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FORMULATION OF THYMOL (*Thymus vulgaris*) AS OIL IN WATER EMULSION (EW) AND DETERMINATION ITS HERBICIDAL EFFECT AGAINST MONO AND DICOTYLEDONOUS PLANTS

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ABSTRACT

An essential oil thymol (crystals) *Thymus vulgaris* prepared as Oil in water Emulsion (EW) 10% and the formula passed successfully through emulsion stability test before and after accelerated storage at 54 ± 2 °C for three days and all the physico-chemical tests. Herbicidal efficacy of prepared 10% EW formulation against mono and dicotyledonous crops (wheat and cucumber) and weeds (*Phalares minor* Retz, *Portulaca oleraceae*) were assessed under laboratory and greenhouse conditions, using Petri-dishes, drench technique as pre emergence, and foliar technique as post emergence herbicidal activity. Germination, roots and shoots growth (length) were assessed as indicators for herbicidal activity. The obtained results indicated that, thymol 10% EW has high herbicidal activity against mono and dicotyledonous crops (wheat& cucumber) and weeds *Phalares minor* Retz and *Portulaca oleracea*. Cucumber was more sensitive than wheat and both crops shoots were more sensitive than roots under laboratory and greenhouse conditions where 400 ppm was the concentration that showed completely inhibition of germination with wheat and cucumber plants under laboratory conditions, while 3200 ppm showed completely inhibition of germination with wheat, cucumber, portulaca, and phalares. However the EC₅₀ values for wheat germination, root length and shoot length under laboratory

conditions were: 104.8, 97.31, and 79.4 ppm respectively. While its values under greenhouse conditions were, 1242.8, 1244.85, 1008.8 ppm respectively. While, for Cucumber were, 86.6, 53.2, and 52.66 ppm respectively under laboratory conditions and 1063.8, 918.97, and 760.04 ppm respectively under greenhouse conditions. These results indicated that thymol 10% EW has pre emergence herbicidal activity against mono, and dicotyledonous weeds.

Key words: Essential oil, EW formulation, Pre emergence herbicide.

INTRODUCTION

Weeds are the major pests of the crop plants; therefore, herbicides are accounted for 50 % of the total pesticides used in the world. Over 75 % of herbicides are used in developed countries. Extensive use of herbicide causes human health and environmental problems. Most of the herbicides found in groundwater and surface water due to their extensive use in agriculture, forests, pastures, lakes, parks, root-sides as well as home lawns. The susceptible annual weeds are killed while the more difficult perennial weeds invade the area.

Synthetic herbicides cannot be used to control weeds in the organic farming systems. Hence, hand hoeing, inter-row tillage, mulching and ground covering are the major approaches to control weeds. However, these techniques are costly and may not adequately control weeds. Therefore, naturally occurring biologically active compounds from plants receive more attention in recent years as a rich source of potential weed-control agents. Essential oils represent a rich potential source of an alternative and environmentally acceptable weed control compounds. **Uremis *et al.* (2009)**

Phalaris minor Retz. Family Poaceae, commonly known as Small-seeded or little seed canary grass, is a troublesome weed of wheat fields in India **Singh. *et al.*, (1999).**

Conventional formulations could cause problems related to environmental protection, leaving residues in ecosystem food, final products, etc. Hence, there is a growing demand for use of environmental friendly water based formulations as oil-in-water emulsions, aqueous suspension concentrates, aqueous capsule suspensions and so on instead of conventional pesticide formulations. These formulations are tended not only to replace toxic, non-degradable ingredients from formulations, but also to increase the

efficacy of products through a proper choice and a balance of all components in the formulation **Knowles, (2005)**.

Oil-in-water emulsions can reduce phytotoxicity, ecotoxicity and dermal toxicity, have a higher flash point than EC and are safer in transport and storage; also EW are more compatible with water based SC formulations for blends of active ingredients. **Salvica Gašić, et al (2012)**.

The aim of this study is preparing of thymol as suitable formulation (oil in water emulsion) and testing its herbicidal effect against both economic crops and weeds to be used as an alternative for conventional herbicides.

