# EFFECT OF SOME ANTIOXIDANTS ON WHITE ROT IN ONION AND GARLIC AND THEIR YIELD

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***Abstract***

Effect of citric acid, tartaric acid, sodium citrate, sodium salicylate, sodium benzoate, and ammonium tartarate, as antioxidants, was tested on the radial growth of *Sclerotium cepivorum* Berk. The effect on disease incidence and yield of onion as well as garlic was determined. The tested antioxidants at 10mM gave the best results in reducing the radial growth, especially sodium benzoate that gave 2.4 cm radial growth compared to 8.75 cm. for the non-treated control. The number of sclerotia was decreased by the used antioxidants regardless of the concentrations. No clear correlation was observed between the effect of the tested antioxidants on the radial growth and the formation of sclerotia.

Field experiments for two seasons, 2002 and 2003, using a concentration of 10 mM in treating onion transplants or garlic cloves for one hour were carried out at Mallawy research station. The efficiency of the antioxidants in controlling white rot was 88.5% and 87.9% in onion, while it was 60% and 70.50% in garlic in 2002 and 2003, respectively. Regarding the yield, the use of the antioxidants increased onion yield by 498.2% and 355.5% compared to the non-treated control as well as 122.1% and 234.9% in garlic in 2002 and 2003. respectively.

The chemical analyses of healthy or infected antioxidant-treated onion bulbs or garlic cloves indicated a higher phenolic content in healthy onion bulbs than in the infected ones and similar trend was observed for garlic. Regarding the amino acids, methionine, cysteine and cystine were found in onion, while cystine and cysteine were detected in garlic. Data indicated higher amounts of the amino acids in healthy samples than in the infected ones both in onion and garlic.

***Keywords:*** *S. cepivorum, white rot, onion, garlic, antioxidants*

## INTRODUCTION

White rot, caused by the fungus *Sclerotium cepivorum* Berk, is a serious disease in onion (*Allium cepae* L.) and garlic (*A. Sativum* L.) in Egypt. Many studies have been conducted on controlling this disease chemically (El-Shehaby, *et al.*, 1992 and Khaled, *et al.*, 1997) and biologically (Abdel-Moity and Shatla , 1981; Reddy *et al.*, 1992, Kay and Stewart, 1994) as well as by solarization (Satour *et al.*, 1989 and Basallote-Ureba *et al.*, 1995).

On the other hand, several investigators reported that antioxidants had an antibacterial and antifungal properties (Okuno et al.,1991, Elad, 1992, Walters *et al.*, 1993 and Palva *et al.*, 1994). Moreover, the toxicity of some antioxidants to some soil-borne or air-borne fungi has been reported frequently. Some of the pathogenic fungi that were affected by the antioxidants were *Fusarium* spp. in cowpea (Galal and Abdo, 1996), *Pytophthora infestans* in potato (Arnoldi *et al.*, 1989) *Sclerotinia sclerotiorum* and *Botrytis cinerea* in tomato and pepper and *Senecia* sp. in bean, eggplant and roses. Many other studies have indicated the role of antioxidants against plant pathogens as shown by the biochemical responses of the host plant following treatment (Reuveni *et al.*, 1993).

The aim of this investigation was to study the effect of some antioxidants on white rot in onion and garlic and their impact on the yield.

## MATERIALS AND METHODS

Effect of some antioxidant substances on the white rot disease in onion (cv. Giza-6) and garlic(cv.Sids-40) was tested *in vitro* and field experiments which were carried out in Mallawi for two seasons (2002 and 2003). The used antioxidants were citric acid, tartaric acid, sodium citrate, sodium salicylate, sodium benzoate, and ammonium tartarate.

***In vitro* experiments**

Each antioxidant was dissolved in sterilized distilled water to make 100 mM stock solutions. Calculated volumes from each stock solution, were added, separately, to a 100 ml. of potato-dextrose-agar (PDA) medium to give the concentration of 1, 5, or 10 mM just before pouring in the Petri dishes. *Sclerotium cepivorum* was grown on PDA at 20° C for five days and a 0.5 cm diam. disk was placed on each PDA-antioxidant-amended Petri dish. Four Petri dishes were used for each treatment and four antioxidant-free Petri dishes were inoculated with *S. cepivorum* disks to serve as control. They were incubated at 20° C. The radial growth of *S. cepivorum* was measured when growth in the control treatment covered the plate. Also, the number of sclerotia in 0.5-cm.-diam.disk taken from each dish was determined using a research microscope (Abdel-Momen, *et al.*, 2000).

