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Antioxidants for Controlling Common Seed-Borne Fungi Attacking Cotton Plants and Scaling up Both Yield and Fiber Quality

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ABSTRACT

There are many fungi could be isolated from cotton seeds. In this research the following fungi were isolated: *Alternaria alternate*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceous*, *Cephalosporium* sp., *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium semitectum*, *Fusarium solani*, *Penicillium* sp., *Rhizoctonia solani*, *Rhizopus* sp., *Stemphylium* sp., *Trichothecium* sp. and *Verticillium* sp. Pathogenicity test showed that *Fusarium oxysporum*, *Fusarium solani*, *Fusarium moniliforme* and *Rhizoctonia solani* were the most devastative fungi on cotton seeds. The use of trisodium orthophosphate (1.9 g L^{-1}) significantly, reduced the incidence of the ungerminated seeds, post-emergence damping off and the abnormal seedlings. Soaked seeds prior to the sawing followed by 3 times of spraying cotton seedlings with the target antioxidant or formulated antioxidants GAWDA® formulation at the rate of 2.2 g L^{-1} significant increased the contents of chlorophyll a, b, carotenoid and total phenols in the plant leaves at the age of (30, 45, 60, 75) days old. Plant height, shoot and root lengths, shoot and root weights, number of cotton bolls, number of branches% and weight of bolls were increased by 30.05, 29.90, 80.34, 82.20, 86.53, 121.08, 168.80, 242.96 and 140.54%, respectively. Plant dry weight was increased by 82.24%, cotton seed yield by 142.77% and lint cotton was increased by 22.09%. The quality of the cotton fibers including; Micronair, length (mm) UHM, Regularity %, Durability gm/tex, Elongation%, the degree of color reflection (Rd) and the degree of yellowing b+ were also increased by 19.49, 4.00, 6.36, 16.95, 9.35, 11.86 and 6.49%, respectively. These results are persuasive evidence directed to cotton growers to apply this method for controlling seed-borne fungi of cotton, improve cotton yield, promote the quality of the cotton fibers, as well as increase the seed cotton production and subsequently the extracted oil.

Key words: Cotton fibers, yield, antioxidants, promotion, pathogenic fungi

INTRODUCTION

Cotton (*Gossypium barabadense*) is one of the major economic crops in a number of countries including African tropics, Australia, China, India, Sudan and Egypt, as well as the wormer regions of Central and South America (Kirkpatrick and Rothrock, 2001; Compendium of Cotton Disease, APS). Several decades back this crop was valued only for its extraordinarily strong, fine and durable fibers. At present, it is found that the improved are Gossypol free seeds, so as to be good and valuable source of food and feed (Alford *et al*., 1996; Gerasimidis *et al*., 2007).

A number of diseases i.e., gray mold, powdery mildew, damping off, *Verticillium* and *Fusarium* wilts are negatively affect the development of cotton growth. Wang *et al*. (1992) recorded a high

frequent incidence of *Fusarium moniliforme* and *F. semitectum* on cotton seedlings and cotton bolls, but *F. oxysporum*, *F. solani, F. equiseti* and *F. compctum* were less frequent. *F. oxysporum* and *F. solani* presented as the most colonizing spp. in the rhizoplane of cotton roots. Wang and Davis (1997) verified the ability of *Rhizoctonia solani* to damage cotton roots.

A friendly environmental approach to stop or retard the development of disease incidence disclosed in the past few decades inducing the use of natural plant defense activators i.e., Salicylic acid and jasmonic acid (Pluskota *et al*., 2007; Tamaoki *et al*., 2008) resulting in the acquired systemic resistance and manifest a long lasting protection against a broad numbers of plant pathogens.

Impact of using antioxidants and antioxidant formulations as plant growth promotors were carried out by a number researchers, who illustrated that some selected antioxidants singly or in formulations increased the productivity of the tested crops and produced high quality seeds (Elwakil and El-Metwally, 2000; Elwakil, 2003; Farouk *et al*., 2008; Abd El-Hai *et al*., 2009).

Based upon the above accumulated data, the objective of this research was planned to study the possible effect of using antioxidants or formulated antioxidant (GAWDA®) for accelerating the systemic acquired resistance in cotton plants and overcome colonization and invasion of the target pathogenic fungi mentioned above on seeds and in roots. This approach, if succeeded, it may be recommended to the cotton growers as an innovative tactic to produce higher yield of quality fibers and seeds.

MATERIALS AND METHODS

Source of cotton seeds: Seed lots of cultivars Giza 86 and Giza 92 were donated by Delta Ginning Company at Mansoura city, Egypt. Also 18 seed samples were collected from the cotton growers at scattered locations in the Nile Delta of Egypt.

Isolation of seed- borne pathogens: Seed Health Testing (SHT) following the rules of the International Seed Testing Association (ISTA., 1996) was carried out to record seed-borne fungi of historical potential to cause wilt disease on cotton plants.

The isolated fungi were subjected to identification in consultation with the description sheets of CABI (formerly CAB International and before that Commonwealth Agricultural Bureaux), England, Danish Government Institute of Seed Pathology (DGISP) publications as well (Booth, 1985; Burrges *et al*., 1988; Chidambaram *et al*., 1973; Moubasher *et al*., 1977; Ellis, 1971; Raper and Fennel, 1965; Singh *et al*., 1991).

