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Comparison of Nanoemulsions and Conservation as a Biopesticide Encapsulation Strategy from Fermentation and Extraction: Review

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Abstract

The increasing demand for sustainable agriculture has accelerated the development of biopesticides as environmentally friendly alternatives to synthetic pesticides. However, their application remains constrained by poor stability, low solubility, rapid degradation, and inconsistent field performance. This review aims to evaluate the relationship between bioactive compound production methods and appropriate encapsulation techniques, focusing on fermentation- and extraction-derived biopesticides. A comparative analysis of recent literature was conducted to assess the compatibility of fermentation and extraction processes with coacervation and nanoemulsion encapsulation technologies. The analysis shows that fermentation-derived bioactive compounds, including microbial spores and protein toxins, are best protected through coacervation, which enhances environmental stability and enables controlled release. Conversely, extraction-derived bioactive compounds, such as essential oils, terpenoids, and phenolics, are more effectively formulated using nanoemulsions, improving solubility, dispersion, bioavailability, and biological efficacy while reducing application rates. Matching bioactive production methods with suitable encapsulation strategies significantly enhances formulation stability, active ingredient delivery, and pest control performance. This integrated approach provides a practical framework for developing effective, stable, and commercially viable biopesticides to support sustainable agricultural systems. This review, based on a comparative analysis of recent studies, highlights that fermentation-derived bioactives are more compatible with coacervation systems, whereas extraction-derived bioactives are better suited for nanoemulsion formulations. The findings emphasise that aligning the characteristics of bioactive sources with suitable encapsulation techniques is crucial for improving stability, active ingredient loading, and pest control performance. This integrated approach provides a strategic framework for designing effective, stable, and commercially viable biopesticide formulations that support sustainable agriculture.

Keywords: Agriculture, Bioactive Compounds, Coacervation, Essential Oils, Microbial Metabolites.

INTRODUCTION

Global pressure to increase food production while lowering the environmental footprint demands more sustainable pest control strategies amid a projected population of close to 10 billion by 2050 (Deguine et al., 2021). Long-term dependence on chemical pesticides raises the problem of pest resistance, environmental contamination, and toxicity to non-target organisms that undermine the resilience of agroecological systems (Angon et al., 2023). This condition is driving a shift towards innovative solutions that are not only effective in controlling pests, but also aligned with ecosystem health and food security (Ahmad et al., 2024). Developing innovative formulations is essential to improve the commercial viability of microbial biopesticides (Ortiz & Sansinenea, 2023). These formulations should improve the shelf life, ease of use, and field performance of microbial products to make them more appealing to end-users (Mehta & Vyas, 2023). This includes incorporating the right carriers, protectors, and stabilizers to extend the viability of microbial cells during storage and transport (Areeshi, 2022). Additionally, innovative delivery systems, such as encapsulation or controlled-release technologies, can enhance the spread, adhesion, and resistance of microbial biopesticides to target pests and crops, thereby improving their efficacy and reliability in the field (Ataei et al., 2022). Additionally, molecular engineering can help address these risks by providing a detailed understanding of their impacts and developing effective mobilizers (Fenibo et al., 2021). However, strict restrictions and regulations are needed to prevent overuse and potential harm to microbial communities and ecosystems (Raimi et al., 2021).

Challenges in production and formulation further complicate the commercialization of microbial biopesticides (Schütz et al., 2018). Increasing the production of microbial strains to a commercial level requires optimizing growth conditions and fermentation processes, both of which are technically complex and costly (Sala et al., 2020). The downstream process, which includes harvesting, stabilizing, and formulating microbial cells into viable and shelf-stable products, is critical to ensuring the quality and consistency of the final product (de Sá Santos et al., 2021). Despite technological advancements, developing formulations that improve shelf life, ease of use, and field performance remains an ongoing challenge (Yusuf et al., 2025). This study focuses on active ingredients obtained through fermentation and extraction processes, aiming to determine the optimal combination of production and encapsulation methods based on stability, active ingredient loading, and biological efficacy against plant pest targets.

