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Efficiency of Absolute Oils of Olive Fruits and Carnation Flowers as New Formulation Against *Tribolium Castaneum* (Herbst) And *Rhizopertha Dominica* (F.)

Abo-Arab, R. B. S.; Amany, A. Khalifa and Nariman and M. El-Tawelh
Plant protection Res. Inst., Agric. Res. Center, Dokki, Giza, Egypt.
Corresponding author: medovenus202020@gmail.com

Abstract

Olive and carnation absolutes are new forms extracted from the concrete produced after extraction of oil. Repellency and fumigation bioassays are of the best methods which used to evaluate the absolutes of olive and carnation against *T. castaneum* and *R. dominica* adults. Results obtained showed that the repellent and fumigant activities of carnation had the strongest effect to the two tested insects in comparison with that of olive. Moreover, the lowest concentration of carnation (5 ppm) achieved 50, 100% repellent activity compared to that of olive which caused zero and 35% after 24 h against *T. castaneum* and *R. dominica*, respectively. Additionally, the affection of oil increased with increasing of exposure period. However, the repellent of the two absolutes against the two insects started to decrease after 24 h post treatment. The time needed for oil to cause LT50 was also estimated at the highest concentration which achieved nearly 100 % mortality (449.95 mg/170 cm^3) for both carnation and olive. Basedon LT 50 values the carnation oil was the premier compared to the olive with LT 50 values of 0.166, 0.01 and 0.688, 50.166 h against *R. dominica* and *T. castaneum* , respectively. Finally, the present study suggests application the absolute oils against stored product insects where they are very cheap, available and easy affordable and they may introduce the program of integrated pest management.

Keywords: *Tribolium castaneum* (Herbst), *Rhizopertha dominica* (F.), olive oil, carnation oil

Introduction

The purpose of storage is to equilibrate of fluctuation between to major factors, supply and demand. Improper management of harvested grain causes significant quantitative and qualitative losses are estimated to range from 9% in the United States **(Pimental, 1991)** up to 50% in some parts in the developing nations. *T. castaneum* and *R. dominica* have also been directly associated with seed damage **(Sinha, 1990).** *R. dominica* (F.) cause the most grain damage because the immature stages develop inside the grain kernel **(Hagstrum and Subramanyam, 2006).** Many tools were used for annihilation or minimizing the numbers of insects on stored commodities. The main tools was the heavy reliance on the synthetic insecticides which had negative side effects on human and the environment included development of insect resistance, disorders to non-target organisms and users.

Botanicals have been used since time immorial for protection of stored products against common pests. They act as repellents, antifeedants, toxicants and behave as chemosterilants/reproduction inhibitors or insect growth and development inhibitors. They are advantageous in many ways as they are naturally occurring, highly specific to target pests, little or no adverse effect on beneficial insects, insect resistance is slow or less common, they have no unknown environmental hazardous, have less residual activity and are effective against insecticide resistance species of insects. Due to all these various reasons botanicals are considered as commercially viable green pesticides and are gaining tremendous impetus **(Trivedi *et al*., 2018).**

Botanical insecticides have been considered as suitable alternatives to conventional insecticides for pest control owing to insecticidal potential of their secondary chemicals in spired by plant insect chemical interactions **(Miresmailli and Isman, 2014).**

Meanwhile, they are relatively low toxic to environment and human health contrasted to synthetic chemical insecticides **(Isman, 2006).** Another benefit of the essential oils would be the slowly development resistance owing to the complex mixtures of the compounds **(Koul *et al*., 2008).** Most of the compounds in essential oils are non-toxic to mammals, birds and fishes **(Stroh *et al*., 1998).**

Botanical pesticides constitute an alternative way of reducing the use of chemical insecticides. On 21 October, 2009, the European and the council of the European Union established directive 2009/128/WE (http: eur_lex.europa.eu) to achieve the sustainable use of pesticides by reducing the risks and impacts of pesticides use on human health and the environment, and by promoting the use of integrated pest management and of alternative approaches or techniques such as non-chemical alternatives of pesticides **(Konecka *et al*., 2018).**

Therefore, the present study performed to evaluate two new forms (absolutes) olive and carnation of plant derived oils (resulted from re-extraction the waste of the first extraction of oils) as fumigant and repellent agents against two important insects, *T. castaneum* and *R. dominica* using two of the best bioassay techniques, repellency and fumigation.

