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Innovative Nano Based Biopesticides Application Strategies for Insect Pest Management

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ABSTRACT: With an increasing world population, the demand for quality food is rising. To meet safe food demand, it is necessary to double or maybe triple agriculture production. Annually, almost 25% of the world crop is destroyed due to pests. During the past few decades, different pesticides, including chemical, synthetic, biological, and botanical have been adopted to achieve adequate results against pests for agriculture interests and plant safety. Globally, more than 200,000 people died every year due to direct chemical and synthetic pesticides poisoning. But these pesticides did not achieve the desired results due to delivery problems, less stability, low biodegradability, less specificity, and high cost. To overcome these problems, the rapidly emerging field of nanotechnology is considered an important achievement in the agriculture sectors in order to improve pest mortality rates and crop production. The nano-biopesticides attained special attention against the insect pests due to their small size (1-100 nm), large surface area, high stability, cost-effectiveness, fewer toxicity, and easy field application. The current chapter highlights the relevance of nano-biopesticides for pest insect management on several crops of agricultural concern. The mechanisms of action, delivery, and environmental sustainability of nano-biopesticides are also discussed

KEYWORDS-nano, biopesticides, agriculture, insect, pest, management

I. INTRODUCTION

Biopesticides are pest-control agents made from microorganisms, plants, and animals. Synthetic pesticides continue to strongly protect agricultural products, but their long-term use has negative effects such as carcinogenicity, long-term stability in the environment, and other similar consequences. To address these issues, the production of new pesticides became necessary [48]. Biopesticides were considered, since they are safe and eco-friendly.

Fungal spores were first used as a biopesticide in the late 1800s to control insect pests. In 1835, Agostine Bassi demonstrated that spores of the white muscardine fungus (*Beauveria bassiana*) could protect silkworms from diseases. This was one of the first documented cases of biopesticide use. Since then, the application of biopesticides has been continuous throughout modern agricultural history, although it has remained minimal compared to conventional crop protection. The primary distinction between biopesticides and synthetic pesticides is their mode of action. While most synthetic insecticides, if not all, are neurotoxic to pests, many biopesticides have other modes of action, such as anti-feeding, mating disturbance, desiccation, and suffocation. Biochemical, microbial, and plant-incorporated protectants (PIPs) are the three types of biopesticides identified by the US Environmental Protection Agency (EPA)[1,2,3]

Microbial pesticides

Microorganisms such as viruses, bacteria, fungi, protozoa, and yeasts are used to produce biopesticides. Microbial pesticides are a more effective alternative to chemical insecticides. Their pathogenicity to the target pest varies depending on the species [67]. The effect of microbial pathogens is induced by the invasion of the pathogen through the skin or the gut of the insect, which results in pathogen multiplication and the death of the host, i.e., the insect. Insecticidal toxins produced by pathogens are critical in their pathogenesis. The majority of toxins produced by microbial pathogens are recognized as peptides, but their structure and toxicity vary considerably. Humans and other non-target species can benefit from the effectiveness and safety of the pesticides. These pathogens leave their meals with little or no residues [68]. Microbial pesticides are ecologically safe and remove threats from other natural pests, resulting in increased biodiversity in managed ecosystems. As a result, microbial agents are highly specific against target pests, allowing beneficial insects to thrive in treated crops. This is the reason why, over the last 3 decades [69], microbial insecticides have been introduced as biological control agents. Different pests can be controlled using different microorganisms as the AIs, such as bacteria, fungi, viruses, or protozoa; however, each active ingredient is relatively specific for its target pest. Some fungi, for example, control certain weeds, while others kill particular insects. As another example, *Bacillus thuringiensis* toxin may be more effective against *Aedes aegypti*, while the sphaericus strain is effective against a variety of insects, including *Culex quinquefasciatus* [70].



Biochemical pesticides

Biochemical pesticides are substances naturally occurring in the environment, which control pests via a non-toxic mechanism. The mechanism of action of biochemical pesticides is different from that of traditional pesticides. Traditional pesticides directly affect and destroy their target, but biochemical pesticides act indirectly. For example, they disrupt the sexual function of their targets. Natural plant-derived products such as terpenoids, alkaloids, phenolics, and other secondary chemicals may be used as biopesticides. Pesticidal properties have also been discovered in certain vegetable oils, such as canola oil [71].