Pathogenicity tests: The isolated fungi with history to attack cotton plants i.e., *Fusarium oxysporum* (2), *F. solani* (3), *F. moniliforme* (4) and *Rhizoctonia solani* (5) were used to study their possible adverse effect on the growth of cotton seedlings. Twenty pots of 20 cm diameter having 1/2 kg each of sandy loam soil were infested with each of the target fungus separately, watered with tab water and left for 3 day to allow the fungus to adapt to the newly soil condition. Seeds were then seeded (5 seeds/pot) and watered again with tap water.

The check (control) presented apparently healthy looking seeds were seeded in pots of sandy loam soil and watered.

The above fungi of potential to attack cotton seedlings were investigated to study their effect on the plant growth at different stages in the presence of selected antioxidants or formulated one (GAWDA® formulation).

Antioxidants and antioxidant formulation: The following antioxidants were used in this research i.e., Trisodium ortho phosphate (1.9 g L^{-1}), Disodium hydrogen ortho phosphate (1.4 g L^{-1}) and Potassium sodium (+) -tartrate (1.4 g L^{-1}) as well as, the formulated antioxidant GAWDA® (2.2 g L^{-1}) (Patent No 23798) consists of (Tartaric acid 2 mM+Hydroxyquinoline 1 mM+Calcium Chloride 6 mM+Magnesium Chloride 5 mM+Calcium Borate 5 mM).

Field experiments: Field plots of (3×3.5) square meter/each were designed to plant cotton seeds treated with the above antioxidants. Plant growth, number and size of cotton balls and quality of cotton fibers and their yield were recorded.

Determination of photosynthetic pigments in cotton leaves: The third upper parts of cotton leaves presenting 30, 45, 60, 75 days old plants were used to determine their contents of the photosynthetic pigments. Method described by Mackinney (1941) was followed.

Determination of total phenols: Samples of fresh shoots of 30, 45, 60, 75 days old plants were collected to determine their contents of total phenols using Foline-ciocalteau reagent and the method described by Singletonand Rossi Jr. (1965) was followed.

Cotton yield: Yield of none ginned cotton collected from the field plots was weighted and recorded. On the other hand, the lint percentage was calculated using the following equation:

$$\text{Lint } (\%) = \frac{\text{Weight of lint cotton yeild (g)}}{\text{Weight of cotton seeds yeild (g)}} \times 100$$

Seed oil measurement: Content of cotton seed oil collected from the experimental field in compare with the check of the same weight were carried out following the method of AOAC (2005).

Analysis of cotton fibers: The following characters of cotton fiber i.e., Micronair, Length, Regularity, Durability, Elongation, degree of color reflection and degree of yellowing were measured or calculated in samples of 150 g cotton lint/in each treatment (ASTM., 1986).

The fiber length (Upper Half Mean (U.H.M) in mm and Uniformity Index (U.I)), fiber bundle tensile properties (fiber strength (g/tex) and fiber elongation percentage), Micronair reading (Mic. Reading), which donates fiber and maturity combination) and color characters (Reflectance (Rd) and yellowness (+ b) were investigated at the cotton testing lab of the Institute of Cotton Research, Agricultural Research Center, Giza, Egypt under a supervision of cotton testing experts.

Statistical analysis: The obtained data were statistically analyzed through Costate 6.311 software of analysis of variance (Gomez and Gomez, 1984). The means were compared using Least Significant Difference (LSD) at p = 0.05 as, outlined by Duncan (1995).

RESULTS

A total of 15 seed-borne fungi were identified. They are: *Alternaria alternate* (Fr.) Keissler, *Aspergillus flavus* Link ex. Gray, *Aspergillus niger* Van Tieghem, *Aspergillus ochraceous* Wilhelm,

Cephalosporium sp., *Fusarium moniliforme* Sheld, *Fusarium oxysporum* Schlecht, *Fusarium semitectum* Berk and Rav, *Fusarium solani* (Mart.) Sacc., *Penicillium* sp., *Rhizoctonia solani* Kühn, *Rhizopus* sp., *Stemphylium* sp., *Trichothecium* sp. and *Verticillium* sp. The occurrence of each fungus on cotton seeds was recorded in terms of percentage.

Pathogenicity tests: Data in Table 1, show that the percentage of pre-emergence damping off, as a result of *F. oxysporum* attack was highly pronounced (25.67%), followed by *R. solani* (18%), while *F. moniliforme* presents 17.67% infection followed by *F. solani* (14.33%). Moreover, the highest post-emergence damping off due the invasion of *F. oxysporum* was 19.33%. While, *R. solani* reported 19.00%, followed by *F. solani* (15.33%) and *F. moniliforme* (13.33%).

The percentage of stunted seedlings due to *R. Solani* attack was 24.67%, *F. solani* (15.67%), *F. oxysporum* (12.67%) and *F. moniliforme* (9.33%) while the check (the healthy looking seeds) recorded 4.00% stunted seedlings.

Data presented in Table 2 show that the antioxidant (Trisodium orthophosphate) at the rate of (1.9 g L^{-1}), significantly reduced the ungerminated seeds by 33.33% and GAWDA® formulation at the rate of (2.2 g L^{-1}) by 34%. No rotted seedlings or abnormal seedlings were observed. Rating the normal seedlings in Trisodium orthophosphate and (GAWDA®) treatments were highly significant (66.67 and 66.00%, respectively).

