

Determination of the effects of food preservatives benzoic acid and sodium nitrate on lifespan, fertility and physical growth in *Caenorhabditis elegans*

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Received: March 2019

Accepted: July 2019

Abstract

Presently, the use of protective food additives such as benzoic acid and sodium nitrate is quite common. However, it was found that these additives, which initially appeared to be harmless, led to the emergence of a number of health problems. Cancer and diseases and deaths with no apparent causes are among the leading concerns. Therefore, the studies which can reveal the genotoxic potential of food preservatives and their negative effects on human health are very important in terms of ensuring food safety. Many model organisms are used to show these negative effects. *Caenorhabditis elegans* (*C. elegans*) is an important model organism, which is frequently used in determining the negative effects and toxic doses of substances that are toxic to or may have toxic effects on humans. In the present study, the aim was to determine the effects of different doses of benzoic acid and sodium nitrate, which are among the protective food additives known to cause certain diseases in humans, on lifespan, fertility and physical growth of *C. elegans*. Within the scope of the study; instead of the standard nutrient, *C. elegans* was supplemented with 5 different doses (0.006 g, 0.01 g, 0.02 g, 0.05 g, 0.1 g/10 mL) of benzoic acid and consequently, sodium nitrate, and lifespan, fertility and physical growth changes were examined in *C. elegans* which were exposed to different doses of benzoic acid and sodium nitrate. The findings were evaluated by reaching to comparisons with the control groups. At the end of the study, it has been determined that 0.01 g, 0.02 g, 0.05 g, 0.1 g/10 mL doses of benzoic acid, and all the administered doses of sodium nitrate (0.006 g, 0.01 g, 0.02 g, 0.05 g, 0.1 g/10 mL) have detrimental effects on lifespan, fertility and physical growth in *C. elegans*. On the other hand, 0.006 g/10 mL dose of benzoic acid did not cause any significant difference in lifespan and when compared to the control group. However, at a dose of 0.006 g/10 mL of benzoic acid, physical growth and fertility was found to be less than that of control group. As a result, it was determined that benzoic acid and sodium nitrate have negative effects on lifespan, fertility and physical growth due to dose increase.

Keywords: Benzoic acid, Sodium nitrate, *Caenorhabditis elegans*, Lifespan, Fertility, Physical growth

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Introduction

Preservatives commonly used in food additives, are used to destroy bacteria, molds and yeasts, any microorganisms with or without pathogens, to prevent their proliferation or activities, and thus delay deterioration (Surekha and Reddy, 2000; Kucukoner, 2006; Altug, 2009; Guzel, 2013). Benzoic acid, which is widely used in foods as preservative additives; a white colored, needle or leaflet-like, benzene ring, vapour volatile compound (Ucar, 2004; Arslan, 2011) and the first chemical preservatives to be legalized for use in food (Ogbadu, 1999; Guzel, 2013). Benzoic acid is mostly used as sodium salt (sodium benzoate) in foods due to its low solubility in water. (Koyuncu, 2006; Yildiz, 2010; Guzel, 2013; Arslan, 2011; Ozturkcan and Acar, 2017). Its antimicrobial activity is high on mold and yeasts, but it is not recommended for use against bacteria. This is because pH values above 4.5, where bacterial growth is high, reduce the mechanism of action of benzoic acid. (Robach, 1980; Baker *et al.*, 1988; Ozturkcan and Acar, 2017). A suitable pH limit in which benzoic acid inhibits microorganisms is an acidic pH ranging from 2.5 to 4.0. (Chichester and Tanner, 1972; Saldamli, 1985). Mostly found in fruit juice, marmalade, margarine, jam, sodas, pickles, sauces, ketchup, jelly and similar products in food industry (Fish *et al.*, 2000; Chayabutra and Ju, 2000; Kahraman and Geckil, 2005). The amount prescribed for use in foods is between 0.2–0.3% and is used only in French rennet. (Arslan, 2011; Cotra, 2016).

Studies have shown that in susceptible individuals, benzoic acid even at low doses has side effects, such as brain injury, hypersensitivity, weight loss, triggering asthma and nervous disorders, hyperactivity and urticaria in children, skin redness, swelling, itching and pain, increasing hormone oestrogen hormones and tumour formation (Wibbertmann *et al.*, 2000; Deshpande, 2002; Omaye, 2004; Qi *et al.*, 2009; Erkmén, 2010; Ozpınar *et al.*, 2013). The symptoms in humans are known to induce trigeminal innervation of taste cells in association with childhood hyperactivity and to cause temporary irritation and itching in the mouth and tingling and skin contact (Otero-Loseda, 2003; Zengin *et al.*, 2011; Guzel, 2013).

