Eco-Friendly Cinnamaldehyde Based Emulsion for Phytopathogenic Bacterial Growth Inhibitor

Tahany G. M. Mohammed¹* and A. F. Abd El-Rahman²

¹Central Agricultural Pesticides Laboratory, Pesticide Formulations Department, Agricultural Research Center (ARC), 12618, Dokki, Giza, Egypt.
²Bacterial Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

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

Article Information
DOI: 10.9734/JAMB/2020/v20i1030285
Editor(s):
(1) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
(2) Dr. C. Graciela Castro Escarpulli, Instituto Politecnico Nacional, Mexico.
Reviewers:
(1) Shekh Mehdihasan, Shenzhen University, China.
(2) Nirmalkumar Natvarlal Patel, Research and Development at Dai Ichi Karkaria Ltd, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/61875

Received 05 August 2020
Accepted 10 October 2020
Published 28 October 2020

ABSTRACT

The formulation plays an essential role in achieving the successful delivery and biological activity of any plant protection products. This study aimed to develop a cinnamaldehyde water-based formulation (oil-in-water emulsion) via a high-shear stirring emulsification method. Cinnamaldehyde emulsion was successfully prepared and characterized using different physicochemical parameters (emulsion stability, persistent foaming, accelerated storage at 54 °C for 2 weeks, and stability at 0 °C for one week, as well as pH, surface tension, flash point, viscosity, and particle size distribution). Also, the antibacterial activity was verified in vitro against some important phytopathogenic bacteria; Erwinia amylovora, Pectobacterium aroidearum, Pseudomonas aeruginosa, and Ralstonia solanacearum using well diffusion method. In addition, the minimum inhibition concentration (MIC) was determined by the twofold dilution method. The results revealed that the prepared formulation showed good storage stability, exhibited non-Newtonian shear-thinning behavior and promising antibacterial activity. The inhibition zones against the tested phytopathogenic bacteria were ranged

*Corresponding author: E-mail: tahanymohamad2@gmail.com, Tahany_20102010@yahoo.com;
from 10.3 mm to 52.0 mm. MICs of the prepared formulation were 15.63, 31.25, 62.5, and 15.63 µl/ml against Erwinia amylovora, Pectobacterium aroidearum, Pseudomonas aeruginosa and Ralstonia solanacearum, respectively. Our results provide an environmentally friendly formulation with promising activity to control the agricultural crop disease.

Keywords: Cinnamaldehyde; an oil-in-water emulsion; formulation; phytopathogenic bacteria.

1. INTRODUCTION

Nowadays, plant phytopathogenic bacteria are responsible for huge economic losses in crop yield and the quality of plant production worldwide [1-3]. Collectively, they cause losses of over $1 billion dollars worldwide every year to the food production sector [4,5]. Avoiding or mitigating crop losses due to plant diseases is an important consideration in plant production. The rapid and effective control of the plant diseases in crop cultivation is generally achieved using synthetic pesticides, which became essential to maximize agricultural productivity. Despite their beneficial role in agriculture, pesticides have negative environmental effects on the other living on-target organisms [3,6,7]. Therefore, there has been a growing interest in research concerning alternative natural pesticides and antimicrobial active compounds with less harmful effects to control plant pathogenic diseases, such as essential oils (EOs) [8]. EOs could be used as alternatives for chemical pesticides as they are naturally biodegradable products, with antimicrobial properties, low mammalian toxicity, and low environmental impact [2,9-11]. The individual constituents of EOs are also considered as a promising natural antimicrobial agent against plant pathogens [12].

Cinnamaldehyde, which represents ~65% of the composition of Cinnamomum sp. EO [13,14] is an example of a natural protectant against plant pathogens. This compound is generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA), and Flavour and Extract Manufacturer's Association (FEMA), and has been granted A status (may be used in foodstuffs) by the Council of Europe [15-17]. Cinnamaldehyde is active against pathogenic bacteria, fungi, and viruses [18], besides other activities related to human health like anti-inflammatory, and antioxidant potentials. Therefore, it is widely used in the food, drug, and cosmetic industries [19]. However, EOs and their individual constituents are generally unstable in the presence of light, heat, oxygen, and humidity. Also, they are highly volatile and present poor water solubility which required using organic solvents for their dilution and delivery [20-22]. Hence, a suitable intervention is necessary to overcome these challenges like using the emulsion technology in which water is used as a preparation medium and a delivery system.

An emulsion is a thermodynamically unstable system consisting of at least two immiscible liquids or phases, one of which is dispersed in the form of droplets in the other [23-25]. Emulsions are categorized into two types: oil-in-water (O/W) and water-in-oil (W/O) emulsions [26]. Oil-in-water (O/W) emulsions have many important industrial applications covering different aspects in food [27], pharmaceuticals [28], petrochemicals [29], cosmetics [30] and agrochemicals [23,31]. Agrochemical oil-in-water emulsion (EW) is a formulation in which water-insoluble liquid pesticides or organic solvents containing dissolved solid pesticides are dispersed as small droplets in water to form oil-in-water emulsions by adding suitable surfactants and energy [32]. Oil-in-water emulsion greatly reduces the concentration of surfactants and/or organic solvents compared with microemulsions (ME, the concentration of surfactants in ME is usually approximately ≥ 20%) and conventional emulsifiable concentrate (EC, which uses a large amount of solvent) [33,34]; Oil-in-water emulsions also improve the delivery and penetration efficacy of bioactive ingredients as pesticide delivery systems. This formulation is generally considered as a safe and environmentally friendly water-based pesticide formulation [35].

