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FORMULATION OF PROSPECTIVE PLANT OILS DERIVED MICRO-EMULSIONS FOR HERBICIDAL ACTIVITY.

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ABSTRACT

The aim of the present study is to prepare some oils as emulsifiable formulations and evaluate their herbicidal activity against certain crop plants and weeds. The prepared formulations include the essential oils of clove, peppermint, lettuce, camphor, castor, chamomile and jojoba. The herbicidal activity of such formulation was tested against the germination, initial shoot and root growth of *Triticum aestivum L* and *Hordeum vulgare L.* as well as weeds of *Phalaris Paradoxa* and *Polyogon monspeliensis* at different doses. Based on $ED_{50;}$ phytotoxicity and biological activity results revealed that clove, jojoba and camphor essential oils were more phytotoxic than peppermint, lettuce, castor and chamomile oils against seeds germination, root and shoot elongation of the tested plants. Meanwhile, prepared the previous oils to emulsions formulations were found to be effective and capable of inhibiting the growth of tested plants than their essential oils. The results indicated that a clear w/o microemulsions was obtained by 10% of oils portion. The successfully formulated microemulsions required oils, co-surfactant, surfactant and water ratio of (1:3:6:1), respectively. Such formulation indicated more active against weeds than both their essential oils and emulsion formulations. The successful castor, camphor, peppermint and jojoba oil microemulsions at 1000µl/L resulted in a significant reduction in total biomass dry weight by 48.9%, 52.2% and 50.97% (*P. paradoxa*) *and* 57.1, 62.9%, 58.9% and 57.0%(*P. monospeliensis*) respectively as compared to the control at 30 days after emergence. Bioassay of castor, camphor, peppermint and jojoba oil microemulsions at 300µl/ml were reduced significantly *Convolvulus arvensis* seedling total biomass fresh weight by 65.27%, 70.0%, 61.23% and 70.48% respectively, over the control. In general, essential oils, emulsions and microemulsions of jojoba, clove and camphor caused the maximum negative effects. However, chamomile and lettuce slightly affected the tested plants growth parameters. This study confirmed that water in oil microemulsions forming size droplets able to higher their herbicidal activity than essential oils alone and their formulated emulsions without change in their chemical structure as described by FTIR studies from their position and number of spectrum in 400–4000 cm^{-1} Mid region that characteristics each type's. The microemulsions successful characteristics carrying bioactive compounds than the emulsions formulation for their ability to solubilize the active constituents in the dispersed oils for herbicidal properties and it will be considered a suitable weeds control application in the future.

Keywords: Plant essential oils; emulsions; microemulsions; phytotoxicity; germination; seedling growth.

INTRODUCTIONS

The usage of microemulsions is very functional in many aspects of crude oil exploitation due to its bioavailability and biocompatibility. Microemulsion is a system of oil, water and an amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (Danielsson and Lindman, 1981). Microemulsions are transparent solutions with particle sizes ranging from 5 to 10 nm, and arise from the spontaneous self-assembly of the hydrophobic or hydrophilic parts of surfactant molecules (Flanagan and Singh, 2006). Microemulsions have found numerous applications but the application in foods is limited by the types of surfactants which are used to facilitate microemulsion formation. In contrast to ordinary emulsions which are kinetically stable but are thermodynamically unstable and will phase separate (Shinoda and Lindman, 1987). Microemulsion using cosurfactants may not be suitable to be used in foods because short or medium-chain alcohols can cause toxicity and irritation (Flanagan and Singh, 2006).Co-surfactants are usually used in conjunction with surfactants due to most single chain surfactants being incapable of reducing the interface tension of oil and water to form a microemulsion. The most common co-surfactants are medium chain alcohols, which reduce the surface tension and increase the fluidity of the oil-water interface, thereby increasing the entropy of the system (Lawrence, 1994). Many difficulties are encountered in creating a suitable microemulsion with temperature gradients required large, many ionic surfactants precipitating when contacted with brine, and most non-ionic surfactants unsuitable (Sjiiblom, *et al.*, 1996). Plant-derived compounds are a potential source for such nematicides, and essential oils are one type of botanical product that has been studied for management of weeds, arthropods, nematodes, and microbial pathogens. These oils are active against a number of pest organisms, which are removed fairly rapidly from the environment, and often have low toxicity to mammals (Bainard *et al.*, 2006).Essential oils have a rich source of bioactive compounds and have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Burt, 2004; Kordali, *et al.,* 2005. Milhau, *et al.,* 1997). This study was intended to design emulsions and microemulsions to some essential oils, and estimated their chemical and physical characterization, in addition to determine their dose-response relationships against the selected weeds and crops.

MATERIALS AND METHODS

Materials: Clove oil, eucalyptus oil (Camphor oil), peppermint oil, chamomile oil, castor oil, (lettuce oil, (El-hawag company for extracting and packing natural oils, Egypt) and sodium dodecyl sulfate (SDS), tween 20 (polyoxyethylene (20) sorbitan monolaurate), tween 80 (POE(20) sorbitan monooleate), span 80 (Sigma aldrich), monoethylene glycol mono-oleate and monoethylene glycol di-oleate (Egyptian company for Starch, Yeast and Detergents. Alexandria) as surfactant isooctane or isoamyl alcohol, and carboxy methyl cellulose (CMC) (Sigma aldrich), as co-surfactant and deionized Water.

Preparation of water-in-oil to emulsions

Emulsions (10% oils) were prepared by mixing four volume of oils, two volume of water containing 0.1% (carboxy methyl cellulose, CMC) and one volume of surfactant triturated with two parts water to form mucilage;

then the four parts liquid oils are added slowly, in portions. While, after all the oil is added, the mixture is triturated for several minutes to form the primary emulsion. The above methods also carried out for all oils emulsion with the same concentrations and for each samples viscosity, conductivity and stability period were measured at 30 ± 5 °C and kept for observation with a time interval of one week for a period of one and half month.

