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Detection of Mycotoxigenic *Fusarium* Species in Poultry Rations and Their Growth Control by Zinc Nanoparticles

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Abstract

In the present study, A total of sixty feed samples poultry rations (20 each of growing, broiler and layer poultry feed) were collected from poultry farms. In addition of, the antifungal activity of zinc nanoparticles, curcumin and probiotic was detected. The Prevalence of *Fusarium* species and their toxins in poultry feed was evaluated. All examined samples gave variable rates of contamination, where, the higher incidence of *Fusarium* species was recovered from layers feed (45%) followed by broilers feed (30%) and starter poultry feeds (25%), respectively. The isolates of *F. equiseti, F. poae* and *F. sporotrichioides* produced a mean level 22.2±4.02, 18.23±1.76 and 40.0±0.0 ppm) of tricothecenes mycotoxins, respectively. While, the detected levels of zearalenone and zearalenole illustrated that *F. equiseti* produced zearalenone at mean level of 7.5± 1.62, while, *F.poae and F. sporotrichioides* produced both zearalenone and zearalenol at mean levels of 4.0± 0.0 ppm for each of Zearalenone and Zearalenol) and 6.6± 1.56 ppm for zearalenone and 4.0± 00 for Zearalenol, respectively. On the other hand, the minimal inhibitory concentration (MIC) of Zinc nanoparticles (ZnNPs) for *F. equiseti, F. poae* and *F. sporotrichioides* were 400, 400 and 600 µg /ml, respectively. As the concentration of Zn NPs increased, the viability and colony count of fungus decreased. The antifungal potentials of curcumin and probiotic in inhibition the growth of mycotoxigenic *Fusarium* sp. revealed that the MIC of curcumin and probiotic were 1% in each**.** It is interesting to report that the MIC of Zn NPs for toxigenic *Fusarium* species was decreased to 100 µg /ml when combined with curcumin or probiotic (0.25% for each). These results are essential to avoid the toxicity of Zn NPs addition in animal feeds. Whereas, the evaluation of these antifungal agents in inhibition of Fusarium mycotoxins production in commercial feed indicated that the metal nanoparticles and herbs as curcumin and probiotic can be assed together in poultry feed to avoid the addition of high doses of Zn NPs. Otherwise, these cause the use of safe and fare doses from toxicity of nanomaterials to animal and poultry.

Keyword: *Trichothecenes, Zearalenone, Fusarium sp., Zinc nanoparticles, rations, Curcumin, Probiotic.*

1. Introduction

Nearly every food or feed commodity can be contaminated by fungal organisms and many of these fungi are capable of producing one or more mycotoxins, which are toxic metabolites of concern to human and animal health. The contamination of harvested crops with mycotoxins is a worldwide problem especially in tropical regions, where, up to 80% of the crops are reported to contain significant amounts of mycotoxins **(Refai and Hassan, 2013 and El-Hamaky *et al.*, 2016).**

Several species of the genus *Fusarium* are responsible for destructive action and results in very economically important diseases of cereal crops and the climatic conditions that favor the development of the fungi caused the fungal infections reached epidemic incidence (**McMu-llen *et al.*, 1997**). Various studies recovered different genera of mold including *Fusarium* species from feed as *F. moniliforme***,** *F. tricinctum, F. solani* **(Ragheb, 1994 and Jand and Singh, 1995).** However, **Hassan and Omran, (1996)** detected that the *Fusarium* species predominantly isolated during winter season from feed. However, *Fusarium* produces three of the most important of mycotoxins, such as fumonisins, trichothecenes or zearalenone, and produce other emerging mycotoxins as well as fusaproliferin, beauvericin, enniatins and moniliformin (**Waalwijk *et al.*, 2017).** On the other hand, the trichothecenes mycotoxins are secondary metabolites produced by some *Fusarium species* as (*F. sporotrichioides*, *F.oxysporum*, *F. equiseti*, *F. poae* and *F. graminearum*).The produced trichothecenes are mainly T2, deoxynivalenol and nivalenol (**Ueno, 1983, Sohn *et al.*, 1999 and Hassan *et al.*, 2010 a and b)**. While, zearalenone and the fumonisin B1 (FB_1) are commonly produced by *F. graminearum* and *F. sporotrichioides***(Molto *et al.*, 1997 and Scumadore and Patel, 2000).** The use of antimicrobial agents directly added to foods or through antimicrobial packaging is one effective approach. The resistance to many of the antifungal agents now in use has emerged and seems to create a huge problem ,while the number of fundamentally different types of antifungal agents that are available for treatment remains extremely limited.

Therefore, there are high significant demands to investigate new antimicrobial agents for controlling the infections caused by *Fusarium* sp. and their toxins. Recently, nanotechnology has been used in pharmaceutical indus-

tries to find new antimicrobial agents (**Gajjar *et al.*, 2009**). Furthermore, several studies evaluating the antifungal activity of metal nanoparticles "of the least hazards to the environment" in culture media particularly Zn NPs (**Hassan *et al.*, 2015a and 2017).** On the other hand, the herbal plants as curcumin has been widely used throughout history for the treatment of diverse ailments that include inflammatory conditions, gastrointestinal disorders, and cancer **(Goel *et al.,* 2008).** Moreover, curcumin is known for its antioxidant properties and acts as a free radical scavenger by inhibiting lipid peroxidation and DNA oxidative damage (**Jayaprakasha *et al.,* 2006**). Also, when the probiotics were administered in adequate amounts as antimicrobial agents, **(Reid *et al.,* 2003 and Matthew *et al.,* 2006).** In addition, a number of microbial products added to the feed may exert beneficial effects on livestock health and production. These products are not attributed with specific nutritional roles and are given the term `probiotics'. *Lactobacillus acidophilus* (**Wallace and Newbold, 1992).** Therefore, the present study was undertaken to evaluate the prevalence of *Fusarium* species and their mycotoxins in feeds and detection the efficacy of zinc nanoparticles (Zn NPs) singly and in combination with curcumin or probiotic for inhibition the growth of mycotoxigenic *Fusarium* and degradation of their toxins in poultry feed.

