Asian Journal of Crop Science

ISSN 1994-7879

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Asian Journal of Crop Science 7 (2): 87-112, 2015
ISSN 1994-7879 / DOI: 10.3923/ajcs.2015.87.112

Influence of Antioxidants on Wheat Productivity, Quality and Seed-Borne Fungi Management under NPK Fertilization Levels

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ABSTRACT

In order to study the effects of seed soaking and foliar spraying with antioxidants under nitrogen, phosphorus and potassium (NPK) fertilization levels on wheat growth, yields and its components, grains quality and diseases management, an experiment was conducted at Tag AL-Ezz, Agricultural Research Station Farm, Dakahlia Governorate, Agricultural Research Center, Egypt, during 2011/2012 and 2012/2013 seasons. A strip-split plot design with four replications was used. Where, the vertical-plots were included seed soaking treatments while the horizontal-plots were devoted to foliar spraying treatments. The sub-plots were allocated to nitrogen, phosphorus and potassium (NPK) fertilization levels. Results showed that highest values of growth, yields and its attributes and grains quality were resulted from seed soaking in Salicylic Acid (SA) at the level of 300 ppm. However, untreated seeds resulted the lowest values of these. Foliar spraying with SA produced the highest values of growth, yields and its attributes and grain quality compared with other foliar spraying treatments. Highest growth, yields and its attributes and grain quality were obtained from increasing NPK fertilization levels up to 100% of the recommended dose. In general, it could be indicated that soaking in SA at the level of 300 ppm and spraying plants with SA at the level of 150 ppm beside fertilization with 80.0 kg N+22.5 kg P_2O_5+24.0 K_2O/fed maximized wheat growth, yields and its components as well as grain quality. However, it could be recommended that soaking seeds in SA and foliar spraying plants with SA and fertilization with 80% of the recommended dose of NPK which exceeded grain yield and its quality upon the control treatment (untreated seeds before sowing and without foliar spraying with antioxidants and mineral fertilization with 100% of the recommended dose of NPK fertilizers) to reduce soil pollution with mineral fertilizers.

Key words: Wheat, seed soaking, foliar spraying, foliar application, antioxidants, salicylic acid, citric acid, ascorbic acid, npk levels, growth, yield, quality, diseases management

INTRODUCTION

Wheat (*Triticum aestivum vulgare* L.) is cultivated worldwide primarily as a food commodity. Because of its importance in the Egyptian diet, wheat is considered a strategic commodity. The total cultivated area of wheat in Egypt reached about 3.37 million feddan in 2013 season and the total production exceeded 9.46 Mt with an average of 18.67 ardab/fed (FAO., 2015). In Egypt, the gap between wheat consumption and production is continuously increased due to steady increases in the human population despite limited cultivated area. Therefore, using suitable agricultural practices such as seed soaking and foliar spraying with antioxidants, in addition using appropriate NPK fertilization level may affect wheat productivity, grain quality and diseases management.

Wheat is subjected to relatively large number of diseases during its growing seasons which attack all plant parts causing serious losses in crop productivity. Isolation and identification of seed-borne fungi were conducted according to standard tests described by the International Seed Testing Association (ISTA., 1999). *Tilletia laevis*, *Tilletia tritici*, *Ustilago tritici*, *Fusarium graminearum*, *Fusarium culmorum*, *Microdochium nivale, Bipolaris sorokiniana, Alternaria alternata, Curvularia* sp., *Aspergillus niger*, *Aspergillus candidus*, *Aspergillus flavus*, *Penicillium* sp., *Mucor* sp. and *Rhizopus* sp. were isolated from wheat seeds (Hajihasani *et al*., 2012; Pathak and Zaidi, 2013). Dal Bello *et al*. (2002) revealed that *Fusarium graminearum* was associated with the seedling blight of wheat complex which reduces germination, seedling stand and yield. *Fusarium* fungi, including *F. graminearum*, cause seedling blight, foot rot and head blight diseases in wheat resulting in yield loss. It survives in soil and is seed-transmitted, it may causes reduction of germination through seed decay and seedling blight (Asran and Amal, 2011). Diagnostic assays based on PCR have been developed for major seed borne diseases of wheat. The PCR-based assays are practiced for species specific detection of *Fusarium culmorum*, *F. graminearum* and *F. avanaceum* from contaminated seeds (Schilling *et al*., 1996).

Because of hazards of pesticides in general and fungicides in specific, on public health and environmental balance (Elad, 1992) a relatively recent direction of pest control management was introduced. Inducing or acquiring the systemic resistance in the host plant became a good target for minimizing disease incidence or severity with least cost and without environmental pollution. Antioxidants which save to human and environment had been used successfully to control some plant diseases such as root and pod rot in peanut (Elwakil, 2003), *Fusarium* wilt in chickpea (Nighat-Sarwar *et al*., 2005), faba bean chocolate spot (Hassan *et al*., 2006), peanut root rot (Mahmoud *et al*., 2006), *Fusarium* wilt in tomato (El-Khallal, 2007; Mohamed *et al*., 2007), root rot and leaf blight in lupine (Abdel-Monaim, 2008), damping-off in pepper (Rajkumar *et al*., 2008). At the same time, Mostafa (2006) reported that application of antioxidants, e.g., ascorbic, salicylic, coumaric, benzoic acids and propylgalate as either seed soaking or soil drenching proved sufficient protection against cumin caused by *Fusarium oxysporum*, F. sp., *cumini* or *Acreromonium egyptiacum*. Abdel-Monaim and Ismail (2010) evaluated four antioxidant compounds (coumaric acid, citric acid, propylgalate and salicylic acid each at 100 and 200 ppm) for their *in vitro* and *in vivo* agonist to *Fusarium* pathogenic isolates caused root rot and wilt diseases in pepper plants. All tested antioxidant compounds reduced damping-off, root rot/wilt and area under root rot/wilt progress curve when used as seed soaking, seedling soaking and soil drench especially at 200 ppm under greenhouse and field conditions compared with untreated plants. All chemicals increased fresh and dry weight of seedling grown in soil drenching or seed treatment with any antioxidants. At the same time, all tested chemicals significantly increase plant growth parameters i.e., plant length, plant branching and total yield per plant in case of seedling soaking or soil drench. On the contrary, all chemicals much reduced spore formation in both *Fusarium* species at 100 or 200 ppm and the inhibitory effect of antioxidants increased with increasing their concentrations.

Foliar fertilization is an effective method to correct soil deficiencies, overcoming the soils inability to transfer nutrients to the plant under low moisture conditions and improve nutrient use efficiency (Mosali *et al*., 2006). Seed soaking treatments in water, nutrients or antioxidants solution speeds up germination by stirring up the process on the seeds which may enhance emergence, growth and yield (Das and Choudhury, 1996).

