Research Article

# Controlling the microbial growth in Rainbow trout (*Oncorhynchus mykiss*) by polylactic acid-based packaging containing *Lippia citriodora* nanoemulsion

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## Abstract

In recent years, many studies were carried out for developing new food packaging systems. There is a variety of synthetic and natural antimicrobial compounds used to control the growth of microorganisms. The use of natural antimicrobial agents, due to the low side effects and high antibacterial potential, has captured the attention of scientists. The main aim of this study was to produce polylactic acid-based biodegradable active films containing lemon verbena Essential oil nanoemulsion to control the growth of two common foodborne pathogenic bacteria (*Staphylococcus aureus* and *Escherichia coli*) in Rainbow Trout. To aim this, the antibacterial activity (in vitro) of the films were assessed under MIC and MBC assays using the microdilution method. The nanoemulsion was prepared by ultrasonic waves. Based on dynamic light scattering, the mean droplet size was reported at about 22.4 nm. Based on the antibacterial results, the growth of both microorganisms was significantly decreased after 0, 3, and 7 days of storage in comparison with the control group ($p < 0.05$). To sum up, the presence of *Lippia citriodora* Nanoemulsion in the matrix of the polylactic acid film showed notable antibacterial activity during 7 days of storage at 4˚C (refrigerator). It can be concluded that this film can be a good candidate for food packaging purposes to control the growth of microorganisms.

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**Keywords:** Antimicrobial packaging, Polylactic acid, *Lippia citriodora*, Nanoemulsion, Rainbow trout, Shelf life

## Introduction

Food safety is one of the major concerns of every country. Seafood is one of the main components of the food chain, of which fish

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owns a large share of this food basket. Seafood is perishable faster than other types of food. Therefore, preventing and controlling the growth of microorganisms can increase the quality and health of marine products (Odeyemi *et al.,* 2018). Rainbow trout is one of the favorite seafood products that mostly are offered fresh and frozen. Maintaining the quality of this product until consumption has a significant impact on consumer health (Hauzoukim and Mohanty, 2020).

Microorganisms are among the most important food pollutants, and their control and removal are regarded as the most significant solutions to retain food health and safety (Cui *et al.,* 2018). This can be fulfilled by employing active food packaging systems. Varieties of natural and synthetic antibacterial agents are utilized to fabricate food packaging systems with antibacterial activity (Peidaei *et al.,* 2021). However, due to the long-term usage of synthetic antimicrobials and their risky impacts on consumers, using natural antimicrobial compounds has attracted the attention of scientists (Ahari and Naeimabadi, 2021). The herbal essential oils (EOs) are known as the most appropriate replacement for chemical preservatives such as metal-based nanoparticles. The EOs contain specific ingredients that can eliminate or reduce the growth of destructive bacteria, yeasts, and fungi and prevent the production of toxins by them (Jebelli Javan *et al.,* 2013).

Further, these compounds are eco-friendly and harmless to human beings. Lemon verbena (*Lippia citriodora*) is a species of Verbenaceae family that grows in South America, Africa, and the middle east. As the leaves of this plant smell like the lemon verbena, it has been entitled lemon verbena (Meshkatalsadat *et al.,* 2011). In addition to EO, lemon verbena leaves, contain alkaloids, flavonoids, mucilage, tannins, and acidic phenols. Lemon verbena is full of flavonoids, and its EO has bactericidal and insecticidal properties (Fitsiou *et al.,* 2018). When EOs are added to foods, their low water solubility and hydrophobic bonding to food ingredients, including fats and proteins, decrease their activity (Fitsiou *et al.,* 2018).