Materials and Methods

1- Test Insects:

Insect culture and rearing conditions:

Tribolium castaneum:

Insects were reared on a mixture of wheat grain mixed with wheat flour. Grain were cleaned and sterilized and put in glass jars each containing 400 gm (30% wheat flour) and provided with 100-200 adult insects. Jars were covered and placed under laboratory conditions of 30±2ºC and 65±5% R.H. The newly emerged adults (1-2 weeks old) were used for different tests.

Rhyzopertha dominica:

Insects were reared on wheat grains, cleaned from dusts, and other inert materials and sterilized by heating at 60ºC for one hour, then grains were put in glass jars each containing 400 gm of wheat and provided with 100-200 adult insects. Jars were covered with muslin cloth secured with elastic rubber bands and placed under laboratory conditions of 30±2ºC and 65±5% R.H. The newly emerged adults (1-2 weeks old) were used for tests.

2- Absolute oils:

The tested absolute oils of olive fruits and carnation flowers were supported by Hashim Brothers Company, Al-Sadat City, Egypt.

Extraction of absolute oils:

Olive absolute:

Absolute olive oil was extracted from the remainder waste after extraction of olive oil from its fruits. The waste of olive fruits was extracted with hexan for 4 times (2 h, 2 h, 2 h and overnight), then the remainder concrete was extracted by ethyl alcohol (97%) using special apparatus and after 5 washes will have olive absolute. Then the absolute was purified and filtered.

Carnation absolute:

The carnation flowers were compressed with hexane for four washes 10 minutes, 10 min, 30 min and overnight. After evaporation of solvent, the concrete (waste) was washed with ethyl alcohol 97% for five washes under water-cooling, then the mixture was filtered for discarding wax and to get absolute.

Methods of applications:

Repellent Assay

The repellent effect of (olive and carnation absolutes) oils against adults of both *R. dominica* and *T. castaneum* was evaluated using the area preferences method as described by **(Me Donald *et al*., 1970).** Briefly, test areas consist of 9 cm Whatman No. 1 filter paper disc cut in half (31.80 cm^2). Different concentrations of 5, 10, 20 and 40 ppm were prepared in 0.5 ml of acetone. The half discs of filter paper were uniformly impregnated with each solution using a micropipette. The other half filter paper discs were treated with pure acetone and used as controls. Treated and control half discs were air-dried for 15 min to evaporate the solvent completely. A full disc was carefully remade by attaching the tested half to the control half with tape care was taken so that the attachment did not prevent free movement of insects from the one half to another, but the distance between the filter paper halves remained sufficient to prevent see page of test sample from one half to anthor. Ten insects of *R. dominica* or *T. castaneum* were released in the center of each filter paper disc and a cover was placed over the petri dish. Three replications were used for each concentration of an oil. Counts of insects present on each strip were made after 1h, 3h, 6h and 24 hour for *R. dominica* and *T. castaneum*. The average of the counts was converted to express the percentage of repulsion (PR) using the formula of **(Talukder and Howse, 1993)**: PR = 2 X (C-50) where C is the percentage of insects on the untreated half of the disc positive value expressed repellency and negative values attractancy.