Salicylic Acid (SA) is considered as a hormone like substance which acting an important role in regulating a number of physiological processes in the plants such as stomata closure, ion uptake and transport, inhibition of ethylene biosynthesis, transpiration, membrane permeability, photosynthesis and growth (Abdel-Wahed *et al*., 2006; Ashraf *et al*., 2010), nitrate metabolism, flowering and stress tolerance (Hayat *et al*., 2007). Application of SA stimulated tolerance in plants to many biotic and abiotic stresses counting fungi, bacteria and viruses, chilling, salinity, drought and heat (Khan *et al*., 2010). The SA can have useful or inhibitory effects on plant physiological processes. In this concern; Khodary (2004) stated that SA enhanced the maize salt tolerance in terms of improving the measured plant growth criteria. Al-Hakimi (2008) showed that application of SA was generally associated with a marked increase in the biosynthesis of cell wall-associated proteins of shoots and roots of wheat plants. Soaking wheat grains in SA had a favorable effect on the accumulation of nutritive cations and ameliorated the effect of more distressing ions, especially Na, accumulated in wheat plants. Erdal *et al*. (2013) stated that foliar applied of SA significantly increased the fresh and dry weights in both root and shoots of wheat plants under salt stress. They added that after the application of SA, increasing tolerance of wheat seedlings to salt stress may be related to increases in antioxidative enzyme activity. Ghafiyehsanj *et al*. (2013) showed that exogenous SA application increased protein, insoluble sugar, shoot and root fresh weight contents, but reduced proline and soluble sugar in presence of salinity. El-Housini *et al*. (2014) reported that foliar spraying with salicylic acid at 100 mg L^{-1} concentration gave the highest significant values for growth characters of stevia plants.

Citric Acid (CA) is an organic compound belonging to the family of carboxylic acids. It presents in practically all plants. It is one of a series of compounds involved in the physiological oxidation of fats, proteins and carbohydrates to CO_2 and water. Many researchers works on effects of citric acid on growth and yield of crops. In this respect, Abd-Allah *et al*. (2007) indicated that plant height, yield and its components as well as protein content in common bean, pea and faba bean were increased with application of citric acid. Sheteawi (2007) stated that ascorbic and citric acids appeared to act in a concert which indicates a complete set of antioxidant defense system, rather than protection by a single antioxidant under stressful conditions. Fawy and Atyia (2012) showed that the yield parameters of wheat were increased with increasing foliar application rates of citric acid from 100 up to 300 ppm. Maleki *et al*. (2013) revealed that foliar spray of citric acid significantly increased shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of sweet basil.

In the antioxidants system which involves antioxidant substances such as Ascorbic Acid (AA) which is a small water-soluble antioxidant molecule, that acts as a primary substrate in the cyclical pathway for detoxification and neutralization of superoxide radicals and singlet oxygen (Noctor and Foyer, 1998). Ascorbic acid (vitamin C) is one of the key products of D-glucose metabolism which synthesized in higher plants. It has been shown to play multiple roles in plant growth and development, such as cell division, cell wall expansion (Pignocchi and Foyer, 2003), the electron transport system (El-Kobisy *et al*., 2005) and other developmental processes. The beneficial effects of ascorbic acid upon growth and productivity have been reported on many field crops i.e., cotton (Ghourab and Wahdan, 2000), sugar beet (Salem *et al*., 2000; Orabi and Mekki, 2008), sunflower (El-Gabas, 2006) and maize (Darvishan *et al*., 2013). Concerning wheat, Al-Hakimi and Hamada (2001) reported that wheat grain soaking in ascorbic acid, thiamin or sodium salicylate could offset the adverse effects of NaCl salinity on the seedlings of wheat plant by suppression of salt stress induced accumulation of proline. Khan *et al*. (2006) concluded that foliar spraying with ascorbic acid contributed in protection the photosynthetic machinery from the damaging effects of salt stress. Fercha *et al*. (2011) stated that the application of ascorbic acid mitigated the adverse effect of salt stress on plant growth which may be due, in part, to increased leaf area, improved chlorophyll and carotenoid contents and enhanced proline accumulation. Bakry *et al*. (2013) found that application of 300 mg L^{-1} ascorbic acid gave the best result for plant height, spike length, grain yield/plant, grain, straw and biological yields and protein%. Hussein and Alva (2014) found that exogenous application of ascorbic acid enhanced vegetative growth which may contribute to increased plant biomass and yield.

Nitrogen is often the most important plant nutrients which influences the amount of protein, protoplasm and chlorophyll formed, consequently increases cell size, leaf area and photosynthetic activity. Wheat crop is very sensitive to inadequate nitrogen and very responsive to nitrogen fertilization (David *et al*., 2005). Many researchers in Egypt and around the world have decided that using nitrogen fertilizer in suitable needed level could improve growth, yield and its components as well as quality of wheat, including Seadh and Badawi (2006), Ibrahim (2007), Mekhemar (2008), Seadh *et al*. (2009), Abedi *et al*. (2010), Tababtabaei and Ranjbar (2012), Atia and Ragab (2013), Seleem and Abd El-Dayem (2013), Haileselassie *et al*. (2014), Khan *et al*. (2014) and Seadh and Abido (2014).

Phosphorus fertilizer is second only to nitrogen fertilizer in importance as an essential crop nutrient. Phosphorus is important in building energy for metabolism of plant growth through cellular productions such as ATP and ADP from the early stages to the end of the plant's life (Marschner, 1995). Phosphorus fertilizer is critical for plant growth, especially in the early jointing stages and for enhancing grain yield and yield components of wheat. Mosali *et al*. (2006) stated that application of phosphorus fertilizer at the rate of 11-22 kg P ha^{-1} as broadcast-incorporated preplanting increased wheat grain yield. Ali *et al*. (2014) reported that wheat grain yield was increased by increasing phosphate fertilizer levels in the form of Nutri-phite. Haileselassie *et al*. (2014) found that application of phosphorus at the rate of 46 kg P_2O_5 ha^{-1} significantly increased grain and straw yields by 38 and 46%, respectively than control (without phosphorus fertilizer).

Potassium (K) is participate in many important functions in plants i.e., photosynthesis, translocation of photosynthates, protein synthesis, control of ionic balance, regulation of plant stomata and water use (Marschner, 1995; Reddy *et al*., 2004), enzyme activation and osmoregulation (Mengel, 2007). Also, potassium enhances the ability of plants to resist stress such as diseases, pests, cold and drought. Potassium performs these roles in all crops and wheat, therefore it is important plant nutrient to sustain high productivity and quality, in equilibrium with other essential plant nutrients (Yu-Ying and Hong, 1997), so it is important to ensure adequate potassium for wheat. In this concern, Tahir *et al*. (2008) indicated that fertilized wheat plants with 90 kg K ha^{-1} gave significantly higher yield, but on the basis of economic analysis fertilized with 60 kg K ha^{-1} gave better results. El-Lethy *et al*. (2013) showed that potassium application could play an important role in alleviation of injury of wheat irrigated with salinized water depend on the level of salinity.