METHOD

This study employed a narrative literature review approach to compare fermentation- and extraction-derived biopesticides with their corresponding encapsulation strategies. Relevant articles were collected from scientific databases such as Scopus, ScienceDirect, SpringerLink, Wiley Online Library, and Google Scholar. The inclusion criteria consisted of peer-reviewed articles published primarily between 2019 and 2025 that discussed biopesticide production through fermentation or extraction and nanoemulsion or coacervation encapsulation techniques. The selected studies were systematically and critically analyzed comparatively based on bioactive source, encapsulation method, particle characteristics, stability, loading efficiency, biological performance, and practical applicability.

RESULTS AND DISCUSSION

Biopesticide

Pesticides are natural and synthetic compounds used to prevent, control, or eradicate insects, weeds, and pest organisms that inhibit plant growth (Ahmad et al., 2024). Microbiological biopesticides are mainly composed of bacteria, fungi, viruses, and protozoa that exhibit antagonistic effects against pests and pathogens. These microbial agents function by competing directly with pathogens for space and nutrients or by producing bioactive compounds that suppress pest growth (Samada & Tambunan, 2020). Pesticides are generally differentiated based on their mode of action, chemical structure, hazard level, and application method (Ahamad & Kumar, 2023).

Since the mid-1940s, global demand for pesticides has increased sharply and consistently, primarily driven by large-scale commercial farming practices (Trellu et al., 2021). However, excessive and uncontrolled use has resulted in food contamination and environmental pollution affecting agricultural land and aquatic ecosystems (Bashir et al., 2020). Pesticide residues have been detected in fruits, vegetables, processed foods, water, air, and soil (Singh et al., 2020). As agricultural production intensifies, pesticide trade has also shifted toward more environmentally friendly products and diverse formulations, including powders, solutions, and emulsion concentrates (Yang et al., 2023).

Acute and chronic health effects resulting from pesticide exposure through agricultural activities and daily consumption have become major public health concerns, particularly in developing countries (Umapathi et al., 2022). Toxicologically, many pesticides exhibit carcinogenic, cytotoxic, and mutagenic properties (Cech et al., 2023). Because their mechanisms of action are often non-specific, pesticides can also affect non-target organisms, including humans (Fang et al., 2020). According to WHO and UNEP estimates, pesticide exposure causes approximately three million poisoning cases and 200,000 deaths annually, with most occurring in developing countries (Boedeker et al., 2020). Pesticides can also induce the formation of reactive oxygen species (ROS), reducing antioxidant capacity and cellular defense mechanisms against oxidative damage (Kaur et al., 2019). This oxidative imbalance damages proteins, lipids, and nucleic acids while disrupting cellular signaling pathways, ultimately contributing to long-term health effects (Rani et al., 2021). Persistent ROS accumulation and oxidative stress have been linked to chronic diseases, including cancers of the brain, breast, prostate, bladder, and colon, as well as Alzheimer's disease, Parkinson's disease, neurotoxicity, infertility, leukemia, and diabetes (Perrin et al., 2021; Rafeeinia et al., 2023; Wang et al., 2024).

Despite these concerns, pesticides continue to provide substantial benefits for agricultural productivity by reducing crop losses caused by pests, diseases, and weeds, thereby improving both yield quantity and quality and supporting global food security (Hernández-Mariano et al., 2022). In the public health sector, pesticides play a crucial role in controlling disease vectors such as mosquitoes, ticks, mites, and fleas, helping to prevent the transmission of dengue fever, malaria, and Lyme disease (Tudi et al., 2021).

Although pesticides remain essential for modern agriculture and public health, their application must carefully balance productivity with environmental and human health protection. Consequently, responsible pest management strategies, particularly Integrated Pest Management (IPM), have been increasingly promoted to minimize pesticide dependence while maintaining effective pest control. This growing emphasis on sustainability has also accelerated interest in environmentally friendly alternatives, particularly biopesticides, which offer effective pest management with reduced ecological and toxicological risks.

