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Test of zinc oxide nanoparticles, honey, honey enriched by Zinc oxide nanoparticles and phylex lotion efficiency in inhibition of Aspergillus flavus growth in the culture media..

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ABSTRACT

This study was done at mycotoxin laboratory, plant protection department, college of agriculture, University of Baghdad to test efficiency of zinc oxide nanoparticles ZnO(NP),Honey, honey enriched by Zinc oidex nanoparticles and phylex lotion in Aspergillus flavus growth inhibition in the culture media (PDA).Available isolation of the fungus A.flavus was brought from plant protection department, College of Agriculture, University of Baghdad. It was noticed that adding ZnO(NP) at 1%,2% and 3% concentrations caused fungus growth inhibition by 98%,100% and 100% rates respectively.Using honey at 10% concentration caused fungus inhibition at 75.3% rate and in the 20% Reached 78% and in the 30% Reached 78.6%. The results showed that the honey enriched by ZnO(NP) was active in fungus inhibition, and at 10% concentration, the inhibition rate was 85.6% while at 20% Reached 87% and in the 30% concentration it was 87.3%.The phylex lotion caused fungus inhibition at rates 74.4%,100% and 100% when the phylex concentrations were 0.1%,0.2% and 0.3% respectively.

Keywords: Aflatoxin B1,ZnO(NP),phylex, honey, inhibition, Aspergillus flavus

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INTRODUCTION

Zea mays crop is considered as a strategic crop in the world and it holds the first position in the production after wheat and rice (FAO,2012).The corn crop is subjected to infect by many fungi even at field or during harvest or storage and the most accompanying problems in corn production are crop contamination by many kinds of fungi which produce the mycotoxins (Pacin,2005).The aflatoxins which are produced from some kinds of Aspergillus genusspecies, such as A.flavus, A. paraciticus and A.nomius which are considered as the most importance and danger of the mycotoxins which include B1,B2,G1 and G2 toxins and they have the ability to cause mutations, cancers, liver poisoning and inhibition of many of the metabolic systems(Alobsoy,2010).Honey has the ability to inhibit the fungus growth and it catalyzes to create inconvenient environment to the microorganism due to low water activity which in turn inhibits growth (Crane,1976).

The Nano technique is a new in fungus growth inhibitors and their toxins production and some of the Nano materials such as ZnO, MgO and SiO2 work to create inconvenient environment to fungi spread and inhibition its growth and destruction the resulted mycotoxins, this directs world intention toward it in the beginning of the 1980s of the twentieth century and it became as forefront of the sciences which have the most importance and excitement due to bearing of the great hopes in developing new kinds of the fungiinhibitors and their toxins and it is considered as a scientific technique which may change the science visage in the near future (Rico et al.2011).

The search in high efficiency and safe means in stop or destroy or remove growth of the toxins produced fungi in the foods is considered as necessary matter to protect from their risks to human, animals and the agricultural crops (Paskevicius et al .2006).

The aim of this research is using environmentally friendly materials which have the ability to resist the dangerous toxins produced fungi such as AFB1 and use zinc oxide (ZnO) (white powder and nearly non soluble in water) compared with the chemical materials which proved their activity in destruction or reduction of the toxins such as the phylex.

MATERIALS AND METHODS

Test ZnO(NP) efficiency in A. flavus growth inhibition in the culture media (PAD) laboratory .

Zinc oxide Nanoparticles ZnO(NP) was mixed by using ultra – sonic homogenizer (Sonicator) which was got from the American SKY spring Nano materials company at 1%,2% and 3% concentrations and in three replicate to each concentration. The tetracycline antibiotic(250mgL^{-1} concentration)was added to each petri dish and then circular moving was done to the petri dish and it was left to be solid. The Aspergillus fungi which was grown in PAD media wasput in middle of each petri dish by using(0.5 diameter) disk and then the closed petridishes were put in an incubator at 25 C^{o}for 7 days.The inhibition percentage was estimated(Shaban and AlMalah ,1993).

Test honey efficiency in A.flavus inhibition in the culture media (PDA) laboratory

The honey was got from college of Agriculture beehive and 200 gram honey was dissolved in 400 ml of (95%) ethyl alcohol and the sample was left for 72 hours with shaking between time and another. The sample was filtered by using Watman filter paper (No.2).The sample was concentrated after filtration at circular evaporator device at 40-50 C^{o} and then it was kept in a refrigerator until test time (Hade, 2014). Different honey concentrations of which were 10%,20% and 30% in three replicates and they were put in petri dishes. PDA culture media was prepared and sterilized at an autoclave at 121 C^{o}for 21 C^{o} and one bar pressure and tetracycline antibiotic was added to each petridish with circular shaking and then the petri dishes were left to let the media to be solid and then the Aspergillus fungus was added to the center of the petri dish and three petri dishes were left without addition as control treatment. The petri dishes were put in an incubator at 25 C^{o} for 7 days and the inhibition percentage was estimated (Shaban and AlMalah,1993).The best concentration gives best inhibition.

