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Behaviour of *Staphylococcus aureus* DSM1104 in Saudi yoghurt and its control by natural plant extracts.

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ABSTRACT

The *Staphylococcus aureus* DSM 1104 (*S. aureus*) pathogen grew rapidly in Brain Heart Infusion broth (BHI broth); Saudi yoghurt during its manufacture and its growth values increased by 5 log cycles; 4 log cycles respectively. It was shown both olive leaves extract (OLE); *Nigella sativa* oil (black seed) (NSO) inhibited *S. aureus in vitro* (BHI broth) and *in situ* (Saudi yoghurt) and distinctive differences in growth values (P-values <0.05) were obtained between treated and un-treated samples, reaching 6 log cycles; 9 log cycles within 48 h in BHI broth and reaching 5 log cycles; 6 log cycles within 48 h in yoghurt during its making respectively. The antimicrobial effect by 3% concentrations of either OLE or NSO were more than that obtained by 2% concentrations of both of them. A mixture of OLE-NSO (3% of each) declined 100% of CFU/mL of *S. aureus* and no growth of the *S. aureus* pathogen was obtained after 24 h of incubation of the inoculated and treated samples of Saudi yoghurt.

Keywords: Saudi yoghurt, *Staphylococcus aureus* (*S. aureus*), Olive leaves extract (OLE), *Nigella sativa* oil (black seed, NSO)

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a Gram-positive, cluster forming cocci, and of yellow colonies on routine agar media, non-motile, non-spore forming, facultative anaerobe, catalase positive, DNase positive and coagulase positive. It grow on Baired Parker agar and forms black colonies with white small edges, tolerate NaCl up to 10% concentration and grow in a wide temperature range (7°C-40°C) [1].

S. aureus causes a wide variety of human diseases such as septicemia, wound and skin infections, pharyngitis, osteomyelitis, scarlet fever, puerperal fever, pneumonia, otitis media, sinusitis, cellulitis and epiglottitis [2, 3, 4]. Many strains of *S. aureus* were showed to resist the action of antibiotics and termed as methicillin resistant *S. aureus* (MRS), vancomycin intermediate *S. aureus* (VISA) and vancomycin resistant *S. aureus* (VRSA) [5, 6, 7, 8]. Therefore, the inhibition of this pathogen is necessary. *S. aureus* was founded to grow in many dairy products, meat product, corn-snacks and other foods [9, 10]. Consequently, the inhibition of such pathogen by natural and safe agents in foods is necessary.

Many authors used natural plant extracts to control *S. aureus* and other food-borne pathogens [4, 12, 13, 14, 15, 16]. Many natural plant extracts inhibited *S. aureus*. *Nigella sativa* (black cumin) oil (NSO) inhibited *S. aureus* and the inhibitory activity was due to thymoquinone and benzoquinone derivatives [17]. Also, olive leaves extract (OLE) exerted antimicrobial activities against many food-borne pathogens including *S. aureus, Salmonella, typhi, E. coli, Bacillus cereus* and *Listeria monocytogenes* [8, 18].

The present work was undertaken to inhibit *S. aureus* DSM1104 (*S. aureus*) by *Nigella sativa* oil (NSO), olive leaves extract (OLE) and mixture of both of them in BHI broth and during manufacture of Saudi yoghurt.

MATERIALS AND METHODS

Bacterial strain and culture media

S. aureus DSM 1104 which was used in this study was imported from DSM culture collection. It was preserved in glass heads and propagated in Brain Heart Infusion agar (BHI, Oxoid). Enumeration of *S. aureus* was conducted on the specific Baired Parker agar medium (Oxoid).

Preparation of olive leaves extract OLE

Olive (*Olea europae*) leaves of flowering olive plant were collected, cleaned and dried in oven at 45°C overnight. The dried leaves were grinded in a blender to form powder; about 100 g were macerated in 1000 ml absolute ethanol and allowed to extraction for 48 h [19]. The dark greenish-brown residue was filtered and the filtrate was concentrated in rotary evaporator (New Brunswek Scientific Company) and to evaporate alcohol. The weight of the concentrated and dried extract was about 11% of the original weight. This dried extract was designated OLE and was used for further experiments.

