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# Effects of date (*Phoenix dactylifera* L.) kernel essential oil on growth performance and innate immunoassay in rainbow trout (*Oncorhynchus mykiss*) juveniles

**Mousa S. M. Gaballah[1], Saleh B. A. Alkhunni[2,3], Nejdet GÜLTEPE[4]***

[1] *Omar Al-Mukhtar University, Veterinary Medicine Faculty, Al-Bayda, Libya*

[2] *Biotechnology Research Center, Authority of Science Research and Technology, Tripoli, Libya*

[3] *Kastamonu University, Institute of Science and Technology, Department of Genetics and Bioengineering, 37200, Kastamonu, Turkey*

[4] *Atatürk University, Fisheries Faculty, Department of Fisheries Fundamental Sciences, 25240, Erzurum, Turkey*

Key words: *Oncorhynchus mykiss*, *Phoenix dactylifera*, date kernel essential oil, growth performance, innate immunity

**Abstract**

This study aimed to investigate the effects of date (*Phoenix dactylifera* L.) kernel essential oil on growth and development performance parameters as well as phagocytic activity, respiratory burst activity, superoxide dismutase, lysozyme activity, and myeloperoxidase activity from immune parameters of rainbow trout (*Oncorhynchus mykiss*). The nutritional study was performed for 45 days to assess the effect of three concentrations (0.5, 1, and 2 mL kg-1) of date kernel essential oil in rainbow trout. β-citronellol, an important compound in many respects, was found 31.70% in date kernel oil. It has been determined that the addition of date kernel essential oil to rainbow trout feed decreases the feed conversion rate, increases the percent body weight gain, and the specific growth rate. It was observed that phagocytic activity, respiratory burst activity, and myeloperoxidase activity values improved in date kernel essential oil added groups, however, superoxide dismutase and lysozyme activity values were not affected. Consequently, date kernel essential oil can be act as a growth promoter, increase innate immunity, and cheap feed additive for the rainbow trout juveniles.

* Corresponding author. Tel.: +905326334769, e-mail: nejdetgultepe@yahoo.com

## Introduction

Aquaculture production is increasing rapidly due to various factors such as the decrease in natural stocks, developments in the field of aquaculture and food industry, and feed additives, etc. Feed evaluation ratios of fish vary between 0.8-2.5 depending on the species and ambient conditions. In aquaculture farms, culturists have to draw advantages from all feed ingredients such as increase digestibility, decrease FCR, regulate microbiota, immune system stimulates and/or support for the decreasing of operation cost of fish production. Therefore, a lot of studies carried out by researchers on feed additives, which are focused on plant and their derivatives, as feed ingredients are limited. Essential oils are generally recognized as safe (GRAS) and include low-molecular-weight aroma chemicals such as terpenes, terpenoids, alcohols, ketones, aldehydes, and oxygenated compounds (Gültepe, 2018, 2020). Essential oils stimulate the digestive system, increase digestive enzymes, and the absorption of substances obtained on digestion with their contents. It also has antimicrobial and antioxidant properties (Gültepe et al., 2019). Researchers are stated that the use of essential oils for growth increase and also for immune system support in fish before diseases occur provides an economic advantage (Zheng et al., 2009). Palm kernel oil can be an alternative to fish oil due to its economic advantages, reliability of supply, and additional benefits over many other oils (Turchini et al., 2010). Palm kernel is considered as a mill by-product in palm processing. The core constitutes approximately 45-48% of the weight of the mold obtained as a whole. Palm oil and palm kernel oil can be refined by splitting them into olein and stearin. Besides, palm kernel essential oil is rich in palmitate and stearate which are the major substrate for the enzyme stearoyl-CoA desaturase. This enzyme is the preferred substrate for the synthesis of triglycerides and other complex lipids in metabolism. Because, stearoyl-CoA desaturase and the related gene have important in the cellular stearate-to-oleate ratio, in lipid metabolism and potentially in also the treatment of obesity and related disorders (Sampath and Ntambi, 2005). Palm kernel oil researches are carried out in terms of fatty acid content, energy level, vitamin E content, carotenoids, cost-effectiveness, and its use is increasing day by day especially in Far Eastern countries (Turchini et al., 2010). It is known that essential oils are effectively contributing to the growth performance of fish as well as against fish diseases due to their antimicrobial, antioxidant properties, and secondary metabolites (Mohamed et al., 2020). Any studies on the effects and/or usage of date kernel essential oil were not found in the literature. Therefore, the aim of the present study is to assess the effects of date (*Phoenix dactylifera* L.) kernel essential oil (DEO) on growth performance parameters as well as phagocytic activity, respiratory burst activity, superoxide dismutase, lysozyme activity and myeloperoxidase activity from immune parameters of rainbow trout (*Oncorhynchus mykiss*).