Fumigant Toxicity:

The fumigant effect of essential oils (olive and carnation absolutes) against adults of both *R. dominica* and *T. castaneum* was evaluated using technique was described by **(Moravvej and Abbar, 2008),** 6 cm diameter pieces of filter paper (Whatman No. 1) impregnated with the desired concentrations of plant oils and attached it to under surface of screw cap of plastic jars (170 cm^3). Ten adult insects of *R. dominica* and *T. castaneum* (7 gays old) were separately transferred into plastic jars covered with their screw caps attached with treated filter paper. Pieces of filter paper (Whatman No. 1) treated with acetone only saved as control. Three replicates for treatments and control were used. Mortality was recorded after 24 and 48 hours for *R. dominica* and *T. castaneum* from the commencement of exposure. Mortality data was corrected by using Abbott's formula **(Abbott, 1925)**. The slope, LC50 and confidence limits values were estimated using method of Finney's analysis **(Finney, 1971).**

Further studies were conducted to determine: the LT 50 of the olive and carnation oil absolutes separately against *T. castaneum* and *R. dominica* insects.

LT 50 assessment:

For all treatments a concentration of 449.5 mg/170 cm^3 for both olive and carnation (the concentration which kills nearly 100 % of exposed population). The oil concentration was prepared by dissolving on amount of oil in acetone to obtain the desirable concentration (449.5 mg/170 cm^3). Treatment without oil (solvent only) was used as control. Three replicates for the concentration and control were used. Ten adults insects of *R. dominica* and *T. castaneum* were transferred into Jars of capacity 170 cm^3 (without medium). % mortality was recorded after 24, 36 and 48 h and corrected by Abbots formula **(Abbott, 1925)**. LT 50 values were estimated using using method of Finney's analysis **(Finney, 1971).**

The all experiments were carried out at stored product pest Research Department, Plant protection Research Institute, Sakh Branch, Kafr El-Sheikh Governorate.

Data analysis:

Median lethal concentration (LC 50) and LT 50 values with their confidence limits were calculated based on Finney analysis (Finney, 1971) using Pc-probit software program.

Results and Discussion

Fumigation activity:

Fumigation is one of the most important bioassay applications used for evaluation the agents against the insect pests especially with stored product insects, where it almost destroy the all stages of insect from egg to adult with minimized side effect on grains. Data summarized in Table (1 and 2) showed the effect of the form absolute for carnation and olive oils. Based on LC_{50}, results revealed that the absolute carnation oil was the excellent against *T. castaneum* adults after 24 and 48 hours with LC_{50} of 261.46 & 102.77 and 461.26 & 448.18 ppm, respectively. Also, there was a significant difference between the effect of the two oils according to the confidence limits of the tested absolute oils.

Similarly, the effect of carnation and olive against *R. dominica* had the same trend with *T. castaneum* where carnation had achieved the best effect compared to olive with LC_{50} of 111.64 & 73.32 and 113.46 & 80.33 ppm at 24 and 48 h post-treatment (Tables 3 & 4). In addition, there was no significant difference between the two oils against the *R. dominica* where the LC_{50} of any oil overlapped between the confidence limits of other oil.

In general, the induction of the two oils increased when the exposure period increased with the two tested insects (Tables 1-4).

Repellent activity:

The repellent bioassay technique has the same significance of fumigation method since it repels the insects far away the grains reducing both the loss or the contamination of stored products. Also, the present study used this method to evaluate the new formulation of the tested oils (absolute form) as stored products protectants against the insect attack. The present data clarified that the two oils showed moderately effect against the tested insects. For *T. castaneum*, results involved in Table (5) elucidate the repellent activity of the two oils. According to the obtained findings carnation oil was the premier since it has the highest percent of repellency at the all time periods of experiment compared to that of olive oil. Moreover, the action of the oils increased as the concentration and exposure period increased till 24 h. After 24 h, the action of the oils obviously reduced.

Alike data acquired in Table (6) accentuated the effect of oils against *R. dominica*. The repellent affection of the two oils had the same trend with *T. castaneum*, where the carnation oil kept with the first rank achieving the highest repellent activity at the all time periods through 24 h. Markedly effect showed with the lowest concentration (5 ppm) of carnation compared to the same concentration of olive which has not any effect against the two tested insects. However, the effect of the two oils started to decrease after 24 h post-treatment.

