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Evaluation of *Agaricus* sp. and Pleurotus sp. Extracts Efficiency in *Aspergillus Flavus* Growth Inhibition and Aflatoxin B1 Reduction

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ABSTRACT
The study was conducted to evaluate the activity of *Agaricus* and Pleurotus fungi extracts, normal and nanoform, on *Aspergillus flavus* growth inhibition, and AFB1 reduction. Results showed that the addition of Agaricus extracts into PDA at 250, 500, 1000 ppm caused inhibition in *A. flavus* growth at 72.94, 66.66, 0.00 % respectively for normal extract, 82.35, 78.03, and 40.78 % respectively for nanoform extract. The addition of Pleurotus extracts at the same above concentrations into PDA caused a reduction in *A. flavus* growth at 47.05, 26.27, and 0.00%, respectively for normal extract, 72.94, 69.41, and 45.88%, respectively for the nanoform extract. The inhibition effect was found temporal, disappeared when *A. flavus* was reinoculated on PDA without extracts. The treatment of *A. flavus* contaminated corn seeds with *Agaricus* and Pleurotus extracts at 250 ppm, the more effective concentration, and stored for 30 days caused *A. flavus* growth inhibition and aflatoxin reduction at 57.59 and 62.28% for Agaricus extract, normal and nanoform, respectively, 37.46 and 38.30% for Pleurotus normal and nanoform extracts, respectively. The treatment of AFB1 contaminated corn seeds with the more active concentration of *Agaricus* and *Pleurotus* extracts (250 ppm) and stored for 30 days caused a reduction in AFB1at 72.04 and 66.34% for *Agaricus* normal and nanoform extracts respectively, 42.70 and 46.32% for *Pleurotus,* normal and nanoform, extracts respectively. The results indicated that nanoparticles of natural compounds may be promising to restrict fungi producing toxins and prevent toxins production.

Keywords: Pleurotus, Agaricus, A. flavus, AFB1, nanoform, mycotoxin, fungal extracts.

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INTRODUCTION
The corn crop is considered as one of the world's main cereals for humans and animals [1]. Corn seeds are subjected to infection with many fungi producing mycotoxins in the field and during the storage that representing a severe high risk to humans and animals and limiting corn production [2,3]. Of the mycotoxins produced by fungi, aflatoxins, trichothecenes, ochratoxins, fumonisins, and zearalenone are found the more prominent [4,5]. Mycotoxins represent serious health risks to humans in prolonged exposure that causes immune disorders, liver damage, and cancer. In children, the aflatoxin content of milk can lead to a delay in growth [6].
Several methods were adopted to avoid mycotoxin contamination through preventing mycotoxin production in the field before harvest and after harvest in storage. These methods include physical treatment (thermal and irradiation), chemical transformation to less toxic (ozonization and ammoniation), and biological (bacterial degradation or absorption) [7-10] .
It has been reported that *Agaricus bisporus* extract was very efficient in the growth inhibition of many bacteria and fungi [11]. *Pleurotus ostreatus* was effective against many diseases and possess antioxidant characters [12]. Recent research reported that nanotechnology is increasingly penetrating the field of agriculture and mycotoxins [13].
This study was conducted to test the activity of *Agaricus* sp., and *Pleurotus* sp. extracts, normal and nanoform, in *Aspergillus flavus* growth inhibition and aflatoxin B1 reduction.

MATERIALS AND METHODS
Evaluation the activity of *Agaricus* sp. and *pleurotus* sp. extracts, to inhibit *Aspergillus flavus* growth on PDA.
The two fungi were air-dried in the shade. The fruit bodies were cut to small pieces and ground. The powder was distributed in flasks of 250ml capacity, 50ml/flask, and 100ml of methanol of 95% concentration were added to each flask. The flasks were agitated for 24hrs, passing the extraction through muslin cloth and filter paper Whatman 1.
The extraction was oven-dried at 40°C, and the precipitate was collected in a box covered with aluminum foil [14]. One gram of the precipitate was dissolved in 1000ml dist—water to obtain the standard solution with 10000 ppm. The extract's nanoform were prepared by exposing the standard solution to the ultrasonic system for 8min and tested by an Atomic Force Microscope (AFM).
The normal and nanoform extracts were added to PDA at 250, 500, and 1000 ppm, and the medium poured in petriplates , 9 cm dim. The plates inoculated at the center with spore's suspension of *A. flavus* by the inoculated needle. The plates were maintained at 25 $\pm$ 2 °C for seven days at three replicates with three non-treated replicates as control. The growing colonies diameter was measured, and the growth inhibition is calculated as follows:

$$\%\ inhibition = \frac{ROC_{control} - ROC_{treatement}}{ROC_{control}} * 100$$