2. Materials and Methods

2.1. Feed samples: A total of sixty feed samples of poultry rations (20 each of growing, broiler and layer poultry feed) were collected from poultry farms. The animals in which suffered from symptoms of toxicity as diarrhea, refuse feed and loss of weight and sudden death may occur in some cases. Five hundred gram of each was collected in clean polyethylene bag and transferred to laboratory for further investigation.

2.2 Antifungal agents.

2.2.1. Zinc Nanoparticles.

Synthesis and characterization of Zn NPs were kindly done by fund of Prof. Dr. H. H. Mansour, Head of Central Laboratory of Elemental and Isotopic Analysis, Nuclear Research Centre, Atomic Energy Authority, Egypt.

2.2. 2. Probiotic vials: Each vial contains 1g of powder which consistes of: *Lactobacillus plantarum* $1X10^8$ CFU, *Lactobacillus acidophilus*$1X10^8$ CFU *and Saccharomyces cerevisiae* $1X10^7$ CFU Carrier-skim milk up to 0.5g. It was obtained from Sigma Chemical Company.

2.2.3. Curcumin powder: were purchased from market of herbs in powder form ready for use (El Captin company (CAP pharm), AL aboor city – Cairo – Egypt).

2.3. Fusarium Mycotoxins standard solution for TLC:

Mycotoxins standard of Trichothecenes (T2, DON, NIV), Zearalenone and Zearalenol were obtained from ALDRIK Sigma Chemical Company, St. LouisU.S.A)

2.4 Isolation and Identification of Molds in Feed Samples

One gram of each feed sample separately transferred aseptically into sterile tubes, to which 9 ml of sterile distilled water were added and tenfold serial dilutions were prepared **(APHA, 2003)**. One milliliter quantities of the previously prepared serial dilutions were inoculated separately into sterile Petri dishes plates, and mixed with Dichloran-Rose Bengal Chloramphenicol agar (DRBC) or Sabouraud's dextrose agar (SDA) medium containing 0.05 mg of Chloramphenicol/ ml. The plates were then left to solidify and dry. The plates were incubated aerobically, in the incubator at 25°C ± 1°C for 5 days. The plates were read between 2 d and 5 d of incubation. The identification of different species particularly members of *Fusarium* species was carried out by observation of their macroscopic and microscopic characteristics of molds colonies according to **(ISO 21527/1, 2008)** and **(Pitt and Hocking, 2009).**

2.5 Evaluation of The Mycotoxigenicity of Fusarium Recovered from Feed and Detection of mycotoxins:

The recovered *Fusarium* species from the present feed samples were grown on PDA (Potato Dextrose Agar) for seven days, at 25ºC**.** Five hundred ml flasks, each containing 100 g of finely ground yellow corn and 40-50 ml of sterilized distilled water was mixed and autoclaved at 121 ºC for one hour. The flasks were shaken to prevent cooking of yellow corn. It was inoculated with spores of each *Fusarium* species and incubated for 4 weeks at 25-28 ºC. Then the flasks were transferred to 8-10 ºC for additional 2 weeks **(D'Mello *et al.*, 199**8). After end of incubation period, the corn was removed from flasks, dried, finely ground and 50 gm of each was subjected to Fusarium toxin extraction and measurement by thin layer chromatography (**Kamimura, *et al.*, 1981; Bottalico, *et al.,* 1983 and 1985).**

2.6. Evaluation of Antifungal Potential of Zinc Nanoparticles Against *Fusariumin* sp. singly and in combination with probiotic and curcumin Using Agar Dilution Method (Jin *et al*., 2009):

5.6.1. Preparation of spore suspension of isolates (Gupta and kohli, 2003)

Cultures of one week old of *Fusarium* sp. isolates , the outer layer of growth were scraped by sterile loop using sterile distilled water. These spores suspension were counted in haemocytometer slide considering the dilution factor and the spores count was adjusted to10^5spores /ml.

5.6.2. Antifungal Potential of Zinc Nanoparticles against *Fusarium* sp. Using Agar Dilution Method (Jin *et al*., 2009):

In a sterile petri dishes, a gradual concentrations ranged from 0-1000 µg / ml of zinc nanoparticles and 0.05 ml of 10^5 spores of tested *Fusarium s*p. were added and overlaid with SDA. The plate's contents were shaken over the table in rotate manner and incubated at 25-28 ˚C for 3-5 days.

2.6.3. Antifungal potential of probiotic and curcumin on the growth of *Fusarium* by agar dilution method (Jeff-Agboola *et al*., 2012):

The powders of probiotic and curcumin were added to sterile petri dishes at gradual concentrations (0.0.25, 0.5, 1, 2, 3%) and 0.05 ml of 10^5 of tested *Fusarium* sp. spore suspensions. The contents of plates were mixed by shaking over the table, then covered with SDA and remained till solidified. After solidification of agar, the plates then were incubated at 25-28 ˚C for 3-5 days.

