Effect of Hydrophilic-Lipophilic Balance (HLB) Values of Surfactant Mixtures on the Physicochemical Properties of Emulsifiable Concentrate Formulations of Difenoconazole

Walaa El-Sayed1* and Tahany G. M. Mohammad2

1Department of Plant Protection, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt.
2Department of Pesticide Formulations, Central Agricultural Pesticides Laboratory, Agricultural Research Center, Giza, Egypt.

Authors’ contributions
This work was carried out in collaboration between both authors. Author WES designed the study and wrote the protocol. Author TGMM performed the study, wrote the first draft of the manuscript and managed the literature searches under the supervision of author WES. Both authors read and approved the final manuscript.

Article Information
DOI: 10.9734/JALSI/2019/v22i430135
Editor(s):
(1) Dr. Vasil Simeonov, Laboratory of Chemometrics and Environmetrics, University of Sofia “St. Kliment Ohridski”, Bulgaria.
Reviewers:
(1) Fábio Henrique Portella Corrêa de Oliveira, Universidade Federal Rural de Pernambuco, Brazil.
(2) S. V. Patil, Shree Santkrupa College of Pharmacy, India.
(3) Anis Yohana Chaerunisa, Padjadjaran University, Indonesia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/44186

Received 10 November 2019 Accepted 13 January 2020 Published 25 January 2020

ABSTRACT
This study was designed to investigate the effect of hydrophilic-lipophilic balance (HLB) values of different surfactant mixtures on the physicochemical properties of emulsifiable concentrate formulations of difenoconazole. Physical tests of emulsion characteristics and storage stability studies were performed for the different samples to predict the stability of these formulations. Different parameters such as active ingredient content, pH, refractive index, surface tension, viscosity, flash point, persistent foam was determined for the prepared samples. The results showed that difenoconazole could be formulated as a stable emulsifiable concentrate by using a mixture of surfactants at HLB values 9.7, 11.9, 12.5 and 13.1. The storage stability test showed that the decomposition rate of the active ingredient content of difenoconazole in different stable formulations was within the acceptable limits of FAO Specifications. The physical and chemical properties of the stable formulations fulfilled the requirements of EC formulation.
1. INTRODUCTION

Pesticides are important agrochemicals that are widely used to control pests, preventing crop loss caused by major diseases and pests and increases crop yield [1,2,3]. Most pesticides need to be formulated using suitable formulations.

The pesticide formulations play an important role in delivering agrochemicals to target sites and enhancing their efficacy [4]. Liquid formulations are preferred by the farmer for preparing spray solutions for several reasons; they can be measured volumetrically, are easy to handle, spontaneously form stable emulsions and provide suitable container design, are usually easy to rinse out of the package and do not cause application problems.

Emulsifiable concentrate formulations of pesticides (also referred to as EC) have been very popular for many years and represent the biggest volume of all pesticide formulations (about 40%) in terms of global volume usage, and are the most commonly used delivery system for increasing the yield and quality of crop production [5]. In addition, this formulation easy to produce, handle, transport, store, possessing excellent thermodynamics and storage stability [6-9]. The advantages of EC formulations are that they require little agitation, they are not abrasive, do not plug screen or nozzles, leave little visible residue on treated surfaces, are useful for water-insoluble active ingredients and are easy to apply with high efficiency [10].

EC formulations are typically optically transparent oily liquid formulations that are prepared by dissolving the active ingredient in organic solvents (such as benzene, toluene, xylene, etc.), and they also contain surfactants and other additives. These systems are then diluted with water before utilization, which leads to the spontaneous formation of an oil-in-water emulsion that contains the active ingredients inside oil droplets [11,12]. The stability of the emulsions may be affected by several factors such as the Hydrophilic-Lipophilic Balance (HLB) value [13], concentration of active ingredients [14], and addition of surfactant type [15], among others [16].