Fermentation

Fermentation is a biotechnological approach that is widely applied in the production of microorganism-based biopesticides (Hermawan & Madyasti, 2023). This process enables microorganisms, such as bacteria and fungi, to be cultivated under control conditions to produce spores, blastospores, microsclerotia, and various bioactive metabolites (Lima et al., 2024). Compared to synthetic pesticides, fermentation-based technologies are more environmentally friendly, as they reduce the risk of pest resistance while minimizing adverse impacts on ecosystems (Verma et al., 2024). During fermentation, microbial activity such as that of Lactobacillus, Aspergillus, or Bacillus facilitates the breakdown of complex substrates into simpler compounds with pesticidal properties, including phenolic compounds, terpenoids, alkaloids, and flavonoids (Tong et al., 2023). Overall, fermentation plays a crucial role in enhancing the bioavailability and stability of active compounds, as well as improving selective toxicity against target pests without causing environmental harm.

In general, there are two main fermentation methods for biopesticide production as shown in Table 1, namely Solid-State Fermentation (SSF) and Submerged Fermentation (SmF) (Chilakamarry et al., 2022). SSF is carried out by cultivating microorganisms on solid substrates with limited moisture, typically using low-cost materials or agricultural organic wastes (Molina-Peñate et al., 2022). The use of the solid fraction derived from hydrolysis of the Organic Fraction Of Municipal Solid Waste (OFMSW) in SSF has been reported to yield 3.9×10^8 viable cells g^{-1} dry matter with a sporulation rate of 33% by *Bacillus thuringiensis* (Molina-Peñate et al., 2022). Similarly, *Trichoderma harzianum* grown on green waste via SSF produced up to 3.03×10^9 spores g^{-1} dry matter, along with indole-3-acetic acid (IAA) acting as a plant biostimulant (López-Espinoza et al., 2022). Despite its advantages in simplicity and waste valorization, SSF generally requires longer production times, presents higher contamination risk, and poses challenges in achieving uniform control of fermentation conditions (Mattedi et al., 2023).

In contrast, SmF is conducted in liquid media within bioreactors, allowing precise control over pH, temperature, aeration, and nutrient availability (Mascarin et al., 2024). SmF enables rapid production of microbial biomass and bioactive metabolites with consistent quality (Myeong et al., 2025). The production of Akanthomyces lecanii via SmF has been shown to increase up to 132 fold compared to SSF, accompanied by enhanced efficacy in controlling the red spider mite (Tetranychus urticae), as indicated by a lower LT_{50} value (1.24 days for SmF versus 2.54 days for SSF) (Medina-Chavarría et al., 2021). Furthermore, SmF can stimulate the synthesis of secondary metabolites with stronger insecticidal activity due to improved nutrient and oxygen availability (Quiroga-Cubides et al., 2024).

Thus, the selection of fermentation methods for biopesticide production strongly depends on the intended production goals and resource availability (Mattedi et al., 2023). SSF offers the advantage of low-cost utilization of organic waste materials (Molina-Peñate et al., 2022), whereas SmF is more suitable for industrial-scale applications due to higher yields, shorter processing times, and greater product quality consistency (Quiroga-Cubides et al., 2024). The integration of both approaches or further process optimization holds strong potential as an effective strategy to support sustainable biopesticide development. Fermentation products are generally obtained in the form of liquids, pastes, or semi-solid precipitates containing a wide range of highly bioactive secondary metabolites, such as organic acids, terpenoids, phenolic compounds, alkaloids, flavonoids, and bioactive enzymes (Ozyigit et al., 2023).

Extraction

Extraction is a crucial step in obtaining bioactive compounds that act as active ingredients in biopesticides. The choice of extraction method significantly determines the yield, stability, and bioactivity of the resulting compound.

Extraction is an essential technique for separating bioactive compounds from natural materials by exploiting the solubility differences between the solvent and the material matrix (Viciniescki et al., 2024). Commonly used materials include leaves, fruits, roots, or seeds, which are rich in secondary metabolites such as saponins, flavonoids, and alkaloids (Ramli et al., 2019). Pretreatment steps such as drying at 40–60 °C and grinding contribute to increasing the efficiency of contact between the solvent and the material (Viciniescki et al., 2024)..

