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Effect of *Nigella sativa* (seed and oil) on the bacteriological quality of soft white cheese

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Abstract

The effect of *Nigella sativa* seed (1% and 3%) and oil (0.3% and 1%) on some food poisoning and pathogenic bacteria as well as on the total bacterial count TBC (cfu/g) in soft white cheese prepared from raw ewe's milk and labratory pasteurized ewe's milk inoculated with *Staphylococcus aureus*, *Brucella melitensis* and *Escherichia coli* at a concentration of 1×10^6 cfu/ml were carried out. Cheese samples were examined for bacterial count at: zero, 2nd, 4th and 6th days of storage at refrigerator temp. Results showed that there was Significant decrease (P<0.05) in TBC, *Staphylococcus aureus*, *Brucella melitensis* and *Escherichia coli* count in cheese samples treated with *N. sativa* seed (1% and 3%) and oil (0.3% and 1%) with pronounced concentration dependent inhibition in contrast to control cheese samples which exerted significant increase in bacterial counts as it reached 2.8×10^7, 2.95×10^6, 2.22×10^6 and 2.885×10^6 cfu/g for TBC, *Staph. aureus*, *Br. melitensis* and *E. coli* respectively at the 6th day of storage at refrigerator temp. *N. sativa* oil (0.3% and 1%) was significantly more affective (P<0.05) as antibacterial agent than seed (1% and 3%) respectively. No significant differences (P<0.05) in the susceptibility of *Staph. aureus*, *Br.melitensis* and *E. coli* to the antibacterial effect of *N. sativa* seed (1% and 3%) and oil (0.3% and 1%) were observed in treated soft white cheese.

Keywords: *Nigella sativa***;** Bacteriological quality; Soft white cheese**.**
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دراسة تأثير بذور وزيت الحبة السوداء *Nigella sativa* على النوعية البكتريولوجية للجبن الأبيض الطري

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الخلاصة

اختبرت الفعالية المضادة للجراثيم لبذور وزيت الحبة السوداء *Nigella sativa* على بعض أنواع الجراثيم المسببة للتسمم الغذائي والجراثيم المرضية وكذلك على أعداد الجراثيم الكلية (TBC) في الجبن الأبيض الطري المصنع من حليب النعاج الخام لغرض التعرف على أعداد الجراثيم الكلية (TBC) ومن حليب النعاج المبستر مختبريا والمحقون بجراثيم المكورات العنقودية *Staph. aureus*، البروسيلا المالطية *Br.melitensis*، والأشريكيا القولونية *E. coli* بتركيز ١×١٠^٦ خلية/مل وتم إجراء العد الجرثومي لكل نوع من أنواع الجراثيم المدروسة المكورات العنقودية *Staph. aureus*، البروسيلا المالطية *Br.melitensis*، والأشريكيا القولونية *E. coli* بالإضافة إلى أعداد الجراثيم الكلية (TBC) في عينات أجبان السيطرة والاجبان المعاملة ببذور الحبة السوداء بتركيز ١% و٣% وبزيت الحبة السوداء بتركيز ٠.٣% و١% في الأيام: صفر, ٢, ٤ و٦ من الخزن في درجة حرارة الثلاجة. أظهرت النتائج انخفاضا معنويا (P<0.05) في أعداد الجراثيم المدروسة في عينات الاجبان المعاملة ببذور (١% و ٣%) وزيت (٠.٣% و ١%) الحبة السوداء وقد اعتمدت درجة التثبيط للجراثيم بشكل واضح على التركيز المستخدم من البذور والزيت. على العكس من ذلك أظهرت عينات أجبان السيطرة ارتفاعا معنويا في أعداد الجراثيم المدروسة حيث وصلت أعداد الجراثيم الكلية، جراثيم المكورات العنقودية، البروسيلا المالطية، والاشريكيا القولونية إلى ٢.٨×١٠^٧, ٢.٩٥×١٠^٦, ٢.٢٢×١٠^٦ و ٢.٨٨٥×١٠^٦ خلية/غم على التوالي في اليوم السادس من