Surfactant emulsifier blends are added to these formulations to ensure spontaneous emulsification into the water in the spray tank. Surfactant suppliers provide advice on the selection of a “balanced pair” emulsifier blend which is frequently necessary to ensure good emulsion stability after dilution in water of varying degrees of hardness. The correct balance of surfactant emulsifiers can be obtained by knowing the Hydrophilic-Lipophilic Balance (HLB) values for the surfactants. Surfactants in the HLB range of 8-18 normally give good emulsions [17,18]. The optimum ratio of surfactants is determined by experimentation to give spontaneous emulsification in water (strike or bloom), and a stable emulsion with minimum creaming and no oil droplet coalescence [19,20]. Therefore, the present study aimed to prepare several emulsifiable concentrate formulations of difenoconazole and study the effect of HLB values of different surfactants on the stability of the prepared formulations.

2. MATERIALS AND METHODS

2.1 The Test Active Compound

Difenoconazole (Technical grade, purity 97%) was purchased from China. Difenoconazole (Cis, trans-3-chloro-4- [4-methyl-2-(1H-1, 2, 4-triazol-1-ylmethyl)-1, 3dioxolan-2-y1 phenyl- 4-chlorophenyl ether), CAS Registry No. [119446-69-3] chemical structure is shown in Fig. 1. The substance is white to light beige crystals with a melting point 82.0 - 83.0°C. The value of Kow logP (the octanol/water partition coefficient) is 4.4 (at 25°C). The solubility is 15 mg/l in water at 25°C and >500 g/l in acetone, dichloromethane, toluene, methanol and ethyl acetate, 3 g/l in hexane, 110 g/l in octanol at 25°C, and it is stable up to 150°C.

![Fig. 1. Structure of difenoconazole](image-url)
2.2 Chemicals

Nonionic Surfactants: Soprophor CY/8 (Ethoxylated tristyryl phenol, HLB 13.7); Alkamuls RC (ethoxylated Castor oil, HLB 10.5); Alkamuls 14/R (Ethoxylated Castor oil, HLB 14.9); Arkapol N 100; nonylphenol polyglycoether (10EO), HLB 13.5 were kindly supplied by Rhodia-Home, Personal Care & Industrial Ingredients, Milano, Italy. Anionic Surfactants: Rodacal 60/BE (dodecyl benzene sulfonate, calcium salt, HB 8.3) and Geronol FF4, mixture of anionic and non-ionic derivatives, HLB12.7 were kindly supplied by Rhodia-Home, Personal Care & Industrial Ingredients, Milano, Italy. Tween 80 (Polyoxyethylene (20) Sorbian monooleate, HLB 15.00); Tween 20 (Polyoxyethylene(20) sorbitanmonolaureate, HLB 16.7), Span 20 (Sorbitanmonolaureate, HLB 8.6) and Span 80 (Sorbitanmonooleate, HLB 4.3) and calcium carbonate were purchased from Sigma-Aldrich Chemie GmbH, Riedstr, Steinheim, Germany. Dimethyl formamide and polyethylene glycol were purchased from ADWIC. El Nasr Pharmaceutical Chemical Co., Egypt. Magnesium oxide, methyl red and Solvesso 100 were purchased from Qualikems Fine Chemicals PVT Ltd. India. Ammonia solution was purchased from Prolabo. All materials were used as received without further purification. Deionized water used to prepare the solutions was obtained from a Milli-Q-system (Millipore).

2.3 Preparation of Difenoconazole EC

Emulsifiable concentrate (EC) formulation of difenoconazole was prepared by simple mixing method. Difenoconazole technical grade, solvent, cosolvent, anti-freezing agent and mixed surfactants. The proportion between the two surfactants was calculated to obtain HLB values in Table 1. The resulting formulation was stored in a glass bottle at room temperature (25 ± 2°C) and protected from light.

2.4 Determination of Difenoconazole Content

The content of active ingredient (AI) of difenoconazole in the prepared formulations was detected using (Unicam pro GC with FID) equipped with Electron Capture Detector (ECD) programmed for external standardization using peak area. Gas Chromatography with the following conditions: Oven: 260°C, Injector: 280°C, Detector (FID): 290°C.

