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Toxicity of the Locally Formulated Diesel Oil Alone and Mixed with Botanical Synthetic Materials against Mealybug *Ferrisia virgate* (Cockerell)

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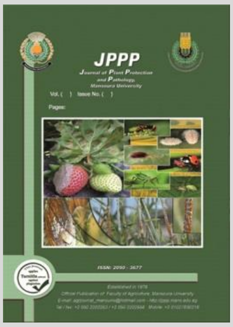

ABSTRACT

Physico-chemical properties of crude diesel oil and botanical synthetic materials (thymol and camphor) were studied and prepared as emulsifiable concentrate formulation (EC). Prepared formulations passed successfully specified testes, then their toxicity were investigated against different stages of mealybug *Ferrisia virgata* (Cockerell) under laboratory conditions. Results indicated that all tested formulations showed high toxicity against all tested insect stages. The mixing, thymol or camphor with diesel oil increased toxicity of oil against mealybug *F. virgate*, whereas mixture, thymol with diesel oil was more effective than camphor with diesel oil. LC_{50} values after two days of application were 4.02, 4.51, 4.86 and 5.33 ml/l for diesel oil, while it were 0.17, 0.17, 0.25, and 0.25ml/l for mixture of oil and thymol, and 0.88, 0.99, 1.12, and 1.24 ml/l for mixture of diesel oil and camphor for first, second, third and adult stages of *F. virgate* respectively. While LC_{50} values after three days of application were: 1.43, 1.49, 1.61 and 1.77 ml/l for diesel oil, while it were 0.03, 0.03, 0.08 and 0.11 ml/l for mixture of oil with thymol, and 0.14, 0.24, 0.21 and 0.16 ml/l for mixture of oil with camphor for 1st, 2nd, 3rd and adult of *F. virgate* respectively. Results showed, efficacy of tested materials increased by increasing concentration and period after application and mixing botanical synthetic materials with diesel oil increased toxicity against all tested stages; moreover, mixtures could be used for controlling mealybug in medical, aromatic and ornamental plants after conducting more semi-field and field experiments.

Keywords: Diesel oil, botanical synthetic materials, ECs, *Ferrisia virgate.*

INTRODUCTION

The most widely recognized type of diesel is petroleum diesel. Diesel is gotten from fossil fuels and refined like gasoline. Diesel oil is delivered from the fractional distillation of unrefined petroleum (oil) at atmospheric pressure, at 200°C to 350°C Celsius degree. These outcomes in a blend of hydrocarbon chains that contains from 8 to 21 carbon atoms per molecule. The thickness and the boiling point of the fuel increase with an expansion in the quantity of carbon molecules. This helps in the partition of the different segments by refining. The chains from C7 to C11 vaporize below 100°C Celsius degree and used as gasoline. The products between of C12 to C15 form kerosene, diesel oil and heavier fuel oils. The No. 2 fuel oil is known as diesel oil and utilized for running cars, trucks and different vehicles. Hydrocarbon chains over the scope of C20 structure solids, like paraffin wax, tar and asphalted bitumen (oil and Gas industry 2010).

Oils have little risks to individuals and most of desirable species, including beneficial natural enemies of insect pests. These advantages may permits oils to coordinate well with organic controls. Harmfulness is insignificant, compared with chemical pesticides, and oil rapidly disseminate through dissipation, leaving little buildup. Oils are suitable to apply with many of spray equipment's and can be mixed with other pesticides to increase their performance Cranshaw and Baxendale, (2011). Mineral oils still have the advantage of being effective against resistance of strains, and development of resistance was not recorded for mineral oils (Micks and Berlin, 1970). Guava mealy bug, *Ferrisia virgata* (Cockerell), is one of the usually happening bugs found to make harm guava (*Psidium guajava*) trees and furthermore to a few host plants. It sucks cell sap from the leaves, delicate shoot and branches (Wabale *et al*. 2010). The mealybug *F. virgata* can be found throughout the world, and is known to feed on more than 100 plant species grown throughout the world. It attacks the foliage, sucks a big amount of plant sap for it's needed of protein and secretes honey dew. Most of these individuals are accumulated around their host branches, foliage, leaves, and twigs and fruits. A large number of these species are secured with white wax and have a distinct fringe of waxy filaments around the circumference of their bodies and the long tails and the presence of two stripes on the body. This species produces an egg mass or ovisac (Ghose and Ghosh, 1990).