## Materials and Methods

*Fish and rearing conditions.* A total of 240 apparently healthy rainbow trout (*Oncorhynchus mykiss*) juveniles with the mean weight 5.77±0.01 g were randomly placed in a free-flow system with 12 aquariums, each with a volume of 100 L (20 fish per aquarium). After a two-week adaptation, the fish were fed a commercial diet containing 45% protein and 15% fat. During the experiment, water properties (mean±SE) were measured daily with the following values obtained: temperature 18.9±0.6 °C, pH 7.7±0.3, dissolved oxygen 7.32±0.28 mg/L, and conductivity 559.3±44.5 μs/S. Four isonitrogenous/isolipidic experimental diets (45 protein/15 fat) were formulated with DEO at 0 mL/kg (control), 0.5 mL/kg (DEO-0.5), 1 mL/kg (DEO-1) and 2 mL/kg (DEO-2) concentrations (**Table 1**) (Gültepe, 2018,2020). The feed was produced with a standard pelleting machine at a size of 2 mm. Pellets were air-dried at 40 °C and stored at -20 °C until use. Fish were ad libitum fed by hand to apparent visual satiety twice a day in morning and midafternoon for 45 days with natural photoperiod. The experiment was performed in accordance with the guidelines for fish research from the animal ethics committees at Atatürk University, Turkey.

**Table 1** Formulations of isonitrogenous/isolipidic experimental diets

| | *Ingredients (g/kg)* | | | |
|---|---|---|---|---|
| | *Control* | *DEO-0.5* | *DEO-1* | *DEO-2* |
| Fish meal | 456 | 456 | 456 | 456 |
| Soy meal | 280 | 280 | 280 | 280 |
| Wheat meal | 80 | 80 | 80 | 80 |
| Corn starch | 40 | 39,5 | 39 | 38 |
| Fish oil (mL) | 104 | 104 | 104 | 104 |
| Vit-Min. Mix[1] | 40 | 40 | 40 | 40 |
| *OEO (mL)* | *0.00* | *0.05* | *0.10* | *0.20* |
| | *Chemical composition (% DM)* | | | |
| Protein | 45.07 | 45.07 | 45.07 | 45.07 |
| Lipid | 15.20 | 15.20 | 15.20 | 15.20 |
| Ash | 8.30 | 8.30 | 8.30 | 8.30 |
| Carbohydrate | 31.43 | 31.43 | 31.43 | 31.43 |

[1] Vit-Min. (Vitamin-Mineral) per kg diet: $4x10^6$ IU vitamin A, $4x10^5$ IU vitamin D3, $4x10^4$ mg vitamin E, 2400 mg vitamin K3, 4000 mg vitamin B1, 6000 mg vitamin B2, 4000 mg vitamin B6, 10 mg vitamin B12, 4000 mg vitamin C, 4000 mg niacin, 4000 mg calcium D-pantothenate, 100 mg D-biotin, 1200 mg folic acid, 6x104 inositol, 270 mg nicotinic acid, 75.3 mg Fe, 12.2 mg Cu, 206 mg Mn, 85 mg Zn, 3 mg I, 0.350 mg Se, 1 mg Co.

*Date kernel essential oil.* Date kernel purchased from a commercial company in Al-Bayda, Libya. The date kernel essential oil (DEO) was obtained from crushed date kernel by hydro-distillation, using a Clevenger system at the boiling temperature on 4 h. DEO (0.1 g) was pretreated with 10 mL of n-hexane, 0.5 mL of 2 N methanolic KOH (potassium hydroxide) solution was added and left for 2 hours in a non-light environment. The formed phase was used for the FAMEs (fatty acid methyl esters) and aroma analysis. Gas chromatography-mass spectrophotometry (GC-MS) analysis of the obtained DEO was conducted by using a Shimadzu GCMS QP 2010 ULTRA and then components identification was performed with Wiley 275 MS data library (Gültepe, 2018, 2020). The FAMEs and aroma analysis results of the active ingredient components of the DEO were given in **Figure 1** and **2**.

