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Chapter

Microemulsion Formulation of Botanical Oils as an Efficient Tool to Provide Sustainable Agricultural Pest Management

Abhishek Sharma, Saurabh Dubey and Nusrat Iqbal

Abstract

Microemulsion formulation is among the most suitable carrier for the delivery of bioactive and, therefore, has excellent potential for industrial applications. The microemulsion system is thermodynamically and kinetically stable. Due to the smaller droplet size of the microemulsion system, the bioactive covers a larger surface of the target pest. Botanicals and essential oils, in particular, are green options to control various soil and seed-borne pathogens. Each oil contains several bioactive constituents that practically avoid microbe-resistance against it. Nevertheless, to improve the handling and shelf-life of botanicals, microemulsion formulation is the best option available. The current chapter provides the insight of a microemulsion system and explores the possibility of botanical oil-based biopesticides for a sustainable agroecosystem. We believe that botanical oil microemulsion could be a better alternative to synthetic pesticides and opens a new corridor for the promotion of the greener way of plant protection in India and across the globe.

Keywords: botanicals, essential oil, microemulsion, pathogen, formulation

1. Introduction

1

Essential oils from aromatic plants are a competent source of pesticides that have a diverse role in pest management like insecticides, fungicides, growth-regulators, deterrent, and repellent activities [1]. Essential oils' role in pharmacology or therapeutic activities and cosmetics is quite known. In recent years, the essential oil is being used in pest management applications and regularly used by the farmers to promote organic farming in an environmentally friendly way [2]. Essential oils of aromatic plants are rich in bioactive compounds viz., terpenes, terpenoids, flavonoids, phenolics, etc. These constituents have different physicochemical properties and stability, but their utilization in pure form is not feasible. Hence, a suitable delivery system is needed to enhance its bio-efficacy and stability during the application.

Various formulations of bioactive compounds are available in the market, but they have certain limitations like un-stability, high in cost, complex compositions, non-targeted delivery, and post-application wastage. It is, therefore, compelling to look upon the carrier system that overcomes the limitations mentioned above. In recent years, researchers have reported that microemulsion (ME) formulation is the

most suitable carrier for the delivery of bioactive constituents of essential oils and has excellent potential for industrial applications. The microemulsion system is not only thermodynamically and kinetically stable but also possesses a small droplet size (preferably below 100 nm). It means the formulation can incorporate a large amount of bioactive essential oil in the disperse phase. Due to these characteristic features, essential oil-based ME favors a stabilized and intelligent approach for the delivery of their active ingredients into the targeted site and results in enhanced bio-efficacy. In this chapter, we will discuss the essential oil-based ME and their role in pest management.

2. Microemulsion systems: a brief background about the origin and characteristic features

Hoar and Schulman gave the concept of microemulsion in the 1940s. They prepared the first microemulsion by dispersing oil in an aqueous solution of surfactants and co-surfactants, which finally provides a transparent solution [3].

The microemulsion, as defined by Danielsson and Lindman, in 1981 [4]. The definition was as given below:

"a microemulsion is a system of water, oil, and an amphiphile which is a single optically isotropic and thermodynamically stable liquid solution."

In microemulsion systems, surfactants and co-surfactant play an essential role, which stabilized the system by reducing the interfacial surface tension between two immiscible liquids and compensates the dispersion entropy and make the system thermodynamically stable.

2.1 Types of microemulsion systems

After the 5 years of microemulsion concept, Winsor studied the phase behavior of water, oil, and surfactant and classified microemulsion system in different phases, also known as Winsor phases as shown in **Figure 1**:

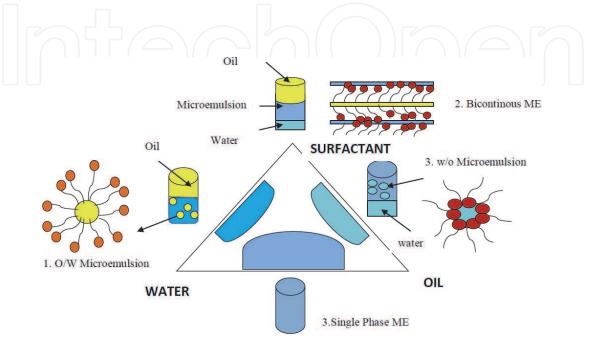


Figure 1.

