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CONTROLLING ONION WHITE ROT BY USING EGYPTIAN FORMULATED ENDO-MYCORRHIZA (MULTI-VAM)

[52]

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ABSTRACT

All tested mycorrhizal treatments to control white rot diseases and increased yield in greenhouse and field., were effective and reduced the diseases infection. Treatment triple No.3 (Application in seed bed 14 days transplanting + Dipping at transplanting time + Soil drench 14 days after transplanting) and treatment single No.5 (Dipping at transplanting time) and treatment double No.6 (Dipping at transplanting time + Soil drench 14 days after transplanting) were higher root colonization of onion plants. The percentage were 80,73.3 and 73.3%, respectively. Sporulation were increase with triple treatment No.3 (Application in seed bed 14 days transplanting + Dipping at transplanting time + Soil drench 14 days after transplanting) and double treatment No.2 (Inoculated with Multi VAM in the seed bed 14 days before transplanting + dipping at transplanting time). In greenhouse experiment, treatment triple No.3 and double treatment No.6 were reduced percentage of white rot disease during two seasons 2001/2002 & 2002/2003 and increase of yield were 4.3,3.4,6.4 and 5.3 folds, respectively. At field experiments treatment triple No.3 and treatment double No.2 and No.6 were higher efficacy to reduce white rot disease during two seasons. The efficacy were 61.7,64.4,57.0,56.1,60.7 and 61.4, respectively and increase of yield were showed by using treatment single No.7 (Soil drench 14 days after transplanting), double No.2 & 6 and triple No.3.

Key words: White rot, *Sclerotium cepivorum*, Arbuscular mycorrhizal fungi (AMF), Egyption formulated endo-mycorrhiza (Multi VAM).

INTRODUCTION

Onion, *Allium cepa* L., is an important crop in many countries of the world including Egypt. According to the Statistical Department of Ministry of Agriculture and Soil Reclamation, the total area cultivated with onion in Egypt is estimated by 102967 feddans (faddan = 4200 m^2) in season 2002. White rot of onion., caused by *Sclerotium cepivorum* Bark., is one of the most serious fungal diseases of onion cultivation in many regions of the world **(Crowe *et al* 1980).** White rot disease is the most widespread soilborne onion disease in Egypt **(Abdel-Moity 1981 and Satour *et al* 1989).**

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Arbuscular mycorrhizal fungi (AMF) increased nutrient uptaking by root and subsequently the growth of many plants **(Stribley, 1990).** Mycorrhization had been proposed as good alternative method for the management of soilborne pathogens **(Schenck, 1981; Dehne, 1982, 1987; Barea *et al* 1984; Hornby, 1990; Perrin, 1990 and Reid, 1990).** Mycorrhizal plants are often less colonized by the pathogens and show reduction in the disease incidence.

Garlic plants inoculated by Vesicular Arbuscular Mycorrhiza (VAM) were larger, had more green leaves, with more photosynthesis rate and had higher fresh and dry weight compared with uninoculated plants. Dry weight of plants treated and untreated with VAM were 51g/bulb and 27g/bulb, respectively **(Koch *et al* 1997).** Mycorrhizal plants showed increase of 22% in onion yield, regardless of the presence of white rot pathogen **(Andrea *et al* 1996).** The defense response of alfa alfa roots to the pathogenic fungi *Rhizoctonia solani* was reduced significantly in roots simultaneously infected with the AM fungus *Glomus intraradices* **(Guenoune *et al* 2001).** Also, *G. clarum* acted to some extent as a bioagent against *Rhizoctonia. solani*, the causal fungus of root rot of cowpea plants. **(Shabana, 2002 and Matsubara *et al* 2001)** revealed that tolerance to fusarium root rot of asparagus caused by *Fusarium oxysporium* f.sp. *asparagi* (FOA) was conditioned by AM fungal infection. **(Abo El-Ela, 2003)** found that formulated mycorrhizal fungi (Multi VAM) and Rhizo-N were the most effective treatments controlling fusarium wilt of gladiolus and increased the yield <2.25 < 13 folds.

This investigation aimed to evaluate the efficiency of the Egyptian formulated endo-mycorrhiza (Multi VAM) in controlling white rot of onion and the yield productivity under different conditions.

