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ROLE OF SOME BIOFERTILIZERS AND ANTIOXIDANTS ON ONION WHITE ROT DISEASE AND YIELD PRODUCTION

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ABSTRACT: ***Single and combined applications of two biofertilizers and/or two antioxidants to Giza 6 onion cultivar seedlings just before transplanting in artificially infested soil with Sclerotium cepivorum reduced white rot disease incidence, (DI) than control. The best significant disease reduction was observed when Biogien, Microbien and Tartaric acid were applied together (6.7% DI). Microbien + Amonium tartrate application reduced (DI) to be 13.3% and this was 20% when Biogien + Tartaric acid treatment was used.***

Under field and natural inoculation conditions in Malawi, Minia governorate at 2005 and 2006 seasons; the applications of biofertilizers and/or antioxidants significantly reduced white rot (w.r.) disease incidence and increased onion bulb yield. Microbien was superior in reducing the disease followed by Amonium tartrate and Biogien + Amonium tratrate. There was a big correlation between (w.r.) disease reduction and increasing onion yield production.

Total phenols were generally increased than control in response to biofertilizers and/or antioxidants application to Giza 6 onion cultivar plants. However, application of Biogien + Microbien + Amonium tartrate gave the highest estimated phenolic compounds. Free phenols were severly lesser than conjugated phenols.

Polyphenol oxidase activity in onion plants was lesser than control ones in response to the applications of any biofertilizer and/or antioxidant. While peroxidase activity was higher than control in all cases except Amonium tartrate treatment.

Key words: ***Biofertilizers, Antioxidants, White rot disease, Sclerotium cepivorum, Phenolic compounds, Polyphenol oxidase and Peroxidase.***

INTRODUCTION

Onion (*Allium cepa* L.) is a very important economic crop in Egypt, both for local consumpition and exportation. It is subjected to the invasion by many pathogens either in the field or storage and transportation. *Sclerotium cepivorum* Berk is the most destructive fungus which cause white rot disease. This fungus completely destroy bulb yield all over the world (Georgy, 1977; Kurtz, 1983; Anonymous, 1985; Goley-Smith, *et al.* 1990; Abd-El-Megid, 1994; Khaled *et al.* 1997; Melero-Vera, *et al.* 2000; Raghuramulu, 2001 and Abd-El-Megid *et al.* 2003). White rot disease was limited in some

places of middle Egypt, but it is now recorded in different governorates of lower Egypt (El-Shennawy, 2004). Such distribution could be due to the transmission of the infected plant materials; especially diseased onion seedlings.

Different trials had been carried out in order to control this disease such as solarization, resistant cultivars, crop rotation, biological control and fungicides, Entwistle (1990a) and (1990 c). However; biofertilizer applications to onion seedlings showed successful role in limitation of this disease and some other soilborne ones (Buonassisi *et al.* (1986); Zaki and Ghaffer, (1987); Saleh and Ahmed, (1988) and Abd-El-Megid *et al.* 2003).

On the other hand; several investigators reported that antioxidants had antifungal properties and toxicity to some soilborne fungi (Arnoldi *et al.* 1989; Okuno *et al.* 1991; Elad, 1992; Reuveni *et al.* 1993; Walters *et al.* 1993; Palva *et al.* 1994; Galal and Abdou, 1996 and Abd-El-Megid *et al.* 2004).

The aim of this work was to study the effect of single and combined application (s) of two biofertilizers and two antioxidants on white rot disease of onion; both under natural inoculation conditions in polluted soil with the pathogen in Mallawi fields; Minia governorate; Egypt.

MATERIALS AND METHODS

Two commercial biofertilizers i.e., Biogien and Microbien which contained the nitrogen fixing bacteria (NFB) were used; respectively at the rates of 20 and 40 g/L; for dipping onion seedlings just before transplanting. These two biofertilizers were manufactured in the Laboratory of Agricultural Research Center, Giza, Egypt. Ammonium tartrat and Tartaric acid were also tried as antioxidants, at the rate of 10M.

I- Greenhouse Experiments:

Formalin sterilized pots (25 cm in diameter) were filled with formalin sterilized clay soil which infested with *S. cepivorum* grown on Barley medium, at the rate of 3% of the soil weight. Seven days later, seedlings of Giza 6 onion cultivar were transplanted. Fifteen seedlings (60 days old) were seperatly treated with any possible single or combined treatment and transplanted in three pots (replicates). Control treatment included three infested potted soil which planted with 15 untreated onion seedlings. Percentage of infection with *S. cepivorum* was estimated at the end of the experiment (90 days after transplanting). Obtained data were statistically analyzed according to Snedecor and Cochran (1972).