Retardation of wilt disease by using, antioxidants and antioxidant formulation (GAWDA®): Effect of antioxidants or GAWDA® formulation on reducing the pre and post-emergence damping-off was recorded on cotton seedlings of 30 days old. The number of stunted and survived plants were also recorded on old plants edged 150 days.

Data in Table 3 show that the antioxidant Trisodium orthophosphate used in forms of seed soaking followed by spraying seedling significantly reduced the pre-emergence damping off down

Table 1: Pre- and post- emergence damping off and stunted seedlings in soil infested with *F. oxysporum*, *F. solani*, *F. moniliforme* and *R. solani*

Fungus	Pre-emergence	Post-emergence	Stunted seedling	Normal seedlings
Fusarium oxysporum	25.67a*	19.33a	12.67b-d	42.33de
Fusarium solani	14.33bc	15.33ab	15.67a-c	54.67b-d
Fusarium moniliforme	17.67bc	13.33ab	9.33cd	59.67bc
Rhizoctonia solani	18.00a-c	19.00a	24.67a	38.33e
Check	5.00d	3.33c	4.00d	87.67a

*Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at p = 0.05

Table 2: Effect of tested antioxidants and antioxidant formulation (GAWDA®) on seed germination

			Germinated seeds		
Chemicals	Concentrations (g L^{-1})	Ungerminated seeds	Rotted seedlings	Abnormal seedlings	Normal seedlings
Check dry		60.00ab*	10.00a	26.00a	4.00e
Check wet		66.33a	10.00a	3.33b	20.33d
Trisodium orthophosphate	1.9	33.33e	0.00d	0.00d	66.67a
Trisodium orthophosphate	1.5	40.00c-e	2.00c	0.00d	58.00ab
Disodium hydrogen orthophosphate	1.4	34.00de	0.00d	0.00d	66.00a
Disodium hydrogen orthophosphate	1.00	50.00bc	6.00b	2.00c	42.00c
Potassium sodium (+) - tartrate	1.4	39.00de	2.00c	0.00d	59.00ab
Potassium sodium (+) - tartrate	1.00	44.00cd	2.00c	2.00c	52.00bc
GAWDA®	2.2	34.00de	0.00d	0.00d	66.00a

*Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at p = 0.05, GAWDA®: A formulated antioxidants and mineral salts

Table 3: Percentage of pre, post emergence damping off and stunted seedlings in the different treatments

Treatments	Pre-emergence	Post- emergence	Stunted seedlings	Normal seedlings
Check				
Sp	43.67a*	23.67a	15.67a	17.00g
So	42.67a	22.67a	14.67a	20.33g
So+Sp	38.67b	18.67b	10.67b	31.33f
Trisodium orthophosphate				
Sp	14.00f	8.00e	2.67ef	75.33b
So	8.33g	2.33f	0.00f	89.67a
So+Sp	4.67g	0.00f	0.00f	95.00a
GAWDA®				
Sp	15.00ef	10.00de	4.00e	70.67bc
So	15.00ef	10.00de	4.00e	71.00bc
So+Sp	14.00f	9.00 e	3.00ef	74.00b
Disodium hydrogen orthophosphate				
Sp	21.00cd	16.00bc	10.00bc	53.00de
So	18.33de	13.33cd	7.33cd	60.67cd
So+Sp	16.00ef	11.00de	5.00de	68.00bc
Potassium sodium (+) -tartrate				
Sp	24.00c	19.00b	13.00ab	43.00e
So	24.00c	19.00b	13.00ab	44.00e
So+Sp	22.00cd	17.00b	11.00b	49.00e

*Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at p = 0.05, Sp: Spraying, So: soaking, GAWDA®: a formulated antioxidants and mineral salts

to be 4.67% and no post-emergence damping off and stunted seedling were observed followed by GAWDA® formulation (14.00%) in the pre- emergence damping off and 9.00% in the post-emergence damping off and the recorded stunted seedlings presented 3.00% of the total number of the tested plants. The check in form of spraying tap water on the seedling came up from soaked seeds in tap water prior to sowing presented highly significant incidence of diseased plants (38.67, 18.67 and 10.67%, respectively). On the other hand, the percentage of normal seedlings were increased in the Trisodium orthophosphate treatment in which seeds were soaked before sawing followed by 3 times of spraying this antioxidant on 30 days old-plants with 15 days interval between each treatment (95.00%) followed by GAWDA® formulation (74%), in compare to 31.33% only in the check.

It was also shown that chlorophyll a, b (chl a, chl b), carotenoid and total phenols content recorded in the leaves of 30, 45, 60, 75 days old plants were significantly increased in the 4 different treatments. However, applying the antioxidant Trisodium orthophosphate in forms of soaking+ three times of spraying plants of 30 days old with 15 days interval between each spray revealed a significant increases in Chl (a), Chl (b), carotenoid and total phenols (Table 4).

Subjected plants to the target antioxidants and GAWDA® formulation revealed significant increases in the growth parameters i.e., plant height, shoot height, root length, plant weight, shoot weight, root weight, number of cotton bolls, weight of bolls, number of branches and plant dry weight as shown in Table 5.

Application of the antioxidant Trisodium orthophosphate in form of (So + Sp) showed significant increases in plant height, shoot height and root height (30.05, 29.90 and 80.34%, respectively) followed by GAWDA® formulation applied as (So+Sp) to show 21.27, 23.81 and 47.54%, increases, respectively compare to the check . the uses of Trisodium orthophosphate in form of (So+Sp) significantly increased plant weight and shoot weight by 82.20 and 86.53%, respectively followed by GAWDA® formulation in form of (So+Sp) to be 20.09 and 24.78%, respectively more than the check.