MATERIAL AND METHODS

Treatments

In this investigation, Egyptian formulated endo-mycorrhiza (Multi VAM, kindly obtained from Dr. Safwat A. El-Haddad, MyCol. & PL. Dis. Surv. Dept, Pl. pathol. Res. Inst., ARC Giza, Egypt) was used in liquid type which consists of propagated units from *Glomus intrardices, G. mosseae, G. clarum, Gigaspora margarita, G. gigantea* and other species in combination (1×10^6 units/liter). Usage Multi VAM in dipping the seedlings before transplanting at 50 ml/liter water dilution and at dilute of 5ml/liter water for soil drench for onion seed bed and after transplanting. Table (1) shows the different treatments in the entire experiments.

Greenhouse experiments

Sclerotium cepivorum inoculum was prepared by growing pure culture on maize meal-sand medium at 20° C for 3 weeks. Pots (25 cm-diam) packed with formalin-sterilized clay and washed sand (1:1 v/v) were infested at the rate 2 % (w/w), 7 days before planting. Three pots for each treatment were used, each planted with 5 onion seedlings and three pots were used as a control 1 with infested soil and without treatments. Another three pots were planted without inoculum control 2. The infection percentage and onion weight were estimated in the end of season (140 days).

Table 1. Design of the treatments in the greenhouse and field experiments during 2001/2002 and 2002/2003 seasons.

TREATMENT	Inoculated with Multi VAM		
	In the seed bed 14 days before trans-planting	Dipping at trans-planting time	Soil drench 14 days after trans-planting
1	+	-	-
2	+	+	-
3	+	+	+
4	+	-	+
5	-	+	-
6	-	+	+
7	-	-	+
(Control 1)8*	-	-	-
(Control 2) 9**	-	-	-

* Application with *S. cepivorum* without VAM.
** Application with neither *S. cepivorum* inoculation nor VAM

Field experiments

Field experiments were carried out in artificially infested soil at the Agricultural Research Stations of Mallawi (El-Minia Governorate) during two successive seasons, 2001/2002 and 2002/2003. Sixty-day old seedlings of onion were transplanted four replicates in 3x3.5 m plots randomized complete block design. The infection percentage of white rot and the obtained yield of onion bulbs were recorded at the end of the growing season, after 160 days from transplanting. All of the mycorrhizal treatments were done using the recommended rates of N and K fertilizers to onion plants and half dose of phosphorus. (Mamatha *et al*, 2002). Rock phosphate was used in this study as a suitable phosphorus source with mycorrhiza as a fertilizer. On the other hand, in all of the treatments without mycorrhizal fungi, the recommended rates of NPK were used **(Daft and Nicolson 1967).**

Clearing and staining of vesicular arbuscular mycorrhizal (VAM) fungi

a. Root Colonization

To study the effect of formulated AMF (Multi VAM) inoculum with differ-

ent execution on the percentage of root colonization of onion plants under field natural infection. Onion hairy root samples were removed from soil, washed by tap water and cutted into 1 cm long pieces. Root pieces were prepared for microscopic observation to determine the percentage of mycorrhizal colonization in the different treatments according to **(Philips and Hayman, 1970)** at the end of season.

b. Sporulation density of VAM in the rhizosphere

250 g of soil samples were collected from the onion experiments (treatment and replicates) and extraction of VAM propagules was done under Lab. conditions using wet sieving and decanting technique as described by **(Gredman and Nicolson, 1963).**

RESULTS AND DISCUSSION

Root Colonization

A subsequent increase in root AMF colonization was observed with triple inoculation by multi VAM. Data in Table (2) indicate that the highest percentage of colonization was 80% in triple inoculation treatment. The dual inoculation in the permanent (soil / field) caused colonization of onion roots higher than dual inoculation in seed bed and permanent soil (73.3 % and 60 , 66.6 % colonization) in treatments No. 6, 2 and 4 respectively. However, the percentage of colonization was recorded in the field inoculation in a single inoculation in the time of transplanting (treatment No. 5 and No. 6) 73.3 % and 60 %, respectively. Also, the lower percentage of root colonization with double treatment (2) and single treatment (7) were 60%.These results are in a harmony with those recorded by **(Andrea *et al* 1996; Johnson 1984; Koch *et al* 1997 and Stribley 1990)** .

