

THE USE OF ANTIOXIDANTS TO CONTROL GREY MOULD AND TO ENHANCE YIELD AND QUALITY OF STRAWBERRY

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ABSTRACT

A variety of commercial and natural antioxidants, *i.e.* butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), *Tert*-butyl hydroquinone (TBHQ), citric acid, ascorbic acid, and benzoic acid were tested for their potential to control grey mould and to enhance yield and quality of strawberry. The laboratory *in vitro* experiment revealed the significant effect of the antioxidants at the three concentrations tested, *i.e.* 0.1 mM, 1.0 mM, and 10.0 mM, to inhibit the colony diameter (2.87% - 21.17%), sporulation (17.06 - 42.93 %), and germination (9.26-64.89%) of the grey mould fungus *Botrytis cinerea*. The 0.1mM concentration showed the most consistent and pronounced effect and, consequently, was used in two field experiments conducted during the 2005-2006 and 2006-2007 growing seasons at the Experimental Station Farm, South El- Tahrir, El-Behera governorate. Spraying of the tested antioxidants onto strawberry plants (cvs. Sweet Charlie, Chandler and Earlibrite) grown in a field naturally infested with the grey mould fungus *Botrytis cinerea* significantly suppressed (19.21% - 69.16%) grey mould incidence on fruits of the treated plants over the two growing seasons. The most pronounced effect was linked to BHT, BHA, Benzoic acid, and Ascorbic acid while TBHQ and Citric acid exhibited the lowest suppressive effect in both seasons. This effect was accompanied with an enhancement on growth, yield, and quality of the tested strawberry cultivars. Antioxidants significantly improved most vegetative and flowering traits

assessed in terms of number of crowns plant⁻¹, number of leaves plant⁻¹, plant leaf area, plant dry mass as well as the flowering time, and number of flower trusses plant⁻¹. Such positive effects were reflected on enhancement of plant yield (332.95 - 498.36 g plant⁻¹), early yield (2.629 - 5.249 ton fed.⁻¹), late yield (9.264 - 13.147 ton fed.⁻¹), total yield (13.175 - 17.721 ton fed.⁻¹), average fruit weight (14.873 - 19.537 g) and the marketable yield (12.532 - 17.305 ton fed.⁻¹), while the non-marketable yield significantly decreased by 44.9% - 79.8%. The antioxidants tested also exhibited a positive effect on fruit quality in terms of their chemical constituents. Total soluble solids (TSS) and vitamin C (V.C.) mostly increased with antioxidants treatments while fruit acidity did not show a distinct response. The water content of fruits, however, significantly decreased compared to the untreated control. On the other hand, total, free and conjugated phenols significantly increased in fruits of the treated plants (cv. Sweet Charlie). The correlation estimates revealed the association of grey mould disease incidence with the fruit quality and their chemical constituents. It was positively correlated with fruit water content ($r= 0.792 - 0.901$), TSS ($r= 0.656 - 0.925$), V.C. ($r= 0.544 - 0.950$), and phenolics content ($r= 0.648 - 0.704$), while it was weakly correlated ($r= 0.214 - 0.557$) with total fruit acidity of the tested strawberry cultivars in this stage of growth. The study confirmed potentials of the tested antioxidants as non-fungicidal environmentally safe means to control grey mould and to enhance yield and quality of strawberry.

Key words: Strawberry, grey mould, antioxidants, benzoic acid, vegetative traits, flowering traits, yield potential, yield components, fruit quality, chemical components.

INTRODUCTION

Strawberry (*Fragaria ananassa* Duch) is one of the most popular vegetable crops. In Egypt, it occupies an important position among the nontraditional vegetable crops due to its multifarious use

for local fresh consumption and food processing. Potentially, it is one of the most profitable horticultural Egyptian exports to Europe (El-Shall *et al.*, 2003). However, strawberry is such vegetable crop which has soft fruits and badly affected with several fungal diseases. Grey mould of strawberry or the so called the ash mould or the botrytis fruit rot caused by *Botrytis cinerea* (Pers. ex Fr.) is a problem wherever strawberry is grown and is probably the most serious fruit rot in strawberry worldwide (Terry *et al.*, 2007). *Botrytis cinerea* is a fungus that overwinters as sclerotia or dormant mycelia in old leaves, petioles, and mummified fruits. Fruit rot symptoms may occur on any portion of the fruit but frequently developed at the calyx end and in tissues contiguous with rotting fruits or diseased flowers. Fruit rot expands rapidly near harvest. In wet, warm seasons, probably no other disease causes a greater loss of flowers and fruits. Fungicides are an important disease management tool in commercial plantings. Growing the public concern over the use of fungicides, in such daily harvested and freshly eaten vegetable fruits, posed a pressure over specialists to seek non-traditional, safer materials to control the grey mould of strawberry. Antioxidants or the so called the free radical scavengers, which are food additives and mostly natural products, were found to have potentials to delay the onset, to inhibit, and to control several plant diseases including *Botrytis* grey mould on several vegetable crops (Prusky, 1988; Elad, 1992; Abd El-Magid *et al.*, 2004; Ramparasad *et al.*, 2004; Terry *et al.*, 2007). Besides, their effects to enhance yield and quality of the treated crops were documented (Abd El-Magid *et al.*, 2004; Namich, 2006). Meantime, the use of antioxidants was reported to enhance antioxidant level in treated fruits which is beneficial for human health (Rikika *et al.*, 2005). The present study therefore, was conducted to investigate the potentials of plant spraying with some recommended commercial and natural antioxidants to control grey mould on strawberry in the field and to investigate their effect on plant growth, yield and quality. Also, the study aimed to investigate the correlation between fruit quality and incidence of grey mould disease on fruits.