Table 1. Fermentation Methods in Biopesticides

Method/Description	Disadvantages	Particle Size	Material	Bioactive Compo-nent	Referen-ces
SSF using *Trichoderma harzianum* produced IAA (101.46 µg g^{-1} DM) and spores (3.03×10^9 g^{-1} DM), with production influenced by tryptophan, substrate moisture, and waste composition	Waste composition depended on season and source; moisture control was required, and maximum spore production occurred at low tryptophan levels, indicating a trade-off with IAA production.	Chopped to a size of <1–2 cm to ensure homogen eity	Green waste (grass, pruning waste) + tryptophan	Indole-3-acetic acid (IAA), *T. harzianum* spores	(Ghoreishi et al., 2023)
SSF using *Bacillus thuringiensis* on OFMSW provided a sugar-rich liquid for other fermentations and a solid fraction for SSF, yielding 3.9×10^8 cells g^{-1} DM with 33% sporulation	Overly harsh hydrolysis reduces the quality of the solid fraction; sporulation is limited (33%); and maintaining substrate consistency at large scale is challenging	Hydrolyz ed solid fraction (<5 mm)	OFMSW (organic fraction of municipal solid waste: kitchen waste, garden waste)	*Bacillus thuringiensis* spores (biopesti-cide)	(Molina-Peñate et al., 2022)
SmF using *Akanthomyces lecanii* in a 14-L bioreactor achieved 132-fold higher production than SSF and enhanced pathogenicity through conidia and toxic metabolite production	The process requires specialised bioreactors, long fermentation time (~10 days), and high initial costs, limiting its industrial adoption compared to simpler SSF	Liquid conidia with a size of appro-ximately 2–3 µm	Liquid medium: mashed potato (35 g/L), malt extract (2 g/L)	*A. lecanii* conidia and toxic compounds (e.g., benzoqui-nones)	(Medina et al., 2021)

The choice of solvent is highly dependent on the polarity of the desired compound, with water and ethanol used for polar compounds, and supercritical CO_2 for nonpolar compounds. In the extraction of Melia azedarach fruit, the use of supercritical CO_2 at 250 bar and 50 °C for 120 minutes resulted in a yield of 3.44% with a high azadirachtin content (Viciniescki et al., 2024). These results indicate that optimizing material and solvent conditions is a key factor in achieving maximum yield and maintaining the stability of the active compound (Ramli et al., 2019). The types of extraction methods used in biopesticide development are described in Tabel 2.

Various extraction methods have been developed as shown in Table 2, including Soxhlet extraction, Microwave-Assisted Extraction (MAE), Supercritical Fluid Extraction (SFE), and Solid-Phase Extraction (SPE) (Ramli et al., 2019). The Soxhlet method is widely used for the extraction of heat-resistant compounds because it can maintain the solvent circulation process at a constant temperature for several hours (Viciniescki et al., 2024). The MAE method uses microwaves with a frequency of 2.45 GHz to accelerate the release of active compounds through direct volumetric heating of plant tissue (Ramli et al., 2019). Meanwhile, SFE uses high-pressure CO_2 solvent, making it fast, selective, and environmentally friendly (Viciniescki et al., 2024). In addition, the cyclodextrin-based miniaturized solid-phase extraction (mini-SPE) method provides a solvent-saving and efficient alternative for extracting biopesticides from complex matrices such as water and fruit juice. This combination of modern extraction technologies is capable of producing bioactive compounds with high purity and minimal degradation (Peng et al., 2017).

Extraction operating conditions such as temperature, pressure, time, and solvent-to-material ratio significantly influence the final result. The use of the response surface methodology approach has proven effective in optimizing parameter combinations to improve extraction yields. In saponin extraction using MAE, optimum conditions were achieved at a temperature of 90°C, a time of 9 minutes, and a solvent ratio of 23.54 mL/g, resulting in a yield of 3.43 wt% (Ramli et al., 2019). In the SFE method, increasing the pressure from 100 to 250 bar increases the solubility of CO_2, thereby enhancing the diffusion of active compounds from the solid to the solvent (Viciniescki et al., 2024). However, excessively high temperatures can damage thermolabile compounds such as azadirachtin and reduce the bioactivity of the extract. Therefore, precise control of temperature and extraction time is necessary to maintain the stability of the chemical structure of the target compound.

