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ANTIBACTERIAL EFFECT OF GARLIC, *NIGELLA SATIVA* AND ANTIBIOTICS ON *BACILLUS CEREUS* AND *STREPTOCOCCUS FAECALIS* ISOLATED FROM READY-TO-EAT MEAT SANDWICHES IN ASSIUT CITY

(With 6 Tables and 2 Figures)

By

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تأثير الثوم وحبة البركة والمضادات الحيوية على الباسيلس سيريس والأستربت فيكاليس المعزولة من سندوتشات اللحوم الجاهزة للأكل فى مدينة أسيوط

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تم تجميع ٧٥ عينة عشوائية من سندوتشات الكبده ، الشاورمة والحواوشى الجاهزة للأكل (٢٥ من كل نوع) من بعض المطاعم والباعة الجائلين بمدينة أسيوط وذلك بهدف التعرف على الحالة البكتيرية لها من حيث العدد الكلى للبكتريا الهوائية والعدد الكلى لكل من ميكروب الباسيلس سيريس والأستربت فيكاليس حيث أظهرت النتائج أن متوسط العدد الكلى فى سندوتشات الكبده ، الشاورمة والحواوشى كان ٢٧±١٨×١٠ ^{٣} ، ٢٩±٤٠٩×١٠ ^{٣} و ٢٠±٢,٦×١٠ ^{٤} خلية/ جم بالترتيب وقد ثبت وجود فروق معنوية بين هذه المتوسطات وكانت نسبة العينات الموجبة لميكروب الباسيلس سيريس ٧٦ ، ٨٨ ، ١٠٠% بمتوسطات ٢ ±٠,٦ × ١٠ ^{٣} ، ٩±٩٠ ، ١,٦ × ١٠ ^{٢} ±٧,٦ على التوالى وقد كانت هناك فروق معنوية بين سندوتشات الكبده من ناحية وكل من الشاورمة والحواوشى. وكانت نسبة العينات الموجبة لميكروب الاستربت فيكاليس ٨٠ ، ٨٨ ، ٨٨% بمتوسطات ٢٦,٧±٦×١٠ ^{٢} ، ٢٨ ±٧×١٠ ^{٢} ، ٤٥,٧±٥,٩×١٠ ^{٣} خلية/جم لكل من سندوتشات الكبده والشاورمة والحواوشى على التوالى. وبدراسة تأثير تسعة من المضادات الحيوية الشائعة الاستخدام وجد أن الباسيلس سيريس ذو حساسية عالية لخمس أنواع من المضادات المستخدمة ومقاوم لنوع واحد "Ampicillin (AM_{10})" بينما قاوم الاستريبت فيكاليس خمسة من المضادات وكان ذو حساسية عالية لـ Danofloxacin ($DFX_{5\ mg}$). وقد تم كذلك دراسة تركيزات مختلفة من حبة البركة المطحونة بتركيز ١ ، ٣ ، ٥% وكذلك الثوم المهروس طازجاً بتركيز ٣، ٤ و ٥% مع اللحم المفروم ضد الميكروبين سالفى الذكر على درجة حرارة الغرفة فى شهر

يناير والتى لا تتعدى ١٥°م. وقد وجد هناك فروق معنوية كبيرة فى التعداد اللوغاريتمى للميكروبات فى آخر أيام التخزين (اليوم الرابع) بين العينات المعاملة بالتركيزات المختلفة والعينات التى لم تعامل. وقد تم مناقشة الأهمية الصحية للميكروبات المعزولة والاحتياطات الواجب إتخاذها لتقليل تواجدها والتخلص منها أثناء اعداد السندوتشات لحماية المستهلك.

