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Research Article
Incidence of Soybean Root and Stalk Rot Diseases as a Result of Antioxidant and Biotic Agents

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Abstract

Background and Objective: Soybean is currently infected by soil-borne fungi, which cause shortage in yield productivity and seed quality. Wherein, the application of bioagent became urgent demand to avoid the side effect of agrochemicals. The aim of study is to investigate the incidence of soybean rot diseases and how to alleviate the causal pathogens of disease. **Methodology:** Infected soybean samples with typical symptoms of root and stalk rot diseases were collected and the causal fungal pathogens were isolated and identified, i.e., *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina*. Antioxidant (benzoic, citric and salicylic acids) and biotic agents (*Bacillus subtilis* and *Saccharomyces cerevisiae*) were used as control agents against fungal pathogens. **Results:** The cultivar Giza 111 was more susceptible to fungal pathogens that cause pre and post damping-off compared to the another one. Interestingly, low concentration of antioxidants showed to be more active compared to higher concentration, however, both *Bacillus subtilis* and *Saccharomyces cerevisiae* showed more active at the higher doses. As well as, the plant heights and branches numbers increased significantly among treatments with both antioxidants and biotic agents in both seasons and cultivars. Seed quality including protein, oil and phosphorus percentages showed increasing as the result of parameters under study. **Conclusion:** Biotic agents, i.e., *B. subtilis* and *S. cerevisiae*, as well as, antioxidants were effective in decreasing pre and post damping-off, increasing plant height and branches number. Seed quality was increased as the result of all treatments.

Key words: Soybean diseases, *Bacillus subtilis*, *Saccharomyces cerevisiae*, antioxidant substances, induce systemic resistance

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Soybean [*Glycine max* (L.) Merr] is one of the most important legumes, rich in protein and oil, which can be used in agriculture and oil extraction industry. It contains up to 40% protein and 20% oil along with calcium, iron, carotene, thiamine and ascorbic acid[1]. Moreover, carbohydrate content is about 30% with total soluble sugars equal to about 10%. Also, soybean can be considered as a friendly crop to the environment related to its supply of the soil by nitrogen fixation. Additionally, it is a good dietary source of calcium and phosphorus that is why soybean is a remarkable plant[2,3]. One of the major constraints in soybean cultivation is the non-availability of high vigor seeds at the time of sowing. Furthermore, poor seed germination is a major constraint for increasing the productivity of soybean, in which the seed longevity is greatly influenced by the relative humidity and storage temperature[4]. In addition, seed yield and quality depend upon multi-criterion, i.e., chemical composition, physical condition, physiological germination, etc.[5]. As well as, soil-borne fungal diseases are limiting factors of soybean growth and productivity, in which root rot and charcoal rot diseases are the most destructive diseases attacking soybean seeds, seedlings and roots as well as lower part of the stem causing serious damage[6,7]. The major fungi that causing soybean seeds diseases are *Rhizoctonia solani*, where causing foot and root rots of young soybean plants, *Macrophomina phaseolina* causing charcoal rot and *Fusarium* spp., causing wilt diseases, which characterized by the browning of the vascular tissue of roots and stems[7,8]. In general, pesticides and fungicides were recommended to control various plant pathogens. However, these agents were found to accumulate their toxicity on the human health and environment. Recently, the use of antioxidant substances were found to be inducible for plant defenses and provide protection against abroad spectrum of diseases-causing organisms[9,10]. Where, the antioxidants enhance the level of plant phenols, which play a major role in plant disease defense, growth and development[11]. The induced systemic resistance is triggered by a number of chemicals such as Salcylic Acid (SA), acetyl salicylic acid (aspirin) and hydroquinone, which induce the systemic resistance in many crops like tomato, tobacco, pea, maize, cotton, rice, potato and other vegetables against viruses, fungi and bacteria[12-14]. On the other hand, the application of biological control using antagonistic microorganisms proved to be a successful tool for controlling various plant pathogens, since *Bacillus subtilis* has a fungicidal effect to *Penicillum* sp. and other 26 fungi associated with soybean seeds. Also, *Bacillus subtilis* reduced stem infection caused by *Phomopsis* sp.[15]. Other studies showed the role of bioagents such *as Saccharomyces cerevisiae*, *B. subtilis*, *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluorescens* against vegetables foliar diseases and faba bean wilt[16,17]. Both *Candida oleophila* and C sake showed antagonist effect toward *Botrytis cinerea* and *Penicillium expansum* on apple, respectively[18,19]. Ultimately, the growing concern over the use of fungicide to human health and environment has brought increasing interest in the use of alternative procedure characterized with negative impact on the environment. Wherein, the present study aimed to controlling soybean root rot and stalk rot diseases during the use of antioxidants (salicylic, benzoic and citric acids) and bio-agents (*Bacillus subtilis* and *Saccharomyces cerevisiae*).

MATERIALS AND METHODS

Source of soybean seeds: Two soybean cultivars (Giza 35 and Giza 111) were kindly obtained from Legume Crop Research Department, Field Crop Research Institute, Agriculture Research Centre, Giza, Egypt.

Chemicals and microorganisms: Three abiotic agents namely, salicylic acid, citric acid and benzoic acid at 15 and 20 mM were used to study their effects in inducing resistance in soybean plants against root rot and stalk rot diseases. Two microorganisms, i.e., *Bacillus subtilis* and *Saccharomyces cerevisiae* were kindly obtained from Microbial Activity Unit, Department of Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt. Rizolex-T 50 at 3 kg^{-1} seeds was used as a control fungicide.

Preparation of inocula of bioagents: Bacterium and yeast strains were grown on nutrient broth and yeast mannitol broth media in Erlenmeyer flasks, respectively. The flasks were incubated on rotary shaker at 25°C and 150 rpm for 24 h (*B. subtilis*) and 72 h (*S. cerevisiae*). The counted number of viable cells of fresh cultures at the time of use for inoculation were adjusted to 10^5 and 10^{10} CFU mL^{-1} (bacterium) and 10^4 and 10^8 cells mL^{-1} (yeast). Haemocytometer slide under light microscope was used for this purpose.

Identification of some isolated soybean fungal pathogens: Soybean plant samples showing typical symptoms of root and stalk rot diseases were collected from Tag EL-EZZ Agricultural Research Station, Dakhlia Government, Egypt and fungal pathogens were isolated on PDA medium. Selected fungi were identified according to their cultural properties, morphological and microscopic characteristics[20,21]. For determination of morphological structures, portions of fungal growth were mounted in lacto-phenol cotton blue stain on clean slides[22].

The prepared slide was examined under a light microscope using the 40× and 100× objectives for vegetative mycelium: septation, diameters, conidiophores (sporangiophores) and the reproductive structures: Conidia and sporangiospores, etc. Fungal colonies were examined under the 10× objective of the microscope. The colonial characteristics of size, texture and colour of the colony were investigated.