الخزن في درجة حرارة الثلاجة. أظهر زيت الحبة السوداء تركيز ٠.٣% و١% فعالية مضادة للجراثيم أكثر معنويا (P<0.05) من بذور الحبة السوداء ذو التركيز ١% و٣% على التوالي. ولوحظ عدم وجود فروقات معنوية (P<0.05) في حساسية كل من جراثيم المكورات العنقودية، البروسيلا المالطية، والاشريكيا القولونية للفعالية المضادة للجراثيم لبذور (١% و ٣%) وزيت (٠.٣% و١%) الحبة السوداء في الجبن الأبيض الطري المعامل.

Introduction

Soft white cheese locally made from sheep's and/ or goat's milk are available in Mosul market during spring season. The cheese is made directly after milking, without any heat treatment. Thus, the traditional way of making this type of cheese lacks the simple hygienic measure even in the way for its display in local market. Frequently, some consumers suffer from diarrhea, gastrointestinal pain and brucellosis (1).

N. sativa seed (Black seed) is a plant which has been used for centuries for medicinal and culinary purposes and reported to possess a number of pharmacological properties, including antimicrobial activity (2).

Staph. aureus is a pathogenic bacteria which can affect any part of the body causing several diseases as septicemia, brain abscess, and enterocolitis (3). Because of the frequent contamination of milk from dairy personnel, and the high incidence of Staphylococcal mastitis in dairy herds, the most commonly occuring type of food poisoning is due to enterotoxin-producing strains of *Staph. aureus* (4).

Br.melitensis is the most common species in the genus Brucella in several parts of the world (5). *Br.melitensis* can infect human and animal causing brucellosis which is a systemic disease which can cause different clinical manifestations (6). The danger of contracting brucellosis through the consumption of unpasteurized raw milk and dairy products drew the attention of public health authorities in many countries to impose quarantine regulations governing the consumption of such products (7).

E. coli also is a food pathogen, strains of this species express potent toxins and cause serious gastrointestinal infections. Additionally it can result in life-threating systemic disease (8).

Although optimum growth temp. is 37˚C for *Staph. aureus*, *Br.melitensis* and *E. coli*, *Staph. aureus* can grow at temperatures slightly above 5˚C, *Br.melitensis* can survive at 5˚C for long periods of time and are part of a so-called "new problem" with dairy products, while *E. coli* exhibit competetive growth in milk at 5˚C (9).

Therefore, this work was planned to build up information on the effect of *N. sativa* seed and oil on growth and survival of total bacteria, *Staph. aureus*, *Br.melitensis* and *E. coli* in soft white cheese during manufacturing and storage at refrigerator temp.

Materials and methods

The bacteria used in this study are known to be the cause of disease in human and some are known to be involved in food poisoning.

Staph. aureus was obtained from Mr. Omar Hashem Sheet, Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul.

Br. melitensis was obtained from Miss Amera Ali Ahmed, Department of Basic Science, Nursing College, University of Mosul.

E. coli was obtained from Mrs. Noor Abd Alhafez Jerjees, Department of Biology, Education College, University of Mosul.

Each isolate was confirmed by reculturing on the selective media and by performing the biochemical tests (10).

Staph. aureus and *E. coli* were propagated in Nutrient broth at 37˚C for 24h. Two transfers were made prior to inoculation, the cfu/ml was determined using Mannitol Salt Agar and MacConkey Agar as a selective media for *Staph. aureus* and *E. coli*, respctively.

Br.melitensis was propagated in Brucella broth at 37˚C for 48h. Also two transfers were made prior to inoculation, the cfu/ml was determined using Brucella Agar as a selective medium (11).

The procedure described by (12) was used for preparation of soft white cheese.

For total bacterial count (TBC), raw ewe's milk was used for preparation of soft white cheese. For other treatments raw ewe's milk was labratory pasteurized at 63˚C for 30 min. Pasteurized milk was divided into three categories, each category was inoculated with one type of bacteria under study (*Staph. aureus*, *Br.melitensis* and *E. coli*) to yield a concentration of about 1×10^6 cfu/ml. Samples from raw and inoculated milk were taken to determine the initial count of bacteria (11).

