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RESEARCH ARTICLE

Green synthesis of Magnesium Oxide nanoparticles and assessing the effect on fungal growth and metabolism of *Aspergillus* species under optimum temperatures

EL-shimaa M.A. Galal [1], M.S. Abd El-sadek [1], Eman G.A. El-Dawy [2] and R. Khalaphallah [3]*

[1] *Nanomaterial Laboratory, Physics Department, Faculty of Science, South Valley University, 83523 Qena, Egypt.*
[2] *Department of Botany, Faculty of Science, South Valley University, 83523 Qena, Egypt.*
[3] *Department of Botany (microbiology), Faculty of Agriculture, South Valley University, 83523 Qena, Egypt.*

Abstract

Using medicinal extracts is one of the most important alternative methods for producing nanoparticles, due to it is safe, biocompatible and eco-friendly. *Hyoscyamus muticus* leaf extract was used in this study for the green synthesis of Magnesium Oxide nanoparticles (MgO NPs) by mixing it with a solution of magnesium nitrate. Several techniques were done to characterize the obtained material, including Scanning Electron Microscopy (SEM), Ultraviolet-Visible (UV-Vis) Spectroscopy, X-ray Diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR). *Aspergillus ochraceous (A.* ochraceous) and *Aspergillus niger* (*A. niger)* were incubated at different temperatures. The results indicated that 27°C and 35°C were the optimum temperatures for the growth, respectively. The effect of MgO NPs on the growth and metabolism (α-amylase) of *A. niger* and *A. ochraceous* were studied under optimum temperatures. It was observed by adding MgO NPs at concentrations of 0.25%, 0.5% and 1%; fungal growth was inhibited with 11%, 19% and 89% for *A. niger* and 67%, 76% and 100% for *A. ochraceous*. The metabolism of *Aspergillus* species as α-amylase completely prevented at all concentrations of MgO NPs. The purpose of this research is to compare the effects of climate change factor and MgO NPs on the growth of *A. niger* and *A. ochraceous* and α-amylase production.

Keywords : *Aspergillus*; Enzyme; Green synthesis; *Hyoscyamus muticus*; Magnesium Oxide nanoparticles.

1. Introduction

Green synthesis techniques make use of moderately pollutant free chemicals to synthesis Nanoparticles and embrace the use of benign solvents such as water, natural extracts (Moorthy *et al.*, 2015). Green synthesis aims to stop pollution before it begins. Preventing waste is better than treating or cleaning up waste after it has already formed. Although physical and chemical approaches are more popular for creating nanoparticles, biogenic fabrication is preferable due to its being eco-friendly (Kavitha *et al.*, 2013).

Magnesium oxide nanoparticles (MgO) NPs are recognized as safe disinfection agents by the U.S. Food and Drug Administration without any harmful byproducts with the advantages of non-toxicity, environmental friendliness, ease of availability, and biocompatibility with human cells. Thus, they hold great promise in both medical therapeutics (Chalkidou *et al.*, 2011; Krishnamoorthy *et al.*, 2012) and water disinfection (Purwajanti *et al.*, 2015). In vitro studies have shown magnesium oxide nanoparticles to be effective as microbicides against gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria (Stoimenov *et al.*, 2002; Makhluf *et al.*, 2005) and fungal pathogens (Chen *et al.*, 2020).

***Corresponding author: Rafat Khalaphallah**
Email: r.shipat@agr.svu.edu.eg
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Aspergillus is a sizable genus of anamorphic fungi. *Aspergilli* are very important in many areas, including those pertaining to plants, animals, and human health. The genus *Aspergillus* includes organisms with highly distinctive characteristics from a pathological, agricultural, industrial, pharmacological, scientific, and cultural perspective. They are crucial in the breakdown of organic substrates, especially plant material (Samson *et al.,* 2009). *Aspergilli* are known for their ability to secrete a variety of biologically active chemical compounds including: immune-suppressants, mycotoxins, antibiotics and cholesterol lowering agents.

