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APPLICATION OF SOME NANOPARTICLES AND VASCULAR ARBUSCULAR MYCORRHIZA (VAM) TO CONTROL ROOT RO/WILT DISEASE OF SAGE (*SALVIA OFFECIALIS* L.) PLANTS

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ABSTRACT

The Sage plant belonging to the family *Lamiaceae* is one of the medicinal and aromatic plants which are receiving considerable attention all over the world due to their vast untapped economic potential, especially in the use of herbal medicines. This study carried out to control root rot and wilt disease of sage plant using copper oxide nanoparticles (CuONPs); zinc oxide nanoparticles (ZnONPs) and vascular arbuscular mycorrhiza (VAM). According to identification procedures, fungal isolates obtained belonged to *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina*. Under laboratory conditions, the growth of the tested fungal isolates (*R. solani* (Rs9), *F. solani* (Fs3) and *M. phaseolina* (Mp11)) was reduced with the application of ZnONPs and CuONPs compared with the control treatment. Also, application of VAM and CuONPs (at 250 ppm) as alone treatment significantly decreased percentage disease incidence and percentage of disease severity of sage root rot/wilt disease, at 30 or 90 days from sowing. Meanwhile, combination between VAM and CuONPs were the most affectivity compared with alone treatment. At the same time, the plant growth parameters and seed yield of sage plants improved.

Key words: Sage, *Salvia officinalis*, wilt/root rot disease, Vascular Arbuscular Mycorrhiza (VAM), Copper oxide nanoparticles (CuONPs), Zinc oxide nanoparticles (ZnONPs).

INTRODUCTION

Salvia officinalis L., also known as sage, is a plant in the *Lamiaceae* family that is prized for its early, mildly peppery flavor and fragrant blooms. One of the most significant indigenous medicinal species found in the ancient world's flora is thought to be this one. The harvest of sage can continue into the autumn because it is typically an evergreen plant. A perennial subshrub that outcrosses, it has violet blossoms and slender, widely branching woody stems. Beyond its native Mediterranean karst in the west Balkans and the Apennine peninsula, it is grown all over the world in a range of Mediterranean and temperate continental temperatures. Avoiding or minimizing the use of pesticides is particularly important with herb crops, due to the restriction on pesticide residues on marketable produce (**Pundt and Smith, 2005)**, nowadays, scientists are attempting to create organic, inorganic, and hybrid nanoparticles with physical, optical, and biological properties. Although there are numerous uses for nanotechnology in the fields of medicine, agriculture, electronics, and textiles. During the last 3 years, common sage has repeatedly shown decline symptoms in several production fields in El Minia Governorate. In the spring of 2018, a serious common sage root rot/wilt disease developed under field conditions resulting in 7 -17.8% loss of plants **(Zakarya *et al*, 2023).**

Nanotechnology in the agriculture field is currently in the experimental stages. From an agricultural standpoint, nanotechnology has become a useful tool for phytopathologists in the detection and treatment of plant diseases through the use of various nanoparticles, the detection of pests through the use of nano sensors, the enhanced ability of plants for nutrient absorption, and on other fields **(Chaudhry *et al*. 2017; Rai and Ingle 2012; Ram *et al*. 2014, Prasad *et al*., 2017, Ismail *et al*. 2017; and Sangeetha *et al.* 2017).** Regarding environmentally friendly agricultural inputs, nanotechnology can help by enhancing the stability and safety of active agents, boosting their pest-control effectiveness, and ultimately increasing producer acceptance **(Nair and Kumar, 2012; Srilatha 2011; Khot *et al*. 2012; Prasad *et al*. 2014, Prasad *et al*., 2007 and Ismail *et al*. 2017).**

Some of the potential key applications in plant pathology include the control of plant diseases through site-targeted delivery of nano-formulated agrochemicals, the development of disease resistant plant varieties through nanomaterial-mediated genetic transformation, and the early detection of plant diseases and pathogens. Agrochemical nanoencapsulation provides effective concentration of the active ingredient with high stability and site-targeted smart delivery with less collateral damage and ecotoxicity **(Nair and Kumar, 2012)**. Efforts to address various environmental issues caused by pesticide overuse resulted in the successful use of some nanoparticles (such as oxides of copper, zinc, magnesium and silver nanoparticles) or a combination of more than one nanoparticles in preventing plant diseases caused by various agents.

Nanosized particles were used in many studies for controlling fungal pathogens, *i.e*. *Rhizoctonia solani*, *Pythium ultimum*, *Magnaporthe grisea*, *Botrytis cinerea* and *Colletotrichum gloeosporioides*, and bacterial pathogens, *vez. Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas syringe*, *Bacillus subtilis*, *Azotobacter chrococcum*, and *Rhizobium tropici* **(Park *et al.,* 2006)** and *Periconia igniaria* (**Abdel-Samad *et al*., 2023).**

Abiotic stresses inhibit plant development and output. Climate change and agricultural malpractices such the excessive use of pesticides and fertilisers have exacerbated the effects of abiotic stress on crop productivity. Additionally, the environment has suffered. There is an urgent need for environmentally friendly management techniques, such as employing arbuscular mycorrhizal fungi (AMF) to boost crop output. It's common to hear people refer to AMF as bio-fertilizers. Almost 90% of plant species, including bryophytes, ferns, and flowering plants, can form symbiotic relationships with AMF (**Zhu *et al*., 2010**). Additionally, it is commonly accepted that AMF inoculation makes host plants more resilient to a variety of stressful conditions, including heat, salt, drought, metals, and extremely low temperatures (**Begum *et al*., 2019**). The oldest and most prevalent symbiotic relationship with land plants is between arbuscular mycorrhizal (AM) fungi and plants. A symbiotic organ made of plant root cells and fungus hyphae is called mycorrhiza. The bidirectional exchange of nutrients between the two partners is made possible by a fully developed mycorrhizal symbiosis. The effects include an increase in the plant's resistance to different stresses such dehydration, contaminated, deficient soils, and pathogenic invasion **(Sikes *et al*., 2009; and Qaddoury, 2017).** Furthermore, as natural root symbionts, arbuscular mycorrhizal fungi (AMF) increase host plant development and yield by supplying them with vital inorganic nutrients. AMF-mediated growth promotion helps the plants absorb more water and nutrients from the nearby soil while also protecting them against fungal and bacterial pathogens (**Smith and Read, 2008; Jung *et al*., 2012).** As a result, AMF are essential endosymbionts that effectively contribute to plant productivity and ecological health. They are crucial for improving crops in a sustainable way (**Gianinazzi *et al*., 2010).**

In this study, we aimed to find an environmentally friendly trials to control root rot/wilt of sage using nanoparticles and arbuscular mycorrhiza under open field conditions**.**