Raw and each category of inoculated milk were divided into five equal parts. The first one was considered as a control, while the 2nd and 3rd parts were mixed with 1% and 3% of *N. sativa* seed and the 4th and 5th parts were mixed with 0.3% and 1% of *N. sativa* oil (cold pressed). These concentrations of *N. sativa* seed and oil were used as 1% and 3% of *N. sativa* seed approximately contain 0.3% and 1% of it's oil respectively.

Samples from finished cheese were examined for TBC, *Staph. aureus*, *Br.melitensis* and *E. coli* count. Finished

cheese were stored at refrigerator temp. (5±2˚C). Sampels from the storage cheese were examined in the 2nd, 4th and 6th days of storage for count of mentioned bacteria according to (11).

The data were Statistically analyzed using Sigma Stat for windows Version 3.10 (2004). Analysis of variance procedures appropriate for either a two-way completely randomized design for data involved in the effect of different concentrations of bacterial inhibition materials (*N. sativa* seed and oil) and storage durations and their interaction and a one-way completely randomized design for data concerned with degree of bacterial sensitivity to each concentration of the inhibitor, according to (13). Significant differences (P<0.05) among treatment means were detected based on Duncan Multiple Range Test (14).

Results

The results illustrated in Table (1) verify that there was significant increase (P<0.05) in TBC in finished (zero day) and stored (2nd, 4th and 6th days of storage at refrigerator temp.) cheese (control) as it reached 2.8×10^7 cfu/g at the 6th day of storage. Also significant differences (P<0.05) in TBC between control (continue increasing) and treated cheese samples were observed. Cheese samples made from milk treated with 1% (1st treatment) and 3% (2nd treatment) of *N. sativa* seed and 0.3% (3rd treatment) and 1% (4th treatment) of *N. sativa* oil also showed an increase in TBC in the finished cheese with a minimum increase (6.2×10^6 cfu/g) was reported in the 4th treatment. This incriment in counts was significantly different (P<0.05) between treatments, except 2nd and 3rd treatments were not significantly different (P<0.05). Results referred that there was a significant decrease (P<0.05) in TBC in treated cheese with *N. sativa* seed and oil during storage at refrigerator temp. until it reached 1.95×10^6, 1.2×10^6, 1.34×10^6 and 9.4×10^5 cfu/g in the 1st, 2nd, 3rd and 4th treatments, respectively at the 6th day of storage with the exception of 4th treatment during the 4th and 6th days of storage which the decrease in TBC was not significantly different (P<0.05). Significant differences (P<0.05) in TBC between treatments at the 2nd day of storage were observed. But no significant differences (P<0.05) between the 1st and 2nd treatments and 2nd and 3rd treatments were recorded. During the 4th and 6th days of storage also there was significant differences (P<0.05) in TBC between treatments except between 1st and 3rd treatments and 2nd and 3rd treatments at the 4th day of storage and between 1st and 3rd treatments and 2nd, 3rd and 4th treatments at the 6th day of storage where TBC were not significantly different (P<0.05). Regardless of storage period results showed significant differences (P<0.05) in TBC between control and between treatments with the exception of 2nd and 3rd treatments which was not significantly different (P<0.05).