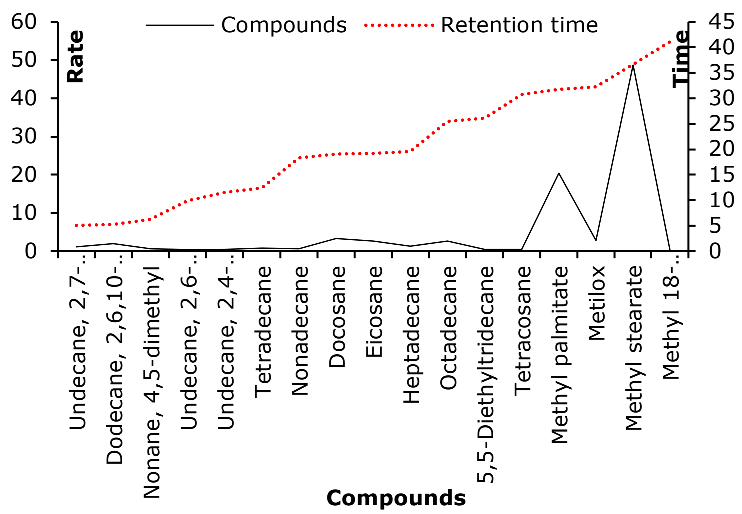


**Figure 1** Aroma analysis result of the DEO

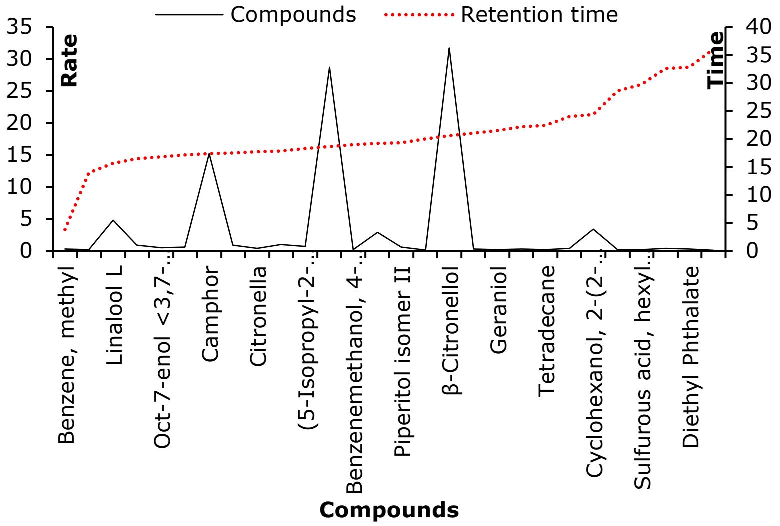


**Figure 2** FAMEs analysis result of the DEO

*Growth performance.* At the end of 45 days feeding trial, fish in each aquarium (n=20) were individually weighed and then growth performance calculated according to follows (Lugert et al., 2016; Gültepe, 2018).

$$AGR = \frac{W_t - W_i}{t}$$

$$RGR = \frac{W_t - W_i}{W_i} \times 100$$

$$IGR = \frac{\log(W_t) - \log(W_i)}{t}$$

$$SGR = \frac{\log(W_t) - \log(W_i)}{t} \times 100$$

$$DFI = \frac{Initial\ Feed\ Consumption}{t}$$

$$FCR = \frac{Feed\ Consumption}{W_t - W_i}$$

Abbreviations: AGR, absolute growth rate; $W_t$, final weight; $W_i$, initial weight; t, experimental day; RGR, relative growth rate; IGR, instantaneous growth rate; SGR, specific growth rate; DFI, daily feed intake; FCR, feed conversion ratio

*Innate immunoassay.* Random ten fish from each aquarium were sampled at the end of the trial and anesthetized with tricaine methanesulfonate (MS-222, SIGMA, United States) for immunological analyses. To reduce stress, the head of fish covered with a wet towel at the blood sampling stage. Blood samples were collected with a sterile plastic syringe from caudal vena. Lysozyme activity (LA), superoxide dismutase (SOD), total myeloperoxidase (MPO), and respiratory burst activity (RBA) were assessed using the turbidometric assay. Phagocytic activity (PA) was determined according to microscopy method (Gültepe et al., 2014a,b).