PIPs

PIPs are pest-control substances made by plants and the genetic material needed for the plant to make the substance [72]. The majority of pesticides in use are synthetic organic compounds with low molecular weights (LMWs, less than 500 g/mol or 0.5 kDa). These pesticides' environmental and analytical chemistry, as well as their potential effect on human and environmental health, have been thoroughly investigated. While LMW-synthetic pesticides have dominated the market, biopesticides are becoming an increasingly important part of the overall pesticide market [73]. The global market for all biopesticides is currently estimated to be worth \$34 billion, accounting for approximately 6% of the total pesticide market. PIPs are biopesticides that are expressed directly in the tissue of genetically modified (GM) crops to protect them from pests such as insects and viruses. Because of the widespread use of insecticidal PIPs around the world, as well as the recent emergence of new PIPs targeting insect pests, this section focuses on PIPs developed against insects [74]. Insect pests consume PIPs when feeding on the transgenic crop tissue. Cry protein and double-stranded ribonucleic acid (dsRNA) PIPs in the insect gut affect insect development or cause insect mortality in various ways [75]. Cry protein PIPs interact with specific receptors on epithelial cells in the insect midgut, insert into the cell membrane, and eventually form transmembrane pores, which lead to cell lysis and pest death. There are several types of Cry proteins, each with a specific structure and toxicity that is unique to certain insect orders. Lepidoptera (e.g., the corn borer) is poisoned by Cry1 proteins, while Coleoptera (e.g., the corn rootworm) is poisoned by Cry3 proteins.

Cry proteins were the first-generation insecticidal PIPs. The next-generation dsRNA PIPs have been recently approved. Following ingestion, the dsRNA PIPs in the pest insect are transported into a target cell. dsRNA is cleaved into small interfering RNA molecules (siRNA, 20 nucleotides) within the cell, which guides the insect's endogenous RNA interference (RNAi) machinery to degrade the complementary mRNA. The degradation of the targeted mRNA prevents it from being translated into basic pest insect proteins, causing sublethal effects (e.g., decreased growth) or pest mortality [4,5,6]. The first dsRNA PIP to be approved by the FDA targets the corn rootworm (*Diabrotica virgifera virgifera*) by interfering with the synthesis of the Snf7 protein, which is an important vacuolar sorting protein [76].

Nano-pesticides

Engineered nanoparticles are now being used, or have the potential to be applied, as novel carriers for pesticide delivery. A range of formulation types has been suggested, including nano-emulsions, nano-encapsulations, nano-vesicles, nano-gels, nano-fibers, etc., which can be used to improve the efficacy of existing pesticide AIs or to enhance their environmental safety profiles, or both [77]. The most common nanoparticles are illustrated in Fig. 7.

Nano-emulsion

Nano-emulsion is a biphasic dispersion system formed by mixing surfactants; AIs dissolved in the oil phase and the water phase [78]. The nanometer-sized droplets (~20–200 nm) make this system kinetically stable and give it a transparent or translucent appearance [79, 80]. Nano-emulsion of pesticides is an oil-in-water (O/W) dispersion, which can dissolve poorly water-soluble pesticides into small oil droplets and greatly improve their bioavailability and efficacy [81, 82]. In addition, nano-emulsion significantly reduces the use of organic solvents and surfactants compared to traditional pesticide formulations, and has attracted considerable attention from researchers in recent years [83, 84]. For example, Jiang et al. presented a nano-emulsion system that encompassed environmentally friendly surfactants, esterified vegetable oils, and 41% (w/w) herbicide glyphosate isopropylamine [85]. This nano-emulsion had a small particle size (<200 nm) and lower surface tension than the commercial cationic surfactant system (Roundup®), which would cause the droplets to be deposited uniformly on leaves with lower contact angle and increase the wetting, spreading, and permeation. Visual injury assessment indicated that the nano-emulsion formulations showed significantly lower effective dose 50 (ED50) than Roundup®, suggesting that they exhibited higher biological efficacy. In another study, Feng et al. developed an abamectin (Abm)-loaded nano-emulsion containing 2% Abm, 5% castor oil polyoxyethylene, and 7.5% hydrocarbon solvent, which conformed to the quality indicators of the Food and