Data obtained in Table 7 and 8 showed that carnation oil was the most toxic against the two tested insect species with LT50 values 0.166 and 0.003 h for *R. dominica* and *T. castaneum.* On the other side, olive oil needed to 0.688 and 50.166 h as LT 50 for *R. dominica* and *T. castaneum,* respectively. The response of both insects significantly differed according to the LT 50. In addition *R. dominica* was more susceptible to olive absolute with LT50: 0.688 mg/170 cm^3) than *T. castaneum* (50.166 mg/170c m^3). While the same insect was more tolerant to carnation with (0.166 mg/170 cm^3) than *R. dominica* which had (0.003 mg/170 cm^3). Our results were in the line of **Maravvej and Abbar (2008),** they indicated that the oil extracted from the peels of four different species of citrus including *Citrus aurantium* had high fumigant activity against *Citrus maculatus* adults, also the showed that the morlatity increased with exposure time from 24 h to 48 h after treatment. The present findings are in the line of **Cao *et al*., (2018)** who reported that Eo extracted from *Evodia lenticellata* had interspecific contact and fumigant toxicity and repellent activity against *T. castaneum*, *Lasioderma serricorne* and *Liposcelis bostrychophila*. **Kumar and Tiwari (2016)** claimed that (Eos) of *Murraya koenigii, Curcuma longa, Calistimone citrinus* and *Citrus reticulate* and their suitable combinations showed fumigant activity against *R. dominica* and *T. castaneum*. So, these essential oils may be utilized for the quick fumigation of stored wheat for eco-friendly and sustainable management of other stored grain insect pests. Essential oils from more than seventy five plant species belonging to different families had fumigant toxicity against several insect pests of stored grains, **(Rajendran and Sriranjinia 2008).** Furthermore, Plant essential oils may act as fumigants, contact insecticides, repellents, deterrents and antifeedants **(Singh and Singh, 1991; Shaaya *et al*., 1997; Isman, 2000; Weaver and Subramanyam, 2000; Shakarami *et al*., 2004; Negahban *et al*., 2007; Kumar, 2016; Kumar and Tiwari, 2017 and Janaki *et al*. (2018)** stated that *Cyperus rotundus* essential oil had repellent activity against *Oryzaephilus surinamensis* L., *Trogoderma granarium* Everts and *C. maculatus* F. The repellency percentage of essential oil was 94%, 94% and 80% after 2 h, and 90%, 92% and 70% after 4 h, on the three abovementioned insects, respectively. Also, they reported that *C. rotundus* oil can be used as a suitable substitute for chemical pesticides to protect the stored products against pests. Essential oils affect the behavioral reactions of insect pests and may have fumigant toxicity **(Zandi-Sohani and Ramezani, 2015),** repellency (**Demirci *et al*., 2017),** ovicidal activity **(Kedia *et al*., 2014),** oviposition deterrence **(Zandi-Sohani and Ramezani, 2012),** contact toxicity **(Gupta *et al*., 2011)** and antifeedant effects **(Kedia *et al*., 2014).** Historically, plant extracts and their parts have been utilized for pest control until the bulk and cheap availability of chemical pesticides.

Romans were earliest to utilize aromatic plants for fumigation of granaries **(Dubey, 2011).** In order to survive, plant produce secondary chemical compounds as a defence mechanism against herbivorous, pests and pathogens **(Khan *et al*., 2017; Pavela, 2016 a and Pavela 2016 b).**

Some plant substances may be toxic for insects and therefore they would be useful as complementary or alternative methods to the heavy use of classical insecticides. Commonly, essential oils can be inhaled, antifeedant and insecticidal effects of essential oils have been observed **(Khater, 2012).**

The presented results are in agreement with that of **Bernardes *et al*., (2018)** investigated the bioactivity of the essential oil of *Chenopodium ambrosioides* L. (Ca-EO), *Ocimum gratissimum* L., (OG.EO) and *Schinus terebinthifolius* Raddi (ST-EO) against *Zabrotes subfasciatus*. After 12-h treatment, CA-EO and OG-EO at 20.0 µl/L of air killed 100% *Z. subfasciatus*, where ST.EO at 100.0 µl/L of air afforded 100% *Z. subfasciattus* mortality. After 24 h CA-EO provided the lowest 24 h LD_{50} (0.8 µl/L of air) and displayed efficiency repellent activity against *Z. subfasciatus*.

