The antifungal activity of methanolic extracts of *Conocarpus erectus* leaves and hybrid nano-methanolic extract solutions against *Fusarium proliferatum* and its zearalenone toxin

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Abstract

This work studied the Comparative Evaluation of Antifungal Activity of Conocarpus erectus Leaf Methanolic Extract and Its Nanohybrid Composite Against *Fusarium proliferatum*. antifungal properties of a methanolic extract of Conocarpus *erectus* leaves and a hybrid nano-methanolic extract solution against *F. proliferatum*, along with its zearalenone synthesis. A 1.5 ml concentration of the methanolic extract of *Conocarpus erectus* leaves showed an inhibitory effect on fungal development, resulting in a growth rate of 4.067 mm. The optimal concentration of the hybrid nano-extract solution was 20 mg/ml, resulting in a fungal growth rate of 0.773 mm, in contrast to the control treatment, which exhibited a growth rate of 5.017 mm. This concentration also reduced the amount of zearalenone produced by the fungus to 16.10 ppb compared to the control treatment, which amounted to 98.92 ppb.

Keywords: *Conocarpus erectus* , Nanohybrid Composite Against *Fusarium proliferatum* .

Introduction

Fusarium proliferatum has been identified in all regions globally that farm maize The fungus in question generates several toxins, which are currently significant due to their association with food and feed products, particularly corn-based commodities [1]. These species can generate substantial quantities of mycotoxins under specific conditions. Global trends are presently transitioning towards the consumption of healthful, natural, and functional foods, as well as sourcing medicine and bioactive compounds from natural origins like plants and microbes. Consequently, greater emphasis has been placed on herbal, medicinal, and unconventional plants. Research indicates that numerous plants, especially medicinal varieties, possess diverse secondary metabolites, including saponins, tannins, alkaloids, phenolics, and flavonoids, inside their roots, barks, stems, leaves, flowers, and seeds [2].

Secondary metabolites are organic compounds mostly produced during the transition from active to stationary growth, playing a crucial role in plant defense [3]. Certain components possess significant biological roles, exhibiting antioxidant effects by directly or indirectly scavenging free radicals and functioning as antioxidants within live cells. Elevating the concentration of these components during oxidative stress

can safeguard cells from oxidation, in conjunction with other antioxidant defense mechanisms [4]. Moreover, polyphenolic chemicals represent one of the most prevalent categories, exhibiting a diverse array of structures, functions, and biological activities [5].

They offer defense against diseases and predators [6]. The assertion regarding the beneficial impact of the conocarpus plant in mitigating plant diseases, corroborated by prior research, indicates that both aqueous and alcoholic extracts of the conocarpus plant demonstrated significant efficacy in diminishing pathogens relative to the control treatment (which consisted solely of the plant). The alcoholic extracts have shown superior efficacy in suppressing pathogens during laboratory tests, decreasing both infection rates and severity in field trials, and enhancing growth metrics relative to aqueous extracts [7].

The compounds generated play a significant role in the management of plant pathogens [8], as these plants have demonstrated efficacy in controlling pathogens or mitigating their effects. Furthermore, they are environmentally benign, cost-effective, and readily accessible. Consequently, their application in managing or diminishing the impact of pathogens lessens the environmental pollution associated with the improper use of pesticides, which can lead to the emergence of resistant strains and contamination of the environment with chemical pesticides [9].

Fusarium sp. is a significant and prevalent soil-borne fungus responsible for numerous illnesses. The extent of loss attributable to this disease is markedly correlated with the concentration of pathogenic fungal inoculum present in the soil, the planting season, and the presence of biological agents [10]. The researchers' interest in employing plant extracts for the management of various plant pathogenic fungi has been amplified by the presence of potent secondary metabolites within these extracts, which possess environmentally favorable characteristics, including rapid degradation, low toxicity and high specificity [11]. Numerous plant extracts exhibit antifungal, antioxidant and antibacterial properties against various illnesses in plants, animals and humans [12].

Nanoparticles have been regarded as one of the most significant and extensively utilized biodegradable polymers in agriculture. Nanoparticles and chitosan-based nanocomposites are preferentially utilized for diverse applications because of their biodegradability, high permeability through biological membranes, non-toxicity to humans, cost-effectiveness, and extensive antifungal properties. Numerous investigations have demonstrated that chitosan exhibits antifungal action due to the affinity of its cationic amino groups (-NH2) for cellular components [13].