Field experiments: The experiments were carried out in a field naturally infection with the causal organisms of damping-off, root rot and stalk rot diseases of soybean during 2013 and 2014 summer seasons at Tag El-Ezz Research Station, Dakhlia Governorate, Egypt. Seeds of soybean (cvs. Giza 35 and Giza 111) were soaked in both abiotic and biotic agents for 15 min, while Rizolex-T 50 was used as seed coating. The wetted seeds were air dried before sowing. Seeds were planted on 5 May, 2 of both seasons, respectively. A split plot design with three replicates was used in these experiments; the main plots represented varieties, while sub-plots were occupied by treatments. The area of each sub-plot was 10.5 m^2 (3.5×3.0 m) sown with 180 seeds. The germination percentage and pre-emergence damping-off were recorded at 20 days from sowing. While post-emergence damping-off was recorded at 40 days from sowing.

Morphological and physiological characters: After 60 days from sowing date, plant height (cm) and number of branched were determined. At the same time, samples were collected to determine photosynthetic pigments and total phenol content.

Determination of photosynthetic pigments: Photosynthetic pigments (chlorophyll a, b and carotenoids) extracted with methanol from the blade of 3rd leaf from tip (terminal leaflet) were determined[23,24].

Determination of phenol content: Total phenolic compounds were determined in fresh shoot using the folin-ciocalteau reagent[25].

Disease assessment: Disease severity of root rot was determined at mature stage[26] as follow:

0 = Healthy plant
1 = The lower part of stem is slight darkened
2 = The region between the knots is heavily darkened
3 = The lower part of the stem is heavily darkened while, the upper is whitened
4 = Damping-off plant

Disease severity of stalk rot disease severity was determined[27] as follow:

0 = 0 cm (no discoloration under the first node)
1 = s cm (discoloration through the first node)
2 = 13 cm (discoloration through the second node)
3 = 17 cm (discoloration through the third node)
4 = 28 cm (discoloration through the fourth node)

Then, the severity of root rot and stalk rot diseases were calculated from the following equation:

$$Sd = \frac{\varepsilon ab}{AK} \times 100$$

Where:
Sd = Severity of disease
a = No. of diseased plants having similar degree of infection
b = Degree of infection
A = No. of examined plants
K = Highest degree of infection
ε = Sum of (ab)

Yield and its components: At harvesting, samples were taken from both varieties to stimulate number of pods per plant, plant yield and weight of 100 seed.

Seed quality: Soybean seeds were chosen from the best treatments in the second season only, dried at 70°C for 48 h, grounded and analyzed for protein and oil content[28] and phosphorus percent[29].

Statistical analysis: All data were subjected to the proper statistical analysis of variance, mean values of treatments were differentiated using Least Significant Difference (LSD). The statistical analysis Soft Ware: CoStat[30] V. 6.4 was used.

RESULTS

Isolation of pathogenic fungi: Soybean plant samples showing typical symptoms of root and stalk rot diseases were collected and subjected to fungal analysis. The causal fungal pathogens were selected and identified, i.e., *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina*, according to their cultural properties, morphological and microscopic characteristics.

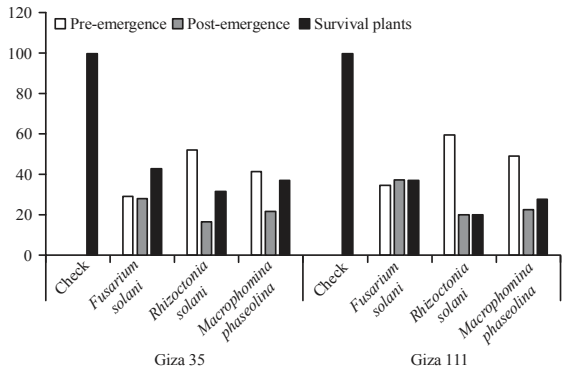

Fig. 1: Pathogenicity test of pre and post-emergence damping-off and survivals of soybean plants infected with isolated fungi under greenhouse conditions

Pathogenicity test: Figure 1 shows that the three tested fungal pathogens that causes pre and post-emergence damping-off were carried out on two soybean cultivars *in vitro*. Giza 111 cultivar was more sensitive to the infection compared to Giza 35. The *R. solani* was the most aggressive pathogen, which causing pre-emergence damping-off in both soybean cultivars, followed by *M. phaseolina*. While *F. solani* caused the highest percentage of post-emergence damping-off. On the other hand, the highest percentage of survival plants occurred in control followed by the treated soil by *F. solani*, *M. phaseolina* and *R. solani*, respectively.

Field experiment

Germination percentage and damping-off: Table 1 showing that Giza 35 cultivar was the highest in germination percentage in the stage of pre-emergence. Whereas, Giza 111 showing less percentage of post-emergence damping-off under natural infection condition.

Concerning the effect of antioxidants and biotic-agents and its interaction with soybean cultivars, it was observed that either antioxidants or biotic agents at any dose used decreased significantly damping-off disease, consequently, the germination percentage of both cultivars increased significantly in both seasons. With respect to antioxidants treatment, the low concentration (15 mM) shows the highest effect compared to the high concentration (20 mM). In this respect, benzoic acid was more effective followed by salicylic acid. On the other hand, the high dose of biotic-agents was more effective than the low dose. *Bacillus subtilis* was the most effective. Generally, *B. subtilis* at the number of 10^8 CFU mL^{-1} came next to fungicide followed by benzoic acid at 15 mM in the first season. However, in the second season, yeast at 100% came in the first followed by *B. subtilis* at 10^8 CFU mL^{-1} and benzoic acid at 15 mM.

Morphological characters: The effect of antioxidants and biotic-agents as well as Rizolex-T 50 fungicide on average plant height and number of branches per plant of both soybean cultivars during two successive growing seasons are presented in Table 2. Plant height significantly increased in Giza 111 compared to Giza 35 in both seasons. While, number of branches showed inverse this. Data also show that, all antioxidants and biotic-agents at any dose used increased significantly plant height and branches number. Whereas, Rizolex did not cause any significant effect on morphological characters in both seasons. The highest values of plant height occurred under the application of low concentration of *B. subtilis* followed by low concentration of yeast. On the other hand, the low level of benzoic acid (15 mM) was the most effective of antioxidants followed by high level of salicylic acid (20 mM). The same trend was observed with the interaction between treatments and cultivars. The highest increase in number of branches under the application of yeast at 50% followed by *B. subtilis* at 10^4 and benzoic acid at 15 mM.

Disease incidence: The disease severity of root rot and stalk rot disease of both soybean cultivars in mature stage were recorded in Table 3. Giza 111 cultivar was more sensitive to infected by root and stalk rots disease than Giza 35 under natural infection in field conditions. All tested materials significantly reduced root rot and stalk rot disease severity in both cultivars of soybean. The maximum reduction was recorded with Rizolex-T 50 fungicide.

