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PEG supported nano formulations of essential oils for agricultural applications.

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Abstract

Imprudent and excessive application of agrochemicals is now a matter of serious concern. The situation has stimulated the researchers to work on viable and nature friendly alternatives. The Polyethylene glycol (PEG) supported nanoparticles has found prevailing applications in various fields including drug delivery systems, nanomedical imaging and nanomedical therapy. Controlled and targeted release of encapsulated material is one of the major advantages of such nanosystems. Many such formulations are being developed for use in food and agricultural applications as they are safe, nontoxic, biocompatible, ecofriendly etc. In this work a comprehensive discussion on essential oil loaded PEG nanoparticles is presented. Different systems, their loading capacity and effective mortality are compared.

Introduction

Essential oils have been traditionally used for various applications including medicine, food and agriculture. However, their efficient and controlled application, particularly in the field of agriculture has always been a challenging task, due to poor water solubility, high volatility and decomposition under different environmental conditions. Synthetic agrochemicals although, highly detrimental in comparison to EOs but were excessively utilized to fulfill increasing demand of crop production and pest control. It has imposed serious threat to environment and human health. Uses of synthetic pesticides have also developed insecticide resistance. To mitigate the problem development of new technology and sustainable agricultural practices is needed.

Despite these challenges the potential solution can be obtained by the application of nanotechnology. In recent years, many nanoformulations have been developed for food and agricultural applications. They are basically nanosized organic or inorganic structures supporting various active ingredients as nanocapsules or nanoemulsions. Stadler et al. recently reported application of nano-alumina against *R. dominica* and *Sitophilus oryzae L.* (rice weevil) (Copeloptera: Curculionidae). Goswami and Debnath prepared various Silica, aluminum oxide, zinc oxide and titanium dioxide NPs and silver NPs using an aqueous leaves extracts. These nanomaterials were successfully applied against *S. oryzae*.
Especially, the organic polymer like polyethylene glycol, pectin, chitosan etc. based nano formulations encapsulating botanical insecticides were extensively explored. PEG is a water soluble, synthetic polymer

where oxygen molecules are bridged by ethylene unit. Biocompatibility and commercial production are major advantages associated with PEG. Commercially PEG is available in a wide range of molecular weights. PEG 200-600 exist as liquid, PEG 1500 semisolid while PEG 3000-20000 as semicrystalline solid.

The focus of this review is to cover recently reported PEG supported nano-formulations loaded with essential oils for agricultural applications. Further, the preparation, properties and its correlation with loading efficiency is discussed in detail.

Essential oils sources and their applications

Essential oils are volatile secondary metabolites produced by plants. It is assumed that during the course of evolution these secondary metabolites have been developed by plants in order to protect themselves against pathogens. **Table 1** summarizes some essential oil and their potential applications. Different studies have shown that essential oils possess antibacterial and antifungal properties.

Target organism	Diseases caused by pathogen	Essential Oil	Property	Reference
Alternaria solani	Early blight	*Angelica archangelica*	Antifungal	Fraternale *et al.*
Aspergillus carbonarius	Ochratoxin producer	*Citrus x limon* L.	Antifungal	Dimi´c, G *et al.*
Bipolaris oryzae	Brown spot	*Piper sarmentosum*	Antifungal	Irshad, M. *et al.*
Phytophthora megakarya	Black pod disease	*Syzygium aromaticum* *Zanthoxylum xanthoxyloides*	Antifungal	Nana, W.L. *et al.*
Fusarium sulphureum	Dry rot	*Zanthoxylum bungeanum*	Antifungal	Xing-dong, L. *et al.*
Fusarium verticillioides	Ear rot on maize	*Curcuma longa*	Antifungal	Brado Avanço, G. *et al.*
Geotrichum citri-aurantii	Sour rot (post-harvest)	*Thymus* spp.	Antifungal	Boubaker, H. *et al.*
Lasiodiplodia theobromae	Rot and dieback (forest species)	*Myrcia lundiana*	Antifungal	Alves,M.F. *et al.*