**Field experiments**

The field experiments were carried out in a naturally infested soil with a history of the disease incidence at Mallawy agricultural research station for two seasons (2002 and 2003). Sixty-day-old onion transplants, cv. Giza-6, were separately dipped in 10 mM solutions of each of the used antioxidants, separately, for one hour. The same treatments were applied to garlic cloves. The experimental design was the completely randomized block design, where onion transplants or garlic cloves were planted in 3.5m x3.0m experimental plots. Each treatment was replicated three times and three control plots were planted with non-treated onion or garlic.

After 150 days from planting, percentage of white rot diseased plants as well as the yield of onion or garlic bulbs was determined for each plot. Data were statistically analyzed using the L S D method.

**Chemical analysis**

**Preparation of samples**

Ten grams of onion bulbs or garlic cloves ,either healthy, infected or non-treated control, were separately bottled in 10 ml. of 95%-ethanol. The sample-ethanol mixture was boiled in a water bath for five minutes, then extracted for 16 hrs. by Soxlet's apparatus. The extracted samples were then dissolved in 5 ml. 70% ethanol, centrifuged at 3,000 rpm for ten minutes at room temperature to separate

the ethanol from sample and let to dry. The remaining dry film was dissolved in 5-8 ml of isopropanol and used in the following analyses.

**Determination of phenolic compounds**

Phenolic compounds were colorimetrically determined using the phosphotungistic-phosphomolybdic acid: (Folin Ciocalteau) phenol reagent according to Snell and Snell (1953).

***Total phenols***

Ten drops of concentrated HCl were added to 0.1 ml of the sample, heated quickly to boiling point and placed in a boiling water bath for 10 min. After cooling, 1.0ml of the folin reagent and 5ml of a 20% Na $CO_3$ were added. The mixture was diluted to 10ml with distilled water and determination was carried out using spectrophotometer (UV2600) at 520 nm after 30 min.

***Free phenols***

Free phenols were determined by adding 1.0ml of the folin reagent and 3 ml of a 20% solution of sodium carbonate to 0.1 ml of the sample, then diluted to 10ml with distilled water. Reading was made using spectrophotometer (UV2600) at 520nm after 30 min.

***Conjugated phenols***

Conjugated phenols were determined by subtracting values of free phenols from the values for total phenols. All these determinations were expressed as mg/g fresh weight of plant sample.

**Determination of total free amino acids**

Total free amino acids were determined in ethanol-extracted samples of non-inoculated or inoculated onion or garlic plants according to the method described by Rosen (1957). A volume of 0.1 ml of ethanol-extracted sample, 0.5 ml of cyanide acetate buffer and 0.5 ml of 3% ninhydrin were added and mixed together. The mixture was then heated in a boiling water bath for 10 min. After cooling the reaction mixture, 5 ml of isopropyl alcohol and water (1:1v/v) was added and the developed color was measured using spectrophotometer (UV2600) at 570 nm . Free amino acids

in different samples were calculated using an arginine standard curve and the results were expressed as mg equivalent of arginine per gram fresh weight sample.

## RESULTS

### Effect of antioxidants on the radial growth

Table (1) shows that all the used antioxidants significantly reduced the radial growth of *S. cepivorum* at the tested concentrations. Also, the radial growth was significantly decreased by increasing the concentration of the antioxidants.

Sodium benzoate at 1mM gave the lowest radial growth (4.54 cm.) and the highest was for tartaric acid (7.93 cm.) at the same concentration. At higher concentration, 5 mM, ammonium tartrate gave the lowest radial growth (3.05 cm.) and the highest (6.2 cm) was for tartaric acid, while at 10 mM sodium benzoate gave the lowest radial growth (2.4 cm) and the highest (4.13 cm.) was found with tartaric acid. The radial growth for the non-treated control was 8.75cm.

