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Insecticidal activity of eucalyptus oil nanoemulsion with karanja and jatropha aqueous filtrates

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ABSTRACT

The aim of this study was to utilize the aqueous filtrate of de-oiled karanja (*Pongamia glabra*) and jatropha (*Jatropha curcas*) cakes left after extracting oil for preparing biodiesel to enhance the activity of eucalyptus oil (*Eucalyptus globulus*) as a pesticide by making a nanoemulsion for the control of *Tribolium castaneum*, a secondary pest of stored grains. The aqueous filtrate obtained from the solid cake possessing insecticidal properties was used in place of distilled water to enhance the shelf life of eucalyptus oil for a longer period of time. The eucalyptus oil nanoemulsion containing karanja and jatropha aqueous filtrate with concentrations of 300 and 1500 ppm gave 88–100% mortality rates against *T. castaneum* adults within 24 h. The LC_{50} values recorded for the nanoemulsions with and without the aqueous filtrate were 0.1646 mg/l and 5.4872 mg l^{-1}, indicating greater toxicity for the nanoemulsion containing aqueous filtrate. The GC–MS analysis also indicated the degradation of the marker compound, i.e., 1, 8-cineole, in both types of nanoemulsions, i.e., with and without the aqueous filtrate. Analysis with ATR-FTIR also confirmed the presence of 1,8-cineole in the nanoemulsion containing the aqueous filtrate. The nanoemulsion formulation with the lowest average particle size, 77 nm, was chosen for extensive efficacy trials against *T. castaneum.* It was found that the loss of eucalyptus oil by volatilization was stabilised in the nanoemulsion containing the aqueous filtrate.

1. Introduction

Food grain losses due to insect infestation during storage are a serious problem, particularly in developing countries. Accumulation of high levels of insect detritus may result in infestation of grain, making it unfit for human consumption, and in loss of food commodities, both in terms of quality and quantity. Insect-infestation-induced changes in the storage environment may cause warm, moist “hotspots” that provide suitable conditions for fungal growth, which causes further grain loss (Rajashekar et al., 2012). It is estimated that more than 20,000 species of field and storage pests destroy approximately one-third of the world’s food production, valued annually at more than $100 billion, and among which the highest losses (43%) occur in the developing world (Jacobson, 1982; Ahmed and Grainge, 1986). Storage pests destroy approximately one third of the world’s food production in developing Asian and African countries (Talukder, 2006). In the USA and Canada, 20–26% of stored wheat was infested by stored-product pests (White et al., 1985). In India, losses caused by insects accounted for 6.5% of stored grains (Raju, 1984). Food grain production in India has reached 250 million tonnes/year in the year 2010–2012; (Pugazhvendan et al., 2012); in which nearly 25% was damaged by pests (Rajashekar et al., 2010). The global post-harvest grain losses caused by insect damage and other bio-agents range from 10 to 40% (Papachristos and Stamopoulos, 2002). Small scale farmers may lose as much as 80% of their stock due to insects after storing for 6–8 months (Kitch et al., 1992; Nukenine et al., 2002). In the northern provinces of Cameroon, where cereals play an essential role as human food, the maize weevil *Sitophilus zeamais Motschulsky* (Curculionidae) is the main pest in granaries (Ngamo Tinkeu et al., 2004).

Integrated pest management has now come up as a widely accepted strategy in pest control; it includes post-harvest

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infestation control, which involves the use of chemical (contact/residual) insecticides along with fumigants (Shaaya et al., 1997). Although synthetic insecticides are commonly used to reduce the losses caused by insects, there is global concern about their negative effects such as environmental pollution, pest resistance and pesticide residues in food (Ogendo et al., 2008). The continuous application and excessive reliance on chemical pesticides have also resulted in toxicity hazards for non-target organisms and users (Isman, 2006). The concept of "biopesticides" refers to products obtained from natural sources such as animals, plants, and microorganisms, including "natural ingredient pesticides," "microorganism pesticides," and "biochemical pesticides" that can help reduce pest populations and increase food production (Koul and Walia, 2009). In recent years, studies have been focussed on the use of plant essential oils and their bioactive chemical constituents as possible alternatives to synthetic insecticides (Rajendran and Sriranjini, 2008). Eucalyptus oil, with its wide range of desirable properties, can be used as a biopesticide for the control of various insect pests (Koul et al., 2008).

Eucalyptus oil has been placed under the GRAS (generally regarded as safe) category by The U.S. Food and Drug Administration and classified as nontoxic (USEPA, 1993) (Mahboubi et al., 2013). Even the Council of Europe has approved use of eucalyptus oil as a flavoring agent in foods (>5 mg kg^{-1}), candies, and confectionary items (<15 mg kg^{-1}) (Council of Europe, 1992). Vilela et al. (2009) also studied the effect of eucalyptus essential oil by contact and head space volatile assay on food-spoiling fungi. The antimicrobial effect of *Eucalyptus globulus* oil against bacteria (gram-negative, gram-positive), fungi, and yeast in liquid as well as in the vapour phase was studied by Tyagi and Malik (2011).