The present study was designed to prepare cinnamaldehyde-in-water emulsion for evaluation as a potential eco-friendly bactericide. Physiochemical properties, stability, and antibacterial activity against some important plant phytopathogenic bacteria will be also studied.

2. MATERIALS AND METHODS

2.1 Chemicals

Magnesium sulfate heptahydrate and cinnamaldehyde were purchased from Sigma
Aldrich (St. Louis, MO, USA), and Polyoxy-35-castor oil (Cremophor-EL, non-ionic surfactant) was purchased from Sigma-Merck (Germany). Agar, glycerol, dipotassium phosphate, sodium phosphate dibasic dodecahydrate, sodium phosphate monobasic dehydrate, propylene glycol, and sodium hydroxide were purchased from ADWIC, El Nasr Company, Egypt. Calcium carbonate was purchased from Sigma-Aldrich, Germany. Magnesium oxide and methyl red were purchased from Qualikems Fine Chemicals. India. Ammonia solution was purchased from Prolabo. Proteose peptone was purchased from Technopharmchem, India. Water was obtained from Milli-Q water purification system (Millipore, LABCONCO Corporation, USA). All other chemicals used were of analytical reagent grade.

2.2 Phytopathogenic Bacterial Strains

The bacterial isolates used in this study were obtained from Bacterial Disease Research Department, Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt. These phytopathogenic bacteria were previously isolated by the second author and others from different hosts. These isolates of phytopathogenic bacteria were; *Erwinia amylovora* isolated from pear trees [36], *Pectobacterium aroidearum* isolated from guava root [37], *Pseudomonas aeruginosa* isolated from onion plants (Unpublished data) and *Ralstonia solanacearum* isolated from potato tubers [38]. These isolates were previously identified using different identification methods. *Erwinia amylovora*, and *Ralstonia solanacearum* were identified using polymerase chain reaction (PCR), using the two primers AMSJ14258 and AMSK14892c [39] for *Erwinia amylovora* [36] and the two primers RS-1-F and RS-1-R [40] for *Ralstonia* [41]. While *Pectobacterium aroidearum* [37] and *Pseudomonas aeruginosa* (Unpublished data) were identified using 16S rRNA analysis.

2.3 Emulsion Preparation

Oil-in-water emulsions were prepared via a high-shear stirring emulsification method. Cinnamaldehyde (10wt %) and surfactant were mixed to form the oil phase. Deionized water, preservative, Arabic gum, and propylene glycol as the aqueous phase. Emulsions were prepared by progressively incorporating the oil phase into the aqueous phase using a high shear dispersion homogenizer Ultra-Turrax homogenizer (T25D IKA, Germany) at 18000 rpm for 5 min at room temperature.

2.4 Characterization of the Emulsion

2.4.1 Stability tests

The formulation stability tests included emulsion characteristics, stability at 0°C, accelerated storage procedure at 54°C, and persistent foaming. All test methods followed the official CIPAC standard method outlined in the CIPAC handbook [42]. In the emulsion characteristics experiment, 5 ml of the emulsion were separately mixed with CIPAC standard water in a 100 ml measuring cylinder to produce 100 ml of diluted emulsion. The stopper was placed on the cylinder, which was subsequently turned upside down 10 times. Subsequently, the amount of free oil or cream that separated at the top or the bottom of the emulsion was observed after the emulsion could stand undisturbed for various intervals (0, 0.5, 1, 2, 24, and 24.5 h). For the stability test at low temperature (0°C), 100 ml of the sample was transferred to a glass tube. For cooling, the tube and its contents were placed in a refrigerator and remained at 0±2°C for 7 days. At the end of 7 days, the tube was removed from the refrigerator, and allowed to remain undisturbed at room temperature for 3 h. The volume of any separated material at the bottom of the tube was subsequently recorded. Accelerated storage procedure was executed by placing the sample (about 50 ml) in the bottle and placing the capped bottle and contents in an oven of 54 ± 2°C for 14 days. 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. The specified amount of the prepared formulation is added to CIPAC standard waters A and D (95 ml) in the measuring cylinder and made up to the mark. The cylinder is stoppered and inverted 30 times. Stand the cylinder on the bench and left undisturbed for the specified time. The volume of foam was noted.