Preparation of water-in-oil to microemulsions.

The microemulsions were determined by titration method as followed, different concentration of surfactant and co surfactant mixture were dissolved in the selected oils in stopper vials and kept in room temperature at 30 ± 5 °C such solution was then titrated with deionized water added drop by drop while mixing on a magnetic stirrer until the appearance of persistent turbidity. Each oils phase; oils and surfactant plus cosurfactant (1:3-8:3-6) mix were combined in different weight ratios so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Slow titration with the aqueous phase was done to each weight ratio of oil and surfactant and co-surfactant mix, and visual observations were made for transparent and easily flowable oil-in-water (o/w) microemulsions.

Characterization of plant oils, emulsions and microemulsions.

1-Percentage of transmittance

Transparency of plant oils, emulsions and microemulsions formulation was determined by measuring percentage transmittance at scan mode with purified water taken as blank using UV-VIS spectrophotometers thermo (Nicolet evolution 300) (Date and Nagarsenker, 2008).

2-Stability studies

Stability study was performed as procedure given in literature (Araya *et al.*, 2005). Preservation stability of essential oils, emulsions and microemulsions was prepared in 10 ml volume and stored at room temperature and elevated temperature (30 ± 5 °C) and shelf life of stored microemulsion system was evaluated by visual inspection (phase separation) for different periods.

3-Determination of Viscosity:

The viscosities were measured with a Brookfield Programmable DV-11+Viscometer: 60RPM where cm/ pouse are the unit of viscosity measurement. The measurement was done at ambient temperature.

4-Determination of pH and conductivity measurements:

The pH values of the samples were measured by a pH meter (model thermo Orion 25 star Instruments, USA), at 25 ± 2°C. Electrical conductivity of essential oil, emulsions and microemulsions was measured using a conduct meter at ambient temperature by ppm.

Pre-emergence phytotoxicity assayed:

The obtained plant oils, emulsions and microemulsion were tested with series and different of concentration according to the oils type against crop seeds of wheat (*Triticum aestivum L)* and barley (*Hordeum vulgare L.*), as well as weed seeds of (*Phalaris Paradoxa and Polyogon monspeliensis*). The tested weed seeds were sterilized using sodium hypochloride (0.3% v/v) for 10-12 min. and washed four times in sterile double-distilled water. The tested weed seeds were sown in 9 cm diameter petri dishes moistened with 5 ml of the emulsions. While the tested weed seeds control were treated with distilled water in equal of the tested oils. Petri dishes were placed in dark at 25 ± 3°C for 10 days. Germination and growth length were recorded at the test end, while each treatment was replicated three times.

Post emergence activity of microemulsions.

Weed seeds were sowed in pots and filled with vermiculites soil, after emergence only two seedlings were kept in each pot. Pots had been initially watered two times weekly until 3-4 leaves stage in the greenhouse. The spray treatment was prepared from microemulsions at (control and 250,500, 1000µl/L). Sprays were applied with a glass sprayer to provide 10 ml of liquid solution to each pot. Control pots were similarly sprayed with micro emulsions without oils. The sprayed pots were arranged in a randomized complete block in greenhouse. The data were obtained in three replicates, the survival of seedlings and dry weight for aboveground parts were recorded after one week of spraying (Heisey, 1996).

Bioassay of essential oils and microemulsions against *C. arvensis* seedlings total biomass.

Bindweeds (*C. arvensis*) seeds surface were sterilized using sodium hypochlorite (0.3% v/v) for 10-12 min and then washed four times in sterile double-distilled water before the use. Seeds were placed on static Murashige and Skoog (MS) and allowed to germinate for seven days in petri dish until roots and shoots emerged. Seven-day-old seedlings were transferred to tissue culture tube containing 5mL of liquid MS media with one seedling in each tube. Seedling was treated with the concentration series of 100, 200,300 µl/5ml media in three replicates. Plant cultures were maintained under the conditions. Total seedling fresh biomasses were recorded after 10 days from treatment and compared over their controls.

The ED_{50} values for each growth parameter were calculated by plotting concentration on a log scale (X) and the response (Y) on probit scale mathematically transformed, the data appear linear and sign the point in a semi-log graph paper. Calculate the percent inhibition of growth rate for each treatment replicate from the equation: I% = (C-T)/(C) ×100, where: I%: percent inhibition in average specific growth rate; C= mean value for the control; T= value for treatments. All experiments were designed in a randomized complete block design with three replicates. Data was statistically analyzed by ANOVA, according to (Snedecor and Cochrán, 1990) and treatment means were compared by Duncan or LSD test at 5% level of probability

FTIR spectra measurement

FTIR spectra was scanned using a Perkin Elmer FTIR spectrometer model L1600300 Spectrum Two LiTa made in UK equipped (S.N: 94754) with DTGS detector with a resolution of 4 cm−1, number of scanning of 32 co-adding in the 400–4000 cm−1 region. Spectra were acquired using FTIR Spectrum 10™ software. All spectra were rationed against a background of air spectrum. After every scan, a new reference air background spectrum was taken. These spectra were recorded as transmition values at each data point for clove, peppermint, lettuce, camphor, castor, chamomile and Jojoba oil, emulsion and microemulsions.

