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EFFECTIVENESS OF NANOMATERIAL OF MAGNESIUM OXIDE, LOCALLY MANUFACTURED, IN ADSORPTION OF AFLATOXIN B_1 IN NATURALLY CONTAMINATED YELLOW MAIZE IN BAGHDAD

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Abstract

In this study, thirty–five samples of yellow maize grain were collected from Iraqi local markets in Baghdad for detection of the contamination Aflatoxin B_1 (AFB_1). Results of Enzyme – Linked Immuno Sorbent Assay (ELISA) was indicated to contamination of all samples with AFB_1 at various levels. Three concentrations of nanomaterial Magnesium Oxide (Nano-Mgo), locally manufactured, (0.1, 0.3, 0.5 gm/100 ml distilled water) were tested for adsorption of AFB_1 in vitro. Results were showed that Nano-MgO has a high adsorption capacity for AFB_1. Reduction ratios were reduced according to increasing concentrations of AFB_1 while it was increased by increasing the concentration of Nano-MgO.

***Keywords*:** Magnesium Oxide, Aflatoxin B1, Yellow Maize

Introduction

Aflatoxins (AFs), including AFB_1 are the most important and dangerous mycotoxins. AFB_1 is the leading cause of the liver cancer in both of human and animal, renal failure, neurotoxicity (Atroshi et al., 2001), and weak immune system (Richard, 2007). Many of methods were used in reducing and destroying of mycotoxin such as biological, chemical and physical methods. Recently, nanotechnology has been widely used in many scientific researches in various fields of life. This technique deals with the matter at the level of atoms and molecules and has distinct physical and chemical properties, therefore, it has a vital role in applying them in medical, industrial and agricultural fields (Huy *et al*., 2011; Fakkruddin *et al*., 2013). Many studies have been shown that Nano-MgO can be used to inhibitio bacterial and fungal growth (Tany and LV, 2013; Falah and Halima, 2016) and also used as adsorption agents for mycotoxins including AFs (Lange and Obendorf, 2012; Hekmati *et al*., 2010). The objectives of the study are:

1. Detection of contamination of maize grain collected from the local markets of Baghdad with AFB_1.
2. Estimation of AFB_1 by ELISA test.
3. Testing efficacy of Nano-MgO, locally manufactured, in adsorption of AFB_1 in maize grain in vitro.

Materials and Methods

Characterization of Nano-MgO locally manufactured by Al-Qaisy, 2015, (in partial fulfillment of the requirements for the degree of doctor of philosophy in Agriculture Science Plant Protection/Plant Diseases (mycotoxins)) College of Agriculture / University of Baghdad according to the method of Ding *et al*., 2001, at size: 58.66 nm and density: 0.18 gm/cm^3.