II. Field Experiments:

Field experiments were carried out at Mallawi Agricultural Research Station, Minia Governorate During 2005 and 2006 Seasons. Used field is

naturally polluted with *S. cepivorum* fungus where it is a partial for the performance of white rot experiments. Three plots of 3 x 3.5 m (1/400 feddan) served as replicates for each treatment. The same rates and concentration of biofertilizers and antioxidants which used in the greenhouse experiment were also followed in the field ones. Sixty days old Giza 6 onion cultivar seedlings were used and application of the antioxidant were perior to the biofertilizers in the untreated seedlings of the same cultivar.

Control plots were planted with untreated seedlings. Percentage of infection with *S. cepivorum* and healthy bulb yield were estimated at harvesting. Data were statistically analyzed according to Snedecor and Cochram (1972).

Biochemical Assay:

1-Phenolic Compounds:

Total, free and conjugated phenolic compounds were colourimetrically determined using phosphotungestic-phosphomolybdic acid (Folin and Ciocaltese) reagents according to Snell and Snell, (1953). A standard curve of P. hydroxyl benzoic acid was used to calculate the amount of phenolic compounds in different tested samples. The obtained results were expressed as mg P. hydroxyl benzoic equivalent per gram fresh weight.

2-Peroxidase Activity:

Peroxides assay (based on oxidation of pyrogallol to purpurogallin in the presence of H_2O_2) was determined according to the methods of Allain and Hollis, (1972).

3-Polyphenol-oxidase Activity:

The activity of polyphenol-oxidase was measured according to the methods described by Matta and Dimond, (1963).

RESULTS AND DISCUSSION

I- Green house Experiments:

Results present in Table (1) clear that all tested compounds reduced white rot disease incidence (DI) than control, under greenhouse and artificial inoculation conditions. Reduction of (DI) was significant in many cases and the best result was observed when Biogien, Microbien and Tartaric acid were applied in combination (6.7% DI). In this respect, Microbien + Ammonium tartrate gave the best second rate of efficacy (13.3% DI) followed by Biogien + Tartaric acid (20% DI). However, significant differences than control were also noticed when Microbien + Tartaric acid, Biogien + Ammonium tartrate and Microbien alone were applied. The best treatments showed either equal result of disease incidence as control i.e. Tartaric acid or insignificant (DI) results, i.e. Biogien A.T., T.A. and biogien + Microbien + A.T. Treatments.

Obvious results indicate that the combined applications of tested biofertilizers and antioxidants gave much better results in controlling the disease than did seperate ones. Also, combination of Biogien with Tartaric

acid showed best results than those of the same biofertilizer with Amonium tartrate and the vice was versa with Microbien biofertilizer. Combination of both biofertilizers with Tartaric acid severly reduced (DI) but did not when Amonium tratrate was instead used.

Table (1): Effect of biofertilizers and/or antioxidants application on white rot disease of onion under greenhouse and artificial inoculation conditions.

Treatment	Diseased plants		Efficacy
	No.	(%)	(%)
Biogien	9	60.0	10.05
Microbien	8	53.6	20.09
Amonium tartrate (AT)	9	60.0	10.05
Tartaric acid (TA)	10	66.7	0.0
Biogien + AT	7	46.7	29.99
Biogien + TA	3	20.1	70.02
Microbien + AT	2	13.3	80.06
Microbien + TA	6	40.2	40.03
Biogien + Microbien + AT	9	60.0	10.05
Biogien + Microbien + TA	1	6.7	89.96
Control	10	66.7	
LSD at 5%	2.8	18.6	

The above results are in agreement with those observed by Schipper *et al.* (1987) who declared that the biofertilizers containe plant growth promoting rhizobacteria (PGPR) produce suppressive antibiotics which affect soil pathogens. In addition, positive effects due to plant nutrition were also observed. Limitation of white rot disease of onion and some other soilborne pathogens by biofertilizers was also indicated by Buonassisi, *et al.*(1986);Zaki and Ghaffer,(1987); Saleh and Ahmed, (1988) and Abd-El-Megid *et al.* (2003).

On the other hand; antifungal properities and toxicity to soilborne fungi by antioxidans were also noticed by Arnoldi *et al.* (1989); Okuno *et al.* (1991); Elad, (1992); Reuveni *et al.* (1993); Walters *et al.* (1993); Palva *et al.* (1994); Galal and Abdou, (1996) and Abd-El-Megid *et al.* (2004).