Different methods were applied to control the behavior of nanodroplets and keep their efficacy at a high level. Strategies such as encapsulation and micro-and nano-emulsions production depicted notable efficacy in this regard (Ahari and Naeimabadi, 2021). These methods decrease the adverse effects of food ingredients and cause the normal distribution of EOs in food. Nanoemulsions of EOs are known as antimicrobial agents (Espitia *et al.,* 2019). This property is due to the reduction of particle size and the increase of EO contact surface with the bacterium. At the same time, interactions between food ingredients with plant EOs and other antimicrobial active ingredients may reduce the activity of these active agents (Ryu *et al.,* 2018). Therefore, higher concentrations of these materials must be added to the food to fulfill the required activity (Yadegarinia *et al.,* 2006). In such conditions, the negative impact of higher concentrations of EO and antimicrobial material on the sensory properties of food materials is a serious challenge that should be noticed. Among the presented solutions, integrating these active agents with



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films and edible coatings has been recently attended (Norcino *et al.,* 2020). The different packaging materials have been evaluated as base polymers for active packaging. Among these polymers, polylactic acid (PLA) has been distinguished as an interesting option for active food packaging due to its biodegradability and acceptable physical and mechanical properties. PLA is a linear chain thermoplastic polyester that has the potential to replace conventional polymers such as polyamide, polyethylene, polypropylene, and polyethylene terephthalate (Singhvi *et al.,* 2019). Lactic acid, as its constituent monomer, is obtained from the fermentation of plant raw materials such as cornstarch. PLA has desirable properties such as high mechanical strength and transparency and inhibition against the passage of ultraviolet light. Obtaining a biological source (derived from corn) puts it in a unique position for dietary plans. Polylactic acid shows good transparency and is confirmed by the Food and Drug Administration of America (FDA) as a packaging material in contact with antimicrobial components (Blasi, 2019). In many studies, different active components such as antimicrobial agents have been added to this film in active packaging design (Jayasena *et al.,* 2013).

The usability of this polymer in the absorption, maintenance, and release of active components in laboratory conditions and different food materials has been proved (Jayasena *et al.,* 2013). Regarding the significance of fisheries in household food baskets and the rapid spoilage of these products, their packaging is of utmost importance. On the other side, the trade, and transportation of these products to remote areas, the significance of creating an appropriate condition to prevent their spoilage has been increased. In this regard, this study aimed to develop a new formulation for the production of nanoemulsion of lemon verbena EO to combine with PLA-based coatings to reduce the microbial load in *Oncorhynchus mykiss*.

## Materials and methods

Rainbow trout were obtained from fish farms of the north part of the country (Plour village). Further, lemon verbena was prepared from the north of the country. Polylactic acids were obtained from Sigma Aldrich Company (USA). Moreover, dichloromethane, Tween 80, lecithin, and used culture mediums were prepared from Merck Company (Germany). The studied microorganisms (*E. coli:* ATCC 25922 and *S. aureus:* ATCC 25923) were obtained from the Institute of Standards and Industrial Research of Iran (ISIRI) (Persian Type Culture Collection (PTCC)) (Code: l 124). These two bacteria are the most common microorganisms that play a vital role in marine food spoilage. Besides, based on the previous studies, these bacteria have been always under evaluation in similar research studies (Guan *et al.,* 2021).

### Food sampling and processing

Rainbow trout samples with an average weight of 800 ± 50 g were collected from fish farms of the north of the country (Plour village), and their health has been confirmed by Veterinarians of Protein Products Laboratory of



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Iran. They were transported to the laboratory inside Styrofoam boxes in fish and ice ratio 2:1 using an ice pack (4 ˚C). Upon arrival, whole fish were washed under running tap water, weighted, headed, gutted, cleaned, rewashed, and filleted (10 g) without skin.

**Essential oil extraction**

The lemon verbena leaves were collected and their genus and species were confirmed by the Phytopathology department of Islamic Azad University, then they were dried using shade-drying for five days. 100 g of dried leaves were ground to a powder and mixed with 800 mL of distilled water to extract its EO. The water distillation method was applied for 3 h by Clevenger-type apparatus (using 1000 ml round bottom flask connected to a 300 mm Liebig condenser) based on European Pharmacopoeia to extract the EO. After dehydration by dried sodium sulfate, they were stored in dark glassware in the refrigerator (4 °C) for subsequent analyses (Moghimi *et al.,* 2016).