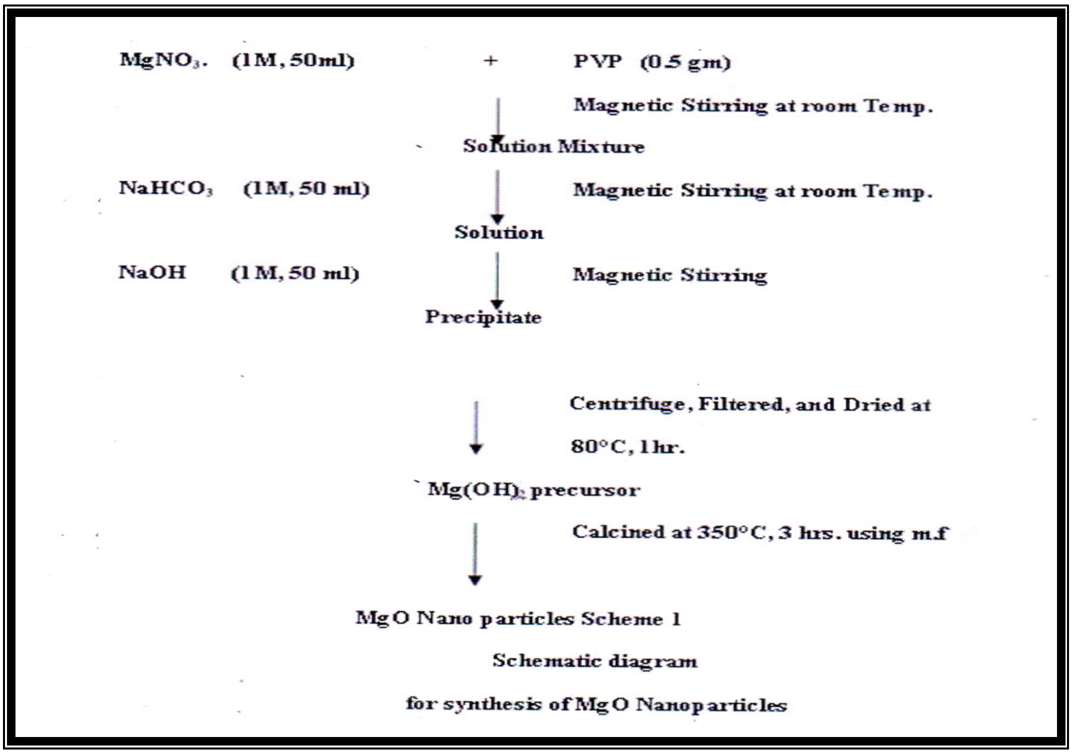
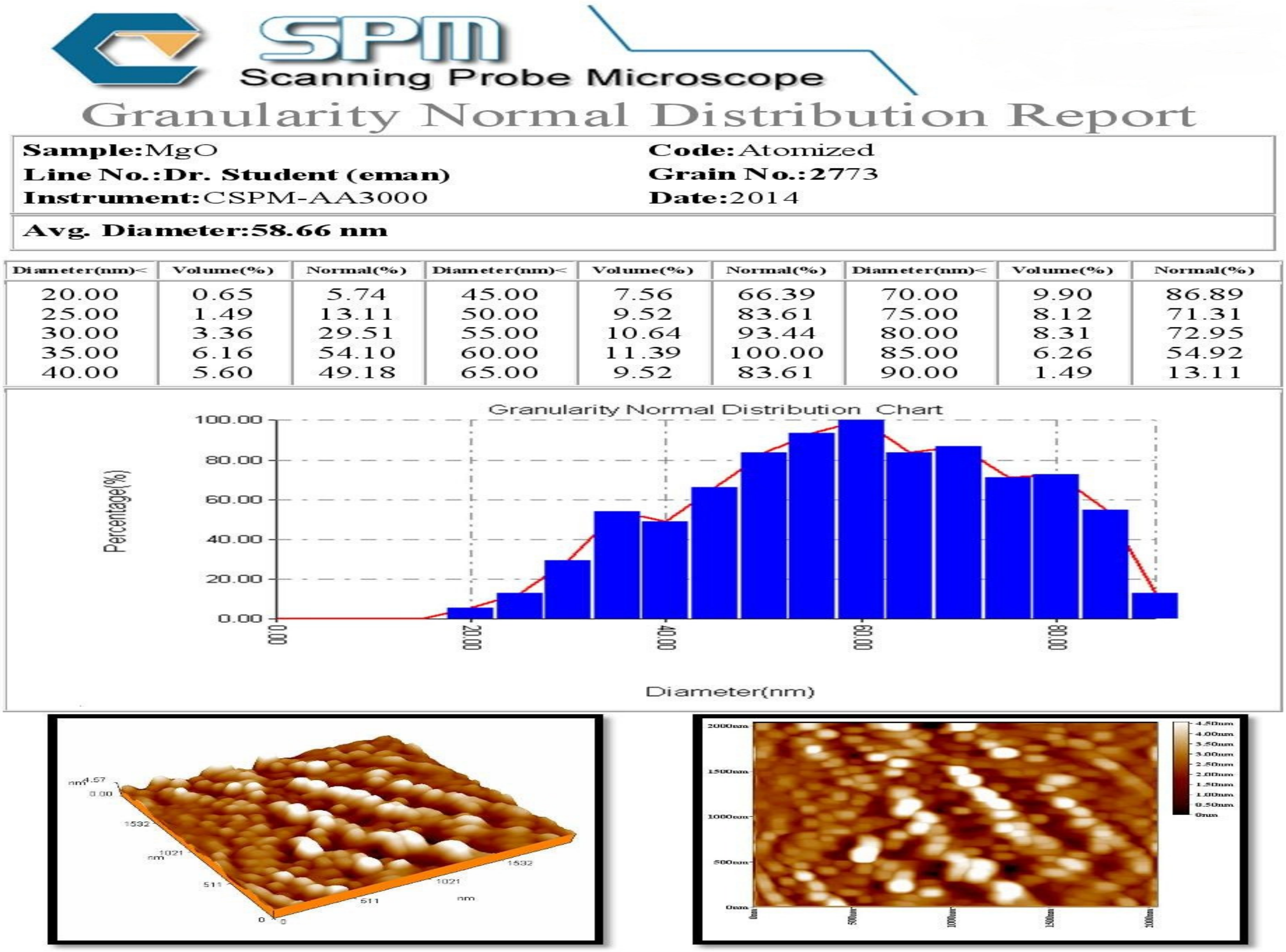