Therefore, the aim of this study was to determine the ability of the alcoholic extract of the *Conocarpus erectus* plant and the nanomaterial to degrade the toxin zearalenone.

Materials and Methods

Isolation and identification of the fungus *Fusarium proliferatum* from maize

The Fusarium fungus was isolated from yellow corn grains. 100 grains were taken randomly from each sample collected from the stores of Karbala Governorate. They were sterilized with 2% sodium hypochlorite for two minutes, then washed three times with sterile distilled water and dried with sterile filter paper. They were transferred using sterile forceps to 9 cm diameter Petri dishes containing 20 cm^3 of PDA culture medium, with 5 seeds per dish and 5 dishes per sample. The dishes were then incubated at 25 ± 2°C for one week. Then, the *Fusarium* isolates were purified and cultured on the same PDA medium and incubated at 2±25°C for a week. Then, the fungal isolates were identified. together with an assessment of its capacity to generate zearalenone by thin layer chromatography [14].

Plant Collection

Leaves were obtained from trees at the University of Kerbala and designated as *Conocarpus erectus L*. The leaves were pulverized with a grinder and preserved at -20°C for subsequent analysis.

Preparation of methanolic extract

Fifty grams of *Conocarpus erectus* leaves were placed in a flask, and 350 milliliters of 70% methanol was added, maintaining a temperature of 40-60 °C for six hours. The solution was filtered using Whitman No. 1 filter paper and evaporated to dryness under vacuum at 40°C to exclude methanol; the extract was subsequently kept in amber glass vials at 4°C until analysis [15].

Preparation of Nanohybrid ZnO-methanolic extract

Nanohybrid methanolic extract. The process employed to synthesize the nanohybrid therapeutic was clearly delineated by[16] with certain modifications

A. Zinc Oxide Solution: Prepare this solution by dissolving 2 g of Zinc Oxide in 50% ethanol and diluting to a final volume of 100 mL with ethanol.
B. A methanolic extract solution: was made by dissolving 1 gram of the extract in 50% ethanol and subsequently adding it to 100 mL of ethanol.
C. Ion exchange technique utilizing methanolic extract solution for the synthesis of nanohybrids composed of zinc oxide layers: The previously described procedures by 17, with some modifications, involve adding the prepared solutions drop by drop into 100 mL of zinc oxide solution simultaneously. Prior to placing the mixture in the incubator, magnetically agitate it for two hours at ambient temperature. Vibrations were conducted for 18 hours at 37°C, followed by incubation for 24 hours at 40°C. After one hour, isolate the precipitate by centrifuging for 20 minutes at 5000 rpm. The precipitate

was subsequently washed with distilled water many times before being dried at 40°C. Subsequently, it was pulverized in a ceramic mortar and preserved.

Prepared Stock solution

The stock solution of methanolic extract and methanolic extract solution nano-hybrids were prepared separately by weighing 1.6 g of each antibody and placing them in two separate test tubes. 10 ml of distilled water was added to the solution to obtain a stock solution with a concentration of 160 mg/ml, which will be used in the subsequent steps to prepare the concentrations used in this study.

Effect of methanolic extract on the growth of fungi *Fusarium proliferatum* on P.D.A. medium

A liquid thick (Stock solution) as the base solution, from which three concentrations (0.5, 1.0, 1.5) % are prepared by dissolving (0.5 ml, 1.0 ml, 1.5 ml) of the solution into (99.5 ml, 99.0 ml, 98.5 ml) of P.D.A. medium. Chloramphenicol was included in the medium, which was subsequently dispensed onto sterile dishes with a diameter of 9 cm. Upon solidification of the medium, the centers of the dishes were infected with a disc (0.5 cm in diameter) derived from the culture media harboring *Fusarium proliferatum* growth. Divided into four groups, one group control and. As for the rest of the groups, each group has a specific focus. At seven days of age, three plates of each fungus were utilized as replicates. The plates were incubated at 25 ± 2°C, and the results were recorded after 7 days [17].