RESULTS AND DISCUSSION

The physical properties and characteristics of the selected plant oils and the prepared emulsions as well as microemulsions were studied under room condition, i.e; viscosity and transmittance (figure 1), pH, conductivity, stability were shown in (Table, 1). Data represented that there was a small difference between pH in plant oils and microemulsions; however, there was a lot of change observed in the formulated emulsions. Microemulsions were characterized by the lower viscosity than essential plant oils. They had a lower viscosity than the formulated emulsions. Garti *et al.* (2005) reported that viscosity depends largely on the microemulsion structure, i.e., the type and shape of aggregates, concentration and interaction between dispersed particles. The stability of plants oils were prolonged to more than 12 months without any change, followed by microemulsions were stable over six months except clove, lettuce and chamomile microemulsions. Moreover, the emulsion stability ranged from 130 to 230 min. under conditions (30±5). Finally, light transmittance for microemulsions is higher than essential plant oils, whereas, their emulsions were slightly transmittance to UV and visible light (VIS). According to visual and the physicochemical studies, it was found that peppermint, castor, camphor, and jojoba microemulsions showed superior stability performance, and gave a better higher transmittance appearance than other formulated microemulsions. It could concluded that microemulsions were characterized by visual view and some physicochemical measuring such as the higher transmittance and stability as well as lower viscosity than their formulated emulsions which described by low conductivity, stability and transmittance as well as high viscosity.

Table 1. Physicochemical properties of plant oils, emulsions and microemulsions.

Oils	PH	Conductivity (ppm)	Stability month	PH	Conductivity (ppm)	Stability Min.	PH	Conductivity (ppm)	Stability month
		Plant oils			**Emulsions**			**Microemusions**	
Clove	6.64	1.29	<12.0	5.631	76.10	140.0	6.73	34.42	1.0
Peppermint	6.13	1.39	<12.0	3.479	72.10	230.0	7.24	37.11	6.0
Lettuce	6.16	1.21	<12.0	5.739	72.00	130.0	7.28	19.11	2.0
Camphor	6.34	1.28	<12.0	3.477	64.30	160.0	5.84	44.50	6.0
Castor	6.29	1.21	<12.0	3.799	64.00	200.0	6.73	33.19	6.0
Chamomile	6.52	1.25	<12.0	3.820	83.00	130.0	7.19	30.18	3.0
Jojoba oil	6.21	1.19	<12.0	3.62	76.00	140.0	6.57	25.15	6.0

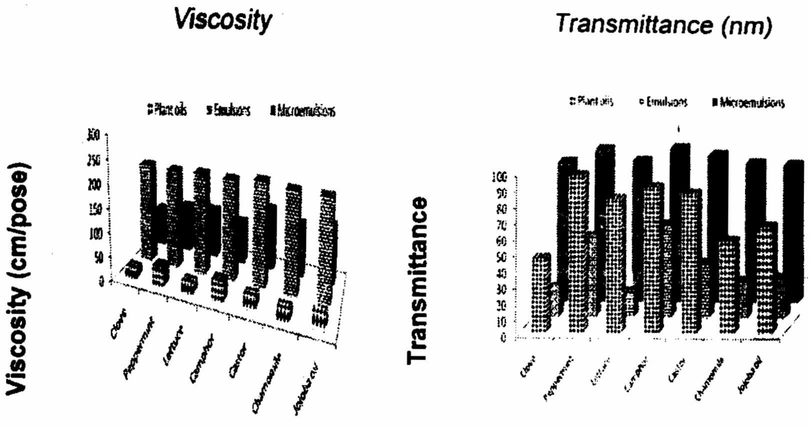


Figure 1. **Viscosity and transmittance of plants oils, emulsions and microemulsions.**

Bioactivity assay against crops germinations and growth.

In order to determine the relative contributions of plant oils on weeds control, the phytotoxicity was studied at series concentrations relative to their activity as shown in (Table 2). Essential oils have a significant effect in crop percentage of seeds germination, shoot and root length that varied depending on oil types, concentration and their formulated emulsions and microemulsions as indicated by ED_{50}. The highest inhibitory achieved from clove oils and their prepared emulsions than peppermint, lettuce, camphor, castor, chamomile and jojoba oils against *T. aestivum* and *H. vulgare* crop seeds germination, root and shoot length depending on an ED_{50} value. In the second ordered came jajoba oil and their emulsion affects crop growth, however, chamomile oil and their emulsions were recorded slightly affect the tested crops. Crop root length was the most sensitive parts due to oils and their emulations as compared to seeds germination and shoot lengths. While, the synergistic effect of formulated emulsions doubled many times than their oils against crop growth parameters.

Table 2. ED_{50} (µl/ml) of natural oils and their emulsions on crops growth parameters.

		Crude oils			Emulsions		
		Germ	**Shoot**	**Root**	**Germ**	**Shoot**	**Root**
Clove	*T. aestivum*	96.8±2.3	82.5±1.6	69.0±1.6	22.5±3.5	16.0±2.2	15.1±2.5
	H.vulgare	380.0±3.4	350.0± 1.1	300.0±1.2	37.5±3.06	35.0±3.5	27.2±1.1
peppermint	*T. aestivum*	122.3±2.0	160.0± 1.5	100.3±.9	33.0±2.9	22.5±4.2	18.5±3.9
	H. vulgare	450.0±3.6	400.4±2.4	320.0± 2.3	55.0±3.2	50.3±1.7	35.2±2.2
letuuce	*T. aestivum*	175.0±2.0	170.0± 1.52	95.6± 1.87	27.1±3.2	21.5± 2.6	18.0±2.3
	H. vulgare	385.0± 3.9	390.0±5.5	313.0± 3.9	62.5±2.1	42.5±1.7	35.4±1.2
Camphor	*T. aestivum*	105.3±1.3	90.7±1.7	88.5±2.0	27.5±2.9	30.0±3.3	23.2±3.1
	H. vulgare	390.0±3.4	360.0±8.1	290.0±4.6	50.0±3.3	46.3±2.2	35.1±0.4
Castor	*T. aestivum*	176.0±1.6	174.0±1.7	120.0±2.0	29.8±3.2	26.4±3.3	23.0± 3.1
	H.vulgare	320.0± 3.5	337.0±3.6	286.0±3.9	48.0±3.2	40.7±2.6	39.3±0.9
Chamomile	*T.aestivum*	180.0±3	120.0±1.9	110.0±1.4	36.2±2.2	23.1±2.3	21.2±1.9
	H.vulgare	460.0±4.5	385.0±1.3	350.0±3.2	68.5±4.3	57.0±2.4	55.3±0.4
Jojoba oil	*T.aestivum*	113.0±2.4	107.0±0.7	93.9±2.0	24.8±3.1	22.5±3.4	21.0±1.9
	H.vulgare	336.0±5.0	349.0±4.3	321.6±4.0	42.5± 2.7	39.0±3.6	35.0±4.6

Bioactivity assay against weeds germination and growth.