Fig. 1 : Stages of Manufactured Nano-MgO according to Ding *et al.* (2001)



Diameter(nm)<	Volume(%)	Normal(%)	Diameter(nm)<	Volume(%)	Normal(%)	Diameter(nm)<	Volume(%)	Normal(%)
20.00	0.65	5.74	45.00	7.56	66.39	70.00	9.90	86.89
25.00	1.49	13.11	50.00	9.52	83.61	75.00	8.12	71.31
30.00	3.36	29.51	55.00	10.64	93.44	80.00	8.31	72.95
35.00	6.16	54.10	60.00	11.39	100.00	85.00	6.26	54.92
40.00	5.60	49.18	65.00	9.52	83.61	90.00	1.49	13.11

Fig. 2 : Size of Nano-MgO (locally manufactured) by Atomic Force Microscope (AFM) according to method by Ding *et al.* (2001)

Collection of Samples :

A 35 samples of maize grain were collected from some local Iraqi markets, included area of A-Rasheed, Abu Ghraib, Jamila, Al-Alawi, Bayaa, and College of Agriculture/ University of Baghdad, at a rate 5kg per sample. Theses samples were kept in plastic cans (with writing number, location and data for each sample) and kept at a temperature of
(-4)^{o}C until the required tests.

Determination of moisture content of maize grain :

Moisture content was determined in a 100 gm for each sample of maize grain by a humidifier.

Preparation of Samples :

Samples of maize grain was weighted at 25 gm for each sample and placed in an oven at a temperature of 105^{o}C for 5 hours to dry and avoid fungal growth and then grinded well and kept at a temperature -4^{o}C until the extraction process.

Extraction and quantitative estimation of AFB_1 in maize grain samples by ELISA test :

AFB_1 was extracted from ground corn samples using ethanol and distilled water (20% : 80%). AFB_1 was measured by Enzyme – Linked Immuno Sorbent Assay (ELISA).

Preparation of AFB_1 titers and mixing with ground corn :

Eleven titers (7 – 2500) ppb of AFB_1 extraction were prepared by using ethanol (95%). 1 ml from each of 11 test tubes containing 1 gm of non-contaminated ground corn, which was obtained from silo in Baghdad, and mixed together (three replicates for each dilution). These tubes were incubated at room temperature 25^{o}C for 30 minutes to allow of AFB_1 to absorption by the ground corn. Another series tubes were left without adding of AFB_1. All these tubes were used in the following experiment.

Testing efficacy of Nano-MgO, locally manufactured, in adsorption of AFB_1 in ground corn :

This experiment included two separate tests to evaluate an efficacy of Nano-MgO, locally manufactured, for adsorption of AFB_1 as following:

(A) The first test (Using one conc. of Nano-MgO) :

In this test 1 ml of Nano-MgO (0.5 g/100 ml distilled water) was added to only 10 tubes, containing ground corn with AFB_1 (7-2000) ppb, and shook for 30 minutes to increasing its ability for adsorption of AFB_1. A same series of tubes (10 tubes) containing ground corn with AFB_1 (7 – 2000) ppb but without adding Nano – MgO as a positive control treatment, and another test tubes containing ground corn with Nano-MgO but without AFB_1, as a negative control treatment. Later AFB_1 was extracted and determined by ELISA.

(B) The second test :

Three concentrations of Nano-MgO (0.1, 0.3, 0.5 gm / 100 ml distilled water) were tested for adsorption of AFB_1 at 2500 ppb. 1 ml of each conc. was added to each of the tubes containing ground corn with AFB_1. The same series of tubes containing ground corn with AFB_1 (2500) ppb but without adding Nano-MgO as a positive control treatment and left another series tubes containing ground corn with Nano-MgO but without AFB_1 as a negative control treatment. Saline solution (10gm of NaCl/1L distilled water) was added for three times to all these tubes (in both of test A and B), which containing of Nano-MgO, for washing and removing of non-adsorbent of Nano-MgO, and then these tubes were placed in the centrifuge to separate and discarded the supernatant and then was added 1ml of distilled water to each of these tubes and later AFB_1 was extracted and estimated by ELISA.