Phases of the microemulsion system.

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- a. Oil in water (o/w) microemulsion: In this system, oil is dispersed in a water medium, and the surfactant is in high concentration in water medium and low surfactant concentration in oil medium.
- b. Water in oil (w/o) microemulsion: In this system, water is dispersed in oil medium. The surfactant is a high concentration in the oil phase and surfactant-low in the aqueous phase (Winsor II).
- c. Bicontinuousmicroemulsion (transition state from o/w to w/o): A three-phase system where a surfactant-rich middle-phase coexists with both water and surfactant (Winsor III or middle-phase microemulsion).
- d. Single-phase homogeneous mixture: single-phase isotropic micelle solution

2.2 Characteristics features of ME

The microemulsion system is a competent and stabilized carrier system for all types of active constituents. The characteristic features of microemulsion are:

- Thermodynamically stable
- Isotropic
- Transparent colloidal system
- Easily penetrate
- Long shelf-life
- Zero interfacial tension
- Easy to prepare
- Droplet size is tiny, i.e., 1–100 nm

2.3 Theories of micro emulsion formation

Three theories explain the microemulsion formation and stability.

i. **Interfacial or Mixed film theory**: According to this theory, the microemulsion is formed due to the formation of oil and water complex interface reduction by surfactant and co-surfactants. This theory depends on the reduction of interfacial tension and expressed as [Eq. (1)]:

$$\Upsilon_{\mathbf{i}} = \Upsilon_{\mathbf{o}/\mathbf{w}} - \mathbf{\Psi} \tag{1}$$

 Ψ = spreading pressure; Υ_i = interfacial tension; $\Upsilon_{o/w}$ = interfacial tension between oil and water.

Interfacial of oil and water reduced to zero and increases the spreading pressure.

- ii. **Solubilization theory:** According to this theory, oil or water reverse micelle structures solubilized and form the monophasic system.
- iii. **Thermodynamic theory:** According to this theory, microemulsion formation is a spontaneous process that depends upon the lowering of interfacial tension on the addition of surfactants and co-surfactants, and mixing of one

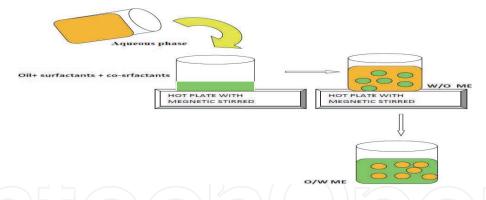


Figure 2.Diagrammatic representation of the phase inversion method of microemulsion.

phase to another contributes in enhancing the entropy which results into reduction of droplet size. The following thermodynamic equation expresses this theory as (Eq. (2):

$$\Delta G_{f} = \gamma \Delta A - T \Delta S \tag{2}$$

 ΔG_f = free energy of formation; γ = surface tension of the oil–water interface; ΔA = change in the interfacial area after microemulsion; ΔS = change in entropy of the system after mixing; T = is the temperature.

2.4 Preparation methods

There are two methods to develop a microemulsion system of very low interfacial tension at the correct ratio of surfactants and co-surfactants. There are two methods of microemulsion preparation:

- I.Phase inversion method. In this method, phase inversion occurs after the addition of excess dispersed phase in the surfactant system under temperature control. During phase inversion, the particle size of any drug or agrochemical reduced, which results in active release kinetics. This method is also called the phase inversion temperature method. Because after the cooling phase, inversion will occur from w/o to o/w. short chains of surfactants promote this inversion (Figure 2).
- II. Phase titration method. This method is also known as a spontaneous emulsification method and can represent with the help of phase diagrams. The phase diagram is handy in studying the various interactions that occur while mixing different components of the microemulsion. The phase diagram is constructed to find out the zones of the microemulsion, and each corner represents 100% of each element. The phase diagram is built at fixed surfactant and co-surfactant weight ratios and titrated with water at room temperature. The formation of the transparent monobasic system is established by physical appearance (Figure 3).

3. Factor affecting the particle size of active entity (drug or agrochemical) in microemulsion

The microcavities in microemulsion by surface-active agents causes a cage-like effect and check the particle agglomeration [5]. The stability of microemulsion drops depends upon the following factors.