**Isolation and identification of EO compounds**

Briefly, the prepared sample was injected to Gas chromatography device (GC-2010 plus, Japan), and the most appropriate thermal planning of column was specified for complete isolating of EO compounds. Then, the percentage of EO constituent components and retention index was calculated for each compound. The EO was injected to Gas chromatography device connected to a mass spectrometer (HP 6890, Japan), and the mass of compounds was obtained (Mazarei and Rafati, 2019).

**Preparation of lemon verbena EO nanoemulsion**

Ultrasonic homogenizer was applied to prepare nanoemulsion of lemon verbena EO. The method involves three phases of adding water, oil, and surfactant and then applying ultrasound. In this method, for synthesis operation, it is necessary to determine the proper ratio of surfactant, water, and EO and be exposed to ultrasound using a sonicator probe for a certain period. To prepare this nanoemulsion, surfactants of Tween 80, lecithin, lemon verbena EO, and water were used. Then, the ultrasound irradiation was applied to the obtained composition. It continued till a homogeneous and transparent composition was obtained (Mazarei and Rafati, 2019).

**Dynamic light scattering test**

A dynamic light scattering test was applied to determine the nanodroplets' size. All emulsion samples were diluted by distilled water up to 10% to prevent multiple radiation scattering. Five mL of the colloidal nanoemulsion solution were poured into special cells of the device and placed in the dynamic light scattering (DLS) device. After the light passed through the sample, a diagram was obtained. According to this diagram, the droplet size distribution was revealed. (Lu *et al.,* 2018).

**Atomic force microscopy**

Atomic force microscope (AFM) was used to evaluate the surface topography of lemon verbena EO nanoemulsion after 90 days. The sample was diluted and a small quantity of that was placed on a clean slide and was allowed to



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be dried completely. The sample surface was imaged in 5×5 μm dimensions, and NOVA_1.26.0.1443_ solver_ eng.exe software was used to analyze the images (Maillard *et al.,* 2019).

**Preparation of polylactic acid film**

The molding method was applied to prepare the film. To do so, 2 g PLA (density 1.33 g/cm$^3$, molecular weight 197000 g/mol) was dissolved in 100 mL dichloromethane. Then, that was mixed at room temperature for 8 hours by using a magnetic stirrer so that the PLA granules dissolved well. The intended amounts of lemon verbena EO nanoemulsion were added by weight to the earlier solution. Next, the solution was poured on 100 mm- diameter glass plates. Then, dichloromethane solvent is evaporated at room temperature by placing the plates under a chemical fume hood. Finally, the film was separated from the glass mold and placed in a desiccator containing silica gel for analysis (Javaherzadeh *et al.,* 2020).

**Assessment of antibacterial activity of lemon verbena nanoemulsion**

The microdilution broth method was used for determining the level of MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) of lemon verbena EO against the investigated pathogenic bacteria. At first, 100 μL Brain Heart Infusion Broth (BHIB) and 100 μl nanoemulsion were poured into the micro-wells. Then, a combination of 100 μl BHIB medium and suspension of investigated bacteria standardized with 0.5 McFarland was added to the wells. The microplate was incubated at 37 °C for 24 hours. When the incubation terminated, the MIC and MBC values were calculated based on μg/mL. The Microbial growth was calculated based on the level of optical absorption at 600 nm (Molchanova *et al.,* 2017).

**Evaluation of the antibacterial activity of polylactic acid films**

To evaluate the antibacterial activity of PLA films, fish fillets soaked in $10^6$ CFU/mL microbial suspension were wrapped in PLA films and refrigerated at 4° C until microbial testing. Microbial tests were performed at 0, 3, and 7 days of refrigerator temperature storage. The microbial tests were performed on sample rainbow trout based on Iran national standard No. 9899. The effective percentage of nanoemulsion was obtained regarding MIC results. It was added to the film when preparing that to evaluate the antibacterial activity of PLA-containing lemon verbena nanoemulsion. Next, two common food pathogen bacteria were used: a gram-positive bacterium, *S. aureus*, a gram-negative bacterium, and *E. coli*. To count these microorganisms, the first one gram of the sample was carefully weighed under sterile conditions. After homogenization, it was transferred to a test tube containing 9 mL sterile ringer's solution and mixed well. The obtained combination was used as a primary suspension for subsequent decimal dilutions. When the dilutions were prepared, using a pipette sterile or sampler, 0.1 ml of a suspension of *S. aureus* and every one of obtained dilutions were transferred to the surface of the sterile plate containing Baird-



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Parker Agar culture medium (that has been prepared before, and egg yolk emulsion and potassium tellurite were added to that). Then, that was quickly spread by a glass stirring rod on the surface of the plates. These plates were incubated at 37 °C for 24 to 48 hours. Tryptone Bile X-Glucuronide culture medium was used to count *E. coli*. The prepared plates were incubated at 44 °C for 24 hours (Shokri *et al.,* 2020).