Results and Discussion

Results of the quantitative estimation of AFB_1 were showed that all the samples of yellow maize contaminated with various levels of AFB_1 (Table 1). AFB_1 Levels were ranged between lowest level at 2.9 mg/kg (ppm) in sample (12) and highest level at 9.72 mg/kg (ppm) in sample (4). The contamination of maize with AFB_1 may be due to presence of the fungus (*A. flavus*) naturally in the soil (Cotty, Tucson, 2009). And the difference in the levels of AFB_1 may be due to difference of genetic susceptibility of *A. flavus* isolates (Amin *et al*., 2009; Reddy *et al*., 2009), and also may to the various of severity of field infection and the lack of suitable storage conditions were affected in the levels of AFB_1 (Kumar *et al*., 2008; Kabak, 2009).

These results were compatible with that of Brown et al., (2010) that maize crop is one of the most crops susceptible to be contaminated with aflatoxin. Also results in table 2 indicated to the high efficiency of Nano-MgO at a conc. of 0.005 g/ml in adsorption and reduction of AFB_1 at various levels from 5 – 2000 ppb. Reduction ratios of AFB_1 were ranged between a highest ratio 100% for each level from 7 – 500 ppb and a lowest ratio 69% at 2000 ppb, and this may results to owing of the Nano-MgO a high interactive surface to adsorption of AFB_1, but the reduction ratios were reduced when increasing the levels of AFB_1 because the interactive surface of Nano-MgO was reached to saturation level (Shi *et al*., 2006; Fandi, Yih, 2006). And this results was agreed with a study by Hekmati *et al*. (2010) about using of $MgO-SiO_2$ nanocomposite at 100 g/L for adsorption and reduction of different levels of aflatoxin (AF) in wheat flour. Results in table 3 were showed that the reduction ratios of AFB_1 were increased with increasing of concentrations of Nano-MgO, obviously that was due to increasing of the effectiveness of surface area of Nano-MgO for adsorption of AFB_1.

Table 1 : Levels of aflatoxin B1 in naturally contaminated corn samples and its moisture content.

Sample number	Sample Site	Moisture content of samples %	Level of AFB_1 mg / kg (ppm)
1	Al-Rashid	**15.2**	**6.2**
2	Jamila	**15.9**	**8**
3	Al-Rashid	**17.3**	**5.6**
4	Al-Baiaa	**18.3**	**9.72**
5	College of Agriculture	**15.6**	**3.1**
6	Al-Baiaa	**17.2**	**7.4**
7	Al-Rashid	**18.6**	**4**
8	College of Agriculture	**16.5**	**9.1**
9	Allawi	**18.4**	**6.6**
10	Allawi	**16.6**	**8**
11	Al-Rashid	**15.8**	**9.4**
12	Allawi	**15.3**	**2.9**
13	Jamila	**18.5**	**5**
14	Allawi	**17.1**	**8.6**
15	Al-Rashid	**15.4**	**7.2**
16	Al-Baiaa	**18.2**	**9**
17	Al-Baiaa	**16.3**	**7.8**
18	Al-Rashid	**15.5**	**5.6**
19	College of Agriculture	**17.4**	**7.3**
20	College of Agriculture	**15.7**	**3**
21	Al-Baiaa	**16.2**	**4.5**
22	Al-Baiaa	**18.1**	**3.6**
23	Al-Baiaa	**17.5**	**3.6**
24	Al-Rashid	**16.3**	**4.2**
25	Jamila	**16.7**	**6.0**
26	Jamila	**18.1**	**4**
27	College of Agriculture	**17.7**	**6.2**
28	Al-Baiaa	**16.4**	**3.1**
29	Allawi	**16.6**	**5.8**
30	Al-Baiaa	**15.8**	**4.2**
31	Al-Baiaa	**17.5**	**5**
32	Al-Rashid	**17.2**	**7.3**

33	Jamila	**15.6**	**4.5**
34	Al-Rashid	**17.2**	**6.1**
35	Allawi	**15.6**	**7.6**

Table 2 : Reduction ratios of aflatoxin B_1 in naturally contaminated corn by using a concentration of Nano-MgO.

