



## Impact of *Funneliformis mosseae* Inoculation on the Growth and Productivity of *Gladiolus grandiflorus* L. under Different Types and Rates of Organic Manures Fertilization



Mohamed A.I. Mansour<sup>(1)#</sup>, Hany M.S. Hassan<sup>(2)</sup>, Mohamed A.I. AbdelKader<sup>(3)</sup>

<sup>(1)</sup>Department of Botany & Microbiology, Faculty of Science, Arish University, Al-Arish 45511, Egypt; <sup>(2)</sup>Plant Production Department, Faculty of Environmental Agricultural Sciences, Arish University, 45511, Egypt; <sup>(3)</sup>Horticulture Department, Faculty of Environmental Agriculture, Zagazig University, 44523, Egypt.

**G**LADIOLUS *grandiflorus* L. cv. White Prosperity corms were cultivated on field experiments during the two consecutive seasons of 2018/2019 and 2019/2020 at the Experimental Farm, Faculty of Environmental Agricultural Sciences, Arish University, Egypt to study the influence of *Funneliformis mosseae* (with and without inoculation) under different organic manures types: Chicken manure (ChM) and Farmyard manure (FYM) and rates (0.0, 10 and 20m<sup>3</sup>/feddan) on *G. grandiflorus* L. cv. White Prosperity on the vegetative growth and productivity. The obtained results showed that chicken manure addition at 20m<sup>3</sup> fed<sup>-1</sup> significantly increased gladiolus vegetative growth, flowering characters and reduced days to spike emergence and days to first floret opening during the two seasons. This treatment enhanced corm values and productivity with improvement of chemical constituent values during the two seasons. *F. mosseae* inoculation significantly increased all vegetative growth, flowering characters (except that of days to spike emergence and days to first floret opening), corms productivity and chemical constituents during both seasons. The interaction between *F. mosseae* and ChM or FYM significantly increased the formation and percentage of *F. mosseae* spore density, root colonization and structure (hyphae, vesicles and arbuscules) compared with control treatment. *F. mosseae* structures significantly increased by adding ChM at two rates (10 or 20m<sup>3</sup> fed<sup>-1</sup>) which gave the highest values at flowering stage compared with after 30 days and harvesting stage in the two seasons.

**Keywords:** Chicken manure (ChM), Farmyard manure (FYM), *Funneliformis mosseae* and *Gladiolus grandiflorus* L.

### Introduction

Gladiolus (*Gladiolus grandiflorus* L. cv. White prosperity) belongs to the Iridaceae. It is a perennial bulbous flowering plant with pure white flowers including ruffled petals. The number of florets per spike is about 13-15 and the floret size is 10-11cm. It is an excellent cormels producer which belongs to late maturity group (Vijay et al., 2018). Gladiolus occupies the second place as a cut flower next to tulip because of it is long vase life and

market value. It is a serious commercial bulbous flower crop as well as its cut flowers have a pivotal place in international markets (Singh, 2006).

Previous studies and the chemical analysis of North Sinai soil proved that the soil is sandy loam in texture. Therefore, organic fertilizers such as chicken manure and farmyard manure play an important role in improving sandy soil's physical, biological and chemical properties. They also influence the availability of nutrients to the plants.

#Corresponding author email: mmansour@sci.aru.edu.eg, ORCID identifier is 0000-0001-6840-5686

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Organic fertilizers are low-priced and eco-friendly that have tremendous prospect of supplying nutrients which can overcome the over dependence on chemical fertilizers (Sezen, 1995; Drinkwater et al., 1995).

*Arbuscular mycorrhizal* Fungi (AMF) symbiosis is the most widespread on earth and is defined as the association between the fungi of the phylum Glomeromycota and most of the terrestrial species (Schüßler et al., 2001). Eight genera include about 150 species of AMF, although the identification is mainly based on the morphological characteristics of asexual spores, molecular methods and various biochemical parameters are now utilized in systematic research (Schüßler & Walker, 2010). AMF play a vital role in the nutrition and development of plants. They also provide plants with other benefits, including increased photosynthesis, nitrogen fixation, drought tolerance, pest resistance, flower development and vase life, and improvement of soil structure (Helgason et al., 2002; Javaid, 2009; Nyoki & Ndakidemi, 2014; Keston et al., 2017; Pereira et al., 2019).

Therefore, the main objective of the present investigation is to study the influence of *Funneliformis mosseae* under different organic manures types and rates on (*Gladiolus grandiflorus* cv. White prosperity) vegetative growth and productivity.

## Materials and Methods

A field experiment was conducted at the Experimental Farm, Faculty of Environmental Agricultural Sciences, Arish University, Egypt, during two consecutive seasons 2018/2019 and 2019/2020 to study the impact of *Funneliformis mosseae* under various organic manures types and rates on *G. grandiflorus* cv. White prosperity vegetative growth and productivity.

Two sources of organic manure i.e., Chicken manure (ChM) and Farmyard manure (FYM) were obtained from Animal and Poultry Production Farm, Fac. Environ. Agric. Sci., Arish University, North Sinai, Egypt. They were stored according to standard methods for applying from season to season at rates (0, 10 or 20m<sup>3</sup> fed<sup>-1</sup>) during soil preparation. ChM contained 2.35% total nitrogen, 1.68% total phosphorus and 1.43% total potassium with 12.9 C/N ratio. While, FYM contained 1.57% total nitrogen, 1.22% total phosphorus and 0.67% total potassium with 14.6 C/N ratio.

Ordinary calcium super phosphate (15.5% P<sub>2</sub>O<sub>5</sub>) and potassium sulphate (48% K<sub>2</sub>O) were added during soil preparation and pots filled at the rates of 200 and 30kg. fed<sup>-1</sup>, respectively. *F. mosseae* was used in two treatments (Inoculation and Non-inoculation) during *G. grandiflorus* planting.

### *Gladiolus* plant materials and cultivation

The gladiolus corms (cv. White prosperity) were acquired from El-Ahaly Farm in El-Qanater El-Khayrea, Qalubia Governorate, Egypt. Corms were planted on 8<sup>th</sup> October during 2018/2019 and 2019/2020 seasons in sandy loam soil (Table 1) of the experimental unites at 50×30 cm distance between plants and rows, respectively under drip irrigation system.

### Soil, plant and evaluation of AMF inoculum

#### Soil sampling

Soil samples were collected from plant rhizosphere at 25-50cm depth of seven mature plants (*Aloe vera*, *Coriandrum sativum*, *Cynara scolymus*, *Helianthus annuus*, *Ocimum basilicum*, *Rosmarinus officinalis* and *Salvia officinalis*) grown in North Sinai Governorate, Egypt. Soil samples were collected at one Kg of soil and kept in labeled plastic bags at 4°C until processing.

