Modeling the growth of *Staphylococcus aureus* as affected by black zira (*Bunium persicum*) essential oil, temperature, pH and inoculum levels

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**Abstract**

Black zira (*Bunium persicum*) is a medicinal plant and spice, naturally grows in Iran. The aim of this study was to investigate the combined effects of different concentrations of *Bunium persicum* essential oil (EO; including 0, 0.08, 0.16 and 0.24%), three incubation temperatures (15, 25 and 35°C), three levels of pH (5, 6 and 7 adjusted by hydrochloric acid), and three inoculum size (10^2, 10^3 and 10^4 CFU mL^-1) on the growth of *Staphylococcus aureus* in the brain heart infusion broth. Black zira EO was extracted and its component was identified using gas chromatography-mass spectrometry (GC-MS) analyses. The experiment was carried out in triplicate. Growth was monitored by visible turbidity during a 30-day period. To evaluate effects of explanatory variable on time to detection (TTD) of bacterial growth, parametric survival models based on Log-normal distribution was used. All explanatory variables had significant association with time to detection (p < 0.05). The final model accurately predicted the growth initiation and inhibition of *S. aureus*. © 2014 Urmia University. All rights reserved.

**Keywords:** Black zira, Essential oil, Predictive modeling, *Staphylococcus* growth
Introduction

*Staphylococcus aureus* is the most common enterotoxigenic staphylococcal species causing foodborne disease. Amongst the reported foodborne illnesses, *S. aureus* is considered the third most important cause of disease in the world. This gram-positive bacterium has no particular nutritional and environmental requirement for its growth and it can grow at a of 0.06, pH above 4.80 and its minimum growth temperature is 8.9 °C. Most strains are capable of producing one or more enterotoxins which are the cause of gastrointestinal symptoms observed during intoxications.

As food safety is of major concern in modern society, the scientific discipline of predictive microbiology gains more and more interest worldwide. An important research topic in this field is the development of mathematical models able to predict the growth of pathogenic micro-organisms in foods. Such models present a valuable tool in risk assessment and HACCP studies.

Predictive microbiology is a young discipline that has developed most of its terminology and methodology in the last two decades. It is based on the premise that the response of microorganisms (e.g., germination, growth and thermal inactivation kinetics) is reproducible, so the results from past observations can be used to predict the response of the same organism at similar environmental conditions.

Essential oils (EOs) are aromatic oily liquids obtained from plant material. Terpenoids and phenolic compounds such as thymol, carvacrol and eugenol are responsible for their antimicrobial activity. Essential oils can be obtained by expression, fermentation, enfleurage or extraction, but the method of steam distillation is most commonly used for commercial production of EOs.

There is considerable interest in the possible use of these compounds as food additives, to delay the onset of food spoilage or to prevent the growth of foodborne pathogens. Among these pathogens *Salmonella enteritidis* and *S. aureus* are of great importance.

*Bunium persicum* [Boiss.] B. Fedtsch is an important medicinal plant and spice belonging to the Apiaceae family. The plant grows wild in the dry temperature regions of Jammu and Kashmir, Himachal Pradesh, Afghanistan, and Iran. It is a small, grassy and perennial plant which produces white or pink flowers on the terminal and lateral stems during the third year of its life.

The seeds, rich in essential oil (EO), are consumed widely as condiment. In the indigenous system of medicines, seeds are regarded as stimulants and carminative and found to be useful in diarrhea and dyspepsia. The extracts of *B. persicum* have hypoglycemic activity and can prevent diabetes and obesity. Also, this plant is used for culinary purposes and for flavoring foods and beverages.

This study was designed to model the combined effects of different levels of *B. persicum* EO, temperature, pH and inoculum levels on the growth of *S. aureus*.

Materials and Methods

**Plant material.** The air-dried seeds of *B. persicum* [Boiss.] B. Fedtsch, were supplied from agricultural research fields of Ferdows University of Mashhad (FUM), Mashhad, Iran. The plant materials were authenticated in FUM herbarium and voucher specimens (No. 36267) deposited in the herbarium.

**Essential oil extraction.** Amount of 100 g of dried material were finely ground in a blender and submitted to FUM hydrodistillation facility. Hydrodistillation was done in 4 hr, using a clevenger-type apparatus. The EOs obtained were separated from water and dried over anhydrous Na2SO4 and stored in dark glass bottles at 4 °C prior to use.