Extraction products can be crude extracts, active fractions, or pure compounds, depending on further purification steps such as filtration, fractionation, and chromatography (Peng et al., 2017). Qualitative and quantitative analysis of bioactive compounds is typically performed using FTIR, HPLC-UV, or UV/Vis spectrophotometry to verify purity and concentration. In biopesticide development, active compounds such as azadirachtin, saponins, and berberine are often formulated into ready-to-use products through an encapsulation process. Spray-drying with coating materials such as gum arabic and whey protein can increase compound stability against oxidation and enable controlled release. Research shows that encapsulated extracts are as effective as synthetic pesticides but more environmentally friendly. Therefore, extraction methods followed by innovative formulations are a strategic step in producing efficient and sustainable natural biopesticides.

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Each extraction method has specific advantages depending on the purpose. MAE excels in energy efficiency and biopesticide activity, $ScCO_2$ offers high purity and sustainability with encapsulation support, while cyclodextrin-based SPE stands out for sensitive residue detection. The choice of method should be tailored to the needs: rapid production, stable commercial formulations, or residue analysis for food safety. Combining techniques, such as integrating $ScCO_2$ with encapsulation or applying MAE at industrial scale, represents a promising research direction for increasing the availability of sustainable botanical biopesticides.

Encapsulation

a. Nanoemulsion

Nanoemulsion is a biopesticide formulation approach that utilizes an oil-in-water colloidal system with very small droplet sizes (<200 nm), thereby increasing the solubility, stability, and bioactivity of lipophilic bioactive compounds (Savitharani et al., 2024). The very fine particle size allows for more efficient penetration into target tissues, both insects and plant pathogens, and slows the volatilization of active compounds.. This is important considering that essential oils and secondary plant metabolites tend to be volatile, degraded by light, and have low solubility in water. Thus, nanoemulsions function not only as carrier systems but also as enhancers of biological activity, thereby reducing the dose of biopesticide application and extending its duration of action in the field (Riaz et al., 2025).

Nanoemulsions are colloidal oil-in-water dispersion systems with droplet sizes less than 200 nm, designed to enhance the solubility, stability, and bioactivity of natural bioactive compounds (Singh & Pulikkal, 2022). Extracted compounds such as essential oils or phenolic compounds are often formulated as nanoemulsions because they are hydrophobic and easily degraded by oxidation or light (Savitharani et al., 2024). In general, several nanoemulsion methods for biopesticide production are shown in Table 3. For example, hydrodistilled essential oil of *Anisomeles indica* yielded 59 bioactive components, and after formulation into a nanoemulsion through sonication, it showed increased stability and antibacterial activity against *Xanthomonas*.

The preparation of nanoemulsions from extraction products generally involves nonionic surfactants such as Tween 80, which stabilize droplets at room temperature (Singh & Pulikkal, 2022). The small particle size allows increased penetration of active compounds into microbial cells, thereby strengthening the biopesticide effect. Therefore, nanoemulsions from extracted materials play a crucial role in improving delivery efficiency of poorly water-soluble active compounds.

Table 2. Extraction Methods in Biopesticides

Method/Description	Disadvantages	Particle Size	Material	Bioactive Component	References
MAE the extraction of saponins from *Furcraea selloa* and tea dregs using microwaves. Yield 3.43 wt%. Bioassay showed 100% mortality of golden apple snails (*Pomacea canaliculata*) within 24 hours and increased rice growth.	Risk of degradation of thermolabile compounds if microwave power is too high; strict temperature control is required.	Not reported (liquid extract)	*Furcraea selloa* biomass and tea dregs; ethanol/water solvent	Saponin	(Ramli et al., 2019)
SPE based on β-Siklodekstrin. Extraction and purification of bioactive alkaloids (sanguinarine, berberine, rotenone, osthole) from food matrices (tomato juice, orange, and water) using β-CD sorbent, followed by UHPLC-Q-TOF/MS.	Limited capacity; better suited to laboratory analysis than to mass production.	Not relevant (residue analysis, not microcapsules)	β-cyclodextrin sorbent; liquid samples (juice, water)	Sanguinarine, berberine, rotenone, osthole	(Peng et al., 2017)
Supercritical CO_2 Extraction ($ScCO_2$) + Spray Drying. Extraction of *Melia azedarach* (chinaberry) fruit at 250 bar, 50 °C (yield 3.44%). The azadirachtin-rich extract has acaricidal activity against Tetranychus urticae. It was then encapsulated by spray drying (gum arabic, whey) for 90-day stability.	Requires high pressure (≥250 bar); high equipment costs; bioactives are susceptible to UV degradation before encapsulation.	Spray-dried microcapsules (1–10 μm)	Melia azedarach fruit; supercritical CO_2 solvent; gum arabic and whey as encapsulation walls	Azadirachtin and terpenoid compounds	(Viciniescki et al., 2024)

