WFL Publisher
Science and Technology
Meri-Rastilantie 3 B, FI-00980
Helsinki, Finland
e-mail: info@world-food.net

Journal of Food, Agriculture & Environment Vol.7 (1) : 219-223. 2009 *www.world-food.net*

Effect of some bioagents on growth and toxin production of *Aspergillus flavus* Link

Saleh A. Kabli
Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box: 80203, Jeddah, Saudi Arabia.
e-mail: sakabli@yahoo.com

Received 3 October 2008, accepted 9 December 2008.

Abstract

The extracts of *Aspergillus niger*, *Lactobacillus lactis* and *Nigella sativa* seeds as aflatoxin and growth inhibitors of *Aspergillus flavus* were screened. Minimal inhibitory (MIC) and minimal fungicidal (MFC) concentrations of the extracts were determined. *N. sativa* seeds extract showed a higher inhibitory effect than the other two bioagents extracts. The results show that *N. sativa* extract inhibits growth and aflatoxin production, which may be due to a reduction in lipid biosynthesis in the toxin producing cultures. The correlation between versicolorin A accumulation and aflatoxin biosynthesis by *A. flavus* Link was also discussed. It could be of significant importance as bio-preservatives in the prevention and control of aflatoxin production.

Key words: Bioagents, *Aspergillus flavus,* aflatoxin inhibitors, versicolorin A.

Introduction

Aflatoxin (AF) is one of the mycotoxins produced by some strains of *Aspergillus flavus*, *Aspergillus nomius* and *Aspergillus parasiticus*. Aflatoxin has been reported in many food and feed raw materials [1, 3-5, 39]. Food contaminated with AF poses a serious health threat when consumed by humans and animals. The toxin has been reported to be carcinogenic, teratogenic and hepatotoxic [10, 12, 14]. The increased demand for safe and natural food, without chemical preservatives, provokes many researchers to investigate the antimicrobial effects of natural compounds. Besides the pure chemicals, some plants and microbes or their active metabolites have been introduced as inhibitors of aflatoxin biosynthesis by the producing fungi [42]. Despite that, the majority of known AF inhibitors suffer from a large limitations concern to their harmful effects on eukaryotic systems at effective concentrations which highlight the need for search about new active principals. Numerous investigations have confirmed the antimicrobial action of different biopreservatives in model food system and real food [25, 34, 35]. *Nigella sativa* is an annual herbaceous plant; seeds are black in color and taste slightly bitter. They are frequently used as a spice and added as a flavoring agent to bread, pickles and other dishes [3]. The seeds of the plant are extensively used in traditional medicine in some countries, for the treatment of several diseases [29]. Recent scientific investigations on the seeds and their oil indicated a number of pharmacological activities including anticarcinogenic [37], antiulcer [16], antibacterial [24], antifungal [18], anti-inflammatory, antipyretic and analgesic [2]. In this study, the effects of *Aspergillus niger, Lactobacillus lactis* and *N. sativa* seed extracts on growth and AF production by *A. flavus* Link were evaluated.

Materials and Methods

Bioagents: Local strains of *A. niger* and *Lactobacillus lactis* were kindly provided by the Microbiology Section, Faculty of Science, King Abdulaziz University. Seed oil of *Nigella sativa* were used to study their inhibition effect on growth and aflatoxin producing strain of *A. flavus* Link.

