., capsules, gels, spheres, liposomes, micelles, and inorganic cages (Graily-Moradi and Lajayer 2021; Shen *et al.* 2023). The latter authors reviewed nanopesticides that are responsive to a group of stimuli affecting controlled-release processes, including several with dual- or triple-responsiveness. General factors that affect controlled release are the type of nanocapsule material, preparation method, shape and dimensions of the matter used, shell thickness and porosity, encapsulation efficacy and the amount of encapsulate load (Nuruzzaman *et al.* 2016). Moreover, environmental factors (e.g., soil, water, air, humidity, temperature) have an impact on this controlled release (Xu *et al.* 2017; Shishir *et al.* 2018; Shen *et al.* 2023). Marella *et al.* (2021) summed up notable merits of nanoencapsulation of insecticides.

They included enhanced solubility with the dispersion rate of AIs, avoidance of harmful solvents, controlled release of AIs, improved stability and mobility with consequently higher activity, minimal environmental pollution during preparation and application relative to classical insecticides. Furthermore, nanogenic formulations of insecticides containing engineered nanomaterials of microbial toxins or EOs can enhance the target species' active ingredient stability, solubility, penetrability, and controlled release traits via nanoencapsulation (Adisa *et al.* 2019).

Numerous devices are used to characterize and assess the favorable outcomes of nanoinsecticides. Important characteristics involve efficacy, stability, bioavailability, toxicity, solubility, and controlled release. Basically, the particle size has a significant role in preparation, loading capacity, application, and insecticidal efficiency. To secure the success of the pesticide product, examining the external surface of the post-preparation NPs before and after AI loading is a key factor**.** Many tools are utilized to test the prepared nanoencapsulated insecticides (Pisal *et al.* 2020). To name a few, X-ray diffraction manifests the prepared products-phase composition, X-ray photoelectron spectroscopy shows the composition of the coating materials and the related chemical bonding, a superconducting quantum interference device can measure the magnetic properties of nanocapsules, Fourier transform infra-red analysis is used to confirm the specific peaks-presence for the functional groups of the materials used, spectrophotometry can assess the loading capacity and efficiency of nanocapsules, and atomic absorption evaluates the concentration of the loaded matters. Also, while scanning electron microscopy (EM) can scout all structures and clusters of the nanomaterials at various scales, transmitting EM can examine their detailed morphology to clearly show the shell/core formation of the nanocapsules.

Conventional and advanced formulations of essential oils as insecticides

Conventional formulations

As secondary metabolites of plants, EOs are complex chemicals, volatile in nature with a strong odor, and lower density than water. Their characteristics enable them to possess a wide array of activities against pests e.g., insecticidal, repellent, antifeedant, growth regulatory, oviposition deterrent, and antivectorial properties (Sarri *et al.* 2024). Therefore, EOs can be used via classical formulations, either liquid (e.g., suspensions and emulsions) or dry (e.g., water-dispersible granules, granules, wettable powders, and powders) formulations. With the passing of classical formulations, novel and reliable ones are enticing stakeholders into their usage. To assure the efficacy of such EOs in ecofriendly approaches even with classical applications, their insecticidal potential has been compared with commercial synthetic insecticides. Spraying of *Foeniculum vulgare* Mill. (Fennel)-aqueous EO (fenchone and transanethole as AIs) could significantly induce high and specific mortality of *Myzus persicae* Sulzer (polyphagous aphid) (Pavela 2018). In contrast, such an effect caused by commercially marketed Vaztak (α-cypermethrin) was linked to damaging effects on nontarget species (Pavela 2018). Using the multicolored Asian lady beetle *(Harmonia axyridis* Pallas) and the manure worm (*Eisenia fetida* Savigny) as positive controls, the insecticidal efficacy of *Schizogyne sericea* L.-EO spray (diluted in dimethyl sulfoxide/acetone or water) was proved against four insect species (Benelli *et al.* 2019). Numerous promising adulticidal, larvicidal, and ovicidal properties of EOs against insect pests were recently reviewed (Gupta *et al.* 2023) to encourage the shift towards these reliable and benign alternatives. However, such classical formulations have several defects as liquid insecticide products are subject to spillage and splashing, leading to direct contact with the skin, wastage, and contamination. Also, dry formulations tend to generate unfavorable dust (Kala *et al.* 2020).

Nanoformulations of insecticidal essential oils

Valid as they are, these classical formulations of insecticidal essential oils face several difficulties in addition to those already mentioned but directly related to the EOs-active ingredients. Major challenges are related to their inherent nature (high volatility/lipophilicity), costly production, and manufacturing obstacles. A promising technique to circumvent such defects and upgrade EO bioactivity is encapsulating EOs in chemical matrices as carriers to ease the controlled release of oils. To develop stable emulsions, nano- and microformulations based on the encapsulation of AIs into a matrix proved to be reliable (Campolo *et al.* 2018; Giunti *et al.* 2021). Moreover, nanosized emulsions are better than micro emulsions because of their long-lasting stability, minimal volatility, and need of a minimum surfactant concentration (Gupta *et al.* 2023). Therefore, numerous investigations have demonstrated the capacity of nanoencapsulation formulas as protective carriers to lessen/prevent rapid evaporation and degradation, which improves their activity (Adel *et al.* 2019). The latter authors appraised this approach since it offers optimum and efficient delivery within the targeted scope via AI-controlled release. In addition to being recognized as less harmful to the targeted ecosystems, EO nanoparticles are much more mobile than the corresponding bulk materials. This trait boosts the penetration rate of the EOs-active molecules into the body of insect pests via both direct contact through the insect's cuticle and insect feeding. Although their nanoencapsulations can enhance the insecticidal effects as they protect the AIs and raise their solubility with controlled release, incorporating such formulations with favorable materials such as chitosan has proved to have additional merits. Chitosan is also utilized to optimize the interaction between microorganisms and the metabolisms of plants. Thus, several chitosan formulations have improved the availability/stabilization of EOs-insecticides (Abenaim and Conti 2023). To evaluate the effectiveness of nanoencapsulated EOs against various groups of insects, different studies are reported as follows.

