

Efficiency of Some Isolates of Mycorrhiza Fungus to Inhibition the White Mold on Eggplant Which Caused by Sclerotinia Sclerotiorum Fungi

Muqdad Salih Al-Darraji

Department of Plant protection, Agriculture College, Tikrit University, Iraq

Email: md@tu.edu.iq

Abstract: *The experiment was carried out in the fields of the College of Agriculture, University of Tikrit, where evaluated the efficiency of five isolates of mycorrhizal fungi Gigaspore (Glomus mosseae, Glomus intaticum, Glomus fasciculatum, Scutellospora mairita) in resisting the white rot disease sclerotia on eggplant caused by the fungus Sclerotinia sclerotiorum, The isolates were activated by growing them in pots with onion roots to produce the primary inoculums, The soil containing the activated primary inoculum was taken with infected onion roots and added to the soil in pots, and the eggplant plant was planted, To study the effect of this inoculation in inhibiting the pathogen and stimulating the growth of eggplant plant, Gigaspore mairita Isolate superiority was had the highest percentage of infection with mycorrhizal fungal filaments, which was 80%, In terms of dry and fresh weight, the Glomus mosseae isolate superiority 2 grams, respectively, superiority the Glomus mosseae isolate in the 6with 23.41 and 200. reducing the percentage and severity of the infection, and it was 21.42 and 11.7%. also the same Glomus mosseae isolate was superiority in phosphorous percentage 0.49 mg/g. while the comparison treatment recorded 0.23 mg / g. In the amount of zinc, it was 82 mg/ kg⁻¹ for Glomus mosseae, while the control treatment reached 37 mg/ kg⁻¹*

Keywords: *Mycorrhiza, White Rot, Zinc, Sclerotinia, Phosphor*

1. INTRODUCTION

Eggplant *Solanum melongena* L is the economic and important vegetable crops in many countries of the world. It belongs to the Solanaceae family and includes about 90 Genus and 2000 species of plants. It is planting at the beginning of spring as a summer crop in open fields. Then its cultivation was introduced into greenhouses to cover the market's need. Eggplant has nutritional importance and It is healthy, as each 100 grams of it contains 91 water, 1.1 grams of protein, 0.2 mg fats, 0.5 mg ash, 5.5 mg carbohydrates, 0.9 mg fiber, 15 mg of calcium, 37 mg of phosphorus, and 0.4 mg of iron. 54 calories 30 IU Vitamin E 0.04 mg Vitamin B1 0.5 mg Vitamin B2 0.6 mg Vitamin Niacin 5 mg Vitamin C(1) The eggplant crop is one of the stressful crops for the soil due to the long period of its growth in open fields or inside greenhouses, as it consumes large amounts of nutrients such as nitrogen, phosphorus and potassium as well as microelements, Nutrients, which contain a group of major and minor nutrients, in addition to other compounds, play an important role in the normal growth

of the plant and in completing its life cycle, and the deficiency of these elements leads to the emergence of various symptoms and has direct effects on the growth and health of the plant yield.

The eggplant plant is affected by many pathogens, including fungi, *Cercospora*, *Botrytis*, *Pythium*, *Fusarium*, *Phoma*, *Necteria*, (Al-kassim and monwar, 2000) *Phytophthora*, *Rhizoctonia*, *Sclerotinia*, (Chupp, 2006) As these fungi cause several diseases, including the death of seedlings and vascular wilt.

In addition, eggplant was infected with white mold caused by the fungus *Sclerotinia sclerotiorum* in greenhouses to affects the vegetative system .

The pathogen It is characterized by the formation of transparent mycelium yarn, divided, branched and multi-nucleated on host plants or agricultural media, the colors change from white to dark when the melanin pigment is collected and as it forms sclerotia stone bodies during vegetative development (Kirk et al, 2001), Several means have been used to reduce the action of this pathogen, including chemical pesticides such as chagazole, rhizolex and others.(Al-shiaah,2005) However, the use of chemicals has been accompanied by the emergence of strains resistant to the effect of these pesticides as well as their risk effect on the environment resulting from this use (Montealegre et al. , 2003).