2.6.4. Antifungal effects of combination treatments of

Zn NPs with probiotic or curcumin for control of *Fusarium* on synthetic SDA (Gupta and Kohli (2003): Combination effects of Zn NPs, probiotic or curcumin were performed as the procedures of (2.6..2 and 2.6.3). With exception that the low doses of Zn NPs (100, 200 µg/ml) were combined with lower doses of probiotic or curcumin (0.25, 0.5 %) to control *Fusarium* species.
All plates were incubated at 25-28 ˚C for 3-5 days. Then, the MIC was determined which was the lowest concentration of ZnNPs, probiotic and curcumin that completely inhibited and prevented the growth of *Fusarium sp.*

2.7 Application of ZnNPs singly and in combination with probiotic or curcumin for control of *Fusarium* species growth and their toxin production on commercial yellow corn (D'Mello *et al*., 1998 **and Gupta and Kohli (2003):**
The same procedures as in (2.5., 2.6, 2.6.3., and 2.6.4.) were repeated using commercial yellow corn contaminated with toxigenic *Fusarium* instead of synthetic SDA. The total colony count of *Fusarium* was evaluated before and after treatment at 5 days and furthermore, the infected corn incubated for 4 weeks at 25-28ºC. Then the flasks were transferred to 8-10 ºC for additional 2 weeks**,** then, the corn as subjected to *Fusarium t*oxin extraction and measurement by thin layer chromatography (**Kamimura, *et al.,* 1981; Bottalico, *et al.,* 1983 and 1985.)**

2.8. Scanning Electron Microscopy of the treated microbial cells (SEM) (Gong *et al*., 2007):
The morphological changes of *Fusarium* sp. which were treated by Zn NPs were observed with a scanning electron microscope (SEM). All the treated fungal spores added to separate tubes and centrifuged. The sediments of each was dehydrated separately through a graded series of ethanol (30, 50, 60, 70, 80, 90, and 100%), each level was applied twice for 15 min each time, then the ethanol: isoamyl acetate (3:1, 1:1, 1:3) and 100% isoamyl acetate applied twice for 30 min). The solutions in wells were dried with a critical-point drier using liquid CO_2 and coated with gold-coater for 5 min. The coated samples were observed under SEM, Model (JSM- 5600 LV) with accelerating voltage of 10 KV.

2.9. Statistical analysis
Data obtained were statistically analyzed for the mean and standard error of the mean as method as Statistical Package for Social Science **(SPSS 14, 2006)**.

3. Results and Discussion
The fungal pollution of animal and poultry feeds and human food by mycotoxigenic fungi contributes a major problem to their health and are responsible for high economical losses in animal production due to decrease in milk and meat production and may be transmitted to human through consumption of contaminated food of animal origin . Such contamination constitutes a public health hazard due to production of mycotoxins, which cause some degree of acute toxicity when consumed in high amounts and are potential carcinogen. In developing countries; it appears that there is a direct correlation between dietary aflatoxins intake and the incidence of liver cancer **(FDA, 2000, Bahtnager and Ehrlich, 2002**). In addition, the outbreaks of food borne pathogens continue to draw public attention to food safety.
The fungi of *Fusarium species* and produced serious carcinogenic adverse effects on animal health. Whereas, the characterization and identification of *Fusarium* species have some difficulty due to similarity in morphological characteristics of macro-conidia and micro-conidia (**Refai *et al.,* 2015**).

Table (1). Prevalence of *Fusarium* Species in Poultry Rations

Poultry Ration	Total moulds			*Fusarium* species		
	No. of +ve	%	Mean of cc	No. of +ve	%	Mean of cc
Starter poultry feed (20)	20	100	$1.5x10^3$	5	25	$1x10^2$
Broiler poultry feed (20)	14	70	$3x10^2$	6	30	1x10
Layers Poultry feeds (20)	18	90	$3x10^3$	9	45	$1x10^2$
Total (60)	52	86.66%	$1.6x10^2$	20	33.3%	$0.7x10^2$

*cc: Colony Count

In the present study, the fungal examination of 60 poultry feed samples (20 of each of starter, broiler and layers) for detection of *Fusarium species* incidence in samples was investigated. The results (Table, 1) revealed that all examined samples gave variable rates of contamination, where, 100% of starter poultry feed were contaminated with different mould species included 33.3% contaminated by *Fusarium* sp. The higher incidence of *Fusarium* species was recovered from layers feed (45%) followed by broilers feed (30%), and starter poultry feed (25%) Similar findings were reported by **Buckley *et al.* (2007); Hassan *et al.* (2015a, 2016, 2017 and 2018) and El-Hamaky *et al.* (2016)**, who recovered most of these fungi from the examined poultry feed samples. In addition, mycotoxigenic *Fusarium* species *cause* significant economic losses in animals' production and *Fusarium* species capable of killing cells by causing damage to cellular membrane **(Abou-Elyazeid *et al.,* 2011).**
However, *Fusarium* sp. was the most common fungi in maize as reported by **Cvetnic *et al.* (2004**). Whereas, it recovered from (60%) of diseased sheep and their used feeds and water at desert districts in Egypt with isolation range of (40-90%) **(Hassan *et al.,* 2010 a)**, Other study **by Ana-Marrija *et al.,* (2005)**, recovered *Fusarium spp., Penicillium spp. and Aspergillus spp*. from maize grain samples .