#### Spore extraction, purification and identification

TABLE 1. Physical and chemical properties of experimental soil

			Physical analysis				Soil texture				
Clay (%)		Silt (%)	Fine sand (%)		Coarse sand (%)	sandy loam					
9.3		12.8	19.9		58.0						
Chemical analysis											
pH	E.C. (dSm <sup>1</sup> )	Organic matter (%)	Soluble cations (meq./L)			Soluble anions (meq./L)			Available (ppm)		
			Mg <sup>++</sup>	Ca <sup>++</sup>	Na <sup>+</sup>	Cl <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>	N	P	K
8.09	1.01	0.164	3.63	3.90	2.54	4.45	4.20	1.42	12	24	0.32

According to the wet sieving followed by floatation centrifugation in 50% sucrose procedure described by Gerdemann & Nicolson (1963) and Oehl et al. (2003). AMF spore extraction was performed on 100g of sampled soil. The number of spores was expressed as the mean of three replicates and spore morphology was studied under a stereoscopic microscope at 100– 400x magnification. According to Blaszkowski (2012) who reported that the isolated spores were mounted in polyvinyl lactoglycerol (PVLG) and identified spores using according to the size, color, wall thickness and opposing hyphae of the spores until the species level and the original description and identification code of the species by Melo et al. (2020) and INVAM (2021).

#### *Root colonization of AMF*

To remove soil debris of the mentioned plants, the roots were washed under water and cleared with 10% KOH for 30- 45 minutes at 90°C and acidified in 1% HCl for 5-10min. The roots were stained with trypan blue (0.05% lacto-glycerol solution) for 10min. The roots were cut into 10-15mm pieces in order to obtain a better staining effect and calculate the percentage of root colonization according to Trouvelot & Kought (1986) as updated by Dalpé & Seguin (2013). The percentage occurrence of various AMF structures *viz.*, hyphae, vesicles and arbuscules were recorded based on their presence and absence. For percentage colonization of these structures, each of the 100 root pieces was observed under  $\times 10$  power of a compound microscope according to the following equation:

$$\text{Percentage root colonization} = \left[ \frac{\text{No. of infected segments}}{\text{Total no. of segments examined}} \right] \times 100$$

*F. mosseae* was selected for further study because of its universal presence in all soil samples.

#### *Multiplication of Funneliformis mosseae inoculum*

In order to increase the regeneration of *F. mosseae*, onion (*Allium cepa* L.) is well-known as a trap plant for a period of 4 months to increase the spore density and root colonization. Before use, soil was sterilized for 60min at 121°C to eliminate indigenous AMF and other microorganisms. Spores were surface sterilized with Chloramine-T (2%) & antibiotics as described by Horii & Ishii (2006). Spores were then injected in autoclaved sandy soil which were used to inoculate onion plants and grown in a greenhouse under natural conditions.

The large number of settled fibrous roots of onion are also cut into small pieces and thoroughly mixed with the relevant rhizosphere soil (including roots such as hyphae, vesicles, arbuscules and spores). According to Menge & Timmer (1982) spores and large number of colonized roots with *F. mosseae* were transferred to the experimental field and mixed into gladiolus plant soil to determine the effects of AMF, 500g air-dried inoculum of *F. mosseae* was mixed into each pot containing culture mycorrhizal plants with an equal amount of sterilized soil as a control.

#### *Data recorded*

##### *Growth parameters*

Two months and a half after planting the gladiolus corms, the following data were recorded: plant height (cm), number of leaves as well as fresh and dry weights of leaves (g).

##### *Flowering parameters*

One month later from vegetative parameters, the following data were tabulated: spike length (cm), number of florets per spike, days to spike emergence and days to first floret opening.

##### *Corms and cormels parameters*

After two months from flowers harvest the following data were recorded: number of cormels, new corm fresh weight (g), fresh weight of cormels (g) and corm diameter (cm).

##### *Chemical constituents of G. grandiflorus*

The oven dried materials of leaves were ground and wet digested by a sulfuric- perchloric acids then total N, P and K % were determined according to the methods of Black (1965), Jackson (1967) and A.O.A.C. (1975).

##### *Funneliformis mosseae parameters*

Spore density/100g dry soil, total root colonization (%) and mycorrhizal structures % after 30 days from gladiolus cultivation, at flowering stage and at harvest stage were determined.

#### *Experimental design and statistical analysis*

The statistical layout of this experiment was a split plot in Completely Randomized Block Design (CRBD) with three replicates. Two organic manures (Chicken manure and farmyard manure) were randomly distributed in the main plots with three rates of each (0, 10 and 20m<sup>3</sup> fed<sup>-1</sup>). The sub plots were entitled to two *F. mosseae* inoculation treatments (inoculated and non-inoculated). All

collected data were analyzed with analysis of variance (ANOVA) producer using program of Statistic version 9 (Analytical Software, 2008). Means differences were compared by using Duncan's multiple range test at 0.05 level (Duncan, 1955).

## Results and Discussions

### *Impact of F. mosseae, ChM and FYM rates, and their interaction on G. grandiflorus vegetative growth*

The illustrated data in Table 2 show that chicken manure addition at 20 m<sup>3</sup> fed<sup>-1</sup> significantly increased gladiolus vegetative growth characters i.e. [plant height (cm), leaf number/plant, leaves

fresh weight (g) and leaves dry weight (g) during both seasons (86.50, 87.67; 8.86, 9.03; 39.50, 40.50 and 8.15, 8.29, respectively].

Regarding the influence of *F. mosseae* inoculation, data concluded that *F. mosseae* inoculation significantly increased all vegetative growth except leaf number/ plant during the two seasons. Concerning the effect of *F. mosseae* inoculation and ChM and FYM, as shown in Table 2 data clear that *F. mosseae* inoculation combined with addition rate of ChM at 20m<sup>3</sup> fed<sup>-1</sup> enhanced vegetative growth and recorded the maximum previous growth parameters during the two seasons (89.33, 90.67; 9.40, 9.56; 40.67, 41.67 and 8.39, 8.53, respectively).

**TABLE 2. Impact of *F. mosseae*, ChM and FYM rates, and their interactions on *Gladiolus grandiflorus* vegetative growth during 2018/2019 and 2019/2020 seasons**