2.5 Stability Tests

Pesticides chemical stability is determined by its ability to withstand (a) the deteriorating effects encountered during storage formulation and (b) the environment to which it is subjected after application [21]. The formulation stability tests included emulsion characteristics, stability at 0°C, accelerated storage procedure at 54°C, and persistent foaming.

2.5.1 Emulsion characteristics and re-emulsification

In the emulsion characteristics test, the emulsion and re-emulsification stability tests were performed according to CIPAC MT 36.3 [22]. The formulation was diluted at 30 ± 2°C with CIPAC Standard A and D waters prepared as per CIPAC MT 18 [23]. In the emulsion characteristics experiment, 5 ml of the formulation sample was separately mixed with 95 ml standard CIPAC A water (20 ppm hardness, pH 5.00-6.00, Ca²⁺: Mg²⁺=1:1) and CIPAC D water (342 ppm hardness, pH 6.00-7.00, Ca²⁺: Mg²⁺= 4:1) in a 100 milliliter measuring cylinder to produce 100 milliliter of aqueous emulsion. The cylinder was then stoppered and inverted once then it was noted if the emulsion was homogenous or not, after 30 sec it was inverted 10 times and left to stand. Emulsion stability was determined according to CIPAC MT 36.3 [22] by recording traces of oil and cream < 2 ml at 0, 0.5, 1, 2, 24, and 24.5 h.

Table 1. Composition of difenoconazole EC

| Formulations code | Mixed surfactants | HLB value |
|-------------------|-------------------|-----------|
| F1                | Alkamuls RC: Rodacal 60/BE | 9.7       |
| F2                | Tween 80: Span 80 | 11.4      |
| F3                | Soprophor Cy/8: Rodacal 60/BE | 11.9      |
| F4                | Arkapol N 100: Alkamuls RC | 12.5      |
| F5                | Alkamuls 14/R: Geronol FF4 | 13.1      |
| F6                | Tween 80: Alkamuls RC | 13.5      |
| F7                | Tween 20: Span 20 | 14        |
2.5.2 Storage stability

Accelerated stability tests at elevated temperatures are designed to increase the rate of chemical degradation or physical change of a product. Accelerated testing was performed at elevated temperatures to obtain information on the shelf life of a product in a relatively short time. Accelerated testing involves extrapolations from higher to lower temperatures and from shorter to longer storage periods. The accelerated storage test at elevated temperatures is performed by placing 50 ml of the product in a tightly capped bottle in the oven at 54 ±2°C for 14 days. The volume of any separated material at the bottom of the tube was then recorded CIPAC MT 46.3 [22].

Storage at low temperatures may result in crystallization of active constituent, significant changes in viscosity or separation of the formulation. The liquid formulation was tested at 0 ± 2°C for 7 days as per CIPAC MT 39.39 [22]. A 100 ml of the sample was transferred to a glass tube and cooled in a refrigerator at 0 ± 2°C for 7 days, the tube was left to stand at room temperature for 3 hours. The volume of any separated material at the bottom of the tube was recorded.

2.5.3 Persistent foam

Persistent foam is a measure of the amount of foam likely to be present in a spray tank or other application equipment following dilution of the product with water. A specified amount of the prepared formulation is added to CIPAC standard waters (95 ml) in the measuring cylinder and made up to the mark. The cylinder is stoppered and inverted 30 times. The cylinder is left to stand undisturbed for the specified time. The volume of foam was noted at 0, 10 s, and 1, 3, and 12 min according to CIPAC MT 47.2 [22].

2.6 Physical Characterization

The pH of a 1% solution of the formulations was measured using a pH meter (Jenway model pH 3510) which was recalibrated before testing; the measurements were carried at 25°C by direct immersion of pH glass electrode into EC samples as per CIPAC MT 75.3 [22].