Effect of methanolic extract solution nanohybrids on the growth of fungi *Fusarium proliferatum* on P.D.A. medium

Three concentrations of methanolic extract solution nanohybrids (0.5, 1, 1.5) mg/ml were prepared and dissolved in 1 liter for each concentration. The medium was then poured into petri dishes. Chloramphenicol was included in the medium, which was subsequently dispensed onto sterile dishes with a diameter of 9 cm. Upon solidification of the medium, the centers of the dishes were infected with a disc (0.5 cm in diameter) derived from the culture media harboring *Fusarium proliferatum* growth. Divided into four groups, including one control group. Each group has a distinct focus. At seven days of age, three plates of each fungus were utilized as replicates. The plates were incubated at 25 ± 2°C, and the results were recorded after 7 days [18]

Effect of methanolic extract on the reduced zearalenone mycotoxin

First group A thick liquid (stock solution) serves as the base solution, with a concentration of 1.5% P.D.B. medium, then the antibiotics Chloramphenicol into the P.D.B. medium inoculated with a disc (0.5 cm in diameter) obtained from culture media containing *Fusarium proliferatum* growth. At seven days old, the second group of flasks inoculated with fungi only the flask was incubated at 25 ± 2°C, and the mycotoxin was extracted from all groups after 21 days [19].

Effect of methanolic extract solution nanohybrids on the reduced zearalenone mycotoxin

A methanolic extract solution of nanohybrids was prepared at a concentration of 20 mg/ml, followed by the addition of the antibiotic Chloramphenicol into the P.D.B. medium, which was inoculated with a 0.5 cm diameter disc derived from culture media containing *Fusarium proliferatum* growth. At seven days of age, the second group of flasks was inoculated with fungi only, the flasks were incubated at 25 ± 2°C, and the mycotoxin was extracted from all groups after 21 days [20].

Extract zearalenone mycotoxin

by adding the same equal amount of medium and chloroform and shaking for 20 minutes after filtering. filter paper and collected into dark vials.

The characterization of the nanohybrid treatment under investigation was conducted using FT-IR

FT-IR (Fourier Transform Infrared Spectroscopy) [21]: The infrared spectrum of Nanohybrid therapy, free-form therapy, zinc oxide (ZnO), LDH, Nano-Ano therapy, and Nano-LDH therapy was obtained by grinding a material of the compound under examination with potassium bromide (KBr) and measuring the infrared spectrum within the 400–4000 cm-1 wavenumber range.

Results and Discussion

The fungus *Fusarium proliferatum* was isolated, described visually and molecularly, and verified to produce zearalenone using fluorescence color and the distance traveled by the toxin relative to a standard toxin solution The *Fusarium* fungus infects maize plants in the field, and the infection progresses in the grains during storage, particularly when stored in the rainy season with air humidity levels reaching 90%. This elevated humidity prolongs the viability of the spores, resulting in increased contamination of the stored grains [22].

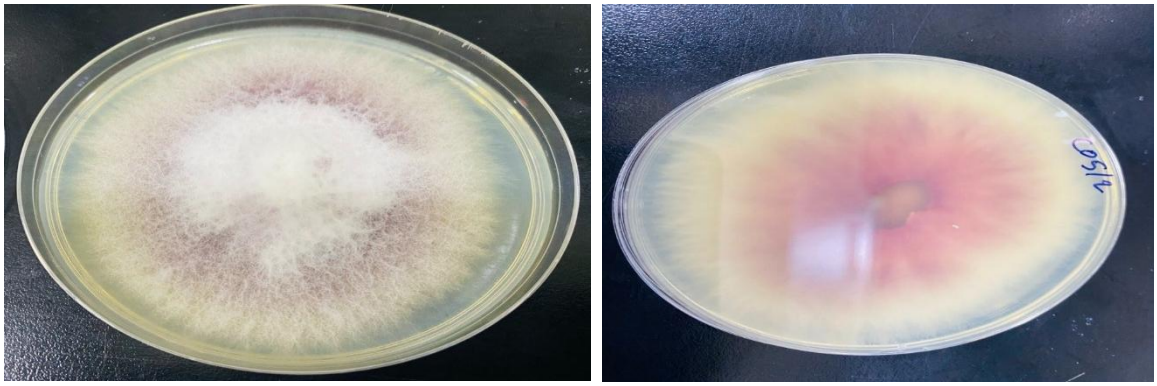

Figure (1): *Fusarium proliferatum* growth

Nanotechnology Study

Nanoparticles have achieved global recognition in diverse medical sectors due to their distinctive physical and biological properties and their ability to facilitate regulated medication delivery. Numerous inorganic nanoparticles exhibiting antifungal properties, such as gold, silver, zinc oxide, and titanium dioxide nanoparticles, have been synthesized in recent years. Exposure to fungus may result in significant membrane damage, disruption of the mycelium surface, increased membrane permeability, and potential cell disintegration [23].