Organic fertilization types and rates (m <sup>3</sup> /fed <sup>-1</sup> )	<i>F. mosseae</i> inoculation					
	Non-inoculation	Inoculation	Mean (O)	Non-inoculation	Inoculation	Mean (O)
	First season (2018-2019)			Second season (2019-2020)		
<b>Plant height (cm)</b>						
Control	64.67d	70.67cd	67.50 d	65.66 d	71.00 cd	68.58 d
10 ChM	69.67 c	73.67 c	71.67 c	71.33 c	74.67 c	73.00 c
20 ChM	83.67 ab	89.33 a	86.50 a	84.67 ab	90.67 a	87.67 a
10 FYM	67.00 cd	72.33 c	69.66 cd	69.00 cd	74.33 c	71.66 c
20 FYM	79.66 b	82.33 b	81.00 b	81.67 b	84.00 b	82.83 b
Mean (M)	72.93 b	77.66 a		74.46 b	78.93 a	
<b>Leaf number/ plant</b>						
Control	7.11 e	7.50 de	7.30 b	7.44 e	8.00 c-e	7.72 b
10 ChM	7.67 de	7.96 b-d	7.81 ab	8.00 c-e	8.33 bc	8.16 ab
20 ChM	8.33 bc	9.40 a	8.86 a	8.50 bc	9.56 a	9.03 a
10 FYM	7.40 de	7.83 cd	7.61 b	7.67 de	8.16 cd	7.91 b
20 FYM	7.83 cd	8.50 b	8.16 ab	8.00 c-e	8.83 b	8.41 ab
Mean (M)	7.66 a	8.23 a		7.92 a	8.57 a	
<b>Leaves fresh weight (g)</b>						
Control	22.67 f	30.00 e	26.33 e	23.33 e	30.66 d	27.00 d
10 ChM	31.67 de	35.67 bc	33.67 c	32.67 cd	36.67 bc	34.67 c
20 ChM	38.33 ab	40.67 a	39.50 a	39.33 ab	41.67 a	40.50 a
10 FYM	29.67 e	33.67 cd	31.67 d	31.00 d	34.67 b-d	32.83 c
20 FYM	35.67 bc	37.67ab	36.67 b	36.33 bc	38.67 ab	37.500 b
Mean (M)	31.60 b	35.53 a		32.53 b	36.46 a	
<b>Leaf dry weight (g)</b>						
Control	4.68 f	6.56 de	5.62 e	4.78 f	6.67 de	5.72 d
10 ChM	6.53de	7.36 bc	6.95 c	6.69 de	7.51 b-d	7.10 c
20 ChM	7.91 ab	8.39 a	8.15 a	8.06 ab	8.53 a	8.29 a
10 FYM	6.12 e	6.95 cd	6.53 d	6.35 e	7.10 c-e	6.72 c
20 FYM	7.36 bc	7.77 b	7.56 b	7.44 b-d	7.92 a-c	7.68 b
Mean (M)	6.52 b	7.41 a		6.66 b	7.55 a	

Means having the same letter (s) are not significantly different according to Duncan's multiple range test at 5% level of probability

In this regard, the obtained results agree with those found by Detpiratmongkol et al. (2014) on *Andrographis paniculata* Nees plants who observed that the maximum leaf area index, total dry weight and dry weight yield were obtained by chicken manure addition. Similarly, the results of Khan et al. (2017) on cucumber cultivars revealed that addition of poultry manure at 20ton ha<sup>-1</sup> recorded the highest number of branches/plant and numbers of leaves/plant.

Concerning the effect of inoculation of AMF on *Zelkova serrata* (Thunb) Makino, it was proposed by Wang et al. (2019) that plant growth and photosynthesis under normal conditions are

affected by AMF strains. The same trend was noticed by Khatun (2020) on *Coleus* plants and Juntahum et al. (2020) on sugarcane plants.

*Impact of F. mosseae, ChM and FYM rates, and their interaction on G. grandiflorus flowering parameters*

Data in Table 3 reveals that flowering parameters are significantly impacted by ChM addition rate. Results showed that ChM at 20m<sup>3</sup> fed<sup>-1</sup> increase flowering characters (spike length and number of flowers per spike) as well as reducing days to spike emergence and days to first floret opening (day) during the two seasons.

**TABLE 3. Impact of *F. mosseae*, ChM and FYM rates, and their interaction on *G. grandiflorus* flowering during 2018/2019 and 2019/2020 seasons**

Organic fertilization types and rates (m <sup>3</sup> fed <sup>-1</sup> )	<i>F. mosseae</i> inoculation					
	Non-inoculation		Mean (O)	Non-inoculation		Mean (O)
	Inoculation			Inoculation		
	First season (2018-2019)			Second season (2019-2020)		
<b>Spike length (cm)</b>						
Control	47.67 e	50.67 de	49.17 d	49.00 e	52.00 de	50.50 d
10 ChM	58.33 c	61.67 bc	60.00 c	59.33 c	63.33 bc	61.33 bc
20 ChM	62.67 bc	71.00 a	66.83 a	63.67 bc	72.67 a	68.17 a
10 FYM	56.33 cd	59.00 bc	57.66 c	57.33 cd	60.33 bc	58.83 c
20 FYM	61.00 bc	65.67 ab	63.33 b	62.33 bc	66.67 ab	64.50 ab
Mean (M)	57.20 b	61.60 a		58.33 b	63.00 a	
<b>Number of florets per spike</b>						
Control	7.33 d	8.33 cd	7.83 c	8.00 d	9.33 cd	8.66 b
10 ChM	8.33 cd	9.67 a-c	9.00 b	9.33 cd	10.67 a-c	10.00 a
20 ChM	9.67 a-c	11.67 a	10.67 a	10.33 a-c	12.00 a	11.16 a
10 FYM	8.33 cd	9.67 a-c	9.00 b	9.33 cd	11.00 a-c	10.16 a
20 FYM	9.33 b-d	11.33 ab	10.33 a	10.00 b-d	12.00 ab	11.00 a
Mean (M)	8.60 b	10.13 a		9.40 b	11.00 a	
<b>Days to spike emergence (day)</b>						
Control	87.67 a	85.67 ab	86.67 a	86.67 a	84.67 ab	85.67 a
10 ChM	82.67 b-d	79.33 de	81.00 b	81.33 b-d	78.00 de	79.66 b
20 ChM	80.67 cd	75.00 e	77.83 c	79.33 cd	74.00 e	76.66 c
10 FYM	86.00 ab	83.00 b-d	84.50 a	85.00 ab	81.67 b-d	83.33 a
20 FYM	84.00 a-c	79.00 de	81.50 b	82.67 a-c	78.00 de	80.33 b
Mean (M)	84.20 a	80.40 b		83.00 a	79.26 b	
<b>Days to first floret opening (day)</b>						
Control	106.67 a	95.67 b	101.17 a	96.67 a	94.33 a-c	95.50 a
10 ChM	92.67 bc	89.67 c	91.17 c	90.67 cd	88.00 d	89.33 c
20 ChM	90.33 c	84.33 d	87.33 d	89.00 d	83.33 e	86.16 d
10 FYM	96.33 b	93.00 bc	94.66 b	94.67 ab	91.33 b-d	93.00 b
20 FYM	93.67 bc	88.67 cd	91.17 c	92.00 b-d	88.00 d	90.00 c
Mean (M)	95.93 a	90.26 b		92.60 a	89.00 b	

Means having the same letter (s) are not significantly different according to Duncan's multiple range test at 5% level of probability

In connection with the impact of *F. mosseae* inoculation, data pointed out that spike length (cm) and number of flowers per spike were increased (61.60, 63.00 and 10.13, 11.00) by *F. mosseae* inoculation during the first and second seasons, respectively, while the same treatments resulted in a significant decrease in the days to spike emergence and days to first floret opening (80.40, 79.26 and 90.26, 89.00 days) during both seasons, respectively.

Concerning the interaction effect between *F. mosseae* inoculation and ChM and FYM, data in the same Table show that both spike length (cm) and number of flowers per spike recorded a significant difference by *F. mosseae* inoculation with addition rate of ChM at 20m<sup>3</sup> fed<sup>-1</sup> during the two seasons and the minimum days for spike emergence (day) and days to first floret opening (day) were decrease during both seasons.

