Preparation Thymolas Microemulsion and Oil/Water Emulsion and Evaluation its Pre-Transplanting Nematicidal Activity against Root-Knot Nematode *Meloidogine incognita*

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Abstract: ***Thymol (crystal) an essential oil prepared as microemulsion(ME10%) and oil/water emulsion (EW10%), the two formulas were passed successfully of all the physico-chemical properties testes, and the particle size analysis. Compared the nematicidal efficacy of both formulas aspost-emergence and pre-transplanting application under greenhouse conditions was determined. The results of post-emergence trial showed severe phytotoxicity of both formulas on cucumber treated seedling, and the ME10% showed more nematicidal efficacy on Meloidogyneincognita than EW10%, it showed as reducing in galls number on cucumber seedling roots comparing with untreated seedling. Pre-transplanting trials results showed no phytotoxicity on cucumber seedling planted after 3 or 7 days of both formulas application. However both of them gave excellent protection for cucumber seedling at 1000 and 2000 ppm as indicating by inhibition percent of formed galls, fresh weight of roots and fresh weight of shoots. In all bioassay trials the microemulsion formulation was better in efficacy against nematode than oil in water emulsion that is due to its smaller particle size and other physical properties like thermodynamically stable.***

Keywords: micro emulsion, o/w emulsion and pre- transplanting application

1. Introduction

A microemulsion is a thermodynamically stable fluid. It is different from kinetically stable emulsions which will be break into oil and water over time. The particle size of micro emulsions ranges from about 10-300 nm. Because of the small particle sizes, microemulsions appear as clear or translucent solutions **Johannes, (2015).**

Emulsion is a heterogeneous system consisting of at least one immiscible liquid dispersed in another in the form of droplet with the help of surfactant. There are two types of emulsions: oil- in-water (O/W) (oil is dispersed phase while water is continuous phase) and water- in- oil (W/O) (water is dispersed phase and oil is continuous phase). Depending on the size of the dispersed particles, emulsions can be classified into: macroemulsion (droplet size – 1.5-100 µm); nanoemlsion (droplet size- 50- 500 nm) and microemulsion (droplet size- 3-50 nm) **Jafari et al.,(2008).**

Microemlsions are thermodynamically stable, fluid, optically clear dispersions of two immiscible liquids such as oil and water. Microemulsions form when a surfactant, or more commonly a mixture of surfactants and cosurfactants, lowers the oil/water interfacial tension to ultra-low values (often less than 0.001 dynes/cm), allowing thermal motions to spontaneous disperse the two immiscible phases**Biais et al.,(1987).**

Root- knot nematodes, Meloidogyne sp., are widely distributed attacking over 2000 different plant species, including crops and wild plants. Root-knot nematodes spend part of their life in soil either as eggs or as second – stage larvae. The latter enter the roots and establish feeding sites in susceptible hosts, inducing roots swelling with a characteristic "knotty" appearance. Root galling can drastically limits water and nutrient uptake leading to several symptoms, like malnutrition, chlorosis, and stunting, causing considerable quantitative and qualitative losses in several crop plants **(Taylor and Sasser 1978).** A greenhouse experiment was performed combining increasing dosages of geraniol at (0, 50, 100, 150, and 200µl/kg soil) and thymolat (0, 50 and 100 mg/kg soil). Ten days after applications, the terpenes showed synergistic effects in suppressing populations of Meloidogyne incognita and increasing bacterial counts. Geraniol and thymol caused significant reduction in populations of fungi and actenomycetes. Population of pseudomonas spp. shifted from15% of total bacteria counts in control pots to 70- 86% in the treated pots. **Soler, et al.,(1993)**

The aim of this study is preparing an essential oil (thymol) crystal in form of microemulsion formulation ME 10% and oil/water emulsion EW10% and comparing their nematicidal efficacy against root knot nematode *M. incogneta* on cucumber seedling under greenhouse condition by two method of application to find natural and safety alternative of chemical nematicide.

2. Material and methods

2.1. Chemicals

2.1.1 Chemical nematicides

Fosthiazate EC (Nemathorin 150) Supplied by Syngenta agro Swiss.

2.1.2Thymol extra pure (crystal) supplied by EL-Gomhoria Co., Cairo, Egypt.

