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Evaluation of the antifungal effect of magnesium oxide nanoparticles on *Fusarium oxysporum* F. Sp. lycopersici, pathogenic agent of tomato

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ABSTRACT

Coping with plant diseases and pests are among the most critical problems of agriculture sector. "Fusarium oxysporum f. sp. lycopersici", the fusariumwilt agent in tomato plant, is one of the important plant pathogens, which enjoys a global significance due to it severe damages. Taking into account the mutation of pathogens and resistance against different pesticides and heavy costs of generating new and resistant races and also potential ability of nanoparticles, the antifungal effect of magnesium oxide nanoparticles on "FOL" will be assessed in the present research. The nanoparticles used in the current research were chemically synthesized and its physical-chemical properties were measured and confirmed using double-beam visible-ultraviolet spectrophotometer (Model: TU-1901), X-ray diffraction device (Model: D/Max-RA) under CuKα emission, and transmission electron microscope (Model: TEM-200CX). Concentrations of 0.5%, 1%, and 2% of magnesium oxide nanoparticles were prepared with deionized water and their effect on the respective fungus was studied in liquid and solid growth media; the results were analyzed by means of Student t-test software at p-value<0.05. The results indicated that controlling effect increases with an increase in the administered dosage of nanoparticles, and, there exists a direct correlation between the administered dosage and controlling effect such that the concentration of 2% had the greatest effect in both liquid and solid growth media. The results are in accordance with other researches concerning effect of nanoparticles on microorganisms, and, it can be also interpreted that the cells are decomposed at a higher rate in presence of nanoparticles.

Key words: Antifungal effect, Magnesium oxide nanoparticles, *Fusarium oxysporum f.sp. lycopersici*

INTRODUCTION

Plant diseases, controlling, and coping with them are among the most crucial challenges of agriculture sector. Various microorganisms are involved in creating pests in agriculture sector. Among these microorganisms, fungi hold a remarkable share such that the percentage of diseases caused by fungi in agricultural systems is very high [1]. Fusarium fungus is an important plant pathogen found in various kinds. *Fusarium oxysporum f.sp. lycopersici* the Fusarium wilt agent in tomato plant. This disease has a global significance owing to the severe damages it inflicts to agricultural products, prolonged survival of fungus in soil, generation of resistant races, and also the problems encountered in combatting this disease. 32 countries have reported its presence around the world [2-3]. In Iran, it was primarily reported in Hormozgan Province in 1964 [4]. As a result of this disease, the plant growth is impaired, old leaves starts shedding, the plant tends to wilt down, the color turns yellowish, and little by little, the whole plant turns yellow. The disease proceeds as far as the plant disintegrates. Color change of vascular system into brown is one of the signs to diagnose the respective disease [5-6]. Control mechanism of this kind of pests and their eradication in a way that no harm is imparted to the crops and also no adverse impact is imposed to the environment have constantly been a concern of agriculture sector and HSE organizations. As of today, use of synthesized fungicides and also application of resistant cultivars have been emphasized for controlling the fungi Mutation in pathogens and resistance against different pesticides as well as high costs of generating new and resistant genes have made it difficult to utilize such pesticides as a solution, compelling the researchers to seek for appropriate and inexpensive solutions which are compatible with environmental regulations. Currently, use of nano-structural materials is expanding and numerous advantages have been so far achieved from organic and inorganic nanoparticles. This suggests the possibility to use nano-materials in different fields including physics, chemistry, pharmacy, surface coaters, biochemistry, textiles, agriculture and farming. Additionally, no report has been published by common chemical sciences on the hazards resulting from nanoparticles [7-8]. With regard to high antibacterial and antifungal potential of nanoparticles, the antifungal effect of magnesium oxide nanoparticles on *FOL* will be analyzed in the present research.

MATERIALS AND METHODS

2-1- The growth media used in the current study as well as the materials such as 0.14-molar magnesium acetate (Mg $(CH_3COO)_2$), poly-vinyl pirolyn ($(C_6H_9NO)n$), trimethylammonium hydroxide ($(CH_3)4NOH$), and ethanol (C_2H_6O) were all prepared from Germany's Merck Company. The strain of F. oxysporumf.slycopersi fungus was supplied by laboratory of Shiraz Agriculture Researches Center. Double-beam visible-ultraviolet spectrophotometer (Model: TU-1901), X-ray diffraction device (Model: D/Max-RA) under CuKα emission, and transmission electron microscope (Model: JEM-200CX) were used in the present study.