2.4.2 pH measurement

The pH of a 1% solution of the prepared formulation was measured using a pH meter (Jenway® model pH 3510), it was recalibrated before testing using a standard buffer solution at pH 7.0 and pH 4.0; the measurements were carried out at 25°C by direct immersion of pH glass electrode into the formulation samples.
2.4.3 Surface tension measurements

The surface tension was measured by the Wilhelmy plate method using a tensiometer “Sigma 700” instrument (Biolin Scientific). The platinum plate was burned under an alcohol flame after being washed by deionized water and alcohol to remove impurities before each measurement. To guarantee the cleanliness of the plate, the surface tension of water was used as a control to calibrate the tensiometer.

2.4.4 Flash point

Measurement of the flash point of the prepared formulation was carried out by the 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.

2.4.5 Viscosity measurements

The viscosity of the prepared formulation was measured at different shear rates, without dilution, 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). Preliminary experiments showed that the appreciated value of torque to obtain reliable results was between 10 and 100% of the measuring range. The flow curve of the prepared formulation was obtained by directly reading the viscosity (mPas) and shear rate (s⁻¹) from the viscometer.

2.4.6 Size distribution of the emulsions

The oil-droplet size of oil-in-water emulsions was measured using a Mastersizer 3000 (Malvern Instruments, Malvern, UK) after preparation at ambient temperature (~25 °C). Distilled water (25°C) was used as the dispersion medium; the refractive index values input was 1.333. The emulsion was added into the water dropwise. When the laser intensity reached 10% to 20%, the measurement was started. The average droplet size was expressed in terms of volume mean diameter D [43].

2.5 Antibacterial Activity of Cinnamaldehyde Formulation

2.5.1 Preparation of bacterial suspensions

Each isolate of the tested phytopathogenic bacteria was separately inoculated in Petri plates containing King’s agar B medium (proteose peptone (20.0 g), dipotassium phosphate (1.5g), magnesium sulfate heptahydrate (1.5g), glycerol 15.0 ml, Agar (20.0 g), distilled water 1000.0 ml and final pH 7.2±0.2). Inoculated plates were incubated at 28°C for 48 hrs. Bacterial growth was harvested using sterilized 10 mM phosphate buffer (2.7 g Na₅H₂PO₄.12H₂O;0.4 g NaH₂PO₄.2H₂O; distilled water to 1.0 liter and final pH 7.2) and the cell suspension was adjusted to 10⁶ CFU/ml using UV/Visible spectrophotometer (MODEL:2000 UV-UNICO INSTRUMENTS CO., LTD,USA). The optical density (at λ=600 nm) is between 0.07 and 0.1 and this is roughly equivalent to 1 x 10⁶ CFU/ml [44].

2.5.2 Antibacterial activity

Antibacterial activity of the prepared cinnamaldehyde-water emulsion (without dilution) was tested against phytopathogenic bacteria using a well diffusion method [45]. YPG agar medium (5 g yeast extract; 5 g peptone; 10 g glucose; 15g agar; distilled water to 1000.0 ml) was prepared. The medium was sterilized in an autoclave for 15 minutes at 121°C. The sterile medium temperature was lowered to 50°C in a water bath. Then, the medium flask containing 250 ml was inoculated with 1.00 ml suspension (10⁶CFU/ml) of a tested isolate of phytopathogenic bacteria to obtain a final concentration of about 4 x 10⁵ CFU/ml of bacteria in the medium. Then, 25 ml of inoculated medium were poured into each sterilized Petri plate (9 cm).Once the agar was solidified, a hole with a diameter of 5 mm has punched aseptically with a sterile cork borer, and then wells were filled with 50 µl of the prepared formulation. Wells filled with 50 µl of sterilized water used as a control. The experiment was performed in three replicates. The Plates were incubated at 28°C for 48 h. Clear zones that formed around the wells were measured to the nearest millimeter.

2.5.3 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of cinnamaldehyde-in-water emulsion formulation against *Erwinia amylovora*, *Pectobacterium aroidearum*, *Pseudomonas aeruginosa*, and *Ralstonia solanacearum* was determined using well diffusion method [46]. Seven serial twofold dilutions ranged from 500.0 to 7.81 µl/ml of the formulation in sterile distilled water were prepared. Eight agar wells (5 mm diameter) were prepared around the center of Petri plates (15 cm) containing the YPG agar medium (60
The experiment was performed in three replicates. Wells filled with 50 µl of sterilized water were used as a control. The experiment was performed in three replicates. Wells filled with 50 µl of sterilized water were used as a control. The plates were incubated at 28°C for 48 h. Clear zones that formed around the wells were measured to the nearest millimeter. The lowest concentration formed clear zones around the wells are considered the minimum inhibitory concentration to the tested isolate of phytopathogenic bacteria.