SUMMARY

Seventy five random samples of ready-to-eat, liver, shawarma and Elhawawshy sandwiches (25 of each) were obtained from food restaurants and street-vendors with different sanitation levels in Assiut City. The samples were examined bacteriologically for aerobic plate count, *Bacillus cereus* and *Streptococcus faecalis* count. The obtained results revealed that the mean values of aerobic plate count for liver, shawerma, and Elhawawshy were $27\pm18x10^3$, $29\pm4.9x10^3$ and $20\pm 2.6x10^4$ CFU/g, respectively. Significant differences were detected among the three different sandwiches. Positive *B. cereus* samples were 76, 88 and 100% with mean values of $2\pm0.6x10^3$, 90 ± 9 and $1.6x10^2\pm 17.6$ CFU/g for liver, shawarma and El-hawawshy sandwiches respectively. There was a significant difference between liver sandwiches and the other two tested sandwiches. *S. faecalis* was isolated from 80, 88 and 88%, with mean values of $26.7\pm6x10^2$, $28\pm7x10^2$ and $45.7\pm5.9x10^3$ CFU/g for liver, shawerma, and El-hawawshy sandwiches, respectively. Determining the resistance of these isolates to 9 antibiotics commonly used show that *B. cereus* was highly sensitive to 5 kinds of the tested antibiotics and resistant to Ampicillin (AM_{10}). In contrast, *S. faecalis* was highly sensitive to Danofloxacin (DFX_{5mg}) only. The effect of different concentrations of both *Nigella sativa* 1, 3 & 5% and freshly crushed garlic 3, 4 & 5% were tested against the isolated *B. cereus* and *S. faecalis* using minced meat stored at room temperature in January (does not exceed 15°C). Highly significant differences were recorded between the control and the treated samples. Public health significance and suggested measures for improving the quality of ready-to-eat meat sandwiches to protect the consumer were given.

Key words: *Garilc, Nigella sativa, antibiotic effect, Bacillus cereusand strept Faecalis, Meat sandwiches*

INTRODUCTION

Food is one of the most important basic human needs, and is essential for living. Ready to eat foods prepared and/or sold in streets or other public places are called "street foods". This type of food supply is an ancient and worldwide phenomenon but has assumed a vital role in the food-supply system of rapidly growing urban centers in developing countries in recent decades (Abdussalam and Kaferstein, 1992). Street foods provide a source of readily available inexpensive, nutritional meals, while providing a source of income for the vendors (Bryan *et al.*, 1988, Dawson and Canet, 1991, Bryan *et al.*, 1992, Swanepoel *et al.*, 1995 and Ekanem, 1998).

In contrast to these potential benefits, concerns over the safety and quality of street-vended foods have been raised because the vendors lack an adequate appreciation of basic food safety issues (Moy *et al.*, 1997). Furthermore, safe food storage temperature are difficult to maintain, since food are often displayed for long periods and may not be reheated before being served. In other cases vendors buy raw materials from dubious source and these may either already be contaminated with food borne pathogens or may be unfit for consumption for other reasons. Such conditions and practices are likely to lead to cross-contamination of cooked ready-to-eat foods (Bryan *et al.*, 1988, Dawson and Camet, 1991 and Ekanem, 1998). The world heath organization statistics indicated that food-borne diseases may be 300 to 350 times more frequent than the reported cases reveal (Saucier, 1999).

The most common ready-to-eat sandwiches sold by street vendors and fast food restaurants are liver, sausage, brain, spleen, El-hawawshy and shawarma which is a popular meat sandwich in Middle East Countries (Ayaz *et al.*, 1985).

B. cereus is an aerobic sporeforming commonly found in the environment and often isolated from a wide range of raw and processed foods (Kramer and Gilbert, 1989). This microorganism has often been incriminated in outbreaks of foodborne illness due to the consumption of contaminated foods (Roberts *et al.*, 1996 and Faille *et al.*, 2002).

Group D streptococci includes, enterococci as *S. faecalis* and *S. faecium* and non enterococci. Enterococci were isolated from shawarma samples collected from fast-food restaurants in Assiut city (Refaie and Moustafa, 1990).

N. sativa seeds (black seeds or Hebet El-Baraka) is a herbaceous plant which is a member of family Ranuculaceae. it is a spicy plant and

used as flavoring agent for bakery products (Saleh *et al.*, 2002). The antimicrobial activity of *N. sativa* seeds were reported by Mahmoud (1993), Sabreen (1996), and Abdel-Kader *et al.* (2001).