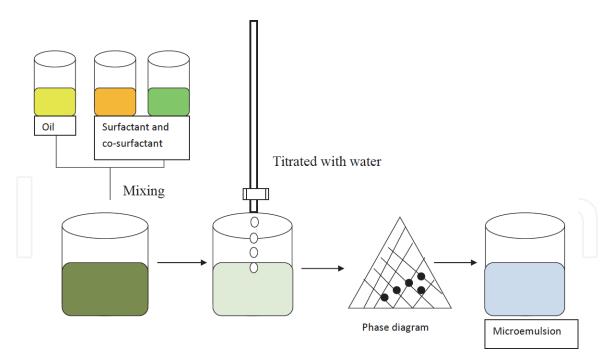


Figure 3.Diagrammatic representation of the titration method of microemulsion.

I. **The viscosity of the microemulsion**: The size of particle in a microemulsion is depended upon the viscosity of the mixture after adding all the surfaceactive agents and water and expressed by the following equation [Eq. (3)]:

$$\eta_{\rm r} = \eta/\eta_{\rm o} = 1 + 5/2\varphi \tag{3}$$

where, η_r = relative viscosity; η = viscosity of the dispersion; η_o = solvent viscosity; ϕ = volume of droplets.

In the microemulsion system, breaking up of droplets gives droplet volume fractions up to 0.2, the expected relative maximum viscosity is 1.5, which results in droplet interactions and destabilizes the microemulsion system [5].

II. **The ratio of water to surface-active agents:** The water level inside the spherical micelles gives the radius measurement by the following expression [Eq. (4)] and **Figure 4**.

$$r = 3 \text{ Vm/s} \tag{4}$$

where, Vm is the dispersed volume of water; s is the interfacial area by surfactant molecules.

As the water level is high, it lowers the stability due to less capability of surface-active agents to protect the more substantial drop — consequently, particles undergo coagulation and flocculation. Therefore, the size of the droplet depends upon the ration of water to surface-active agents (6).

- III.Nature of surface-active agents and concentration of aqueous reactants: An increase in surface-active agents decreases the particle size. The surface-active agents stabilized the microemulsion by reducing the ration of water to surface-active agents (\(\omega\)). Therefore, surface-active agents control the droplet size as well as provide stability to the microemulsion system.
- IV.**Temperature**: Temperature plays a significant role in droplet size reduction. As temperature decreases, the viscosity of the microemulsion increases, which results in particle agglomeration. Elevated temperature decreases the solubility

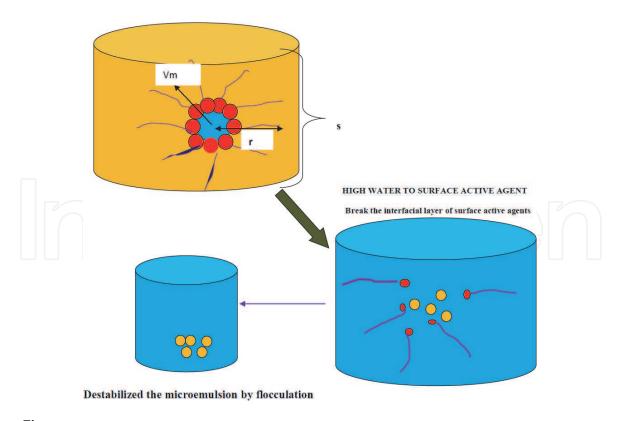


Figure 4.Diagrammatic representation of the effect of water on the surface-active agent on the stability of the microemulsion.

of non-ionic surfactants due to de-hydration of hydrophilic groups at high temperature. In the oil phase, on the other hand, active agents' solubility increases in the oil phase. Therefore temperature optimization is critical in droplet size reduction and microemulsion stabilization.

4. Microemulsion evaluation parameters

4.1 Physical appearance

For Physical appearance, microemulsion can be checked visually by the fluidity, optical clarity, and uniform appearance.

4.2 Transmittance test

Transparency of microemulsion is the first sign of the microemulsion system. The percent transmittance is measured by UV–visible spectroscopy.

4.3 Scattering Techniques

Microemulsion structures can be studied by scattering of x-ray radiations. This technique identifies the nature of microemulsions weather; it is a diluted monodispersed or polydispersed system.

