

SUPPRESSION OF AFLATOXINS PRODUCTION IN ARTIFICIALLY INFESTED MAIZE GRAINS WITH *ASPERGILLUS FLAVUS* DURING STORAGE CONDITIONS

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ABSTRACT

Maize is the one of important crops in Egypt. Aflatoxins (AFs) are the foremost cancer present compounds by *Aspergillus flavus* (*A. flavus*) and cause health risks to human and animals. This study aimed to suppression of aflatoxins production by *A. flavus* by using different concentrations of natural substances (carnation oil, lemongrass oil, propolis and beeswax) and chemical substances (salicylic acid and potassium sorbate) on maize grains. The strains of *A. flavus* were isolated from local maize grains on Potato Dextrose Agar (PDA) and detect its ability of aflatoxins production on coconut agar media. Samples (100g) of sterilized maize grains were treated individually with different concentration of carnation oil, lemongrass oil, salicylic acid and potassium sorbate (0.25, 0.5, 1.0, 2.0, 4.0 and 6.0%) and at concentration 1% and 4% for propolis and beeswax each, then inoculated with *A. flavus* and stored for 30 days at 28±2°C. AFs reduction was determined by using High Performance liquid Chromatography (HPLC). All the tested substances had active effect in inhibition of AFs production by *A. flavus* in stored maize grains. The production of Aflatoxin B₁ and B₂ (AFB₁ and AFB₂) was decreased to about 93% and 99% at concentration of 0.25% carnation oil. Lemongrass oil almost completely inhibited AFB₁ and AFB₂ production (99.12% - 99.98% and 99.98% -99.99%, respectively) at concentration of 2% - 6%. Potassium sorbate and salicylic acid (0.25%- 6%) that were significantly effective controlling aflatoxins production on maize grains compared with control. While, the propolis and beeswax found to be the most active to protect maize grains against fungi. Natural substances, carnation oil, lemongrass oil, beeswax and propolis had higher active effect at low concentration on aflatoxin production more than salicylic acid and more safe for human used.

Keywords: Aflatoxins, *Aspergillus flavus*, natural substance, chemical substance, maize

INTRODUCTION

Maize (*Zea mays*) is that the third field crop within the world, also considered as one of important crops in Egypt. It's planted on 378,000 Fadden of land (FAO, 2013). Within the storage, amount several pests and parasites attack maize. However, fungi also are vital and thought of because the second explanation for grain losses (Ominski *et al.*, 1994 and Kumari *et al.*, 2019).

Aspergillus species could be a quite common fungus within the setting, and may be a problem in hold on grains. Fungus genus, *A. parasiticus* and *A. flavus* synthesize aflatoxins once they grow on a variety of vulnerable food and feed crops. These fungi are reported together of the extreme contaminants of varied plants and plant material like maize, peanuts, rice, cotton seeds and spices, in addition to exploit product (El-Nagerabi *et al.*, 2012). Aflatoxins are among the foremost cancer present compounds acknowledged (or identified) and that they cause vital health risks to humans and animals (Elshafie *et al.*, 2011 and Qureshi *et al.*, 2015).

Over the past few years, there are varieties of approaches that may be taken to attenuate aflatoxins contamination in grains and these involve prevention of fungal growth and therefore aflatoxins formation to reduce or eliminate aflatoxins from contaminated grains. It's a well-established undeniable fact that, some plant based mostly essential oils contain compounds that are able to inhibit plant growth, there's appreciable interest in these essential oils from aromatic plants with antimicrobial properties to manage pathogens and toxin-producing molds (Soliman and Badeaa, 2002 and Tepe *et al.*, 2005). Though the bulk of the essential oils are classified as usually Generally Recognized As Safe (GRAS), their use in foods as preservatives is usually restricted because of flavor issues (Lambert *et al.*, 2001). At the present many product are used as antifungal agents together with common preservatives as essential oils like carnation and lemongrass (Smith-palmer *et al.*, 2001), organic acid (salicylic acid) (Rajesh and Mubasshirin, 2018), organic salt (potassium salt) (Merck, 2015) and natural products as beeswax and propolis (Buchta *et al.*, 2011). Propolis is that