MATERIALS AND METHODS

Laboratory experiment

1.The *in vitro* effect of antioxidants on *Botrytis cinerea*.

The tested fungus.

The fungal pathogen *Botrytis cinerea* used in the present study was obtained by direct isolation from strawberry fruits showing grey mould disease collected from affected strawberry fields grown during the early 2005 at the Agricultural Experimental Farm, South El-Tahrir, El-behera governorate. The fungus was isolated and maintained on Czapek-Dox agar (Stewart, 1974). Developed cultures were purified by the single spore isolation technique and identified according to Barnett and Hunter (1987).

The antioxidants tested.

Three commercial antioxidants, *i.e.* butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), and *Tert*-butyl hydroquinone (TBHQ) were purchased from the Agrochemicals Co. India. Also, three other natural antioxidants, *i.e.* ascorbic acid, citric acid, and benzoic acid were obtained from El-Nasr Co., Egypt. These antioxidants were reported to have potential to control several fungal diseases on several crops (Elad, 1992; Abdel Rahman, 2005; Namich, 2006).

Fungal growth assessments.

Antioxidants were dissolved in 0.1% ethanol, 0.1% Triton B, suspended in sterile distilled water and applied to molten Czapek-Dox agar medium to obtain the desired concentrations (0.1, 1.0, and 10.0 mM) before pouring the plates according to Elad (1992). Mycelium disks (0.5 mm in diameter), taken from the actively growing margin of 7-day-old cultures, were centric inoculated on to amended plates and incubated at 22°C in darkness. Control plates were free of antioxidants. Four replicate plates were prepared for each treatment. Seven days after inoculation, diameter of the developed colonies on amended plates and control were measured. In addition, conidia of the developed colonies were harvested and counted according to Kulakiotu *et al.* (2004). This was conducted by flooding plates with

20 ml distilled water and gentle rubbing of culture surface with sterile L-shaped glass rod. The resulting conidial suspension was filtered through cheesecloth to remove hyphae. Then, density of conidia (sporulation) was determined under light microscope in four replicates and expressed as conidia per milliliter of water suspension. Furthermore, conidial suspensions (10^3 / ml), prepared from 2-week-old cultures as previously described, were spread over plates (1ml /plate, in four replicates) amended with the tested antioxidants to test the antioxidant effect on germination of conidia according to Elad (1992). Germination was observed under a light microscope within 24 h. and expressed as percent of the plated conidia.

Field experiments.

Strawberry cultivars.

Three cultivars of strawberry widely cultivated into Egypt were chosen. These cultivars were: the most sugary with excellent flavor cv. Sweet Charlie and the universally grown, high yielding cvs. Chandler and Earlibrite. Fresh transplants of the tested strawberry cvs. were obtained from Strawberry and Non-traditional Crops Research Station, Nobarria, El-Behera governorate, Egypt, and transplanted into the field at the 1st of October, and the 25th of September in the first and the second growing seasons, respectively.

Experimental procedure.

Two field experiments were conducted during the 2005-2006 and 2006-2007 growing seasons at the Experimental Station Farm, Horticultural Research Institute (South El-Tahrir), El-behera governorate, under drip irrigation system. Physical and chemical properties of the two experimental sites were determined according to methods described by Black (1965) and presented in Table (1). The experimental layout was a split-plot system in a randomized complete blocks design with four replications. Strawberry cultivars were arranged as the main plots and the antioxidants treatments were considered as the sub-plots. All horticultural procedures were applied as recommended for strawberry commercial production, except no fungicides were used.

Table 1: Physical and chemical analysis of of the two experimental sites of the 2005-2006 and 2006-2007 growing seasons.

Season	Physical characteristics			Chemical characteristics							
	Sand	Silt	Clay	EC (ds/mg)	pH	Soluble cations (mg/L)			Soluble anions (mg/L)		Total N (%)
						Ca	Mg	K	HCO ₃	SO ₄	
2005-2006	94	4	2	0.16	8.7	0.74	0.54	0.22	0.57	0.59	0.007
2006-2007	94	5	1	0.20	8.9	0.69	0.56	0.19	0.54	0.57	0.009

Antioxidants preparation and application.

All antioxidants tested, *i.e.* BHA, BHT, TBHQ, ascorbic acid, citric acid, and benzoic acid were dissolved in 0.1% ethanol plus 0.1% Triton B and suspended in sterile distilled water to obtain the desired concentration. The most consistent and effective concentration which inhibited growth, sporulation, and germination of the grey mould fungus *B. cinerea* in the laboratory *in vitro* tests was used in the conducted field studies. Antioxidants were sprayed onto plants until run-off at flowering stage 40 days after transplantation and repeated 6 times at 2-week-intervals according to Morsy and El-Bana (2000) and Namich, (2006).

2. Incidence of grey mould Disease.

Grey mould incidence was determined as percentage of plants showed grey mould on fruits in the four replicate sub-plots of each treatment. This was conducted at 60, 90, and 120 days after transplantation in each of the two growing seasons and the means of disease incidence were calculated.

3. Vegetative growth traits.

Five randomly selected plants were taken from each sub-plot at the full blooming stage. Measurements for the number of crowns plant⁻¹, the number of leaves plant⁻¹, plant leaf area (cm), and plant dry mass (g) were recorded.

4. Flowering traits

Ten randomly chosen plants from each sub-plot were labeled to record the time of flowering as the number of days from transplantation till flowering of 25% of the plants, and to count the number of flower trusses per plant till the end of experiment.