Sporulation density of VAM in the rhizosphere

Increasing in the percentage of sporulation was observed in all treatments. Table (3) indicates that the highest sporulation was recorded in triple adding multi VAM (T3) and in dual adding (T2). Inoculation with multi VAM as a single treatment in the seed bed 14 days before transplanting (T1) resulted in an increase of sporulation more than in the time of transplanting or 14 days after transplanting (T5 and T7), Table (3). These results were agreement with **(Reid 1990 Azcon-Aguilar and Barea, 1996 and Shaltout, 1998).**

Table 2. Effect of formulated AMF inoculum (Multi VAM) with different applications on the percentage of root colonization of onion plants under field natural infection with white rot pathogen at the end of 2002/2003 growing season

Treatment 1	1	2	3	4	5	6	7	8	9*
Percentage of root colonization **(calculated from 15 samples)	66.6	60	80	66.6	73.3	73.3	60	53.3	- -

* Not tested

** The percentage of root colonization was estimated as average of 15 samples.

Table 3. Sporulation density number of spores in the rhizosphere of onion plants under greenhouse at the end of the 2002/2003 season

Treatment	1	2	3	4	5	6	7	8	9
No. of spores/1gm dry soil	25	128	144	14	18	17	15	11	9
Increasing of sporulation %	178	1322	1500	56	100	89	67	22	-

$$Sporulation\ \% = \frac{Treatment - Control}{Control} X\ 100$$

Greenhouse experiments

Data of the season 2001/2002 presented in Table (4) shows that all of the Mycorrhizal Multi VAM treatments significantly reduced the infection percentage of onion white rot compared with artificial infestation (control). Treatments triple No.3 followed treatment double No. 6 resulted in the best values in controlling the white rot of onion. Non significant results were recorded between treatments single No. 4, 5, treatment double 6 and treatment single 7. Higher efficacy was resulted in by using formulated AMF (Multi VAM) triple No.3 and double No.6 ranged between 58.8% and 52.2%. Higher yield was recorded by using treatments No.3,6. Also, significant results were showed between the three treatments 3,6 and 7 compared with the other treatments and control. Non significant results in the yield were showed between the treatments double No. 2 and 4 Table (4).

These results were agreement with those obtained by **(Azcon-Aguilar and Barea, 1996)** and they explain the result as follow; 1- Both mycorrhizal fungus and pathogen depend on host photosynthates for their growth, mycorrhiral fungus has primary access to photo synthates, the higher C demand many inhibit pathogen growth. If VAM fungus colonizes the root then this would limit the colonization of the pathogenic fungi to areas of the root which had not been colonized, thus offording protection **(Goncalves *et al* 1991).** 2-Mycorrhizal symbiosis increases the nutrient uptake and results in more vigorous plants, the plant itself may thus become more resistant tolerant to pathogen attack **(Azcon-Aguilar and Barea, 1996).** 3-Modification of the basic physiology of plant roots may contibute to disease suppression. This promotion of water and nutrient uptake by mycorrhizal fungi affect the course of pathogensis. VAM root have higher respiratory activity than non-VAM roots **(Dehne, 1987 and Snellgrove *et al* 1982).** Increased respiration rate of VAM roots is an indication for the higher metabolic activity which might enable plants to react more rapidly and more effectively against root pathogens **(Dugassa *et al* 1996).** Furthermore, VAM roots showed an increase of ethylene production

Table 4. Effect of Formulated AMF (Multi VAM) on percentages of white rot infection and onion bulb yield under greenhouse conditions 2001/2002 growing seasons.

Treatments	Infection %	Efficacy %	Weight of bulb yield (gm / pot)	Increase of yield %
1	65	23.5	27.2	54.98
9	60	29.4	32.6	85.75
3	35	58.8	94.6	439.03
4	45	47.0	45.6	159.8
5	55	35.2	36.0	105.1
6	40	52.2	77.85	343.58
7	50	41.0	49.4	181.48
8	85	0.0	17.55	0.0
9	Zero	-	76.0	333.04
L.S.D 5%	18.2		6.3	

$$* \textit{Efficacy} = \frac{\textit{Treatment of Multi Vam} - \textit{Treatment of control}}{\textit{Treatment of control}} X\ 100$$

$$\textit{Increase of yield} = \frac{\textit{Weight of bulb yield treatment}}{\textit{Weight of bulb yield in control}} - \textit{Weight of bulb yield in control } X\ 100$$

and DNA methylation (**Dugassa *et al* 1996**) Colonization with VAM fungi also results in an increase of amino acid production especially arginine which inhibits the sporulation of the pathogen (**Baltuschat and Schönkbeck, 1975**). Exudation pattern of root also changes both qualitatively as well as quantitatively (**Graham *et al* 1981**).

Data of all Multi VAM treatments showed increasing of the yield, being 54.98% to 439.03%. The higher yield were obtained in the treatments No.3,6 and 7. These increments were 439.03 %, 343.58% and 181.48%, respectively compared with control 1 Table (4).