The Refractive index is an optical measurement of a material's ability to bend a beam of light. Also, the refractive index may be used to determine the purity of the material; the refractive index of the EC samples was measured using a digital ABBE Refractometer, ATAGO, Co., LTD, Japan by placing one drop of the EC formulation on the slide at 25°C. Distilled water was used as a standard, it had a refractive index of 1.3330 [24].

The viscosity was measured using "Brookfield DV II+ Pro" digital Viscometer (Brookfield, USA) UL rotational adaptor, the temperature was kept at 25°C during the measurement by water bath (Model: TC-502 USA) and each reading was taken after equilibrating the sample. The data were acquired via a personal computer using Rheolac Software developed by Brookfield Engineering Lab. Five replicates were conducted for the sample and the average was reported and expressed as millipascal-second (mPa.s) [25].

Surface tension was measured using “Sigma 700” by Wilhelmy plate method, the instrument was recalibrated before testing and the sample measured was clean, homogenous, and free from any bubbles and has a stable surface. The surface tension of the EC samples was recorded [26].

Density was measured using a digital density meter model DDM2910 with a touch screen (Rudolph Research Analytical, USA) [27].

Measurement of the flash point of the prepared EC was carried out by a tag open cup method by Koehler instrument company, INC, USA. The flash point was recorded as the temperature at the thermometer when a flash appeared [28].

3. RESULTS AND DISCUSSION

3.1 Storage Stability

Storage stability is an effective factor in determining the shelf-life of pesticide formulations. Delamination and degradation of AIs in pesticides occurred particularly during long-term storage, leading to reduced effectiveness; hence, whether agrochemical formulations can maintain excellent stability is important for practical applications [12].

We investigated the stabilities of difenoconazole samples including stability at 0 ± 2°C for 7 days and accelerated storage in the oven at 54 ± 2°C for 14 days, and the experimental results are summarized in Table 2. The AI contents of difenoconazole in freshly prepared, 0°C for 7 days and 54°C for 14 days was within the
acceptable range of defined specification (+/-5%) [29] and the stable formulations had no phase separation and no sedimentation, which indicated that formulated EC is thermodynamically stable.

### 3.2 Emulsion Stability

The most important factor in emulsion preparation is the choice of appropriate surfactants that will emulsify the selected ingredients satisfactorily and preserve their stability. Surfactant mixtures play a promising role in surface chemical applications and often exhibit interfacial properties more pronounced than those of the individual surface-active components of the mixture [30,31]. The hydrophilic-lipophilic balance (HLB) value of surfactants plays an important role in the determination of its functionality. Emulsions with surfactants of HLB < 6 tend to be oil soluble and stabilize water-in-oil, whereas emulsions with surfactants of HLB >10 tend to be water soluble and stabilize oil-in-water [11].

The emulsion and re-emulsification stability test state that the formulation when diluted at 30 ± 2°C with CIPAC standard waters A and D shall comply with the specification of emulsification of emulsifiable concentrate formulation [29]. The maximum level of cream and precipitate layer didn’t exceed 2 ml after 0.5, 2 and 24.5 hrs from dilution.

Results in Tables 3-5 showed that the effect of HLB values of mixed surfactants on the emulsification and re-emulsification stability test before and after storage when diluted with CIPAC Standard Waters A and D shall comply with the specification of emulsification of emulsifiable concentrate formulation [29]. The maximum level of cream and precipitate layer didn’t exceed 2 ml after 0.5, 2 and 24.5 hrs from dilution.

The pH is a very important parameter because it can reflect a chemical change in the components present in the formulation. Also, the changes in pH over long storage periods can indicate degradation of the active component, a proliferation of bacteria, instability or incompatibility of certain compounds [34]. The pH values of the EC formulations were in the range of 5.45 to 6.61, which indicated that the formulations had acidic character.

