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Effectiveness of chitosan and some essential oils as maize grain edible coating films on the growth of *escherichia coli* and *staphyllococcus aureus* and some mycotoxins produced by *fusarium verticilloides* and *aspergillus flavus*.

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ABSTRACT

Composite film designed as new innovative transparent edible film coating grains was prepared by casting film-forming colloidal emulsions covering maize grains which was based on chitosan and / or cinnamon, basil and dill oils or extracts added to chitosan HMW (high molecular weight).

Chitosan individual coating film succeeded as antibacterial and detoxifying agent, the reduction rate of *E.coli* growth was the highest followed by Fumonisin B_1 then aflatoxin B_1production at the same(Minimum Inhibitory concentration(MIC). In case of *Staphyllococcus aureus*: Cinnamon extract at its both tested concentrations showed the best results (79.9 and 75.46 successively in inhibiting *Staphyllococcus aureus* growth) followed by chitosan at concentration 0.5% which realized (74.29%) growth inhibition. All tested EOs individual coating films succeeded at their MICs as antibacterial and detoxifying agents.

The composite coating films are more effective as antibacterial than detoxifying agents except the film composited of chitosan and cinnamon oil which was highly succeeded as antibacterial and detoxifying agent in case of *E.coli* ,AflatoxinB_1 and fumonisin$B_1$the inhibition ratios were 94.145%, 91.217% and 90.01% respectively .Also, the composite film of chitosan and cinnamon extract inhibited *E.coli*, AflatoxinB_1 and fumonisin B_1 being: 97.943%,87.259% and 88.370% respectively.

Synergistic Effects are more dominant in Bacterial growth inhibition than mycotoxins inhibition whereas, The antagonistic effect was often appeared in case of mycotoxins inhibition except the compound of chitosan and cinnamon oil, which gave the best results in the inhibition of mycotoxins.The composite film of chitosan and basil oil exhibited the highier inhibition ratio onE.coli growth being 98.418% .

It is clear from the obtained results that the usage of composite film of chitosan and cinnamon oil can be used effectively to cover and preserve maize grains during storage.

Keywords: essential oils, chitosan, individual film, composite film,bacterial growth, mycotoxin inhibition, stored maize grains.

INTRODUCTION

Edible coatings are an environmental friendly technology that is applied on many products to control moisture transfer, gas exchange and oxidation processes. Edible coatings can provide an additional protective coating to produce and can also give the same effect as modified atmosphere storage in modifying internal gas composition. One major advantage of using edible films and coatings is that several active ingredients can be incorporated into the polymer matrix and consumed with the food, thus enhancing safety or even nutritional and sensory attributes (Dhall,2013).

The spoilage of grains each year all over the world incurs great economic losses during storage . Grains are usually contaminated by molds secreting mycotoxins . (Bankole andMabekoje, 2004, Youssef, 2009 and Abbas *et al*.,2009).

Escherichia coli among other Coliform bacteria are usually associated withfecal contaminations.(Farkas,2000). Staphylococci rarely contaminate plants (Kunene *et al*.,1999), but Abo Donia. (2008) registered several cases of plants contaminated with *staphylococcus aureus*. In order to preserve grains from microorganisms; various antimicrobial processes have been developed. Polymeric bioactive films laced with assortment of antimicrobial agents have been found very effective and practical in applications (Coma *et al*., 2002). Till date, Edible films or coatings have been investigated for their ability to retard moisture, oxygen, aromas and solute transports (Dutta *et al*., 2008).

Chitosan, a linear polysaccharide consisting of (1,4)-linked 2- amino- deoxy-β- D- glucan, is a deacetylated derivative of chitin , which is the second most abundant polysaccharide found in nature after cellulose. Chitosan has been found to be nontoxic, biofunctional and biocompatible in addition to having antimicrobial characteristics (Tripathi *et al*., 2008 and Terbojevich *etal*.2011).

Also, essential oils are complex volatile compounds, naturally synthesized by various parts of the plant during secondary metabolism . A wide range of plants having the medicinal properties have been explored and used for the extraction of essential oils worldwide due to their antimicrobial properties against bacteria, fungi and virus (Hyldgaard *et al*.,2012). The presence of a large number of alkaloids, phenols, terpenes derivatives compounds and other antimicrobial compounds makes the essential oils more précised in their mode of action against the ample variety of pathogenic microorganisms. Thus, essential oils could be used as supplements against pathogenic microorganisms. (Henri *et al*.,2012 and Akhtar *et al*.,2014). Several studies have shown that the antimicrobial and antioxidative effect of chitosan was greatly enhanced by the addition of essential oils (Georgantelis *et al*. 2007; Kanatt *et al.,* 2008 and Ahmed *et al*., 2014).