Rhodococcus fascians	Leafy gall disease	*Ocimum ciliatum*	Antibacterial	Moghaddam, M. *et al.*
Clavibacter michiganensis	Ring rot disease	*Achillea biebersteinii* *Achillea millefolium*	Antibacterial	Kotan, R. *et al.*
Xanthomonas campestris	Black rot and leaf spot	*Zataria multiflflora*	Antibacterial	Amini, L. *et al.*
Pseudomonas aeruginosa	Soft rot	*Vetiveria zizanioides*	Antibacterial	Atif, M. *et al.*
Pseudomonas syringae *Pseudomonas spp.*	Bacterial canker	*Thymus vulgaris*	Antibacterial	Oliva, M.D.L.M. *et al.*

Table 1. Essential oils and their properties

Preparation of nano formulation:

Usually the melt dispersion method is utilized for the preparation of EO loaded nanoparticles. The method involves melting of PEG 6000 on hot plate followed by addition of essential oils in portion with high stirring of 15000 rpm for the duration of 10 to 30 minutes. Further, cooling of mixture at temperature ranging from -4 °C to 25 °C for 45 minutes to 12 h is reported. On melting the PEG molecules orient them randomly. Cooling initiates the nucleation which leads to the formation of amorphous or crystalline particles. When the faster cooling is carried out in presence of foreign material it leads to the formation of amorphous solid which is good in encapsulation property and storage ability. The grounding of mixture in a refrigerated mortar yields fine powder of different particle size. The powder thus obtained on passing thorough sieve (230 mesh) yields desired nanoparticles.

Properties of nanoparticles:

The concept of loading essential oil over PEG nanoparticle is based on ability of polymer matrix to encapsulate the material. Another important criterion for the selection of support is the chemical stability of encapsulated material under polymer matrix. Generally, good encapsulation ability and stability is reported with PEG. The loading efficiency can be estimated by preparing the solutions of different concentrations following serial dilution method. The absorbance was determined using UV spectrophotometer. The standard curve of concentration versus absorbance gives a comparative picture of loading efficiency. Yang *et al* in their work observed the oil loading efficiency of 80% at 10% optimal ratio of garlic oil to PEG. Nanoparticles have shown unimodal size distribution with no variation in mean size and size distribution.

Properties and activity of some nanoformulations are listed in Table 2. A relationship between nanoparticle size, PDI and degree of encapsulation has been observed in various reports (Zuidam and Shimoni, 2010). Peppermint loaded nanoparticles of high PDI values have irregular shapes and wide range of sizes. This has led to low encapsulation and quick release of essential oils. The NPs were of the size 380 nm and loading efficiency 89.7%. However, Palmarosa essential oil encapsulating nanoparticles have low PDI values. They have shown higher levels of encapsulation and slower oil release rate as well. The nanoformulations with low PDI show a more controlled release of essential oils from the polymeric matrix. In case of Palmarosa essential oil loaded NPs all the components were maintained even after 7 day of formulation. Da Rosa *et al.* reported that the difference in properties of nanoparticles may be attributed to the varying chemical behavior of essential oils.

Essential oil	**Size of NP**	**PDI**	**Loading Efficiency**	**Pest species/Insect**	**Ref**
Clove oil	179±1.69	0.24	77	*Tribolium castaneum*	Ikawati *et al.*
Palmarosa oil	191±5	0.232±0.015	89.75±2.5	*Plodia interpunctella*	Werdin-González *et al.*
Geranium	259±12	0.228±0.007	90.5±2.32	*Plodia interpunctella*	Werdin-González *et al.*
	234	0.253±0.027	83±3	*R. dominica T castaneum*	Werdin-González *et al.*
Peppermint	281±29	0.532±0.013	72.25±1.6	*Plodia interpunctella*	Werdin-González *et al.*
	331±12.84	0.547±0.015	85		Kumar *et al.*
Bergamot	184±18	0.279±0.037	78±3	*R. dominica T castaneum*	Werdin-González *et al.*
Garlic oil	233±10.8		80.46±4.94	*T castaneum*	Yang *et al*.
Lemon	240±2.51	0.34	96	*Tuta absoluta*	Campolo *et al.*
Mandarin	212.05±0.04	0.26	92	*Tuta absoluta*	Campolo *et al*.
Sweet orange	216.6±0.63	0.23	88	*Tuta absoluta*	Campolo *et al*.