3. RESULTS AND DISCUSSION

3.1 Emulsion Stability

Creaming is best defined as the tendency of droplets for moving upward, which occurs if the droplets have a lower density than the liquid phase [47]. The oil-in-water emulsions stability can be described and monitored during a lifetime. An emulsion system is stable if no phase separation occurs and a single phase is formed over the whole period. The emulsion stability with different levels of water hardness is an important factor for a product of agricultural use due to the type of water hardness present in different agricultural regions. Some agricultural regions use groundwater that often has significant levels of hardness, but some surface water supplies also have the same issue. Calcium concentrations up to and exceeding 100 mg. L\(^{-1}\) is common with groundwater. In contrast, magnesium usually occurs at lower concentrations compared to calcium in groundwater (about 50 mg. L\(^{-1}\) and rarely about 100 mg. L\(^{-1}\)), and calcium-based hardness usually predominates [48]. Considering its importance, the water of different hardness was used, called waters CIPAC A and D [49]. The data presented in Table 1 showed the emulsion stability and re-emulsification of the prepared cinnamaldehyde-in-water emulsion after storage at 54°C for 14 days when diluted with the different hardness of water (CIPAC A and D) presented appropriated bloom, formed milky bluish emulsion, and did not show creamy layer until 24 h. Such signs are a preliminary indication of physical stability. In the experiment of low temperature stability, the formulation was stored in a freezer (0±2°C) for 7 days, no precipitation or a separate material was observed in the formulation which indicated that the prepared formulation was stable without phase separation; these results demonstrated that the emulsion would have a satisfactory shelf life either in the tropics or in temperature zones and maintain the quality of the emulsion. Finally, the volume of foam from the samples in CIPAC standard waters A and D is low and passed through the recommended rat of foam.

3.2 Physical Properties

The physical properties of the prepared formulation are important to expand the applicability. pH plays an important role in the stability of the emulsion system [50]. pH results revealed that no obvious chemical degradation occurred in the prepared formulation. The pH values of the prepared formulation were in the range of (4.12-4.16), indicating that the prepared formulation in the different storage conditions having acidic character implying that it will have good biological activity.

The prepared formulation had a value of surface tension range (33.65-34.87 mN/m): the lower surface tension of a solution indicates a higher wetting property and further promotes the deposition and retention of pesticide droplets on target surfaces [51,52]. Flash point is an important parameter to measure product safety for the storage and transportation, the prepared formulation in the storage conditions having the high value of flash point more than 65°C, which showed good security for storage and transportation, thus demonstrating that the cinnamaldehyde-in-water emulsion formulation had higher flash points suitable for spraying.

Rheological properties are some of the most vital factors for characterizing emulsions, because they are directly related to formation, creaming stability, the sensory attributes, and the shelf life [53]. For instance, the creaming of oil particles in O/W emulsions was effectively dependent on the viscosity of the water phase. Fig. 1 displays the emulsion exhibited the typical non-Newtonian behavior of shear-thinning in the shear rate (reduced in apparent viscosity with enhancing shear rate). The rheological behavior of the emulsions was attributed mainly due to the presence of polysaccharides in aqueous solutions. When the shear rate was increased, the entanglements between the polysaccharide-chains were disrupted, and the molecular chains were more randomly orientated. Finally, the interactions between the adjacent chains were reduced, and the viscosity was decreased [54-56]. Based on the obtained results the particle size distribution varied from 1.19 µm to 1.24 µm.

Mohammed and El-Rahman; JAMB, 20(10): 1-12, 2020; Article no.JAMB.61875
Table 1. Emulsion characteristics of the cinnamaldehyde-in-water emulsion formulation

| Test code | Temperature (25±1 °C) | Accelerated storage (54±2 °C, 14 days) |
|-----------|------------------------|----------------------------------------|
| Water hardness | A | D | A | D |
| Emulsion stability | 0h | 0/0 | 0/0 | 0/0 |
| | 0.5h | 0/0 | 0/0 | 0/0 |
| | 1h | 0/0 | 0/0 | 0/0 |
| | 2h | 0/0 | 0/0 | 0/0 |
| | 24h | 0/0 | 0/0 | 0/0 |
| | 24.5 h (REE) | 0/0 | 0/0 | 0/0 |

0/0: no creamy or oily layer was observed
REE: 24.5 h : 30 minutes after reemulsification

Table 2. Physicochemical properties of the cinnamaldehyde-in-water emulsion formulation

| Test Code | Temperature (25±1 °C) | Accelerated storage (54±2 °C, 14 days) |
|-----------|------------------------|----------------------------------------|
| pH value (1%) | | 4.12 | 4.16 |
| Surface tension (mN/m) | 33.65 | 34.87 |
| Flash point (°C) | Over 60 | Over 60 |
| particle size distribution (µm) | 1.24 | 1.19 |

Fig. 1. Rheological properties of the cinnamaldehyde-in-water emulsion formulation