Table 1: Effect of antioxidants and biotic-agents on germination percentage, pre and post-emergence damping-off of soybean plant under field condition

	Germination (%)		Pre-emergence		Post-emergence	
Treatments	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Variety						
Giza 35	92.44^{a}	88.47^{a}	7.56^{b}	11.53^{b}	4.77^{A}	5.86^{A}
Giza 111	90.42^{b}	84.69^{b}	8.61^{a}	15.31^{a}	4.17^{B}	5.06^{B}
Treatments						
Saly-15	92.00^{cd}	86.17^{c}	8.00^{de}	13.83^{c}	4.67^{De}	5.67^{De}
Saly-20	90.00^{f}	83.67^{de}	10.00^{abc}	16.33^{ab}	5.33^{Bc}	6.50^{Bc}
Citric-15	90.50^{ef}	86.00^{c}	9.50^{bc}	14.00^{c}	5.00^{Cd}	6.17^{Cd}
Citric-20	88.83^{g}	83.83^{de}	11.17^{ab}	16.17^{ab}	5.67^{B}	6.83^{B}
Benzoic-15	92.83^{c}	86.83^{c}	7.17^{ef}	13.17^{c}	4.17^{Ef}	5.17^{Ef}
Benzoic-20	89.83^{fg}	84.50^{d}	10.17^{ab}	15.50^{b}	4.67^{De}	5.50^{E}
Bacillus subtilis 10^{8}	90.67^{ef}	89.17^{b}	9.33^{bc}	10.83^{d}	3.33^{Gh}	4.00^{Hi}
Bacillus subtilis 10^{4}	92.00^{cd}	87.17^{c}	8.00^{de}	12.83^{c}	4.33^{Ef}	4.83^{Fg}
Yeast 100%	91.17^{de}	90.00^{b}	8.83^{d}	10.00^{d}	3.00^{H}	3.67^{Ij}
Yeast 50%	87.67^{h}	86.83^{c}	13.33^{a}	13.17^{c}	3.83^{Fg}	4.50^{Gh}
Rizolex	94.00^{b}	92.00^{a}	6.00^{fg}	8.00^{e}	2.33^{I}	3.17^{J}
Control	90.00^{f}	82.83^{e}	10.00^{abc}	17.1^{a}	7.33^{A}	9.50^{A}
Interaction						
Giza 35						
Saly-15	92.67^{Cdef}	87.67^{ef}	7.33^{fghi}	12.33^{fg}	5.33^{cde}	6.00^{Ef}
Saly-20	91.33^{Fgh}	86.00^{fghi}	8.67^{def}	14.00^{cdef}	5.67^{bcd}	7.00^{Cd}
Citric-15	92.00^{Efg}	88.00^{de}	8.00^{efgh}	12.00^{gh}	5.33^{cde}	6.33^{De}
Citric-20	91.00^{Ghi}	86.67^{efgh}	9.00^{def}	13.33^{defg}	6.00^{Bc}	7.33^{C}
Benzoic-15	93.33^{Bcde}	88.33^{cde}	6.67^{ghij}	11.67^{ghi}	4.67^{Ef}	5.67^{e-g}
Benzoic-20	91.33^{Fgh}	87.33^{efg}	8.67^{def}	12.67^{efg}	5.33^{cde}	6.33^{De}
Bacillus subtilis 10^{8}	94.00^{bc}	91.00^{b}	6.00^{ij}	9.00^{j}	3.33^{ghi}	4.33^{i-k}
Bacillus subtilis 10^{4}	91.67^{fgh}	90.00^{bc}	8.33^{efg}	10.00^{ij}	4.00^{Fg}	5.00^{g-i}
Yeast 100%	93.67^{bcd}	90.33^{b}	6.33^{hij}	9.67^{j}	3.00^{Hi}	4.00^{i-l}
Yeast 50%	91.33^{fgh}	88.00^{de}	8.67^{def}	12.00^{gh}	3.67^{Gh}	4.67^{h-j}
Rizolex	96.67^{a}	93.33^{a}	3.33^{l}	6.67^{k}	2.67^{Ij}	3.67^{Kl}
Control	90.33^{hij}	85.00^{hij}	9.67^{cde}	15.00^{bcd}	8.33^{A}	10.33^{A}
Giza 111						
Saly-15	91.33^{fgh}	84.67^{ij}	8.67^{def}	15.33^{bc}	4.00^{Fg}	5.33^{f-h}
Saly-20	88.67^{k}	81.33^{K}	11.33^{bc}	18.67^{a}	5.00^{De}	6.00^{Ef}
Citric-15	89.00^{jk}	84.00^{J}	11.00^{bc}	16.00^{b}	4.67^{Ef}	6.00^{Ef}
Citric-20	86.67^{l}	81.00^{K}	13.33^{a}	19.00^{a}	5.33^{cde}	6.33^{De}
Benzoic-15	92.33^{defg}	85.33^{hij}	7.67^{fghi}	14.67^{bcd}	3.67^{Gh}	4.67^{h-j}
Benzoic-20	88.33^{k}	81.67^{K}	11.67^{ab}	18.33^{a}	4.00^{Fg}	5.00^{g-i}
Bacillus subtilis 10^{8}	94.00^{bc}	87.33^{efg}	6.00^{ij}	12.67^{efg}	3.33^{ghi}	3.67^{Kl}
Bacillus subtilis 10^{4}	89.67^{ijk}	84.33^{Ij}	10.33^{bcd}	15.67^{bc}	4.67^{Ef}	4.67^{h-j}
Yeast 100%	94.33^{b}	89.67^{bcd}	4.00^{kl}	10.33^{hij}	3.00^{Hi}	3.33^{Lm}
Yeast 50%	91.00^{ghi}	85.67^{ghij}	9.00^{def}	14.33^{bcde}	4.00^{Fg}	4.33^{i-k}
Rizolex	94.67^{b}	90.67^{B}	5.33^{jk}	9.33^{j}	2.00^{J}	2.67^{M}
Control	85.00^{m}	80.67^{K}	5.00^{jkl}	19.33^{a}	6.33^{B}	8.67^{B}
LSD						
Varieties	0.11	0.66	0.31	0.97	0.11	0.63
Treatments	1.06	1.18	1.22	1.20	0.60	0.59
V×T	1.50	1.67	1.73	1.70	0.92	0.27

Means within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test

With respect to antioxidants, the low concentration of benzoic acid came in the first order followed by high concentration of salicylic and citric acids, respectively . On the other side, low dose of biotic-agents showed to have a great reduction for root rot and stalk rot disease severity compared with high dose. However, *B. subtilis* at 10^{4} was more superior to other one.