Evaluation the activity of *Agaricus* sp. and *Pleurotus* sp. extracts on *A. flavus* growth and prevention AFBI production on corn seeds under Lab. conditions

Corn seeds dipped in water for 2hrs, and the seeds are distributed in glass containers of 200g /container. The seeds in the containers were autoclaved at 121 °C,1.5 kg/ cm^2 for 2omin. The normal and nanoform extracts of *Agaricus* and *Pleurotus* were added into the container at 250 ppm. The seeds were inoculated with *A. flavus* inoculum on millet seeds, two weeks old, at 5g /container.

The containers were thoroughly agitated and maintained at the lab. Temperature for 30 days at 3 replicates with three non-treated replicates as control. The seeds were oven-dried, 50g were taken from each treatment, and ground for AFB1 determination. The reduction of AFB1 was calculated using the following equation.

$$\%AFB1_{reduction} = \frac{AFB1_{control} - AFB1_{treatement}}{AFB1_{control}} * 100$$

The treatments T1 represent corn seeds inoculated with *A.flavus* as control, T2 is for seeds treated with normal *Agaricus* extract and inoculated with A. flavus, T3 is the seeds treated with nanoform *Agaricus* extract and inoculated with *A.flavus*, T4 is seeds treated with normal *Pleurotus* extract and inoculated with *A .flavus*, and T5 is for seeds treated with nanoform *Pleurotus* extracts and inoculated with *A.flavus.*

Evaluation the efficacy of *Agaricus* sp. and *Pleurotus* sp. extracts in AFB1 degradation on corn seeds under Lab. conditions

Corn seeds, prepared as described above, were distributed in a glass container, 200g/container, and autoclaved at 121°C and 1.5kg/cm2 for 20 min. The seeds are contaminated with *A.flavus* on millet seeds,5g/container. The containers were tight closed and maintained at 25$\pm$2 for 21 days with agitation and aerification every five days. The containers autoclaved at 121 °C and 1.5kg/cm2 for 20 min. The extracts were then added into containers, thoroughly agitated, and maintained at Lab. conditions for 30 days at three replicates with three untreated containers as control.

The seeds were oven-dried, 50g of each treatment was ground for ABF1 estimation, and the aflatoxin reduction was calculated. The detection and estimation of AFB1 were carried out, as described in [15].

The treatments were, T1= AFB1 contaminated seeds (control), T2= AFB1 contaminated seeds, treated with normal *Agaricus* extract, T3 = AFB1 contaminated seeds, treated with nanoform Agaricus extract, T4 = AFB1 contaminated seeds, treated with normal *Pleurotus* extract, T5= AFB1 contaminated seeds, trembled with nanoform *Pleurotus* extract.

RESULTS AND DISCUSSION

Efficiency of *Agaricus* sp. extracts in *A. flavus* growth inhibition on PDA

The results illustrated in Table (1), Figure (1) showed that the addition of normal and nanoform (72.27nm) of *Agaricus* extract into PDA at 250, 500, l000 ppm caused high growth inhibition of *A. flavus* with inhibition percentages, 72.94, 66.66,0.00 % respectively with normal extract, 82.35, 78.03,40.78 % respectively with nanoform extract.