Agriculture Organization (FAO) [86]. Compared with commercial oil/water emulsions (EW) and microemulsions (ME), the nano-emulsion exhibited various advantages such as a smaller dynamic contact angle on cabbage leaves, higher insecticidal activity, and lower cytotoxicity. Although a nano-emulsion of pesticides with remarkable physical stability can be readily obtained, the most common approach is high-energy emulsification, and the oil phase and emulsifiers may still be toxic [87, 88]. To overcome these challenges, green nano-emulsions and low-energy methods (e.g., self-emulsification, phase transition, phase inversion temperature methods, etc.) are promising strategies [89, 90]. Du et al. developed a green nano-emulsion of β -cypermethrin using renewable fatty acid methyl ester (methyl laurate) as the oil phase, “green surfactant” alkyl polyglycoside, and the non-ionic surfactant polyoxyethylene 3-lauryl ether (C12E3) as the mixed surfactant [89]. The nano-emulsion incorporating β -cypermethrin had a nearly monodisperse droplet size distribution (polydispersity index (PDI) < 0.2 Mw/Mn), which indicated excellent wetting and spreading properties on the hydrophobic surface compared with the commercial β -cypermethrin nano-emulsions, suggesting a biocompatible strategy for pesticide delivery.[7,8,9]

Nano-encapsulation

Nano-encapsulation of pesticides is a delivery method in which AIs are encapsulated in various nanomaterials and released in a controlled way [91]. Encapsulation by nanomaterials can protect AIs from premature degradation (e.g., photolysis, hydrolysis, biodegradation, etc.) and unnecessary losses by leaching and volatilization, and it is more effective in practical applications than traditional pesticide formulations [92, 93]. After the rational design, nanomaterials can also enable the encapsulation system to exhibit sustained release behavior to prolong the control period or give it stimulus-responsive release properties to achieve precision control [94,95,96].

Various nanomaterials including organic materials (e.g., synthetic and natural polymers [97,98,99,100,101,102], lipids [103, 104], plant-derived nanoparticles [105,106,107], etc.), and inorganic materials (e.g., silica-, carbon-, calcium-, and clay-based nanoparticles, etc.) have been applied for nano-encapsulation of pesticides [95, 108]. AIs are often encapsulated into nanocarriers via incorporation, electrostatic complexation interactions, or covalent bonding, which will improve uptake, dispersibility, mobility, adhesion, and controlled or target release, thus leading to increased bioavailability and a longer effective lifetime of AIs. Due to their excellent biocompatibility and biodegradability, as well as their abundant functional groups, various forms of polymer-based nano-encapsulated pesticides, including nano-capsules, nano-spheres, nano-micelles, nano-gels, and nano-fibers, have been extensively investigated [100]. Lipid-based nano-encapsulation of pesticides not only can lead to effective encapsulation of hydrophobic or hydrophilic AIs without the need to use organic solvents but also can enhance their penetration and absorption in insect epidermis, resulting in an increased insecticidal activity. More recently, several published articles have demonstrated that the nano-encapsulation of the pesticides based on plant viral nanoparticles (red clover necrotic mosaic virus, tobacco mild green mosaic virus) could increase the mobility or distribution of Abm, a nematocide, within the soil compared to the free Abm, leading to enhanced crop protection against plant-parasitic nematodes in the soil [106]. Although nano-encapsulation of pesticides based on low-cost organic materials has been widely investigated, many challenges still need to be addressed, such as physicochemical instability and acid monomers formed by polymer degradation leading to the decomposition of AIs. Deteriorative reactions occur when polymers are subjected to heat, oxygen, and mechanical stress, and during the useful life of the materials when oxygen and sunlight are the most important degradative agencies. Moreover, degradation may be induced by high-energy radiation, ozone, atmospheric pollutants, mechanical stress, biological action, hydrolysis, and many other influences [109].

