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Effect of Some Biochemical Compounds on Onion White Rot Disease Incidence and Yield Production

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Under greenhouse and artificial soil infestation conditions, application of Plant Guard (5 ml/l), Clean roots (4 g/l), Root Most (10 ml/l) and Multi VAM (50 ml/l) reduced the incidence of white rot disease of onion (cv. Giza 20) than the control free treatment. Under field and natural soil infestation conditions, disease reduction was also higher than the control treatment when Root Most, Multi VAM, Plant Guard, Clean roots and Alga 600 were applied, individually at 2009/10 season. Folicur fungicide (20 ml/l) reduced the disease incidence by 72.6%. Yield production was responded positively to the commercial compounds application. Root Most and Multi VAM recorded the highest yield production in both 2009/10 and 2010/11 growing seasons. They were more effective for yield increments than Folicur. Activity of peroxidase and catalase enzymes in onion plant tissues were highly increased in response to different treatments application. On the other hand, polyphenol oxidase activity was severely decreased than control, as a result of various compound applications. The total phenolic compounds were increased in onion tissues of the plants treated with each of the tested compounds.

Keywords: Biocides, biofertilizers, onion, VAM and white rot.

Onion (*Allium cepa* L.) is a very important vegetable crop all over the world. Many fungal diseases attack this plant in the field causing severe reduction in plant stands and yield losses. White rot disease caused by the soil-borne fungus *Sclerotium cepivorum* Berk is the most dangerous problem on onion and garlic; where its sclerotia are able to survive in the soil up to 10 years (Coley-Smith, 1967). Its sclerotia only germinate and attack *Allium* species hosts where their root exudates stimulate sclerotia germination (Ammar, 2003). Disease severity depends on sclerotia population in the soil which disseminate by infected transplants as well as irrigation water and soil. White rot disease symptoms include leaf yellowing, dieback, root-rot and complete plant death which may reach 100% loss of the diseased plants (Kurtz, 1983 and Entwistle, 1990).

No data are available on the use of resistant genotypes in Egypt. Nevertheless, the use of some bioagents, *i.e. Trichoderma* spp. are recommended (Abd El-Moity, 1981; El-Shennawy, 2003 and Abd-Elrazik *et al.*, 1988). When the disease was first recorded in Middle Egypt, plant quarantine was followed to prevent the extension of the disease outside this area. However, the disease was recorded outside this area and became a serious problem.

Beside the recommended fungicides, biofertilizers could be used to increase soil fertility, improving plant growth and protecting plants from pests and diseases

(El-Yazid *et al.,* 2004). However, Rokhazadi *et al.* (2004) mentioned that biofertilizers are products containing living cells of microorganisms which colonize the rhizosphere or plant by converting nutrient important elements from unavailable to available form through biological process such as solubilization of phosphate rock. Saleh (2008) proved that single and combined applications of biofertilizers and/or antioxidants reduced white rot disease incidence of Giza 6 onion cultivar, significantly.

On the other hand, marine algae and their extracts were used as crop fertilizers by Abd El-Megid *et al.* (2004) and Strik *et al.* (2004). Thirumaran *et al.* (2009) proved that seaweed fertilizers are preferred, not only for their NPK content, but also because of the presence of trace element metabolites similar to growth regulators. Seaweed extracts as liquid fertilizers (SLF) are well known in the agriculture markets. They contain many growth promoting hormones like auxin, gibbrellin, trace elements, vitamins, amino acids and micronutrients.

Vesicular arbuscular mycorrhizae (VAM) have the role of storage organs for water and minerals which release them into the host cell. Such process improves plants growth and tolerance to variable diseases (Ammar, 2003). El-Fiki *et al.* (2007) tested four VAM isolates of *Glomus* spp. for controlling some maize fungal diseases both under greenhouse and field conditions. They found that VAM treatments significantly improved maize growth parameters and decreased the diseases incited by *Cephalosporium maydis, Fusarium moniliforme* and *Rhizoctonia solani.* However, Khalifa *et al.* (2012) reported that inoculation of VAM fungus *Glomus macrocarpum* in combination with different rates of calcium chloride as dipping treatments were significantly effective for reducing onion white rot disease incidence both under greenhouse and field conditions (artificial and natural soil infestation).