2.2. Preparation of Magnesium Oxide Nanoparticles

At first, in order to form the nano-structure of magnesium hydroxide, 50 ml of 0.14-molar magnesium acetate together with required amount of polyvinyl pyrolin (PVP) were sonicated for 30 minutes, and then, sufficient volume of 0.34-molar trimethylammonium hydroxide (TMAH) solution was gradually added. The nano-structure of magnesium hydroxide was formed in this stage according to the reaction below:

$$Mg\,(CH_3COO)_{2(aq)} + 2(CH_3)4N(OH)_{(aq)} \rightarrow Mg(OH)_{2(s)} + 2(CH_3COO)N(CH_3)_{4(aq)} \quad (1)$$

After passing through filter, the precipitated magnesium hydroxide was washed three times with ethanol and distilled water. Then, 50 ml of ethanol was added to the precipitation, and, the acquired mixture was sonicated for 30 minutes. The sonicated mixture was subsequently passed through filter. The final acquired precipitation was dehydrated for 4 hours at 550℃ according to the following reaction:

$$Mg\,(OH)_{2(s)} \rightarrow MgO_{(s)} + H_2O_{(g)} \quad (2)$$

To get rid of the bulk state, magnesium oxide nanoparticles were sonciated for 30 minutes in ethanol. At the last stage, the acquired mixture was filtered and dehydrated at 110˚C. Ultimately, the magnesium oxide nanoparticles were obtained as powder which were preserved for the subsequent stages of the research.

2.3. Analysis of effect of Magnesium Oxide Nanoparticles on the Respective Fungus

Potato dextrose agar growth medium was used for culturing the respective fungus in analysis of the antifungal properties of these nanoparticles. The respective fungus was cultured on this media and preserved for 6 days at 25-27˚C. Initially, 6-day culture of *FOL*was used for preparing the fungal suspension. Following removal and culturing in potato dextrose broth medium, the spores were separated after 3 days, and finally, a suspension with a concentration of $5*10^6$ spores per milliliter was prepared using hemocytometer lam [9]. In experiment of *FOL* sensitivity to magnesium oxide nanoparticles, two different estimation methods were employed. In the first method, different concentrations of magnesium oxide nanoparticles from PDA media were used for analyzing the antifungal properties. To do so, 0.5%, 1%, and 2% concentrations of magnesium oxide nanoparticles prepared from deionized water were added to autoclaved growth medium after its temperature reached approximately 40-50℃, and then, they were divided in sterile plates. Nanoparticle-free PDA growth medium was used as the control group. One-centimeter discs were separated from 6-day culture of *FOL*and were placed onto the plates containing nanoparticles and the plate containing pure growth medium as the control group. The plates were kept for 6-day in incubator at 28℃. Radial growth of the respective fungus in the plates containing nanoparticles and also the plate without nanoparticles were separately analyzed afterwards. SDB medium was used in the second method. For this purpose, a similar amount of the respective fungus was added to SDB growth medium. This solution was kept at 28˚C for 2 days. Optical Density (OD) of the abovementioned solution was measured every two hours at wavelength of 530 nm in spectrophotometer and its diagram was plotted. The acquired results were compared with those of control group to which no nanoparticles have been added. All tests were conducted in three replications and Student t-test was applied for determining and assessing significance of results.

RESULTS AND DISCUSSION

3.1. Analysis of Properties of Synthesized Magnesium Oxide Nanoparticles

3.1.1. X-ray Diffraction model of the generated nanoparticles

Figure 1 illustrates an example of X-ray diffraction for the magnesium oxide nanoparticle sample. Diffraction peaks are absorbed at 2θ value. Sharp peaks are used to estimate the grain size of the sample via scherers equation i.e. $D=K\lambda/(\beta\cos\theta)4$, where K is constant and equals (0.9), λ is wavelength, β is the full width at the half-maximum, and θ is diffraction angle. Grain size is estimated using peak intensity ratio. A grain size of 70 nm was obtained for magnesium oxide nanoparticles. Increase in sharpness of XRD peaks indicates that the particles are essentially crystalline.

