Green synthesis and characterization of Zinc oxide nanoparticles using plant *Asparagus officinalis* and effect on *Aspergillus* species

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Abstract

This research aims to evaluate the effectiveness of green nano zinc oxide in inhibiting some fungi producing aflatoxin, Mycotoxin producing fungi were detected using coconut media and TLC plates, Zinc oxide nano showed inhibitory efficacy against the fungi studied. The nanomaterial was diagnosed using XRD, UV, SEM, FT-IR and AFM, The fungicidal activity of green nano was tested against aflatoxin producing fungi, *A.flavus ,A.niger, A.oryzae , A.fumigatus* with excellent antifungal action

Keywords: Zinc oxide. *Asparagus officinalis* .*Green* synthesis. A tifungal activity. aflatoxin

Introduction

Practising greener pathway in nanotechnology usually inculcates utilization of natural sources, biodegradable and biocompatible materials, non-toxic solvents in a productive and cost effective manner [1–4]. Green chemistry inspires the design of new products and processes that cut back or eliminate the utilization and generation of hazardous substances [5–8]. Nanotechnology has expanded vastly in various fields, involving the synthesis of nanoparticles, nanotubes, and nanowires due to their surface-enhanced Raman scattering (SERS) and surface plasmon resonance (SPR). Metal oxide nanoparticles have widespread consideration because of their demand in various felds, including biomedical sciences, chemical industry, electronics, drug–gene delivery and biosensor, etc. [9]. Asparagus (*Asparagus officinalis* L.), a perennial herb originated from the eastern Mediterranean and Asia Minor and has been cultivated for over 2000 years. At present, more than 60 countries around the world grow asparagus including the United States, Italy, Netherlands, Canada, Germany and other western developed countries. Currently, production, scientific research & development of asparagus have extended from the developed to developing countries, and China has become one of the major destinations. Peru, the world's leading exporter of asparagus [10], has a two-thirds market share with China. Asparagus can be simply classified into green asparagus, white asparagus, purple green asparagus, purple-blue asparagus and pink asparagus based on the color differences. White asparagus turns into green asparagus when exposed to sunlight after harvesting. Asparagus can be processed into canned food and marketed as a fresh peeled product and green asparagus to meet the increasing demand of vegetables [11]. In recent years, the nutritional and bioactive components in various

plants, including Rambutan (Nephelium lappaceum L.)[12], Rosa roxburghii [13], the genus Spinacia [14], lettuce (Lactuca sativa L.) [15], etc. have been well reviewed. Asparagus is rich in protein, fat, vitamins and minerals, and the contents are five times higher than other normal vegetables [16]. As a traditional Chinese herb, according to the famous medical book "Compendium of Materia Medica", asparagus contains a variety of bioactive phytochemicals, including bioactive polysaccharides, steroidal saponins, flavonoids, dietary fiber and bioactive oligosaccharides [17]. The physiological properties such as antioxidant, antiepileptic, antitumor, hypolipidemic, decompression, immunomodulatory, and antifungal effects all have been reported [18-22]. Al-Snafi reviewed the chemical constituents and the pharmacological effects of *A. officinalis,* but the influence of storage and progress of asparagus on bioactive components and its application have not been reviewed [23]. Due to its high nutritional value, medicinal value and special therapeutic effect, asparagus has become one of the world's top ten dishes and is named as "the King of Vegetables" internationally. This paper specifically addresses the nutritional value, bioactivities, the influence of storage and processing of asparagus on bioactive components and its application, to provide the theoretical basis for the development of *A. officinalis L* [24]. The discovery of aflatoxin was at the time of high mortality ratio of turkeys (about 100,000) in England, the United States of America, and Brazil, while the cause of death was not clear, and thus, the disease was called X [25]. However, a research in 1960 on the pollutants in the peanut meal revealed that these contaminates was with fungi [26]. Analysis of the peanut meal showed four types of spats on chromatographic plates under ultraviolet rays emitted green and blue colors [27]. The fungi that produced the toxins were detected, and it was found that the contaminated fungus was *A. flavus* [28], and the toxins were called aflatoxins [29]. Aflatoxins are secondary metabolites produced naturally by certain species of *Aspergillus spp* [30]. The properties of aflatoxins are colorless, odourless and tasteless; therefore, it is difficult to detect with the naked eye [31]. More than 20 types of aflatoxins have been identified, but the most common and toxic are B1, B2, G1, G2, where B and G refer to the fluorescent after separation on chromatographic plates and exposed to ultraviolet radiation appeared blue and green colors. The numbers (2-1) are the symbol of the (RF) caused by spots on the chromatographic plates [32]. In addition, Aflatoxin M1 and M2 are derived from AFB1 and AFB2, respectively, and are excreted in cattle milk after feed consumption contaminated with aflatoxin [33]. Contaminated food is the main source of exposure to aflatoxin, which adversely affects human and animal health [34], and toxicity affects animals according to species, sex, age, nutritional status, chemical effects, toxicity and length of exposure to the organism [35].