#### Table 2. Active ingredient content of difenoconazole EC formulations

| Test period | Fresh Form. | 7 days | 14 days |
|-------------|-------------|--------|---------|
| Formulations code | AI | % loss | AI | % loss |
| F1 | 25.94 | 3.97 | 24.65 | 4.97 |
| F3 | 25.67 | 2.37 | 25.04 | 2.45 |
| F4 | 24.37 | 1.27 | 24.03 | 1.39 |
| F5 | 25.09 | 0.67 | 24.63 | 1.83 |
| F6 | 24.89 | 0.52 | 24.52 | 1.48 |
| F7 | 25.03 | 0.44 | 24.30 | 2.92 |
Table 3. Emulsion characteristics at ambient temperature - measurement of creamy layer – CL in ml for samples made with CIPAC water A & D

| Time  | F1  | F2  | F3  | F4  | F5  | F6  | F7  |
|-------|-----|-----|-----|-----|-----|-----|-----|
|       | CL*/ml in CIPAC water A | CL*/ml in CIPAC water D | CL*/ml in CIPAC water A | CL*/ml in CIPAC water D | CL*/ml in CIPAC water A | CL*/ml in CIPAC water D | CL*/ml in CIPAC water A |
|       |     |     |     |     |     |     |     |
| 0.0 h | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| 0.5h  | 0   | 0.1 | 0   | 0   | 0   | 0   | 0.1 |
| 1h    | 0   | 0.3 | 0   | 0   | 0   | 0   | 0.5 |
| 2h    | 0.5 | 0.5 | 8   | 0   | 0   | 0   | 0.2 |
| 24h   | 1   | 1   | 15  | 6   | 0.1 | 0   | 1   |
| REE   | 0   | 1   | 0   | 9   | 0.2 | 0   | 0.5 |

*CL: Creamy layer (ml)

Table 4. Emulsion characteristics at 0±2°C after 7 days - measurement of creamy layer - CL in ml for samples made with CIPAC water A & D

| Time  | F1  | F2  | F3  | F4  | F5  | F6  |
|-------|-----|-----|-----|-----|-----|-----|
|       | CL*/ml in CIPAC water A | CL*/ml in CIPAC water D | CL*/ml in CIPAC water A | CL*/ml in CIPAC water D | CL*/ml in CIPAC water A | CL*/ml in CIPAC water D |
|       |     |     |     |     |     |     |
| 0.0 h | 0   | 0   | 0   | 0   | 0   | 0   |
| 0.5h  | 0   | 0   | 0   | 0   | 0   | 0   |
| 1h    | 0   | 0   | 0   | 0   | 0   | 0   |
| 2h    | 0.5 | 0   | 0   | 0   | 0   | 0   |
| 24h   | 0   | 0   | 0   | 0   | 0.1 | 0   |
| REE   | 0   | 0   | 0   | 0   | 0   | 0   |

*CL: Creamy layer (ml)
Table 5. Emulsion characteristics at 54±2°C after 14 days measurement of creamy layer CL in ml for samples made with CIPAC water A & D

| Time  | F1       | F2       | F3       | F4       | F5       |
|-------|----------|----------|----------|----------|----------|
|       | CL*/ml in CIPAC water A | CL*/ml in CIPAC water D | CL*/ml in CIPAC water A | CL*/ml in CIPAC water D | CL*/ml in CIPAC water A | CL*/ml in CIPAC water D |
| 0.0 h | 0        | 0        | 0        | 0        | 0        | 0        |
| 0.5h  | 0        | 0        | 0        | 0        | 0        | 0        |
| 1 h   | 0        | 0        | 0        | 0        | 0        | 0        |
| 2 h   | 0        | 0        | 0        | 0        | 0        | 0        |
| 24 h  | 1        | 0        | 0        | 0        | 0        | 0        |
| REE   | 0        | 0        | 0        | 0        | 0        | 0        |