For insect pests of stored grains

Nanoencapsulation of EOs possesses a broad spectrum of bioactivities including repellent, fumigation, oviposition inhibitory, antifeedant, toxicity, and insecticidal performance. The technique can diminish the dosage; enhance the permeability, stability, and persistence of the residual activity via controlled release towards targeted pests in stored grain. Adequate homogenization of 100% glycerol and 2.5% surfactant of nanoencapsulated oils derived from purslane, mustard, and castor achieved 93.61, 79.10, and 59.39% *Ephestia cautella* Walker-larval mortalities, respectively. Their corresponding mortalities were 70.04, 43.1, and 20.13%, respectively, when using bulk oils, i.e., using the same material but not as nanoforms. Thus, although the oil doses were reduced, their insecticidal activities were enhanced due to the creation of stable droplets and increased retention of the low released nanoencapsulated oils (Sabbour and Abd El-Aziz 2016). In another study, *Tribolium castaneum* Herbst-adult mortality was 78.3% for free oil and 100% for nanoencapsulated oil at the same rosemary oil concentration (Khoobde *et al*. 2017). The authors concluded that nanoencapsulation can not only decrease the applied doses but also reduce the number of oil applications. Likewise, the nanoencapsulated gel formula of cumin oil has better toxicity than pure oil against two stored product pests (Ziaee *et al*. 2014). While the free cumin oil used entirely lost its fumigant toxicity after 12 days, the nanoencapsulated oil lost only 15% of its toxicity for *Tribolium confusum* Jacquelin du Val and 60% for *Sitophilus granarius* L. at the same period due to the different stability of the oil formulation used. Khanahmadi *et al*. (2017) utilized *Artemisia* sp.-nanoencapsulated EOs to attain 100% mortality of *Sitophilus oryzae* L., and *T. confusum* Jacquelin du Val with LC_{50} of nanoencapsulated oil as 30.29 and 45.82 ppm, respectively, relative to 550.1 and 713.2 using a classical fumigant (Phosphine), respectively. Garlic oil loaded on synthetic polymer (polyethylene-glycol) as nanoencapsulated oil induced 80% *T. castaneum*–mortality with prolonged activity due to the slow release from the capsule matrix, and/or persistence of Ais. However, free garlic oil, not in nanoform, at a similar concentration caused only 11% mortality (Yang *et al*. 2009). Similarly, Werdin-Gonzalez *et al*. (2014) noted that the EOs-polyethylene glycol NPs increased the bioactivity of the volatile components and boosted the contact toxicity 3.6 and 7.8 times against *T. castaneum* and *Rhizopertha dominica* Fabricius, respectively, relative to free EOs. Interestingly, they found that the size of oil-containing capsules was not significantly altered but the amount of free EOs was decreased by 25% at 6 months post-storage. Remarkable increases of the residual contact toxicity generated by nanoencapsulated EOs against various species of insect pests have been shown in numerous other studies (e.g., Martín *et al*. 2010; Kumar *et al*. 2019). Consequently, favorable effects of these nanocapsules are reflected on the stored products. For instance, Abdel-Halim and Attia (2018) reported that nanoencapsulated clove oil (using solid lipid NPs as a carrier) was more (4.86-fold) toxic for *T. castaneum* than free clove oil after 7 days. Furthermore, the impact of the formulated EOs on the biochemical contents of the stored wheat grains was significantly higher in the total protein and carbohydrate contents as well as percentages of germination than the control group.

The difference in the effect of the active ingredient in EO of one plant to another must be considered within this nanoencapsulation approach. Both nanoencapsulated and free EOs of rosemary, garlic, citronella, and catnip were compared for efficacy against two key pests, *Callosobruchus maculatus* Fabricius and *Callosobruchus chinensis* L. Results showed that the nanoencapsulated oils were more toxic than free oils (Sabbour 2019). However, nano-oils of rosemary and garlic were more effective than the others against the two pests. The former oils caused accumulative mortalities of 82.2 and 66.3% in adult *C. maculatus* but the other oils induced 61.2 and 46.6% mortalities, respectively, at 7 days post-treatment. For *C. chinensis*, these mortalities were 68.9 and 49.9% after treatment with free rosemary and garlic oils, but were significantly enhanced to 89.7 and 70.9% using rosemary and garlic oils, respectively. From an economic point of view, weight loss of the tested seeds was significantly reduced after nano-oil treatments for both pests, 90 days post-storage. In addition, a repellency test of these nano-oils found that rosemary and garlic were the highest repellant oils. These and similar results can be helpful and possibly extended to control many other insect pests of stored grains. Recent studies recommended that using such nanoencapsulated EOs should rely on basic factors governing their activity and toxicity (Bayramzadeh *et al.* 2019). These factors include the EO-extraction technique, formulation structure and chemical composition, encapsulation method, target insect species/stage, EO-controlled release action, and application systems. The latter authors found that EO extracted from *Cuminum cyminum* L. was more effective against the pest species *Oryzaephilus surinamensis* L., *T. castaneum*, and *S. granarius* L., than EO of *Lavandula angustifolia*. Since their report was the first for nano-capsulate formulation through emulsion solvent evaporation using poly-ethersulfone polymer, they attributed the relatively low toxicity to the formation method. Also, Chenni *et al.* (2020) found that the toxicity of encapsulated clove oil had a higher toxic effect against *R. dominica* than *S. oryzae* L. Furthermore, for the application method, they recorded that the contact test was more toxic to the insects than the ingestion regime whereas the adults of *T. castaneum* were generally insensitive to the treatments. Such toxicity variation may be ascribed to various factors such as chemical compositions of the capsule shell, EO-extraction technique, and bioassay approaches of the formulation. Ikawati *et al.* (2020) clarified that the lower nanoencapsulated clove oil toxicity for *Cryptolestes ferrugineus* Stephens adults than that of corresponding bulk oil was related to the controlled release action and the loading efficiency in the capsule which reached 77%. Moreover, as the nanoencapsulation diminished the volatility of clove oil constituents and kept it from degradation/evaporation, the impact of the nanoencapsulation lasted for a longer time. In another study, the encapsulation efficiency of EOs for geranium and palmarosa against *Plodia interpunctella* Hübner was 90% but was reduced to 72% in peppermint (Jasser *et al.* 2020). Besides, when these EOs of bulk and nanocapsules were applied through contact or fumigation, the temperature had a significant effect on their insecticidal activity by contact only. Attia *et al.* (2020) used cinnamon oil-loaded mesoporous silica NPs, pure cinnamon oil, and silica gel against *Corcyra cephalonica* Stainton larvae and recorded a high reduction in all biological aspects of the pest in all treatments compared with the untreated control. The percentage of adult emergence was 0 and 28% in treatment of cinnamon oil-loaded silica NPs and silica gel, respectively, compared to 87.5% in the control group. Moreover, their results indicated that the total protein contents of the treated larvae were 28.88, 28.65, 28.2 mg/ml after treatment with silica NPs, nanoencapsulated oil, and cinnamon oil, respectively, compared to 32.56 mg · ml^{-1} in the untreated control.

