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Insecticidal activity of diesel oil and synthetic plant materials against pink sugar cane mealybug *Saccharicoccus sacchari* (Hemiptera: Pseudococcidae)

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Abstract:

The pink sugar cane mealybug *Saccharicoccus sacchari* (Cockerell) (Hemiptera: Pseudococcidae) is considered one of the dangerous pests of sugarcane (*Saccharum officinarum* L.). The infestation of this pest caused poor growth and yellowing of the stem and leaves. 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 was investigated against different stages of the pink sugar cane mealybug *S. sacchari* 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 *S. sacchari*, whereas mixture, thymol with diesel oil was more effective than camphor with diesel oil. LC50 values after two days of application were 4.46 and 5.01 ml/l for diesel oil, while it was 0.20 and 0.24 ml/l for mixture of oil and thymol, and 1 and 1.23 ml/l for mixture of diesel oil and camphor for nymph and adult stages of *S. sacchari* respectively. While LC50 values after three days of application were: 1.51 and 1.69 ml/l for diesel oil, while it was 0.05 and 0.12 ml/l for mixture of oil with thymol, and 0.20 and 0.17 ml/l for mixture of oil with camphor for nymph and adult stages of *S. sacchari*, respectively. Results showed that the efficacy of tested materials increased by increasing concentration and period after application. Mixing botanical synthetic materials with diesel oil increased toxicity against pink sugar cane mealybug *S. sacchari*.

Introduction

Saccharicoccus sacchari (Cockerell) (Hemiptera: Pseudococcidae) is the major pest in Egypt and the world. It lives on sugarcane both under-ground and on aerial stem tissue. Crawlers infest upper internodal tissue behind leaf sheaths and develop through a series of juvenile instar stages before becoming adult. It obtains nutrition from the plant's phloem and excess carbohydrate is exuded as honeydew and the ability of *S. sacchari* to transmit virus particles. Inkerman *et al*. (1986) and Beardsley (1960) stated that the numbers of pink sugar cane mealybug increased in stem tissue develops and heavy infestations of *S. sacchari* effect of crop yield. Later Mohamed *et al*. (2009), it effects of a reduction in juice quality. *S. sacchari* is considered as a pest of continued commercial interest (Lockhart *et al.,* 1992).

Petroleum oils are highly refined, parrafinic oils that are used to manage pests and diseases of plants. Similar parrafinic oils are found in automotive and household lubricants and cleansers. Petroleum oils may be referred to many names, including horticultural oil, spray oil, dormant oil, summer oil, supreme oil, superior oil, Volck oil or white mineral oil.

Oils have been used as pesticides for centuries and are some of the most effective, safe alternatives to synthetic insecticides and fungicides. Pesticides with mineral oil as the active ingredient are used as insecticides and acaricides for treating trees and shrubs. They are available as concentrates to be diluted before use (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).Plant essential oils have been long been used in perfumes and foods, and are now being used for the development of reduced risk pesticides. Essential oil-based insecticides are considered relatively safe to humans and mixtures of essential oil constituents have been proven to be effective against many insects.

The aim of this work is to study the effect diesel oil alone and its mixture with botanical synthetic materials (thymol and camphor) against different stages of the mealy bug *S. sacchari* under laboratory conditions.

Materials and methods

1- Tested chemicals:

1.1. Active ingredient:

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

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

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

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

1.3. 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:

2.1. Active ingredient:

2.1.1. 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).

2.1.2. Free acidity or alkalinity:

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

2.2. Surface active agents:

2.2.1. Surface tension:

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

2.2.2. Critical micelle concentration (CMC):

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

2.2.3. Hydrophilic-lipophilic balance (HLB):

The (HLB) of the tested surfactants were measured by using the method reported by (Lynch and Griffin, 1974). Free acidity or alkalinity: It was measured by using the method that mentioned above.

2.3. 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.

2.4. Determination of the physico-chemical properties of the prepared emulsifiable concentrates formulation (EC):

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

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

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

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

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

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

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

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

2.5.4. 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 Eskander *et al*. (2020) with some modifications. Samples of infested sugar cane collected randomly from infested internodes and transferred to the laboratory. The nymphs and adult individuals of pink sugar cane mealybug, *S. sacchari* 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 internodes were kept in a jar, four replicates were done for each treatment and four replicates treated by water as a control. Inspection was conducted after 24, 48 and 72 hours from treatment by counting dead and a live of the different stages of *S. sacchari* by the aid of stereomicroscope. Also, a precount was taken for each treatment as an index; percentages of mortalities were calculated according to Abbot's formula (1925).

4. 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

1. 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 % H2SO4 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 % H2SO4 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 ºC 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 % H2SO4	Emulsion stability (ml. cream. Sep.)		Foam (cm^3)		Free acidity as % H_2SO_4
	H.W	S.W	H.W	S.W		H.W	S.W	H.W	S.W	
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

2. Bioassay part:

The efficacy of prepared ECs formulations against *S. sacchari* **.** 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 and 6) indicated that diesel oil + thymol mixture was the most effective formulation against the nymph and adults pink sugar cane mealybug, *S. sacchari* 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.

Table(5):The insecticidal efficacy of formulated tested materials against pink sugar cane mealybug ***Saccharicoccus sacchari.***

Material	Con. m/L	*Saccharicoccus sacchari*											
		1st nymph			2nd nymph			3rd nymph			Adult		
		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 *Saccharicoccus sacchari.*

Material	Parameter	% Mortality of *Saccharicoccus sacchari*			
		Nymphs		Adults	
		48 hrs.	72 hrs.	48hrs.	72 hrs.
Diesel oil 90% (EC)	LC_{50}	4.46	1.51	5.01	1.69
	LC_{90}	51.60	10.40	55.44	12.32
	Slope	1.2077	1.5316	1.2012	1.4101
Diesel oil(65%) + thymol (10%)	LC_{50}	0.20	0.05	0.24	0.12
	LC_{90}	3.27	0.40	7.1	1.12
	Slope	1.0406	1.3879	0.8744	1.3214
Diesel oil (70%) + camphor (10%)	LC_{50}	1.00	0.20	1.23	0.17
	LC_{90}	8.58	0.95	13.25	2.01
	Slope	1.3721	1.8741	1.231	1.0623

On the other hand there was slight differences in efficacy among the different developmental insect stages, where the LC50 values of diesel oil + thymol for nymph and adults after two days were: 0.20 and 0.24 ml/L respectively, while they were: 0.05 and 0.12 ml/L after three days of application. While the LC50 values of diesel oil + camphor for nymphs and adults after two days were: 1 and 1.23 ml/L respectively but they were: 0.20 and 0.17 ml/L respectively after three days of application. Whereas the LC50 values of diesel oil for nymph and adults after two days were: 4.46 and 5.01 respectively but were: 1.51 and 1.69 ml/L respectively after three days of application. The insecticidal efficacy of formulated tested materials against *S. sacchari* (Table 5).

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 feed. Dissolve the external waxy layer on the insect body causing dehydration (Helmy *et al.*, 2012 and Eskander *et al.*, 2020).

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prepared the materials , Diesel oil (EC) , Diesel oil + thymol (EC) 3_ Diesel oil +Camphor as emulsifiable concentrates.

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