Garlic (*A. sativum*), family lilaceae is widely distributed and used in all parts of the world as a spice and herbal remedy for prevention and treatment of variety of diseases due to its active substance Allicin which possess its characteristic odour (Kamal and Daoud, 2003). Allicin has a broad spectrum antimicorbial effect, it appears to inhibit sulfhydryl enzyme in wide variety of bacteria (Council for Agricultural Science and Technology, 1998).

Antibiotics are chemical substances which have effect on microorganism causing inhibition, some of them act only on Gram positive or Gram negative bacteria and other have a broad spectrum effect. Some people fed the antibiotic annually to cattle and poultry to increase its weight but dosing animals with subtherapeutic concentration of antibiotics promotes the development of antimicrobial resistance of pathogens in meat and processed meat products (Manie *et al.*, 1999).

Therefore this study was aimed to:

1 - Determine of total bacterial count in some popular ready-to-eat sandwiches (Liver, Shawarma and El-hawawshy) in Assiut City.
2 - Investigate the occurrence of *B. cereus* & *S. fecalis* in the examined sandwiches.
3 - Determine the antibiotic resistance of the isolated *B. cereus* & *S. faecalis*.
4 - Study the effect of different concentrations of fresh minced garlic and black seed on the viability and survival of the isolated strains, *B. cereus* and *S. faecalis*.

MATERIALS and METHODS

1- Collection of samples:

Seventy five ready-to-eat sandwiches were obtained from food restaurants and street-vendors with different sanitation levels in Assiut City. Sandwiches types evaluated were liver, shawarma and El-hawawshy (25 of each). All samples were obtained aspectically in sterile polyethylene bags. All samples were analyzed immediately after transporting to the laboratory for enumeration of:

a- Aerobic plate count (ICMCF, 1978).
b- *B. cereus* count (Harmon and Goepfert, 1984).
c- *S. faecalis* count (Deibel and Hartman, 1984).

2- Preparation of sample:

This was done as recommended by (APHA, 1984).

3- Antibiotics sensitivity test:

The applied method and inhibitors zones were measured and recorded according to (Quinn *et al.*, 1994).

- Experimental test:

The effect of different concentrations of garlic and *N. sativa* on the growth rate of *B. cereus* and *S. faecalis* in minced meat stored at room temperature in January (ca. 15°C) for 4 d to study the effect of garlic and *N. sativa* on the viability and survival of both pathogens in minced meet.

Minced meat samples were prepared according to (Hefnawy *et al.*, 1993). Meat samples were purchased from local supermarket aseptically in sterile polyethylene bags and divided aseptically into two equal parts each part was transferred into a sterile glass blender jar. One part was inoculated with strain culture of *B. cereus* and the other part with *S. faecalis*.

Test organism:

The organism was grown in 10 ml of nutrient broth at 37°C/24 h. The culture was decimally diluted and plated to enumerate the organisms present. The culture was diluted to achieve an inoculum level of 10^4/g for *B. cereus* and 10^6/g for *S. faecalis*.

Each strain culture was thoroughly mixed with minced meat, then divided into 7 parts each part (100g) in sterile beaker. The first beaker was considered as a control, while the 2nd, 3rd and 4th were mixed with 3, 4 and 5% of garlic (freshly crushed garlic) and the 5th, 6th and 7th beakers were mixed with 1, 3 and 5% of *N. sativa* (freshly ground seeds), respectively. All beakers were covered with aluminium foil and stored at room temperature (ca. 15°C) and daily examined for *B. cereus* and *S. faecalis*.

5 - Statistical analysis:

The data were statistically analyzed according to (Michigan State University, 1988).

RESULTS

Results were explained in Tables 1-6, Fig. 1 and 2.

DISCUSSION

The results recorded in Table 1 reveal that the aerobic plate count of the examined ready-to-eat liver sandwiches ranged from $2x10^2$ to $44x10^4$ with a mean value of $27\pm18x10^3$ cfu/g. Higher counts were recorded by (Hegazy, 1999, and Ebraheem, 2001).

Concerning shawarma sandwiches the APC varied from $3x10^3$ to $12x10^4$ with a mean value of $29\pm4.9x10^3$ cfu/g. These results were also lower than those of Ayaz *et al.* (1985) who reported 10^2 to $3.0x10^6$, Refaie & Moustafa (1990) detected $24.6x10^7$ cfu/g and Ebraheem (2001) recorded a mean value of $67.88\pm31.7x10^6$ cfu/g. Our results were higher than those of Morshdy *et al.* (1986) who recorded $38x10^2$/g, of ready-to-eat shawarma sandwiches.