Porous inorganic materials such as silica and calcium carbonate are ideal carriers for pesticides because of their large surface area, tunable pore size, high loading capacity, and good biocompatibility [110,111,112,113,114,115,116]. The controlled release properties and anti-photolysis ability of AIs are significantly enhanced after encapsulation in those materials. Some articles have reported that the release behavior of encapsulated AIs could be regulated through controlling the pore structures (e.g., 2D hexagonal channel, 3D open network structure, single large pore, etc.), thus further improving the efficacy in practice [117, 118]. In addition, some inorganic materials with specific shapes or structures have been reported for controlling the encapsulated AI loss. For example, the Wu group reported a high-energy electron beam (HEEB)-modified natural nano-clay that could effectively encapsulate chlorpyrifos and increase its adhesion on crop leaves through the 3D network structure [119]. In another study, Sharma et al. used graphene oxide decorated with copper selenide nanoparticles for the encapsulation of chlorpyrifos, which led to enhanced adhesion to cauliflower leaf due to the resistance of graphene oxide to aqueous runoff, the ability of carbon to bind to the organic surface, and the piercing effect by a sharp sheet on the plant leaf [120].

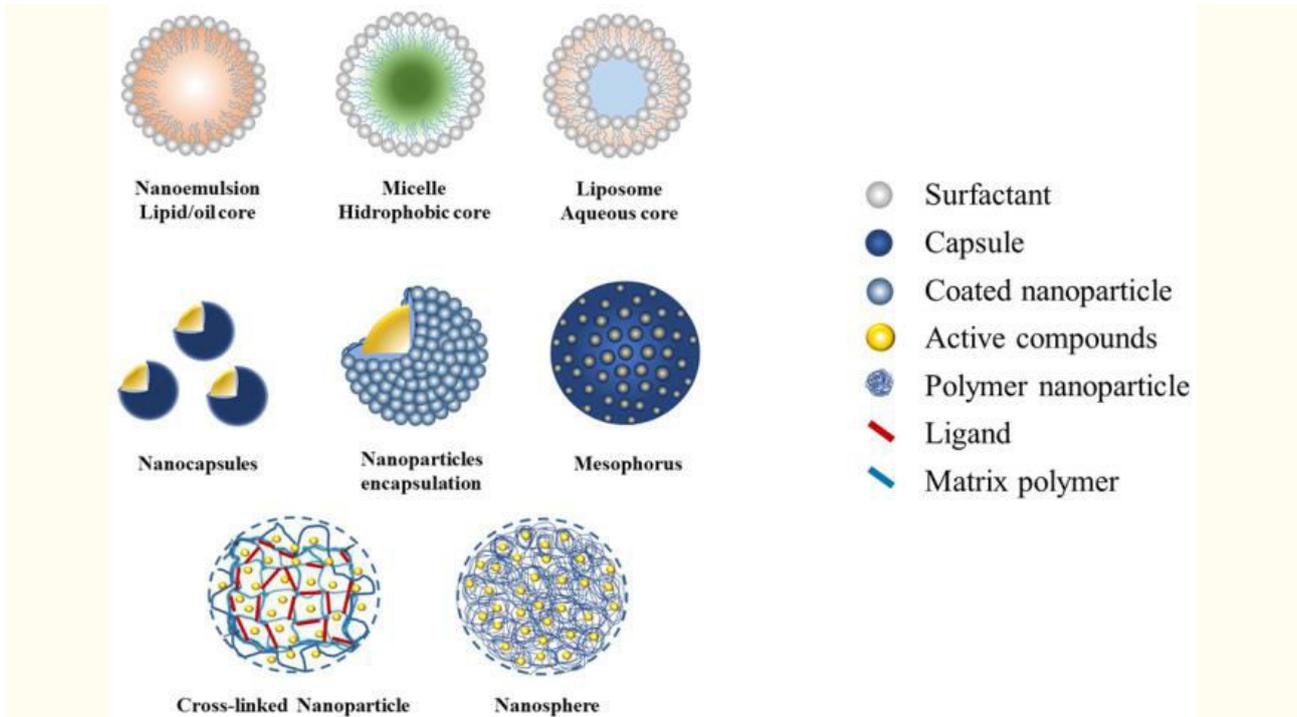


In general, nano-encapsulated pesticides have two typical release behaviors: (i) sustained (slow) release and (ii) stimuli-responsive release. For the sustained release of nano-encapsulated pesticides, AI concentrations remain within an effective control window against pests for a long period, avoiding frequent pesticide application. To achieve optimal control efficacy, developing stimuli-responsive nano-encapsulated pesticides that can precisely deliver AIs to the target is crucial. So far, various stimuli-responsive (e.g., pH-, enzyme-, temperature-, redox-, light-, ionic strength-, and humidity-responsive) nano-encapsulated pesticides have been reported for promoting the smart release of AIs in response to biotic (plant pathogens, insects, and weeds) or abiotic (temperature, sunlight, drought, soil texture, flooding, and salinity) stimuli, and have been discussed in detail in the previous reviews [94, 121,122,123,124,125,126]. These controlled release properties indicate the potential of nano-encapsulated pesticides for improving pesticide-use efficiency.

Vesicles such as liposomes and niosomes are versatile drug vehicles that are composed of various phospholipids or non-ionic surfactants [127, 128]. In general, they could carry both lipophilic and hydrophilic drugs anchored into their bilayer or encapsulated in their cavity, respectively. These vesicles have the potential to be used as carriers for pesticide delivery. Some studies have utilized vesicles for pesticide delivery. Kang et al. examined the impact of the nanof orm of pyrifluquinazon on the destruction of the