Physiological characters

Photosynthetic pigments and phenol content: Chlorophylls a and b are known as a good parameters reflecting the plant healthy. Moreover, carotenoids is known that a highly effective antioxidants. In addition to, the accumulation of phenols at the infection site is considered a first step of the plant defense mechanism. Data presented in

Table 2: Effect of antioxidants and biotic-agents on growth of soybean plant under field condition

	Plant height (cm)		No. of branches per plant	
Treatments	Season 1	Season 2	Season 1	Season 2
Variety				
Giza 35	116.92^{b}	111.44^{b}	6.69^{a}	7.19^{A}
Giza 111	124.31^{a}	118.92^{a}	6.17^{b}	6.36^{B}
Treatments				
Saly-15	123.67^{d}	113.33^{fg}	6.5^{cde}	6.83^{Cde}
Saly-20	129.83^{c}	122.33^{de}	7.0^{bc}	7.33^{Bc}
Citric-15	104.17^{f}	107.83^{h}	6.0^{e}	6.5^{E}
Citric-20	119.67^{d}	111.00^{g}	6.33^{de}	7.0^{Cde}
Benzoic-15	130.17^{c}	120.17^{e}	7.17^{ab}	7.17^{Bcd}
Benzoic-20	112.00^{e}	113.83^{f}	6.33^{de}	6.67^{De}
Bacillus subtilis 10^{8}	137.33^{b}	126.50^{c}	6.17^{e}	7.0^{Cde}
Bacillus subtilis 10^{4}	143.67^{a}	136.00^{a}	7.17^{ab}	7.67^{Ab}
Yeast 100%	130.83^{c}	123.83^{cd}	6.83^{bcd}	7.17^{Bcd}
Yeast 50%	139.00^{ab}	129.83^{b}	7.67^{a}	8.00^{A}
Rizolex	88.67^{g}	89.33^{i}	5.17^{f}	5.17^{F}
Control	88.33^{g}	88.17^{i}	4.83^{f}	4.83^{F}
Interaction				
Giza 35				
Saly-15	121.33^{jkl}	110.33^{hi}	6.67^{bcd}	7.33^{Bcd}
Saly-20	131.33^{efgh}	120.67^{ef}	7.33^{ab}	7.67^{Abc}
Citric-15	96.33^{o}	104.67^{j}	6.00^{def}	6.67^{Def}
Citric-20	117.67^{klm}	108.00^{ij}	7.00^{bc}	7.33^{Bcd}
Benzoic-15	130.00^{efgh}	117.33^{fg}	7.33^{ab}	7.67^{Abc}
Benzoic-20	109.33^{n}	111.67^{hi}	6.67^{bcd}	7.00^{Cde}
Bacillus subtilis 10^{8}	132.33^{efg}	123.00^{e}	6.67^{bcd}	7.33^{Bcd}
Bacillus subtilis 10^{4}	140.00^{bcd}	130.67^{bc}	7.33^{ab}	8.00^{Ab}
Yeast 100%	125.33^{hij}	120.67^{ef}	7.00^{bc}	7.33^{Bcd}
Yeast 50%	134.00^{def}	127.33^{cd}	8.00^{a}	8.33^{A}
Rizolex	82.67^{p}	82l5.00^{gh}	6.00^{Fg}	
Control	82.67^{p}	81l5.33^{fg}	5.67^{G}	
Giza 111				
Saly-15	126.00^{ghij}	116.33^{g}	6.33^{cde}	6.33^{Efg}
Saly-20	128.33^{fghi}	124.00^{de}	6.67^{bcd}	7.00^{Cde}
Citric-15	112.00^{mn}	111.00^{hi}	6.00^{def}	6.33^{Efg}
Citric-20	121.67^{ijk}	114.00^{gh}	6.67^{bcd}	6.67^{Def}
Benzoic-15	130.33^{efgh}	123.00^{e}	7.00^{bc}	6.67^{Def}
Benzoic-20	114.67^{lmn}	116.00^{g}	6.00^{def}	6.33^{Efg}
Bacillus subtilis 10^{8}	142.33^{abc}	130.00^{bc}	5.67^{efg}	6.67^{Def}
Bacillus subtilis 10^{4}	147.33^{a}	141.33^{a}	7.00^{bc}	7.33^{Bcd}
Yeast 100%	136.33^{cde}	127.00^{cd}	6.67^{bcd}	7.00^{Cde}
Yeast 50%	144.00^{ab}	132.33^{b}	7.33^{ab}	7.67^{Abc}
Rizolex	94.67^{o}	96.67^{k}	5.33^{fg}	4.33^{H}
Control	94.00^{o}	95.33^{k}	4.33^{h}	4.00^{H}
LSD				
Varieties	2.81	0.11	0.31	0.41
Treatments	4.89	2.74	0.54	0.56
V×T	6.91	3.88	0.77	0.79

Means within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test

Table 4 and 5 show that there was a marked differences between the tested materials of both cultivars regarding photosynthetic pigments and phenol content. Giza 35 cultivar give the higher amounts of chlorophyll a, b, total chlorophyll and carotenoids as well as phenol content compared to Giza 111.

Regarding to the effect of antioxidants and biotic-agents as well as Rizolex-T 50, data reveal that all treatments at any concentration increased significantly all of photosynthetic pigments in soybean leaves and total phenol in fresh shoot. Salicylic acid at 20 mM had the highest significant increase followed by benzoic acid 20 mM then citric acid 20 mM in both cultivars. On the other hand, the yeast bio-agent was more effective than *Bacillus*. In this respect low level of yeast (50%) was the most effective.

Table 3: Effect of antioxidants and biotic-agents on root rot and stalk rot diseases of soybean plant under field condition