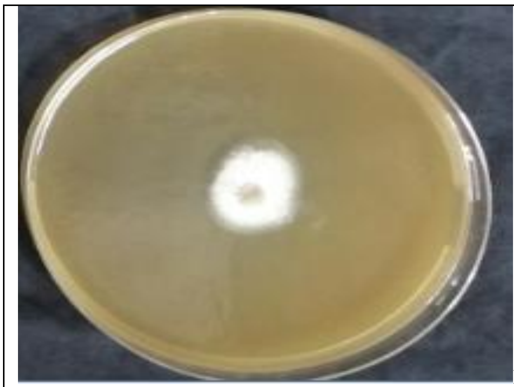
It was observed that *A. flavus* growth inhibition decreased with increasing extract concentration for both normal and nanoform, where the higher growth inhibition was at 250 ppm,72.94, and 82.35%, respectively. High growth may be due to the high content of nutritional elements in the extract that activate fungal growth. The inhibition effect of *Agaricus* extract against *A. flavus* may be due to the presence of active antifungal compounds. It was reported that *A. bisporus* contains many compounds including, polysaccharides, lipopolysaccharides, peptides, phenolic compounds, nucleosides, and amino acids [16]. Of the phenolic compounds found in *A.bisporus* extract are benzoic acid derivatives (p-hydroxybenzoic, protocatechuic acid, gallic acid), cinnamic acid derivatives (cinnamic acid, p-coumaric acid, ferulic and chlorogenic acid), and ergosterol, that used as bio-fungicide [17-20].

The promotion of *A. flavus* growth at a high concentration of *Agaricus* extract may came from its contents of nutritional elements and compounds promoting fungal growth. It has been reported that *A.bisporus* extract contains proteins, amino acids, carbohydrates, lipids, and vitamins [21-23] as well as containing micro and macro-elements [24]. Therefore, increasing *Agaricus* extract concentration, in PDA induced *A. flavus* growth promotion that overcomes the inhibition effect of active antifungal compounds in the extract.

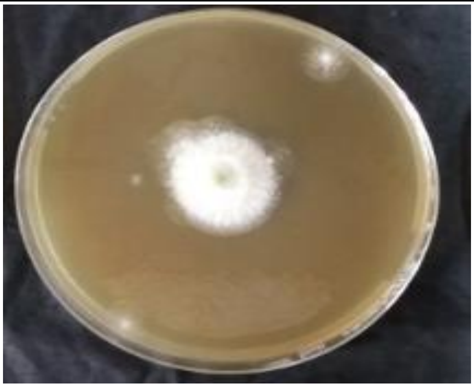
It was recently reported that methanol and ethanol extracts of *A.bisporus* are considered as a source of active biological compounds including, unsaturated fatty acids, phenolic compounds, ergosterols showing antifungal activities [25].

Table 1: Efficiency of normal and nanoform Agaricus sp. extracts in A. flavus growth inhibition on PDA.

Concentration (ppm)	% inhibition of normal *Agaricus* sp. extracts	% inhibition of nanoform *Agaricus* sp. extracts
0	0	0
250	72.94	82.35
500	66.66	78.03
1000	0.00	40.78
L.S.D.5%	10.25**	10.72**



nanoform *Agaricus* sp. extracts of 250ppm



normal *Agaricus* sp. extracts at 250ppm

Control

Figure 1: Effect of the more effective concentration of Agaricus sp. normal and nanoform extracts in A. flavus growth inhibition .

Activity of *Pleurotus* sp. extract in *Aspergillus flavus* growth inhibition on PDA

Results in Table 2 and Figure 2 indicated that addition of normal and nanoform (53.06 nm) *Pleurotus* extracts into PDA at 250,500,1000 ppm induced *A. flavus* growth inhibition at 47.05, 26.27 ,0.00 % respectively for normal extract: 72.94, 69.41, and 45.88%, respectively for nanoform extract.

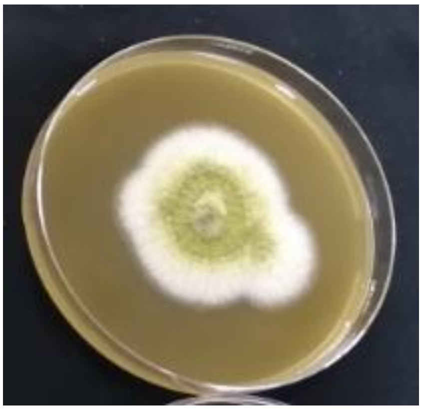
As in *Agaricus* extract effect, it was observed that the growth inhibition of *A. flavus* decreased with increasing extract concentration. The fungal growth inhibition may Came from the presence of active antifungal compounds inhibiting *A. flavus* growth as previously described. The ineffectively of the extract on *A. flavus* growth at high concentration may be due to its, contents of nutritive compounds promoting the fungal growth and leading to overcome the inhibition effect of the active compounds [26,27,16] . It was reported that petroleum ether and acetone extract of *Pleurotus* showed growth inhibition activity for many pathogenic bacteria and fungi [28].