5. Yield potentials.

- a. Early fruit yield was calculated in ton fed.⁻¹ as the fresh weight of harvested fruits from the first four pickings.
- b. Late fruit yield was calculated in ton fed.⁻¹ as fresh weight of harvested fruits after the first four pickings until the end of the season.
- c. Total yield was calculated in ton fed.⁻¹ as the fresh weight of all harvested fruits over the growing season.
- d. Yield plant⁻¹ (g) was calculated in a random sample of ten plants per sub-plot at each harvest.

6. Yield components.

- a. Average fruit weight (g), was calculated in a random sample of ten plants per sub-plot at each harvest.
- b. Non-marketable yield (ton /fed.), including splitted, malformed, green shouldered, damaged and rotted fruits was determined at each harvest.
- c. Marketable yield (ton /fed.) was calculated at each harvest.
- d. Percentage of recovery (%R) was calculated as [non-marketable yield in untreated control – non-marketable yield in a certain treatment] / non-marketable yield in untreated control x100.

7. Fruit quality.

Random samples of ten fruits were taken from each sub-plot, at monthly intervals starting from the first harvest, to determine the following characteristics:

- **Water content (%)**: was determined on fresh weight basis by calculating the difference between fruit fresh weight and oven dried (70°C until a constant weight) fruits/ fruit fresh weight x 100.
- **Total soluble solids (TSS%)**: was determined using a Carl Zeiss hand refractometer.
- **Titrateable acidity**: according to the methods of AOAC (1992) and expressed as (%) citric acid.
- **Vitamin C (V.C.) content**: was determined according to the methods of AOAC (1992) and expressed as (mg /g fresh weight).

8. Total , free and conjugated phenols.

Phenolics content: were determined according to the colourimetric methods of analysis of Snell and Snell (1953), using the Folin-Ciocalteu phenol reagent. Total and free phenols were qualified in 10g homogenate samples and concentrations were calculated by preparing a chlorogenic acid standard curve and expressed as mg/g fresh weight. Conjugated phenols were determined as the difference between the total and free phenols.

Statistical analysis. The obtained data were statistically analyzed using the American SAS/STAT Software, version 6 (SAS Institute Inc., Cary, USA) and means were compared by the least significant difference test (LSD). Correlation analyses were conducted using the data analysis program on the Microsoft Office Excel, 2003.

RESULTS

1. The *in vitro* effect of antioxidants on growth characteristics of *Botrytis cinerea*.

All the tested antioxidants showed an *in vitro* inhibition effect, to different degrees, on colony growth, sporulation, and germination of the grey mould fungus *Botrytis cinerea*. Colony diameter of *Botrytis cinerea* developed on agar amended with the tested antioxidants was 6.59 - 8.12 cm compared to 8.36 cm for the unamended control. This reflected an inhibition effect of 2.87% - 21.17% on fungus colony growth, and the inhibition rate generally increased with increasing concentration of applied antioxidants. Antioxidants also affected sporulation of the fungus in a more obvious manner. Numbers of conidia developed on the amended plates were 9.33 - 13.56 $\times 10^4$ per each cm^2 of the developed colony compared to 16.35 $\times 10^4/\text{cm}^2$ for the unamended control. This reflecting inhibition effect of 17.06 - 42.93% relative to the unamended control. Meantime, percentages of conidia germination on amended agar were 21.96-77.59% which reflected inhibitions of 9.26-64.89% with the different antioxidants concentrations tested. The 1.0 mM concentration, however, showed the most consistent and pronounced effect with most used antioxidants for most growth parameters tested compared to the other two concentrations, *i.e.* 0.1 mM; 10.0 mM, which gave an advantage for the 1.0 mM concentration to be used in the field investigations (Table 2).

Table 2: The *in vitro* effect of certain antioxidants on growth, sporulation and germination of *Botrytis cinerea* the causal fungus of grey mould of strawberry.

Tested antioxidants	Conc. (mM)	Growth (cm)		Sporulation ($\times 10^4/\text{cm}^2$)		Spore Germination	
		C. D.	% IH	S. V.	% IH	% Ger.	% IH
BHA	0.1	7.94	05.02	12.94	20.85	69.54	17.31
	1.0	6.83	18.30	11.92	27.09	37.68	49.17
	10.0	6.69	19.97	12.37	24.34	52.21	34.64
BHT	0.1	6.98	16.50	11.28	31.00	54.73	32.12
	1.0	6.87	17.82	9.33	42.93	21.96	64.89
	10.0	6.59	21.17	9.62	41.16	25.64	61.21
TBHQ	0.1	8.02	04.06	13.19	19.32	71.46	15.39
	1.0	7.40	11.48	12.47	23.73	52.01	34.84
	10.0	7.54	09.80	10.12	38.10	45.66	41.19
Citric acid	0.1	8.12	02.87	13.56	17.06	77.59	09.26
	1.0	7.01	16.14	11.96	26.85	65.60	21.25
	10.0	7.83	06.33	12.21	25.32	68.53	18.32
Ascorbic acid	0.1	7.84	06.22	12.13	25.81	64.18	22.67
	1.0	6.77	19.01	11.04	32.47	29.91	56.94
	10.0	6.63	20.69	9.89	39.51	43.35	43.50
Benzoic acid	0.1	7.87	05.86	10.85	33.63	51.34	35.51
	1.0	6.62	20.81	9.73	40.48	22.13	64.72
	10.0	6.75	19.25	9.54	41.65	31.26	55.59
Control		8.36	0.0	16.35	0.0	86.85	0.00
LSD		0.81		3.16		6.37	

C.D.= Colony diameter, S.V.=Sporulation value as number of conidia developed on one cm^2 of the fungal colony, %IH= Percentage of inhibition relative to the nonamended control, %Ger.= Percentage of spore germination relative to the total number of plated conidial spores. Spore germination was estimated on water agar as mean of five replicates counted under Zeiss inverted microscope.