the resinous substance collected honey bees from numerous plant sources. The antifungal activity of propolis has been evaluated by Quiroga *et al.*, 2006; Aly and Elewa, 2007; Ghasem *et al.*, 2007 and Yang *et al.*, 2010. The chemical composition of propolis is extremely complex, containing over 150 elements like flavonoids, phenolic resin acids and their esters, alcohols, ketones, amino acids, and inorganic compounds (Hegazi *et al.*, 2000; Banskota *et al.*, 2001; Marcucci *et al.*, 2001 and Bankova, 2005a). The honeybee's wax has a very wide spectrum of useful applications, cosmetics, food process (food packaging, process and preservation – natural additive) and medication (coating pills, antibiotic properties) (Krell, 1996).

Therefore, in the present investigation, suppression of aflatoxin production of *A. flavus* by several substances either natural as carnation oil and lemongrass oil, propolis and beeswax or chemical as salicylic acid and potassium sorbate were evaluated for their efficacy as preservative against aflatoxigenic fungi in maize grains.

MATERIALS AND METHODS

Isolation and identification of *A. flavus*

Maize grains were collected from local markets in Egypt. The surface sterilized maize grains were placed on Potato Dextrose Agar (PDA) medium and incubation at 28 ±2°C for 7 days. At the end of the incubation period, *Aspegillia* isolates of fungal were identified based on light macroscopic and microscopical characteristics. *Aspegillia* isolates of fungal colonies were transferred onto fresh PDA plates to study their morphological characteristics. The isolates were identified using the taxonomic key prepared by using fungal keys and manuals (Klich, 2002 and Samson *et al.* 2004).

Mycotoxycological analysis of *A. flavus* isolates

The ability of *A. flavus* isolates for production of AFs was examined using coconut agar media (CAM) (100 g of sliced coconut was homogenized for 5 min. with 300 ml of hot water. Then filtrate and adjust pH to 7.0 using 2N NaOH and adding 20g/L agar then autoclave, Davis *et al.*, 1987). Plug from *A. flavus* (39 isolates) was placed on the center of CAM plates and incubated for 7 days. After the incubation period, plates were examined under (UV) lamp in a dark room for fluorescence to detect the presence of aflatoxin production. If the mould fluoresced under UV light was considered to be aflatoxin positive and confirmed as an aflatoxigenic form of *A. flavus*.

Screening and detecting of aflatoxins produced by different isolates of *A. flavus*

To confirm the correlation between fluorescence and aflatoxin production, five of toxigenic strains of *A. flavus* (NOs.1, 2, 5, 7 and 8) were used in this test. The colonies were grown on Yeast Extract Sucrose (YES; 2 % yeast extract and 20% sucrose) (Abdollahi and Buchanan, 1981). Spore suspensions of the isolates were prepared and adjusted to approximately 10^6 spores /ml by using a hemocytometer. One ml spore suspension was inoculated into 50 mL of sterile YES and incubated at 25 °C for 14 days. The entire culture was filtrated by filter paper No.3, the filtrate was transferred to separating funnel and extracted with chloroform (50 mlx3) (Fente *et al.*, 2001). The chloroform extracts were evaporated and redissolved in 1 ml chloroform (Kumar *et al.*, 2010). Aflatoxins were qualitatively detected by thin layer chromatography (TLC, 20x20 cm). 10 µl of the extracts and standard aflatoxins (sigma CO) were spotted on the TLC. The spotted plates were developed in developing system (toluene: ethyl acetate: formic acid, 6:3:1, v/v/v), and examined under long wave UV light (365 nm) (Abarca *et al.*, 1994). Compare sample aliquot to aflatoxins standard for the presence of blue (AFB₁ and AFB₂) fluorescent spots and Rf similar to those in standards.