Infrared spectrum (FT-IR)

The FT-IR spectrum of zinc oxide (ZnO) showed non-distinct frequencies at 3500–500 cm-1, which are attributed to the vibration of the Zn-O metal bond, as zinc oxide is an inorganic oxide, as shown in Figure 2 [24]

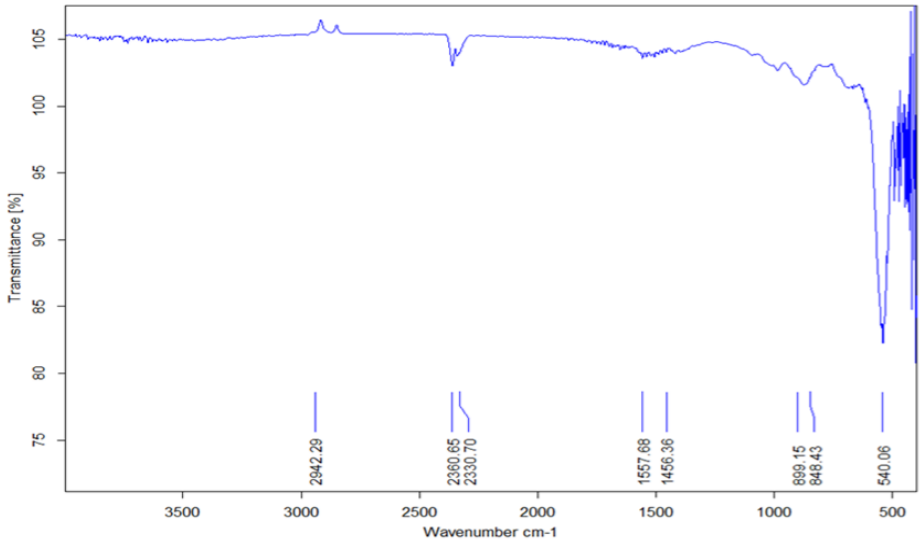

Figure (2): FTIR analysis of zinc oxide (ZnO)

The composition of the methanolic extract can be confirmed from the FTIR spectrum in Figure 3 and as shown below:

The broad and strong absorption band at 3352 cm-1 is due to the stretching of the phenolic and alcoholic hydroxyl (O-H) groups in flavonoids, tannins, glycosides, saponins, and Phyto steroids, which overlaps with the aromatic C-H stretching bands. It is unlikely to be due to the carboxyl group (carboxylic acid) because the strong carbonyl stretching band does not appear near the frequency of 1750. The two weak absorption bands at approximately frequencies (2850 and 2930) are due to the stretching of aliphatic (C-H) bonds. The strong absorption band at 1636 nm is due to the overlap of stretching of the carbonyl groups (C=O) exchanged (ketone and ester) in flavonoids, tannins, and Phyto steroids. The weak absorption bands in the fingerprint region between 1400 and 1000 nm are due to stretching of C-O single bonds. The absorption bands at 653 and 600 cm-1 are due to the bending of the aromatic (C-

H) bonds out of plane. The absorption band at 558 cm-1 is due to the stretching of the (Zn-O) bond [25].