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Test honey enriched byzinc oxide NanoparticlesZnO(NP) in A.flavus inhibition in the culture media PDA laboratory.

The honey which was brought from college of agriculture beehive, University of Baghdad was mixed by ultra- sonic homogenizer (Sonicator) device and 200g.of the honey enriched by ZnO(NP) was dissolved in 400ml of 95% ethyl alcohol and the sample was left for 72 hour with shaking between time and another.

Three honey enriched by ZnO(NP) concentrations (10%,20% and 30%) in three replicates were taken and put in petri dishes and to each petrridish, the last prepared PDA culture media was added at (1) bar pressure and the dish was round moved and it was left to be solid and then the Aspergillus fungi were added in the middle of the petrri dish at it was put in an incubator at 25 C^o for 7days.The inhibition percentage was estimated (Shaban and AlMalah,1993).

Test efficiency of phylex lotion in inhibition of A. flavus fungus in the culture media (PDA) laboratory.

Three concentrations (0.1,0.2 and 0.3 ml) in three replicates of phylex lotion which is a liquid produced by the Dutch company (SELKO) and it was formed from group of the organic acids, propionic acid, lactic acid, orthophosphoric acids, citric acid, formic acid, sorbet acid and other materialssuch as anti-evaporation materials, disseminated materials, water and ammonia, were taken to each concentration. They were sterilized at autoclave at 121 C^o and for 21 minutes and at (1) bar pressure and the tetracycline antibiotic was added at 250 mgL^{-1}.The media was added to each petri dish with round moving and it was left to be solid and then the fungi was added in the middle of the petridish with using three petri dishes to be as control treatment and then all the petri dishes were put in an incubator at 25 C^o for 7 days.

The inhibition percentages of all treatments which were ZnO(NP)honey, honey enriched by ZnO(NP) and phylex lotion were estimated by using the following equation (Shaban and AlMalah,1993).

The inhibition percentage = **Error!***100

RESULTS AND DISCUSSION

Test efficiency of ZnO (NP) particles in A. flavus fungus inhibition in the culture media PDA laboratory:

The results showed that zinc oxide Nanoparticles was active in inhibition of A.flavus fungus in the 1%,2% and 3% As it wasPDA concentrations and the inhibition percentages were 1% is 98%,2%,3% is 100% in the three concentrations respectively. The Nanoparticles of the mineral oxides are considered unique in their capability in fungus inhibition and destroying their mycotoxins due to the high surface area of the nanoparticle and to the sharp structure of the nanoparticles surface milestones

(Klabunde et al.1996) compared with the well-known non-Nano organic and non-organic materials,such as Zinc oxide which has effect and bear forces and increase in its selectivity (do specified functions in limited medium) and increase in its heat resistance (Padmavathy and Vijayaraghavan,2008).

The studies provide zinc oxide activity as anti-bacterial and anti-fungus material in agriculture field (Yamamoto,2001; Sawai and Yoshikawa, 2004).Zinc compounds are also used as fungicides (Waxman,1998).

Control Concentration 2 %

Fig(1): Effect of ZnO(NP) on A. flavus growth inhibition on PDA media.

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Table (1): Test efficiency of ZnO(NP) on inhibition of the diameter growth of A. flavus fungi on PAD media

inhibition ratio%	Rate of colony diameter fungus(cm)	concentration%
98	0.13	1
100	0.0	2
100	0.0	3
0.0	0.0	Compared
NS:11.624	not significant	L.S.D value
(P<0.05)		

Test of honey efficiency in A. flavus inhibition in the PDA media laboratory.