II- Field Experiments :

Nearly similar results were obtained under field and natural inoculation conditions at 2005 and 2006 (Table 2). Results of both years indicate that all tested biofertilizers and/or antioxidants sharply reduced white rot disease incidence. Microbien, however, was superior in reducing the disease followed by Amonium tartrate, Biogien + Amonium tartrate, Biogien and Microbien + Tartrate acid. Highly significant differences were also noticed between the other tested treatments and control. Disease reduction ranged from 48.15 to 79.26% at 2005 and from 38.13 to 69.17% at 2006. In both investigation years; the best disease reduction was observed when Microbien was applied while the worst one was recorded when Biogien + Tartratic acid was used.

Table (2): Effect of biofertilizers and/or antioxidants application on white rot disease of onion and yield production under field and natural inoculation conditions (2005 and 2006 seasons).

Treatment	2005			2006		
	Diseased Plants (%)	Efficacy (%)	Yield (Ton/fed)	Diseased Plants (%)	Efficacy (%)	Yield (Ton/fed)
Biogien	21.8	67.70	5.27	18.6	61.25	5.4
Microbien	14.0	79.26	5.13	14.8	69.17	5.5
Amonium tartrate (AT)	15.6	76.89	4.80	14.9	68.96	5.2
Tartaric acid (TA)	27.6	59.11	4.93	24.9	48.13	5.0
Biogien + AT	18.6	72.44	5.23	16.6	65.42	5.4
Biogien + TA	35.0	48.15	5.00	29.7	38.13	4.5
Microbien + AT	23.7	64.89	5.33	21.4	55.42	4.5
Microbien + TA	22.0	67.41	5.07	17.6	63.33	5.8
Biogien +Microbien +AT	34.5	48.89	5.00	27.2	43.33	5.2
Biogien +Microbien + TA	31.3	53.63	4.60	27.2	43.33	4.7
Control	67.5		2.70	48.0		3.1
LSD at 5%	3.4		0.45	4.4		0.8

Yield production was highly affected by either disease incidence (control) or different tested treatments. Onion bulb yield at 2005 was 2.70 to 5.33 Ton/fed. respectively for control and Microbien + Tartrate acid applications. Bulb yield at 2006 of the same two treatments was 3.1 and 5.8 Ton/feddan respectively. It could be noticed that white rot natural infection was higher at 2005 than 2006 where they recorded 67.5 and 48.0% respectively. This could be due to climatic factors as mentioned by Anonymous, 1985.

Results of Table 2 also clear that there are big correlation between white rot disease incidence and yield reduction of onion bulbs. This is logic where

this disease partially or completely destroys bulbs of *Allium* crops all over the world as reported by Georgey, (1977); Kurtz, (1983); Goley-Smith, 1990; Entwistle, *et al.* (1990); Abd-El-Megid, (1994); Khaled *et al.* (1997) and Abd-El-Megid *et al.* (2003).

Biochemical Analysis:

1- Phenolic compounds:

Samples of onion plants (different treatments of 2006 season) were chemically analysed to determine total- free,and conjugated phenols. Results shown in Table (3) clear that total phenols were increased than control in response to all tested biofertilizers and/or antioxidants except those of Biogien + Amonium tartrate. Total phenols (9.43 mg/g fresh weight) were recorded in the case of Biogien + Microbien + Amonium tartrate followed by Microbien + Tararic acid treatment (5.45 mg/g fresh weight). It was noticed that most of the determined phenols were conjugated. They were 8.29 and 4.56 mg/g fresh weight; respectively for the abovementioned treatments. However, free phenols of these two treatments were 1.14 and 0.89 mg/g fresh weight respectively. Increasing phenolic compounds is a reaction of the diseased plants as a physiological control method (Ammar, 2003). Application of Amonium tratrate with either Biogien or Microbien slightly reduced total phenols than control. This could be due to chemical reactions affected pH of the rhizosphere.

Table (3): Phenolic compounds (mg/g fresh weight) in onion plants as affected by biofertilizers and/or antioxidants under field and natural inoculation conditions with *S. cepivorum* in Malawi at 2006 season.

