International Research Journal of BIOLOGICAL SCIENCES

Volume 02 | Issue 01 | 2020

SciRange
PUBLICATIONS
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SciRange Open Access

International Research Journal of Biological Sciences Research Article

pISSN: 2663-5968, eISSN: 2663-5976

Effect of Some Essential Oils on *Rhyzopertha dominica* (Coleoptera: Bostrichidae)

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ARTICLE INFORMATION

Received: February 07, 2020

Accepted: March 29, 2020

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ABSTRACT

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) are very harmful insect pests to wheat. The aim of this study was to test the effect of natural essential oils on stored insects of *R. domenica.* Three oils tested on these pests were, citronella, moringa and goatweed under laboratory and store conditions. Usage of the three natural oils to decrease the chemical insecticides against *R. dominica*, which were used against stored products and pollute the environment. The polymerization technology was made to obtain nano-encapsulation. Nano-oils were experimented and tested at the concentration of (0.5%) in order to evaluate the insecticidal activities on the 3rd instar larvae of *R. dominica*. Results showed that, the percentage of the accumulative insects were decrease significantly and increased when increasing the time of exposure to the treated foam and also treated with the tested essential citronella oil from *Cymbopogon* (lemongrass), moringa oil from (*Moringa oleifera*) and goatweed extracted from *Ageratum conyzoides*. Nano-oils showed a highest accumulative mortality (99.0%, 89, 87%) when treated with citronella, moringa and goatweed, respectively. The reduction of the eggs laid per female was scored 99.9% eggs/female after treatments with nano Citronella. Under store conditions the percentage of infestations were significantly decreased to 2, 7 and 10% after treating the sacs with citronella, moringa and goatweed oils respectively as compared to 99% in the control sacs (untreated). The three oils and its nano capsules decreased the infestations numbers of *R. dominica* under laboratory and store conditions.

Key words: Natural oils, *Rhyzopertha dominica*, citronella, moringa, goatweed

INTRODUCTION

The lesser grain borer, *Rhyzopertha dominica* (F.), is a major cosmopolitan insect pest of stored wheat. The females lay eggs on the outside of the wheat kernel and the first-instars hatch and bore into the kernel. The larvae feed and develop inside the kernel and upon reaching the adult stage, bore out of the kernel and create a large exit hole. Because the majority of the development occurs inside the kernel, *R. dominica* is hardly controlled by the usage of insecticides applied on the stored wheat[1,2,3]. *Rhyzopertha dominica* considered as a serious harmful insect of wheat. It contaminates the stored seed and cause a problem for stored product seeds. In developmental countries the industries there having a zero allowance for pests in food grains[4,5]. *Rhyzopertha dominica* is a widespread pest of stored products-especially cereals- and found around the world. The adult moths do not feed. The immature stages of the stored products larvae are the most harmful and destructive stages. The feeding pollutes stored products with faeces and webbings and causing spoil of the product[5,6]. Plants played a significant role in Integrated Pest Management strategies because they are important[7,8,9]. Essential oils display a broad spectrum of biological activities against insect pests especially of stored products including fumigation, toxicity, insecticidal activities, repellent, antifeeding and ovipostion inhibitory[10-13]. Each of nanopesticides, nanofungicides and nanoherbicides have been utilized in agriculture[14,15,16]. 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. This study also aimed to investigate the effect of some essential oils and nano-capsulated oils, on *R. dominica* under laboratory and during storage.

MATERIALS AND METHODS

Rearing of insects: *Rhyzopertha dominica* reared at National Research Centre under laboratory conditions, Agriculture Division, on beans seeds and wheat seed. Cultures made at 26±2°C and 60-70% (RH), with 16 h light and 8 h dark.

