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Research Article
Use of Antioxidants as Green Chemicals to Control Soybean Diseases and Scaling up the Yield and Quality

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Abstract

Background and Objective: Soybeans are a globally important crop consumed by both man and animal. Worldwide production in 2019/2020 was 358 million metric t. The current research aimed at testing the possible correlation between treating seeds and plants of soybean with antioxidants and retreading the disease severity that occurred by the pathogenic fungi. **Materials and Methods:** Soybean seeds collected from different locations in Egypt were used to isolate seed-borne plant pathogenic fungi. Antioxidants were selected for further testing that produced the greatest growth reduction of target fungi from *in vitro* tests. Effective antioxidants were then applied as seed treatments (soaks) and sprays to soybean plants in field tests. Plant growth parameters and seed yield and quality were then recorded. An analysis of variance was performed on the collected data using CoStat 6.311. Means were compared using Least Significant Difference (LSD) according to Duncan. **Results:** Soaking seeds in salicylic acid (3 g L^{-1}), citric acid (6 g L^{-1}), benzoic acid (6 g L^{-1}) and a combination of these before planting, followed by two plant spray applications at the same concentrations reduced disease incidence and increased yield by 48%. **Conclusion:** The results showed a strong correlation between use of antioxidants and decreased disease incidence and resulted in increased yields. This research suggests the potential for benefits using antioxidants as seed treatments and plant sprays to control soybean diseases resulting in increased yields of high quality seeds.

Key words: Soybean, diseases, control, antioxidants, green chemicals, salicylic acid, citric acid, benzoic acid, yield, quality

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plants can develop a significant and long lasting resistance to pathogens infection when treated with chemicals of antioxidants properties. These chemicals are currently named green chemicals. They are safe and have friendly environmental properties. In this regard, a number of these chemicals were tested for their ability to scavenge the free radicals produced in the plants during their metabolic process or due to the effect of biotic or abiotic stress occurred during the life span of the growing plants.

Soybeans (*Glycine max)* are globally important crop consumed by both man and animal. Worldwide production in 2019/2020 was 358 million metric tons. Soybeans are versatile crop and substitute meat and milk as they are rich in oil, protein and vitamins, in flavones, omega-6-fatty acids and linolenic acid[1]. In Egypt, this crop was grown intensively in Bani-Swaif, Assiut and El-Minia in the 1970s, but since 1980 the cultivation decreased dramatically and substituted with maize[2].

Soybeans are subjected to a number of fungal diseases reducing the plant growth and subsequently the production and its quality. However, the common fungi affecting this crop are *Phytophthora* species the cause of damping-off disease, *Fusarium oxysporum* the cause of wilt disease, *Sclerotium bataticola* the cause of charcoal rot[3], *Cylindrocladium parasiticum* the cause of red crown rot and *Diaporthe phaseolorum* which causes stem canker, these fungi are mainly harbored in the residue of both soil and crop and enlarged in the wet weather.

The *in vitro* studies show that Benzoic acid, salicylic acid and ascorbic acid significantly reduce the linear growth of *Fusarium oxysporum*, *F. solani* and *Rhizoctonia solani* as well as the formation and germination of *Fusarium* spp. spores. It was also reported that damping off of tomatoes was significantly inhibited by these antioxidants used in form of soil dressing[4].

Since scanty information regarding the use of green chemical in the control of plant diseases, the present investigation was carried out to evaluate the potential of a number of green chemicals of antioxidant prosperities i.e.: salicylic acid, citric acid and benzoic acid in the control of the common fungal diseases which invade and attack soybeans during their life span and to study their role in increasing yields of high quality seeds.

The possible correlation between treating seeds and plants of soybean with antioxidants and retreading the disease severity occurred by the pathogenic fungi was the second objective of this research.

MATERIALS AND METHODS

Study area: Studies on the prevalence of seed-borne fungi of soybean and their pathogenicity were conducted in the laboratories of Plant Pathology Department, Faculty of Agriculture, Mansoura University during the years of 2014-2018.

Collection of seed samples: A total of 15 soybean seed samples presenting Giza.111 and Giza 21 cultivars were collected from Sakha Research Station, the Agriculture Research Centre, Ministry of Agriculture, Egypt during 2014 and 2015 harvested seasons to be used in preset studies.

Isolation and identification of seed borne fungi: The testing method described by Shovan *et al*.[5] was carried out to detect soybean seed-borne fungi. In this method two blotter papers were soaked in sterilized water and placed in the petri-dish. Two-hundred seeds were randomly taken from each sample and placed onto the wet papers in the dishes (10 seeds per petri-dish). The -dishes were divided in two groups; the first group was incubated at 25°C for 7 days while the second group was kept in a deep-freezer for 24 hrs and transferred later on to an incubator at 25°C for 7 days. Seed germination and fungal association with the tested seeds were recorded. The growing fungi were examined under a stereo-binocular microscope for tentative identification. Since the slow growing fungi do not show up within the seven days, they kept incubated for longer time. Microscopic slide preparations were used to confirm the tentative identification while the standard keys described by Parmeter[6]; Dhingra *et al*.[7]; Nelson *et al*.[8] and Booth[9].

Pathogenicity test: Four commonly fungi grown on the tested seeds were isolated to study their pathogenicity on the soybean plants i.e.: *Fusarium oxysporum*, *F. moniliforme*, *Sclerotium bataticola* and *Rhizoctonia solani*. The highly pathogenic isolates that cause damping-off, root rot and wilting on the variety Giza 21 were selected to be used in this investigation. Sorghum grains were used as a growth substrate for preparation of the inoculums of these fungi. The grains were soaked overnight in water and then transferred to the 500 mL Erlenmeyer flasks, autoclaved under 1.5 kg cm^{-2} pressure at 121°C for 20 min. A separate group of flasks was used for the preparation of each isolate. Ten mycelium discs of 5 mm diameter each were cut from the edges of 7 days old PDA culture grown in the petri-dish and transferred under aseptic condition to the sorghum grain in the flasks while one flask presents one isolate. Flasks were kept incubated at 25°C

for 20 days. The pathogenicity of each fungus was carried out by infesting the soil before seating seeds in the pots. Each pot was filled with 3.0 kg sterilized sandy soil (1:1 w/w). Inoculum of *Fusarium oxysporum* was thoroughly mixed in sterilized soil at 40 g kg^{-1}. While, *Sclerotium bataticola* and *Rhizoctonia solani were* mixed in the soil at a rate of 20 g kg^{-1} soil. The controls were grown in sterilized soil only. Five seeds of soybean were sown in each pot while each treatment was presented by 5 replicated pots. Damping-off syndrome was observed regularly 10 and 20 days after planting. The average of infection between 80-100% is graded as it caused by a highly virulent isolate, 70 up to 79% infection was graded as caused by a virulent isolate, 50 up to 69% infection was graded as caused by a moderately virulent while the less than 50% infection considered the infection caused by a weak isolate. This key of disease development was expressed by Shovan *et al.*[5].