As regarded to El-hawawshy ready to eat sandwiches the total aerobic count varied from $5x10^4$ to $4x10^5$ with a mean value of $20\pm2.6x10^4$ cfu/g. El-Daly *et al.* (1987) recorded low results ($7\pm3x10^3$/g). Table 1 also showed that there were significant differences ($P>0.05$) among the three different sandwiches studied. The highest contaminated sandwiches were El-hawawshy. Bacterial counts in the sandwiches analyzed in the present study were found to be lower than those reported by Fathi (1988), Nassar (1988) and Refaie and Moustafa (1990). However, the APC recorded by Mosupye and Holy (1999) in meat samples collected from street-vendors in Johannesburg, South Africa were lower than ours (3.1 ± 0.9 log cfu/g). However, in the other countries as Pakistan, Zambia and Nigeria higher results were recorded for street-vended cooked meals (Bryan *et al.*, 1992, Ekanem, 1998 and Mosupye and Holy, 1999).

According to the microbiological standard for hot meals recorded by Lufthansa and Swiss Air Service (1986) it is clearly evident that the means of APC for liver, shawarma and El-hawawshy were less than 10^5 cfu/g which considered within the permisable limits.

Table 2 shows that the minimum, maximum and the means of *B. cereus* counts in liver ready-to-eat sandwiches were 10, $9x10^3$ and $2\pm0.6x10^3$ cfu/g, respectively. The results obtained in this study showed no significant differences ($P>0.05$) between the mean values in case of shawarma and El-hawawshy but a significant difference was observed between liver and both shawarma and El-hawawshy.

B. cereus was isolated from 76% of liver sandwiches. Ebraheem (2001) recorded 100% positive samples with a mean value

99.6±7.9/cfu/g. Hegazy (1999) recorded little higher count 10^4±6x10^3 cfu/g. The recorded data in Table 2 showed that the positive samples were 88% of examined ready-to-eat shawarma sandwiches with count ranged from 10 to 2x10^2 and a mean value of 90±9 cfu/g. Mousupye and Holy (1999) detected *B. cereus* in 7 out 30 cooked meat samples.

B. cereus was isolated from all the examined El-hawawshy ready-to-eat sandwiches with a count ranged from 60 to 3.5x10^2 and a mean value of 1.6x10^2±17.6 cfu/g. It is observed from the achieved results that *B. cereus* contaminated very high percentage of the examined ready-to-eat sandwiches and this may due to the fact that *B.cereus* is widely distributed in nature. Kramer and Gilbert (1989) stated that *B. cereus* population between 10^5 and 10^7 cells/g of food required to produce intoxication. Accordingly, the level of contamination with *B. cereus* appeared to be not significant to public health. As recorded in Table 3 *S. faecalis* were existed in the examined ready to eat liver sandwiches in numbers varied from 10 to 12x10^3 with a mean value of 26.7±6x10^2, the count of *B. cereus* in shawarma ranged from 10^2 to 11.4x10^3 with a mean value of 28±7x10^2 and from 12x10^3 to 12x10^4 with a mean value of 45.7±5.9x10^3 cfu/g in the tested ready-to-eat El-hawawshy sandwiches respectively.

Significant difference in the means of *S. faecalis* count between El-hawawshy and both liver and shawarma sandwiches was observed. But no significant difference between liver and shawarma was recorded (Table 3).

A higher results in both the count and percentage of positive samples were recorded by Elwi (1988) which recorded 5x10^5/g for cooked liver, Ebraheem (2001) and Morshdy *et al.* (1986) recorded low count 6x10^2 in shawarma. El-Daly (1987) also recorded 0.4x10^2 ± 0.15x10^2 in El-hawawshy sandwiches.

Mohs (1972) reported a level of <10^3/g of *S. faecalis* as an acceptable bacteriological quality in cooked foods. The examined liver and shawarma sandwiches comply with this limit.

