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Research Article

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Eco-Friendly Nano-emulsion Formulation of *Mentha piperita* Against Stored Product Pest *Sitophilus oryzae*

Magdy A Massoud[1], Manal M Adel[2*], Osman A Zaghloul[1], Magdy IE Mohamed[3] and Khaled H Abdel-Rheim[3]

[1]Department of Plant Protection, Faculty of Agriculture-Saba Basha, Alexandria University, Egypt

[2]Department of Pests and Plant Protection, National Research Center, Cairo, Egypt

[3]Department of Stored Products and Grains Pests, Plant Protection Research Institute, Agricultural Research Center, Sabahia, Alexandria, Egypt

[*]**Corresponding author:** Manal M Adel, Department of Pests and Plant Protection, National Research Center, Cairo, Egypt, Tel: 01224214021; E-mail: mhassanein11@hotmail.com

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Abstract

Nanotechnology is not just involved with small things. Nanotechnology is a multi-disciplinary science. In Egypt, the annual loss in wheat due to stored insects is estimated as equivalent to half a million tons of which 12% is caused by the rice weevil alone. The nanotechnology can be one of the rational solution especially with utilizing essential oils. The efficacy of nano-emulsion of *Mentha piperita* was formulated using ultrasonic emulsification and compared with its essential oil against Rice weevil, *Sitophilus oryzae*. The resulted nano-emulsion droplets were in size of 43.55 nm. Insecticidal activity of *M. piperita* nano-emulsion and the free *M. piperita* EO were evaluated by film residue contact toxicity. *M. piperita* nano-emulsion caused high mortality of insect adults with low concentrations compared to the bulk oil. Moreover, the mortality in *S. oryzae* was increased with increasing exposure time and concentration. The results also showed significant differences of adult emergence between *M. piperita* (EO) and *M. piperita* nano-emulsion after 6 weeks from treatment. The results concluded that *M. piperita* nano-emulsions may be used as an alternative for the control of other stored-product insect pests. They have the advantage of promising insecticidal activity and being eco-friendly and less toxic than synthetic pesticides.

Keywords: Rice weevil; *Sitophilus oryzae*; *Mentha piperita* L.; Nano formulation; EO nano-emulsion

Introduction

Stored-grain insects in developing countries cause huge losses of stored-grain products, amounting to 5-10% loss in temperate regions and 20-30% in the tropical regions [1]. In Egypt, the annual loss in wheat due to stored insects is estimated as equivalent to half a million tons of which 12% is caused by the rice weevil alone [2].

The rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) is a major primary pest, of stored grain-based products, including maize, rice and wheat, particularly in the tropic regions [3,4]. This pest can cause considerable damage to stored grains and completely destroy kernels. Moreover, the product quality is affected by presence of eggs and dead insects, and holes on the grains [5]. According to the global economic estimation, the related costs of pests to stored food stuffs could reach to about 500 million USD per year [6].

Massive applications of conventional pesticides to control these insects result in adverse effects on bene icial organisms, leaves their residues in the food leads to severe risks to human health and environment, it reduced the populations of natural enemies and developed the insect resistance to synthetic insecticides [7,8].

Recently scientists pointed to use naturally occurring insecticides. many of these compounds are secondary plant substances (allelochemicals) including alkaloids, quinones and essential oils [9]. These active substances extracted from plants are effective against wide range of insects and act as toxicants, as insect growth regulator (IGR), repellents or as phagodeterrent [10]. These properties make them suitable bioinsecticides for organic agriculture and could be an alternative to those chemical insecticides.

Owing to the fact, the most essential oils used as flavoring agents possessing insecticidal properties showed ovicidal, larvicidal, adulticidal against several insect species [11,12]. One of the most effective plants against stored pests is *Mentha piperita* L. or peppermint, a plant from the Lamiaceae family, *Mentha piperita* oil possesses diversified potential in the areas of food, cosmetics, medicines, and pest control [13]. The essential oils extracted from *M. piperita* have also been reported as a source of botanical insecticides [14].