The objective of this study is to investigate the effectiveness of chitosan HMW(high molecular weight) film coating maize grains used alone or in combination with certain essential oils as antibacterial and antifungal agents and discuss their subsequent applicability on grains as preservative during storage.

MATERIALS AND METHODS

Microbial strains:

- **Bacterial strains:** *Staphylococcus aureus* (ATCC 29213) as gram positive bacteria and *Escherichia coli* (ATCC 25922) as gram negative bacteria were obtained from Microbiology Dept (CAICC), Faculty of Agriculture, Cairo University.
- **Fungal isolates:**

Mycotoxigenic Fungal isolates previously isolated,purified and identified from infected maize grains, purified and identified as *Fusarium verticilloides* N97 and *Aspergillus flavus* N367 in Agriculture Faculty,Dept. of Plant Disease, Alexandria University, then tested for their mycotoxins production abilities.

-Acetic acid: 96% ADWIC, El Nasr Pharmaceutical Chemicals Co.

-Chitosan: chitosan HMW (High molecular weight) was obtained From Sigma Aldrich .

-Cinnamon, Dill and basil oils were obtained from El – Captain Company for extracting oils.El Obour City, Cairo, Egypt. All purchased oils were checked to ensure that they are free from mycotoxins.

- Preparation of herbal extracts: Cinnamon, Dill and basil ethanolic extracts were prepared from fresh purified plant free from mycotoxins then the plant extracts were prepared using the following method of Matkowski and Piotrowska ,2006) modified by Dambolena *et al*., (2010). Ten grams of the dried powder from the plant were soaked separately in 100 ml of ethanol (98.8%), then, were refluxed in a water bath in a dark room at 45 °C. The extracts were filtered through Whatman filter paper No. 42. The Collected filtrates were dried under vaccum at 40 °C using a rotary evaporator (Buchi, Switzerland); the extraction was repeated twice. The resulting residue was re-dissolved in ethanol for the determination of the main phenolic components using GC apparatus. Active components were determined in all oils and extracts. Data were registered as shown inTable(1). All oils, extracts and maize grains were tested for the presence of bacterial and fungal strains under study before starting the experience by using specific media.

Preparation of Chitosan Emulsion :

Chitosan colloidal emulsion 1% was prepared as[1% chitosan: 0.8% acetic acid] according to Bonilla *et al.,* (2012) and, this ratio was adjusted in case of chitosan 0.5% as [0.5% chitosan: 0.4% acetic acid].

Preparation of Bacterial inocula:

The bacterial isolates were grown separately for activation in Brain heart broth for 24h.*S.aureus* cells were harvested by centrifugation at 600rpm for 10 minutes and then washed twice with phosphate buffer. The washed cells were re-suspended in the buffer and the turbidity was adjusted to an optical density (O.D.) of 0.85 at wave length 600nm using spectrophotometer.An aliquot of 0.2 ml of the cells suspension was inoculated into nutrient agar and incubated at 37°C for 48 h.for *staphylococcus aureus* according to Hazan *et al.*,(2012) and at 44°C for 48 h. for *Escherichia coli* after applied standard method using serial dilution according to Arashisara *et al*., 2004. The plate count was determined after the incubation period using Colonies Forming Units (CFU), results were registered and the obtained counts were adjusted to be $210x10^6$ CFU/ml and 280x106 CFU/ml of *staphylococcus aureus* and *Escherichia coli* respectively .

The Experiment

Preparation of treated maize grains:

Testing the occurrence of *Escherichia coli and Staphyllococcus aureus* and other bacteria in the tested maize grains:

The natural occurrence of both tested bacteria and other bacteria in maize grain samples was tested .Three Erlenmyer flasks containing 1L. peptone water were sterilized. Twenty grams of maize grains were transmitted into each Erlenmyer flask under aseptic conditions according to Arashisara *et al*., 2004. The first Erlenmyer flask was incubated at 44°C for 48hrs then serially diluted down to 10^{-7} were made and 1 ml from each dilution was transfered to MacConkey agar plates with three replicates and incubated for 48hrs at 44°C. At end of incubation period, the viable number of *E.coli* was enumerated. The second Erlenmyer flask that tested *Staphylococcus aureus* occurrence was incubated at 37°C for 24 hours, serial dilution was made and 1 ml from each dilution was transfered to Baired Parker plates with three replicates and incubated for 48hrs at 37°C. At end of incubation period, the viable number of *Staphyllococcus aureus* was enumerated according to Farkas, 2000. The third flask that tested other bacteria occurrence was incubated at 37°C for 24 hours, serial dilution was made and 1 ml from each dilution was transfered to plates containing plate count medium with three replicates and incubated for 48hrs at 25°C.The resulted Data were recorded and free grains were selected to be used in the study .

Testing maize grains for the presence of aflatoxins and fumonisins:

Both total aflatoxins and fumonisins were estimated in maize grains samples using TLC (Thin Layer Chromatography) technique (Frisvad and Thrane,1995 and Jouany, 2007) and the results were recorded and free grains were used in this study.