Table (2). Identification of members of fusarium that isolated from poultry Rations

Fusarium sp.	Starter poultry feed (20)		Broiler poultry feed (20)		Layers Poultry feeds (20)		Total (60)	
	No. of +ve	%	No. of +ve	%	No. of +ve	%	No. of +ve	%
F. equiseti	1	5	2	10	2	10	5	7
F. poae	1	5	1	5	2	5	4	6
F. columarum	1	5	-	-	-	-	1	1.5
F. graminarum	1	5	-	-=	1	5	2	3
F. sporotrichioides	1	5	1	5	4	20	6	10
F. avenactum	-	-	1	5	-	-	1	1.5
F. verticillioides	-	-	1	5	-	-	1	1.5
Total	5	25	6	30	9	45	20	33.3

Currently, the characterization of *Fusarium species* members yielded that the *F. equiseti was* recovered from broiler and layers poultry feed at relatively higher incidence (10% for each) (Table, 2). Whereas, the incidence in starter poultry feed was relatively lower (5%). While, *F. sporotrichioides* was recovered from (20%) of examined layers feed , (5%) of each of broiler and starters feed samples, respectively. Regarding *F. poae,* it was detected in (5%) of each of starters, broilers and layers poultry feeds. While, a total (33.3 %) of all examined feed samples yielded *Fusarium* sp., the majority of isolates were recovered from layers feed (45%) followed by broiler feeds and starter feed(30% and 25%) respectively (Table, 2). These differences in these levels of contamination may be due to the exposure of the examined samples to different climatic condition either during preparation, transportation or storage.

The members **of** *Fusarium* sp. recovered only from 2.6% of poultry concentrated feed and **Buckley *et al*., (2007) and** from 6% of animal feed mainly corn seed, barley and corn silage samples **Khosravi *et al*., (2008)**. But, **Abou-Elyazeid *et al.,* (2011)** recovered *F. verticillioides*; *F. anthophilum* and *F. proliferatum* from poultry feedstuffs *and* **Chu *et al.* (1995) and Hassan *et al.* (2010 b)** isolated *F. oxysporum* from feeds produced tibial dyschondroplasia and immune suppression in poultry. Moreover, **Pan *et al.* (2009),** detected *Fusarium* spp. in wheat samples at Uruguay particularly during spring rains occur**.**

Furthermore, *the Fusarium* sp. have a potential public health hazard due to mycotoxin production which are of carcinogen effects on liver cells when animal exposed to these mycotoxins for long periods (**FDA, 2000**). While, mycotoxin contamination can occur during pre-harvest and storage periods of cereals at 37°C and high humidity during prolonged storage times which potentiated the mold growth and mycotoxin production in cereals **(Alshannaq and Yu, 2017).**

Moreover, the mycotoxin-producing species are *F. sporotrichioides, F. graminearum* and *F. verticillioides,* which produce toxins such as zearalenone, zearalene, deoxynivalenol or nivalenol, T-2 toxin and diacetoxyscirpenol **(Atanasova-Penichon *et al.,* 2012)**. These toxins generate diverse diseases to crops and contamination to diverse types of cereals mainly to maize being of toxicological concern the ear rot **Atanasova-Penichon *et al.,* 2014)**. However, trichothecenes produced by *Fusarium* species alter immune-mediated activities in ruminants **(Black *et al.,* 1992)** and have a potent inhibitor of eukaryotic protein biosynthesis, inducing vomiting, diarrhea, anemia and food refusal in larg animals **(Coulombe, 1993 and Osweiler, 2000).**

Table (3). Mean levels of Fusarium mycotoxins produced by isolated *Fusarium sp.* from poultry rations (mg/kg of feeds) (ppm).

Fusarium sp.	Levels of Trichothecenes (mg/kg of feeds)(ppm)												Total Trich.	levels of zearalenone and zearalenole in feeds (mg/kg of feeds)(ppm)					
	*T2			*DON			*DAS			*NIV				Zearalenone			zearalenole		
	no of +ve	%	mean levels± SE	no of +ve	%	mean levels ±SE	no of +ve	%	mean lev-els±SE	no of +ve	%	mean lev-els±SE	mean lev-els±se	no of +ve	%	mean lev-els±SE	no of +ve	%	mean lev-els±SE
F.equiseti (5)	4	80	30± 2.3	-	-	-	3	60	16.6±3. 33	2	28. 5	20± 1.0	22.2±4. 02	4	80	7.5± 1.62	-	-	-
F.poae(4)	3	75	14.7±5 .30	-	-	-	1	25	20 ±0.0	1	25	20± 0.0	18.23± 1.76	1	25	4.0±0	1	25	4.0± 0.0
F.culmorum(1)	1	100	10± 00	-	-	-	-	-	-	-	-	-	-	-	-	-	1	100	1.0± 00
F.graminarum(2)	-	-	-	-	-	-	-	-	-	-	-	-		1	50	4.0± 00	2	100	2.2± 00
F.sporotrichoides (6)	-	-	-	-	-	-	1	16.6	40±00	1	16. 6	40± 00	40± 00	6	100	6.6± 1.56	1	16. 6	4.0± 00

*** T_2, Deoxynivalenol (DON), diacetoxy-scirpenol (DAS) and nivalenol (NIV)**
*permissible limits of fusarium toxins in animal and poultry feeds according to **(European Commission, (2013)** were: 0.25-0.5 ppm for T-2 and 2-8 ppm for Deoxynivalenol (DON) and 2-3 ppmfor Zearalenone.