The most popular fungi used to produce α-amylase are those from the genus *Aspergillus*. There are different extracellular enzymes produced by the *Aspergillus* species, but amylases are the ones with the most significant industrial importance (Hernández *et al.,* 2006). Enzymes produced in large numbers by filamentous fungi like *Aspergillus oryzae* and *Aspergillus niger* are widely used in the industry (Tiwari *et al.,* 2015). Alpha amylase (α-1,4-D-glucan glucanohydrolase) is a starch digesting enzyme that randomly cleaves α - 1,4D-glucosidic linkages in starch molecules. Alpha amylase is widely distributed in nature. It is the most important enzyme and has a significant role in modern biotechnology. The enzyme is used in a wide variety of applications in many industries, including paper, textiles, brewing, food, sugar, and starch liquefaction. Additionally, it has outstanding uses in fields like pharmaceutical, clinical, medicinal, and analytical chemistry (Batlle *et al.*, 2000); because both cell growth and the production of enzymes and other metabolites are often sensitive to temperature, the incubation temperature is probably the most significant factor among all the physical variables influencing the performance (Krishna, 2005). Production of α-amylase by fungi is related to growth, which in turn depends on the incubation temperature (Irfan *et al.,* 2012). Therefore, the optimum temperature is determined by whether the culture is thermophilic or mesophilic (Sivaramakrishnan *et al.,* 2006; Shah *et al.,* 2014). The objective of this study is to evaluate the effect of climate change factors and MgO NPs on the growth of *A. niger* and *A. ochraceous* and α-amylase production.

2. Materials and methods

2.1. Collection of fungi

Aspergillus niger and *Aspergillus ochraceous* were obtained from the Botany Department, Faculty of Science, Aswan University. The collected isolates were cultured on Potato Dextrose Agar (PDA) in slant tubes for 5 days at 27°C. Then they were preserved in a refrigerator at 4°C until use (Bedan *et al.,* 2014). The (PDA) media was prepared by boiling 200g of potatoes in 1-liter distilled water for 30 min, filtered through cheesecloth, and then mixed with 20g of Dextrose, 20g of Agar, and water to get a 1-liter media and boiled to dissolve. It was then autoclaved for 20 minutes at 121 °C.

2.2. Preparation of MgO NPs

2.2.1. Hyoscyamus muticus leaf extract preparation

Hyoscyamus muticus leaves as a reducing agent were used and collected from South Valley University (SVU), Qena. Fresh *H. muticus* leaves were washed using distilled water to remove unwanted impurities such as dust and other materials. Then dried at room temperature, and finally ground into powder and stored at room temperature. leaf extraction was conducted with reference to the method reported in (Palanisamy *et al.,* 2017). *H. muticus* leaf powder sample of 6 g was mixed with 100 mL of distilled water. The mixture was then heated to 95 °C for 1 hour, stirring until all the powder was evenly mixed. After heating, the solution was allowed to cool and filtered using cheesecloth to remove solid particulates, followed by centrifugation for 5 min at 15000 rpm. The resulting filtrate was used as a stock solution for the synthesis of MgO

nanoparticles. The filtered extracts were stored in a refrigerator (5°C) for further use (Fatiqin *et al.*, 2021).

2.3. Green synthesis of MgO NPs from H. muticus

The synthesis of MgO nanoparticles was commenced by adding 5 mL of the *H. muticus* extract to 50 mL of (0.1M) Mg $(NO_3)_2.6H_2O$ solution and stirred at 600 rpm at 60℃. After the temperature of 60 ℃ has been reached, NaOH solution was added dropwise to the mixture to set the pH to 13.6, and the mixture was left for 1 h aging process in order to optimize the formation of the Mg $(OH)_2$ precipitate. The precipitate formed was separated from the mixture by centrifugation with a rotation of 7500 rpm at room temperature for 20 min and the precipitate was washed three times with distilled water and once with alcohol (70%). The precipitate was dried to remove any residual water and alcohol. After drying, the precipitate was calcined at 600 ℃ for 3 hours (Palanisamy *et al*., 2017).

2.4. MgO NPs characterization

The synthesized of MgO nanoparticles of *H. muticus* was observing by UV-Visible spectrophotometer. An aliquot of 1 mL of reaction mixture was placed in a glass cuvette and the absorbance of the sample was scanned from 200 to 800 nm wavelength. The MgO nanoparticles produced were characterized using different instrumental techniques. The optical and morphology property was studied using Scanning Electron Microscopy (SEM, FEI Inspect-S50). The crystal structure was studied using X-ray diffraction (XRD, PAN Analytical Expert Pro) techniques. To detect surface functional groups, the samples were characterized using a Fourier Transform Infrared spectrophotometer (FTIR, Shimadzu IR Prestige 21) (Palanisamy *et al*., 2017).