For crop-insect pests

Various nanotechnological approaches are being used for plant protection under field conditions. Nanoparticles can be formed as delivery carriages for genetic matter, agrichemicals, and probes as well as biosensors for diagnostics of plant diseases (Elmer and White 2018). Among them, NP encapsulations with essential oils or microbial toxins are examples of promising insecticidal techniques. With essential oils, nanoencapsulation is emerging as an unconventional tool to enhance agricultural insect pest management. A key variety of insect species was tested to assess the role of nanoencapsulated EOs in plant protection. Solid lipid nanoparticles were used to evaluate the activity and persistence of the geranium (*Pelargonium graveolens* L'Hér) EO against *Phthorimaea operculella* Zeller (Adel *et al.* 2015). Their nanocapsules were more effective on different *P. operculella* developmental stages, and more stable under field conditions. Furthermore, they gave higher insect mortality than the free oil used. Also, nanoencapsulated EO of cinnamon (*Cinnamomum zeylanicum* Blume) was more toxic than the pure oil against *P. operculella* at all stages of fumigant bioassays (Mahdavi *et al.* 2017); the LC_{50} values of nano-formula and pure oil were 4.92 and 1.76 μl · l^{-1} air for eggs, 0.4 and 0.2 μl · l^{-1} air for females, and 0.4 and 0.19 μl · l^{-1} air for males, respectively. Moreover, while the residual impacts of the nano-formula lost their insecticidal efficacy 47 days after application, such a loss in the case of pure oil was recorded at only 15 days. Similarly, the oil-loading lipid nanocapsules of the geranium EO were more stable under field conditions and gave a high percentage of black cutworm (*Agrotis ipsilon* Hufnagel) mortality relative to the free geranium EO at the two concentrations used (Adel *et al.* 2019). Another technique evaluated the citrus peel oil-loaded polyethylene NPs against *Tuta absoluta* Meyrick (Campolo *et al.* 2017). They found that the nanoencapsulated EOs had a significant insecticidal activity and brought about high mortality with a significant decrease in the visible adverse effects on the plants. Hence, they recommended nanoformulated oil as a novel approach for managing *T. absoluta*. Also, managing the silver leaf whitefly (*Bemisia tabaci* Gennadius) on tomato plants with the EO of prickly ash (*Zanthoxylum rhoifolium*. Lam.) showed better efficacy of the nanoencapsulated oil in reducing egg-laying females than its natural oil (Christofoli *et al.* 2015). To avoid the high vulnerability of borage seed oil (BSO) to oxidation, ultrasound-aided BSO-loaded nanoemulsions were furnished with adjusted starch. This technique could incorporate different concentrations of peppermint oil as a natural antioxidant (Rehman *et al.* 2020). Such superior formulation due to its high physical and oxidative stability properties should be expanded to other nanoemulsion oils for further applications against insect pests. Adel *et al.* (2019) used nanoencapsulation of geranium EO by solid lipid nanoparticles against the third instar larvae of the cotton leaf worm, *Spodoptera littoralis* Boisd., and found that the EO encapsulated into solid lipid NPs with concentrations of 5.0 and 2.5% were more stable and effective than the corresponding free oil in both field and laboratory settings.

For disease-vector insect pests

Vector insect species are very important since they can inflict numerous diseases to human and animals. In contrast to chemical insecticides, biological vector control represents a key tool as an environmental and durable technique since it possesses a slow developmental rate for pest resistance (Fukruksa *et al.* 2017). Due to the presence of these insect species in houses, the use of repellents is an obvious, practical, and economical means of preventing the transmission of diseases by them to humans. EOs can be used as management agents for these species because they tend to be selective and have low side effects (Kamsuk *et al.* 2007). Various EO formulations using microencapsulated and non-encapsulated forms were designed for topical usage and sprays against mosquitos (Solomon *et al.* 2012). These authors suggested that an encapsulation formula of citronella oil is adequate to enhance the duration of its effective repellent for mosquitoes. Their formula could both diminish the oil-membrane permeability by about 50% and control the volatile release. Likewise, Kumar *et al.* (2011) recorded a highly toxic effect of a peppermint oil-loaded polymer against the house fly larvae when compared to non-capsulated oil. Using chitin NPs with negramina (*Siparuna guianensis* Aublet) EO exhibited promising potential as a biocontrol agent (BCA) too. Ferreira *et al.* (2019) detailed its management programs against the yellow fever mosquito (*Aedes aegypti* Hasselquist) responsible for spreading dengue fever, achieving 100% mortality of *A. aegypti* larvae at 7 days post-treatment. Moreover, it showed prolonged action due to the improved contact and controlled release by chitosan NPs that maintained the AI activity. Due to its importance in boosting the availability and stabilization of insecticides and EOs, chitosan was recently reviewed in terms of its favorable traits and formulations for insect pest control (Abenaim and Conti 2023).

