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Larvicidal Effect of Petroleum Oils, Tar oil and Surfactants against the Mosquito, *Culex pipiens* (Dipetra: Culicidae) Larvae

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ABSTRACT

Culex pipiens in Egypt is widely distributed and is the main vector of lymphatic filariasis, West Nile virus, and Rift Valley fever. The Present study was laboratory conducted to evaluate the larvicidal effects of some local materials: petroleum oils (odourless kerosene, kerosene, solar, lubrication cut of petroleum oil and motor oil), emulsifiable oils such as CAPL2 (Central Agricultural Pesticides Laboratory) and commercial petroleum oil, tar oil, and surfactant such as Sisi6 against the mosquito, *Culex pipiens* larvae under two different conditions; at winter (18 ± 2 °C) and summer (38 ± 2 °C). The obtained results indicated that odourless kerosene, kerosene, surfactant (Sisi6) and the commercial petroleum oil (Zed-oil) were considered successful larvicides under both temperatures since they gave mortality percentage of >90% after 24 hours exposure, while solar and CAPL2 succeeded only under summer temperature but lubrication cut of petroleum oil, motor oil, and tar oil didn't succeed under both temperatures. Also, the study indicated that there is an inverse correlation of viscosity of any petroleum oil and its larvicidal effect. Moreover, toxicity was indirectly related to material viscosity. As a conclusion, for controlling larvae under any condition, it is recommended that the petroleum oil should have a viscosity of =< 12 milli-poises.

INTRODUCTION

In Egypt, *Culex (Cux.) pipiens* L. is widely distributed and is the main vector of periodic lymphatic filariasis, West Nile virus, and Rift Valley fever (Taylor *et al.*, 1956; Farid *et al.*, 2001; Abdel-Hamid *et al*., 2011 and Kenawy *et al*., 2018).

The avoidance of diseases transmitted by *Culex pipiens* requires either targeting the mosquito larvae through spraying of breeding sites or by killing the adult mosquitoes by using insecticides. Larviciding is a successful way of reducing the mosquito population in their breeding places before they emerge into adults. Using chemical insecticides in water, introduces many risks to people and environment. Furthermore, many of these insecticides such as pyrethroids are very toxic to fish and should not be used in fish or crustacean's ecosystem (Rozendaal, 1997).

The frequent use of such insecticides leads to a subversion of ecosystem and enhance resistance to chemical insecticides resulting in rebounding vectorial capacity (Kranthi *et al.,* 2002; Liu *et al.,* 2006 and Ahmed *et al.,* 2007). Recently, the environment friendly natural insecticides have been receiving attention as an alternative measure of control of arthropods of public health importance (Nathan *et al.,* 2005).

Initially, petroleum products such as kerosene, motor oil, and waste oil, have used as 'ancestral insecticides' which produced spectacular results as larvicides in several communities and were advocated for vector control by several national malaria control programmes. Their chemical composition appears to confer them immense insecticidal properties. Their properties should be properly researched as they could potentially be exploited in areas of insecticide resistance. The spreading of kerosene on mosquito breeding sites was among the first strategy adopted by early malaria control programmes (Burton, 1967). This traditional vector control method is still in use in communities where the populations are poor and do not have the financial resources to employ conventional insecticides (Djouaka *et al.,* 2007).

In the present study, the toxicity of mineral oils such as odourless kerosene, kerosene, solar, lubrication cut of petroleum oil, motor oil and CAPL2 and a surfactant (Sisi6) were examined against *Cx. pipiens* larvae in comparison with commercial petroleum oil in an attempt to find alternative pesticides and control measures that pose no risk or posing minimal risk to human health and the environment. These materials are natural, inexpensive and locally available.

MATERIALS AND METHODS

Test Mosquito:

A colony of *Culex pipiens* was established from larvae originally collected from stagnant breeding water at Al-Marg city, Cairo Governorate and laboratory maintained at 27 ± 2 °C temperature, 70 ± 10% RH and 12: 12 light and dark photoperiod following the methods described by El-Sisi *et al.* (2019). After breeding of several generations, larval specimens were selected for running the bioassay experiments.