Recently, various biological agents have been used to reduce this disease and other diseases, including *Pseudomonas fluorescens*, *Bacillus* and *Trichoderma* spp. It gave encouraging results in controlling it, (jabur,2004 : Aabduljaleel, 2004: Alaashoor,2005), The term *Micorrhizae* was also used by scientists on the symbiotic relationship between certain groups of fungi and the roots of vascular plants under natural conditions, this relationship is symbiotic unsatisfactory, i.e. the host plant responds to it, improving its physiological and morphological qualities and increasing its resistance to diseases and many inappropriate or optimal factors such as freezing, drought and salinity, and this state of coexistence and symbiosis remains throughout life (Barea et al, 2011), in order produce healthy plant without disease therefore our research aims to compare and evaluate the efficiency of different isolates of mycorrhizae fungi in affecting the disease on eggplant Replacing chemical control with biological resistance for comprehensive environmental safety.

2. MATERIALS AND METHODS

1. Materials

1.1 Solutions

Formalin aceto alcohol solution

This solution was prepared by mixing 50 ml of formalin, 50 ml of acitic acid and 900 ml of ethanol and kept at a temperature of 4 until use, and this solution was used to preserve root models until the implementation of the pigmentation process, as it maintains the structures of mycorrhizic fungi without any morphological change. (virheiling et al, 1998).

Potassium hydroxide solution (10%)

Prepare the solution by dissolving 100 g of potassium hydroxide (KOH) in 900 ml of distilled water and keeping at 4 °C until use.

Acetic acid solution (5%)

The solution was prepared by adding 50 ml of 95% Acitic acid to 950 ml of distilled water and was preserved at 4 °C until (virheiling et al, 1998).

Tincture (Triplan Blue)

0.02 g of which was dyed and the roots of the eggplant appeared in the color of purple in microscopic examination.

Methods

Preparation of the Mycorrhizal vaccine

The Mycorrhiza vaccine was prepared by the trap cultures described in (stuz and Morton, 1996) We took mixed soil and it was sterilized with an Autoclave device and placed in plastic anvils and we added the spuree stuck to it for each species separately except for the control treatment Onion plant *Allium cepa* was planted as a host and grew for three months, then the roots were examined and it was confirmed microscopically and according to the percentage of infection, as ten root filaments were taken from the onion roots on a slice and the percentage of infection with mycorrhiza fungi was calculated

Preparation of the Mycorrhizal inoculum

The Micorrhizal inoculum was prepared by the trap cultures described in (stuz and Morton, 1996) We took mixed soil and it was sterilized with an Autoclave device and placed in plastic anvils and we added the spuree stuck to it for each species separately except for the control treatment Onion plant *Allium cepa* was planted as a host and grew for three months, then the roots were examined and it was confirmed microscopically and according to the percentage of infection, as ten root filaments were taken from the onion roots on a slice and the percentage of infection with mycorrhiza fungi was calculated.(Liu and Luo ,1994).

:Method of dyeing the roots to detect infection with mycorrhiza
Its secondary roots were cut into small pieces of 1 cm each, with 30 samples per pot, washed
.a second time with distilled water and kept in test tubes
We added to it a 10% potassium hydroxide solution, which was prepared (from dissolving -
10 g of potassium hydroxide KOH in 100 ml distilled water) and then placed in a water bath
The root pieces were washed at a temperature of 90 ° C for a period of time of 30-60 minutes
with running water after being extracted from the water bath
-We added to it a 10% HCl acid solution prepared from (add 10 ml of concentrated HCl acid
in 90 ml of distilled water) 2-3 minutes and then poured the acid without washing the roots,
The dye solution (Trypan blue) was added to the test tube and then placed in the water bath at
a temperature of 90 ° C for 15 times -20 minutes
We added lactic acid after extracting it from this dye and then it was examined
microscopically, The pre-prepared mycorrhiza inoculum was added according to the
prescribed method (Morton, 2000) and the inoculum was roots from onion plants infected
with mycorrhiza with soil to the predetermined treatments in the anvils intended for growing
eggplants under seeds with an area of 5 cm and a depth of 5 cm.
The severity of the injury was then calculated according to a gradient (Doodson and Dixon,
1971).

0 =i.e. there is no injury.

1=Surround the stem with mildew by less than 50% of the stem circumference.

2= Surround the stem with fungal rot by 50% to less than 100% of the stem circumference.

3= Surround the stem with fungal rot by 100% of the stem circumference.