Preparation of coating films:

Mycotoxins free maize grains were divided into subsamples of 100g each which then was entirely coated by different treatments under study which are chitosan emulsion at concentration 1% and/ or 0.5%, acetic acid at 0.8% and / or 0.4%. Herbal oils and /or extracts (basil, dill and cinnamon) at concentrations 0.5% and 1%.Each treated portion was placed in sterilized plastic bags and homogenized using stomacher apparatus then placed in oven at 45°c for 2days for best drying according to Terbojevich *et al*.,2011 Bonilla *et al.*,2012.After that, treated samples were ready to be inoculated with the specified bacterial and fungal strains.

Fungal infection:

Fungal spore suspensions were prepared using haemocytometer to have approximately ($2.5\text{-}3.0x10^6$ /ml) 40µl of each fungal suspesion was added to each treatment All treatments were homogenized by using stomacher according to Bonilla *et al*, 2012 and Ahmed *et al*, 2014. Moisture content was adjusted for all tested samples to be 14% by adding a calculated volume of sterilized distilled water according to the method approved by the American Association of Cereals (Anon, 1962 and Fahmy 1980).This equation calculates the volume of water required for 100 g grains:

$$S° = \frac{\text{Required moisture content-Initial moist. X100}}{\text{100- Required moisture content}}$$

Where:

S° = x ml sterilized deionized water for 100g grains

N.B.=the volume of added bacterial or fungal suspension was measured and was considered as initial moisture.

The experiment was carried out by placing each treated infected samples group in incubators for 6weeks at 44°c, 37°c,30°c and 25°c for *Escherichia coli* , *Staphylococcus aureus, Aspergillus flavus* an *Fusarium verticilloides* respectively.

At the end of the incubation period, samples were taken for bacterial viable count and mycotoxins estimation to determined the MIC (minimum inhibitory concentration) of each treatment.

Determination of effect of used Treatments and their MICs after first storage period.

Viable plate count:

The effectiveness of each treatment was estimated by determination of living bacterial cells in each treatment after 6 weeks of storage. Each treatment(20g.) was serially diluted after preparation as mentioned before ,total viable bacterial counts were determined by using plate count agar method (MPCA) Arashisara *et al*.(2004). The plates were incubated for 48hrs at 37°C and 44°C for total viable counts of *E.coli* and *Staph.aureus* respectively . All counts were expressed as log10 cfu/ml and performed in duplicate. The MICs were determined according to Wiegand *et al*.,2008).

Mycotoxins estimation after first storage period:

AflatoxinB_1 or fumonisinB_1 were estimated in all maize grain samples infected with fungus using TLC (Thin layer chromatography) techniques (Frisvad *et al*,.2007 and Dambolena *et al*.,2008).Both of afltoxin and fumonisin plates were examined under U.V at 366nm and 254nm respectively . The obtained results were confirmed by using HPLC-UV technique according to Samapundo *et al*.,(2006) in Central Lab at Faculty of Pharmacy ,Alexandria University.

Storage experiment:

Purified mycotoxin's free maize grains samples(100g) each was entirely coated by each tested treatment separetly as the following: chitosan at concentration 1% and/ or 0.5%, acetic acid at 0.8% and / or 0.4%. Each chitosan concentration was added to100g maize grain sample in sterilized plastic bag and homogenized using stomacher apparatus then placed in incubator at 45°c for 2days for best drying grains.After that, herbal oils and /or extracts (basil, dill and cinnamon) were added individually at concentrations 0.5% and 1%.Each treatment was replaced in sterilized plastic bags and homogenized using stomacher apparatus then placed in incubator at 45°c for 24hrs. according to Terbojevich *et al*.,2011and Bonilla *et al*.,2012.The dried grain samples were ready for the inoculation process with each tested bacterium and/or fungus individually then they were incubated at 25°C or/ and 30°C for fungi and 44°C and/or 37°C for bacteria for six weeks .At the end of incubation period, grain samples were taken for bacterial enumeration and mycotoxins detection.

Statistical analysis:

The experiment was laid out in completely randomized design with three replicates. The data were subjected to statistical analysis using Costat computer package (CoHort Software, Berkeley, CA, USA). Least significant difference (LSD) using Duncan's Multiple Range test was applied to compare the treatment mean values. According to McDonald, (2009).

RESULTS AND DISCUSSION

Active components in the tested herbal plant oils and their extracts:

Resuls in Table (1) showed clearly that herbal plant ethyl extracts posses more active components than herbal plant oils that's may be due to the method of oil extraction, the degree of essential oil volatality ,the season of herbal plant collection and the time of collection during the day .All these reasons are typically coinceeded with those of Lee and Shibamoto. 2002 , Masotti *et al*.,2003 and Verma *et al*.,2010. Data showed also that cinnamon extract posses the higher contents of active components which indicate its powerness as antimicrobial agents, this remark is coincided with those of Velluti *et al*.,2003 , 2004 and Maurya *et al*.,2013.