	Root rot		Stalk rot	
Treatments	Season 1	Season 2	Season 1	Season 2
Variety				
Giza 35	16.20^{b}	16.67^{b}	17.78^{a}	19.56^{B}
Giza 111	14.42^{a}	18.92^{a}	18.81^{a}	21.58^{A}
Treatments				
Saly-15	20.00^{c}	23.50^{c}	23.17^{c}	26.17^{C}
Saly-20	13.5^{g}	16.33^{f}	16.00^{fg}	17.67^{F}
Citric-15	20.83^{b}	24.83^{b}	24.83^{b}	27.33^{B}
Citric-20	14.67^{f}	17.17^{f}	17.00^{ef}	20.00^{E}
Benzoic-15	12.5^{h}	14.17^{g}	15.00^{g}	17.00^{F}
Benzoic-20	18.67^{d}	21.17^{d}	21.67^{cd}	23.00^{D}
Bacillus subtilis 10^{8}	15.33^{f}	17.00^{f}	18.33^{e}	19.83^{E}
Bacillus subtilis 10^{4}	9.17^{j}	10.67^{i}	11.67^{hi}	13.33^{H}
Yeast 100%	16.83^{e}	19.17^{e}	20.17^{d}	22.5^{D}
Yeast 50%	10.33^{i}	12.5^{h}	13.17^{h}	14.67^{G}
Rizolex	7.00^{k}	8.83^{j}	10.83^{i}	12.67^{H}
Control	24.83^{a}	28.17^{a}	27.67^{a}	32.67^{A}
Interaction				
Giza 35				
Saly-15	19.67^{de}	22.67^{de}	22.67^{cd}	25.67^{D}
Saly-20	12.67^{l}	15.33^{lm}	15.67^{j-l}	16.33^{I}
Citric-15	20.00^{d}	23.67^{cd}	24.00^{bc}	26.33^{D}
Citric-20	14.33^{jk}	16.67^{ijk}	16.67^{h-j}	18.33^{H}
Benzoic-15	11.00^{mn}	12.67^{n}	14.33^{k-m}	15.00^{Ij}
Benzoic-20	18.33^{f}	20.33^{f}	21.33^{de}	22.67^{Ef}
Bacillus subtilis 10^{8}	15.00^{ij}	16.33^{jkl}	18.00^{g-i}	20.00^{G}
Bacillus subtilis 10^{4}	7.67^{p}	9.00^{p}	10.67^{op}	12.67^{Kl}
Yeast 100%	16.33^{h}	18.67^{gh}	19.67^{e-g}	22.33^{Ef}
Yeast 50%	9.00^{o}	10.67^{o}	12.67^{m-o}	13.00^{Kl}
Rizolex	5.67^{q}	7.67^{q}	10.00^{p}	11.67^{L}
Control	23.33^{b}	26.33^{b}	27.67^{a}	30.67^{B}
Giza 111				
Saly-15	20.33^{d}	24.33^{c}	23.67^{bc}	26.67^{D}
Saly-20	14.33^{jk}	17.33^{ij}	16.33^{i-k}	19.00^{Gh}
Citric-15	21.67^{c}	26.00^{b}	25.67^{ab}	28.33^{C}
Citric-20	15.00^{ij}	17.67^{hi}	17.33^{h-j}	21.67^{F}
Benzoic-15	14.00^{k}	15.67^{kl}	15.67^{j-l}	19.00^{Gh}
Benzoic-20	19.00^{ef}	22.00^{e}	22.00^{cd}	23.33^{E}
Bacillus subtilis 10^{8}	15.67^{hi}	17.67^{hi}	18.67^{f-h}	19.67^{Gh}
Bacillus subtilis 10^{4}	10.67^{n}	12.33^{n}	12.67^{m-o}	14.00^{Jk}
Yeast 100%	17.33^{g}	19.67^{fg}	20.67^{d-f}	22.67^{Ef}
Yeast 50%	11.67^{m}	14.33^{m}	13.67^{l-n}	16.33^{I}
Rizolex	8.33^{op}	10.00^{op}	11.67^{n-p}	13.67^{Jk}
Control	26.33^{a}	30.00^{a}	27.67^{a}	34.67^{A}
LSD				
Varieties	0.86	0.54	1.33	0.66
Treatments	0.68	0.91	1.86	1.00
V×T	0.97	0.91	2.24	1.42

Means within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test

Yield and its components: It is clear that from Table 6, soybean Giza 35 cultivar produced the best plant yield components (No. of pods per plant, plant seed yield and weight of 100 seeds) as compared with Giza 111 in both seasons.

With regard to the effect of both antioxidants and biotic agents on yield and its components, results showed that in all treatments, significant increase these parameters. The highest values occurred under the application of biotic-agents at low concentration. Yeast was better than *Bacillus* in this respect.

Table 4: Effect of antioxidants and biotic-agents on photosynthetic pigments of soybean plant under field condition

	Chloropyll a		Chloropyll b		Total chloropyll		Carotenoids	
Treatments	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Variety								
Giza 35	1.310^{a}	1.399^{a}	0.787^{a}	0.840^{a}	2.099^{a}	2.239^{a}	0.256^{a}	0.273^{A}
Giza 111	1.270^{b}	1.356^{b}	0.728^{b}	0.776^{b}	1.999^{b}	2.131^{b}	0.25^{b}	0.266^{B}
Treatments								
Saly-15	1.175^{j}	1.253^{j}	0.675^{f}	0.718^{f}	1.85^{i}	1.973^{i}	0.18^{j}	0.190^{J}
Saly-20	1.470^{a}	1.568^{a}	0.892^{a}	0.953^{a}	2.362^{a}	2.518^{a}	0.362^{a}	0.383^{A}
Citric-15	1.358^{h}	1.450^{d}	0.778^{c}	0.830^{c}	2.137^{d}	2.28^{d}	0.295^{d}	0.315^{D}
Citric-20	1.388^{c}	1.480^{c}	0.827^{b}	0.883^{b}	2.215^{c}	2.363^{c}	0.313^{c}	0.333^{C}
Benzoic-15	1.22^{hi}	1.30^{hi}	0.697^{f}	0.743^{f}	1.917^{h}	2.045^{h}	0.207^{h}	0.218^{H}
Benzoic-20	1.422^{b}	1.517^{b}	0.835^{b}	0.890^{b}	2.257^{b}	2.407^{b}	0.332^{b}	0.352^{B}
Bacillus subtilis 10^{8}	1.292^{f}	1.378^{f}	0.762^{cd}	0.813^{cd}	2.053^{e}	2.188^{e}	0.245^{f}	0.265^{F}
Bacillus subtilis 10^{4}	1.232^{h}	1.313^{h}	0.730^{e}	0.780^{e}	1.962^{g}	2.092^{g}	0.217^{h}	0.228^{H}
Yeast 100%	1.208^{i}	1.288^{i}	0.688^{f}	0.732^{f}	1.897^{h}	2.022^{h}	0.195^{i}	0.205^{I}
Yeast 50%	1.332^{e}	1.422^{e}	0.772^{cd}	0.823^{cd}	2.103^{d}	2.245^{d}	0.275^{e}	0.295^{E}
Rizolex	1.262^{g}	1.345^{g}	0.745^{de}	0.796^{de}	2.007^{f}	2.141^{f}	0.228^{g}	0.245^{G}
Control	1.142^{k}	1.218^{k}	0.687^{f}	0.732^{f}	1.828^{i}	1.95^{i}	0.192^{i}	0.202^{Ij}
Interaction								
Giza 35								
Saly-15	1.203^{j}	1.283^{k}	0.727^{ghij}	0.773^{ghi}	1.93^{jk}	2.06^{jk}	0.173^{k}	0.183^{O}
Saly-20	1.500^{a}	1.600^{a}	0.92^{a}	0.983^{a}	2.42^{a}	2.58^{a}	0.37^{a}	0.393^{A}
Citric-15	1.390^{d}	1.483^{d}	0.793^{def}	0.847^{def}	2.183^{de}	2.33^{de}	0.303^{d}	0.323^{Ef}
Citric-20	1.413^{c}	1.507^{cd}	0.86^{bc}	0.920^{b}	2.273^{bc}	2.427^{bc}	0.317^{cd}	0.337^{De}
Benzoic-15	1.243^{i}	1.323^{j}	0.717^{ijk}	0.767^{hi}	1.960^{ijk}	2.09^{ijk}	0.213^{hi}	0.227^{Kl}
Benzoic-20	1.427^{bc}	1.523b^{bc}	0.85^{bc}	0.907^{bc}	2.277^{bc}	2.43^{bc}	0.340^{b}	0.36^{Bc}
Bacillus subtilis 10^{8}	1.313^{f}	1.403^{g}	0.79^{def}	0.843^{def}	2.103^{fg}	2.24^{fg}	0.250^{g}	0.27^{Ij}
Bacillus subtilis 10^{4}	1.250^{hi}	1.333^{ij}	0.757^{fghi}	0.807^{fgh}	2.007^{hi}	2.14^{hi}	0.220^{h}	0.233^{K}
Yeast 100%	1.240^{i}	1.32^{j}	0.733^{ghij}	0.78^{ghi}	1.973^{ij}	2.103^{ij}	0.197^{j}	0.207^{Mn}
Yeast 50%	1.350^{e}	1.44^{ef}	0.8^{de}	0.853^{de}	2.150^{ef}	2.297^{ef}	0.267^{f}	0.287^{Hi}
Rizolex	1.283^{g}	1.37^{h}	0.767^{efg}	0.818^{efg}	2.050^{gh}	2.188^{gh}	0.24^{g}	0.26^{J}
Control	1.133^{l}	1.207^{m}	0.73^{ghij}	0.78^{ghi}	1.863^{lm}	1.987^{lm}	0.187^{jk}	0.197^{No}
Giza 111								
Saly-15	1.147^{l}	1.223^{m}	0.623^{m}	0.663^{l}	1.770^{n}	1.887^{n}	0.187^{jk}	0.197^{No}
Saly-20	1.44^{b}	1.537^{b}	0.863^{b}	0.923^{b}	2.303^{b}	2.457^{b}	0.353^{b}	0.373^{B}
Citric-15	1.327^{f}	1.417^{fg}	0.763^{efgh}	0.813^{efg}	2.090^{g}	2.23^{g}	0.287^{e}	0.307^{Fg}
Citric-20	1.363^{e}	1.453^{e}	0.793^{def}	0.847^{def}	2.157^{ef}	2.30^{e}	0.31^{cd}	0.33^{De}
Benzoic-15	1.197^{jk}	1.277^{kl}	0.677^{kl}	0.72^{jk}	1.873^{lm}	2.00^{l}	0.2^{ij}	0.21^{Lmn}
Benzoic-20	1.417^{c}	1.51^{c}	0.82^{cd}	0.873^{cd}	2.237^{cd}	2.383^{cd}	0.323^{c}	0.343^{Cd}
Bacillus subtilis 10^{8}	1.27^{gh}	1.353^{hi}	0.733^{ghij}	0.783^{ghi}	2.003^{hi}	2.137^{hi}	0.24^{g}	0.26^{J}
Bacillus subtilis 10^{4}	1.213^{j}	1.293^{k}	0.703^{jk}	0.753^{ij}	1.917^{kl}	2.043^{kl}	0.213^{hi}	0.223^{Klm}
Yeast 100%	1.177^{k}	1.257^{l}	0.643^{Lm}	0.683^{kl}	1.82^{mn}	1.94^{mn}	0.193^{j}	0.203^{N}
Yeast 50%	1.313^{f}	1.403^{g}	0.743^{ghij}	0.793^{ghi}	2.057^{gh}	2.193^{gh}	0.283^{e}	0.303^{Gh}
Rizolex	1.240^{i}	1.320^{j}	0.723^{Hij}	0.773^{ghi}	1.963^{ijk}	2.093^{ijk}	0.217^{h}	0.230^{K}
Control	1.150^{l}	1.230^{m}	0.643^{Lm}	0.683^{kl}	1.793^{n}	1.913^{N}	0.197^{j}	0.207^{Mn}
LSD								
Varieties	0.008	0.017	0.074	0.031	0.077	0.041	0.006	0.008
Treatments	0.015	0.009	0.029	0.081	0.037	0.082	0.010	0.012
V×T	0.022	0.024	0.041	0.044	0.053	0.058	0.015	0.017