Materials Used:

1. Glass beakers of 500 ml capacity.
2. Different micro-pipettes measuring from 5 µl to1000 µl.
3. Disposable tips suitable for micropipettes for measuring different doses for each tested material.
4. Plastic Pasteur pipettes for collecting mosquito larvae
5. Disposable plastic cups where mosquito larvae were collected from the colony to be tested.
6. Graduated measuring cylinders of 25 ml and 250 ml capacity
7. Dechlorinated water: Removing of chlorine from water by filling a bucket with tap water and leaving the bucket without cover at room temperature for 24 hours to allow the chlorine to evaporate naturally.

Tested Materials:

1. Petroleum oils supplied from Petrol stations such as odourless kerosene, kerosene, solar and motor oil.
2. Lubrication cut of petroleum oil: produced by the Amreya Petroleum Refining Company, Alexandria, Egypt.
3. Tar oil: a crude material from the waste distillation of charcoal under 550 °C at the New & Renewable Energy Authority (NREA), Seventh district, Nasr City, Egypt.
4. Surfactant (Sisi6, 10% SL.): a surface-active agent capable of reducing the surface tension of water. It was prepared by Dr. Ahmed G. El-Sisi through the neutralization of sulphuric acid with a suitable alkaline.
5. Emulsifiable oils:
    a. CAPL2 (96.6% EC): a lubrication cut of petroleum oil formulated as an emulsifiable concentrate by Dr. Ahmed G. El-

Sisi. It was registered at no. 502 for controlling scale insects infesting citrus crops.

b. Commercial petroleum oil: ZED-OIL 94% (Kafr El-Zayat company for pesticides & chemicals) containing: Mineral oil 94%, Geronal FF/4 (surfactant) 2%, Rhodacal 60/BE (spreading agent) 1% and Amesolv CME (solvent) up to 100 ml.

Measuring Viscosity of the Tested Materials:

The viscosity of each material was measured using a viscometer (Stalgometer) at two temperatures of 18 ± 2 °C (winter) and 38 ± 2 °C (summer).

Testing the Toxicity of Materials against *Cx. pipiens* Larvae:

The bioassays tests were conducted on third instar larvae in Mosquito Research Department at Research Institute of Medical Entomology according to the standard procedure (WHO, 2003 & 2005) with some modifications. Two experiments were carried out. The 1st one was to examine the toxicity of the different materials by using 500 µl of the material and counting the larval mortality. Materials that showed low toxicity (or nontoxic) were canceled and the rest were exposed to the 2nd experiment that examined the larval susceptibility level as follow: for each material, a wide range of doses that gave 10% to 95% mortality were tested to determine the LD_{50} and LD_{90} (the doses of the material that produce 50% and 90% mortality, respectively in the exposed larvae after 24 hours). Each test was conducted twice in three replicates, at winter where the temperature was 18 ± 2 °C and summer where the temperature was 38 ± 2 °C. For each material dose, a 500 µl volume was added to 250 ml of dechlorinated water in a beaker as testing media to which 25 late third instar larvae and traces of larvae food (grinded rusk and yeast) were added. A control replicate was prepared by keeping 25 larvae in a beaker containing only 250 ml of water. Mortality was counted after 24 hours of exposure. Larvae that have pupated during the test period were canceled. If more than 10% of the control larvae pupated in the course of the experiment, the test was discarded and repeated. When the control mortality was between 5% and 20%, it was corrected according to Abbott's formula (Abbott, 1925):

Mortality (%) = $(X – Y)/ Y \times 100$, where X = percentage survival in the untreated control and Y = percentage survival in the treated sample.

Statistical Analysis:

The obtained toxicity data were fitted to the log-probit model according to Finney (1971) using an LDP line program (Ehab_Soft, http://www.ehabsoft.com/ldpline) and LD_{50} and LD_{90} were determined for each material. The toxicity index was calculated as follows: LD_{50} (of the most effective material) / LD_{50} (of the required material) x 100. The Simple Regression analysis was used to examine the relation of toxicity with the viscosity of the tested materials. The regression equation was in the form of Toxicity= a + b viscosity, where (a) is a constant (intercept), (b) is the slope (regression coefficient). The slope was tested for deviation from 0 by t-test. The SSP (Smiths Statistical Package) software (Smith, 2004) was used for such analysis.