Treatment Number	AFB_1 level before adsorption (ppb)	Nano-MgO conc. gm/ml	AFB_1 level after adsorption (ppb)	Reduction ratios %
T1 (Positive Control)	**7**, 15, 25, 50, 75 , 100, 250, 500, 1000, 1500, 2000	0	**7**, 15, 25, 50, 75, 100, 250, 500, 1000, 1500, 2000	**--**
T2	7	0.005	0	100
T3	15	0.005	0	100
T4	25	0.005	0	100
T5	50	0.005	1.2	97.6
T6	75	0.005	2.5	96.6
T7	100	0.005	3.7	96.3
T8	250	0.005	32.4	87.4
T9	500	0.005	81.8	83.6
T10	1000	0.005	229.6	77
T11	1500	0.005	431.2	71.25
T12	2000	0.005	620	69
T13 (Negative Control)	0	0.005	0	**--**

- Results of an analysis rate for three replicates.

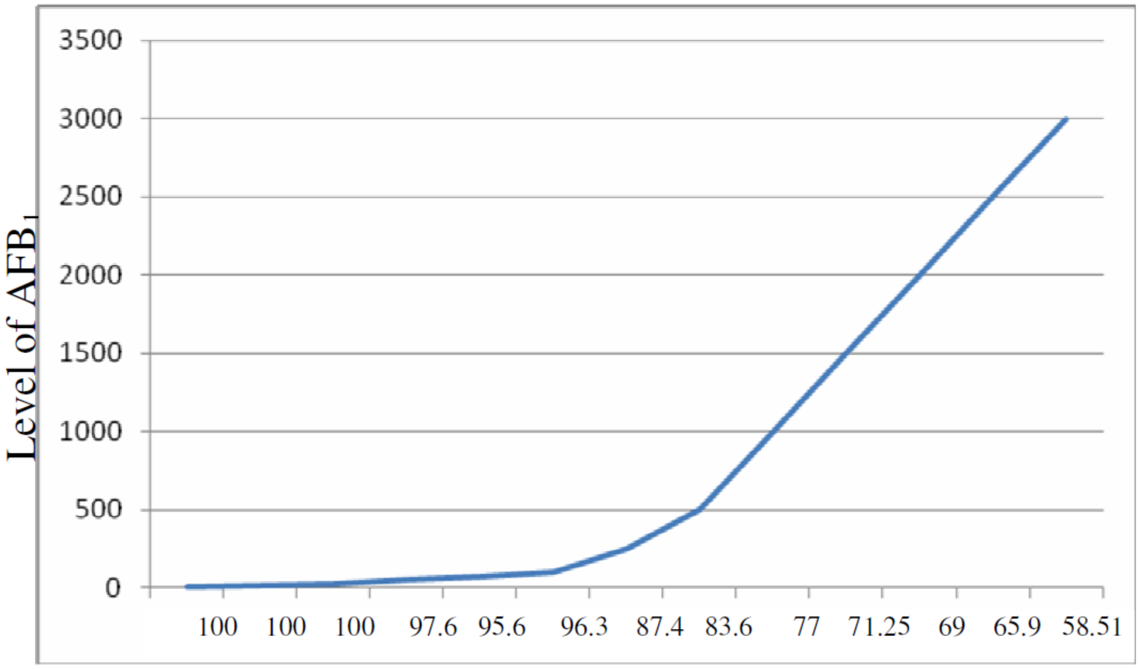


Fig. 3 : Reduction ratio of aflatoxin B_1 in naturally contaminated corn by using a concentration of Nano-MgO

Table 3 : Reduction ratios of aflatoxin B_1 in naturally contaminated corn by using three concentrations of Nano-MgO

Treatment Number	**AFB_1 level Before adsorption (ppb)**	**Nano-MgO conc. gm/ml**	**AFB_1 level After adsorption (ppb)**	**Reduction ratios %**
T1 (Positive Control)	2500	0	2500	**--**
T2	2500	0.001	2113	29.6
T3	2500	0.003	1632.2	45.6
T4	2500	0.005	852.3	65.9
T5 (Negative Control)	0	0.001, 0.003 0.005	0	**--**

* Results of an analysis rate for three replicates.