Efficacy of antioxidants on the soybean growth: Field experiments were carried out at the Plant Pathology Department, Faculty of Agriculture, located at the Campus of Mansoura University to confirm the *in vitro* results. Split plot design of six replicates was applied. Soybean seeds cv. Giza 21 were sown in ridges of 20 cm apart in hills spaced 60 cm apart on one side of the ridge.

Determination of photosynthetic pigments content in soybean leaves: The third upper parts of a number of soybean leaves was collected to determine their content of the photosynthetic pigments as described by Mackinney[10].

Total phenols: Fresh leaves of soybean plants were collected to determine their content of the total phenols using Folin-Ciocalteu reagent as described by Singleton and Rossi[11].

Plant growth characters and yield components: At the maturity stage, the following characters were measured:

- Shoot fresh weight (g), root fresh weight (g), shoot dry weight (g), root dry weight (g), shoot length (cm), root length (cm) and number of branches
- Number of pods, pods fresh weight (g) and pods dry weight (g), as well as yield per fed (kg), were recorded. The gathered seeds from the field experiment were recorded as Ton per feddan

Seed chemical analysis: Seed samples collected from each treatment were oven dried, ground finely while oil percentage was determined in Soxhelt's apparatus using petroleum hexane 30. Nitrogen percentage was estimated using micro Kjeldahl apparatus and multiplied by the converting factor (6.25) to get percentage protein in seed.

Statistical analysis: Data analyzed using CoStat 6.311 software of analysis of variance. Means were compared by Least Significant Difference (LSD) at $p \leq 0.05$ as outlined by Duncan[12].

In all tables, the different letter(s) in the column are significantly different according to Duncan's multiple range tests ≤ 0.05.

RESULTS

Seed-borne fungi of soybean

Seed Health Testing (SHT): Blotter and deep-freezing methods were applied. Non-surface sterilized and surface sterilized soybean seeds in sodium hypochlorite were used to detect the seed-borne fungi incidence. Eighteen seed samples collected from commercial markets of Egypt were used.

A total of 16 seed-borne fungi (11 genera) were identified. They are: *Alternaria alternate* (Fr.) Keissler, *Aspergillus niger* Van Tieghem, *Aspergillus flavus* Link ex. Gray, *Aspergillus ochraceous* Wilhelm, *Cephalosporium* sp., *Macrophomina phasoleina*, *Fusarium oxysporum* Schlecht, *Fusarium moniliforme* Shield, *Fusarium semitectum* Berk and Rav, *Fusarium solani* (Mart.) Sacc., *Penicillium* sp., *Rhizopus* sp., *Rhizoctonia solani* Kuhn, *Stemphylium* sp., *Trichothecium* sp. and *Verticillium* sp. The occurrence of each fungus on soybean seeds was recorded in terms of percentage as shown in Table 1. It was found that the blotter method presented the greatest number of fungi followed by the deep-freezing method either when seeds were surface sterilized or not.

Detection of internal seed-borne fungi

Non-surface sterilized seeds

Standard Blotter Method (SBM): Table 1 show that the standard moist blotter method (SBM) enhanced the growth of the fast growing saprophytes viz., *A. niger*, followed by *Rhizopus* sp., *A. flavus*, *Penicillium* sp., *Trichothecium* sp., *Stemphylium* sp. and *A. alternata* while, the growth of slow growing seed-borne fungi were less than the fast growing saprophytes viz., *F. oxysporum* followed by *Verticillium* sp., *Cephalosporium* sp., *S. bataticola*, *F. solani*, *A. ochraceous*, *F. moniliforme*, *R. solani* and *F. semitectum*.

Table 1: Percentage of detected seed-borne fungi in 20 random samples of soybean cultivars

	Non-sterilized seeds		Sterilized seeds	
Fungi	Standard blotter method	Deep freezing method	Standard blotter method	Deep freezing method
Alternaria alternate	25.82	15.45	21.45	13.81
Aspergillus flavus	64.16	42.36	48.35	24.18
Aspergillus niger	88.86	35.44	60.52	22.90
Aspergillus ochraceous	7.08	6.00	4.37	4.37
Cephalosporium sp.	12.36	22.17	25.26	35.61
Fusarium moniliforme	5.84	10.35	9.70	17.06
Fusarium oxysporum	12.81	25.57	21.26	42.53
Fusarium semitectum	3.26	7.72	13.62	16.89
Fusarium solani	7.08	23.44	11.99	40.35
Macrophomina phasoleina	8.70	12.51	13.66	21.00
Penicillium sp.	50.70	23.44	32.17	15.26
Rhizoctonia solani	5.45	11.99	8.72	19.63
Rhizopus sp.	65.43	39.80	30.54	19.63
Stemphylium sp.	27.27	9.81	43.62	14.18
Trichothecium sp.	29.44	18.53	7.08	4.37
Verticillium sp.	12.55	15.26	25.07	30.54

Table 2: Pathogenicity test of the isolated fungi on soybean plant and the percentage of survival seedlings

Fungi	Pre emergence damping-off (%)	Post emergence damping-off (%)	Stunt seedlings (%)	Survival (%)
Control	0.00^{e}	0.00^{e}	0.00^{e}	100.00^{a}
F. moniliforme	17.93^{d}	8.97^{d}	7.97^{d}	65.13^{b}
F. oxysporum	26.90^{c}	17.93^{c}	8.97^{c}	46.20^{c}
Sclerotium bataticola	36.71^{b}	19.02^{b}	12.24^{b}	32.03^{d}
Rhizoctonia solani	40.62^{a}	20.31^{a}	15.78^{a}	23.29^{e}

Deep-Freezing Method (DFM): Deep-Freezing Method (DFM) presented the following fungi: *A. flavus* at the higher rate followed by *Rhizopus* sp., *A. niger*, *F. oxysporum*, *F. solani*, *Penicillium* sp., *Cephalosporium* sp., *Trichothecium* sp., *A. alternata*, *S. bataticola, R. solani*, *F. moniliforme, Stemphylium* sp., *F. semitectum* and *A. ochraceous*.

Surface sterilized seeds

Blotter method: It was shown that the growth of the fast growing saprophytes on seed were lower percentage compared to the non-surface sterilized seeds.

Deep-freezing method: In this method, the presence of fast growing saprophytes was recorded at a lower percentage when compared with the non-surface sterilized ones.

Pathogenicity tests: The percentage of pre-emergence damping off as a result of *R. solani* attack was highly pronounced (40.62%), followed by *S. bataticola* (36.71%) while, *F. oxysporum* symptoms presents 26.90% and *F. moniliforme* (17.93%). Moreover, the highest post-emergence damping off due the invasion of *R. solani* was 20.31%. While, *S. bataticola* reported 19.02%, followed by *F. oxysporum* (17.93%) and *F. moniliforme* (8.97%). The percentage of stunted seedlings due to *R. solani* attack was 15.78%, *S. bataticola* (12.24%), *F. oxysporum* (8.97%) and *F. moniliforme* (7.97%) while, the check (healthy looking seeds) recorded zero (%) stunted seedlings as shown in Table 2.

