ISSN 110-2047 ALEX. J. VET. Med. SCIENCE 2010 VOL. 29 NO. 1: (51-59)

EFFECT OF *NIGELLA SATIVA* OIL ON THE MICROBIAL QUALITY OF CHILLED CHICKEN FILLETS.

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ABSTRACT

The present study was conducted to evaluate the effect of addition of different concentration of *Nigella sativa* oil on microbial quality of chicken fillets during refrigeration for 2, 4, 7, and 10 days storage depending on bacteriological assessments. There is no effect on sensory properties (odour and appearance) of treated chicken meat fillets during storage as compared with control one. Bacteriological examinations recorded high initial bacterial counts, Most Probable Number of coliforms, *Staphylococcus aureus* and yeast & molds counts). During refrigeration period, there was increase in bacterial counts .

A series of five different oil concentrations were screened for their antibacterial and antifungal effects at 0.5, 1.0, 1.5, 2.0 and 2.5% (w /w)The results revealed that all oil percentages showed antibacterial activity against bacteria, yeast and molds on this assay. The oil at 2.0% concentration was more effective as compared to other concentrations on the bacterial count, coliforms count (MPN/gm), *Staphylococcus aureus*and yeast and molds counts.

Therefore, *Nigella sativa* oil may be used as an antimicrobial agent in food products to prevent spoilage and may be used as preservative agent.

INTRODUCTION

The direction to use natural herbs and spices as decontaminants in poultry industry as well as in poultry products have large attention to avoid the human health hazards.

The seeds of *Nigella sativa* are used by the Egyptian public as carminative and flavoring agents in bread. An anti-asthmatic compound, nigellone, was isolated from the volatile oil of the seeds of N. sativa **(Mahfouz, and El-Dakhakhny. 1960).**

The seeds have been thoroughly studied scientifically in the last 3-4 decades and have been reported to possess a number of medicinal properties **(Randhawa and Al-Ghamdi, 2002; Ali and Blunden, 2003).** Their crude extracts and essential oil have been shown to possess antibacterial activity against several bacteria **(Mouhajir and Pedersen, 1999; Halwani et al. 1999 and Ali et al. 2001)**.

Nigella sativa (family Ranunculaceae) is commonly known as black cumin or black seed. The seed or its oil is used as a carminative, diuretic, lactagogue and vermifuge **(Akgul, 1989; Ali and Blunden, 2003).** The dried seeds from black cumin are also used for sprinkling on bread or flavouring foods, especially bakery products and cheese **(Ustum et al., 1990; Takruri and**

Dameh, 1998). *Nigella sativa* seeds contain 36-38% fixed oils, proteins, alkaloids, saponin and 0.4-2.5% essential oil **(Ali and Blunden, 2003).**

The antioxidant, antibacterial and antifungal activities of spices and their derivatives have been investigated by some researchers **(De et al., 1999; Sagdic et al., 2002; Sagdic, 2003).** Many bioactive properties have been attributed to black cumin seed, fixed oil and/or essential oil, including antibacterial **(Akgul, 1989; Hanafy and Hatem, 1991; Farrag et al., 2000),** antifungal **(Akgul, 1989; Khan et al., 2003)** and antioxidant activities **(Burits and Bucar, 2000).**

Therefore, the present study has been made to investigate the antibacterial effect of *Nigella sativa* oil on the microbial quality of chilled chicken fillets; as well as to establish a traditional role for black cumin as preservative, antiseptic and disinfectant.

MATERIALS AND METHODS

1- Sampling:

Twenty five whole fresh broiler chicken fillets (each weight 500 g) were collected from chicken retail shops (poultry's shops) at Cairo and Giza Governorates and individually packed in polyethylene bags. The samples were transferred in an ice box to the laboratory.

2- Preparation of samples:

Samples were divided into five groups; first used as control and the others were mixed with different concentrations of *N. sativa* oil (w.w) (0.5, 1.0, 1.5, 2.0 and 2.5%) and stored at refrigerator (10^{o}C). The samples were stored and periodically examined at zero time, then at 2^{nd}, 4^{th}, 7^{th} and 10^{th} days.

***3. Preparation of N. sativa oil* (Ozcan, 1998).**

One hundred grams of *N. sativa* seeds were ground in an omni mixer and extracted for 10 hours in a Soxhlet extractor with 500 ml n-hexan (Merck-Darmstadt, Germany) at 70^{o}C. The fixed oils were pooled and concentrated in a rotary evaporator (Buchi Rotavapor-RE 111), and then kept in small (10 ml) sterile bottles under refrigerated conditions until use.