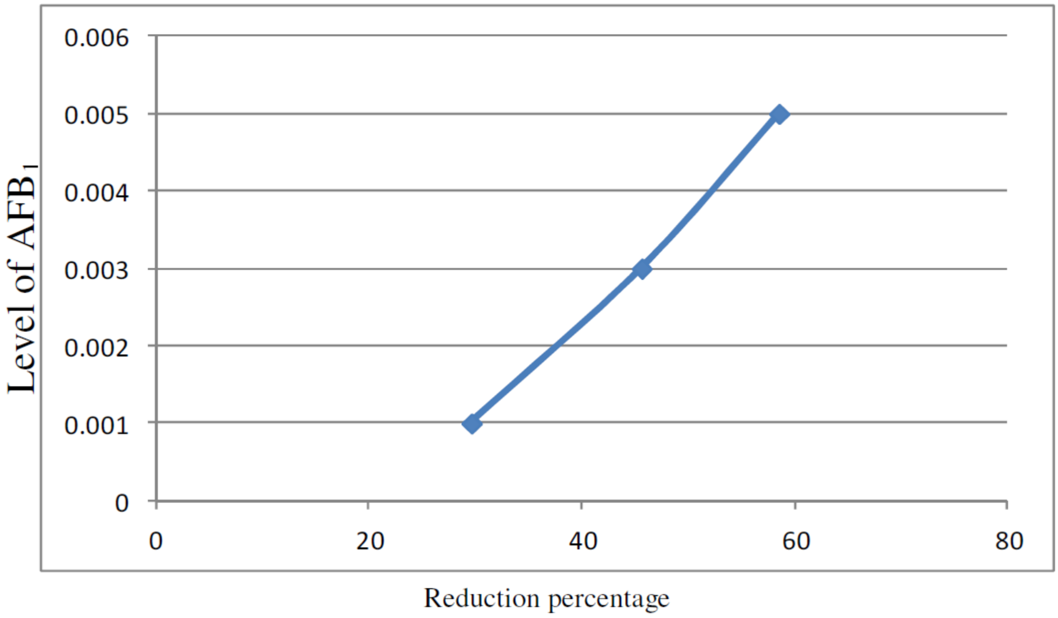


Fig. 4 : Reduction ratios of aflatoxin B_1 in naturally contaminated corn by using three concentrations of Nano-MgO

References

Amin, O.; Elzupir; Suzan, Makawi, Z.A. and Abdelrahman, E.M. 2009. Determination of aflatoxin and ochratoxin A in dairy cattle feed and milk in Wad Medani , Sudan . Journal of Animal and Veterinary Advances., 8 (12) : 2508-2511.

Atroshi, F.; Rizzo A.; Tuomas Westermarck, T. and Ali–Vehmas, T. (2002). Antioxidant nutrients and mycotoxins, Toxicology, 180 : 151-167.

Brown, R.L.; Zhi–Yuan, C.; Marilyn, W.; Meng, L.; Abebe, M.; Ahmed, F.; Deepak, B. (2010). Discovery and Characterization of Proteins Associated with Aflatoxin – Resistance : Evaluating their Potential as Breeding Markers. Toxins, (2): 919 – 923.

Fakruddin, Md, Hossain, Z. and Afroz, H. (2013). Prospects and applications of nanobiotechnology: a medical perpective. J. Nanobiotechnol, 10: 31.

Falah Abdul–Hasan and Halima Z. Hussein (2016). Evaluation of Antifungal Effect of Nanoparticles (Magnesium Oxide, Silver) and Chemical (Phylax) on *Fusarium solani* f. sp Cucurbita, Pathogenic Agent of Melon. International Journal of Agriculture Innovations and Research. 5(2): 2319-1473.

Jo, Y.K.; Kim B.H. and Jung G. (2009). Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Dis. 93: 1037-1043.

Kabak, B. and Dobson, A.D. (2009). Biological strategies to counteract the effects of mycotoxins. Journal of Food Production, 72: 2006-2016.

Kumar, O. and Rai, T. (2008). Nanotechnology in milk and its products Indian Dairyman, 37-40.

Le, A.T.; Huy, P.T.; Tam, P.D.; Hieu, N. and Huy, T. (2011). Novel Silver Nanoparticles : Synthesis, Properties and Applications. International Journal of Nanotechnology, 8: 278-290.

Reddy, K.R.N.; Abbas, H.K.; Abel, C.A.; Shier, W.T.; Oliveira, C.A.F. and Raghavender, C.R. (2009). Mycotoxin contamination of commercially important agricultural commodities. Toxin Reviews, 28(2-3): 154-168.

Richard, J.L. (2007). Some major mycotoxins and their mycotoxicoses–An overview. International Journal of Food Microbiology, 199: 3-10.

Tang, Z.-X. and L.V., B.-F. (2013) MgO Nanoparticles as Antibacterial Agent Preparation and Activity. Brazilian Journal of Chemical Engineering, 31: 591–601.

Tucson, A.Z. and Cotty, P.J. (2006). Crop rotation influences aflatoxin producing potential of *Aseprgillus* communities in South Texas : Beltwide Cotton Conferences, San Antionio, Texas, 3(6): 60-64.