Aflatoxin B1 is the most toxic type other types, and its presence causes several losses in agriculture, industry and poultry breeding, where it increases the losses of animals and has a harmful effect human and animal health [36]. Due to the widespread prevalence of aflatoxin producing fungi in food products, FAD has now allowed 20 ppm of aflatoxin in the food as the maximum allowed level [37].

Materials and Methods

Plant material and extraction

Preparation of asparagus extract: *Asparagus* herb was purchased dry from the local market for herbalists; the plant was ground and took 12.5 grams of it and placed in a unique bag, then placed in a dissolute device, then took 250 ml of ethanol alcohol at a concentration of 96% and the device was operated for two hours, and then the extract was filtered by unique filter papers, then placed in bottles and kept in the refrigerator until use.

Green synthesis of Zinc oxide nano

The biosynthesis of zinc oxide nanoparticles followed the method described by [38] ,With some modification in the preparation of the hybrid nanocomposite, the preparation of ZnO NPs by plant synthesis method, 34 g of ZnCl2 was added in 500 ml of deionized water, and the solution was placed on a magnetic motor, then 50 ml of asparagus extract was added drop by drop by buret with continuous stirring by magnetic mixer, heating at 45 °C for two hours and adding ammonia and potassium hydroxide until a pale yellow precipitate was formed with grounding control. pH by special papers to measure PH until it reached PH=12 and left the nano solution for a whole day in the laboratory and then we filtered the solution by filter papers and washed the precipitate by deionized water several times and then washed ethanol alcohol, and the precipitate was dried at a temperature of 60 °C for 8 hours and then ground with ceramic mortar well to obtain a fine powder and kept in the refrigerator until used in the study

Characterization of Zinc oxide nano

Diagnosis of nanocomposites Five methods were used to diagnose zinc oxide nanohybrids under study, including ultraviolet, visible spectrophotometer (UV) Fourier transform infrared spectroscopy (FTIR), as well as the use of X-ray diffraction (XRD), scanning electron microscope (SEM) and atomic fors macroscopic (AFM).

Fungi isolation and purification

Three small pieces of each sample were taken and planted by sterile needle in Petri dishes with a diameter of (9cm) containing the PDA culture medium prepared in advance at a rate of three repeaters for each sample in the center of each dish in sterilization conditions and then incubated the dishes in the incubator at a temperature of (25_ + 2 C) for five days with follow-up, after the appearance of fungal growth the plantation was purified by taking a swab from the edge of the colony by a sterile needle and regrown On the same medium and incubated for another seven days at the same temperature as the previous and so pure cultures were obtained, the samples were kept in clean and sterile plastic test tubes containing the PDA media in an oblique slant tablets were planted on the culture medium for five days, and these media were prepared for the purpose of preserving the fungi for a more extended period and preserving them without contamination until the completion of the study, and kept in the refrigerator at a temperature of 4 m.

Detection of the susceptibility of fungal isolates to the production of toxins

Using coconut medium and ammonia solution A distinction was made between isolates that produce toxins and non-toxin isolates, by growing them on a pre-prepared coconut medium and inoculated with pure colonies grown on a seven-day-old PDA medium of isolated fungi and incubated for seven days in the incubator at a temperature of 27-25 ° C. After the appearance of mycos, wet filter papers were placed with drops of ammonia solution at a concentration of 20% on the lid of the dishes on which the fungus grew and re-incubated for four days upside down and the fungi producing toxins were distinguished from others by coloring the bases of the colonies in red or orange instead of transparent color (39).

