Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(4): 3728-3737. **OPEN ACCESS**

Essential oils, organic acids and salts treatments for inhibiting mycotoxigenic fungal invasion to maize grains under storage conditions

Mokhtar Mohamed Abdel-Kader*, Nehal Samy El-Mougy and Mohamed Saied Ali Khalil

*Plant Pathology Dept., National Research Centre, Giza 12622, **Egypt.**

***Correspondence: mokh_nrc@yahoo.com** **Received: 24-09-2019, Revised: 18-11-2019, Accepted: 29-11-2019 e-Published: 26-12-2019**

A study was undertaken to screen the efficacy of some essential oils (carnation, lemongrass), organic acids (Salicylic, Ascorbic) and organic salt potassium sorbate that can inhibit fungal invasion to artificially inoculated Maize grains cultivars with *Aspergillus flavus* and *Aspergillus niger* stored for 30 days. It was observed that fungal infection reduced depending on the used treatments of essential oil, organic acid, salt as well as their concentration. Also, it was observed that the fungal infection of treated grains differed significantly compared with untreated ones. Moreover, the percentage of Maize grains infection was decreased by increasing the concentration used of each treatment. The highest percentage of infected Maize grains was observed at carnation oil followed by lemongrass oil and ascorbic acid treatments for both yellow (cv. M84) and white (Giza310) cultivars. Announced low infection was recorded at concentration of 2.0% of salicylic acid for both Maize cultivars with either *A. flavus* or *A. niger.*

Keywords: Essential oils, organic acids, organic salt, mycotoxigenic fungi, stored maize grains.

INTRODUCTION

Maize (*Zea mays* L.) as a monocotyledonous angiosperm plant that belongs to the grass family (Poaceae) (Park, 2001) is cultivated representing proportion for the use of the world's population (Anderson et al., 2004). In Egypt, maize plays a very significant role in grain production. Maize diseases caused considerable losses in its productivity worldwide (Oerke, 2005). These losses were estimated as 4% from Northern Europe and 14 % from West Africa and South Asia (Balint-Kurti and Johal, 2009). Maize diseases that affect roots, stalks, ears, and kernels are caused by fungi (White, 2000). In all countries wherever corn is grown, ear rot is one of the most important diseases. Xiang et al. (2010) reported that the fungi *Aspergillus*, *Fusarium* and *Gibberella* ear are the most predominant types of maize ear rot diseases in the world. In many parts of the world contaminated agricultural products, *e.g*. sorghum, corn, and wheat grains with mycotoxin have become a natural phenomenon. Favorable environmental conditions in parallel to convention cultivation method, harvesting, handling could lead to mycotoxigenic fungal invasion to those products and subsequently mycotoxin production (Soares and Rodriguez Amaya, 1985).

Aspergillus is considered as one of the most economically important of the fungal genera. Some of fungal isolates are used for the production of soy sauce through food fermentation processes, and production of several organic acids and enzymes in pharmaceutical and biotechnological industries and also biologically active metabolites as lovastatin (Varga et al., 2008). *Aspergillus niger* and *A. flavus* are the most regular fungal pathogens attacking plants, and causing various diseases in susceptible plants. A small number of *Aspergillus* spp. can attack plant tissues, and mostly observed as contaminants of post-harvest agricultural products. Aspergilli can taint horticultural items at various stages, including harvest, preparing and handling. The most significant part of nourishment decay brought about by these fungi is forming mycotoxins, which may affect human and farm animal health. Mycotoxins as Aflatoxins, ochratoxins, fumonisins and patulin were recorded to contaminate various agricultural products and identified as economically important. Under storage conditions Maize grains could be considered favorable medium for mycotoxigenic fungi to grow, develop and activity as well. (Lacey, 1990) such as *Aspergillus flavus* and *Aspergillus niger*. Such fungi invade grains and subsequently change their chemical and nutritional characteristics, reduce their germination and produce mycotoxins, which are toxic to man and animals (Bennett and Klich, 2003). The health and environmental problems due to intensive use of synthetic fungicides in agriculture led to intensification of efforts to find safe, effective alternatives. In such manner plant-based fungicides can be less harmful to man, promptly biodegradable, suitable for use and capable of protecting the cultivated crops. One of such alternatives is the use of natural plant preservatives such as essential oils, organic acids and salts to prevent the development of fungi and protect food from decay

In this concern, the present investigation was designed to screen the prevention effect of some essential oils, organic acids and salts against mould fungal invasion and development to maize grains under storage conditions.

MATERIALS AND METHODS

Grains and mycotoxigenic organisms

Freshly harvested white and yellow maize grains of local varieties, yellow (cv. M84) and white (cv. Giza310) obtained from the Agriculture Research Centre (ARC), Giza, Egypt were used in the present investigation. Maize grain samples analyzed using Infrared Grain Analyzer 1241, Foss Infratec TM 1241 Analyzer, Sweeden as stated in Table (1).

The strains of *Aspergillus flavus, Aspergillus niger* previously isolated from maize grains obtained from ARC, Egypt and tested for their mycotoxin production at Toxins **&** Food Pollutants Dept., National Research Centre, Egypt.