Antifungal analysis: Antifungal analysis and determination of the minimal inhibitory (MIC) and minimal fungicidal (MFC) concentrations, and fungicidal kinetics of the bioagents extracts were performed. The disc diffusion method was used for antifungal screening as follows: Sterile Sabouraud Dextrose Agar (Merck) was inoculated with *A. flavus* Link spores (10^6 spores/ml) and distributed into Petri plates of 70 mm diameter. Whatman No.1 sterile filter paper discs (6 mm) containing 5 µl and 15 µl from each of the tested extracts were placed on the agar plates. The plates were incubated at 28±2°C for 10 days. Three replicates were used for each treatment. Diameters of inhibition zones were measured using Vernier calipers. The percentage of mycelial inhibition was calculated by the equation: I = 1-T/C×100 [where I is the inhibition (%), C is the colony diameter from a control Petri plate (mm) and T is the colony diameter from a test Petri plate (mm)] was used. MIC and MFC [32] were determined by broth dilution method as follows. Various concentrations of the bioagent extracts were added to 5 ml of YES broth tubes containing 10^6 spores/ml. The tubes were then incubated in incubator shaker as to evenly disperse the extracts throughout the broth in tubes. The highest dilution (lowest concentration), showing no visible growth, was regarded as MIC. Cells from the tubes showing no growth were sub-cultured on Sabouraud Dextrose Agar plates to determine if the inhibition was reversible or permanent. MFC was determined as the highest dilution (lowest concentration) at which no growth occurred on the plate.

Fungicidal kinetics of the oils: Of the bioagent extracts 20 µl exhibiting lethal properties over the spores at the dilution determined by MFC was added to 5 ml of spore suspension tubes containing 10^6 spores/ml in triplicate and were then incubated at 28±2°C, samples were taken after 0, 10, 20, 40, 60, 80, 100, 120, 140, 160 and 180 min and then cultured on Sabouraud Dextrose Agar plates for 48 h at 28±2°C. The control tube had no bioagent extract. Fungal colonies were counted after the incubation period and the total number of viable spores per ml was calculated.

Aflatoxin analysis

Toxin production: An inoculum of 1ml spore suspension of *A. flavus* Link from 2 weeks old culture was transferred to 250 ml Erlenenmeyer flasks, each containing 50 ml of glucose-ammonium nitrate medium [8] and the desired concentration of each *A. niger* and *L. lactis* spores and *N. sativa* seed extract. Triplicate flasks were prepared for each bioagent and for control. These flasks were incubated at 28°C for the desired periods for aflatoxin extraction and analysis.

Thin layer chromatographic analysis of aflatoxins: The chloroform extracts were analyzed for the presence of aflatoxins (B_1, B_2, G_1, G_2), using TLC plates according to the methods previously described [41]. Standard aflatoxin references were kindly provided by Dr. Peter M. Scott, Sir Frederick Banting Research Division, Tunneys Pasture, Ottawa, Ontario, Canada KJAOLZ.

Biochemical analysis

Estimation of versicolorin: *A. flavus* Link was maintained and grown as previously described [11]. The organism was cultivated in peptone-salt-glucose medium of the following composition: 0.5% bactopeptone, 10 mM $MgPO_4 \cdot 7H_2O$, 50 mM citric acid, 4 mM $MgCl_2$, 0.1 mM $MnCl_2$, 0.5 mM $CaCl_2$, 0.2 mM iron citrate, 10 µM $ZnCl_2$, 0.2 M glucose, pH 6.2. Of the medium 100 ml in 500 ml Erlenmeyer flasks was inoculated with $1x10^8$ conidial spores and grown at 25°C under shaking at 250 rpm. Under these conditions filamentous, nonpellicular growth is achieved. Mycelia were harvested by vacuum filtration and lyophilized for the determination of dry weight, and versicolorin quantification was done spectrophotometrically, after methanol extraction of 50 mg of lyophilized mycelia [27].

Estimation of lipid content and total protein: Lipids were extracted from known weights of dried mats with a mixture of methanol and chloroform (2:1, v/v) as described by Bligh and Dyer [6]. The phospholipids and neutral lipids were also estimated according to the method previously described of Kates [17]. The total protein in mycelium was estimated according to the method of Lowry *et al.* [22].

Determination of mycelial weight: Flasks containing mycelia were filtered through preweighed Whatman filter No.1 and then washed with distilled water. The mycelia were placed on preweighed Petri plates, allowed to dry at 50°C for 6 h and at 40°C over night and the net dry weight of mycelia was determined.