RESULTS AND DISCUSSION

Toxicity of the Tested Materials:

Laboratory studies were carried out under two different conditions; at winter where the temperature was 18 ±2 °C and at summer where the temperature was 38 ±2 °C to evaluate the toxicity of each material to *Cx. pipiens* larvae.

Preventing the larvae, which live underwater but come periodically to the surface to breathe, from developing into full-grown mosquitoes is by spraying the surface of the water with suitable material with high larvicidal activity. Such materials penetrate the siphon (breathing-tubes) of the larvae, and kill them either by suffocation or by true toxic action (Lord, 1941).

Results (Table 1) indicate that all the tested materials gave different mortalities at

500 µl/250 ml water. These larval mortalities after 24 hours of exposure increased in summer than in winter for most of the tested materials. Odourless kerosene, kerosene, surfactant as Sisi6 and commercial petroleum oil as Zed-oil were considered successful larvicides under both temperatures since they gave mortality percentages of >90%, while solar and CAPL2 succeeded only under summer temperature but lubrication cut of petroleum oil and motor oil gave low mortality. The tar oil had no toxic effect, so it was excluded from testing the susceptibility level.

Table 1: The Toxicity of the tested materials against *Culex pipiens* larvae and their viscosities (milli-poises, m.p.) in winter and summer:

Materials	Winter		Summer	
	Toxicity (%)	Viscosity (m.p.)	Toxicity (%)	Viscosity (m.p.)
Odourless kerosene	100	10	100	9. 7
Kerosene	94.7	12	98.7	10.7
Solar	76	12.7	94.7	10.8
Lubrication cut of petroleum oil	40	23.7	69.3	15.1
Motor oil	4	303.2	68	131.2
Tar oil	2.7	9.7	0	9.4
CAPL2	24	29.1	100	21
Sisi6	98.7	10.7	100	10
Commercial petroleum oil	94.7	24.6	100	15.3

Correlation of Viscosity of the Tested Materials with Their Toxicity against Mosquito Larvae:

Viscosity is a measure of the resistance of a fluid to a force applied to it or that experienced by a body moving through it. In other words, it indicates the thickness of a liquid. Thus, water is low viscous while honey is highly viscous. By heating liquids, its thickness gets reduced or in other words, its viscosity is reduced. This indicates that viscosity is a function of temperature (Soliman, 1987). Therefore, viscosity was measured for each material at the two experimental conditions (Table 1).