Table 1: percentage of some constituents of maize grains

Maize cultivars	percentage of some constituents (%) of maize grains			
	oil	protein	starch	moisture
Yellow (M84)	4.5	8.3	71.2	11.4
White (Giza 310)	4.0	10.2	70.5	11.0

One isolate of each *A. flavus* and *A. niger* among tested Apergllі proved to be high aflatoxins B_1 and B_2 producers were used in present study as test microorganisms. The two fungal cultures were then grown on Sabouraud dextrose agar (Difco, Detroit, MI) plate at 25°C for 10 days. Ten milliliters of sterilized distilled water supplemented with1% Tween 20 were added for spore's collection. The spore suspensions were further adjusted with sterile distilled water to give a final concentration of 10^6 spores/ml. Spore concentration was determined with the aid of a haemocytometer slide. The suspensions were stored at 4°C until used.

Tested materials

Essential oils, carnation and lemongrass oils and organic acids, salicylic and ascorbic as well as the salt potassium sorbate at concentrations of 0.25, 0.5, 1.0 and 2% (v:v or w:v with distilled water) were used. A volume of 1% Tween 20 was added to essential oils to obtain emulsifier solution). All tested materials were prepared at high concentration and stored into black bottles in refrigerator (4°C) until used. Referring to previous work (unpublished data), the tested essential oils, organic acids and salt were evaluated for their growth inhibitor effect against the same isolates of *A. flavus* and *A. niger*. In that work, superior complete inhibition in fungal growth was observed at concentration of 1 and 2% of both Ascorbic acid and Potassium sorbate. Meanwhile, complete inhibition of fungal growth was observed at 1% of *Aspergillus flavus* and *A. niger* of tested lemongrass and carnation essential oils. Therefore, the tested concentrations from 0.25 up to 2% of used materials were based on these previous results.

Maize grains inoculation and treatment

Maize gains distributed in several lots each of 200g quantities into tightly stopper glass bottles and sterilized for 20 min at 121°C in autoclave. Then, autoclaved maize grains treated separately with individual essential oil or organic acids and salts at certain proposed concentrations and shacked thoroughly to ensure even distribution of added tested agents. Two days after, all treated maize grains were inoculated individually with 2 ml (10^6 spores/ml) of conidial suspension of both tested fungi. The final moisture content of the grains was reconstituted to 17% by adding sterile distilled water. The bottles containing maize grains were incubated at ambient temperature (25±2°C) for 30 days then examined. A set of either untreated & inoculated or untreated & un-inoculated samples were used as control treatments.

Evaluation of fungal infection

Maize grains samples, in triplicate, were tested for grains infection by the inoculated fungi after 30 days of storage by the method used by Chatterjee (1990).

For each treatment, approximately 50 surface-sterilized (by 2% NaOCl) grains were plated aseptically on autoclaved PDA plates (22 cm-diameter) supplemented with the antibiotic chloramphenicol. After 7 days incubation at 25± 2°C, the percentages of infected grains were recorded.

Statistical Analysis

The obtained data were subjected to IBM SPSS software version 14.0. Analysis of variance was determined and the mean values were compared by Duncan's multiple range test at $P < 0.05$.

RESULTS

The present investigation was concerned to screen the efficacy of some essential oils, organic acids and salts that can inhibit fungal invasion to maize grains under *in vivo* conditions. Essential oils, carnation and lemongrass oils and organic acids, salicylic and ascorbic as well as the salt potassium sorbate at concentrations of 0.25, 0.5, 1.0 and 2% were used. The obtained results showed that during storage no fungal infection was observed in the un-inoculated and untreated grains (control samples). Meanwhile, observation of fungal infection was detected within 30 days for the untreated inoculated grains. Data in Tables (2, 3) and Fig (1) showed the inhibition percentages of fungal infection. Also, the recorded fungal infection showed positive reduction related to the used essential oil, organic acid, salt as well as their concentrations. Therefore, the percentage of Maize grains infection was decreased by increasing the concentration used of each treatment (Table 2). The highest percentage of infected Maize grains was observed at carnation oil treatment, whereas, yellow Maize grains (cv. M-84) recorded an infection with *Aspergillus flavus* and *Aspergillus niger* at a range of 60.0-76.7%, 60.0-73.4% at the used concentrations, respectively. Meanwhile, white Maize grains (cv Giza 310) revealed a range 63.4-76.7% and 60.0%-73.4% of grains infected with *A. flavus* and *A. niger*, in respective order.

These figures followed by lemongrass oil and ascorbic acid. Lower Maize grains infection was observed at salicylic acid and potassium sorbate treatments. Announced low infection was recorded at concentration of 2.0% of salicylic acid for both *A. flavus* and *A. niger* whereas 3.4%, 13.4% and 10.0%, 16.7% recorded for the two Maize cultivars, M-84 and Giza 310, in respective order. Meanwhile, it was observed that the fungal infection differed significantly in treated grains compared with untreated ones. On the other hand, data in Table (3) revealed that the reduction percentage of Maize grains fungal infection was observed in ascending order in parallel to rising concentration from 0.25% up to 2.0% of each treatment. Also, data in Table (3) showed the highest effect of salicylic acid for reducing Maize grains infection.