MATERIALS AND METHODS

1. Chemicals

a) Active ingredient:

Thymol extra pure ($C_{10}H_{14}O$) supplied by EL-Gomhoria Co., Cairo, Egypt.

b) Surface active agents:

1. Sodium dodecyl sulfate, supplied by EL-Gomhoria Co., Cairo, Egypt.
2. Tween 20 and Tween 80, supplied by EL-Gomhoria Co., Cairo, Egypt.
3. Poly ethylene glycol 600 di-oleate, produced by the National Co., for yeast and detergent, Alexandria, Egypt.

c) **Solvents:** Xylene (Dimethyl benzene), acetone and N,N-dimethyl formamide (DMF), Supplied by EL-Nasr pharmaceutical, chemicals co.

2. Determination of the physico- chemical properties for the constituents of formulation.

Active ingredient:

a) **Solubility (w/v):** it was determined by measuring the volume of distilled water, xylene, acetone and dimethyl formamide (DMF) for complete solubility of 0.5 gram of each material. Then % solubility calculated according to this equation.

% solubility= weight of material/ solvent or water volume x 100

b) **Free acidity or alkalinity:** it was determined according to **CIPAC (2002)**

Surface active agents:

- a) **Free acidity or alkalinity:** it was determined according to **WHO specifications (1979)**
- b) **Hydrophilic- lipophilic Balance (HLB):** the solubility of a surfactant in water can use a guide in approximating their HLB and their usefulness **Lynch and Griffin, (1974)**
- c) **Critical micelle concentration (CMC):**

The concentration of surfactant which no decrease in surface tension obtained by increasing the surfactant concentration. it was determined according to **Osipow, (1964)** Surface tension was measured by Du-Nouy tensiometer for solutions containing 0.1- 1 % surfactant according to ASTM- 1331.

3. Preparation of thymol as Oil in water Emulsion (EW) 10%.

Several trials were conducted to prepare thymol as oil in water emulsion (EW) according to **Salvica Gašić, et al (2012)**, emulsion stability, foam and free acidity or alkalinity were studied before and after storage at $54 \pm 2^\circ\text{C}$ for three days to determine the successful formula according to **CIPAC (2002)**.

4. Determination the physico- chemical properties of the prepared EW formulation.

- a) **Emulsion stability:** it was measured according to **CIPAC (2002)**
- b) **Foam:** it was measured according to **CIPAC (2002)**.
- c) **Free acidity or alkalinity:** it was determined as mentioned before.
- d) **Stability at 0 °C. (Cold storage)** It was measured according to **CIPAC (2002)**
- e) **Stability at elevated temperature $54 \pm 2^\circ\text{C}$ (accelerated storage)**
It was measured according to **CIPAC (2002)**

5. Determination the physico- chemical properties of the spray solution at the expected application rate (0.5%) of formulated thymol 10% EW.

- a) **Surface tension:** it was determined as mentioned before.
- b) **Viscosity:** determined according to **ASTM (2005)**.
- c) **pH value:** it was determined by Cole – Parmer pH/ Conductivity meter 1484-44 according to the method of **Dobrat and Martijn, (1995)**
- d) **Electrical conductivity:** it was determined according to of **Dobrat and Martijn, (1955)**

6. Bioassay:

a- under laboratory condition (Petri-dishes).

The inhibitory effect of formulated essential oil thymol 10 % EW was studied in a preliminary test according to the procedure described by **Sharkhiz, et al. (2008)** with some modifications as described below:

Seeds Wheat as pattern for monocotyledonous and cucumber as pattern for dicotyledonous were germinated in petri dishes (9 cm diam.) on filter paper (whatman No. 3) ten seeds put on a sterilized filter paper, the concentrations of the 10% EW (50, 100, 200, and 400 ppm were prepared by dilution in tap water and 6 ml. of each concentration pipetted on sterilized filter paper, 4 replicate of each concentration (for wheat and cucumber), 4 replicate for each crop were wetted only by 6 ml. tap water as control. Petri dishes containing treated seeds were sealed with (PVC) electrical insulating tape, and then incubated under laboratory conditions in dark. The number of germinated and non-germinated seeds and radical length for roots and shoots were recorded.

b - Under greenhouse conditions (drench technique):

1) Efficacy against sensitive plants:

This experiment conducted according to **Hussein, (1989)**. With some modifications as described below:

Four small plastic pots for each concentration were filled till up to 2 cm down their lower surface by sand + peat mouse (3:1) then irrigated at field capacity ten grains of wheat or cucumber seeds were planted in each pot and filled with soil mixture. The tested concentrations were 400, 800, 1600, and 3200 ppm which prepared by dilution in tap water and 10 ml. of each concentration pipetted on top of pot as a drench technique. 4 replicate of each concentration (for wheat and cucumber), 4 replicate for each crop were wetted only by 10 ml. tap water as control. The number of germinated seeds and radical length for roots and shoots were recorded.