Effect of the selective antioxidants on the fungal growth (*in vitro* study): Data in Table 3 showed that salicylic acid at the concentrations of 1, 1.5, 2 and 2.5 g L^{-1}, citric acid and benzoic acid at 2, 2.5, 3 and 3.5 g L^{-1} and the combinations of salicylic acid at 2 g L^{-1}+citric acid at 3 g L^{-1}, salicylic acid at 2 g L^{-1}+ benzoic acid at 3 g L^{-1} as well as salicylic acid at 2 g L^{-1}+citric acid at 3 g L^{-1}+benzoic acid at 3 g L^{-1}, significantly reduced the *in vitro* growth of *F. moniliforme*, *F. oxysporum*, *Sclerotium bataticola* and *R. solani*. It was also noticed that the reduction in the fungal growth was correlated to the concentration of the tested antioxidants.

Field experiment: Role of the tested antioxidants and their combinations aligned in different methods of application on controlling soybean diseases.

Table 3: Effect of different concentrations of the antioxidants and their combination on the linear growth (cm) of the tested fungi

Treatments	Concentration (g L^{-1})	*Fusarium moniliforme*	*Fusarium oxysporum*	*Sclerotium bataticola*	*Rhizoctonia solani*
Salicylic acid	1	6.63^{bcd}	5.37^{bcd}	4.28^{bcde}	3.65^{def}
	1.5	5.15^{def}	4.31^{def}	2.85^{efg}	2.91^{efg}
	2	3.50^{gh}	3.37^{fg}	2.47^{fg}	2.20^{fg}
	2.5	2.51^{hi}	2.41^{gh}	1.77^{g}	1.57^{g}
Citric acid	2	8.60^{a}	8.29^{a}	5.14^{b}	8.17^{a}
	2.5	7.74^{ab}	6.49^{b}	4.44^{bcd}	6.33^{b}
	3	6.99^{bc}	5.85^{bc}	3.65^{cdef}	4.83^{cd}
	3.5	5.84^{cde}	4.89^{cde}	3.05^{defg}	4.04^{cde}
Benzoic acid	2	7.62^{ab}	6.39^{b}	5.10^{bc}	5.32^{bc}
	2.5	5.22^{def}	4.37^{def}	2.95^{efg}	4.26^{cde}
	3	5.10^{ef}	4.27^{def}	2.67^{fg}	2.88^{efg}
	3.5	4.32^{fg}	3.62^{efg}	2.26^{fg}	2.44^{fg}
Salicylic acid + citric acid	2+3	1.35^{ijk}	0.84^{i}	0.00^{h}	0.00^{h}
	2+3	0.93^{jk}	0.62^{i}	0.00^{h}	0.00^{h}
	3+3	1.82^{ij}	1.32^{hi}	0.00^{h}	0.00^{h}
	2+3+3	0.00^{k}	0.00^{i}	0.00^{h}	0.00^{h}
		9.00^{a}	9.00^{a}	9.00^{a}	9.00^{a}

Treatment		Pre emergence damping-off (%)	Post emergence damping-off (%)	Stunt seedlings (%)	Survival seedlings
Salicylic acid+benzoic acid	2+3	0.93^{jk}	0.62^{i}	0.00^{h}	0.00^{h}
Citric acid+benzoic acid	3+3	1.82^{ij}	1.32^{hi}	0.00^{h}	0.00^{h}
Salicylic acid+citric acid+benzoic acid	2+3+3	0.00^{k}	0.00^{i}	0.00^{h}	0.00^{h}
Check		9.00^{a}	9.00^{a}	9.00^{a}	9.00^{a}

Table 4: Impact of tested antioxidants on retarding pre-emergence damping off syndromes of soybean grown under infested with selected fungi

Fungi		Pre emergence damping-off (%)	Post emergence damping-off (%)	Stunt seedlings	Survival seedlings
Salicylic acid (2 g L^{-1})	Soaking	7.00^{b-e}	5.33^{b-f}	6.00^{b-d}	81.33^{e-h}
	Spraying	4.00^{e-g}	3.33^{d-g}	3.67^{c-f}	88.67^{b-f}
	Soaking+ Spraying	2.67^{f-h}	2.67^{e-h}	2.33^{e-g}	92.33^{a-d}
Citric acid (3 g L^{-1})	Soaking	8.67^{bc}	6.67^{a-c}	6.67^{a-c}	78.00^{g-i}
	Spraying	7.00^{b-e}	5.00^{b-f}	6.33^{a-c}	82.00^{d-h}
	Soaking+Spraying	4.67^{d-g}	3.33^{d-g}	4.00^{c-f}	88.33^{b-g}
Benzoic acid (3 g L^{-1})	Soaking	8.00^{b-d}	6.00^{b-d}	6.67^{a-c}	79.67^{f-h}
	Spraying	5.67^{c-g}	4.33^{c-g}	5.33^{c-e}	84.67^{d-g}
	Soaking+Spraying	3.33^{e-h}	2.33^{f-h}	2.67^{d-g}	91.67^{a-e}
Salicylic acid (2 g L^{-1}) + Citric acid (3 g L^{-1})	Soaking	6.33^{b-f}	4.67^{b-f}	5.33^{c-e}	83.67^{d-h}
	Spraying	4.33^{d-g}	3.33^{d-g}	3.67^{c-e}	88.67^{b-f}
	Soaking+Spraying	0.00^{h}	0.00^{h}	0.00^{g}	100.00^{a}
Salicylic acid (2 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	6.00^{c-f}	4.33^{c-g}	5.00^{c-f}	85.00^{c-g}
	Spraying	4.33^{d-g}	3.33^{d-g}	4.00^{c-f}	88.67^{b-f}
	Soaking+Spraying	0.00^{h}	0.00^{h}	0.00^{g}	100.00^{a}
Citric acid (3 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	7.00^{b-e}	5.67^{b-e}	6.33^{a-c}	82.00^{d-h}
	Spraying	4.00^{e-g}	3.00^{d-h}	3.67^{c-f}	89.33^{b-f}
	Soaking+Spraying	0.00^{h}	1.33^{gh}	1.67^{fg}	97.33^{ab}
Salicylic acid(2 g L^{-1}) + Citric acid (3 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	5.00^{c-g}	4.00^{c-g}	3.67^{c-f}	87.67^{b-g}
	Spraying	2.00^{gh}	1.33^{gh}	1.67^{fg}	95.33^{a-c}
	Soaking+Spraying	0.00^{h}	0.00^{h}	0.00^{g}	100.00^{a}
Check	Soaking	12.67^{a}	9.67^{a}	9.67^{a}	68.67^{i}
	Spraying	10.00^{ab}	7.67^{ab}	9.00^{ab}	73.67^{hi}
	Soaking+Spraying	6.67^{b-e}	4.67^{b-f}	5.67^{b-e}	83.33^{d-h}

Disease assessment: Data in Table 4 show the result of soaking and spraying the tested antioxidants on seeds and seedlings and the possible effect in decreasing of pre, post emergence damping off under natural infection. By using a combination of Salicylic Acid, Citric Acid and Benzoic Acid (SA+CA+BA) in the form of soaking+spraying, a significant retard in the incidence of the pre, post-emergence damping off and stunted seedlings were shown.