Meanwhile, nanoemulsions based on fermented materials are developed from secondary metabolites of microorganisms produced during biotechnological processes such as lactic acid bacteria or fungal fermentation (Riaz et al., 2025). Fermentation produces bioactive compounds such as organic acids, antimicrobial peptides, and enzymes that can be formulated into nanoemulsions to increase stability and broaden the spectrum of biological activity (Savitharani et al., 2024). The process of making nanoemulsions based on fermented materials often utilizes low-energy emulsification or phase inversion composition approaches to maintain the integrity of thermolabile compounds (Singh & Pulikkal, 2022). Research shows that fermentation of plant materials such as soybean seeds or fruit pulp can produce extracts with high phenolic components, which, after encapsulation in nanoemulsions, show up to a 40% increase in antioxidant activity compared to their free form. Furthermore, fermented materials tend to have lower viscosity and better chemical stability, making them suitable for formulation as nanoemulsions. The combination of bioactive characteristics resulting from fermentation and the nanoemulsion system produces a natural preparation that is more stable, easily absorbed, and environmentally friendly.

From a technical perspective, the main difference between fermented and extracted nanoemulsions lies in the initial properties of the compounds. Fermented compounds are generally more polar and temperature-sensitive, requiring low-energy emulsification to prevent degradation. Conversely, extracted compounds, such as essential oils or terpenoids, are nonpolar and therefore require surfactants with high HLB values to maintain emulsion stability (Singh & Pulikkal, 2022). For example, D-limonene from citrus extraction encapsulated in a nanoemulsion with the surfactant EL-40 (HLB 13.5) produced 112 nm droplets with high stability during storage. This nanoemulsion demonstrated up to a 60% increase in fungicidal activity against Rhizoctonia solani compared to the non-encapsulated form. Therefore, both fermented and extracted materials can be formulated into nanoemulsions, but the treatment mechanisms and stability differ depending on the chemical nature of the active compounds.

Based on various research results, nanoemulsions from extracted materials show superior physical stability, droplet size, and antimicrobial activity compared to nanoemulsions from fermented materials (Riaz et al., 2025). Extracted compounds such as essential oils and terpenoids tend to increase the efficiency of nanoemulsion formulations due to their good compatibility with surfactants and the oil phase. Nanoemulsions from fermented materials tend to have broad biological activity, but their stability is lower due to the presence of polar compounds that are easily degraded. Furthermore, fermented products require strict pH and temperature optimization during the emulsification process to prevent denaturation of the active compounds. Considering the stability, bioavailability, and effectiveness of biopesticides, it can be concluded that nanoemulsions are more suitable for application on extracted materials than fermented materials (Lina et al., 2023). Nanoemulsion formulations from extracted materials are also more easily adapted to the needs of sustainable agriculture.