The influence of the selected essential oils and their emulsions were compared according to an ED_{50} value on *P. monosplensis and P. Paradoxa* growth and germination as shown in (Table, 3). Firstly, plant oils were recorded inhibition in weeds shoot and root height and seed germination as compared with water controls, under laboratory conditions. The ED_{50} value proved that jojoba oil was highly phytotoxic followed by clove oil, while camphor oil exhibit a moderate phytotoxicity. However, the growth and germination of weeds were affected slightly by the other plant oils. Secondly, clove oil emulsion clearly has superiority herbicidal activity followed by jojoba emulsions than others emulsions on the tested weeds. However, peppermint oil and their emulsion showed lower reduction activity against weed growth parameters. Further, weeds root growth was more severely affected to plant oils and their emulsions than shoot heights and seeds germination. Thereafter, preparing emulsions from plant oils increased their phytotoxicity against weed growth parameters double times approximately than using plant oils. In this respect, crops parameters were more sensitive than weeds parameters regardless of oils and their emulsions bioactive constituents (Table, 3).

Table 3. ED50 (µl/ml) of plant oils and their emulsions on weeds growth parameters.

		Plant oils			Emulsions		
		Germ	Shoot	Root	Germ	Shoot	Root
Clove	*P. paradoxa*	73.5±1.73	60.7±0.6	56.7±0.8	14.2±1.7	12.5±1.2	12.0±2.4
	P.monospeliensis	93.4± 2.7	90.6±1.2	73.5±1.1	10.5±3.2	10.0±1.3	8.5±0.4
Peppermint	*P. paradoxa*	83.9±2.3	77.7± 2.5	71.9±1.5	14.0±2.3	14.8±0.8	12.5±1.3
	P. monospeliensis	140.3±1.5	110.5± 0.7	90.5±0.6	18.0±1.3	15.0± 0.4	16.0± 1.4
Lettuce	*P. paradoxa*	76.9±1.0	70.4± 0.2	60.3±0.3	16.0±1.6	12.3±0.5	12.0±2.8
	P. monospeliensis	89.3± 1.2	78.9±1.7	80.5± 0.3	20.5±6.3	13.5±0.6	11.6±0.3
	H. vulgare	385.0± 3.9	390.0±5.5	313.0± 3.9	62.5±2.1	42.5±1.7	35.4±1.2
Camphor	*P. paradoxa*	75.1±1.6	62.5±0.5	60.3±0.4	11.5±1.5	9.5±0.4	9.0±1.4
	P. monospeliensis	95.0±0.8	94.3±0.2	82.3± 0.3	16.5±3.5	14.2±0.5	16.0±0.2
Castor	*P. paradoxa*	77.4± 1.7	84.3±0.4	64.3±0.8	14.9±1.0	12.0±0.3	14.0±2.7
	P.monospeliensis	85.9± 0.7	82.3± 0.2	66.3±0.5	16.6±2.1	17.0±1.3	12.0±0.3
	H.vulgare	320.0± 3.5	337.0±3.6	286.0±3.9	48.0±3.2	40.7±2.6	39.3±0.9
Chamomile	*P. paradoxa*	75.0±1.4	80.0±1.4	68.0±0.4	7.5±2.0	9.2±0.5	7.0±3.8
	P.monospeliensis	83.0±1.2	81.5±0.5	67.1±0.6	17.5± 2.5	17.0±0.4	12.8±0.3
Jojoba oil	*P. paradoxa*	87.0±.8	76.0±0.8	63.8±0.6	13.9±1.2	11.3±0.7	10.8± 2.3
	P.monospeliensis	89.0±1.2	39.0±1.2	75.2±0.9	13.5±1.0	12.8±0.9	11.2±2.1

Assay of microemulsions against weed seeds and growth parameters.

The formulated microemulsion was tested with series concentration against the selected crops and weed seeds germination and seedling growth under laboratory conditions. Firstly, ED_{50} value for the crops trait's guided to the highly toxicity of microemulsion as compared with their essential oils and emulsions. Furthermore, it was observed that *H. vulgar* was susceptible to the formulated microemulsions than *T. aestivum;* these results are well correlated and similar with the previous reported using plants oil and emulsions formulation. Secondary, jojoba oils microemulsion caused the highest activity against *P. monosplensis* and *P. Paradoxa* followed by castor, camphor, clove and peppermint oil microemulsions; Further, lettuce and chamomile microemulsions exhibit a moderate herbicidal activity on the tested weeds growth parameters. However, castor oil microemulsion showed lowest phytotoxic activity. It was found that *P. Paradoxa* growth was more sensitive to all formulated microemulsions than *P. monosplensis*. Weed seeds germination and root length were more susceptible than shoot lengths to all formulated microemulsions as compared with its control (Table, 4).

Table 4. ED_{50} (µl/ml) of microemulsions on crops and weeds seeds growth parameters.