Currently, as illustrated in Table (3), the screening of recovered *Fusarium* species from feeds for production of mycotoxins was undertaken. The isolates of *F. equiseti, F.poae* and *F. sporotrichioides* produced a mean levels 22.2±4.02, 18.23±1.76 and 40.0±0.0 ppm respectively of tricothecenes mycotoxins,. Regarding, members of trichothecenes, T2 mycotoxins produced by *F. equiseti*, *and F.poae* at a mean levels of 30±2.30 and 14.7±5.30 ppm respectively and T2 toxin not produced at all in case of *F.graminarum and F. sporotrichioides.* Whereas, DAS and NIV detected only during screening of *F.equiseti , F.poae and F. sporotrichioides* at a mean levels of 16.6±3.33 ppm DAS and 20±1.0 ppm NIV, 20±00 ppm DAS and 20±00 ppm NIV and 40±00 ppm DAS and 40±00 ppm NIV, respectively. Regarding the detected levels of zearalenone and zearalenol, all tested members of *Fusarium* produced these toxins. The fungus of *F.equiseti* produced zearalenone (7.5± 1.62), but not produced zearalenol. While, *F.poae and F.sporotrichioides* produced *both* zearalenone *and* zearalenol (4.0± 0.0 ppm for each of zearalenone. and zearalenol.) and (6.6± 1.56 ppm for Zearalenone. and 4.0± 00 for Zearolenol, respectively)

Whereas, **Hassan *et al.*,(2010 a)** detected the *Fusarium* toxins in feed samples, the largest amount estimated in crushed yellow corn (60%) namely FB_1, T_2 and zearalenone with the mean levels of 48.4±1.0; 3.0±0.1 and 0.84±0.03 ppm, respectively. Also, **Ana-Marrija *et al.*, (2005)** detected that *F. graminearum*(the producer of ZEA) was isolated from all samples of maiz and the most frequent mycotoxins were FB1 (459.5 ppm) and ZEA (1.70 ppm), respectively,

The most important type of trichothecenes for poultry is DON (vomitoxin) which has numerous adverse health effects, with neural, gastrointestinal tract and immune system being the most sensitive organs **(Canady, *et al.*, 2001).** In addition, the low to moderate dose of trichothecene cause gastrointestinal irritation or necrosis, haematological disorders, diarrhoea, vomiting and feed refusal and decreased body weight gain. Whereas, the exposure to higher dose levels of DON are mainly expressed as severe reduction in body weight, severe damage to the haematopoietic systems in bone marrow, spleen, thymus and lymph nodes, and impaired 88 resistance to infection, particularly bacterial infection **(Ueno, 1984).**

All the previous literatures of fungal diseases caused by *Fusarium* infection and their toxins recorded that the pollution by these fungi affected upon the growth rate. They potentiated several health problems of human and animals including anemia, stunted growth, carcinogenic, tremor genic, hemorrhagic, dermatitis, pulmonary edema, immunosuppressive and hormonal effects (**Mogeda *et al.*, 2002, Hassan *et al.*, 2010 a and Abou-Elyazeid *et al.*, (2011).**

Regarding, Zearalenone mycotoxin produced mainly by *F. graminearum, F. semitectum, F. equiseti* and *F. culmorum.* It has estrogenic effects in animals, which may be due to its binding to oestrogene receptors and in woman causes precocious menarche in tropical and mild climates (**Saenz and de Rodriguez, 1984 and Szuetz et al., 1997).** The general, the direct ingestion of contaminated food and feed was the main rout of the human and animal exposure to mycotoxins (**Abd-Allah and Hassan, 2000 and Hassan, 2017 *et al.*, and 2018).**

Recently, the antibiotic-resistance of some fungi is resulted in problem for control fungal diseases and potentiated production of novel antifungals. Today, the uses of metals nanoparticles as antifungal agent were found to inhibit microbial growth as nanoparticles of Zn NPs which showed strong antibacterial and antifungal activity **(Reddy, 2007 and Hassan *et al.*, 2014).** They have gained more attention due to its special properties and its fewer hazards to environment **(Violeta *et al.*, 2011)** and they are effective in inhibiting the growth of toxigenic fungi and their ability for toxins production **(Mohamed *et al.*, 2015).**

In addition, Nano Zn improves the immunity of the animals, for an instance, a reduction in somatic cell count in subclinical mastitic cow and an increase in the milk production was observed due to supplementation of Zn-NPs (**Rajendran, 2013)**

Table (4). Minimal inhibitory concentration of Zn NPS on toxigenic *Fusarium* species isolated from present feed samples

Member of *Fusarium* Species	Colony count of *Fusarium sp.* at gradual concentrations of Zn NPS (µg /ml) in Sabaroud dextrose agar media (SDA)						
	0	100	200	400	600	800	1000
F .equiseti	$2X10^5$	$5\ X10^2$	7X 10	00	00	00	00
F.poae	$2X10^5$	$4X10^2$	3X10	00	00	00	00
F.sporotrichioides	$3X10^5$	$3X10^3$	$4X10^2$	1X10	00	00	00

In the present study, the MIC of Zn NPs for *F. equiseti, F.poae and F. sporotrichioides* were 400, 400 and 600 µg /ml, respectively. As the concentration of ZnNPs increased, the viability and colony count of fungus decresed (**Table 4**).

The recent reports illustrated the efficacy of nanoparticles of metal oxides as most potent antifungal and antibacterial agents against all fungal and bacterial animal pathogens **(Hassan *et al.*, 2014, 2013 a & b & 2015 a, b)**.