It causes reduction in infection calculated as a range of 40.0%, 43.3% up to 96.6%, 86.6% for the yellow Maize cultivar M-84. As well as from 46.6%, 53.3% up to 90.0%, 83.3% for the white Maize cultivar Giza 310, arranged from the lowest (0.25%) to the highest (2.0%) concentration used, respectively. The other tested treatment followed the similar trend concerning potassium sorbate, ascorbic acid, carnation and lemongrass oils, respectively.

Furthermore, illustrated data in Fig. (1) showed the mean reduction of the tested treatments. Mean reduction in Maize grains invasion with *A. flavus* and *A. niger* calculated in descending order as 63.3, 51.6, 44.1, 39.9, 29.9% and 68.2, 51.6, 44.1, 40.8, 33.3% for yellow Maize cultivar M-84 at concentrations 0.25, 0.5, 1.0 and 2.0% of salicylic acid, potassium sorbate, ascorbic acid, lemongrass and carnation oils, respectively. For white Maize cultivar, another arrangement was observed. Maize grains invasion with *A. flavus* and *A. niger* calculated as 67.4, 50.0, 43.3, 39.9, 30.7% and 66.6, 54.9, 46.6, 45.8, 31.6% at the same previous concentrations of salicylic acid, potassium sorbate, lemongrass, ascorbic acid and carnation oils, in relevant order.

Table 2: Fungal invasion (%) to maize grains treated with essential oil, organic acids and salt stored for 30 days

Tested materials	Concentration %	Fungi			
		Aspergillus flavus		*Aspergillus niger*	
		Maize cultivar			
		Yellow (c.v M-84)	White (c.v Giza310)	Yellow (c.v M-84)	White (c.v Giza310)
Carnation oil	0.25	76.7±15.2 b	76.7 ± 11.5b	73.4 ± 11.5b	73.4 ± 11.5b
	0.5	73.4 ±15.2b	73.4 ± 11.5b	66.6 ± 15.2c	70.0 ± 10.0b
	1.0	70.0 ± 10.0b	63.4 ± 15.2c	66.6 ± 15.2c	70.0 ± 10.0b
	2.0	60.0 ± 10.0c	63.4 ± 15.2c	60.0 ± 20.0c	60.0 ± 20.0c
Lemongrass oil	0.25	63.4 ± 15.2c	60.0 ± 10.0c	63.4 ± 15.2c	53.4 ± 15.2d
	0.5	56.7 ± 15.2d	53.4 ± 15.2d	56.7 ± 15.2d	46.7 ± 15.2e
	1.0	60.0 ± 20.0c	66.3 ± 15.2c	56.7 ± 15.2d	60.0 ± 10.0c
	2.0	60.0 ± 15.2c	46.7 ± 15.2e	56.7 ± 11.5d	53.4 ± 20.8d
Salicylic acid	0.25	60.0 ± 10.0c	53.4 ± 15.2d	56.7 ± 15.2d	46.7 ± 15.2e
	0.5	56.7 ± 15.2d	56.7 ± 11.5	43.4 ± 11.5e	40.0 ± 17.3e
	1.0	26.7 ± 15.2g	20.0 ± 10.0g	13.6 ± 15.2h	30.0 ± 20.0f
	2.0	3.4 ± 5.7hi	10.0 ± 10.0h	13.4 ± 15.2h	16.7 ± 15.2h
Ascorbic acid	0.25	63.4 ± 5.7c	63.4 ± 15.2c	56.7 ± 11.5d	60.0 ± 20.0c
	0.5	56.7 ± 15.2d	60.0 ± 20.0c	53.4 ± 20.8d	56.7 ± 20.8d
	1.0	53.4 ± 15.2d	56.7 ± 15.2d	56.7 ± 20.8d	50.0 ± 20.0d
	2.0	50 .0 ± 20.0d	60.0 ± 10.0c	56.7 ± 20.8d	50.0 ± 20.0d
Potassium sorbate	0.25	63.4 ± 20.8c	60.0 ± 10.0c	63.4 ± 15.2c	60.0 ± 20.0c
	0.5	53.4 ± 15.2d	50.0 ± 20.0d	50.0 ± 20.0d	53.4 ± 20.8d
	1.0	40.0 ± 15.2e	50.0 ± 20.0d	46.7 ± 15.2e	40.0 ± 10.0e
	2.0	36.7 ± 15.2f	40.0 ± 10.0e	33.3 ± 15.2f	26.7 ± 15.2g
Control (Autoclaved grains)	inoculated	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
	Un-inoculated	0.0 ±0.0i	0.0 ±0.0i	0.0 ±0.0i	0.0 ±0.0i

Means ± standard deviations within a column followed by the same letter are not significantly different by Duncan multiple range test at $P < 0.05$.