2. Incidence of grey mould disease.

Spraying of the antioxidants tested onto strawberry plants (cvs. Sweet Charlie; Chandler and Earlibrite) grown in a field naturally infested with grey mould fungus (*Botrytis cineria*) during the 2005-2006 and 2006-2007 growing seasons, significantly, affected incidence of grey mould on fruits of the treated plant cultivars (Table 3). In the first season of 2005-2006, disease incidence on cv. Sweet Charlie, significantly, decreased to 14.47-26.94% compared to

46.35% on the untreated control. This was reflected in suppression in grey mould incidence ranged between 41.87% and 68.78% for the different antioxidants compared to the untreated control. The most pronounced suppression effect (68.78%) was recorded with BHT application and followed by BHA, benzoic acid, ascorbic acid, TBHQ, and citric acid where the disease suppressions were 64.33%, 62.63%, 57.15%, 56.59%, and 41.87%, respectively. Less suppressive effects were recorded, however, with antioxidant applications on the other two strawberry cultivars tested. These were 24.68-54.66% and 38.94-58.98% for cvs. Chandler and Earlibrite, respectively, and the manner of suppression with the different antioxidants was almost similar to that on cv. Sweet Charlie. In the second season of 2006-2007, disease incidence occurred on the three tested cultivars also decreased with the antioxidant applications. This was in the range of 9.12% - 17.15% compared to 21.23% - 29.58% in the untreated control which reflected suppression of 19.21- 69.16% on the developed disease. This was in a manner almost similar to the suppression effect recorded in the 2005-2006 growing season with the different antioxidants tested. The cv. Sweet Charlie exhibited the most pronounced response to antioxidant application while cvs. Chandler and Earlibrite exhibited less obvious responses over the two growing seasons (Table 3).

3. Vegetative growth traits.

Spraying growing plants of the three tested strawberry cvs., *i.e.* Sweet Charlie; Chandler and Earlibrite, with the tested antioxidants showed significant increments on most studied vegetative growth parameters during the 2005-2006 growing season (Table 4). Higher averages in number of crowns plant⁻¹ (3.748-5.954), number of leaves plant⁻¹ (21.24-27.81), plant leaf area (332.74-431.93 cm²), and plant dry mass (8.93-12.18 g) were recorded with antioxidant applications on tested cultivars. This was compared to 3.196-3729, 19.29-23.68, 309.72-331.65 cm² and 8.12-9.14 g for the same traits, respectively, in the untreated control (Table 4). It was also evident that BHT antioxidant exhibited the most pronounced effects on most parameters. This was followed, mostly, by BHA, ascorbic acid, benzoic acid, TBHQ, and citric acid. However, the obtained effects were not always significant with TBHQ and Citric acid on several vegetative parameters.

Results of the second growing season (2006-2007) were generally consistent with results of the first growing season and showed similar trends on the three tested strawberry cultivars (Table 4).

4. Flowering traits.

Data in Table (5) revealed that the various antioxidants applied during the 2005-2006 growing season significantly affected both of the flowering traits studied. This was reflected as an earlier flowering date ranged between 46.754 and 54.846 day for the three cvs. tested compared to 55.241 - 61.722 days for the untreated control. The most pronounced effects were linked to BHA and benzoic acid, followed mostly with BHT, ascorbic acid, TBHQ, and citric acid, respectively. This was generally consistent with results obtained in the second season.

Meantime, application of antioxidants enhanced number of flower trusses plant⁻¹ in both seasons. This ranged between 4.835 - 7.356 plant⁻¹ and 4.391- 6.986 plant⁻¹ compared to 3.772-4.364 plant⁻¹ and 3.823 - 4.125 plant⁻¹ for the first and the second seasons respectively. The enhancing effect was most obvious with BHA, BHT, and benzoic acid followed by ascorbic acid, TBHQ, and citric acid on the three tested strawberry cultivars which were, almost, equally responded in this respect (Table 5).

5. Yield potentials.

Data presented in Table (6) showed that field application of the antioxidants tested to strawberry cvs. grown in a field naturally affected with the grey mould disease significantly enhanced strawberry yield potentials during the first season of 2005-2006. This was revealed in terms of mean plant yield (338.26 – 483.41 g plant⁻¹), early yield (2.629 – 5.249 ton fed.⁻¹), late yield (9.264 – 12.966 ton fed.⁻¹) and total yield (13.427 – 16.139 ton fed.⁻¹). compared to 293.11 – 331.87 g plant⁻¹, 2.518 – 3.268 ton fed.⁻¹, 8.463 – 10.239 ton fed.⁻¹, and 11.261 – 12.757 ton fed.⁻¹, respectively, in the untreated control. The BHT applications exhibited the most pronounced effects on the three cvs. tested.

This was mostly followed by benzoic acid, BHA, ascorbic acid, TBHQ, and citric acid, respectively. These effects were most obvious on cv. Sweet Charlie compared to cvs. Chandler and Earlibrite. Results obtained in the second season of 2006-2007 were, generally, in consistent with those of the first season. The BHT treatment however, exhibited an even more obvious effect (Table 6).

6. Yield components.

Data in table (7) showed that antioxidants, tested in the 2005-2006 growing season, had a significant effect on yield components of the strawberry cvs. tested. This was reflected in a higher average fruit weight ranged between 15.635 g and 19.469 g compared to 13.692 g and 14.743 g for the untreated control. Ascorbic acid, BHT, BHA, and benzoic acid showed the most pronounced effects while TBHQ and citric acid showed the lowest effect. Meantime, antioxidant applications significantly decreased non-marketable yield to 0.411-0.978 ton fed.⁻¹ in the tested cvs. compared to 1.803-2.039 ton fed.⁻¹ in the untreated control. Such a result was reflected in yield loss recovery ranged between 47.1% and 79.8% of the untreated control which positively increased the obtained marketable yield in the range of 12.629 ton fed.⁻¹ - 15.728 ton fed.⁻¹ for the different applications. The results of the second season (2006-2007) confirmed those of the first one and showed similar trends with the different yield components (Table 7).