The aim of this study was preparing the crude diesel oil alone and its mixture with botanical synthetic materials (thymol and camphor) in the form of emulsifiable concentrates formulation (ECs) and comparing the toxicity of mineral oil (Diesel oil) alone and its mixture with botanical synthetic materials against different stages of the mealy bug *F. virgate* under laboratory conditions as alternatives of chemical insecticides.

MATERIALS and METHODS

1- Tested chemicals:

1- active ingredient:

1-Diesel oil: It is a light medium cut of petroleum oil (Solar cut), It was bought from fuel station, Cairo, Egypt.

2- Thymol extra pure ($C_{10}H_{14}O$): It was supplied by El-Gomhoria Co., Cairo, Egypt.

3-Camphor ($C_{10}H_{16}O$): was supplied by El- Gomhoria Co., Cairo, Egypt.

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2- Solvents: Acetone, xylene and dimethyl formammide., were supplied by El- Gomhoria Co., Cairo, Egypt.

3- Surface active agents: Toximol, Toximol-R and Toximol-H., were supplied by El- Gomhoria Co., Cairo, Egypt. Poly ethylene glycol 600 di- lurate., were supplied by the Egyptian Starch, Yeast and Detergents Co., Alexandria, Egypt.

2. The physico-chemical properties of the basic formulation components:

1. Active ingredient:

a) Solubility:

It was measured by calculating the volume of distilled water, acetone and xylene for complete solubility or miscibility of one gram of active ingredient at 20 ˚C (Nelson and Fiero, 1954).

b) Free acidity or alkalinity:

It was measured by using the method described by WHO specification (1979).

2. Surface active agents:

a) Surface tension:

It was measured using surface tensiomate for solutions at 0.5 % (W/V) surfactant according to ASTM D-1331 (2001).

b) Critical micelle concentration (CMC):

The (CMC) of the tested surfactants was measured by using the method reported by (Osipow, 1964).

c) Hydrophilic-lipophilic balance (HLB):

The (HLB) of the tested surfactants were measured by using the method reported by (Lynch and Griffin, 1974).

d) Free acidity or alkalinity:

It was measured by using the method that mentioned above.

3. Preparation of diesel oil and mixed with 10% thymol and 10% camphor oil as Emulsifiable concentrates (EC):

Emulsifiable concentrates a liquid formulation contains technical material, organic water-immiscible solvents and emulsifiers (surface active agents). When EC formulations are diluted with water in a spray tank they form a spontaneous emulsion. For preparing diesel oil, diesel oil + thymol and diesel oil + camphor in form of emulsifiable concentrate formulation, several trials were carried out as follow:

Diesel oil and its mixtures prepared as ECs by adding different weights to other different weights of suitable emulsifier or blend of emulsifier and stirring to homogeneity. Emulsion stability test was done for all formulated formulas by using the method reported by CIPAC MT 36.1 (2002) to determine which of them will pass and suitable for application.