Tested oils: Three essential oils were used in the bioassay tests, citronella, moringa and goatweed. The essential oils were obtained by steam distillation of dried plants[17]. Emulsions were prepared as follows: 5 drops of TritonX-100 as emulsifier were mixed thoroughly with 5 mL of each tested oils and then water was added to obtain the desired concentrations (2%) in percent of (v/v). The emulsifier was mixed at the corresponding concentrations and used as check. Insecticidal activity of tested oils: The experiment was planned to evaluate the premier as well as the continual effect of the tested oils on the stored product weevils as accumulative mortality during sequential, times (24, 48, 96 and 168 h). Foam granules about 1 cm in diameter were treated at time (zero time) with tested oils (2% conc. dried and provided with heat sterilized rice seeds (100 g each) fastened each with a string. Then all experiments were made by immediately as non-choice examination. The particles of treated foam with the examined essential oils mixed with the tested stored seeds at (2 g foam/100 g seeds) according to Sabbour and Hussein[15] and Sabbour and Abd-El-Aziz[16]. A pair of newly emerged weevils was placed with treated or untreated rice seeds in glass jars (250 mL capacity) covered with muslin. The dead larvae numbers of the *R. dominica* calculated in each jar every day and then the mortality percentages were corrected by Abbott formula[18]. This experiment was achieved under the National Research Centre laboratory conditions of 27±2°C and 60-65% R. H. The experiment was replicated four times[19]. The Nano encapsulation is the chemical slowly permission of the chemical substances and released efficiently to the especial host for *R. dominica* control. "Release mechanisms were includes dissolution, biodegradation, diffusion and osmotic pressure with specific pH"[20].

The nano encapsulation process was carried out by polymerization technology. The tested oils were used as a core material and urea (U) and formaldehyde (F) as shell materials. Sulfuric acid solution (10% w/w) was prepared in the laboratory to control the pH (4.4) of emulsion and tween 80 (polysorbate 80) was used as emulsifier (Merck, Germany). The obtained suspension of nano capsules was cooled down to ambient temperature, rinsed with deionized water, filtered and finally dehydrated by freeze-drying using a LIO-5P, which is a Freeze Dryers for Laboratory Use. (Apparatus CinquePascal, Trezzano SN, Milan, Italy). Nano-emulsion was 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 pest protection time[21]. For each tested bulk essential oils, four concentrations were prepared (3, 1.5, 0.5 and 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) were mixed with water and used as check. The tested oils (Bulk and Nano) were experimented at tested concentrations for their insecticidal activities against the 3rd instar larvae of *R. dominica*. According to Abd El-Aziz[22], the foam granules were sprayed with the tested oils (Bulk and Nano) and were mixed with wheat (2 g foam/100 g wheat). For each tested concentration, four glass jars as replicates were used. Introduce ten 3rd larval instars of *R. dominica* in each glass jar then, covered with a muslin for aeration. The control, experiments were made by adding twelve replicates without treatments under the same laboratory conditions. After seven days of exposure, mortality percentages were calculated in the treated and untreated control. All tests were carried at 27±2°C and 65±5% Relative Humidity (RH). The dead larvae numbers were estimated in each jar used and the mortalities percent were determined. Each experiment was replicated four times.

The tested oils (Bulk and Nano) were sprayed to the foam granules and were mixed with wheat (2 g foam/100 g wheat) for testing the oviposition inhibitory effects of tested oils[22]. Two pairs of *R. dominica* males and females (2-3 days old) placed with treated or controlled wheat seeds with particles of foam in a glass jars (250 cc) in (no-choice test), then covered with a piece of muslin. Adults female moths were left to lay their eggs, hen then deposited the eggs numbers and treated or untreated the grains/female were estimated in each of the tested jars. Four glass jars replicates for each tested concentration, were used and the test was repeated three times. 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, two newly emerged males and females moths placed in a jar (2 L cc). Then remove the moths which were died after lying egg. Then count the new emerged adult moths after one hundred and twenty days about (four months).

Statistical analysis: The data was analyzed using analysis of variance (ANOVA), where significant differences between the treatments were observed. Mean values were significantly separated by using the Least Significant Difference (LSD) test at 5% level[23]. The corrected mortality was due to Abbot formula[18].

