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Attempts for improving mycological quality of chicken carcasses Ghada A. Abd Elhameed, Asmaa R. Ahmed

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ABSTRACT n old fashion poultry plant in Menoufia governorate, seventy five chicken carcasses were divided into 5 groups (15 for each) and packed into plastic bags after sprayed with water, potassium sorbate (2% and 2.5%) and natamycin (0.1% and 0.2%). They were kept in a refrigerator at 4° C. After a day, the samples were analyzed to determine how these treatments had affected their mycological profile. When natamycin (0.1% and 0.2%) was used, there was a greater reduction percentage in the total mold counts (91.1% and 97.2%). While, lower reduction percent in the total molds count by using of potassium sorbate (2% and 2.5%) were 69.4% and 82.6%, respectively. A. flavus (40%), A. fumigatus (6.7%), A. niger (13.3%), A. ochraceus (13.3%), and A. tereus (6.7%) were the Aspergillus species isolated from the control group; A. flavus (33.3%), A. fumigatus (6.7%), A. niger (13.3%), A. ochraceus (6.7%), and A. tereus (6.7%) were the species isolated from the potassium sorbate (2%) groups. A. flavus (20%), A. niger (13.3%), Aochraceus (6.7%), and A. tereus (6.7%) were the isolation percentages in the potassium sorbate (2.5%) group, A. flavus (20%), A. niger (6.7%), A. ochraceus (6.7%) in the natamycin (0.1%) group while A. flavus (13.3%) and A. niger (6.7%) were isolated from the natamycin (0.2%) group. Toxigenic A. flavus isolated from control, potassium sorbate (2%), potassium sorbate (2.5%) and natamycin (0.1%) treated groups were 26.7%, 26.7%, 13.3% and 6.7%, respectively but there are no toxigenic strains of A. flavus isolated from 0.2% Natamycin treated group. Aflatoxins B1, B2, G1 and G2 were extracted from control and potassium sorbate (2%) groups but B1 and B2 were extracted from potassium sorbate (2.5%) group. While, in 0.1% Natamycin group B1 is the only aflatoxin that extracted. Generally, natamycin proved to be more efficient than potassium sorbate in suppression of mold growth in chicken carcasses and higher concentrations are better. So, the use of natamycin (0.2 %), as it is safe antifungal agent, is recommended to improve safety of chicken carcasses.

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INTRODUCTION:

As an excellent source of high-quality animal protein, chicken has a high concentration of minerals like magnesium, sodium, potassium, iron, copper, and zinc, as well as a balanced supply of most B vitamins and essential amino acids. However, chicken is less expensive and simpler to prepare and consume than beef. Furthermore, chicken has less fat than beef (Zhang et al. 2001 and Akl 2002). So poultry meat is an excellent food product and is considered a good supplement to dietary deficiencies in animal protein. The consumption of chicken has increased and the general public regardless of social status and age consume chicken meats that become contaminated by different types of microorganisms that harbor dangerous effects on consumer health. Therefore, it is important to protect consumers from infection by these pathogens (Abdel hameed, 2018).

According to **Saleem (2008)**, tiny filamentous fungi frequently contaminate food items, posing a risk to human health and the wellbeing of slaughterhouse animals. Therefore, it's critical to stop the growth of these dangerous molds in food and stop them from producing mycotoxin in order to protect people (**Davidson and Parish 1989)**.

Mycotoxins are natural secondary toxic metabolite products of fungal origin, which contaminate our foodstuff (Dancea et al. 2004).

Mycotoxins include aflatoxin B1, B2, G1, and G2. Aflatoxins (AFs) are highly significant. Aflatoxins are produced by different pathways with many strains of *Aspergillus spp*. as secondary metabolites. Less hygienic and proper handling of carcasses during preparation after slaughtering and also improper evisceration, results in high bacterial contamination. Moreover, bad cooling leads to spread of mold contamination, so food corruption occurs, which increases the amount of toxin like aflatoxin (**Iacumin et al. 2009**).

In the food industry, Potassium sorbate and

natamycin are used as preservatives. Potassium sorbate and natamycin are antifungal substances approved by FDA for use on the surface of some food items to inhibit the mold spoilage (Eldaly, 2000).