2) Efficacy against harmful weed seeds (drench technique):

The efficacy of thymol 10% EW were assessed against *Phalres minor Retz.* as pattern for monocotyledons and *Portulaca oleracea* as pattern for dicotyledons weeds by the same method that mentioned before. While the weed seeds were difficult to count so 0.15 gr seeds

of *Portulaca oleracea* and 0.25 gr seeds of *Phalres minor* Retz was planted in experimental pots.

3) Efficacy against sensitive plants (spraying technique)

Four plastic pots for each concentration were filled with sand + peat mouse (3:1) ten grains of wheat or cucumber seeds were planted in each pot and irrigated, left until the seeds grown up then the pots were sprayed by concentration of spray solution of the thymol 10 EW, left for about seven days, irrigated with water daily according to need, then compared with untreated pots taken as control (Hussein, 1989).

Statistical analysis:

Inhibition percentages were corrected using **Abbott's formula (1925)**, and the concentration inhibition regression lines were drawn according to the method of **Finney (1952)**.

RESULTS AND DISCUSSION

1. Formulation Part:

a) Physico- chemical properties of formulation constituents:

1) Active ingredient:

Data in **Table (1)** indicated that thymol crystals non soluble in water, while, soluble in xylene and miscible in acetone and dimethyl formamide. It has low acidity and its value was % 0.098, and low melting point (50 °C) so it suitable for EW formulation.

Table (1) the physico- chemical properties of the active ingredient (thymol).

% Solubility at 25 °C (w/v.) in				Free acidity % as H ₂ SO ₄	Melting point °C
Water	acetone	Xylene	DMF		
N.S	142.85	100	125	0.098	50

2) Surface active agent:

Data in **Table (2)** showed that sodium dodecyl sulfate soluble in water but it was non soluble in acetone and xylene. PEG 600DO soluble in acetone and xylene, while give emulsion in water, (SDS, tween 80 and tween20 soluble in water, acetone and xylene. Surfactant soluble in water such as tween 80 and tween 20 should be

used as wetting and spreading agent, while those give emulsion in water and soluble in acetone and xylene should be used as emulsifiers for emulsifiable concentrates.

SDS, tween 80 and tween 20 had high HLB value so it suitable to be used as dispersing and suspending agent, while PEG 600DO had HLB value (10- 12) suitable to be used as emulsifying agent. Also SDS and PEG 600DO were alkaline and the alkalinity %as NaOH were 0.027 and 0.3 respectively. While tween 80 and tween 20 showed slightly acidic. .

PEG 600DO had the height CMC value, followed by tween 80, SDS and tween 20 were their CMC values were 0.9, 0.5 ,0.3 and 0.2 %(w/v) respectively. It could be stated that local surfactant proved effectiveness in stabilizing the formed emulsions and diminishing the high surface tension of water (**Eskander, 2008**) SDS was the best one in reducing surface tension of water followed by PEG 600 DO, tween 20 and tween 80.

Table (2) the physico- chemical properties of the suggested surface active agent.

Surfactant	% Solubility at 25 °C (w/v)			HLB	Free		CMC at 25 °C % (w/v)	Surface tension at 0.5%
	water	acetone	Xylene		Acidity % as H ₂ SO ₄	Alkalinity % as NaOH		
Sodium dodecyl sulfate	36	N/S	N/S	18	-	0.027	0.3	29
PEG 600 di-oleate	Emulsion	24	23.9	10-12	-	0.3	0.9	39.1
Tween 80	26	58.7	56.3	15	0.06	-	0.5	46.3
Tween 20	42	67.1	61.4	16	0.42	-	0.2	41.2

b) Preparation of an essential oil (thymol) as Oil in water Emulsion (EW) 10 %.