MATERIALS AND METHODS

1- Pathogenic fungi:

Three highly virulent isolates of sage root rot/wilt fungi, *Fusarium solani* (isolate Fs3), *Rhizoctonia solani* (isolate Rs9) and *Macrophomina phaseolina* (isolate Mp11), which were isolated from natural infected sage plants showed root rot and wilt symptoms, collected from private farms in different districts of Minia Governorate during 2018-2019 winter season and tested for their ability to induce sage root rot/wilt, were used in these experiments. These isolates were highly aggressive and induced the highest percentages of sage infection either 30 or 90 days after sowing, ranged between 86.11 – 94.44 DI%, and 67.78 - 74.44% DS%, 90 days after sowing, were chosen to carry out the other experiments (**Zakarya *et al*., 2023**). Fungi were cultured on PDA medium,7 days before using it for preparation of inocula. Pot experiment was carried out in Plant Pathology Department Experimental Farm of Faculty of Agriculture, Minia University in 2021 winter season.

Inocula of the isolated fungi were prepared, separately, on sterilized barley grains (150 g of grains + 200 ml water in Erlenmeyer flask,1000-ml). Inoculated flasks were kept at 25±2^{o}C for 15 days then used for soil infestation. Disinfected clay pots (30 cm in diameter) were filled with sterilized soil (Nile clay and sand, 1:1 v/v), as 4 Kg/pot. Pots and soil were sterilized using formalin 5% and aerated for 15 days until the evaporation of formalin was completed. Soil infestation was applied one week before planting, by thoroughly mixing 2% of the inoculum, representing a barley culture of one fungus, with the soil in each pot as described by **Papavizas and Devey (1962)**. Before planting, the infested soil was watered daily for a week in order to encourage fungal development and ensure its establishment.

2- Vascular Arbuscular Mycorrhiza (VAM):

A mixture of formulated mycorrhizal fungi was purchased from the Mycological Research and Plant Disease Survey Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt, and were previously identified using VANS1/VALETC and VANS1-NS21 primers **(Sabet *et al*., 2013).** This Mixture consists of propagated units of *Glomus mosseae* (Nicol. And Gerd) Gerd. and Trappe, *G. intraradices* Schenck and Smith, *G. etunicatum* W.N. Becker & Gerd., and *G. coronatum* Giovann. They were isolated from the rhizosphere of various plants grown in Egypt. Every three to four months, the VAM propagules were re-cultured and kept alive on the roots of Sudan grass (*Sorghum sudanesis* Pers.) and renewed every 3-4 months. The hemacytometer (Reichert-Jung, Cambridge Instruments Inc. Buffalo. New York 14215) was used to set the concentration of mycorrhizal fungus in their suspensions to 1x10^{6} propagules/ml.

3 - Nanoparticles

Copper oxide nanoparticles (CuONPs) and Zinc oxide nanoparticles (ZnONPs) were purchased from Nanotechnology & Advanced Nano-Materials Laboratory (NANML), Mycology & Disease survey Research Department, ARC, Giza, Egypt.

According to the source, the particles size was 35±5 nm for CuONPs and 15±3 nm for ZnONPs and the shape is spherical for both. The Characterization and properties of CuONPs and ZnONPs were confirmed as described in Table (1).

4- Effect of zinc oxide- and copper oxide- nanoparticles on the growth of sage root rot/wilt pathogens *in vitro*:

Different concentrations of ZnO- and CuO-NPs (0, 50, 150 and 250 ppm) were prepared by diluting the original stock solution (1000 ppm) using sterile deionized water. Then, different ZnONPs and CuONPs concentrations (50, 150, and 250 ppm) were applied to freshly produced PDA. Isolates of *R. solani* (Rs9), *F. solani* (Fs3) and *M. phaseolina* (Mp11) were grown in PDA medium at 25 ◦C for 7 days. The fungal growth medium was then placed into Petri dishes when the fungal media had cooled to about 45^{o}C and NPs had been added. The center of the 9-cm-diameter Petri plates were inoculated with 5mm discs of fungal inoculum (*Fusarium solani* (isolate Fs3), *Rhizoctonia solani* (isolate Rs9) and *Macrophomina phaseolina* (isolate Mp11), which were cut with a cork borer from the culture's active margins and incubated at 25°C for 5-7 days. As a control, PDA plates cultivated in identical circumstances but without any nanoparticle compounds were employed (**Oussou-Azo *et al*., 2020)**. The fungal linear growth was assessed, and growth inhibition percentages were computed.

5 -Effect of copper oxide nanoparticles and Vascular Arbuscular Mycorrhiza (VAM) alone or in combination on sage root rot and wilt disease, under artificial inoculation conditions:

5-1. Host:

Sage 10-12 cm cuttings were taken from healthy two-years plants cultivated in Experimental Farm of Plant Pathology Department, Faculty of Agriculture, Minia University. The cuttings were surface superficially disinfected for 2 minutes in sodium hypochlorite (2%) before being washed three times for 3 minutes each in sterile distilled water. Cuttings were kept under greenhouse conditions, 2 weeks before planting, to encouragement uprooting at the end of this period.

5.2. Inoculum preparation and soil infestation:

Pot experiment was carried out in the greenhouse, Department of Plant Pathology, Faculty of Agriculture, Minia University during 2022 and 2023 winter seasons. Sterilized clay pots, 30 cm in diameter, were filled with about 4 Kg disinfected soil (Nile Loamy and sand, 1:1v/v), were artificially inoculated with *F. solani* (isolate Fs3), *R. solani* (isolate Rs9) or *M. phaseolina* (isolate Mp11) as described before. After one week, soil was inoculated with VAM solution

The VAM inoculum was mixture (*Glomus mosseae*, *G. intraradices*, *G. etunicatum.* and *G. coronatum*) of spores (40-70 spores), mycelium and colonized root fragments of Sudan grass. This mixture was added to the soil at the rate of 30g/pot. Mycorrhizal mixture was added directly into the planting hole immediately before the cuttings were planted. The adding of mycorrhiza was repeated three times with 15 days interval, by pouring the mycorrhizal soil around the roots and then covered with mulch to prevent the inhibitory effect of sunlight.

The CuONPs solution (250 ppm+ 0.2% of the tween 80) was prepared and used. The basal parts (about 10-15 cm) of surface sterile sage cuttings were immersed in the CuONPs solution prepared, 15 minutes before planting. The treatment was repeated by spraying plants after 30 days of planting with the same concentration of the compound.