2.5. Effect of Temperature on the growth of A. niger and A. ochraceous

The effect of temperatures on the growth *Aspergillus* species was tested in incubators set at 27° C, 35° C and 40° C. The PDA media plates were inoculated with a disc from the mycelia and spores of *A. niger* or *A. ochraceous* that were previously cultured on PDA medium at 27° C for 5 days. The fungal inoculum was obtained using a sterile cork-borer (10 mm diameter). These were then incubated at three different temperatures. Fungal growth was measured after 7 days of incubation and the average of three replicate plates was taken as the growth rate for each species of fungi (Shehu *et al.*, 2011).

2.6. Effect of MgO Nps on the growth of A. niger and A. ochraceous under optimum temperature

Three concentrations of MgO nanomaterials (0.25, 0.5, and 1 g per 100 ml of PDA medium after sterilization) were used to determine the effect of these concentrations on the growth of *A. niger* and *A. ochraceous*. Each concentration was mixed with the medium, poured into 9 cm plates and cultivated with fungal isolates. The untreated control treatment was done on the same medium in Petri dishes by growing one disc of the fungus. The fungal inoculum was obtained using a sterile cork-borer. Three replicates were used. All plates were incubated at the optimum temperature for each isolate for 7 days after inoculation. Data was collected after 7 days of incubation and compared with the control group by measuring the fungal growth to calculate the inhibition percentage. The inhibition % was calculated according to the following equation:

$$Inhibition = \frac{Diameter\ of\ control - Diameter\ of\ treated\ colony}{Diameter\ of\ control} x\ 100$$

(Hussein *et al.*, 2020; El-Sheshtawi *et al.*, 2009).

2.7. Effect of temperature on the metabolism (α-amylase) of A. niger and A. ochraceous

Two isolates of *Aspergillus* were screened for their ability to produce extracellular α-amylase. Isolates were cultured on solid starch yeast extract agar (SYE) medium with a composition (in g/L) of soluble starch, 5.0; Bacto-yeast extract, 2.0; KH2 PO4, 1.0; MgSO4. 7 H2O, 0.5

and agar, 15. Cultures were incubated at 27º C for 6 days.

The inoculums were obtained by using a sterile cork-borer (10 mm diameter). One sterile 250 ml Erlenmeyer flask containing 100 ml of the broth SYE was prepared for each fungal species. Cultures were incubated at 27° C, 35° C and 45° C without shaking for 7 days. After that, the mycelium was collected by filtration. The amylolytic activity of *Aspergillus* species was determined by using the filtrates as the method of the Society of American Bacteriologists (1957). Briefly, (0.1 ml) of a culture filtrate was put into 10 mm pores that were cut in SYE agar plates. After 24 h of incubation at 22ºC, plates were immersed by iodine solution (KI, 15 g; I2, 3 g per liter of distilled water). The formation of a clear zone around cavities indicates amylase production. The mean diameter of clear zones (in mm) of the triplicates for each isolate was recorded (Barnett *et al.*, 1971; Gherbawy *et al*., 2019).

2.8. Effect of MgO Nps of H. muticus on the α-amylase production of A. niger and A. ochraceous under optimum temperature

Isolates were cultured on starch Yeast Extract Agar (SYE) medium. Cultures were incubated at 27ºC for 6 days. One sterile 250 ml Erlenmeyer flask containing 100 ml of the broth SYE was prepared for each fungal species. Three concentrations of MgO nanomaterials (0.25, 0.5 and 1 g per 100 ml) were used to study the effect of these concentrations on the ability of *A. niger* and *A. ochraceous* to produce extracellular α-amylase. Each concentration was mixed with the broth, placed in a flask for each isolate and incubated at different temperatures. The untreated control treatment was done on the same medium. Cultures were incubated at the optimum temperature for each isolate for 7 days. After that, the mycelium was collected by filtration. 0.1 ml of a culture filtrate was put into 10 mm pores which were cut in SYE agar plates. After 24 h of incubation at 22º C, the plates were immersed in diluted iodine solution. The formation of a clear zone around cavities indicates amylase production. The mean diameter of clear zones (in mm) of the triplicates for each isolate was recorded.

3. Results

3.1. Effect of different temperatures on the growth of A. niger and A. ochraceous

Temperature affected the growth of tested fungi (*A. niger* and *A. ochraceous*) differently. They were incubated at different temperatures (Figure 1).