green peach aphid, *Myzus persicae*. The nanotype of pyrifluquinazon was constructed using liposomes and was subsequently covered with chitosan to make the unstable core materials more durable [129]. Moreover, to decrease the toxicity of paraquat, which is a widely used herbicide, photo-responsive and user-friendly vesicles loaded with paraquat were utilized. In this formulation, paraquat could only be released upon UV or sunlight irradiation [130]. Zhang et al. used liposomes to deliver the dsRNA of the P0 gene to the tick *Rhipicephalus haemaphysaloides* and evaluate the anti-tick characteristics of this system.[10,11,12]

II. DISCUSSION

Currently, nanotechnology is the breakthrough of various innovations in the development of bioinsecticide formulas [9,36]. Biopesticide formulas established through nanotechnology improve delivery performances and enhance their application efficiencies. It is well known that the smaller size of particles serves to increase the surface of the active ingredient and, consequently, improve the solubility. Moreover, the challenges involved are preparing the synthesis of the water-based medium, formula stability, mobility, and ensuring the delivery target system [76]. A broad variety of natural materials are used in the assembly of pesticide nanoformulations. There are two types of formulations—nano-particle pesticides and nano-carrier systems—to allow delivering active compounds to the target site. The structure of the delivery system includes the encapsulation of active compounds inside, a nanoparticulate polymeric shell, adsorption onto the nanoparticle surface, attachment onto the nanoparticle core via ligands, and entrapment within the polymeric matrix [77]. The properties of these various types of nanocarrier formulations are known to enhance the efficacy and efficiency of biopesticides against insect pests, i.e., a nanoemulsion loaded with essential oil from various plants products [78,79,80], plant extracts loaded in micelle with a hydrophobic core [58] and liposome with a hydrophilic core [81], as shown in Figure 2. Recently, materials from natural polysaccharides, proteins, alginates, silica, and other types of polymers have been utilized as nanoparticle encapsulants, such as chitosan, zein, gum arabic, and silica nanoparticles [31,78,82].



The types of nano-delivery biopesticide formulations.

Botanical active compounds have also been reported to be successfully loaded in a nanocapsule and being mesoporous for the slow-release system as well as being entrapped in the matrix polymer and the cross-linked nanoparticles mediated by specific ligands [83,84,85,86]. It is well known that the characteristic content of organic active compounds inherent in botanical ingredients is that they are easily degraded and, consequently, have a lower long-term potency [12]. The various types of nanocarrier systems offer the appropriate properties to improve the efficacy and efficiency performance of plant-derived nano-pesticides' delivery .