4. Determination of the physico- chemical properties of the prepared Emulsifiable concentrates formulation (EC):

a) Emulsion stability: It was measured by using the method reported in CIPAC MT 36.1 (2002)

b) Persistent foam: it was determined by using the method reported in CIPAC MT 47.2(2002).

c) Free acidity or alkalinity: It was determined by using the same method that mentioned above.

e) Stability at elevated temperature 54 ± 2 ºC (accelerated storage): It was determined by using the method reported in CIPAC MT 46.3 (2002)

5. Determination of the physico-chemical properties of the spray solution of the local prepared formulation at the field dilution rate:

a) Surface tension: It was measured by using the method that mentioned above.

b) pH: It was measured by using Cole-Parmer pH conductivity meter 1484-44 by using the method described by Dobrat and Martijn (1995).

c) Viscosity: It was measured by using Brookfield Viscometer Model DVII+Pro, by using the method reported in ASTM D-2196 (2005).

d) Electrical Conductivity: It was measured by using Cole-Parmer pH/Conductivity using the method described by Dobrat and Martijn (1995).

3- Bioassay.

Laboratory experiment:

Toxicity of the tested materials was conducted by using the method reported by EL- Hefny, *et al*., (2011) with some modifications.

Samples of infested acacia leaves were collected randomly from infested shrubs (each consisted of 8 leaves / treatment), kept in paper bags and transferred to the laboratory. Leaves were sprayed by using the concentrations (1, 0.5, 0.25 and 0.125 %) for the three locally prepared formulations used in the study and every concentration. Treated leaves were kept in a petri- dishes, four replicate were done for each treatment and four replicate treated by water as a control. Insepectation was conducted after 24, 48 and 72 hours from treatment by counting dead and a live of the different stages of *F. virgata* by the aid of stereomicroscope. Also, a pre count was taken for each treatment as an index; percentages of mortalities were calculated according to Abbot's formula (1925).

Statistical analysis:

Mortality percentages were calculated according to Abbot's formula (1925).

To estimate the LC_{50} values, the corrected mortality percentages were subjected to probit analysis according to Finney (1952).

RESULTS AND DISCUSSION

a- Formulation part.

Results in Table (1) showed that diesel oil, thymol and camphor were non-soluble in water, while diesel oil was miscible in xylene and acetone but immiscible with dimethyl formamide (DMF), however thymol and camphor were soluble in acetone, xylene and dimethyl formamide. All of them cleared that slightly acidic property, the acidity values as % H_2SO_4 were 0.049, 0.098 and 0.059 for diesel oil, thymol and camphor respectively.

Table 1. Physico-chemical properties of the active ingredients

Compounds	Solubility % (W/V)				Free acidity as % H_2SO_4
	water	xylene	Acetone	DMF	
Diesel oil	N.S	Miscible	Miscible	Immiscible	0.049
Thymol	N.S	142.85	100	125	0.098
Camphor oil	N.S	100	100	100	0.059

N.S = insoluble.

Data presented in Table (2) showed the physico-chemical properties for the suggested surfactants called Toximol R, Toximol H, Tween 80 and polyethylene glycol 600 di-oleate (PEG 600DO) were measured to determine if they were suitable to prepare the tested materials (diesel oil and synthetic plant materials) as emulsifiable concentrates. According to HLB values, Toximol R, Toximol H, and polyethylene glycol 600 di-oleate were considered as emulsifying agent (emulsifiers) where its values were 10- 12, whereas tween 80 was greater than 13 it could be considered as dispersing or emulsifying agent. On the other hand, these surfactants reduced the surface tension values compared with water, their values were 32, 36, 39.2 and 38.6 dyne/cm for Toximol R, Toximol H, Tween 80 and PEG 600DO respectively. All of them were acidic and the acidity values as % H_2SO_4 were: 0.49, 0.2, 0.5 and 0.88 respectively. PEG600DO had the highest CMC value followed by Tween 80, toximol H and Toximol R. the mentioned results showed clearly that, the tested surfactants were suitable to formulate diesel oil and synthetic plant materials (thymol and camphor) as emulsifiable concentrates because it acts as emulsifiers.

Table 2. Physico- chemical properties of surface active agents.