The corresponding results obtained from cheese prepared from milk inoculated with *Staph. aureus* at a level of 1×10^6 cfu/ml (control) and stored at refrigerator revealed that *Staph. aureus* count significantly increased (P<0.05) from 2.7×10^6 cfu/g in the finished cheese (zero day) to 2.95×10^6 cfu/g at the 6th day of storage. It was obvious that there was significant differences (P<0.05) in *Staph. aureus* counts between control and *N. sativa* seed and oil treated cheese samples in the finished cheese and during it's storage at refrigerator temp. Firstly *Staph. aureus* counts increased in finished cheese prepared from milk inoculated with the bacteria and treated with *N. sativa* seed and oil with a minimum increase (1.19×10^6 cfu/g) observed in the 4th treatment. Significant differences (P<0.05) in *Staph. aureus* counts between treatments were recorded at zero time except between 1st and 2nd treatments and between 2nd and 3rd treatments which increment in counts was not significantly different (P<0.05). After that, *Staph. aureus* counts significantly decreased (P<0.05) in treated cheese with *N. sativa* seed and oil during storage at refrigerator temp. - in contrast to the control cheese samples- as it reached 2.57×10^5, 1.5×10^5, 1.74×10^5 and 8.5×10^4 cfu/g in the 1st, 2nd, 3rd and 4th treatments, respectively at the 6th day of storage except 4th treatment at the 4th and 6th days of storage where decrease in counts was not significantly different (P<0.05). At the 2nd and 4th day of storage results showed significant differences (P<0.05) in *Staph. aureus* counts between treatments with the exception of 1st and 3rd treatments and 2nd and 3rd treatments which differences were not significant (P<0.05). No significant differences (P<0.05) in *Staph. aureus* counts were observed between treatments at the 6th day of storage of treated cheese except 1st and 4th treatments which *Staph. aureus* count was significantly different (P<0.05). As mentioned in table (1), results showed that there was significant differences (P<0.05) in counts of *Staph. aureus* between control and between treatments regardless of storage period. But no significant differences (P<0.05) in counts between 2nd and 3rd treatments was noticed (Table 2).

From data in Table (3) it was evident that *Br.melitensis* counts increased as it reached 2.23×10^6 cfu/g at the 2nd and 4th days of storage, then slightly declined to reach 2.22×10^6 cfu/g at the 6th day of storage of control cheese samples prepared from milk inoculated with *Br.melitensis* at a conc. of 1×10^6 cfu/ml. This incriment and decline in counts of *Br.melitensis* was not significant (P<0.05), where as there was significant differences (P<0.05) in *Br.melitensis* counts between control and treated cheese samples in the finished cheese and during storage at refrigerator temp. *Br.melitensis* counts also increased in the finished cheese prepared from milk inoculated with the bacteria and treated with *N. sativa* seed and oil to record a minimum increase in counts (1.29×10^6 cfu/g) in the 4th treatment at zero time. *Br.melitensis* counts were significantly different (P<0.05)

between treatments at zero time. During storage at refrigerator temp., results showed significant decrease (P<0.05) in counts of *Br.melitensis* in treated cheese sampels until it reached 4.5×10^5, 2.9×10^5, 3.2×10^5 and 2.2×10^5 cfu/g in the 1st, 2nd, 3rd and 4th treatments, respectively at the 6th day of storage. Significant differences (P<0.05) in *Br.melitensis* counts were observed between treatments of cheese at each storage period in refrigerator, except between 1st and 3rd treatments, 2nd and 3rd treatments and 2nd, 3rd and 4th treatments at the 2nd, 4th and 6th day of storage, respectively where differences in counts were not significant (P<0.05). Control and treated cheese samples showed significant differences (P<0.05) in *Br.melitensis* counts between them, regardless of storage period.

Table 1: Effect of *Nigella sativa* (seed and oil) on the means of TBC (cfu/g) during manufacturing and storage of soft white cheese at refrigerator temp.

Storage Period (days)	Treatments (cfu/g)				
	Control	1% Seed	3% Seed	0.3% Oil	1% Oil
0	1.8×10^7 a	8.5×10^6 b	7.4×10^6 c	7.6×10^6 c	6.2×10^6 d
2	2.2×10^7 e	4.7×10^6 f	4.1×10^6 gf	3.8×10^6 g	2.8×10^6 h
4	2.4×10^7 i	3×10^6 j	2.2×10^6 k	2.5×10^6 kj	1.5×10^6 o
6	2.8×10^7 m	1.95×10^6 n	1.2×10^6 o	1.34×10^6 on	9.4×10^5 o
Treatments effect	2.3×10^7 A	4.5375×10^6 B	3.725×10^6 C	3.81×10^6 C	2.86×10^6 D

- Horizontally different small letters within each storage period are significantly different (P<0.05).
- Vertically different small letters within each treatment are significantly different (P<0.05).
- Different capital letters within last raw (treatments regardless of storage period) are significantly different (P<0.05).