The results were in line as obtained by Preetham et al. (2019) on (*Polyanthus tuberosa*) var. Shringar where they found that, total number of florets per spike improved by treatment poultry manure at (0.5kg/ m<sup>2</sup>). For the addition of AMF Liu et al. (2018) on *Medicago truncatula* Gaertn they reported that, the total flowers were significantly influenced by AMF treatment.

*Impact of F. mosseae, ChM and FYM rates, and their interaction on G. grandiflorus corms and cormels productivity*

As shown in Table 4 data suggest that corms values were significantly impacted by ChM addition rate. Results stated that, number of cormels per plant, corms fresh weight (g), cormels fresh weight (g) and corms diameter (cm) recorded the maximum values by ChM fertilization at 20 m<sup>3</sup> fed<sup>-1</sup> during the two seasons (17.66, 18.83; 38.00, 39.83; 15.60, 14.31 and 4.11, 4.25, respectively).

**TABLE 4. Impact of *F. mosseae*, ChM and FYM rates, and their interactions on *G. grandiflorus* corms and cormels productivity during 2018/2019 and 2019/2020 seasons**

Organic fertilization types and rates (m <sup>3</sup> fed <sup>-1</sup> )	<i>F. mosseae</i> inoculation		Mean (O)	<i>F. mosseae</i> inoculation		Mean (O)
	Non-inoculation	Inoculation		Non-inoculation	Inoculation	
	First season (2018-2019)			Second season (2019-2020)		
<b>Number of cormels per plant</b>						
Control	8.26 e	9.67 de	8.96 d	8.67 e	11.00 de	9.83 d
10 ChM	11.33 c-e	17.00 b	14.16 b	12.33 c-e	18.33 b	15.33 bc
20 ChM	15.00 bc	20.33 a	17.66 a	16.00 bc	21.67 a	18.83 a
10 FYM	9.33 de	15.67 bc	12.50 c	10.67 de	17.00 bc	13.83 c
20 FYM	12.33 b-d	16.67 b	14.50 b	13.67 b- d	18.33 b	16.00 b
Mean (M)	11.25 b	15.86 a		12.26 b	17.26 a	
<b>Corms fresh weight (g)</b>						
Control	25.16 f	29.33 de	27.25 d	26.67f	31.00 d-f	28.83 d
10 ChM	29.00 d-f	36.67 b	32.83 b	30.33 d-f	38.67 b	34.50 b
20 ChM	32.67 cd	43.33 a	38.00 a	34.33 b-d	45.33 a	39.83 a
10 FYM	26.33 ef	32.33 d	29.33 c	27.67 ef	35.00 b-d	31.33 c
20 FYM	30.33 d	36.33 bc	33.33 b	32.00 c-e	37.67 bc	34.83 b
Mean (M)	28.70 b	35.59 a		30.20 b	37.53 a	
<b>Cormels fresh weight (g)</b>						
Control	7.67 f	9.66 ef	8.66 d	8.67 f	11.00 ef	9.83 b
10 ChM	11.00 c-e	12.67 b-d	11.83 c	12.33 c-e	13.67 b-d	13.00 ab
20 ChM	13.33 bc	17.00 a	15.16 a	10.63 ef	18.00 a	14.31 a
10 FYM	10.33 d-f	13.67 bc	12.00 c	11.33 d-f	14.67 bc	13.00 ab
20 FYM	11.67 c-e	15.33 ab	13.50 b	13.00 c-e	16.33 ab	14.66 a
Mean (M)	10.80 b	13.66 a		11.19 b	14.73 a	
<b>Corms diameter (cm)</b>						
Control	2.96 e	3.16 de	3.06 d	3.06 e	3.33 de	3.20 c
10 ChM	3.60 b-d	3.73 bc	3.66 bc	3.70 b-d	3.86 bc	3.78 b
20 ChM	3.90 ab	4.33 a	4.11 a	3.96 ab	4.53 a	4.25 a
10 FYM	3.40 c-e	3.70 bc	3.55 c	3.53 c-e	3.90 bc	3.71 b
20 FYM	3.60 b-d	3.96 ab	3.78 b	3.67 b-d	4.10 ab	3.88 b
Mean (M)	3.49 b	3.78 a		3.58 b	3.94 a	

Means having the same letter (s) are not significantly different according to Duncan's multiple range test at 5% level of probability

Inoculation with *F. mosseae* significantly increased all corms and cormels productivity data during the first and second season (15.86, 17.26; 35.59, 37.53; 13.66, 14.73 and 3.78, 3.94, respectively). The impact of interaction between *F. mosseae* inoculation and ChM & FYM confirm that *F. mosseae* inoculation coupled with the addition of ChM at 20 m<sup>3</sup> fed<sup>-1</sup> attained the highest values in all corms production values during the two seasons (20.33, 21.67; 43.33, 45.33; 17.00, 18.00 and 4.33, 4.53, respectively). The obtained results are in a harmony with those found by Srivastava et al. (2014) on tuberoso who found that fertilization with poultry manure at 0.5kg/ m<sup>2</sup> is the best treatment for increasing diameter and weight of bulb and bulbs number/m<sup>2</sup>. Furthermore, Ronsheim (2012) on *Allium vineale* reported that more bulbils and larger offsets were obtained by mycorrhizal-inoculated plants compared with un-inoculated plants. Also, Juntahum et al. (2020) observed that initial AMF colonization after 4 months was significantly correlated with soil properties, biomass and productivity in sugarcane plant.

*Impact of F. mosseae, ChM and FYM rates, and their interaction on G. grandiflorus chemical constituents*

Data in Table 5 indicate that chemical constituents, i.e., total N (%), total P (%) and total K (%) increased significantly by ChM fertilization at 20m<sup>3</sup> fed<sup>-1</sup> during the two seasons (2.00, 2.16; 0.281, 0.295 and 2.20, 2.40, respectively). As regarded in the same table, data present that, *F. mosseae* inoculation significantly increased all the previous parameters during the first and second season (1.92, 2.14; 0.260, 0.278 and 2.00, 2.30, respectively).

Regarding the interaction effect between *F. mosseae* inoculation and ChM & FYM, data in the same table confirm that *F. mosseae* inoculation with addition rate of ChM at 20 m<sup>3</sup> fed<sup>-1</sup> recorded the maximum values in chemical constituents values during the two seasons (2.50, 2.66; 0.290, 0.310 and 2.48, 2.71, respectively). Similar results were obtained by Lin et al. (2016) they found that, poultry litter influenced plant nutrient uptake with slightly negative effect for N uptake, while, significant positive impacts for P and K.