7. Fruit quality.

Data in Table (8) showed that antioxidant applications during the 2005-2006 growing season had significant effects on most parameters of fruit quality of the three strawberry cvs. tested. Water contents of fruits of the treated plants were significantly affected and decreased to 89.172-91.453% compared to 91.632-93.248% in the untreated control of the tested cultivars. The most pronounced effects were mostly recorded with benzoic acid and BHT applications. Meantime, TSS significantly increased to 7.254-9.793 % with antioxidant applications in the tested cvs. compared to 5.346 - 6.192 % in the untreated control.

The most pronounced increase was recorded mostly with ascorbic acid, BHT, benzoic acid, and citric acid applications while TBHQ and BHA exhibited the lowest effect. The titratable acidity, however, did not reflect a distinct trend except that with citric acid antioxidant application where significant increments were recorded in the three strawberry tested cultivars. On the other hand, vitamin C (V.C.) content was affected in a manner almost similar to that of antioxidants on TSS. Results of the second season of 2006 - 2007, generally, supported the aforementioned 2005-2006 results and revealed similar trends (Table 8).

8. Correlation between fruit quality and grey mould disease incidence

Correlation estimates reflected the associations of fruit quality of the tested strawberry cvs. with the percentage of grey mould incited on their fruits in the field. There were proportionate increases in disease incidence on fruits with the increased water content and acidity of fruits. However, disease incidence appeared to be highly correlated ($r=0.792 - r=0.901$) with fruit water content while a low correlation ($r=0.214 - r=0.557$) was revealed with fruit acidity of the three tested cultivars (Fig. 1). On the other hand, inverse proportions were revealed between disease incidence and both of fruit V.C. and TSS content. A correlation coefficient of $r=0.44-0.950$ was recorded for fruit V.C. and $r=0.656 -0.925$ for fruit TSS with the highest correlation values were linked to cv. Sweet Charlie (Fig. 1).

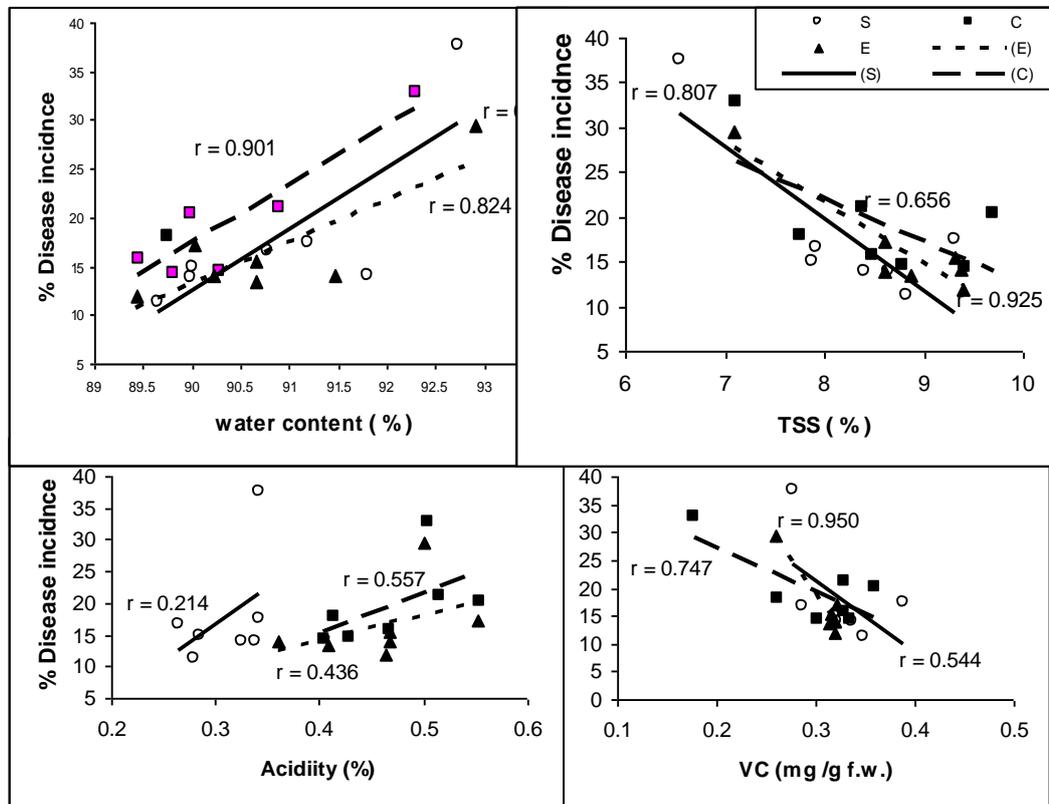


Fig. 1: Correlations between fruit quality of strawberry (cvs. Sweet Charlie, Chandler and Earlibrite) as affected by antioxidant field applications and grey mould disease incidence on strawberry fruits calculated from the 2005-2006 & 2006-2007 combined data. S= cv. Sweet Charlie, C= cv. Chandler, E= cv. Earlibrite.