b. Coacervation

Coacervation is one of the biopesticide encapsulation techniques that utilizes the interaction of oppositely charged polymers to produce a protective capsule. In the Na-alginate–chitosan formulation for *Bacillus thuringiensis* (Bt-127), the active ingredient is obtained through liquid fermentation, producing spores and δ-endotoxin crystal proteins (Duraimurugan et al., 2024). The fermentation compounds are then mixed with alginate solution as a negatively charged polymer (Saberi-Riseh et al., 2021). Chitosan is added as a positively charged polymer, triggering electrostatic interactions that lead to coacervate formation (Nezamdoost-Sani et al., 2024). Borate or multivalent ions are used as cross-linkers to strengthen capsule walls (Duraimurugan et al., 2024). After the coacervation process, dry microcapsules sized 10–100 μm are obtained and applied by spraying on leaves to suppress larval populations such as *Spodoptera litura* (Saberi-Riseh et al., 2021). This Na-alginate–chitosan coacervation method has also been developed to protect *Bacillus thuringiensis* var. kurstaki (Bt-127) (Duraimurugan et al., 2024). This system utilizes interactions of oppositely charged polymers, namely anionic alginate and cationic chitosan (Saberi-Riseh et al., 2021). This mechanism produces microcapsules sized 10–100 μm (Duraimurugan et al., 2024). The capsules maintain spore viability and δ-endotoxin crystal proteins against environmental degradation (Nezamdoost-Sani et al., 2024). The limitation of this formulation is the low active ingredient concentration, around 10% a.i. (Duraimurugan et al., 2024). In addition, capsule stability decreases under UV radiation and high temperatures (Saberi-Riseh et al., 2021).Coacervation is one of the biopesticide encapsulation techniques that utilizes the interaction of oppositely charged polymers to produce a protective capsule. Coacervation can be classified into simple coacervation and complex coacervation. Simple coacervation involves phase separation induced by changes in environmental conditions, such as pH adjustment, temperature variation, or the addition of salts or non-solvents, using a single polymer system. In contrast, complex coacervation occurs through electrostatic interactions between two or more oppositely charged polymers, resulting in the spontaneous formation of a polymer-rich coacervate phase that surrounds the active ingredient. Because most biopesticide formulations rely on interactions between oppositely charged biopolymers to improve capsule stability and encapsulation efficiency, complex coacervation is more commonly applied than simple coacervation. In the Na-alginate–chitosan formulation for *Bacillus thuringiensis* (Bt-127), the active ingredient obtained through liquid fermentation produces spores and δ-endotoxin crystal proteins, providing enhanced environmental protection, prolonged shelf life, and controlled bioactive release (Duraimurugan et al., 2024)

In gelatin–gum arabic formulations for plant essential oils such as neem, thyme, and oregano, the active compounds are extracted through steam distillation or organic solvent extraction (Zhang et al., 2022). The essential oils are then dispersed in a positively charged gelatin solution (Napiórkowska & Kurek, 2022). Gum arabic is added as a negatively charged polymer, and the pH is adjusted to trigger coacervate formation (Guo et al., 2022). The process continues with the addition of formaldehyde as a cross-linker to strengthen capsule walls (Székely-Szentmiklósi et al., 2024). The gelatin–gum arabic coacervation method to the encapsulation of essential oils such as neem, thyme, and oregano (Guo et al., 2022). Interactions between positively charged gelatin and negatively charged gum arabic produce liquid capsules (Székely-Szentmiklósi et al., 2024).

The size of the formed capsules ranges from 1–10 μm (Guo et al., 2022) This size distribution supports widespread dispersion on leaf surfaces (Napiórkowska & Kurek, 2022). The formulation is effective against small pests such as Aphis gossypii and Tribolium castaneum (Guo et al., 2022) The main drawback of this system is the vulnerability of essential oils to oxidation (Napiórkowska & Kurek, 2022). Capsule stability is highly dependent on the use of a cross-linker ((Mascarin et al., 2024)). The addition of formaldehyde has been shown to strengthen the capsule walls (Guo et al., 2022). However, the use of formaldehyde poses a potential risk of toxic residues (Székely-Szentmiklósi et al., 2024).

In gelatin–CMC–collagen formulations for seed coating applications, essential oils as the active ingredient are first extracted using the steam distillation method (Fernandes et al., 2024). The extracted oil is mixed into a solution of gelatin and CMC-Na, which serves as the main polymer (Li et al., 2022). Hydrolyzed collagen is added to improve the adhesion of the capsule wall to the seed surface (Fernandes et al., 2024). The coacervation process is triggered by adjusting the pH, and the wall is then strengthened using 4–6 mmol/g glutaraldehyde as a cross-linker (Li et al., 2022).