### Effect of antioxidants on the number of sclerotia

Table (1) shows that number of sclerotia in 0.5cm disks was decreased by different treatments; however, some concentrations within the same treatment had no effect and some increase in the sclerotial production was observed. Ammonium tartrate at 10 mM gave the lowest number of sclerotia per disk (57.63) compared to the highest number (93.38) recovered at 1 mM. It is noteworthy to mention that no relation could be drawn between the used treatments on radial growth and sclerotia formation of *S. cepivorum*.

Table 1. Effect of some antioxidant compounds on the radial growth and number of sclerotia of *S. cepivorum in vitro*.

| Antioxidant | Radial growth (cm) | | | Number of sclerotia per 0.5-cm-disk | | |
|---|---|---|---|---|---|---|
| | 1mM | 5mM | 10mM | 1mM | 5mM | 10mM |
| Citric acid | 6.08 | 3.78 | 3.40 | 77.75 | 55.63 | 76.88 |
| Sodium benzoate | 4.54 | 4.43 | 2.40 | 78.75 | 72.13 | 59.75 |
| Sodium citrate | 6.93 | 4.43 | 3.90 | 65.88 | 59.25 | 78.13 |
| Ammonium tartrate | 6.68 | 3.05 | 2.70 | 93.38 | 79.50 | 57.63 |
| Sodium salicylate | 6.45 | 4.20 | 2.83 | 67.38 | 66.50 | 86.25 |
| Tartaric acid | 7.93 | 6.20 | 4.13 | 81.75 | 84.00 | 73.50 |
| Control | 8.75 | 8.75 | 8.75 | 86.00 | 86.00 | 86.00 |
| L.S.D. 0.01 | Treatments 0.54<br>Concentrations 0.36<br>Treat. X Conc. 0.99 | | | Treatments 13.25<br>Concentrations N.S<br>Treat. X Conc. 22.94 | | |

**Effect of antioxidants on the infection by *S. cepivorum* and yield in field**

Data in Table (2) indicate that all tested antioxidants significantly reduced the percentage of white rot disease in onion and garlic. The efficacy reached 88.5% in onion and 70.5% in garlic. In the onion experiments, ammonium tartrate, tartaric acid and citric acid were the most effective treatments giving 88.5%, 84% and 83.8% efficacy, respectively, in the first season. In the second season, the efficacy of tartaric acid was 87.9% compared to ammonium tartarate (84.6%) and sodium salicylate (83.6%). These are considered the most effective treatments in reducing the white rot disease (Table 2).

In the garlic experiments, sodium citrate and ammonium tartarate were the most effective treatments giving 60% efficacy for each (Table 3). In the second season, tartaric acid and ammonium tartarate showing efficacies of 70.5% and 70.0%, respectively (Table 3).

It could be observed that percentage of the yield increase of onion in the first year was relatively higher than that recorded in the second one. The increase ranged from 337% to 498.2% for sodium benzoate and sodium citrate respectively, despite the high percentage of infected plants in these treatments.

Table 2 Effect of some antioxidants on the white rot incidence and onion yield (Mallawy research station).

| Antioxidant (10mM) | Season of 2002 | | | | Season of 2003 | | | |
|---|---|---|---|---|---|---|---|---|
| | Infected Plants (%) | Efficacy (%) | Yield Kg per 1/400 feddan | Incease of yield (%) | Infected Plants (%) | Efficacy (%) | Yield kg per 1/400 feddan | Increase of yield (%) |
| Citric acid | 9.9 | 83.8 | 21.24 | 427 | 14.2 | 74 | 26.754 | 355.5 |
| Sodium benzoate | 15.3 | 74 | 17.628 | 337 | 15.3 | 72 | 16.95 | 188.5 |
| Sodium citrate | 28.3 | 54 | 24.108 | 498.2 | 8.9 | 83.9 | 19.8 | 237 |
| Ammonium tartrate | 7.00 | 88 .5 | 18.972 | 370 | 8.50 | 84.6 | 21.72 | 269.7 |
| Sodium salicylate | 13.9 | 77.2 | 23.392 | 480 | 9.1 | 83.6 | 22.204 | 278 |
| Tartaric acid | 9.7 | 84 | 22.743 | 464 | 6.7 | 87.9 | 22.753 | 287 |
| Control | 61.20 | | 4.03 | 0.0 | 55.5 | 0.0 | 5.874 | 0.0 |
| L.S.D. 0.05 | 4.54 | | 4.8 | | 1.24 | | 4.56 | |