Using the method of thin chromatography sheets

fungal isolates were developed on the medium (PDA) by placing tablets of the studied fungi with a diameter of 5 mm at the age of one week in the center of each dish and repeated the process three (repeaters) for each fungal isolation after which it was incubated at a temperature of 25-37 ° C for a week, then a dish was chosen from each isolation and the agricultural medium was cut on the isolation of the mushroom by a sterile knife in the form of small pieces, then the pieces were transferred by a sterile needle to the electric mixer containing 20 ml of chloroform and then mix the mixture for 10 minutes, then the mixture was filtered by filter paper, then took the filter and placed in a clean and sterile flask and placed in an electric oven with a temperature of 40 m, where the amount was concentrated to approximately 1 ml only. The presence of aflatoxin B1 was detected, using the technique of thin chromatographic sheets (TLC) with dimensions (10 * 20) cm, where the plates were activated in the electric oven at a degree of (120) m for an hour before use, where the method was followed [40]. The chloroform separation system was used: methanol 98:2.

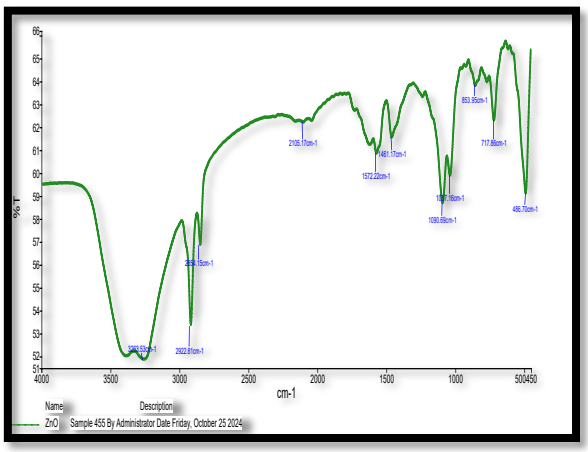
Testing of the effect of zinc oxide nanoparticles ZnO and asparagus plant extract on the radial growth of fungi *A. flavus, A.niger, A.oryzae and A. fumigatus*

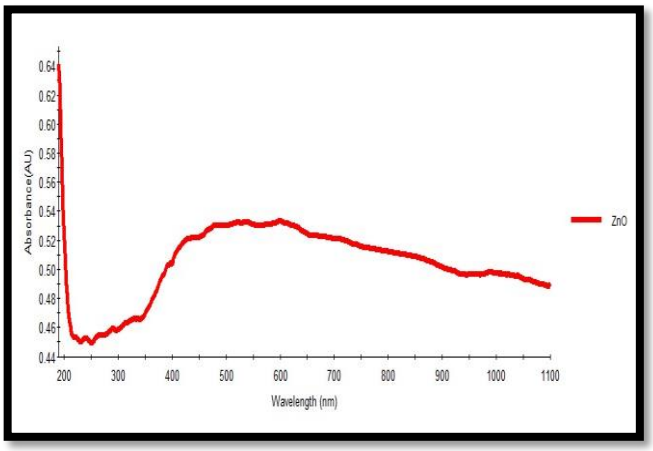
An evaluation of zinc oxide nanotechnology and alcoholic extract of asparagus plant was conducted, where the Stock Solution was prepared from a suspension of 2 g of nanomaterial prepared in 10 ml of deionized water and a little DMSO solvent was added to it at a concentration of 1% and completed the volume to 100 ml and trek on the Magnetic Stirrer device for two hours and took concentrations (5, 10, 15, 20 and 25) mg/ml added to the culture medium (PDA) which sterilized autoclave for 20 minutes Under pressure of 1.5 atmospheres and a temperature of 121 °C, then the dishes were inoculated with a 5 mm tablet of the fungi *A.flavus, A.niger, A.oryzae and A. fumigatus* taken from pure colonies at the age of 7 days separately, as well as inoculation of dishes free of nanomaterial and extract for comparison, knowing that all treatments and comparison have been repeated three times. All dishes were incubated at a temperature of 27 for a week.

Results and Discussion

Characterization of green synthesized Zinc oxide nano

When experimenting, we noticed a change in the colour of the solution from dark green to pale yellow due to the peak of plasmon resonance, which is located between 200 and 400 nm, in order to examine the type of plasmon in the surface of the nano-material, the function that is the absorbency is drawn in terms of the wavelength, so we see a peak height as shown in Figure (1) The technique of spectroscopy of visible and ultraviolet rays is characterized as a simple, sensitive and fast technique that provides valuable information about nanomaterials and is used to examine The size and oxidation state of carbon nanotubes or to determine the size and shape of metal nanoparticles.