In the second season during 2002/2003, significant decrease in the infection percentage of the white rot were showed in all Multi VAM treatments. Treatments triple No.3 and double No.6 reduced the infection percentage of white rot, ranging between 40-45% compared with control, being 95%. Also, significant results were showed between treatments No.3 and 6 compared with treatment sin-

gle No.1. However, not significant results were observed between the other treatments. The same treatments showed with application No.3, No.6 and No.7, resulted in 57.8%, 52.6% and 47.3%. All Multi VAM treatments recorded an increase in the obtained yield, being 34.31 % to 640.44%. Multi VAM treatments No.3 and No.6 recorded an increase of the yield, being 4.0 and 6.0 folds compared with the control Table (5).

These results were interpreted by **(Gerdemann, 1968 and Boatman *et al* 1978)** who stated that *Allium spp*, including garlic are extremely responsive to VAM symbiosis and stunting of onion growth in the absence of VAM fungi has been well documented. Also, **(Koch *et al* 1997)** found that VAM inoculation garlic plants greatly increased the rate of photosynthesis including higher fresh and dry weights compared with non inoculated plants The increased growth of the Mycorrhizal plants may attributed to the increase of photosynthesis. Also, higher photosynthesis in Mycorrhizal plants in pot grown plants of a variety of species has been found by several workers **(Johnson, 1984; Brown & Bethlenfalvay, 1987 and Allen *et al* 1981).**

Field experiments

Data in Table (6) show a significant decrease in the infection percentages of white rot compared with the of the control by using all Multi VAM treatments during 2001/2002 and 2002/2003 growing seasons. Treatments triple No.3, double 6,2, single No.7 and treatment double 4 recorded the highest efficacy ranged between 53.1% and 61.7% during the first season 2001/2002. On the other hand, efficacy of these treatments ranged between 49.5% to 64.4% during 2002/2003 season. Also, treatments No.3 and No.6 decreased percentage of infection between 11.6%, 8.0% and 11.9%, 8.8% during the two seasons, respectively.

Table 5. Effect of Formulated AMF (Multi VAM) on the infection percentages of white rot and onion bulb yield under greenhouse conditions during 2002/2003 growing season.

Treatments	Infection %	Efficacy %	Weight of bulb yield (gm / pot)	Increase of yield %
1	70	26.3	16.87	34.31
9	60	36.8	28.2	124.52
3	40	57.8	93.0	640.44
4	55	42.1	26.77	113.13
5	60	36.8	29.6	135.66
6	45	52.6	79.25	530.97
7	50	47.3	43.4	245.54
8	95	0.0	12.56	0.0
9	Zero	-	54.0	329.93
L.S.D 5%	20.4		5.4	

Table 6. Effect of Formulated AMF (Multi VAM) on percentages of white rot infection on onion during 2001/2002 and 2002/2003 growing season. under field conditions of Mallawi Research Station, Minia governorate

Treatments	2001/2002		2002/2003	
	Infection %	Efficacy %	Infection %	Efficacy %
1	14.8	51.1	11.8	48.2
9	13.0	57.0	10.0	56.1
3	11.6	61.7	8.0	64.4
4	14.2	53.1	11.5	49.5
5	16.4	45.8	12.1	46.9
6	11.9	60.7	8.8	61.4
7	13.7	54.7	10.7	53.0
8	30.3	0.0	22.8	0.0
L.S.D 5%	1.1		5.7	

The lowest efficacy was showed by using treatments single No.5 ranged between 45.8% and 46.9% during the two seasons Table (4). These results agree with **(Andrea *et al* 1996)** who stated that, Mycorrhizaion infection delayed onion white rot epidemics by 2 weeks and provided a significant protection against the disease for 11 weeks after transplanting.

Therefore, there was an observation of a decrease in the incidence of *S. cepivorum* disease from 92 to 28.7% at natural infestation. Furthermore, as reported by **(Zhengjia and Xiangdong, 1991)** inoculation with Mycorrhizal fungi before planting conferred more complete protection against pathogens.

The present observations that Mycorrhizal plants suffer less colonization by pathogens and that disease incidence is reduced with the addition of *Glomus confirms* what has been found and suggested by other researchers **(Dehne 1982 and Hag *et al* 1987).**

All Mycorrhizal (MV) treatments significantly increased bulb yield than the control in both field experiments. Application of Multi VAM No.6 was the higher yield and increase of yield ranged between 135.4%, 106.5% and No. 3 107.5%, 92.3% Table (7).