4= Plant death.

$$\text{Severity Injury} = \frac{(\text{Number of plants in grade } 0 \times 0 + \dots + \text{number of plants grade } 4 \times 4)}{\text{Total number of plants examined} \times 4}$$

$$\text{infection rate} = \frac{\text{Number of plants infected with the pathogen}}{\text{Total number of plants}} \times 100$$

Method of digestion of plant specimens

The digestion process is done by taking 0.2 g of dried and ground vegetative total and we put it in a 50 ml conical flask and we put it 4 ml of concentrated H₂SO₄ acid related to analyzes using the buret, and left for 24 hours, the flask is placed in a sand bath, preferably the temperature does not exceed 250 m and has been gradually increased until it bursts, these flasks were cooled and 1 ml of pyrochloric acid was added to the buret and returned to the sand bath until it became clear in color and then transferred the contents of this flask to a volume flask of 50 ml, and complete the volume with water to the mark and then estimated phosphorus ions.

Phosphorus Determination Method

The concentration of phosphorus element in the plant extract was estimated using SP spectrophotometer according to the method described by the scientist (Watanabe, Olsen et al, 1982) using sodium bicarbonate (0.5) standard, The color was developed using ammonium and ascorbic acid molybdates and measuring the phosphorus concentration using the SP spectrometer at a wavelength of 840 nm.

Zinc Estimation

Measured using SHIMADZU AA-6200 atomic absorption spectrometer

3. RESULTS & DISCUSSION

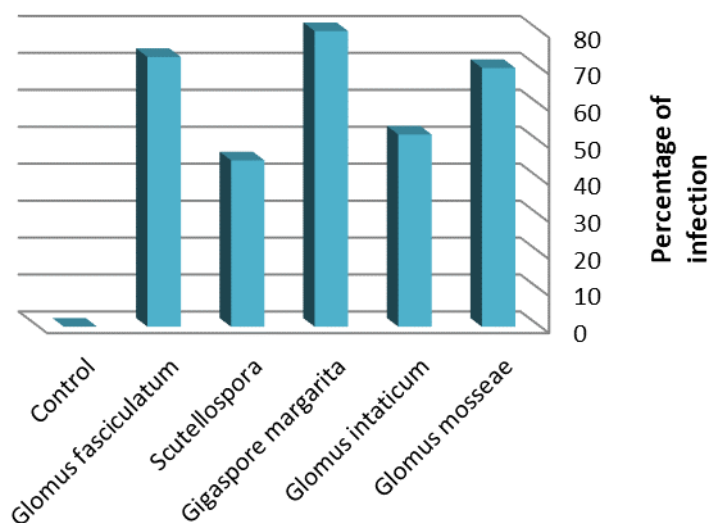


figure)1(Percentage of the infection by Mycorrhizal hyphae of eggplant plant roots

The results of the figure (1) showed that the isolation *Gigaspora margarita* was the most infected with mycorrhiza fungi for eggplant roots, as the infection rate reached 80% while it was 0% in the control treatment, and the superiority of this isolation over the rest of the isolates is attributed to genetic reasons that made the surrounding environmental conditions have a greater preference than the rest of the isolates.



Figure 2 Mycorrhiza and dendrites in eggplant plant roots

LSD	Control	<i>Glomus fasciculatum</i>	<i>Scutellospora</i>	<i>Gigaspora margarita</i>	<i>Glomus intaticum</i>	<i>Glomus mosseae</i>	Isolations
1.28	19.83	21.54	22.38	20.22	22.25	23.51	Dry plant weight
5.57	175.12	188.43	183.62	194.85	194.28	200.62	Soft plant weight

Table (1) Effect isolations Mycorrhiza in the dry and soft weight of the eggplant

The table (1) on the weight of the dry and soft plant shows the superiority of the *Glomus mosseae* treatment, as it amounted to 200.62 and 23.51, while the control treatment came at 175.12 and 19.83, respectively, and it may be likely that there will be a disparity in the readiness of the elements between the coefficients and the difference in their ability to retain water, which may result from the replacement of gypsum soil particles in place of clay and silt particles, which leads to a decrease in the surface area, as well as a decrease in the exchange capacity of those soils and a lack of organic matter as a result of climate conditions and lack of vegetation cover, all of these factors led to poor vital activity in the soil and stimulates the fungi Mycorrhiza readiness of some elements to be absorbed by the roots as well as improving the exchange surface capacity (Al-Karaki et al., 1998).

