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RESEARCH ARTICLE

A COMPARATIVE STUDY ON ANTIFUNGAL ACTIVITY OF FE_2O_3, AND FE_3O_4 NANOPARTICLES.

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Abstract

Nanoparticle is a new innovation of using particles in a new size which ranged from1- 100 nanometers that equal 1 $X10^{-6}$ meter. In this study we evaluated the antifungal effect of Fe_2O_3 and Fe_3O_4 nanoparticles on Aspergillus flavus (KP137700) selected for the study isolated from broiler feed was isolated from broiler feed by Mycology Department of the Animal Health Research Institute, Giza, Egypt. Fe_2O_3 and Fe_3O_4 nanoparticles synthesized by co-precipitate method. The size of synthesized nanoparticles was 45 nanometer for Fe_2O_3 and 9 nanometer for Fe_3O_4. The antifungal effect Fe_2O_3 and Fe_3O_4 nanoparticles against Aspergillus flavus measured by MIC method and evaluated by scanning electron microscope. The results revealed that Fe_2O_3 nanoparticles have antifungal effect more than and Fe_3O_4 nanoparticles.

Introduction:-

Nanotechnology has offered great possibilities in various fields of science and technology (Adibkia et al., 2007). Pharmaceutical nanotechnology with numerous advantages has growingly attracted the attention of many researchers. Nanoscale, Size range from approximately from 1 nm to 100 nm. This definition is accompanied by two notes: Note 1: Properties that are not extrapolations from a larger size will typically, but not exclusively, be exhibited in this size range. For such properties the size limits are considered approximate. Note 2: The lower limit in this definition (approximately 1 nm) is introduced to avoid single and small groups of atoms from being designated as nano-objects or elements of nanostructures, which might be implied by the absence of a lower limit (ASTM 2006).

Nanoparticles and nanocomposites prepared chemically by precipitation or in situ formation in a given matrix through the sol–gel processes (Sanchez et al., 1996). The conditions determinants of nanoparticle growth are changed in the dependence on method preparation of nanoparticles. Materials scientists and engineers have made significant developments in the improvement of methods of synthesis of nanomaterial solids (Hansany et al., 2012).

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Aspergillus flavus is one of the most important pathogenic fungi that contaminate grains or feed mixtures and cause serious problems in such as mycotoxicosis due to its aflatoxins production (Hassan et al., 2014). Development of new and effective antimicrobial agents seems to be of paramount importance, through the antimicrobial activity of metals having various properties, potencies and spectra of activity, has been known and applied for centuries especially in nano-size (Malarkodi et al., 2014).

The aim of this study is the founding of a new effective antifungal compounds through the nanotechnology against Aspergillus flavus which is the most dangerous fungi on animal productions.

Materials and Methods:-

Synthesis of nanoparticles:-

Fe_2O_3 nanoparticles synthesized by co-precipitation technique method by Using titra-hydrate ferrous chloride FeCl2.4H2O (> 99:9%) dissolved in distilled water. The ammonium hydroxide NH4OH were added drop-wise to the mixture with stirring under strong ultrasonic agitation was added to the solution to adjust the pH value at 9 or10 till precipitation occurred. Dark brown precipitate is formed and washed using distilled water for several times to remove excess ammonia (10 times) the precipitate was dried at 400°C for 4 hours (Kandpal et al., 2014).

Synthesis of Fe_3O_4 nanoparticles, Fe_3O_4 nanoparticles were synthesized by co-precipitation technique method of titra-hydrate ferrous chloride $FeCl_2$.4H2O (> 99:9%) , about 1.9881g, and hexa-hydrate ferric chloride FeCl3 .6H2O (> 97:9%) , about 5.406gm (Ozkaya et al., 2009).

Characterization of synthesized nanoparticles:-

X-ray diffraction analyses were carried out to identify the previously prepared nanoparticles in pure single phase. Also it confirms the successful formation of these nanoparticles according to (Cullity et al., 2001)
Infrared Spectra were recorded on a Perkin – Elmer (FT-IR):

FT-IR were carried out to identify the synthesized nanoparticles and to determine its size according to (Guan et al., 2003).