The oil-in-water emulsion (EW) was prepared by progressively adding oil phase in water phase under stirring. The oil phase was prepared by dissolving the active ingredients in suitable solvent.

Data in **Table (3)** indicated that, the prepared EW formulation passed successfully through Emulsion stability test with hard and soft

water at the concentration 5%(v/v), where no oily or cream separation formed, also passed form cold storage $0\text{ }^{\circ}\text{C} \pm 2$ for 24hours, the foam formed was less than WHO specifications. No observable changes in emulsion stability; foam and free acidity of prepared (EW) formulation after accelerated storage at $54 \pm 2\text{ }^{\circ}\text{C}$ for three days only slightly decrease in acidity.

Table (3) the physico- chemical properties of the prepared Oil in water emulsion (EW).

Type of water	Emulsion stability (ml.cream sep.)	Foam (CM3)	Free acidity % as H_2SO_4	Cold storage at $0\text{ }^{\circ}\text{C} \pm 2$ for 24hours	Accelerated storage at $54 \pm 2\text{ }^{\circ}\text{C}$ for 3 days		
					Emulsion stability (ml.cream sep.)	Foam (CM3)	Free acidity % as H_2SO_4
Hard water	0.0	6	0.277	passed	0.0	7	0.19
Soft water	0.0	4			0.0	5	

Data in **Table (4)** indicated that, spray solution of thymol prepared as 10%EW formulation had low surface tension value 32.47 dyne/cm than water and high viscosity value 1.94 cm poise, while it has acidic pH value 5.99. These results agreeable with **Ryckaert *et al.*, (2007)** who indicated that, the reduction in surface tension of spray solution cause a good wettability, spreading and depositing of the particles of the solution on the treated surfaces.

Table (4) the physico- chemical properties of the prepared EW 10% spray solution at 0.5% concentration

Materials	Surface tension (dyne/cm.) at $25\text{ }^{\circ}\text{C}$	pH value	conductivity μ mhos	Viscosity (cm poise)
EW 10%	32.47	5.99	93.4	1.94
Water	72	7.8	396	1.0

2. Herbicidal Efficacy Bioassay:

a) Under laboratory condition (Petri-dishes technique):

Table (5) % inhibition of germination and growth of shoots and roots in wheat and cucumber under laboratory conditions.

Plants	Conc. (ppm)	%inhibition of germination after		% inhibition of growth After 7 days	
		3 days	7 days	Roots	shoots
Wheat	50	21.5	6.25	8.2	24.31
	100	75.26	47.91	53.35	63.69
	200	93.54	89.58	95	91.78
	400	100	100	100	100
cucumber	50	18.57	24.28	47.75	48.97
	100	57.14	57.14	78.77	79.00
	200	85.71	90.71	97.55	98.76
	400	100	100	100	100

Table (6) EC₅₀, EC₉₀ and Slopes of Germination, roots length, and shoots length inhibition under laboratory conditions.

plants	EC ₅₀			EC ₉₀			Slope		
	G	RL	SL	G	RL	SL	G	RL	SL
Wheat	104.8	97.31	79.4	198.57	189.63	185.89	4.616 ± 0.4258	4.4234 ± 0.3782	3.466 ± 0.371
cucumber	83.73	53.2	52.66	204.22	133.9	126.7	3.3100 ± 0.3596	3.1982 ± 0.4164	3.3603 ± 0.4350

G = germination

RL= root length

SL = shoot length

Data in **Table (5&6)** indicated clearly that an essential oil thymol prepared as 10% EW formulation had complete (100%) inhibition in germination, root and shoot growth on wheat and cucumber seeds (mono and dicotyledonous crops) at the high tested rate 400 ppm. Data in **Table (5)** showed that its effect on wheat after 3 and 7 days was delaying in germination, however with cucumber was complete % inhibition after 3 days. Data in **Table (6)** showed that cucumber was more sensitive than wheat in germination, roots and shoots growth under laboratory conditions. Where the EC₅₀ values for

wheat germination, root length and shoot length were, 104.8, 97.31, and 79.4 ppm respectively. While its values for cucumber was 83.73, 53.2, and 52.66.

