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Screening effects of three natural oils and their Nano against *Ephestia kuehniella*(Lepidoptera-Pyralidae)in laboratory and store

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In the current study, three essential oils (Bulk and Nano phase) were evaluated in vitro and in store for their efficacy against *E. kuehniella* larvae and moths. The extreme insecticidal efficacious oil was Purslane oil (bulk) against larvae of *E. kuehniella* with 66.64, 55.21, 45.32 and 18.61 % mortality gained at 3, 1.5, 0.5 and 0.2 % concentrations, respectively. However, the mortality values amounted to 96.64, 88.68, 78.79 and 60.61% when the larvae were exposed to 0.1, 0.5, 0.05 and 0.005% concentrations of Nano-Purslane, respectively. The least impact oil was Mustard oil against *E. kuehniella* larvae. Nano Purslane became highest significantly more efficient as sterilizer against adult moths with 1.3±3.4, 2.0±7.8 and 4.4±3.4 eggs/female at 0.1,0.05 and 0.005% concentrations, respectively comparing with other tested Nano-oils and control. The tested oils (Bulk and Nano) vapors had bioresidual efficacy against *E. kuehniella* moths. The percentage of emerged moths was extremely significantly decreased with Purslane oil in Bulk phase (10%) in comparison with untreated control (57%) during tested storage interval (120 days). While, Purslane oil in Nano phase completely suppressed the percentage of emerged moths (zero %) comparing with untreated control (66%). These results displayed that treated foam with Bulk and Nano Purslane oil and covering gunny bags provided many efficient activities, toxic, sterilizer, suppressing moth's populations and persevere for appreciable periods for protecting the stored wheat grains and could be utilized as control agents in post-harvest treatment program.

Keywords: mill moth, essential oils, Purslane, Mustard, Castor oil

INTRODUCTION

Almost all dried food products are susceptible to insect attack. Insects of stored products are main reasons of degeneration in dried products and grains (Rajendran, 2002). The Mediterranean flour moth, *Ephestia kuehniella* (family: Pyralidae), and also known as (mill moth). It is a widespread pest of stored products – especially cereals – and found around the world. The adult moths do not feed. The caterpillar larvae are the damaging stage and their feeding pollutes stored products with faeces and webbings and causing spoil of the product (Athanassiou et al. 2008). Plants played a significant role in Integrated Pest Management strategies because they possess an important source of insecticides (Golob and Webley, 1980). Essential oils are distinguished by a low toxicity to mammalian, high volatility, and toxicity to stored product insects (Regnault Roger et al. 1993). Essential oils display a broad spectrum of biological activities against insect pests especially of stored products including fumigation, toxicity, insecticidal activities,repellent , antifeeding ,ovipostion inhibitory (Abd El-Aziz and Sharaby,1997; Abd El-Aziz and Ismail ,2000; Abd El-Aziz, 2001; Sabbour and Abd El-Aziz ,2002; Ketoh et al. 2006; ; Sabbour and Abd El-Aziz ,2007 a&b ; Abd El-Aziz and Ezz El-Din,2007;

Abd El-Aziz *et al*,2007; Sabbour and Abd El-Aziz ,2010; Zapata and Smagghea,2010; Abd El-Aziz ,2011; Sabbour and Abd El-Raheem,2013; Abd El-Aziz et al. 2015). Each of nanopesticides, nanofungicides and nanoherbicides have been utilized in agriculture (Owolade et al. 2008; Sabbour and Hussein, 2015; Sabbour ,2016; Sabbour and Abd El-Aziz ,2016 a&b ; Sabbour and Hussein, 2016; Sabbour and Solieman,2016). Nanoencapsulated pesticide formulation is able to minimize the dosage of pesticides and human exposition to them, which is environmentally friendly for crop protection. Botanical essential oils have efficient stored product pest management properties. Therefore, this study investigated some botanical oils comparing with Nano-botanical oils, against *Ephestia kuehniella* under laboratory and during storage.