**Gas chromatography and GC-MS analysis.** The components of the EO sample were identified by GC and GC-MS analyses. The GC-MS apparatus was a Varian GC-MS spectrometer consisting of a Varian Star 3400 GC equipped with a fused-silica column (DB-5, 30 m length × 0.25 mm inner diameter, 0.25 μm film thickness; J and W Scientific Inc., Folsom, USA), interfaced with a mass spectrometric detector (Model Varian Saturn 3; Agilent Technology Inc., Santa Clara, USA). The operating conditions were as follows: oven temperature 60-240 °C with a rate of 3 °C per min, injector temperature 280 °C, injector mode: split injection, with carrier gas, Helium, flow rate 2 mL min⁻¹, mass spectra: electronic impact, ionization potential 70 eV, ion source temperature 250 °C, ionization current 1000 μA, resolution 1000 and mass range 40-300 unit. The GC was a Shimadzu GC-17 equipped with a FID detector, fused silica column (BP-5, 25 m length × 0.22 mm inner diameter, 0.25 μm film thickness). The operating conditions were: oven temperature 60-280 °C with a rate of 8 °C per min, injector temperature 280 °C, split ratio 1:10, with carrier gas, nitrogen, and detector temperature 300 °C.

**Identification of components.** The oil components were identified from their retention indices (RI) obtained with reference to the n-alkane series (Sigma, Gillingham, UK) on the DB-5 column, mass spectra with those of authentic samples, composition of their mass spectra and fragmentation patterns reported in the literature, and computer matching with MS-data bank (Saturn version 4; Agilent Technology Inc., Santa Clara, USA). Quantification of the relative amount of the individual components was performed according to the area percentage method.

**Test organism.** *Staphylococcus aureus* ATCC 25923 (Mast International Inc., Merseyside, UK) was used as the test organism in this study.

**Experimental design.** To assess the effects of *B. persicum* EO, pH, temperature, and inoculum level on growth initiation of *S. aureus*, the experiment was arranged in a factorial design in brain heart infusion broth (BHI; Merck, Darmstadt, Germany). This design (4 × 3 × 3 × 3 equal to 324 combinations) included four concentrations of EO (0, 0.08, 0.16 and 0.24%), three levels of pH (5, 6
and 7) adjusted by hydrochloric acid, three incubation temperatures (15, 25 and 35 °C), three inoculums size (10³, 10⁴ and 10⁵ CFU mL⁻¹), three replicate of all combinations and repeated observations (daily) for growth in BHI broth for up to 30 days.

**Preparation of inoculum.** The reference bacterium was plated on Baird Parker agar (Merck, Darmstadt, Germany) agar medium and incubated at 37 °C for 24 hr. After confirmation typical colonies as *S. aureus* by biochemical tests, inoculums were prepared by transferring a loop full of the bacterial colonies to isotonic saline solution in a sterile cuvette to adjust the absorbance of 0.02 at 600 nm using a spectrophotometer (Model 6105; Jenway, Essex, UK). This adjustment gave a cell concentration of 1.2 × 10⁹ CFU mL⁻¹. The numbers of cells in the suspension were estimated by duplicate plating from 10-fold serial dilutions on BHI agar and counting the colonies after 24 hr incubation at 37 °C.

**Performing the experiment.** Amount of 3.7 g BHI broth powder (Merck, Darmstadt, Germany) was dissolved in 90 mL distilled water in a 250 mL flask by mild heating. In order to produce and maintain a stable oil-water emulsion in broth substrate during the period of study (30 days), the method explained by Mann and Markham were used, with some modifications. Briefly, we added 5% (v/v) dimethyl-sulfoxide (Merck, Darmstadt, Germany) as an emulsifier and 0.05% (w/v) agar (Merck, Darmstadt, Germany) as a stabilizer to the broth substrate. The pH was adjusted using hydrochloric acid to designate pH values. The values of pH were adjusted using a pH meter (Jenway, Staffordshire, UK). The final volume of broth substrate was brought to 100 mL with additional distilled water. The content of each flask was autoclaved at 121 °C for 15 min. After cooling, the pH of each combination in broth medium was measured and adjusted again to the considered pH values using 1 M filter sterilized HCl or NaOH. Then filter sterilized EO was added in different amounts to satisfy the experimental design. The content of flask containing sterile BHI broth was dispensed in portions of 3 mL into 16 × 100 mm sterile capped tubes (Becton Dickinson, San Jose, USA). The tubes were inoculated with *S. aureus* culture (10³, 10⁴ and 10⁵ CFU mL⁻¹). For each combination the inoculated tubes were stored at 15, 25 and 35 °C for up to 30 days. During these periods all the tubes were observed for visible growth (turbidity) daily up to 30 days. The number of tubes (combinations) showing growth at a particular observation were recorded. For each combination a negative control (un-inoculated tube) was used. All experiments were conducted in independent triplicate.