Two AFB₁ and B₂ producing *A. flavus* isolates (5 and 7) were inoculated with sterilized maize grains and incubated for 30 days. The extraction, clean up and determination of aflatoxins were done according to AOAC (2016).

Preparation of test materials

Natural substance

Essential oils (carnation, lemongrass): they were purchased from Cairo Company for oils and aromatic extractions CID, Egypt. Essential oils were stored in dark glass bottles at 4°C until use. Emulsified stocks at high concentration of tested materials were prepared by dissolving in sterilized distilled water (dH₂O). A few drops of the emulsifier Tween 80 (Sigma Co.) were added the prepared emulsifier volumes (Awadall *et al.*, 2008).

Natural products (propolis and beeswax): purchased from local market. Propolis sample was kept at room temperature in dark. Crude propolis was grounded into powder and macerated in acetic acid (4g propolis + 96 ml acetic acid) and lactic acid (4g propolis + 96 ml lactic acid). The extract of propolis was filtered through Whatman No. 1 filter paper to obtain a stock solution (4%). Meanwhile beeswax was melted at 65°C in hot water, prior to use, at the rate of 4g beeswax: 96 ml water (Badawy, 2016 and Abdel-Kader, *et al.*, 2019).

Chemical substance

Organic acid (salicylic acid), Organic salt (potassium sorbate) were purchased from (Sigma- Aldrich).

Prior maize grains treating, dilutions of working solutions were prepared at concentration 0.25, 0.5, 1.0, 2.0, 4.0 and 6.0% for carnation oil, lemongrass oil, salicylic acid and potassium sorbate and 1% and 4% for propolis and beeswax.

Evaluation of aflatoxins production in treated, stored maize grains

One hundred grams of maize grains was placed in flasks (500 ml) with 10 ml of dH₂O and sterilized for 20 min in an autoclave. Each sterilized flask of maize grains were treated individually with the tested materials at the proposed concentrations and shaken for few min. The day after, all the treated maize grains were inoculated with 2 mL *A. flavus* (isolate No. 5 that recorded the highest aflatoxin production) spore suspension (10^6 spore /ml), shaken well. The treated and un-treated inoculated maize grains were stored for 30 days at 28±2°C. After incubation period, the moldy maize grains were autoclaved at 100 °C for 30 min and used for the extraction of aflatoxins according to CB method (AOAC, 2016).

Determination of aflatoxins in maize grains

The determination of Aflatoxins (AFs) was performed using High Performance liquid Chromatography (HPLC), according to (AOAC, 2016). The HPLC system used for AFs determination was ultimate 3000 Thermo Fisher system (Germany)

equipped with auto sampler, pump, fluorescence detector and a C18 column chromatography Phenomenex (250x4.6mm, 5µm). The mobile phase, water: methanol: acetonitrile (60:30:10, v/v/v), was isocratically flowed at 1.2 ml/min. AFs were measured at 360 nm excitation and 440 nm emission wave length.

Statistical analysis

Results were subjected to one-way analysis of variance (ANOVA) of the general liner model (GLM) using SAS (1999) statistical package. The results were the average of three replicates ($p \leq 0.05$).

RESULTS

Screening and detecting of aflatoxins produced by different isolates of *A. flavus*

Based on the cultural and physiological characteristics, thirty-nine isolates were referred to as *A. flavus* from maize grain samples using a taxonomical key and species represented by (Klich, 2002 and Samson *et al.*, 2004). The detection of aflatoxigenic and non aflatoxigenic *A. flavus* isolates by using ultraviolet light (UV) revealed that five (12.82%) of isolates (NOs. 1,2,5,7 and 8) were aflatoxigenic (positive) and 34 (87.18%) of isolates were non-aflatoxigenic (negative). The detection by UV at 365 nm recognized aflatoxigenic by turn out blue fluorescent colonies within the center of glass petri dish of CAM.