4- Examination of samples:

4.1.Organoleptic examination:

The samples were organoliptically examined for changes in colour appearance scores during storage according to method recommended by **Ogunbanwo and Okanlawon (2006).** The treated chicken meat parts were judged by score a nine-point hedonic scale.

4.2 Bacteriological examination:

***4.2.1. Preparation of sample* (APHA, 1992).**

Twenty five grams from each sample were aseptically placed in a sterile blender with 225 ml of peptone water (1%) and homogenized for two minutes then serial dilution were prepared in sterile peptone water (1%) then, subjected to the following examination:

4.2.2. Bacteriological examination:

4.2.2.1. Total colony count:

From each of the previously prepared serial dilution 0.1 ml was plated onto

standard Plate Count agar in duplicate and incubated at 37°C for 28 hours, and at 10°C for 7 days for mesophilic and Psychrophilic count respectively according to the method recommended by **APHA, (1992).**

4.2.2.2. Coliforms count (MPN/g):

Using methods recommended by **APHA, (1992).**

4.2.2.3. Staphylococcus aureus count (Coagulase positive):

Staphylococcus aureus (coagulase positive) were enumerated on Baird Parker agar medium according to **APHA, (1992).**

4.2.2.4. Yeast and mould count:

Enumeration of yeast and mould were performed on Sabaroud's dextrose agar medium supplemented with chloramephnicol 0.05mg/ml and incubated at 25°C for 5 days as described by **Koneman et al., (1994).**

RESULTS

Table (1) Effect of different concentrations of *N. sativa* on the total bacterial counts at chilled storage

Conc. / Time	Untreated sample (Control)	Treated samples with different conc. of *N. sativa* (w.w)				
		0.5%	1.0%	1.5%	2.0%	2.5%
		Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
0 day	4.6×10^5 $\pm2.0\times10^3$	4.6×10^5 $\pm2.0\times10^3$	4.6×10^5 $\pm2.0\times10^3$	4.6×10^5 $\pm2.0\times10^3$	4.5×10^5 $\pm2.0\times10^3$	4.6×10^5 $\pm2.0\times10^3$
2 days	4.8×10^5 $\pm2.1\times10^3$	4.7×10^5 $\pm2.0\times10^3$	4.7×10^5 $\pm2.1\times10^3$	4.0×10^5 $\pm1.9\times10^3$	3.2×10^5 $\pm1.5\times10^3$	3.3×10^5 $\pm1.4\times10^3$
4 days	5.2×10^5 $\pm2.2\times10^3$	4.6×10^5 $\pm2.0\times10^3$	4.2×10^5 $\pm1.8\times10^3$	4.0×10^5 $\pm1.6\times10^3$	3.0×10^4 $\pm2.0\times10^3$	2.8×10^4 $\pm2.0\times10^3$
7 days	6.8×10^5 $\pm3\times10^3$	6.8×10^5 $\pm3.3\times10^3$	6.4×10^5 $\pm4.1\times10^3$	6.2×10^5 $\pm2.1\times10^3$	$4..2\times10^5$ $\pm3.2\times10^3$	5.8×10^4 $\pm1.5\times10^3$
10 days	6.6×10^6 $\pm2.2\times10^4$	6.4×10^6 $\pm2.4\times10^4$	6.2×10^6 $\pm3.0\times10^4$	6.1×10^6 $\pm2.5\times10^4$	6.0×10^6 $\pm2.6\times10^4$	6.0×10^6 $\pm2.3\times10^4$

Table (2) Effect of different concentrations of *N. sativa* on the total coliforms (MPN) count at chilled storage

Conc. Time	Untreated sample (Control)	Treated samples with different conc. of *N. sativa* (w.w)				
		0.5%	1.0%	1.5%	2.0%	2.5%
	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
0 day	2.9×10^4 $\pm1.1\times10^3$	2.6×10^4 $\pm2.1\times10^3$	1.9×10^4 $\pm2.0\times10^3$	2.0×10^4 $\pm2.0\times10^3$	2.0×10^4 $\pm2.0\times10^3$	2.1×10^4 $\pm2.0\times10^3$
2 days	3.2×10^4 $\pm2.1\times10^3$	3.3×10^4 $\pm2.0\times10^3$	3.5×10^4 $\pm2.1\times10^3$	3.0×10^4 $\pm1.9\times10^3$	2.2×10^4 $\pm1.5\times10^3$	2.2×10^4 $\pm1.4\times10^3$
4 days	3.6×10^4 $\pm2.2\times10^3$	3.8×10^5 $\pm2.0\times10^3$	4.0×10^5 $\pm1.8\times10^3$	4.0×10^5 $\pm1.6\times10^3$	3.0×10^4 $\pm2.0\times10^3$	3.1×10^4 $\pm2.0\times10^3$
7 days	4.0×10^5 $\pm3.0\times10^3$	4.0×10^5 $\pm3.0\times10^3$	4.2×10^5 $\pm4.1\times10^3$	3.8×10^5 $\pm2.1\times10^3$	3.1×10^5 $\pm3.2\times10^3$	3.2×10^4 $\pm1.5\times10^3$
10 days	6.2×10^6 $\pm2.0\times10^4$	6.1×10^6 $\pm2.2\times10^4$	6.1×10^6 $\pm2.2\times10^4$	5.8×10^6 $\pm2.0\times10^4$	5.4×10^6 $\pm2.2\times10^4$	5.4×10^6 $\pm2.2\times10^4$