Table 5: Role of the tested antioxidants and their combinations aligned in different methods of application on the growth parameters of soybean plants

Treatments	Method of application	Growth parameters: Shoot length (cm)	Root length (cm)	Number of branch	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Salicylic acid (2 g L^{-1})	Soaking	458^{h-j}	82.3^{d-f}	25.3^{k-m}	533^{i-k}	109^{k-m}	240^{i-k}	68.0^{k-m}
	Spraying	527^{f-h}	107^{c-f}	29.7^{h}	625^{g}	142^{e-i}	281^{g}	88.3^{f-i}
	Soaking+Spraying	608^{c-f}	125^{cd}	34.0^{ef}	723^{ef}	171^{cd}	325^{ef}	107^{c-e}
Citric acid (3 g L^{-1})	Soaking	372^{k}	90.7^{d-f}	22.0^{n}	457^{l}	102^{m}	205^{l}	63.7^{m}
	Spraying	525^{f-h}	65.7^{ef}	28.0^{h-k}	583^{g-i}	130^{h-j}	263^{g-i}	81.0^{h-j}
	Soaking+Spraying	678^{a-c}	94.0^{d-f}	36.0^{de}	762^{de}	174^{c}	343^{de}	108^{cd}
Benzoic acid (3 g L^{-1})	Soaking	392^{jk}	114^{c-e}	23.7^{l-n}	499^{j-l}	105^{lm}	225^{j-l}	65.3^{lm}
	Spraying	494^{hi}	107^{c-f}	28.0^{h-k}	593^{gh}	132^{h-j}	267^{gh}	82.3^{h-j}
	Soaking+Spraying	644^{b-d}	84.0^{d-f}	34.0^{ef}	718^{ef}	173^{cd}	323^{ef}	107^{c-e}
Salicylic acid (2 g L^{-1}) + Citric acid (3 g L^{-1})	Soaking	432^{i-k}	133^{cd}	26.3^{i-l}	557^{h-j}	109^{k-m}	251^{h-j}	68.0^{k-m}
	Spraying	627^{b-d}	106^{c-f}	34.3^{ef}	723^{ef}	147^{r-h}	326^{ef}	91.3^{f-h}
	Soaking+Spraying	744^{a}	93.7^{d-f}	39.3^{c}	827^{c}	196^{b}	373^{c}	122^{b}
Salicylic acid (2 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	469^{h-j}	94.0^{d-f}	26.0^{j-m}	556^{h-j}	126^{i-k}	250^{h-j}	78.3^{i-k}
	Spraying	583^{d-g}	153^{bc}	34.3^{ef}	726^{ef}	156^{c-f}	327^{ef}	97.3^{d-g}
	Soaking+Spraying	733^{a}	189^{ab}	43.3^{b}	910^{b}	204^{b}	410^{b}	127^{b}
Citric acid (3 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	394^{jk}	104^{c-f}	23.3^{mn}	491^{kl}	120^{j-l}	221^{kl}	75.0^{j-l}
	Spraying	512^{g-i}	101^{c-f}	28.7^{h-j}	604^{gh}	141^{f-i}	272^{gh}	88.0^{g-i}
	Soaking+Spraying	696^{ab}	129^{cd}	38.7^{cd}	814^{cd}	174^{c}	367^{cd}	108^{c}
Salicylic acid(2 g L^{-1}) + Citric acid (3 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	534^{e-h}	115^{c-e}	30.3^{gh}	640^{g}	159^{c-e}	288^{g}	99.0^{c-f}
	Spraying	599^{c-f}	208^{ab}	38.0^{cd}	796^{cd}	194^{b}	358^{cd}	121^{b}
	Soaking+Spraying	761^{a}	239^{a}	46.7^{a}	986^{a}	223^{a}	444^{a}	139^{a}
Check	Soaking	400^{jk}	56.0^{f}	21.3^{n}	450^{l}	95.7^{m}	203^{l}	59.0^{m}
	Spraying	535^{e-h}	82.0^{d-f}	29.0^{hi}	609^{gh}	139^{g-i}	274^{gh}	86.7^{g-i}
	Soaking+Spraying	619^{b-e}	90.7^{d-f}	33.0^{fg}	700^{f}	156^{d-g}	315^{f}	97.0^{e-g}

Growth parameters: Table 5 illustrated the effect of the tested antioxidants and their combinations as seed soaking, 3 times of spraying started on 30 days old plants with 15 days interval on the growth parameters of soybean plants. By using of a combination of salicylic acid , citric acid and benzoic acid (SA+CA+BA) on soybean plants in the form of soaking +spraying , increases in the shoot length, root length, number of branches, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight were obvious and recorded 761 and 239 cm, 46.7, 986, 223, 444 and 139 g, respectively compared to the check (619 and 90.7 cm, 33.0, 700, 156, 315 and 97.0 g, respectively).

Yield components: Data in Table 6 showed the effect of the tested antioxidants and their combinations on yield components i.e., number of pods, pods fresh weight, pods dry weight and yield/fed of soybean plants. The application methods were carried out as seed soaking, 3 times of spraying started on 30 days old plants with 15 days interval and soaking +spraying.

The combination of SA+CA+BA in the form of soaking +spraying showed to be the most significant effect on increasing the number of pods, pods fresh weight, pods dry weight and yield/fed to record 88.7, 356 g,180 and 1555 kg, respectively compared to the check which recorded 62.0, 249 and 126 g and 1048 kg, respectively.

Photosynthetic pigments

Chlorophyll a: Data presented in Table 7 show the effect of the selected antioxidants and their combinations on chlorophyll a content in the leaves of 30, 45, 60 and 75 days old soybean plants. The treatments were carried out in form of seed soaking followed by 3 successive times of spraying started on 30 days old plants with 15 days interval.

The combination of SA+CA+BA in the form of soaking+spraying was the highest effective treatment on scaling up chlorophyll a content in the leaves of 30, 45, 60 and 75 days old plants. They recorded 2.10, 2.41, 2.54 and 1.98 mg g^{-1} fresh weight, respectively compared to check which recorded 1.49, 1.71, 1.81 and 1.41 mg g^{-1} fresh weight, respectively.

Chlorophyll b: Data presented in Table 8 show the effect of the selected antioxidants and their combinations on chlorophyll b content in the leaves of 30, 45, 60 and 75 days old soybean plants. The treatments were carried out in form of seed soaking followed by 3 successive times of spraying started on 30 days old plants with 15 days interval.