	Crops	Germ	Shoot	Root	Weeds	Germ	Shoot	Root
Clove	*T. aestivum*	17.00±2.0	18.00±2.3	12.00±2.3	*P. paradoxa*	7.00±1.5	8.00±0.5	7.00±1.2
	H.vulgare	8.00±3,0	9.00±1.5	6.00±4.8	*P.monospeliensis*	8.00±2.5	9.00±0.8	7.00±3.2
Peppermint	*T. aestivum*	24.00±2.0	17.00±1.5	15.00±1.5	*P. paradoxa*	9.00±2.0	9.00±0.4	8.00±0.3
	H.vulgare	22.00±4.0	16.00±2.5	13.00±3.0	*P. monospeliensis*	11.00±3.0	13.00±0.3	8.00±2.6
Lettuce	*T. aestivum*	31.00±5.0	32.00±0.8	19.00±2.3	*P. paradoxa*	8.00±1.0	12.00±0.3	8.00±0.3
	H.vulgare	15.00±3.0	17.00±2.0	14.00±2.5	*P. monospeliensis*	8.00±2.0	13.00±0.2	9.00±2.3
Camphor	*T. aestivum*	20.00±6.0	23.00±4.0	17.00±2.0	*P. paradoxa*	9.00±3.0	10.00±0.5	7.00±0.2
	H.vulgare	16.00±3.0	12.00±1.0	13.00±3.5	*P. monospeliensis*	11.00±1.0	8.00±0.2	7.00±3.3
Castor	*T. aestivum*	24.00±3.0	22.00±2.0	21.00±2.0	*P. paradoxa*	6.00±0.5	9.00±0.5	6.00±0.5
	H.vulgare	14.00±2.7	18.00±3.0	12.00±2.7	*P.monospeliensis*	7.00±1.3	10.00±0.3	11.00±2.2
Chamomile	*T. aestivum*	40.00±4.0	43.00±2.0	30.00±0.5	*P. paradoxa*	6.00±2.0	8.00±0.2	5.00±1.2
	H.vulgare	23.00±1.0	22.00±2.0	17.00±1.0	*P.monospeliensis*	11.00±1.6	12.00±0.1	9.00±0.2
Jojoba oil	*T. aestivum*	24.00±2.3	32.00±4.0	17.00±2.0	*P. paradoxa*	6.00±0.9	8.00±0.2	5.00±0.3
	H.vulgare	13.00±4.5	21.00±5.0	14.00±2.6	*P.monospeliensis*	8.00±1.5	9.00±0.3	6.00±2.0

Post emergence bioassay for microemulsions.

Greenhouse efficacy evaluation was conducted on pots for microemulsions formulation at 0, 250,500, 1000µl/L (figure, 2). Control received only tap water and after 30 day from emergence and one week from treatment 10 plants were weighted. The higher concentration of microemulsions (1000 µl/L) exhibited potent post emergence activity and the abilities to inhibited *T. aestivum*, *P. Paradoxa* and *P. monspeliensis* total biomass of the tested plants as the comparison with other concentrations and its control. Whereas, clove oil microemulsion decreased total biomass dry weight of *T. aestivum, P. paradoxa* and *P.monospeliensis* by 41.0%, 62.04% and 71.2%, respectively as compared to the treated control (30 day after emergence). Thereafter, it is noteworthy that treatments of camphor oil microemulsions at 1000 µl/L resulted a significant reduction in *T. aestivum, P. paradoxa* and *P. monospeliensis* total biomass dry weights by 25.9%, 52.2% and 62.9% respectively over the control. Furthermore, peppermint oil microemulsions at 1000 µl/L reduced significantly *P. paradoxa* and *P. monospeliensis* total biomass dry weight by 56.90% and 58.90% respectively as compared to the control. However, chamomile and Lettuce microemulsions achieved the lower activity for plants dry weights, finally, jojoba microemulsions at 1000 µl/L resulted in a significant reduction in *P. monospeliensis* dry weight by 57.0% over the controls. The successful castor, camphor, peppermint and jojoba microemulsions at 1000µl/L decreased significantly total biomass dry weight by 48.9%, 52.2% and 50.97%, (*P. paradoxa*) *and* 57.1, 62.9%, 58.9% and 57.0%, (*P.monospeliensis*) respectively after 30 days from emergence, as compared to the control. The present results clarified that the tested microemulsions significantly inhibited plant growth than emulsions formations. Meanwhile, the growth of tested weeds was more sensitive to plant oils, and their formulated emulsions and microemulsions due to the specific characteristics of formulation specially microemulsions. The studied peppermint, camphor, jojoba and castor oil microemulsions system exhibited a potent stability towards times and enhanced it's biological activity against plants in post- emergence stage as compared with essential oils and their formulated emulsions.

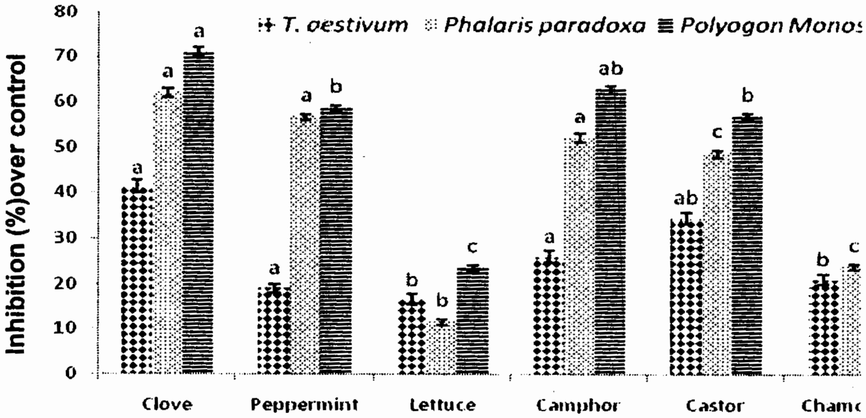


Figure 2. Post emergence activity of microemulsions on weeds total

Assay on *C. arvensis* seedlings total biomass fresh weights.