9. Total, free and conjugated phenols contents

The cv. Sweet Charlie, which was the most responding strawberry cultivar for antioxidant application, was subjected to further investigation for phenolics contents which are most associated with resistance and susceptibility to grey mould disease in plants. In the first season of 2005-2006, total phenols significantly increased

with antioxidant applications in fruits of the treated plants. This was in the range of 1.493 - 2.746 mg/g f.w. compared to 0.827 mg/g f.w. for the untreated control. This was most pronounced with benzoic acid followed by BHT, ascorbic acid, BHA, TBHQ, and citric acid, respectively. Meantime, free phenols and conjugated phenols increased to 1.129 - 2.186 mg/g f.w. and 0.349 - 0.619 mg/g f.w., respectively compared to 0.589 mg/g f.w. and 0.238 mg/g f.w. in the untreated control. It was also obvious that both free and conjugated phenols were affected in a manner, almost, parallel to that of the total phenols. On the other hand, the 2006-2007 recorded data showed similar trends with the antioxidant applications for the total, free, and conjugated phenols in fruits of the treated strawberry plants (Table 9).

Table 9: Effect of plant spraying with certain antioxidants on fruit phenolics content of strawberry plants (cv. Sweet Charlie) grown in a field naturally infested with the grey mould fungus (*Botrytis cineria*) during the 2005-2006 and 2006-2007 growing seasons.

Antioxidants	2005-2006 season			2006-2007 season		
	Total	Free	Conjugated	Total	Free	Conjugated
BHA	1.983	1.634	0.349	0.981	0.637	0.344
BHT	2.735	2.163	0.572	1.482	1.214	0.268
TBHQ	1.715	1.334	0.381	0.846	0.621	0.225
Citric acid	1.493	1.129	0.364	0.683	0.462	0.221
Ascorbic acid	2.716	2.186	0.530	1.367	1.058	0.309
Benzoic acid	2.746	2.127	0.619	1.530	1.198	0.332
Control	0.827	0.589	0.238	0.437	0.313	0.124
LSD	0.646	0.447	0.111	0.296	0.318	0.092

Total, free and conjugated phenols were expressed as mg /g f.w.

10. Correlation between phenolics content and grey mould disease incidence.

Incidence of grey mould on strawberry fruits (cv. Sweet Charlie) was found to be correlated with their phenolics content. An inversely proportional relationship was revealed between phenolics content and grey mould disease incidence where a decrease was

recorded in disease incidence with increasing any of the total, free, and conjugated phenols. However, free and conjugated phenols exhibited very close correlation values being $r = 0.704 - 0.701$, respectively, which both were higher than that of the total phenols where a lower correlation value of $r = 0.648$ was recognized (Fig 2).

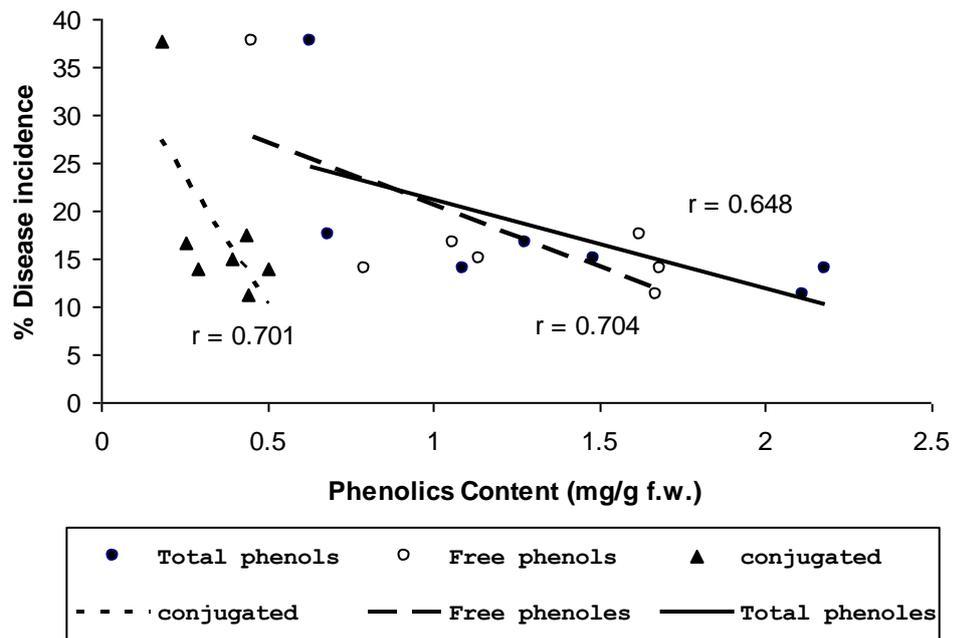


Fig. 2: Correlation between total, free, and conjugated phenol contents of strawberry (cv. Sweet Charlie) fruits, as affected by antioxidant field applications, and incidence of grey mould disease on fruits estimated from the 2005-2006 & 2006-2007 combined data.