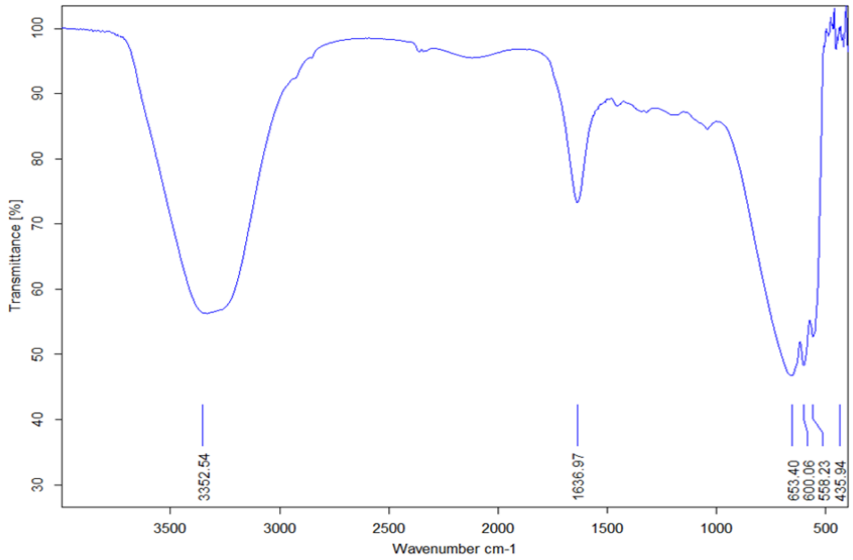

Figure (3): FTIR analysis of methanolic extract free

Figure 4 shows the successful formation of methanolic extract solution nanohybrids, In the nanoform, a change in the intensity and position of the absorption bands occurred, as the absorption band due to the stretching of the phenolic and alcoholic hydroxyl groups (O-H) in flavonoids, tannins, glycosides, saponins, and phytosteroids, which overlaps with the aromatic C-H bond stretching bands, shifted towards the higher frequency at 3366 cm^{-1} with a noticeable decrease in their intensity. Meanwhile, the two weak absorption bands due to the stretching of the aliphatic (C-H) bonds shifted towards the lower frequencies with a relative increase in their intensity.

The strong absorption band due to the overlap of stretching of the carbonyl groups (C=O) exchanged (ketone and ester) in flavonoids, tannins, and phytosteroids was significantly shifted towards the lower frequency at 1576.

The weak absorption bands in the fingerprint region between 1400-1000 due to stretching of single C-O bonds had a significant increase in absorption intensity. The absorption bands at frequencies of 653 and 600 cm-1, which are due to the out-of-plane aromatic (C-H) bonds, showed a significant decrease in absorption intensity. Meanwhile, the absorption intensity of the Zn-O bond band increased and its frequency decreased, appearing at 543 cm^{-1}[25].

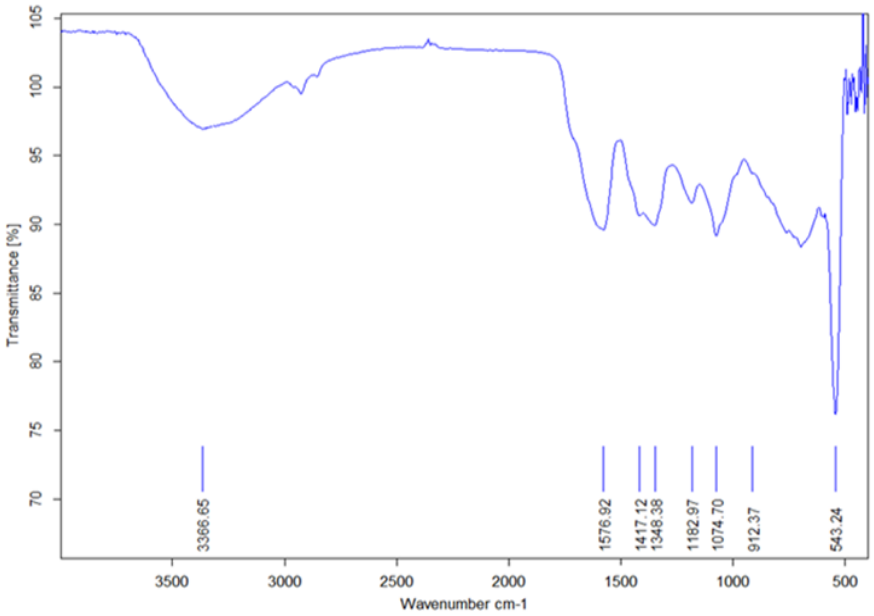

Figure (4): FTIR analysis of methanolic extract solution nanohybrids

Progression of the impact of methanolic extract solution on the proliferation of the fungus *Fusarium proliferatum* in P.D.A. medium

The data presented in the table provide insight into the effect of increasing concentrations of a treatment on the growth of *Fusarium proliferatum*, a significant fungal pathogen known for its mycotoxin production. The concentrations tested include 0 (control), 0.5, 1.0, and 1.5 ml, with corresponding fungal growth values of 5.017, 4.450, 4.117, and 4.067, respectively. A Least Significant Difference (L.S.D.) value of 0.5070 is provided to assess whether differences between group means are statistically meaningful. At a concentration of 0, representing the untreated control, fungal growth is at its highest (5.017), reflecting the typical proliferation of *F. proliferatum* in the absence of any inhibitory agent.