Surface active agent	Surface tension at 0.5% (dyne/cm)	CMC %	HLB	Free acidity as % H2SO4
Toximol- R	32	0.3	10-12	0.49
Toximol-H	36	0.3	10-12	0.2
Tween 80	39.2	0.5	>13	0.50
PEG 600 DO	38.6	0.9	10-12	0.88

PEG 600 DO: poly ethylene glycol 600 dioleate.

According to data presented in Table (3), the local prepared formulations (ECs) passed successfully from storage at 54 ^{0}C for three days where no observable changes in emulsion stability and foam were before and after storage. On the other hand there was a little increase in free acidity values with all prepared formulations.

Table 3. Physico-chemical properties of the locally prepared Emulsifiable concentrates (ECs) before and after accelerated storage.

Compounds	Before storage					After storage				
	Emulsion stability (ml. cream. Sep.)		Foam (cm^3)		Free acidity as	Emulsion stability (ml. cream. Sep.)		Foam (cm^3)		Free acidity as
	H.W	S.W	H.W	S.W	% H2SO4	H.W	S.W	H.W	S.W	% H_2SO_4
Diesel oil 90% EC	0	0	0	0	0.059	0	0	0	0	0.11
Diesel oil + thymol	1	0	0	0	0.186	2	1	0	0	0.24
Diesel oil + Camphor oil	0	0	1	0	0.166	0	0	2	1	0.22

H.W: Hard water (342 ppm as $CaCO_3$) S.W: Soft water (57 ppm)

As shown in Table (4) the properties of prepared emulsifiable concentrate at the field concentration (0.5%) were measured and the results obtained that, the spray solution possesses low values of surface tension and high values of viscosity compared with water values. Reducing in surface tension value of pesticide spray solution give an indication of rising spreading on the treated surface with a consequence increase in pesticide efficacy (Ryckaert *et. al.,* 2007). Increasing the viscosity of spray solution causes reducing drift, retention sticking and insecticidal efficacy (Spanoghe *et. al.,* 2007). On the other hand spray solution for all prepared formulations had the same percentage of salinity values as same as water these lower values increased the formulations biological activity, whereas all of them showed a little increase in the electrical conductivity values.

Table 4. Physico-chemical properties of spray solution of prepared emulsifiable concentrates (ECs) at field dilution rate (0.5 %).

Compounds	Viscosity (Centipoise)	Electrical conductivity (µ mhos)	% Salinity	Surface tension (Dyne/cm)
Diesel oil	2.0	436	0.2	44
Diesel oil + thymol	1.94	442	0.2	35.2
Diesel oil + Camphor	1.94	439	0.2	39.5
Water	1.0	417	0.2	72.0

b- Bioassay part:

The efficacy of prepared ECs formulations against *F. virgate*.

The toxicity of diesel oil, diesel oil + thymol and diesel oil + camphor emulsifiable concentrate formulations (ECs) were measured by conducting a laboratory experiment using serial concentrations. The results in

Table (5 & 6) indicated that diesel oil + thymol mixture was the most effective formulation against the different developmental stages of mealybug *F. virgata* followed by diesel oil + camphor and diesel oil, it means that the formulated mixture of synthesized plant oils (thymol or camphor) with mineral oil (diesel oil) was more effective than the formulated diesel oil. However the efficacy for all tested formulations was increased by increasing concentrations and the period after application.

On the other hand there was slight differences in efficacy among the different developmental insect stages, where the LC_{50} values of diesel oil + thymol for 1st, 2nd , 3rd and adults after two days were:0.17, 0.17, 0.25 and 0.25 ml/L respectively, while they were: 0.03, 0.03, 0.08 and 0.11 ml/L after three days of application. While the LC_{50} values of diesel oil + camphor for 1st, 2nd, 3rd and adults after two days were: 0.88, 0.99, 1.12 and 1.24 ml/L respectively but they were: 0.14, 0.24, 0.21 and 0.16 ml/L respectively after three days of application. Whereas the LC_{50} values of diesel oil for 1st, 2nd, 3rd and adults after two days were: 4.02, 4.51, 4.86 and 5.33 respectively, but were: 1.43, 1.49, 1.61 and 1.77 ml/L respectively after three days of application.