Thin layer chromatography analysis for aflatoxins production by Aflatoxigenic isolates on (YES) medium showed blue fluorescing spots (Fig 1) under long wave UV (365 nm) parallel to AFB₁ and AFB₂ standards. The results of TLC showed that, five tested of *A. flavus* isolates (NOs. 1, 2, 5, 7 and 8) were able to produce one or more types of aflatoxin(s). In this respect, isolates NOs.1, 2 and 8 produced AFB₁ only, whereas, isolates NOs. 5 and 7 produced AFB₁ and B₂ (Fig 1). From the quantitative method of HPLC the concentration of AFB₁ and AFB₂ produced by isolates (NOs 5 and 7) were calculated. It was found that the concentration of AFB₁ was calculated as 1060 and 608 µg/kg maize grains for strain 5 and 7, respectively. Meanwhile, the concentration of AFB₂ was 460 and 700 µg/kg maize grains for strain 5 and 7, respectively. So, the total production of AFB₁ and B₂ calculated as 1520 and 1308 µg/kg maize grains for the two isolates 5 and 7, respectively, on the basis of capability to produce.

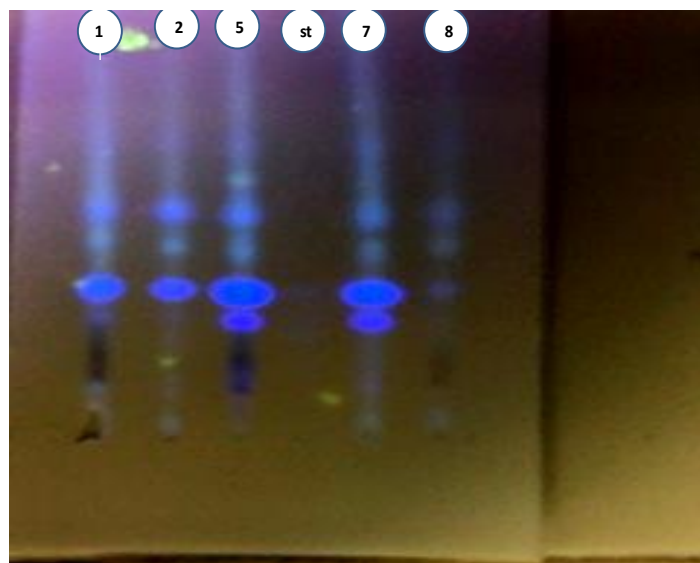


Figure 1 Thin layer chromatography screening for AFB₁ and AFB₂ production by *A. flavus* isolates (No.1, 2, 5, 7 and 8).St: standard of AFs

Determination of aflatoxins production in treated maize grains

Table 1 represented the effect of essential oils (carnation, lemongrass) on AFB₁ and AFB₂ production by *A. flavus* in stored maize grains. The highest decrease in AFB₁ production was observed in carnation oil at concentration 2-4% (0.21-0.01µg/kg) while; AFB₂ was highly decreased at 1- 4% of carnation oil. However, carnation oil suppressed the production of AFB₂ to more than 99% from the concentration of 0.25%. The complete reduction of AFB₁ and AFB₂ production was recorded at 6% (Figs 2 and 3).

Figs 2 and 3 illustrated the reduction percentage of AFB₁ and B₂ after treated with carnation oil and lemongrass oil. AFB₁ production rate was reduced about 75.34%, 90.88% and 97.06% following treatment with lemongrass oil at a concentration of 0.25%, 0.5% and 1%, respectively. However, AFB₁ and AFB₂ production by *A. flavus* was approximately complete inhibited by treatment with 2% of lemongrass oil (99.12%-99.98%), respectively and by 6% of lemongrass oil (99.98%- 99.99%), respectively (Figs 2 and 3). There was a significant

difference in aflatoxins production of control compared with the carnation oil and lemongrass oil treated samples (**Table 1**). In the current study, carnation oil had the highest reduction effect on AFB₁ and B₂ production were recorded from concentration (0.25% - 1%) compared with lemongrass oil.