It was found that the effect of *Agaricus* and *Pleurotus* normal and nanoform extracts was temporal and directly disappeared when reinoculation the fungus on PDA without extract.

Table 2: Efficiency of normal and nanoform Pleurotus sp. extracts in A. flavus growth inhibition on PDA.

Concentration (ppm)	% inhibition of normal *Pleurotus* sp. extracts	% inhibition of nanoform *Pleurotus s* sp. extracts
0	0	0
250	47.059	72.941
500	26.275	69.412
1000	0.000	45.882
L.S.D.5%	6.593**	3.079**

nanoform *Agaricus* sp. extracts



normal *Agaricus* sp. extracts at 250ppm

Control

Figure 2: Effect of the more effective concentration of pleurotus sp. normal and nanoform extracts in A. flavus growth inhibition .

Activity of *Agaricus* and *Pleurotus* extracts in *Aspergillus flavus* growth inhibition and prevention of AFB1 production in corn seeds

Results, Table 3, showed that the addition of more effective concentration of *Agaricus* and *Pleurotus* extract at 250 ppm on corn seeds contaminated with *A. flavus* and stored for 30 days, caused *A.flavus* growth inhibition and reduced aflatoxin production attained to 57.59 and 62.28 % for normal and nanoform *Agaricus* extracts respectively, 37.46md 38.30% for normal and nanoform *Pleurotus* extracts respectively. The reduction in aflatoxin came mainly from *A.flavus* growth inhibition and the capacity of compounds in the extract to adsorb and degrade the aflatoxin.

Variation in the extract's activity was observed; this may depend on the constituents of the extract and the targeted organisms. It was reported that the inhibition activity of fungi extracts against microorganisms depend on fungal species and fungal constituents [29-31].

Table 3: Efficiency of Agaricus and Pleurotus normal and nanoform extracts in Aspergillus flavus growth inhibition in stored corn seeds and prevention of AFB1 production

Treatment	AFB1 concentration ppm	% AFB1 reduction
Control	21.2	0
Normal *Agaricus* sp. extract	9.01	57.59
Nanoform *Agaricus* sp. extract	7.39	62.28
Normal *Pleurotus* sp. extract	13.36	37.46
Nanoform *Pleurotus* sp. extract	13.17	38.30
L.S.D. 5%		6.160**

Activity of *Agaricus* and *Pleurotus* extracts in AFB1 degradation in corn seeds under Lab. conditions

Results in Table 4 indicates that the addition of *Agaricus* and *Pleurotu*s extracts, normal and nanoform, to corn seeds contaminated with AFB1 at 250 ppm and stored for 30 days caused a reduction in AFB1 at 66.34 and 72.04% six respectively, with *Agaricu*s extract, 42.70 and 46.32% with *Pleurotus* extract.

The reduction of AFB1 may be due to the adsorbent capacity of the extract that binds AFB1 by functional groups on extract particles' surface. The high activity of nanoform extract compared with normal extract, especially with *Agaricu*s, maybe due to their high surface area to the volume that enables the binding of higher concentration of aflatoxin as well as the use of nanoparticles induced modification of the surface by functional groups [32].

Table 4: Efficiency of normal and nanoform Agaricus and Pleurotus extracts in AFB1degradation in stored corn seeds.

Treatment	AFB1 concentrate ppm	% AFB1 reduction
Control	59.12	0
Normal *Agaricus* sp. extract	16.47	66.34
Nanoform *Agaricus* sp. extract	19.83	72.04
Normal *Pleurotus* sp. extract	33.75	42.70
Nanoform *Pleurotus* sp. extract	31.7	46.32
L.S.D.		4.209**

CONCLUSION

The results of this study indicated that nanoparticles of flesh fungi, *Agaricus* and *pleurotus*, exhibited high activity in reduction AEB1 produced by *A.flavus* on corn seeds .The reduction of AFB1 may be due to restriction *A. flavus* growth as proved on PDA, and to the capacity of active compounds in the extract to adsorb and converted the aflatoxin less toxic compounds or degradation the toxin as proved by the reduction of AFB1 on contaminated corn seeds. These results suggested that nanoparticles of natural innocuous compounds may be promising in restriction of *A.flavus* growth and elimination of AFB1 from food and feeds.

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