3.3 Antibacterial Activity of Cinnamaldehyde Formulation

In the present study, in vitro antibacterial activity of cinnamaldehyde formulation (without dilution) was determined as the diameter of the inhibition zone against phytopathogenic bacteria using well diffusion method Table 3 and Fig.2. The diameter of the inhibition zone mostly indicates the degree of antibacterial effect that exists in the product or sample; in this case, a higher zone means that the antimicrobial is more effective. The antimicrobial activity based on these inhibition diameters was determined in the tested treatment. Among the plant pathogenic bacteria, Ralstonia solanacearum was the most sensitive to the prepared formulation with 52.0 mm inhibition zone diameter. The prepared formulation showed inhibition zones of 29.7 and 25.7 mm against Erwinia amylovora and Pectobacterium aroidearum, respectively. However, Pseudomonas aeruginosa displayed less sensitivity to the oil-in-water emulsion formulation with a 10.3 mm zone diameter. The antibacterial mechanism of cinnamaldehyde was studied by [57-59]. The target of cinnamaldehyde is mainly the bacterial membrane; the contact of cinnamaldehyde with the bacterial membrane might cause the loss of membrane functionality or cause the loss of channel proteins in the
membrane, resulting in the death of bacterial cells. Also [60,61] suggested an interaction with the cell membrane induces rapid inhibition of energy metabolism. The disruption of the proton motive forces results in leakage of small ions without the leakage of large components such as ATP accompanied by the inhibition of ATP generation and inhibition of membrane-bound adenosine triphosphatase (ATPase) activity. Other mechanisms of cinnamaldehyde action include perturbing the cell membrane and altering the lipid profile of the membrane [62,63].

3.4 Minimum Inhibitory Concentration (MIC)

Results of the inhibitory effect of the prepared formulation against *Erwinia amylovora*, *Pectobacterium aroidearum*, *Pseudomonas aeruginosa*, and *Ralstonia solanacearum* are given in Table 4 and Fig. 3. The MIC value is the lowest concentration of the prepared formulation when a clear zone is formed around the well. MICs of the prepared formulation were 15.63, 31.25, 62.5, and 15.63 µl/ml against *Erwinia amylovora*, *Pectobacterium aroidearum*, *Pseudomonas aeruginosa*, and *Ralstonia solanacearum*, respectively. *Ralstonia solanacearum* was the most affected bacterium by the prepared formulation followed by *Erwinia amylovora*, *Pectobacterium aroidearum* and, *Pseudomonas aeruginosa*, respectively. None of the four tested species was affected by the concentration of 7.81 µl/ml of cinnamaldehyde cinnamaldehyde formulation inhibits the growth of *Erwinia amylovora* and *Ralstonia solanacearum*.

### Table 3. Inhibition zone (mm) of cinnamaldehyde-in-water emulsion against some phytopathogenic bacteria

| Phytopathogenic bacteria          | Inhibition zone diameter (mm) |
|-----------------------------------|-------------------------------|
| *Erwinia amylovora*               | 29.7±2.08                     |
| *Pectobacterium aroidearum*       | 25.7±1.52                     |
| *Pseudomonas aeruginosa*          | 10.3±0.58                     |
| *Ralstonia solanacearum*          | 52.0±2.00                     |
| Control (sterile distilled water) | 0.0±0.00                      |

*Means of three replicates; values expressed as mean ± Standard Deviation (SD)*

**Fig. 2.** Antibacterial activity of cinnamaldehyde -in water emulsion against some phytopathogenic bacteria

*C = Control i.e. Sterile distilled water, Ci = cinnamaldehyde formulation*
### Table 4. Minimum Inhibitory Concentration (MIC) of cinnamaldehyde-in-water emulsion against some phytopathogenic bacteria using well diffusion method

| Phytopathogenic bacteria | Diameter of inhibition zone (mm) at different concentrations (µl/ml) | MIC (µl/m) |
|--------------------------|------------------------------------------------------------------|------------|
|                         | 500      | 250     | 125   | 62.5   | 31.25  | 15.63  | 7.81  | 0.0**  |
| *Erwinia amylovora*      | 26.3±    | 22.3±0  | 20.0±  | 16.3±  | 14.0±  | 9.3±0  | 0.0±0 | 0.0±0  | 1.563  |
|                         | 1.53     | .58     | 0.00   | 1.15   | 1.00   | 58     | 00    | 0.00   |         |
| *Pectobacterium aroidearum* | 22.7±    | 19.3±0  | 16.7±  | 11.7±  | 9.7±0  | 0.0±0  | 0.0±0 | 0.0±0  | 3.125  |
|                         | 1.15     | .58     | 1.53   | 1.53   | 58     | 00     | 00    | 0.00   |         |
| *Pseudomonas aeruginosa* | 14.3±    | 11.7±1  | 10.0±  | 8.0±1  | 0.0±0  | 0.0±0  | 0.0±0 | 0.0±0  | 6.25   |
|                         | 0.58     | .53     | 0.00   | 0.00   | 00     | 00     | 00    | 0.00   |         |
| *Ralstonia solanacearum* | 51.3±    | 46.3±2  | 35.0±  | 22.7±  | 14.7±  | 11.3±  | 0.0±0 | 0.0±0  | 1.563  |
|                         | 3.21     | .30     | 0.00   | 2.08   | 1.15   | 1.53   | 00    | 0.00   |         |

*Means of three replicates; values expressed as mean ± Standard Deviation (SD); **Control (sterile distilled water)*

---

**Fig. 3. Minimum Inhibitory Concentration (MIC) of cinnamaldehyde – in-water emulsion formulation against some phytopathogenic bacteria.** Different concentrations of formulation (500, 250, 125, 62.5, 31.25, 15.63 and 7.81 µl/ml) and Control (0 µl/ml i.e. Sterile distilled water)

*solanacearum*, and inhibition zones were 9.3 and 11.3 mm, respectively. While this concentration of 15.63 µl/ml was not inhibited the growth of *Pectobacterium aroidearum* and *Pseudomonas aeruginosa*.