A mixture of (VAM) and CuONPs as well as the integration between them in comparison to the chemical fungicide (Ridomil Plus 44 WP) against sage root rot/wilt diseases caused by *F. solani*, *R. solani* and *M. phaseolina*, was estimated under artificial infection conditions. The treatments applied in this study can be summarized as follows:

Pathogen (P); Pathogen + VAM (PM); Pathogen + CuONps (PN), Pathogen + VAM + CuONPs (PMN); Pathogen + fungicide (PF); VAM (CM); CuONPs (CN), and Control (C) are the various treatments used in this investigation.

The reference fungicide (Ridomil Plus 44 WP), prepared in water at the dose recommended by the manufacturer (3.3 g. l^{-1}) was used to soil treatment immediately before sowing.

5.3. Disease assessments:

The percentages of disease incidence were calculated according to the number of infected plants after 30 and 90 days of planting, as follows:

Disease incidence (DI%) = (Total No. of sown cuttings / No. of infected plants) x 100. According to **Rowe (1980) and Liu *et al.* (1995),** sage root rot and wilt severity (DS,%) was graded 30 and 90 DAS in the following degrees: No root or leaf discoloration is coded as “0”, No discoloration with superficial lesions (25%) on the tap root is coded as “1”, Slight internal discoloration is coded as “2”, Moderate internal discoloration is coded as “3”, and Severe internal and external discoloration is coded as “4” (more than 75%). Disease severity (DS, %) = {[Sum (n×r0)+(n×r1)+....+ (n×r4)]/ 4N}x100

Whereas: "N" is the total number of plants, and "n" is the number of plants in each category (r0–r4).

The reduction on percentages of disease incidence and disease severity were also calculated.

6 -Influence of nanoparticles and mycorrhiza on the sage vegetative parameters:

The sage plant vegetable parameters, viz, plant fresh weight (g), No. of leaves/plant, leaves fresh and Dry wt./ plant (g), shoot height (cm), root length (cm), No. of branches/plant, and number of inflorescence/ a plant, were measured after 160 days of sowing.

7. Statistical analysis:

Data from three replicates for each treatment were organized and presented as the mean. All tests used entirely random experimental designs. Utilizing analysis of variance, data were statistically evaluated for significance in Statistic (8th edition, Analytical Software, USA, **Steel *et al.*, 1997).** (ANOVA). The significances between averages wer tested using Duncan's multiple range test with a probability of 0.05 in accordance with the **Gomez and Gomez (1994)** method.

RESULTS

1- Effect of ZnONPs and CuONPs on the of sage root rot /wilt pathogens growth, in laboratory study:

Data in Figures (1 and 2) points to affect the growth of tested pathogens, *Fusarium solani* (isolate Fs3), *Rhizoctonia solani* (isolate Rs9) and *Macrophomina phaseolina* (isolate

Mp11), with all concentrations of ZnO and CuO nanoparticles. The growth of the pathogens was reduced with the application of nanoparticle materials . The maximum reduction of *F. solani R. solani* and *M. phaseolina* growth was shown at the maximum nanoparticle's concentration (250 ppm) used, 80.44,84.11 and 80.78% reduction when ZnONPs was used, and 82.67, 85.56 and 84.44% by applying CuONps, respetively. Also, the growth of pathogens was gradually reduced with increasing nanoparticle material concentration. Data also showed that CuNPs is slightly more affected on pathogens mycelial growth than ZnONPs.

2- Effect of VAM and CuONPs alone or in combination on severity of sage root rot/wilt disease, under artificial inoculation conditions:

In this study, pot experiment was conducted to study the efficacy of VAM, CuONPs, VAM integrated with CuONPs, and Ridomil plus fungicide as a positive control against sage root rot/wilt caused by *F. solani*, *R. solani* and *M. phaseolina* under open field conditions of El-Minia Governorate, Egypt. They were estimated by determining the percentage of infected plants and disease severity, 30 and 90 days after cutting sowing. Experiments were carried out in two successive seasons, 2022 and 2023. Data presented in figures (3- 6) show that the VAM and CuONPs (at 250 ppm) treatments significantly reduced the percentages of both DI% and DS% in both plant ages. In most cases, 30 days after sowing, the percentages of reduction for both disease incidence and severity were lowest when CuONPs was applied than VAM treatments. Inverse versa data were obtained after 90DAS, where VAM treatments insignificantly decreased DI% and DS% more than CuONPs. The maximum reduction was observed when the VAM and CuONps were integrated together. Also, Redomil plus fungicide and the integration between VAM and CuONps were in most cases, the most effective treatments gave the lowest infection and severity percentages in comparison with the infected control.

3-Effect of VAM, CuONPs and their combination on sage vegetable parameters:

Sage vegetable parameters (fresh wt/plant (g), leaves No/plant, fresh and dry wts. of leaves / plant (g), shoot and root length (cm), No. of branches/ plant and Number of inflorescence/ plant) were determined after 160 days of sowing. Data in Tables (2 and 3) showed that all vegetable parameters of sage affected by either VAM or CuONPs treatments. The minimum parameters values were obtained when pathogen infected plants (T1), whereas the maximum vegetable parameters were recorded in treatment 4 when plants were treated with VAM and CuONPs compared with the control.

In sage maximum plant fresh weight 72.57, 62.9, 72.83 g when treated with VAM and CuONPs was highly significant than control or pathogen treatments. VAM and CuONPs increased the plant fresh weight between 1.2 – 5.3 folders, 1.07-4.58 and 1.24 -5.43 folders when plants infected with *F. solani*, *R. solani* and *M. phaseolina*, respectively

In most cases, the tested vegetable parameters of sage were significantly increased when plants treated with VAM (T2), followed with that treated with

CuONPs (T3) comparing with control (non-treated ones). The minimum vegetable parameters were recorded in plants artificially inoculated with *R. solant* followed with that inoculated with *M. phaselina* and *F. solani.*

DISCUSSION

Root rot /wilt disease caused by *Fusarium solani, Rhizoctonia solani* and *Macrophomina phaseolina* is considered as a common disease attacking plants of sage (*S. officinalis* L.) in El-Minia Governorate and other areas of the world. Sage root rots may cause severe damage and they were reported in a few countries, such as India **(Mallesh *et al*., 2009)**, Italy (**Garibaldi *et al*., 2015),** Poland **(Zimowska, 2008)** and Turkey **(Çarkacı and Maden, 1998).** Some isolates of *F. solani*, *F. moniliforme, F. oxysporum* and *R. solani* produced 90%-100% damping-off of sage **(Çakir *et al*., 2017).** Also, *Phytophthora cryptogea* was reported as the pathogen causing sage root and crown rots in Turkey **(Çarkacı and Maden, 1998; Bayram, 2001 and Çakir *et al*., 2017). Mallesh *et al.* (2009),** in India, reported that root rot of sage was mainly caused by *F. solani* and *R. solani* and sage was so vulnerable against root rot.