Table 3. Effect of some antioxidants on white rot incidence and garlic yield (Malawiresearch station).

| Antioxidant (10mM) | Season of 2002 | | | | Season of 2003 | | | |
|---|---|---|---|---|---|---|---|---|
| | Infected Plants (%) | Efficacy (%) | Yield kg 1\400 feddan | Increase of yield (%) | Infected Plants (%) | Efficacy (%) | Yield kg 1\400 feddan | Increase of yield (%) |
| Citric acid | 29.6 | 43.00 | 14.748 | 117 | 35.30 | 25.10 | 13.39 | 103.7 |
| Sodium benzoate | 25.20 | 51.00 | 14.79 | 105 | 16.50 | 64.90 | 21.142 | 221.6 |
| Sodium citrate | 20.80 | 60.00 | 13.852 | 82 | 15.80 | 66.50 | 19.984 | 204 |
| Ammonium tartrate | 20.80 | 60.00 | 13.631 | 79 | 14.1 | 70.00 | 22.016 | 234.9 |
| Sodium salicylate | 30.00 | 43.00 | 14.924 | 122.1 | 28.70 | 39.00 | 15.105 | 129.8 |
| Tartaric acid | 33.10 | 37.00 | 13.936 | 116.6 | 13.8 | 70.50 | 19.665 | 199.2 |
| Control | 52.2 | 0.0 | 4.608. | 0.0 | 47.1 | 0.0 | 6.572 | 0.0 |
| L.S.D. 0.05 | 3.24 | | 3.12 | | 2.83 | | 2.88 | |

In the second season, the yield showed 355.5% increase for citric acid compared to 188.5% increase for sodium benzoate. For garlic (Table 3), the yield increase ranged from 122.1% with sodium salicylate to 79% for ammonium tartrate in the first season (2002). In the second season (2003), the highest yield increase was for ammonium tartrate being 234.9% and sodium benzoate at 221.6% The lowest yield, however, was obtained with citric acid treatment giving only 103.7% increase (Table 3).

**Chemical analyses**

Data in Table (4) indicate that total phenols were higher in infected onion bulbs than in the healthy ones in all treatments and the control. The highest amount (23.67 mg/ gm sample) of total phenols in healthy onion bulbs was obtained from those treated with sodium citrate, while in the infected bulbs, citric acid treatment gave the highest amount of total phenols (45.93 mg/ gm sample). A similar trend was observed for the free phenols where they were higher in healthy than in infected onion bulbs in most treatments. Total phenols of non-treated healthy onion (control) were lower than most of the treated ones, while free phenols were higher than in most treatments.

Table 4. Determination of phenolic compounds in healthy and white rot-infected onion plants treated with some antioxidants.

| Antioxidant (10mM) | Healthy onion samples | | | Infected onion samples | | |
|---|---|---|---|---|---|---|
| | Total phenols | Free phenols | Conjug. phenols | Total phenols | Free phenols | Conju. Phenols |
| Citric acid | 9.35 | 4.04 | 5.31 | 45.93 | 3.09 | 42.90 |
| Sodium benzoate | 10.95 | 4.42 | 6.53 | 13.03 | 3.16 | 9.87 |
| Sodium citrate | 23.67 | 1.86 | 21.81 | 24.21 | 1.64 | 22.57 |
| Ammonium tartrate | 8.33 | 1.55 | 6.78 | 37.10 | 1.93 | 35.17 |
| Sodium salicylate | 10.84 | 2.06 | 8.78 | 42.18 | 1.22 | 40.96 |
| Tartaric acid | 11.23 | 1.91 | 9.41 | 29.90 | 1.57 | 28.33 |
| Non-treated | 9.70 | 3.60 | 6.10 | - | - | - |

In garlic, the total phenols were higher in the healthy plants treated with ammonium tartrate, tartaric acid, sodium citrate, or citric acid compared to the infected ones under the same conditions. However, total phenols were higher in infected plants treated with sodium benzoate and sodium salicylate than in the healthy garlic plants receiving the same treatments (Table 5). Data in Table (5) also indicate that some treatments increased the free phenols in healthy samples than in the infected ones, while other treatments showed the opposite. Total phenols of the healthy non-treated garlic were variable with respect to the treatments, while free phenols were always lower. (Table 5).