Preparation of *Nigella sativa* oil (NSO)

NSO is the one of *Nigella sativa* seeds. It was obtained from local Saudi Market, Riyadh City, Saudi Arabia. This oil was mixed in distilled water by mechanical stirring and about 2% and 3% concentrations were used in the experiments [20].

Saudi yoghurt making and inhibition of *S. aureus* by both OLE and NSO

Mixture of fresh buffalo's and cow's milk (1:1) was heated at 80-82°C for 20 min and cooled to 40°C. Yoghurt starters (1:1 v/v of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) were inoculated into 100 mL containers. Also 3% concentrations; 2% concentrations of either OLE or NSO were added. In another containers, mixture of 3%-3% of OLE-NSO was added in triplicate. Controls and treated samples were incubated at 38°C. After appropriate time intervals, 1 g samples were withdrawn and analysed for CFU/mL of *S. aureus* onto Baired Parker agar [21].

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Statistical analysis

Values of CFU/mL were taken from three replicates and were expressed by mean values. One way ANOVA analysis with Duncan pasthac multiple comparison test was used using SPSS statistical software programme. Results were considered significant at P-value <0.05.

RESULTS

In the preliminary studies, it was shown that growth of the starter cultures used for Saudi yoghurt making was not affected by either 3% OLE or 3% NSO; therefore, these natural extracts (OLE, NSO) were used to inhibit *S. aureus* in Saudi yoghurt.

The *in vitro* (BHI broth) effect of either OLE or NSO on *S. aureus* growth was studied. Results are given in Figures 1,2. In control experiments (Samples without treatments), *S. aureus* grew rapidly and CFU/mL increased by 5 log cycles within 48 h, but in treated samples, CFU/mL of *S. aureus* decreased by 1 log cycle; 2 log cycles; 2 log cycles; 4 log cycles in samples treated by 2% OLE; 3% OLE; 2% NSO; 3% NSO respectively (Figures 1, 2). A distinctive differences in colony counts (P-value <0.05) were observed among treated samples and controls after 24-48 h of incubation, reaching 6 log cycles. It is of interest to find out that 3% NSO prevented *S. aureus* growth within 48 h (Figure 2).

Saudi yoghurt was made as described in Materials and Methods. The inhibition of *S. aureus* by either OLE or NSO was studied in Saudi yoghurt samples through their making. Results are given in Figures 3, 4. *S. aureus* grew rapidly in yoghurt and colony counts increased in un-treated samples from 2 x 10^4 CFU/mL at zero time to 1.2 x 10^8 CFU/mL; by 4 log cycles increase within 48 h. However, growth of *S. aureus* in yoghurt samples treated by either OLE or NSO was decreased and distinctive differences in CFU/mL values (P-value <0.05) were observed between treated and un-treated samples, reaching 5 log cycles; 5 log cycles; 6 log cycles; 6 log cycles in samples treated by 2% OLE; 3% OLE; 2% NSO; 3% NSO respectively. No growth of *S. aureus* was detected from yoghurt treated by either 3% NSO or 3% NSO after 96 h of incubation (Figure 4).

A mixture of 3%-3% of OLE-NSO was made and Saudi yoghurt samples inoculated by the *S. aureus* strain was treated by this mixture. Growth of *S. aureus* was analysed from the inoculated and treated samples throughout 96 h of incubation. Results are given in Figure 5. A distinctive differences in colony counts were observed between treated and un-treated yoghurt samples (P-value <0.05), reaching 3 log cycles within 12 h of incubation. No growth of *S. aureus* was observed after 48 h of incubation, indicating on high antimicrobial activity of OLE-NSO mixture. No growth of *S. aureus* was detected in treated yoghurt by this mixture throughout further storage of Saudi yoghurt up to 7 days, indicating on bactericidal activity of OLE-NSO mixture.