The NPK fertilization is among the vital factors affecting growth, yield and quality of wheat. Thus, application the suitable level of NPK is one of the favorable factors for increasing wheat productivity and quality. In this respect, Laghari *et al*. (2010) found that application of 180-60-60 NPK kg ha^{-1} produced tall plants, maximum nodes/stem, internode length, grain weight/spike and grain index. Meena *et al*. (2013) pointed out that significant improvement in plant height, number of tillers, dry matter production, number of productive tillers and grain and straw yields/ha with application of 100% NPK. Youssef *et al*. (2013) reported that application of 288 kg N+53 kg P_2O_5+120 kg K_2O ha^{-1} recorded the highest values for protein content in wheat grains and total chlorophyll content. Haileselassie *et al*. (2014) revealed that combined application of 46 kg N+46 kg P_2O_5 ha^{-1} was recommended to achieve sustainable wheat production on the sandy soils. Khan *et al*. (2014) showed that application of 180 kg N ha^{-1} in combination with 90 kg K_2O ha^{-1} enhanced grain yield by (47.4%), biological yield by (28.5%), 1000 grain weight by (29.2%) and grains weight/spike by (24.6%) as compared with control.

Now-a-days, there is no accurate and comprehensive information regard to the effect of antioxidants as foliar application in combination with NPK fertilization levels on quantity and quality characters of wheat. So, this study aimed to study the effects of seed soaking and foliar spraying treatments with antioxidants under NPK fertilization levels on wheat growth, yields and its components, grain quality and diseases management under the environmental conditions of Dakahlia Governorate, Egypt.

MATERIALS AND METHODS

Laboratory experiment

Seed health testing: Seed Health Testing (SHT) of seed-borne fungi following the rules of International Seed Testing Association (ISTA., 1999) was carried out. Standard blotter method was selected in this study while sample of 200 seeds was randomly taken from each sample for SHT.

Blotter method: Replicates of ten seeds were plated in 11 cm diameter Petri-dish containing three layers of water-soaked blotters. The plates were incubated at 20±2°C for 7 days under 12 h alternating cycles of cool white fluorescent light and darkness. Seeds were then ready for examination under a stereoscopic binocular microscope (6-50 X) for the presence of seed-borne fungi and to study their habit characters. Wherever, necessary the compound microscope was used for confirming the identification after having examined the morphology of conidia and conidiophores.

Moreover, fungi presented on infested seeds were identified in consultation with the description sheets of Commownealth Mycological Institute, Kew, Surrey, England (CMI), Danish Government Institute of Seed Pathology (DGISP) publications as well as (Raper and Fennel, 1965; Ellis, 1971; Booth, 1985; Burrges *et al*., 1988; Singh *et al*., 1991).

Pathogenicity tests of important seed-borne fungi on wheat

Seed borne fungi used in the pathogenicity tests: Four fungal species namely, *Cephalosporium* sp., *Fusarium culmorum*, *F. moniliforme* and *F. graminearum* were tested for their pathogenic effects on wheat seeds and seedlings.

Soil inoculation with pathogenic fungi: Fungal inocula were prepared by growing each fungus on media initiated from wheat seeds and incubated at 24±2°C for 14 days. Potted soils were infested with the fungal preparation at a rate of 1% (w/w). Pots containing soil mixed with the same amounts of non-infested medium served as checks. Three replicates were used per treatment. These pots were kept in greenhouse for seven days to allow the fungi to adapt before sowing seeds. Ten seeds were sown in 20 cm plastic pots containing combined steamed sterilized sandy soil. Other 3 pots were prepared using the same manner but without adding the fungus (non-inoculated medium were used) and served as control.

Study the effect of antioxidants on seed-borne fungi isolated from wheat seeds: Salicylic acid, citric acid and ascorbic acid were used. The antioxidants were dissolved in water to obtain the desired concentration (100, 200 and 300 ppm) from salicylic acid, citric acid and ascorbic acid in order to test their direct effect on the 4 selected fungi.

Linear growth test (*in vitro* studies): Mycelium disks (0.9 mm diameter) from 7 days old cultures of each fungus were landed onto the center of Czapek's agar media supplemented with different concentration of the promising antioxidant to study its inhibitory effect on the isolated fungi. The linear growth of each fungus was recorded.

Data of laboratory experiment was statistically analyzed according to the technique of analysis of variance (ANOVA) for completely randomized design as published by Gomez and Gomez (1984) using MSTAT statistical package. Least Significant Difference (LSD) method was used to test the differences between treatment means at 5% level of probability as described by Snedecor and Cochran (1980).

Greenhouse experiment: Pots of 20 cm diameter were infested with fungal inocula. Pots containing soil mixed with the same amounts of noninfested medium were served as a control. Replicates of three pots were used per treatment (10 seeds per each). Pots were kept in greenhouse for 7 days to allow the fungi to adapt before sowing seeds. Then study the effect of seed soaking and foliar spraying treatments with antioxidants and nitrogen, phosphorus and potassium (NPK) fertilization levels as well as their interactions on damping-off. A daily observation for damping off were recorded.

Field experiment

Study site and objective: Two field experiments were conducted at Tag Al-Ezz, Agricultural Research Station Farm, Dakahlia Governorate, Agricultural Research Center, Egypt (+7 m altitude, 31° 36' latitude and 30° 57' longitude), during 2011/2012 and 2012/2013 seasons to study the effect of seed soaking and foliar spraying treatments with antioxidants and nitrogen, phosphorus and potassium (NPK) fertilization levels as well as their interactions on growth, yields and its components as well as grain quality characters of wheat cultivar Sakha 93. Seeds of Sakha 93 cultivar was obtained from Wheat Research Section, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.

Experimental design and treatments: The experiments were carried out in a strip-split plot design with four replicates. Each experiment included seventy five treatments comprising, five seed soaking treatments, five foliar spraying treatments and three NPK fertilization levels. The vertical-plots were included five seed soaking treatments as following:

- Without treatment (control)
- Soaking seeds in tap water
- Soaking seeds in Salicylic Acid (SA) at the level of 300 ppm
- Soaking seeds in Citric Acid (CA) at the level of 300 ppm
- Soaking seeds in Ascorbic Acid (AA) at the level of 300 ppm

Wheat seeds were soaked for 12 h in all soaking treatments and then air dried before sowing in the field. Ascorbic Acid, salicylic acid and citric acid anhydrous were produced by El-Nasr Pharmaceutical Chemicals Co., Abu Zaabal, Egypt and obtained from El-Gomhouria Company for Trading Pharmaceutical Chemical and Medical.

The horizontal-plots were devoted to five foliar spraying treatments as follows:

- Without foliar spraying (control)
- Foliar spraying with water
- Foliar spraying with Salicylic Acid (SA) twice after 40 and 55 Days From Sowing (DFS) at the level of 150 ppm in each one
- Foliar spraying with Citric Acid (CA) twice after 40 and 55 DFS at the level of 150 ppm in each one
- Foliar spraying with Ascorbic Acid (AA) twice after 40 and 55 DFS at the level of 150 ppm in each one

The foliar solution volume was 200 L/fed and spraying was conducted by hand sprayer (for experimental plots) until saturation point.