2.2 Surface active agent

2.2.1. Sodium dodecyl sulfate, supplied by EL-Gomhoria Co., Cairo, Egypt.

2.2.2. Tween 20 and Tween 80, supplied by EL-Gomhoria Co., Cairo, Egypt.

2.2.3. Poly ethylene glycol 600 di-oleate, produced by the National Co., for yeast and detergent, Alexandria, Egypt.
2.3) Solvents: Xylene (Dimethyl benzene), acetone and N, N-dimethyl formamide (DMF), Supplied by EL-Nasr pharmaceutical, chemicals co.

1) Determination of the physico- chemical properties for:

a) Active ingredient

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

b) Surface active agents

- **Solubility (w/v):** it was determined for the tested surfactant as mentioned above.
- **Free acidity or alkalinity:** it was determined according to **WHO specifications (1979)**
- **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 surface tensiomat for solutions containing 0.1- 1 % surfactant according to**D-1331- ASTM (2001).**

2) Preparation of thymol as Oil/water Emulsion (EW) 10%

Several trials were conducted to prepare thymol as oil in water emulsion (EW) according to **Salvica, et al., (2012)**, emulation stability ,foam and free acidity or alkalinity were determined before and after storage at 54 ± 2ºC for three days to determine the successful formula according to **CIPAC (2002).**

3) Determination the physico- chemical properties of the prepared EW formulation.

- **Emulsion stability**: it was measured according to **CIPAC (2002)**
- **Foam**: it was measured according to **CIPAC (2002).**
- **Free acidity or alkalinity**: it was determined as mentioned above.
- **Stability at 0 ºC. (Cold storage)**
  It was measured according to **CIPAC (2002)**
- **Stability at elevated temperature** 54 ± 2 ºC (accelerated storage)
  It was measured according to **CIPAC (2002)**

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

- **Surface tension**: it was determined as mentioned before.
- **Viscosity**: determined according to **ASTM (2005).**
- **pH value**: it was determined by Jenway pH meter according **CIPAC (2002)**
- **Electrical conductivity**: it was determined according to **CIPAC (2002)**

5) Preparation of thymol as microemulsion 10% ME:

Thymol prepared as microemulsion using high stirring mixer, it takes 3 stages to reach the final particle size but from the first step it was translucent or clear. But to get the lowest particle size the formulas were sonicated using a 20 khzsonicator(Sonic vibra cell) with maximum power output of 500 w for 30and 60 minutes at 25 ± 2 C. according to Periasamy, et al (2016) the formulated microemulsion morphology, droplet size ,emulsion stability, pH, acidity and alkalinity, viscosity, surface tension, electrical conductivity, and accelerated storage were measured as mentioned before.

6) *Meloidogyne incognita* egg masses were obtained from inoculated cucumber plants and the egg were extracted using sodium hypochlorite 1% NaOCl (Hussey and Janssen 2002)

7) Post emergence trials :(drench application).

3000 eggs of *M.incognita* added to every mini drench pot (4cm diameter) in 2ml water, 7days after cucumber seed sowing, then treated by 5ml of tested products diluted at the tested concentrations (50, 100, and 200 ppm) forthymol EW10% and thymol ME 10%, and 20 ppm for Nemathorin EC 150 .four replicate for every treatment and four replicate inoculated by eggs but not treated as control. After that, treated and untreated plants incubated in greenhouse at 25 ± 2 C for 14 days, evaluation of % control based on the average of gall index according to **(Zeck 1971)**then % of inhibition of gall was corrected using **Abbott's formula (1925)**, and the concentration inhibition regression lines were drawn according to the method of **Finney (1952).**

8) Pre- transplanting trials :(soil application)

Two greenhouse experiments were conducting according to (Walker and Melin 1996) with some modifications. A mixture of sand, peat-moss, and clay (2:1:1) prepared and sterilized in autoclave , four plastic pot /2kg for every treatment and four replicate as control for every trial were filled by sterilized soil mixture and irrigated at field capacity ,then inoculated by 20,000 eggs /pot (in 2 ml) and the tested concentrations of EW10 and ME 10 were diluted in water at (500,1000,and 2000 ppm) four replicate for every trial and four replicate without inoculation or treated a(free control) .

The treated pot incubated in greenhouse at 25 ± 2 C for three days for the first trial and seven days for the second trial, then cucumber seedling havingthree leaves were transplanted in treated and untreated pots placed in complete randomized in greenhouse at 25 ± 2 C for 24 days. Seedlings were uprooted and weight of fresh roots and shoots were recorded, and root galling indices were recorded.