Sodium nitrate (NaNO_3), which is the sodium salt of nitrate; is one of the preservative food additives used in foods (especially meat, meat products and fish) due to its characteristic flavour, natural colour preservation and antimicrobial properties (Erkmén, 2010; Arslan, 2011). Although it varies by country, the incorporation of sodium nitrate or equivalent compounds of around 500 ppm (mg/kg) into processed meat products has become standard (Sanlı and Kaya, 1998; Kaya, 2011).

Nitrate ions have no direct toxic effect (Bories ve Bories, 1995; Ozdestan and Uren, 2010). However, it was stated in studies that nitrate taken together with food can be reduced to nitrite by bacterial nitrate reductase activity and nitrite, on the other hand, is a precursor of nitrosamine compounds that induce tumour formation in humans and animals, plays a role in the

formation of cancers of the liver, lung, kidney, larynx, stomach and pancreas. (Omaye, 2004; Erkmen and Bozoglu, 2008; Erkmen, 2010; Aschebrook-Kilfoy *et al.*, 2011). In addition, besides all these negativities, dietary nitrate may cause methemoglobinemia, shortness of breath, dizziness, hypotension and circulatory collapse (Omaye; 2004; Erkmen, 2010).

The present study aims to determine the effects of protective food additives known to cause some diseases in humans; of different doses of benzoic acid and sodium nitrate (0.006 g, 0.01 g, 0.02 g, 0.05 g, 0.1 g/10 mL) on lifespan, fertility and physical growth in a model organism *C. elegans*. *C. elegans* is a model organism, showing 60-80% gene homology with humans, providing experimental advantages because of its basic features such as simplicity, transparency and short life cycle and frequently used in basic biological studies and toxicity studies for years.

Material and methods

N2 wild type *C. elegans* and *Escherichia coli* OP50 strain were used in the study and stock cultures were obtained from Caenorhabditis Genetic Center (CGC) of the University of Minnesota. Stock cultures were maintained in the dark and at appropriate temperatures (20-25°C for *C. elegans*; 4°C for *Escherichia coli* OP50 strain).

Purification of E. coli OP50 strain on TBX Agar medium

9.125 g of TBX Agar (Tryptone Bile Glucuronide Agar) was weighed on a

sensitive balance and 250 mL of distilled water (distilled water, dH₂O) was added. The agar was thawed in a magnetic stirrer and placed in an autoclave at 125°C for 15 minutes. The medium exiting the autoclave was cooled to 55°C. The cooled TBX Agar medium was poured into approximately 60 mL petri dishes and allowed to solidify. After solidification, TBX Agar media which were ready for use were added *E. coli* OP50 strain with burner flame with the help of the loop and incubated at 37°C for 24 hours. After colony formation was observed, specific blue colonies belonging to the strain *E. coli* OP50 were identified.

Preparation of liquid medium for E. coli OP50 strain

After weighing 9.125 g Lauryl Sulfate Tryptose Broth (LST Broth) on a precision balance, 250 mL of distilled water was added thereto and autoclaved at 120°C for 15 minutes. The autoclave sterilized medium was cooled to 37°C and then a colony *E. coli* OP50 strains was taken from TBX Agar medium with the help of a loop and seeded into LST Broth in a laminar flow (sterile cabinet). After transfer to the liquid medium, it was incubated for 24 hours in an oven at 37°C. After reproduction, it was raised to 4 °C for use in the study.

Preparation of Nematode Growth Media (NGM)

After weighing 2.5 g of Bacto-peptone, 3 g of NaCl and 20 g of Agar, it is dissolved in 1 L of distilled water until it reaches the boiling temperature and autoclaved at 120 °C for 15 minutes. After leaving the

autoclave, it was cooled to 55°C. To ensure homogenisation of the cooled NGMs, previously prepared supplements were added to the medium; 1 mL MgSO₄ (1M), 1 mL Cholesterol (5 mg/mL), 1 mL CaCl₂ (1M) and 25 mL KPO₄ buffer (pH: 7) were added to the medium by filtration through 0.2 µm porous cellulose filters.

NGM to be used in lifespan analysis was added to fluorodeoxyuridine (FUdR) in order to prevent the egg development of *C. elegans*. For fertility and physical growth analysis, NGMs without FUdR were used. Subsequently, the preservative food additives (benzoic acid and sodium nitrate) used in the study were added to NGM at different doses (0.006 g, 0.01 g, 0.02 g, 0.05 g, 0.1 g/10 mL) and approximately 10 mL in 60 mm petri dishes were expected to obtain agar consistency. For the control group, no additives were added.