*Statistics.* Statistical parameters were examined by ANOVA. Levels of significance were determined using Tukey's HSD test, with critical limits being set at P<0.05. Values are expressed as means ± standard deviation (SD) for each measured variable.

## Results

A total of 17 different compounds were found in 50 different retention times in the FAMEs analysis of DEO. Among the compounds defined, methyl stearate constitutes the largest part with a rate of 48.70%. Methyl palmitate takes second place with 20.43%. Similarly, a total of 28 different compounds were found in 50 different retention times in the aroma analysis of DEO. Within the identified compounds, β-citronellol constitutes the largest part with a ratio of 31.70%, and secondly terpinene-4-ol with a value of 28.73%.

**Table 2** shows the growth performance and immunostimulatory effects of DEO on rainbow trout juveniles. Survival at the end of the experiment was 100% in all experimental groups. FCR was performed as 1.56±0.01, 1.47±0.02, 1.23±0.01, and 0.89±0.03 at the control, DEO-0.5, DEO-1, and DEO-2 groups, respectively ($P<0.05$). DEO added experimental diets gradually decreased FCR compared to the control diet. In the DEO-2 group, AGR, IGR, RGR, SGR, and DFI were significantly higher than other groups ($P<0.05$). The LA values in fish fed with the DEO-2 diet were significantly different from those of the other three groups ($P<0.05$). The MPO level in fish fed with DEO-1 and DEO-2 diets was significantly higher than both the control and DEO-0.5 treated groups ($P<0.05$). The RBA was significantly higher in the DEO-2 group when compared to others ($P<0.05$) except for the DEO-1 group.

**Table 2** Growth performance and immunostimulatory effects of DEO on fish after 45 days of feeding the experimental diets

| Parameters | Control | DEO-0.5 | DEO-1 | DEO-2 |
|---|---|---|---|---|
| $W_i$ (g) | 5.77 ± 0.01 | 5.77 ± 0.01 | 5.77 ± 0.01 | 5.77 ± 0.01 |
| $W_t$ (g) | 10.22±2.49 | 10.55±2.13 | 11.70±1.80 | 14.80±2.93 |
| *DFI (g)* | 0.155 | 0.156 | 0.162 | 0.177 |
| *AGR* | $0.0098^{a}$ | $0.106^{a}$ | $0.132^{b}$ | $0.201^{c}$ |
| *IGR* | $0.0055^{a}$ | $0.0058^{a}$ | $0.0068^{b}$ | $0.0091^{c}$ |
| *RGR* | $77.12^{a}$ | $82.84^{a}$ | $102.77^{b}$ | $156.50^{c}$ |
| *SGR* | $0.55^{a}$ | $0.58^{a}$ | $0.68^{b}$ | $0.91^{c}$ |
| *FCR* | $1.56^{c}$ | $1.47^{c}$ | $1.23^{b}$ | $0.89^{a}$ |
| *LA (μg/mL)* | 20.15±0.30 | 21.45±1.20 | 20.95±2.05 | 22.10±1.15 |
| *SOD (u/L)* | 83.11±5.05 | 87.25±5.28 | 86.06±4.15 | 89.05±6.25 |
| *MPO (u/L)* | $0.65±0.10^{a}$ | $0.60±0.15^{a}$ | $0.75±0.10^{b}$ | $0.77±0.15^{b}$ |
| *PA (%)* | $28.28±2.23^{a}$ | $29.12±1.20^{a}$ | $30.42±3.62^{ab}$ | $40.10±2.43^{c}$ |
| *RBA (mg formazan/mL)* | $2.80±0.10^{a}$ | $2.99±0.15^{a}$ | $3.78±0.05^{b}$ | $3.97±0.06^{bc}$ |

* $W_i$, initial weight; $W_t$, final weight; DFI, daily feed intake; AGR, absolute growth rate; IGR, instantaneous growth rate; RGR, relative growth rate; SGR, specific growth rate; FCR, feed conversion ratio, LA, lysozyme activity; MPO, total myeloperoxidase; SOD, superoxide dismutase; PA, phagocytic activity; RBA, respiratory burst activity. The values are expressed as means±SD (n=20 for growth parameters and n=10 for immunological analysis). Means with different superscripts in rows are significantly different ($P < 0.05$).