Doubtless, application of biofertilizers, biocides and/or fungicides make changes in plant metabolism which led to the increase of phenolic compounds and the oxidative enzymes (Margo, 1984; McLusky *et al.*, 1999; Saleh, 2008 and Saleh *et al.*, 2009).

The objective of this study was to evaluate the individual role of some commercial compounds in reducing onion white rot disease incidence and increasing yield production in comparison with Folicur fungicide both under greenhouse and field conditions.

Materials and Methods

Greenhouse experiment was carried out at Plant Pathology Research Institute, ARC, Giza, Egypt, while the field experiments were conducted in the Research Station Meet-Khalaf, Minufiya Governorate in naturally heavily infested soil with *Sclerotium cepivorum*.

Tested compounds:

The tested compounds for controlling white rot disease of onion are present in Table (1).

Table 1. The biochemical tested compounds and used doses

Commercial name*	Component	Dose
Alga 600	Mixture of alginic acid and manitol (600 ppm), water soluble powder extracted from three marine algae, *i.e. Sorgassum*, *Laminaria* and *Ascophyllum*	2 and/or 4 g/l
Root Most	Amino acids + extracts of marine algae and seaweeds.	5 and/or 10 ml/l
Clean roots	*Bacillus subtilis*, $30x10^6$ CFU/g	2 and/or 4 g/l
Plant Guard	*Trichoderma harzianum*; $30x10^6$ CFU/g	2.5 and/or 5 ml/l
Multi VAM	Vesicular Arbuscular Mycorrhizae $30x10^3$ CFU/g	50 ml/l
Folicur	a-(2-4-dichlorphenyl)-a-(1,1-dimetly lethyl)-1H- 1,2,4-triazole-1-ethanol	20 ml/l

* Alga 600 and Root Most were obtained from Technogreen Company, Ismailia Square, Heliopolis, Egypt. The rest compounds and Folicur were obtained from Plant Pathology Research Institute, ARC, Giza, Egypt.

Greenhouse experiment:

This experiment was carried out in sterilized pots (30 cm in diameter) containing sterilized clay soil. Pots and soil were sterilized by 5% formalin and left up to two weeks before using, for formalin evaporation. *Sclerotium cepivorum* was grown on sterilized barley seeds medium (75 g barley + 25 gram sand + 100 ml distilled water) for two weeks at 25°C. Soil infestation was conducted at the rate of 2% soil weight by mixing the inoculum into the soil. The infested potted soil was irrigated as required; for a week before planting to enhance the fungal growth. Five pots were used as replicates for each treatment and ten seedlings were sown in every pot (50 plants/treatment). Control free treatment was planted with seedlings dipped for a minute in sterilized distilled water only. Data were recorded 2 months after planting as average number of diseased plants, percentage of infection and tested compounds efficacy were determined.

Field experiment:

Field experiment was carried out in naturally infested soil with *S. cepivorum* at Meet Khalaf Research Station, Shebin El-Kom, Minufiya governorate. Three plots of 3x3.5 m were used as replicates per each treatment in complete randomized block design. All cultural practices were followed as recommended by Ministry of culture, *i.e.* irrigation and fertilization. Data were recorded after five months (harvesting time) as percentage of disease incidence, compounds efficacy, yield production (weight of 100 bulbs) and yield increment in response to the compound applications.

Disease assessment:

White rot disease was assessed as the number of the dead plants to the total number of the plants as following:

$$\text{White rot incidence (\%)} = \frac{\text{Number of dead plants}}{\text{Total number of plants}} \times 100$$

In both greenhouse and field experiments; seedlings of onion (cv. Giza 20) were dipped; individually in each preparation for one minute just before planting.