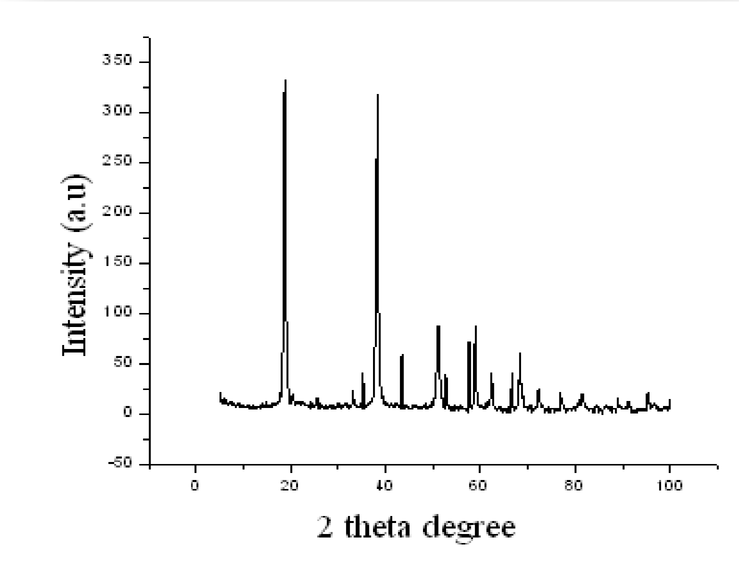

Figure 1: Example of X-Ray Diffraction (XRD) model of synthesized magnesium oxide nanoparticles

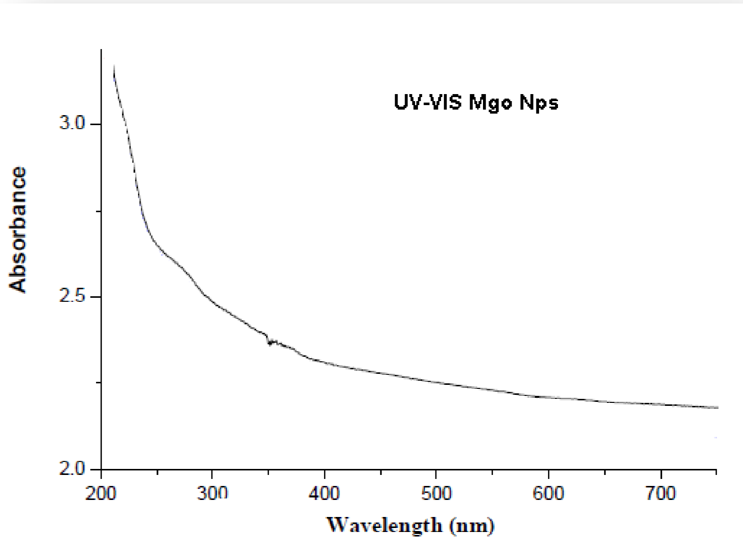

Figure 2: Visible-Ultraviolet spectrum of synthesized magnesium oxide nanoparticles

3.1.2. Properties of Visible-Ultraviolet Spectrum of Magnesium Oxide Nanoparticles
Figure 2 represents the visible-ultraviolet spectrum using chemical method. As observed in the figure, absorption peak lies in "300-900" (nm) interval. Absence of sharp peaks implies generation of nanoparticles at different sizes in this method and confirms the visible-ultraviolet spectrum and electron microscopy results. This information proved the specific and quantum properties of the nanoparticles.

3.1.3. Electron Microscopy Analysis of Magnesium Oxide Nanoparticles
Figure 3 illustrates transmission electron microscope (TEM) image of the synthesized magnesium oxide nanoparticles. Due to increase in surface-to-volume ratio with reduction in size of smaller nanoparticles, these nanoparticles are able to play highly significant roles along immobilization processes. According to the results of TEM studies, diameter of the synthesized magnesium oxide nanoparticles is around 70 nm.

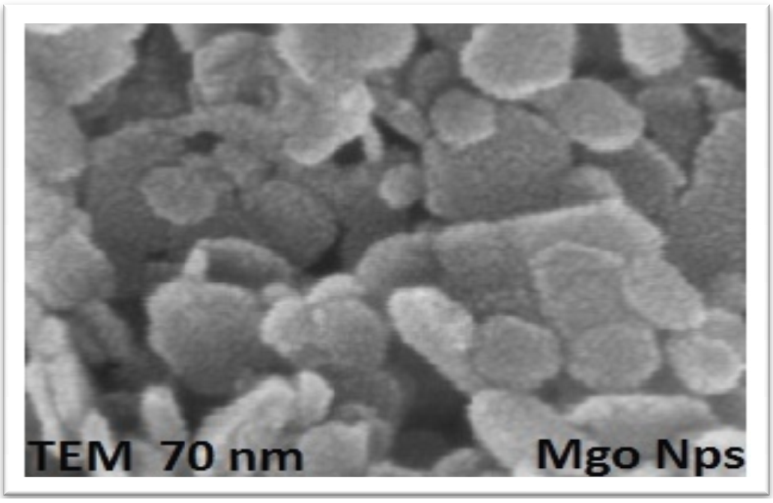

Figure 3: transmission electron microscope image of the synthesized magnesium oxide nanoparticles