**TABLE 5. Impact of *F. mosseae*, ChM and FYM rates, and their interactions on *G. grandiflorus* chemical constituents during 2018/2019 and 2019/2020 seasons**

Organic fertilization types and rates (m <sup>3</sup> fed <sup>-1</sup> )	<i>F. mosseae</i> inoculation					
	Non-inoculation		Mean (O)	Inoculation		Mean (O)
	First season (2018-2019)			Second season (2019-2020)		
<b>Total nitrogen (%)</b>						
Control	0.93 h	1.19 g	1.06 e	1.10 f	1.45 d	1.28 c
10 ChM	1.32 f	1.81 c	1.56 c	1.43 d	1.95 b	1.69 b
20 ChM	1.51 e	2.50 a	2.00 a	1.65 c	2.66 a	2.16 a
10 FYM	1.18 g	1.71 d	1.44 d	1.26 e	2.03 b	1.65 b
20 FYM	1.33 f	2.38 b	1.86 b	1.51 d	2.61 ab	2.06 a
Mean (M)	1.25 b	1.92 a		1.39 b	2.14 a	
<b>Total phosphorus (%)</b>						
Control	0.207 f	0.235 de	0.221 d	0.217 f	0.253 d	0.235 c
10 ChM	0.230 e	0.259 bc	0.244 bc	0.236 e	0.276 bc	0.256 b
20 ChM	0.273 b	0.290 a	0.281 a	0.280 bc	0.310 a	0.295 a
10 FYM	0.210 f	0.250 cd	0.230 cd	0.226 ef	0.266 cd	0.246 bc
20 FYM	0.247 c-e	0.266 bc	0.257 b	0.270 b-d	0.286 b	0.278 a
Mean (M)	0.233 b	0.260 a		0.246 b	0.278 a	
<b>Potassium (%)</b>						
Control	1.33 e	1.63 d	1.48 d	1.53 e	1.84 de	1.68 d
10 ChM	1.60 d	1.85 c	1.72 c	1.75 de	2.31 bc	2.03 bc
20 ChM	1.92 c	2.48 a	2.20 a	2.10 b-d	2.71 a	2.40 a
10 FYM	1.60 d	1.88 c	1.74 c	1.66 e	2.14 b-d	1.90 c
20 FYM	1.80 c	2.16 b	1.98 b	1.88c- e	2.50 ab	2.19 b
Mean (M)	1.65 b	2.00 a		1.78 b	2.30 a	

Means having the same letter (s) are not significantly different according to Duncan's multiple range test at 5% level of probability

Regarding the impact of AMF inoculation, Abu-Elsaoud et al. (2017) found that compared with non-mycorrhizal plants (NM), mycorrhizal wheat has a higher content of P, K, Ca, S and Fe in the buds. However, in the root and shoot system of mycorrhizal wheat, the content of copper and zinc is low. Also, Nafady et al. (2018) stated that AMF symbiosis can provide mineral nutrients for faba bean plant growth and improved the structure of the soil. Moreover, Jangra et al. (2019) on *Ocimum basilicum* L. demonstrated that the co-inoculation of *Pseudomonas fluorescens* with AMF increased nutrient absorption especially phosphorus. Also, Khatun (2020) indicated that the P content of leaf tissue remains higher in AMF *Coleus* plants as compared to non-mycorrhizal ones and there was an increase in the amount of phosphorus with increase in age of the plant. Furthermore, Juntahum et al. (2020) found that both AMF inoculation alone and combined with 50% P fertilization increased plant nutrient uptake of NPK as compared with the

control in sugarcane plant.

*Distribution, species of AMF, spore density, root colonization % and AMF structures in seven plants*

This study shows AMF diversity and root colonization rates of some medicinal plants from different plants grown in North Sinai Governorate, Egypt. AMF have been found in soil rhizospheres and invade the roots of seven mature plants (*Aloe vera*, *Coriandrum sativum*, *Cynara scolymus*, *Helianthus annuus*, *Ocimum basilicum*, *Rosmarinus officinalis* and *Salvia officinalis*).

As shown in Table 6, eight species belonging to five genera of AMF were detected by morphological spore identification: *Acaulospora bireticulata*, *Acaulospora laevis*, *Funneliformis caledonium*, *Funneliformis mosseae*, *Gigaspora margarita*, *Glomus etunicatum*, *Rhizophagus clarus* and *Rhizophagus intraradices*.

**TABLE 6. Distribution, diversity, isolation, species of AMF, spore density, root colonization (%) and AMF structures in seven plants during 2018-2019 and 2019-2020 seasons**

Plant species	Family	Species of AMF	Spore density (in 100g dry soil)	Root colonization (%)	AMF structures		
					Hyphae	Vesicles	Arbuscules
Aloe ( <i>Aloe vera</i> L.)	<i>Asphodelaceae</i>	<i>A. laevis</i>	55.0	44.0	+	+	+
		<i>F. mosseae</i>			+	+	+
		<i>G. margarita</i>			+	+	+
		<i>R. clarus</i>			+	+	+
		<i>F. mosseae</i>			+	+	+
Basil ( <i>Ocimum basilicum</i> L.)	<i>Lamiaceae</i>	<i>G. etunicatum</i> ,	70.0	55.0	+	+	+
		<i>R. clarus</i>			+	+	+
		<i>A. bireticulata</i>			+	+	+
		<i>F. mosseae</i>			+	+	+
Common sage ( <i>Salvia officinalis</i> L.)	<i>Lamiaceae</i>	<i>F. mosseae</i>	61.0	52.0	+	+	+
		<i>F. caledonium</i>			+	+	+
		<i>G. margarita</i>			+	+	+
		<i>F. mosseae</i>			+	+	+
		<i>G. etunicatum</i>			+	+	+
Coriander ( <i>Coriandrum sativum</i> L.)	<i>Apiaceae</i>	<i>G. margarita</i>	48.0	36.0	+	+	+
		<i>R. intraradices</i>			+	+	+
		<i>A. laevis</i>			+	+	+
Globe Artichoke ( <i>Cynara scolymus</i> L.)	<i>Asteraceae</i>	<i>F. mosseae</i>	50.0	40.0	+	+	+
		<i>R. clarus</i>			+	+	+
		<i>A. bireticulata</i>			+	+	+
		<i>F. caledonium</i>			+	+	+
		<i>F. mosseae</i>			+	+	+
Rosemary ( <i>Rosmarinus officinalis</i> L.)	<i>Lamiaceae</i>	<i>R. clarus</i>	78.0	57.0	+	+	+
		<i>R. intraradices</i>			+	+	+
		<i>A. laevis</i>			+	+	+
		<i>F. caledonium</i>			+	+	+
Sunflower ( <i>Helianthus annuus</i> L.)	<i>Asteraceae</i>	<i>F. mosseae</i>	57.0	47.0	+	+	+
		<i>G. margarita</i>			+	+	+
					+	+	+
Average			59.86	47.29	+	+	+



The genus *Funneliformis* was the most abundant and was found in all sites followed *Rhizophagus*, *Acaulospora*, *Gigaspora*, and *Glomus*. Moreover, *Funneliformis mosseae* was the dominant mycorrhizal species in all seven plants. AMF spores have been identified with the help of keys suggested by Melo et al. (2020), INVAM (2021) which reported that that genus *Funneliformis mosseae* Synonym: *Glomus mosseae*. Our findings agree with many authors who found that genus *Funneliformis* is a predominantly distributed genus in the soil all over the world (Shokr, 2006; Mansour, 2010; Sarwade et al., 2011; Nafady et al., 2016). On the other hand, Songa et al. (2019) found that the dominant genera of AMF across the sampling sites were *Glomus*, *Septoglomus*, *Rhizophagus*, *Kamienskia* and *Sclerocystis*.

