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Phytochemical-Based Nano Emulsions for Stored Grain Protection

Moshe Kostyukovsky*, Elazar Quinn, Gilad Golden, Aviv Rapaport, Eli Shaaya, Elena Poverenov

ARO, the Volcani Center, HaMaccabim Road 68, POB 15159, Rishon-LeZion 7528809, Israel
*Corresponding author: inspect@volcani.agri.gov.il
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Abstract

Stored grain losses caused by pest insects contribute significantly to the global food crisis. Currently, there are two main chemical control methods against stored product insect pests: fumigation with very toxic gases and grain protection by residual contact insecticides. Today, the global tendency is to prevent/reduce the wide use of insecticides, which have high toxicity to humans and harm the environment. Therefore, there is an urgent need to develop alternative eco-friendly approaches for stored insect pest control.

Essential oils from *Micromeria fruticosa* and *Mentha rotundifolia* (Fam. Labiatae) and their main constituent pulegone which previously were shown by us as very active against stored product insect pests, were encapsulated into coarse and nano emulsions. The insecticidal activity of the developed formulations against primary internal insect rice weevil (*Sitophilus oryzae* L.) and secondary external pest red flour beetle (*Tribolium castaneum* Herbst) was evaluated in laboratory and pilot experiments.

It was found that the phytochemical-based nano emulsions offered significant advantages and provided powerful and prolonged biological activity compare with the coarse formulations. The developed nano emulsions could serve as a natural, effective, low-toxify for human, and environmentally preferred method for protection stored grain and dry food products from pest insects.

Keywords: essential oils**,** nano emulsions, pulegone, stored product insects, stored product protection.

Introduction

Insect damage in stored grain contributes significantly to the global food crisis (Philips and Throne, 2010, Nopsa et al, 2015). Today, the use of fumigants and protectants are common chemical control methods for stored product protection against pest insects. In spite of their high efficacy, both these methods have well known disadvantages (Kostyukovsky and Shaaya, 2012, Opit et al, 2012, Nayak et al, 2013, Daglish et al, 2014). The use of plant essential oils (EOs) and their constituents may be one of alternative eco-friendly approaches for stored insect pest control (Shaaya et al., 1991, 1993, Shaaya and Kostyukovsky, 2006, Kostyukovsky and Shaaya, 2011, 2012). However, for the implementation of the essential oils, suitable formulations are needed.

Encapsulation of essential oils allow even distribution of the active agents, slow evaporation of volatile compounds, and avoid the undesired odors (Onvulata et al, 2012, Majeed et al, 2015). Oil-in-water emulsions are utilized to introduce the water insoluble ingredients into aqueous solutions. In addition to the coarse emulsions, nanoemulsions can be prepared utilizing high or low energy methods. Due to a higher ratio of droplet surface area per mass unit, nano emulsions typically have increased kinetic stability and higher encapsulation capacity (Silva et al., 2012, Salvia-Trujillo et al., 2015).

Essential oils extracted from the *Micromeria fruticose* and *Mentha rotundifolia* (Labiatae) plants content 70-98% of monoterpenes (pulegone and SEM respectively), which were found to possess excellent insecticidal activity, include against stored product insect pests (Shaaya et al., 1993, Franzios et al., 1997, Kostyukovsky and Shaaya, 2011). However, their effective use is limited by the absence of suitable formulations.

In this research, we aimed to develop an appropriate formulation for effective delivery of EOs, the nature-sourced pest-managing agents for the grain storage. For this purpose, EOs were encapsulated into coarse and nano emulsions. The prepared emulsions where characterized by spectroscopic and microscopic methods and their stability and release ability at various environmental conditions were examined. The insecticidal activity against *S. oryzae*, the primary insect pest, and *T. castaneum*, the secondary insect pest, was studied under the laboratory and pilot conditions.

Materials and Methods

Oil-in-water emulsions were formed by stirring pulegone/SEM with sunflower oil in 1:1 ratio at various concentrations with Tween 80 (1.0% v/v) and double distilled water at 1000 rpm for 30 min. Microemulsions were homogenized for 3 min using Power Control Unit homogenizer (Kinematica, Switzerland). Nano emulsions were prepared by ultrasonicating the coarse emulsion with Vibracell ultrasonicator (Sonics&materials, Inc. Newtown, CT, USA) at 70% intensity for 30 min. The average droplet size and polydispercity index (PDI) were measured by dynamic light scattering (DLS) on a Zetasizer Nano ZS laser diffractometer (Malvern Instruments Ltd, Worcestershire, UK) working at 633 nm at 25°C and equipped with a backscatter detector (173°), which is appropriate for measuring submicron particles (Brar and Verma, 2011). Emulsions with pulegone concentrations of 1, 5, 10% and an emulsifier concentration of 1%, were placed at room temperature and checked over the period of a month to examine changes in particle size (published by Golden et al., 2018). The reported values represent an average of three measurements and standard deviation.