Statistical analysis:

Obtained data were statistically analyzed according to the standard procedures mentioned by Snedecor and Cochran (1976). The averages were compared at 5% level using the least significant differences LSD according to Fisher (1948).

Chemical analysis:

a) Enzymes activity:

Samples of onion bulbs were taken just after harvest and prepared to determine peroxidase, polyphenol oxidase and catalase activities as described by Maxwell and Bateman (1967).

1- Peroxidase assay:

Peroxidase activity was estimated according to the methods of Allam and Hollis (1972) by measuring the oxidation of pyrogallol in the presence of H_2O at 425 nm. The sample cuvette contained 0.5 ml of 0.1 M potassium phosphate buffer (pH 7) 0.1 ml enzyme extract, 0.3 ml of pyrogallol and 0.1 ml % H_2O_2 brought to 3 ml with distilled water.

2- Polyphenol oxidase assay:

The activity of polyphenol oxidase was measured using the colorimetric method of Maxwell and Bateman (1967). The reaction mixture contained 0-2 ml enzyme extract, 0.5 ml sodium phosphate buffer at pH 7 and 0.5 ml of catechol and brought to a final volume of 3.0 ml with distilled water. The activity of polyphenol oxidase was expressed as the change in absorbance/ ml of extract per min at 495 nm.

3- Catalase assay:

Catalase activity was determined using the spectrophotometer method of Maxwell and Bateman (1967). The sample cuvette contained 0.5 ml of 0.2 m sodium phosphate buffer at pH 7.6, 0.3 ml of 0.5% H_2O_2 and 0.4 ml tissue extract, and brought to a final volume of 3.0 ml with distilled water. Data were expressed as the change in absorbance/ml of extract per min at 240 nm.

b) Determination of phenolic compounds:

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. Obtained results were expressed as mg P. hydroxyl benzoic equivalent per gram fresh weight.

Results

Under greenhouse and artificial soil infestation conditions; all tested compounds significantly reduced the incidence of white rot disease on onion (cv. Giza 20) than the control (Table 2). Plant Guard (5 ml/l) was the most effective one where it showed 68.6% disease reduction; in comparison with free control treatment (control 2). This was followed by Clean Roots (4 g/l), Root Most (10 ml/l) and Multi VAM (50 ml/l) where their efficacy in disease reduction than control (2) reached

Table 2. Effect of biofertilizers, biocides and Multi VAM on white rot disease incidence of onion (cv. Giza 20) under greenhouse and artificial soil infestation conditions (2009/10)

Treatment	Concentration	Average No of diseased plants	Infection (%)	Efficacy (%)
Alga 600	2 g/l	5.6	11.2	21.4
	4 g/l	3.8	7.6	45.8
Root most	5 m/l	4.8	9.6	31.4
	10 m/l	2.8	5.6	60.0
Clean roots	2 g/l	4.0	8.0	43.0
	4 g/l	2.4	4.8	65.7
Plant Guard	2.5 ml/l	3.6	7.2	48.6
	5 ml/l	2.2	4.4	68.6
Multi VAM	50 ml/l	2.8	5.6	60.0
Folicur (control 1)	20 ml/l	3.4	6.8	51.4
Control (2) (Treatment free)		7.0	14.0	
L.S.D. at 5 %		0.80	0.65	

65.7, 60.0 and 60.0%, respectively. The tested fungicide Folicur (20 ml/l) reduced the incidence of white rot disease by 51.4%.