Synchronization of C. elegans

1 g of NaOH was weighed on a precision balance and 5 mL of distilled water was added to dissolve. Then, 1 mL of sodium hypochlorite and 0.5 mL of NaOH solution were transferred to the centrifuge tube. The previously prepared *C. elegans* were cultured in NGMs by pipetting with distilled water (approximately 2 mL) to ensure that the eggs were washed. The washed eggs were transferred to the centrifuge tube by micropipette and centrifuged at 3000 rpm for 10 minutes. After centrifugation, the supernatant was discarded and the remainder was transferred to new NGMs. The transferred eggs form synchronized fry.

Lifespan analysis

Approximately 400 µl of *E. coli* OP50 strain was added to the middle of the NGMs containing FUdR and different doses of protective food additives were prepared for lifespan analysis and left to dry in sterile environment for approximately 1-2 days. When the bacterial solution was dried, 50 individuals from *C. elegans* in adult form (L4) synchronized to the each petri dishes were transferred under a stereomicroscope. The same procedure was done for control petri dishes. At the same time each day after transfer, live *C. elegans* in all petri dishes were counted with the control group under a 4x objective under a light microscope. Counting process continued until *C. elegans* died (Sutphin and Kaerberlein, 2009).

Fertility analysis

NGMs without FUdR were used for fertility analysis. Egg counting was performed according to Koelle (2005) protocol. According to this; after adding *E. coli* OP50 strain to each petri dishes prepared with different doses of food additives (0.006 g, 0.01 g, 0.02 g, 0.05 g, 0.1 g/10 mL) used in the study, they were transferred from 15 well-fed L4 stage *C. elegans*. After 36 hours, 10 of them were transferred to new petri dishes and incubated for 30 minutes at 20°C. At the end of the period, eggs were counted under a light microscope with a 4x objective, and after counting; adult *C. elegans* were introduced into new petri dishes. This process was repeated for 3 days. In addition, *C. elegans* hatched eggs were

counted one day later in the petri containing eggs and non-cracking eggs were detected. Egg yield and egg yield were calculated and compared with the control group.

Physical growth analysis

Fluorodeoxyuridine (FUDR) in NGM containing the same doses of food additives prepared for the control of physical growth was not added and an equal amount of *C. elegans* eggs was added to the petri dishes. Petri dishes including 50-100 eggs in each, at the end of the 3rd day, images were taken with the control group under a 4x objective under a light microscope. And the body sizes of *C. elegans* were measured from these images.

Statistical analysis

All analyses were repeated at least three times. In lifespan analysis findings were determined by Log-Rank Test using OASIS (Online Application for Survival Analysis) software (Yang *et al.*, 2011). For fertility and physical growth research findings, statistical analyses were performed using SPSS 22.0 (IBM Corporation, Armonk, New York, United States) program. Differences between means were determined to be $p < 0.05$ by Tukey test.

Results

The results of Log-Rank test showing the effect of different doses of benzoic acid on lifespan in *C. elegans* are given in Table 1 and the percentage of survival is given in Fig. 1. In the comparison between the benzoic acid doses and control group; there

is a significant difference for the application doses 0.1 g, 0.05 g, 0.02 g, 0.01 g/10 mL ($p < 0.05$). This difference increases with increasing dose. On the other hand, the 0.006 g/10 mL dose of benzoic acid was not significant when compared to the control group ($p > 0.05$). As a result, all application doses (0.1 g, 0.05 g, 0.02 g, 0.01 g/10 mL), except for the dosage of 0.006 g/10 mL of benzoic acid, reduced toxicity for *C. elegans* (Table 1).

The lowest percentage of survival was seen at 0.1 g/10 mL, the highest dose of benzoic acid; the highest survival percentage was seen at the lowest dose of 0.006 g/10 mL. At the dose of 0.1 g/10 mL, half of *C. elegans* individuals (50%, 25 individuals) died at the end of day 4. At the end of the 10th day, no *C. elegans* survived at this dose. For *C. elegans*, individuals known to have a lifespan of about 14-20 days, this dose of benzoic acid (0.1 g/10 mL) significantly reduced the lifespan (about 50%). In other application doses (except 0.006 g/10 mL), significant reductions in percentage of survival and increased dose of benzoic acid increased the toxic effect were determined (Fig. 1).

Table 2 shows the statistical results of the effect of different doses of benzoic acid on fertility in *C. elegans*. When the number of eggs and egg hatching with egg productivity obtained from 10 *C. elegans* individuals for 3 days were evaluated, it was seen that all dose groups of benzoic acid had negative effects on fertility.

Table 1: Effect of benzoic acid on lifespan in *C. elegans*.

Control-dose comparison	Chi-Square	<i>p</i> - value	Bonferroni <i>p</i> -value
Control v.s. 0.006 g/10 mL	1.32	0.2507	1.0000
Control v.s. 0.01 g/10 mL	17.87	2.4e-05	0.0001
Control v.s. 0.02 g/10 mL	30.38	3.6e-08	1.8e-07
Control v.s. 0.05 g/10 mL	68.90	0.0e+00	0.0e+00
Control v.s. 0.1 g/10 mL	98.26	0.0e+00	0.0e+00

p-value 0.00E+00 is provided when $p < 1.0 \times 10^{-10}$. *p* values are significant for $p < 0.05$.