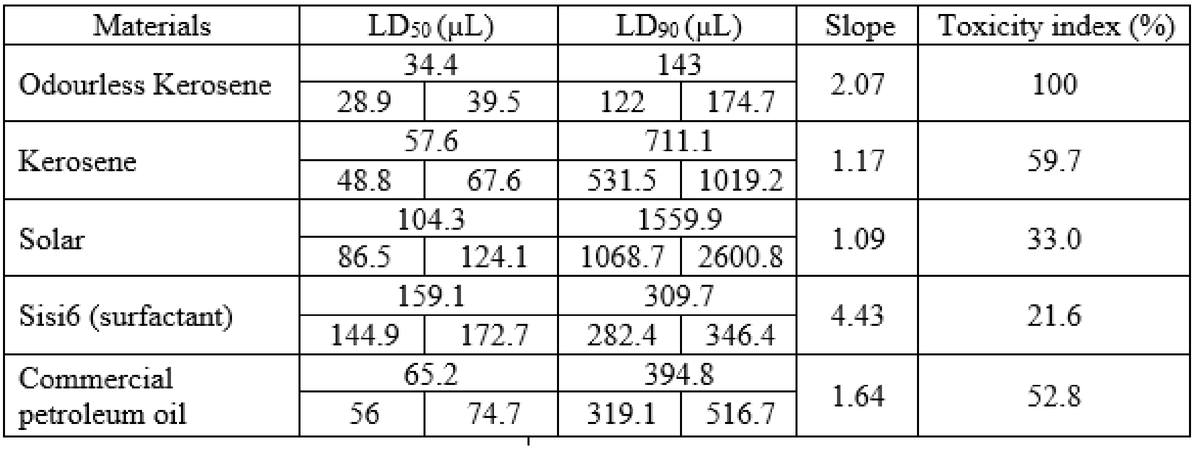
Comparing the viscosity of each petroleum oil (odourless kerosene, kerosene, solar, lubrication cut of petroleum oil and motor oil) in summer and winter showed that it varies with temperature. Generally, viscosity was lowered by increasing temperature, *i.e.* it increased in winter (18 ±2 °C) and decreased in summer (38 ±2 °C) in agreement with Soliman (1987) and Sorab (1991) observations. Also, results illustrate that odourless kerosene was the highest toxic oil when tested at 500 µl/250 ml water against larvae although it has lower viscosity in summer (9.7 m.p) and winter (10 m.p.). The motor oil was the lowest toxic oil as well as the highest viscous oil in winter (303.2 m.p) and summer (131.2 m.p). The viscosities of emulsifiable oils such as CAPL2, commercial petroleum oil, and surfactants as Sisi6, were not proportional to the toxicities as these three materials were effective against larvae in summer (100% mortality) although they have different degrees of viscosities, while their toxicities decreased and their viscosities increased in winter. The commercial petroleum oil with its different contents was higher in toxicity (94.7%) than CAPL2 (24%) had a lower viscosity (24.6 m.p) than CAPL2 (29.06 m.p). Although Tar oil was the lowest viscous oil in winter and summer, it had no toxic effect against *Cx. pipiens* larvae. Therefore, with these results, it can be concluded that the efficacy of petroleum oils, emulsifiable oils, and surfactants against *Cx. pipiens* larvae increased while their viscosity decreased so their efficacy against larvae increased in summer than in winter, also petroleum oils with viscosities that were equal or lower than 12 m.p., had a highly toxic effect against larvae. Moreover, the regression analysis indicated that toxicity had an indirect relation with viscosity, *i.e.* it decreases as viscosity increased (b= -0.27 and -0.10, P>0.05 in winter and summer, respectively). The computed values of correlation coefficient (R) indicated that the regression model of the viscosity was accounted for 0.52 and 0.14 of the variance in larval toxicity and the remaining 48.0% and 86% in winter and summer, respectively are attributed to other factors. However, as far as we are aware, no available observations on the toxicity- viscosity relationship.

Susceptibility Levels of Larvae to the Tested Materials:

. According to Lord (1941), if the viscosity of the oil was reasonably low, penetration into the siphon of the larva becomes available. The results showed that by testing the petroleum oils against *Cx. pipiens* larvae, when the viscosity was equal or lower than 12 m.p., the dose required for high toxicity will be decreased, thus this can minimize the usage of larvicidal oils in summer than in winter.

The results (Table 2 and Fig. 1) show toxicity of the different materials at winter and indicate that the odourless kerosene was the highest toxic material (LD_{50} = 34.4 µl and toxicity index= 100%) followed by kerosene and commercial petroleum oil which showed LD50 values of 57.6 µl and 65.2 µl and lower slopes of 1.17 and 1.64 for kerosene and commercial petroleum oil, respectively. However, Solar gave high toxicity at larger doses than other lower viscous petroleum oils where its LD_{50} was 104.3 µl and slope of 1.09 which was lower than that of odourless kerosene (2.07). The Sisi6 as a surfactant required a higher dose to achieve toxicity against larvae (LD50 of 159.1 µl and toxicity index of 21.6%) in comparison to odourless kerosene (Table 2 and Fig. 2). While other materials (lubrication cut of petroleum oil, motor oil, tar oil, and CAPL2) were excluded to be tested as their toxicities were lower than 50% (Table 1).