**Statistical analysis**

SPSS 25 software was used for analyzing the data. A one-way ANOVA test was used to evaluate DLS. A repeated measurement model of ANOVA was used for analyzing the bacterium results.

## Results

**Chemical composition of lemon verbena EO**

GC-MS analyzed the chemical composites of lemon verbena EO. Totally, 15 chemical composites were recognized in lemon verbena EO that the most composites of EO included: Geranial (%21.19), Neral (%18.63), Spathulenol (%10.56), Limone (%9.40), 6-Methyl-5-hepten-2-one (%7.40), caryophyllene oxide (%6.72, α-Curcumene (%4.36), and Nerolidol (%3.10).

**Table 1.** Composition determined by GC-MS analysis of the EOs from lemon verbena

| Composite | Composition (%) | Retention time (min) | Kovalts index |
|---|---|---|---|
| **6-Methyl-5-hepten-2-one** | 7.40 | 15.20 | 974.00 |
| **Sabinen** | 1.28 | 15.37 | 981.00 |
| **Limonene** | 9.40 | 16.74 | 1,045.00 |
| **3-Methyl-2-methyl-2-butenyl** | 0.73 | 17.95 | 1,102.00 |
| **α-Terpineol** | 1.87 | 20.26 | 1,209.00 |
| **Z-Citral (Neral)** | 18.63 | 21.17 | 1,252.00 |
| **E-Citral (Geranial)** | 21.19 | 21.77 | 1,280.00 |
| **Geranyl acetate** | 2.66 | 23.77 | 1,373.00 |
| **Trans-Caryophyllene** | 1.92 | 25.28 | 1,443.00 |
| **α-Curcumene** | 4.36 | 25.99 | 1,476.00 |
| **γ- Cadinene** | 1.62 | 26.83 | 1,516.00 |
| **Nerolidol** | 3.10 | 27.30 | 1,537.00 |
| **Spathulenol** | 10.56 | 27.98 | 1,569.00 |
| **caryophyllene oxide** | 6.72 | 28.10 | 1,575.00 |
| **δ-Cadinene** | 2.77 | 28.89 | 1,612.00 |

**Results of dynamic light scattering test**

Table 2 represents the results of analysis of lemon verbena EO nanoemulsion accomplished by DLS method, particles size average, and manner of particles distribution.

**Table 2.** DLS results of lemon verbena EO nanoemulsion

| Treatment | PI | Mean(nm) | Lecithin (w/w%) | Tween 80 (w/w%) | EO (w/w%) |
|---|---|---|---|---|---|
| **Lemon verbena EO nanoemulsion** | 0.39 | 22.4 | 2 | 8 | 10 |



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**Atomic Force Microscopy data**

Two-dimensional and three-dimensional images of the AFM microscope were taken 90 days after the production of the nanoemulsion of lemon verbena. The fuzzy picture of the sample represents the chemical difference of the materials on the surface by different colors. As shown in the pictures, the particle size is below 100 nm after 90 days, indicating nanoemulsion stability during the storage period (Figure 1).