DISCUSSION

The use of antioxidants (the free radicals scavengers) proved to have the potential to decrease the grey mould disease incidence on strawberry. The three commercial antioxidant tested, *i.e.* Botylated hydroxy anisole (BHA), botylated hydroxy toluene (BHT) and *Tert*-butyl hydroquinone (TBHQ) as well as the three natural antioxidants, *i.e.* benzoic acid, ascorbic acid, and citric acid showed *in vitro* inhibition effects in different degrees on colony growth (2.87% - 21.17%), sporulation (17.06 - 42.93 %) and conidia germination (9.26 - 64.89 %) of the grey mould fungus *Botrytis cinerea* compared to the unamended control. The 1.0 mM concentration tested showed the most consistent and pronounced effect with most antioxidants compared to the other tested concentrations tested, *i.e.* 0.1mM; 10.0 mM, which gave an advantage for 1.0mM concentration to be used in the field investigations. The field studies conducted confirmed this effect as plant spraying with the tested antioxidants, significantly, suppressed incidence of grey mould (19.91% - 69.16%) on the three tested strawberry cultivars, *i.e.* Sweet Charlie; Chandler and Earlibrite, compared to the untreated control. The most pronounced suppression effects were mostly linked to BHT application which was, mostly, followed by BHA, benzoic acid, ascorbic acid, TBHQ, and citric acid, respectively. These effects were positive on the three tested cvs. while cv. Sweet Charlie, generally, exhibited the highest susceptibility to grey mould and the highest response to antioxidant applications compared to the other two cultivars. These findings were in harmony with results obtained with antioxidants against several fungal pathogens on several plant crops by different investigators (Elad, 1992; Galal and Abdou, 1996; Morsy and El-Bana 2000; Shahda, 2000; Abd El-Magid *et al.*, 2003; El-Shazly and Morsi, 2003; Fouly, 2004; Shaat and Galal, 2004; Abdel Rahman, 2005; Namich, 2006). The disease suppressive effect with antioxidant could be explained, in a way, in view of that most antioxidants are phenolic compounds known of their antifungal potential (Arnoldi *et al.*, 1989; Rice-Evans, *et al.*, 1997; Amborabe *et al.*, 2003), or it might be attributed to the potential of antioxidants to inhibit fungal differentiation through different enzymatic processes (Georgiou *et al.*, 2000). It may also be explained in view of the effect of antioxidants

to manipulate strawberry fruit softening by anti-senesce expression of pectate lyase gene (Bermudez, 2002), or by their effect as free radical scavengers to eliminate or stabilize free radicals generated either by infected tissues or the pathogen during pathogenesis to form harmless compounds which could control deterioration of host tissues and retard disease development (Edlich, 1989; Elad, 1992; Rikika *et al.*, 2005).

On the other hand, results of the present study showed an enhancement effect for antioxidant on growth characteristics and yield of the tested strawberry cultivars grown in a field naturally affected with the grey mould disease. Field plant spraying with the tested antioxidants showed significant increments on most studied vegetative. Higher averages in number of crowns plant⁻¹ (3.242 - 5.956), number of leaves plant⁻¹ (21.24 - 27.81), plant leaf area (326.52 - 431.93 cm²) and plant dry mass (8.12 - 12.46 g) were recorded with antioxidant applications on tested cultivars compared to untreated control. It was also evident that BHT antioxidant exhibited the most pronounced effect for most parameters. This was followed, mostly, by BHA, benzoic acid, ascorbic acid, TBHQ and citric acid, respectively. Also, antioxidants application significantly affected the various flowering traits studied. This was reflected in an earlier flowering date (46.163 - 57.821 days), and higher number of flower trusses plant⁻¹ (4.835-7.356). This was most obvious with BHT, BHA and benzoic acid followed by ascorbic acid, TBHQ and citric acid, respectively. This was positively reflected on higher yield potentials and improved yield components of the tested cultivars. Antioxidants significantly increased early yield (2.629- 5.249 ton fed.⁻¹), late yield (9.264 - 13.147 ton fed.⁻¹) and total yield (13.175 - 17.721 ton fed.⁻¹) compared to the untreated control. Meantime, antioxidants improved yield components of the strawberry cvs. tested reflected in a higher average fruit weight (14.873 - 19.537 g), increased marketable yield (12.532 - 17.305 ton fed.⁻¹) and decreased non-marketable yield by 44.9% - 79.8% of the untreated control. This could be explained in view of the revealed effects of antioxidants to suppress the developed disease. This could be also explained in view of potential of such antioxidants as free radicals scavengers to eliminate the deleterious effects of free radicals evolved by plants during their metabolism or by pathogen after infection which definitely was reflected in a good

health in strawberry plants, higher yield potentials and better yield components. These findings were in agreement with Elad (1992), Hancock (1999), Morsy and El-Bana (2000), El-Araby *et al.* (2003), El-Shazly and Morsi (2003), Ghoneim *et al.* (2003), Navapour *et al.* (2003), Abd El-Magid *et al.* (2004), Fouly (2004), Karabulut *et al.* (2004), Lima *et al.* (2005) and Namich (2006).

The antioxidant effect as a safer means to control grey mould in strawberry was evaluated in view of their effect on strawberry fruit quality. Spraying antioxidants onto strawberry plants grown in fields affected with the grey mould, in the present study, produced more firmer fruits with relatively lower water content of 89.172 - 91.453 % where BHT and benzoic acid antioxidants exhibited the most pronounced and consistent effect in this respect. Also, TSS content significantly increased (7.062-9.793 %) in the tested cvs. with the most pronounced increase was recorded with ascorbic acid, BHT, and benzoic acid. The V.C. content also increased and was affected in a manner almost similar to that of TSS while the titratable acidity did not reflect a distinct trend in the three tested cultivars. Meantime, total, free and conjugated phenols in fruits of the treated plants (cv. Sweet Charlie), significantly increased (1.8 - 3.3 fold) with antioxidants applications. This was most pronounced with benzoic acid which was followed by BHT, ascorbic acid, BHA, TBHQ, and citric acid, respectively. Correlation coefficient estimates revealed the association of the fruit quality, *i.e.* their chemical constituents, with the grey mould disease incidence on fruits. A proportionate increase in disease incidence on fruits was revealed with increasing their water content and acidity. However, disease incidence appeared to be highly correlated ($r=0.792 - 0.901$) with fruit water content while low correlation values ($r=0.214 - 0.557$) were revealed with fruit acidity. On the other hand, an inverse proportional relationships were revealed between disease incidence and both of fruit V.C. and TSS content. Correlation coefficients of $r=0.544 - 0.950$ were recorded with fruit V.C. while correlations of $r=0.656 - 0.925$ were obtained for fruit TSS of the tested strawberry cultivars. Meantime, inversely proportional relationships ($r=0.648 - 0.704$) were revealed with phenolics contents and grey mould disease incidence. Phenolics compounds are known of having potentials as antifungal compounds to provide protection against fungal attacks in plants (Arnoldi *et al.*, 1989; Gil, *et al.*, 1997),