The bandwidth of the absorption spectrum and intensity and the wavelength value of the surface plasmon resonance of metal nanoparticles depend on their composition, shape, agglomeration state and size (41), where Beatty and his group presented a linear relationship and estimated the size of the zinc oxide nanoparticles from the full mid-intensity width of the peak

Figure (1): UV ZnO NPs **Figure (2) :FT-IR ZnO NPs**

Figure (2): FT-IR beams of Zinc Oxide ZnO Nps from the alcoholic extract of the Asparagus plant in powder form FT-IR beams start with a range of 450-500.

This peak refers to the expansion vibrations of the Zn-O bond (zinc with oxygen), which is the main feature that confirms the presence of zinc oxide nanoparticles, if the top is clear and sharp at this range, ZnO has been successfully formed. In the case of nanoparticles, the peak may be slightly wider due to the small size of the particles and their surface effects, and the area 3000-3500 (o-H groups) these peaks indicate the vibrations of hydroxylic bonds (O-H), which may be caused by water absorbed on the surface of the nanoparticles. Hydroxylated functional groups are found in the plant extract used for the synthesis of nanoparticles; the broad peaks in this area often indicate water absorption or the presence of covalent hydroxyl groups; if the synthesis was done using asparagus plant extract, it is natural that these peaks appear as a result of the remaining plant compounds, and the area of 1600-1700 (c=o or c=c) peaks in this region belong to...

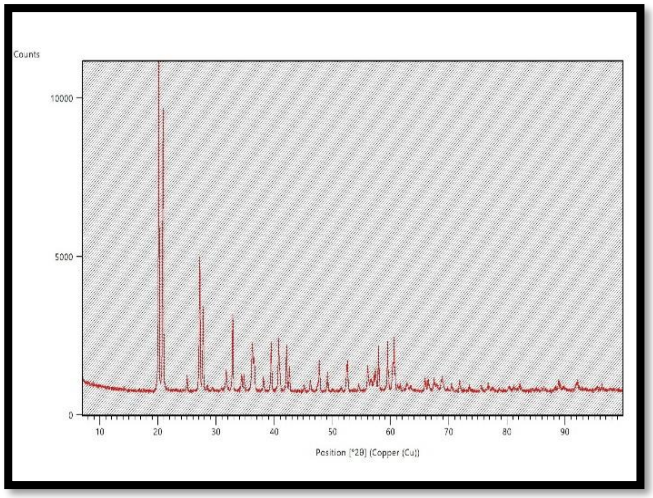


Figure (4): XRD ZnO NPs

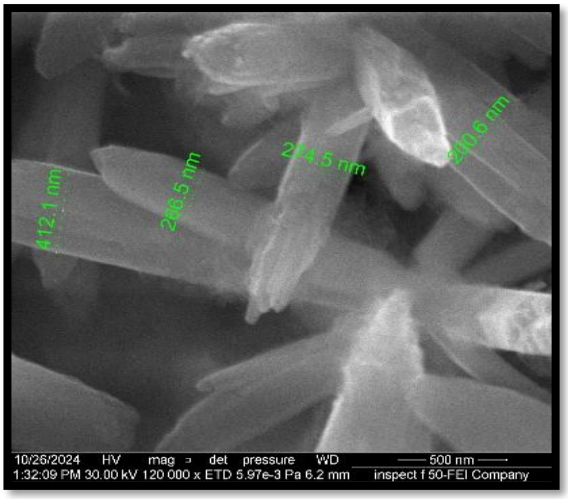


Figure (5): SEM ZnO NPs

The biosynthesized zinc oxide nanoparticles from the alcoholic extract of asparagus plant were examined with an X-ray diffraction device to obtain the crystal structure and the average particle size, and Figure (3) shows the XRD x-ray diffraction spectrum of zinc oxide particles and shows the appearance of peaks and angles that indicate the cramming of zinc oxide nanoparticles between the layers of the alcoholic extract of the asparagus plant, where the prominent peaks appeared with Braque reflections at the value of 2 theta (2Ɵ). X-ray diffraction (XRD) is an analytical technique used to determine the crystal structure of nanomaterials. When X-rays are shining on the crystalline material, the rays are scattered at specific angles that depend on the atomic arrangement within the material This dispersion produces a distinctive pattern known as the diffraction pattern, which can be analyzed to determine the material's properties. The surface phenotypic shape of the biosynthetic zinc oxide nanoparticles from the alcoholic extract of the asparagus plant Asparagus officintiol was detected by SEM electron microscopy Figure (4) Pictures Crystal structures appear in prismatic shapes or Rod rods and these shapes indicate that ZnO may be formed in an organized crystalline way, and the regular structure indicates that the synthesis conditions (temperature, extract concentration) were suitable for the formation of clear crystalline structures, and assemblies may be due to biological processes (organic matter in the extract). vegetation) Alternatively, due to the rapid drying of the sample preparation, porosity is a plus because it increases the effective surface area, enhancing properties such as absorbency or activity Catalyzed activity, and the images confirm the success of ZnO particle biogenesis with the appearance of regular crystalline structures.