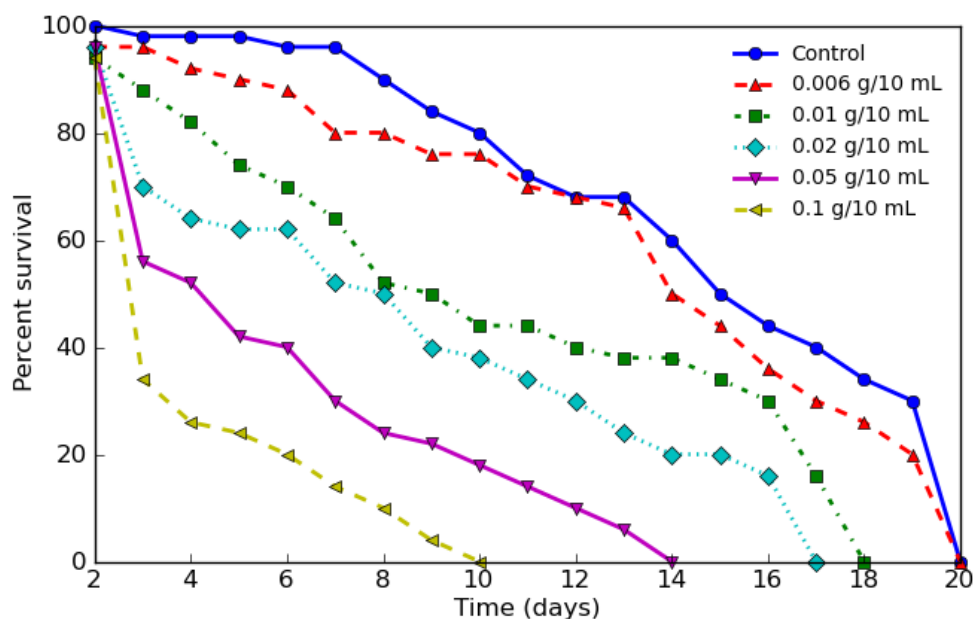


Figure 1: The effect of different doses of benzoic acid on the percentage of survival in *C. elegans*.

In the comparison with the control group, the greatest decrease in the number of eggs and individuals was for the dose of 0.1 g/10 mL. Although the dose of benzoic acid decreased while the number of eggs and hatching individuals increased, the effect on fertility was found to be significant even at the lowest benzoic acid dose (0.006 g/10 mL) ($p < 0.05$). When the dose groups were compared with each other, statistical differences were also observed. Each dose group had a significant effect on fertility differently from each other (Table 2). Egg

productivity of *C. elegans* were found to be 45.8%, 60.9%, 66.4%, 76.8% and 81.7% for application doses of 0.1 g, 0.05 g, 0.02 g, 0.01 g and 0.006 g/10 mL of benzoic acid, respectively. In the control group, egg productivity was 98.4%. In comparison with the control, the decrease in egg productivity caused by benzoic acid doses was statistically significant ($p < 0.05$). Statistically significant differences were also found when dose groups were compared among themselves.

Table 2: The effect of benzoic acid on fertility in *C. elegans*.

Doses (g/10 mL)	Number of eggs	Number of individuals	Egg productivity (%)
Control	985.33±2.51 ^a	973.33±1.52 ^a	98.4 ^a
0.006	642.0±3.60 ^b	528.66±2.08 ^b	81.7 ^b
0.01	502.33±2.51 ^c	386.33±1.52 ^c	76.8 ^c
0.02	349.66±4.04 ^d	232.66±2.08 ^d	66.4 ^d
0.05	266.0±3.0 ^e	160.66±1.52 ^e	60.9 ^e
0.1	71.66±2.51 ^f	31.33±1.52 ^f	45.8 ^f

The numbers indicated by different letters are statistically different from each other ($p < 0.05$).

There is a difference of about 36% between the highest (0.1 g/10 mL) and the lowest (0.006 g/10 mL) dose of benzoic acid (Fig. 2).

The measured body sizes (micrometers, μm) of *C. elegans* are shown in Fig. 3. The statistical data obtained from the analysis of the effects of benzoic acid on physical growth are given in Table 3. The effect of all doses of benzoic acid on physical growth in *C. elegans* was found to be significant compared to the control group ($P < 0.05$). The increase in the amount of benzoic acid reduces the body size in *C. elegans* individuals. Therefore, growth increases with retardation due to dose increase. The lowest average length measured between benzoic acid dose groups was 9.42 μm , while the highest average length was 15.73 μm . The mean length of the control group was calculated as 22.26 μm (Table 3).