Fusarium solani, Rhizoctonia solani, and *Macrophomina phaseolina* were the three most prevalent isolated fungus from naturally root-rotted and wilted sage plants gathered from various areas in Minia Governorate, Egypt during the 2018 growing season. In this investigation, three extremely virulent isolates of Macrophomina phaseolina (isolate Mp11), Rhizoctonia solani (isolate Rs9), and Fusarium solani (isolate Fs3) were employed. These isolates have demonstrated the potential to infect sage plants and cause root rot/wilt symptoms **(Zakarya et al., 2023).**

Fungicide use is undoubtedly the most important aspect of pest and disease management programmes in horticultural production systems, especially when it comes to horticulture and orchard crops. This is due to the possibility of horticultural crops being destroyed by fungal diseases, rendering them unsaleable.
The environment, however, may be at ris k from the routine use of fungicides, particularly if residues linger in the soil or migrate off site and into waterways, for example, as a result of spray drift or run off (**Kooka**na *et al.,* 1998; **Wightwick and Allinson, 2007**; **Kibria *et al*, 2010; and Komarek *et al.*, 2010).** Aquatic and terrestrial ecosystems might negatively suffer effects if this happens. For instance, concerns have been voiced regarding the prolonged use of fungicides based on copper, which can lead to copper buildup in the soil **(Wightwick *et al.*, 2008; and Komarek *et al*., 2010).** Additionally, this may pose a threat to harmless soil organisms like microorganisms and earthworms. Use hence, proper usage as well as mycorrhizae and nanoparticles disposal is important from the environmental aspect.

Due to the excellent antimicrobial capabilities of nanoparticles, which reduce the risk of fungicide-resistant pathogens, research into the possible use of these substances functioning as nano-fungicides in sustainable agriculture is fast advancing.

Data in this work showed that the growth of *F. solani*, *R. solani* and *M. phaseolina*, the causal agents of sage root rot/wilt, reduced with application of all tested concentrations of ZnO- and CuO-nanoparticles. The maximum inhibition of the fungal growth was shown at the maximum nanoparticle's concentration (250 ppm) used. Also, the growth of pathogens was gradually reduced with increasing nanoparticle material concentration. Data also showed that CuONPs is more affected on pathogen's mycelial growth than ZnONPs. This result is in agreements with that obtained by several authors. **El-Argawy *et al* (2017)** found that MgO-, TiO_{2-} and ZnO-NPs reduced the radial growth and increasing the inhibition of *F. oxysporum* f. sp. *betae*, *S. rolfsii* and *R. solani* growth, the causal pathogens of sugar beet root rot and damping off, *in vitro* and the maximum effect was showed at 100 ppm, the highest concentration tested.

Due to their high electrical conductivity, electronic correlation effect, and unique physical properties, CuONPs (cupric oxide nanoparticles) are utilised extensively in a variety of fields. Recent investigations have demonstrated that CuO nanoparticles with specific antifungal activity have promising application potential in agricultural productivity. CuONPs have been shown to exhibit antifungal action (**Ren *et al.*, 2009, Delgado *et al*., 2011, Al-Johani *et al*., 2017, Arendsen *et al*., 2019 and Oussou-Azo *et al*., 2020).**

For instance, CuONPs cause tobacco to become resistant to the soil-borne *Phytophthora nicotianae* fungal disease **(Chen *et al*. 2022).** The authors found that CuONPs greatly interfered with the reproductive growth process of *P. nicotianae*, repressing hyphal growth, germination of spores and production of sporangia**.** CuONPs increase watermelon yield by preventing the growth of *F. oxysporum*, which causes Fusarium wilt **(Elmer *et al*., 2018)**. CuONPs further guards against cucumber downy mildew **(Zhiheng *et al*., 2022).** Similar to this, *Aspergillus niger* and *Mucor piriformis* are more resistant to CuONPs' antifungal effects (**Hsd *et al*., 2021)**. Recent investigations revealed that CuONPs have good application possibilities against *Magnaporthe oryzae*, the cause of rice blast disease (**Chen *et al*., 2023).** CuONPs treatment raises H_2O_2 and •OH in duckweed by 56% and 57%, respectively (**Simkhada and Thapa, 2022**). **Hou *et al*. (2017)** noticed production of hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) in the roots and leaves of *Arabidopsis* when treated with CuONPs (2-100 mg L^{-1}). Additionally, rice roots exposed to 125 mg L^{-1} CuONPs dramatically boosted their super oxide dismutase (SOD) activity, while rice leaves treated with 250 mg/L^{-1} CuONPs saw a decrease in CAT and SOD activities (**Tsuda and Katagiri, 2010**). CuONPs treatment greatly increases the basal resistance against *M. oryzae* in part because of the ROS buildup that is generated.

In this study, application of the VAM and CuONPs (at 250 ppm) treatments significantly reduced the percentages of both sage root rot/wilt incidence (DI%) and severity (DS%), 30 and 90 days after sowing (DAS). In most cases, the percentages of DI% and DS% reduction, 30 days after sowing, were lowest with CuONPs than VAM treatments. Inverse versa data were obtained at 90 DAS, where VAM treatments insignificantly decreased the DI% and DS% more than CuONPs. The maximum reduction was observed when the VAM and CuONps were integrated together. Also, Redomil plus fungicide and the integration between VAM and CuONPs were in most cases, the most effective treatments gave the lowest infection and severity percentages in comparison with the infected control. The same results were obtained by **El-Argawy *et al* (2017).** The author reported that the tested Mg O-, TiO_2- and ZnO-NPs compounds significantly decreased the developed and severity of sugar beet root rot and damping off under greenhouse conditions. According to **Gogos *et al.* (2012),** significant progress has recently been made in the field of nanomaterials for agricultural applications as innovative anti-microbial agents with better effectiveness. The direct fungistatic or fungicidal effects of a multitude of inorganic metal and metal oxide nanoparticles and carbon-based nanomaterials on phytopathogenic agents, *e.g.* CuO, ZnO, TiO_2, Ag NPs, and carbon nanotubes, are well studied (**Chen *et al*. 2016; and Shenashen *et al.* 2017).** These nanomaterials include copper oxide nanoparticles (CuONPs), which are straightforward, affordable, stable, and readily available copper compounds with a high surface area and crystal shape. CuONPs was recommended using foliar sprays or dip applications as inhibitors with effective antimicrobial effects to control root fungal and bacterial diseases **(Meghana *et al*. 2015; Devipriya and Roopan, 2017; Borgatta *et al*., 2018; Elmer *et al*., 2018; Hao *et al*. 2019).** CuONPs had potent antibacterial and antifungal effects on soilborne *Ralstonia solanacearum*, the cause of bacterial wilt, as well as *Verticillium dahliae* and *Fusarium oxysporum* infections on tomato and aubergine (eggplant) plants cultivated in diseased soil **(Elmer and White, 2016).** They reported also that CuONPs showed high fungitoxic action than the corresponding compound used in bulk. **Hao *et al.* (2017 and 2019)** demonstrated that CuNPs and CuONPs showed strong fungitoxic activity against several foliar and soil-borne plant fungal pathogens, such as *Podosphaera pannosa* and *Botrytis cinerea*, *in vitro* and in the detached tests upon foliar application, compared to treatment with TiO_2 and Fe_2O_3 in bulk. The most significant role that copper plays in plants is in activating their defence mechanisms against pathogens, insects, and abiotic stress (**Sathiyabama and Manikandan, 2018).** This result is a result of copper's engagement in plants' redox reactions, which produces cuprin, a component of defense enzymes and a participant in photosynthesis (**Sathiyabama and Manikandan 2018; Shang *et al.* 2020).** Although Cu nanotoxicity has been documented, low-dose Cu-based nanomaterials have been shown to significantly boost Cu absorption and translocation in greenhouse and field trials, as well as the growth and yield of a number of crops