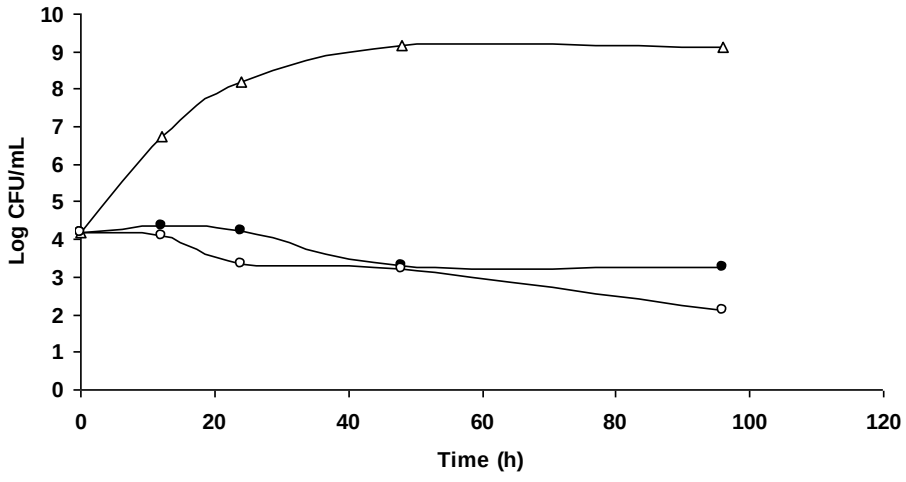

Figure 1. Growth (CFU/mL) of *S. aureus* DSM1104 in BHI broth without treatment (Δ); in BHI broth plus 2% OLE (•) and in BHI broth plus 3% OLE (o).

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Figure 2. Growth (CFU/mL) of *S. aureus* DSM1104 in BHI broth without treatment (Δ); in BHI broth plus 2% NSO (•) and in BHI broth plus 3% NSO (o).

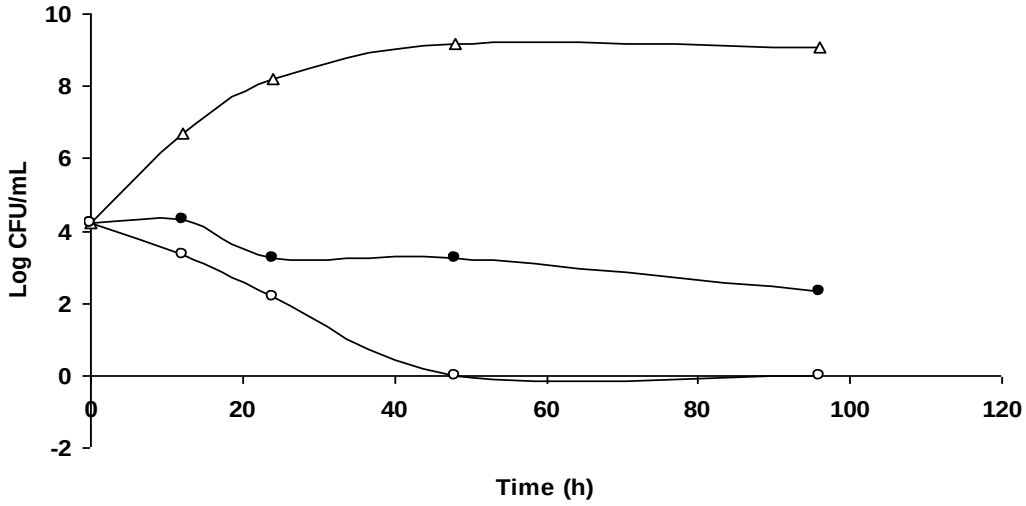

Figure 3. Growth (CFU/mL) of *S. aureus* DSM1104 during Saudi making of yoghurt (•); (Δ); (□), CFU/mL during incubation of yoghurt only; yoghurt plus 2% OLE; yoghurt plus 3% OLE respectively.