Other globally serious household and public health pests could be controlled using nanoencapsulations of EOs. Werdin-Gonzalez *et al.* (2014) assessed the activity of two EO formulations and found that NPs loaded with geranium and bergamot oils boosted the contact toxicity between 8 and 10 times on adults of the German cockroach, *Blattella germanica* L. relative to the free oil. Additionally, when the EOs of both peppermint and palmarosa were nanoencapsulated in a nanopolymeric matrix, nanoencapsulated peppermint had eight times more insecticidal activity than the free EO and 10 times more than the free EO activity of palmarosa, with no significant difference between the two NPs (Yeguermana *et al.* 2020). Their results showed a significant reduction in the nymphal growth rate and food consumption in both nanoencapsulated oils relative to their free oils. Furthermore, the free oils of both peppermint and palmarosa were repellent for only 12 and 36 h but the nanoencapsulation tactic enhanced the bioactivity of EOs which increased the repellent impacts up to 36 and 72 h, respectively.

Modes of action of essential oils

Due to the previously mentioned variety of EO activities against different pests under various settings (Sarri *et al.* 2024), the degree of intake, relocation, and accumulation of their NPs also depend on environmental conditions, formulation type, and application technique. Basically, many studies have examined their mechanisms of toxicity via their AIs as a way of insect pest management. Monoterpenes, the main components of numerous EOs, have a cytotoxic impact on insect tissues. They can reduce cell membrane permeability, minimize the number of sound mitochondria and Golgi bodies, and damage respiration tissues of the insects (Gupta *et al.* 2023). Since the nervous system is crucial to the functional integrity of insects, most EOs found it to be an easy target of action (Kostyukovsky *et al.* 2002). Many recognized receptors for EO activity have been described, such as receptors for octopamine, adenosine triphosphatases, acetylcholinesterase (AChE), butyrylcholinesterase, gamma-aminobutyric acid-gated chloride channels, and nicotinic acetylcholine (Yeom *et al.* 2015; Gaire *et al.* 2019; Gupta *et al.* 2023). For instance, since AChE causes breakdown of the neurotransmitter acetylcholine, the activity of AChE could be inhibited by EOs of *Cyclotrichium niveum* Boiss. Manden and Scheng, *Anethum graveolens* L., and *Thymus praecox* subsp. *caucasicus* Willd. (Ronniger) Jalas var. *caucasicus* (Gupta *et al.* 2023). Also, monoterpenes of EOs can damage AChE and induce arthropods-neurotoxicity. Neuron activity of another essential neurotransmitter, octopamine, could also be modified by EOs via octopamine receptors, leading to a collapsed nervous system of the insect (Tripathi *et al.* 2009). Likewise, tyramine is another neuroactive compound with similar properties to octopamine. The interactions between insect tyramine receptors and monoterpenes may lead to neuromodulatory impacts too (Ohta and Ozoe 2014). Generally, EOs can act as receptor antagonists and strengthen or suppress the insect nervous system (Jankowska *et al.* 2017). Al-Harbi *et al.* (2021) found that EOs from lavender (*L. angustifolia* Mill), and basil (*Ocimum basilicum* L.) up-regulated the expression of genes associated with the detoxification system against *S. oryzae* L., thus showing the biopesticidal efficacy of these oils. Additional examples showing the origin of EOs, encapsulation methods that were used, and modes of action on specific pests are given (Table 1). These mechanisms do not negate the fact that such superior bioactivity of these nanoforms is ascribed to the small size of EOs polymeric nanoparticles that boost the contact surface, enhance absorption, and efficiently interact with behavioral aspects of the pest (Yeguermana *et al.* 2020).

Further optimization of EOs with nanotechnology

Notwithstanding the superior bioactivity of EO nanoforms, optimizing their biocontrol efficiency has yet to be realized. As EOs are insoluble in water, large quantities of toxic solvent may be needed to dissolve the oil. Important milestones of solvent-free methods for the nanoencapsulation of EOs are expected to deal with this issue (Lima *et al.* 2021). In contrast, a wide range of polymeric carriers are being developed for the encapsulation of EOs, which have several advantages, especially the EOs-controlled delivery during storage/application (Chiriac *et al.* 2021). Composites with encapsulated NPs can be set via coacervation (with gelatin/arabic gum), emulsification, ionic gelation with chitosan, spray drying with maltodextrin, complexation (with cyclodextrin), film hydration, and nanoprecipitation (Maes *et al.* 2019). Intriguingly, encapsulation with chitosan or lipid-based carriers against *Spodoptera frugiperda* Smith manifested not only protected release but also better efficacy (toxicity) than commercially available pesticides (Lopes *et al.* 2020). To meet other challenges in optimizing insecticidal EOs, researchers are paying attention to the fact that the nanoemulsion size and stability are also ruled by the EO source and concentration. For instance, nanoemulsions of *Artemisia* sp.-EOs were stable 28 weeks post-storage at a ratio of 3 oil: 1 surfactant (Campolo *et al.* 2020).