Table (2) Effect of Mycorrhiza Fungi Isolates on Percentage and Severity of Infection

LSD	Control	<i>Glomus fasciculatum</i>	<i>Scutellospora</i>	<i>Gigaspora margarita</i>	<i>Glomus intaticum</i>	<i>Glomus mosseae</i>	Isolations
3.21	85.50	31.48	29.37	22.39	25.77	21.22	Infection percentage
2.7	78.4	18.92	17.68	13.52	14.73	11.7	Infection severity

Table (2) shows that *Glomus mosseae* recorded the lowest rate of infection and a significant difference with the isolates *Glomus intaticum*, *Scutellospora* and *Glomus fasciculatum* which

came in 21.22%, 25.77%, 29.37% and 31.48% respectively, while the percentage of infection in the comparison treatment was 85.50%.

As for the severity of the injury, the same isolation recorded the lowest percentage in severity and a significant difference for the same isolates, reaching 11.7%, while the comparison treatment was 78.4%, and this disparity between isolates in the percentage of mycorrhizal filaments, the proportion and severity of the injury, may be attributed to genetic factors as well as in the amount of absorbent substances and the technique of inducing resistance (Al-Hmoud and Al-Momany, 2015).

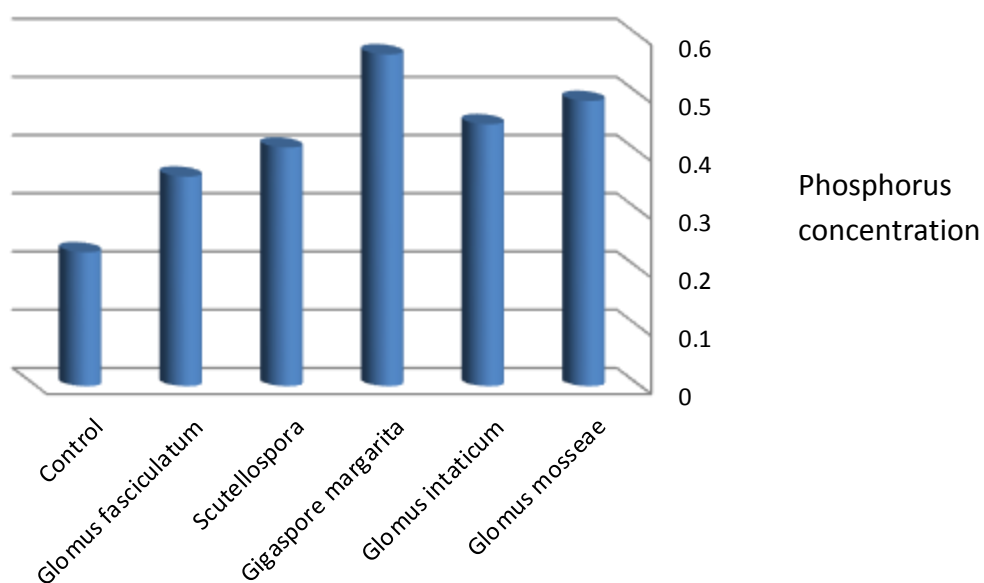


Figure 3 effect of mycorrhiza isolates on the concentration of phosphorus in the vegetative part of the eggplant plant mg/kg⁻¹

The results of scheme (3) showed the superiority of the treatment of *G. margarita* over the rest of the isolates, as it amounted to 0.49 mg / g, while the control treatment amounted to 0.23 mg / g, and this may be due to the fact that there is a direct proportion between the incidence of filaments and mycorrhizal vesicles and the amount of phosphorus in the plant (Lin and Marschner 1991).

Zinc concentration

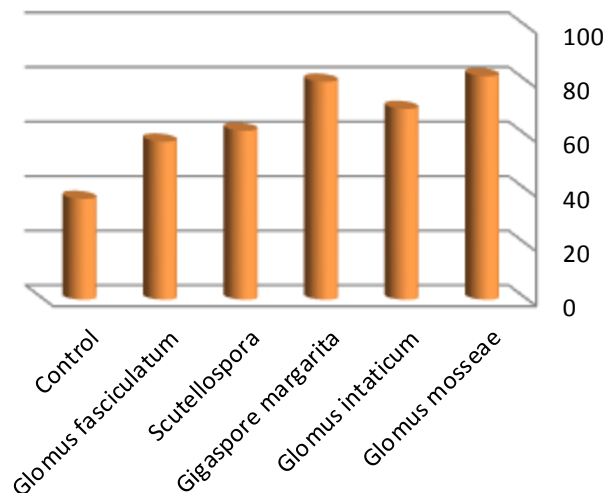


figure (4) shows the amount of zinc in the vegetative part of the eggplant plant mg/kg^{-1} figure (4) showed superiority the treatment of *Glomus mosseae* as it reached 82 mg/kg^{-1} while the control treatment reached 37 mg/kg^{-1} Zinc has an effect on the defense system and inhibition of plant diseases (Cheruiyot, 2013) and pollination with mycorrhiza fungi raises the level of plant growth and encourages it to find an effective root system in its absorption of the necessary nutrients and tolerance to inappropriate conditions and thus increase yields for many crops. (Alkurtany,2006)