However, the major inconvenience of the use of essential oils are their chemical instability in the presence of air, light, moisture and high temperature that can determine the rapid evaporation and degradation of some active components [15]. A method to overcome these problems is the incorporation of essential oils into a controlled-release nano-formulation which prevents rapid evaporation and degradation, enhances stability and maintains the minimum effective dosage/application [16]. In addition, this nano-formulation compared with bulk formulation is expected to be more effective, showed less toxicity towards non-target organisms and increased persistence of the active ingredient [17,18].

Regarding, the peppermint is considered a medicinal plant and its extracted essential oils have been reported as a source of botanical insecticides. Formulating the essential oils may improve its efficacy as pesticide delivery system. Moreover, the small droplet sizes of EO nano-emulsion with less organic solvents can enhance the kinetic stability and permeability as a result of low surface tension than conventional emulsifiable concentrates. So, this study aims to evaluate the efficiency of *M. piperita* (EO nano-emulsion) as possible as protecting agents of wheat grains against infestation by the rice weevil, *Sitophilus oryzae*.

Materials and Methods

Insect rearing

Cultures of the rice weevil, *Sitophilus oryzae* (L.), was maintained in (Stored Products Department, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.) over 5 years without exposure to insecticides and reared on sterilized whole wheat. Insect rearing and all experimental procedures were carried out at 26.1°C and 65.5% R.H. Adults used in studies was two weeks post-eclosion.

Materials

Tween 80, Acetone and Peppermint oil was purchased as pure oil from Department of Botany and microbiology, Faculty of Science, Alexandria University, Egypt.

Preparation of nano-emulsion

Preparation of nano-emulsion was conducted in National Research Center laboratory, the oil-in-water nano-emulsion was formulated using *M. piperita* (peppermint) essential oil, non-ionic surfactant (tween 80) and deionized water, according to Ghotbi et al. [19] and Sugumar et al. [20]. The concentration of *M. piperita* (EO) (4%, v/v) was prepared. Initially, coarse emulsion was prepared by adding water to organic phase containing oil and surfactant in ratios 1:5 (v/v) using a magnetic stirrer, which was then subjected to ultrasonic emulsification using a 20 kHz Sonicator (*BANDELIN Sonopuls*).

M. piperita nano-emulsion characterizations

Droplet size determined at central lab in National Research Center: The emulsion droplet size and size distribution was determined using particle size analyzer (Malvern-UK, 4700 model) Droplet size was analyzed using dynamic light scattering (DLS) technique [21]. Prior to all the experiments, the nano-emulsion oil formulations were diluted with water to get rid of the multiple scattering effects. The droplet size and the polydispersity index (PDI) of the formulated nano-emulsion oil were measured.

Morphology of *M. piperita* nano-emulsion: To visualize the shape and morphology of the formulated *M. piperita* nano-emulsion oil, transmission electron microscopy (TEM) at the EM Unit in the Faculty of Science, Alex. Univ. was carried out. One drop of emulsion was negatively stained with ethanol and was positioned on a copper grid. The TEM micrographs were acquired using a transmission electron microscope (JEOL JEM-1400Plus) with a tungsten source and operating at 80 kV.

Bioassay technique

Contact toxicity bioassay using thin ilm residue: The insecticidal activity of the tested essential oils against the adults of *S. oryzae* was determined by direct contact application [22,23]. A series of dilutions of *M. piperita* essential oils were prepared using acetone as a solvent. Aliquots of 1 ml of the dilutions were applied on the bottom of a glass Petri dish (9 cm diameter) to give different concentrations from the bulk of *M. piperita* and *M. piperita* nano-emulsion. A ter evaporation of the solvent for 2 min, 20 adults of tested insects were separately placed into each Petri dish. Control dishes with and without solvent were used. All treatments were replicated three times. Mortality percentages were recorded after 24, 48 and 72 h of treatment and LC_{50} values were calculated according to Finney [24]. The toxic index (TI) of the tested EO nano-emulsion or free EO was calculated by the following equation according to Yamamoto et al. [25] and Sun [26]:

The toxic index (%) (TI) was calculated (based on LC_{50} after 72 hrs.)

$$Toxicity\ index\ (TI) = \frac{LC_{50}\ of\ EO\ nanoemulsion\ or\ EO}{LC_{50}\ of\ EO}$$

The compound has TI less than 1 (TI1) have high toxicity.