The sub-plots were allocated to three nitrogen, phosphorus and potassium (NPK) fertilization levels as follows:

- 60% of the recommended doses (48.0 kg N+13.5 kg P_2O_5+14.4 K_2O/fed)
- 80% of the recommended doses (64.0 kg N+18.0 kg P_2O_5+19.2 K_2O/fed)
- 100% of the recommended doses (80.0 kg N+22.5 kg P_2O_5+24.0 K_2O/fed)

Calcium super phosphate (15.0% P_2O_5) as a source of phosphorus fertilizer was applied during soil preparation. Potassium fertilizer in the form of potassium sulphate (48.0% K_2O) was applied in one dose before the first irrigation. Nitrogen fertilizer in the form of ammonium nitrate (33.5% N) was used in two equal doses prior the first and the second irrigations.

Soil samples for both physical and chemical analysis of the experimental soil were taken before sowing and the corresponding data are presented in Table 1. Each experimental unit area was 3×3.5 m occupying an area of 10.5 m^2 i.e., 1/400 feddan (one feddan = 4200 m^2). The preceding summer crop was rice (*Oryza sativa* L.) in both seasons.

Table 1: Physical and chemical soil characteristics at the experimental sites during 2005/2006 and 2006/2007

	Physical characteristics							
Seasons	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Soil texture	$CaCO_3$ (%)	Field capacity (%)	Real density (g cm^{-3})
2011/2012	6.2	32.6	25.7	35.5	Clay loam	2.45	34.3	2.66
2012/2013	5.8	33.2	25.3	35.7	Clay loam	2.54	35.2	2.65

	Chemical characteristics					
				Available nutrients (ppm)		
Seasons	pH soil paste	EC (dS m^{-1})	Organic matter (%)	N	P	K
2011/2012	7.6	2.2	1.72	32.3	7.1	232
2012/2013	7.8	2.4	1.83	36.4	7.3	240

Agricultural practices: Sakha 93 seed was sown at November 26th and 28th in the first and second seasons, respectively. Wheat grains at the rate of 60 kg/fed were broadcasted using method (Afir). The other recommended agricultural practices for growing wheat in the studied area were followed according to the recommendations of Ministry of Agriculture, except the factors under study.

Studied characters

Disease characters: A daily observation for damping off percentages were recorded.

Growth characters:

- Total phenolic compounds. After 70 days from sowing, wheat fresh shoots were used to determine total phenolic compounds using the Folin-Ciocalteau reagent according to Singleton and Rossi Jr. (1965)

After 120 days from sowing, five plants from one square meter was randomly choice from each sub-plot to estimate:

- Total chlorophyll (SPAD). Chlorophyll content in flag leaf was assessed by SPAD-502 (Minolta Co. Ltd., Osaka, Japan)
- Flag leaf area (cm^2). It was determined using Field Portable Leaf Area Meter AM-300 (Bio-Scientific, Ltd., Great Am well, Herefordshire, England).
- Plant height (cm)

Yield and its attributes: At harvesting, one square meter was randomly selected from each sub-plot to estimate the following characters:

- Number of spikes/m^2
- Spike length (cm)
- Number of grains/spike
- Grains weight/spike (g)
- 1000 grain weight (g)
- Grain yield (ardab/fed). It was calculated by harvesting whole plants in each experimental plot and air dried, then threshed and the grains at 13% moisture were weighted in kg and converted to ardab per feddan (one ardab = 150 kg)
- Straw yield (t/fed). The straw resulted from previous sample was weighted in kg/plot and then it was converted to ton per feddan

Grain quality:

- Carbohydrates percentage in grains. It was estimated using the anthrone method as described by Sadasivam and Manickam (1996)
- Crude protein percentage in grains. It was estimated by the improved Kjeldahl-method according to AOAC (1990), modified by distilling the ammonia into saturated boric solution and titration in standard acid. Crude protein percentage was calculated by multiplying the total nitrogen values in wheat flour by 5.75

- Phosphorous in grains and straw was determined by the methods described by Cooper (1977)
- Potassium in grains and straw was determined using flamephotometer as described by Peterburgski (1968)

Statistical analysis: All obtained data of greenhouse and field experiments was statistically analyzed according to the technique of analysis of variance (ANOVA) for the strip-split plot design as published by Gomez and Gomez (1984) using MSTAT statistical package (MSTAT-C with MGRAPH version 2.10, Crop and Soil Sciences Department, Michigan State University, USA) developed by Russell (1986). Least Significant Difference (LSD) method was used to test the differences between treatment means at 5% level of probability as described by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Laboratory experiment: Results presented in Table 2 show that the wheat seed samples used in the experiments during 2011/2012 and 2012/2013 seasons were infected with 7 genera (11 species) of fungi. Of these, *Alternaria alternate*, *Aspergillus flavus*, *Aspergillus niger*, *Cephalosporium* sp., *Fusarium culmorum*, *Fusarium moniliforme*, *Fusarium semitectum*, *Fusarium graminearum*, *Penicillium* sp., *Stemphylium* sp. and *Verticillium* spp.

Pathogenicity tests of important seed-borne fungi on wheat

Pathogenicity tests: Results in Table 3 show that the four tested fungi viz., *Cephalosporium* sp., *Fusarium culmorum*, *F. moniliforme* and *F. graminearum* were subjected to pathogenicity tests to determine the most aggressive one. The results indicated that all tested fungi have the potency to cause damping-off disease on wheat seedlings. The most aggressive one is *F. graminearum* (36.51% damping off and 73.35% survival seedlings) followed by *F. moniliforme* (31.41% damping off and 68.49% survival seedlings), *Fusarium culmorum* (28.23% damping off and 71.77% survival seedlings) and *Cephalosporium* sp. (26.65% damping off and 73.35% survival seedlings) compared with control (11.00% damping off and 89.00% survival seedlings).