The Experiment:

Table (1): Active components in the ued herbal plant oils and extracts:

Components	Cinammon oil		Cinammon extract		Basil oil		Basil extract		Dill oil		Dill extract	
	Amount (ppm)	Occurrence %	Amount (ppm)	Occurrence %	Amount (ppm)	Occurrence %	Amount (ppm)	Occurrence %	Amount (ppm)	Occurrence %	Amount (ppm)	Occurrence %
α pinene	1233	15.9839	236.61	0.50386	7.52	0.07498	392.7405	2.93373			258.2318	2.9337
Cinole	97.47	1.2635517										
Carvacrol	0.13	0.0016852	0.93	0.00198	0.31	0.003100	4.01	0.02995	4.00	0.1343	35.0	0.3976
Eugenol	5602.53	72.62836	3036.92	6.46708	5556.46	55.403	603.90	4.51106	2023.377	67.933	6029.01	68.49478
Estragol	----		11292.32	24.04685	274.61	2.73812	2549.254	19.0426	930.5	31.224	904.03	10.27056
Caryophellene	661.58	8.576388	416.52	0.88697	3322.82	33.1316	546.44	4.08184			2.7666	0.0314
Elenalol	---				24.086	0.24016	3.545	0.02648				
E .m.c	---				1.4	0.01396	0.706	0.005274				
Mercyne					602.65	6.00899	412.5450	3.08167			289.289	3.28657
Fenchone	52.88	0.685509			27.15	0.27071	74.489	0.55642			2.318	0.02633
Camphor	----				0.11	0.00101	57.0522	0.42617			713.5595	8.10665
Carvone	----		31947.09	68.0309	6.034	0.06016	222.2506	1.66130			377.552	4.28932
Cinamaldhyde	6.78	0.087892	1.382	0.00294	1.29	0.01286	2.3160	0.01730			2.7125	0.0308
Terpinol	Trs				2.3625	0.02356	8263.790	61.7295			174.178	1.9788
Verbenone	-----				108.34	1.08025	17.8467	0.13331			4.4450	0.0505
Anthole	-----				94.00	0.93727	235.9157	1.76226			9.0529	0.10285
Cinnapinene	9.6	0.124449	27.89	0.05939								
Benzyl benzoate	Trs											
Phellendrene	-----								2.2375	0.0751		
Limonene	50.0	0.6481747							18.734	0. 6289		
Total	7713.97	99.99993	46959.66	99.9997	10029.142	99.99973	13387.0897	99.99439	2978.486	99.995	8802.145	99.99987

Where:
E.m.c=Ester methyl cinnamate.

Table (2): Effect of used treatments on the growth of *Escherichia coli* and*Staphylococcus aureus* :

Treatment	Tested concentration %	Tested Bacteria: *Escherichia coli* Total count (CFU per 3repl.)	E.R%	MIC	*Staphylococcus aureus* Total count (CFU per 3repl.)	E.R%	MIC
Control	0.0	0.0 **	--	--	0.0 **	--	--
Contaminated Control		632 x $1o^6$	0.000	--	599 x $1o^6$a	0.000	--
Acetic acid	0.5	824 x $1o^6$a	-30.379	1	569 x $1o^6$b	5.008	1
	1	607 x $1o^6$c	3.9557		351 x $1o^6$c	41.402	
Chitosan	0.5	577 x $1o^6$d	8.703	1	154 x $1o^6$j	74.290	0.5
	1	58 x $1o^6$ o	90.823		241 x $1o^6$g	59.766	
Basil oil	0.5	627 x $1o^6$ b	0.791	1	284 x $1o^6$d	52.588	1
	1	318 x $1o^6$g	49.683		229 x $1o^6$i	61.770	
Basil Extract	0.5	455x x $1o^6$ f	28.006	1	275 x $1o^6$e	54.090	1
	1	284 1 x $1o^6$i	55.063		236 x $1o^6$gh	60.60	
Dill oil	0.5	303 x $1o^6$h	52.057	1	354 x $1o^6$c	40.901	1
	1	238 x $1o^6$l	62.342		250 x $1o^6$f	58.264	
Dill Extract	0.5	450 x $1o^6$ f	28.797	1	350 x $1o^6$c	41.569	1
	1	249 x $1o^6$k	60.601		199 x $1o^6$k	66.778	
Cinnamon oil	0.5	197 x $1o^6$m	68.823	0.5	235 x $1o^6$h	60.768	1
	1	115 x $1o^6$n	81.804		175 x $1o^6$l	70.785	
Cinnamon Extract	0.5	268 x $1o^6$j	57.595	0.5	120 x $1o^6$n	79.967	0.5
	1	502 x $1o^6$e	20.570		147 x $1o^6$m	75.459	
L.S.D.$_{0.5}$		5.32384			5.82734		

Where:
****= other bacteria number in control =$61x10^{-3}$**
ER= Efficacy ratio of each treatment.
MIC= Minimum inhibitory concentration of each treatment.