Table 1. Fumigation effect of two essential oils against *Tribolium castaneum* after 24 h.

Oil obsolete	Conc. Mg/170 cm^3	% Mortality	LC_{50} Mg/170 cm^3	Slope	Confidence limits	
					Upper	Lower
Olive	56.2 112.4 224.8 449.95	14.67 49.44 84.66 98.02	461.26	2.96	576.05	393.77
Carnation	56.2 112.4 224.8 449.95	12.52 56.39 45.39 65.74	261.46	1.72	329.58	217.47

Table 2. Fumigation effect of two essential oils against Tribolium *castaneum* after 48 h.

Oil absolute	Conc. Mg/170 cm^3	% Mortality	LC_{50} Mg/170 cm^3	Slope	Confidence limits	
					Upper	Lower
Olive	56.2 112.4 224.8 449.95	4.30 12.62 28.39 50.11	448.18	1.90	619.95	359.001
Carnation	56.2 112.4 224.8 449.95	42.90 51.05 59.17 66.90	102.77	0.68	156.41	47.33

Table 3: Fumigation effect of two essential oils against *Rhizopertha dominica* after 24 h.

Oil absolute	Conc. Mg/170 cm^3	% mortality	LC_{50} Mg/170 cm^3	Slope	Confidence limits	
					Upper	Lower
Olive	56.2 112.4 224.8 449.95	14.67 49.44 84.66 98.02	113.46	3.44	137.2	93.71
Carnation	56.2 112.4 224.8 449.95	22.02 50.31 48.42 94.12	111.64	2.59	138.38	90.00

Table 4. Fumigation effect of two essential oils against *Rhizopertha dominica* after 48 h.

Oil absolute	Conc. Mg/170 cm^3	% mortality	LC_{50} Mg/170 cm^3	Slope	Confidence limits	
					Upper	Lower
Olive	56.2	30.26	80.33	3.33	92.34	69.82
	112.4	68.65				
	224.8	93.16				
	449.95	99.36				
Carnation	56.2	39.36	73.32	2.34	85.02	63.18
	112.4	66.78				
	224.8	87.23				
	449.95	96.67				

Table 5. Repellent effect of two essential oils against *T. castaneum*

Plant oil absolute	Conc. ppm	1 hour %	3 hours %	6 hours %	24 hours %	% repellency over the 24 h duration*
Olive	5	0	0	0	0	0.00
	10	20	35	50	60	41.25
	20	50	70	90	90	75.00
	40	60	80	100	100	85.00
Carnation	5	10	30	40	50	32.50
	10	20	50	60	75	51.25
	20	50	70	90	100	77.50
	40	70	80	100	100	87.50

***** % repellency over the 24 h duration = PR = 2 X (C-50)

Table 6. Repellent effect of two essential oils against *R. dominica*

Plant oil absolute	Conc. Ppm	1 hour %	3 hours %	6 hours %	24 hours %	% repellency over the 24 h duration *
Olive	5	0	0	0	35	8.75
	10	20	35	50	70	43.75
	20	60	80	90	100	82.50
	40	70	80	100	100	87.50
Carnation	5	40	70	90	100	75.00
	10	40	80	90	100	77.50
	20	60	90	100	100	87.50
	40	80	100	100	100	95.00

***** % repellency over the 24 h duration = PR = 2 X (C-50)

Table 7. LT 50 values of oil absolutes against *Rhizopertha dominica* exposed to the indicated concentrations at different periods using fumigation method.