## Discussion

Plant essential oils have attracted the attention of researchers in recent years due to the positive effects of terpenic hydrocarbons, alcohols, ketones, aldehydes, and oxygenated compounds they contain on living things, and many studies have been conducted. Although some of these studies were made with known medicinal and aromatic plants, some of them were made with waste products generated as a result of various industrial processes. Kernel of date is considered as a mill by-product in date processing. The kernel constitutes approximately 45-48% of the weight of the mold obtained as a whole. The amount of stearin of palm kernel oil, which is rich in lauric acid content, is higher than palm oil. Due to its fatty acid profile that can provide unique melting properties, it has a wide application area in the food industry as a special oil (Zhang et al., 2017). In recent studies, palm oil and crude date kernel oil are used as an alternative to fish oil in feed formulations in fish feed making and have been evaluated as potential alternatives. Especially in Far Eastern countries, researches are carried out in terms of fatty acid content, energy level, vitamin E content, and carotenoids, especially in the Far-Eastern countries, and its use is increasing day by day (Turchini et al., 2010). There are used a lot of growth performance calculation methods in aquaculture. Although aquaculturists have focused on the final biomass of farms, these growth performance calculation methods are really needed for the monitoring, sustainability, and productivity of aquaculture. The absolute growth rate (AGR) is a calculation method, which is the quickest, simplest, and frequently used method, for growth. The instantaneous growth rate (IGR) is based on the absolute growth rate and uses the natural logarithm. The relative growth rate (RGR) is based on the absolute growth rate as a percentage increase over time. Specific growth rate (SGR) gives us the growth performance as a percentage increase per day with natural logarithm, it is also a more flexible method than RGR (Lugert et al., 2016). Scientists often use SGR and feed conversion ratio (FCR) in their work and also weight gain (WG) is used instead of RGR. Actually, it is important to each of these methods use in long-term studies and production conditions in the farms in terms of both forecast and foresight of the real production cycle. AGR, IGR, RGR, and SGR was significantly increased fish fed with DEO-1 and DEO-2 ($P<0.05$). These rates on the DEO-0.5 group were slightly increased compared with the control group in this study. FCR was started to decrease due to the use of date kernel essential oil and showed a clear difference, especially in the DEO-2 group ($P<0.05$). Gilthead seabream juveniles fed with palm oil-treated diet was improved Wt at the high temperature, and also researchers pointed out that plant-based diets will be important to the sustainable production and welfare of aquatic animals in a future climate change (Balbuena-Pecino et al., 2021). Similarly, palm oil replacing 25% of fish oil was improved growth performance such as FCR, SGR, WG, and the survival rate of both Atlantic salmon (Ng et al., 2004) and rainbow trout (Fonseca-Madrigal et al., 2005). The date kernel essential oil used in this study contains 48.70% methyl stearate and 20.43% methyl palmitate, which are fatty acid methyl ester of palmitic acid, palmitoleic acid, and stearic acid. Many studies have shown that palmitic acid, palmitoleic acid, and stearic acid constitute the majority of the fatty acid composition in both freshwater fish and marine fish (Moreno et al., 1979; Ng et al., 2004; Özogul et al., 2009). However, plant essential oils have antimicrobial and immunological effects against fish pathogens while improving the growth performance of fish (Gültepe, 2020). In addition, β-citronellol-containing essential oils have an anesthetic effect (Toni et al., 2014) and anthelmintic activity (Hierro et al., 2004; Barros et al., 2009) on fish that do not disrupt the metabolic ion regulation. Nevertheless, Morales-Serna et al. (2020) do not recommend the use of β-citronellol as anti-monogenean in fish, since it is impractical. Moreover, citronellol has anti-carcinogenic effects and decreases leukopenia and neutropenia (Zhuang et al., 2009). Besides the positive effects of β-citronellol, the most common fatty acids in the body structures of fish are palmitic and stearic acids. Especially the presence of these acids in the cell structure positively affected the growth of rainbow trout juveniles, which was determined in all growth parameters, including FCR. This situation suggests that it is caused by the continuous cell proliferation on the body whole of juvenile's fish during the growing stage.