3.2. Analysis of different concentrations of magnesium oxide nanoparticles on *FOL* in solid growth medium
Figure 4 shows impact of magnesium oxide nanoparticles on radial growth of fungus mycelia. As the results suggest, the concentrations under analysis had different impacts compared to the control group such that the concentration of 2% exhibited the greatest effect on the respective fungus among the three selected concentrations in the present study. As demonstrated, a significant difference is observed compared to the control group. Concentrations of 1% and 0.5% respectively exhibited smaller effects, and, the concentration of 0.5% had the lowest impact in comparison with other values.

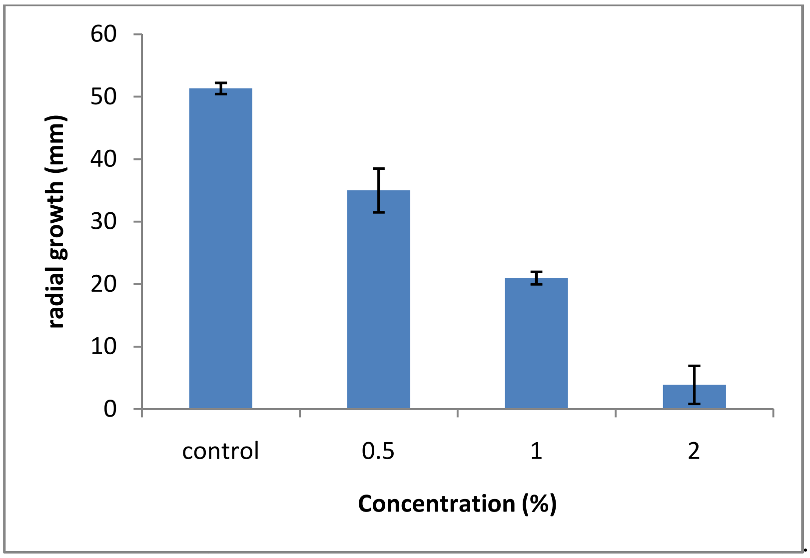

Figure 4: Effects of magnesium oxide nanoparticles on radial growth *FOL* in solid growth medium

3.3. Analysis of different concentrations of magnesium oxide nanoparticles on *FOL* in liquid growth medium
Regarding the measured optical density of the respective fungus suspension in the presence of different concentrations of nanoparticles and according to its plotted diagram in Figure 5, it was concluded that the concentration of 0.5% did not have considerable impact compared on the control group but two other concentrations exhibited significant impact ($P<0.05$) in comparison with the control group. The largest effect in liquid growth medium also belonged to concentration of 2%.

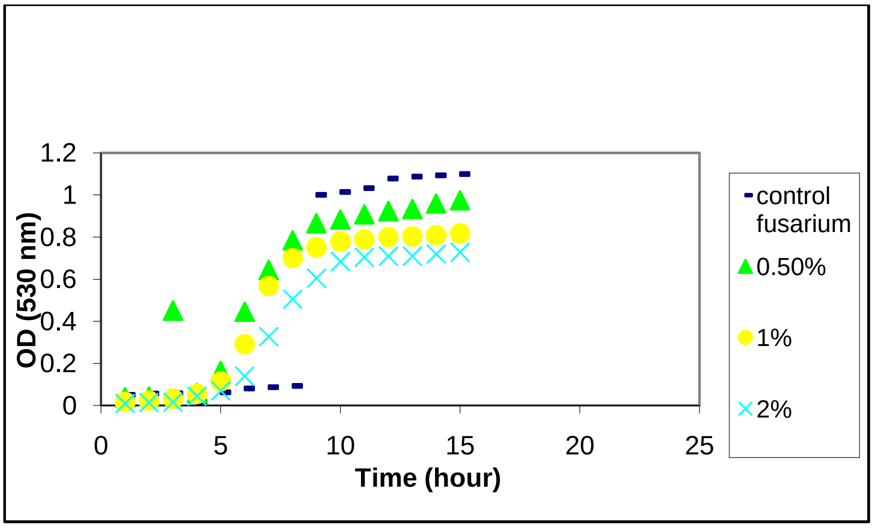

Figure 5 – Measured optical density of *FOL* in presence of magnesium oxide nanoparticles