The combination of SA+CA+BA in the form of soaking+spraying was the highest effective treatment on increasing the chlorophyll b content in the leaves of 30, 45, 60 and 75 days old plants. They recorded 1.53, 1.76, 1.85 and

Table 6: Effect of the tested antioxidant and their combinations aligned in different methods on the yield components of soybean plants

Treatments	Method of application	Yield components			
		Number of pods/plant	Pods fresh weight per plant (g)	Pods dry weight per plant (g)	Yield per fed (kg)
Salicylic acid (2 g L^{-1})	Soaking	43.3^{l-n}	174^{k-m}	88.3^{j-l}	760^{j-l}
	Spraying	56.7^{f-j}	226^{e-i}	115^{e-h}	993^{e-h}
	Soaking+Spraying	68.0^{c-e}	274^{cd}	138^{cd}	1192^{cd}
Citric acid (3 g L^{-1})	Soaking	40.7^{n}	163^{m}	82.7^{l}	708^{l}
	Spraying	51.7^{i-k}	208^{h-j}	105^{g-i}	907^{g-l}
	Soaking+Spraying	69.0^{cd}	277^{c}	140^{cd}	1209^{cd}
Benzoic acid (3 g L^{-1})	Soaking	42.0^{mn}	168^{lm}	84.7^{kl}	725^{kl}
	Spraying	53.0^{i-k}	212^{h-j}	107^{g-i}	924^{g-l}
	Soaking+Spraying	69.0^{cd}	276^{cd}	139^{cd}	1200^{cd}
Salicylic acid (2 g L^{-1}) + Citric acid (3 g L^{-1})	Soaking	43.0^{mn}	174^{k-m}	87.8^{kl}	751^{kl}
	Spraying	58.3^{f-i}	234^{e-h}	118^{e-g}	1019^{e-g}
	Soaking+Spraying	78.0^{b}	313^{b}	159^{b}	1373^{b}
Salicylic acid (2 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	50.0^{j-l}	201^{i-k}	102^{h-j}	881^{h-j}
	Spraying	62.3^{d-g}	250^{c-f}	126^{c-f}	1088^{c-f}
	Soaking+Spraying	81.3^{b}	326^{b}	165^{b}	1425^{b}
Citric acid (3 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	47.7^{k-m}	193^{j-l}	97.3^{i-k}	838^{i-k}
	Spraying	56.0^{g-j}	225^{f-i}	114^{f-h}	985^{f-h}
	Soaking+Spraying	69.7^{c}	278^{c}	140^{c}	1209^{c}
Salicylic acid(2 g L^{-1}) + Citric acid (3 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	63.3^{c-f}	254^{c-e}	128^{c-e}	1105^{c-e}
	Spraying	77.3^{b}	311^{b}	157^{b}	1356^{b}
	Soaking+Spraying	88.7^{a}	356^{a}	180^{a}	1555^{a}
Check	Soaking	37.3^{n}	151^{m}	76.7^{l}	662^{l}
	Spraying	55.3^{h-j}	222^{g-i}	112^{f-h}	967^{f-h}
	Soaking+Spraying	62.0^{e-h}	249^{d-g}	126^{d-f}	1048^{d-f}

Table 7: Effect of the tested antioxidants and their combinations aligned in different methods on the chlorophyll a content in the leaves of soybean plants

Treatments	Method of application	Chlorophyll a (mg g^{-1} fresh weight)			
		Before spray	After 1st spray	After 2nd spray	After 3rd spray
		30 days old plants	45 days old plants	60 days old plants	75 days old plants
Salicylic acid (2 g L^{-1})	Soaking	1.14^{i-k}	1.30^{i-k}	1.37^{i-k}	1.07^{i-k}
	Spraying	1.33^{g}	1.53^{g}	1.61^{g}	1.26^{g}
	Soaking+Spraying	1.54^{ef}	1.77^{ef}	1.86^{ef}	1.45^{ef}
Citric acid (3 g L^{-1})	Soaking	0.97^{l}	1.12^{l}	1.18^{l}	0.92^{l}
	Spraying	1.24^{g-i}	1.43^{g-i}	1.50^{g-i}	1.17^{g-l}
	Soaking+Spraying	1.62^{de}	1.86^{de}	1.96^{de}	1.53^{de}
Benzoic acid (3 g L^{-1})	Soaking	1.06^{j-l}	1.22^{j-l}	1.29^{j-l}	1.00^{j-l}
	Spraying	1.26^{gh}	1.45^{gh}	1.53^{gh}	1.19^{gh}
	Soaking+Spraying	1.53^{ef}	1.76^{ef}	1.85^{ef}	1.44^{ef}
Salicylic acid (2 g L^{-1}) + Citric acid (3 g L^{-1})	Soaking	1.19^{h-j}	1.36^{h-j}	1.44^{h-j}	1.12^{h-j}
	Spraying	1.54^{ef}	1.77^{ef}	1.86^{ef}	1.45^{ef}
	Soaking+Spraying	1.76^{c}	2.02^{c}	2.13^{c}	1.66^{c}
Salicylic acid (2 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	1.18^{h-j}	1.36^{h-j}	1.43^{h-j}	1.12^{h-j}
	Spraying	1.55^{ef}	1.78^{ef}	1.87^{ef}	1.46^{ef}
	Soaking+Spraying	1.94^{b}	2.22^{b}	2.34^{b}	1.83^{b}
Citric acid (3 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	1.05^{kl}	1.20^{kl}	1.26^{kl}	0.99^{kl}
	Spraying	1.29^{gh}	1.48^{gh}	1.56^{gh}	1.21^{gh}
	Soaking+Spraying	1.73^{cd}	1.99^{cd}	2.10^{cd}	1.63^{cd}
Salicylic acid (2 g L^{-1}) + Citric acid (3 g L^{-1}) + benzoic acid (3 g L^{-1})	Soaking	1.36^{g}	1.57^{g}	1.65^{g}	1.29^{g}
	Spraying	1.70^{cd}	1.95^{cd}	2.05^{cd}	1.60^{cd}
	Soaking+Spraying	2.10^{a}	2.41^{a}	2.54^{a}	1.98^{a}
Check	Soaking	0.96^{l}	1.10^{l}	1.16^{l}	0.90^{l}
	Spraying	1.30^{gh}	1.49^{gh}	1.57^{gh}	1.22^{gh}
	Soaking+Spraying	1.49^{f}	1.71^{f}	1.81^{f}	1.41^{f}

Table 8: Effect of the tested antioxidants and their combinations aligned in different methods on the chlorophyll b in the leaves of soybean plants