Bioassay comparison between essential oils and their microemulsions were conducted with sequence concentrations of 100, 200, 300 µl/5ml MS media against *Convolvulus arvensis* seedlings in tissue *culture tubes for 10 days and total biomass fresh weight was recorded and* compared over its control. The result revealed that clove oil, camphor and jojoba essential oils caused a significant reduction in *C. arvensis* seedling total biomass fresh weights by 50.22%, 44.59% and 51.24%, respectively, compared to its control. However, the successful castor, camphor, peppermint and jojoba oil microemulsions at 300µl/L showed a significant reduction in total biomass fresh weight 65.27%, 70.0%, 61.23% and 70.48% than the untreated control. Followed by clove oil microemulsion caused a significant reduction in *C. arvensis* seedling total biomass fresh weight by 58.9%, over the control. Furthermore, chamomile and lettuce oil microemulsions showed slightly decreasing *C. arvensis* seedling by significantly, 25.82%, 26.7% respectively compared to control (Table, 5).

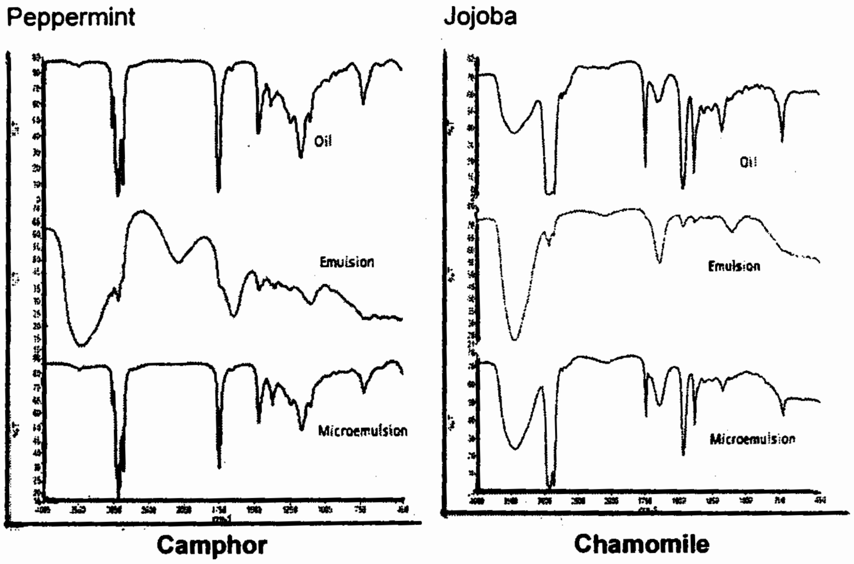
Table (5). A comparison between essential oils and their microemulsions against *C. arvensis* seedlings.

	Essential oils				Microemulsions			
Conc.	Control	100	200	300	Control	100	200	300
Clove	0.23	0.18	0.17	0.113	0.24	0.17	0.15	0.097
	(0.00)	(20.35)	(23.45)	(50.22)	(0.00)	(27.12)	(36.86)	(58.9)
Peppermint	0.24	0.22	0.20	0.19	0.23	0.19	0.16	0.088
	(0.00)	(8.51)	(15.74)	(19.57)	(0.00)	(16.30)	(30.40)	(61.23)
Lettuce	0.23	0.22	0.20	0.19	0.23	0.20	0.18	0.17
	(0.00)	(2.65)	(11.50)	(15.49)	(0.00)	(14.66)	(23.28)	(26.7)
Camphor	0.23	0.20	0.20	0.128	0.24	0.17	0.13	0.072
	(0.00)	(12.99)	(15.58)	(44.59)	(0.00)	(28.29)	(45.00)	(70.0)
Castor	0.23	0.21	0.19	0.16	0.24	0.18	0.15	0.083
	(0.00)	(10.09)	(18.42)	(28.95)	(0.00)	(26.27)	(38.91)	(65.27)
Chamomile	0.24	0.22	0.20	0.19	0.24	0.18	0.18	0.17
	(0.00)	(6.81)	(14.47)	(21.28)	(0.00)	(23.31)	(23.83)	(25.8)
Jojoba oil	0.23	0.21	0.19	0.113	0.23	0.17	0.13	0.07
	(0.00)	(10.73)	(18.03)	(51.24)	(0.00)	(26.78)	(41.41)	(70.48)
LSD0.05	0.081				0.11			

Values between brackets are the inhibition percentage.

FTIR analysis of oils, emulsions and microemulsions.

IR spectrum for clove, peppermint, lettuce, camphor, castor, chamomile and jojoba essential oils, emulsions and microemulsions indicate that at higher frequencies between 3400 and 3550 cm^{-1}, the bands observed in the spectrum of oil can be related to the –OH stretching vibration of water (H–OH), hydroperoxides (ROOH) and their breakdown products, namely alcohols (ROH). The 3007.6-2850–cm^{-1} region is known as the absorption zone of vinylic C–H stretching vibration of methylene and terminal methyl groups of fatty acid chains. In the center of the spectrum, a band due to the C=O stretching absorption of the triglyceride ester linkages is present. The spectral region between 2700 and 2000 cm^{-1} and the band at 1900- 1700cm^{-1} is known functional groups C-O-C asymmetric stretching undergo by several changes during the oxidation process of oil samples heated at various temperatures or/and exposed to UV radiation. At lower frequencies (1655–1500 cm^{-1}), the carbonyl absorption bands of aldehydes (R–CHO) and ketones (R–CO–R) can be observed. The next region (1500–597 cm^{-1}) is the so-called "fingerprint region", it described by C-H in-plane bending because the pattern of the bands is particularly characteristic of molecular composition and can be used to identify minor substances which differentiate the microemulsions formulation than the crude oils beside the number of spectrum that characteristics each oil types. Moreover, in emulsions formulation most of oils spectrum disappear in IR profile due to the high amount of water in this formulation. The most important issues observed from FTIR analysis is the similarity between IR profile in most of the successfully microemulsions formulation and their essential oils expect in the fingerprint region1400–500 cm^{-1} and the quantity of to the –OH region (Fig 3Aand 3B).