Nano-emulsified carriers are emerging as the most intensively investigated of plant-derived pesticides (Table 2). This system is suitable to be adapted to EO and crude extracts of plant-derived pesticides by applying a simple emulsification method, requiring low energy and with suitable surfactants [23,92]. Emulsion-based formulations are designed to increase dispersion or solubility of ingredients, improve stability, and increase bioactivity and efficiency, especially in controlling insect crop pests [31]. Nanoemulsion formulas are extensively investigated for EO plant-derived nano-pesticides' delivery to obtain desired properties due to the nano-sized droplet dispersion uniformity and the stability into two liquid phases by the fundamental role of the surfactants. Thus, the engineering characteristic and the properties of the delivery system can provide a slow-release performance [23]. Micelles are ideal nanocarriers for encapsulating, especially for insoluble-organic compounds such as plant extracts [93]. This allows the nano-sized insoluble-organic suspension dispersed in the water system that enhances the wettability and bioefficacy toward targeted insect pests [9]. Liposomes are vesicular to nanoscale structures, and which consist of a lipid bilayer covering an aqueous phase in the core [93]. The preparation of a liposomal nano-carrier has emerged as a promising aspect of nano-delivery biopesticides due to separate compartments that can encapsulate both the hydrophilic and hydrophobic active compounds that are effective against targeted insect pests [81].

The encapsulation involves a vesicular composed of the biodegradable matrix/polymer that encloses the active compounds in the inner core [9]. Nanocapsule and nanoparticle encapsulation increase the targeting delivery, and shell degrades slowly by environmental conditions, thus improving the chemical stability of organic compounds, such as volatile compounds commonly containing types of EO [93]. Mesoporous nanoparticles with hollow silica were adapted for water-soluble and lipid-dispersed controlled release biopesticide delivery systems. While nanospheres are designed as dense spherical vesicular systems in which active compounds are evenly distributed via adsorption or trapping in the nano-matrix/polymer, the cross-linked nanoparticles of the entrapped active compounds are mediated by ligands that



act as sensors or markers for specific receptor molecules in targeted delivery. These efficient encapsulations and smart entrapped nano-carrier systems were confirmed to load the EO or pure active compounds with quite a high loading capacity with lethal and sublethal bioactivities due to a controlled slow-release mechanism [78,84,85,86,88].

Plant-derived nano-pesticides have been tailored for desired properties, involving the use of matrix types [94]. Studies have reported carrier systems prepared by organic and inorganic matrices/polymers and suitable surfactants as a means of delivering various extracts, EO, and their active compounds [88,94]. The utilization of nature/organic matrices' resources matter is growing rapidly to compete with the non-organic matrices, such as chitosan, gum arabic, and zein. This carrier system maintains the susceptibility of active organic compounds to degradation so that they can be persistent for a longer period. Thus, these efficiently increase toxicity, fumigants, repellency, attractants, antifeedant, growth development, and oviposition inhibition [88,95].

The evaluation of studies shows that a compatible nanocarrier adopted in crude EO can even outperform or be comparable with the effectiveness of pure active compounds [78,89]. Nanocarrier biopesticide formulas can also enhance the effectiveness of pure active compounds to be comparable or more effective than synthetic insecticides in an in vitro bioassay test [81]. The performance of nanocarrier formulas of EO and plant extract can reduce the level of toxicity, indeed enhancing sublethal bioactivities such as the impact of antifeedant and repellency, and inhibiting growth regulation [58,88,91]. The advantages of the nanocarrier formula compared to conventional or synthetic insecticide formulas are determined through increased efficiency performance, such as the solubility and dispersion, formula stability, and release control mechanism offered by the nano-delivery system. This factor has a significant impact on increasing its efficacy against target insect pests. Plant-derived pesticides from abundant plant extracts resources are the most studied pesticides in the investigation of crop pest management. However, the potential compatibility of nanocarrier formulas for application is less explored.