The most common species was *F. mosseae* which has been recorded in all plants, followed by *F. geosporum*. A total of spore density for all plants were 425/100g soil and AMF colonization rates were widely varied from 62 to 90%, with the highest being recorded at plant Abu-Elsaoud et al. (2017). On the other side Abdel-Azeem et al. (2007) reported that the AMF spores obtained belonged to eight species from three genera (*Acaulospora*, *Gigaspora*, and *Glomus*). The identified species of AMF were *G. clarum*, *G. clavisporum*, *G. etunicatum*, *G. mosseae*, *G. sinuosum* and *Gigaspora margarita*.

Spores of *G. clarum* and *G. mosseae* dominate root zone soils of six selected host plants percentage of spore density per 100g soil, ranging from 27.06 to 52.31%. AMF isolates were distributed in 5 families (*Glomeraceae*, *Gigasporaceae*, *Acaulosporaceae*, *Entrophosporaceae* and *Paraglomeraceae*) and 14 species were recovered from different plant species.

AMF spores were isolated from different soil samples and morphologically identified. There were 48 to 78 spores in 100g dry soil collected from the different soil samples and the average at 59.86 in 100g dry soil. The highest number of spore count was found in *R. officinalis* (*Lamiaceae*) at 78 spores in 100g dry soil while the lowest was observed in *C. sativum* (*Apiaceae*) at 48 in 100g dry soil. The average of mycorrhizal colonization rate reached 47.29%; the highest level of colonization was recorded in *R. officinalis*, *O. basilicum* and *S. officinalis*

(*Lamiaceae*) at 57.0, 55.0 and 52.0, respectively. The lowest mycorrhizal colonization rate was found in *C. sativum* (*Apiaceae*) at 36.0%. Many similar studies have targeted associations between endophytic fungi and medicinal plants (Jia et al., 2016; Bakker et al., 2018).

Songa et al. (2019) found that AMF colonization rates ranged from 33% to 98%. There were 62 to 275 spores in 50g dry soil collected from the different soil samples, 26 species were detected by morphological spore identification, belonging to 10 genera of AMF. In contrast to the present results, the highest root colonization % was observed in *Asteraceae* (100%), while the lowest value was observed in families *Labiataceae*, *Lamiaceae* showing 10% and 15% colonization, respectively (Yaseen et al., 2020).

#### *Funneliformis mosseae* multiplication

*Funneliformis mosseae* was the dominant mycorrhizal species in all seven plants of North Sinai Governorate, Egypt during 2019-2020. Therefore, *F. mosseae* was isolated, purified and chosen for use in this experiment. In particular, the two elements related to AMF's inoculum potential (spore density as count/100g dry soil, and root colonization rate %) were used. The inoculum potential of *F. mosseae* as spores have been increased by growing onion is recommended as a trap plant grown in sterilized sandy soil for a period of four months to increase the spore density and root colonization. Data in Table 6 indicate that average spore count was 59.86 spores/100g dry soil and 47.29% average of root colonization. This result is considered very low because these soils are classified as arid & semi-arid and consequently very poor in nutrients. After four months from using onion plant as trap culture, the spore counts reached 225 spores/100g dry soil and 88.00% of root colonization. After the end of the trapping and cultivation experiment, the soil and fibrous roots of the onion crops are taken out and used to grow gladiolus plants in pots. These results were in harmony with those found by Yaseen et al. (2016), Nafady et al. (2018).

#### Impact of ChM and FYM rates on spore density and total root colonization on *G. grandiflorus*

The obtained data in Figs. 1, 2 demonstrate that the un-inoculated *G. grandiflorus* plants showed no *F. mosseae* spore density and root colonization. However, there are differences in AMF spore density and root colonization

between mycorrhizal inoculated plants. Inoculum *F. mosseae* increased significantly spore density and root colonization in *G. grandiflorus* plant singly or combined with ChM and FYM by the time. *F. mosseae* spore density showed the highest value at harvest stage while the root colonization showed the highest value at flowering stage. The combination of *F. mosseae* and ChM and FYM fertilizers increased significantly spore density

and root colonization in *G. grandiflorus* plant. The combination with chicken manure showed that the highest AMF spore density at two concentrations (10 ChM and 20 ChM) in all stages (after 30 days, flowering stage and harvest stage) at two different seasons compared with the combination between *F. mosseae* and FYM at the same concentrations and control treatment.

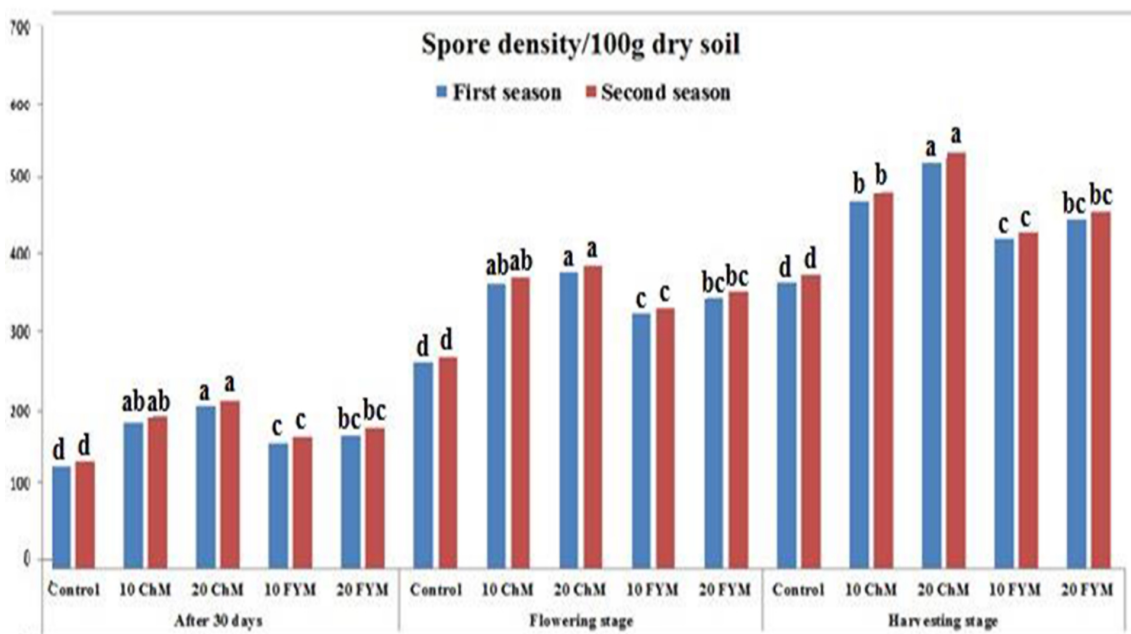


Fig. 1. Impact of rates of application of chicken manure (ChM) and farmyard manure (FYM) on *Funneliformis mosseae* spore density/100g dry soil during 2018/2019 and 2019/2020 seasons