Bioinformatic tools can advance nanoencapsulation of EOs at a rapid pace. They can depict/survey the interactions of EOs/their active constituents with their bio-targets to tackle multifactorial pests/diseases. Significant contributions have been made, for example, via a docking-based virtual survey to define the insecticidal capacity of active compounds from the genus *Calceolaria* towards insect target proteins (Loza-Mejía *et al.* 2018). Using bioinformatic tools, the capability of AIs from "Negramina" EO to control aphids was validated by their reaction to the transient receptor potential channels of the insects (Toledo *et al.* 2019). Similarly, a docking study enabled researchers to describe the assignment of insect tyramine receptors in explaining the responses of EOs towards insect pests (Ocampo *et al.* 2020). In contrast, mosquitoes' detoxification response against EO components could be revealed via molecular docking too (Sierra *et al.* 2021). Hence, the toxicity removal of a xenobiotic component, p-cymene, via chemosensory proteins found in *A. aegypti* larvae verified the function of these proteins in inhibiting the natural larvicidal impact of *Eucalyptus camaldulensis* Dehnh. EO (Liao *et al.* 2016). Thus,

Table 1. Examples of plant essential oil (EO)-based nanoencapsulated bioinsecticides showing their origin, encapsulation method, and modes of action on specific pests

Origin (Source of EO)	Encapsulation method	Mode of action	Target pest	Reference
syzygium aromaticum (L.) Merr. & L.M.Perry	melt-dispersion method to produce dry nanocapsules	toxicity affected both nutritional indices and feeding deterrent index of insects	*Rhyzopertha dominica* Fabricius	Ibrahim (2022)
Lippia multiflora Mold.	low-energy emulsification	the aqueous solubility was enhanced by chitosan resulting in acute toxicity or repellence	several species but so effective against *Plutella xylostella* L. & *Brevicoryne brassicae* L.	Tia *et al.* (2021)
Foeniculum vulgare Mill., *Mentha piperita* L. & *Citrus sinensis* L. (Osbeck)	spontaneous emulsification	repellent activity against insects	*Rhyzopetha dominica* Fabricius	Giunti *et al.* (2021)
Cinnamomum zeylanicum Blume	lyophilization	toxicity on development and reproduction parameters was enhanced by nanoencapsulation	*Phthorimaea operculella* Zeller	Elbehery and Ibrahim (2022)
Citrus sinensis L. (Osbeck)	sol-gel microencapsulation	contact toxicity against *S. littoralis* & Suppress fertility of *A. gossypii*	*Spodoptera littoralis* Boisduval & *Aphis gossypii* Weed	Sciortino *et al.* (2021)

bioinformatic devices heavily figure for research on related biopesticides. They can accurately hypothesize a huge amount of physiological research ahead of time using combined resources. Consequently, they offer researchers the choice of doing multiple tests to define adequate interactions. Likewise, probing the molecular foundation of the insecticidal activity of many EOs related to profiles of insect transcriptome has been progressing (Liao *et al.* 2016; Gao *et al.* 2020). The use of RNA sequencing and studies of gene expression could detect interesting molecular targets for plant protection of pests. Therefore, transcriptome analyses have allowed quantifying related gene expression and identifying novel genes. Furthermore, overexpression of specific genes after oil exposure could shed light on the key role of such genes in the systemic metabolic reactions to insects (Huang *et al.* 2020). Gupta *et al.* (2023) reviewed previous studies of numerous gene-distinctive responses relevant to EO treatment for controlling species of insect pests. They confirmed that future research focusing on more transcriptomic investigations would explore the molecular targets of applied EOs in certain insects. This focus can lead to discovering new, efficient, and eco-friendly alternatives of such control schemes for the examined insect species.

Nanoencapsulation of microbial toxins with specific mechanisms

Researchers and stakeholders have been promoting bioinsecticides, living BCAs and their bioactive compounds, as replacements for unhealthy synthetic chemicals. Upgrading bioinsecticides efficacy via nanotechnologies can truly transform their use. Many studies are directed to achieve this target in terms of preparing and assessing the effectiveness of nanoencapsulated microbial toxins against insects. As microbial toxins are biotoxic substances generated or excreted by a microorganism, their effectiveness chiefly depends on specific factors. These may involve the formulation type, sedimentation, stability, application method, solubility, and ecological settings (Peralta and Palma 2017). Once encapsulated, the toxins of entomopathogenic microorganisms (e.g., bacteria, viruses, fungi, nematodes, and protozoa) will basically be protected by the encapsulating materials. Further merits due to their nano-size are increased dispersion and solubility which can consequently cover large surface areas and enhance their interaction with the target insects (Vidallon and Teo 2020). Yet, they still have a small uptake in the pesticidal market (Shen *et al.* 2023; Machado *et al.* 2023) and therefore applying related nanoencapsulations is being researched to resolve other relevant pesticidal obstacles/issues (Abd-Elgawad 2024).

Bacterial toxins

Bacteria that generate insect-specific toxins are related to key species of a few taxonomic families, such as Bacillaceae, e.g., *Bacillus thuringiensis* (Berliner) (*Bt*); and Enterobacteriaceae, or symbiotic bacteria (Sharma *et al.* 2021a). More than 40 *Bt* strains and their toxin-based commercial products are found to share about 2% of the worldwide insecticide market (Sharma *et al.* 2021b), representing the most widely distributed BCA. In contrast to other species, *Bt* has insecticidal protein crystals, named δ-endotoxins (Cry endotoxins), against specific insect orders. They are generated during the sporulation phase (Hofte and Whiteley 1989). Once ingested by the target insects, the endotoxin is activated by the alkaline conditions in the insect's stomach (Tran *et al.* 2021). It then attaches to specific receptors on the gut wall, causing break down in the gut cells. This method normally allows the spores to enter the hemolymph where the bacterium can proliferate causing septicemia and insect death. Despite the appearance of insect resistance to *Bt*, it has achieved valuable results in insect control (Chattopadhyay *et al.* 2017). But using *Bt* is limited by the low efficacy of their preparations under field conditions. Many reasons are responsible for this limitation such as sedimentation, drifting, and stability. Photo-inactivation seems to be one of the main limitations affecting the *Bt* endotoxin stability (Jhones *et al.* 2021). Hence, many trials have studied encapsulation of the *Bt* toxins as a protectant to improve the toxin efficiency. For instance, Tamez-Guerra *et al.* (2000) tested about 80 of such formulations using natural polymers as lignin with/without photo-protective agents. In a field trial, some encapsulated formulae showed enhanced insecticide activity in cabbage 7 days post-application against *Ostrinia nubilalis* Hübner (European corn borer) relative to commercial formulations. Additional advantages could be obtained for insect control with various nanoencapsulations of *Bt* toxins. Examples are: high persistence in inducing increased insect mortality using cellulose derivatives as photoprotective agents (Ramírez-Lepe *et al.* 2003), supreme efficacy against *Helicoverpa armigera* with a low dosage of *Bt*-crude powder submitted to high-pressure homogenization (Murthy *et al.* 2014), prolonged toxic effect against insects even when exposed to sunlight, boosted protection and stability with efficient release of the toxins encapsulated in chitosan nanoparticles as a carrier (Ureña-Saborío *et al.* 2017), and elevated virulence and UV resistance when $Mg(OH)_2$ nanoparticles were used as protective cover (Pan X *et al.* 2013).