Furthermore, the prominent role of the nano-delivery plant-derived pesticides formula is to reduce the level of toxicity so that the antifeedant and other potent sub-lethal bioactivities can be enhanced due to nano-delivery reserves. Especially for safety products in crop management, a plant-derived pesticides formula is hindered by toxicant residues and resistance problems. The challenges are compatibility with nanocarriers and resources for appropriate bioactivity on target insect pests and cost-effective formulation to allow the field or practical application of recent advanced technological development.

Nano-Delivery System of Antifeedant Formulation

As antifeedant is potentially received from plant-derived bioactivity, it becomes an interesting object of study as an important component of integrated pest management, especially in crop pest insect control [4,96]. Further noted is that the antifeedant mode of action is determined by a feeding mechanism, which is induced by special taste receptors in insects that stop feeding activity. Antifeedants are generally obtained from the resources of plant extracts or essential oils that contain ingredients sensitive to insect taste receptors [46,97]. The biodiversity of potentially bioactive phytochemicals is the main source in formulating nanobiopesticides. Nanobiopesticides have been shown to have a significant impact on improving plant-derived pesticide properties, including antifeedant performance [27,36]. The efficiency and effectiveness of nanobiopesticides including antifeedants are enhanced by using nanoformulation polymers, metal oxides, active particles combined with micelles, etc. [36].

III. RESULTS

The world's population is continuously increasing; therefore, food availability will be one of the major concerns of our future. In addition to that, many practices and products used, such as pesticides and fertilizers have been shown harmful to the environment and human health and are assumed as being one of the main factors responsible for the loss of biodiversity. Also, climate change could aggravate the problem since it causes unpredictable variation of local and regional climate conditions, which frequently favor the growth of diseases, pathogens and pest growth. The use of natural products, like essential oils, plant extracts, or substances of microbial-origin in combination with nanotechnology is one suitable way to outgrow this problem. The most often employed natural products in research studies to date include pyrethrum extract, neem oil, and various essential oils, which when enclosed shown increased resistance to environmental factors. They also demonstrated insecticidal, antibacterial, and fungicidal properties.



However, in order to truly determine if these products, despite being natural, would be hazardous or not, testing in non-target organisms, which are rare, must start to become a common practice. [13,14] Therefore, this review aims to present the existing literature concerning nanoformulations of biopesticides and a standard definition for nanobiopesticides, their synthesis methods and their possible ecotoxicological impacts, while discussing the regulatory aspects regarding their authorization and commercialization. As a result of this, you will find a critical analysis in this reading. The most obvious findings are that i) there are insufficient reliable ecotoxicological data for risk assessment purposes and to establish safety doses; and ii) the requirements for registration and authorization of these new products are not as straightforward as those for synthetic chemicals and take a lot of time, which is a major challenge/limitation in terms of the goals set by the Farm to Fork initiative.

IV. CONCLUSION

Post-harvest pest control can rely on few approved pesticides and tools; hence, there is a rising interest in new sustainable, eco-friendly approaches. In this study, eight commercial essential oils (EOs) (anise *Pimpinella anisum*, artemisia *Artemisia vulgaris*, fennel *Foeniculum vulgare*, garlic *Allium sativum*, lavender *Lavandula angustifolia*, mint *Mentha piperita*, rosemary *Rosmarinus officinalis*, and sage *Salvia officinalis*) were selected for their bioactivity and commercial availability, and then formulated in nano-emulsions. Repellency and acute toxicity of the developed nano-formulations were tested against a key stored product pest, *Tribolium confusum* (Coleoptera: Tenebrionidae). All the developed nano-emulsions presented optimal physical characteristics (droplet dimension = 95.01–144.30 nm; PDI = 0.146–0.248). All the formulations were repellent over time tested against adult beetles, in area preference bioassays. The best repellent was the anise EO-based formulation (RC₅₀ = 0.033 mg). Mortality values from cold aerosol trials showed that the majority of tested EOs caused immediate acute toxicity, and garlic EO nano-emulsion caused the highest mortality of *T. confusum* adults (LC₅₀ = 0.486 mg/L of air). EO-based nano-insecticides, used as cold aerosol and gel, are promising control methods against stored product pests, which can be integrated and combined with other sustainable biorational approaches.[15]