Table 2: The toxicity data of the tested oils against *Culex pipiens* larvae at winter:



Materials	LD_{50} (µL)		LD_{90} (µL)		Slope	Toxicity index (%)
Odourless Kerosene	34.4		143		2.07	100
	28.9	39.5	122	174.7		
Kerosene	57.6		711.1		1.17	59.7
	48.8	67.6	531.5	1019.2		
Solar	104.3		1559.9		1.09	33.0
	86.5	124.1	1068.7	2600.8		
Sisi6 (surfactant)	159.1		309.7		4.43	21.6
	144.9	172.7	282.4	346.4		
Commercial petroleum oil	65.2		394.8		1.64	52.8
	56	74.7	319.1	516.7		

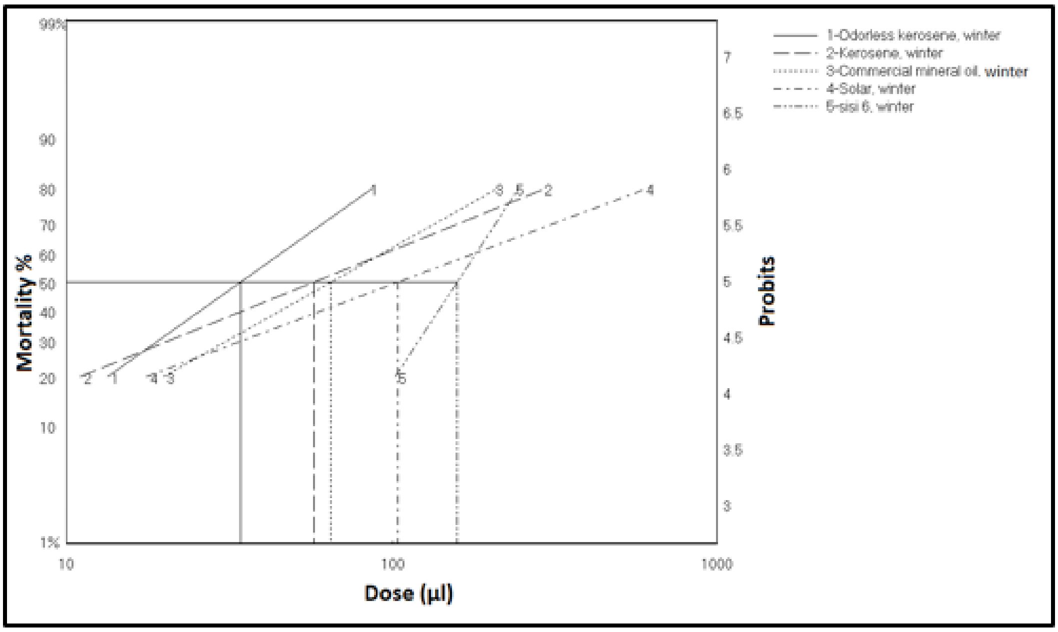


Fig. 1: Toxicity lines of different materials tested against *Culex pipiens* larvae at winter showing LD_{50} differences:

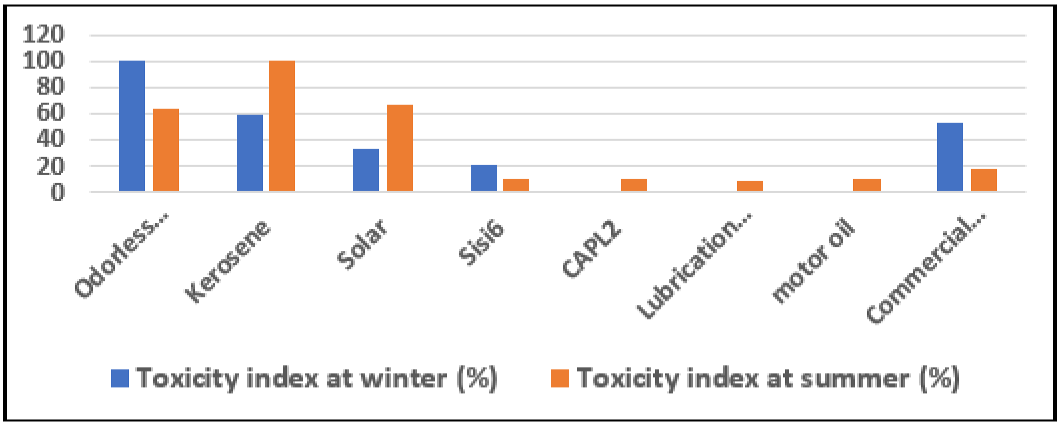


Fig. 2: Toxicity indices of different materials tested against *Culex pipiens* larvae

Results in Table (3) and Figure (3) illustrate the toxicity of the different materials in summer. Generally, the toxicity of the tested materials in winter differed from that in summer and LD50 values in summer were lower than those in winter. Furthermore, the highly viscous materials that were excluded in winter showed higher toxicity in summer with large doses.