MATERIALS AND METHODS

Larvae of *E. kuehniella* were used in the experiments. The tested insect was reared under laboratory conditions on semi-artificial diet (fine wheat with some adherent endosperm) with 20% glycin and 5% yeast powder. All cultures and experiments were held at 26 ± 2 °C and 70-80% R.H. with 16 hours light and 8 hours dark (Abd El-Aziz et al. 2012) .

Three essential oils were used in the bioassay:, (*Portulaca oleracea* L.), belonging to(family : Portulacaceae); Castor bean oil, (*Ricinus communis* L.), belonging to (family : Euphorbiaceae);Black mustard (*Brassica nigra*), belonging to(family:Brassicaceae). The essential oils were obtained by steam distillation methods of dried plants (Guenther, 1961).The tested oil emulsions were prepared according to (Sabbour and Abd El-Aziz, 2016 a).

The Nano encapsulation is a process through which a chemical is slowly but efficiently released to the particular host for insect pests control. “Release mechanisms include dissolution, biodegradation, diffusion and osmotic pressure with specific pH” (Vidyalakshmi et al. 2009). Encapsulated of the three oils tested (Castor oil, Mustard and Purslane) Nano-emulsion is prepared by high-pressure homogenization of 2.5% surfactant and 100% glycerol, to create stable droplets which that increase the retention of the oil and cause as low release of the nanomaterials. The release rate depends upon the protection time; consequently a decrease in release rate can prolong insect pests protection time (Sakulk et al. 2009). For each tested bulk essential oils, four concentrations were prepared (3, 1.5, 0.5and 0.2%).While, in case of Nano-essential oils, the tested concentrations were (0.1, 0.5, 0.05, and 0.005%).Three drops of emulsifier (Triton X-100)was mixed with water and used as check.The tested oils (Bulk & Nano) were experimented at tested concentrations for their insecticidal activities against the 3rd instar larvae of *E. kuehniella* .According to (Abd El-Aziz,2001),the foam granules were sprayed with the tested oils (Bulk &Nano) and were mixed with wheat (2g foam / 100 g wheat). For each tested concentration, four glass jars as replicates were used. Thereafter, ten third instar larvae were introduced into each glass jar and was covered with muslin for suitable ventilation. In case of untreated control, twelve replicates were kept under the same conditions without any treatments. After seven days of exposure, mortality percentages were calculated in the treated and untreated control. All tests were carried at 27±2oC and 65±5% relative humidity (RH). The number of dead larvae in each jar was assessed and the percentages of mortality were calculated. The experiment was repeated 4 times.

The tested oils (Bulk &Nano) were sprayed to the foam granules and were mixed with wheat (2g foam / 100 g wheat) for testing the oviposition inhibitory effects of tested oils (Abd El-Aziz,2001). In (no-choice test), two pairs of mixed sex of Mediterranean flour moth adults(2-3 days old) were placed with treated or untreated wheat grains with foam particles in glass jars (250 cc capacity) covered with muslin. The moths were left to lay eggs, and then the numbers of deposited eggs on treated or untreated grains/ female were calculated in the tested jars. Four glass jars as replicates for each tested concentration, were used and the test was repeated three times (Abd El-Aziz and Ismail, 2000). The persistence effect of tested oils (Bulk and Nano) on foam as surface protectant was evaluated after storage interval 120 days (4 months) against *E. kuehniella* moths' emergence. Hundred gram of heat sterilized wheat grains were introduced to gunny sacks (10x10 cm each) closed each with a string. The foam granules (about 1 cm in diameter) were sprayed with treatments, dried and provided as a layer between sacks. Then, two pairs of newly emerged moths were placed in a jar (2 l capacity with two gunny sacks). The dead moths were removed after egg lying. The emerged adult moths were counted after 120 days (4 months). Data were displayed to analysis of variance (ANOVA) and means were compared by a least significant different test.