Among all food preservatives, potassium sorbate has the lowest potential for causing allergies and is a naturally occurring unsaturated fatty acid. It is completely safe for human health (**Alrabadi et al. 2013**). Potassium sorbate inhibit microbial activity at average pH values of 5.5 to 6.5 which are close to the pH value of the meat (Dzieak, 1986) and because of its high water solubility, making the use of potassium sorbate useful (**Sofos and Busta**, **1981**).

The fermentation of the bacteria Streptomyces natalensis yields natamycin, a naturally occurring antifungal drug. It is effective in very low level and affect the growth of mould and yeast, it also slow down their growth for up to six months, but it cannot completely inhibit it (Zeuthen and Sorensen, 2003). According to Food Standards (2004), natamycin exhibits potent cidal activity against susceptible microorganisms and is especially efficient against fungi that have the potential to create mycotoxins. Since it has no color or smell, it is most frequently chosen above the other preservatives. After a thorough evaluation, the European Union has classified it as a natural preservative and assigned it the E number (EEC No. 235), as well as the Food and Drug Administration (FDA), the World Health Organization (WHO), and the European Food Safety Authority (EFSA) have listed it as generally recognized as safe (GRAS) status (Mahima et al. 2021).

So, the aim of the current study was planned out to evaluate antifungal and antimycotoxin effect of potassium sorbate (2% and 2.5%) and natamycin (0.1% and 0.2%) in chicken carcasses to find out which is better.

MATERIAL and METHODS

1- Preparation of suspensions:

Four suspensions of 2% and 2.5% of potassium sorbate as well as 0.1% and 0.2% natamycin "delvocid®" were prepared in water

2- Preparation of samples:

Inside old fashion poultry plant in Menoufia governorate; seventy five chicken carcasses have a high degree of similarity after complete preparations (slaughtering-scalding-DE feathering-evisceration) were divided to five groups. Control group of chicken carcasses sprayed with water and the other 4 groups of chicken carcasses sprayed with suspension of potassium sorbate at levels of 2% and 2.5% as well as natamycin at levels of 0.1% and 0.2% (15 of each group). Both of control and treated samples were drained for 10 min. then packed into polyethylene bags, labeled and stored at 4°C. Mycological analysis was conducted after 24 hours from preparation. Swabs were taken from the surfaces of chicken carcasses. Swabs were represented by sterile cotton screw capped plastic tubes ready for use. All swabs of chicken surfaces were examined to study the effect of such treatments on their mycological profile.

3- Mycological examination:

3-1- Preparation of swabs (Elgazzar et al. 2005):

Triple swabs were taken from a limited area (10 cm^2) over the chicken skins. The first was moistened by 0.1% peptone water (diluent), however, the other two swabs were dry. The sticks of these 3 swabs were applied on the target area then put in sterile test tube containing 10 ml of the diluent. The triple swabs were then homogenized to give an original dilution of 1: 1 from which serial dilutions were prepared.

3-2- Determination of total mold count (Hocking, 2001):

The prepared sterile plates of dichloran Rose Bengal Chloramphenicol agar (DRBC) were separately inoculated with 0.1 ml of each dilution. The inoculated plated were incubated at 25°C for 5 days after which the total mold count/ cm² of the swabbed surfaces were calculated and recorded.

3-3- Identification of isolated molds:

The identification of mold genera was performed according to **Samson et al. (1995**) while identification of genus *Aspergillus* was carried out according to **Pestka (1996).**

3-4- Testing of *A. flavus* for aflatoxin production (Hara et al. 1984):

3-4-1- Cultivation and observation for the fluorescence:

A single inoculation from each *A. flavus* culture was done on the center of coconut agar plates which incubated at 25°C for 5 days. The plates were examined under ultra violet light (365nm) for detection of fluorescence in the agar surrounding colonies (Davies et al. 1987).

3-4-2- Verification and typing of aflatoxin production (Pestka, 1996):

Aspergillus flavus strains which exhibit blue fluorescence were inoculated in liquid medium of Yeast Extract Sucrose (YES) and incubated at 25°C for 7 days then filtered. Chloroform was added by a volume equal to the culture filtrate and the mixture was thoroughly homogenized and centrifuged at 3000 rpm for 10 minutes. The lower chloroform layer was separated and filtered through Whatman filter paper containing 10 g anhydrous sodium suphate. The types and concentrations of aflatoxins in the chloroform extract were determined using Thin Layer Chromatography (TLC). The concentrations of 0.5 μ g/ ml for B1 and G1 as well as 0.1 μ g/ ml for B2 and G2 were prepared using the reference standards of aflatoxins B1, B2, G1 and G2 (LGC Ltd Co., UK).