Toxicity increase (%)=(TI of EO-TI of EO nano-emulsion) × 100

Contact toxicity bioassay using treatment wheat grains with free *M. piperita* (EO) and *M. piperita* (EO) nano-emulsion: The essential oils were admixed with grains according to Qi and Burkholder [22]. Wheat grains were treated with both *M. piperita* (EO) and (EO nano-emulsion) at different concentration (0.8, 1.6, 3.3, and 13.3 ml/kg). The *M. piperita* (EO) and (EO nano-emulsion) were dissolved in acetone (2 ml) then mixed manually with grains (60 gm in 0.4-Litter) glass jars and were divided into three equal replicates. Notably, it has been previously elucidated that when the solvent evaporates, the nano-emulsion retains its properties [27,28]. A ter evaporation of acetone, the treated grains were infested by newly emerged adults (10 pairs). Mortality was recorded every week for two weeks. The number of progeny was recorded a ter six weeks of infestation.

Statistical analysis

Mortality rate was estimated and corrected according to formula [29] as follows:

$$Corrected\ Mortality = \frac{Mortality\%\ of\ treated\ insects - Mortality\%\ of\ control}{(100 - Mortality\%\ of\ control)} \times 100$$

The toxicity data was analyzed using probit analysis to estimate the LC_{50} (Ldp line).

Results

M. piperita nano-emulsion characterizations

Nano-emulsion droplet size distribution and surface morphology: The nano-emulsion was obtained after sonicating coarse emulsion for 45 min Figure 1 the results showed that emulsion droplets were in the range of 43.55 nm. The average of droplets size recorded 43.55 nm with poly dispersity index (PDI) of 0.787, Figure 2 the morphology of peppermint oil *M. piperita* nano-emulsions was optically transparent or translucent appearance compared with micro-emulsion with the same formulation. The nano-emulsion was visualized using transmission electron microscopy (TEM). Figure 3 show the particles appeared round, spherical in shape, a good dispersion and narrow size distribution, when *M. piperita* (EO) used at 4% concentration.

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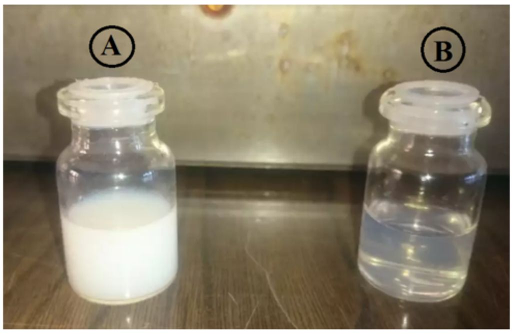

Figure 1: Nano-emulsion (O/W) of peppermint essential oil *M. piperita* (4%) obtained by (A) Before Sonication, (B) After Sonication.

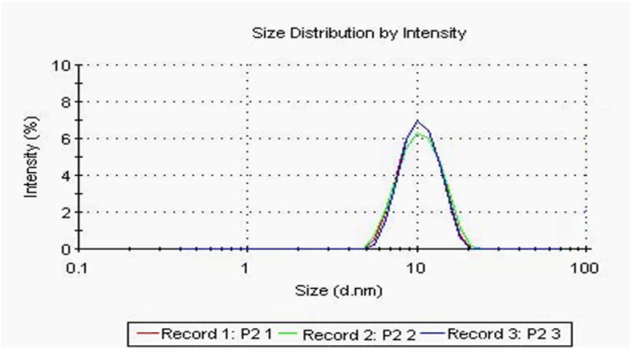

Figure 2: Density distribution diagram of *M. piperita* nano-emulsion sonicated for 45 min with oil and surfactant ratio of 1:4.