4.4 Droplet size

The droplet size of the microemulsion is measured by dynamic light scattering experiments or electron microscopy. Along with droplet size, it also gives polydispersity values of the microemulsion system.

4.5 Active ingredient stability

The active ingredient stability is quantified by suitable analytical techniques like HPLC, GC–MS, LC–MS, and others. According to the active constituents after formulation development. The active constituent's stability is crucial after formulation development.

4.6 Viscosity measurement

Viscosity is the fundamental property of the microemulsion system. If any type of viscosity change occurs, it will destabilize the microemulsion and leads to floculation or phase separation.

4.7 Electrical conductivity

The electrical conductivity of formulated samples in microemulsion form was checked after adding a surfactant, oil, and water components. A conductometer does this measurement at ambient temperature and 1 Hz frequency.

4.8 In-vitro drug release

The bioactive content release study was carried out in Franz diffusion cell of volume 20 ml. Two compartments are present- one is a receptor compartment, and the other is the donor compartment. Receptor compartment is filled with buffer, and the donor compartment is filled with a microemulsion sample and covered by a cellophane membrane. At certain intervals of time the donor compartment is analyzed for active ingredient content.

4.9 Advantages

- Solubilize water-insoluble active constituents
- Enhanced bio-efficacy
- Slow-release delivery system
- Effective in both contact as well as systemic delivery
- Easy to form
- Smell masking of unpleasant active ingredients
- Easy to develop
- Protect the active constituents from hydrolysis and oxidation.

4.10 Disadvantages

- Use of high amount of surfactants and co-surfactants
- Limiting solubilizing capacity for high melting active constituents
- Microemulsion stability influenced by temperature and ph.

5. Applications of microemulsion

Microemulsion are being used in many areas.

- a. **Drug delivery**: Microemulsion formulation has been extensively used in drug delivery. From the last few decades, the microemulsion system has been used in a variety of drug delivery systems like oral, topical, parenteral, and oral.
- b. **Analytical applications**: The microemulsion system is the suitable solubilizing medium for hydrophobic constituents and then can be analyzed for various constituent detection.
- c. **Biotechnology applications**: Enzymatic reaction, immobilization of proteins, and bioseparation can be done successfully in microemulsion systems.
- d. **Oil recovery**: The microemulsion system also helps in the improvement of oil due to the high amount of surfactants.

Besides, the applications mentioned above, microemulsion formulation also find its applications in the following fields:

- Cosmetics
- Coating of Textiles
- Detergents
- Environmental Remediation
- Agrochemicals
- Food

6. Botanical oil microemulsion in crop protection with emphasis on essential oil

Several botanicals sources of phytochemicals offer great promise for insect pest control. Six plant families with several representative species, Asteraceae, Cladophoraceae, Labiatae, Meliaceae, Oocystaceae, and Rutaceae, appear to have the most significant potential for providing future insect control in crops [6].

A most crucial source of phytochemical compounds against insect pests is the essential oil. Essential oil is the mixture of volatile compounds generally produced by Plant as secondary metabolites, constituted by hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, and phenols) [7]. Bioactive chemical compounds of essential oils have repellent properties against mosquitoes. Previous studies revealed that terpenoid groups are biologically active compounds for mosquito repellency. There are 20 active terpenoids with a functional group of negatively charged and containing ester/ether bonds or an ethanol hydroxyl group and other positively charged end containing alkane groups [8]. These bio-active chemicals have several properties, which are categorized as repellents, feeding deterrents, toxins, and growth

regulators. Five major categories of bioactive compounds are (1) nitrogen compounds (primarily alkaloids), (2) terpenoids, (3) phenolics, (4) proteinase inhibitors, and (5) growth regulators [9].

Many bioactive compounds (phytochemicals) have been discovered by the researchers who have good repellent activity against insect pests [10]. These botanical-based repellents not only have excellent repellency but also influences the physiology of insect. These botanical bioactive agents (phytochemicals) can effectively replace the presently available synthetic pesticides against the mosquito.