Table 5.The insecticidal efficacy of formulated tested materials against *F. virgata*.

materials	Conc. ml/L	% Mortality of *F. vergata*											
		1 St			2 nd			3 rd			Adults		
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Diesel oil 90% (EC)	1.25	19.6	26.8	46.3	16.4	24.8	45.4	15.8	24.4	43.4	14.6	21.9	41.2
	2.5	32.4	40.0	64.8	31.6	37.7	63.4	30.7	36.7	61.3	28.3	34.3	58.7
	5.0	35.2	54.6	80.3	34.2	52.2	78.8	34.1	50.6	77.1	32.9	48.6	74.7
	10.0	40.8	68.6	90.7	38.7	66.4	89.5	39.2	64.4	88.4	38.4	63.2	86.6
Diesel oil(65%) + thymol (10%)	1.25	50.7	81.7	97.9	49.4	80.6	97.9	47.2	78.6	96.9	41.4	72.95	91.1
	2.5	75.4	88.4	99.2	74.2	87.7	99.2	72.8	87.1	99.1	66.7	80.90	95.9
	5.0	84.6	93.3	99.7	83.1	92.7	99.7	81.7	92.9	99.8	74.3	87.23	98.3
	10.0	88.7	96.4	100	87.1	96.0	100	86.9	96.4	100	77.3	91.94	99.4
Diesel oil (70%) + camphor (10%)	1.25	35.8	57.8	96.1	34.4	55.4	93	32.4	52.8	91.0	30.3	50.2	84.9
	2.5	61.2	72.3	98.9	58.9	70.6	98.2	56.7	69.5	96.9	51.2	64.6	91.6
	5.0	70.4	83.7	99.8	69.4	82.8	99.7	67.3	82.9	99.2	63.6	77.2	95.8
	10.0	78.9	91.6	100	78.2	91.2	100	77.1	91.7	99.8	73.4	86.8	98.1

Table 6. LC$_{50}$, LC$_{90}$ and Slope values of ECs formulations against *F. virgata*.

Material	parameter	Developmental stages of *F. virgata*							
		1 St		2 nd		3 rd		Adults	
		Day 2	Day 3	Day 2	Day 3	Day 2	Day 3	Day 2	Day 3
Diesel oil 90% EC	LC$_{50}$	4.02	1.43	4.51	1.49	4.86	1.61	5.33	1.77
	LC$_{90}$	44.82	9.4	50.55	10.39	59.43	11.41	58.66	13.16
	Slope	1.2234	1.5682	1.2212	1.5188	1.1784	1.5077	1.2304	1.4706
Diesel oil(65%) + thymol (10%)	LC$_{50}$	0.17	0.03	0.17	0.03	0.25	0.08	0.25	0.11
	LC$_{90}$	3.04	0.33	3.34	0.33	3.42	0.53	7.3	1.11
	Slope	1.0186	1.2883	0.9827	1.2883	1.1204	1.5872	0.8746	1.3116
Diesel oil (70%) + camphor (10%)	LC$_{50}$	0.88	0.14	0.99	0.24	1.12	0.21	1.24	0.16
	LC$_{90}$	8.44	0.68	8.86	1.00	8.45	1.16	13.57	2.10
	Slope	1.3081	1.8335	1.3487	2.0492	1.4595	1.7396	1.2330	1.1562

Mineral oil mode of action:

Mineral oils block the insect air holes (spiracles) which insects breathe, causing them to die from asphyxiation. Also, the spread of oil through the respiratory pores and block the insect's trachea which leads to insect death. Oils may act as poisons, interacting with the fatty acid of insect and interfering with normal metabolism. Also may disrupt how an insect feeds. Dissolve the external waxy layer on the insect body causing dehydration. (Helmy *et. al.*, 2012)