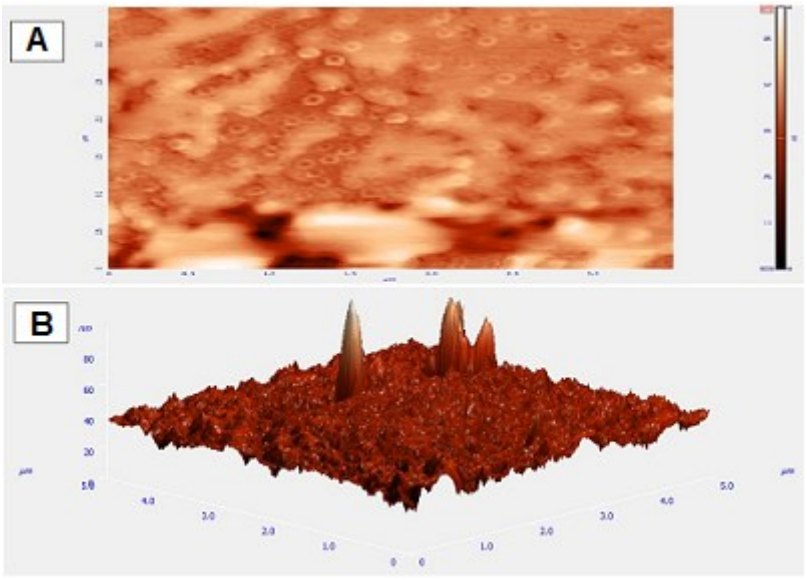


**Figure 1.** The Atomic Force Microscope images. A) 2-dimensional, and B) 3-dimensional images of the scanned area of the nanoemulsion (lemon verbena nanoemulsion kept at room temperature).

**Results of the assessment of antibacterial activity of lemon verbena EO**

MIC and MBC values of lemon verbena EO against the investigated bacteria have been presented in Table 3. This table shows the effect of nanoemulsion in inhibiting two pathogenic bacteria of *E. coli* and *S. aureus*. Lemon verbena Nanoemulsion showed higher antimicrobial activity against *E. coli*.

**Table 3.** Antibacterial activity of lemon verbena EO nanoemulsion

| Treatment | MIC (µg/mL) | | MBC (µg/ mL) | |
|---|---|---|---|---|
| | ***E. coli*** | ***S. aureus*** | ***E. coli*** | ***S. aureus*** |
| **Nanoemulsion of lemon verbena EO** | 0.78 ± 0.05 [a] | 1.56 ± 0.05 [b] | 0.78 ± 0.04 [a] | 1.57 ± 0.04 [b] |

The different lowercase letters represent significant differences ($p< 0.05$).

**Results of the assessment of antibacterial activity of polylactic acid films**

The effect of PLA films integrated with the nanoemulsion of lemon verbena EO on the bacterial load of rainbow trout (fillet) in comparison to the control (without film) sample at refrigerator temperature after 0, 3,



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and 7 days has been shown in Table 4. The lemon verbena nanoemulsion combined with PLA could decrease the number of *S. aureus* at 0 and 3 days of experiment 0.4 log CFU/g and 1.19 log CFU/g compared to the control group. On the seventh day of storage in the refrigerator, the film containing lemon verbena nanoemulsion could act as the strongest composition against *S. aureus* in the fish samples (1.68 log CFU/g) in comparison to the control group ($p< 0.05$). In the control group, *E. coli* could not be stable at refrigerator temperature since their number was less than 1 log CFU/g on the third and seventh days. The number of *E. coli* in the rainbow trout samples wrapped in PLA film containing nanoemulsion in fillets inoculated with bacteria decreased to less than 1 log CFU/g on the seventh day. In the samples wrapped in pure PLA film. The *S. aureus* grew less than the control sample, but the difference was insignificant ($p> 0.05$).

**Table 4.** Comparison of bacteria load (log CFU/g) in rainbow trout fillets under different treatments

| Day | Strain | Control | PLA | PLA film containing nanoemulsion |
|---|---|---|---|---|
| **0** | *E. coli* | Nd | Nd | Nd |
| | *S. aureus* | $3.00\pm0.00^{aA}$ | $2.64\pm0.00^{aA}$ | $2.54\pm0.00^{aA}$ |
| **3** | *E. coli* | <1.0 | Nd | Nd |
| | *S. aureus* | $3.11\pm0.00^{cA}$ | $2.25\pm0.01^{bB}$ | $1.92\pm0.01^{aB}$ |
| **7** | *E. coli* | <1.0 | <1.0 | <1.0 |
| | *S. aureus* | $2.89\pm0.00^{bA}$ | $1.80\pm0.00^{aB}$ | $1.68\pm0.00^{aB}$ |

The different lowercase in each row indicates a significant difference ($p< 0.05$).
Different capital superscripts in each column indicates a significant difference ($p< 0.05$).
Nd: not detected.