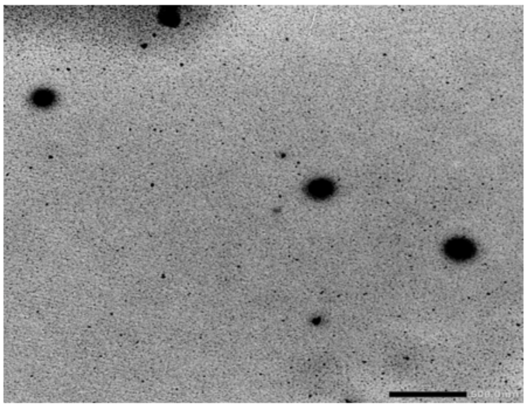

Figure 3: Round, spherical in shape, a good dispersion and narrow size distribution of peppermint oil M. piperita nano-emulsion at 4% by Transmission Electron Microscopy (TEM).

Contact toxicity bioassay using thin film residue

Insecticidal activity of *M. piperita* nano-emulsion and the free *M. piperita* EO were evaluated by film residue contact toxicity against *S. oryzae*. Results showed that both of them have toxic effect against *S. oryzae* after, 24, 48 and 72 h of treatment. The lethal concentration (LC_{50}) accounted 0.181, 0.147 and 0.136 µl/cm^2 of free *M. piperita* EO against *S. oryzae* after 24, 48 and 72 h post exposure, respectively, as show in Table 1. However, the lethal concentration (LC_{50}) of *M. piperita* nano-emulsion accounted 0.127, 48 h 0.106 and 0.095 µl/cm^2 after 24, 48 and 72 h of exposure, respectively, as show in Table 2.

Time	LC_{50} µl/cm^2	Confidence Limits		Slope	X^2	Toxicity Index (TI)
		Lower	Upper			
24 hr	0.181	0.159	0.199	3.861 ± 0.451	1.132	
48 hr	0.147	-	-	3.696 ± 0.565	12.065	
72 hr	0.136	0.109	0.155	4.063 ± 0.674	3.668	1

Table 1: Insecticidal effect of *M. piperita* on *Sitophilus oryzae* adults using thin film residue after 24, 48, and 72 hr post-exposure.

M. piperita nano-emulsion caused high mortality and toxicity with less concentration compared to the bulk oil. The toxicity index (TI) accounted for *S. oryzae* 0.698 based on LC_{50} after 72 h of exposure as shown in Table 2.

Time	LC_{50} µl/cm^2	Confidence limits		Slope	X^2	Toxicity Index (TI)	Toxicity Increase (%)
		Lower	Upper				
24 hr	0.127	0.097	0.145	5.102 ± 1.043	0.001		
48 hr	0.106	0.031	0.123	5.224 ± 1.737	0.576		
72 hr	0.095	0.023	0.123	5.576 ± 2.004	1.859	0.698	30.2

Table 2: Insecticidal effect of *M. piperita* EO Nano-emulsion on *Sitophilus oryzae* adults using thin film residue after 24, 48, and 72 h post-exposure.

However, the mortality in *S. oryzae* was increased with increasing exposure time and concentration of *M. piperita* nano-emulsion or the bulk EO in a concentration-dependent manner. The mortality in *S. oryzae* as shown in Table 2 account 68.33, 91.67 and 100% after 24 hr and 86.67, 98.33 and 100% after 48 hr and 88.33, 100 and 100% after 72 hr of exposure to nano-emulsion at concentration 10, 15 and 30 µl (Figure 4). In contrast, the free *M. piperita* EO showed nearly the same effect with high concentrations ranged from 10-30 µg as shown in Table 2. These observations demonstrated that *M. piperita* nano-emulsion in lower concentrations compared with essential oil has a considerably greater effect on the adult of *S. oryzae*. The results concluded that, the toxicity effects were significantly more pronounced for the *M. piperita* nano-emulsion formulations compared with the bulk *M. piperita* oil (Figure 5).