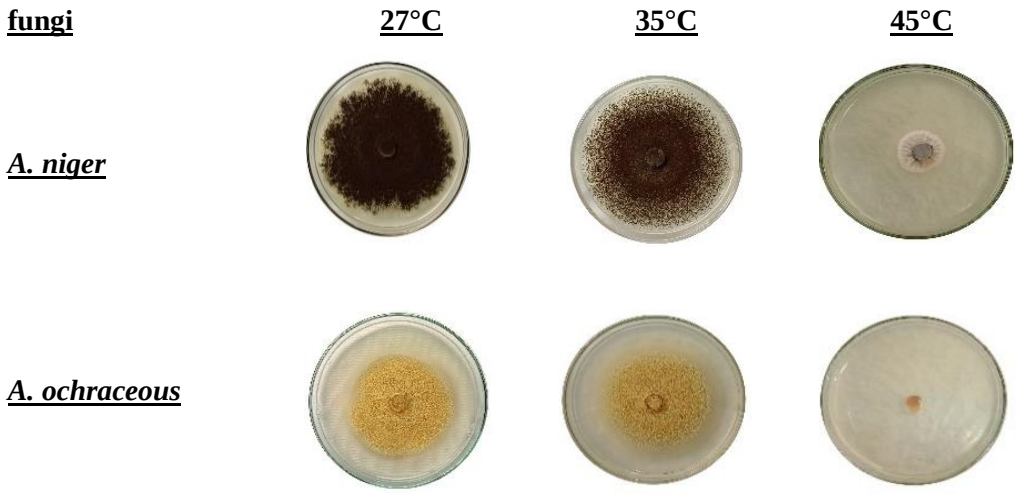

Figure 1. The effect of different temperatures on the growth of *A. niger* and *A. ochraceous* on PDA medium.

A. niger incubated at 27°C, it gave fungal growth, but it was observed that increasing the temperature to 35°C enhanced the growth of *A. niger* and the optimum growth was achieved at this temperature (colony diameter = 90 mm), but when incubating at 45°C, it led to a decrease in the growth (colony diameter = 30 mm). While incubating *A. ochraceous* at 27°C gave the highest fungal growth (colony diameter = 70 mm), which was relatively decreased when the incubation temperature was increased to 35°C, while there was no growth when incubated at 45°C; the temperature completely inhibited the growth (Table 1).

Table 1. The fungal growth rate of *A. niger* and *A. ochraceous* under the influence of different temperatures (Diameter at mm)

Fungal species	Temperature	Fungal growth rate (mm)
A. niger	27°C	80
	35°C	90
	45°C	30
A. ochraceous	27°C	70
	35°C	60
	45°C	0

3.2. Effect of MgO Nps on the growth of Aspergillus niger and A. ochraceous under optimum temperature

The MgO nanoparticles exhibited inhibitory activity against *Aspergillus* spp. on PDA medium. The results in (Table 2), showed the inhibitory activity of MgO nanoparticles against *A. niger* and *A. ochraceous* at different concentrations. It was observed by adding MgO NPs at concentrations of 0.25%, 0.5% and 1%, the inhibition of fungal growth with 11%, 19% and 89% for *Aspergillus niger* and 67%, 76% and 100% for *Aspergillus ochraceous* respectively. MgO Nps showed this inhibitory activity when fungi were incubated at the optimum temperature for each isolate (35 and 27°C) (Figure 2).

Table 2. The different concentrations of MgO nanoparticles inhibitory activities against *A. niger and A. ochraceous* growth under optimum temperature.

No. of isolate	Morphological identification	Concentration of MgO nps	Temperature	Inhibition percentage %
1	*A. niger*	0.25	35°C	11
		0.5		19
		1		89
2	*A. ochraceous*	0.25	27°C	67
		0.5		76
		1		100