3-4-3- Application of TLC:

Solutions of reference standards of aflatoxins B1, B2, G1 and G2 were prepared by dissolving their dry films in benzo-acetonitrile (9+1) for preparation of stock concentrations of 10 micrograms/ ml. The spotting concentrations of 0.5 μ g/ ml for B1 and G1 as well as 0.1 μ g/ ml for B2 and G2 were prepared.

The concentration of each aflatoxin through the rate of flow of TLC besides fluorescent color and intensities of aflatoxin spots of sample compared with those of the standard spots. Accordingly, the concentration of the detected aflatoxin was estimated and recorded as μ g /L medium. The experiment was performed in triplicate

4- Statistical analysis:

The obtained results were statistically evaluated according to Feldman et al., (2003).

RESULTS

Table 1. Analytical results of total mold counts/cm² in control and treated chicken carcass surfaces (n=15 each) after 24 hours from refrigeration at 4^{0} C.

| Surface treatment | Min | Max | Mean \pm S.E [*] | R% |
|-------------------------|-------------------|-------------------|---|------|
| Control | 3×10^{3} | 8×10 ⁴ | $3.14 \times 10^4 \pm 0.27 \times 10^{4}$ A | |
| 2 % Potassium sorbate | 7×10^{2} | 5×10^{4} | $9.62 \times 10^4 \pm 1.15 \times 10^{3 \text{ B}}$ | 69.4 |
| 2.5 % Potassium sorbate | 6×10^{2} | 2×10^{4} | $5.47{\times}10^{4}\pm0.49{\times}10^{3}{}^{\rm C}$ | 82.6 |
| 0.1% Natamycin | 3×10^{2} | 1×10^{4} | $2.81{\times}10^3 \ \pm 0.22 \ {\times}10^{3} \ ^{\rm D}$ | 91.1 |
| 0.2% Natamycin | 1×10^{2} | 6×10 ⁴ | $8.75 \times 10^2 \pm 0.64 \times 10^2 {}^{\rm E}$ | 97.2 |

*Mean values with different superscripts in the same rows are significantly different at (P<0.05). R%= Reduction%

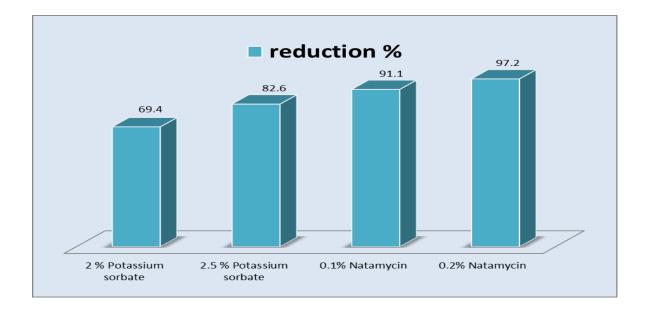


Figure 1. Effect of certain treatments on average mold counts/cm² of chicken carcass surfaces.

| Treatment | Control | | Pot. Sorbate 2% | | Pot. Sorbate 2.5% | | Natamycin 0.1% | | Natamycin 0.2% | |
|---------------------|---------|------|-----------------|------|-------------------|------|-------------------|------|-------------------|-----|
| Mold genera | | | | | | | | | | |
| | No. | % | No. | % | No. | % | No. | % | No. | % |
| Aspergillus species | 12 | 80 | 10 | 66.7 | 7 | 46.7 | 5 | 33.3 | 3 | 20 |
| Penicillium species | 8 | 53.3 | 6 | 40 | 4 | 26.7 | 3 | 20 | 1 | 6.7 |
| Paecilomyces | 3 | 20 | 1 | 6.7 | 1 | 6.7 | 0 | 0 | 0 | 0 |
| Fusarium spp. | 3 | 20 | 4 | 26.7 | 1 | 6.7 | 0 | 0 | 0 | 0 |
| Cladosporium spp. | 5 | 33.3 | 3 | 20 | 2 | 13.3 | 2 | 13.3 | 0 | 0 |
| Rhizopus | 1 | 6.7 | 1 | 13.3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mucor species | 2 | 13.3 | 2 | 13.3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Absidia spp. | 2 | 13.3 | 1 | 6.7 | 1 | 6.7 | 0 | 0 | 0 | 0 |
| Thamnidium | 4 | 26.7 | 2 | 13.3 | 1 | 6.7 | 1 | 6.7 | 0 | 0 |

Table 2. Incidence of mold genera isolated from the surfaces of control and treated chicken (n=15 each).