Effect of used treatments on the growth of *Escherichia coli* and *Staphylococcus aureus*:

Data in Table (2 clearly showed that chitosan at concentration of 1% showed the best result (90.8%) in inhibiting E.coli growth followed by cinnamon oil at both tested concentrations (1% and 0.5%) which realized growth inhibition efficacies (81.8 %and 68.8%) respectively. Our findings were relatively in agreement with those of Oussalah *et al*.,2006 and Muzzarelli, 2010. On the other hand, results showed that cinnamon extract at concentration of (0.5% and1%) realized the best results (79.9% and 75.46%) in inhibiting *Staphyllococcus aureus* growth respectively followed by chitosan at concentration of 0.5% which realized (74.29%) growth inhibition.These results are in harmony with those of Mohsenzadeh, 2007. Oussalah *et al*.,2007 and Sun *et al*.,2014. Futhermore, results indicated that *S.aureus* exhibited different response against inhibitory treatments than *E.coli*, where *S.aureus* was less resistance than *E.coli*. For meanwhile,in some cases such as chitosan 1%,Dill oil 0.5% and cinnamon oil 0.5% and1%, *E.coli* was less resistance than *S.aureus*. These findings are totally in agreement with those of Gutierrez *et al*.,2008 who reported that generally, gram-positive bacteria were more sensitive to EOs than gram-negative bacteria.

The resulted minimum inhibitory concentration (MIC) of chitosan for E.coli and S.aureus at

concentrations 0.5% and 1.0% respectively indicated that *S.aureus* was more sensitive to chitosan than *E.coli* but at the same time chitosan was more efficace against E.coli at 1% .This observation was coincided with those of Ahmed *et al*.,2014 who declared that the effect of chitosan is on the cell wall . Differences between cell wall structure ,quorum sensing factors (QS) and their signaling molecule categories of Gram negative(E.coli) and Gam bacteria positive (S.aureus)(Høiby and Givskov. 2005 and KokGan *et al*.,2011) explained our obtained results .For the MICs of other treatments (hebal oils and their extracts), our results were in line of those declared by Lambert, 2001 and Hood *et al*., 2003 who mentioned that essential oils which having the hydroxyl group at a different location on phenol, both substances appear to make the cell membrane permeable and also are able to disintegrate the outer membrane of Gram negative bacteria, releasing lipopolysaccharids (LPS) increasing the cytoplasmic membrane permeability and caused increasing membrane fluidity. El-Meleigy *et al*.,2010 found that *Staphylococcus aureus* is very sensitive to the most essential oils used, whereas no growth was obtained by using essential oils of cinnamon, corundum and marjoram.

Data in Table (5) showed the effectiveness of the tested treatments on aflatoxin B1and fumonisinB_1 inhibition. Results showed that the inhibition of aflatoxin B_1 was affected with cinnamon oil(0.5%) > cinnamon oil (1%) >cinnamon extract (0.5%) >dill oil (1%) > dill extract (0.5%) > basil extract (1.0%) > basil oil (0.5%) > dill extract (1%) > basil oil (1%) > basil extract (0.5%) > cinnamon extract (1%) > chitosan (1%) > chitosan (0.5%) >dill oil (0.5%) >acetic acid (1%) >acetic acid (0.5%).Our findings are in agreement with those of Bluma and Etcheverry,(2008), and Deabes *et al*.,(2012).They reported that the extent of inhibition of aflatoxin production and fungal growth depent on the type and concentration of essential oil used ,the type of mycotoxin and the fungal genera. These results are in harmony with those of Gujar and Talwankar, 2012 and El-Habib ,2012 who mentioned that dill oil is the most effective against aflatoxin production, while both EOs of thyme and basil delayed the growth of *A. flavus* and complete inhibition was observed at 150 µl /100 ml medium. The data also showed that the essential oils added to the culture media were an effective practical way to control the aflatoxin B_1 production and consequently to avoid its toxic effect.

For fumonisin B_1 inhibition: it can be observed from data that cinnamon oil (1%) > cinnamon oil (0.5%) > dill oil (0.5%) > dill oil (1%) > dill extract (0.5) = cinnamon extract (0.5) > chitosan (1%) > basil extract (0.5) > chitosan (0.5%) > basil extract(1%) >dill extract (1%) > cinnamon extract (1%) > basil oil(1%) > basil oil(0.5%) >acetic acid (1%) > acetic acid (0.5%).Our findings in fumonisin B_1 inhibition are in line with those of Fandohan *et al*.(2004) and Dambolena *et al*.(2010). They showed that *Ocimum basilicum* essential oil posses significant inhibitory effect on FB1 production (78%).Furthermore,our findings are coincided with Sumalan *et al*.(2013) who reported that cinnamon and dill essential oils showed inhibitory effect on fusaria and Aspergilla contaminated wheat seeds.