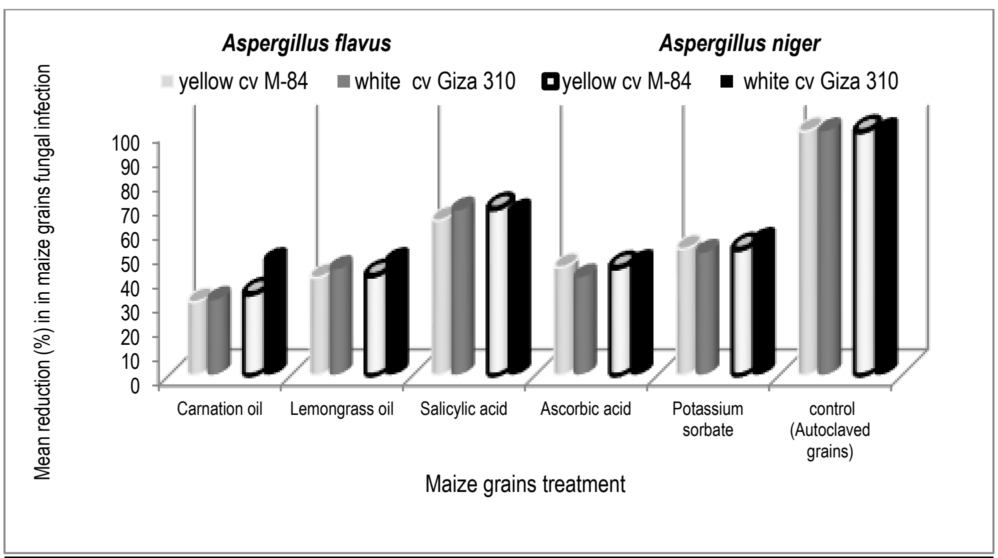

Figure 1: Mean reduction (%) of fungal infection in Maize grains treated with essential oil, organic acids and salt stored for 30 days

Table 3: Reduction in the fungal infection to maize grains treated with essential oil, organic acids and salt stored for 30 days

Tested materials	Concentration %	Fungi			
		Aspergillus flavus		*Aspergillus niger*	
		Maize cultivar			
		Yellow (c.v M-84)	White (c.v Giza310)	Yellow (c.v M-84)	White (c.v Giza310)
Carnation oil	0.25	23.3 ±15.2 i	23.3 ± 11.5 i	26.6 ± 11.5 i	26.6 ± 11.5 i
	0.5	26.6 ± 15.2 i	26.6 ± 11.5 i	33.3 ± 15.2 h	30.0 ± 10.0 h
	1.0	30.0 ± 10.0 h	36.6 ± 15.2 h	33.3 ± 15.2 h	30.0 ± 10.0 h
	2.0	40.0 ± 10.0 g	36.6 ± 15.2 h	40.0 ± 20.0 g	40.0 ± 20.0 g
	Mean	29.9	30.7	33.3	31.6
Lemongrass oil	0.25	36.6 ± 15.2 h	33.3 ± 15.2 h	36.6 ± 15.2 h	40.0 ± 10.0 g
	0.5	43.3 ± 15.2 g	40.0 ± 10.0 g	43.3 ± 15.2 g	40.0 ± 10.0 g
	1.0	40.0 ± 20.0 g	46.6 ± 15.2 g	43.3 ± 15.2 g	46.6 ± 20.8 g
	2.0	40 .0 ± 15.2 g	53.3 ± 15.2 f	43.3 ± 11.5 g	53.3 ± 15.2 f
	Mean	39.9	43.3	40.8	46.6
Salicylic acid	0.25	40.0 ± 10.0 g	46.6 ± 15.2 g	43.3 ± 15.2 g	53.3 ± 15.2 f
	0.5	43.3 ± 15.2 g	53.3 ± 11.5 f	56.6 ± 11.5 f	60.0 ± 17.3 e
	1.0	73.3 ± 15.2 d	80.0 ± 10.0 bc	86.6 ± 15.2 bc	70.0 ± 20.0 d
	2.0	96.6 ± 5.7 b	90.0 ± 10.0 b	86.6 ± 15.2 bc	83.3 ± 15.2 bc
	Mean	63.3	67.4	68.2	66.6
Ascorbic acid	0.25	36.6 ± 5.7 h	36.6 ± 15.2 h	43.3 ± 11.5 g	40.0 ± 20.0 g
	0.5	43.3 ± 15.2 g	40.0 ± 20.0 g	43.3 ± 20.8 g	43.3 ± 20.8 g
	1.0	46.6 ± 15.2 g	43.3 ± 15.2 g	43.3 ± 20.8 g	50.0 ± 20.0 f
	2.0	50 .0 ± 20.0 f	40.0 ± 10.0 g	46.6 ± 20.8 g	50.0 ± 20.0 f
	Mean	44.1	39.9	44.1	45.8
Potassium sorbate	0.25	36.6 ± 20.8 h	40.0 ± 20.0 g	36.6 ± 15.2 h	40.0 ± 20.0 g
	0.5	46.6 ± 15.2 g	50.0 ± 20.0 f	50.0 ± 20.0 f	46.6 ± 20.8 g
	1.0	60.0 ± 15.2 e	50.0 ± 10.0 f	53.3 ± 15.2 f	60.0 ± 10.0 e
	2.0	63.3 ± 15.2 e	60.0 ± 10.0 e	66.6 ± 15.2 e	73.3 ± 15.2 d
	Mean	51.6	50.0	51.6	54.9
Control (Autoclaved grains)	inoculated	100 ± 0.0 a	100 ± 0 a	100 ± 0.0 a	100 ± 0.0 a

Means ± standard deviations within a column followed by the same letter are not significantly different by Duncan multiple range test at $P < 0.05$

DISCUSSION

The efficacy of carnation and lemongrass essential oils, organic acids, salicylic and ascorbic as well as the salt potassium sorbate at different concentrations were evaluated against Maize grains fungal invasion with either *Aspergillus flavus* or *Aspergillus niger* under aseptic *in vivo* conditions in post-harvest stored maize grains for 30 days. A significant inhibition of fungal infection to treated grains compared with untreated ones was observed. Also, it was noticed that the reduction of fungal infection was increased positively affected with the tested agents of essential oil, organic acid, salt and their concentrations as well. Moreover, the percentage of Maize grains infection was decreased by increasing the concentration used of each treatment.