The adults of coleopterans *S. oryzae* and *T. castaneum* served as the test insects. The insects were reared in the Volcani Center, Department of Food Quality and Safety under the controlled climatic conditions: the air temperature 28 ± 1°C, the air relative humidity 65 ± 5% in prolonged darkness. After the laboratory treatments, the tested insects were maintained in an incubator under the same controlled conditions. The cultures of the tested insects have been reared for many years without any contact with insecticides. For rearing of *S. oryzae*, the whole wheat grains of 12% moisture content were used. *T. castaneum* was reared on the wheat flour (egg laying) and on the ground wheat grain.

The laboratory experiments were conducted in the incubator at the temperature of 28°C. The glass chambers of 600 ml volume were filled with 100 g of wheat grains with the moisture content of 12%. To each chamber, 200 µl emulsions containing pulegone/SEM at concentrations of 0.1, 1, 5, 7.5, 10 and 20% were added (2-400 ppm on the wheat kernels). Coarse and nano emulsions were examined. The chambers were closed and the grains were mixed for a few minutes. Ten unsexed adults of each test insect 10-15 days old were introduced into the chambers and the chambers were closed hermetically. The control grains were treated with an emulsion without pulegone/SEM. The insect mortality was checked weekly. The grains were sieved by the 10-mesh sieve and were re-infested with the new adults of the same species. Before grain sieving, the concentration of carbon dioxide (CO_2) was measured by Oxybaby instrument (Witt-gasetechnik-Germany). The experiments were conducted in three replicates and were continued until the loss of the emulsions efficacy.

The pilot experiments were conducted in the bins of 60 l volume with 45 kg of the treated and untreated wheat grain. The tested insects were placed in cages with holes in three depths locations. The nano and coarse emulsions contained 10 or 20% pulegone/SEM were tested. The insect mortality was checked every two weeks and the grain was re-infested with the new adults of the same species.

Statistical analyses were done using the ANOVA and TUKEY student range test, which were performed with the JMP 13 software (Statistical Discovery™ from SAS, Cary, NC).

Results

The optimal content for the stable emulsions was istablished. The concentrations of SEM/pulegone of 5 and 10% v/v and Tween 80 of 1% v/v resulted in stable emulsions with insecticidal activity. The droplet size of nanoemulsions was depended on the pulegone concentration and stabilized after a week. The initial amount of the pulegone released from the nano formulation (after 4 days) was 3-3.5 times higher than that of the coarse emulsion. The nano emulsions provide significantly higher amounts of pulegone for prolonged period in comparison to coarse emulsion (0.15-0.35 mol/l vs 0.05-0.1 mol/l). After 200 days, in 10% pulegone at 32°C, coarse emulsions released all the pulegone whereas from nano emulsions the pulegone was still released (published by Golden et al., 2018).

The nano emulsions were found much more active compared with the coarse formulations. The total mortality of *S. oryzae* adults was recorded for nano emulsion of 10% pulegone for 5 weeks (exposure of a week) compared with one week in the coarse emulsion. The same tendency was observed in *T. castaneum*. Mortality of above 90% was observed for over 5 weeks, with 10% pulegone nanoemulsion compared with one week in the coarse emulsion (published by Golden et al., 2018).

In the case of 10% SEM emulsions, the high efficacy against *S. orysae* was recorded for 5 months with nano- and for 2 months with coarse emulsions, and against *T. castaneum* for only 1 week (nano).

In parallel to the re-infestation process, the levels of CO_2 were checked weekly. At the first few weeks, concentrations of CO_2 in the control reached 4-14%, elevating to 15% and more, that are lethal for the insects. In contrast, in the pulegone/SEM coarse and nano emulsions that caused insect mortality, the concentrations of CO_2 were at low levels of 0.1-4%.

In the pilot experiments, 10% pulegone emulsions were active against *S. orysae* for 3 months, against *T. castaneum* for a month with the advantage for nano emulsion. 10% SEM emulsions were active against *S. orysae* for 6 and 5 months (nano and coarse), against *T. castaneum* for two weeks.

Discussion

The release studies show the advantage of nano emulsions, and the effective prolonged release of pulegone. These properties are necessary when considering slow release and prolonged exposure of food storage insects to the pest-managing agent. In the most treatments, the quantity of the released pulegone/SEM from nanoemulsions were higher for prolonged period compared to the coarse emulsions. Nanoemulsions have a higher surface area in comparison to coarse emulsions and therefore can release a higher amount of pulegone. They are more stable than coarse emulsion (Arnon-Rips and Poverenov, 2016). After 200 days of application, the pulegone concentration in nanoemulsions was still high.