The results showed activity of the honey in A. flavus fungus inhibition on the PDA media at the three concentrations,10% 20% and 30%.The honey caused an inhibition in the fungi growth on the PDA media compared with the control treatment. in the 10% concentration the inhibition ratio was 75.3% in the 20% Reached 78% and in the 30% Reached 78.6%.Honey content of saccharide and potassium gave it the high ability to kill the different microbes (Abo Shawar,2003).The studies refer that nearly 80species of microbes were inhibited by honey and other honey products, and the modern researches referredthat honey products contain many antibiotics which are capable to inhibit different kinds of bacteria and morbidity fungi (Abo Ayana and AlMizen,2009)

Fig(2): Effect of honey on A. flavus fungi inhibition on the PDA media

Control Concentration 20 %

Table (2): Test efficiency of honey on A. flavus diametric growth inhibition on PDA Media

inhibition ratio%	Rate of colony diameter fungus(cm)	concentration%
75.3	2.16	10
78.0	1.89	20

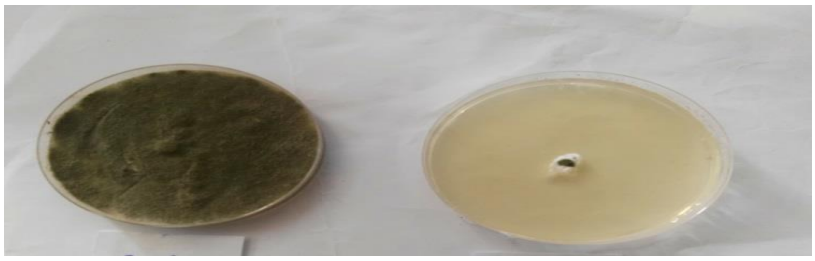
78.6	1.86	30
0.0	0.0	Compared
NS:6.933	not- significant	L.S.D value
(P<0.05)		

Test of efficiency of the honey enriched by ZnO(NP) on A. Flavus fungus inhibition on the culture media PDA laboratory.

The results showed activity of honey enriched byZnO(NP) in A. flavus inhibition on the PDA media at the three concentrations 10%,20% and 30%.In the 10% concentration the inhibition ratio was 85.6%, and in 20% Reached 87%, and in 30% Reached 87.3%, and it was clear that the honey inhibition ratio increased with ZnO(NP) addition.The honey takes good role in inhibition of many germs growth and it destroys them due to the high osmosis or to presence of enzymes or to presence of the bacterial growth inhibition materials (Frobisher et al,1974).

Ability of each type of honey type in inhibition and kill fungi differed with the plant and geographic honey differences even the chemical structure which causes presence of different inhibition factors. (AboAyana and AlMazen,2009).

Fig(3): Effect of the honey enriched by ZnO(NP) in A. flavus fungi growth inhibition on PDA media.



Control Concentration 20 %

Table (3): Test efficiency of honey enriched by ZnO(NP) in A.flavus diameter growth inhibition on PDA media

inhibition ratio%	Rate of colony diameter fungus(cm)	concentration%
85.6	1.23	10
87.0	1.09	20
87.3	1.09	30
0.0	0.0	Compared
NS: 6.021	not- significant	L.S.D
(P<0.05)		

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Test efficiency of phylex lotion in A.flavus fungus inhibition on PDA media laboratory.

Results of adding phylex lotion in A.flavus inhibition on PDA media showed that phylex lotion caused complete growth inhibition of the fungi on the PDA media compared with the control treatment and at 0.1 concentration,the inhibition percentage was 74.4,while it was 100% at both of 0.2 and 0.3 concentrations .This high inhibition activity caused complete inhibition and no fungus growth appeared during incubation periods,this result agrees with finding of AlHete (1977) who found that the brucel material which contains 99% propionic acid inhibited the fungi which infect the zea mays at rate reached 100%.In addition to the many results that used acids and their compounds for fungi growth inhibition,citric acid was used at 0.5% concentration and lactic acid at 0.75% concentration against A.parasiticus fungus to prevent fungi growth and to produce the aflatoxin.Salome (2007); AlHamere (2007) ;AlKase;AlBaldawe,(2007);AlBldawe(2012);AlGobore (2016) and AbidAlHassn (2017) recognized the high efficiency of phylex lotion in inhibition of Fusarium gramineram, A.ochaeuse, A.flavus and F.solani fungi respectively.

Fig(4): Effect of phylex lotion in A.flavus fungi growth inhibition on PDA media.

Control Concentration 0.2%

Table (4): Test efficiency of phylex lotion in A. flavus fungi diameter growth inhibition on PDA media

inhibition ratio%	Rate of colony diameter fungus(cm)	concentration%
74.4	2.23	0.1
100	0.0	0.2
100	0.0	0.3
0.0	0.0	Compared
NS:7.522	not- significant	L.S.D value
(P<0.05)		

CONCLUSIONS

Based on the results presented, efficiency of ZnO(NP), Honey, honey enriched by Zinc oidex nanoparticles and phylex lotion in A.flavus growth inhibition in the culture media (PDA).

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