It is suggested that the antimicrobial potentials of metals nanomaterials related to its doses of treatment and particles size **(Violeta *et al.*, 2011).** While**, Hassan *et al.*, (2013a, b, c)** detected that the growth of aflatoxigenic moulds and aflatoxins production were inhibited by addition of 8 µg/ml of ZnO NPs and ochratoxin A and fumonisin B1 producing moulds and mycotoxins production were inhibited by addition of 10 µg/ml of ZnO NPs to tested medium. Similarly, **Hassan *et al*., (2014),** detected the antifungal activities of ZnO NPs against

C.*albicans* which was more sensitive for lower concentrations (100 ug/ml) than *A. niger, A. flavus* and *A. ochraceus* (200, 300 and 300 µg /ml) to inhibit their growth, respectively. The antimicrobial potentials of ZnO NPs may be due to the formation of hydrogen bond between hydroxyl group of cellulose molecules of fungi with oxygen atom of ZnO NPs leading to inhibition of the microbial growth. In addition, the release of Zn 2+ may occur which causes damages cell membrane and interacts with intraocular contents **(Moraru *et al.,* 2003).**

Currently, when the treated *Fusarium* sp. were subjected to SEM, the damage and rupture of their cell wall were detected in the area surrounding growth. The normal conidial cell of *Fusarium* sp. has a macro-conidia abundant in sporodochia, long, slender, dorsoventral curvature, several septa, apical cell elongate and tapering, basal cell foot-shaped and micro-conidia are oval and elongated oval, smooth cell wall and intact cell membrane **(Refai *et al.,* 2015).** The effect of high concentration of Zn NPs on the treated *Fusarium* sp. was observed as membrane damage of cells and some pits that have been caused in inter cellular components, leading to leakage and finally cell death. **(Fig. 1).** Similar findings were also reported by **(Shawky *et al.,* 2014 and Violeta *et al*., 2011).**

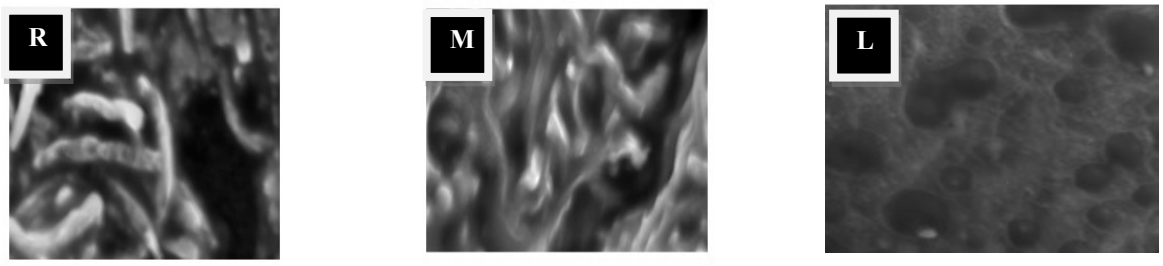

Figure (1). Photogram of Scanning Electron Microscopy of *Fusarium Sp.* (R) Normal conidia without treatment of Zn NPs (M) After treatment with MIC of Zn NPs (400 µg/ml) (L) After treatment with high dose of (600Ug/ml)

Table (5). Minimal inhibitory concentration of Probiotic and Curcumin on toxigenic *Fusarium* species isolated from present feed samples

Member of *Fusarium*	Colony count of *Fusarium sp.* at gradual concentrations of Probiotic and Curcumin (%)										
	No. treat.	0.1%		0.25%		0.5%		1%		2%	
	No treat.	Probi-otic	Curcu-min	Probi-otic	Curcu-min	Probi-otic	Curcu-min	Probi-otic	Curcu-min.	Probi-otic	Cur-cumin .
F .equiseti	$2X10^5$	$5X X10^2$	$2.5X10^3$	1X 10	5X10	00	0.3X10	00	00	00	
F.poae	$2X10^5$	$4X10^2$	$1.5X10^2$	3X10	3X10	00	0.1X10	00	00	00	
F.sporotrichioides	$3X10^5$	$3X10^3$	$2.0X10^2$	$4X10^2$	2.0X10	00	0.2 X10	00	00	00	

Up to date, the natural oils that extracted from herbal plants are widely used as antimicrobial potential. Moreover, Curcumin has been widely used throughout history for the treatment of inflammatory conditions, gastrointestinal disorders, and cancer **(Goel *et al.,*2008).** It is known for its antioxidant properties and acts as a free radical scavenger by inhibiting lipid peroxidation and DNA oxidative damage (**Jayaprakasha *et al.,* 2006**)

Also, the probiotic enhance immune response and nutrition of host species through the production of supplemental digestive enzymes **(Abdel-Kader, 2009).** It was indicated that dietary probiotic had antioxidant activity and had a protective effect against dietary aflatoxin, this results agreed with **Chen *et al*., (2013a & b) and Nabawy *et al*., (2014).**

the dietary probiotic detected to have antioxidant activity and had a protective effect against dietary aflatoxin this results agreed with **Chen *et al*., 2013 a** and reduced oxidative stress and inflammatory response **(Chen *et al.,* 2013 b and Zhao *et al.,* 2013).**

Ouwehand *et al.* (2002) explored the immune-modulator effect of probiotics to improve phagocytosis by increasing the number of natural killer cells. In the present study, we evaluate Curcumin and probiotic in inhibition the growth of mycotoxigenic *Fusarium sp.* The obtained results in (Table, 5)**.**revealed that the MIC of curcumin and probiotic were 1% in each Several studies were reported similar findings to our results as**, Hassan *et al.,* (2008),** who used *Rhamnus cathtica* plant extract**, Hassan *et al.,* (2012b)** who used clove, onion and garlic oils and **Yage *et al.,* (2012) and Taha *et al.,* (2014),** who used clove oil and all these studies revealed that these plants extracts were possessed antifungal activities against *A. ochraceus* and *A. niger*.