The nanoencapsulation of other bacterial toxic metabolites is in progress. Spinosad is a derivative from the fermentation of *Saccharopolyspora spinosad* Mertz and Yao. It has a mix of two Spinosoids, Spinosyn A, the main part, and Spinosyn D (the minor part), at about a 17:3 ratio. Spinosad could be used singly or potentially by another BCA for better insect control. It is highly active via both contact and ingestion and kills the insect through hyperexcitation of the nervous system (Sparks *et al.* 2012). EI Badawy *et al.* (2016) used spinosad-loaded chitosan against *Culex pipiens* (L.) larvae and reported 100% larval mortalities in both stagnant and running water via 0.05 g loaded capsules at 24 h post-treatment. It then declined to 40% at 25 days with no mortalities at 48 days post-exposure to loaded spinosad in running water. In contrast, they recorded 100% mortality in stagnant water for up to 7 months. Similarly, spinosad-loaded chitosan nanoparticles were more effective and had a longer lasting residual effect against *Drosophila melanogaster* Meigen than free spinosad (Sharma *et al.* 2019).

Abamectin is another bio-pesticide with a broad spectrum of activity against a variety of pests such as insects and plant-parasitic nematodes. It is a mixture of avermectins excreted by the bacteria *Streptomyces avermitilis* Burg. As it is poorly soluble in water and highly sensitive to ultraviolet light that can induce biodegradation, nanoencapsulation of abamectin is a key factor to boost its persistence, stability, and toxicity. Thus, Li *et al.* (2007) could limit UV light degradation of avermectin via hollow silica nanoparticles for loading avermectin with a loading efficiency of 63.6% and medium release up to 30 days. Yu *et al.* (2017) synthesized three different forms of abamectin nanoparticles with various cucumber leaf sticky properties. In a bioassay on cucumber aphids, they found no difference between sticky PLA nanoparticles, commercial granules, and emulsifiable concentration. Wang *et al.* (2018) reported an increase in the solubility and dispersion rate of nano avermectin with higher activity and lower LC_{50} values than commercial formula. They attributed the great efficacy to the nano-sized particles that enhanced the avermectin adherence on the surface of crops, minimizing pesticide loss and drifting during spraying. As an insect feeds on a plant portion with this chemical, abamectin enters the digestive system and moves to the nerve system to induce paralysis followed by death. Sun *et al.* (2020) set two abamectin nanoformulations with average particle sizes of 140 nm and 150 nm against the pea aphid *Acyrthosiphon pisum* Harris. The two forms demonstrated similar insecticidal effects to commercial abamectin formulations with no significant differences. Finally, encapsulated bacterial toxins are easier to use in IPM programs. The encapsulation can protect them from direct contact with other AIs and adjuvants in mixing tanks during application. Thereafter, the encapsulation acts as an efficient device in decreasing the negative impacts of ecological factors that affect their efficiency.

Entomopathogenic virus

Although most viral preparations for insecticidal production are unstable when exposed to ultraviolet radiation, promising results have been obtained with viral encapsulation. This technique used different encapsulation materials such as maize flour and lignin via a spray-drying process to control different insect species, e.g., *Anagrapha falcifera* (Kirby.) (Behle *et al.* 2006), *Autographa californica* Speyer. (McGuire *et al.* 2001), *Cydia pomonella* L. (Arthurs *et al.* 2006), and *S. frugiperda* (Behle and Popham 2012). The activity of the encapsulation formula was tested in both laboratory and field studies. All results indicated efficiency of the encapsulation technique to boost the virus activity, tolerate climatic adversities, particularly UV light exposure, and sustain residual activity against pests. Interestingly, when the AI (baculovirus) is micro-encapsulated in wax particles combined with a UV absorbent (titanium dioxide, TiO2), it could protect the sensitive viral DNA from degrading due to UV, dissolve in the alkaline insect gut to release the virus, pass the basal lamina to migrate and infect other pest tissue, and continue to grow fast and finally kill the host (Wilson *et al.* 2020). Chariou *et al.* (2019) examined virus-based models and synthetic nanopesticides for their mobility in soil with computational modeling. They recorded that both tobacco mild green mosaic virus and cowpea mosaic virus could move into the soil to a depth of at least 30 cm; therefore, they delivered pesticides to pests found at that depth of the rhizosphere.

Entomopathogenic fungi (EPF)

Striking examples of fungi that cause a significant reduction in insect pest populations under favorable settings are *Beauveria bassiana* Vuill. and *Metarhizium anisopliae* Metchnikoff. Usually, EPF enter the host during insect feeding or contact, grow and proliferate within the insect's body, interact with its defense mechanisms to kill it, and finally sporulate within and on the cadaver. As the host dies due to water loss, its body hardens. Advantages of EPF as BCAs include broad-spectrum activity with multiple metabolic types, inexpensive mass-production, specific insect pathogenicity without side effects on human health, easy application, and high effectiveness as host resistance is unlikely to occur. The mycotoxins that act as BCAs are cuticle-degrading enzymes and a complex cyclo-depsi-peptide structure, e.g., destruxins, beauvericins, beauverolides, and bassinolide (Qasim *et al.* 2020; Wu *et al.* 2021). For instance, destruxin can damage insect cells by binding with arginine tRNA synthetase (BmArgRS) protein which precludes cell growth and leads to insect death (Wang *et al*. 2020). Because such insecticidal activities can be damaged by ecological conditions (Moraga 2020), it is imperative to look for novel formulations that are more effective, durable, and stable.