The presence of *S. faecalis* in the examined ready-to-eat sandwiches may be due to postprocessing contamination or heat resistant character of organism.

Table 4 shows the antibiotic susceptibility of the isolated *B. cereus* and *S. faecalis* from ready-to-eat examined sandwiches. *B. cereus* was found to be highly sensitive, moderately sensitive and resistance to [Rfaampicin (RFa_{30}), Erythromycin (E_{15}) Chloramphemicol (C_{30}), Gentamicin (GN_{10})], Danofloxacin (DFX_{5mg}), Garmaycin (GM_{10}) and

Doxycycline (D_{30})] and [Ampicillin (AM_{10}), respectively. In the same Table the results show that *S. faecalis* was resistant to (RFa_{30}) (GM_{10}), (D_{30}), (C_{30}) and (GN_{10}). It was highly sensitive, moderately sensitive and weak sensitive to (DFX_{5mg}), (E_{15}) and Norafloxacin and (AM_{10}), respectively.

Pavia *et al.* (2000) found that *S. faecalis* and *S. faecium*, were susceptible to ampcillin (80%) and resistant to methicillin (88.9%). They also found that resistance of *S. faecalis* and *S. faecium* to varomycin was significantly associated with the same antibiotics including Chloramphenicol and Erythromycin.

Tables 5, 6 and Fig. 1, 2 show the effect of different concentrations of garlic and *N. sativa* on *B.cereus* and *S. faecalis* isolated from ready-to-eat sandwiches, in minced meat stored at room temperature (ca. 15°C). The effect of 3, 4 and 5% garlic decreased the numbers of *B. cereus* by 4.5 orders of magnitude in the 4th, 3rd and 3rd days of storage comparable to zero time and 6.5 and 6.7 orders of magnitude in the 3rd and 4th days of storage comparable to control respectively. While these concentrations decreased the numbers of *S. faecalis* by 1.3, 1.5 and 1.6 orders of magnitude in the 4th day of storage comparable to zero time and 3, 3.2 and 3.3 orders of magnitude comparable to control in the last day of storage, respectively (Table 6).

No significant differences were observed between the different concentrations of *N. sativa* on the 3rd day of storage on *B. cereus* (Table 5) and of the numbers of organisms in the last day of storage decreased by 4.5 orders of magnitude comparable to zero time and by 6.8 orders of magnitude comparable to the control regardless to the concentration of *N. sativa* used with *B. cereus*. Finally, Table 6 and Fig. 2 show that the numbers of *S. faecalis* decreased by 0.3, 1.0 and 1.3 log cycles at concentrations of 1, 3 and 5% of *N. sativa* comparable to zero time and by 2, 2.7 and 3 log cycles as compared with control in the last day of storage, respectively.

Our results are in agreement with those obtained by Allatif and Ibraheem (1996). The antibacterial effect of both garlic and *N. sativa* was recorded in many other studies (Mahmoud, 1993, Council for Agricultural Science and Technology, 1998 and Abdel-Kader *et al.*, 2001).

The information given by the achieved results proved that most of the examined ready-to-eat sandwiches were highly contaminated with large number of aerobic plate count. High percentage of these samples contained high number of *B. cereus* and *S. faecalis*, it indicated improper

sanitation and neglected hygienic measures during preparation causing sandwiches of inferior quality and unfit for consumption and index of fecal contamination and possibility of presence of enteric pathogens.

From the previous results we concluded that garlic and *N. sativa* play a significant role in prevention of bacterial growth, so must be added to sandwiches to improve the quality of ready-to-eat sandwiches. Services the ten golden rules drawn by (WHO, 1989). Educational programs and training courses should be recommended to the meat handlers and workers. Periodical cleaning of clothes, utensils, hands especially after visiting toilet. Protect foods from dust, insects, avoid keeping of worm below 60°C, food may only be stored for a limited time-avoid over use of antibiotics in animal husbandry and man.

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Table 1: Statistical values of aerobic plate count/g of the examined ready-to-eat sandwiches. (n= 25 of each).