Lysozyme is an antimicrobial peptide and plays an important role in the disease defense mechanism. LA was slightly increased at the groups fed with DEO ($P>0.05$). Similarly, LA was insignificantly increased in rainbow trout (O. mykiss) fed with yeast β-glucan (Kunttu et al., 2009), and D-limonene derived from orange peel essential oil (Gültepe, 2020). Nevertheless, tilapia (*Oreochromis mossambicus*) fed with rosemary (*Rosmarinus officinalis*) and thyme (*Thymus vulgaris*) showed identical results (Gültepe et al., 2014a). Although SOD, which catalyzes the dismutation of superoxide radicals to hydrogen peroxide and oxygen, tends to increase, this was not significantly different between groups in the present study ($P>0.05$). Ayisi et al. (2018) reported that serum SOD decreased when palm oil was used instead of fish oil in tilapia feeds. In contrast, Huang et al. (2016) reported that as in this study, juvenile chu's croaker (*Nibea coibors*) as the amount of palm oil in the diet increases, the SOD value tends to increase in the liver and muscle. However, oregano essential oil in channel catfish (Zheng et al., 2009), and orange peel essential oil in rainbow trout (Gültepe, 2020) were increased superoxide anions. This suggests that date kernel essential oil supports the innate immune system in juvenile fish. Myeloperoxidase plays a role both as an antimicrobial in metabolism and as a marker of polymorphonuclear leukocyte influx in inflammation. MPO was slightly lower in the DEO-0.5 group compared with the control group (P>0.05) but significantly differ from DEO-1 and DEO-2 groups (P<0.05) in this study. Similarly, the authors reported that MPO is increased in *O. mykiss*, *O. mossambicus*, *Seriola rivoliana* when essential oils were used in the diets (Gültepe et al., 2014a,b; Gültepe, 2020; Hernández-Contreras et al., 2020; Shourbela et al., 2021). Neutrophils of individuals with MPO enzyme deficiency can normally phagocytosis. However, they cannot kill the phagocytosed agent and are therefore extremely prone to chronic bacterial infections. Therefore, MPO is directly related to PA. PA is the main function of renal leukocytes of teleost's, and phagocytosis is the first line of defense of the immune system to eliminate most invading pathogenic microorganisms and is an essential part of tissue homeostasis and remodeling, which offers protective defense to bodies (Ringø et al., 2012). PA was increased fish fed with DEO, nonetheless only significantly different in the DEO-2 group when compared to the control group (P<0.05). According to recent studies, PA was increased when essential oils were used in the diets, such as sweet orange and lemon peels essential oils in Nile tilapia (Mohamed et al., 2020), oregano essential oil in common carp (Abdel-Latif et al., 2020), thyme essential oil in African catfish (Euony et al., 2020), orange peels essential oil in rainbow trout (Gültepe, 2020). RBA is an indicator of innate immunity and manifests as high oxygen consumption during phagocytosis (Biller-Takahashi et al., 2013; Biller and Takahashi, 2018). Additionally, widely used to evaluate the defense ability of a host against different pathogens. In the present study, RBA was increased in all DEO fed groups and was highest in the DEO-2 group (P<0.05). Researchers similar results reported that essential oil of bitter lemon fruit peels in juvenile *Labeo victorianus* (Ngugi et al., 2017), *Ducrosia anethifolia* essential oil in rainbow trout (Vazirzadeh et al., 2017), and *Ocimum americanum*, *Cymbopogon flexuosus*, and *Melaleuca alternifolia* essential oils in red drum (*Sciaenops ocellatus*) (Sutili et al., 2016).

In conclusion, the results of this current study indicated that the inclusion of date (*Phoenix dactylifera* L.) kernel essential oil has no negative effects on rainbow trout (*Oncorhynchus mykiss*) juveniles. Additionally, date (*P. dactylifera* L.) kernel essential oil has increased growth performance, improved innate immunity. According to the study results, 2‰ date (*P. dactylifera* L.) kernel essential oil supplemented diets can be applying to as feed additives and immunostimulants in rainbow trout farming.

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