When the treatment is applied at a concentration of 0.5, growth declines to 4.450. This reduction of 0.567 units compared to the control exceeds the L.S.D. threshold, indicating a statistically significant suppression of fungal growth due to the treatment with a further increase in concentration to 1.0, fungal growth continues to decline, reaching a value of 4.117.

This decrease is also statistically significant relative to the control, as the difference (0.900) is well above the L.S.D. However, the difference between the 0.5 and 1.0 concentrations (0.333) is less than the L.S.D., suggesting that the additional reduction in fungal growth is not statistically significant. At the highest concentration tested (1.5), growth slightly decreases again to 4.067. Although this level remains lower than the control and is still significantly different from it, the difference between 1.0 and 1.5 concentrations (0.050) is minimal and statistically in significant .In conclusion, the treatment shows a clear inhibitory effect on the growth of *Fusarium proliferatum*, with significant reductions observed at all concentrations compared to the control.

The presence of harmful components in the extract of *Conocarpus erectus L.* leaves, referred to as secondary compounds, includes alkaloids, tannins, flavonoids, and saponins. These secondary metabolites infiltrate the fungal cell wall, resulting in its demise [26]. proposed that the tannins in *Conocarpus erectus* extracts predominantly account for the plant's antimicrobial properties. also, the extract of *Conocarpus erectus* leaves is rich in phytochemicals that contribute to its therapeutic properties, providing advantageous effects in combating many ailments [27].

Table (1): Relationship between concentration of methanolic extract and free diameter growth for *F. proliferatum*

Concentration (ml)	Growth of *F. proliferatum* (mm)
0	5.017
0.5	4.450
1	4.117
1.5	4.067
L.S.D. (0.05) = 0.5070	

Progression of the impact of methanolic extract solution nanohybrids on the proliferation of the fungus *F. proliferatum* in P.D.A. medium

The data presented in the table provide a clear evaluation of the impact of different treatment concentrations (measured in mg/ml) on the growth of *F. proliferatum*, a pathogenic fungus associated with crop diseases and mycotoxin production. The tested concentrations include 0, 10, 15, and 20 mg/ml, with fungal growth values of 5.017, 1.650, 1.300, and 0.773, respectively. A Least Significant Difference (L.S.D.) value of 0.4021 is given to determine whether the observed differences in growth are statistically significant. At a concentration of 0 mg/ml, which serves as the untreated control, fungal growth reaches its maximum value of 5.017.

This baseline measurement represents the normal proliferation of *F. proliferatum* in the absence of any inhibitory agent. When the treatment is applied at 10 mg/ml, fungal growth dramatically decreases to 1.650. This significant reduction, approximately 67% compared to the control, strongly suggests that the treatment possesses potent antifungal properties. The difference of 3.367 units between the control and the 10 mg/ml treatment far exceeds the L.S.D. value, indicating a statistically significant effect.

As the concentration increases to 15 mg/ml, fungal growth continues to decline, reaching 1.300. Although this decrease demonstrates a continuing trend of inhibition, the difference between 10 mg/ml and 15 mg/ml (0.350) falls below the L.S.D. threshold. Therefore, the additional reduction in fungal growth at this concentration is not statistically significant, suggesting a possible plateau in the treatment's efficacy. At 20 mg/ml, fungal growth drops further to 0.773, representing an 84.6% reduction compared to the control.

The difference between 15 mg/ml and 20 mg/ml (0.527) exceeds the L.S.D. value, confirming that this decrease is statistically significant. This result indicates that while the inhibition may be slightly at intermediate concentrations, higher doses continue to provide additional antifungal effects. In conclusion, the treatment exhibits a strong dose-dependent inhibitory effect on the growth of *Fusarium proliferatum*. Statistically significant reductions in fungal growth are observed at both 10 mg/ml and 20 mg/ml concentrations, highlighting the treatment's efficacy. While the response at 15 mg/ml does not differ significantly from that at 10 mg/ml, the continued suppression at 20 mg/ml supports its potential as an effective antifungal agent. The results indicated that the methanolic extract solution nanohybrids inhibited fungal growth.