Results and Discussion

Plants and microorganisms provide unexplored natural sources for development of potentially new antimicrobial bioagents in the preservation of food and feed. The ability of extracts of *A. niger*, *L. lactis* and *N. sativa* to inhibit aflatoxin production by *A. flavus* was investigated. The results in Table 1 show that both fungal growth and aflatoxin biosynthesis were inhibited by extracts of *N. sativa, L. lactis* and *A. niger*. The inhibitory effects of these bioagents increased in proportion to their concentration. At 2.0% (w/v) of *N. sativa* seed extract, aflatoxin production was completely inhibited, and the dry weight showed a decrease of 79.3%. Experimental studies indicate that inhibition of aflatoxin biosynthesis and fungal development by different plants and microorganisms are continually discovered during the food biopreservation processes [15, 20, 21, 25, 28, 35].

The results in Table 2 show that the inhibitory effect of *A. niger, L. lactis* and *N. sativa* extracts was increased in proportion to their concentrations. At 15 µl extract/disc, *A. niger* and *L. lactis* extracts showed inhibitory effect until 5 and 7 days of incubation, respectively, while *N. sativa* completely inhibited the growth after the 4th day of incubation. In addition, *N. sativa* extract showed higher inhibitory effects compared with the other two extracts. *N. sativa* extract has been reported to be among the most active compounds against a number of food spoilage and pathogenic microorganisms [3, 18, 19]. The antimicrobial activity of *N. sativa* was also demonstrated [23, 31, 43].

MIC and MFC techniques were employed to assess fungistatic and fungicidal properties of the bioagent extracts. *N. sativa* was strongly active at MIC of 800 ppm and at MFC of 2200 ppm, while *A. niger* and *L. lactis* showed a fungistatic activity against *A. flavus* at MIC of 1800 and 1200 ppm, respectively. These extracts had no fungicidal activity on spores of *A. flavus* (Fig. 1). *N. sativa* killed more than 50% of spore population after 60 min, and 90-100% lethal effects were observed within 100 min of the exposure to the oil (Fig. 2). One of the characteristics of aflatoxin deactivation processes is that it destroys the mycelia and spores of the toxic fungi, which may proliferate under favorable conditions [26]. The results (Table 2, Figs 1 and 2) comply with the above specified characteristics, that bioagent extracts retarded mycelial growth and significantly decreased the aflatoxin production (Table 1). It is evident that 2% (w/v) *N. sativa* extract was significantly effective in inhibition of aflatoxin production (Table 1). Aflatoxin production was significantly inhibited at lower fungistatic concentrations of both oils (Tables 1 and 2). These inhibitory effects are interesting in connection with the prevention of aflatoxin contamination in many foods.

The effects of 2.0% (w/v) *N. sativa* seed extract on total carbohydrates, total proteins and total lipids in relation to versicolorin A and aflatoxin biosynthesis by *A. flavus* are presented

Table 1. Effect of *A. niger*, *L. lactis* and *N. sativa* extracts on the growth and aflatoxin production by *A. flavus.*

Extract	Conc.	Dry weight g/50 ml	Aflatoxin µg/ml	% of aflatoxin inhibition
A. niger	0.0	2.9	589	0.0
	50%	2.6	432	26.6
	100%	2.2	327	44.5
L. lactis	0.0	2.9	589	0.0
	10^4/ml	2.4	356	39.5
	10^6/ml	1.9	228	61.2
N. sativa	0.0	2.9	589	0.0
	1.5% (w/v)	1.8	206	65
	2.00% (w/v)	0.6	ND	100

ND = not detected

Table 2. Antifungal effect of *A. niger*, *L. lactis* and *N. sativa* bioagents on the growth of *A. flavus* (% of inhibition zone).