According to the resulted data in Table (3) , only chitosan has the same MIC in inhibiting the production of the two tested mycotoxins . The other treatments varied in their MIC values which indicated that these treatments differed in their action against the tested mycotoxins. This is in coincidence with those of Hammer *et al.* (1999), Juglal *elal*. (2002), Kalemba and Kunicha. (2003), and Sumalan *et al.* (2013) which announced that the essential oil's antimicrobial ability was dose-dependent and depends also on essential oils contents and its antioxidant properities.

Effectiveness of treatments as composite coating film on bacterial growth inhibition:

Results as shown in Table (4) illustrated that the efficacy of the complex film in inhibiting bacterial growth was more intense than the efficacy of each treatment alone, it may be due to a sorte of synergism between the tested treatments except for the complex film of cinnamon oil and chitosan in case of *S.aureus*. Results are relatively coincided with those of Rhim *et al*.,2006, and Mulyaningsih *et al*.,2010. Results also are in line with those of Dicko, 2010 ,Hylgaard *et al*.,2012 and Dhall,2013. They mentioned that in some cases, edible coatings were not successful. The success of edible coatings for fresh products totally depends on the composition of the used edible coating film as carriers of functional ingredients on fresh fruits and vegetables and includes also the incorporation of antimicrobials to maximize their quality and shelf life.

Effectiveness of treatments as composited coating film on tested mycotoxins inhibition:

Data in Table (5) showed that the coating film composited of chitosan and cinnamon oil was the best composite film in realizing the highest inhibition ratios for both tested mycotoxin followed by coating film composited of chitosan and dill extract or chitosan and cinnamon extract in case of AFB_1 . Only the last metioned composite film in case of FB_1. The film composited of chitosan and basil oil was the worst inhibitor for the two tested mycotoxin. Furthermore, most composited film exhibited a sort of antagonism comparing with individual coating film except the composited films of chitosan and cinnamon oil and chitosan and dill extract in case of AFB_1 and chitosan and cinnamon extract in case of FB_1 . Our findings are in agreement with Pereda *et al*.2012 and relatively in harmony with those of Pichersky *et al*.,2006, Ait-Ouazzou *et al*.,2011 and Dahll, 2013 who mentioned that in some cases between EOs, edible coatings were not successful.

Table (3): Effect of the tested treatments concentrations on aflatoxinB_1(AFB1) and fumonisinB_1(FB_1).

Treatment	Tested concentrations %	Tested Mycotoxins: AflatoxinB$_1$(AFB$_1$) Conc. /ppb	ER%	MIC	Fumonisin B$_1$(FB$_1$) Conc.	ER%	MIC
Control	0.0	N.D	------		N.D	------	
Positive Control	0.0	41.122 a	------		43.322 a	------	
Acetic acid	0.5	32.370 b	21.280	1	34.995b	19.221	1
	1	16.426 b	60.055		17.715b	59.108	
Chitosan	0.5	13.405 c	67.400	1	09.728c	77.545	1
	1	06.300 c	84.680		05.869 c	86.453	
Basil oil	0.5	04.574d	88.877	0.5	15.136 c	65.062	1
	1	04.681c	88.617		13.994 c	67.698	
Basil Extract	0.5	05.556d	86.489	1	08.731 c	79.846	0.5
	1	04.508c	89.037		10.143 d	76.587	
Dill oil	0.5	15.540 d	62.210	1	04.638 c	89.294	0.5
	1	04.250 c	89.665		04.969 e	88.530	
Dill Extract	0.5	04. 329 c	89.473	0.5	05.285 c	87.803	0.5
	1	04. 673 c	88.636		10.803 e	75.063	
Cinnamon oil	0.5	03.853 d	90.630	0.5	04.415 c	89.809	1
	1	03.909 c	90.494		04.185 e	90.340	
Cinnamon Extract	0.5	03.932 d	90.438	0.5	05.284 c	87.803	0.5
	1	05.817 c	85.854		11.235 c	74.066	
L.S.D.$_{0.5}$	Conc.0.5	2.470			2.139		
	Conc.1	2.375			2.214		

Where: ER= Efficacy ratio of each treatment.
MIC= Minimum inhibitory concentration of each treatment.