Table 1 Effect of carnation oil and lemongrass oil on AFB₁ and AFB₂ production by *A. flavus* in stored maize grains

Treatment	Con. %	Aflatoxins concentration (µg/kg) (mean±SE)	
		B ₁	B ₂
Control	-	1060.0 ± 6.928 ^a	460.0 ± 7.506 ^a
Carnation oil	0.25	67.0 ± 1.155 ^b	1.7 ± 0.116 ^b
	0.50	9.22 ± 0.231 ^c	0.27 ± 0.023 ^b
	1.0	0.69 ± 0.020 ^d	0.0 ± 0.0
	2.0	0.21 ± 0.023 ^d	0.0 ± 0.0
	4.0	0.01 ± 0.001 ^d	0.02 ± 0.002 ^b
	6.0	0.0 ± 0.0	0.0 ± 0.0
Lemongrass oil	0.25	261.3 ± 1.819 ^b	8.71 ± 0.687 ^b
	0.50	96.7 ± 0.693 ^c	3.68 ± 0.116 ^b
	1.0	31.2 ± 2.424 ^d	0.14 ± 0.017 ^b
	2.0	9.3 ± 0.058 ^e	0.07 ± 0.002 ^b
	4.0	0.27 ± 0.014 ^f	0.19 ± 0.082 ^b
	6.0	0.18 ± 0.017 ^f	0.01 ± 0.001 ^b

* Con%.: Concentration%

Data are presented as the mean ± SE, different superscript letters within column are significantly different (p=0.051).

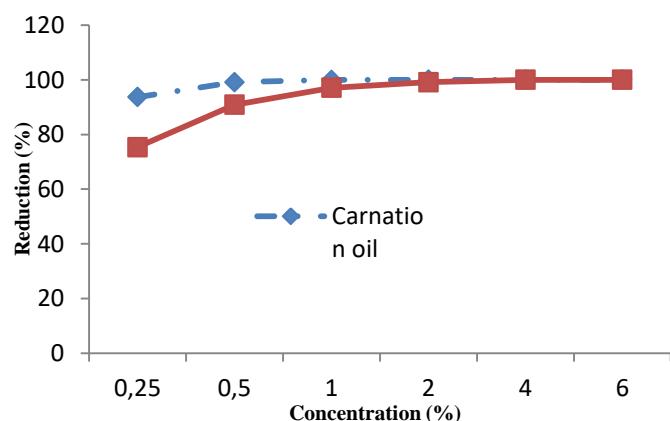


Figure 2 Reduction percentage of AFB₁ production in stored maize grains after treatment with carnation oil and lemongrass oil

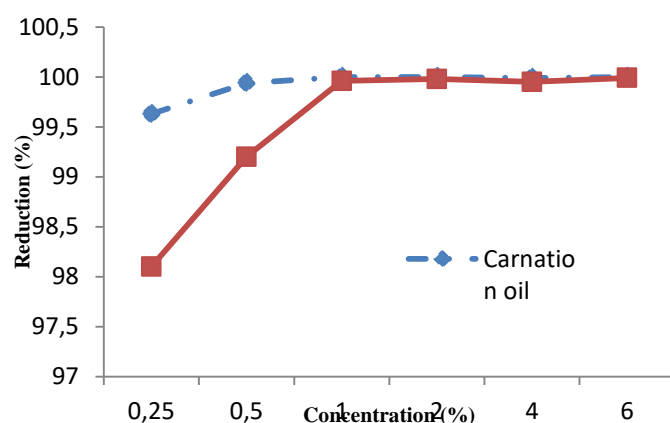


Figure 3 Reduction percentage of AFB₂ production in stored maize grains after treatment with carnation oil and lemongrass oil

Data presented in **Table (2)** showed that potassium sorbate and salicylic acid had a highly effect on the reduction of AFB₁ and B₂ at all concentration. Both potassium sorbate and salicylic acid at different concentrations from 0.25% to 6% that were significantly effective in controlling aflatoxin production on maize grains caused by *A. flavus* compared with control. The highest decrease of AFB₁ and AFB₂ were observed in 4% of potassium sorbate (99.97% and 99.98%) and salicylic acid (98.27% - 99.95%). While, the completely reduction of AFB₁ and AFB₂ production observed at 6% of both.