Also, when the probiotics were administered in adequate amounts as antimicrobial agents, resulted in a huge benefit on the animals and human health **(Reid *et al.,* 2003, Matthew *et al.,* 2006 and Syed El Ahl *et al.,* 2006).**

Table (6). Combination between treatments of ZnNPs with probiotic or curcumin for control of *Fusarium* on SDA.

Member of Fusarium	Colony count of *Fusarium sp.* at gradual concentrations of								
	Non treated (N.T.)	ZnNPs (100 µg /ml)		ZnNPs (100 µg /ml)		ZnNPs (200 µg /ml)		ZnNPs (200 µg /ml)	
		Probiotic (0.25%)	Curcumin (0.25%)	Probiotic (0.5%)	Curcumin (0.5%)	Probiotic (0.25%)	Curcumin (0.25%)	Probiotic (0.5%)	Curcumin (0.5%)
F. equiseti	$2X10^5$	0.5X10	1.5X10	00	00	00	00	00	00
F. poae	$2X10^5$	0.3X10	0.6X10	00	00	00	00	00	00
F. sporotrichioides	$3X10^5$	0.1X10	0.2X10	00	00	00	00	00	00

Table (7). Influence of Combination treatments of ZnNPs with probiotic or curcumin in control of *Fusarium* in contaminated commercial yellow corn.

Member of Fusarium	Colony count of *Fusarium sp.* at gradual concentrations of								
	Non treated (N.T.)	ZnNPs (100 µg /ml)		ZnNPs (100 µg /ml)		ZnNPs (200 µg /ml)		ZnNPs (200 µg /ml)	
		Probiotic (0.25%)	Curcumin (0.25%)	Probiotic (0.5%)	Curcumin (0.5%)	Probiotic (0.25%)	Curcumin (0.25%)	Probiotic (0.5%)	Curcumin (0.5%)
F. equiseti	$2X10^5$	$1X10^2$	1X10	O.7X10	0.1X10	00	00	00	00
F. poae	$2X10^5$	$0.3X10^2$	0.5X10	0.5X10	00	00	00	00	00
F. sporotrichoides	$3X10^5$	1X10	0.1X10	00	1X10	00	00	00	00

Regarding the combination antifungal potential of Zn NPs with curcumin or probiotic on synthetic medium of SDA, it is interesting to report here that the MIC of Zn NPs against toxigenic *Fusarium* sp. was decreased to100 µg /ml when combined with curcumin or probiotic (0.25% for each).These results are essential to avoid the toxicity of Zn NPs addition in animal feeds (Table, 6).

Similar findings were obtained when applied these combination between Zn NPs and curcumin or probiotic on natural yellow corn, but, the MIC required elevation of used concentrations of curcumin and probiotic (0.5% for each) and ZnNPs (100 µg /ml) (Table, 7).

Hence, the synergistic, combination therapy of ZnNPs with other traditional drugs was urgently required to decrease the used concentration of nanoparticles, overcome the microbial resistant to traditional antibiotics and resulted more efficient antimicrobial activity for the treatment of human and animal diseases.

Regarding the beneficial effects of the curcumin, **Pinlaor *et al*.(2010)**, reported that it can alleviate fibrosis in skin affection due to its anti-inflammatory property .While **,Lin et al. 2009**suggested that curcumin exerted antifibrotic effects depending on its concentrations , at lower concentrations curcumin exerted antifibrogenic effects, whereas at higher concentrations curcumin induce fibrosis through induction of apoptosis in hepatic satellite cells.

The feed additives are essential for biological functions of the animal and poultry including growth promoters, digestion, absorption, antimicrobial agent, metabolic modifiers, probiotic and prophylactics, amelioration the toxic effects of mycotoxins if existed in feed (**Namur *et al*.,1998 and Nabawy, 2015 and Hassan *et al*., 2017).**

In poultry, the adverse effects of *Fusarium t*oxins, particularly DON have serious adverse effects for broiler chicken, since concentrations of 10–12 mg DON/kg feed causedsigns such as reduced feed intake and reduced body weight gain. In addition, this toxicity characterized by extensive ecchymotic hemorrhages, deposition of urates, alteration of the nervous system, and inflammation of the upper gastrointestinal tract **(Huff *et al.,* 1981).**

Where, the T-2 toxin effects in poultry has genotoxic and cytotoxic effects, immunomodulatory effects, effects on the cells of the digestive system and liver, effects on the nervous system and skin and impairment of performance. **(Tobias *et al.,* 1992).**

Therefore, detoxification strategies have been developed to protect livestock animals and poultry against mycotoxin contaminated feed.

However, the elimination of trichothecenes from contaminated feedstuffs is an unsolved problem and once contamination has occurred in grains, few strategies can be adopted for limiting adverse effects in livestock **(WHO, 2001)**.