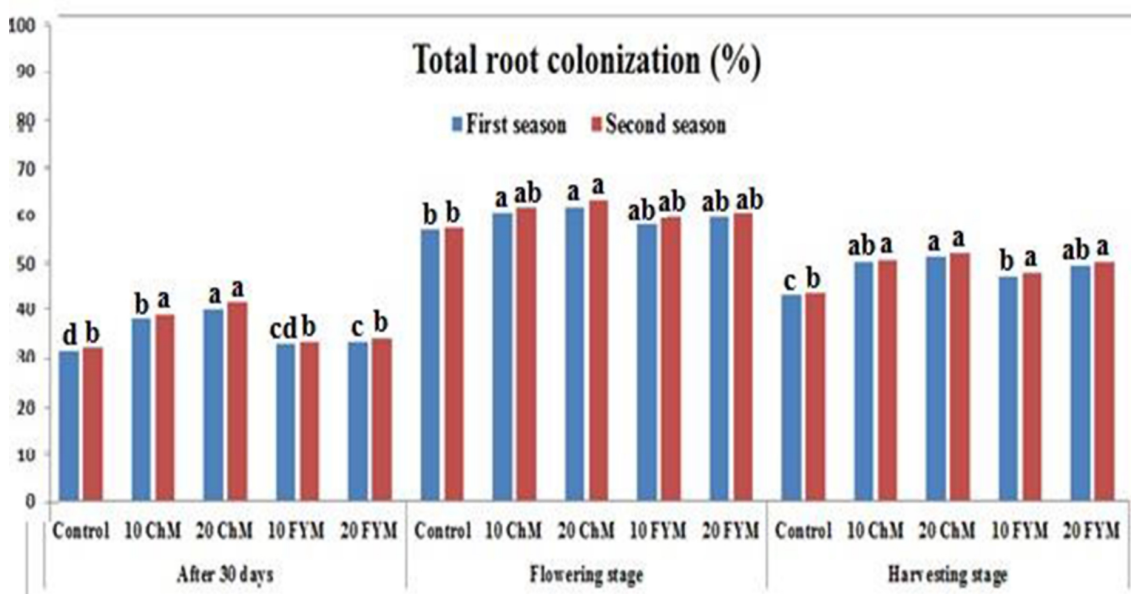


Fig. 2. Impact of rates of application of chicken manure (ChM) and farmyard manure on *Funneliformis mosseae* total root colonization (%) during 2018/2019 and 2019/2020 seasons

These results are in line with those of Juntahum et al. (2020) who observed no spores of *F. mosseae* in the uninoculated treatment. The number of spore changes with time, and the spore density is highest after eight months. In the unfertilized and non-inoculated controls, the spore density was the lowest. Nafady et al. (2018) found that the uninoculated faba bean plants showed no colonization and significant differences between colonization patterns in the tested roots. Intercellular, intracellular hyphae, arbuscules and vesicles occupy the colonized roots. Javaid et al. (1995) reported that mycorrhizal colonization was enhanced in *T. alexandrinum* co-cultured with *Brassica campestris* compared with monoculture crops.

The combination of *F. mosseae* and 20m<sup>3</sup> fed<sup>-1</sup> ChM noticed that the best treatment among all treatments in spore density 663 spores/100g dry soil at harvest stage in second season while, the root colonization 91.80% at flowering stage. On the other hand, the lowest value of spore density (457.33 spores/100g dry soil in first season) was found in control treatment at harvest stage, while the root colonization 63.36 % in the same stage Fig. 2. In this connection, Juntahum et al. (2020) noticed that AMF colonization was significantly higher in both AMF treatments (*F. mosseae* inoculation and *F. mosseae* inoculation with half dosage of P fertilizer) compared with the uninoculated treatments in sugarcane plant. In the treatment of inoculation, the number of spores tends to be higher, which is mainly due to the spores of *F. mosseae*. Riaz & Javaid (2017) showed that both *Gladiolus* and *Narcissus* exhibited positive effects on mycorrhizal colonization. AMF colonization was significantly higher in mixed cropped *Gladiolus* and *Narcissus* plants than corresponding mono cultivated plants.

On the other hand, the lowest treatment in spore density at harvest stage found that in control treatment 457.33 spores/100g dry soil in first season while, the root colonization 63.36 % in the same stage Fig. 2. In this connection Juntahum et al. (2020) noticed that AMF colonization was significantly higher in both AMF treatments (inoculation with *F. mosseae* with native inoculum and inoculation with *F. mosseae* with native inoculum and with half dosage of P fertilizer) compared to the uninoculated treatments in Sugarcane plant. In the treatment of inoculation, the number of spores tends to be higher, which is mainly due to the spores of *F. mosseae*. Riaz & Javaid (2017) showed that both *Gladiolus* and *Narcissus* exhibited positive effects on mycorrhizal

colonization. AMF colonization was significantly higher in mixed cropped *Gladiolus* and *Narcissus* plants than corresponding mono cultivated plants.

#### *Impact of ChM and FYM rates on the formation and percentage of F. mosseae structures on G. grandiflorus*

Data in Table 7 show that *F. mosseae* structures increased by the time of growth in *G. grandiflorus* plant. The combination of *F. mosseae* and ChM & FYM significantly increased the formation and percentage of mycorrhizal structures (hyphae, vesicles and arbuscules) compared with control treatment. The greatest values of hyphae, vesicles and arbuscules colonization were found in plants treated with dual inoculations compared with the control treatment.

*F. mosseae* structures significantly increased by adding ChM and FYM fertilizer while, using ChM at two concentrations (10 and 20m<sup>3</sup> fed<sup>-1</sup> ChM) proved to be much better than using the same concentrations of FYM at all stages throughout the two different seasons. All mycorrhizal structures observed that the highest value at flowering stage compared with after 30 days and at harvest stage by using ChM & FYM fertilizer.

Hyphae showed the highest result at flowering stage with the addition of 20 ChM at 89.90% and 91.80 % in 1<sup>st</sup> and 2<sup>nd</sup> seasons, respectively. Vesicles followed the same trend at 42.33% and 44.40% in 1<sup>st</sup> and 2<sup>nd</sup> seasons, respectively at the same stage. On the other hand, the formation and percentage of arbuscules was less than hyphae and vesicles at 20.10 and 21.80% in 1<sup>st</sup> and 2<sup>nd</sup> seasons respectively at the same stage.

The obtained results were in a harmony with those found by Juntahum et al. (2020) who observed increased AMF root colonization rate % in all the treatments (AMF and AMF + 50% P). AMF root colonization rate was characterized by intercellular and intracellular hyphae, arbuscules and vesicles. The non-inoculated control had significantly lower colonization rate than the non-inoculated P fertilizer treatment. Nafady et al. (2018) showed that the highest values of hyphal and arbuscular colonization were found in plants inoculated with dual inoculations with *Acaulospora laevis* and *Glomus caesaris*, while the vesicular colonization reached the minimum amount in dual inoculated *Vicia faba* plants. Riaz & Javaid (2017) observed that mycorrhizal colonization differed among *Gladiolus* varieties.