(Rastogi *et al.* 2017; Toqeer *et al*. 2020; Wang *et al*. 2020).

Our results showed, also, that either VAM or CuONPs treatments affected all vegetable parameters of sage. The minimum parameters values were obtained when pathogen infected plants (treatment 1), whereas the best vegetable parameters were recorded in treatment 4 when plants were treated with VAM and CuONPs compared with the control. Many investigators reported that VAM has been observed to protect plants from pathogens relative to their non-mycorrhyzal in experimental studies. According to **Smith and Read (2008)**, VAM, this obligatory symbiotic root fungus, increase the biomass and stress tolerance of many flowering plant species. Numerous studies have shown their beneficial effects on banana growth and nutrient uptake, as well as their enhanced resistance to abiotic stresses like aluminium toxicity **(Rufyikiri *et al*., 2000)** and biotic stresses like nematodes **(Jaizme-Vega *et al*., 1997; Elsen *et al.*, 2001)** and *Fusarium oxysporum* f. sp. *cubense*, the cause of Panama disease **(Jaizme-Vega *et al*., 1998).** Many studies show that mycorrhizal colonization enhances plant resistance against pathogenic fungi. **Song *et al.* (2015)** reported that the *Alternaria solani*, the causal agent of tomato early blight disease was considerably reduced by mycorrhizal inoculation with AMF *Funneliformis mosseae*. They are primarily thought to aid in phosphorus, zinc and other minor nutrient elements uptake in plants, but they can also perform a variety of other beneficial functions such as enhanced plant protection from root pathogens **(Sikes, 2010).** Through their extra-radical mycelium (ECM), these ECM fungi contribute to the restitution of soil nutrients such as C, N, P, K, Ca, and Fe.

Previous studies showed that mycorrhiza promotes plant growth by increasing the above ground biomass and root system (**Smith and Read, 2008 and Essahibi *et al.*, 2017).** The mycorrhiza help restore soil nutrients such as C, N, P, K, Ca, Fe etc., by their extraradical mycelium (**Marschner and Dell, 1994; Ekblad *et al*., 2013)**. It is essential for recycling, and as result, for the wellbeing of the soil eco-system (**Smith and Read, 2008**). Conifer and horticultural plant roots may be stimulated by ecto-mycorrhizal symbiotic fungi, according to some studies. This is mostly caused by the indole-3-acetic acid production abilities of several fungi. Numerous investigations have reported on auxin and auxin mimics produced by various ecto-mycorrhizal fungus (**Niemi *et al*., 2002; Krause *et al*., 2015; and Vayssie`res *et al*., 2015)**. **Zouari and El Mtili (2020)** reported that soil inoculation with *Pisolithus tinctorius* supported carbo (*Ceratonia siliqua* L.) growth and rooting, it improved all tested vegetable plant parameters.

In addition to spores and hyphae in the rhizosphere, VAM also forms vesicles, arbuscules, and hyphae in roots. Plant growth is improved when a hyphal network is formed by the VAM in conjunction with plant roots, greatly enhancing the roots' access to a vast soil surface area (**Bowles *et al*., 2016**). By enhancing the availability and transport of different nutrients, VAM promote plant nutrition **(Rouphael *et al*., 2015).** VAM enhances soil quality by affecting the texture and structure of the soil, which benefits plant growth (**Zou *et al*., 2016; Thirkell *et al*., 2017).** Organic materials in soil can decompose more quickly to fungus hyphae **(Paterson *et al*., 2016**). Additionally, by enhancing the "sink effect" and moving photons from the host plants to the atmosphere, mycorrhizal fungus may impact how much ambient CO_2 is fixed by the hosts. **Song *et al.* (2015)** reported that the *Alternaria solani*, causing early blight of tomato, was considerably reduced by mycorrhizal inoculation with *Funneliformis mosseae* (AMF). The Jasmonic acid signalling pathway is crucial for mycorrhiza-primed disease resistance, and mycorrhizal colonisation boosted tomato resistance to the pathogen by boosting systemic defence response.

Following pathogen inoculation of tomato leaves, AMF pre-inoculation caused a considerable rise in the activities of phenylalanine ammonia-lyase (PAL), 1,3-glucanase, lipoxygenase (LOX) and chitinase enzymes. The majority of the genes evaluated did not have their transcripts altered by mycorrhizal inoculation alone. The three genes PR1, PR2, and PR3 that encode pathogenesis-related proteins as well as the defense-related genes, *i.e*., AOC, LOX, and PAL all responded vigorously to pathogen attack in AMF-inoculated plants. When a pathogen infection was present, AMF pre-inoculated plants' defensive responses were induced considerably more strongly and quickly than those of uninoculated plants **(Song *et al*., 2015)**.

Conclusion: According to the obtained results, using CuONPs and/or VA mycorrhiza fungi could be the best and environmentally friendly substitute for fungicides in preventing sage wilt / root rot.