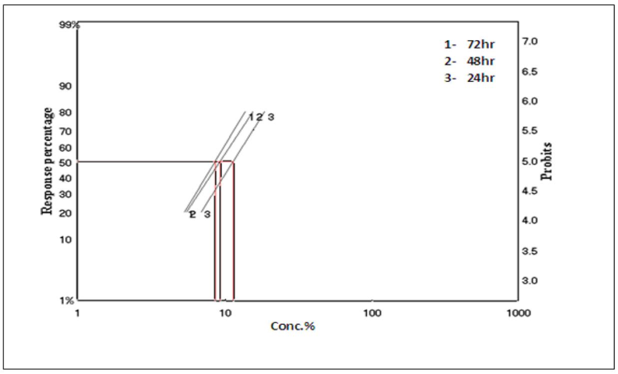

Figure 4: Efficacy of free *M. piperita* EO against *S. oryzae* adults after 24, 48 and 72 hr.

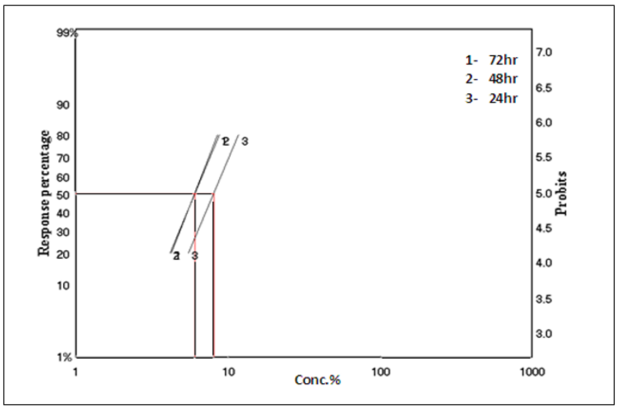

Figure 5: Efficacy of the *M. piperita* Nano-emulsion against *S. oryzae* adults after 24, 48 and 72 hr.

Contact toxicity bioassay using treatment wheat grains with *M. piperita* (EO) and (EO nano-emulsion)

Mortality of *S. oryzae* via Contact toxicity using treatment with wheat grains method, were significantly affected by the exposure time, all main effects (formulations and concentrations) and associated interactions (Table 3).

After one week of exposure, the highest mortality was recorded for *S. oryzae*, exposed to 13.3 ml/kg of *M. piperita* nano-emulsion, where all exposed adults were dead. After two weeks of exposure, 8.3 mL/kg of the tested oil nano-emulsion formulations caused 100% mortality of exposed adults (Table 3).

Among the bulk of *M. piperita* (EO) the highest mortality recorded after one week for *S. oryzae* exposed to 13.3 ml/kg and after two weeks of exposure, 1.6 ml/kg. The results also showed significant differences in the adult emergence of *S. oryzae* in *M. piperita* (EO) and *M. piperita* nano-emulsion after 6 weeks from treatment (Table 3).

Essential oil	Conc. mL/kg	Mortality (%) after			Mean of emerged adults after	
		1 week ± SD	2 weeks ± SD	Mean	6 weeks ± SD	Mean
M. piperita (EO nano-emulsion) 4%	1.6	0.0 ± 0.0	44 ± 3.13	78.58	1.67	0.84
	3.3	88 ± 0.57	98.33 ± 0.58		1.67	
	8.3	98.33 ± 0.58	100 ± 0.0		0.0	
	13.3	100 ± 0.0	100 ± 0.0		0.0	
M. piperita (EO)	0.8	82.5 ± 2.52	95 ± 1.73	94.06	2.5	0.83
	1.6	85 ± 1.73	100 ± 0.0		2.5	
	3.3	90 ± 2.64	100 ± 0.0		0.0	
	13.3	100 ± 0.0	100 ± 0.0		0.0	
Control	0.0	0.0	0.0		115	

Table 3: Effect of *M. piperita* (EO), (EO nano-emulsion) on mortality and emergence of *S. oryzae* adults.