### 4. CONCLUSION

It can be concluded that cinnamaldehyde could be successfully formulated in the form of an oil-in-water emulsion. The formulation can be used as a natural antimicrobial agent; it had potent antibacterial activity against various species of bacterial pathogens, could be a new alternative to chemical bactericide. Also, it is safe to be used. However, more studies should be done to check the storage stability of the emulsion and further work will focus on the evaluation of the toxicity of the prepared formulation and comparing the activity with the traditional and commercial formulation currently used.
DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENT

The authors are thankful for Dr. Shady Selim for providing cinnamaldehyde.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Benali T, Bouyahya A, Habbadi K, Zengin G, Khabbach A, Achbani EH, Hammani K. Chemical composition and antibacterial activity of the essential oil and extracts of Cistus ladaniferus subsp. ladanifer and Mentha suaveolens against phytopathogenic bacteria and their ecofriendly management of phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;28:101696.

2. Kotan R, Cakir A, Ozer H, Kordali S, Cakmakci R, Dadasoglu F, Dikbas N, Aydin T, Kazaz C. Antibacterial effects of Origanum onites against phytopathogenic strains isolated from soybean. Journal of the Science of Food and Agriculture. 2015;17(3):758-765.

3. Pedrotti C, Marcon ÂR, Delamare APL, Echeverrigaray S, Da Silva Ribeiro RT, Schwambach J. Alternative control of grape rots by essential oils of two Eucalyptus species. Biocatalysis and Agricultural Biotechnology. 2019;108:442-450.

4. Isman MB. Plant essential oils for pest and disease management. Crop Protection. 2000;19(8):603-608.

5. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

6. Isman MB. Plant essential oils for pest and disease management. Crop Protection. 2000;19(8):603-608.

7. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

8. Isman MB. Plant essential oils for pest and disease management. Crop Protection. 2000;19(8):603-608.

9. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

10. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

11. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

12. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

13. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

14. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

15. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

16. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

17. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

18. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

19. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

20. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

21. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

22. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

23. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

24. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.

25. Oliva MDLM, Carezzano ME, Giuliano M, Daghero J, Zygadlo J, Bogino P, Giordano W, Demo M. Antimicrobial activity of essential oils of Thymus vulgaris and Origanum vulgare on phytopathogenic bacteria. Biocatalysis and Agricultural Biotechnology. 2020;108:442-450.
14. Bevilacqua A, Corbo MR, Sinigaglia M. Combined effects of low pH and cinnamaldehyde on the inhibition of Alicyclobacillus acidoterrestris spores in a laboratory medium. Journal of Food Processing and Preservation. 2008;32(5):839-852.

15. Doyle AA, Stephens JC. A review of cinnamaldehyde and its derivatives as antibacterial agents. Fitoterapia. 2019;139:104405.

16. Friedman M. Chemistry, Antimicrobial Mechanisms, and Antibiotic Activities of Cinnamaldehyde against Pathogenic Bacteria in Animal Feeds and Human Foods. J Agric Food Chem. 2017;65(48):10406-10423.

17. Yildiz ZI, Kilic ME, Durgun E, Uyar T. Molecular Encapsulation of Cinnamaldehyde within Cyclodextrin Inclusion Complex Electrospun Nanofibers: Fast-Dissolution, Enhanced Water Solubility, High Temperature Stability, and Antibacterial Activity of Cinnamaldehyde. Journal of Agricultural and Food Chemistry. 2019;67(40):11066-11076.

18. Mousavi F, Bojko B, Bessonneau V, Pawliszyn J. Cinnamaldehyde Characterization as an Antibacterial Agent toward E. coli Metabolic Profile Using 96-Blade Solid-Phase Microextraction Coupled to Liquid Chromatography–Mass Spectrometry. Journal of Proteome Research. 2016;15(3):963-975.

19. Mateen S, Rehman MT, Shahzad S, Naeem SS, Faizy AF, Khan AQ, Khan MS, Husain FM, Moin S. Anti-oxidant and anti-inflammator effects of cinnamaldehyde and eugenol on mononuclear cells of rheumatoid arthritis patients. Eur J Pharmacol. 2019;852:14-24.

20. Günöldu SDS, De Godoi, SN, Santos RCV, Da Silva JT, Leite, LBdM, Amaral AC, Ourique AF. In vivo antifungal activity of nanoemulsions containing eucalyptus or lemongrass essential oils in murine model of vulvovaginal candidiasis. Journal of Drug Delivery Science and Technology. 2020;57:101762.