Peppermint

Jojoba

Camphor

Chamomile

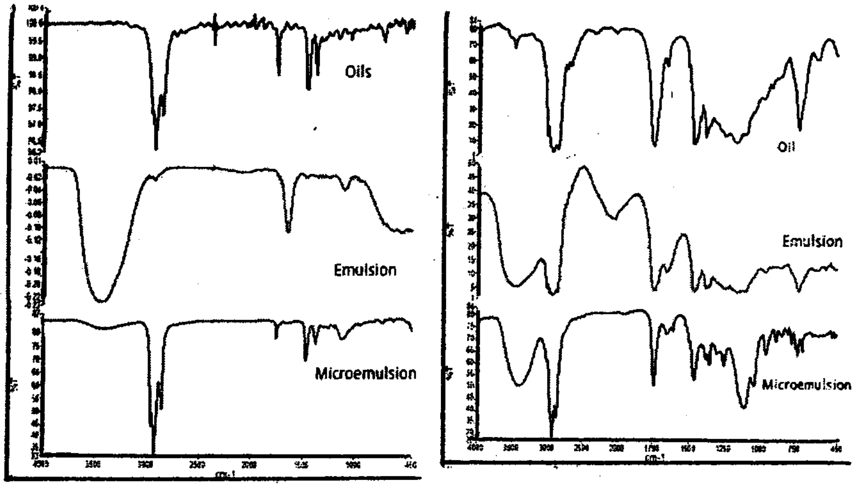
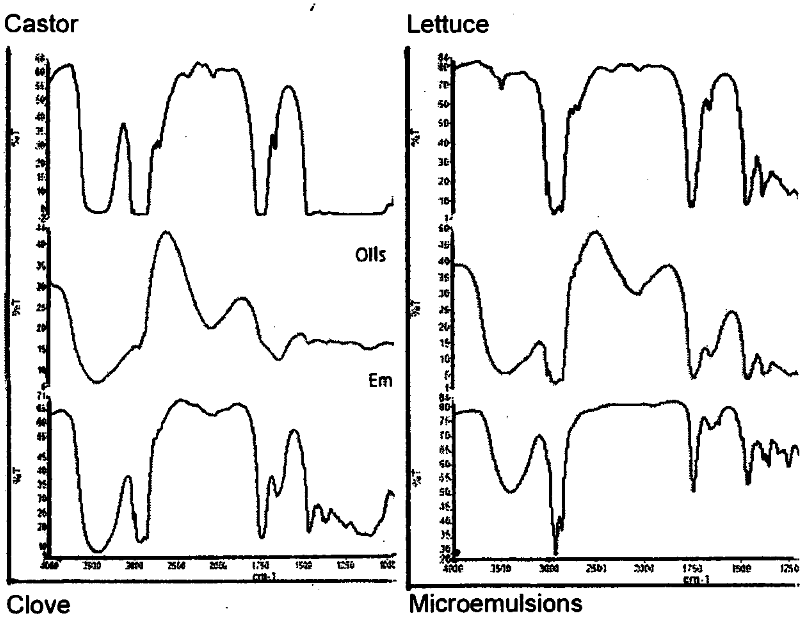


Fig 3A: FTIR spectrum of clove, peppermint, lettuce, camphor, castor, chamomile and jojoba essential oils, emulsions and microemulsions.



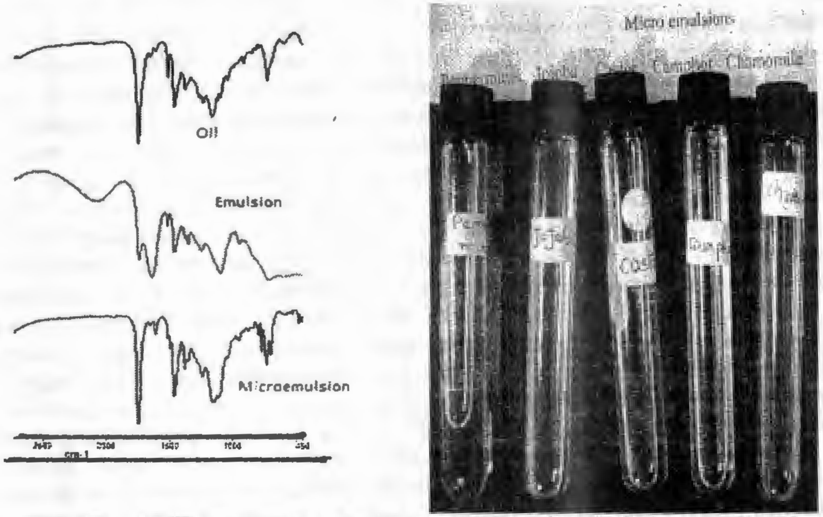


Fig 3B: FTIR spectrum of clove, peppermint, lettuce, camphor, castor, chamomile and Jojoba essential oils, emulsions and microemulsions.

The above mentioned results indicated the herbicidal properties and exhibited inhibition of plant oils in the tested weeds, this activity depending on many factors such as plant species, oil types, the tested concentrations and the prepared formulation. Plant oils have a rich source of bioactive compounds and have exhibited potent herbicidal properties. The obtained results agree with this finding; all oils were active against germination and early radical growth of *Lepidium sativum, Raphanus sativus* and *Lactuca sativa,* but in different levels of activity (Vokou, 1992). Clove oil caused greater inhibition of seedling growth in broccoli, common lambsquarters, and redroot pigweed species (Bainard, *et al.*, 2006). The observed inhibitory effect of eucalyptus volatile oils on seed germination is not new as essential volatile oils from a number of species are well-known as germination inhibitors (Dudai *et al.*, 1999; Mao *et al.*, 2004; Singh *et al.*, 1991). In a comparison with the prepared formulation; there were lower phytotoxic effect of the essential oils than the formulated emulsions and microemulsions in the germination percentage and growth traits of all plants among treatments. Further, improvements in essential oils to produce microemulsions were found to expand the activity and enhanced their potential uses as shown in peppermint, jojoba, camphor and castor microemulsions as compared with the most active clove oil. These results supported by (Boyd and Brennan, 2006); clove oil phytotoxic can occur and are dependent upon factors such as plant species, oil concentrations tested, volume of clove oil formulation applied, and the size of plants. Stable and a clear O/W microemulsions could be obtained by utilizing nonionic surfactant with the mix between oils and a cosurfactant that can be formed transparent microemulsions. The results *indicated that w/o microemulsions containing portion 10% of oils was*