Discussion

Few reports are available on the insecticidal activity of *M. piperita* essential, with fast and high mortality, against stored-product pests. This study showed an increase in the insecticidal effect of *M. piperita* essential oil against the insect species when formulated as a nano-emulsion. In the present study, the results are in almost agreed with the results stated by earlier investigators. The differences in chemical components may be due to variations in environmental, climatic and geographical which effect on chemical composition of *M. piperita*. Formulating essential oils (EOs) into nano-emulsion which is transparent and can be used in food and beverage products, thereby, decreasing the amount of EOs required [30]. The TEM image of *M. piperita* confirmed the results that show the spherical shape and a good dispersion of droplets nano-emulsion agreed with Sugumar et al. [20] and Ostertag et al. [31] who reported that good nano-emulsion had droplets size between 20-200 nm. The tested *M. piperita* coarse emulsion was turbid and milky white in color due to droplet size in micrometer range. After sonication, the emulsion became optically transparent. This decrease in turbidity was due to minimized droplet diameter after sonication which results in relatively weak scattering making the emulsion system optically transparent agreed with McClements [32]; Pey et al. [33]; Abouelkassem et al. [34].

The bioassay of the nano-emulsion formulations of *M. piperita* demonstrated toxicity effects on *S. oryzae*. The insecticidal effects of the formulations varied with the insect species, concentration of the formulations, exposure time and the method of application. The results of contact toxicity in thin film residue method using glass Petri dish and contact toxicity using treatment with wheat grains method, showed that increasing mortality with increasing concentration and the exposure time for the tested insect [35-38]. The possible explanation for these results is the absorption of the toxic substance increases through insect's body by increase time and concentration. The results indicated that the *S. oryzae* very sensitive when exposed to *M. piperita* (EO) and (EO nano-emulsion) [39-44]. Results agreed with the effectiveness of *M. piperita* EO with concentration 1.0% against *S.*

oryzae evaluated by Magdy et al. [45], which lead to about 96.6% mortality after 24 hr and 100% mortality after 84 hr of exposure using thin film residue method.

The insecticidal activity of *M. piperita* EO is due to the presence of menthol, menthone, methyl acetate, mentho-furan and 1,8-cineole that found as major components. Moreover, the minor compounds can play an important role in EO toxicity. Previous studies showed that insecticidal and biological activity of *M. piperita* EO could be due to the present of major constituents such as menthol, menthone, and menthofuran [46-50]. It has been reported that *M. piperita* EO, have insecticidal activity against many insects such as *S. oryzae* [51-54].

Among the nano-emulsion formulations, the highest and fastest toxic effect were observed with *M. piperita* (4%) nano-emulsion against *S. oryzae* via thin film residue method and treatment with wheat grains method, because of smaller particle size and increase biological activity due to increased surface area of emulsion droplets therefore more opportunity of the formulation to come in contact with the target insect. Whereas, the lower mortality caused by *M. piperita* EO with the biggest particle size indicates that the smaller the particle size, the greater the probability of higher efficacy.

This finding is consistent with the studies of Anjali et al. [17]; Nenaah [55]; Pant et al. [56]; Sugumar et al. [20]; Abouelkassem et al. [34]; Nenaah et al. [28]; Oliveira et al. [57,58]; Choupanian et al. [59]; Mossa et al. [60]; Choupanian et al. [61]. The study showed that *M. piperita* essential oil based nano-emulsion formulations were able to increase the mean mortality rate of *S. oryzae* compared to the free *M. piperita* EO.

Conclusion

The nano-emulsion formulations of *M. piperita* essential oil containing surfactants were successfully created via the high-energy emulsification method. The formulation provided a nano particle-size, with the smallest size being 43.55 nm. The *M. piperita* with the smallest particle size was found to be most effective in controlling *S. oryzae* adults. Overall, the present study proved that *M. piperita* nano-emulsions are effective in controlling *S. oryzae* adults. These nano-emulsions may be used as an alternative for the control of other stored-product insect pests. They have the advantage of promising insecticidal activity and being eco-friendly and less toxic than synthetic pesticides.

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