21. Efrati R, Natan M, Pealah A, Haberer A, Banin E, Dotan A, Ophir A. The effect of polyethylene crystallinity and polarity on thermal stability and controlled release of essential oils in antimicrobial films. Journal of Applied Polymer Science. 2014;131(11).

22. Yazgan H. Investigation of antimicrobial properties of sage essential oil and its nanoemulsion as antimicrobial agent. LWT. 2020;130:109669.

23. Zhang X, Liu J. Effect of Arabic Gum and Xanthan Gum on the Stability of Pesticide in Water Emulsion. Journal of Agricultural and Food Chemistry. 2011;59(4):1308-1315.

24. Elekiaeih, Navvab Kashani M, Biggs MJ. Modelling of immiscible liquid-liquid systems by Smoothed Particle Hydrodynamics. Journal of Colloid and Interface Science. 2017;508:567-574.

25. Feng J, Chen Q, Wu X, Jafari SM, McClements DJ. 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.

26. Wang B, Tian H, Xiang D. Stabilizing the Oil-in-Water Emulsions Using the Mixtures of Dendrobium Officinalis Polysaccharides and Gum Arabic or Propylene Glycol Alginate. Molecules. 2020;25(3):759.

27. Zhai X, Lin D, Liu D, Yang X. Emulsions stabilized by nanofibers from bacterial cellulose: New potential food-grade Pickering emulsions. Food Research International. 2018;103:12-20.

28. Silva BFB, Rodriguez-Abreu C, Vilanova N. Recent advances in multiple emulsions and their application as templates. Current Opinion in Colloid & Interface Science. 2016;25:98-108.

29. Kundu P, Kumar V, Misra IM. Modeling the steady-shear rheological behavior of dilute to highly concentrated oil-in-water (o/w) emulsions: Effect of temperature, oil volume fraction and anionic surfactant concentration. Journal of Petroleum Science and Engineering. 2015;129:189-204.

30. Ferreira A, Vecino X, Ferreira D, Cruz JM, Moldes AB, Rodrigues LR. Novel cosmetic formulations containing a biosurfactant from Lactobacillus paracasei. Colloids Surf B Biointerfaces. 2017;155:522-529.

31. Chen K, Yu G, He F, Zhou Q, Xiao D, Li J, Feng Y. A pH-responsive emulsion stabilized by alginate-grafted anisotropic silica and its application in the controlled release of λ-cyhalothrin. Carbohydrate polymers. 2017;176:203-213.

32. Liu Y, Wei F, Wang Y, Zhu G. Studies on the formation of bifenthrin oil-in-water nano-emulsions prepared with mixed surfactants. Colloids and Surfaces A:
Physicochemical and Engineering Aspects. 2011;389(1):90-96.

33. Chin C-P, Lan C-W, Wu H-S. Application of biodiesel as carrier for insecticide emulsifiable concentrate formulation. Journal of the Taiwan Institute of Chemical Engineers. 2012;43(4):578-584.

34. Da Costa JT, Forim MR, Costa ES, De Souza JR, Mondego JM, Boiça Junior AL. Effects of different formulations of neem oil-based products on control Zabrote subscafacius (Bohemian, 1833) (Coleoptera: Bruchidae) on beans. Journal of Stored Products Research. 2014;56:49-53.

35. Iqbal N, Kumar N, Saini MK, Dubey S, Agrawal A, Kumar J. Role of high shear mixing in improving stability and bioefficacy of botanical oil in water formulation for early stage mosquito eradication. Heliyon. 2020;6(2):e03380.

36. Abd El-Rahman AF, Mohammed TGM. Soluble concentrate formulation of oxalic acid and N-Acetyl-L-cysteine: Potential of use in controlling Ralstonia solanacearum. J. of Plant Protection and Pathology, Mansoura Univ. 2020;11(9):427-434.

37. Abd El-Rahman AF, Balabel NM, Abd-El-Aziz RM. Isolation and identification of bacteria associated with Guava decline in Egypt. Journal of American Science. 2020;16(3):51-62.

38. Abd El-Rahman AF, Shaheen HA. Biological control of the brown rot of potato, Ralstonia solanacearum and effect of bacterization with antagonists on promotion of potato growth. Egyptian Journal of Biological Pest Control. 2016;26(4):733-739.

39. Mohammadi M, Moltmann E, Zeller W, Geider K. Characterisation of naturally occurring Erwinia amylovora strains lacking the common plasmid pEA29 and their detection with real-time PCR. European Journal of Plant Pathology. 2009;124(2):293-302.

40. Pastrik KH, Elphinstone JG, Pukall R. Sequence Analysis and Detection of Ralstonia solanacearum by Multiplex PCR Amplification of 16S–23S Ribosomal Intergenic Spacer Region with Internal Positive Control. European Journal of Plant Pathology. 2002;108:831-842.

41. Abd El-Rahman AF, Mohammed TGM. Soluble concentrate formulation of oxalic acid and N-Acetyl-L-cysteine: Potential of use in controlling Ralstonia solanacearum. J. of Plant Protection and Pathology, Mansoura Univ. 2020;11(9):427-434.