Table 2: Percentages of seed-borne fungi associated with wheat seeds before sowing during 2011/2012 and 2012/2013 seasons

	Seed-borne fungi associated with wheat seeds before sowing (%)	
Fungi	2011/2012	2012/2013
Alternaria alternata	69.6	57.3
Aspergillus flavus	5.4	5.2
Aspergillus niger	3.4	3.7
Cephalosporium sp.	7.2	6.7
Fusarium culmorum	9.8	9.4
Fusarium moniliforme	34.4	29.6
Fusarium semitectum	12.8	11.5
Fusarium graminearum	4.5	3.6
Penicillium sp.	3.9	2.5
Stemphylium sp.	15.7	14.3
Verticillium sp.	4.5	2.2

Table 3: Percentage of damping-off on wheat seedlings under greenhouse condition

Treatments	Damping-off (%)	Survival seedlings (%)
Cephalosporium sp.	26.65	73.35
Fusarium culmorum	28.23	71.77
Fusarium moniliforme	31.40	68.59
Fusarium graminearum	36.50	63.49
Control	11.00	89.00
LSD at 5%	2.02	2.02

Table 4: Effect of salicylic acid, citric acid and ascorbic acid on linear growth of fungi isolated from wheat seeds

Treatments	*Cephalosporium* sp.	*Fusarium culmorum*	*Fusarium moniliforme*	*Fusarium graminearu*
Salicylic acid (ppm)				
100	5.4	6.4	6.8	7.4
150	4.2	4.3	3.2	3.5
300	0.0	0.0	0.0	0.0
Citric acid (ppm)				
100	6.7	7.3	7.8	8.5
150	5.1	5.2	5.5	6.0
300	2.4	1.6	2.6	2.8
Ascorbic acid (ppm)				
100	6.1	6.8	7.2	7.9
150	4.5	4.6	4.9	5.4
300	0.0	0.0	0.0	0.0
Control	9.0	9.0	9.0	9.0
LSD at 5%	0.1	0.1	1.0	0.1

Table 5: Damping-off percentages as affected by seed soaking and foliar application treatments of antioxidants and NPK fertilization levels as well as their interactions on wheat seedlings under greenhouse condition

	Damping-off				
Treatments	*Cephalosporium* sp.	*Fusarium culmorum*	*Fusarium moniliforme*	*Fusarium graminearum*	Control
Seed soaking treatments					
Without	15.82	26.52	12.88	19.51	10.63
Water	16.07	26.54	13.08	19.82	10.81
Salicylic Acid (SA)	1.78	2.82	1.44	2.19	1.19
Citric Acid (CA)	1.29	2.06	1.05	1.59	0.87
Ascorbic Acid (AA)	2.36	3.76	1.91	2.90	1.58
LSD at 5%	0.35	0.26	0.28	0.43	0.24
Foliar application treatments					
Without	8.33	13.98	6.78	10.27	5.60
Water	8.42	14.02	6.84	10.38	5.65
Salicylic Acid (SA)	6.73	11.03	5.48	8.30	4.52
Citric Acid (CA)	6.56	10.71	5.33	8.10	4.41
Ascorbic Acid (AA)	7.28	11.96	5.92	8.97	4.89
LSD at 5%	0.37	0.36	0.30	0.46	0.25
NPK fertilization levels					
60% NPK	7.83	12.73	6.37	9.65	5.26
80% NPK	7.44	12.31	6.05	9.17	5.00
100% NPK	7.13	11.98	5.80	8.79	4.79
LSD at 5%	0.10	0.16	0.08	0.13	0.07
Interactions					
A×B	*	*	*	*	*
A×C	NS	NS	NS	NS	NS
B×C	*	*	*	*	*
A×B×C	NS	*	NS	*	*

*Interaction is significant, NS: Not significant interaction

Study the effect of antioxidants on seed-borne fungi isolated from wheat seeds

Linear growth test (*in vitro* studies): Data in Table 4 showed that the effect of Salicylic acid, Citric acid and Ascorbic acid at (100, 150 and 300 ppm) on linear growth of *Cephalosporium* sp., *Fusarium culmorum*, *F. moniliforme* and *F. graminearum* isolated from wheat seeds. It was also noticed that the reduction in linear growth was correlated to the increase in compounds concentrations.

Greenhouse experiment: Results in Table 5 indicated that all tested antioxidants reduced damping-off caused by artificial infection with the tested fungi, when applied any application methods (seed soaking and spraying on plant shoot) in greenhouse conditions. Salicylic acid treatment gave the maximum reduction in damping-off caused by all tested fungi compared with the control treatment.

Field experiment: Results presented in Table 6 indicated that all tested antioxidants reduced percentages of damping-off and seed-borne fungi associated with wheat seeds after harvest when applied any application methods (seed soaking or spraying on plant shoot) under field conditions. Soaking seeds before sowing in Salicylic Acid (SA) solution at the level of 300 ppm for 12 h produced the maximum reduction in percentages of damping-off and seed-borne fungi associated with wheat seeds after harvest. Spraying wheat plants twice (after 40 and 55 DFS) with Salicylic Acid (SA) at the level of 150 ppm of each spraying gave the maximum reduction in percentages of damping-off and seed-borne fungi associated with wheat seeds after harvest. Wheat plants fertilized with 100% of the recommended dose of NPK (80.0 kg N+22.5 kg P_2O_5+24.0 K_2O/fed) resulted in the maximum reduction in percentages of damping-off and seed-borne fungi associated with wheat seeds after harvest. Similar conclusions were reported by Galal and Abdou (1996) they found that application of salicylic or ascorbic acid as a soil drenching was better than foliar application to control fusarial diseases of cowpea. Also, Mostafa (2006) reported that soaking cumin seeds or soil drenching with antioxidant solutions (salicylic, ascorbic, coumaric, benzoic acids and propylgalate) before planting resulted in resistant cumin seedlings against infection with the *Fusarium oxysporum cumini* and *Acremonium egyptiacum*. Abdel-Monaim (2008) showed that soaking lupine seeds in antioxidant solutions reduced the damping-of and root rot diseases caused by *Fusarium solani* and *Macrophomina phaseolina. In vitro* all antioxidants show less effect on dry weight. Propylgalate had the highest effect on spore formation especially highly concentration (200 ppm). The antioxidants mode of action was reported in many host-pathogen interactions i.e., many oxidative enzymes such as peroxidase, catalase, ascorbate oxidase and polyphenol oxidase were detected as a result of infection with many pathogens (Clarke *et al*., 2002) or as a result of treatments with various antioxidants (Takahama and Oniki, 1994; El-Khallal, 2007; Abdel-Monaim, 2008). Moreover, Lyon and McGill (1989) reported that the phenolic acids benzoic, ferulic, coumaric and protocatechoic acid inhibit *in-vitro* activity of polygalacturonase and polygalacturonic acid lyase from *Erwinia carotovora*. Chen *et al.* (1993) reported that SA binds to, or inhibits catalase. Also in the present study shows that all tested antioxidants reduced the damping-off in greenhouse and field conditions and increased the growth characters, yield and its attributes and grain quality in both seasons. The increase in wheat yield may be due to the role of antioxidants in stimulation of physiological processes which reflect the improving vegetative growth that followed by active translocation of the photo as simulation. In this respect, antioxidants might be right being regulating plant growth by increasing enzyme activity as α-amylase and nitrate reductase which accelerate the sugar translocation from the leaves to developing fruit (Sharma *et al*., 1986). In addition, application of SA inhibits ethylene production leading to an increase in fruit number and consequently increases fruit yield per plant (Leslie and Romani, 1986). Abdel-Monaim (2008) found that lupine seed soaking in antioxidant solutions increases of chlorophyll and carotenoids content in leaves and this reflects the health condition of the plant.