However, under field and natural soil infestation conditions both at 2009/10 and 2010/11 growing seasons; significant disease reduction was observed when any of the tested compounds was individually applied (Table 3). In comparison with control 2 treatment; disease reduction showed 70.9, 70.8, 67.6, 66.6 and 66.1%, respectively, when the higher concentrations of Root Most, Multi VAM, Plant Guard, Clean roots and Alga 600 were individually applied at 2009/10 growing season. The least effective compound was Alga 600 (2 g/l) which reduced the disease incidence by 55.6%. Folicur fungicide (20 ml/l) showed more significant action of disease reduction in this season (72.6%).

In 2010/11 growing season; similar results of disease reduction were achieved with little variations in the compound efficacy. Clean roots (4 g/l) and Multi VAM (50 ml/l) showed superior effect than Folicur where they reduced white rot disease incidence by 72.2 and 71.9%, respectively.

Results present in Table (4) indicated that the individual application of different tested compounds associated with highly increase peroxidase enzyme activity in onion plants than those of control (2) treatment. The most increase was achieved when the higher concentrations of Multi VAM, Alga 600 and Plant Guard were applied. They recorded 10.52, 10.01 and 9.07 mg/g fresh weight (f.w.), respectively. On the contrary; polyphenol oxidase activity was severely decreased than control in response to the separate application of the tested compounds. The most reduction was observed when Folicur and/or Root Most were applied. The least reduction of this enzyme was obtained when Alga 600 (4 g/l) was used. Catalase activity in onion plants was highly increased by variable treatments. The highest activity

Table 3. Evaluation of some biochemical biofertilizer biocides and Multi VAM application on white rot disease incidence and yield production of onion (cv. Giza 20) under field and natural soil infection conditions at 2009/10 and 2010/11

Treatment	Concentration	2009/10				2010/11			
		Disease incidence (%)	Efficacy (%)	100 bulb weight	Increment (%)	Disease incidence (%)	Efficacy (%)	Yield production kg/100 bulb	Increment (%)
Alga 600	2 g/l	13.3	55.6	16.60	10.6	12.9	58.5	17.10	16.3
	4 g/l	10.3	66.1	18.45	23.0	10.0	67.8	19.05	32.6
Root most	5 m/l	11.6	61.7	21.600	44.0	11.1	64.3	20.20	37.4
	10 m/l	8.8	70.9	22.85	52.3	10.0	67.8	20.85	41.3
Clean roots	2 g/l	12.2	59.3	17.40	16.0	11.8	62.1	17.85	21.4
	4 g/l	10.0	66.6	18.85	25.6	7.7	72.2	18.90	28.5
Plant Guard	2.5 ml/l	11.1	63.6	18.00	20.0	12.2	60.8	18.00	22.4
	5 ml/l	9.1	67.6	20.25	35.0	9.8	68.4	19.65	33.6
Multi VAM	50 ml/l	8.9	70.8	21.30	42.0	9.5	71.9	20.85	41.8
Folicur (control 1)	20 ml/l	8.3	72.6	18.39	22.6	7.9	69.5	19.30	31.3
Control (2) Treatment free		30.3		15.00		31.1		14.70	
L.S.D. at 0.05		0.66		0.65		0.81		0.65	

Table 4. Effect of biofertilizers, biocides and Multi VAM on the content of peroxidase, polyphenol oxidase and catalase enzymes in onion bulbs (cv. Giza 20) grown under field conditions (2010/11)

Treatment	Enzyme activity			
	Concentration	Peroxidase (%)	Polyphenol oxidase (%)	Catalase (%)
Alga 600	4 g/l	10.01	0.653	11.08
Root most	10 m/l	5.59	0.098	11.48
Clean roots	4 g/l	7.12	0.140	6.93
Plant Guard	5 ml/l	9.07	0.120	9.45
Multi VAM	50 ml/l	10.52	0.120	13.11
Folicur (control 1)	20 ml/l	5.81	0.095	9.85
Control (2) (Treatment free)		1.19	1.22	2.73

(13.11 mg/g f.w.) was achieved when Multi VAM was applied. It was followed by Root Most and Alga 600 treatments, which recorded 11.48 and 11.08 mg/g f.w, respectively. Clean roots caused the least activity of catalase increment than control (6.93 mg/g Fresh weight).