## Discussion

**Chemical composition of lemon verbena EO**

The results of GC-MS analysis of the composition of lemon verbena EO collected from the north of the country revealed that, out of 15 recognized components, Geranial (21.19%), Neral (18.63%), Spathulenol (10.56%), and Limonene (9.40%) had the most EO components. The data show that monoterpenes and sesquiterpenes were the most abundant compounds in EO. Another research was done by Kaskoos (2019) on the chemical composition of lemon verbena revealed a result similar to the present study. In their study, *E. citral* and *Z. citral* had the percentage of 20.21% and 14.37 % respectively. Based on these results and by comparing with that of our study, it can be concluded that E. citral and Z. citral are the main components of *L. citriodora* EO.

**Nanoemulsion of lemon verbena EO**

Whereas the activity of EOs decreases when they are added to the food material due to their low water solubility and their hydrophobic connection to the food ingredients, including fat and protein, the use of EOs nanoemulsion reduces the adverse effects of food materials and causes the normal distribution of EO in them. The EO nanoemulsion is an appropriate



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antibacterial compound due to the reduction of particle size and increase of contact surface of EO with the bacterium (McClements, 2013). The analysis of lemon verbena EO nanoemulsion by DLS test and AFM microscope revealed that the particle size was less than 100 nm (22.4), indicating the nanoemulsions stability. Considering the Nanoemulsion, longer stability is known as a critical feature. When Nanoemulsion is stable for a long time it means there is no agglomeration and the droplets keep their small size and no size enhancement happens. The smaller droplets, the higher the antibacterial activity (Pongsumpun *et al.,* 2020). Ghosh *et al*. (2013) studied the formulation of food-grade nanoemulsions by ultrasonic emulsification and evaluated their microbial activity. The results showed that the surfactant concentration negatively correlated with the droplet diameter. In contrast, the emulsification time had a positive relationship with the droplet diameter and the inherent stability of the emulsion. Nanoemulsions with 29.3 nm droplets were formulated by ultrasonic emulsification (Ghosh *et al.,* 2013), which are in good agreement with the present research results. In 2016, Ehsani *et al.* investigated the effect of *Echinophora platyloba* and lycopene EOs on the stability of pasteurized cream made from cow's milk. Their study showed that creams treated with EO and lycopene had better microbial and chemical properties and more stability during storage than control (Ehsani *et al.,* 2016). In another investigation, it was shown that the size of curcumin nanoemulsion was less than 11 nm after 15 days. The smaller particle size in each emulsion makes it more resistant to aggregation and dispersion (Khoshbouy Lahidjani *et al.,* 2020). Emulsions with larger particle sizes, on the other hand, have higher PDI (polydispersity index) values (Masarudin *et al.,* 2015), indicating particle incompatibility (Clayton *et al.,* 2016). A PDI value greater than 0.7 indicates a wide distribution for particle size, and the DLS method for determining their size would be inappropriate (Danaei *et al.,* 2018). The results of the DLS test in this study showed that the PDI was 0.39, which indicates the stability of the nanoemulsion. The results of AFM microscopy performed after 90 days confirm these results. In another study, the MIC of L. citriodora/ AgNPs against *S. aureus*, *E. coli,* and *Salmonella Typhi* were 90, 20 and 50 µg/mL, respectively (Elemike *et al.,* 2017). The MBCs of lemon verbena EO against *E. coli* and *S. aureus* were 12.48 and 9.73 mg /mL, respectively (Oukerrou *et al.,* 2017). This lower efficiency may be due to differences like the extract and EO used in this study. In another study, the antimicrobial activity of silver nanoparticles on gram-negative bacteria was shown to be higher than that of gram-positive bacteria, which could be due to the thinner cell walls (Seong and Lee, 2017). The results of MIC and MBC in this study also showed that nanoemulsion of lemon verbena EO had more antimicrobial activity against *E. coli* strains.