The lower viscous petroleum oils; odourless kerosene, kerosene, and solar showed the lowest values of LD_{50} (9.6 µl, 6.2 µl, and 9.3 µl, respectively). While the higher viscous petroleum oils: lubrication cut of petroleum oil and motor oil exhibited a higher LD_{50} value of 68.6 µl and 154.5 µl for the two materials, respectively**.** These results agree with those of Jouaka *et al.* (2007).

The emulsifiable oils exhibited lower toxicity indices in comparison to the lower viscous petroleum oils, but commercial petroleum oil showed higher slope (2.08) and lower LD_{50} (34.1 µl) than CAPL2 (slope = 1.67 and LD_{50} of 60.2 µl) therefore commercial petroleum oil was more toxic than CAPL2 at lower doses**.**

The bioassay of Sisi6 as a surfactant indicated that it had a toxic effect against *Cx. pipiens* larvae at summer and winter (Figs.1 and 3), with a lower dose, was adequate at summer to kill larvae than at winter (LD_{50}

values of 57.2 µl and 159.1 µl at summer and winter, respectively) where its viscosity differed at summer and winter (10 m.p. and 10.7 m.p., respectively) in agreement with Soliman (1987) and Sorab (1991) reports.

Similarly, odourless kerosene, kerosene, and solar showed lower LD_{50} values in summer than those in winter (Figs. 1 and 3) therefore it could be recommended to use them in summer with lower doses than in winter. Also, they displayed the lowest LD_{50} values at summer while odourless kerosene, kerosene, and commercial petroleum oil displayed the lowest LD_{50} values in winter (Tables 2 and 3) in comparison with the other materials

Table 3: Toxicity data of the tested materials against *Culex pipiens* larvae at summer:

Tested materials	LD_{50} (µL)		LD_{90} (µL)		Slope	Toxicity index (%)
odourless Kerosene	9.6		76.7		1.42	64.6
	7.5	11.8	61.6	100.9		
Kerosene	6.2		32.5		1.78	100
	4.7	7.6	26.7	41.9		
Solar	9.3		54		1.68	66.7
	6.5	12	44.5	69.5		
Sisi6	61.9		97.4		6.50	10.0
	58.6	65.1	90.5	107.4		
CAPL2	60.2		351		1.67	10.3
	51.7	69	280.8	468.4		
Lubrication cut of petroleum oil	68.6		4830.8		0.69	9.0
	50.1	89.1	2845.7	9957.9		
Motor oil	154.5		2008.6		1.15	4.0
	132.5	180.5	1449.1	3027.6		
Commercial petroleum oil	34.1		140.6		2.08	18.2
	29.9	38.6	114.19	185.4		