Data was analyzed using ProcANOVA in SAS means separation was conducted using Duncan multiple range test in SAS. **(SAS Institute 1988).**

3. Results and Discussion

3.1. Formulation Part:

3.1.1.Physico- chemical properties of formulation constituents:

3.1.1.1. Active ingredient:

Data in Table (1) indicated that thymol crystals non soluble in water, while soluble in acetone,xylene and dimethyl formamide and its solublity values were 145.5, 100 and 127 % respecteviely. It was acidic and its acidity value was % 0.1, and low melting point (52 ºC) so it was suitable formicroemulsion andoil in water emulsion formulation.

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

% Solubility at 25 ºC (w/v.) in				Free acidity % as H2SO4	Melting point ºC
Water	acetone	Xylene	DMF		
N.S	145.5	100	127	0.1	52

3.1.1.2 Surface active agent

The results obtained in Table (2) showedthat SDS soluble in water andnon-soluble in acetone and xylene. Polyethelene glycol 600 dioleate soluble in acetone, xylene and give emulsion in water.Tween 20 also soluble in water, acetone and xylene. Surfactant soluble in water 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.

Tween 80, tween 20 and SDS had high HLB value so it could be used as dispersing agent, while Polyethelene glycol 600 dioleate had HLB value (10- 12) so it could be used as emulsifier. Also sodium dodecyl sulfate and Polyethelene glycol 600 dioleate were alkaline and the alkalinity %as NaOHwas 0.024 and 0.3 respectively. While tween 80 and tween 20 were slightly acidic. .

PEG 600DO had the heighest CMC value, followed by tween 80, SDS and tween 20. 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 ºC (w/v)			HLB	Free		CMC at 25 ºC %(w/v)	Surface tension at 0.5%
	water	acetone	Xylene		Acidity % as H_2SO_4	Alkalinity %as NaOH		
Sodium dodecyl sulfate	40	N/S	N/S	17	-	0.024	0.3	31
PEG 600 di-oleate	Emulsion	27	25.6	10- 12	-	0.3	0.9	35.3
Tween 80	24	58.7	56.3	15	0.05	-	0.5	42.3
Tween 20	38	64.4	59.4	16	0.39	-	0.2	38.9

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

The oil-in-water emulsion (EW) was prepared by adding oil phase to the water phase under high stirring. The oil phase was prepared by dissolving thymol crystal in suitable solvent. Then mixed with water containing suitable emulsifier.

Data in Table (3) indicated that, the prepared microemulsion ME10% and oil in water emulsion EW10% formulation passed successfully through emulation stability test in hard and soft water at the concentration 5 %(v/v), where no oily or cream separation formed, also passed through cold storage at 0 ºC± 2 for 24hours, the foam formed was less than WHO specifications. No observable changes in emulsion stability; foam and free acidity of both prepared formulation after accelerated storage at 54 ± 2 ºC for 14 days only slightly decrease in aciditywith EW formulation from 0.25 to 0.22and slightly increase in ME acidity value from 0.098 to 0.1% .

Table 3: The physico- chemical properties of the prepared microemulsion 10%ME and Oil in water emulsion (10%EW)

	Emulsion stability (ml.creamsep.)		Foam (CM3)		Free acidity % as H_2SO_4	Accelerated storage at 54 ± 2 ºC for 14 days				
						Emulsion stability (ml.creamsep.)		Foam (CM3)		Free acidity % as H_2SO_4
	Hard water	Soft water	Hard water	Soft water		Hard water	Soft water	Hard water	Soft water	
EW10%	0.0	0.0	5	2	0.25	0.0	0.0	6	3	0.22
ME10%	0.0	0.0	3	2	0.098	0.0	0.0	3	2	0.1

Data in Table (4) indicated that, spray solution of thymol prepared as microemulsion ME10%and EW10% formulation had low surface tension value 32.22 and 33.45 dyne/cmrespectively.High viscosity value 1.99 and 1.75 cm poise for EW 10% and ME 10%, while they had acidic pH and their values were6.82 and 6.1.the prepared microemulsion had high electrical conductivity value 366µ mhos whilethe EW 10% had lower value 98 µ mhos compared with water alone.