Oil absolute	Conc. Mg/170 cm^3	% mortality			LT 50 hours	C.L.		S.V
		24 h	36 h	48 h		Upper	Lower	
Olive	449.95	98.02	98.69	99.36	0.688	1.167	0.07	1.39
Carnation	449.95	94.12	95.41	96.69	0.166	0.23	0.02	0.7

Table 8. LT 50 values of oil absolutes against *Tribalium castaneum* exposed to the indicated concentrations at different periods using fumigation method.

Oil absolute	Conc. Mg/170 cm^3	% mortality			LT 50 hours	C.L.		S.V
		24 h	36 h	48 h		Upper	Lower	
Olive	449.95	48.69	49.01	50.11	50.166	52.14	47.3	0.11
Carnation	449.95	65.74	66.30	66.90	0.003	0.01	0.001	0.102

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كفاءة الزيوت الحرة لثمار الزيتون وزهور القرنفل كتجهيزات حديثة ضد خنفساء الدقيق الصدئية وثاقبة الحبوب الصغرى

رأفت بدر سعد أبو عرب ، أمانى عبد الحكيم خليفة ، ناريمان محمد الطويلة

معهد بحوث وقاية النباتات . مركز البحوث الزراعية . الدقي . الجيزة . مصر

أجريت الدراسة الحالية لتقييم صورتين جديدتين من الزيوت وهى الزيوت الحرة absolute oils والتى تم إستخلاصها من المواد المتخلفة بعد إستخلاص الزيت الأساسى من النبات المستهدف.

أستخدمت طريقتين من طرق التقييم الحيوى وهما النشاط الطارد repellency وتأثير التبخير Fumigation وذلك لتقييم صور الزيوت الحرة تحت الدراسة لثمار الزيتون Olive fruits وزهور القرنفل Carnation flower ضد حشرتى خنفساء الدقيق الصدئية الحمراء *T. castaneum* و ثاقبة الحبوب الصغرى *R. dominica*.

أظهرت النتائج المتحصل عليها أن زيت القرنفل الحر أظهر تأثيراً طاردا ونشاطا كمادة تبخير أقوى من زيت الزيتون ضد الحشرتين موضع الدراسة.

أظهر أقل تركيز لزيت القرنفل الحر (5 ppm) ونسبة نشاط طارد 50 ، 100% مقارنة بنسبة صفر ، 35% بعد 24ساعة عند خنفساء الدقيق ، وثاقبة الحبوب الصغرى.

بالإضافة إلى ذلك فإن تأثير الزيوت المختبرة إزداد بزيادة وقت التعرض وبالرغم من ذلك فإن التأثير الطارد للزيوت الحرة ضد الحشرتين بدأت فى الإنخفاض بعد 24 ساعة من التعرض.

وقد تم أيضا حساب الوقت اللازم لقتل نصف عدد الحشرات المعرضة للزيوت المستخدمة (L T 50) لكل من زيت الزيتون الحر وكذلك زيت القرنفل الحر وذلك عند التركيز القاتل تقريبا لـ 100 % وهو 449.95 مجم/170سم^3. كان زيت القرنفل الحر هو الأكثر تأثيرا مقارنة بزيت الزيتون وذلك على أساس الوقت اللازم لقتل نصف عدد الحشرات المعرضة والتي أعطت 0.166 ، 0.01 و 0.688 ‘ 50.166 ساعة لكل من زيت القرنفل والزينون ضد حشرتي ثاقبة الحبوب الصغرى وخنفساء الدقيق الصدائية الحمراء على التوالي.

تقترح الدراسة تطبيق الزيوت الحرة المختبرة ضد حشرات الحبوب المخزونة نظرا لإنخفاض تكلفتها وسهولة الحصول عليها وكذا يمكن إدخالها كأحد عناصر برنامج المكافحة المتكاملة.