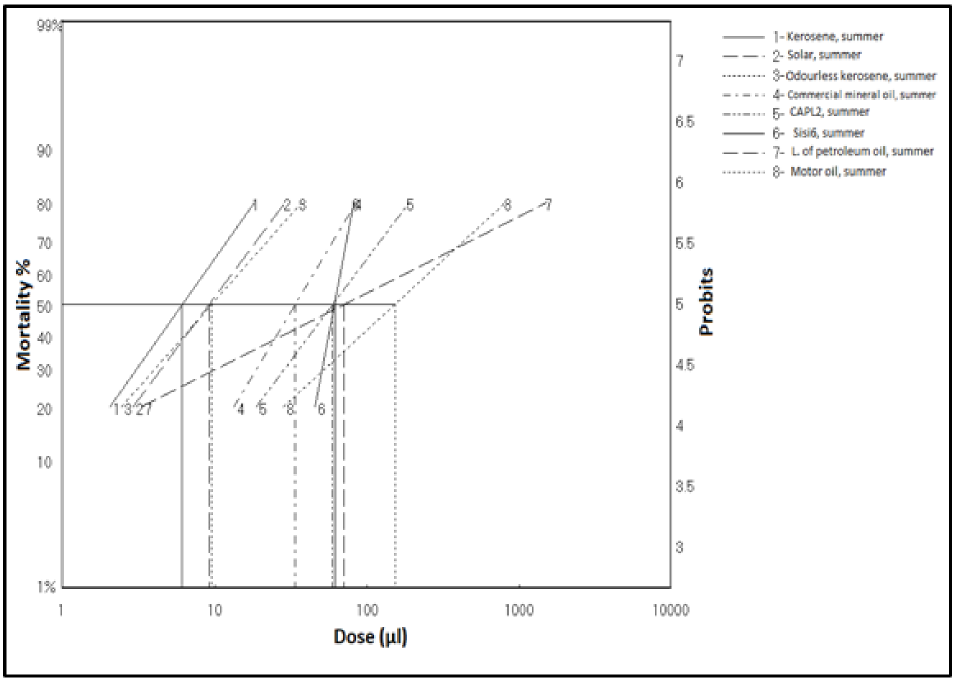


Fig. 3: Toxicity lines of different materials tested against *Culex pipiens* larvae at summer showing LD_{50} differences

Generally, for all tested petroleum oils, viscosity is the factor on which their larvicidal action depends. The penetration into the siphons of the larva is important, which can only take place if the viscosity of the oil is reasonably low. It is possible, for example, for larvae to live several days under a film of highly toxic aromatic extract from a lubricating oil base, because the viscosity of the oil is too high for effective penetration into the trachea (Lord, 1941).

The insecticidal efficacy of any surfactant increased by its ability in decreasing the surface tension of water (El-Hariry and El-Sisi, 1991), since it might solve the epi-cuticle layer of insects as a result of its emulsifying effect then cause mortality (Rizk et al., 1999).

CONCLUSION

It can be concluded that most of the tested materials had remarkable larvicidal efficacy against mosquito, *Culex pipie*ns larvae but with different doses according to seasonal conditions and their toxicities, thus intensive studies are needed to test their toxicity on other beneficial organisms as well as their efficacy under field conditions.

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ARABIC SUMMARY

تقييم سمية الزيوت البترولية والزيت القطراني والمواد ذات النشاط السطحي ضد يرقات بعوضة الكيولكس بيبينز

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أجريت تجارب معملية لتقييم سمية بعض المواد البترولية صيفاً وشتاءً مثل الكيروسين, الكيروسين عديم الرائحة, السولار, قطفة زيت بترولي, زيت الموتور وكابل2 وأيضآ مادة لتقليل التوتر السطحي مثل سيسي6 ومقارنتهم بسمية زيت بترولي تجاري. أوضحت النتائج أن أغلب المواد المختبرة لها تأثير سام على يرقات البعوض عند جرعات محددة لكل منهم طبقاً لاختلاف السمية ودرجة اللزوجة وتناسب درجة اللزوجة عكسياً مع درجة الحرارة, كذلك تناسب درجة اللزوجة عكسياً مع السمية ضد يرقات البعوض وذلك للزيوت البترولية. أما الزيت القطراني فليس له تأثير سام على يرقات البعوض.

وقد تبين من هذه النتائج أن الكيروسين النقي والكيروسين والسولار وسيسي6 والزيت البترولي التجاري كان لكل منها تأثير سام على اليرقات صيفاً وشتاءً حيث سجلت LD_{50} شتاءً لكل منها 34،4 , 57،6 , 104،3 , 159،1 , 65،2 ميكرو ليترعلى التوالي. بينما سجلت LD_{50} صيفاً 9،6 , 6،2 , 9،3 , 61،9 , 60،2 , 68،6 , 154،5 , 34،1 ميكرو ليتر لكلٍ من الكيروسين النقي والكيروسين والسولار وسيسي6 وكابل وقطفة الزيت البترولي وزيت الموتور والزيت المعدني التجاري على التوالي. وثبت أن الزيوت البترولية الناجحة التي تعطي نسبة موت >= 90% هي تلك التي لها لزوجة تعادل 12 ملليبواز أو أقل تحتظر وف التقييم سواء صيفاً أو شتاءً.