The values of the oral and acute LD_{50} of eucalyptus oil and 1,8-cineole for rats is 4440 mg kg^{-1} body weight (BW) and 2840 mg kg^{-1} (BW), respectively (Regnault-Roger, 1997), rendering it much less toxic than pyrethrins (with LD_{50} values of 350–500 mg kg^{-1} BW [USEPA, 1993]) and even technical-grade pyrethrum (LD_{50} value − 1500 mg kg^{-1} BW [Batish et al., 2008]).

The preparation of a submicron emulsion, called a nanoemulsion or miniemulsion, has emerged as a promising alternative for both intravenous and dermal application. Nanoemulsions are fine oil-in-water dispersions having a droplet size ranging from 100 to 600 nm (Solans et al., 2003). Nanoemulsions are not only kinetically stable but are also physically stable for a long time, with no apparent flocculation or coalescence (Bouchernal et al., 2004). In addition to specific shelf life or stability depending on the individual system or mixture of ingredients (active and inert), low viscosity and droplet transparency make them an attractive system for many industrial applications, such as drug delivery systems in the pharmaceutical field; they can also be used in cosmetics and in pesticide delivery systems (Sonneville-Aubrun et al., 2004).

An emulsion is a thermodynamically unstable two-phase system consisting of at least two immiscible liquids, one of which is dispersed in the form of small droplets throughout the other, and an emulsifying agent. The dispersed liquid is known as the internal or discontinuous phase, whereas the dispersion medium is known as the external or continuous phase. If the aqueous phase constitutes more than 45% of the total weight with a hydrophilic emulsifier, oil/water emulsions are formed (Wang et al., 2007). Water/oil emulsions are generally formed if the aqueous phase constitutes less than 45% of the total weight and a lipophilic emulsifier is used (Abren and Massimo, 2008).

Nanoemulsions are metastable systems, and their stability depends upon the method of synthesis or preparation (Gutiérrez et al., 2008). The most common approach is high-energy emulsification, which involves high-shear stirring, high-pressure homogenizers, and ultrasound generators. Recently, a new low-energy emulsification method has been developed by taking advantages of phase behaviour and properties to promote the formation of ultra-small droplets with constant vigorous stirring, a "top-down" process (Ostertag et al., 2012). These low-energy techniques include self-emulsification, phase transition, and phase inversion temperature (Izquierdo et al., 2005). In contrast, solid nanoparticles are usually produced in a "bottom-up" process. A recently developed process for generating nanometer-scale oil droplets in water has been reported in the journal *Angewandte Chemie* by Japanese researchers, who have developed a technique they named MAGIQ (monodisperse nanodroplet generation in quenched hydrothermal solution). This technique requires generally high temperature and pressure for the preparation of nanoemulsions (Deguchi and Ifuku, 2013). In our nanoemulsion system, this technique is inapplicable because of the highly volatile nature of the eucalyptus oil.

De-oiled seed cake of jatropha showed molluscicidal, insecticidal, fungicidal, and nematicidal activity. De-oiled seed cake of karanja also possesses insecticidal and nematicidal activity (Kesari et al., 2010; Sharma et al., 2011). Neem and karanja oil cakes in combination also showed insecticidal properties against mosquito vectors (Shanmugasundaram et al., 2008). Aqueous extract of karanja and jatropha seed cakes had a significant effect on the termite *Odontotermes obesus* (Sharma et al., 2011). But there is no relevant information regarding the use of these aqueous extracts to formulate an essential oil to increase their shelf life and effectiveness.

The LC_{50} value is the concentration of a pesticide that is lethal to 50% of a population of test animals and is usually determined for a specific exposure period. A pesticide with a lower LC_{50} is more toxic than a pesticide with a higher number because it takes less of the pesticide to kill half of the test animals (Mikhaiel, 2011).

This paper focuses on enhancing the bioefficacy of the highly volatile essential oil for a longer period of time by decreasing its volatility and also increasing its effectiveness using karanja and jatropha aqueous filtrate in the eucalyptus oil nanoemulsion formulation.

2. Materials and methods

Eucalyptus globulus essential oil was purchased from Gogia Chemicals, Delhi, India. Karanja and jatropha cakes were collected from the Centre for Rural Development Technology, I.I.T, Delhi; the butanol was from Merck; and Suprol T-60 (commercial Polysorbate-80 or Tween-80) was supplied by Supreme Surfactant Limited, Sonipat, Haryana, India.

2.1. Test insect

Chosen as the test insect was the red flour beetle, *Tribolium castaneum;* it is a worldwide pest of stored products, particularly food grains, and a model organism for ethological research. The source of the insect was infested flour collected from the Department of Entomology, Indian Agricultural Research Institute, Delhi. A pure line culture of *T. castaneum* was maintained at 30–34 °C and 70% RH. The paper transfer method was used to transfer the beetles from the existing stock to the fresh jar. A clean paper strip was put in the glass jar containing beetles along with the flour. It was inserted 1/3–1/4th of its length; adult beetles were collected from the lower side of the strip. The strip was then withdrawn carefully and inserted into a jar of fresh flour. The strip was discarded and a new one was used to repeat the subculture. The method selects the healthiest and most vigorous beetles and prevents any contamination.