Incubation	*A. niger*		*L. lactis*		*N. sativa*	
period (days)	5 µl/disc	15 µl/disc	5 µl/disc	15 µl/disc	5 µl/disc	15 µl/disc
3	17.2	43	22	51	39.4	61.9
4	14	35.3	31.6	69.2	67	91
5	0.0	16.3	19.2	57	64	100
6	0.0	0.0	10	19.1	26.7	100
7	0.0	0.0	0.0	12	13.4	100
8	0.0	0.0	0.0	0.0	6.4	100
9	0.0	0.0	0.0	0.0	0.0	100
10	0.0	0.0	0.0	0.0	0.0	100
11	0.0	0.0	0.0	0.0	0.0	100
12	0.0	0.0	0.0	0.0	0.0	100

0.0 = resistance, % of inhibition = diameter of inhibition zone/diameter of Petri plates

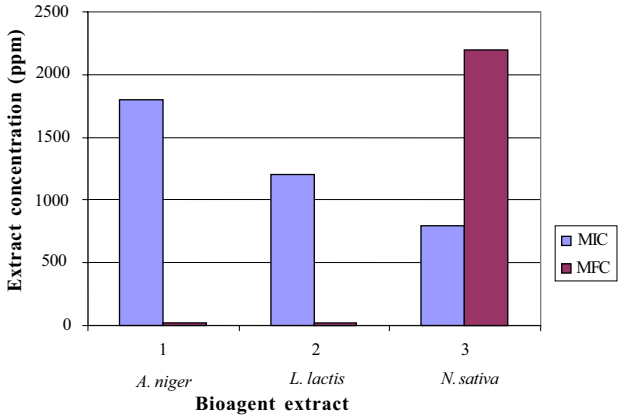

Figure 1. MIC and MFC (ppm) of *A. niger*, *L. lactis* and *N. sativa* extracts against *A. flavus.*

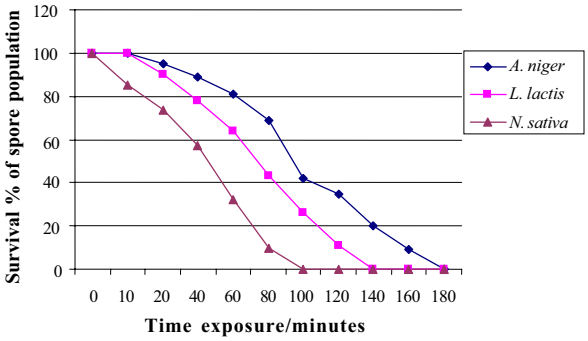

Figure 2. Survival % of *A. flavus* spores (initial spore concentration 10^5/ml) during incubation with *A. niger, L. lactis* and *N. sativa* extracts.

in Table 3. The concentration of *N. sativa* extract used in this investigation did not have an appreciable effect on the carbohydrate content of *A. flavus*. The protein content of *A. flavus* was affected by addition of *N. sativa* extract to the growth medium. It showed a decrease from 6 to 41% of the control. Total lipid content of fungal biomass was more sensitive to this concentration (2.0%) accounting collectively decrease from 2.0 to 66%. The results indicate that *N. sativa* extract inhibits the growth as a result of a reduction in lipid biosynthesis. Similar observations have been reported also earlier [3, 19, 43].

Table 3. Effect of *N. sativa* extract (2.0%, w/v) on carbohydrate, protein and lipid contents of *A. flavus,* as well as on its versicolorin A and aflatoxin production at different incubation periods (decrease % in total protein and lipids in parentheses).