Table 4: Chlorophyll a, b, carotenoid and total phenols in the upper leaves of 30, 45, 60, 75 days old plant pre-treated with different antioxidants or GAWDA® formulation effect of the number of spraying the antioxidants during the life span of plants of all treatments are tested

	30 day old plants (Check)								1st spraying (45 days after sowing)							
Treatments	Chl a	Chl a (%)	Chl b	Chl b (%)	Carotenoid	Carotenoid (%)	Tpc	Tpc (%)	Chl a	Chl a (%)	Chl b	Chl b (%)	Carotenoid	Carotenoid (%)	Tpc	Tpc (%)
Chemical																
Check	0.72c*	0.00	0.59c	0.00	0.03e	0.00	352.43d	0.00	0.79d	0.00	0.65d	0.00	0.07e	0.00	424.34e	0.00
Trisodium orthophosphate	1.00a	38.89	0.82a	38.98	0.15a	400.00	875.18a	148.33	1.16a	46.84	0.98a	50.77	0.17a	142.86	886.43a	108.90
GAWDA®	0.90b	25.00	0.79ab	33.90	0.11b	266.67	774.30ab	119.70	1.04b	31.65	0.92ab	41.54	0.11b	57.14	786.28b	85.29
Disodium hydrogen orthophosphate	0.84b	16.67	0.75b	27.12	0.08c	166.67	645.64bc	83.20	0.99bc	25.32	0.87b	33.85	0.09c	28.57	685.90c	61.64
Potassium sodium (+) -tartrate	0.81bc	12.50	0.64c	8.47	0.05d	66.67	517.64c	46.88	0.95c	20.25	0.80c	23.08	0.08d	14.29	573.70d	35.20
Method of application																
Check	0.72	0.00	0.59	0.00	0.03	0.00	352.43	0.00	0.79	0.00	0.65	0.00	0.07	0.00	424.33	0.00
Sp	0.83b	14.75	0.70a	19.32	0.07b	133.33	586.45b	66.40	0.95b	20.76	0.83a	27.04	0.10a	36.36	634.23b	49.47
So	0.85ab	17.51	0.72a	22.73	0.08b	166.67	633.47ab	79.75	0.97b	23.31	0.84a	28.57	0.10a	36.36	668.45b	57.53
So+Sp	0.89a	23.04	0.74a	26.14	0.09a	200.00	678.89a	92.63	1.03a	30.93	0.87a	33.16	0.11a	50.00	711.31a	67.63
	2nd spraying (60 days after sowing)								3rd spraying (75 days after sowing)							
Treatments	Chl a	Chl a (%)	Chl b	Chl b (%)	Carotenoid	Carotenoid (%)	Tpc	Tpc (%)	Chl a	Chl a (%)	Chl b	Chl b (%)	Carotenoid	Carotenoid (%)	Tpc	Tpc (%)
Chemical																
Check	0.91d*	0.00	0.07e	0.00	0.05e	0.00	432.11e	0.00	0.94d	0.00	0.80d	0.00	0.04e	0.00	450.14e	0.00
Trisodium orthophosphate	1.22a	34.07	0.17a	142.86	0.15a	200.00	904.09a	109.23	1.38a	46.81	1.18a	47.50	0.27a	575.00	949.06a	110.84
GAWDA®	1.07b	17.58	0.11b	57.14	0.12b	140.00	794.06b	83.76	1.19b	26.60	1.03b	28.75	0.14b	250.00	817.51b	81.61
Disodium hydrogen orthophosphate	1.00c	9.89	0.09c	28.57	0.10c	100.00	700.06b	62.01	1.11c	18.09	0.99bc	23.75	0.09c	125.00	716.82c	59.24
Potassium sodium (+) - tartrate	0.91d	0.00	0.08d	14.29	0.08d	60.00	598.92d	38.60	1.08c	14.89	0.92c	15.00	0.07d	75.00	624.23d	38.67
Method of application																
Check	0.91	0.00	0.07	0.00	0.05	0.00	432.15	0.00	0.94	0.00	0.80	0.00	0.05	0.00	450.14	0.00
Sp	0.99b	8.79	0.10a	36.36	0.09c	80.00	651.09c	50.66	1.11b	18.09	0.93b	15.77	0.09c	92.86	672.26c	49.35
So	1.04a	14.29	0.10a	36.36	0.10b	100.00	683.11b	58.07	1.141ab	21.38	1.01a	25.73	0.11b	135.71	717.95b	59.50
So+Sp	1.05a	15.38	0.11a	50.00	0.11a	120.00	723.58a	67.44	1.17a	24.47	1.01a	25.73	0.15a	221.43	744.45a	65.38
	30 day old plants (Check)								1st spraying (45 days after sowing)							
Treatments	Chl a	Chl a (%)	Chl b	Chl b (%)	Carotenoid	Carotenoid (%)	Tpc	Tpc (%)	Chl a	Chl a (%)	Chl b	Chl b (%)	Carotenoid	Carotenoid (%)	Tpc	Tpc (%)
Check																
Sp	0.677g*	0.00	0.567f	0.00	0.030j	0.00	277.623i	0.00	0.717f	0.00	0.617f	0.00	0.067e	0.00	373.800i	0.00
So	0.737fg	0.00	0.590ef	0.00	0.033ij	0.00	355.767hi	0.00	0.763f	0.00	0.637f	0.00	0.067e	0.00	433.243hi	0.00
So+Sp	0.750fg	0.00	0.603ef	0.00	0.033ij	0.00	423.890g-i	0.00	0.880e	0.00	0.703ef	0.00	0.077de	0.00	465.963gh	0.00
Trisodium orthophosphate																
Sp	0.950bc	31.70	0.807ab	37.56	0.130bc	333.33	830.680a-c	135.70	1.127ab	43.26	0.957ab	46.70	0.137bc	94.79	844.623b	76.21
So	0.977ab	26.17	0.820ab	28.46	0.150ab	400.00	856.667ab	58.86	1.147ab	31.42	0.993a	34.31	0.167ab	57.88	868.61ab	99.05
So+Sp	1.087a	50.69	0.847a	44.38	0.167a	466.67	938.207a	166.21	1.197a	52.16	0.993a	52.22	0.203a	188.63	946.050a	122.95
GAWDA®																
Sp	0.877b-e	21.58	0.790ab	34.66	0.097de	233.33	731.837b-d	107.66	1.007cd	28.01	0.917a-c	40.57	0.103c-e	46.45	747.720cd	51.15
So	0.890b-e	23.38	0.790ab	34.66	0.117c-e	300.00	784.537a-c	122.61	1.017cd	29.28	0.917a-c	40.57	0.103c-e	46.45	795.893bc	87.56
So+Sp	0.927b-d	28.51	0.797ab	35.85	0.120cd	300.00	806.527a-c	128.85	1.083bc	37.67	0.930a-c	42.57	0.113cd	60.66	815.240bc	92.12