6.1 Issues with essential oils in agriculture

Essentials are potent pesticides. But still, exploitation of them in pure form is not encouragable in the agriculture because of the following reasons:

- a. **Water solubility:** Essential oils are water-insoluble due to fatty acids and cannot be dispersed uniformly in water before application [11]. Non-uniformity during use creates the problem of dispersion over the applied areas. It is the main ineffectiveness of essential oil application.
- b. **Penetration:** Essential oils in a pure form not penetrate in the targeted area and wash off by rain or evaporated quickly by air [12].
- c. **Shelf life:** In the pure form, shelf life of essential oil decreases and their constituents starts degrading in the open atmosphere by light, pH, temperature variations, etc. [13].

6.2 Microemulsion system: a practical approach to solve the inefficiencies of essential oil in crop protection

Microemulsions are the thermodynamically stable isotropic solution of nanodispersions of size 10–200 nm. These are highly stabilized solutions with surfactants and co-surfactants [14, 15]. These microemulsion systems provide effective delivery systems, give extended shelf life, easy to prepare, and can easily scalable by low input of energy [16].

In microemulsion physio-chemical parameters of essential oil changes like improvement insolubilization, better bioavailability, and enhance the rate of penetration in targeted sites without any wastage [14, 17] in addition to this essential oil bio-constituents spreading capacity in aqueous solution improves and gives uniform dispersion on targeted sites after application [18] and provide good bioefficacy [19]. Moreover, in microemulsion formulation, essential oil active ingredient degradation rate decreases and enhances their shelf life for an extended period [20]. Further, due to the smaller droplet size in microemulsion also enhances wetting, spreading, and permeability and uniformly deposited over leaf surfaces [21, 22]. Characteristic features of essential oil microemulsion have been highlighted in **Figure 5**.

6.3 Available essential oil microemulsions and targeted pest

Microemulsions can incorporate the natural oil or essential oil in a high amount in water without any destabilization of active constituents. Essential oil gives a superstability performance in microemulsion form as compared to emulsion forms [23]. The essential oil has many potential active ingredients that give very good bioefficacy in insect pests. Hence, essential oil could be a better alternative to

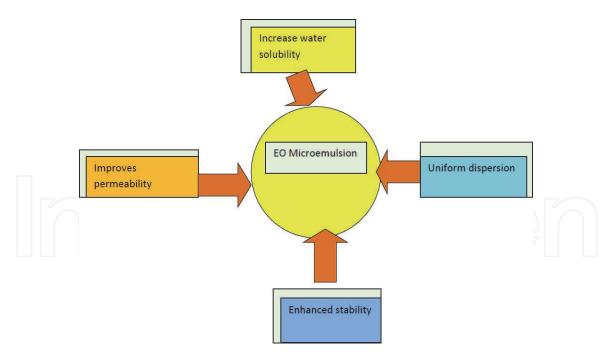


Figure 5.
Characteristics of essential oil microemulsion.

synthetic pesticides. In microemulsion systems, essential oil active constituents are protected in micelles and give a useful model of the delivery system against different pest populations.

In the microemulsion system, droplet size is very small, i.e., in the range of 10–100 nm. So, the translocation through vascular tissues is very easy in systemic, and due to good adherence and spreadability, it gives equally good results in the non-systemic mode of action. **Table 1** shows the available microemulsions in crop protection.

Cinnamon (CM) essential has been reported as a potential alternative to chemical fungicides. In CM microemulsion formulation rate of control of gray mold was increased up to 20% with 500ug L-1 in comparison with the non-microemulsion formulation. CM microemulsion postponed the ascorbic acid loss and cause no significant influence on pear qualities such as color and taste [24].

The microemulsion system, along with stabilizers and surfactants, gives stable physicochemical characteristics and excellent stability. Insecticidal bioassay indicated that the acute LC50 to P. xylostella was 12.477 mg/L. It will be environment-friendly and shows potential alternative features to synthetic pesticide against P. xylostella [25].

Neem, along with Karanja oil, has been termed as a natural pesticide microemulsion system. This combination has been proven economical as well as the stabilized formulation for an extended period and gives good bioefficacy against different types of crop pest populations [26].

Essential oils give combinatory properties along with synthetic pesticides. Along with stability, it also enhances the bio-efficacy against different insect pests.

Neem oil microemulsion by using biodiesel waste as co-solvent has been developed as an effective delivery system against different pests. These microemulsions are eco-friendly, economical, and safe for non-targets. The developed microemulsions highly stabilized and efficient at a low dose [27].