RESULTS

After seven days of exposure interval to tested essential oils, the larvicidal efficiency (% mortality) of tested (Bulk and Nano) oils was experimented against *E.kuehniella* larvae (Table 1&2).The larvicidal efficiency increased with the increase in concentration. Purslane oil (Bulk and Nano) produced a highly significant increase in the mean mortality percent followed by Castor oil against *E. kuehniella* larvae. The least impact oil was Mustard oil against *E. kuehniella* larvae. The Nano oils were more effective than bulk tested oils at all tested concentrations

Purslane essential oil as Bulk phase was extreme significantly efficient oil as ovipositional deterrent against *E. kuehniella* moths with 4.1±3.8, 20.4±9.9 and 48.4+5.6 eggs/female at 3, 1.5 and 0.5% concentrations, respectively compared with control (199.5±9.9 eggs/female) (Table 3). However , Nano Purslane became highest significantly more efficient as sterilizer against adult moths with 1.3±3.4, 2.0±7.8 and 4.4±3.4 eggs/female at 0.1,0.05 and 0.005% concentrations, respectively comparing with other tested Nano-oils and control (Table 4). There were no significant effects between Mustard and Castor (Bulk and Nano) oils at tested concentrations. Both Mustard and Castor had moderate ovipositional deterrent effect compared with control against moths (Table3 and 4).

The tested oils (bulk and nano) vapours had bioresidual efficacy against *E. kuehniella* moths (Figs. 1 and 2). The percentage of emerged moths was extremely significantly decreased with Purslane oil in Bulk phase (10%) in comparison with untreated control (57%) during tested storage interval (120 days). While, Purslane oil in Nano phase completely suppressed the percentage of emerged moths (zero %) comparing with untreated control (66%).

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Table 1: Mortality percentages of tested bulk essential oils against *E. kuehniella*larvae under laboratory conditions.

Treatments oils	Concentrations	% of larval mortality of *E. cautella*
Castor oil	**3** **1.5** **0.5** **0.2**	**49.13** **33.22** **22.43** **10.41**
Mustard	**3** **1.5** **0.5** **0.2**	**23.11** **16.16** **8.12** **4.21**
Purslane	**3** **1.5** **0.5** **0.2**	**66.64** **55.21** **45.32** **18.61**
Control	**0**	**0.0**
F test		**27.5**
LSD 5%		**10.7**

Table 2: Mortality percentages of tested nano essential oils against *E. kuehniella*larvae under laboratory conditions.

Treatments oils	Concentrations	% of larval mortality of *E.kuehniella*
Castor oil	0.1 0.5 0.05 0.005	72.12 61.21 37.41 25.37
Mustard	0.1 0.5 0.05 0.005	31.49 27.56 12.19 6.28
Purslane	0.1 0.5 0.05 0.005	96.64 88.68 78.79 60.61
Control F test LSD 5%	0	0.0 26.2 9.4

Table 3:The ovipositional deterrent efficacy of tested bulk oils against ,*E. kuehniella* moths

Oils used	Mean number of eggs/female ±S.E.		
	Concentration 0.5%	1.5%	3%
Castor oil	107.1±9.3	84.4±5.5	42.6±7.7
Mustard	136.1±1.4	111.1±5.1	88±3.8
Purslane	48.4+5.6	20.4±9.9	4.1±3.8
Control	199.5±9.9		
F value LSD	30.00 19.10		

Table 4:The ovipositional deterrent efficacy of tested nano oils against, *E. kuehniella* moths

Oils used	Mean number of eggs/female ±S.E.		
	Concentration 0.1%	0.05%	0.005%
Castor oil	15.0±4.4	23.6±4.8	28.5±3.7
Mustard	33.3±3.3	33.8±5.9	42.4±9.4
Purslane	1.3±3.4	2.0±7.8	4.4±3.4
Control	199.6±4.9		
F value LSD	31.99 16.99		