Growth characters and yield attributes: Soaking wheat seeds before sowing in different treatments i.e., without, soaking in tap water, Salicylic Acid (SA), Citric Acid (CA) and Ascorbic Acid (AA) at the level of 300 ppm of each one exhibited significant effects on wheat growth

Table 6: Percentages of damping-off and seed-borne fungi associated with wheat seeds after harvest as affected by seed soaking and foliar application treatments of antioxidants and NPK fertilization levels as well as their interactions during 2011/2012 and 2012/2013 seasons

			Seed-borne fungi associated with wheat seeds after harvest (%)									
	Damping-off		*Alternaria alternate*		*Aspergillus flavus*		*Aspergillus niger*		*Cephalosporium* sp.		*Fusarium culmorum*	
Treatments	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013
Seed soaking treatments												
Without	26.55	25.80	67.56	65.55	3.853	3.733	2.753	2.662	5.580	5.418	7.589	7.369
Water	26.55	25.80	67.61	65.60	3.918	3.800	2.791	2.709	5.676	5.504	7.598	7.369
Salicylic acid (SA)	2.06	1.93	5.45	5.29	0.311	0.311	0.207	0.200	0.460	0.451	0.600	0.556
Citric acid (CA)	3.60	3.57	9.93	9.64	0.580	0.562	0.387	0.380	0.827	0.804	1.060	1.047
Ascorbic acid (AA)	3.04	3.04	7.59	7.36	0.444	0.436	0.304	0.302	0.627	0.607	0.818	0.778
LSD at 5%	0.25	0.26	0.69	0.67	0.059	0.046	0.063	0.062	0.128	0.123	0.077	0.079
Foliar application treatments												
Without	13.93	13.66	35.63	34.57	2.027	1.967	1.442	1.398	2.940	2.853	4.004	3.882
Water	13.95	13.66	35.68	34.62	2.040	1.987	1.451	1.411	2.971	2.880	4.009	3.891
Salicylic acid (SA)	10.77	10.51	27.38	26.57	1.609	1.560	1.127	1.091	2.316	2.251	3.073	2.976
Citric acid (CA)	12.13	11.62	30.46	29.56	1.769	1.716	1.262	1.231	2.569	2.496	3.418	3.313
Ascorbic acid (AA)	11.02	10.68	28.98	28.12	1.662	1.613	1.160	1.122	2.373	2.304	3.160	3.056
LSD at 5%	0.62	0.52	0.94	0.97	0.092	0.098	0.069	0.066	0.138	0.132	0.153	0.151
NPK fertilization levels												
60% NPK	12.82	12.38	32.46	31.50	1.907	1.848	1.333	1.293	2.759	2.680	3.645	3.531
80% NPK	12.33	12.05	31.92	30.97	1.821	1.773	1.289	1.253	2.625	2.547	3.531	3.417
100% NPK	11.93	11.65	30.50	29.60	1.736	1.684	1.243	1.205	2.517	2.444	3.423	3.323
LSD at 5%	0.18	0.16	0.40	0.39	0.027	0.029	0.021	0.021	0.037	0.036	0.045	0.044
Interactions												
A×B	*	*	*	*	*	*	*	*	*	*	*	*
A×C	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
B×C	*	*	*	*	*	*	*	*	*	*	*	*
A×B×C	*	*	*	*	NS	NS	NS	NS	NS	NS	*	*

Table 6: Continue

	Seed-borne fungi associated with wheat seeds after harvest (%)											
	F. moniliforme		*F. semitectum*		*F. graminearum*		*Penicillium* sp.		*Stemphylium* sp.		*Verticillium* sp.	
Treatments	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013
Seed soaking treatments												
Without	27.82	27.00	10.78	10.47	3.687	3.580	2.353	2.276	13.45	13.05	2.051	1.993
Water	28.27	27.45	10.81	10.48	3.749	3.636	2.391	2.324	13.67	13.27	2.087	2.029
Salicylic Acid (SA)	2.27	2.21	0.84	0.80	0.307	0.304	0.191	0.184	1.10	1.06	0.147	0.147
Citric Acid (CA)	4.14	4.02	1.52	1.47	0.551	0.536	0.340	0.329	2.00	1.94	0.293	0.293
Ascorbic Acid (AA)	3.16	3.07	1.15	1.11	0.411	0.400	0.242	0.231	1.50	1.46	0.220	0.211
LSD at 5%	0.62	0.60	0.11	0.10	0.088	0.082	0.047	0.056	0.29	0.28	0.052	0.041
Foliar application treatments												
Without	14.65	14.22	5.68	5.52	1.944	1.889	1.242	1.200	7.09	6.88	1.076	1.056
Water	14.79	14.36	5.70	5.52	1.962	1.909	1.253	1.220	7.15	6.94	1.087	1.064
Salicylic Acid (SA)	11.58	11.25	4.36	4.23	1.527	1.480	0.967	0.938	5.58	5.41	0.847	0.822
Citric Acid (CA)	12.80	12.43	4.86	4.72	1.698	1.656	1.084	1.042	6.19	6.00	0.944	0.913
Ascorbic Acid (AA)	11.83	11.49	4.48	4.35	1.573	1.522	0.971	0.944	5.72	5.55	0.844	0.818
LSD at 5%	0.66	0.64	0.22	0.21	0.089	0.089	0.052	0.055	0.32	0.31	0.050	0.048
NPK fertilization levels												
60% NPK	13.77	13.38	5.18	5.02	1.825	1.773	1.144	1.108	6.65	6.45	0.991	0.967
80% NPK	13.08	12.70	5.01	4.86	1.735	1.683	1.103	1.071	6.32	6.13	0.964	0.940
100% NPK	12.54	12.17	4.87	4.72	1.663	1.617	1.064	1.028	6.06	5.88	0.924	0.897
LSD at 5%	0.19	0.18	0.06	0.05	0.026	0.025	0.016	0.016	0.09	0.08	0.015	0.014
Interactions												
A×B	*	*	*	*	*	*	*	*	*	*	*	*
A×C	NS	NS	NS	*	NS	NS	NS	*	NS	NS	*	*
B×C	*	*	*	*	*	*	*	*	*	*	*	*
A×B×C	NS	*	*	*	NS	NS	*	*	NS	*	NS	NS

*Significant interaction, NS: Non significant interaction

Table 7: Total phenolic compounds, total chlorophyll, flag leaf area and plant height as affected by seed soaking and Foliar spraying treatments of antioxidants and NPK fertilization levels as well as their interactions during 2011/2012 and 2012/2013 seasons