Table 3. Identification of *Aspergillus species* isolated from the surfaces of control and treated chicken (n=15 each).

| Treatment | Cor | ntrol | | orbate % | | orbate 5% | Natar 0.1 | nycin 1% | | nycin 2% |
|------------------|-----|-------|-----|-------------|-----|--------------|--------------|-------------|-----|-------------|
| Aspergillus Spp. | No. | % | No. | % | No. | % | No. | % | No. | % |
| A. flavus | 6 | 40 | 5 | 33.3 | 3 | 20 | 3 | 20 | 2 | 13.3 |
| A. fumigatus | 1 | 6.7 | 1 | 6.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| A. niger | 2 | 13.3 | 2 | 13.3 | 2 | 13.3 | 1 | 6.7 | 1 | 6.7 |
| A. ochraceus | 2 | 13.3 | 1 | 6.7 | 1 | 6.7 | 1 | 6.7 | 0 | 0 |
| A. tereus | 1 | 6.7 | 1 | 6.7 | 1 | 6.7 | 0 | 0 | 0 | 0 |

Table 4. Detection of Toxigenicity of *Aspergillus flavus* isolated from the surfaces of control and treated chicken (n=15 each).

| Surface treatment | | solates of <i>flavus</i> | | genic <i>la vus</i> |
|-------------------------|-----|-----------------------------|-----|------------------------|
| | No. | % | No. | % |
| Control | 6 | 40 | 4 | 26.7 |
| 2 % Potassium sorbate | 5 | 33.3 | 4 | 26.7 |
| 2.5 % Potassium sorbate | 3 | 20 | 2 | 13.3 |
| 0.1% Natamycin | 3 | 20 | 1 | 6.7 |
| 0.2% Natamycin | 2 | 13.3 | 0 | 0 |

| Surface treatment | | Levels of A. flavu | s aflatoxins (μg/L) | |
|-------------------------|-----------------|--------------------|---------------------|---------------|
| | B1 | B2 | G1 | G2 |
| Control | 28.3 ± 4.2 | 15.8± 1.4 | 7.7 ± 0.5 | 3.4± 0.2 |
| 2 % Potassium sorbate | 19.7 ± 3.1 | 10.2 ± 0.9 | 4.9 ± 0.3 | 2.6 ± 0.1 |
| 2.5 % Potassium sorbate | $7.4 {\pm}~0.6$ | 3.5± 0.2 | | |
| 0.1% Natamycin | 2.09 | | | |
| 0.2% Natamycin | | | | |

Table 5. Average concentrations of aflatoxins (μ g/L) extracted from toxigenic strains of *A* flavus in control and treated chicken surfaces

DISCUSSION

An indicator of good cleanliness and highquality products is the total fungal count. In addition to assisting in the putrefactive processes, molds can give food a moldy taste and odor. Additionally, as mold grows in a very broad range of temperatures, it can be found on practically any food at any temperature that it is kept. Furthermore, mold has the ability to promote putrefactive processes and generate poisonous compounds called mycotoxins, which are dangerous to both humans and animals (Algabry et al. 2012). Hence, the prevention of moulds to gain access the meat is absolutely necessary. Natamycin and potassium sorbate are antifungal substances approved by FDA (Griffin, 1994).

Recently, the European Food Safety Authority (EFSA) issued a positive scientific opinion on the use of natamycin as a food additive (EFSA, 2009). Its advantage over other natural antifungal agents is that it has a broad spectrum of antifungal activity at low concentrations. Since it is a white, tasteless and odorless powder, its action does not change the sensory properties of food (Dzigbordi et al. 2013). The sensory panel also found that potassium sorbate treatment didn't affect sensory proparities adversely, supporting evaluations reported by cunningham (1979).