RESULTS

Tested oil on *R. dominica* accumulations: During the first week of board the accumulations mortality of *R. dominica* adults oils, showed a highest accumulative mortality obtained (51.2, 34.3 and 44.3%) after two days of treated seeds with citronella, moringa and goatweed, respectively. After seven days the accumulative mortality of the corresponding oil calculated (90.0%, 89.0, 87.3%), respectively as compared to 0.1 in the control (Table 1).

When *R. dominica* adults treated with the nanocapsules of essential oils 55.8, 40.9 and 33.2% after treated with nano oils of citronella, moringa and goatweed, respectively as compared to zero in the control. After seven days of treatments of the corresponding oils the accumulative mortality reached to 98.7, 83.8 and 90.9 as compared to 0.5% in the control (Table 2).

Table 1: Accumulations mortality of *R. dominica* adults during the first week of broad bean seeds in treated foam with different oils

Tested oil	Time (days)	*Rhyzopertha dominica*
Citronella	0	30.9
	2	51.2
	4	68.2
	7	90.2
Moringa	0	29.9
	2	34.3
	4	66.1
	7	89.0
Goatweed	0	31.2
	2	44.3
	4	59.1
	7	87.3
Untreated	0	0.0
	2	0.0
	4	0.2
	7	0.1
F-test		26.0
LSD 5%		10.8

Table 2: Accumulations mortality of *R. dominica* adults during the first week of broad bean seeds in treated foam with different nano oils

Tested oil	Time (days)	*Rhyzopertha dominica*
Nano citronella	0	36.9
	2	55.8
	4	64.9
	7	98.7
Nano moringa	0	29.8
	2	40.9
	4	48.5
	7	83.8
Nano goatweed	0	31.1
	2	33.2
	4	55.3
	7	90.9
Untreated	0	0.0
	2	0.0
	4	0.3
	7	0.5
F test	38.9	
LSD 5%	19.7	

Table 3: Effect of tested oils on number of laid eggs/female and % of adult emergence (F1) of *R. dominica* during storage periods

Tested materials	% of reduction of eggs laid/female	% adult emergence (F1)	% of malformation
Nano citronella	99.9	1	100
Nano moringa	94.1	10	100
Nano goatweed	91.4	7	99
Control	100.0	100	0
Ftest	38.7		
LSD 5%	19.9		

Effect of tested oils on number of laid eggs/female and % of adult emergence (F1) of *R. dominica* during storage periods. The reduction of the eggs laid per female was scored 99.9% eggs/female after treatments with nano Citronella. Table 3, showed the effect of oils and its nano on number of laid

Table 4: Effect of nano tested oils on number of laid eggs/female and % of adult emergence (F1) of *R. dominica* during storage periods

Tested materials	No. of eggs /♀±SE	% adult emergence (F1)	% of malformation
Nano citronella	13.9±7.1	0.1	98
Nano moringa	28.9±5.218	2	99
Nano goatweed	20.4±5.38	1	98
Control	295.7±9.19	100	0
F test	36.7		
LSD 5%	18.7		

eggs/female and % of adult emergence (F1) of *R. dominica* during storage periods. The percentages of eggs laid/female significantly decreased to 99.3, 94.1 and 91.4 after Nano Citronella, Nano moringa and Nano Goatweed treatments as compared to 100% in the control (untreated).

Effect of nano tested oils on number of laid eggs/female and % of adult emergence (F1) of *R. dominica* during storage periods. Table 4 show that the nano compositions of the three tested oil affect on the means number of eggs laid per female. The means number significantly decreased to 13.9±7.1, 28.9±5.218 and 20.4±5.38 eggs/female after Nano Citronella, Nano moringa and Nano Goatweed as compared to 295.7±9.19 eggs/female in the control. The percentages of adult emergence significantly decreased to 0.1% in case of Nano theme treatments as compared to 100 in the control. Also the malformation of the Nano Citronella, Nano moringa and Nano Goatweed reached to 98, 99 and 98% as compared to zero in the control (Table 4).