	Total phenolic compounds		Total chlorophyll (SPAD)		Flag leaf area (cm^2)		Plant height (cm)	
Treatments	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013
Seed soaking treatments								
Without	58.00	56.37	50.43	50.98	28.48	28.94	85.93	87.06
Water	58.15	56.42	51.13	51.46	28.82	28.95	86.46	87.77
Salicylic acid (SA)	73.75	71.57	55.64	55.98	33.23	33.41	98.95	100.20
Citric acid (CA)	60.91	59.04	53.22	53.55	31.12	31.20	92.20	93.80
Ascorbic acid (AA)	62.88	60.95	54.66	53.62	32.42	32.58	95.44	96.91
LSD at 5%	0.55	0.45	0.44	0.55	0.32	0.33	0.75	0.70
Foliar spraying treatments								
Without	60.40	58.62	50.68	51.13	28.46	28.68	84.95	86.35
Water	60.77	59.04	51.34	51.88	28.95	29.25	86.68	88.11
Salicylic acid (SA)	67.44	65.42	55.52	55.84	32.96	33.12	98.11	99.73
Citric acid (CA)	61.91	60.06	53.15	52.04	31.52	31.66	93.46	94.37
Ascorbic acid (AA)	63.17	61.22	54.40	54.70	32.19	32.36	95.77	97.17
LSD at 5%	0.90	0.89	0.25	0.38	0.39	0.46	0.89	0.73
NPK fertilization levels								
60% NPK	60.92	59.09	44.86	45.18	29.58	29.86	86.86	88.21
80% NPK	62.66	60.85	55.44	55.88	30.92	31.26	92.92	94.18
100% NPK	64.64	62.68	58.75	58.30	31.95	31.93	95.61	97.05
LSD at 5%	0.31	0.32	0.22	0.32	0.17	0.15	0.51	0.44
Interactions								
A×B	*	*	*	*	*	*	*	*
A×C	*	*	*	NS	NS	NS	*	*
B×C	*	*	*	*	*	*	NS	NS
A×B×C	*	*	*	NS	NS	NS	NS	*

*Interaction is significant, NS: Not significant interaction

characters and yield attributes in both seasons as shown from results presented in Table 7 and 8. An overview of the obtained data it is clear that seed soaking treatments in water and antioxidants (SA, CA and AA) significantly increased wheat growth and yield attributes characters as compared with the control treatment (untreated seeds) in both seasons. Soaking seeds before sowing in Salicylic Acid (SA) solution at the level of 300 ppm for 12 h produced the highest values of total phenolic compounds (73.75 and 71.57), total chlorophyll (55.64 and 55.98 SPAD), flag leaf area (33.23 and 33.41 cm^2), plant height (98.95 and 100.20 cm), number of spikes m^{-2} (398.6 and 400.8 spikes m^{-2}), spike length (12.01 and 12.01 cm), number of grains/spike (68.78 and 69.01 grains/spike), weight of grains/spike (3.265 and 3.280 g) and 1000-grain weight (42.36 and 42.63 g) in the first and second seasons, respectively. Whereas, soaking seed in Ascorbic Acid (AA) solution at the level of 300 ppm for 12 h also ranked after previously mentioned treatment and followed by concerning growth and yield attributes with significant differences between them in both seasons. However, soaking seed in Citric Acid (CA) solution at the level of 300 ppm for 12 h ranked third and followed by soaking seed in tap water for 12 h in both seasons.

Foliar spraying treatments (without, spraying with water and antioxidants i.e., SA, CA and AA) had a significant effect on wheat growth characters and yield attributes in both seasons (Table 7 and 8). Spraying wheat plants twice (after 40 and 55 DFS) with Salicylic Acid (SA) at the level of 150 ppm of each spraying significantly exceeded other foliar spraying treatments and produced the highest values of growth characters and yield attributes in both seasons. It is worth mentioning that spraying with SA increased total phenolic compounds by 11.66 and 11.60%, total chlorophyll by 9.55 and 9.21%, flag leaf area by 15.81and 15.48%, plant height by 15.49 and 15.50%, number of spikes m^{-2} by 7.20 and 7.03%, spike length by 5.29 and 5.31%, number of grains/spike

Table 8: Number of spikes/m^2, spike length, number of grains/spike, grains weight/spike and 1000 grain weight as affected by seed soaking and foliar spraying treatments of antioxidants and NPK fertilization levels as well as their interactions during 2011/2012 and 2012/2013 seasons

	No. of spikes/m^2		Spike length (cm)		No. of grains/spike		Grains weight/spike (g)		1000 grain weight (g)	
Treatments	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013
Seed soaking treatments										
Without	340.00	343.20	11.23	11.20	64.72	65.07	2.878	2.900	37.98	38.26
Water	344.60	346.70	11.36	11.39	65.02	65.30	2.921	2.943	38.46	38.76
Salicylic Acid (SA)	398.60	400.80	12.01	12.01	68.78	69.01	3.265	3.280	42.36	42.63
Citric Acid (CA)	376.60	379.30	11.69	11.66	67.79	68.04	3.167	3.203	41.02	41.30
Ascorbic Acid (AA)	384.40	386.00	11.88	11.92	68.19	68.52	3.224	3.254	41.68	41.89
LSD at 5%	2.85	2.07	0.06	0.06	0.18	0.24	0.032	0.030	0.13	0.14
Foliar spraying treatments										
Without	355.80	358.50	11.34	11.31	64.95	65.24	2.862	2.888	39.35	39.69
Water	358.60	361.10	11.40	11.41	65.26	65.49	2.905	2.935	39.64	39.85
Salicylic Acid (SA)	381.40	383.70	11.94	11.91	68.67	68.94	3.327	3.353	41.10	41.42
Citric Acid (CA)	370.80	373.20	11.69	11.71	67.58	67.94	3.138	3.165	40.57	40.81
Ascorbic Acid (AA)	377.50	379.50	11.80	11.84	68.04	68.33	3.225	3.239	40.85	41.08
LSD at 5%	1.53	1.45	0.02	0.05	0.15	0.18	0.023	0.024	0.10	0.12
NPK fertilization levels										
60% NPK	362.40	364.50	11.33	11.38	65.21	65.47	2.848	2.875	39.83	40.05
80% NPK	370.00	372.20	11.70	11.63	67.19	67.47	3.141	3.166	40.36	40.64
100% NPK	374.00	376.90	11.87	11.90	68.30	68.62	3.285	3.307	40.71	41.01
LSD at 5%	0.82	0.81	0.02	0.03	0.13	0.14	0.021	0.016	0.06	0.07
Interactions										
A×B	NS	*	*	*	*	*	*	*	*	*
A×C	*	NS	*	*	*	*	*	*	*	*
B×C	*	NS	*	*	*	*	*	*	NS	NS
A×B×C	*	*	NS	*	*	*	*	*	*	*

*Significant interaction, NS: Non significant interaction

by 5.73 and 5.67%, weight of grains/spike by 16.25 and 16.10 and 1000 grain weight by 4.45 and 4.36% compared with control treatment (without foliar spraying) in the first and second seasons, respectively. The other foliar spraying treatments can be arranged as follows; spraying with AA, spraying with CA, spraying with water and lastly control treatment in the two growing seasons.