Samples	% of positive Samples	Aerobic plate count/g***			
		Min.	Max.	Mean*	S.E.**
Liver	100	$2x10^2$	$44x10^4$	$27x10^3$ C	$18x10^3$
Shawarma	100	$3x10^3$	$12x10^4$	$29x10^3$ B	$4.9x10^3$
El-hawawshy	100	$5x10^4$	$4x10^5$	$20x10^4$ A	$2.6x10^4$

* Means have the same letters are not significant ($P<0.05$).

** S.E. = Standard Error.

*** Average of 3 trials.

Table 2: Statistical values of *B. cereus* count/g of the examined ready-to-eat sandwiches. (n= 25 of each).

Samples	% of positive samples	*B. cereus* count/g**			
		Min.	Max.	Mean*	S.E.**
Liver	76	10	$9x10^3$	$2x10^3$ E	$0.6x10^3$
Shawarma	88	10	$2x10^2$	90 F	9
El-hawawshy	100	60	$3.5x10^2$	$1.6x10^2$ F	17.6

* Means have the same letters are not significant (P<0.05).
** S.E. = Standard Error.
*** Average of 3 trials.

Table 3: Statistical values of *S. faecalis* count/g of the examined ready-to-eat sandwiches. (n= 25 of each).

Samples	% of positive Samples	*S. faecalis* count/g***			
		Min.	Max.	Mean*	S.E.**
Liver	80	10	$12x10^3$	$26.7x10^2$ D	$6x10^2$
Shawarma	88	10^2	$11.4x10^3$	$28x10^2$ D	$7x10^2$
El-hawawshy	88	$12x10^3$	$12x10^4$	$45.7x10^3$ B	$5.9x10^3$

* Means have the same letters are not significant (P<0.05).
** S.E. = Standard Error.
*** Average of 3 trials.

Table 4: Sensitivity tests of isolated *B. cereus* & *S. faecalis*.

Antibiotics	*B. cereus*	*S. faecalis*
1- Danofloxacin (DFX_{5mg})	++	+++
2- Riaactan Rfa Ampicin (RFa_{30})	+++	-
3- Erythromycin (E_{15})	+++	++
4- Ampicillin (AM_{10})	-	+
5- Garamycin (GM_{10})	++	-
6- Doxy cycline (D_{30})	++	-
7- Chloramphenicol (C_{30})	+++	-
8- Gentamicin (N_{10})	+++	-
9- Norafloxacin	+++	++

(-) = Resistant
(++) = Moderately sensitive
(+) = Weak sensitive
(+++) = Highly sensitive

Table 5: Fate of *B. cereus* isolated from ready-to-eat sandwiches in minced meat stored at room temperature (ca. 15°C) as affected by different concentrations of garlic and *Nigella sativa*.

Log_{10} cfu/g							
Storage period /days	*B. cereus* + Garlic				*B. cereus* + *Nigella sativa*		
	Control	3%	4%	5%	1%	3%	5%
0	4.5 E	4.5 E	4.5 E	4.5 E	4.5 E	4.5 E	4.5 E
1	5.3 D	4.0 F	3.7 F	3.3 G	3.3 E	1.5 L	2.7 I
2	5.8 C	3.7 F	1.3 M	1.6 KL	3.0 H	1.7 K	2.5 J
3	6.5 B	3.0	0.0 H	0.0 N	0.0 N	0.0 N	0.0 N
4	6.7 A	0.0	0.0 N	0.0 N	0.0 N	0.0 N	0.0 N

Log_{10} cfu/g have the same letters are not significant ($P<0.05$).

Table 6: Fate of *S. faecalis* isolated from ready-to-eat sandwiches in minced meat stored at room temperature (ca. 15°C) as affected by different concentrations of garlic and *Nigella sativa*.

Log_{10} cfu/g							
Storage period /days	*S. faecalis* + Garlic				*S. faecalis* + *Nigella sativa*		
	Control	3%	4%	5%	1%	3%	5%
0	6.3 F	6.3 F	6.3 F	6.3 F	6.3 F	6.3 F	6.3 F
1	6.5 D	6.6 C	6.3 F	6.3 F	6.3 F	6.3 F	6.0 G
2	6.6 C	6.5 D	6.3 F	5.6 I	6.4 E	5.8 H	5.6 I
3	7.5 B	5.3 J	5.3 J	5.6 I	6.00 G	5.6 I	5.3 J
4	8.0 A	5.0 K	4.8 L	4.7 M	6.0 G	5.3 J	5.0 K

Log_{10} cfu/g have the same letters are not significant ($P<0.05$).