42. CIPAC, Collaborative International Pesticides Analytical Council. Disponivel em: <Disponivel em: http://www.cipac.org/>. Acesso em: 1 jun. 2016; 2016.

43. Du Z, Wang P. Gelatin Hydrolysate Hybrid Nanoparticles as Soft Edible Pickering Stabilizers for Oil-In-Water Emulsions. Molecules. 2020;25(2):393.

44. Daly SM, Sturge CR, Greenberg DE. Inhibition of Bacterial Growth by Peptide-Conjugated Morpholino Oligomers. Methods Mol Biol. 2017;1565:115-122.

45. Mohammad TGM, Abd El-Rahman AF. Environmentally friendly synthesis of silver nanoparticles using Moringa oleifera (Lam) leaf extract and their antibacterial activity against some important pathogenic bacteria. Mycopath. 2015;13(1):1-6.

46. Bonev B, Hooper J, Parisot J. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. The Journal of antimicrobial chemotherapy, 2008;61(6):1295-1301.

47. Keowmaneechai E, McClements DJ. Effect of CaCl2 and KCl on Physiochemical Properties of Model Nutritional Beverages Based on Whey Protein Stabilized Oil-in-Water Emulsions. Journal of Food Science. 2002;67(2):665-671.

48. WHO, Hardness in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality, Washington, DC, USA; 2011.

49. Alvarado aguilar MC, Recalde coronel PC, Leal alvarado DA, Villa sanchez FE, Tamayo alcivar R. Oil-in-water (o/w) emulsionable concentrate of ishpink (ocotea quixos) with thermodynamic stability. Revista Caatinga. 2019;32:590-598.

50. Karthik P, Ezhilarasi PN, Anandharamakrishnan C. Challenges associated in stability of food grade nanoemulsions. Crit Rev Food Sci Nutr. 2017;57(7):1435-1450.

51. Cao C, Song Y-Y, Zhou Z-L, Cao L-D, Li F-M, Huang Q-L. Effect of adhesion force on the height pesticide droplets bounce on impaction with cabbage leaf surfaces. Soft Matter. 2018;14(39):8030-8035.

52. Li Y, Feng Y, Yu G, Li J, Zhou Y, Liu Y. Preparation and characterization of oil-in-water emulsion based on eco-friendly emulsifiers. Colloids and Surfaces A:
Physicochemical and Engineering Aspects. 2020;602:125024.

53. McClements DJ. Food emulsions: principles, practices, and techniques; 2016.

54. Zhong L, Oostrom M, Truex MJ, Vermeul VR, Szecsody JE. Rheological behavior of xanthan gum solution related to shear thinning fluid delivery for subsurface remediation. Journal of Hazardous Materials. 2013;244-245:160-170.

55. Xu J-L, Zhang J-C, Liu Y, Sun H-J, Wang J-H. Rheological properties of a polysaccharide from floral mushrooms cultivated in Huangshan Mountain. Carbohydrate Polymers. 2016;139:43-49.

56. Qiao L, Li Y, Chi Y, Ji Y, Gao Y, Hwang H, Aker WG, Wang P. Rheological properties, gelling behavior and texture characteristics of polysaccharide from Enteromorpha prolifera. Carbohydrate Polymers. 2016;136:1307-1314.

57. Song Y-R, Choi M-S, Choi G-W, Park I-K, Oh C-S. Antibacterial Activity of Cinnamaldehyde and Estragole Extracted from Plant Essential Oils against Pseudomonas syringae pv. actinidiae Causing Bacterial Canker Disease in Kiwifruit. The Plant Pathology Journal. 2016;32(4):363-370.

58. Di Pasqua R, Hoskins N, Betts G, Mauriello G. Changes in membrane fatty acids composition of microbial cells induced by addition of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol in the growing media. J Agric Food Chem. 2006;54(7):2745-9.

59. Gill AO, Holley RA. Mechanisms of bactericidal action of cinnamaldehyde against Listeria monocytogenes and of eugenol against L. monocytogenes and Lactobacillus sakei. Applied and environmental microbiology. 2004;70(10):5750-5755.

60. Gill AO, Holley RA. Inhibition of membrane bound ATPases of Escherichia coli and Listeria monocytogenes by plant oilaromatics. 2006;111:170−174.

61. Gill AO, Holley RA. Disruption of Escherichia coli, Listeriamonocytogenes and Lactobacillus sakei cellular membranes by plant oilaromatics. Int. J. Food Microbiol. 2006;108:1-9.

62. Berthold-Pluta A., et al., Antibacterial activities of plant-derived compounds and essential oils against Cronobacter strains. European Food Research and Technology. 2019;245(5):1137-1147.

63. Vasconcelos NG, Croda J, Simionatto S. Antibacterial mechanisms of cinnamon and its constituents: A review. Microb Pathog. 2018;120:198-203.

© 2020 Mohammed and El-Rahman; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/61875