Lemongrass oil has been proved a right stabilizer as well as a dispersant in the neem oil microemulsion system. The primary specialty of this formulation is that these Neem ME formulations are free from any co-solvents. In the presence of lemongrass stability of active ingredient, i.e., azadirachtin also increased, and HPLC data shows a very less degradation after 14 days of storage at 54°C [28].

S.No.	Essential oil	Active constituents	Mode of action	Insect pest	References
1.	Cinnamon oil	Cinnamaldehyde Cinnamyl Acetate Caryophyllene Linalool Eugenol Cinnamaldehyde (3-phenyl-2- propanal)	Fungicide	Gray mold of pears	[24]
2.	Castor Oil	Ricinoleicacid	Herbicide	Convolvulus arvensis	[23]
	Camphor Oil	Pinene Camphene Limonene 1,8-Cineole p-Cymene	Herbicide	Convolvulus arvensis	[23]
	Peppermint Oil	Menthol Menthone 1,8-Cineole Menthyl acetate Isovalerate Pinene Limonene	Herbicide	Convolvulus arvensis	[23]
	Jojoba Oil	Gondoic acid	Herbicide	Convolvulus arvensis	[23]
3	Peppermint Oil	Menthol Menthone 1,8-Cineole Menthyl acetate Isovalerate Pinene Limonene	Insecticide	Sitophilus oryzae	[29]
4.	Thyme oil	Borneol Carvacrol Linalool Thymol Tannin Saponins Triterpenic Acids	Fungicide	Geotrichum citri (citrus sour rot)	[30]
5.	Eucalyptus oil	Eucalyptol or 1,8- cineol	Insecticide	Sitophilus oryzae (L.) and Tribolium castaneum (Herbst)	[31]
6.	Neem oil	Azadirachtin	Acaricides	Tetranychus urticae	[32]
7.	Neem oil + lemon grass oil	Myrcene Citral Citronellal Geranyl Acetate Nerol Geraniol Limonene	Insecticides	_	[28]
8.	Natural pyrethrin	Pyrethrin I Pyrethrin II Cinerin I Cinerin II Jasmolin I Jasmolin II	Insecticides	Aphis gossypii	[33]

S.No.	Essential oil	Active constituents	Mode of action	Insect pest	References
9.	Betel leaf (<i>Piper betle</i> L.) essential oil	Cadinene Sesquiterpene Chavicol Geraniol A-Thujene Terpinolene Chavibetol Phenyl Propane Trans B-Ocimene Safrole Caryophyllene Cineole Cadinol Eugenol Camphene Limonene Pinene Eugenyl Acetate	Fungicide	Aspergillus species	[34]
10.	Chlorpyrifos + jatropha and karanj oil	Karanjin Pongamol Oleic Acid	Insecticides	Stored Grain Pest	[35]
11.	Clove and lemongrass oil	Myrcene Citral Citronellal acetyl eugenol beta-caryophyllene vanillin crategolic acid tannins Flavinoids	Fungicide	Fusarium oxysporum	[36]
12.	Eucalyptus oil	Eucalyptol or 1,8-cineol	Insecticides	Tribolium castaneum	[37]

Table 1.Literature survey on essential oil microemulsion for crop protection.

Natural oil microemulsion along with botanical synergist (*Prosopis juliflora*) have been proved the stability of active constituents in ME formulation. The HPLC data showed that botanical synergist lowers the degradation rate of active components of natural oil. Further, the bioefficacy results showed a prominent biocontrol against Spodopteralitura at 400 PPM [38].

Clove (CO) and lemongrass oil (LGO) ME showed potential antifungal agents against *Fusarium oxysporum* f.sp. *lycopersici* (FOL) without showing any sign of phytotoxicity in tomato plants [36].

Eukalyptus (*Eucalyptus globules*) oil ME was developed along with Karanja (Pongamiaglabra) and jatropha cakes (*Jatropha curcas*) to enhance the bioefficacy against Triboliumcastaneum, a stored grain pest. The mortality data shows that Eucalyptus oil ME with Karanja and jatropha cakes extract gives LC50 at 50 ppm, and without extract, it LC50 at 100 PPM. The GC-ms data shows that degradation % of a marker compound, i.e., 1, 8-cineole, also reduced in filtrate based ME. So, this study discovered that essential oil stability and bioefficacy could be improved in microemulsion by using these types of botanical extracts [37].