Means within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test

On the other side, from antioxidants benzoic acid at 15 mM gave the maximum increase in pods number and plant seed yield followed by salicylic acid 20 mM. There is no significant differences in both. In contrast, Rizolex-T 50 had no significant effect on yield and its components.

Seed quality: Soybean proved more useful than other grain legumes by virtue of its dual purpose (protein and oil). While, it is not a major source of minerals.

From the above mentioned results, the best treatments were taken to determine seed quality. Figure 2 illustrated that soybean Giza 111 cultivar was the best in seed quality (protein%, oil% and phosphorus%) compared to Giza 35. Generally, antioxidants gave the highest values of seed protein percentage as compared with biotic-agents. Salicylic acid 20 mM came first followed by citric acid 20 mM then benzoic acid at 15 mM. Of biotic-agents, *B. subtilis* at 10^{4} was better than yeast at 50%. Meanwhile, Rizolex-T 50

Table 5: Effect of antioxidants and biotic-agents on total phenols of soybean plant under field condition

Treatments	Total phenols	
	Season 1	Season 2
Variety		
Giza 35	108[a]	115[A]
Giza 111	105[b]	112[B]
Treatments		
Saly-15	97[j]	104[J]
Saly-20	121[a]	129[A]
Citric-15	112[d]	120[D]
Citric-20	114[c]	122[C]
Benzoic-15	101[hi]	107[Hi]
Benzoic-20	117[b]	125[B]
Bacillus subtilis 10^8	107[f]	114[F]
Bacillus subtilis 10^4	102[h]	108[H]
Yeast 100%	100[i]	107[I]
Yeast 50%	110[e]	117[E]
Rizolex	104[g]	111[G]
Control	94[k]	101[K]
Interaction		
Giza 35		
Saly-15	99[j]	106[J]
Saly-20	124[a]	132[A]
Citric-15	115[d]	122[D]
Citric-20	116[cd]	124[Cd]
Benzoic-15	103[i]	109[I]
Benzoic-20	118[bc]	126[Bc]
Bacillus subtilis 10^8	109[f]	116[F]
Bacillus subtilis 10^4	103[hi]	110[Hi]
Yeast 100%	102[i]	109[I]
Yeast 50%	111[e]	119[E]
Rizolex	106[g]	113[G]
Control	93[l]	100[L]
Giza 111		
Saly-15	95[l]	101[L]
Saly-20	119[b]	127[B]
Citric-15	109[f]	117[F]
Citric-20	112[e]	120[E]
Benzoic-15	99[j]	105[Jk]
Benzoic-20	117[bc]	125[C]
Bacillus subtilis 10^8	105[gh]	112[Gh]
Bacillus subtilis 10^4	100[j]	107[J]
Yeast 100%	97[k]	104[K]
Yeast 50%	109[f]	116[F]
Rizolex	102[i]	109[I]
Control	95[l]	101[L]
LSD		
Varieties	1.25	0.78
Treatments	1.33	1.39
V×T	1.89	1.97

Means within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test

Table 6: Effect of antioxidants and biotic-agents on yield components of soybean plant under field condition