CONCLUSION

The physico- chemical properties diesel oil and botanical synthetic materials (thymol& camphor) were tested as active ingredients to determine the type of formulation and if it could be miscible together or not. These materials were prepared as emulsifiable concentrates as solo or in mixtures. The prepared emulsifiable concentrates passed successfully all specified testes, then its insecticidal activity against the mealy bug *F. virgata* developmental stages under laboratory conditions were examined. The results indicated that all prepared formulations showed good insecticidal activity against the tested insect but with variable value, where diesel oil + thymol was the most effective followed by diesel oil + camphor and diesel oil alone. It means that mixing the synthetized plant oils with mineral oils increased the efficacy and decreased the effective concentration; moreover they acts as perfume to bad smell of diesel oil so these mixtures could be used for controlling mealybug in medical, aromatic and ornamental plants after conducting more semi- field and field experiments.

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سمية زيت الديزل المجهز محليا منفردا ومخاليطة مع المواد النباتية المخلقة ضد البق الدقيقى *Ferrisia virgate* (Cockerell)

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[1]المعمل المركزى للمبيدات ، مركز البحوث الزراعية ، دقى ، جيزة ، مصر .

[2]معهدبحوث وقاية النباتات ، مركزالبحوثالزراعية ، الدقى ، جيزة ، مصر .

تم دراسة الخواص الفيزيائية – الكيميائية لزيت الديزل الخام والمواد النباتية المخلقة (ثيمول – كافور) ثم تم تحضيرها فى صورة مركزات قابلة للاستحلاب فى الماء.المستحضرات المجهزة اجتازت بنجاح الاختبارات الخاصة بالمركزات القابلة للاستحلاب فى الماء .تمت دراسة سمية المستحضرات المجهزة على الاطوار المختلفة لحشرة البق الدقيقى تحت الظروف المعملية، واوضحت النتائج ان كل المستحضرات تحت الدراسة اظهرت سمية عالية على الاطوار المختلفة للحشرة المختبرة. على الجانب الاخر خلط زيت الديزل بالمواد النباتية المخلقة رفع من كفاءة زيت الديزل على البق الدقيقى بينما مخلوط زيت الديزل مع الثيمول اظهر كفاءة اعلى من مخلوط زيت الديزل مع الكافور.قيم التركيز النصفى المميت بعد يومين من المعاملة كانت ٤,٠٢، ٤,٥١، ٤,٨٦ و ٥,٣٣ مل/ لتر مع زيت الديزل بينما كانت ٠,١٧، ٠,١٧، ٠,٢٥ و ٠,١٥ مل/ لتر مع مخلوط زيت الديزل و الثيمول وكانت ٠,٨٨، ٠,٩٩، ١,١٢ و ١,٢٤ مل/ لتر مع مخلوط زيت الديزل و الكافور، وذلك على العمر اليرقى الاول والثانى والثالث والحشرة الكاملة على التوالى.بينما كانت قيم التركيز النصفى المميت بعد ثلاث ايام من المعاملة هى: ١,٤٣، ١,٤٩، ١,٦١ و ١,٧٧ مل/ لتر لزيت الديزل بينما كانت ٠,٠٣، ٠,٠٣، ٠,٠٨ و ٠,١١ مل/ لتر مع مخلوط زيت الديزل والثيمول، وكانت ٠,١٤، ٠,٢٤، ٠,٢١ و ٠,١٦ مل/ لتر لمخلوط زيت الديزل مع الكافور وذلك على العمر اليرقى الاول والثانى والثالث والحشرة الكاملة على التوالى.اوضحت النتائج ان كفاءة المستحضرات تزيد بزيادة التركيز والفترة بعد المعاملة، كذلك خلط المواد النباتية المخلقة مع زيت الديزل رفعت الكفاءة الابادية لزيت الديزل على الحشرة المختبرة.