Table 6. Persistent foam difenoconazole EC formulations made with CIPAC water A & D

| Time  | Persistent foam in CIPAC water A | Room temperature | Persistent foam in CIPAC water D | Room temperature |
|-------|---------------------------------|------------------|---------------------------------|------------------|
|       | 0-22°C for 7 days                | 54-52°C for 14 days | 0-22°C for 7 days                | 54-52°C for 14 days |
| F1    | F2                               | F3 | F4 | F5 | F6 | F7 | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F1 | F2 | F3 | F4 | F5 | F6 | F7 |
| Foam in Foam | ml | Foam in Foam | ml | Foam in Foam | ml | Foam in Foam | ml | Foam in Foam | ml | Foam in Foam | ml | Foam in Foam | ml | Foam in Foam | ml | Foam in Foam | ml | Foam in Foam | ml | Foam in Foam | ml | Foam in Foam | ml | Foam in Foam | ml | Foam in Foam | ml | Foam in Foam | ml |
| 0s    | 0                                | 3  | 2  | 2  | 3  | 0  | 3  | 2  | 2  | 2  | 4  | 2  | 3  | 3  | 3  | 3  | 3  | 4  | 0  | 3  | 2  | 2  | 3  | 0  | 3  | 2  | 2  | 3  | 0  | 3  | 2  |
| 10s   | 0                                | 2  | 2  | 1  | 2  | 3  | 0  | 3  | 2  | 1  | 1  | 1  | 4  | 0  | 1  | 3  | 2  | 1  | 3  | 3  | 0  | 2  | 1  | 1  | 2  | 0  | 1  | 1  | 1  | 1  |
| 1 min | 0                                | 2  | 1  | 0  | 1  | 1  | 0  | 3  | 2  | 1  | 1  | 3  | 0  | 0  | 2  | 1  | 1  | 2  | 2  | 1  | 1  | 2  | 1  | 1  | 1  | 1  | 1  | 1  |
| 3 min | 0                                | 1  | 1  | 0  | 1  | 2  | 0  | 2  | 1  | 0  | 0  | 0  | 3  | 0  | 0  | 1  | 1  | 1  | 1  | 2  | 1  | 1  | 2  | 1  | 1  | 1  | 1  | 1  |
| 12 min| 0                                | 1  | 0  | 0  | 0  | 0  | 1  | 0  | 1  | 0  | 0  | 0  | 2  | 0  | 0  | 1  | 0  | 0  | 1  | 1  | 0  | 1  | 1  | 0  | 1  

*CL: Creamy layer (ml)
Table 7. Physical properties of difenoconazole EC formulations at room temperature

| Physical properties                  | F1    | F3    | F4    | F5    |
|-------------------------------------|-------|-------|-------|-------|
| pH (1%)                             | 6.06  | 5.88  | 6.36  | 5.75  |
| Surface Tension (mN/m)              | 33.51 | 32.92 | 32.72 | 33.98 |
| Density (g/cm³)                     | 0.9800| 1.0036| 1.0014| 1.0033|
| Flash point (°C)                    | 54    | 55    | 55    | 54    |
| Refractive Index                    | 1.5115| 1.5097| 1.5077| 1.5077|
| Viscosity (mPa)                     | 4.95  | 4.65  | 4.13  | 4.96  |

Table 8. Physical properties of difenoconazole EC formulations at 0±2°C for 7 days

| Physical properties                  | F1    | F3    | F4    | F5    |
|-------------------------------------|-------|-------|-------|-------|
| pH (1%)                             | 6.32  | 6.16  | 6.29  | 5.88  |
| Surface Tension (mN/m)              | 33.62 | 32.85 | 32.65 | 33.86 |
| Density (g/cm³)                     | 1.0010| 1.0038| 1.0017| 1.0037|
| Flash point (°C)                    | 54    | 55    | 55    | 55    |
| Refractive Index                    | 1.5044| 1.5081| 1.5095| 1.5075|
| Viscosity (mPa)                     | 4.34  | 4.10  | 3.81  | 4.96  |