**Statistical Analysis.** The time to visible growth (TTD: time-to-detection or time to the nearest visible growth detection) considered as outcome variable in the present study. Since some combinations did not grow until the end of the study (30 days), event-time (survival) analysis was employed.

Kaplan-Meier survival curves for each level of an explanatory variable were plotted and the homogeneity of the curves between levels tested using the log rank statistic. Explanatory variables that showed a significant association with TTD were selected for inclusion in the multivariate analysis.

Parametric survival model based on accelerated failure time (AFT) approach was used to quantify the effect of each of the prescribed explanatory variables on time to detection of bacterial growth. The general form of the accelerated failure time model is:

$$\log(t) = (\alpha + \beta_1x_1 + ... + \beta_mx_m) + log(\tau)$$

where $\log(t)$ is the natural logarithm of the time to 'failure' (growth), $\alpha$ an intercept term, $\beta_1x_1 + ... + \beta_mx_m$ is a linear combination of the $m$ explanatory variables and their regression coefficients, and $log(\tau)$ is an error term. Using this approach the accelerated failure time coefficients represent the expected change in $log(t)$ for changes in the predictor levels.

In the present study the fit of the exponential, weibull, log-normal and log-logistic distribution to the current data was evaluated using mean square error (MSE) values as below. The smaller MSE value indicates a better fit.

$$MSE = \frac{\sum(predicted - observed)^2}{n - p}$$

where $n$ is the number of observations and $p$ is the number of parameters to be estimated.

To select those explanatory variables that best explained time to detection a backward stepwise approach was used. Explanatory variables that were not statistically significant were removed from the model one at a time, beginning with the least significant, until the estimated regression coefficients for all retained variables were significant at $p < 0.05$. All analyses were carried out using Stata Statistical Software, version 10 (Stata-Corp, College Station, Texas, USA).

**Results**

**Chemical composition of *B. Persicum* Boiss. EO.** The components of essential are presented in Table 1. The yield of oil from *B. persicum* was 9.10% (v/w). GC/MS identified 35 compounds, representing 95.50% of the oil content. y-terpinene was the main monoterpenic hydrocarbon, with a content of 44.20%. The cumin-aldehyde and p-cymene contents determined as 16.90 and 8.00%, respectively.

**Description of growth and no growth.** About 84.75% of combinations (270 out of 324) showed growth during the study period and 15.25% of combinations (54 out of 324) did not grow and were considered as censored observations.
Table 1. Phytochemical composition of B. persicum essential oil.