In this regards, the antifungal activities of essential oils were studied by several workers. They found that these activities are related to their chemical constituents which known to have antifungal properties (Didry et al., 1993; Viollon and Chaumont 1994; Periago et al., 2001). Essential oils were reported to play an economic role in many industries such as food, pharmaceutical and perfumery. Variation in the quantity and quality of essential oils and their chemical composition and biological properties were subjected to a number of studies which has been increasing recently (Simões et al., 2003). Patkar et al. (1995) stated that one gram of rice grains mixed with 9µl of cinnamon oil could prevent the fungal growth and the mycotoxins production as well. Also, Kaur and Arora (1999) reported that some Plant products which had antimicrobial spectrum and used as food preservatives systems are directly used as main antimicrobial compounds or indirectly for improving the action of other antimicrobial compounds. Recently some studies explained the mode of action of some essential oils against microbial microorganisms through their effect on permeability and disruption the cell membranes of bacteria and fungi (Cox et al., 1998; Lambert et al., 2001; Bennis et al., 2004). Moreover, some plants contain compounds acting as suppressing factor against microbial growth (Naqui et al., 1994). These compounds differed in their structures and their mode of action compared with traditional antimicrobials (Nascimento et al., 2000). Also, such compounds are synthesizing Alkaloids, flavonoids, isoflavonoids, tannins, cumarins, glycosides, terpens, phenylpropannes, organic acids (Nychas, 1996). Generally, volatile, lipophilic, liquid and odiferous substances were found as complex mixtures and described as volatile oils, ethereal oils and essences (Simões et al., 2003). The mode of action on mould fungi by essential oils involves cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intercellular and extracellular enzymes which could take place directly or indirectly with mycelium germination inhibition (Campo et al., 2003). Also, it is reported that plant lytic enzymes act in the fungal cell wall causing breakage of b-1,3 glycan, b-1,6 glycan and chitin polymers (Brull and Coote, 1999). It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability and unavailability of vital intracellular constituents (Juven et al., 1994). Moreober, Kim et al. (1995) indicated that essential oils containing carvacrol, eugenol and thymol (phenolic compounds) had the highest antibacterial performances.

Since the mould fungus *A. flavus* causes serious damage to agricultural products, therefore, effective control against the pathogen is essential to be found. Moreover, control methods should not have side effects neither on human health nor the environment. Recently, there are some natural products having antifungal effects and used effectively in seeds or grains, fruits and vegetables storage. In this concern, some preservatives such as different organic acids and their salts that had antibacterial and antifungal properties are successfully used in the food-processing industry and therefore also could be useful to be recommended as postharvest treatment against decay control of agricultural products (Al-Zaemey et al., 1993; Olivier et al., 1999). In the same manner, several investigators reported that postharvest diseases of tomato, apple, carrots and potato ware controlled by using organic salts such as sodium benzoate or potassium sorbate (Ryu and Holt, 1993; Saleh and Huang, 1997; Oliver et al., 1998).

Hall (1988) found similar fungicidal activity against citrus green mould when fruits artificially inoculated with green mould incident *Penicillium digitatum* and treated with potassium sorbate or sodium benzoate. He concluded that postharvest citrus fruit decay could be controlled by using these food preservatives. Furthermore, the use of organic acids and salts for controlling various fungal diseases in plants during vegetative growth or at postharvest and storage was reported. Salicylic acid as an organic acid is a natural compound present in many plant tissues. It is known as a phytohormone (Hayat and Ahmad, 2007) since it plays a vital role in important physiological processes such as photosynthesis (Khodary, 2004), growth, development and ion uptake (Glass, 1973). However, induction systemic acquired resistance (SAR) in different plant tissues is considered the most critical role of salicylic acid (Amborab´e et al., 2002).