Table 2: Effect of *N. sativa* (seed and oil) on the means of *Staphylococcus aureus* (cfu/g) during manufacturing and storage of soft white cheese at refrigerator temp*.

Storage Period (days)	Treatments (cfu/g)				
	Control	1% Seed	3% Seed	0.3% Oil	1% Oil
0	2.7×10^6 a	1.56×10^6 b	1.49×10^6 bc	1.4×10^6 c	1.19×10^6 d
2	2.74×10^6 a	8.2×10^5 f	6×10^5 g	6.9×10^5 fg	3.2×10^5 h
4	2.83×10^6 an	5.1×10^5 j	3.2×10^5 k	4.4×10^5 jk	1.7×10^5 mp
6	2.95×10^6 n	2.57×10^5 o	1.5×10^5 op	1.74×10^5 op	8.5×10^4 p
Treatments effect	2.805×10^6 A	7.8675×10^5 B	6.4×10^5 C	6.76×10^5 C	4.4125×10^5 D

- Horizontally different small letters within each storage period are significantly different (P<0.05).
- Vertically different small letters within each treatment are significantly different (P<0.05).
- Different capital letters within last raw (treatments regardless of storage period) are significantly different (P<0.05).

* Bacteria was added to the milk used for preparation of soft white cheese at a conc. of 1×10^6 cfu/ml.

Results recorded in Table (4) revealed that *E. coli* counts increased significantly (P<0.05) from 2.585×10^6 cfu/g in the control cheese samples at zero time to 2.885×10^6 cfu/g at the 6th day of storage of cheese prepared from milk inoculated with *E. coli* at a level of 1×10^6 cfu/ml. *E. coli* counts were significantly different (P<0.05) between control and treated cheese samples in the finished cheese at zero time and during storage at refrigerator temp. As in control cheese sampels, at beginning *E. coli* counts increased in the finished cheese prepared from milk inoculated with the bacteria and treated with *N. sativa* seed (1% and 3%) and oil (0.3% and 1%) to record a minimum

increase (1.45×10^6 cfu/g) in the 3rd treatment at zero time. *E. coli* count was only significantly different (P<0.05) between 1st and 3rd treatments and 1st and 4th treatments at zero time. Later, *E. coli* counts significantly decreased (P<0.05) during storage of *N. sativa* seed and oil treated cheese at refrigerator temp. as it reached 7×10^5, 4.5×10^5, 4.5×10^5 and 2.5×10^5 cfu/g in the 1st, 2nd, 3rd and 4th treatments, respectively at the 6th day of storage. But no significant decrease (P<0.05) was observed in the 1st, 2nd, 3rd and 4th treatments at the 4th and 6th days of storage and in the 2nd and 4th treatments at the 2nd and 4th day of storage. Every storage period showed no significant differences (P<0.05) in *E. coli* counts between treatments with the exception of 1st and 4th treatments at the 2nd day of storage, 1st and 3rd treatments and 1st and 4th treatments at the 4th day of storage, and 1st and 4th treatments at the 6th day of storage where differences in counts were significant (P<0.05). As mentioned in Table 1 and 2 significant differences (P<0.05) in *E. coli* counts between control and between treated cheese samples were observed, regardless of storage period, except 2nd and 3rd treatments which difference was not significant (P<0.05).

Table 3: Effect of *N. sativa* (seed and oil) on the means of *Brucella melitensis* (cfu/g) during manufacturing and storage of soft white cheese at refrigerator temp*.