Evaluation of Fe_2O_3 and Fe_3O_4 nanoparticles effect on the growth of A. flavus:-

Selection of test pathogen:
Pathogenic Aspergillus flavus (KP137700) selected for the study isolated from broiler feed was obtained from the Mycology Department of the Animal Health Research Institute, Giza, Egypt. Preparation of dilutions of synthesized compounds 10 mg of the each nanoparticle (Fe2o3 and Fe_3O_4) were weighed accurately and dissolved in 10 ml dimethyl sulfoxide giving a solution of 1mg/ml concentration. 1 ml of the above solution was again diluted to 10 ml with dimethyl sulfoxide giving a solution of 100 µg/ml concentration. Determination of minimum inhibitory concentration and minimum fungicidal concentration Minimum inhibitory concentration (MIC) was determined for Fe_2O_3 and Fe_3O_4 nanoparticles showing antifungal activity against test pathogen by serial dilution method. Broth microdilution method was followed for determination of MIC values.

Sterilized loop wire was used to transfer A. flavus to sabouraud dextrose agar and incubated at 25 °C for 5 days. From the A. flavus strain, small portion was transferred to 3ml of sabouraud dextrose broth media separately and incubated at 25°C for 24 hrs.0.1 ml of the above five medias were transferred to five different stoppered conical flasks containing 0.9% NaCl solution.1ml of media was taken in a test tube, to which, 1ml of test solution (100 µg/ml) was added. Thereafter, 0.1ml of the microbial strain (A. flavus) prepared in 0.9% NaCl was added to the test tube containing media and test solution. Serial dilution were done five times giving concentrations of 50, 25, 12.5, 6.25, 3.75, 1.5 µg/ml. The test tube were stoppered with cotton and incubated at 25°C for one week. The MIC values were taken as the lowest concentration of the particles in the test tube that showed no turbidity after incubation. The turbidity of the contents in the test tube was interpreted as visible growth of microorganisms. The minimum fungicidal concentration (MFC) was determined by sub culturing 50µl from each test tube showing no apparent growth. Least concentration of test substance showing no visible growth on sub culturing was taken as MFC.

Scanning electron microscope examination of A. flavus after the exposure to Fe_2O_3 and Fe_3O_4:
In conical flasks one liter containing 200 ml of yeast extract broth (YEB) than the flasks were autoclaved for 15 minutes at 121 °C cooled at room temperature. The Fe2O3 and Fe3O4 nanoparticles were taken separately at different concentrations of 1.5, 3, 4.5 and 6 mg/100 ml, each concentration dissolved in sterilized distilled water

using an ultrasound bath. Preparation of spore suspensions of A. flavus was standardized to 10^6 spores/ml then adding one millimeter of spore suspension of A. flavus and different concentrations of Fe_2O_3 and Fe3O4 nanoparticles were add to Yeast extract broth (YEB). All the inoculated flasks were incubated at 25°C for 21 days. After the end of incubation period, the content of each flask was filtrated. The treated fungi mycelia sections were collected, fixed with formaldehyde, washed with phosphate buffer solution and dehydrated with alcohol solution (30, 60, 80, 90 and 100%, maintaining the mycelia at 100%) and then submitted to critical point drying according to (Al-othman et al., 2014). Aspergillus flavus became ready for scanning electron microscopy (SEM) using JEOL (JSM-6380 LA) instrument.

Results:-

Phase identification and structural analysis were carried out by XRD and FTIR spectra at room temperature for Fe_2O_3 and Fe_3O_4 nanoparticles showed that the investigated sample crystallized in a single phase 45nm size of Fe_2O_3 nanoparticles and 9nm size of Fe_3O_4 nanoparticles as shown in Fig 1, 2, 3 and 4.

Minimum inhibitory concentration (MIC) of Fe_2O_3, and Fe_3O_4 nanoparticles was 25mg and 50mg respectively against Aspergillus flavus.

SEM examination confirmed the anti-fungal effect of Fe_2O_3 and Fe_3O_4 nanoparticles through different degree of fungi cell membrane rupture fungal spores were observed damage such as reduce in spores number, malformations and hypertrophy these effects lead to destroyed and damaged of spores resulting in possible reduction of multiplication and the enzymatic activity of the micro-organism. This aspect is great important because Aspergillus reproduction involves mainly formation of spores. The severity of damage and malformation of Aspergilus flavus is high with Fe_2O_3 nanoparticles and low with Fe3O4 nanoparticles as in Fig 5 and 6.