In general, several authors investigated the effect of organic acids, *i.e*. salicylic and ascorbic acids, against fungal growth which contaminates food and feed. Acidic medium as a result of organic acid application affects the fungal cell membrane permeability and the activity of enzymes responsible for degrading the substrate (Kristiansen and Sinclair, 1979). The effect of organic acids against microbial growth was tested by many researchers. They recorded reduction in cytoplasmic pH and suppression in metabolic activities by these organic acids and finally death by the susceptible organisms was observed. Moreover, Dalie et al. (2010) stated that organic acids act on the plasmic membrane by changing its electrochemical properties and increasing its permeability. A greater concentration of protons is resulted due to the reduction of pH which increasing the diffusion of acid across the plasmic membrane and cytoplasm. Therefore, the need to continue acid solubility with the need to achieve maximal activity by pH reduction should be balanced (Lopez et al., 2012; Pelaez et al., 2012). Generally, the acidity phenomenon is considered the mechanism of organic acids for inhibiting the fungal growth. It is well established that fungal growth and its morphology are influenced by the acidity of media (Higgins and Brinkhaus, 1999). Explain of the inhibitory mode of organic acids have been suggested as various mechanisms. The most reported explanation was the decreasing in pH value that influence the growth by acidifying the cell, which will consume a great amount of energy to maintain the intracellular pH homeostasis (Kang et al., 2003). The other proposed explanations include the membrane disruption, the interruption of metabolic reactions, and the accumulation of toxic anions.

In the present study salicylic acid and potassium sorbate showed superior effect against *A. flavus* and *A. niger* Maize infection percentage compared with ascorbic acid and carnation or lemongrass oils. Our results are agreement with other previous reports which demonstrated the ability of similar natural products for suppressing microbial strains (Rusul and March, 1987; Sofos, 1992; Sebti and Tantaoui-Elaraki, 1994; Stead, 1995). Also, Olivier et al. (1998) reported that the growth and sporulation of *Helminthosporium solani* were effectively inhibited by using organic and inorganic salts. Also, Al Zaemey et al. (1993) recorded that growth of the banana pathogen *Colletotricum musae* completely suppressed *in vitro* using the food preservatives, sorbic acid and potassium sorbate (Al Zaemey et al., 1993). Sebti and Tantaoui-Elaraki (1994) reported that sorbic acid was able to inhibit the growth of 15 different fungi isolated from ‘‘Pastilla’’ papers, a wheat-based dough. Many explanations were reported regarding the mode of action of organic acids or their salts against pathogenic microorganisms. in this manner, sorbic acid and its salts was found to cause inhibition of microorganisms by several ways, 1) alternate the function of cell-permeability function, 2) inhibit the enzymes which involved in the glycolytic pathway or tricarboxylic acid cycle by suppressing RNA, DNA, 3) synthesis of protein, 4) uncoupling of the oxidative phosphorylation in mitochondria (Sofos et al., 1986; Sofos, 1992). Also, it was recorded that conidial spores of different moulds fungi exposed to sorbic acid exhibited reduction in quantity of ATP (Cheng and Piper, 1994).

CONCLUSION

In the present study, the recorded reduction of fungal development in maize grains during storage in response to tested essential oils as well as organic acids and salts suggest their use as phytochemical compounds to control post-harvest decay fungi. Further studies for characterization the active compounds, definition and evaluation their toxicity and feasibility are needed for these biological agents. Also, it should be taken in consideration that future researches is needed to better understanding the ways of contamination the raw fruits and vegetables by pathogens and moreover the mechanisms for eliminate them once they are present, either on the surface or in internal tissues.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

This research was supported in part by In-House Project No. 11030132 of National Research Centre, Egypt entitled “Integrated Management of Diseases Affecting Maize Crop during Vegetative Growth and Storage Periods”

AUTHOR CONTRIBUTIONS

MMA designed and performed the experiments and also wrote the manuscript. NSE and MSAK performed storage experiment, follow isolation and bio-assay Lab. experiments, and data analysis. MMA, NSE and MSAK shared in designed experiments and reviewed the manuscript. All authors read and approved the final version.

REFERENCES

Al-Zaemey, AB, Magan, N, Thompson, AK. 1993. Studies on the fruit coating polymers and organic acid on growth of *Colletotrichum musae in vitro* and postharvest control of anthracnose of bananas. Mycological Research 97: 1463 – 1468. https://doi.org/10.1016/S0953-7562(09)80218-9

Amborab´e, BE, Fleurat-Lessard, P, Chollet, JF, Roblin, G. 2002. Antifungal effects of salicylic acid and other benzoic acid derivatives towards *Eutypa lata*: structure–activity relationship. Plant Physiology and Biochemistry 40:1051–1060. https://doi.org/10.1016/S0981-

9428(02)01470-5

Anderson, PK, Cunningham, AA, Patel, NG, Morales, FJ, Epstein, PR, Daszak, P. 2004. Emerging infectious diseases of the plants: pathogen pollution, climate change and agrotechnology drivers. Trends in Ecology and Evolution 19:535-544. DOI: 10.1016/j.tree.2004.07.021

Arora, D, Kaur, J. 1999. Antimicrobial activities of species. International Journal of Antimicrobial Agents 12: 257–262. https://doi.org/10.1016/S0924-8579(99)00074-6

Balint-Kurti, PJ, Johal, GS. 2009. Maize Disease Resistance. (eds.), Handbook of Maize: Its Biology, 229 DOI: 10.1007/978-0-387-79418-1-12

Bennett, JW, Klich, M. 2003. Mycotoxins. Clinical Microbiology Review 16: 497-516. DOI: 10.1128/cmr.16.3.497-516.2003

Bennis, S, Chami, F, Chami, N, Bouchikhi, T, Remmal, A. 2004. Surface alteration of *Saccharomyces cerevisiae* induced by thymol and eugenol. Letters in Applied Microbiology 38: 454–458. DOI:10.1111/j.1472-765X.2004.01511.x