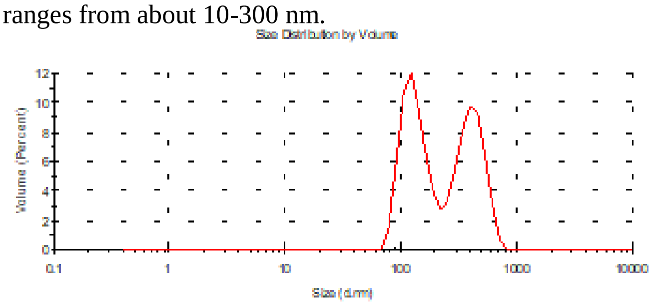
These results agreeable with **Ryckaert et al., (2007)** who reported that, the decrease in surface tension of spray solution cause a good wettability, spreading and depositing of the particles of the solution on the treated surfaces.

Table 4: The physico- chemical properties of the prepared microemulsion 10%ME and EW 10% spray solution at 0.5% concentration

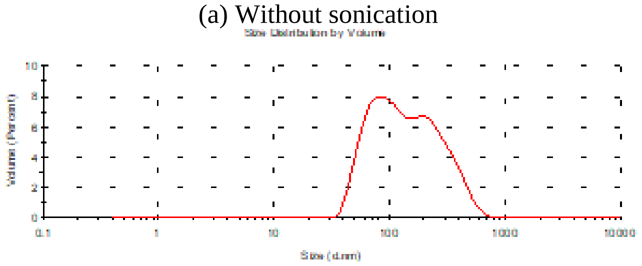
Materials	Surface tension (dyne/cm.) at 25 °C	pH value	Conductivity µ mhos	Viscosity (cm poise)
EW 10%	33.45	6.10	96.7	1.99
ME 10%	32.22	6.82	366	1.75
Water	72	7.8	396	1.0

3.1.3. Preparation of an essential oil (thymol) as microemulsion 10% ME

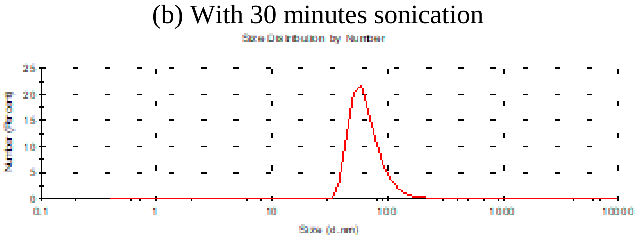
Thymol prepared as microemulsion using high stirring and sonication, it takes 3 stages to reach the final particle size but from the first step it was translucent or clear. however to get the lowest particle size the formulas were sonicated using a 20 khzsonicator(Sonic vibra cell)with maximum power output of 500 w for 30and 60 minutes at 25+/-2 C, the results indicated that 2 peak and particle size was 133.5nm about 51.7% and 407.3nm about 48.3% by stirring the mixture of the microemulsion constituents but after 30 minutes sonication the particle size was 97.16 nm about 60.1% and 274.1 nm about 39.9%, however the lowest particle size obtained after 60 minutes of sonication 66.7nm about 100%.these results agreeable with Johannes, F. (2015) which reported that The particle size of micro emulsions ranges from about 10-300 nm.



(a) Without sonication



(b) With 30 minutes sonication



(c) With 60 minutes sonication

Table 5: The particle sizeanalysis of thymolmicroemulsion ME. 10%

	steps		PDI	Size (d.nm)	% number	Width (d.nm)
a	Without sonication	Peak1	0.267	133.5	51.7	34.87
		Peak2		407.3	48.3	112
		Peak3		0	0	0
b	30 minutes Sonication	Peak1	0.231	97.16	60.1	35.87
		Peak2		274.1	39.9	103.7
		Peak3		0	0	0
c	60 minutes Sonication	Peak1	0.241	66.7	100	26.27
		Peak2		0	0	0
		Peak3		0	0	0

PDI = polydispersity index

3.2. Bioassay part (Nematodicidal efficacy)

3.2.1 Post- emergence (7 day post sowing):

Data in table 6 and 7indicated that local formulated thymol EW10% showed low nematicidal effect at 200 ppm (44.28%) and the $EC_{50}$and EC_{90} values were 249.6 and 2509.2ppm, and showed phytotoxic effect in form plant stunting 60% and the $EC_{50}$and EC_{90} values were 142.59 and 727.17 ppm.