Effect of Zinc Oxide Nanoparticles ZnO NPs with Asparagus Choleretic Extract on the Growth of *A.flavus, A.niger, A.oryzae and A. fumigatus*

The present study examined the antifungal activity of zinc oxide nanoparticles prepared using medicinal asparagus plant extract Table (1) against four types of Aspergillus fungus (*A. flavus, A. niger, A. oryzae, and A. fumigatus*) on Akar PDA. Inhibition regions were measured in mm and evaluated at five different concentrations of

nanoparticles prepared: 5 mg/ml, 10 mg/ml, 15 mg/ml, 20 mg/ml and 25 mg/ml. Control samples, which did not include any addition, showed the largest inhibition areas for all types of fungi, ranging from 50.0 mm for A. flavus to 65.0 mm for A. oryzae, when applying nanoparticles at a concentration of 5 mg/ml, large inhibition areas were observed in all species, with A. flavus showing 40.25 mm and A. fumigatus showing 35.5 mm**.**

Table (1): Effect of Zinc Oxide Nanoparticles with *Asparagus* Alcoholic Extract on the Growth of *A.flavus, A.niger, A.oryzae and A.fumigatus*

Extract (mg/mL)	*A. Flavus* (Mean ± S.D.)	*A.niger* (Mean ± S.D.)	*A. Oryaze* (Mean ± S.D.)	*A. fumigatus (Mean ± S.D.)*
Control	50.0 ± 0.13 (a)	55.0 ± 0.18 (a)	65.0 ± 0.36 (a)	41.25 ± 0.18 (a)
5	40.25 ± 0.19 (b)	37.5 ± 0.14 (b)	37.0 ± 0.32 (b)	35.5 ± 0.27 (b)
10	35.0 ± 0.30 (c)	29.5 ± 0.22 (c)	35.0 ± 0.25 (c)	30.0 ± 0.33 (c)
15	34.0 ± 0.25 (c)	25.0 ± 0.12 (c)	30.0 ± 0.36 (c)	15.0 ± 0.25 (d)
20	30.0 ± 0.13 (d)	23.5 ± 0.32 (d)	29.25 ± 0.34 (c)	0.0 ± 0.0 (e)
25	20.0 ± 0.20 (e)	19.75 ± 0.21 (e)	16.25 ± 0.25 (d)	0.0 ± 0.0 (e)
LSD	3.14	3.11	6.19	5.14

These results demonstrate the effectiveness of nanoparticles in lower concentrations in inhibiting fungal growth. A gradual decrease in the inhibition zones has been observed. For example, in A. flavus, inhibition areas decreased from 40.25 mm at 5 mg/ml to 20.0 mm at 25 mm/ml, while in A. fumigatus, inhibition areas decreased from 35.5 mm at 5 mg/ml to 0.0 mm at 20 mg/ml and 25 mg/ml, indicating complete resistance to treatment at higher concentrations. Similar trends have been observed in other fungal species, where inhibition areas have steadily diminished as the concentration of nanoparticles increases. Of the four types, A. oryzae showed relatively larger inhibition areas at lower concentrations but showed a significant decrease at higher concentrations. In contrast, A. fumigatus showed the lowest overall inhibition regions and complete resistance at higher concentrations of nanoparticles. The results indicate a variation in sensitivity between fungal species to zinc oxide nanoparticles. Statistical analysis using the Least Significant Difference (LSD) test confirmed significant differences between inhibition zones at different concentrations. The letters were assigned to indicate statistical significance, indicating that higher concentrations consistently led to smaller inhibition zones. For example, in A. flavus, the inhibition zones at 5 mg/ml (b) were significantly larger than those at 20 mg/ml (d) and 25 mg/ml (h). The results indicate that zinc oxide nanoparticles prepared with medical asparagus extract possess antifungal properties against all four types of Aspergillus. However, their effectiveness decreases at higher concentrations, which may be attributed to factors such as nanoparticle aggregation, reduced bioavailability, or self-determination mechanisms at high doses. This pattern suggests that nanoparticles are most effective at low concentrations (42)