Treatment	Total phenols	Free phenols	Conjugated phenols
Biogien	2.93	0.73	2.20
Microbien	2.45	0.64	1.81
Amonium tartrate (AT)	3.55	0.78	2.77
Tartaric acid (TA)	2.25	0.68	1.57
Biogien + AT	1.10	0.25	0.85

Biogien + TA	**2.68**	**0.54**	**2.14**
Microbien + AT	**1.04**	**0.26**	**0.78**
Microbien + TA	**5.45**	**0.89**	**4.56**
Biogien + Microbien + AT	**9.43**	**1.14**	**8.29**
Biogien + Microbien + TA	**2.48**	**0.63**	**1.85**
Control	**1.48**	**0.49**	**0.99**
LSD at 5%			

2- Activity of two oxidative enzymes:

The activity of two enzymes; i.e., polyphenol oxidase and peroxidase in relation to the tested treatments for controlling white rot disease was also determined. Results present in Table (4) indicate that polyphenol oxidase was less active than control in response to all tested treatments, except Microbien which resulted only 0.01% higher activity. The least activity of polyphenol oxidase was recorded when Tartaric acid was applied (0.03%). This was followed by Biogien + Tartaric acid (0.05%) and Microbien + Tartaric acid (0.07%). Generally, all the treatments had Tartaric acid showed less activity of polyphenol oxidase.

On the other hand; peroxidase activity was higher than control plants in all cases, except with Amonium Tartrate which was severly low (0.18%) and Microbien (0.85%). The best peroxidase activity was recorded when Biogien was applied (1.57%) and this was followed by the application of Biogien + Microbien + Amonium tartrate (1.40%).

In general; respiration rate increased in diseased than healthy plants and the oxidative enzemes could be a factor for disease situation (Ammar, 2003).

Table (4): Activity of polyphenol oxidase and peroxidase (%) in relation to white rot disease infection (2006) as affected by biofertilizers and antioxidants application under field and natural inoculation conditions.

Treatment	Polyphenol oxidase	Peroxidase	Disease incidence (% at 2006)
Biogien	**0.11**	**1.57**	**18.6**
Microbien	**0.15**	**0.85**	**14.8**
Amonium tartrate (AT)	**0.12**	**0.18**	**14.9**
Tartaric acid (TA)	**0.03**	**1.03**	**24.9**
Biogien + AT	**0.10**	**1.06**	**16.6**
Biogien + TA	**0.05**	**1.20**	**29.7**
Microbien + AT	**0.13**	**1.10**	**21.4**

Microbien + TA	0.07	1.20	17.6
Biogien + Microbien + AT	0.08	1.40	27.2
Biogien + Microbien + TA	0.09	0.66	27.2
Control	0.14	0.89	48.0

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تأثير بعض المخصبات الحيوية ومركبات الأكسدة على مرض العفن الأبيض فى البصل وإنتاج المحصول

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الملخص العربى

أدت المعاملة المنفردة أو المشتركة بمركبين من المخصبات الحيوية وآخرين من مضادات الأكسدة لشتلات صنف البصل جيزة ٦ قبل الزراعة مباشرة فى تربة معداه صناعيا بالفطر (سكليروشيوم سيبيفورام) الى تقليل الإصابة بمرض العفن الأبيض ، وكانت أفضل النتائج عند المعاملة بمركبات بيوجين + ميكروبين + حمض الطرطريك مجتمعه (٦.٧% إصابة) . أما المعاملة بالميكروبين + طرطرات الأمونيوم فقد أظهرت ١٣.٣% إصابة والتى كانت ٢٠% عند معاملة بالبيوجين + حمض الطرطريك .

وقد أدت المعاملات الحقلية بمركز ملوى محافظة المنيا تحت ظروف العدوى الطبيعية عامى ٢٠٠٥ ، ٢٠٠٦ بهذه المركبات الى النقص المعنوى لحدوث المرض والزيادة المعنوية فى المحصول . وكان مركب ميكروبيين هو الأفضل فى اختزال المرض وزيادة المحصول تلاه طرطرات

الأمونيوم ، بيوجين + طرطرات الأمونيوم . كما لوحظ الارتباط الوثيق بين انخفاض الإصابة بالمرض وزيادة المحصول .

لوحظ زيادة الفينولات الكلية فى النباتات المعاملة مقارنة بالغير معاملة ، وكانت أفضل التقديرات عند إضافة بيوجين + ميكروبين + طرطرات الأمونيوم . وبصفة عامة كانت الفينولات الحرة أقل من تلك المرتبطة . كما وجد أن نشاط انزيم بولى فينول أكسيديز كان أقل كاستجابة للمعاملة بالمخصبات الحيوية وكذلك مضادات الأكسدة ، فى حين كان نشاط انزيم بيرأوكسيديز أعلى باستثناء المعاملة بطرطرات الأمونيوم .