Table 4: Continue

Treatments	30 day old plants (Check) Chl a	Chl a (%)	Chl b	Chl b (%)	Carotenoid	Carotenoid (%)	Tpc	Tpc (%)	1st spraying (45 days after sowing) Chl a	Chl a (%)	Chl b	Chl b (%)	Carotenoid	Carotenoid (%)	Tpc	Tpc (%)
Disodium hydrogen orthophosphate																
Sp	0.823d-f	14.09	0.730b-d	24.43	0.067gh	133.33	597.537d-g	69.55	0.977de	24.19	0.867b-d	32.91	0.090de	27.96	668.287de	57.49
So	0.840c-f	16.45	0.763a-c	30.06	0.070gh	133.33	655.640c-f	86.04	0.993cd	26.23	0.837b-d	28.31	0.093c-e	32.23	693.707de	63.48
So+Sp	0.843c-f	16.87	0.770a-c	31.25	0.093ef	200.00	683.740b-e	94.01	1.000cd	27.12	0.903a-c	38.43	0.097c-e	37.91	695.710de	63.95
Potassium sodium (+) - tartrate																
Sp	0.790e-g	9.52	0.610ef	3.98	0.040ij	33.33	496.067f-h	40.76	0.930de	18.22	0.770de	18.04	0.083de	18.01	536.700g	26.48
So	0.810d-f	12.29	0.637d-f	8.58	0.043h-j	33.33	514.7567f-h	46.06	0.940de	19.49	0.817c-e	25.24	0.083de	18.01	550.787fg	29.80
So+Sp	0.850d-f	17.84	0.680c-e	15.91	0.057g-i	100.00	542.103e-g	53.82	0.970de	23.31	0.820c-e	25.70	0.083de	18.01	633.603ef	49.32
Treatments	2nd spraying (60 days after sowing) Chl a	Chl a (%)	Chl b	Chl b (%)	Carotenoid	Carotenoid (%)	Tpc	Tpc (%)	3rd spraying (75 days after sowing) Chl a	Chl a (%)	Chl b	Chl b (%)	Carotenoid	Carotenoid (%)	Tpc	Tpc (%)
Check																
Sp	0.863h*	0.00	0.637f	0.00	0.047f	0.00	380.467j	0.00	0.893g	0.00	0.757f	0.00	0.037i	0.00	393.800i	0.00
So	0.937g	0.00	0.677f	0.00	0.050f	0.00	443.243ij	0.00	0.943fg	0.00	0.837d-f	0.00	0.040i	0.00	453.243h	0.00
So+Sp	0.927gh	0.00	0.715ef	0.00	0.063ef	0.00	472.730i	0.00	0.990e-g	0.00	0.813ef	0.00	0.057hi	0.00	503.367h	0.00
Trisodium orthophosphate																
Sp	1.163b	27.94	0.977ab	132.32	0.127c	138.13	883.277b	104.39	1.357ab	44.06	1.067b	32.99	0.183c	309.70	909.383b	102.02
So	1.243a	26.87	0.997a	65.18	0.157b	66.03	876.290b	50.68	1.383a	31.89	1.230a	34.77	0.250b	82.13	952.56ab	52.74
So+Sp	1.243a	36.74	0.997a	229.27	0.180a	237.50	952.717a	120.46	1.403a	48.94	1.243a	54.92	0.353a	690.30	985.24a	118.88
GAWDA®																
Sp	1.023de	12.54	0.927a-c	119.51	0.120c	125.00	754.837de	74.67	1.133cd	20.28	1.003bc	25.01	0.117e	161.94	799.297c	77.57
So	1.073cd	18.04	0.927a-c	125.00	0.123c	55.63	805.893cd	86.49	1.183cd	25.58	1.027b	28.00	0.143d	220.15	821.943c	82.60
So+Sp	1.110bc	22.11	0.950a-c	125.00	0.123c	63.13	821.907bc	90.19	1.247bc	32.38	1.063b	32.49	0.147d	229.10	831.29c	84.68
Disodium hydrogen orthophosphate																
Sp	0.970e-g	6.71	0.887b-d	51.83	0.083de	119.38	684.953f	58.50	1.103de	17.09	0.96b-d	19.65	0.07gh	56.72	698.040de	55.07
So	1.003ef	10.34	0.897b-d	59.15	0.087d	25.63	699.377ef	61.84	1.103de	17.09	0.997bc	24.26	0.090fg	101.49	721.720de	60.33
So+Sp	1.023de	12.54	0.923a-c	114.02	0.117c	50.00	717.04ef	65.93	1.123cd	19.21	1.003bc	25.01	0.107ef	139.55	730.710d	62.33
Potassium sodium (+) - tartrate																
Sp	0.950fg	4.51	0.790de	15.24	0.067d-f	25.63	552.367h	27.82	1.06d-f	12.53	0.887c-f	10.55	0.067gh	50.00	560.787g	24.58
So	0.957fg	5.28	0.847c-e	46.34	0.080de	50.00	590.787gh	36.71	1.090de	15.71	0.940b-e	17.16	0.067gh	50.00	640.270f	42.24
So+Sp	0.967e-g	6.38	0.850c-e	51.83	0.083d-f	55.63	653.603fg	51.25	1.093de	16.03	0.947b-e	18.03	0.070gh	56.72	671.620ef	49.20

*Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at p = 0.05. Sp: Spraying, So: Soaking

Table 5: Growth parameters in cotton plants treated with different antioxidants and GAWDA® formulation

Treatments	Plant height	Plant height (%)	Shoot height	Shoot height (%)	Root length	Root length (%)	Plant weight	Plant weight (%)	Shoot weight	Shoot weight (%)
Check										
Sp	179g*	0.00	158.00g	0.00	20.00d	0.00	276.00g	0.00	240.00g	0.00
So	193.33fg	0.00	158.00g	0.00	20.00d	0.00	293.33fg	0.00	265.00fg	0.00
So+Sp	196.67f	0.00	176.67f	0.00	21.00d	0.00	303.33e-g	0.00	272.33e-g	0.00
Trisodium orthophosphate										
Sp	236.67a-c	24.78	211.67ab	28.89	33.33a-c	63.92	356.67bc	22.61	323.33bc	24.78
So	238.33ab	25.66	213.33a	29.90	35.33ab	73.75	363.33b	24.90	326.67b	26.07
So+Sp	246.67a	30.05	213.33a	29.90	36.67a	80.34	530.00a	82.20	483.33a	86.53
GAWDA®										
Sp	226.67b-d	19.51	201.67a-d	22.80	26.67a-d	31.16	343.33b-e	18.03	311.00b-e	20.03
So	226.67b-d	19.51	203.33a-c	23.81	30.00a-d	47.54	345.00b-e	18.60	313.33b-d	20.93
So+Sp	230b-d	21.27	203.33a-c	23.81	30.00a-d	47.54	349.33b-d	20.09	323.33bc	24.78
Disodium hydrogen orthophosphate										
Sp	220de	15.99	195.00b-e	18.74	25.00b-d	22.95	310.00d-g	6.57	286.67c-f	10.64
So	220de	15.99	200.00a-e	21.79	25.00b-d	22.95	320.00c-f	10.01	286.67c-f	10.64
So+Sp	223.33cd	17.75	200.00a-e	21.79	26.67a-d	31.16	331.67b-f	14.02	298.33b-f	15.14
Potassium sodium (+) - tartrate										
Sp	206.67ef	8.96	183.33ef	11.63	21.67d	6.57	305.00e-g	4.85	276.67d-g	6.78
So	206.67ef	8.96	185.00d-f	12.65	23.33cd	14.74	310.00d-g	6.57	278.33d-g	7.42
So+Sp	216.67de	14.24	186.67c-f	13.67	23.33cd	14.74	310.00d-g	6.57	286.67c-f	10.64