| Phytochemicals       | RIa | Percentage |
|----------------------|-----|------------|
| α-Thujene            | 925 | 0.4        |
| α-Pinene             | 932 | 1.0        |
| Camphene             | 946 | 0.1        |
| Sabinene             | 970 | 1.2        |
| β-Pinene             | 975 | 1.6        |
| Myrcene              | 990 | 1.0        |
| δ-2-Carene           | 1002| trb        |
| Isosylvestrene       | 1013| 0.3        |
| ρ-Cymene             | 1019| 8.0        |
| Limonene             | 1025| 2.0        |
| 1,8-Cineole          | 1032| 2.9        |
| Z-β-Ocimene          | 1037| 0.1        |
| γ-Terpinene          | 1055| 44.2       |
| 3-methylbenzaldehyde | 1059| tr         |
| cis-Sabinene hydrate | 1061| tr         |
| Terpinolene          | 1085| 0.7        |
| Linalool             | 1093| 0.1        |
| trans-Sabinene hydrate | 1095| 0.1        |
| Borneol              | 1162| 0.1        |
| Terpinen-4-ol        | 1170| 0.4        |
| α-Terpinol           | 1189| tr         |
| meta-Cuminalde     | 1217| tr         |
| p-Cuminaldehyde     | 1231| 16.9       |
| trans-p-Menth-2-en-7-ol | 1261| 0.2        |
| Perillaldehyde       | 1265| 0.2        |
| Bornyl acetate       | 1280| 2.9        |
| α-Terpinen-7-al      | 1281| 0.4        |
| γ-Terpinen-7-al      | 1287| 10.5       |
| Thymol               | 1289| 0.1        |
| 9-epi-β-Caryophyllene| 1413| tr         |
| ar-Curcumene         | 1474| tr         |
| Germacrene D         | 1476| 0.1        |
| α-Zingiberene        | 1490| tr         |
| EE-α-Farnesene       | 1503| tr         |
| β-Sesquiphellandrene | 1518| 0.1        |
| Total identified     | 95.5|            |

a Retention index relative to n-alkane series on the DB-5 column.

b Trace (< 0.05%)

Evaluation of time to detection of bacterial growth.

Median time to detection of bacterial growth was 7 days. Kaplan-Meier survival curve for different levels of explainatory variables are presented in Figure 1.

On the basis of MSE value the log normal model provided the best fit to the data. The MSE value of the log normal model was 207.97. While the MSE values were 211.27, 250.10 and 584.70 for log-logistic, Weibull and exponential models, respectively. The final model showed that all explanatory variables had significant (p < 0.001) association with time to detection, (Table 2).

On average, time to detection for combinations with 0.08%, 0.16% and 0.24% of B. persicum essential oil was 1.72, 2.92 and 5.49 times greater than those without it, respectively. Time to detection (TTD) for those combinations with pH levels of 6 and 5 was 1.53, and 4.28 times greater than those with pH level of 7. Also, this time for combinations with inoculum size, temperature and pH.
with inoculum level of $10^3$ and $10^2$ was 1.38 and 2.15 times greater than combinations with inoculum level of $10^4$. Furthermore, this period for combinations with incubation temperature of 25 °C and 15 °C was 1.59 and 3.95 times greater than combinations with those by incubation temperature of 35 °C.

The results of the present study showed that the major compound of B. persicum EO was phenolic monoterpene γ-terpinene (44.20%). Other important compounds were cuminaldehyde (16.90%) and ρ-cymene (8.00%).

The model predicts the value of TTD describing the growth of S. aureus, as environmental factors change. From these models the values of predicted TTD can be calculated from any combination of EO, T, pH and IL with the limits studied. Observed and predicted time to detection of bacterial growth for each combination presented in Figure 2.

### Table 2. Accelerated failure time model of factors influencing time to detection of bacterial growth.

| Variable           | β(SE)  | p-value | Time ratio (95% CI) |
|--------------------|--------|---------|---------------------|
| Intercept          | -0.361(0.068) | < 0.001 |                      |
| Essential oil      |        |         |                     |
| 0                  | 0      | 1       |                     |
| 0.08               | 0.547(0.058) | < 0.001 | 1.72 (1.54-1.94)    |
| 0.16               | 1.07(0.059)  | < 0.001 | 2.92 (2.60-3.28)    |
| 0.24               | 1.70(0.063)  | < 0.001 | 5.49 (4.85-6.23)    |
| pH                 |        |         |                     |
| 7                  | 0      | 1       |                     |
| 6                  | 0.424(0.051) | < 0.001 | 1.53 (1.38-1.70)    |
| 5                  | 1.45(0.055)  | < 0.001 | 4.28 (3.84-4.77)    |
| Inoculum level     |        |         |                     |
| $10^4$             | 0      | 1       |                     |
| $10^3$             | 0.327(0.053) | < 0.001 | 1.38 (1.25-1.54)    |
| $10^2$             | 0.765(0.052) | < 0.001 | 2.15 (1.94-2.39)    |
| Temperature        |        |         |                     |
| 35°C               | 0      | 1       |                     |
| 25°C               | 0.463(0.051) | < 0.001 | 1.59 (1.44-1.75)    |
| 15°C               | 1.37(0.054)  | < 0.001 | 3.95 (3.55-4.40)    |
| sigma              | 0.369(0.016) |         |                     |

Several researchers have investigated antibacterial and antifungal effect of B. persicum EO. It has been proposed that the antifungal activity of B. persicum EO is due to cuminaldehyde. Previous studies have shown that B. persicum essential oil had strong activity against S. aureus.