Discussion:-

(Gehan et al., 2014) supported results of this study through evaluation of the antifungal potential Fe_2O_3 nanoparticles in inhibiting the growth of Aspergillus flavus. There was antifungal potential of prepared Fe_2O_3 nanoparticles in disc diffusion test. When the treated fungi with Fe_2O_3 nanoparticles were subjected to SEM, the damage and rupture of their cell wall were detected in the area surrounding growth. The normal conidial cell of Aspergillus flavus has a spherical shape and smooth cell wall and intact cell membrane. The effect of high concentration of Fe_2O_3 nanoparticles on the treated fungi was observed as membrane damage of cells and some pits that have been caused in intercellular components, leading to leakage and finally cell death.

The antifungal effect of Fe_3O_4 nanoparticles that evaluated in our study is agreed with (Bhupendra et al., 2013) who studied the minimum inhibitory concentration (MIC) values of Fe_3O_4–Ag nanoparticles is less than Ag nanoparticles against Aspergillus glaucus. The fungal growth declines as the concentration of Fe_3O_4–Ag is raised and when the latter reaches MIC, no growth is observed. Presence of Fe_3O_4 in composite with Ag gives the Ag more antifungal effect.

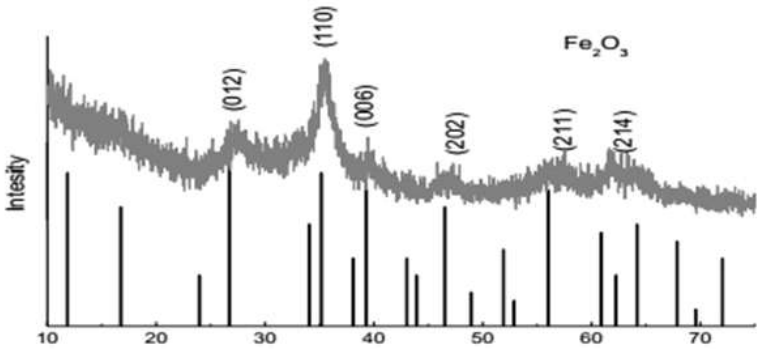

Figure (1):- XRD pattern of Fe_2O_3 nanocrystalline revealed *single phase 45nm size*.

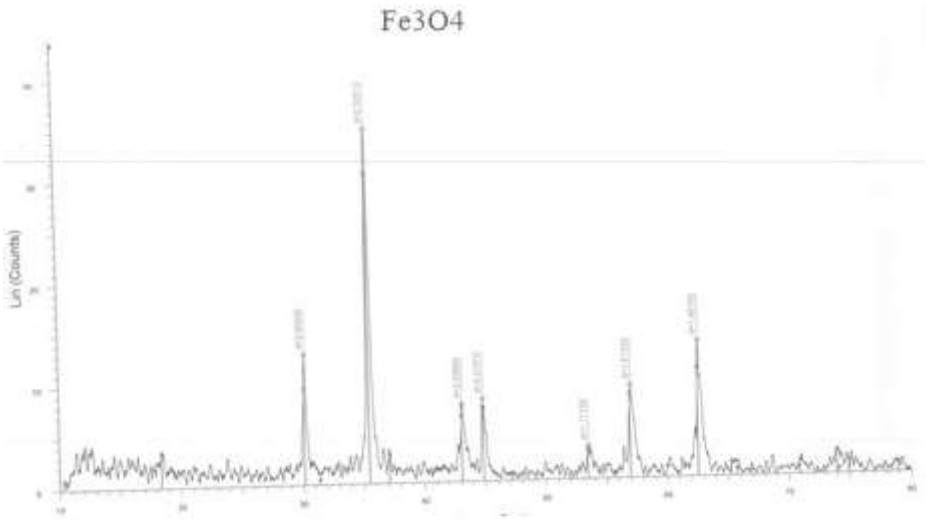

Figure (2):- XRD pattern of Fe_3O_4 monocrystalline revealed *single phase 9 nm size*.