**TABLE 7. Impact of rates of application of chicken manure (ChM) and farmyard manure (FYM) on the formation and percentage of *Funneliformis mosseae* structures in *Gladiolus grandiflorus* during 2018/2019 and 2019/2020 seasons**

Organic fertilization types and rates (m <sup>3</sup> /fed <sup>-1</sup> )	<i>F. mosseae</i> structures					
	After 30 days		Flowering stage		Harvesting stage	
	2018/2019 season	2018/2019 season	2018/2019 season	2018/2019 season	2018/2019 season	2018/2019 season
	<b>Hyphae (%)</b>					
Control	46.40 d	47.23 b	82.96 b	84.43 b	63.36 c	64.50 b
10 ChM	55.93 b	57.23 a	88.36 a	89.76 ab	73.20 ab	74.50 a
20 ChM	59.16 a	60.76 a	89.90 a	91.80 a	74.90 a	76.10 a
10 FYM	48.13 cd	48.56 b	85.46 ab	86.93 ab	69.36 b	70.67 a
20 FYM	49.16 c	50.00 b	87.00 ab	88.23 ab	71.86 ab	73.06 a
	<b>Vesicles (%)</b>					
Control	21.90 a	23.20 a	35.86 c	37.06 c	26.63c	28.00 c
10 ChM	24.30 a	25.43 a	41.16 ab	42.40 ab	31.23 ab	32.40 a-c
20 ChM	25.63 a	27.03 a	42.33 a	44.40 a	33.56 a	34.86 a
10 FYM	22.00 a	23.26 a	37.46 bc	38.76 bc	28.73 bc	30.20 bc
20 FYM	22.80 a	24.20 a	38.26 a-c	39.83 bc	30.96 ab	32.76 ab
	<b>Arbuscules (%)</b>					
Control	7.16 a	8.33 b	15.26 b	16.26 b	9.96 a	10.63 a
10 ChM	9.56 a	10.63 ab	19.36 a	20.63 a	11.60 a	13.00 a
20 ChM	10.93 a	12.56 a	20.10 a	21.80 a	12.10 a	13.86 a
10 FYM	7.90 a	9.10 ab	17.83 ab	18.80 ab	10.33 a	11.00 a
20 FYM	8.90 a	10.46 ab	19.23 ab	20.60 a	11.30 a	12.43 a

Means having the same letter (s) are not significantly different according to Duncan's multiple range test at 5% level of probability

Colonization rates of different mycorrhizal structures (mycelial, arbuscular and vesicular) in *Gladiolus* varieties was stimulated in mixed cropping with *Narcissus*. The mycorrhizal colonization reaction on the roots of Fado and Chinon were the most responsive where the percentage of mycelial, arbuscular and vesicular infections increased significantly due to the mixing with *Narcissus*. Vesicular colonization was very low in all treatments, possibly because plants were harvested at early growth stage and vesicles are generally formed in large numbers at the end of the growing seasons to serve as storage organs (Bethlenfalvay & Linderman, 1992).

On the other hand, Riaz et al. (2007) found that *Fusarium oxysporum* inoculation adversely affected all the mycorrhizal structures (mycelium, arbuscules and vesicles). The incorporation of residues of different allelopathic plants reduced the percentage of mycelium and arbuscular colonization by 46-59% and 50-88%, respectively.

### **Conclusion**

Inoculation with *Funneliformis mosseae* is an important factor in promoting *Gladiolus grandiflorus* growth and productivity. Vegetative growth, flowering characters, corms values and productivity and chemical constituents of *G. grandiflorus* are significantly enhanced by *F. mosseae* inoculation with chicken manure addition at 20 m<sup>3</sup> fed<sup>-1</sup> alone or in combination.

*Conflict of interests:* The authors confirm that there is no conflict of interest to disclose

*Author contribution:* MAIM, HMSH, and MAIA proposed the idea of this study. All authors contributed equally in designing the experimental work, making the measurements, analyzing and interpreting the data, and writing the manuscript. MAIM performed the calculations and statistical analysis. All authors participated in the revision of the manuscript.

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## تأثير التلقيح بفطر *Funneliformis mosseae* على نمو وإنتاجية أبصال الجلادبولس تحت أنواع ومعدلات مختلفة من التسميد العضوي

محمد أحمد ابراهيم أحمد منصور<sup>(1)</sup>، هاني محمد سامي حسن<sup>(2)</sup>، محمد أحمد ابراهيم عبد القادر<sup>(3)</sup>  
<sup>(1)</sup>قسم النبات والميكروبيولوجي- كلية العلوم- جامعة العريش - العريش 45511 - مصر، (2) قسم الإنتاج النباتي- كلية العلوم الزراعية البيئية- جامعة العريش- 11554- مصر، (3) قسم البساتين - كلية الزراعة - جامعة الزقازيق - 32544 - مصر.

تم زراعة أبصال الجلادبولس خلال موسمين متتاليين 2018/2019 و 2019/2020 في المزرعة التجريبية ، كلية العلوم الزراعية البيئية ، جامعة العريش ، مصر لدراسة تأثير أحد أجناس الفطريات الجذرية الداخلية (الميكوريزا) وهو فطر *Funneliformis mosseae* من خلال معاملتين (ملقح وبدون تلقيح) مع أنواع مختلفة من الأسمدة العضوية: مثل سماد الدجاج والسماد البلدي وبمعدلات إضافة (صفر ، 10 و 20 م<sup>3</sup>/فدان) على النمو الخضري والإنتاجية لأبصال الجلادبولس. وأظهرت النتائج أن إضافة سماد الدجاج بتركيز 20 م<sup>3</sup>/فدان أدت إلى زيادة كبيرة في النمو الخضري ، بالإضافة إلى الصفات المرغوبة للأزهار مثل تفتح الأزهار بوقت قصير مقارنة بالمعاملات الأخرى علي مدار الموسمين . وكذلك زيادة ملحوظة في الإنتاجية وتركيز بعض العناصر المهمة مثل: النيتروجين ، الفوسفور ، البوتاسيوم في النبات . عزز التلقيح بفطر *F. mosseae* زيادة كبيرة في النمو الخضري ، والإنتاجية وتركيز العناصر السابق ذكرها خلال كلا الموسمين. التفاعل بين فطر *F. mosseae* وإضافة سماد الدجاج والسماد البلدي أدت إلى زيادة كبيرة في تكوين وكثافة الجراثيم للفطر ، وأيضاً استعمار جذور الجلادبولس مقارنة باستخدام المعاملات كلاً علي حده. إلا أن التفاعل بين التلقيح بفطر *F. mosseae* و سماد الدجاج خصوصاً وبمعدلات (10 و 20 م<sup>3</sup>/فدان) أعطت أعلى القيم مقارنة بباقي المعاملات علي مدار الموسمين.