Incubation period (days)	Treatment	Total carbohydrate (% of dry weight)	Total protein (% of dry weight)	Total lipids (% of dry weight)	Versicolorin A (µmol/g dry weight)	Aflatoxin (µg/g dry weight)
2	Control	81.4	4.5	4.3	0.0	0.0
	N. sativa	80.5	4.2 (7%)	4.2 (2%)	0.0	0.0
4	Control	81.4	5.3	4.5	18	0.0
	N. sativa	80.7	4.4 (17%)	4.1 (8%)	22	2.3
6	Control	81.8	6.3	4.8	36	4.6
	N. sativa	80.9	5.1 (19%)	3.4 (29%)	47	1.9
8	Control	82.0	7.6	5.3	45	7.3
	N. sativa	81.3	5.9 (22%)	3.7 (30%)	71	2.6
10	Control	82.0	7.7	5.5	68	9.6
	N. sativa	81.5	5.9 (23%)	3.7 (33%)	87	1.4
12	Control	82.0	7.9	5.6	81	10.2
	N. sativa	81.6	5.8 (27%)	3.7 (34%)	69	0.7
14	Control	82.0	8.6	6.1	117	9.8
	N. sativa	82.4	5.4 (37%)	2.8 (54%)	145	0.5
16	Control	82.0	8.7	6.1	112	8.6
	N. sativa	81.7	5.1 (41.3%)	2.3 (62%)	178	ND
18	Control	82.3	8.7	6.2	94	8.2
	N. sativa	81.9	5.1 (41.3%)	2.1 (66%)	196	ND

Major information is available on the correlation between versicolorin accumulation and aflatoxin biosynthesis by *A. flavus* in the medium supplemented by *N. sativa* seed extract. The results in Table 3 show that versicolorin A accumulated in the presence of *N. sativa* seed extract more than in control and it increased with increasing the incubation period, accounting collectively the maximum amount when the aflatoxin biosynthesis was lost after 16 and 18 days of incubation. On the other hand, aflatoxin production decreased with the time of incubation. In this connection, Rabie [30] pointed out that *A. flavus* begins to decrease aflatoxin production after 20 days of incubation, while versicolorin A biosynthesis continually increases until the end of incubation period in the medium supplemented with 1.8% (w/v) alcoholic extract of *N. sativa*. It suggests that *N. sativa* seed extracts probably mask a step of versicolorin A formation which is generally accepted to be a key intermediate in the biosynthesis of aflatoxin.

References

[1]Al-Garni, S.M. 1999. Nut-borne fungi and aflatoxins production. Proceeding of the 9th International Conference on Environmental Protection is a Must, Alexandria, Egypt, pp. 169-177.

[2]Al-Ghamdi, M.S. 2001.The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. Journal of Ethnopharmacology **76**:45-48.

[3]Aljabre, S.H.M., Randhawa, M.A., Akhtar, N., Alakloby, O.M., Alqurashi, A.M. and Aldossary, A. 2005. Antidermatophyte activity of ether extract of *Nigella sativa* and its active principle, thymoquinone. Journal of Ethnopharmacology **101**:116-119.

[4]Al-Hazmi, N.A., Al-Garni, S.A. and El-Sayed, F. 2008. Aflatoxin M1 determination in powder and infants milk collected from different markets in Jeddah, Saudi Arabia. Journal of King Abdulaziz University (Science). In press.

[5]Aycicek, H., Aksoy, A. and Saygi, S. 2005. Determination of aflatoxin levels in some dairy and food products which consumed in Ankara, Turkey. Food Control **16**:263-266.

[6]Bligh, E. and Dyer, W. 1959. A rapid method for total lipid extraction and purification. Gen. J. Biophys. **37**:9-11.

[7]Boyraz, N. and Ozcan, M. 2006. Inhibition of phytopathogenic fungi by essential oil, hydrosol, groundmaterial and extract of summer savory (*Satureja hortensis* L.) growing wild in Turkey. International Journal of Food Microbiology **107**:238-242.

[8]Brian, P.W., Dawkins, W., Crove, F., Hemming, G., Lowe, D. and Norris, G. 1961. Phytotoxic compounds produced by *Fusarium equiseti.* J. Exptl. Botany **12**:1.

[9]EL-Sayed, Z.G.M. 1994. Mycotoxins Production on Some Egyptian Medicinal Herbs and Inhibition of Microbial Growth by Gamma Radiation. Ph.D. thesis, Microbiology, Women´s College, Ain-Shams University.

[10]Erdogan, A. 2004.The aflatoxin contamination of some pepper types sold in Turkey. Chemosphere **56**(4):321-325.