Table (1): Characterization and properties of CuO- and ZnO-NPs

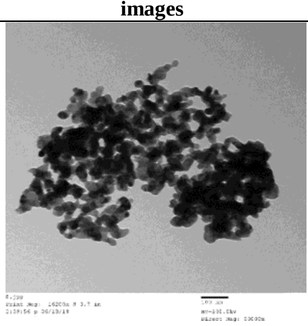
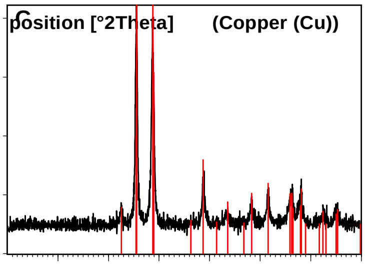
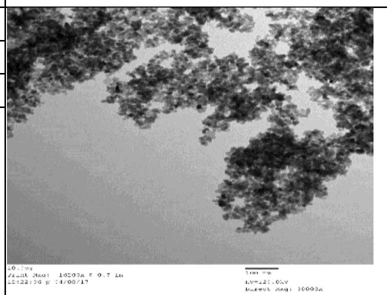
Compound	General properties, and character		images
Copper oxide Nanoparticles	Appearance Color	Dark brown to black	 **TEM images of CuO nanoparticles**
	Appearance Form	Powder	
	Molecular wt.	79.545 gm/mol	
	TEM images were performed on JEOL, JEM-2100 high resolution transmission electron microscope at an accelerating voltage of 200 KV, respectively.	The TEM image of CuO NPs revealed that the compound was produced with average size 35±5 nm and spherical like shape.	
	XRD	An XRD pattern has been performed using XPERT-PRO Powder Diffactometer system, with 2 theta (20^o – 80^o), with Minimum step size 2Theta: 0.001, and atwave length (Kα)= $1.54614^{o.}$	**XRD pattern of CuO nanoparticles** 
Zinc oxide Nanoparticles	Appearance Color	White to yellow	 TEM images of ZnO nanoparticles
	Appearance Form	Powder	
	Molecular wt.	81.408 gm/mol	
	TEM images were performed on JEOL, JEM-2100 high resolution transmission electron microscope at an accelerating voltage of 200 KV, respectively.	The TEM image of ZnO NPs revealed that the compound is in nanoscale with average size 15±3 nm and spherical like shape.	

Table (2): Effect of VAM, Nanoparticles and their combination on vegetable parameters of sage plant, during 2022 season, after 160 days.

Treatment	Fresh wt/plant (g)	Leaves No/Pl	Fresh wt. of leaves / plant (g)	Dry wt. of leaves / plant (g)	Shoot length (cm)	Root length (cm)	No. of branches	No of inflorescence
F. solani (T1)	13.08e	7.0i	2.67g	0.63i	14.2g	3.40b	3.6f	0.0i
F. solani +VAM (T2)	35.63p	82.33fg	25.57c-e	6.88b	29.1c-e	7.53a	11.7cd	4.33fg
F. solani + CuONPs (T3)	39.18d	84ef	24.47c-e	5.33de	29.3c-e	7.90a	12.6b-d	6.33d
F. solani + VAM +CuONPs(T4)	72.57a	93.67bc	50.47a	9.29a	34.56ab	9.60a	13.3bc	11.67a
F. solani + Redomil (T5)	35.0d	80fgh	24.47c-e	4.05gh	30.97cd	3.20b	8.7e	0.0i
R. solani (T1)	13.73e	8.33i	2.20g	0.41i	9.77h	3.30b	4.3f	0.0i
R. solani +VAM (T2)	37.7d	78gh	26.70cd	6.15c	28.93c-e	7.43a	11.3d	3.33gh
R. solani +CuO (T3)	37.35d	81fgh	21.62ef	4.87ef	27.8def	8.00a	11.6-d	6.66d
R. solani +VAM+CuOPs (T4)	62.9b	91cd	42.20b	9.48a	31.5bc	10.47a	15.3a	10.33b
R. solani + Redomil (T5)	34.53d	77.67gh	17.10f	3.71h	27.7ef	8.80a	11.3d	0.0i
M. phaseolina (T1)	13.39e	8.3i	3.36g	0.98i	13.5g	3.50b	3.6f	0.0i
M. phaseolina +VAM (T2)	46.3c	76.33h	22.20de	4.52fg	25.57f	8.13a	12.7b-d	3.0h
M. phaseolina +CuO (T3)	37.88d	81.3fg	23.57c-e	4.64e-g	28.17d-f	7.90a	12.6b-d	6.33d
M. phaseolina +VAM+ CuO (T4)	72.83a	101a	47.13a	9.69a	35.86a	8.60a	15.7a	8.66c
M. phaseolina + Redomil (T5)	36.2d	79.33f-h	27.20c	5.71cd	26.26ef	9.83a	11.7cd	0.0i
VAM (T6)	58.8b	97ab	26.83c	6.91b	34.9a	10.10a	14.0ab	6.0de
CuONPs (T7)	38.89d	87.6de	25.45c-e	5.97cd	29.2c-e	8.80a	13.3bc	5.0ef
Control (T8)	38.12d	83.6ef	24.07c-e	5.71cd	28.7c-f	8.36a	11.3d	0.0i
LSD 5%	5.60	4.68	4.62	2.04	3.20	3.59	1.90	1.23

Table (3): Effect of VAM, Nanoparticles and their combination on vegetable parameters of sage plant, during 2023 season, after 160 days.

Treatment	Fresh wt/plant (g)	Leaves No/Pl	Fresh wt. of leaves / plant (g)	Dry wt. of leaves / plant (g)	Shoot Length (cm)	Root length (cm)	No. of branches	No of inflorescence
F. solani (T1)	12.28h	7.67f	2.33f	0.66g	13.03g	2.73f	3.33i	0.0g
F. solani +VAM (T2)	40.83e	81.33bc	26.87c	6.23bc	32.10c	7.03d	12.67b-d	2.7f
F. solani + CuONPs (T3)	39.18ef	76.7c--e	23.63cd	6.24bc	29.70c-e	7.97cd	14.00bc	4.66d
F. solani + VAM +CuONPs(T4)	75.87a	98.33a	48.47a	8.35a	37.23b	10.7b	14.33b	11.33a
F. solani + Redomil (T5)	37.8e-g	76.67c-e	21.83de	4.94e	30.23cd	4.53ef	8.33h	0g
R. solani (T1)	12.70h	9.67f	2.97f	0.71g	8.73h	2.93f	3.67i	0g
R. solani +VAM (T2)	38.8ef	71.67de	23.90cd	6.42bc	27.93d-f	7.57cd	12.33cd	2.67f
R. solani +CuO (T3)	42.4de	70.33e	19.34e	5.22de	27.10ef	7.00d	12.33cd	3.33ef
R. solani +VAM+CuOPs (T4)	67.43b	97.67a	43.33b	9.15a	40.33a	11.3b	18.00a	9.67b
R. solani + Redomil (T5)	32.53g	72.67de	17.87e	3.38f	26.37f	7.13d	10.00f-h	0g
M. phaseolina (T1)	12.44h	9.33f	3.07f	0.88g	11.87g	3.30ef	3.33i	0g
M. phaseolina +VAM (T2)	48.40c	72.33de	23.80cd	5.57c-e	31.23c	8.23cd	11.33d-f	5.33d
M. phaseolina +CuO (T3)	42.7de	78.0b-e	25.20cd	4.98e	27.10ef	7.47d	12.00de	3.66e
M. phaseolina +VAM+ CuO (T4)	77.3ia	104.67a	40.87b	9.14a	39.20ab	14.3a	16.67a	10b
M. phaseolina + Redomil (T5)	34.23fg	73.67c-e	26.73c	6.04cd	29.47c-e	4.83e	8.67gh	0g
VAM (T6)	63.70b	85.00b	26.13c	6.94b	36.37b	9.43bc	12.33cd	7c
CuONPs (T7)	46.7cd	79.00b-d	24.00cd	6.01cd	27.20d-f	7.8cd	10.33e-g	7.33c
Control (T8)	39.0ef	75.00c-e	18.93e	5.21de	26.07f	7.37d	8.33h	0g
LSD 5%	5.28	7.77	4.14	0.85	3.07	1.88	1.70	0.90