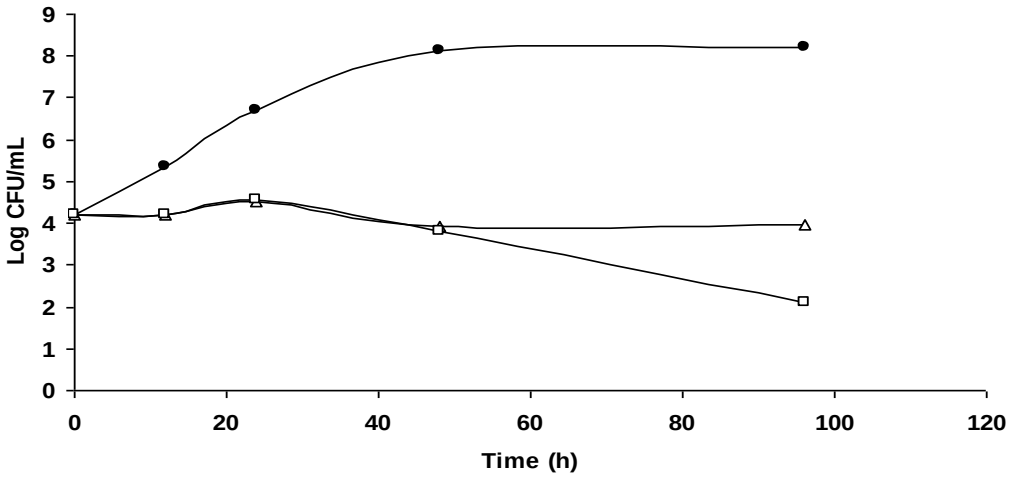

Figure 4. Growth (CFU/mL) of *S. aureus* DSM1104 during yoghurt making. (•); (Δ); (□), samples, without treatment, treate with 2%; treated with 3% NSO respectively.