Research has also shown the efficiency of these fungi in the field of biological control as they fortify infected root cells through fungal structures that are inside the cortex cells and give local immunity against fungal filaments of pathogens as the root cells gain induced immunity against infection with pathogens whatever they are (Pearson and Gianninazzi, 2000) that mycorrhiza fungi have an inhibitory effect on plant diseases, as mycorrhiza protects the plant from many pathogens by inhibiting the organisms causing it and in several ways, including: Antagonism with the pathogen, increasing the level of nutrients, acquiring plant hosts the characteristic of resistance to diseases, and making a structural and biological change in the tissues of the plant host, as well as acting as a mechanical barrier against the entry of pathogens into the host (Kehri and Chandra, 1993). It can be said that the reason for the readiness of the elements, whether phosphorus or zinc, in plant leaves is due to the extension of mycorrhizal hyphae to more distant areas, absorbing minerals, as well as increasing the surface area of the roots, as well as the role of microelements in inhibiting organisms. pathogen by contrasting with it as well as its effectiveness in improving soil construction through its secretions.

4. CONCLUSIONS

Mycorrhiza fungi have proven to be of varying efficacy against the pathogen *S. sclerotinia* Mycorrhiza fungi have a significant effect on a number of studied traits.

Recommendations

Carrying out research examining the effect of mycorrhiza fungi on the amount of eggplant production

Study the effect of this fungus on the qualitative characteristics of eggplant fruit

Study of the synergistic effect of these fungi with other organisms against pathogens

Preparing periodic publications and awareness seminars towards the use of mycorrhiza to reduce the impact of pathogens as an alternative to chemical agents harmful to the environment and humans

5. REFERENCES

- [1] Abduljaleel, Adnan.(2004). Resistance to tomato seedling rot and death caused by the fungus *Pythium aphanidermatum* using the interaction of some chemical pesticides and the biocide fluramyl. Master Thesis – Faculty of Agriculture – University of Kufa.
- [2] Al-aashoor, Ali Jabir Jasim.(2005). The possibility of producing a bio-preparation from *Bacillus cereus* bacteria to control some fungi that cause Damping off . Master Thesis - College of Science. University of Kufa. 77 pages.
- [3] Alabouvette, C.; Hoepfer H.; Lemanceau P. and Steinberg C. (1996) Soil suppressiveness to disease induced by soil borne plant pathogens. *Soil Biochemistry*, 9, 371- 413.
- [4] Al-Ansi, Kamel Abdul Ghani. (1999) . Integral resistance to *Fusarium oxysporum* p.sp *Lycopersici* disease. Master Thesis - College of Agriculture, University of Basra. 97 pages.
- [5] Al-Hmoud G. and A. Al-Momany 2015. Effect of Four Mycorrhizal Products on *Fusarium* Root Rot on Different Vegetable Crops. *J Plant Pathol Microb* 6: 255 doi:10.4172/2157-7471.1000255, 5pp
- [6] Al-Karaki, G.N., A. Al-Raddad and R.B. Clark, 1998. Water stress and mycorrhizal isolate effects on growth and nutrient acquisition of wheat. *J. Plant Nutr.*, 21: 891-902.
- [7] Alkurtany, 2006. Abdulkareem erebi Sabea. The effect of pollination of some fungi and their interaction with pollination with fungi on the growth of eggplant plants and the incidence of mycorrhiza and phosphorus absorption. *Diyala Journal of Applied Research*, 2(2) 53-65
- [8] Alrikaby, Fakher mohammed and Abduljabbar jamishaal, Vegetable production. Technical Institutes Foundation, ministry of higher aducation and scientific research. Iraq. 126.129. 1981.
- [9] Alsahhaf, Fadhil Hussein, Applied plant nutrition, Beit Alhikma, University of Baghdad, ministry of higher aducation and scientific research, Iraq.1989
- [10] Al-shilaah, Lubna Abdulmuttalib.(2005). Study the effect of some environmental factors on a number of physiological aspects of fungi, Master Thesis - College of Science. University of Babylon.
- [11] Arora NK, Khare E, Oh H, Kang SC, Maheshwari DK (2008) Diverse mechanisms adopted by *Pseudomonas* fluorescent PGC2 during the inhibition of *Rhizoctonia solani* and *Phytophthora capsici*. *World J Microbiol Biotechnol* 24: 581-585