[11]Failla, L.J. and Niehaus, W.G. 1986. Regulation of Zn^+ uptake and versicolorin A synthesis in mutant strain of *Aspergillus parasiticus*. Ex. Mycol.**10**:35.

[12]Fazekas, B., Tar, A. and Kovacs, M. 2005. Aflatoxin and ochratoxin A content of spices in Hungary. Food Additives and Contaminants **22**(9):856-863.

[13]Ghali, R., Hmaissia-Khalifa, K., Ghorbel, H., Maaroufi, K. and Hedili, A. 2008. Incidence of aflatoxins, ochratoxin A and zearalenone in Tunisian foods. Food Control **19**:921-924.

[14]Gursoy, N., Sarac, B., Durmus, N., Parlak, A., Yildirim, S., Kaya, T. and Bagcivan, I. 2008. Changes in spontaneous contractions of rat ileum by aflatoxin *in vitro*. Food and Chemical Toxicology **46**:2124-2127.

[15]Horn, B. and Dorner, J. 2001. Effect of competition and adverse culture conditions on aflatoxin production by *Aspergillus flavus* through successive generations. Mycologia **94**(5):741-751.

[16]Kanter, M., Demir, H., Karakaya, C. and Ozbek, H. 2005. Gastroprotective activity of *Nigella sativa* L. oil and its constituent, thymoquinone against acute alcohol-induced gastric mucosal injury in rats. World Journal of Gastroenterology **11**:6662-6666.

[17]Kates, M. 1972. Techniques of lipidology. In Work, T.S and Work L.E. (eds). Laboratory Techniques in Biochemistry and Molecular Biology. Elsevier North, Amsterdam, Holland.

[18]Khan, M.A., Ashfaq, M.K., Zuberi, H.S., Mahmoud, M.S. and Gilani, A.H. 2003. The *in vivo* antifungal activity of the aqueous extract from *Nigella sativa* seeds. Phytotherapy Research **17**:183-186.

[19]Khattak, K.F., Simpson, T.J. and Ihasnullah, T. 2008. Effect of gamma irradiation on the extraction yield, total phenolic content and free radical-scavenging activity of *Nigella sativa* seed. Food Chemistry **110**:967-972.

[20]López, P., Sanchez, C., Battle, R. and Nerín, C. 2007. Vapor-phase activities of cinnamon, thyme, and oregano essential oils and key constituents against food borne microorganisms. Journal of Agricultural and Food Chemistry **55**:4348-4356.

[21]López-Malo, A., Maris-Alzamora, S. and Palou, E. 2005. *Aspergillus flavus* growth in the presence of chemical preservatives and naturally occurring antimicrobial compounds. International Journal of Food Microbiology **99**:119-128.

[22]Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265.

[23]Machmudah, S., Shiramizu, Y., Goto, M., Sasaki, M. and Hirose, T. 2005. Extraction of *Nigella sativa* L. using supercritical CO_2: A study of antioxidant activity of the extract. Separation Science and Technology **40**:1267-1275.

[24]Morsi, N.M. 2000. Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria. Acta Microbiologica Polonica **49**:63-74.

[25]Mutungi, C., Lamuka, P., Arimi, S., James, G. and Onyango, C. 2008. The fate of aflatoxins during processing of maize into muthokoi - a traditional Kenyan food. Food Control **19**:714-721.

[26]Namazi, M., Allameh, A., Aminshahidi, M., Nohee, A. and Malekzadeh, F. 2002.Inhibitory effects of ammonia solution on growth and aflatoxin production by *Aspergillus parasiticus* NRRL-2999. Acta Poloniae Toxicologica **10**(1):65-72.

[27]Niehaus, W. and Failla, I. 1984. Effect of zink on versicolorin production by a mutant strain of *Aspergillus parasiticus*. Ex. Mycol. **8**:80.

[28]Niles, E.V., Joanna, A.N. and Pimbley, D. 1985. Growth and aflatoxin production of *Aspergillus flavus* in wheat and barley. Trans. Br. Mycol. Soc. **84**(2):259.