Table 4 shows the log-rank test results of different doses of sodium nitrate and the lifespan analysis data of the control group. The differences between 5 different doses of sodium nitrate (0.006 g, 0.01 g, 0.02 g, 0.05 g, 0.1 g/10 mL) and the control group were significant ($p < 0.05$). It was determined that the survival time was

shortened in parallel with the dose increase (Table 4).

Fig. 4 shows the percentage survival of the different doses of sodium nitrate and the control group. While on day 1 of additive exposure, all *C. elegans* individuals (100%) in all application doses were alive, at the end of day 12 at 0.1 g/10 mL, at the end of day 13 at 0.05 g/10 mL, 0.02 g/10 mL, at the end of day 14, at the end of day 16 at 0.01 g/10 mL and at the end of day 17 at 0.006 g/10 mL, all *C. elegans* individuals died. In the control group, *C. elegans* survived for 19 days after the transfer. In this case, the dose increase in sodium nitrate decreased the percentage of survival in *C. elegans* individuals (Fig. 4).

Table 5 shows the statistical results of the effect of 0.006 g, 0.01 g, 0.02 g, 0.05 g, 0.1 g/10 mL doses of sodium nitrate on fertility in *C. elegans*. When Table 5 is evaluated, which shows the effect of different doses of sodium nitrate on fertility, it seen that the statistically significances for each dose group are important ($p < 0.05$).

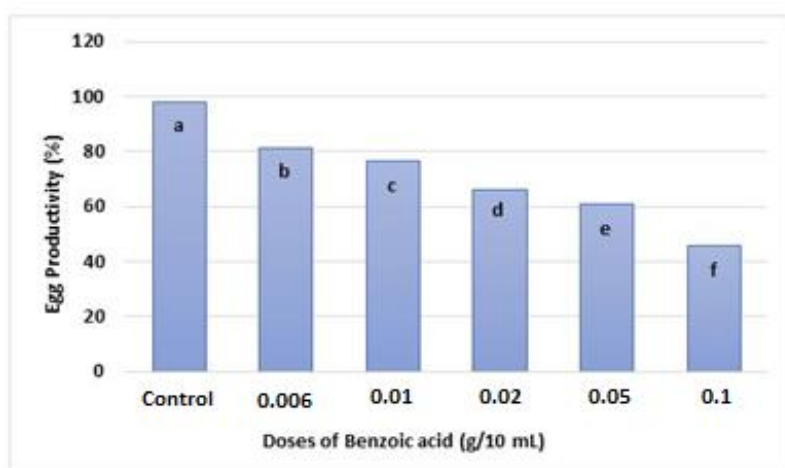


Figure 2: The effect of benzoic acid on egg productivity in *C. elegans*.

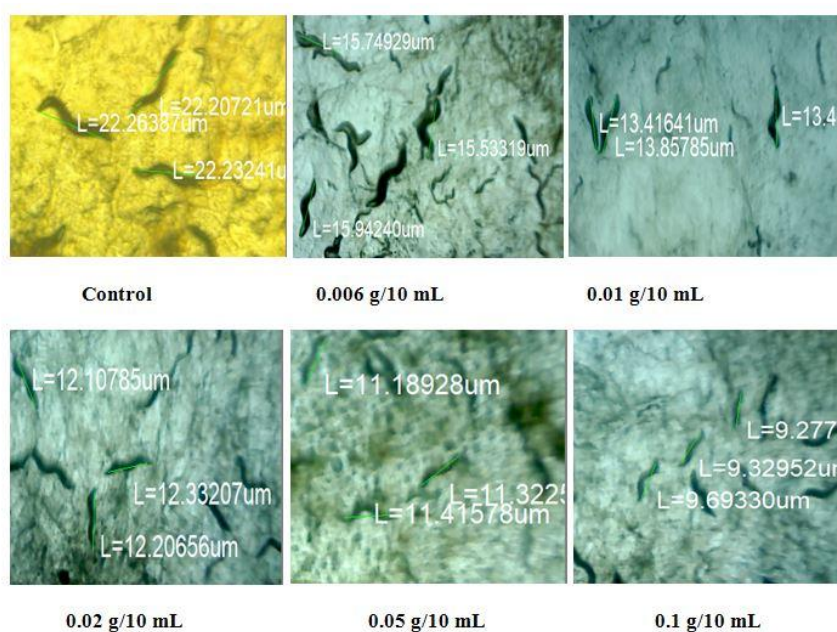


Figure 3: Body sizes measured by the application of different doses of benzoic acid.

Table 3: The effect of benzoic acid on physical growth in *C. elegans*.