The results suggest that the plant extract is most effective at lower concentrations, especially against A. flavus and A. oryzae. This highlights its potential as a natural antifungal agent for controlling Aspergillus species in agricultural or clinical applications (43). Zinc oxide nanoparticles are known to generate reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), hydroxylated radicals (•OH) and superoxygen anions ($O_2•^-$), when exposed to light or in aquatic environments. These reactive types of oxygen cause oxidative stress in fungal cells, damaging critical cellular components such as lipids, proteins, and DNA. This oxidative stress disrupts fungal cell membranes and metabolic processes, ultimately inhibiting fungal growth and causing observed inhibition zones (44).

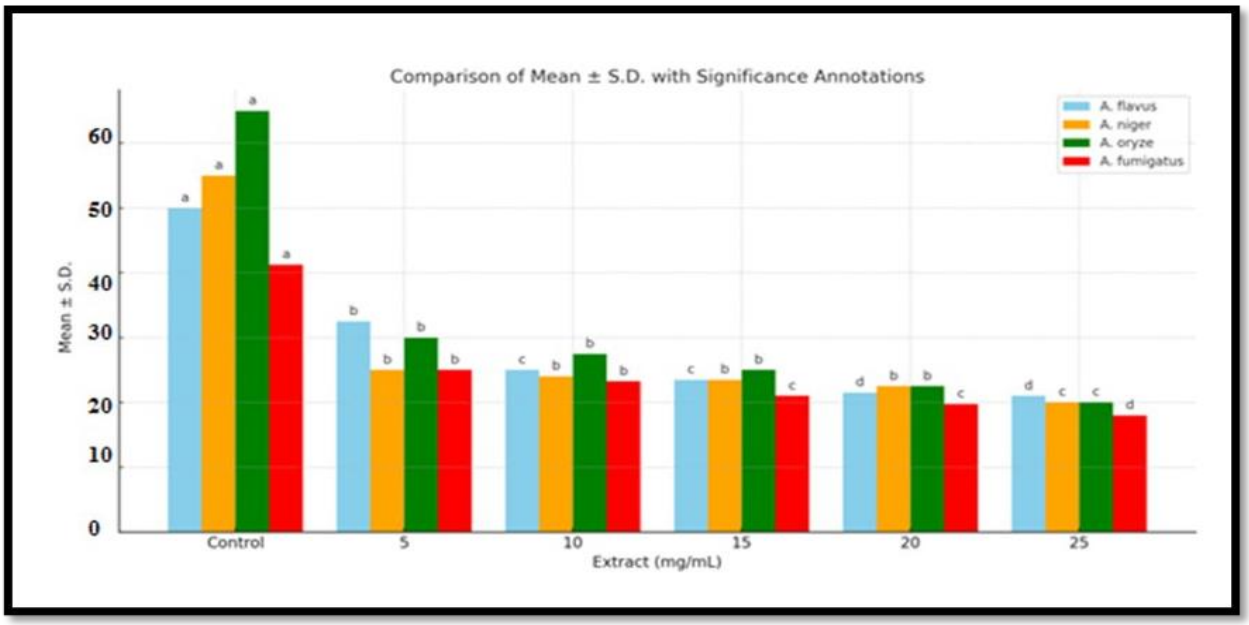


Figure (6): Effect ZnO NPs on fungi *A.fumigatus , A.niger ,A.oryzae ,A.flavus*

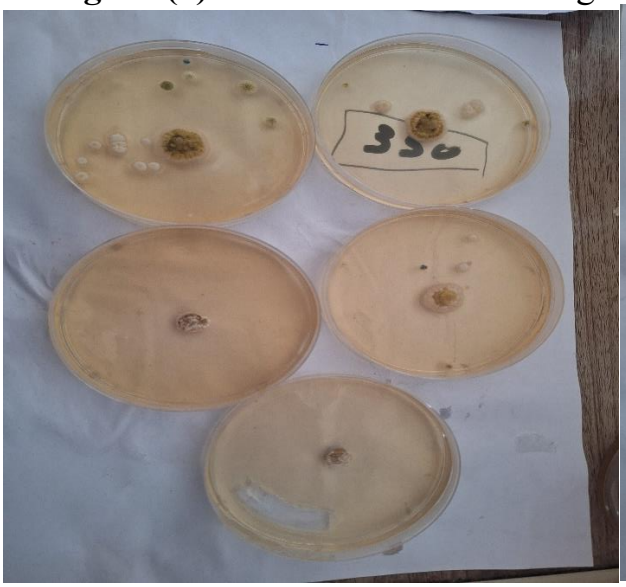


Figure (7): Effect ZnO NPs on the *A.fumigatus*

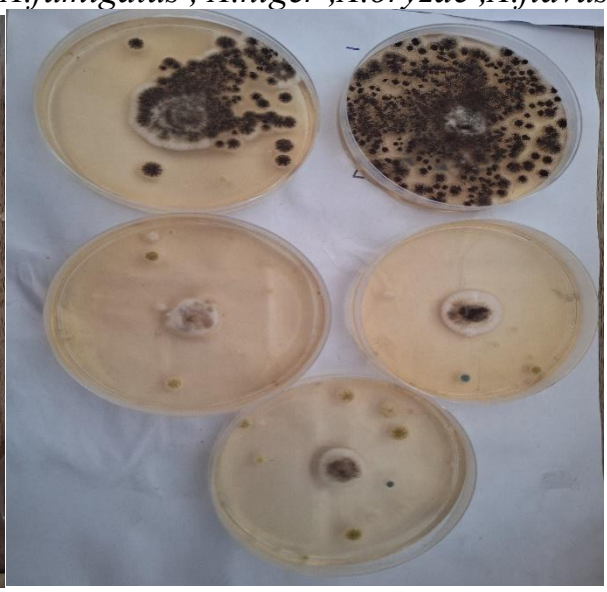


Figure (8): Effect ZnO NPs on *A.niger*

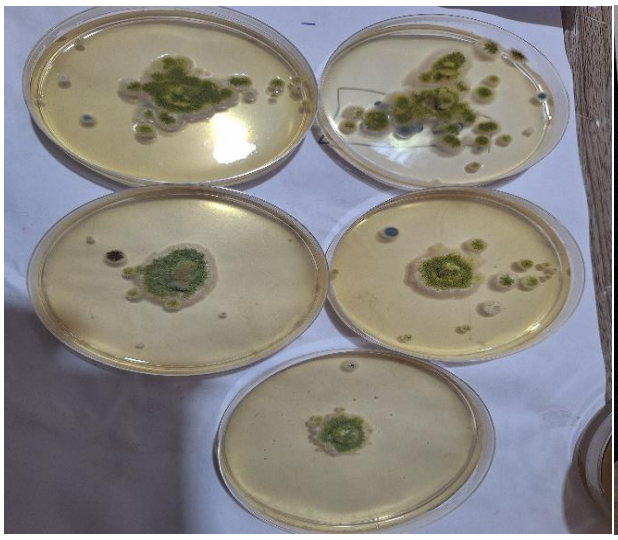


Figure (9): Effect ZnO NPs on *A.oryzae*

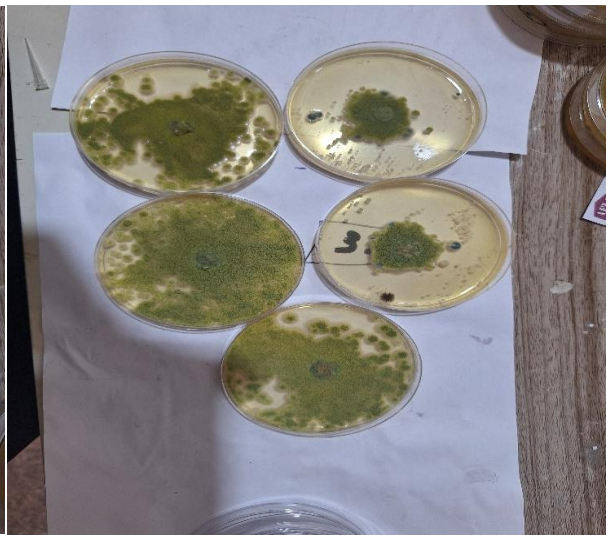


Figure (10): Effect ZnO NPs on *A.flavus*

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