However thymol ME 10% showed high nematode control at 200ppm (64.28%) compared with untreated andit's$EC_{50}$and EC_{90}values were 123.03 and 650.24ppm. while showed severe plant stunting (80%)and the $EC_{50}$and EC_{90}values were 78.45 & 284.23 ppm. These results showed that ME 10% more effective than EW10% against nematode *M. incognita* and has more toxic effect (plant stunting), these results agreeable with**Eskander, (2018)** who reported that thymol 10%EW formulation acts as non-selective herbicide where it showed completely inhibition of germination, root and shoot length of wheat and cucumber seeds under laboratory conditions and the EC_{50}values were: 104.8, 97.31, and 79.4ppm respectively for wheat, and86.6, 53.2, and 52.66ppm respectively for cucumber .also **Said, et al., (2006)**they reported that carvacrol, thymol, and linalool at 1, 2 and 4 mg liter-1 concentrations were the most toxic against *M. incognita* second-stage juveniles (J2s), and hatching was completely inhibited at low concentrations (2, 4 mg liter-1) of carvacrol, thymol, and linalool.

Table 6: %Nematicidal efficacy of thymol formulated as EW10% and ME 10% against M.incognita, 7 days post sowing

Treatment	Conc. Ppm.	Average % Root galls	% Nematode control	%phyto. Stunting
Control	-	5.6	-	-
Thymol EW 10%	200	3.12	44.28	60
	100	3.8	32.14	40
	50	4.6	17.85	20
Thymol ME 10%	200	2	64.28	80
	100	3.12	44.28	65
	50	4.25	24.11	30
Nemathorin EC 15%	20	0.1	98.2	0

Table 7: EC_{50}, EC_{90}, and slopes of the efficacy of thymol EW10% and ME10% against M. incognita 7 days post sowing.

Parameter		EW10%	ME10%
Nematode Efficacy	Slope	1.2788+/- 0.3180	1.7725+/- 0.3118
	EC_{50}	249.6	123.03
	EC_{90}	2509.2	650.24
Cucumber Plants Phytotoxicity	Slope	1.8114+/- 0.3180	2.2925+/- 0.3255
	EC_{50}	142.59	78.45
	EC_{90}	727.17	284.23

3.2.2. Pre- transplanting application

Data obtained in table (8) indicated thatthymol ME10% and EW10% treated in a mixture of soil after inoculated by *M. icognita*eggs and incubated in greenhouse at 25 ± 2 C for three and seven daysbefore transplanting cucumber seedling in the second leaf stage. The results showed that transplanting after seven days of application gave excellent control of nematode better than that after three days with both tested formulation.However, microemultion formulation was better than oil in water emulsioin in 3 and 7 days after application, where the % controlof nematode were 95.76 and 84.61 for ME10% and EW10% respectively at 3 days, and they were 96.29 and 87.03 for ME10% and EW10% respectively at 7 days with 2000ppm.

Noany phytotoxicitywas observed oncucumber seedling with two tested formulas at the two periods of transplanting. Data analysis showed that no significant differences in activity between the concentrations 1000 and 2000ppm inside EW10% or ME10%, while there are significant difference in nematode control between ME and EW at all tested concentrations and after 3 or 7 days of applications.

Table 8: % Nematicidalefficacy of thymol formulated as EW10% and ME 10% against M. incognita 3 and 7 days pre-transplanting.

Treatment	Conc. Ppm.	Average % root galls		% *M. incognita* control	
		3 days	7days	3 days	7 days
Control	-	65 a	67.5 a	-	-
Thymol EW10%	2000	10 cd	8.75 cd	84.61	87.03
	1000	15 c	11.25 c	76.92	83.33
	500	46.25 b	23.75 b	28.84	64.81
Thymol ME10%	2000	2.75 d	2.5 e	95.76	96.29
	1000	13.75 c	4.25 de	78.84	93.7
	500	45 b	12.5 c	30.76	81.48

Data in table (9) indicated the effect of thymol formulated as ME10% and EW10% on cucumber seedling fresh roots is due to its nematode efficacy which was high in concentration 2000 ppm that gave the best significant decrease in fresh weight rootsOF treated seedling comparing with untreated in case of ME10% and EW10% tested formulations and with both time of transplanting. Where the decrease in fresh root weight is due to the decrease in forming nematode galls on roots, it means the decrease in root weight due to the efficacy of tested products. However data analysis showed that there was significant difference in activity between ME10% and EW10% in case of 3 and 7 days of application and also in the concentrations inside both tested products.