Brull, S, Coote, P. 1999. Preservative agents in foods: mode of action and microbial resistance mechanisms. International Journal of Food Microbiology 50: 1–17. DOI:10.1016/s0168-1605(99)00072-0

Campo, J, Nguyen-The, C, Sergent, M, Amito, MJ. 2003. Determination of most bioactive phenolic compounds from rosemary against *Listeria monocytogenes*: influence of concentration, pH and NaCl. Journal of Food Science 68: 2066–2071.https://doi.org/10.1111/j.1365-2621.2003.tb07019.x

Chatterjee, D. 1990. Inhibition of fungal growth and infection in maize grains by spice oils. Letters in Applied Microbiology 11(3): 148-151. https://doi.org/10.1111/j.1472-765X.1990.tb00146.x

Cheng, L, Piper, PW. 1994. Weak acid preservation block the heat shock response and heat shock-element-directed lacZ expression pf low pH *Saccharomyces cerevisiae* cultures an inhibitory action partially relieved by respiratory deficiency. *Microbiology* 140:1085- 1096. DOI: 10.1099/13500872-140-5-1085

Cox, SD, Gustafson, JE, Mann, CM, Markham, JL, Liew, YC, Hartland, RP. 1998. Tea tree oil causes K+ leakage and inhibits respiration in *Escherichia coli*. Letters in Applied Microbiology 26, 355–358. DOI:10.1046/j.1472-765x.1998.00348.x

Dalie, DKD, Deschamps, AM, Forget, FR. 2010. Lactic acid bacteria –potential for control of mold growth and mycotoxins: A review, Food Control 21:370–380. https://doi.org/10.1016/j.foodcont.2009.07.011

Didry, N, Dubreuil, L, Pinkas, M. 1993. Activité antibactérienne du thymol, du carvacrol, et de l'aldhehyde cinnamique seuls ou associés. *Pharmazie* 48: 301–304. http://europepmc.org/abstract/med/8321882

Glass, ADM. 1973. Influence of phenolic acids on ion uptake. I. Inhibition of phosphate uptake. Plant Physiology 51:1037–1041. https://www.jstor.org/stable/4263267

Hall, DJ. 1988. Comparative activity of selected food preservatives as citrus postharvest fungicides. Proceedings of Florida State Horticulture Society 101:184 – 187. http://www.hdhagriproducts.com/pdfs/oldpub-david-hall-passed/FoodPreservatives88.pdf

Hayat, S, Ahmad, A. 2007. *Salicylic Acid: A Plant Hormone*. Springer, Berlin. https://www.springer.com/gp/book/9781402051838

Higgins, C, Brinkhaus, F. 1999. Efficacy of several organic acids against molds. Journal of Applied Poultry Research 8:480-487. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.880.8281&rep=rep1&type=pdf

Juven, BJ, Kanner, J, Sched, F, Weisslowicz, H. 1994. Factors that interact with the antibacterial of thyme essential oil and its active constituents. Journal of Applied Microbiology 76: 626–631. DOI:10.1111/j.1365-2672.1994.tb01661.x

Kang, HC, Park, YH, Go, SJ. 2003. Growth inhibition of a phytopathogenic fungus, *Colletotrichum* species by acetic acid. Microbiological Research 158: 321–326. DOI:10.1078/0944-5013-00211

Khodary, SEA. 2004. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants. International Journal of Agriculture and Biology6:4-8. https://pdfs.semanticscholar.org/7841/ae6488ae777eb390b04bae635cb3ccc8029b.pdf

Kim, J, Marshall, MR, Wei, C. 1995. Antibacterial activity of some essential oils components against five foodborne pathogens. Journal of Agricultural and Food Chemistry 43: 2839–

2845. https://doi.org/10.1021/jf00059a013

Kristiansen, B, Sinclair, CG. 1979. Production of citric acid in continuous culture. Biotechnology and Bioengineering 11: 279-315. **https://doi.org/10.1002/bit.260210214**

Lacey, J. 1990. Mycotoxins in UK Cereals and their control. Aspects of Applied Biology 25: 395405https://www.cabdirect.org/cabdirect/abstract/19922313968

Lambert, RJW, Skandamis, PN, Coote, PJ, Nychas, GJE. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of Applied Microbiology 91: 453–462. https://pdfs.semanticscholar.org/32fc/a7925bb62e5a622a4f51f8b73e95e0da6500.pdf

Lopez, EM, Garcia, HS, Malo, AL. 2012. Organic acids as antimicrobials to control *Salmonella* in meat and poultry products. Food Research International 45: 713–721. https://doi.org/10.1016/j.foodres.2011.04.043

Nascimento, GG, Locatelli, J, Freitas, PC. 2000. Antibacterial activity of plants extracts and phytochemical on antibiotic resistant bacteria. Brazilian Journal of Microbiology 31: 247–256http://dx.doi.org/10.1590/S1517-83822000000400003

Nychas, GJE. 1996. Natural antimicrobial from plants. p. 235–258. In: “New Methods of Food Preservation”. Gould G.W. (Ed.). CRC Press, Londres, pp:235-258.