Fig1. Effect of tested bulk oils on% moths emergence under store conditions

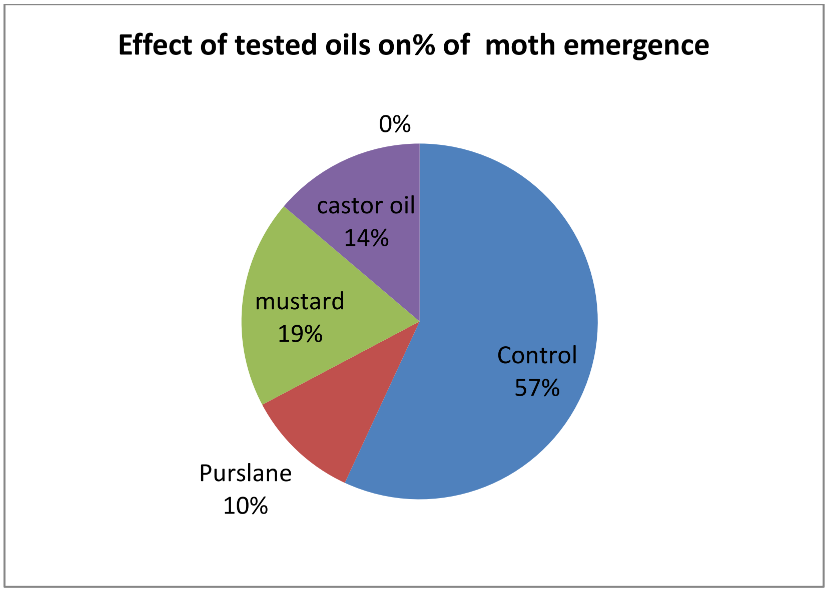
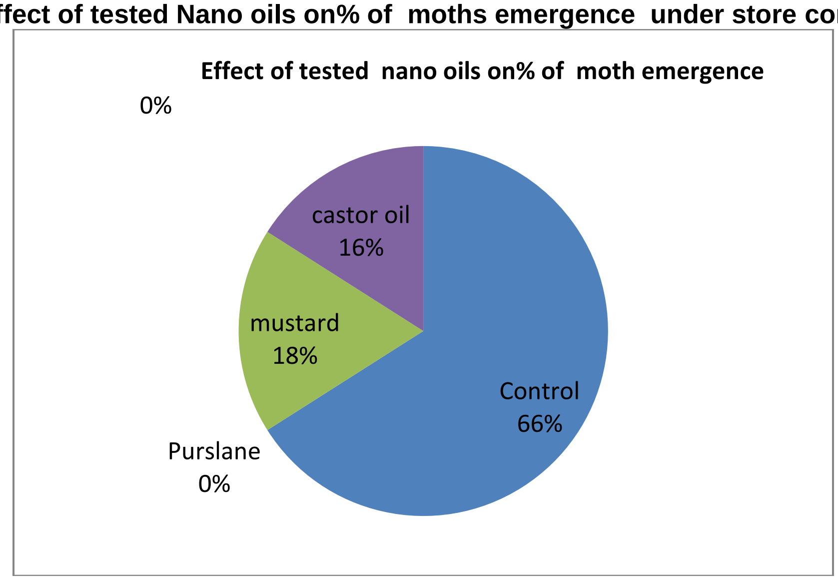