Table 9: Average weight values of fresh roots of cucumber plants after 24 days of transplanting.

Treatment	Conc. Ppm.	Average weight values of fresh roots in gr	
		3 days	**7days**
Control	-	26.57a	28.5a
Thymol EW10%	2000	18.9 d	21.3 d
	1000	22.0 c	24.7bc
	500	24.3abc	25.9 b
Thymol ME10%	2000	19.5d	23.9c
	1000	bc 23.6	25.4bc
	500	24.9ab	26.1b

Data in table (10) showed the effect of thymol formulated as ME and EW on fresh weight shoots of cucumber seedling after 24 days of transplanting in inoculated and treated soil. The results showed that transplanting after 7 days of application was better than 3 days, where there is significant increase in shoot weight with both formulation and with all concentration compared with untreated seedling shoot weight. Also there are significant differences between the concentrations and all of them gave shoot weight bigger than untreated with two tested formulations but there is increase in weight by decrease the concentration.

Table 10: Average weight values of fresh shoots of cucumber plants after 24 days of transplanting.

Treatment	Conc. ppm.	Average weight values of fresh shoots in gr.	
		3 days	7 days
Control	-	60.3 e	65.15 e
Thymol EW10%	2000	57.69 f	70.4 d
	1000	66.7 b	73.17 c
	500	63.47 c	74.57 b
Thymol ME10%	2000	61.8d	72.2c
	1000	69.15a	75.8b
	500	66.12b	77.9a

Generally from data in table (11) which indicated the factorial analysis of obtained data with general mean for treatments, concentrations and the two time of application, it showed clearly that, ME10% was better than EW10% in reducing forming galls, and increasing weight of fresh shoot for cucumber seedling comparing the untreated seedling, while EW10% was better than ME10% in reducing the weight of fresh roots comparing with untreated.

In other hand there is no significant difference between the concentrations 1000 and 2000 ppm in reducing forming galls, while there are significant differences between them in case of weight of fresh roots and shoots.

Finally these data clearly confirmed that, transplanting after 7 days of application was better than 3 days with forming galls, weight of fresh roots and shoots.

Table 11: Factorial analysis of obtained data

Factor	*level*	*%galls Mean*	*Weight of fresh Roots mean*	*Weight of fresh shoots mean*
Treatment	1-check	66.25 a	27.5375 a	62.7375 c
	2-EW10%	19.167 b	22.875 c	67.6667 b
	3-ME10%	13.458 c	23.9667 b	70.5042 a
	F-value	154.35	37.35	64.37
	P-value	<0.0001	<0.0001	<0.0001
	LSD value	5.635	0.989	1.274
Concentration	0	66.250 a	27.5375 a	62.7375 c
	500	31.875 b	25.3188 b	70.5188 a
	1000	11.063 c	24.0125 c	71.2063 a
	2000	6.000 c	20.9313 d	65.5313 b
	F-value	53.10	46.47	52.98
	P-value	<0.0001	<0.0001	<0.0001
	LSD value	5.976	1.050	1.351
Time	3	28.25 a	22.8857 a	63.6071 b
	7	18.643 b	25.132 b	72.7500 a
	F-value	22.81	40.43	403.98
	P-value	<0.0001	<0.0001	<0.0001
	LSD value	4.041	0.7096	0.9137

4. Conclusions

An essential oil thymol(pure crystal) was prepared as microemulsion formulation ME10% using sonication technique in three steps to get smallest particle size 66.7 nm and pass successfully from all specified tests which showed clearly that the prepared formula was thermodynamically stable , clear or translucent and particle size in microemulsions range. Also thymol prepared as oil in water emulsion EW10% and the formula pass successfully from all specified testes which indicated the stability under accelerated storage and the emulsion stability in water.

The bioassay trials were conducted in two methods post emergence and pre - transplanting and its results showed clearly that ME10% was better in efficacy against root knot nematode than EW10%, and transplanting after 7 days of application was better than 3 days of application. Also the data analysis showed that the concentration 1000 ppm treated soil pre-transplanting was good for nematode control.

From this study and the previous study on weeds **Eskander, (2018)** and the study on soil born fungi **Eskander, (2019)** we can conclude that thymol formulated as micoemulsion ME10% or oil in water emulsion EW10% could be used as pre-plant pesticide or as soil sterilize 7 days before sowing or transplanting.

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