Previous studies revealed that essential oil microemulsion formulation with optimum surfactants and botanical synergists or stabilizers could improve the

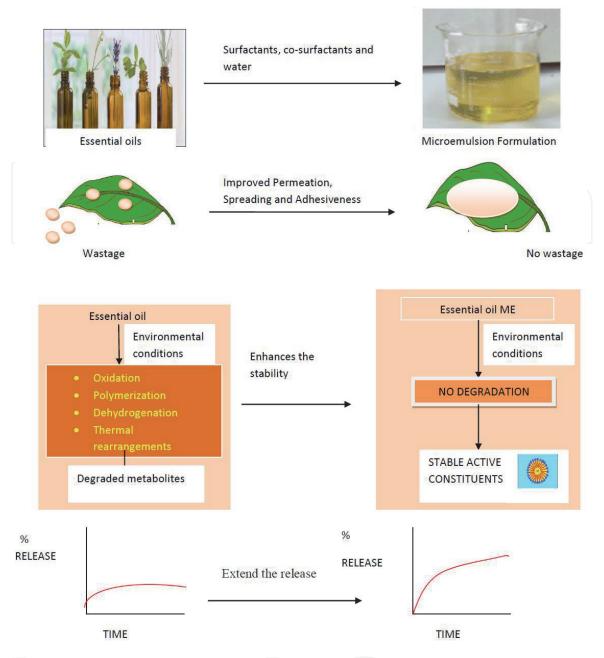


Figure 6.Advanced features of essential oil ME formulations over essential oil.

stability as well as bioactivity against different crop pests. Therefore essential oil ME could be the safest mode of a delivery system shortly. Advanced features of essential oil microemulsion are depicted in **Figure 6**.

6.4 Scope of work in promoting essential oil microemulsion in crop protection

Essential oil based microemulsion formulation is a promising tool for the biocontrol of the pests of economic importance. However, there are still some areas of improvement that should be focused upon in order to promote oil based microemulsion system in the agriculture sector.

1. The need for combinatory botanicals or synergists: Essential oil ME in pure form requires a higher rate of application for controlling pest species, which will increase the cost of formulation and limits its usage. So, there is an urgent need for discovering new combinatory or synergistic constituents, which will be very helpful in promoting essential oil ME.

- 2. Extraction and refinement of active components: There are many active components present in the essential oils, and each gives some specific property. So, many research studies are required to extract, isolate, and identify these constituents. After refinement of most active components, Microemulsion formulation will be straightforward and give much fold of efficacy against various insect pests in an economical way.
- 3. **Regulatory approval:** The developed essential oil ME with high efficiency shouldbe approved by regulatory systems so that the commercialization will be easy and successfully used by the farmers.
- 4. **Availability of raw material:** Essential oil plant or tree sources should be cultivated so that enough amount of raw material is available to scale up the essential oil ME formulation.
- 5. **Needs awareness:** As we know, people are very used to synthetic pesticides and less aware of these types of formulations due to lack of knowledge about these formulations.

All these advanced formulations are only up-to the lab level. So, a proper system of endorsement and awareness is required for the promotion of essential oil ME formulation.

7. Conclusion

Microemulsion formulation is the most suitable thermodynamically stable formulation for essential oils. Essential oils give maximum stability in microemulsions with optimized surfactants and co-surfactants. Various essential oil microemulsions have been formulated and found very useful for controlling multiple agricultural pests. Many studies revealed that essential oil stability and bio-efficacy could be further improved by using eco-friendly stabilizers and botanical synergists. Essential oil microemulsion could be a better alternative to synthetic pesticides and opens a new corridor for the promotion of the greener way of plant protection in India and across the globe.

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Conflict of interest

The authors declare no conflict of interest.

Appendix and nomenclature

O/W oil in water w/o water in oil

 Υ_i interfacial tension

 ΔG_f free energy of formation

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 η_r relative viscosity

HPLC high pressure liquid chromatography

GC-MS gas chromatography-mass spectrophotometer LC-MS liquid chromatography-mass spectrophotometer



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