The microencapsulation technique of *B. bassiana*-fungal conidia using protein-based polymers as encapsulation materials was developed by Mishra *et al.* (2013) to conserve them from adverse ecological conditions, enhance shelf-life, and potentiate bio-efficacy. This encapsulation induced 54.8% mortality in fresh preparations and 30.6% at 1 year post-storage of housefly, *Musca domestica* L., larvae in simulated field conditions. In contrast, the non-capsulated formula caused 51.6% mortality in fresh preparations and 19.9% in a 1 year stored formula. Thus, they concluded that the protected formula raised not only the fungal efficiency, but also the general persistence, with a considerable increase in shelf life. Another way to use nanoencapsulation via chitosan is to coat the fungal metabolite from *nomureae rileyi* Farl.. Chandra *et al*. (2013) confirmed better efficacy of this technique than the uncoated fungal metabolite and spores. The LC_{50} value was 2.45 µg for coated fungal metabolites, compared to 3.97µg and 5.55×10^{10} spores · ml^{-1} for uncoated fungal metabolites and fungal spores, respectively, against *Spodoptera litura*-larval instars. Also, LT_{50} for the adult longevity of coated, uncoated, and free fungal spores were 2.17 ± 0.2, 4.21 ± 0.2, and 32.7 ± 0.2 h, respectively. Furthermore, similar nanoencapsulation of insecticidal metabolites known as Beauvericin against *S. litura* Fabr. showed improved performance (Bharani *et al.* 2014). While their related loading efficiency was 85%, cumulative release from the capsules was about 91% after 24 h indicating the biocompatibility of chitosan nanoparticles as a mycotoxin's carrier. Beauvericin activities showed that all *S. litura*-life stages were sensitive to the nano formulation.

Other entomopathogenic microorganisms and their optimization

Continuous efforts are underway for other nanoencapsulated insecticidal toxins from various microorganisms that are or are likely to soon become widely available. To name but few, applying nanoencapsulation to the complex of entomopathogenic nematode (EPN)-symbiotic bacterium or their individual symbiotic bacteria alone to boost their formulations in pest control is being researched. Such formulations are key factors to fit their deliveries and settings. Because EPNs-infective juveniles (IJs) are extremely susceptible to definite edaphic factors, e.g., soil moisture and texture, and temperature, their biocontrol efficacy can be damaged. Therefore, their nanoencapsulations are needed to enhance the persistence of IJs for adequate field efficacy and to boost their shelf-life. Related to this, biopreparation of copper (Cu) NPs combined with the EPN *Steinernema feltiae* Steiner could effectively suppress the mealworm beetle, *Alphitobius diaperinus* Panzer, population. Yet, the mortality of EPN-IJs was affected by both the time of exposure and concentrations (5, 2, and 0.5 ppm) of nano-Cu. The highest Cu concentration decreased the ability of *S. feltiae* to enter, develop, and proliferate inside the insect host (Kucharska *et al.* 2011). Nonetheless, the insect mortality after the EPN contact with nano-Cu was significantly higher than that in the untreated check (IJs in water). Usually, nematodes release their mutualistic bacteria that cause septicemia in the insect body. Also, Taha and Abo-Shady (2016) studied the efficacy of different nano-silver (Ag) concentrations on the EPNs *Steinernema abbasi* Elawad, Ahmad & Reid, *S. arenarium* Artyukhovsky, and *Heterorhabditis indica* Poinar mortality, reproduction, and pathogenicity where the IJ mortality depended on the Ag concentration and exposure time. This was also reflected in their pathogenicity to *Galleria mellonella* L.-last instar larvae. The reproduction of these EPN species was considerably affected at the two highest nano-Ag concentrations, 500 and 1500 ppm. Taha and Abo-Shady (2016) concluded that the EPN biocontrol efficacy depends on the concentration of the used element, biopreparation, exposure time, and EPN species/strain. Generally, the nematodes are harmed at low concentrations. Furthermore, EPNs were formulated via individual EPN coating with titania (TiO_2) NPs and mineral oil using oil-in-water Pickering emulsions (Kotliarevski *et al.* 2022). Consequently, IJ yields obtained from infected *G. mellonella* L. cadavers for 150 days showed no significant ($p > 0.05$) differences using the tested emulsions relative to the control of aqueous suspension. However, this coating obviously enabled IJs to manifest high levels of not only pest control potential but also stability and shelf-life. Additionally, IJ virulence/viability was not reduced even after UV exposure for 20 minutes.

Thus, it is clear that nanoencapsulations of the addressed AIs can boost the efficiency and persistence of nanoinsecticides. Their proper uses have proved to be durable during ecological stresses. Fewer doses are needed with lesser toxicity and cost-effective applications against various insect pests (Fig. 1). Nonetheless, further optimization of insecticidal toxins via nanotechnology will basically depend on the ratio/concentration of the AIs and related carriers, the nanocolloidal element involved and biopreparation, exposure time, as well as relevant microbial species/strain. Additionally, advanced technology like bioinformatics and RNA-Sequencing, should be incorporated.