Potassium sorbate was recorded a reduction percent (99.83% - 100%) for AFB₁ and (99.74% - 100%) for AFB₂ production. While, Salicylic acid, the minimum

inhibition of AFB₁ (83.28%) and AFB₂ (91.86%) caused by salicylic acid in 0.25%, whereas the maximum reduction of AFB₁ and AFB₂ were (100%) that exhibited by 6% salicylic acid, these results shown in **Figs 4 and 5**. Nevertheless, the propolis and beeswax found to be the most active substance as preservative material on maize grains.

Table 2 Effect of potassium sorbate and salicylic acid on the production of AFB₁ and AFB₂ by *A. flavus* in stored maize grains

Treatment	Con. %	Aflatoxins concentration (µg/kg) (mean±SE)	
		B ₁	B ₂
Control	-	1060.0 ± 6.928 ^a	460.0 ± 7.506 ^a
Potassium sorbate	0.25	1.76 ± 0.023 ^b	1.18 ± 0.017 ^b
	0.50	0.99 ± 0.069 ^b	0.28 ± 0.017 ^b
	1.0	0.79 ± 0.017 ^b	0.00 ± 0.000
	2.0	0.41 ± 0.017 ^b	0.17 ± 0.017 ^b
	4.0	0.24 ± 0.023 ^b	0.09 ± 0.010 ^b
	6.0	0.00 ± 0.000	0.00 ± 0.000
Salicylic acid	0.25	177.22 ± 3.47 ^b	37.44 ± 1.755 ^b
	0.50	108.20 ± 1.790 ^c	36.72 ± 0.837 ^b
	1.0	49.66 ± 2.373 ^d	31.20 ± 1.270 ^b
	2.0	36.80 ± 1.386 ^e	13.30 ± 0.231 ^c
	4.0	18.32 ± 0.751 ^f	0.19 ± 0.017 ^d
	6.0	0.00 ± 0.000	0.00 ± 0.000

Data are presented as the mean ± SE, different superscript letters within column are significantly different (p=0.051).

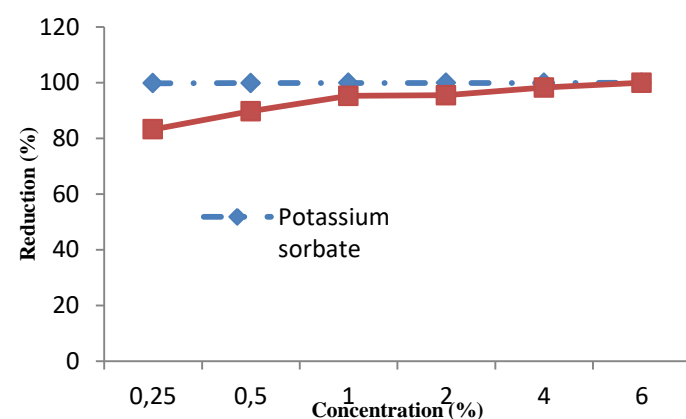


Figure 4 Reduction percentage of AFB₁ production in stored maize grains after treatment with potassium sorbate and salicylic acid