DISCUSSIONS

Results showed that the reduction in number of eggs laid/female decreased to 10.3±0.0, 20.4±1.21 and 20.4±5.38 eggs/female after Nano Citronella, Nano Moringa and Nano Goatweed as compared to 297.9±7.89 eggs/female in the control, this meet of Abd El-Aziz[22], Sokal and Rohlf[23] and Shaaya *et al.*[24], who reported that the entomopathogenic fungus *B. bassiana* and botanical oils, *N. sativa, T. distichum* and *B. carterii* decreasing the infestations of *Plodia interpunctella* and *Ephestia cautella* as compared to the control group. Many studies found that such as, Shaaya *et al.*[24] reported that natural edible oils have a potency control agents against *S. granarius* and also play a serious and remarkable effect in stored-grain protection. Abd El-Aziz[22], Sokal and Rohlf[23] and Shaaya *et al.*[24] mentioned that, Nano Citronella, Nano Moringa and Nano Goatweed clove and eucalyptus oil vapors impaired the fecundity of Bruchid beetles data proved promising oviposition deterrence toxicity and suppression of eggs and adult emergence[19].

Lisansky[25] recorded that the LC_{50} for some formulations of *B. bassiana* was reduced to 97% after the addition of coconut oil. It was suggested that the cutinophilic properties of the oil could allow a greater number of fungal conidia to penetrate the mouth parts of insects. Oil transporter could distribute the inoculum over the stored product insect pests thin skin membranes, which were more easily penetrated by entomopathogenic fungi[25]. The increase in the pathogenicity of *B. bassiana* combined with mustard oil to *C. maculatus* beetles may be attributed to some degradation occurring at the structural level of the integument, which could have facilitated the penetration of the cuticle by the germ tube of the fungus. Similar results were obtained by Hassan and Charnley[26] in Manduca sexta treated with *M. anisopliae* and the chitin-synthesis inhibitor dimiline. Hassan and Charnley[26] reported that Synergistic effects of a combined application of *B. bassiana* and the chloronicotinyl insecticide imidiaclopride on *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae). Similar results obtained by Sabbour and Abd El-Aziz[13] and Vidhyalakshmi *et al.*[27]. Chander and Ahmed[28] reported the applications of the different doses of natural essential oils like the *Acorus catamus* seeds oils of green beans to protect them from the dangerous pest infestation. Abd El-Aziz[22] reported that foam sprayed with clove oil (5%) and placed between sacks caused the highest mortality to *C. maculatus*. Similar results obtained by Hassan and Charnley[26], Vidhyalakshmi *et al.*[27], Chander and Ahmed[28] and Saxena *et al.*[29]. The fungus *B. bassiana* and plant extracts reduced insect infestations of *Cassida vittata* and *Callosobruchus maculatus*. Accordingly, Sabbour and Shadia[30], Sabbour[31] and Sabbour[32], who used two entomopathogenic fungi alone or in combination with modified diatomaceous earth to Control of Bruchidius incarnates and *R. dominica*. Accordingly Hanafi[33], who reported that biological and natural insecticides were more functional in preventing seeds losses by pests in stores in cases which contain the premier levels of pest infestation, was relatively low.

CONCLUSIONS AND RECOMMENDATIONS

This research indicate that some the natural essential oils are very important and useful for managing and control the harmful pests in enclosed stores places because of their effective repellant and fumigant action. Moreover, natural oils are highly eclectic to pests. Incorporation of natural oils as a controlled release nano materials formulation which was prevented the rapid vaporization and degradation. These oils also, causing a constant increase and decrease the effective dosage/application. Treated foam particles with the nano citronella, moringa oils gave many efficient effects on the lesser grain borer, *R. dominica*. The present experiments showed that the essential oils tested are obviously very important and also, showed that the tested nano oils have been found significantly efficient against tested insects of *R. dominica* as more insect mortality, a smaller number of eggs, less adult emergence, high percentage of adult malformation and less percentage of weight loss after 90 days of storage. These results based on the criteria that help the farmers to decrease the loss of stored seeds and give a good method to stored the grains. We recommended to use the natural oils as bio-products as they do not pollute the environment.

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