In the present study on the basis of MSE value, the log normal model provided the best fit to the data. We used the concepts of the TTD in a factorial design study to quantify the effect of B. persicum EO, pH, and temperature and inoculum level on the growth responses of S. aureus in BHI broth medium. Our results indicated that in the final model all explanatory variables had significant ($p < 0.05$) association with time to detection.

To sum up, evidence provided in this study showed that the values of TTD were higher at low levels of temperature, pH, and inoculums but high level of EO. The same results have been reported by Valero et al. and Jamshidi et al. In agreement to results of Goudarzi et al. and Barros et al., the present study showed that the antimicrobial properties of plant EO are dose-dependent. According to our results, increasing the B. persicum EO concentration had a significant effect ($p < 0.001$) on time to detection of S. aureus. By increasing the concentration of...
EO, the growth initiation of organism and also proportion of no growth combinations increased.

Temperature is the most common hurdles used to control microbial growth. According to our results, decreasing the incubation temperature had a significant effect on growth initiation of inoculated bacteria. Probably the most important effect of temperature on growth of a micro-organism is on the shape of enzymes required for metabolism and they will have the proper shape only within a relatively narrow range of temperatures or it may be due to the lower metabolic activity at the lower temperature.

Tassou et al. showed the effect of temperature and different concentrations (1.2% v/v) of mint EO on the growth/survival of *S. aureus* in nutrient broth. Their results showed that both factors significantly affected the detection time.7

Basti et al. studied the effects of *Zataria multiflora* Boiss. EO, pH and temperature on *S. Typhimurium* and *S. aureus*.49 They showed that TTD of both organisms were significantly affected by temperature, EO and pH, (*p* < 0.01).

The growth and metabolism of microorganisms are influenced by pH, because acidity or alkalinity of an environment has a profound effect on the activity and stability of macromolecules.32

According to our results the growth of *S. aureus* was significantly affected by pH. When the pH was decreased, the growth initiation of *S. aureus* and proportion of no growth combinations were increased. This can be attributed either the direct effect of pH or to the better dissolving of the EO in the lipid phase of the bacterial membrane at the low pH.33 In agreement to these results Basti et al. have shown the inhibitory action of *Zataria multiflora* Boiss. EO on the growth initiation of *S. Typhimurium* and *S. aureus* was enhanced by decreasing the pH value at each defined temperature.19

In this study we used three levels of inoculation. According to our results the growth of *S. aureus* was affected significantly by the inoculum size. Our results showed that the TTD for combinations with inoculum level of $10^3$ and $10^2$ was 1.38 and 2.15 times greater than combinations with inoculum level of $10^4$. Several studies have indicated the importance of inoculum size on the ability of a microbial population to initiate growth.34-37

Skandamis et al. have modeled the effect of inoculum size and acid adaptation on growth/no growth interface of *E. coli* O157:H7.38 They found that regarding the effect of inoculum concentration, the lower was the initial population, the higher were the pH levels allowing growth, especially at low temperatures (i.e., 10 and 15 °C).

Zhao et al. developed linear regression model with polynomial terms for analyzing the effect of environmental factors on time to detection. Their results showed when temperature was increased, the TTD was increased and when the pH and inoculums size were increased, the TTD was decreased. They also described a very good correlation of predicted and observed values of TTD.39 This was in agreement with our results were obtained for the effects of the designated factors on the TTD of *S. aureus* within the studied limits.

The results showed the observed time to detection of bacterial growth and value that predicted by log normal model equation for TTD of *S. aureus* in designated combinations. The graph demonstrated good agreement between predicted and observed values. So our model adequately predicted the growth initiation, and inhibition conditions of *S. aureus* as affected by EO, pH, temperature, and inoculum level.

The predicted values may not match with whatever would occur in any special food system. This means that before the models could be used in such a manner, the user would have to validate the models for each specific food of interest.