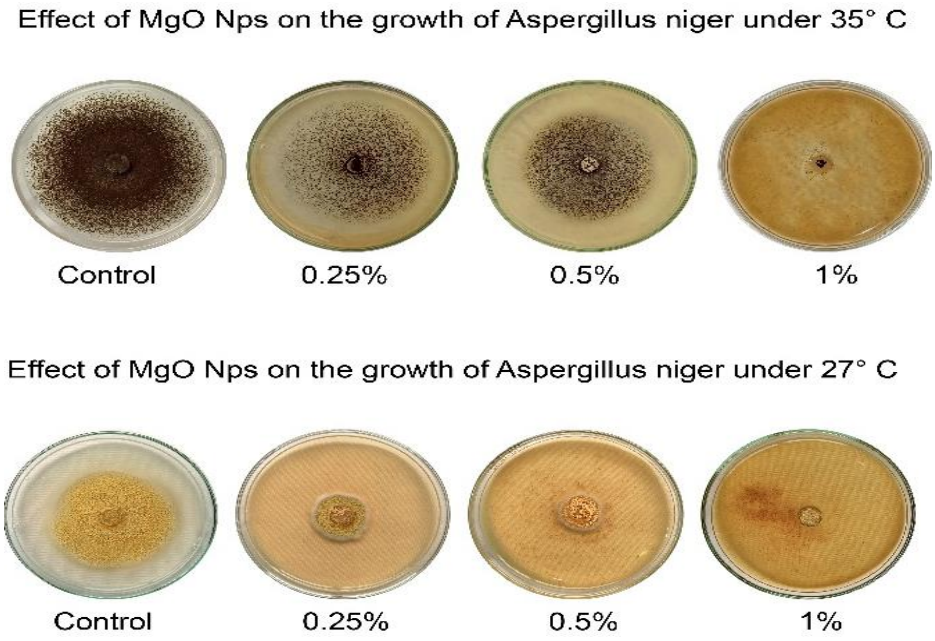

Figure 2. MgO nanoparticles activity on the inhibition of *A. niger* and *A. ochraceous* under optimum temperatures on PDA medium.

3.3. Effect of temperature on the metabolism (α-amylase) of A. niger and A. ochraceous

The results showed the effect of temperature on the metabolism (α-amylase) production of both fungi *A. niger* and *A. ochraceous* (Figure 3). Fungi tested in this study to be screened for the production of α-amylase qualitative assay, which depends on the color change of the iodine indicator from blue to colorless. The results were recorded in (Table 3) and each sample was selected in three replicates.

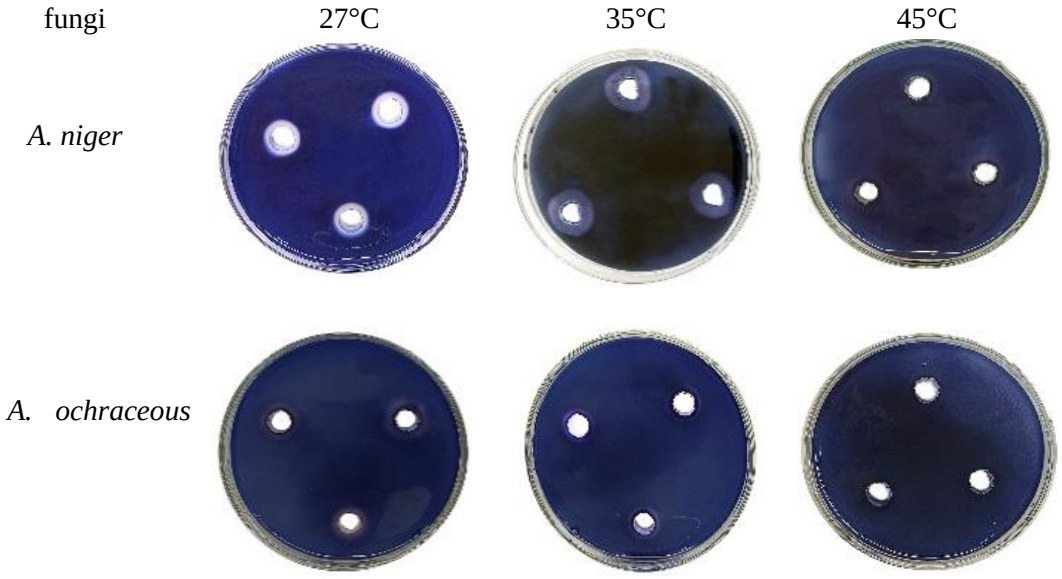

Figure 3. Clear zone representing amylase activity produced by *Aspergillus* spp.

Table 3. Screening of *Aspergillus* spp. for α-amylase production under different temperatures.