Oerke, E. 2006. Crop losses to pests. The Journal of Agricultural Science 144(1): 31-43. DOI: https://doi.org/10.1017/S0021859605005708

Olivier, C, Halseth, DE, Mizubuti, ESG, Loria, J. 1998. Postharvest application of organic and inorganic salts for suppression of silver scurf on potato tubers. Plant Disease 82:213 – 217.https://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS.1998.82.2.213

Olivier, C, Macneil, CR, Loria, J. 1999. Application of organic and inorganic salts to field-grown potato tubers can suppress silver scurf during potato storage. Plant Disease 83:814 – 818. Doi: 10.1094/PDIS.1999.83.9.814.

Park, KJ. 2001. Corn Production in Asia: China, Indonesia, Thailand, Philippines, Taiwan, North Korea, South Korea, and Japan. ASPAC Food & Fertilizer Technology Center, Taipie. https://www.cabdirect.org/cabdirect/abstract/20023194968

Patkar, KL, Usha, CM, Shetty, HS, Paster, N, Lacey, J. 1995. Effects of spice oils treatment of rice on moulding and mycotoxin contamination. Crop Protection 13: 519-524. https://doi.org/10.1016/0261-2194(94)90104-X

Pelaez, AML, Catano, C.AS, Yepes, EAQ, Villarroel, RRG, Antoni, GLD, Giannuzzi, L. 2012. Inhibitory activity of lactic and acetic acid on *Aspergillus flavus* growth for food preservation. Food Control 24: 177-183. https://doi.org/10.1016/j.foodcont.2011.09.024

Periago, PM, Palop, A, Fernandez, PS. 2001. Combined effect of nisin, carvacrol and thymol on the viability of Bacillus cereus heat treated vegetative cells. Food Science and Technology International 7: 487–492. https://journals.sagepub.com/doi/pdf/10.1106/JE3P-NYKQ-4UDQ-TJC6

Rusul, G, March, EH. 1987. Growth and aflatoxin production by *Aspergillus parasiticus* NRRL 2999 in the presence of potassium benzoate or potassium sorbate and at different initial pH values. Journal of Food Protection 50:820–825. Doi: 10.4315/0362-028X-50.11.909.

Ryu, D, Holt, DL. 1993. Growth inhibition of *Penicilium expansum* by several commonly used food ingredients. Journal of Food Protection 56:862–867. DOI: 10.4315/0362-028X-56.10.862

Saleh, OI, Huang, JS. 1997. Bacterial soft rot disease of tomato fruits in Florida, USA: Identification, response of some American and Egyptian cultivars of solanaceous plants and chemical control. Assuit Journal of Agricultural Science 28: 11 – 26. https://www.cabi.org/isc/abstract/19981004816

Sebti, F, Tantaoui-Elaraki, A. 1994. *In vitro* inhibition of fungi isolated from ‘‘Pastilla’’ papers by organic acids and cinnamon. *Lebensmittel-Wissenschaft und Technolgie* 27:370–374. https://doi.org/10.1006/fstl.1994.1075

Simões, CMO, Schenkel, EP, Gosmann, G, Mello, JCP, Mentz, LA, Petrovick, PR. 2003. Farmacognosia da Planta ao Medicamento**.** 5th ed. UFRGS/UFSC: Porto Alegre/Florianópolis.

Soares, LM, Rodriguez-Amaya, DB. 1985. Screening and quantitation of ochratoxin A in corn, peanuts, beans, rice and cassava. Journal - Association of Official Analytical

Chemists 68(6): 1128-1130. http://europepmc.org/abstract/med/4086437

Sofos, JN, Pierson, MD, Blocher, JC, Busta, FF. 1986. Mode of action of sorbic acid on bacterial cells and spores. International Journal of Food Microbiology 3:1 – 17. https://doi.org/10.1016/0168-1605(86)90036-X

Sofos, JN. 1992. Sorbic acid, mode of action. In: Ledeberg J, editor. Encyclopedia of microbiology. San Diego, CA: Academic Press. pp 43 – 52.

Stead, D. 1995. The effect of hydroxycinnamic acids and potassium sorbate on the growth of 11 strains of spoilage yeasts. Journal of Applied Bacteriology 7:82 – 87. https://doi.org/10.1111/j.13652672.1995.tb01677.x

Varga, J, Houbraken, J, Samson, RA, Frisvad, JC. 2008. Molecular diversity of *Aspergillus* and *Penicillium* species on fruits and vegetables. In: R Barkai-Golan, N Paster, Eds., Mycotoxins in fruits and vegetables. Academic Press, New York, pp 205-223.

Viollon, C, Chaumont, JP. 1994. Antifungal properties of essential oils and their main components upon *Cryptococcus neoformans.* Mycopathologia 128, 151-153. https://link.springer.com/content/pdf/10.1007%2FBF01138476.pdf

White, GD. 2000. Compendium of corn diseases, 3rd ed n.st Paul MN: APS Press, 78pp.

Xiang, K, Zhang, ZM, Reid, LM, Zhu, XY, Yuan, GS, Pan, GT. 2010. A meta-analysis of QTL associated with ear rot resistance in maize. Maydica 55: 281-290. http://www.agr.gc.ca/eng/abstract/?id=21727000000509