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**References**

1. Desmarchelier MP, Higgs GM, Mills L, et al. Incidence of coagulase positive *Staphylococcus* on beef carcasses in three Australian abattoirs. Int J Food Microbiol 1999; 47(3): 221-229.

2. Zhang S, Iandolo J, Stewart C. The enterotoxin D plasmid of *Staphylococcus aureus* encodes a second enterotoxin determinant (sej). FEMS Microbiol Lett 1998; 168(2): 227-233.

3. Normanno G, Firinu A, Virgilio S, et al. Coagulase-positive *Staphylococcus* and *Staphylococcus aureus* in food products marketed in Italy. Int J Food Microbiol 2005; 98(1):73-79.

4. Vereecken KM, Dens EJ, Van Imp JF. Predictive modeling of mixed microbial populations in food products: Evaluation of two-species models. J Theor Biol 2000; 205(1): 53-72.

5. Roos T, McMeekin TA. Predictive microbiology. Int J Food Microbiol 1994; 23(3): 241-264.

6. Burt S. Essential oils: Their antibacterial properties and potential applications in foods - A review. Int J Food Microbiol 2004; 94(3): 223-253.

7. Tassou C, Koutsoumanis K, Nychas GJE. Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil. Food Res Int 2000; 33(3): 273-280.

8. Omidbaigi R. Approaches to production and processing
of medicinal plants [Farsi]. 1st ed. Tehran, Iran: Tarhahan-e-Nasrh 1998; 424.

9. Abduganiew BE, Abdullaev UA, Aripov KN, et al. Composition of the essential oil of *Bunium persicum* (Boiss.) B. Fedtsch. from Tajikistan. J Essent Oil Res 1997; 9(5): 597-598.

10. Sofi PA, Zeerak NA, Singh P. Kala zeera (*Bunium persicum* Bivss.): A Kashmirian high value crop. Turk J Biol 2009; 33(3): 249-258.

11. Pourmortazavia SM, Ghadiri M, Hajmirsadeghi SS. Supercritical fluid extraction of volatile components from *Bunium persicum* Boiss. (Black cumin) and *Mespilus germanica* L. (medlar) seeds. J Food Composit and Anal 2010; 18(5): 439-446.

12. Thappa RK, Ghosh S, Agarwal SG. Comparative studies on the major volatiles of Kalazira (*Bunium persicum*) seed of wild and cultivated sources. Food Chem 1991; 41(2): 129-134.

13. Adams RP. Identification of essential oil components by gas chromatography and mass spectrometry. 3rd ed. Carol Stream, USA: Allured Publishing Corporation 2001:455.

14. Mann CM, Markham JL. A new method for determining the minimum inhibitory concentration of essential oils. J Appl Microbiol 1998; 84(4): 538-544.

15. Kleinbaum D, Klein M. Survival analysis: A self-learning text. 2nd ed. New York, USA: Springer 2005:266-286.

16. Tienungoon S, Ratkowsky DA, Mcmeekin TA, et al. Growth limits of *Listeria monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. Appl Environ Microb 2000; 66(11): 4979-4987.

17. Bagamboula CF, Uyttendaele M, Debevere J. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragole, linalool, and p-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microbiol 2004; 21(1): 33-42.

18. Kanzadi S, Gharibzadeh S, Raoufy M, et al. Application of artificial neural networks to predict *clostridium botulinum* growth as a function of *Zataria multiflora* essential oil, pH, NaCl and temperature. J Food Safety 2010; 30(2): 490-505.

19. Basti, AA, Misaghi A, Khaschabi D. Growth response and modelling of the effects of *Zataria multiflora* Boiss. essential oil, pH and temperature on *Salmonella Typhimurium* and *Staphylococcus aureus*. LWT Food Sci Technol 2007; 40(6): 973-981.

20. Oroojian F, Kasra-Kermanshahi R, Azizi M. Phytochemical composition of the essential oils from three *Apiaceae* family and their antibacterial effects on foodborne pathogens. Food Chem 2010; 120(3): 765-770.

21. Valero M, Salmeron MC. Antibacterial activity of 11 essential oils against *Bacillus ceruse* in tyndalized carrot broth. Int J Food Microbiol 2003; 85(1): 73-81.