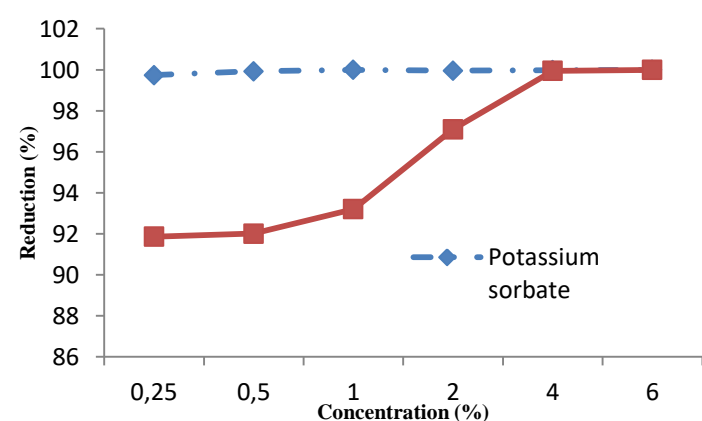


Figure 5 Reduction percentage of AFB₂ production in stored maize grains after treatment with potassium sorbate and salicylic acid

DISCUSSION

In the present study, five strains of *A. flavus* isolated from maize grains were used to evaluate their ability to produce aflatoxin(s) in synthetic medium (YES). The most aflatoxigenic isolates of *A. flavus* was used to estimate the production of AFB₁ and AFB₂ in stored maize grains for 30 days treated with different concentrations of carnation oil, lemongrass oil, salicylic acid, potassium sorbate, beeswax and propolis. It is well known that aflatoxigenic fungi are frequently found in foodstuffs and animal feeds and are associated with a wide spectrum of

stored agricultural commodities. However, not all *Aspergillus* species are able to produce aflatoxins.

In this concern, regional variations in biological weapon contamination of crops is also thanks to atmospheric condition and to agricultural practices that increase condition of plants to invasion by *A. flavus* and ratio plays an important role within the development and unfold of plant life contaminations (Nawar, 2008 and Fountain et al., 2014) and pre-harvest conditions of temperature and humidity in the field and improper postharvest handling and storage.

Over the past few years, a lot of effort has been place into research on new antifungal agents to regulate the expansion of *Aspergillus* species in cereals supposed for human and animal consumption (Soliman and Badeaa, 2002; Sukatta et al., 2008 and Abdel-Rahman et al., 2019). One methodology of preventing mycotoxin contamination is to inhibit the expansion of mycotoxin-producing molds by totally different ways. The essential oils (carnation and lemongrass), salicylic acid and potassium sorbate lead to a decrease in aflatoxins (B_1 and B_2) produced by *A. flavus* down to zero level by increasing their concentrations up to 6% compared with positive control. Several previous reports elucidate the antimicrobial activity of essential oils including lemongrass, citronella, clove, peppermint, thyme and oregano oils against different fungal species (Viuda-Martos et al., 2007 and Leyva salas et al., 2017). Also, some of the natural products, such as cinnamon and clove oil (Sukatta et al., 2008), phenols (Pizzolitto et al., 2015), some spices (Hasan and Mahmoud, 1993 and Ibrahim et al., 2017) and many essential oils (Soliman and Badeaa, 2002 and Youssef et al., 2016) have been reported as effective inhibitors of fungal growth and aflatoxin production.

Considering the massive variety of various groups of chemical compounds present in essential oils, it's possibly that their antimicrobial activity isn't due to one specific mechanism however to the existence of many targets within the cell (Carson et al., 2002). According to Pawar and Thaker (2006), the physical nature of essential oils, i.e. low mass combined with pronounced lipophilic tendencies enable them to penetrate cell wall additional quickly than alternative substances. Conner and Beuchat (1984 a, b) and Chouhan et al. (2017) suggested that the antimicrobial activity of essential oils or their constituents like thymol, carvacrol, vanillin may well be the results of injury to the catalyst cell system, as well as those related to energy production and synthesis of structural compounds.