Total phenolic compounds content in onion (cv. Giza 20) were highly increased in response to different tested control methods of white rot disease (Table 5). Root Most and Plant Guard resulted the highest contents of total phenols (35.98 and 33.81 mg/g f.w.), respectively. However, Folicur gave the lowest estimated total phenols (17.62 mg/g f.w.) which, in the meantime, was more than two folds of control (2) treatment phenols (8.20 mg/g f.w.). Generally; free phenols were higher than conjugated phenols in all cases except that of Root Most application, which resulted so high conjugated phenolic compounds (22.77 mg/g Fresh weight).

Table 5. Phenolic compounds in onion bulbs (cv. Giza 20) as affected by biofertilizers, biocide and Multi VAM under field and natural soils infestation conditions (2010/11)

Treatment	Concentration	Free phenols	Conjugated phenols	Total phenols
Alga 600	4 g/l	14.07	8.37	22.44
Root most	10 m/l	13.21	22.77	35.98
Clean roots	4 g/l	15.19	11.24	26.43
Plant Guard	5 ml/l	16.96	16.95	33.81
Multi VAM	50 ml/l	16.14	12.71	28.85
Folicur (control 1)	20 ml/l	9.14	8.48	17.62
Control (2) (Treatment free)		7.40	0.80	8.20

Yield production of onion (cv. Giza 20) was also positively responded due to various compound applications (Table 3). At 2009/10 growing season; Root Most (10 ml/l) increased the yield of onion (cv. Giza 20) by 52.3% than control (2) treatment. This was the best action followed by root most (5m/1) and Multi VAM (50 ml/1) which resulted 44.0 and 42.0% yield increment than control (2) .However, application of Folicur fungicide at the rate of 20 ml/l (control (1) treatment) increased bulb yield by 22.6%. In the second season (2010/11); Root Most (10 ml/l) and Multi VAM (50 ml/l) resulted 41.8% yield increment. They were followed with Root Most (5 ml/l) which increased the yield by 37.4%. Folicur fungicide treatment led to yield increment than control (2) treatment by 31.3%.

Discussion

White rot is the most dangerous disease on onion plants in Egypt. It appeared on onion in Middle Egypt; but in the last few years it covered many parts of the country (Coley-Smith, 1967 and El-Shennawy, 2003). The causal organism (*Sclerotium cepivorum*) is a very dangerous because it produces sclerotia resistant to the severe environmental conditions which remain viable in the soil for several seasons (Ammar, 2003). On the other hand, data revealed that resistant onion genotypes are not available and no successful breading program for resistant has been started in Egypt (Abd El-Megid *et al.,* 2004). Therefore, other methods are being used to reduce the incidence of the disease: Biological methods, *i.e.* using bioagents (Abd El-Moity, 1981), chemical control and agricultural methods, plant quarantine.

The successful method of disease management has been the effective method for controlling the disease. However the disease was recorded outside Middle Egypt which complicated the problem. The application of growth regulators, antioxidants and fertilizers are used for disease control.

Both under greenhouse and field conditions; the tested compounds (biofertilizers, biocides and Folicur) reduced the incidence of onion white rot disease significantly. This action could be attributed to the antagonistic effect of *Trichoderma harzianum* in (Plant Guard treatment) or *Bacillus subtilis* (Clean roots) as reported by Abd El-Moity, 1981; Abd Elrazik *et al.,* 1988 and El-Shennawy, 2003). It could be also due to improving plant growth and protecting them from the disease when biofertilizers were applied, *i.e.* Alga 600, Root Most and Multi VAM; as reported by Ammar (2003); El-Yazid *et al.* (2004) and Rokhazadi *et al.* (2004). However, Strik *et al.* (2004) and Thirumaran *et al.* (2009) preferred seaweed fertilizers not only for their NPK content but also for the presence of trace element metabolites similar to growth regulators. Such compounds and VAM promote the growth by converting nutrient important elements from unavailable to available form through biological process (Ammar, 2003; Abd El-Megid *et al.,* 2004 and El-Yazid *et al.,* 2004). It is of logic that increasing the concentration of any used compound; within the recommended doses; should gave more beneficial effects in both plant growth and disease reduction.