No. of isolate	Morphological identification	Temperature	Amylase activity (Clear zone, mm)
1	*Aspergillus niger*	27°C	19
		35°C	22
		45°C	0
2	*Aspergillus ochraceous*	27°C	18
		35°C	15
		45°C	0

It was observed that *A. niger* and *A. ochraceous* produced α-amylase at different levels depending on the incubation temperature. In *A. niger* α-amylase production was lower than (20 mm) when incubated at 27°C, while at 35°C (best temperature for the growth of *A. niger*) α-amylase production was more than 20 mm, but at 45°C, no α-amylase was produced. In *A. ochraceous* when incubated at 27°C (best temperature for the growth of *A. ochraceous*) and incubated at 35°C, the production of α-amylase ranged from 15-18 mm, while at 45°C, no α-amylase was produced. These results indicated that the temperature required to produce a large amount of amylase enzyme was the same as the optimum temperature for fungal growth.

3.4. Effect of MgO Nps on the metabolism of (α-amylase) A. niger and A. ochraceous under optimum temperature

The results in (Figure 4) showed the effect of MgO Nps on α-amylase production under optimum temperatures. *A. niger* and *A. ochraceous* showed the same results. When different concentrations of MgO Nps added, it resulted in a complete inhibition of α-amylase production compared to the control (free from MgO Nps). All results were recorded in (Table4**)**.

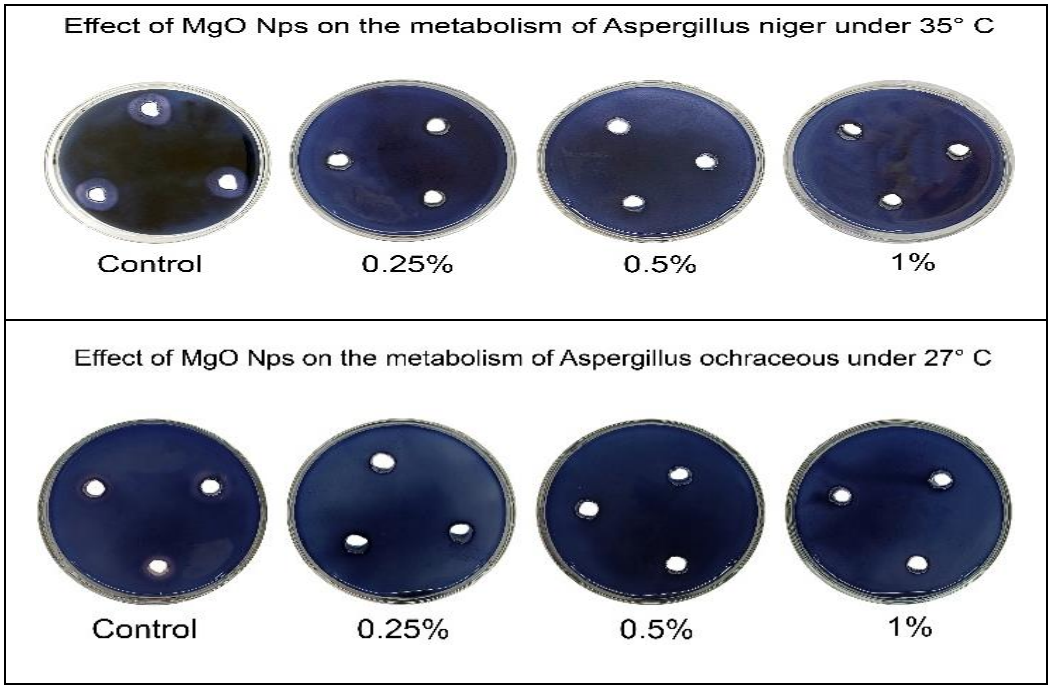

Figure 4. Clear zone representing amylase activity produced by *Aspergillus* spp in the presence of MgO Nps under optimum temperature

Table 4. Screening of *Aspergillus* spp. for α-amylase production in the presence of MgO nps under optimum temperature.

No. of isolate	Morphological identification	Concentration of MgO nps	Temperature	Amylase activity (Clear zone, mm)
1	*A. niger*	0.25	35°C	0
		0.5		0
		1		0
2	*A. ochraceous*	0.25	27°C	0
		0.5		0
		1		0

4. Discussion

In the current work, it was found that temperature affects the growth of fungi and every fungal species showed the optimum growth at an optimum temperature. found that 27°C and 35°C were the optimum temperatures for the growth of *A. niger* and *A. ochraceous,* this result agree with *(Palacios-Cabrera et al.,* 2005) . Temperatures above the optimum of growth generally represent a stress for fungi (Leong *et al.,* 2006) . That is, the higher of temperature, result in the lower of growth of fungi (Passamani *et al.,* 2014),like what happened with *A. niger* and *A. ochraceous*, increasing the temperature to 45°C inhibited growth.