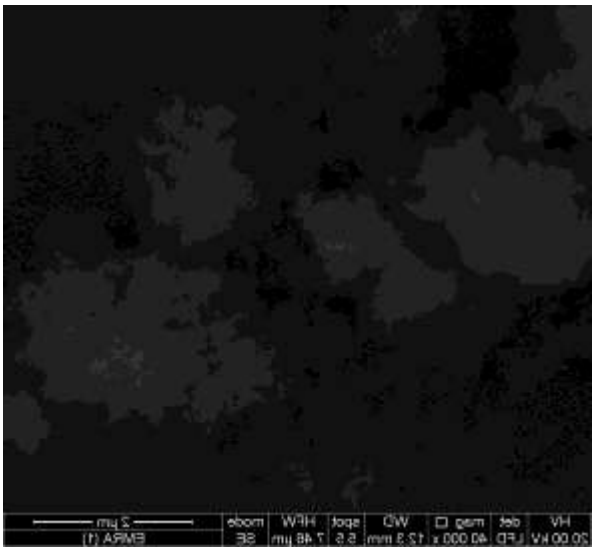

Figure (4):- FTIR pattern of Fe_2O_3 monocrystalline.

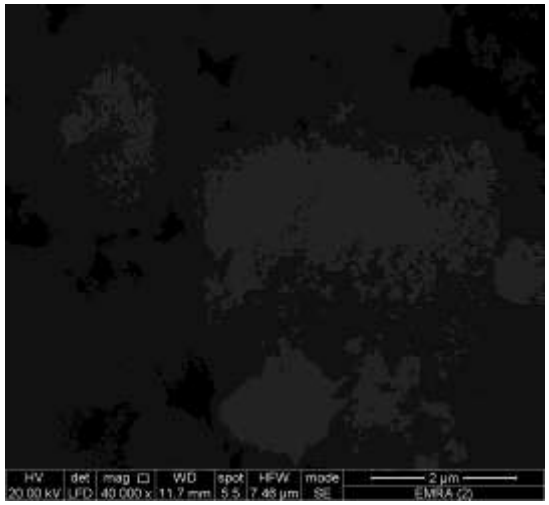

Figure (4):- FTIR pattern of Fe_3O_4 monocrystalline.

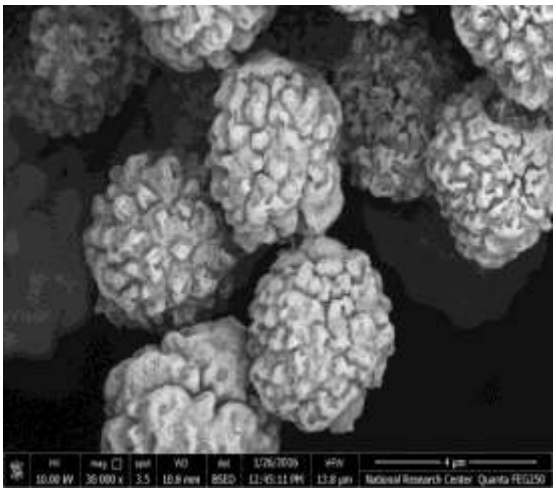

Figure (5):- SEM Aspergillus flavus with Fe_3O_4 monocrystalline *slight degree of fungi cell membrane rupture*.

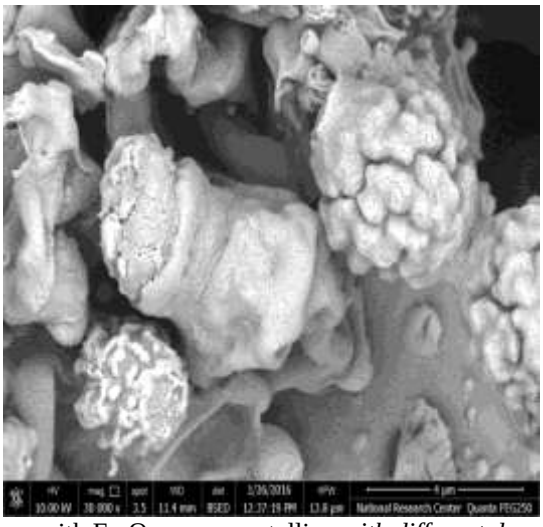

Figure (6):- SEM Aspergillus flavus with Fe_2O_3 monocrystalline *with different degree of fungi cell membrane rupture and fungal spore's damage.*

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