Table 3. Nanoemulsion Method in Biopesticides

Method/Description	Disadvantages	Particle Size	Material	Bioactive Component	References
***Anisomeles indica* nanoemulsion** by sonication. Application against Xanthomonas oryzae and X. citri increased antibacterial effectiveness, suppressed biofilm formation, and reduced the incidence of rice leaf blight by up to 84%.	Requires high sonication energy; stability still needs to be evaluated during long-term storage.	<100 nm	Anisomeles indica leaf essential oil, non-ionic surfactant	Terpenoid compounds, antibacterial phenolics	(Savitharani et al., 2024)
***Cymbopogon winterianus* nanoemulsion** formulated into a nanoemulsion to control Tribolium castaneum. Toxicity was nearly doubled compared to pure oil; it was safe for wheat seed viability.	Requires a relatively high amount of surfactant; potential for bioactive degradation during storage.	80–150 nm	Citronella oil, Tween 80 surfactant, water	Citronellal, geraniol, citronellol	(Riaz et al., 2025)
Aromatic essential oil nanoemulsion (general) formulated using high-energy emulsification. Stable, with uniform granules, it is effective as a biopesticide against insect pests.	Relatively high surfactant concentration; higher production costs than conventional formulations.	50–200 nm	Aromatic essential oil, surfactant, water	Thymol, carvacrol, linalool (depending on oil source)	(Ayllón-Gutiérrez et al., 2023)

After the process, the capsules are applied directly as a seed coating so that active ingredients like thymol, carvacrol, and linalool are gradually released in the rhizosphere to protect plants from soil pests such as Agriotes sp. (Fernandes et al., 2024). The gelatin–CMC–collagen coacervation method is applied for seed coating applications (Li et al., 2022). The combination of gelatin, CMC-Na, and hydrolyzed collagen produces viscous capsules with high adhesion (Míguez-González et al., 2023). These characteristics allow the active ingredients to adhere well to the seeds (Li et al., 2022). The bioactives used are thymol, carvacrol, linalool, and eugenol (Fernandes et al., 2024). These active ingredients are gradually released in the rhizosphere (Li et al., 2022). This formulation is effective against soil pests such as Agriotes sp. and Melolontha melolontha (Fernandes et al., 2024). The formulation's efficacy rate is about 50% compared to synthetic insecticides like imidacloprid (Michel et al., 2023) Capsule stability is achieved through the use of 4–6 mmol/g glutaraldehyde (Duraimurugan et al., 2024). However, the use of glutaraldehyde raises residue issues that need to be addressed (Li et al., 2022).

The comparison of the three methods in the table shows variations in results, methods, and products. The Na-alginate–chitosan system is more suitable for protecting microbes such as *B. thuringiensis* (Duraimurugan et al., 2024). The gelatin–gum arabic system is better suited for volatile essential oils (Guo et al., 2022). The gelatin–CMC–collagen system excels for seed coating due to its adhesive properties (Li et al., 2022). However, each method has different limitations. Na-alginate–chitosan produces low active content (Saberi-Riseh et al., 2021). Gelatin–gum arabic is prone to oxidation (Michel et al., 2023). Gelatin–CMC–collagen is still less effective compared to synthetic insecticides (Lima et al., 2024). These differences emphasize that coacervation formulation design must be tailored to the bioactive properties, target pests, and application purposes (Nezamdoost-Sani et al., 2024), considering formulation stability, release behavior, environmental conditions, and practical field requirements.

CONCLUSION

The development of effective biopesticides requires integration between bioactive production methods and suitable encapsulation strategies. Fermentation-derived biopesticides containing microbes, spores, or protein toxins are more compatible with coacervation systems, which enhance stability, protect bioactive components, and enable controlled release under diverse environmental conditions during field applications. In contrast, extraction-derived compounds such as essential oils and terpenoids are better suited for nanoemulsion formulations due to their ability to improve solubility, reduce volatilization, and enhance bioactivity. These differences highlight that selecting encapsulation techniques based on the characteristics of bioactive sources is crucial for developing stable and efficient biopesticide formulations. Future research should focus on optimizing encapsulation parameters, improving storage stability under field conditions, and evaluating scalability for commercial applications, including the development of sustainable cross-linking agents and advanced formulation approaches.

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