Table (3) Effect of different concentrations of *N. sativa* on *S. aureus* count at chilled storage

Conc. Time	Untreated sample (Control)	Treated samples with different conc. of *N. sativa* (w.w)				
		0.5%	1.0%	1.5%	2.0%	2.5%
	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
0 day	3.5×10^4 $\pm10^3$	3.1×10^4 $\pm2.1\times10^3$	2.8×10^4 $\pm2.0\times10^3$	9.4×10^3 $\pm2.0\times10^2$	3.3×10^3 $\pm2.0\times10^2$	3.2×10^3 $\pm2.0\times10^2$
2 days	3.5×10^4 $\pm2.3\times10^3$	3.0×10^4 $\pm10^3$	2.5×10^4 $\pm1.1\times10^3$	2.0×10^4 $\pm10^3$	1.4×10^3 $\pm\times10^2$	1.2×10^3 $\pm10^2$
4 days	3.8×10^4 $\pm1.2\times10^3$	3.6×10^4 $\pm1.3\times10^3$	3.1×10^4 $\pm1.8\times10^3$	2.2×10^4 $\pm1.2\times10^3$	2.1×10^3 $\pm10^3$	2.4×10^3 $\pm1.1\times10^2$
7 days	4.0×10^4 $\pm1.2\times10^3$	3.8×10^4 $\pm1.1\times10^3$	3.8×10^4 $\pm1.1\times10^3$	3.2×10^4 $\pm10^3$	2.3×10^3 $\pm10^2$	2.3×10^3 $\pm10^2$
10 days	4×10^5 $\pm10^4$	4.1×10^5 $\pm10^4$	3.8×10^5 $\pm1.1\times10^4$	3.6×10^5 $\pm10^4$	4.0×10^4 $\pm10^3$	4.2×10^4 $\pm1.1\times10^3$

Table (4) Effect of different concentration of N. sativa on the Yeast and mould count at chilled storage

Conc. Time	Untreated sample (Control)	Treated samples with different conc. of N. sativa (w.w)				
		0.5%	1.0%	1.5%	2.0%	2.5%
		Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
0 day	5.5×10^3 $1.1\times\pm10^2$	5.4×10^3 $\pm2.1\times10^2$	5.4×10^3 $\pm2.0\times10^2$	5.0×10^3 $\pm2.0\times10^2$	5.1×10^3 $\pm2.0\times10^2$	5.2×10^3 $\pm2.0\times10^2$
2 days	5.6×10^3 $\pm2.3\times10^2$	5.7×10^3 $\pm10^2$	5.7×10^3 $\pm1.1\times10^2$	5.0×10^3 $2.2\times\pm10^2$	4.8×10^3 $2.4\times\pm\times10^2$	4.8×10^3 3.1 ± 10^2
4 days	5.8×10^4 $\pm1.2\times10^3$	5.6×10^4 $\pm1.1\times10^3$	5.5×10^4 $\pm1.1\times10^3$	5.2×10^4 $\pm10^3$	4.1×10^3 $\pm10^2$	4.1×10^3 $\pm10^2$
7 days	6.0×10^4 $\pm1.5\times10^3$	5.8×10^4 $\pm1.1\times10^3$	5.8×10^4 $\pm1.2\times10^3$	5.2×10^4 $\pm10^3$	4.3×10^3 $\pm10^2$	2.3×10^3 $\pm10^2$
10 days	1.2×10^5 $\pm10^4$	1.2×10^5 $\pm10^4$	1.0×10^5 $\pm1.1\times10^4$	3.6×10^5 $\pm10^4$	3.4×10^4 $\pm10^3$	3.4×10^4 $\pm1.1\times10^3$

DISCUSSION

There is no marked changes in each of odour and appearance of samples during chilled storage. This may be attributed to that the extraction of *Nigella sativa* is a volatile oil with slightly odour. However the storage time has a great effect on stability of odour.