Three amino acids, namely, methionine, cysteine and cystine, were detected in healthy and infected onion bulbs. The amounts of these amino acids were higher in healthy than in infected samples. Citric acid treatment gave the highest amounts of the determined amino acids in both healthy and infected samples; however, cysteine was the highest in infected samples treated with ammonium tartrate (Table 6).

Healthy non-treated onion showed higher amounts of such amino acids than those found in the treated ones (Table 6).

Table 5. Determination of phenolic compounds in healthy and white rut infected garlic Treated with some antioxidants(mg/g fresh wt.).

| Antioxidant (10mM) | Healthy garlic samples | | | Infected garlic samples | | |
|---|---|---|---|---|---|---|
| | Total phenols | Free phenols | Conjug. phenols | Total phenols | Free phenols | Conjug. Phenols |
| Citric acid | 12.06 | 9.36 | 2.70 | 11.83 | 10.10 | 1.73 |
| Sodium benzoate | 12.23 | 11.63 | 0.60 | 15.23 | 12.60 | 2.63 |
| Sodium citrate | 14.00 | 10.81 | 3.19 | 12.37 | 10.07 | 2.30 |
| Ammonium tartrate | 20.49 | 15.08 | 5.41 | 18.59 | 12.24 | 6.35 |
| Sodium salicylate | 16.89 | 12.57 | 4.32 | 17.89 | 11.61 | 6.28 |
| Tartaric acid | 19.40 | 13.89 | 5.51 | 18.38 | 13.95 | 4.43 |
| Non-treated | 15.6 | 3.5 | 12.1 | - | - | - |

Table 6. Determination of some amino acids in healthy and white rot –infected onion treated with antioxidants(mg/g fresh wt.).

| Antioxidant (10mM) | Healthy onion samples | | | Infected onion samples | | |
|---|---|---|---|---|---|---|
| | Methionine | Cysteine | Cystine | Methionine | Cysteine | Cystine |
| Citric acid | 5.21 | 3.34 | 7.18 | 5.11 | 2.41 | 7.00 |
| Sodium benzoate | 4.66 | 3.02 | 6.88 | 4.40 | 2.52 | 6.70 |
| Sodium citrate | 4.78 | 2.98 | 6.95 | 4.55 | 2.46 | 6.77 |
| Ammonium tartrate | 4.32 | 3.33 | 7.05 | 4.26 | 3.20 | 6.93 |
| Sodium salicylate | 4.56 | 3.33 | 7.06 | 4.25 | 2.44 | 6.95 |
| Tartaric acid | 5.01 | 2.25 | 6.93 | 4.89 | 2.45 | 6.80 |
| Non-treated | 5.25 | 3.40 | 7.20 | - | - | - |

Healthy garlic showed higher amounts of the determined amino acids than those obtained from the infected ones. In healthy samples, ammonium tartrate gave the highest amounts of cysteine while in the infected samples, sodium benzoate gave the highest amount of cystine in infected garlic samples and sodium salicylate gave the highest amount of cystine in the healthy garlic samples (Table 7). In healthy non-treated garlic, cystine was higher than the treated ones except for that treated with sodium salicylate, while cysteine was lower than all treatments except for samples treated with sodium benzoate and sodium citrate (Table 7).

Table 7. concentrations of some amino acids in healthy and white rot –infected garlic treated with antioxidants (mg arginine equivalent/ fresh wt.).

| Antioxidant (10mM) | Healthy garlic samples | | Infected garlic samples | |
|---|---|---|---|---|
| | Cystine | Cysteine | Cystine | Cysteine |
| Citric acid | 0.23 | 4.02 | 0.18 | 0.84 |
| Sodium benzoate | 0.06 | 1.78 | 0.24 | 0.41 |
| Sodium citrate | 0.14 | 1.16 | 0.00 | 2.21 |
| Ammonium tartrate | 0.60 | 6.70 | 0.08 | 1.56 |
| Sodium salicylate | 5.25 | 6.40 | 0.05 | 2.59 |
| Tartaric acid | 0.56 | 6.45 | 0.05 | 2.54 |
| Non-treated | 4.29 | 2.38 | - | - |