FOL a terrigenous fungus, a species of Fusarium fungus having prolonged durability in soil. At present, the only way to cope with such plant pathogen is utilization of chemical pesticides which has no remarkable impact despite its environmental contamination and prolonged fungus survival [10]. Keeping in mind the global prevalence of this fungus, numerous researchers in entire world are attempting to propose effective solution for combatting such fungi. Acquisition of materials with antibacterial, antiviral, and antifungal properties can have applications in many applications. During the recent decades, inorganic nanoparticles with advanced physical, chemical, and biological properties have been developed. These nano-materials have attracted attention of endless number of researchers all over the world thanks to their potential ability to attain the selective stage in biological, medicine, and agricultural processes [11]. Nanotechnology has been developed for controlling materials in very small dimensions and exploiting the emergent phenomena at nano scales [12-13]. Nanotechnology science with its potential ability is capable of developing the production process toward providing products with suitable features and novel applications [14]. In fact, nanotechnology is an interdisciplinary subject which involves different sciences including physics, chemistry, biology, agriculture, and engineering and is linked with microscopic production techniques [15]. In the current study, antifungal effect of magnesium oxide nanoparticles at concentrations of 0.5%, 1%, and 2% were analyzed on *FOL* fungus in solid and liquid growth media. The acquired results are in agreement with former researches which have used nanoparticles against bacteria and fungi [16]. In an analogous research, effect of silver nanoparticles on Fusariummoniliforme fungus was analyzed leading to the inference that silver nanoparticles in short term contributed to remarkable reduction in radial growth of fungus [17]**.**

CONCLUSION

Findings of the present research are suggestive of the fact that magnesium oxide nanoparticles have considerable impact on*FOL* such that controlling effect of nanoparticles improves in both solid and liquid media with increase in the administered dosage, and actually, there is a direct relationship between the dosage value and controlling effect of nanoparticles. The results acquired in the present study can be effective for proposing a synthetic compound based on biologic effects of biological nanoparticles for coping with diseases caused by different species of Fusarium fungus.

REFERENCES

[1] Hadian, SH., Shamluo, P., Monazam, K. and KHanduoz, A. *Journalof Plant Science*. **2011** 6(21): 68-77.
[2] Ahmadvand, b. and Vzrbkhsh, A.S. *Water,soil,agricultural*. **2008**(1): 65- 77.
[3] Walker, J.C. Fuzarium wilt of Tomato. Monograf NO.6, *APS Press*,**1981**:86 p.
[4] EloquentA.S. *Journal of Plant Diseases.***1985** :1:29-32.

[5] Etebarian H.R. Vsyfy vegetable diseases and methods of combatingit,*Tehran University*. **2002*:600 pages.***
[6] Jones, J. B., Jones, J. P., Stall, R.E. and Ziter, T. A. *APS Press*.**1991**: P: 73-90.
[7] Xua, X. N., Wolfus, Y., Shaulov, A. and Yeshurunb,Y. *Journal Applied Physics*.**2008**, 91(7): 28-39.
[8] Kahru, A., Dubourguier, H.C., Blinova, I., Ivask, A. and Kasemets, K. *Aminireview. Sensors*.**2008**, 8(8):5153-70.
[9] Banihashemi, z. *Journal of PlantDiseases*. **1982**, 18:1-6.
[10] Dimond, A.E. and Wagonner, P.E. *Phytopatology*. **1953**, 43: 663-669.
[11] Wu, X., Liu, h., Liu, J., Haley, K.N., Treadway, JA., Larson JP. *Nat Biotech* . **2003**, 21: 41-6.
[12] Hussain, N., Jani, P.U. and Florence, A.T. *Pharm Res*. **1997**, 14(5): 6-13.
[13] Oberdörster, G., Oberdörster, E., *J Nanotoxicology*. **2005**, 113(7): 8-23.
[14] Ravi, M.N., Bakowsky, U. and Lehr, C.M. *Biomaterials*. **2004**, 25(10): 17-71.
[15] Kompella, U.B., Bandi, N. and Ayalasomayajula S.P. *Drug Deliv.Technol*. **2001**, 1(2): 1-7.
[16] Service, R.F. *Science* **2004**, 304:1732-1734.
[17] Katuoly, N. and Rahnema, k. *Journal of plant food*. **2007**, 1: 6-14.