In the most experiments, pulegone/SEM nano emulsions provided prolonged efficacy against both primary and secondary grain pests *S. oryzae* and *T. castaneum* compared to coarse emulsions. The high efficacy of the emulsions was observed for longer periods against adult stage of *S. oryzae* compared with *T. castaneum*. This finding consist with our earlier experiments (Shaaya et al, 1993) in which *T. castaneum* was found the most tolerant insect to a wide range of essential oils and their constituents, including pure pulegone and SEM, compared with the other stored product insect pests.

EOs-based emulsions applied to wheat grain may serve as both fumigant and protectant. The insects inhale released from the kernels fumes, contact with the treated kernels and ingest them.

EOs have good properties for fumigation and may be applied for a wide range of insect pests (Shaaya and Kostyukovsky, 2006, Isman and Akhtar, 2007, Rajendran and Sriranjini, 2008, Kostyukovsky and Shaaya, 2011, 2012). On the other hand, Germinara et al (2017) performed topical application of EO and reported a high mortality rate of adult granary weevils due to contact activity of the EOs.

The current global tendency is to decrease the wide use of toxic chemicals in food. EOs-based nanoemulsions applied to stored wheat grain may serve as an alternative eco-friendly approuch for stored product insect control.

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Anti-termitic properties of Jatropha (Jatropha curcas L.) on wood termites (Macrotermes bellicosus (Smeathman)

Okweche Simon Idoko*, Nnah Comfort Gordon

Department of Forestry and Wildlife Resources Management, University of Calabar
*Corresponding author: idokosi@yahoo.com
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Abstract

The efficacy of *Jatropha curcas* in the management of wood termites, (*Macrotermes bellicosus*) was carried out in the Teaching and Research Farm of the Department of Forestry and Wildlife Resources Management. University of Calabar. The experiment consisted of 5 levels of *J. curcas* oil (0, 0.5, 1.0, 1.5 and 2.0) and a corresponding quantity in powder, replicated 4 times and arranged in Randomized Completely Block Design (RCBD). Each concentration was tested on 20 unsexed adult wood termite placed in grave yard of 8cm x 8cm. Data on mortality rate was taken at 12 hourly up to 72 hours. The result from the experiment showed that *J. curcas* oil was significantly efficacious compared with *J. curcas* powder both in the field and in the laboratory. It was observed that there was progressive increase in mortality rate due to increased concentration and time duration. The management of termite using *J. curcas* should be encouraged due to its environmental friendliness and should also be incorporated into integrated pest management (IPM).

Key words: Jatropha curcas, Macrotermes bellicosus, oil, powder, mortality

Introduction

Termites (*Macrotermes bellicosus)* are social insects living in colonies, they are sometimes called white ants but are not ants, because the true ants belong to the order hymenoptera, while termites belong to the order isoptera (Grimaldi and Engel, 2005). Termites occur in all temperate an d tropical countries of the world, many of which cause extensive damage to wooden structure and to manufactured goods made of wood, paper and cloth. Occasionally, they cause significant damage to growing trees such as teak and agricultural crops such as cotton (Solomon, 1995). The economic importance of a few pest species is so dramatic that the importance of termite breaking down woody tissue and returning nutrient to the soil is obscured (Truman and Robinson, 1982). Termites are responsible for some of the degradation of wood and other cellulose material in the terrestrial environment, mainly in the tropics and subtropics (Bulthman, 1979). Cellulose being the principal food of termites, wood and wood product such as paper, fabrics and wood structures are consumed and destroyed by termite, and hence a constant effort is directed towards their control. Field and laboratory test indicated that some woods are not resistant, but are susceptible to attack by African wood termite causing significant damage. Factors affecting wood consumption by termites are numerous and complexly related. Among the most important of these factors are: wood species, hardness of the wood Presence of toxic substance, feeding inhibitors or deterrents, Presence or absence of fungi or fungal decay, Moisture content of the wood and soil (Carter *et al*., 1974; Peralta *et al.,* 2004).

Termites of the *Macrotermes* spp are fungus growing termite belonging to the family rhinotermitidae, they are mostly mound builders and are the largest termite species (Osipitan and Oseyemi, 2012). The species of the termite under the genus *Macrotermes*, impact the economy negatively by causing damage to various agricultural crops, rangeland, wooden portions of buildings, furniture, books, utility poles and fences in several parts of Africa (Wong *et al.,* 2001*;* Mitchell, 2002; Cox, 2004). It has been reported that *Macrotermes* causes a complete damage of between 80 to 100 % on stored products (Michael, 2000; UNEP and FAO, 2000; Sekematte, 2001;