successfully formulated, it required; oils, cosurfactant, surfactant and water ratio of (1:3-8:3:1) or little bit higher. Peppermint, camphor, castor and jojoba microemulsions have a successful physicochemical characteristic than others prepared microemulsions and more active against weeds than their essential oil and emulsions formulations. Formulated oils emulsions also are useful against several types of weeds and can be an effective control for these common weeds and easy prepared as compared with microemulsions. The main limitation of spray oils and their formulation may be caused plant injury (phytotoxicity) in some situations. Its preferred to apply with direct sprayed on broad weeds types with avoidance to the crop plants that tend to be oils and their formulations-sensitive. The results of the current work showed that it was possible to obtain stabilized oil-in-water microemulsions. Also, it was concluded that microemulsions forming small size droplets would have higher herbicidal activity than essential oils and their emulsions without change in its chemical structure as described by FTIR studies from the position and the number of spectrum in the range of 400–4000 cm^{-1} mid region. These results clearly confirmed that the microemulsions are stable, transparent formulation with effective killing rate against weeds growth. So the use of microemulsions as herbicide agent is promising and new innovation. Future experiments may be needed to focus on the selective properties of the used material in prepared formulation from the essential oils and the possible effects against other weeds, crops selectivity and the biological mode of action as well as to be considered weeds control application under field conditions.

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تجهيز بعض الزيوت النباتية الواعدة لمستحلبات دقيقة لإداء الكفاءة الابادية للحشائش

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إستهدف هذة الدراسة تجهيز بعض الزيوت النباتية فى صورة مستحضرات قابلة للإستحلاب وتقيم نشاطها الابادى العشبى تجاة بعض المحاصيل والاعشاب. أشتملت المستحضرات المجهزة على الزيوت الاساسية مثل القرنفل, النعناع, الخس, الكافور,الخروع ,الجوجوبا والبابونج. تم إختبار الخصائص الابادية بسلسة من التركيزات لهذة المستحضرات على نمو وانبات محصولى القمح والشعيروحشائش الفلارس وديل القط . حيث أظهرت النتائج سمية نباتية وكفاءة بيولوجية للمستحضرات المختبرة بناء على الجرعة المؤثرة النصفية. وكان زيت القرنفل والجوجوبا والكافور اكثر فاعلية وسمية نباتية عن زيت النعناع والخس والخروع والبابونج. كما أثبتت الدراسة ان تجهيزهذة الزيوت النباتية الاساسية السابقة لمستحضرات من مستحلبات الماء فى الزيت كانت ذات كفاءة ابادية و فاعليه أكبر على نمو وانبات النباتات المختبرة. اكدت الدراسة على إمكانية الحصول على مستحلبات دقيقة ماء فى زيت بتركيز 10% زيت. وكان من أهم متطلبات المستحلبات الدقيقة (ميكرواملشن) الناجحة هو ان نسبة الزيت , المواد المساعدة, المواد النشطة سطحيا مع الماء تقريبا (1:6:3:1) على الترتيب, وقد تميزت بالفاعلية فى اخماد الحشائش عن الزيوت الاساسية وعن تجهيزات المستحلبات.بينما كان مستحضرات زيوت الخروع والكافوروالنعناع والجوحوبا افضل ميكرواملشن حيث ادت الى نقص الوزن الجاف للحشائش بنسب52.2,48.2 ,50.9%(للفلارس),58.9,62.9,57.1% على التوالى (ديل القط) بالقارنة بالكنترول بعد 30 يوم من انبثاقها. كما تمت المقارنة بين كفاءة الزيوت الاساسية و مستحضرات المستحلبات الدقيقة على بادرات العليق بعد 7 ايام من نموها حيث انقص مستحضرات الميكرواملشن للخروع والكافوروالنعناع والجوحوبا وزن الكتلة الحيوية الخضرى للعليق بعد 10 ايام من المعاملة الى 70.48,61.23,70.0,65.27 %,على التوالى بالمقارنة بالكنترول. عموما كانت الزيوت الاساسية والمستحلبات والمستحلبات الدقيقة لكل من الجوجوبا والقرنفل والكافور أعلى فاعلية وتاثير فى حين ان الخس والبابونج كان لهم تاثير ضعيف وغير معنوى على نمو وانبات النباتات المختبرة. أكدت الدراسة امكانية الحصول على مستحلبات ميكرواملشن حقيقية قابلة على تكوين قطرات صغيرة الحجم فعالة وقادرة على زيادة الخصائص الابادية العشبية بالمقارنة بالزيوت الاساسية او تجهيزاتها للمستحلبات العادية بدون تغير فى تركيبها الكيماوى حيث تم توصيف وتميزكل زيت عن الاخر بإستخدام جهاز FTIR فى المنطقة المتوسطة 400 الى 4000 سم $^{-1}$. حيث ان تجهيزات الميكرواملشن لديها خصائص قادرة على حمل المكونات و المركبات الفعالة المنتشرة فى الزيت وذلك لمقدرتها العالية على الإذابه فتكون ذات فاعلية يمكن ان تؤخذ فى الاعتبار كأحد وسائل مكافحة الحشائش فى المستقبل.

كلمات مفتاحية: زيوت نباتية أساسية, مستحلبات دقيقة, سمية نباتية, انبات ونموالبادرات.

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