Treatments	Root weight	Root weight (%)	No. of boll	No. of boll (%)	Weight of boll	Weight of boll (%)	No. of branches	No. of branches (%)	Plant dry weight	Plant dry weight (%)
Check										
Sp	16.67e*	0.00	6.00c	0.00	43.33c	0.00	1.00a	0.00	110.33g	0.00
So	23.33de	0.00	9.67bc	0.00	49.33c	0.00	1.00a	0.00	117.33fg	0.00
So+Sp	2 3.33de	0.00	10.00bc	0.00	51.67bc	0.00	1.33a	0.00	121.33e-g	0.00
Trisodium orthophosphate										
Sp	38.33ab	81.57	18.00a-c	110.36	131.67a-c	173.69	2.00a	80.18	142.67bc	22.64
So	40.00ab	89.48	21.33ab	149.28	155.00ab	222.18	2.00a	80.18	145.33b	24.93
So+Sp	46.67a	121.08	23.00a	168.80	165.00a	242.96	2.67a	140.54	212.00a	82.24
GAWDA®										
Sp	33.33b-d	57.89	15.00a-c	75.30	100.00a-c	107.86	2.00a	80.18	137.33b-e	18.05
So	33.33b-d	57.89	15.67a-c	83.13	100.00a-c	107.86	2.00a	80.18	138.00b-e	18.63
So+Sp	36.00a-c	70.54	16.00a-c	86.99	121.67a-c	152.90	2.00a	80.18	139.67b-d	20.06
Disodium hydrogen orthophosphate										
Sp	32.67b-d	54.76	11.67a-c	36.38	81.67a-c	69.76	1.67a	50.45	127.33c-f	9.46
So	33.33b-d	57.89	13.33a-c	55.78	93.00a-c	93.31	1.67a	50.45	128.00c-f	10.03
So+Sp	33.33b-d	57.89	15.00a-c	75.30	99.00a-c	105.78	2.000a	80.18	132.67b-f	14.05
Potassium sodium (+) - tartrate										
Sp	25.00c-e	18.43	10.33a-c	20.72	64.67a-c	34.42	1.33a	19.82	124.00d-g	6.59
So	28.33b-e	34.20	11.00a-c	28.55	66.67a-c	38.58	1.33a	19.82	124.00d-g	6.59
So+Sp	31.67b-d	50.02	11.33a-c	32.41	76.67a-c	59.36	1.67a	50.45	124.00d-g	6.59

*Means followed by different letter(s) in the column are significantly different according to Duncan's multiple range test at p = 0.05, Sp: Spraying, So: soaking, GAWDA®: A formulated antioxidants and mineral salts

Regarding the other parameters of the plant growth, it was shown that Trisodium orthophosphate in form of (So+Sp) significantly increased root weight, number of cotton bolls/plant and weight of bolls by 121.08, 168.80 and 242.96%, respectively, followed by GAWDA® formulation in form of (So+Sp) to be 70.54, 86.99 and 152.90%, respectively, more than the check.

The number of branches and dry weight were also increased significantly when Trisodium orthophosphate applied in form of (So+Sp) to present 140.54 and 82.24%, increases, respectively, followed by GAWDA® formulation in form of (So+Sp) to show 80.18 and 20.6% increase, respectively, more than the check.

Moreover, Trisodium orthophosphate in form of (So+Sp) significantly increased the yield of cotton seed and weight of seed yield by 142.77 and 76.49%, respectively, followed by GAWDA® formulation in form of (So+Sp) treatment to show increases of 111.11 and 70.40%, respectively, rather than the check. Based on the above, it was shown also that the increases in weight of yield of cotton lint and cotton lint% were significant to present 196.56 and 22.09%, increases, respectively, followed by GAWDA® formulation to show 144.16 and 11.95%, respectively, rather than the check treatment.

In respect to the percentage of oil in seed, it was increased in Trisodium orthophosphate treatment by 36.81 and by 21.58%, when GAWDA® formulation was applied when compared with the check treatment as shown in Table 6.

Effect of Antioxidant and formulated antioxidants on the quality of the cotton fibers: Table 7 presents the tested characters of cotton fibers including, the Micronair, length (mm) UHM, Regularity%, Durability (gm/tex), Elongation, the degree of color reflection (Rd) and the degree of yellowing (b+).

The results show that antioxidant Trisodium orthophosphate in form of (So+Sp) increased the Micronair and length (mm) (UHM), as well as the Regularity by 19.49, 4.00 and 6.36%, respectively, followed by GAWDA® formulation in form of (So+Sp) by 19.49, 1.69 and 3.96%, respectively compared with the check.

Durability (gm/tex) and Elongation also increased by 16.95 and 9.35%, respectively followed by GAWDA® formulation by 10.41 and 6.54%, respectively.

The degree of color reflection (Rd) and the degree of yellowing (+b) were also increased by 11.86 and 6.49%, respectively followed by GAWDA® formulation (5.33 and 3.05%, increases, respectively).

DISCUSSION

A significant number of fungi were associated with cotton seeds while some had potential to injury the plants and cause pre and post-emergence damping off. These pathogens are *F. oxysporum*, *F. solani*, *F. moniliforme* and *R. solani*. The damage they cause significantly affects the quality and quantity of the cotton fibers.

These pathogens when invade the vascular system of the plants, they results in weak and immature balls.

So far, the objective of this research was planned to use a friendly environmental method to control the above disease, scale up the fibers production and the cotton seed yield. Also, protect the soil microflora from the adverse effect of the intensive uses of pesticides.

The results presented in this research show that when uses the antioxidant Trisodium orthophosphate (1.9 g L^{-1}) or formulated antioxidant (GAWDA® 2.2 g L^{-1}) for soaking cotton seed before sowing or soaking seeds plus 3 times of spraying plants significantly improve the cotton yield of fibers and increase the seed oil quantity.