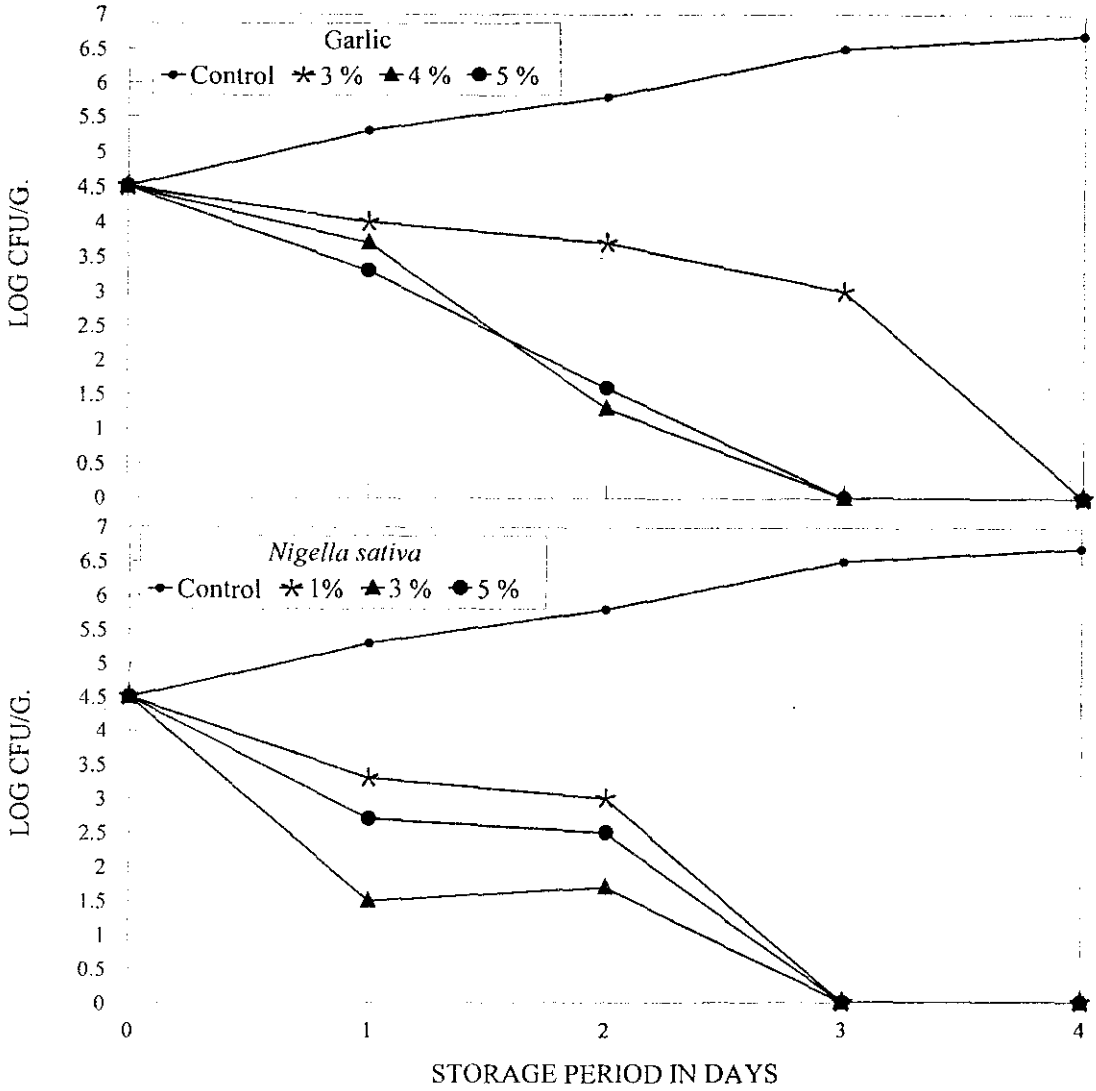

Fig. (1): Fate of *B. cereus* isolated from ready- to- eat sandwiches in minced meat stored at 15 °C as affected by different concentrations of Garlic and *Nigella sativa* .