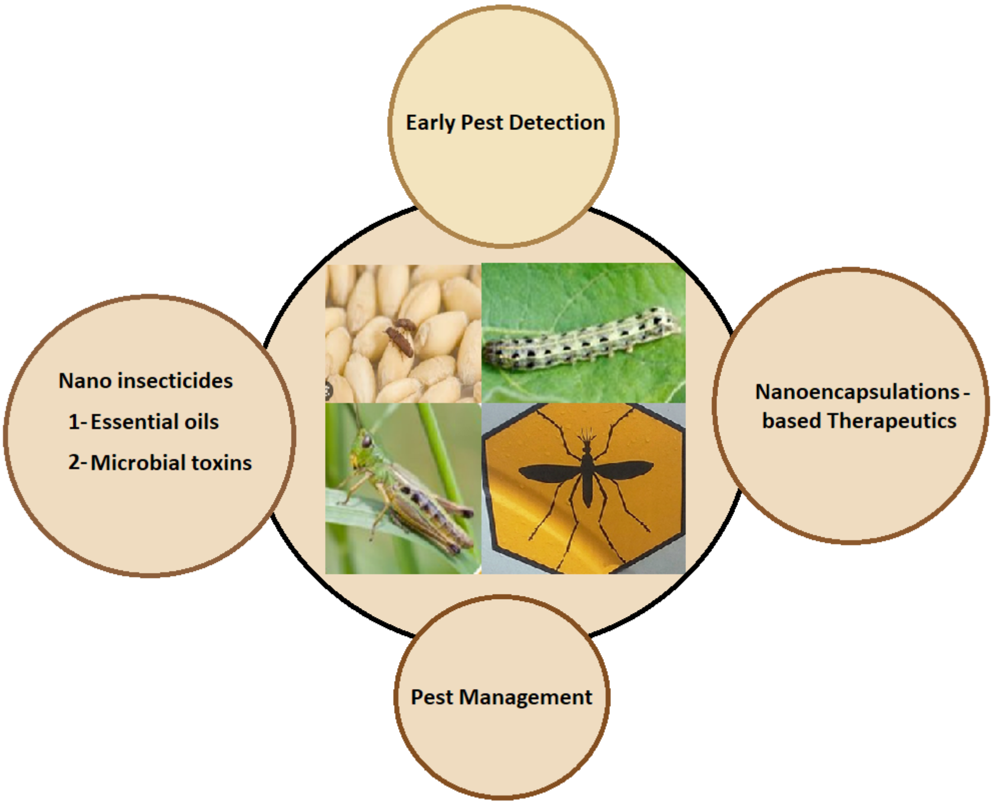

Fig. 1. Nanotechnology-based strategy against various groups of harmful insects including stored grain/farming pests as well as disease-vectors

A repetitious consensus among researchers throughout the mentioned tests assures that nanoencapsulation of essential oils and microbial toxins will upgrade various aspects of insect pest control. However, more interest should be dedicated for their safe production and usage with cost-effective techniques (Abd-Elgawad 2024).

Other mechanisms of nano-insecticides

On using these insecticides, the released AIs may enter into insects via physical contact (through insect tissues by a diffusion process) or via stomatal/natural openings and then move to various cells as the cell membrane allows passing 5–20 nm nanoparticles (Nair *et al*. 2010), ingestion, and inhalation. In physical contact nanoparticles penetrate the exoskeleton, binding the nanomaterials to protein or phosphorus from DNA in intracellular space, causing deterioration of cell contents and consequent cell death (Abd-Elsalam and Prasad 2018). Other types of NPs can dehydrate the insect body by attaching to their cuticle wax layer. Nano-clay and nano-silica are attached to insect cuticles and absorb the water from the insect's body where fissures consequently occur. Also, inhalation of NPs may lead to midgut deterioration with reductions in protein, lipid, and glucose levels, finally causing insect death (Gustafson *et al*. 2015). Moreover, inhalation may also alter the activity of nervous system enzymes and membrane potential as done by the insecticidal organo-phosphorous and carbamate group.

The need to develop standard guidelines for using nanoinsecticides

Having presented the benefits of NPs, it is essential to avoid their possible side effects, if any, especially for large-scale applications. With the current progress in high-technology, environmental risk assessment of nanoencapsulated insecticides is crucial before a product is marketed. As nanoparticles have specific characteristics, such as their minute size and catalytic properties, they require further testing beyond their toxicity levels. Nanoparticles in these pesticides can enter the human body through the nose/mouth and then go to the respiratory tract with possible harm to the lungs. Therefore, such an assessment should include their behavior, persistence, and fate in the settings prior to their usage. Jasrotia *et al*. (2022) confirmed that these regulatory terms are needed to validate their safe release. This does not negate the fact that boosting the accuracy and precision of the allocation of their AIs should always be earnestly attempted to further diminish the load of material released into the system, minimizing unwanted impacts on the non-targets.

The current revision of risk assessment calls for an immediate need to design novel ecotoxicological assays relevant to physicochemical properties of these insecticides to assess the risks linked to their usage (Awashra and Młynarz 2023). Furthermore, it is suggested to apply these assays on a case-by-case basis to comply with the given variables and scenarios. This will make it possible to follow their fate and optimize pest control according to the studied variables. It would be possible to precisely define any of their merits/demerits and combine them with other pest control strategies into integrated management. Overall, other issues such as climate change and rapid population growth lends more urgency to the need to exploit sound nanoinsecticidal usage in farm management to transform cropping systems.

Conclusions

Emerging insecticidal applications of nano encapsulated essential oils and microbial toxins have impressive favorable features such as remarkable efficacy, high conductivity, minimal AIs with consequent cost-effective uses, and sober chemical reactivity against their various target pests. They can also be set in a variety of forms and synthesized as organic (e.g., polymers) and/or inorganic components (e.g., single or composite metallic oxides). Their control effects on a variety of insect pests have exceeded that of traditional pesticides under many practical conditions, such as cultivated fields and stored grain as well as against disease-vector insect pests. Therefore, such types of nanoinsecticides can potentially shift the present pest management systems to upgrade the agricultural sector with the aid of precision of their distribution and applications to avoid any unwanted effects while boosting agricultural sustainability.

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