Storage Period (days)	Treatments (cfu/g)				
	Control	1% Seed	3% Seed	0.3% Oil	1% Oil
0	2.2×10^6 a	1.85×10^6 b	1.41×10^6 c	1.59×10^6 d	1.29×10^6 e
2	2.23×10^6 a	1.12×10^6 g	8.9×10^5 h	1.06×10^6 g	6.3×10^5 i
4	2.23×10^6 a	7.5×10^5 k	5.1×10^5 m	5.9×10^5 m	3.6×10^5 n
6	2.22×10^6 a	4.5×10^5 p	2.9×10^5 q	3.2×10^5 q	2.2×10^5 q
Treatments effect	2.22×10^6 A	1.0425×10^6 B	7.75×10^5 C	8.9×10^5 D	6.25×10^5 E

- Horizontally different small letters within each storage period are significantly different (P<0.05).
- Vertically different small letters within each treatment are significantly different (P<0.05).
- Different capital letters within last raw (treatments regardless of storage period) are significantly different (P<0.05).

* Bacteria was added to the milk used for preparation of soft white cheese at a conc. of 1×10^6 cfu/ml.

Table 4: Effect of *N. sativa* (seed and oil) on the means of *E. coli* (cfu/g) during manufacturing and storage of soft white cheese at refrigerator temp*.

Storage Period (days)	Treatments (cfu/g)				
	Control	1% Seed	3% Seed	0.3% Oil	1% Oil
0	2.585×10^6 a	1.85×10^6 b	1.65×10^6 bc	1.45×10^6 c	1.55×10^6 c
2	2.735×10^6 aj	1.1×10^6 e	8.5×10^5 ef	8.5×10^5 ef	6×10^5 fi
4	2.83×10^6 aj	8.5×10^5 h	6.5×10^5 hif	5.5×10^5 ik	4×10^5 im
6	2.885×10^6 j	7×10^5 kh	4.5×10^5 kmi	4.5×10^5 km	2.5×10^5 m
Treatments effect	2.75875×10^6 A	1.125×10^6 B	9×10^5 C	8.25×10^5 C	7×10^5 D

- Horizontally different small letters within each storage period are significantly different (P<0.05).
- Vertically different small letters within each treatment are significantly different (P<0.05).
- Different capital letters within last raw (treatments regardless of storage period) are significantly different (P<0.05).

* Bacteria was added to the milk used for preparation of soft white cheese at a conc. of 1×10^6 cfu/ml.

The information obtained by the achieved results in Table (5) proved that there was no significant differences (P<0.05) in the mean values of examined bacterial counts (*Staph. aureus*, *Br. melitensis*, and *E. coli*) within the same treatment in cheese samples prepared from milk inoculated with these bacteria and treated with *N. sativa* seed (1% and 3%) and oil (0.3% and 1%) after 6th days of storage at refrigerator temp.

Table 5: Comparison of the antibacterial activity of *N. sativa* seed and oil towards bacterial types under study in soft white cheese.

Bacterial type	Mean ± Se			
	1% Seed	3% Seed	0.3% Oil	1% Oil
Staph. aureus	$7.8675\times10^5 \pm 1.8658\times10^5$ a	$6.4\times10^5 \pm 1.96086\times10^5$ a	$6.76\times10^5 \pm 1.72758\times10^5$ a	$4.4125\times10^5 \pm 1.66539\times10^5$ a
Br. melitensis	$1.0425\times10^6 \pm 1.98104\times10^5$ a	$7.75\times10^5 \pm 1.60732\times10^5$ a	$8.9\times10^5 \pm 1.82795\times10^5$ a	$6.25\times10^5 \pm 1.55878\times10^5$ a
E. coli	$1.125\times10^6 \pm 1.70115\times10^5$ a	$9\times10^5 \pm 1.75895\times10^5$ a	$8.25\times10^5 \pm 1.50641\times10^5$ a	$7\times10^5 \pm 1.92251\times10^5$ a

- Vertically similar letters are not significant (P<0.05).

Discussion

It was clear from the forementioned results there was an obvious increase in bacterial counts in finished cheese at zero time (Tables 1-4), this incriment primarily belong to that all the organisms present in the milk become part of the fresh curd flora, being concentrated in the curd (4).