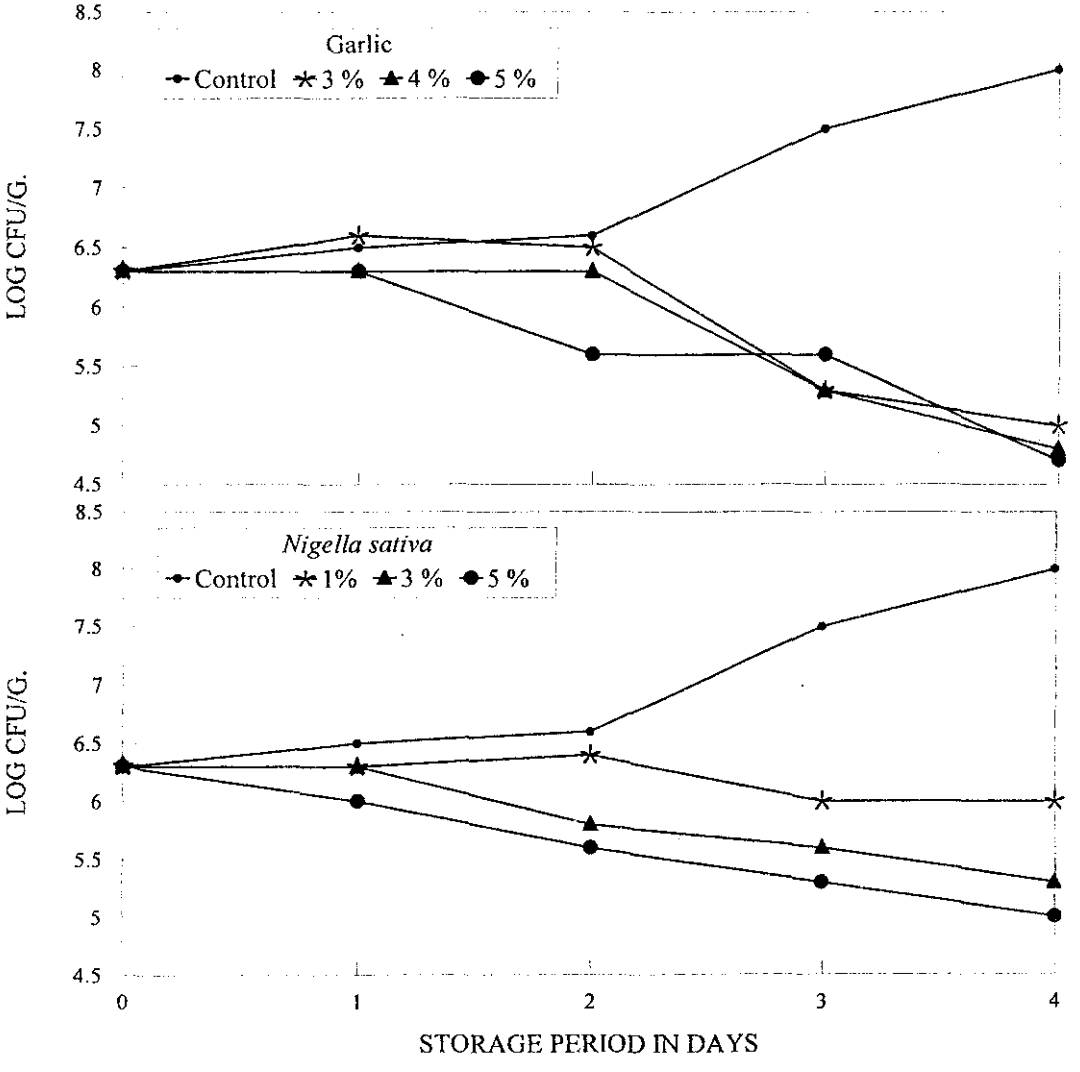

Fig. (2): Fate of *S. faecalis* isolated from ready- to- eat sandwiches in minced meat stored at 15 °C as affected by different concentrations of Garlic and *Nigella sativa* .