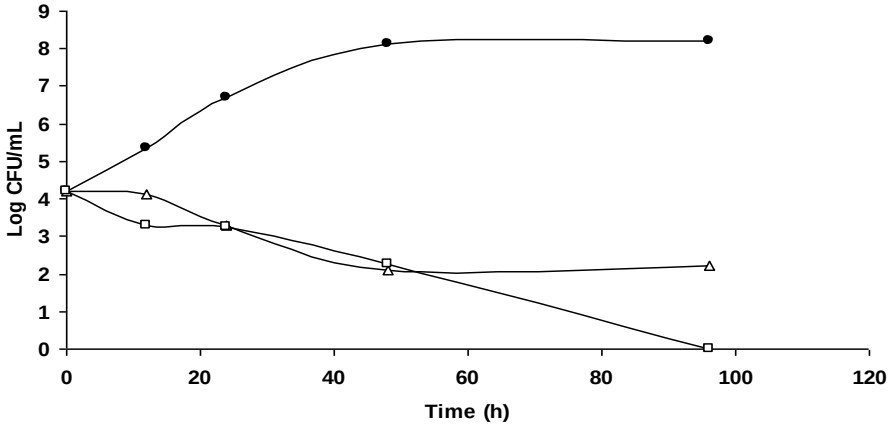

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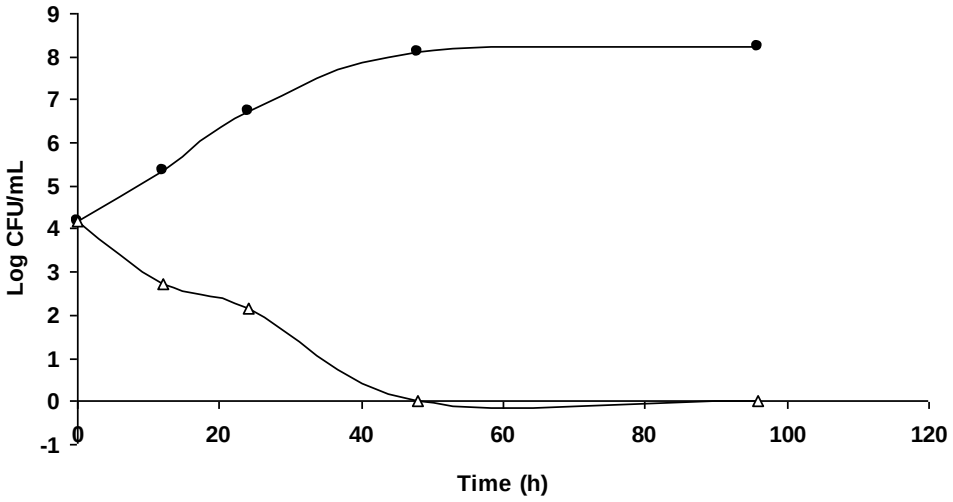
Figure 5. Growth (CFU/mL) of *S. aureus* DSM1104 during yoghurt making without treatment (●) and with treated with mixture of 3% OLES plus 3% NSO (Δ).



DISCUSSION

S. aureus remains a dominant cause of bacteremia and a common human pathogen for many human infections around the world [7, 8]. In addition most strains of *S. aureus* cause food poisoning and serious skin infections [22]. Therefore, the control of this pathogen in foods is necessary. Latter studies have showed that *S. aureus* can grow and survive in many foods including dairy products [23]. In a previous study [9], 50 samples of yoghurt were analysed for existence of coagulase-positive *Staphylococcus aureus*. It was found that it was isolated from 8% of examined samples. Also, a total of 30 samples of yoghurt and found a 26% incidence of *S. aureus* in them. This makes a necessary challenge to control *S. aureus* dusing yoghurt manufacture [24].

It is of interest to control food-borne pathogens during food-making by safe natural extract; both OLE and NSO were used in this study and interestingly, they didn't affect growth of starter cultures used for Saudi yoghurt manufacture. This study coupled with latter studies in this respect [21]. Hence, both OLE and NSO were used in this study to inhibit *S. aureus* during yoghurt manufacture. It was necessary to check the effect of starter cultures used on growth of the *S. aureus* or other pathogens in foods. The starter cultures used in this study for Saudi yoghurt making (*Lb. bulgaricus, Strept. thermophilus*) didn't inhibit *S. aureus* in control samples and *S. aureus* grew rapidly and increased almost 5-7 log cycles within 48 h in yoghurt samples. This is possible because the ability of starter cultures used for food making to inhibit food-borne pathogen depend on the strain used and nature of this strain regarding its ability to produce a metabolite with antimicrobial activity [25].

Either 2% and 3% concentrations inhibited *S. aureus* growth *in vitro* and during Saudi yoghurt making in this study and latter published work support these results [26, 27]. The antimicrobial activity of OLE is due the polyphenol eleuropein which is abundant in leaves [28]. Also many phenolic compounds are existed in olive leaves which have antimicrobial activities such as caffeine, leuteolin 7-O-glucoside, rutin, apigenin and luteolin-4-O-glucoside [29]. The antimicrobial activity of NSO is due to its content of thymoquinone volaatiule oil; this substance inhibit RNA and protein synthesis of bacteria, giving a bacterial effect [30].

Interestingly, a mixture of OLE-NSO (3% of each) inhibited *S. aureus* during manufacture of Saudi yoghurt within 48 h; both of two extracts act in a synergistic effect and this is in agreement with latter published work in this respect [15]; such mixture is recommended to be used as food additive.

CONCLUSION

Saudi yoghurt samples were made by both *Lb. bulgaricus* and *St. thermophillus* as an starter cultures; such starters didn't affect by either OLE or NSO used. *S. aureus* grew rapidly in both BHI broth and *in situ*

during Saudi yoghurt making. Either 2% or 3% concentrations of either OLE or NSO inhibited *S. aureus* growth *in vitro* and *in situ*. A mixture of both of them (3% for each) prevented growth of *S. aureus* in yoghurt within 48 h.

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