[29]Nostro, A., Germano, M.P., D'Angelo, V. and Cannatelli, M.A. 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in Applied Microbiology **30**:279-284.

[30]Rabie, G.H. 1997. Prevention and control of aflatoxins in wheat grains during storage. Egypt. J. Microbiol. **32**(2):245-268.

[31]Randhawa, M.A. and Al-Ghamdi, M.S. 2002. A review of the pharmaco-therapeutic effects of *Nigella sativa*. Pakistan Journal of Medical Research **41**:77-83.

[32]Rasooli, I. and Mirmostafa, S.A. 2003. Bacterial susceptibility to and chemical composition of essential oils from *Thymus kotschyanus* and *Thymus persicus*. Journal of Agricultural and Food Chemistry **51**(8):2200-2205.

[33]Rasooli, I. and Razzaghi-Abyaneh, M. 2004. Inhibitory effects of thyme oils on growth and aflatoxin production by *Aspergillus parasiticus.* Food Control **15**:479-483.

[34]Rasooli, I., Rezaei, M.B. and Allameh, A. 2006.Growth inhibition and morphological alterations of *Aspergillus niger* by essential oils from *Thymus eriocalyx* and *Thymus x-porlock*. Food Control **17**:359-364.

[35]Razzaghi-Abyaneh, M., Shams-Ghahfarokhi, M., Yoshinari, T.,

Mohammad-Bagher, R., Jaimand, H., Nagasawa, K. and Sakuda, S. 2008. Inhibitory effects of *Satureja hortensis* L. essential oil on growth and aflatoxin production by *Aspergillus parasiticus*. International Journal of Food Microbiology **123**:228-233.

[36]Razzaghi-Abyaneh, M., Allameh, A., Tiraihi, T., Shams-Ghahfarokhi, M. and Gorbanian, M. 2005. Morphological alterations in toxigenic *Aspergillus parasiticus* exposed to neem (*Azadirachta indica*) leaf and seed aqueous extracts. Mycopathologia **159**:565-570.

[37]Rooney, S. and Ryan, M.F. 2005. Effects of alpha-hederin and thymoquinone - a constituent of *Nigella sativa*, on human cancer cell lines. Anticancer Research **25**:2199-2204.

[38]Sakuda, S., Ono, M., Ikeda, H., Nakamura, T., Inagaki, Y., Kawachi, R., Nakayama, J., Suzuki, A., Isogai, A. and Nagasawa, H. 2000. Blasticidin A as an inhibitor of aflatoxin production by *Aspergillus parasiticus*. Journal of Antibiotics (Tokyo) **68**:407-412.

[39]Sarımehmetoglu, B., Kuplulu, O. and Elik, T.H. 2004. Detection of aflatoxin M1 in cheese samples by ELISA. Food Control **15**:287-290.

[40]Schnürer, J. and Magnusson, J. 2005. Antifungal lactic acid bacteria as biopreservatives. Trends in Food Science & Technology **16**:70-78.

[41]Subtabhaha, S. and Rao, A.S. 1972. Occurrence of aflatoxins and sitrinin in groundnut (*Arachis hypogaea* L.) at harvest in relation to pod condition and kernel moisture content. Current Sci. **43**:707.

[42]Yoshinari, T., Akiyama, T., Nakamura, K., Kondo, T., Takahashi, Y., Muraoka, Y., Nonomura, Y., Nagasawa, H. and Sakuda, S. 2007. Dioctatin A is a strong inhibitor of aflatoxin production by *Aspergillus parasiticus*. Microbiology **153**:2774-2780.

[43]Zeinab, E.B., Hala, A.F., El-Fouly, M.E.D. and Seham, Y.M.T. 2001. Inhibitory effect of gamma radiation and *Nigella sativa* seeds oil on growth, spore germination and toxin production of fungi. Radiation Physics and Chemistry **60**:181-189.