Treatments	Method of application	Chlorophyll b (mg g^{-1} fresh weight) Before spray 30 days old plants	After 1st spray 45 days old plants	After 2nd spray 60 days old plants	After 3rd spray 75 days old plants
Salicylic acid (2 g L^{-1})	Soaking	0.83^{i-k}	0.95^{i-k}	1.00^{i-k}	0.78^{i-k}
	Spraying	0.97^{g}	1.11^{g}	1.17^{g}	0.91^{g}
	Soaking+Spraying	1.12^{ef}	1.29^{ef}	1.36^{ef}	1.06^{ef}
Citric acid (3 g L^{-1})	Soaking	0.71^{l}	0.81^{l}	0.86^{l}	0.67^{l}
	Spraying	0.90^{g-i}	1.04^{g-i}	1.09^{g-i}	0.85^{g-l}
	Soaking+Spraying	1.18^{de}	1.36^{de}	1.43^{de}	1.11^{de}
Benzoic acid (3 g L^{-1})	Soaking	0.77^{j-l}	0.89^{j-l}	0.94^{j-l}	0.73^{j-l}
	Spraying	0.92^{gh}	1.06^{gh}	1.11^{gh}	0.87^{gh}
	Soaking+Spraying	1.11^{ef}	1.28^{ef}	1.35^{ef}	1.05^{ef}
Salicylic acid (2 g L^{-1}) + Citric acid (3 g L^{-1})	Soaking	0.86^{h-j}	0.99^{h-j}	1.05^{h-j}	0.81^{h-j}
	Spraying	1.12^{ef}	1.29^{ef}	1.36^{ef}	1.06^{ef}
	Soaking+Spraying	1.28^{c}	1.47^{c}	1.55^{c}	1.21^{c}
Salicylic acid (2 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	0.86^{h-j}	0.99^{h-j}	1.04^{h-j}	0.81^{h-j}
	Spraying	1.13^{ef}	1.29^{ef}	1.36^{ef}	1.06^{ef}
	Soaking+Spraying	1.41^{b}	1.62^{b}	1.71^{b}	1.33^{b}
Citric acid (3 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	0.76^{kl}	0.87^{kl}	0.92^{kl}	0.72^{kl}
	Spraying	0.94^{gh}	1.08^{gh}	1.13^{gh}	0.88^{gh}
	Soaking+Spraying	1.26^{cd}	1.45^{cd}	1.53^{cd}	1.19^{cd}
Salicylic acid (2 g L^{-1}) + Citric acid (3 g L^{-1}) + benzoic acid (3 g L^{-1})	Soaking	0.99^{g}	1.14^{g}	1.20^{g}	0.94^{g}
	Spraying	1.24^{cd}	1.42^{cd}	1.49^{cd}	1.16^{cd}
	Soaking+Spraying	1.53^{a}	1.76^{a}	1.85^{a}	1.44^{a}
Check	Soaking	0.70^{l}	0.80^{l}	0.84^{l}	0.66^{l}
	Spraying	0.95^{gh}	1.08^{gh}	1.14^{gh}	0.89^{gh}
	Soaking+Spraying	1.09^{f}	1.25^{f}	1.31^{f}	1.02^{f}

1.44 mg g^{-1} fresh weight, respectively compared to check which recorded 1.09, 1.25, 1.31 and 1.02 mg g^{-1} fresh weight, respectively.

Total chlorophyll: Table 9 illustrates the effect of tested antioxidants on the content of total chlorophyll in the leaves of 30, 45, 60 and 75 days old soybean plants. The treatments were carried out in the form of seed soaking followed by 3 successive times of spraying started on 30 days old plants with 15 days interval.

The combination of SA+CA+BA in the form of soaking +spraying was the highest effective treatment on total chlorophyll content in the leaves of the plant of 30, 45, 60 and 75 days old. They recorded 3.63, 4.17, 4.39 and 3.42 mg g^{-1} fresh weight, respectively compared to the check (2.58, 2.96, 3.12 and 2.43 mg g^{-1} fresh weight, respectively.

Carotenoids: Data presented in Table 10 show the effect of the tested antioxidants and their combinations on the carotenoids content in the leaves of 30, 45, 60 and 75 days old plants. The treatments were carried out in form of seed soaking followed by 3 successive times of spraying started on 30 days old plants with 15 days interval.

The combination of SA+CA+BA in the form of soaking + spraying showed the highest effective treatment on carotenoids content in the leaves of 30, 45, 60 and 75 days old plants. They recorded 0.36, 0.41, 0.43and 0.34 mg g^{-1} fresh weight, respectively compared with the check which recorded 0.25, 0.29, 0.31 and 0.24 mg g^{-1} fresh weight, respectively.

Total phenols, protein and oil: Data presented in Table 11 illustrate the role of antioxidants on the total phenols content in the leaves of soybean plants. The treatment was carried out in form of seed soaking+spraying started on 30 days old plants followed by three interval times of spraying on plant leaves. Also, protein and oil content in seeds were determined.

The combination of SA+CA+BA in the form of soaking +spraying was the highest effective treatment in scaling up the content of total phenols in the leaves of 30, 45, 60 and 75 days old plants. They recorded 338, 495, 668 and 765 mg catechol per 100 g compared to the check which recorded 240, 351, 474 and 543 mg catechol per 100 g.

The content of protein and oil in the soybean seeds was significantly increased. Application of the combination of SA+CA+BA in form of soaking+spraying was the highest effect treatment in increasing their percentage to record 39.3 and 51.1%, respectively compared to the check which showed 32.0 and 36.3%, respectively.

Table 9: Effect of the tested antioxidants and their combinations aligned in different methods on the total chlorophyll in the leaves of soybean plants

		Total chlorophyll (mg g^{-1} fresh weight)			
		Before spray	After 1st spray	After 2nd spray	After 3rd spray
Treatments	Method of application	30 days old plants	45 days old plants	60 days old plants	75 days old plants
Salicylic acid (2 g L^{-1})	Soaking	1.96^{i-k}	2.25^{i-k}	2.37^{i-k}	1.85^{i-k}
	Spraying	2.30^{g}	2.64^{g}	2.79^{g}	2.17^{g}
	Soaking+Spraying	2.66^{ef}	3.05^{ef}	3.22^{ef}	2.51^{ef}
Citric acid (3 g L^{-1})	Soaking	1.68^{l}	1.93^{l}	2.03^{l}	1.58^{l}
	Spraying	2.15^{g-i}	2.46^{g-i}	2.60^{g-i}	2.02^{g-l}
	Soaking+Spraying	2.81^{de}	3.22^{de}	3.39^{de}	2.64^{de}
Benzoic acid (3 g L^{-1})	Soaking	1.84^{j-l}	2.11^{j-l}	2.22^{j-l}	1.73^{j-l}
	Spraying	2.18^{gh}	2.51^{gh}	2.64^{gh}	2.06^{gh}
	Soaking+Spraying	2.64^{ef}	3.03^{ef}	3.20^{ef}	2.49^{ef}
Salicylic acid (2 g L^{-1}) + Citric acid (3 g L^{-1})	Soaking	2.05^{h-j}	2.35^{h-j}	2.48^{h-j}	1.93^{h-j}
	Spraying	2.66^{ef}	3.06^{ef}	3.22^{ef}	2.51^{ef}
	Soaking+Spraying	3.05^{c}	3.49^{c}	3.68^{c}	2.87^{c}
Salicylic acid (2 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	2.05^{h-j}	2.35^{h-j}	2.47^{h-j}	1.93^{h-j}
	Spraying	2.67^{ef}	3.07^{ef}	3.23^{ef}	2.52^{ef}
	Soaking+Spraying	3.35^{b}	3.84^{b}	4.05^{b}	3.16^{b}
Citric acid (3 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	1.81^{kl}	2.07^{kl}	2.19^{kl}	1.70^{kl}
	Spraying	2.23^{gh}	2.55^{gh}	2.69^{gh}	2.10^{gh}
	Soaking+Spraying	3.00^{cd}	3.44^{cd}	3.63^{cd}	2.82^{cd}
Salicylic acid(2 g L^{-1}) + Citric acid (3 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	2.36^{g}	2.70^{g}	2.85^{g}	2.22^{g}
	Spraying	2.93^{cd}	3.36^{cd}	3.55^{cd}	2.76^{cd}
	Soaking+Spraying	3.63^{a}	4.17^{a}	4.39^{a}	3.42^{a}
Check	Soaking	1.66^{l}	1.90^{l}	2.00^{l}	1.56^{l}
	Spraying	2.24^{gh}	2.57^{gh}	2.71^{gh}	2.11^{gh}
	Soaking+Spraying	2.58^{f}	2.96^{f}	3.12^{f}	2.43^{f}