The efficacy of methanolic extract solution nanohybrids is attributed to their diminutive size; smaller particles possess greater electric charge, enhanced melting properties, increased surface area, heightened attractiveness, and superior stability towards their targets [28]. Numerous scientific sources indicate that nanoparticle size is a critical characteristic influencing their behavior and toxicity, with smaller particles exhibiting greater effectiveness in inhibiting microbial activity [29]. Consequently, it is proposed to utilize nanoparticles as efficient fungicides for the protection of plant crops during cultivation and post-harvest, as well as in food safety applications.

Table (2): Relationship between the concentration of methanolic extract solution nanohybrids the diameter growth of *F. proliferatum*

Concentration mg/ml	Growth of *F. proliferatum* (mm)
0	5.017
10	1.650
15	1.300
20	0.773
L.S.D. (0.05) = 0.4021	

Evolution of the effect of methanolic extract solution nanohybrids on the reduced amount of zearalenone mycotoxin

The table provides an assessment of the effectiveness of different treatment methods in reducing the concentration of zearalenone, a potent mycotoxin produced by *Fusarium* species. The treatments compared include a control (no treatment), a methanolic extract solution at a concentration of 1.5 mg/mL, and a nanohybrid formulation of the same methanolic extract. The zearalenone concentrations observed after each treatment were 98.92 ppm for the control, 28.96 ppm for the methanolic extract, and 16.10 ppb for the nanohybrid formulation.

A Least Significant Difference L.S.D. (0.05) value of 0.860 was used to determine the statistical significance of the differences among these treatments. In the control group, the zearalenone level remained at 98.92 ppb, indicating the typical concentration of the toxin in the absence of any intervention. This value serves as a baseline for evaluating the efficacy of the applied treatments. The high level of residual toxin demonstrates the persistence of zearalenone under normal conditions and un-

derscores the need for effective detoxification strategies. When treated with the methanolic extract solution, the zearalenone concentration decreased substantially to 28.96 ppb. This reduction reflects a 70.7% decline compared to the control and indicates a significant detoxification effect. The difference between the control and the methanolic extract treatment (approximately 69.96 ppb) far exceeds the L.S.D. (0.05) value, confirming the statistical significance of this reduction. This result suggests that the methanolic extract contains active compounds capable of binding to or breaking down zearalenone, thereby reducing its presence.

The nanohybrid formulation of the methanolic extract exhibited the most pronounced effect, reducing the zearalenone concentration further to 16.10 ppm. This represents an 83.7% reduction relative to the control and a 44.4% reduction compared to the methanolic extract alone. The difference between the nanohybrid and the other treatments exceeds the L.S.D.(0.05), indicating that the nanohybrid form is significantly more effective. This enhanced performance is likely due to the advantages of nanotechnology, such as increased surface area, improved solubility, and better interaction between the active compounds and the toxin.

In conclusion, the findings clearly demonstrate that both the methanolic extract and its nanohybrid formulation are effective in reducing zearalenone levels, with the nanohybrid providing superior results. demonstrated that nanohybrid compounds greatly inhibited radial growth, effectively lowering fungal proliferation while inducing morphological and ultrastructural alterations in the pathogen, hence indicating their potential application as antifungal agents [30]. Nanohybrids, as a broad-spectrum fungicide, have demonstrated fungicidal efficacy against numerous harmful fungi. Copper-CNPs at a concentration of 0.12% resulted in 70.5% and 73.5% inhibition of mycelium growth in *Alternaria solani* and *F. oxysporum*, respectively [31]. This result same as another study that used the Antifungal properties of chitosan, CNPs, and CAgNCs against *F. oxysporum* within the treated cohorts. In treatment samples, irregular hyphal morphology and enlarged vesicles within the mycelium were noted. and mycelium exhibiting a cylindrical morphology with a smooth exterior. Both CNPs and CAgNCs have caused significant, irreversible membrane damage and disruption to the mycelium surface, resulting in increased membrane permeability and cellular disintegration [32].

Table (3): Relationship between the Methanolic extract solution free and the Methanolic extract solution nanohybrids in reduced Zearalenone

Treatment	Zearalenone ppb
Control	98.92
Methanolic extract solution 1.5 ml	28.96
Methanolic extract solution nanohybrids	16.10
L.S.D.(0.05)	0.860

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