Nychas (1995) and Pillai and Ramaswamy (2012) indicated that phenolics might denature the enzymes to blame for spore germination or interfere with the amino acid concerned in germination. Rasooli and Owlia (2005); Helal et al. (2007) and Sharifi-Rad et al. (2017) showed irreversible injury in cell membrane, cytomembrane and cellular organelles once *A. parasiticus* and *A. flavus* were exposed to totally different essential oils.

It was found that organic acids such as salicylic acid have antimicrobial effects (Kupferwasser et al., 2003). It seems that salicylic acid prevented aflatoxin production due to reduced fungal growth and could delay AFB₁ aggregation for a few days. In this regards, Hassan et al. (2015) studied the result of eight organic acids (propionic, acetic, formic, lactic, tartaric, citric, oxalic and malic acids) as antifungal agents on the expansion and toxins production of 4 fungi (*Aspergillus flavus*, *Penicillium purpurogenum*, *Rhizopus nigricans* and *Fusarium oxysporum*). They found that the highest inhibition (50%) of AFB₁ production was observed by *R. nigricans* in the presence of formic acid (10%). Panahirad et al (2014) stated that salicylic acid has been shown to inhibit the mycelial growth and mycotoxin production of *A. flavus* and could be an alternative to fungicidal agents. Bullerman (1983), Shi-Jenq (1986), Mahjoub and Bullerman (1986) and Al-Ashmawy and Ibrahim (2009) reported that Aflatoxin production was reduced and inhibited mold growth by potassium sorbate and calcium propionate. Beeswax and propolis had a highly effect on the aflatoxins production that agreement with few investigations demonstrated an antimicrobial viability of beeswax against overall *Staphylococcus aureus*, *Salmonella enterica*, *Candida albicans* and *Aspergillus niger* (Fratini et al., 2016). Propolis had antimicrobial, antiviral, antifungal and anti-inflammatory properties, its use in drugs dates back to earlier period (Wulandari et al., 2013 and Freires et al., 2016). Extending fruits shelf life and preventing fungal decay during storage as a result of using propolis were reported (Candir et al., 2009 and Özdemir et al., 2010). The propolis contains about 50% resins, 30% wax, 10% essential oils, 5% pollen and 5% other organic compounds (Falcão et al., 2010). Many investigation demonstrated the highly antimicrobial effect of propolis at different concentration of its extracts as Bankova (2005b), Soyulu et al., (2004, 2008), Candir et al., (2009), Curifuta et al., (2012), Matny et al., (2014, 2015) and Hegazi et al., (2014). The presence of phenolic compounds, waxes, vitamins and essential oils could have the most significant biological action of propolis in inhibiting the microbial activity (Burdock, 1998).

In this concern, there are some papers about the application of beeswax based edible films and coatings in the preservation of fruits and vegetables (Navarro-Tarazaga and Pérez-Gago, 2006; Galič, 2009 and Abdel-Kader et al., 2019). However, according to our knowledge, there are no many papers about the incorporation of natural biologically active compounds in the beeswax coating. The beeswax could be an advanced mixture (more than three hundred components) of hydrocarbons, free fatty acids, esters of fatty acids and fatty alcohol, di-esters and exogenous substances (Tulloch, 1980). Crude beeswax showed antifungal (Al-

Waili, 2004) and antibacterial activity against Gram-positive and Gram-negative bacteria (Nevine, 2011). Also, Fagundes et al. (2015) recorded that edible composite coating of sodium benzoate based on either hydroxypropyl methylcellulose or beeswax was the most effective than sodium methyl paraben and sodium ethyl paraben against *Alternaria alternata* black spot on artificially inoculated cherry tomatoes.

CONCLUSION

Our results indicate that food preservative from natural sources as carnation oil, lemongrass oil, beeswax and propolis were more effective against aflatoxigenic fungi and its toxins production during storage than chemical preservative as salicylic acid and potassium sorbate, at the concentrations (0.25 to 6%). It is suggested that natural substances could be used to prevent growth of aflatoxigenic fungi and its toxins production in stored maize grains.

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