These results agreeable with (Ibáñez and Blázquez 2017) Oregano (*Origanum vulgare L.*),essential oil showed completely inhibiting the seed germination of the three weeds at all doses (0.125, 0.25, 0.50 and 1 $\mu\text{L mL}^{-1}$) applied against *Portulaca oleracea*, *Lolium. multiflorum* and *Echinochloa. Crus-galli* seed germination.

b) Under greenhouse conditions (drench Technique) with crops seeds.

The efficacy of thymol 10% EW as herbicidal against sensitive crops under greenhouse conditions by drench technique as pre emergence application was conducted and results in **Table (7&8)** showed that thymol gave complete inhibition in germination, root and shoot growth with both wheat and cucumber at the highest tested concentration 3200ppm, these results was comparable with lab results, where, cucumber more sensitive than wheat to thymol EW formulation in germination, roots and shoots growth. Also shoots of both crops more sensitive than roots. Where the EC_{50} values for wheat germination, roots, and shoots length were, 1242.8, 1244.85, and 1008.8 ppm respectively. While the EC_{50} values for Cucumber were, 1063.8, 918.97, and 760.04 ppm respectively.

Table (7) % inhibition of germination and growth of shoots and roots in wheat and cucumber under greenhouse conditions (Drench technique).

Plants	Conc. (ppm)	%inhibition of germination After		% inhibition of growth After 8 days	
		4 days	8 days	Roots	shoots
Wheat	400	20	10	5.1	14.12
	800	25	15	15	23.96
	1600	95	70	70.58	81.03
	3200	100	100	100	100
cucumber	400	10.52	5.26	2.08	15.7
	800	52.63	26.31	41.20	49.38
	1600	84.20	78.94	87.73	91.22
	3200	100	100	100	100

Table (8) EC_{50} , EC_{90} and Slopes of Germination, roots length, and shoots length inhibition under greenhouse conditions(Drench technique).

	EC_{50}			EC_{90}			Slope		
	G	RL	SL	G	RL	SL	G	RL	SL
Wheat	1242.8	1244.85	1008.8	3075.03	2618.1	2435.4	3.2572± 0.3849	3.9694± 0.4397	3.3483± 0.3652
Cucumber	1063.8	918.97	760.04	2159.13	1636.7	1625.6	4.1691± 0.4211	5.1126± 0.4720	3.8814± 0.3811

G = germination RL= root length SL = shoot length

c) Under greenhouse (foliar application) crop seeds (Cucumber):

Data in **Tables (9&10)** and **figures (1&2)** showed the results of the efficacy of thymol 10%EW as foliar herbicide on sensitive crops wheat as (monocotyledons) and cucumber as (di cotyledons), where it has great efficacy on cucumber and the EC_{50} and EC_{90} values were 1724.87 and 3586.82 ppm respectively after 8 days of application. while the efficacy on wheat as foliar application was low and only at 4000 ppm showed the symptoms in **figure(2)** then recovery during the time of experiment, but showed dry and wilt for the treated plants after nearly 15 days.

Table (9) % Efficacy of Thymol prepared (EW10%) formulation on Dicotyledonous plants (Cucumber) as spraying Technique.

plants	Conc. (ppm)	% Efficacy on cucumber after	
		3 days	8 days
Cucumber	500	0	0
	1000	12.5	18.75
	2000	31.25	56.25
	4000	75	93.75

Table (10) EC₅₀, EC₉₀ and Slopes of the efficacy of Thymol prepared (EW10%) formulation on Dicotyledonous plants (Cucumber) as spraying Technique.

cucumber	EC ₅₀ (ppm)	EC ₉₀ (ppm)	Slope
3 days	2532.94	6425.51	3.1701 ± 0.3078
8 days	1724.87	3586.82	4.0308 ± 0.3316



Figure (1)Thymol 10% EW (4000ppm) on Cucumber (Foliar)



Figure (2) Thymol 10% EW (4000 ppm) on wheat (Foliar)

d) under greenhouse conditions (drench Technique) with weed seeds(*Phalaris minor* Rtz, *Portulaca oleracea*)

Data in Table (11 & 12) and figures (3&4) indicated that thymol 10 % EW formulation has great activity as pre emergence herbicide against both mono and dicotyledonous weeds showed in % inhibition of germination seeds of *Phalaris minor* Rtz and *Portulaca oleracea* under greenhouse conditions, and *P. oleracea* (dicotyledonous) was more sensitive than *P.minor* Retz (monocotyledonous), where the EC_{50} values for phalares and portulaca were 737.25 and 457.29 ppm respectively. These results comparable with results on sensitive crops (wheat& cucumber) mentioned before, however the EC_{50} values for wheat and cucumber were 1242.8 and 1063.8 ppm respectively.