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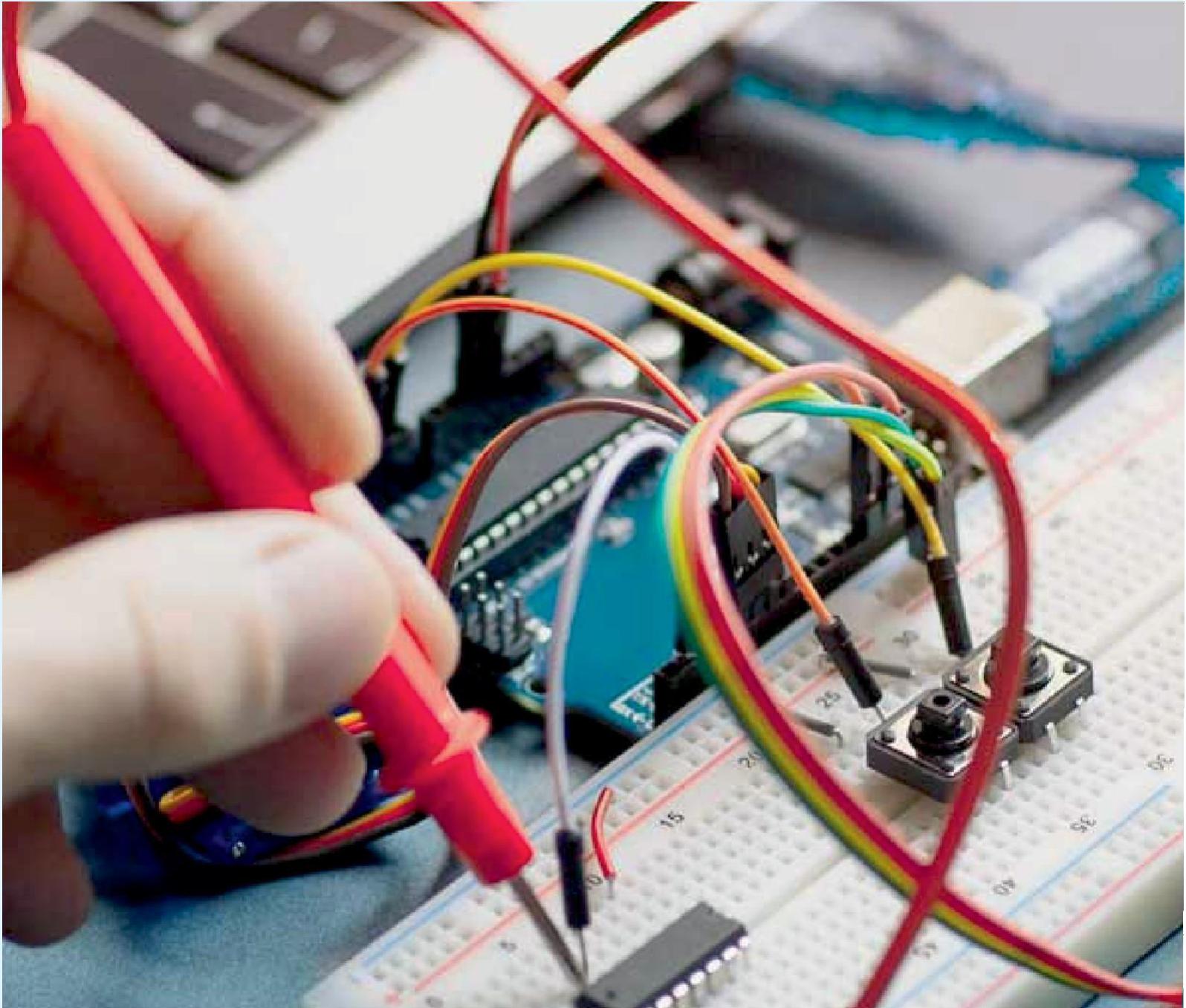
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