Table 9. Physical properties of difenoconazole EC formulations at 54±2°C for 14 days

| Physical properties                  | F1    | F3    | F4    | F5    |
|-------------------------------------|-------|-------|-------|-------|
| pH (1%)                             | 6.00  | 5.97  | 6.61  | 5.45  |
| Surface Tension (mN/m)              | 33.76 | 33.10 | 32.58 | 33.86 |
| Density (g/cm³)                     | 1.0014| 1.0040| 1.0010| 1.0042|
| Flash point (°C)                    | 55    | 55    | 55    | 55    |
| Refractive Index                    | 1.5063| 1.5097| 1.5054| 1.5049|
| Viscosity (mPa)                     | 4.26  | 4.15  | 4.10  | 4.92  |

The prepared EC formulations had a surface tension range (32.58-33.98 mN/m); lower surface tension is a desirable characteristic for most agricultural sprays because it facilitates the spreading of droplets upon impact on leaves or other target surfaces, to increase the surface-active area and improve penetration and uptake of the product into the plants [35]. The variation of density was (0.98-1.0042 g/cm³).

The flash point is a measure of the tendency of a sample to form flammable mixtures with air in controlled laboratory conditions and is a parameter for storage and handling when considering as flammable materials [5]. The prepared formulations in all the storage conditions showed high flash points values (54-55°C).

The variation of refractive index was in the range of 1.5044 to 1.5122, it reflects the EC formulations appear nearly transparent in the visible spectrum and the viscosity of the prepared formulations ranged from 3.81 to 4.96 mPa.

4. CONCLUSION

Emulsifiable concentrate formulations of difenoconazole were prepared by mixing surfactants with different HLB values and characterized according to their active ingredient content, pH, refractive index, surface tension, viscosity, flash point, density, persistent foam, and emulsion stability. Difenoconazole could be formulated as a stable emulsifiable concentrate showing good physical characteristics at the different HLB values 9.7, 11.9, 12.5 and 13.1. Further studies could be performed to evaluate the Fungicidal activity of the prepared formulations and compare the activity with commercial formulation available in the market.