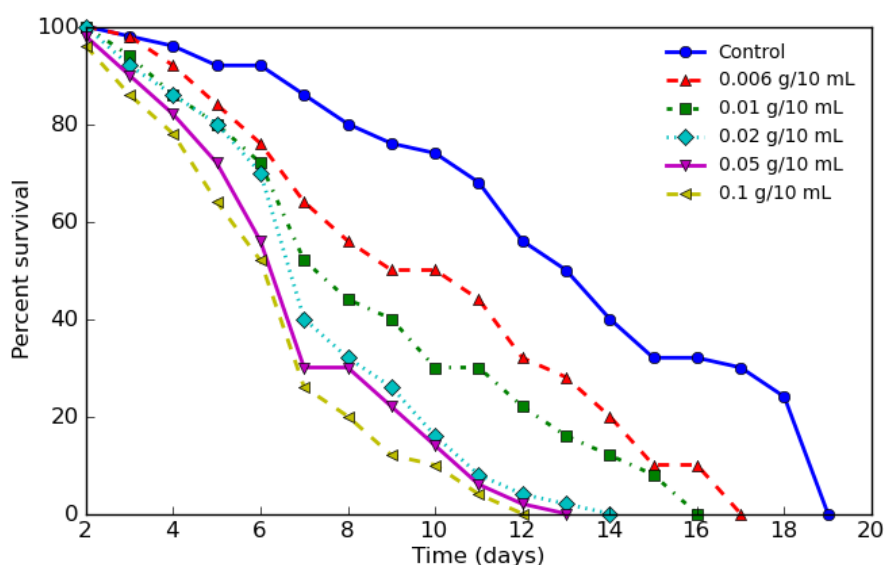
Doses (g/10 mL)	Physical growth
Control	22.23±0.03 ^a
0.006	15.73±0.20 ^b
0.01	13.57±0.23 ^c
0.02	12.21±0.11 ^d
0.05	11.30±0.11 ^e
0.1	9.42±0.22 ^f

The numbers indicated by different letters are statistically different from each other ($p < 0.05$).

Table 4: Effect of sodium nitrate on lifespan in *C. elegans*.

Control-dose comparison	Chi-Square	<i>p</i> -value	Bonferroni <i>p</i> -value
Control v.s. 0.006 g/10 mL	14.79	0.0001	0.0006
Control v.s. 0.01 g/10 mL	24.29	8.3e-07	4.1e-06
Control v.s. 0.02 g/10 mL	48.40	0.0e+00	0.0e+00
Control v.s. 0.05 g/10 mL	54.09	0.0e+00	0.0e+00
Control v.s. 0.1 g/10 mL	60.92	0.0e+00	0.0e+00

p-value 0.00E+00 is provided when $p < 1.0 * 10^{-10}$. *p* values are significant for $p < 0.05$.

**Figure 4: Effect of different doses of sodium nitrate on the percentage of survival in *C. elegans*.**

The sodium nitrate dose of 0.1 g/10 mL was determined as the lowest observed number of eggs (436.33 ± 1.52) and the number of individuals (146.33 ± 2.51). As the dose of sodium nitrate decreases, there is an increase in the number of eggs and individuals hatching from these eggs. However, this increase does not make the difference between the control group insignificant when compared. Even at the lowest sodium nitrate dose (0.006 g/10 mL), there were significant differences in the number of eggs and individuals compared to the control ($p < 0.05$). Therefore, it is clear that all application

doses of sodium nitrate adversely affect fertility (Table 5).

The egg productivity (%) graph of the different doses of sodium nitrate and the control group is presented in Fig. 5. When Fig. 5 is examined; 98.4% yield was found in the control group in terms of egg productivity, while the yield decreased to 34.0% at 0.1 g/10 mL dose. Egg productivity (%) differences between the control group and all dose groups were found to be statistically significant ($p < 0.05$). In addition, increases in the dose of sodium nitrate reduce egg productivity in *C. elegans*.

Table 5: Effect of sodium nitrate on fertility in *C. elegans*

Doses (g/10 mL)	Number of eggs	Number of individuals	Egg productivity (%)
Controls	975.33±1.52 ^a	959.66±2.08 ^a	98.4 ^a
0.006	781.33±2.51 ^b	523.66±2.51 ^b	67.1 ^b
0.01	723.66±2.08 ^c	442.66±1.52 ^c	61.1 ^c
0.02	600.33±2.08 ^d	345.33±0.57 ^d	57.3 ^d
0.05	551.33±2.51 ^e	223.66±3.05 ^e	41.1 ^e
0.1	436.33±1.52 ^f	146.33±2.51 ^f	34.0 ^f

The numbers indicated by different letters are statistically different from each other ($p < 0.05$).

When the dose groups were compared, it was determined that all doses were statistically different from each other in terms of egg productivity (Fig. 5). Body size measurements of *C. elegans* exposed to doses of 0.006 g, 0.01 g, 0.02 g, 0.05 g and 0.1 g/10 mL of sodium nitrate and taken on a light microscope with a 4x objective 3 days after hatching are shown in Fig. 6.