The oxidative enzymes were severely affected by various treatments. High increments were noticed in peroxidase and catalase activities and the vice was versa in polyphenol oxidase activity. Such results were also noticed by Margo (1984), McLusky *et al.* (1999), Ammar (2003) and Saleh *et al.* (2009) who declared the relations between the oxidative enzymes and disease incidence in side and respiratory rate of the host in other side.

Phenolic compounds were increased in response to the application of biofertilizers, biocides and/or fungicide which explain plant tolerance to the disease as mentioned by McLusky *et al.* (1999) and Saleh (2008). The above facts could explain the significant increase of onion yield production obtained in this study especially in the case of white rot disease where the infected plants are completely destroyed. Therefore, a good correlation could be noticed between the disease reduction and increasing of bulb yield production. In the meantime; the application of biofertilizers improve plant growth characters which consequently increase yield production as reported by Ammar (2003), Rokhazadi *et al*. (2004), Strik *et al.* (2004), El-Fiki *et al.* (2007), Thirumaran *et al.* (2009) and Khalifa *et al.* (2012).

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تأثير بعض المركبات البيوكيماوية على مرض العفن الأبيض في البصل وإنتاج المحصول

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تحت ظروف الصوبة والعدوى الصناعية للتربة ، أدت المعاملة بكل من بلانت جارد (٥ ملل/لتر) ، كلين روتس (٤ جم/لتر) ، روت موست (١٠ ملل/لتر) ومالتى فام (٥٠ ملل/لتر) إلى نقص حدوث الإصابة بمرض العفن الأبيض في البصل (صنف جيزة ٢٠) عن تلك الغير معاملة بمعدل ٦٨.٦ ، ٦٥.٧ ، ٦٠.٠ و ٦٠.٠% على التوالى. في حين أدت المعاملات تحت ظروف الحقل والعدوى الطبيعية للتربة إلى نقص حدوث المرض – عن القطع التجريبية غير المعاملة – بمعدل ٧٠.٩ ، ٧٠.٨ ، ٦٧.٦ ، ٦٦.٦ و ٦٦.١% على التوالى مع المعاملات الفردية بكل من روت موست ، مالتى فام ، بلانت جارد ، كلين روتس وألجا ٦٠٠ ، في موسم ٢٠٠٩/ ٢٠١٠ . وأدى استخدام المبيد الفطر فوليكيور (٢٠ ملل/لتر) إلى نقص المرض بمعدل ٧٢.٦% . كما أدت مختلف المعاملات إلى زيادة إنتاج المحصول ، حيث كانت أفضل النتائج في ذلك ترجع إلى المعاملة بأى من روت موست (١٠ ملل/لتر) ، مالتى فام (٥٠ ملل/لتر) في كلا الموسمين ولوحظ أن المعاملة بأى منهما قد أدت إلى زيادة أكبر في المحصول من تلك المتحصل عليها عند استخدام المبيد الفطرى فوليكيور. وإزداد نشاط الانزيمين بيرأوكسيديز ، كاتاليز في نباتات البصل كاستجابة للمعاملات المختلفة مقارنة بالنباتات غير المعاملة. في حين نقص نشاط انزيم بولى فينول أكسيديز بشدة نتيجة لمختلف المعاملات. وسجلت الفينولات الكلية ارتفاعاً ملحوظاً في أنسجة نباتات البصل المعاملة بكل من المركبات المختبرة ، مقارنة بتلك الغير معاملة.