Table (4): Effect of the used treatments as composited coating film on the growth Escherichia coli and *Staphylococcus aureus*:

Treatment at theirMICs	*Escherichia coli* Total count (CFU per 3repl.)	CER%	IE.R%	AV.of IER+Chit ER	CFER (CER-IER)	K.T.EF.	*Staphylococcus aureus* Total count (CFU per 3repl.)	CER%	IE.R%	AV.of IER+Chit ER	CFER (CER-IER)	K.T.EF
Control	0.000	--			--		0.000	--	-	-		
Positive Control	632 X1o^6	0.000	0.000	0.000	--	----	599 X1o^6	0.000	0.000	0.000	0.000	------
chitosan +basil oil	10 X1o^6	98.418	49.683	70.253	48.735	Synerg.	170 X1o^6	71.619	61.770	67.03	9.849	Synerg.
chitosan +basil extract	138 X1o^6	78.164	55.063	72.942	23.101	Synerg.	71 X1o^6	88.147	60.60	66.445	27.547	Synerg.
chitosan +dill oil	142 X1o^6	77.532	62.342	76.582	15.19	Synerg.	61 X1o^6	89.816	58.264	65.277	31.552	Synerg.
chitosan +dill extract	43 X1o^6	93.196	60.601	75.712	32.595	Synerg.	115 X1o^6	80.80	66.778	69.534	14.022	Synerg.
chitosan+cinnamon oil	37 X1o^6	94.145	81.804	86.313	12.341	Synerg.	199 X1o^6	66.778	70.785	71.785	-4.007	Antago.
chitosan +cinnamon extract	13 X1o^6	97.943	57.595	74.209	40.348	Synerg.	116 X1o^6	80.634	79.967	76.128	0.667	Synerg.
L.S.D.$_{0.5}$	2.782						2.093					

Where: IER= individual efficacy ratio of each tested treatment.
CER = complex efficacy ratio of compound coating film.
Chit.ER= efficacy ratio of chitosan as shown in table(2).
K.T.EF.= Kind of each treatment effect.

Table (5): Effect of composited coats on stored infected maize grains with two mycotoxigenic fungi on aflatoxinB$_1$(AFB1) and fumonisinB$_1$(FB$_1$).

Treatments at their MICs	Tested Mycotoxins: AflatoxinB$_1$(AFB$_1$) Conc. /ppb	ER%	AV.of IER+ Chit ER	CFER (CER-IER	K.T.EF	Fumonisin B$_1$(FB$_1$) Conc./ ppm	ER%	AV.of IER+ Chit ER	CFER (CER-IER	K.T.EF.
Positive Control	41.694	0.0000	0.000	0.000		43.191	0.000	0.000	0.000	
Chitosan+ Basil oil	10.736	74.250	86.778	12.53	Antagon.	15.587	63.911	77.075	13.164	Antagon.
Chitosan+ Basil Extract	7.679	81.582	86.858	5.276	Antagon.	9.6515	77.654	83.149	5.495	Antagon.
Chitosan+ Dill oil	7.068	83.048	87.172	4.124	Antagon.	13.215	69.403	87.874	18.471	Antagon
Chitosan+ Dill Extract	5.180	87.576	87.075	-0.501	Additive	11.255	73.941	87.128	13.187	Antagon
Chitosan+ Cinnamon oil	3.662	91.217	87.655	-3.562	Synergis.	4.3149	90.010	88.397	-1.613	Synergis.
Chitosan+cinnamon Extract	5.312	87.259	87.559	0.3	Antagon.	5.0232	88.370	87.173	-1.197	Synergis.
L.S.D.$_{0.5}$	1.9835					2.9600				

Where: IER = individual efficacy ratio of each tested treatment
CER = complex efficacy ratio of compound coating film.
Chit.ER= efficacy ratio of chitosan as shown in table 4.
K.T.EF. = Kind of each treatment effect.

CONCLUSIONS

According to the obtained results it is concluded that:

- Chitosan individual coating film succeeded as antibacterial and detoxifying agent.
- The reduction rate of *E.coli* growth was the highest followed by Fumonisin B_1 then aflatoxin B_1production at the same MIC . On the opposite side, the reduction of *S.aureus* growth was the worst ratio at it's MIC value.
- All tested EOs individual coating films succeeded at their MICs as antibacterial and detoxifying agents.
- The composite coating films are more effective as antibacterial than detoxifying agents except the film composited of chitosan and cinnamon oil which was highly succeeded as detoxifier and antibacterial agent in case of *E.coli* .
- The use of composite film as antimicrobial and detoxifying agents are likely to grow steadily in the future because of greater consumer demands for foods and feed with the possibility of ain lossesdue gr tomicrobial infections and mycotoxins production during storage, our findings are considered a serious attempt to fill in the gap between the quantity produced and consumed by reducing grains wastage during storage.

It is recommended to use a film composited of chitosan and cinnamon oil because it achieves efficiency more than a simple or individual films . Futhermore, it could be considered composite film as an alternative sheap way to make modified atmosphere in silos and grain's store places and at the same time easy for handling ,discharging and transmission from ship to silos across sives.