Table 6: Effect of different treatments on the yield of cotton (hair and oil) in percentage

Treatments	Yield cotton seed	Yield cotton seed (%)	Weight of seed yield (g)	Weight of seed yield (%)	Yield of lint cotton (weight)	Yield of lint cotton (weight) (%)	Lint percentage	Lint (%)	Fat in sample (%)	Fat increase of sample (%)
Check										
Sp	551.33m*	0.00	240.53o	0.00	310.80k	0.00	54.03k	0.00	18.81h	0.00
So	567.00l	0.00	253.60n	0.00	313.40k	0.00	55.28j	0.00	22.55fg	0.00
So+Sp	587.00k	0.00	269.87m	0.00	317.13k	0.00	56.38i	0.00	23.62c-g	0.00
Trisodium orthophosphate										
Sp	1253.16c	120.45	439.09c	72.42	814.06c	159.44	64.96b	17.62	23.90c-g	11.37
So	1266.80b	122.85	441.89b	73.52	824.91b	162.90	65.12b	17.91	27.12ab	26.37
So+Sp	1380.00a	142.77	449.45a	76.49	930.55a	196.56	67.43a	22.09	29.36a	36.81
GAWDA®										
Sp	990.67f	74.28	374.48f	47.05	612.47f	95.19	59.82g	8.31	22.85e-g	6.48
So	1018.67e	79.20	378.19e	48.50	644.18e	105.30	60.86f	10.19	25.05b-f	16.73
So+Sp	1200.05d	111.11	433.94d	70.40	766.11d	144.16	61.83e	11.95	26.09bc	21.58
Disodium hydrogen orthophosphate										
Sp	702.38i	23.56	294.26i	15.55	408.12i	30.07	58.11h	5.21	21.48g	0.09
So	780.00h	37.22	313.43h	23.07	466.57h	48.69	59.82g	8.31	24.78b-f	15.47
So+Sp	820.00g	44.25	320.99g	26.04	499.01g	59.03	60.86f	10.19	25.57b-d	19.15
Potassium sodium (+) -tartrate										
Sp	664.00j	16.81	279.12l	9.60	384.88j	22.66	57.96h	4.94	23.29d-g	8.53
So	665.63j	17.10	279.88k	9.90	385.74j	22.93	57.95h	4.92	24.73b-f	15.24
So+Sp	669.33j	17.75	280.37j	10.09	388.96j	23.96	58.11h	5.21	25.43b-e	18.50

*Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at p=0.05, Sp: Spraying, So: soaking , GAWDA®: A formulated antioxidants and mineral salts

Table 7: Characters of the quality of cotton fibers as result of treating seeds, plants or seeds and plants with antioxidants and formulated antioxidant (GAWDA®)

Treatment	Micronair	Micronair (%)	Length (mm) UHM	Length (mm) UHM (%)	Regularity	Regularity (%)	Durability/ g/tex	Durability/ g/tex (%)	Elongation	Elongation (%)	Degree of color reflection Rd	Degree of color reflection Rd (%)	Degree of yellowing b+	Degree of yellowing b+ (%)
Check														
Sp	3.80d*	0.00	33.00c	0.00	78.80f	0.00	40.30g	0.00	7.10f	0.00	71.30g	0.00	8.70e	0.00
So	3.90d	0.00	33.50bc	0.00	85.10e	0.00	41.2f	0.00	7.10f	0.00	72.20f	0.00	8.70e	0.00
So+Sp	4.10c	0.00	33.80b	0.00	86.00d	0.00	42.40e	0.00	7.20e	0.00	72.40f	0.00	8.80d	0.00
Trisodium orthophosphate														
Sp	4.70a	19.49	34.00b	1.69	87.30	4.80	45.60c	10.41	7.60b	6.54	76.30bc	6.02	9.10b	4.20
So	4.70a	19.49	34.00b	1.69	87.30	4.80	47.00b	13.80	7.80a	9.35	76.50bc	6.30	9.10b	4.20
So+Sp	4.70a	19.49	34.77a	4.00	88.60	6.36	48.30a	16.95	7.80a	9.35	80.50a	11.86	9.30a	6.49
GAWDA®														
Sp	4.30b	9.32	33.90b	1.40	86.00d	3.24	43.80	6.05	7.30d	2.34	73.30d	1.85	8.80d	0.76
So	4.40b	11.86	33.90b	1.40	86.20cd	3.48	43.90	6.30	7.40c	3.74	74.00d	2.83	9.00c	3.05
So+Sp	4.70a	19.49	34.00b	1.69	86.60c	3.96	45.60c	10.41	7.60b	6.54	75.80c	5.33	9.00c	3.05

*Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at p = 0.05, Sp: Spraying, So: soaking, GAWDA®: a formulated antioxidants and mineral salts

This trend in controlling plant diseases with antioxidant of potential to affect the growth of several fungi attacking other crops including: Peanut, Sunflower and Cucumber are in agreement with the results presented in this research (Elwakil and El-Metwally, 2000; Elwakil, 2003; Farouk *et al*., 2008; Abd El-Hai *et al*., 2009).

The results of this research are also novel for controlling seed borne fungi affecting cotton plants as well as produce quality fibers free from pesticides.

According to the available literature no research was carried out to overcome the problem of accumulating of pesticides in the cotton fibers.

Since, increasing the demand on organic cotton fibers is raised day after another especially in the industrial countries. This tactic of using antioxidants for producing high quality fibers, high yield and fibers free from toxic pesticides may be appreciated to fabricate underwear profoundly requested for children having allergy against the commercial cotton fibers.

This tactic also highlights the possible production of high quantity of cotton fibers with lower cost compare the traditional organic ones.

On the other hand, these results may encourage the cotton growers and the fiber producers to replace pesticides used for controlling the diseases by antioxidants of potential to retard the adverse effect of cotton pathogens and protect the environment including the soil microflora from the adverse effect of the toxic pesticides. However, the results show that the antioxidants used in the research are applied in very low concentrations and have not side effect or potential to negatively affect the environment. Moreover, produce low cost cotton fibers free from the accumulated pesticides in cotton hairs and higher yield of fibers and oil seed.

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