Table 10: Effect of the tested antioxidants and their combinations aligned in different methods on the carotenoids content in the leaves of soybean plants

		Carotenoids (mg g^{-1} fresh weight)			
		Before spray	After 1st spray	After 2nd spray	After 3rd spray
Treatments	Method of application	30 days old plants	45 days old plants	60 days old plants	75 days old plants
Salicylic acid (2 g L^{-1})	Soaking	0.19^{i-k}	0.22^{i-k}	0.23^{i-k}	0.18^{i-k}
	Spraying	0.23^{g}	0.26^{g}	0.27^{g}	0.21^{g}
	Soaking + Spraying	0.26^{ef}	0.30^{ef}	0.32^{ef}	0.25^{ef}
Citric acid (3 g L^{-1})	Soaking	0.16^{l}	0.19^{l}	0.20^{l}	0.16^{l}
	Spraying	0.21^{g-i}	0.24^{g-i}	0.26^{g-i}	0.20^{g-l}
	Soaking + Spraying	0.28^{de}	0.32^{de}	0.33^{de}	0.26^{de}
Benzoic acid (3 g L^{-1})	Soaking	0.18^{j-l}	0.21^{j-l}	0.22^{j-l}	0.17^{j-l}
	Spraying	0.21^{gh}	0.25^{gh}	0.26^{gh}	0.20^{gh}
	Soaking + Spraying	0.26^{ef}	0.30^{ef}	0.31^{ef}	0.24^{ef}
Salicylic acid (2 g L^{-1}) + Citric acid (3 g L^{-1})	Soaking	0.20^{h-j}	0.23^{h-j}	0.24^{h-j}	0.19^{h-j}
	Spraying	0.26^{ef}	0.30^{ef}	0.32^{ef}	0.25^{ef}
	Soaking + Spraying	0.30^{c}	0.34^{c}	0.36^{c}	0.28^{c}
Salicylic acid (2 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	0.20^{h-j}	0.23^{h-j}	0.24^{h-j}	0.19^{h-j}
	Spraying	0.26^{ef}	0.30^{ef}	0.32^{ef}	0.25^{ef}
	Soaking + Spraying	0.33^{b}	0.38^{b}	0.40^{b}	0.31^{b}
Citric acid (3 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	0.18^{kl}	0.20^{kl}	0.21^{kl}	0.17^{kl}
	Spraying	0.22^{gh}	0.25^{gh}	0.26^{gh}	0.21^{gh}
	Soaking+Spraying	0.29^{cd}	0.34^{cd}	0.36^{cd}	0.28^{cd}
Salicylic acid(2 g L^{-1}) + Citric acid (3 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	0.23^{g}	0.27^{g}	0.28^{g}	0.22^{g}
	Spraying	0.29^{cd}	0.33^{cd}	0.35^{cd}	0.27^{cd}
	Soaking+Spraying	0.36^{a}	0.41^{a}	0.43^{a}	0.34^{a}
Check	Soaking	0.16^{l}	0.19^{l}	0.20^{l}	0.15^{l}
	Spraying	0.22^{gh}	0.25^{gh}	0.27^{gh}	0.21^{gh}
	Soaking+Spraying	0.25^{f}	0.29^{f}	0.31^{f}	0.24^{f}

Table 11: Effect of the tested antioxidant and their combinations aligned in different methods on the total phenols in form of mg catechol per 100 g fresh weight

		Total phenols (mg catechol per 100 g fresh weight)					
		Before spray	After 1st spray	After 2nd spray	After 3rd spray		
Treatments	Method of application	30 days old plants	45 days old plants	60 days old plants	75 days old plants	Protein	Oil
Salicylic acid (2 g L^{-1})	Soaking	182^{i-k}	268^{i-k}	361^{i-k}	414^{i-k}	23.6^{h-k}	27.6^{i-k}
	Spraying	214^{g}	314^{g}	423^{g}	485^{g}	27.2^{f-h}	32.4^{g}
	Soaking+Spraying	247^{ef}	363^{ef}	489^{ef}	561^{ef}	31.4^{c-f}	37.4^{ef}
Citric acid (3 g L^{-1})	Soaking	156^{l}	229^{l}	309^{l}	354^{l}	19.2^{k}	23.6^{l}
	Spraying	200^{g-i}	293^{g-i}	395^{g-i}	453^{g-i}	27.1^{f-h}	30.2^{g-i}
	Soaking+Spraying	261^{de}	382^{de}	516^{de}	591^{de}	35.0^{a-c}	39.5^{de}
Benzoic acid (3 g L^{-1})	Soaking	171^{j-l}	250^{j-l}	338^{j-l}	387^{j-l}	20.2^{jk}	25.8^{j-l}
	Spraying	203^{gh}	298^{gh}	401^{gh}	460^{gh}	25.5^{hi}	30.7^{gh}
	Soaking+Spraying	246^{ef}	361^{ef}	486^{ef}	557^{ef}	33.3^{b-d}	37.2^{ef}
Salicylic acid (2 g L^{-1}) + Citric acid (3 g L^{-1})	Soaking	190^{h-j}	280^{h-j}	377^{h-j}	432^{h-j}	22.3^{i-k}	28.9^{h-j}
	Spraying	247^{ef}	363^{ef}	489^{ef}	561^{ef}	32.4^{b-d}	37.4^{ef}
	Soaking+Spraying	283^{c}	415^{c}	560^{c}	641^{c}	38.5^{a}	42.8^{c}
Salicylic acid (2 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	190^{h-j}	279^{h-j}	376^{h-j}	431^{h-k}	24.2^{h-j}	28.8^{h-j}
	Spraying	248^{ef}	364^{ef}	492^{ef}	563^{ef}	30.1^{d-g}	37.6^{ef}
	Soaking+Spraying	311^{b}	456^{b}	616^{b}	705^{b}	37.9^{a}	47.1^{b}
Citric acid (3 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	168^{kl}	246^{kl}	332^{kl}	380^{kl}	20.3^{jk}	25.4^{kl}
	Spraying	207^{gh}	303^{gh}	409^{gh}	469^{gh}	26.5^{g-i}	31.3^{gh}
	Soaking+Spraying	279^{cd}	409^{cd}	551^{cd}	631^{cd}	36.0^{ab}	42.2^{cd}
Salicylic acid(2 g L^{-1}) + Citric acid (3 g L^{-1}) + Benzoic acid (3 g L^{-1})	Soaking	219^{g}	321^{g}	433^{g}	497^{g}	27.6^{e-h}	33.2^{g}
	Spraying	272^{cd}	399^{cd}	539^{cd}	617^{cd}	31.0^{c-f}	41.2^{cd}
	Soaking+Spraying	338^{a}	495^{a}	668^{a}	765^{a}	39.3^{a}	51.1^{a}
Check	Soaking	154^{l}	226^{l}	305^{l}	349^{l}	20.7^{jk}	23.3^{l}
	Spraying	209^{gh}	306^{gh}	412^{gh}	472^{gh}	27.6^{e-h}	31.6^{gh}
	Soaking+Spraying	240^{f}	351^{f}	474^{f}	543^{f}	32.0^{b-e}	36.3^{f}