Some studies evaluated biological degradation of fusarium toxins and reported that DON is degraded by *Eubacterium* sp. which transforms DON into its metabolite DOM-1 the non- toxic de-epoxide of DON **(Binder *et al.,* 1997, 2000).** While, **Fuchs *et al.* (2002)** detected that the *Eubacterium* sp. is capable of detoxifying Trichothecenes. Additionally,

Awad *et al.* (2006) found that *Eubacterium* is beneficial in counteracting the toxicity of DON in commercial broilers at the gut level. DON has been reported to be completely transformed to de-epoxy-DON after incubating for 96 h with the content of the large intestine of hens **(He *et al.*, 1992).**

Currently, efforts have been done to screen microorganisms from different origins, which could detoxify trichothecene mycotoxins and this approach is defined as biodegradation **(Schatzmayr *et al.*, 2006**). *While, the organism of Gliocladium roseum* detoxified zearalenone by opening the ring structure with subsequent decarboxylation in yields ranging between 80 and 90% **(El-Sharkawy and Abul-Hajj 1988**).

Table (8). Influence of low doses of ZnNPs singly and in combination with probiotic or curcumin on Fusarium toxin production on commercial yellow corn.

Levels of *Fusarium* mycotoxins at gradual concentrations of treatments with								
Total levels of toxins before treat. (mg/kg-ppm)	**ZnNPs (100 ug/ml)**		**ZnNPs (100 ug/ml)**		**ZnNPs (200 ug/ml)**		**ZnNPs (200 ug/ml)**	
	Prob. (0.25%)	**Curc. (0.25%)**	**Prob. (0.5%)**	**Curc. (0.5%)**	**Prob. (0.25%)**	**Curc. (0.25%)**	**Prob. (0.5%)**	**Curc. (0.5%)**
22.2±4.02 (Total tricho.)	5.2±0.1	7.4±0.0	ND	ND	ND	ND	ND	ND
7.5±1.62 (Zearralenone)	1.0± 0.0	2.4±0.02	ND	ND	ND	ND	ND	ND
18.23±1.76 (Total tricho.)	3.0±0.0	5.1±0.1	ND	ND	ND	ND	ND	ND
4.0±0 (Zearralenone)	ND	ND	ND	ND	ND	ND	ND	ND
4.0±0 (Zearalenole)	ND	ND	ND	ND	ND	ND	ND	ND
40±00 (Total tricho.)	8.1±0.0	9.3±0.1	ND	ND	ND	ND	ND	ND
6.6±1.56 (Zearralenone)	0.4±0.0	ND	ND	ND	ND	ND	ND	ND
4.0±00 (Zearalenole)	0.1±0.0	ND	ND	ND	ND	ND	ND	ND

*ND: Not Detected

As the first steps in this direction, the authors here applied the obtained results in this work on commercial poultry feeds that highly contaminated with different molds included mycotoxigenic *Fusarium sp.*. The obtained results evidenced also higher antimycotoxin potential effects of Zn NPs in combination with other commercial antifungals (Table, 8). Whereas, the evaluation of these antifungal agents in inhibition of *Fusarium* toxins production in commercial feed indicated that the metal nanoparticles and herbs as curcmin and probiotic can be assed together in poultry feed to avoid the addition of high doses of ZnNPs and their toxicity . Currently, the synergistic actions of ZnNPs with curcumin or probiotic preparation at low doses (ZnNPs (100 ug/ml)+Prob.(0.5%)or Curc. (0.5%), resulted complet detoxification of *Fusararium* mycotoxins).

Similar results to our work were obtained by **Kaul *et al.*, (2012)**; **Yage *et al.*, (2012) and Taha *et al.*, (2014), Mohamed *et al.*, 2015 and Nabawy, (2015),** who detected the efficacy of ZnNPs, propionic acid and probiotic preparation in detoxification of ochratoxin and AFs in feeds. no effect of clove oils and metal nanoparticles when added to the ochratoxicated feeds. Generally, until now the metals as zinc, iron, cadmium, selenium and cupper are used as feed additive for their antioxidant and growth promoters for animals and poultry **(Frank *et al.*, 1984)**.

A high affinity addition of these compound to feedstuffs (Zn NPs, probiotic and curcumin) contaminated with *Fusarium* and their toxins has been shown to have a protective effect against the development of mycotoxicosis in farm animals and poultry **(Syed El-Ahl *et al.*, 2006 , Nabawy *et al.*, 2014 and Hassan *et al.*, 2017 and 2018).** The major advantages of these additives include expense, safety and easy administration through addition to animal and poultry feed and the addition of probiotic or curcumin with Zn NPs resulted significant de-

crease the used doses of nanomaterials which caused safe and fare doses from toxicity of nanomaterials to animal and poultry **(Hassan *et al.*, 2016).**

4- Conclusion

In this study mould of *Fusarium* species which recovered from poultry feeds were recorded to produce mycotoxins caused some degree of acute toxicity when consumed in high amounts and are potential carcinogens in animal and poultry. It also resulted in significant losses in animal health, which is an important contributor to the country's economy in the form of meat, milk, wool and leather. The results today suggest that ZnNPs , the probiotic and curcumin singly and in combination have potentials in treatments of mycotoxions producing *Fusarium* sp. and significantly decreased their ability for mycotoxin production. Whereas, the results of combination detected the requirement of lower concentrations from both to obtain the antimicrobial and antimycotoxins effects. Therefore, the synergistic, combination therapy are urgently required to decrease the used concentration of nanoparticles, overcome the microbial resistant to traditional antibiotics and resulted more efficient antimicrobial and antoimycotoxins activities for the treatment of human and animal diseases. Therefore, frequent testing program of the animal feeds and other environmental factors for fungi and mycotoxin contamination and use of feed. Hence, advanced and further investigations are required for direct treatment of farm animals by metal nanoparticles in combination with other safe herbs and biological compound to avoid toxic effects of nanomaterials which may result from misusing of nanoparticles. Hence, significance improvements in animal health and their productivity can be controlled.

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