It could be noticed the high counts of the bacterial types (TBC, *Staph. aureus*, *Br.melitensis* and *E. coli*) in control soft white cheese in contrast to the rapid reduction of bacterial counts in cheese containing *N. sativa* seed and oil added during preparation. Results are in agreement with those observed by (15) who showed similar antibacterial activity of *N. sativa* seed on *Staph. aureus* in Domiati cheese and with the results obtained by (16) who mentioned that *E. coli* was not detectable after three and two weeks of storage at room and refrigerator temperatures respectively for cheeses perpared from milk inoculated with the bacteria (2×10^4 cfu/ml) and treated with 5% and 10% of sod. chloride and *N. sativa* seeds at a concentration of 1% and 3%. Also the results agree to a certain extent with those reported by (17) who indicated that adding of *N. sativa* seed at a conc. of 1% to the processed cheese inhibited microbial growth and with the results recorded by (18) who noticed an inhibitory effect of essential oil of *N. sativa* on microorganisms especially on the total number of bacteria, coliform bacteria, lipolytic and proteolytic bacteria during storage of soft white cheese treated with the oil at a ratio of 0.5% of curd weight at 5˚C for 28 days. Similar inhibitory influence induced by *N. sativa* on microorganisms in yoghurt was reported by (19) who showed that addition of alcoholic extract of black seeds in a conc. of 3% was caused prolongation of it's keeping quality from 7 to 14 days. Another study (20) indicated that milk treatment with oil of black seed (0.5%) gave good results for holding the raw milk quality in acceptable level for 19 hours at temp. 25˚C and for 5 days at 5˚C. The results of water extract of *N. sativa* seed treatment (0.5%) and the oil treatment (0.2%) showed less shelf time 17 hours at 25˚C and 4 days at 5˚C while water extract treatment at a conc. of 0.2% gave an acceptable result in the milk quality preservation 11 hours at 25˚C and 3 days at 5˚C comparable with control which gave shelf time 13 hours at room temp. and 1 day at 5˚C. Finally the investigator recomended the use of water extract and oil of *N. sativa* as natural preservative materials for raw milk.

Several studies *in vitro* refered to the antibacterial effect of extracts and oil of black seed against *Staph. aureus* and *E. coli* (2,21-24). A great inhibitory effect of concentrated crude aqueous extract of *N. sativa* seed on *Brucella* bacteria was reported by (25). Whereas (26) recorded a slight antibacterial effect of *N. sativa* oil on *E. coli* while aqueous and alcoholic extracts showed no effect on this bacteria.

Our results are in agreement with those obtained by (2,27) who showed pronounced concentration dependent inhibition of all the bacteria tested (Table 1-4).

The present findings in Table 1-4 revealed that *N. sativa* oil (0.3% and 1%) was significantly more affective (P<0.05) as antibacterial agent than seed (1% and 3%) respectively. This is primarily belongs to the presence of Thymoquinone TQ (2-isopropyl-5-methyl-benzoquinone) which considered as one of the major components of *N. sativa* volatile oil, but which is also present in the fixed oil (28,29). Antibacterial effect of TQ was due to the inhibition of RNA and protein synthesis (22), as well as α-Pinene (The unsaturated bicyclic monoterpene hydrocarbon) which

also present in *N. sativa* volatile oil and exerts antibacterial action (30). So bacterial types present in cheeses treated with *N. sativa* oil will be more exposed to the antibacterial action than those present in *N. sativa* seed treated cheeses.

Results showed no significant differences ($P<0.05$) between test bacteria in their susceptibility to the antibacterial effect of *N. sativa* seed and oil (Table 5). These results were in contrast to those indicated that the antibacterial activity of *N. sativa* seed and oil was more against gram +ve bacteria than gram –ve bacteria (2,24). This perhaps because those studies were performed in plates (*in vitro*), while our research included study the antibacterial effect of *N. sativa* seed and oil in cheese prepared from milk inoculated with the test bacteria and treated with it. Cheese constituents may affect on the susceptibility of bacteria to seed and oil of *N. sativa* as sod. chloride at a conc. of 5% was added during manufacturing of soft white cheese, this may adversely affect on growth of *Br.melitensis* and *E. coli* and then increase susceptibility of these bacteria to the *N. sativa* seed and oil (16,31) comparable to *Staph. aureus* which can grow at high concentrations of Nacl may reach 10% (32).

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