DISCUSSION

The results presented here clearly demonstrate the potential of antioxidants to control soil and seed borne fungi attacking soybean during their life span.

The *in vivo* results were supported with *in vitro* studies while the following antioxidants were used i.e.: salicylic acid, citric acid, benzoic acid and their combination. The growth of following fungi, *F. oxysporum*, *F. moniliforme*, *Sclerotium bataticola* and *R. solani* were severely depressed.

Based on these experiments, it appears that antioxidants could significantly contribute in the control of diseases attacking a number of important food crops. However, this inclusion closely resemble those found in a number of plants including soybean[13-15], sugar beet[16] and table beet[17].

So far, these results are an additional basis for the potential of antioxidants to control the fungal diseases with significant impact on increasing the yield and quality.

However, it was found a strong correlation between the use of antioxidants; salicylic acid, citric acid and benzoic acid and disease suppression of the above fungi. The treatments also improved the seed quality and gained a higher yield.

The application of the antioxidants in this research was done as seed soaking before sawing seeds followed by spraying the vegetative growth of 30, 45, 60 and 75 days old plants at three interval times. These treatments presented an increase in the growth parameters (shoot length, root length, number of branches, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight); yield components (No. of pods, pods fresh weight and pods dry weight); photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids); total phenols in leaves of soybean plants and the percentage of protein and oil content in the seeds when salicylic acid with citric acid and benzoic acid were applied at concentrations of 1, 1.5, 2, 2.5 g L^{-1}, citric acid at 2, 2.5, 3, 3.5 g L^{-1}, benzoic acid at 2, 2.5, 3, 3.5 g L^{-1}, respectively. The same direction was reported by Ibrahim[18] who found a reduction in the percentage of abnormal seedlings and seed rot, besides an enhancement in the growth parameters compared with the control treatments when soybean seeds was treated with salicylic acid at a concentration 10 mM for 12 hrs.

In the meanwhile, the combination of the three above antioxidants when used the same way as carried in each one solely in formulation of salicylic acid at 2 g L^{-1}+citric acid at 3 g L^{-1}, salicylic acid at 2 g L^{-1}+benzoic acid at 3 g L^{-1} proved to the best of all treatment as the yield increased by 48% and oil content increased by 40.8% and the protein by 22.8%.

It is worth mentioning that the pathogenic fungi isolated from Soybean seeds represented only in three genera i.e., *Fusarium*, *Rhizoctonia*, *Sclerotium* out of 11 isolated species. In this research, the effects of these 3 fungi on seeds, seedlings and mature plants growing in infested soil with each of them were tested. Data revealed their passive effects on the pre and post seed emergence as well as the seedlings growth.

It was also found that the diseased plants due to the attack of *R. solani* was pronounced and reached 40% of the growing plants. Destroyed plants due to the attack of *S. bataticola* reached 37% while *F. oxysporum* showed its effect on 30% of the plants. *F. moniliforme* was restricted in its effect on 18% of the plants.

The snapshot survey of symptoms on the mature plants showed that they steeped to 20% in case of *R. solani*, 19% in case of *S. bataticola*, 17% in case of *F. oxysporum* while 9% infected plants was shown due to *F. moniliforme* infection.

These results direct attention to consider these above fungi as highly pathogenic on the Plants during their early stage of the growth and less effective on the mature ones.

With regard to the role of combination of the 3 tested antioxidants (SA+CA+BA) used in the control of the diseases that occurred by the isolated pathogenic fungi, it was clearly shown that this formulation was the best of all in controlling the target diseases. In this context, this friendly technology used to control soybean fungal diseases verified that antioxidants play their known role in enhancing the acquired resistance in the plants to overcome the passive effects of free radicals accumulation in the reaction pool within the plant cells, so far, reduces the environmental stress and keep the plants grow under optimal conditions.

It is obvious from this research that using chemicals of antioxidant properties as seed soaking with interval spray on the growing plants scale up the growth parameters, the contents of phenols and carotenoids in the leaves as well as vigor the plant growth which enable them to overcome the stress of the invaded fungi during the life span of the plants, these results were to encourage the soybean growers to reform their classical methods of managing the diseases of this crop and to avoid the detrimental effect of using pesticides on the plants and the environment. So far increasing their income when producing a crop of higher yield and quality seeds.

This trend in controlling plant diseases with antioxidant of potential to limit the invasion of the growth of several fungi attacking other crops was reported on sunflower and cucumber[19,20]. Moreover, these antioxidants performed well in scaling up protein and oil in soybean Seeds.

In this direction, the research did not stand at these stages but extended to study their effect on the yield components including the number of pod per plant, pod fresh weight, pod dray weight and subsequently seed yield per Faddan. Although the fungicides remain useful tool in controlling plant diseases, the perusal of date precisely revealed the potential of the antioxidants to narrow down the demand on pesticides in the agriculture programs designed to control plant diseases, Finally date were analyzed using Costat program and verified the significance of using antioxidants for controlling soybean diseases and scaling up the production and the seed quality.

CONCLUSION

The results showed a strong correlation between use of antioxidants and decreased disease incidence and resulted in increased yields. This research suggests the potential for benefits of using antioxidants as seed treatments and plant sprays to control soybean diseases resulting in increased yields of high quality seeds. The combination of Salicylic acid (2 g L^{-1})+Citric acid (3 g L^{-1})+Benzoic acid (3 g L^{-1}) as seed soaking, 3 times of spraying started on 30 days old plants with 15 days interval on plant leaves. This treatment was the highest in decreased disease incidence and resulted in increased yields.

SIGNIFICANCE STATEMENT

This study discovered that antioxidants have the potential to suppress the growth of the pathogenic fungi attacking crops and thrives the growth of the plants as well as show higher yield and quality seeds. Also, keep the environment clean, produce seeds free from the toxic used fungicides which may use intensively far from the international regulation. This research suggests the potential for benefits of using antioxidants as seed treatments and plant sprays to control soybean diseases resulting in increased yield of high-quality seeds.

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