The average initial bacterial count was 4.6×10^5 $\pm2.0\times10^3$cfu/g (0 day) Table (1). Fresh broilers fillets can be expected to have initial counts of 10^4 to 10^5 cfu/g **(Brune and Cunningham, 1971) .**

The present results fell within this range and were nearly similar to that obtained by **Bailey et al., (2000) and Patsias et al., (2006).** In this study total bacterial count reached 6.6×10^6 $\pm2.2\times10^4$ cfu, 6.8×10^5 $\pm3\times10^3$, 5.2×10^5 $\pm2.2\times10^3$ and 4.8×10^5 $\pm2.1\times10^3$ on day 10^{th}, 7^{th} and 4^{th} of chilled storage respectively and it considered as maximum acceptability limit for fresh poultry meat as defined by **ICMSF (1986)**. In this respects **Capita et al., (2001) and Patsias et al. (2005)** showed that the initial microbial count was affected on shelf life of chilled poultry.

The antibacterial effects of the different concentration of N. sativa oil were nearly similar to each other but, the most effective concentration of the oil on the bacterial count were 2 and 2.5% at4th day which reduce the counts; each conistituting; 3.0×10^4 $\pm2.0\times10^3$, $2.8\times10^4\pm2.0\times10^3$ cfu/g respectively. These results agreed with **Ozcan et al., (2003) and Sagdic et al., (2002).** In contrast, the obtained results were higher than those obtained by **Umit et al., (2005)** and **Akgul, (1989)** who found that 1% concentration was more effective.

Coliform bacteria usually gain access to chicken meat during evisceration, as they constitute part of the normal intestinal flora of poultry **(Notermans et al., 1980).**

Mean value of coliform (MPN/g) were relatively constant during storage chilled storage. The growth took place at reasonable rate, especially at the4th day of storage. The chilled samples reached the unacceptable limit of coliforms count during storage after 2 day. Nearly similar results were obtained by **Russell, (1997) and Bailey et al., (2000)**. The most effective concentration of N. sativa oil on the coliforms count (MPN/g) was 2% at day 4^{th} which was 3.0×10^4 $\pm2.0\times10^3$ (Table 2). However **Farag et al., (2000)** found that the oil of N. sativa had an inhibitory effect against Gram negative bacteria.

S. aureus is important in relation to poultry meat hygiene because of its ability to produce enterotoxins which may cause food poisoning in human beings. Staphylococcal food poisoning is one of the major cause of food borne illness throughout the world. **(Waldroup, 1996; Jablonski and Bohach, 1997).**

The initial *S. aureus* counts obtained in this study was $3.5\times10^4\pm10^3$ cfu/gm and began to increase at day 7^{th} ($4.0\times10^4\pm1.2\times10^3$) and reach its maximum at day 10^{th} ($4\times10^5\pm10^4$). The most effective concentration of N. sativa oil on *S. aureus* count was 2% at day 7^{th} which was 2.3×10^3 $\pm10^2$, (Table 3). Similar results were obtained by **Umit et al., (2005).**

The average initial yeast and mould counts were ($5.5\times10^3\pm1.1x\ 10^2$ cfu). The mean values of yeast count showed an increase in samples stored chilling till4th day while, it were highly increased during at day 10^{th}. These finding was higher than those obtained by **Gardner and Golan (1976) and Izat et al., (1989)**. Nearly similar levels were obtained by **Viljoen et al., (1998).** The most effective concentration of *Nigella sativa* oil on yeast and mould count was 2.5% at day 7^{th} which was 2.3×10^3 $\pm10^2$, (Table 4). Similar results were obtained by **De et al., (1999).**

De et al. (1999), **Khan et al. (2003**) and **Al-Jabre et al., (2003)** reported that the extract from *Nigella sativa* seeds had antifungal activity against *Aspergillus parasiticus*, *Candida albicans* and *Aspergillus niger*, respectively.

Many components of black cumin were characterized by **Burits and Bucar (2000)** using GC-MS, but the major ones were thymoquinone, p-cymene and carvacrol. All of these compounds had antibacterial effect **(Ali and Blunden, 2003).** Hence, the antibacterial effects of our samples may be closely related to their high percentage of these compounds.

In conclusion, antibacterial activities of the *Nigella sativa* oil against food spoilage is an important finding. Therefore, the oil of *Nigella sativa* may be used in food as a preservative.

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Received: December 29, 2009**.**
Accepted for Publ. : January 23, 2010.