Also in this study, an attempt was made to determine the efficacy of different MgO nanoparticle concentrations on the growth of fungi such as *A. niger* and *A. ochraceous*. It was clear from the results that different MgO NPs concentrations led to a significant inhibition of fungal growth when compared to controls. The highest inhibition was observed at highest concentration of nanoparticles followed by lower concentration of nanoparticles repetitively. Fungal growth was inhibited by 11%, 19% and 89% for *A. niger* and 67%, 76% and 100% for *A. ochraceous* at concentrations of 0.25%, 0.5% and 1%, respectively. This study agrees with (Hussein and Al-wahab, 2020), they also found higher concentrations more effective than lower concentrations. Number of studies indicated the high inhibitory activity of MgO Nps against plant pathogens (Rico *et al.,* 2011; Abdul *et al.,* 2016). Whereas, (Wani *et al.,* 2012) found that using MgO nanoparticles inhibited *Fusarium* spp. causing wilt diseases in the plant.

As indicated in the present study, the inhibitory effect was found to be dependent on temperature and the concentration of MgO nanoparticles. MgO Nps was tested in order to know its effect on fungi. In some other studies, MgO Nps has also been investigated for other antimicrobial agents (Huang *et al.,* 2005). As shown in the case of the fungus *Trichoderma reesei* and other fungi, the inhibitory effect of nanoparticles may be caused by inhibiting the production of extracellular enzymes and metabolites that act as agents for their own survival when exposed to stress from toxic materials and temperature variations (Vahabi *et al.,* 2011).

Regarding the effect of temperatures on α-amylase, lower and higher temperatures show a gradient in the activity values. Lower temperatures decrease particular activities because they are unsuitable for fungal growth, which in turn reduces enzyme production, while higher temperatures lead to minimize medium water content by vaporization, which in turn affects cell growth, so the production of the enzyme (Bedan *et al.,* 2014). (Kumari *et al.,* 2012), they found that 30 °C was the optimal temperature for the production of the amylase enzyme, giving this enzyme the highest level of activity (Suganthi *et al.,* 2011). (Ugoh *et al.,*

2013) reported that temperatures at 30, 37 and 40°C were the optimum for amylase activity by *Aspergillus* spp. However, (Spier *et al.,* 2006) reported that *Aspergillus* species showed amylase activity at its highest at 45°C. But in this study, it was found that increasing the temperature to 45°C inhibited the production of α-amylase. This is probably due to the fact that the cell activity of the isolate gradually increases with an increase in temperature until it reaches the maximum growth of mycelium to capture nutrients and growth is retarded beyond optimum temperature. When the temperature is increased, the moisture content decreases below the ideal level for the isolate to grow, which has a significant impact on the production of enzymes (Behailu *et al.,* 2018). When adding different concentrations of MgO NPs, this stopped the production of the enzyme. Some studies showed that the percentage of amylase production inhibition increased as the concentration of nanoparticles increased (Chouhan *et al.,* 2020; Debnath *et al.,* 2019).

5. Conclusion

Magnesium oxide was synthesised by green synthesis method from magnesium nitrate Mg $(NO_3)_2.6H_2O$. MgO NPs can be produced using alternative method by plant *H. muticus* leaf extract. MgO NPs had an inhibitory effect on the growth and the production of α-amylase of both *A. niger* and *A. ochraceous*. The optimum temperature on fungal isolates was 35 °C for *A. niger* and 27 °C for *A. ochraceous,* the growth inhibition was highly when the temperature was raised, the α-amylase enzyme activity is affected by the temperature, when temperature increased led to the cessation of amylase production.

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Institutional Review Board Statement
All Institutional Review Board Statements are confirmed and approved.
Data Availability Statement
Data presented in this study are available at fair request from the respective author.
Ethics Approval and Consent to Participate
This work carried out at the Botany department (microbiology) Faculty of Agriculture and Physics and Botany departments, Faculty of Science, South Valley University and followed all the departments instructions.
Consent for Publication
Not applicable.
Conflicts of Interest
The authors declare no conflict of interest.

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