COMPETING INTERESTS

Authors have declared that no competing interests exist.
REFERENCES

1. Qin H, et al. Preparation and properties of lambda-cyhalothrin/polyurethane drug-loaded nanoemulsions. RSC Advances. 2017;7:52684-52693.
2. Wang C, et al. Fabrication and evaluation of Lambda-Cyhalothrin nanosuspension by one-step melt emulsification technique. Nanomaterials. 2019;9:145.
3. Cui B, et al. Improving abamectin bioavailability via nanosuspension constructed by wet milling technique: Abamectin nanosuspension constructed by wet milling. Pest Management Science. 2019;75.
4. Shao H, Xi N, Zhang YL. Microemulsion formulation of a new biopesticide to control the Diamondback moth (Lepidoptera: Plutellidae). Scientific Reports. 2018:8.
5. Encinar JM, González JF, Rodríguez-Reinares A. Biodiesel from used frying oil. Variables affecting the yields and characteristics of the biodiesel. Industrial & Engineering Chemistry Research. 2005;44(15):5491-5499.
6. Sarwar M. Commonly available commercial insecticide formulations and their applications in the field. International Journal of Materials Chemistry and Physics. 2015;1(2):116-123.
7. Vanitha S. Developing new formulation using plant oils and testing their physical stability and antifungal activity against Alternaria chlamydospora causing leaf blight in Solanum nigrum. RJAS. 2010;4:385-390.
8. Rajmani P, et al. Formulation development, standardization and antimicrobial activity of Ageratum conyzoides extracts and their formulation. International Journal of Pharmacy and Pharmaceutical Science. 2014;6.
9. Thakur LK, et al. Development and evaluation of basil emulsifiable concentrates. African Journal of Science and Research. 2014;3(1):06-09.
10. Kozuks Y, Ohtsubo TA. Predictive solubility tool for pesticide emulsifiable concentrate formulations. ASTM International Mem. of Cross Ref; 2009.
11. Feng J, et al. Application of nanoemulsions in formulation of pesticides; 2018.
12. Aguilar M, et al. Oil-in-water (o/w) emulsionable concentrate of Ishpink (Ocotea quixos) with thermodynamic stability. Revista Caatinga. 2019;32:590-598.
13. Losada-Barreiro S, Sánchez-Paz V, Bravo-Díaz C. Effects of emulsifier hydrophilic–lipophilic balance and emulsifier concentration on the distributions of gallic acid, propyl gallate, and α-tocopherol in corn oil emulsions. Journal of Colloid and Interface Science. 2013;389(1):1-9.
14. Hallouard F, et al. Preparation and characterization of spironolactone-loaded nano-emulsions for extemporaneous applications. International Journal of Pharmaceutics. 2015;478(1):193-201.
15. Feng J, et al. Effect of emulsifying process on stability of pesticide nanoemulsions. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2016;497:286-292.
16. Feng J, et al. Formulation of oil-in-water emulsions for pesticide applications: Impact of surfactant type and concentration on physical stability. Environmental Science and Pollution Research. 2018;25(22):21742-21751.
17. Dennis RH. Encyclopedia of agricultural, food and biological engineering. CRC Press; 2003.
18. Knowles A. Recent developments of safer formulations of agrochemicals. The Environmentalist. 2008;28(1):35-44.
19. Knowles A. New developments in crop protection product formulation. Agrow Reports UK: Tand F Informa UK Ltd. 2005:153-156.
20. Ferreira MR, et al. Development and evaluation of emulsions from Carapa guianensis (Andiroba) oil. AAPS PharmSciTech. 2010;11(3):1383-90.
21. Allawzi MA, Allaboun H, Qazaq AS. Formulation, emulsion, and thermal stability of emulsifiable malathion concentrate using ethanol as a solvent. International Journal of Applied Engineering Research. 2016;12:221-231.
22. CIPAC CI. Collaborative international pesticides analytical council. Available:http://www.cipac.org/ (Accessed on 2016)
23. CIPAC. Dobrat W, Martijn A, Editors. Preparation of standard waters A and D. In: CIPAC Handbook F. Physico-chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd. 1995;59-62.
24. ASTM. Standard test method for refractive index and refractive dispersion of hydrocarbon liquids. American Society of Testing and Materials. 2016;D1218-12.

25. ASTM. Standard test methods for rheological properties of Non-Newtonian materials by rotational viscometer. American Society of Testing and Materials. 2018;D2196.

26. ASTM. Standard test methods for surface and interfacial tension of solutions of paints, solvents, solutions of surface-active agents and related materials. American Society for Testing and Materials. 2014;D1331.

27. ASTM. Standard test method for density, relative density, or API gravity of crude petroleum and liquid petroleum products by hydrometer method. 2017;D1298.

28. ASTM. Test methods for flash point by small scale closed cup tester. American Society for Testing and Materials. 2016;D3828.

29. FAO/WHO J. Specifications, food and agriculture organization of the united nations/world health organization - FAO/WHO. Manual on Development and Use of FAO and WHO Specifications for Pesticides; 2018.

30. Rai N, Pandey IP. Study of some physiochemical factors determining emulsion stability with mixed emulsifiers. Journal of Industrial Research & Technology. 2013;3(1):12-16.

31. Myers D. In surfactant science and technology. VCH Publishers, New York; 1998.

32. Tadros. Rheology of dispersions: Principles and applications. First Edit; Wiley-VCH, Weinheim, Germany; 2010.

33. McClements. Food emulsion: Principle, practices, and techniques. 2nd Ed.; CRC Press, Boca Raton, FL, USA; 2005.

34. Campelo PH, et al. Stability of lime essential oil emulsion prepared using biopolymers and ultrasound treatment. International Journal of Food Properties. 2017;20(Sup 1):S564-S579.

35. Giardino L, et al. Surface tension comparison of four common root canal irrigants and two new irrigants containing antibiotic. Journal of Endodontics. 2006;32(11):1091-1093.