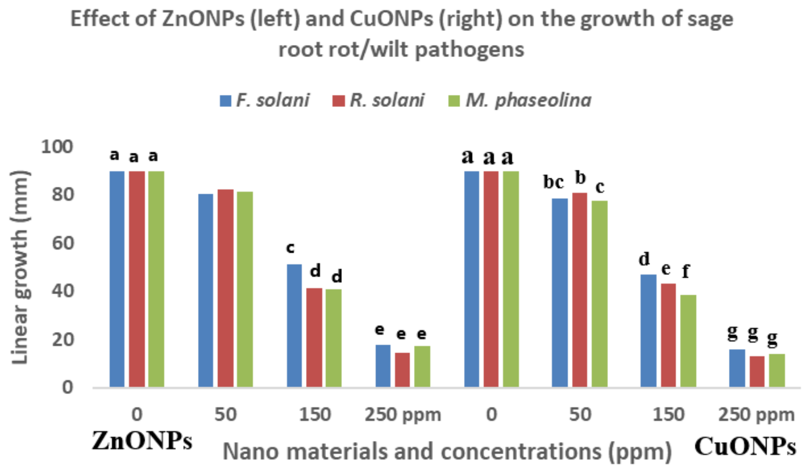


Figure (1): Efficacy of ZnO- and CuO-nanoparticles on the linear growth of sage root rot/wilt pathogens pathogenic fungi, *in vitro.*

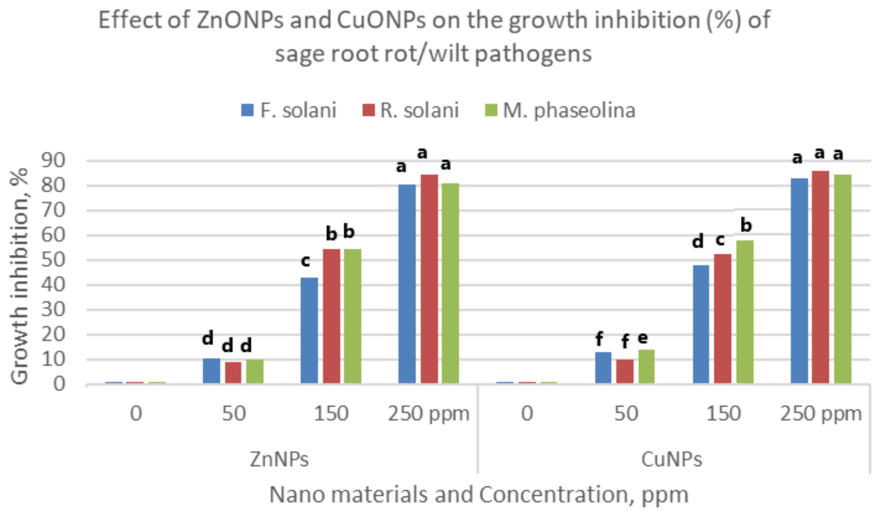


Figure (2): Efficacy of ZnO and CuO nanoparticles on the inhibition of *F solani*, *R solani* and *M. phaseolina* growth, *in vitro*.

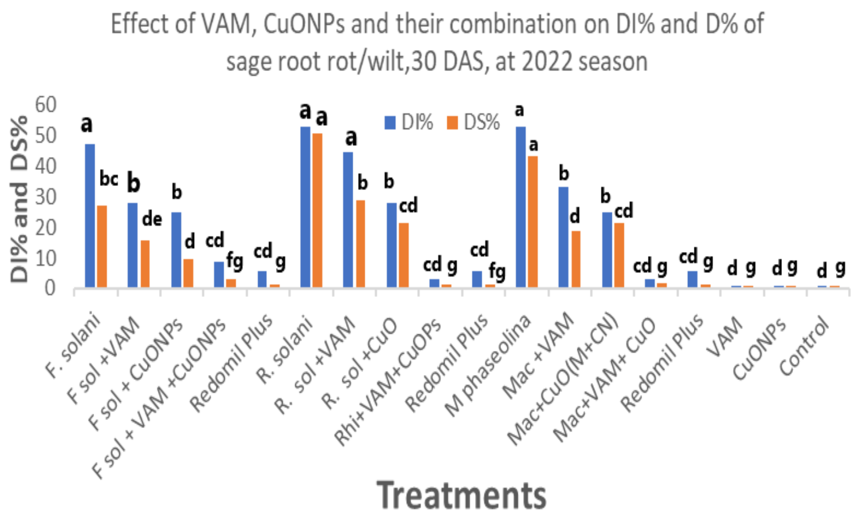


Figure (3): Efficacy of VAM, CuO NPs and their combination on severity of sage root rot/wilt disease, after 30 days, during 2022 season