Figure (3) (*Phalares Minor*) Check 400ppm 800ppm 1600ppm



Figure (4) (*Portulaca oleracea*) Check 400ppm 800ppm 1600ppm

Table (11) % inhibition of germination in *Phalaris minor* Rtz and *Portulaca oleracea* under greenhouse conditions (Drench technique).

Conic. ppm.	<i>Phalaris minor</i> Rtz After		<i>Portulaca oleracea</i> after	
	3 days	7 days	3 days	7 days
400	10	5	67.7	40
800	98.5	65	99.5	85
1600	100	93.75	100	98.25
3200	100	100	100	100

Table (12) EC₅₀, EC₉₀ and Slopes of the efficacy of Thymol prepared (EW10%) formulation on *Phalaris minor* Rtz and *Portulaca oleracea* under greenhouse conditions (Drench technique).

Plants	EC ₅₀	EC ₉₀	Slope
<i>Phalaris minor</i> Rtz	737.25	1282.11	5.3201 ± 0.4855
<i>Portulaca oleracea</i>	457.29	945.11	4.0649 ± 0.4860

Conclusion:

Generally, it could be concluded that the formulated thymol as oil in water emulsion (10 % EW) had high herbicidal efficacy against any plant either crops or weeds if it used before planting or spraying on vegetative growth, therefore it could be used as pre emergence treatment before planting crops or spraying weeds in horticulture crops, therefore thymol 10% EW considered as a general herbicides.

Recommendations:

The formulated thymol as 10% EW showed high herbicidal effect when tested in laboratory and greenhouse experiments, therefore other researches should be conducted under open field conditions according to Ministry of agriculture protocols to determine the suitable application rate when used pre or post planting of different crops.

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تحضير الثيمول كمستحلب (زيت / ماء) وتقييم كفاءة كمبيد حشائش علي النباتات ذوات الفلقة و النباتات ذوات الفلقتين

مجدي عدلي اسكندر

قسم بحوث مستحضرات المبيدات، المعمل المركزي للمبيدات، مركز البحوث الزراعية

تم تحضير الثيمول (زيت نباتي مخلوق) علي صورة مستحلب زيت في ماء (10%) واجريت لة اختبارات الخواص الطبيعية التي تثبت قابليته للتطبيق وكذلك ثبات المستحضر اثناء التخزين سواء علي درجات الحرارة المرتفعة او المنخفضة، كذلك ثبات المستحلب اثناء التخفيف بالماء العسر او اليسر، وقد اجتاز المستحضر كل الاختبارات التي اكدت انه مستحضر ناجح قابل للتطبيق الحقلي0 ايضا تم دراسة فعالية كمبيد حشائش علي النباتات ذوات الفلقة الواحدة والنباتات ذوات الفلقتين، وظهرت النتائج ان لة تاثير تثبيطي لانبات كلا من بذور القمح والخيار تحت الظروف المعملية حيث اظهر التركيز 400 جزء في المليون تثبيط كامل لانبات كلا من بذور القمح والخيار 100% عدم انبات0

تم دراسة تاثير المستحضر علي انبات بذور القمح والخيار وايضا بذور الحشائش (الفلارس و الرجله) تحت ظروف الصوبة حيث اظهر التركيز 3200 جزء في المليون تثبيط كامل لانبات بذور القمح،الخيار، الفلارس و الرجله0

تم دراسة تاثير المستحضر رشا علي اوراق نباتات القمح والخيار وقد اظهرت النتائج ان التركيز 4000 جزء في المليون قضي تماما علي اكثر من 90% من نباتات الخيار المعاملة

بينما اظهر تاثير متأخر علي نباتات القمح.