Results given in table (1) indicated that mold counts in control chicken carcass surfaces were ranged from 3×10^3 to 8×10^4 with an average of 3.14 x $10^4 \pm 0.27$ x 10^4 . Furthermore potassium sorbate (2%), potassium sorbate (2.5%), natamycin (0.1%) and natamycin (0.2%) reduced mold counts in chicken carcass surfaces from $3.14 \times 10^4 \pm 0.27 \times 10^4$ in control group to $9.62 \times 10^3 \pm 1.15 \times 10^3$, $5.47 \times 10^3 \pm 0.49 \times 10^3$, $2.81 \times 10^3 \pm 0.22 \times 10^3$ and $8.75 \times 10^2 \pm 0.64 \times 10^2$, respectively. A higher reduction percent in the total mold counts by using of natamycin (0.1%) and natamycin (0.2%) were 91.1 % and 97.2%, respectively. While, lower reduction percent in the total moulds count by using of potassium sorbate (2%), potassium sorbate (2.5%) were 69.4% and 82.6% in treated chicken carcass surfaces.

It is evident from these results recorded in table (2) the Incidence of mold species isolated from examined swabs of control chicken surfaces were *Aspergillus* 80%, *Penicillium* 53.3%, *Paecilomyces* 20%, *Fusarium* 20%, *Cladosporium* 33.3%, *Rhizopus* 6.7%, *Mucor* 13.3%, *Absidia* 13.3%, and *Thamnidium* 26.7%.

In regard to potassium sorbate (2%) treated chicken surfaces, the incidence of mold genera was Aspergillus, Penicillium, Paecilomyces, Fusarium, Cladosporium, Rhizopus, Mucor, Absidia and Thamnidium at percentage of 66.7%, 40%, 6.7%, 26.7%, 20%, 13.3%, 13.3%, 6.7% and 13.3%, respectively. Moreover, the mold genera isolated from potassium sorbate (2.5%) treated chicken surfacwere Aspergillus 46.7%, Penicillium es 26.7%, Paecilomyces 6.7%, Fusarium 6.7%, Cladosporium 13.3%, Absidia 6.7% and Thamnidium 6.7%. However, Aspergillus (33.3%), Penicillium (20%), Cladosporium (13.3%), and *Thamnidium* (6.7%) represent mold genera isolated from natamycin (0.1%) treated chicken surfaces. While, *Aspergillus* (20%) and *Penicillium* (6.7%) were detected in the examined swabs of natamycin (0.2%) treated chicken surfaces.

The results achieved in table (3) declared that Aspergillus species isolated from the examined swabs of control chicken surfaces were A flavus (40%), A fumigatus (6.7%), A niger (13.3%), A ochraceus (13.3%) and A tereus (6.7%). Concerning the swabs isolated from potassium sorbate (2%) treated chicken surfaces A flavus (33.3%), A fumigatus (6.7%), A niger (13.3%), A ochraceus (6.7%) and A tereus (6.7%) were isolated and identified. In the case of examined swabs of potassium sorbate (2.5%) treated chicken surfaces, the isolation percentages of A flavus, A niger, A ochraceus and A tereus were 20%, 13.3%, 6.7% and 6.7%, respectively.

Regarding natamycin (0.1%), the incidence of *Aspergillus species* isolated from treated chicken surfaces were *A. flavus* (20%), *A. niger* (6.7%) and *A. ochraceus* (6.7%). While, *Aspergilli* isolated from natamycin (0.2%) treated chicken surfaces were *A. flavus* (13.3%) and *A. niger* (6.7%).

Table (4) showed that toxigenic *A. flavus* isolated from control chicken surfaces, potassium sorbate (2%), potassium sorbate (2.5%) and natamycin (0.1%) treated chicken carcasses were 26.7%, 26.7%, 13.3% and 6.7%, respectively. In the other hand toxigenic *A. flavus* was not detected in natamycin (0.2%) treated chicken carcasses.

It is obvious from the results recorded in table (5) the type and the average levels of aflatoxins B1, B2, G1 and G2 (μ g/L) extracted from toxigenic strains of *A. flavus* isolated from control chicken surfaces were 28.3± 4.2, 15.8± 1.4, 7.7± 0.5 and 3.4± 0.2, respectively.

On the other hand, the average levels of aflatoxins B1, B2, G1 and G2 (μ g/L) extracted from toxigenic strains of *A. flavus* isolated from potassium sorbate (2%) treated chicken surfaces were 19.7± 3.1, 10.2± 0.9, 4.9± 0.3 and 2.6± 0.1, respectively. Furthermore, aflatoxins extracted from toxigenic strains of *A*.

flavus in potassium sorbate (2.5%) treated chicken surfaces were B1 and B2 with levels of 7.4 ± 0.6 and 3.5 ± 0.2 , respectively.