In general, increase of yield was recorded in the different applications MV treatments during the two seasons compared with control. These increase were 37.9 to 135.4 % during the first season and 32.6 to 106.5% during the second season. The same results were recorded by **(Andrea *et al*, 1996),** who stated that Mycorrhizal plants showed increase of onion bulb yield 22%, regardless of the presence of the white rot pathogen.

Table 7. Effect of Formulated AMF (Multi VAM) on onion bulb yield 2001/2002 and 2002/2003 growing season. under field conditions of Mallawi Research station Minia governorate

Treatments	2001/2002		2002/2003	
	Yield (kg/plot)	Increase of yield %	Yield (kg/plot)	Increase of yield %
1	12.9	63.2	13.5	46.7
9	13.2	67.0	14.7	59.7
3	16.4	107.5	17.7	92.3
4	10.9	37.9	12.2	32.6
5	14.6	84.8	15.8	71.7
6	18.6	135.4	19.0	106.5
7	17.0	115.1	18.6	102.1
8	7.9	0.0	9.2	0.0
L.S.D 5%	3.46		2.8	

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استخدام مستحضر مصري من فطريات الميكوريزا الداخلية (مالتي فام) في مكافحة مرض العفن الأبيض في البصل

[٥٢]

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١- معهد بحوث أمراض النبات - مركز البحوث الزراعية - الجيزة - مصر

٢- قسم النبات الزراعي - كلية الزراعة - جامعة القاهرة - فرع الفيوم- مصر

استخدمت عدة معاملات من التركيبة المصرية للميكوريزا (مالتي فام) والتي تحتوي على خليط من عدة انواع من الميكروهيزا الداخلية في مقاومة مرض العفن الأبيض في البصل (في كل من الصوبة و الحقل) خلال موسمين متتالين من زراعة البصل ٢٠٠١/٢٠٠٢- ٢٠٠٢/٢٠٠٣ وأشارت النتائج الى نقص معنوي في شدة الاصابة و زيادة المحصول. و وجد ان المعاملة الثلاثية (المعاملة رقم ٣) معاملة الشتلات في المشتل قبل الشتل ب ١٤ يوم + معاملة غمس الشتلات قبل الزراعة + معاملة التربة و المعاملة الفردية (المعاملة رقم ٥) غمس الشتلات قبل الزراعة و المعاملة الثنائية (المعاملة رقم ٦) غمس الشتلات + معاملة التربة كانت أكثر المعاملات احتلال لجذور البصل بفطريات الميكوريزا بنسبة تراوحت بين (٨٠ - ٣ ، ٧٣ - ٣،٧٣ على التوالي) تحت ظروف حقل مصابا طبيعيا.

وقد أعطت النتائج زيادة في معدل التجرثم في المعاملة الثلاثية (٣) و المعاملة الثنائية (٢) معاملة الشتلات في المشتل + غمس الشتلات قبل الزراعة. في تجارب الصوبة وجد أن المعاملة الثلاثية (٣) والمعاملة الثنائية (٦) غمس الشتلات + معاملة التربة قللت من نسبة الاصابة بمرض العفن الأبيض خلال عامين متتاليين من الدراسة ، وفي نفس الوقت حدثت زيادة في المحصول بمقدار ٣،٤ - ٤،٣ - ٤، ٦- ٣،٥ ضعفا على التوالي منسوبة للمقارنة.

و في تجارب الحقل وجد ان اكثر المعاملات كفاءة في تقليل مرض العفن الأبيض في البصل خلال عامي ٢٠٠١/٢٠٠٢ ، ٢٠٠٢/٢٠٠٣ كانت باستخدام المعاملة الثلاثية (٣) معاملة الشتل+ غمس الشتلات قبل الزراعة + معاملة التربة.

و المعاملة الثنائية (٢) معاملة الشتل + غمس الشتلات قبل الزارعة ، و المعاملة

(٦) غمس الشتلات + معاملة التربة سجلت كفاءة ٦١٧-٤، ٦٤ – ٥٧ – ١ ، ٥٦ – ٧، ٦٠– ٤، ٦١ % على التوالي خلال العامين.

و تم تسجيل زيادة في الوزن باستخدام المعاملة الفردية (٧) معاملة التربة المعاملة (٥) غمس الشتلات قبل الزراعة والمعاملة الثنائية (٢) معاملة المشتل + غمس الشتل والمعاملة (٦) غمس الشتلات قبل الزراعة + معاملة التربة و المعاملة الثلاثية (رقم ٣).

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