Treatments	No. of pods per plant		Seed yield per plant		100-seed weight	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Variety						
Giza 35	131.75[a]	129.42[a]	44.12[a]	40.19[a]	16.95[a]	17.56[A]
Giza 111	120.17[b]	118.36[b]	40.69[b]	39.31[b]	15.33[b]	15.22[B]
Treatments						
Saly-15	126.50[ef]	121.50[f]	41.33[f]	37.03[g]	17.47[Ab]	17.22[D]
Saly-20	129.17[de]	125.17[e]	42.73[e]	39.87[e]	17.73[a]	17.92[C]

Table 6: Continue

Treatments	No. of pods per plant		Seed yield per plant		100-seed weight	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
Citric-15	122.67^{g}	117.67^{gh}	3900^{g}	36.47^{gh}	15.20^{E}	15.1^{G}
Citric-20	126.00^{f}	119.5f^{g}	40.28^{f}	36.80^{g}	15.65^{D}	15.35^{G}
Benzoic-15	131.17^{cd}	127.67^{de}	43.17^{e}	41.35^{d}	16.42^{c}	16.05^{F}
Benzoic-20	116.33^{h}	116.00^{h}	38.38^{g}	34.55^{j}	15.32^{de}	15.2^{G}
Bacillus subtilis 10^{8}	134.17^{c}	129.5^{cd}	44.43^{d}	38.67^{f}	16.45^{c}	16.68^{E}
Bacillus subtilis 10^{4}	139.00^{ab}	135.33^{b}	48.23^{b}	46.30^{b}	17.27^{b}	18.48^{B}
Yeast 100%	137.33^{b}	131.50^{c}	46.27^{c}	43.97^{c}	16.73^{c}	17.3^{D}
Yeast 50%	141.17^{a}	138.00^{a}	52.10^{a}	48.85^{a}	17.82^{a}	18.88^{A}
Rizolex	104.00^{i}	111.50^{i}	36.55^{h}	35.53^{i}	13.58^{g}	13.98^{I}
Control	104.00^{i}	113.33^{i}	36.38^{h}	35.97^{hi}	14.08^{f}	14.47^{H}
Interaction						
Giza 35						
Saly-15	131.33^{cde}	126.67^{fg}	42.13^{def}	38.03^{i}	18.23^{ab}	18.03^{D}
Saly-20	134.33^{cd}	130^{ef}	43.60^{d}	40.3^{h}	18.5^{ab}	18.6^{B}
Citric-15	127.67^{efg}	124.33^{gh}	41.37e^{fg}	37.4^{ij}	16.1^{gh}	16.47^{G}
Citric-20	130.67^{def}	124.33^{gh}	42.23d^{ef}	37.53^{ij}	16.43^{fg}	17.13^{F}
Benzoic-15	135.67^{c}	132.33^{de}	43.03^{de}	40.33^{h}	17.43^{cd}	17.3^{Ef}
Benzoic-20	122.00^{hi}	121.67^{hij}	41.20^{fg}	36.77^{jkl}	16.07^{gh}	16.2^{Gh}
Bacillus subtilis 10^{8}	140.33^{b}	134.67^{cd}	45.80^{c}	34.57^{o}	17.1^{de}	18.27^{Bcd}
Bacillus subtilis 10^{4}	146.00^{a}	140.33^{b}	49.50^{b}	47.3^{b}	17.97^{bc}	19.43^{A}
Yeast 100%	143.67^{ab}	136.33^{c}	46.90^{c}	44.4^{de}	17.33^{D}	18.43^{Bc}
Yeast 50%	147.67^{a}	144.33^{a}	54.70^{a}	51.63^{a}	18.70^{a}	19.67^{A}
Rizolex	110.00^{j}	118^{jkl}	39.73^{ghi}	36.97^{jk}	14.50^{L}	15.30^{I}
Control	111.67^{j}	120^{ijk}	39.27^{hi}	37.1^{j}	15.07^{jk}	15.83^{H}
Giza 111						
Saly-15	121.67^{hi}	116.33^{kl}	40.53^{fgh}	36.03^{lm}	16.7^{ef}	16.4^{G}
Saly-20	124.00^{gh}	120.33^{ij}	41.87^{def}	39.43^{h}	16.97^{def}	17.23^{Ef}
Citric-15	117.67^{i}	111.00^{mn}	36.63^{jk}	35.53^{mn}	14.40^{L}	13.73^{L}
Citric-20	121.33^{hi}	114.67^{lm}	38.33^{ij}	36.07^{klm}	14.87^{jkl}	13.57^{L}
Benzoic-15	126.67^{fg}	123.00^{ghi}	43.3^{d}	42.37^{g}	15.40^{ij}	14.80^{J}
Benzoic-20	110.67^{j}	110.33^{no}	35.57^{k}	32.33^{p}	14.57^{kl}	14.20^{K}
Bacillus subtilis 10^{8}	128.0^{efg}	124.33^{gh}	43.07^{de}	42.77^{fg}	15.80^{hi}	15.10^{Ij}
Bacillus subtilis 10^{4}	132.0^{cde}	130.33^{ef}	46.97^{c}	45.3^{cd}	16.57^{efg}	17.53^{E}
Yeast 100%	131.0^{def}	126.67^{fg}	45.63^{c}	43.53^{ef}	16.13^{gh}	16.17^{Gh}
Yeast 50%	134.67^{cd}	131.67^{de}	49.5^{b}	46.07^{c}	16.93^{def}	18.1^{Cd}
Rizolex	98.00^{k}	105.00^{p}	33.37^{l}	34.10^{o}	12.67^{M}	12.67^{N}
Control	96.33^{k}	106.67^{op}	33.5^{l}	34.83^{no}	13.10^{M}	13.10^{M}
LSD						
Varieties	0.94	1.40	1.01	0.35	0.33	0.39
Treatments	3.07	2.60	1.25	0.65	0.4	0.26
V×T	4.34	3.69	1.78	0.92	0.56	0.37

Means within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range test

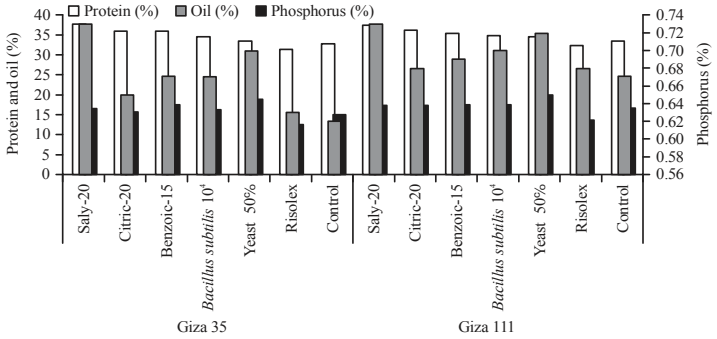

Fig. 2: Effect of antioxidants and biotic-agents on soybean seed quality in the second season only

caused a decreasing effect on seed protein and oil percentage. The highly increase in seed oil percentage occurred under the application of yeast at 50% followed by benzoic acid at 15 mM then salicylic acid at 20 mM. Moreover, the highest percentage of seed phosphorus percentage was observed with salicylic acid followed by yeast at 50% then *B. subtilis*.