In regard to 0.1% Natamycin treated chicken surfaces, B1 is the only aflatoxin extracted and its level was 2.09 μ g/L. Moreover, there are no toxigenic strains of *A. flavus* isolated from 0.2% Natamycin treated chicken surfaces.

From all previous results, it is obtained that natamycin had a higher inhibitory effect than that obtained by potassium sorbate. Furthermore, higher concentrations of natamycin and potassium sorbate (0.2%, 2.5%) had a higher antifungal effect than that obtained by lower concentrations (0.1 %, 2%), respectively as higher concentrations not only effected on growth of mold but also effected on mold toxigenicity.

The obtained results are agree with those reported by Hassan (2004), who studied 90 swabs from the surface of sheep carcasses treated with potassium sorbate, natamycin, and control (30 of each). The author reported that the incidence of total mold count (log cfu/g) decreased from 4.75 in the control group to 3.45 and 2.81 in the groups treated with potassium sorbate and natamycin, respectively. Additionally, the incidence of Aspergillus species decreased in the groups treated with potassium sorbate and natamycin, from 56.67% in the control group to 30% and 13.33 percent, respectively.

These results were in line with those of Hassan and El-Lawandy (2006), Peter Pipek et al. (2010), Hussein et al. (2012), and El-Tawab (2014), who found that total mold count (log cfu/g) decreased from 4.69 in the control group to 2.96 and 3.39 in the natamycin and potassium sorbate treated groups, respectively, indicating that natamycin (0.1%) had a higher inhibitory effect than it was in the other groups.

In addition, these findings agreed with **Salem et al. (2016)** who reported that natamycin (300 ppm) had a higher antifungal effect than that obtained by (100 and 200 ppm). Furthermore, the high concentration of potassium sorbate (0.3%) had a higher inhibitory effect than that obtained by lower concentrations (0.1% and 0.2%) and natamycin (300 ppm) proved to be more efficient than potassium sorbate (0.3%) in suppression of mold growth in minced meat.

Furthermore, these results agreed with those obtained by **Shaltout et al. (2016)** who said that natamycin causes high reduction percent in the total fungal and yeast count in chicken carcasses than potassium sorbate into broiler carcasses.

Results also agree with those obtained by **EL-Matary et al. (2017)** who said that natamycin 0.1% are efficient in reduction of total mold and yeast count at 0, 5, 10 and 15 day of storage. So the use of natamycin, are recommended to improve safety of meat and meat products.

Potassium sorbate exhibited good antimicrobial properties against Gram-negative and Gram-positive bacteria as well as against the fungi. These results would assist food industry to use potassium sorbate at allowable levels in products that are easily contaminated with bacteria and fungi (Nurul et al. 2023). Potassium sorbate interferes with the spore germination, inhibits the activity of several enzyme systems and interferes with substrate and electron transport mechanisms and heat-resistance apparatus of the spores (Davidson et al. 2005).

Natamycin causes defects in the permeability of membrane of the fungi as natamycin interacts with sterols, which are present in the cellular membrane of the fungal cells and thus it destructs the selective permeable membrane (Jay, 2000). When a solution with a higher concentration of natamycin is used, potassium ions move out of the cell first, then amino acids (Robert and Hui, 2001). Natamycin is a broad-spectrum antimicrobial preservative that is effective against molds and yeasts, even at very low concentrations. Strong cidal activity against sensitive bacteria is exhibited by natamycin, which is especially effective against fungi that have the potential to produce mycotoxins and risk public health (El-Diasty et al. 2009).

CONCLUSSION

rom the present study, it was concluded that a wide range of molds coming from different sources is introduced to the surfaces of chicken meat which contain abundant nutrients and have high water availability. The obtained results showed that natamycin proved to be more efficient than potassium sorbate in suppression of mold growth in chicken carcasses. Furthermore, the high concentration of natamycin (0.2%) and potassium sorbate (2.5%) had a higher inhibitory effect than that obtained by lower concentrations (0.1% and 2%), respectively. So, the use of natamycin (0.2 %), as it is safe antifungal agent, is recommended to improve safety of chicken carcasses.

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