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تأثير مدي فاعلية الأغلفة المأكولة المحيطة لحبوب الذرة والمكونة من الكيتوزان وبعض الزيوت النباتية على النمو البكتيرى للبكتيريا إشيريشيا كولاى وإستافيلوكوكس اوراس وإنتاج السموم الفطرية الافلاتوكسين ب١ والفيومنسين ب١ بواسطة كل من الإسبرجلس فلافس والفيوزاريوم فيرتسيلويدس

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الهدف من هذه الدراسة: إيجاد تقنية جديدة سهلة التطبيق وإقتصادية و تحافظ على الذرة المخزنة من بعض مصادر التلوث الحادث أثناء التخزين مثل النمو البكتيرى للبكتيريا إشيريشيا كولاى وإستافيلوكوكس اوراس وإنتاج السموم الفطرية الافلاتوكسين ب١ والفيومنسين ب١ مما يقلل نسبة الفقد فى الحبوب وبالتالى يقلل الفجوة الغذائية ويقلل خطر هذة الملوثات على صحة كل من الإنسان والحيوان بتقليل وصولها الى السلسلة الغذائية .

يتم تطبيق هذه التقنية بعمل غطاء أو فيلم للحبوب من مادة الكيتوزان والذى يحفظ الحبوب بعزلها عن الرطوبة والحرارة الموجودة فى المخزن وفى نفس الوقت يطهر الحبوب من أى ملوثات حيوية و يزود هذا الغطاء الذى يؤكل مع الحبوب بمستخلصات أو زيوت بعض النباتات الطبية والعطرية مثل الشبت والقرفة والريحان مما يزيد من القيمة الغذائية لهذه الحبوب ويتم اختبار هذه المعاملات كل على حدة على الملوثات المذكورة (كغلاف بسيط لحبوب الذرة) وعلى تركيزين ٠,٥% و١% وبعد التخزين لمدة ٦ أسابيع تم تعيين أقل تركيز مثبط لكل من النمو البكتيرى وانتاج السمان الفطريان

تم الإستعانة بعزلات معرفة للبكتيريا المذكورة وبعزلتين فطريتين هما إسبرجلس فلافس و فيوزاريوم فيرتسيلويدس منتجتين للسمين الفطريين المذكورين.

تم التأكد من خلو الحبوب من الملوثات محل الدراسة وذلك قبل إجراء التجربة.

تم تحضير عينات ١٠٠جم حبوب ذرة لكل معاملة وتغطية الحبوب بالتركيزات المختبرة وبعد التغطية والتجفيف تحت ظروف معقمةعلى ٤٥°م تم عدوى الحبوب بكل ملوث على حدة والتخزين للمدة المذكورة .

بعد تعيين أقل تركيز مثبط تم تحضير عينات ١٠٠جم حبوب ذرة لكل معاملة وتغطية الحبوب بغلاف مركب من الكيتوزان وأحد المعاملات الأخرى من زيوت أو مستخلصات النباتات العطرية المختبرة والتخزين لمدة ٦ أسابيع أخرى تحت الظروف المثالية لنمو كل بكتيريا وإنتاج السموم الفطرية محل الاختبار.

تمت دراسة تأثير إضافة هذه المواد مع بعضها من حيث حدوث تأثير تعاضدى أو مضاف أو مضاد وأثبتت النتائج ان التأثير التعاضدى كان غالبا فى حالة تثبيط البكتيريا أما التأثير المضاد فقد كان غالبا فى حالة تثبيط إنتاج السموم الفطرية ما عدا الغلاف المركب من الكيتوزان وزيت القرفة الذى اعطى افضل النتائج فى تثبيط كل من نموالإشيريشيا كولاى وسمي الافلاتوكسين ب١ والفيومنسين ب١ وذلك بالنسب ١٤٥, ٩٤%و٩١,٢١٧%و٩٠,٠١% على التوالى تلاه الغلاف المركب من الكيتوزان ومستخلص القرفة والذى كانت نتائج تثبيطه لكل من نموالإشيريشيا كولاى وسمي الافلاتوكسين ب١ والفيومنسين ب١ وذلك بالنسب٩٧,٩٤٣%و٨٧,٢٥٩%و٨٨,٣٧٠% على التوالى بينما كان للغلاف المركب من زيت الريحان والكيتوزان فاعلية كبيرة على تثبيط نموالإشيريشيا كولاى بنسبة٩٨,٤١٨ %.

لذلك فإننا نوصى بإستخدام الغلاف المركب من الكيتوزان وزيت القرفة لأنه فى حالة الذرة يكون مثاليا.لا سيما أن إحتمال إصابة الذرة بالإستافيلوكوكس أوراس يكون نادرً إذا لم يتواجد ملوث له فى المخزن.