## DISCUSSION

The reduction of *S. cepivorum* radial growth and the decreased number of sclerotia by antioxidants indicated that these antioxidants exhibited an antifungal effect on the fungus. This finding is in consistence with the report by Galal and Abdo (1996) on some *Fusarium* spp. and with the finding of Nesci et al. (2003) on *Aspergillus flavus* and *A. parasiticus.* This antifungal effect differed from one antioxidant to the other depending on the concentration and the possible difference in the nature and mechanisms of the antifungal effect. This antifungal effect could be due to the inhibition of sclerotia biogenesis and inhibition of fungal growth by accumulated toxic byproducts as a result of antioxidants application.( Georgious *et. al.,* 2000). The effect of the antioxidants on the radial growth of *S. cepivorum* was not correlated with its effect on the sclerotia formation indicating the different effect of these antioxidants on the two parameters.

The significant decrease in the disease incidence on onion and garlic plants, compared to the non-treated control, indicated the efficiency of such antioxidants *in vivo.* The strong effect of most antioxidants on onion than that on garlic could be attributed to different response of the two crops and this finding is similar to that by Elad (1992).

In the same crop, some antioxidants were more effective in one season than in the other and this could be due to a change in the environmental conditions. The yield increase by antioxidants in onion and garlic can be attributed to their role in suppressing disease as they combine with other compounds produced by plant or pathogen giving plant-harmless compounds (Elad ,1992). The higher total phenol content in the infected onion bulbs can be attributed to the response of the host to the fungal invasion. In garlic, however, such response was not as clear as in onion and that may be attributed to the lower initial phenolic content in garlic. Additionally, garlic may respond differently to infection by producing other inhibitory material (s) along with phenols. This phenomenon has been reported by other investigators such as Elad (1992) and Georgious *et. al.* (2000).

The determined amino acids were higher in healthy onion and garlic than in the infected ones. This may be due to the depletion of these substances by the invading fungus. Our results, indicate that antioxidants may be used as a component of a system approach for disease control; however, some modifications in the means of application and the used concentrations should be considered.

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# تأثير بعض مضادات الأكسدة على العفن الأبيض في البصل و الثوم وانتاجهما

**مدحت سعد عبد المجيد ، صلاح محمد عبد المؤمن ، نجوى على إبراهيم**

*معهد بحوث أمراض النباتات – مركز البحوث الزراعية – جيزة – مصر*

تم دراسة تأثير المواد المضادة للاكسدة مثل حمض الستريك– حمض الطرطريك– سترات الصـــوديوم – سلســـيلات الصـــوديوم – بـــنزوات الصوديوم – طرطرات الأمونيوم على نمو فطر سكليروشـــيوم سيبفورم في المعمل.وجد إن تركيز ١٠ ملي مول أعطى أفضل النتائج في تقليل نمو الفطر باستخدام كل معاملات المواد المضادة للاكسدة و خاصة بنزوات الصوديوم مقارنة بالكنترول. أيضـــا وجد إن معاملات المواد المضادة للاكسدة تقلل من معدل تكوين الأجسام الحجرية.في تجارب الحقـــل أوضحت النتائج أن استخدام ١٠ ملي مول في معاملة شتلات البصل و فصوص الثوم لمدة ســـاعة تقلل من الإصابة بالعفن الأبيض بدرجة كفاءة تتراوح ما بين ٨٧,٩% ، ٨٨,٥%على البصل و معـــدل كفـــاءة ٦٠% إلـــى ٧٠,٥% على الثوم خلال العامين ٢٠٠٢/٢٠٠٣ وقد سجلت المواد المؤكســـدة زيادة في المحصول تراوحت ما بين ٣٥٥,٥% ، ٤٩٨,٢ % على البصل أما الثوم كانت الزيادة ما بين ١٢٢,١% ، ٢٣٤,٩% خلال عامي ٢٠٠٢ ، ٢٠٠٣.

وقـــد أظهـــرت التحالـــيل الكيميائية زيادة في الفينولات بالنسبة للنباتات السليمة عن المصابة سواء للبصـــل او الـــثوم وان هناك زيادة في الأحماض الأمينية في النباتات السليمة عن المصابة باستخدام مضادات الأكسدة على المحصولين مقارنة بالكنترول