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

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the 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]. 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 using a 20 kHz Sonicator (*BANDELIN Sonopuls*).

M. piperita nano-emulsion characterizations

Droplet size determined at central lab in National Research Center: 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 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. TEM micrographs were acquired using a transmission electron microscope (JEOL JEM-1400Plus) with (‡ c Ag a ή A s

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

Insecticidal activity of *M. piperita* nano-emulsion and the free *M. piperita* EO were evaluated by .. residue contact toxicity against *S. oryzae*. Results showed that both of them have toxic against *S. oryzae* 24, 48 and 72 h of treatment. lethal concentration (LC_{50}) accounted 0.181, 0.147 and 0.136 µl/cm^2 of free *M. piperita* EO against *S. oryzae* 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 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

for the *M. piperita* nano-emulsion formulations compared with the bulk *M. piperita* oil (Figure 5).

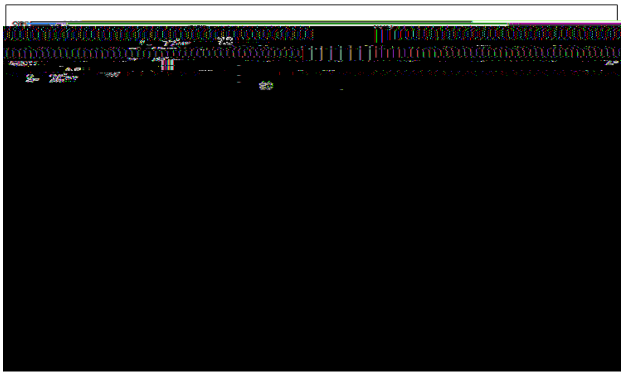

Figure 4: of free *M. piperita* EO against *S. oryzae* adults 24, 48 and 72 hr.

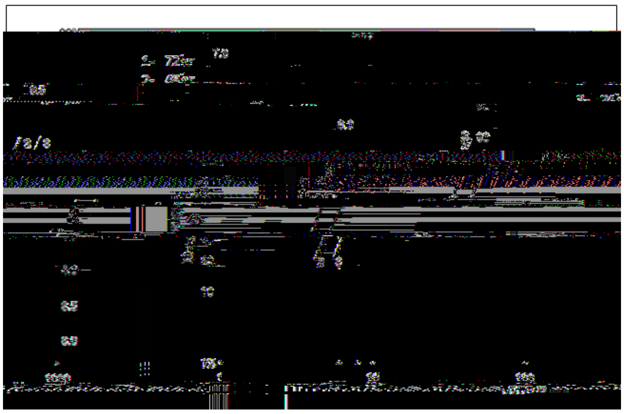

Figure 5: of the *M. piperita* Nano-emulsion against *S. oryzae* adults 24, 48 and 72 hr.

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