DISCUSSION

Root and stalk rots disease that caused by several soil borne fungi are wide spread and acting serious problems in many crops cultivated in different soils. Soybean is one of the most important legumes, that has been attacked by soil borne pathogens, which are limiting factors of soybean growth and productivity. Root rot and charcoal rot diseases are the most destructive disease attacking soybean seeds, seedlings and roots, as well as, lower part of the stem causing damage[6,7]. The investigation trials to controlling such diseases are considered important, especially their occurrence have prevalence in Egypt. Fungicides are considered one of several factors involving in controlling fungal diseases, however, their occurrence is responsible for environmental pollution. So, there is a growing need to develop alternative approaches for controlling plant diseases rather than fungicides. Bio-agents, are risk free factors both for environment and non-target organisms and could reduce the use of agrochemicals. Most bio-agents have varied performance in different environmental conditions, some of these variabilities have been attributed to differences in physical and chemical properties[31,32]. In the present study, the infected samples of soybean plant were collected and the casual agents of fungal pathogens were isolated and identified as *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina* according to their cultural properties, morphological and microscopic characteristics[20-22]. Pathogenicity test has been carried out on two cultivars, i.e., Giza 111 and 35. Generally, Giza 111 cultivar was more susceptible to the pathogens compared to Giza 35, where *R. solani* was the most aggressive pathogen which causes pre-emergence damping-off in both cultivars followed by *M. phaseolina*, whereas, *F. solani* showed the highest percentage of post-emergence damping-off. Moreover, obtained data showed the response of germination of both cultivars to both antioxidants and biotic-agents, wherein, damping off- decreased significantly in both seasons. Interestingly, the low concentration of antioxidants used was more effective compared to the higher concentration in decreasing both of pre and post damping-off. In this context, other studies pointed out that antioxidant substances, i.e., salicylic and acetyl salicylic acids were found to be active as antimicrobial agents, which induced plant resistance against TMV[9], soil-borne fungal root rot and wilt diseases[33,34]. Another investigation showed that the percentage of seed germination of soybean was decreased with the increasing level used of salicylic acid[35]. On the other hand, both *B. subtilis* and *S. cerevisiae* were effective in decreasing damping-off in two cultivars. These results are coinciding with previous study of Mahmoud[17] who found that *S. cerevisiae* has antagonistic effect against *Fusarium oxysporum*, which decreased the incidence of disease under green house condition as well as, *S. cerevisiae* suppressed seed-borne fungi of faba bean[36]. Moreover, the antibiosis competition for colonization sites and nutrients, degradation of the pathogen toxins and enzymes by yeasts involved in the suppression of growth of pathogenic fungi *in vitro* and/or *in vivo*[37]. As well as, the mechanism of yeast against plant pathogens depends upon production of proteinaceous killer toxins lethal to susceptible yeast and fungi strains[38]. On the other side, several studies investigated the role of *Bacillus* spp., as antagoinistic agents against *Fusarium solani* in tomato crop[39], against *Penicillium* sp. and other 26 fungi associated with soybean seeds[15], as well as, against foliar disease and faba bean wilt[16,17]. The response of plant height and branches number to all antioxidant and biotic-agents has been occurred with significant increase. Contrarily, Rizolex showed no significant effect on the morphological features in both seasons. Interestingly, the highest value of plant height has been obtained among the low numbers of both *B. subtilis* and *S. cerevisiae*, respectively. Similarly, benzoic acid (15 mM) was the most active concentration followed by high level of salicylic acid. As well as, the highest increase in branches number was occurred under the low doses of *S. cerevisiae*, *B. subtilis* and benzoic acid. Regarding disease severity of root rot and stalk rot of both cultivars, Giza 111 was more sensitive to pathogens infection *in vivo*. All parameters tested significantly reduced the incidence of diseases. It is obvious that, both of bio-agents and antioxidants played a major role in inducing plant resistance toward the pathogens, in which the photosynthetic pigments, carotenoids and total phenols were increased in both cultivars but in different ratios These results were confirmed from the role of antioxidants that enhance the level of plant phenols, which play a major role in the plant disease defense, growth and development[11]. Additionally, the induced systemic resistance is triggered by a number of chemicals such as salicylic acid and acetyl salicylic and hydroquinone against fungi, viruses and bacteria[13,14]. Other studies investigated the ability of *B. subtilis* in production of plant growth hormones such as indole acetic acid and auxin like substances[40]. As well as, *B. subtilis*, *B. cereus*, *B. pumilus* and *B. amyloliquefaciens* were found to be antagonistic

against *F. solani in vivo* and/or *in vitro* and also shoot and root length were significantly longer compared to control in tomato crop[39]. Other investigators, showed that *B. amyloliquefaciens* produced indole acetic acid which enhanced potato crop[41]. Buensanteai *et al.*[42] studied the enhanced growth of soybean plant by *B. amyloliquefaciens* KPS46, which produced auxin, surfactin and extracellular proteins. Giza 35 was found to have more contents of physiological features in relative to Giza 111. All the measured parameters were reflected on the numbers of pods per plant, seed yield and weight of 100 seeds. Interestingly, the lower concentrations of biotic and antioxidant agents showed significant increase in these parameters. However, Rizolex showed no significant effect on yield and its components. Ultimately, the response of seed quality (protein%, oil% and phosphorus%) to biotic and abiotic-agents was occurred in two cultivars. Giza 111 was the best in seed quality. *Bacillus subtilis* at 10^4 was better than yeast at 50%. Rizolex-T 50 caused a decrease in seed protein and oil percentage. The high content of oil percentage achieved under the dose of yeast at 50% followed by benzoic acid and salicylic acid.

CONCLUSION

The fungal pathogens, i.e., *F. solani*, *R. solani* and *M. phaseolina* were found to associate with symptoms of soybean root and stalk rot diseases. Cultivar Giza 111 was more susceptible to infection by damping-off pathogens compared to Giza 35. Biotics, *B. subtilis* and *S. cerevisiae*, as well as, antioxidants were effective in decreasing pre and post damping-off, increasing plant height and branches number, as well as, increase number of pods per plant, seed yield and weight of 100 seeds. Seed quality as the contents of protein, oil and phosphorus were increased as the result of all treatments but these responses are different according to the doses of antioxidants and microbial numbers used. Lately, it can be recommended that these biotic and abiotic could be used as antimicrobial agents against soybean pathogens, which its safe compared to agrochemicals.

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SIGNIFICANCE STATEMENT

Soybean crop is influenced by soil-borne fungal pathogens, which are the major limiting factors of soybean growth and productivity. Pesticides and fungicides were accumulated their toxicity on human health and environment. In the present study, antioxidant agents were effective in decreasing the pre and post damping-off. Biotic-agents were also increasing the productivity and improving seed quality.

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