besides, their benefits for human health as an anticarcinogen and to reduce coronary heart disease in human were reported (Mass *et al.*, 1990). These results in general were in harmony with findings of several investigators (Rice-Evans *et al.*, 1997; Shahda, 2000; Bermudez *et al.*, 2002; Navapour *et al.*, 2003; Abd El-Magid *et al.*, 2004; Abdel Rahman, 2005; Namich, 2006; Terry *et al.*, 2007). However, variability in the effect of the antioxidants can be attributed to differences in their activity or variation in the response of host plants, or to the ability of compounds to penetrate host tissues. It is still not clear whether the antioxidant applications suppressed grey mould disease which was reflected in the enhancement obtained in growth and quality of strawberry or that the antioxidant applications enhanced growth and quality of strawberries and, consequently, plants getting more tolerant to the grey mould disease. More studies are still needed to verify these ideas and to elucidate the mode of action of antioxidants on both host plant and pathogen and to find cheaper antioxidants for a wider applicable use. Farmers are strongly encouraged to adapt such environmentally sound measures for management of strawberry diseases. The use of antioxidants may not control diseases to a level which may replace the use of fungicides, however, their integration into current disease management practices could reduce the fungicide use and the associated environmental problems.

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الملخص العربي استخدام مضادات الأكسدة في مكافحة العفن الرمادى وتحسين محصول وجودة الفراولة

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فى دراسة معملية وحقلية تم إختبار مجموعة من مضادات الأكسدة التجارية والطبيعية ، وهى بيوتيلاتيد هيدروكسي تولوين (BHT) ، بيوتيلاتيد هيدروكسي انيزول (BHA) ، وتيرت بيوتيل هيدروكينون (TBHQ) ، حمض البنزويك ، حمض الاسكوربيك ، وحمض الستريك ، كمواد آمنة لمكافحة مرض العفن الرمادى فى الفراولة ، وتتبع تأثيرها على النمو والإزهار والمحصول والجودة والتركيب الكيماوى فى ثلاثة من أصناف الفراولة التجارية وهى ، سويت شارلى ، ايرلى برايت ، تشاندلر ، أوضحت الدراسة المعملية فعالية مضادات الأكسدة المختبرة بتركيزات 0.1 مللى مول ، 1 مللى مول ، 10 مللى مول ، فى تثبيط نصف قطر نمو الفطر المسبب للمرض (*Botrytis cinerea*) وذلك على بيئة تشابك ، بنسبة 87 ، 2 ، 17 ، 21 % ، وكذا تثبيط عدد الجراثيم الكونيدية المتكونة بنسبة 17 ، 06 – 93 ، 42 % ، وكذا تثبيط إنبات الجراثيم الكونيدية بنسبة 26 ، 9 – 89 ، 64 % ، وكان التركيز 1 مللى مول هو الأكثر ثباتا وفعالية . وفى دراسة حقلية على مدى الموسمين 2005-2006 ، 2006-2007 بالمزرعة التجريبية بجنوب التحرير معهد بحوث البساتين أظهرت المعاملة بمضادات الأكسدة بتركيز 1 مللى مول رشاً على نباتات الفراولة المنزرعة فى حقل مصاب طبيعياً بفطر العفن الرمادى ، تثبيطاً للمرض على الأصناف الثلاثة المختبرة ، بنسبة 19 ، 21 – 69 ، 16 % ، وكان ترتيب مضادات الأكسدة من حيث تأثيرها لمكافحة المرض هى BHT ، BHA ، حمض البنزويك ، حمض الاسكوربيك ، بينما كان حمض الستريك و TBHQ ، الأقل تأثيراً وكذا فقد أدى استخدام مضادات الأكسدة إلى زيادة معنوية فى صفات النمو الخضرى من حيث ، عدد التيجان ، عدد الأوراق ، المساحة الورقية ، والوزن الجاف للنبات ، وكذا عدد العناقيد الزهرية ، كما أدى إلى التذكير فى الإزهار وذلك فى كلا الموسمين فى الأصناف المختبرة ، وقد أدى هذا إلى زيادة معنوية فى محصول النبات الواحد (95 ، 332 - 36 ، 498 جرام) و المحصول المبكر (2 ، 629 - 5 ، 249 طن للفدان) و المحصول الكلى (13 ، 175 - 17 ، 721 طن للفدان) ، بينما حدث نقص معنوى فى المحصول التالف بنسبة 9 ، 44 - 8 ، 79 % مقارنة بالكنترول وكان ذلك مصحوباً بزيادة معنوية فى المواد الصلبة الذاتية و فيتامين ج ، بينما حدث نقص معنوى فى المحتوى الرطوبى للثمار مقارنة بالكنترول فى الأصناف المختبرة ، كما أدى استخدام مضادات الأكسدة إلى زيادة معنوية فى محتوى الثمار من الفينولات الكلية والحررة والمرتبطة ، وقد أظهر تحليل الارتباط وجود علاقة طردية مع ارتباط قوى بين نسبة الإصابة والمحتوى الرطوبى للثمار (معامل ارتباط 0.901 – 0.792) ، والمحتوى من الفينولات (0.648 – 0.704) . بينما كان الارتباط ضعيفا مع نسبة الحموضة فى مرحلة النمو المختبرة . وأظهرت الدراسة فعالية مضادات الأكسدة كمواد آمنة لمكافحة العفن الرمادى فى الفراولة وتحسين النمو والمحصول والجودة ، وتجنب المشاكل البيئية المرتبطة باستخدام المبيدات الزراعية .