Table 6 shows the statistical results obtained from the analysis of the effects of sodium nitrate on physical growth are given. It was determined that the effects of

all doses of sodium nitrate on physical growth were important and sodium nitrate doses caused growth retardation in *C. elegans* ($p < 0.05$). While the body sizes measured in the control group was 22.2 μm , the average body sizes in sodium nitrate dose groups were from the lowest dose group to the highest dose group,; 17.75; 16.17; 15.14; 14.43; 13.57 μm , respectively. The increase in the sodium nitrate dose amount decreased the body sizes values of *C. elegans* individuals (Table 6).

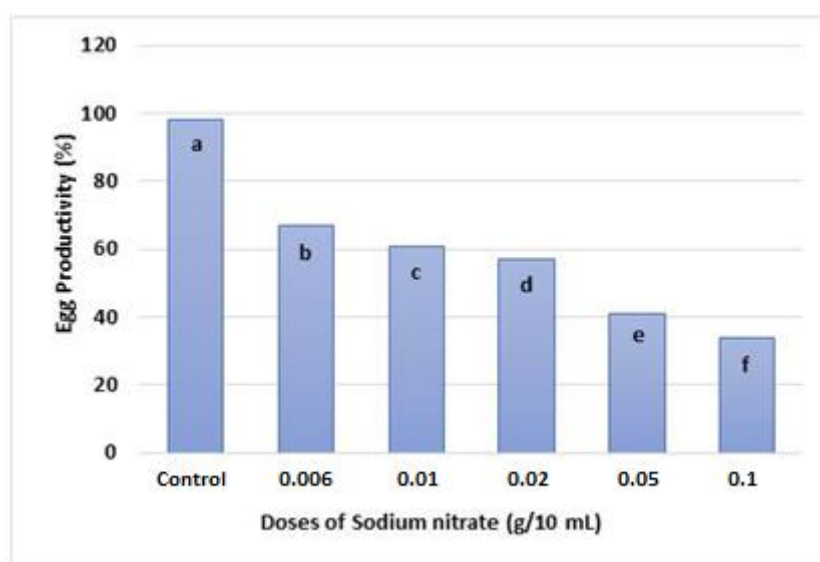


Figure 5: Effect of sodium nitrate on egg production in *C. elegans*.

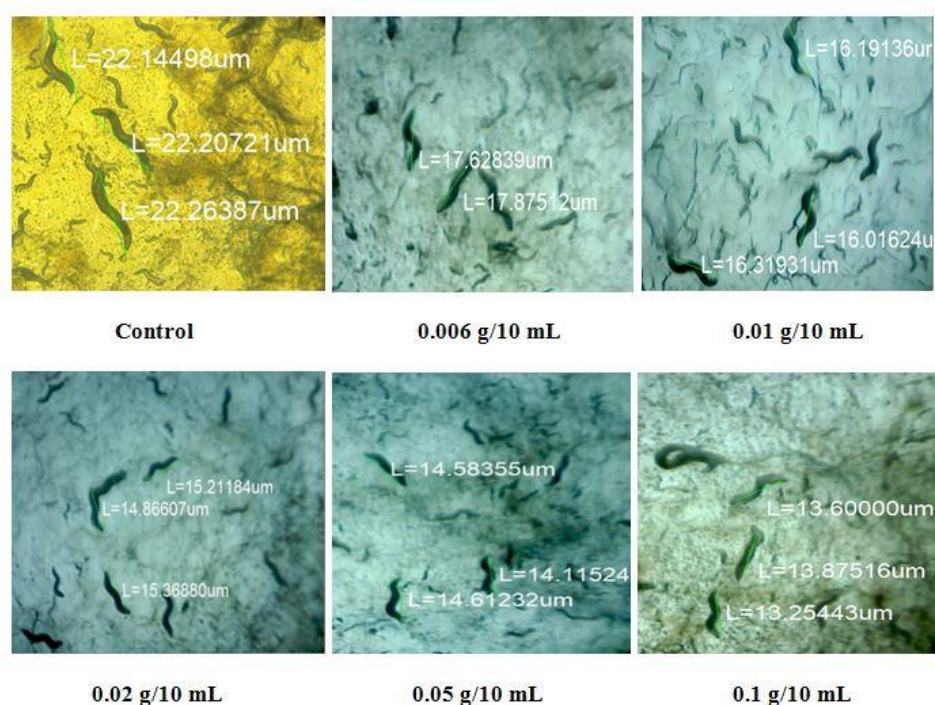


Figure 6: Body sizes measured by the application of different doses of sodium nitrate.

Table 6: The effect of sodium nitrate on physical growth in *C. elegans*.