Table 2. Average size, polydispersity index (PDI), loading efficiency and Pest species of the various nanoformulations.

Werdin-González *et al*. reported 83% and 78% loading of Geranium and Bergamot essential oils respectively at 10 % optimal ratio. At this ratio best results were obtained with respect to size, loading efficiency and low PDI. Spectropotometric determination of loading efficiency for lemon, mandarin and sweet orange EO-NPs was found 96%, 92% and 88%(w/w), respectively.

Nanoparticles of Mentha piperita essential oil at varying oil doses (5-10%, w/v) shows the encapsulation efficiency of 78.2-83.4%. The PDI of nanoparticles varies between 0.547 and 1.000. The average particle size of the nanoparticles synthesized was found between 226 and 331 nm, Ikawati *et al* studied the insecticidal activity of clove oil encapsulated NPs over red flour beetle (*Tribolium castaneum*). The NPS obtained are of irregular shape but good dispersion. The encapsulation efficiency of these nanoparticles is found to be 77%.

Chemical compositions of nanomaterials of different life time were also examined to understand environmental stability of encapsulated essential oils. Yang *et al* reported no significant change in composition between pre-encapsulated and post-encapsulated nanoparticles. Even over zero to five months no change in composition was observed except the variation in diallyl sulfide, diallyl trisulfide and dialyl bisulfide concentrations. Werdin-González *et al.* also reported no significant change in loading over 6 months in essential oil composition for Geranium and Bergamot. Ikawati *et al* observed the contact toxicity for 16 weeks. Even after 16 weeks of storage, more than 70% and 90% mortality at 15.2% concentration was observed. Surprisingly, for all concentration level there is decrease in residual contact toxicity of clove oil NPs in comparison to free clove oil for one weeks of storage. Yang *et al* reported the insecticidal activity of Garlic EO loaded NPs against adult *T castaneum*. The greater decrease in efficiency was observed with nanoparticles in comparison to free essential oil.

Nanoparticle Bioassay

A remarkable residual toxicity of the EO was reported by Werdin-González et al. gainst *T. castaneum* and *R. dominica*. The contact toxicity in case of nanoparticles loaded with geranium and bergamot last for 16 and 12 weeks while the EOs alone just for 4 and 2 weeks, respectively. When bergamot NPs were applied the mortality for *R. dominica* was observed for 20 weeks however, The geranium NP has shown the activity during all the experiments. In another study same group reported the NPs of peppermint, palmarosa, geranium, lavender and rosemary against the German cockroach. Out of these the results of NPs of germanica and palmarosa were most promising. The *menthe piperita* loaded nanoparticles when utilized against housefly larvae have shown considerable mortality under laboratory conditions. While under field the impact was less which further decreased to 93% and 57% after first and sixth week, respectively. Campolo *et al* utilized different citrus oil based nanoparticles against invasive tomato pest *tuta absoluta.* The formulation is found to less effective for eggs than larvae and on treatment less that 50 percent of egg population was affected even at maximum application rate.

.**Conclusions:**

Essential oils have been extensively studied for their pesticide activity. However, *in situ* studies are still scarce due to limitations related to physical properties of essential oils. Recent investigations based on nanotechnology have shown promising results, especially with PEG. It can be established that essential oils loaded on PEG support have better biocompatibility, high loading capacity, high encapsulation efficiency, better environmental safety, low PDI and good mortality against pests over other organic polymer networks. The results are promising enough and have potential to be developed as successful technology.

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