Fig2. Effect of tested Nano oils on% of moths emergence under store conditions



DISCUSSION

The Mediterranean flour moth, *Ephestia kuehniella* is a widespread pest of stored products - especially cereals – and found around the world. The caterpillar larvae are the damaging stage and their feeding pollutes stored products with faeces and webbings and causing spoil of the product (Athanassiou et al. 2008). Purslane oil (Bulk and Nano) produced a highly significant increase in the mean mortality percent followed by Castor oil against *E. kuehniella* larvae. These results in agreement with(Xi Zhou et al. 2015). They isolated the chemical constituents of Purslane essential oil. These constituents are terpenoids, flavonoids, alkaloids, sterols and others. Flavonoids have biological efficiency like antibacterial, antivirus and anti- inflammation properties. Also, the toxic effects of the tested essential oils may come through prohibiting feeding of the larvae (Rodrigues and Vedramin, 1998)or through devastating the larval mid(Zabata *et al* .,2006), leading to decrease the food ingested by larvae, retardation in growth and reducing moths emergence(Richter et al. 1997) .Also, these results are in agreement with (Sabbour and Abd El-Aziz, 2016b). They found that Purslane oil (Bulk and Nano) was the most effective oil followed by Mustard and the least one was Castor against larvae of *E. cautella .* In laboratory evaluation of castor oil , the mortality percentage of *Callosobruchus analis* beetles reached 97.5% (Aheer et al. (1996). At the highest dose (9 ml kg-1) of castor oil and cottonseed oil, mortality percentage of *Acanthoscelides obtectus* beetles reached 99.1 and 74.1% , respectively after120 hr of exposure (Nana *et al.* ,2014).The % mortality of *Sitophilus zeamais* (Mots.) weevils grew with growing dose of castor oil . At the dose (2ml), mortality percentage became (53%) after one hour interval. However, doses higher than 2 ml (>2 ml)gave (>85%) mortality of the weevil (Wale and Assegie, 2015). Subsequent 168 hrs, oil of mustard was highly toxic against beetles of *Bruchidius incarnatus* followed up with clove and cumin with mortality percentages (76%, 63% and42.8%), respectively (Sabbour and Abd-El-Aziz, 2010). Nano Purslane became highest significantly more efficient as sterilizer against adult moths. It can be attributed to the location of appropriate oviposition sites by Lepidopteran female is very serious for the survival of their offspring according to its chemical and physical stimuli (Nansen and Phillips (2003). As the female fecundity based on host-plant stimuli, any modifications of the microenvironment of the oviposition site (as using essential oils)can driving to a blockage of oogenesis and egg retention in the lateral oviduct (Pouzat, 1978). Broad bean seeds treated with Mustard and *Paecilomyces fumosoroseus* fully prevent egg deposition of *Bruchidius incarnatus* during different storage intervals. Coverage gunny bags with treated foam with mustard oil and *P. fumosoroseus* allow favorable safeguard against *B. incarnatus* attack during 120 days of storage (Sabbour and Abd-El-Aziz, 2010).The mean number of deposited eggs reduced with rising tested oils concentrations (Sabbour and Abd El-Aziz, 2016 b).

The tested oils (bulk and nano) vapours had bioresidual efficacy against *E. kuehniella* moths. Purslane oil in Nano phase completely suppressed the percentage of emerged moths (zero %) comparing with untreated control (66%). This mode of action could be referred to the sterilizing impact of Purslane oil (Bulk and Nano). The efficacious constituents of nanoparticles turn into much effective according to their delayed and constant release. These results in agreement with (Yang et al. 2009). The nanoparticles and bulk of garlic essential oil were tested against *Tribolium castaneum* adults. The control efficacy of both nanoparticles and free garlic essential oil were recorded 80% and 11%, respectively (Yang et al. 2009). Moreover, The efficacious constituents of nanoparticles convert into much effective according to their delayed and constant release. Therefore, integral of aromatic oils into a controlled release nano-formulation stop fast volatility and regression, increase persistence and, maintain the lower effective dosage/application **(**Ghormade et al. 2011).

Treated foam with Bulk and Nano Purslane oil and covering gunny bags provided many efficient (toxic, sterilizer, suppressing *E. kuehniella* populations and persevere for appreciable periods) for protecting the stored wheat grains. The high activity of tested Bulk and Nano Purslane against *E. kuehniella* could activate it a possibility replace for methyl bromide in diverse utilizes in stored-product control programs and can be applied in harmony with microbial insecticides , attractants and traps and biological control agents as a component of the Integrated Pest Management.

CONCLUSION

Treated foam with Bulk and Nano Purslane oil and covering gunny bags provided many efficient (toxic, sterilizer, suppressing *E. kuehniella* populations and persevere for appreciable periods) for protecting the stored wheat grains. The high activity of tested Bulk and Nano Purslane against *E. kuehniella* could activate it a possibility replace for methyl bromide in diverse utilizes in stored-product control programs and can be applied in harmony with microbial insecticides , attractants and traps and biological control agents as a component of the Integrated Pest Management.

CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest”

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AUTHOR CONTRIBUTIONS

Sabbour, M.M., share in putting the idea, designed the experiments, make the laboratory and store experiments share in statistical analysis and writing the research, reviewed the manuscript. shadia E, abd El Aziz, also, share in putting the idea, designed the experiments , make the laboratory and store experiments share in statistical analysis and writing the research, reviewed the manuscript. All authors read and approved the final version.

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