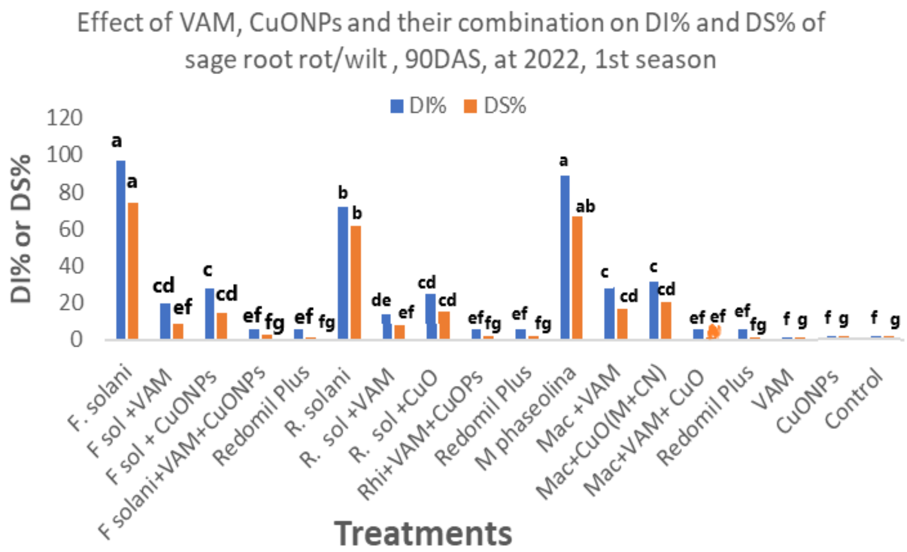


Figure (4): Efficacy of VAM, CuO NPs and their combination on severity of sage root rot/wilt disease, after 90 days, during 2022 season

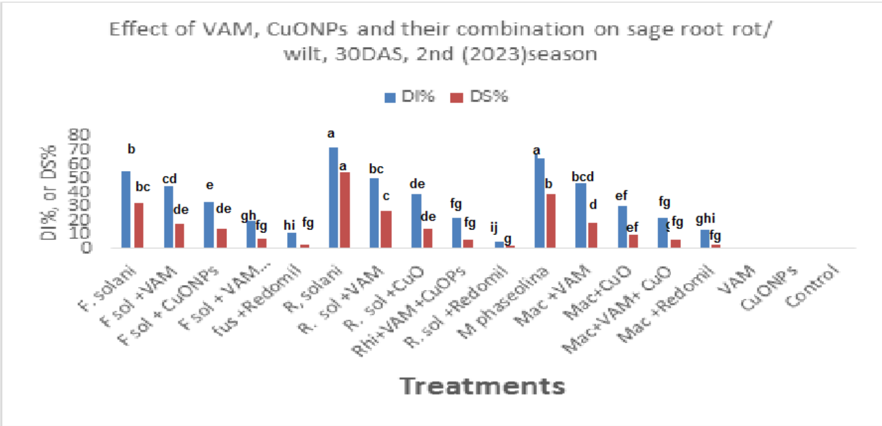


Figure (5): Efficacy of VAM, CuO NPs and their combination on severity of sage root rot/wilt disease, after 30 days, during 2023 season

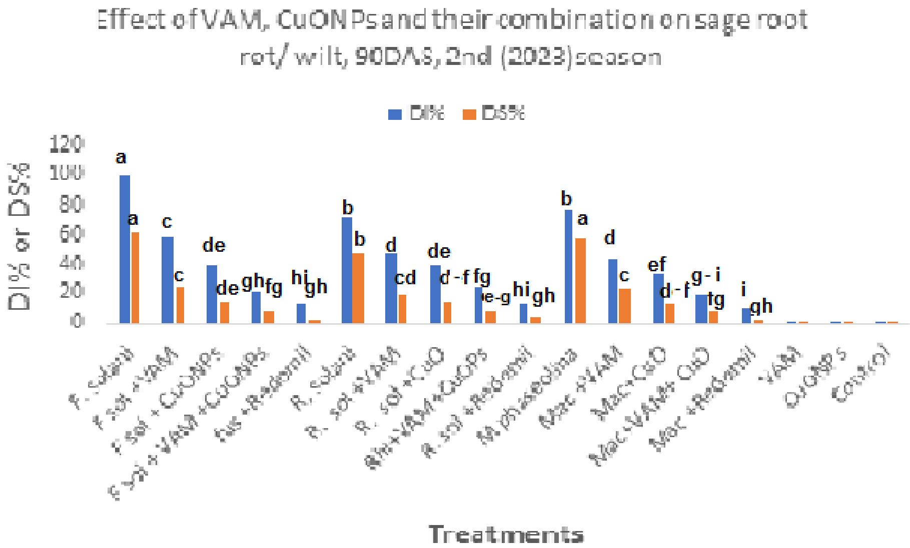


Figure (6): Efficacy of VAM, CuO NPs and their combination on severity of sage root rot / wilt disease, after 90 days, during 2023 season

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الملخص العربي

استخدام بعض المركبات النانوية والميكرهيزا داخلية التطفل لمكافحة مرض عفن الجذور والذبول فى نباتات المرمية

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تعتبر نباتات الميرمية (*Salvia officinalis* Linn.) والتي تنتمي لعائلة *Lamiaceae* واحدة من النباتات الطبية والعطرية الهامة والتي تحظى باهتمام كبير في جميع أنحاء العالم بسبب عدم استغلال امكانياتها الطبية والاقتصادية الهائلة. تتعرض نباتات الميرمية للاصابة بالعديد من الأمراض والتى من أهمها عفن الجذور والذبول المتسبب عن فطريات التربة فيوزاريوم سولاني، ريزوكتونيا سولانى وماكروفومينا فاصيولاي والتى تسبب خسائر كبيرة فى المحصول. خلال السنوات الماضية ظهرت إصابات مستمرة ومتكررة فى حقول انتاج الميرمية فى مراكز محافظة المنيا، أجريت تلك الدراسة بغرض مكافحة مرض عفن جذور وذبول نبات الميرمية مستخدما مركبات أكسيد النحاس والزنك النانوية وميكرهيزا داخلية التطفل منفردة أو متداخلة. ومن أهم النتائج المتحصل عليها تم عزل فطريات الـ *Fusarium solani, Rhizoctonia solani* and *Macrophomina phaseolina* من نباتات الميرمية المصابة والتى جمعت من محافظة المنيا وأظهرت العزلات المتحصل عليها من الفطريات شدة مرضية مختلفة. فى تجربة المعمل خفض نمو الفطريات المختبرة (*R. solani* (Rs9), *F. solani* (Fs3) and *M. phaseolina* (Mp11) عند زيادة التركيزات المختلفة بمركبي النانو لاكسيدي النحاس والزنك. وقد خفضت بشكل معنوي كل من نسبة المرض وشدته بعد الزراعة بـ 30 و90 يوم عند استعمال كل من الميكرهيزا ومركبي النانو (بتركيز 250 جزء/مليون). ووجد أن معاملة التكامل بين المركبات النانوية والميكرهيزا الداخلية التطفل كانت الأكثر فعالية من المعاملة المنفردة. بالإضافة إلي تحسين الخصائص الخضرية وزيادة معنوية لكمية المحصول الخضرى والبذور لنباتات الميرمية

كلمات افتتاحية: الميرمية، عفن الجذور والذبول للميرمية، الميكرهيزا داخلية التطفل، مركب النانو من أكسيد النحاس وأكسيد الزنك