22. Palmer AS, Steward J, Fyfe L. The potential application of plant essential oils as natural preservatives in soft cheese. Food Microbiol 2001; 18(4): 463-470.

23. Yamazaki K, Yamamoto T, Kawai Y, et al. Enhancement of anti-listerial activity of essential oil constituents by nisin and diglycerol fatty acid ester. Food Microbiol 2004; 21(3): 283-289.

24. Moghtader M, Mansori Al, Salari A, et al. Chemical composition and antimicrobial activity of the essential oil of *Bunium persicum* Boiss. Seed. Iranian J Med Aromatic Plants 2009; 25(1): 20-28.

25. Sekine T, Sugano M, Azizi M, et al. Antifungal effects of volatile compounds from Black Zira (*Bunium persicum*) and other spices and herbs. J Chem Ecol 2007; 33(11): 2123-2132.

26. Syed M, Hanif M. Antimicrobial activity of the essential oil of the *umbelliferae* family. Part 1. *Cuminum cyminum*, *Coriandrum sativum*, *Foeniculum vulgare* and *Bunium persicum* oils. Pakistan J Sci Ind Res 1985; 55: 116-120.

27. Syed MM, Khalid R, Chaudhary FM, et al. Antimicrobial activity of essential oils of the *Umbelliferae* family, Part V: *Carum carvi*, *Petroselinum crispum* and *Dorema ammoniacum* oils. Pakistan J Sci Ind Res 1987; 30(2): 106-110.

28. Valero A, Rodríguez M, Carrasco E, et al. Studying the growth boundary and subsequent time to growth of pathogenic *Escherichia coli* serotypes by turbidity measurements. Food Microbiol. 2010; 27(6): 819-828.

29. Jamshidi A, Kazerani HR, Seifi HA, et al. Growth limits of *Staphylococcus aureus* as a function of temperature, acetic acid, NaCl, and inoculum level. Iranian J Vet Res. 2008; 9(4):353-359.

30. Goudarzi GHR, Saharkhiz MJ, Sattari M, et al. Antibacterial activity and chemical composition of *Ajowan* (*Carum cuminum* Benth. & Hook) essential oil. J Agr Sci Technol. 2011; 13: 203-208.

31. Barros JC, Conceicao ML, Neto NG, et al. Interference of *Origanum vulgare* L. essential oil on the growth and some physiological characteristics of *Staphylococcus aureus* strains isolated from foods. LWT Food Sci Technol 2009; 42(6): 1139-1143.

32. Adams MR, Moss MO. Food Microbiology. 3rd ed. Cambridge, UK: RSC Company 2008;115-167.

33. Koutsoumanis K, Lambropoulou K, Nychas GJE. A predictive model for the non-thermal inactivation of *Salmonella enteritidis* in a food model system supplemented with a natural antimicrobial. Int J Food Microbiol 1999; 49(1): 63-74.

34. Razavilar V, Genigeorgis G. Prediction of *Listeria* spp. growth as affected by various levels of chemicals, pH, temperature and storage time in model broth. Int J Food Microbiol 1998; 40(3): 149-157.

35. Masana MO, Baranyi J. Growth/no growth interface of *Brochothrix thermosphacta* as a function of pH and water activity. Food Microbiol 2000; 17(5): 485-493.

36. Robinson TP, Aboaba OO, Kaloti A, et al. The effect of inoculum size on the lag phase of *Listeria monocytogenes*. Int J Food Microbiol 2001; 70(1): 163-173.
37. Pascual C, Robinson TP, Ocio MJ. The effect of inoculum size and sublethal injury on the ability of *Listeria monocytogenes* to initiate growth under suboptimal conditions. Lett Appl Microbiol 2001; 33(5): 357-361.

38. Skandamis PN, Stopforth JD, Kendall PA, et al. Modeling the effect of inoculum size and acid adaptation on growth/no growth interface of *Escherichia coli* O157:H7. Int J Food Microbiol 2007; 120(3): 237-249.

39. Zhao L, Montville TJ, Shaffner DW. Time-to-detection, percent growth-positive and maximum growth rate models for *Clostridium botulinum* 56A at multiple temperatures. Int J Food Microbiol 2002; 77(3): 187-197.