**Assessment of antibacterial activity of polylactic acid films**

PLA prevents oxidation by good barrier properties such as reducing oxygen permeability, removing moisture, and releasing



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volatiles. These properties were improved by adding the nanomaterials to PLA due to their antibacterial properties. This was the main reason for the higher efficiency of PLA films containing nanoemulsion of the nanoemulsion of lemon verbena EO compared to the control treatment.

Few studies have been done about the antimicrobial influence of EO in the food model. Most research has been accomplished in determining their chemical composition and antimicrobial influence in a laboratory environment. There are varieties of synthetic and natural antimicrobial compounds used in antimicrobial packaging. The use of natural antimicrobial materials is increasingly developed due to the side effects of synthetic compounds and the popularity of natural compounds. The method of integrating these active agents with films and edible coatings has been recently attended. Among these polymers, PLA has been distinguished as an interesting option to be used in food-active packaging due to its biodegradability and good physical and mechanical properties. The effect of lemon verbena EO nanoemulsion in PLA films for rainbow trout storage at a temperature of 4°C on the bacterial load indicates a significant difference on 0, 3, and 7 days of storage.

Further, a significant difference was observed between the control sample (without film) treatment and the sample containing nanoemulsion on 3 and 7 days of storage ($p< 0.05$). Bonilla *et al*. (2013) investigated the effect of Chitosan on the physicochemical and antimicrobial properties of PLA films. According to the results, PLA: CH composite showed significant antimicrobial activity against the aerobic and coliform bacteria, especially when chitosan particle size decreased. Talebi *et al*. (2018) researched to increase the shelf life of minced beef. They used polylactic acid film containing peppermint EO, black cumin, and nitrocellulose. This study showed that both EOs have more effective antimicrobial activity against the investigated microorganisms than the control sample. These results were in good agreement with that of our study confirming the antibacterial activity of nanoemulsion.

Another study showed that the number of *Staphylococcus aureus* bacteria on the sixth day of treatment of rainbow trout fillets with chitosan-ZnO layers containing 1.5% pomegranate peel extract reached 2 log CFU/g (Shahbazi and Shavisi, 2018). The study of Zhaleh *et al* also showed that the number of *S. aureus* and *E. coli* bacteria in chilled salmon coated with sodium alginate and *Prosopis farcta* extract and curcumin nanoparticles reached 2.7 and 3.2 log CFU/g, respectively (Zhaleh *et al.,* 2019). These findings of strains number were significantly higher than the results of the present study. Our results showed that nanoemulsion of lemon verbena has a high antibacterial potential, which can be attributed to the size of nanoparticles and their good stability for up to 90 days. In addition to the direct role of antibacterial properties of EOs and nanoemulsions of some plants, insufficient oxygen delivery to bacteria due to PLA resistance to oxygen diffusion can delay lipid



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oxidation and thus bacterial growth (Heydari-Majd *et al.,* 2019).

In the present research, the active compound of lemon verbena EO nanoemulsion developed the biodegradable active films of PLA and increased the rainbow trout shelf life from 3 days to 7 days. Lemon verbena nanoemulsion can be effectively used in the green biosynthesis of PLA nanocomposite films. This study showed that using lemon verbena nanoemulsion can help produce a new active film with more antimicrobial activity than pure PLA film. The DLS test showed that the particle size of the emulsion obtained for the lemon verbena nanoemulsion was 22.4 nm. The results of MIC and MBC tests confirmed the antibacterial activity of lemon verbena nanoemulsion. When the coverage containing lemon verbena nanoemulsion was exposed to the gram-negative and positive bacteria in the treatments, they showed considerable antibacterial activity. Regarding the consumers' demand for natural preservatives and the need to replace the oil-based plastic packaging with biodegradable, eco-friendly packaging, it can be recommended that, due to its effective antibacterial properties, the development of PLA-based active packaging containing lemon verbena nanoemulsion proposed in this study can be a promising method for storage of rainbow trout (fillet).

## Conflict of interest

Authors have no conflict of interest on this work.

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