Doses (g/10 mL)	Physical growth
Control	22.20±0.06 ^a
0.006	17.75±0.12 ^b
0.01	16.17±0.15 ^c
0.02	15.14±0.25 ^d
0.05	14.43±0.28 ^e
0.1	13.57±0.31 ^f

The numbers indicated by different letters are statistically different from each other ($p < 0.05$).

Discussion

Most of the studies on food additives show that the majority of food additives can be toxic to humans and may cause some diseases to occur in humans. Considering that the consumption of food additives is quite high today this can be said to pose a threat to millions of people in terms of both health and food safety. In the study, it was determined that doses of 0.01 g, 0.02 g, 0.05 g, 0.1 g/10 mL of benzoic acid had toxic effects on lifespan. On the other

hand, the dose of benzoic acid of 0.006 g/10 mL was found to have no adverse effect on lifespan. The effects of benzoic acid on fertility and physical growth were significant in all benzoic acid doses (0.006 g, 0.01 g, 0.02 g, 0.05 g, 0.1 g/10 mL). It was found that all of the benzoic acid dose groups decreased the number of eggs and individuals in *C. elegans* and decreased egg productivity. In addition, it was determined that all of the benzoic acid doses caused growth retardation by means

of body sizes measurement values in *C. elegans*. In all analyses (lifespan, fertility, physical growth), adverse effects also increase with increasing dose. In the literature review, similar studies have been found which are largely in line with our results for benzoic acid.

Ozpinar *et al.* (2003), in the study of the physiological and lifespan alterations in *C. elegans* exposed to different doses of benzoic acid (0.1 g, 0.06 g, 0.02 g, 0.008 g and 0.001 g/10 mL) examined and in the result of study, 0.1 g, 0.06 g and 0.02 g/10 mL doses of benzoic acid had negative effects on lifespan, fertility and physical growth in *C. elegans* determined. The doses of 0.008 g and 0.001 g/10 mL did not cause any difference in lifespan, yet they detected that fertility and physical growth were less in these doses than in the control group. On another study, Sarikaya and Solak (2003) evaluated the genotoxic effect of benzoic acid in *Drosophila melanogaster* by Somatic Mutation and Recombination Test (SMART). They found that 50, 75 and 100 mM doses of benzoic acid in *Drosophila melanogaster* cause increased mutations and shorten their lifespan.

All doses of sodium nitrate (0.006 g, 0.01 g, 0.02 g, 0.05 g, 0.1 g / 10 mL), which are the other preservative additives used in the study, were found to have negative effects on lifespan, fertility and physical growth in *C. elegans*. As a result, on this study sodium nitrate was found to shorten the lifespan, decrease the number of eggs and hatching individuals, decrease egg productivity (%) and lead to growth retardation. Sarikaya *et al.* (2006) in their

studies, four food additives (sodium nitrite, sodium nitrate, potassium nitrate and potassium nitrate) investigated the effect of 75 mM doses on lifespan in *Drosophila melanogaster* (mwhxflr). In the results of study from all experimental groups consisting of sodium nitrite, sodium nitrate, potassium nitrate and potassium nitrate comparing to the control it is detected that significant reduction in life expectancy.

With the studies which have been done so far, lifespan of nitrates and nitrites, its effect in terms of physical growth has not been fully revealed. On the other hand, in science world, because nitrate and meat products are deformable there are also those who argue that it is healthier. For colour preservation and prevention of spoilage in meat products no alternative substance to nitrates and nitrites was found. Because, nitrates and nitrites have extensive activity on cured meat products and there is no single alternative that is effective enough to perform all the functions of these additives yet. Therefore, current studies in meat products formulations use of nitrate and nitrite and the amount of residual nitrite in the product reduction with combinations of various additives or these additives should not be used at all, in recent years benefiting from microbial sources, organic acids, high pressure, irradiation and obstacle technology applications is also noteworthy. The lifespan of benzoic acid and sodium nitrate as preservative food additives, other dosages of benzoic acid, except the dose of 0.006 g/10 mL, had a greater effect on lifespan than sodium nitrate, significantly

shortening lifespan when comparing effects on fertility and physical growth. In fertility analysis, although egg productivity values were lower in sodium nitrate, since the number of eggs and individuals determined in the doses of benzoic acid is lower than those determined in the sodium nitrate doses, the negative effect of benzoic acid on fertility is greater. In physical growth analysis all doses of benzoic acid caused more growth retardation than sodium nitrate. Therefore, it is detected that benzoic acid has more harmful effects in all three parameters (lifespan, fertility and physical growth).

This study is considered to be the source of similar studies to be done from now on. In the future, studies can be planned to determine the effects of untested doses of additives or other additives used in this study on lifespan, fertility and physical growth in *C. elegans*.

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