- [12] Barea, J.M., J. Palenzuela., P. Cornejo., I. Sánchez-Castro., C. Navarro-Fernández., A. López-García., B. Estrada., R. Azcón., N. Ferrol and C. Azcón-Aguilar, 2011. Ecological and functional roles of mycorrhizas in semi-arid ecosystems of Southeast Spain. *J. Arid Environ.*, 34: 1-10. 7
- [13] Cardon Z.G and J.L. Whitbeck., 2007. The rhizosphere. Elsevier Academic Press., 235 pp
- [14] Cheruiyot, D. J., Boyd, R. S., Moar, W. J. (2013). Exploring lower limits of plant elemental defense by cobalt, copper, nickel, and zinc. *J. Chem. Ecol.* 39, 666–674. doi: 10.1007/s10886-013-0279-y
- [15] Chupp, C. (2006), *Manual of Vegetable Plant Disease*. Discovery Publishing.
- [16] Clark, D. J. Durner, D. Navarre and D. Klessig (2000). Nitric oxide inhibition of tobacco catalase and ascorbic peroxidase. *MPMI.*)13(: 1380-1384.
- [17] Digson, A,B. 1993. *Diseases of vegetable crops*. Translated by Abd al-Nabi Muhammad Abu Ghania - Saleh Mustafa al-Nuwaisri. Adar Alarabya for for Publishing & Distributio. 647 page.
- [18] Dillard, H.R. 1987. Characterization of isolates of *Rhizoctonia solani* from lima bean grown in New York state. *Phytopathology.* 77:748-751.
- [19] Jabnoun-Khiareddine H, Aydi Ben Abdallah R, El-Mohamedy RSR, Abdel-Kareem F, Gueddes-Chahed M, Hajlaoui A, et al. (2016) Comparative efficacy of potassium salts against soil-borne and air-borne fungi and their ability to suppress tomato wilt and fruit rots. *J Microb Biochem Technol* 8: 45-55.
- [20] Jabur, Sanaa Ghaaly. (2004). Evaluation of the Efficiency and Integration of Some Biological and Chemical Factors in the Control of Wheat Seedling Death Disease Caused by *Pythium Fitz. (Edson) aphanidermatu*. Kufa University. 118 p.
- [21] Kehri, H.K. and Chandra, S. (1993). Effects of Bavistin spray on soil microorganism and AVM formation in greengram in relation to its yield. *Journal of India Botanical Society* 72:55-57.
- [22] Nishikawa, J. ;Kobayashi ,T. ; Shirata,K. ;Chibana,T. and Natsuak,K.T. (2006) Seed borne fungi detected on stored solanaceous berry seeds and their biological activities . *Journal of General Plant Pathology*, 72: 305- 313.
- [23] Nishikawa, J. ;Kobayashi ,T. ; Shirata,K. ;Chibana,T. and Natsuak,K.T. Olaniyi JO, Akanbi WB, Adejumo TA, Akande OG (2010) Growth, fruit yield and nutritional quality of tomato varieties. *Afr J Food Sci* 4: 398-402
- [24] Olaniyi JO, Akanbi WB, Adejumo TA, Akande OG (2010) Growth, fruit yield and nutritional quality of tomato varieties. *Afr J Food Sci* 4: 398-402
- [25] Pearson-Gianinazzi, V. and S. Gianinazzi.(2000). Modulation of defence responses and induced resistance by mycorrhizal fungi. In: *Am in plant health and revegetation and restoration processes*. Book of abstracts. Ed. By Martins, M.A.
- [26] Ramezoni, H. (2008). Biological control of root-rot of Eggplant caused by *Macrophomina phaseolina*. *American-Eurasian Journal of Agricultural & Environmental Sciences* , 4(2):218-220.
- [27] Vierheilig H, Coughlan AP, Wyss U, Piche,Y. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology* 64, 5004–5007