Such desirable effects of seed soaking and/or foliar spraying with antioxidants (SA, CA and AA) might be due to critical role of SA in regulating stomata closure, ion uptake and transport, inhibition of ethylene biosynthesis, transpiration, membrane permeability, photosynthesis and growth (Abdel-Wahed *et al*., 2006; Ashraf *et al*., 2010), nitrate metabolism and stress tolerance (Hayat *et al*., 2007). In addition, the vital role of AA in cell division, cell wall expansion (Pignocchi and Foyer, 2003), the electron transport system (El-Kobisy *et al*., 2005) and other developmental processes. Besides, the important role of CA in the physiological oxidation of fats, proteins and carbohydrates to CO_2 and water. Similar results were reported by Al-Hakimi (2008), Fawy and Atyia (2012), Bakry *et al*. (2013), Ghafiyehsanj *et al*. (2013), El-Housini *et al*. (2014) and Hussein and Alva (2014).

Results presented in Table 7 and 8 reveal that, wheat growth characters and yield attributes were significantly increased by increasing NPK fertilization levels (60-80 and 100% of the recommended dose) in both growing seasons. Wheat plants fertilized with 100% of the recommended dose of NPK (80.0 kg N+22.5 kg P_2O_5+24.0 K_2O/fed) had the highest values of growth characters and yield attributes, followed by plants fertilized with 80% of the recommended dose of NPK (64.0 kg N+18.0 kg P_2O_5+19.2 K_2O/fed) and lastly that fertilized with 60% of the recommended dose of NPK (48.0 kg N+13.5 kg P_2O_5+14.4 K_2O/fed) with significant differences among them in both seasons. Noteworthy, the percentage of increasing values of most growth characters and yield attributes due to increasing NPK levels from 60-80% of the recommended dose was more than those of increasing NPK levels from 80-100% of the recommended dose. This reflected that the response of wheat to increase NPK levels from 80-100% of the recommended dose was less than that when NPK levels increased from 60-80% of the recommended dose.

These results might be ascribed to the essential role of nitrogen fertilizer in formation of proteins, protoplasm and chlorophyll, consequently increases cell size, leaf area, photosynthetic activity and growth characters accordingly yield attributes. Also, the function of phosphorus in building energy for metabolism of plant growth through cellular productions such as ATP and ADP from the early stages to the end of the plant's life (Marschner, 1995). In addition, the role of potassium in photosynthesis, translocation of photosynthates, protein synthesis, control of ionic balance, regulation of plant stomata and water use (Marschner, 1995; Reddy *et al*., 2004), enzyme activation and osmoregulation (Mengel, 2007). These results are in agreement with those obtained by Youssef *et al*. (2013) and Khan *et al*. (2014).

Yield and grains quality: Results presented in Table 9 demonstrate that the effect of seed soaking treatments on wheat grain and straw yields/fed and grains quality was significant in both seasons. There were considerable differences in wheat yields and grain quality among seed soaking treatments (without, soaking in tap water, SA, CA and AA at the level of 300 ppm of each) as compared with control treatment (untreated seeds) in both seasons. Wheat seed soaking in Salicylic Acid (SA) at the level of 300 ppm produced the highest values of grain yield (21.02 and 21.10 ardab/fed), straw yield (4.557 and 4.589 t/fed), carbohydrates (71.75 and 71.82%), crude

Table 9: Grain and straw yields/fed, carbohydrates, crude protein, phosphorus and potassium percentages in grains as affected by seed soaking and Foliar spraying treatments of antioxidants and NPK fertilization levels as well as their interactions during 2011/2012 and 2012/2013 seasons

	Grain yield (ardab/fed)		Straw yield (t/fed)		Carbohydrates (%)		Protein (%)		P (%)		K (%)	
Treatments	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013	2011/2012	2012/2013
Seed soaking treatments												
Without	18.67	18.72	4.168	4.192	70.93	70.98	12.86	12.90	0.259	0.261	1.288	1.299
Water	18.85	18.92	4.237	4.272	71.00	71.08	12.92	12.97	0.264	0.267	1.304	1.316
Salicylic Acid (SA)	21.02	21.10	4.557	4.589	71.75	71.82	13.38	13.41	0.297	0.300	1.364	1.374
Citric Acid (CA)	20.46	20.52	4.393	4.417	71.42	71.49	13.24	13.31	0.289	0.292	1.340	1.353
Ascorbic Acid (AA)	20.78	20.82	4.486	4.508	71.60	71.64	13.30	13.33	0.294	0.296	1.353	1.364
LSD at 5%	0.06	0.04	0.040	0.041	0.06	0.11	0.03	0.02	0.003	0.002	0.005	0.008
Foliar spraying treatments												
Without	19.50	19.55	4.196	4.222	70.81	70.88	12.84	12.89	0.265	0.268	1.302	1.313
Water	19.63	19.67	4.238	4.264	70.94	71.01	12.90	12.94	0.269	0.272	1.314	1.325
Salicylic Acid (SA)	20.35	20.39	4.530	4.563	71.82	71.89	13.39	13.44	0.293	0.295	1.355	1.365
Citric Acid (CA)	20.06	20.18	4.408	4.436	71.47	71.52	13.23	13.27	0.285	0.287	1.333	1.343
Ascorbic Acid (AA)	20.23	20.28	4.470	4.494	71.65	71.71	13.34	13.39	0.290	0.292	1.346	1.360
LSD at 5%	0.05	0.05	0.015	0.016	0.11	0.09	0.04	0.04	0.002	0.002	0.006	0.007
NPK fertilization levels												
60% NPK	19.64	19.70	4.234	4.261	70.40	70.45	12.84	12.89	0.270	0.272	1.316	1.326
80% NPK	20.00	20.06	4.381	4.412	71.63	71.69	13.21	13.25	0.282	0.284	1.330	1.342
100% NPK	20.22	20.29	4.490	4.514	71.99	72.06	13.37	13.41	0.290	0.293	1.344	1.355
LSD at 5%	0.03	0.04	0.013	0.012	0.05	0.05	0.02	0.02	0.001	0.001	0.002	0.002
Interactions												
A×B	*	*	*	*	*	*	*	*	*	*	NS	NS
A×C	*	*	*	*	NS	NS	*	*	*	*	*	*
B×C	*	*	NS	NS	*	*	NS	NS	*	*	NS	NS
A×B×C	*	*	NS	NS	*	*	*	*	*	*	NS	NS

*Significant interaction, NS: Non significant interaction

protein (13.38 and 13.41%), phosphorus (0.297 and 0.300%) and potassium (1.364 and 1.374%) in grains in the first and second seasons, respectively. On the other hand, control treatment (untreated seeds) resulted in the lowest values of these characters in the two growing seasons. Though, wheat seed soaking in Ascorbic Acid (AA) at the level of 300 ppm came in the second rank after those soaked in SA, followed by those soaked in CA and that soaked water in both seasons.

Wheat yields (grain and straw/fed) and grain quality parameters (carbohydrates, crude protein, phosphorus and potassium percentages) show significant response to foliar spraying treatments (without, spraying with water and antioxidants i.e., SA, CA and AA) in both seasons as shown in Table 9. Foliar spraying wheat plants with Salicylic Acid (SA) twice after 40 and 55 DFS at the level of 150 ppm in each one resulted in the highest values of wheat yields and grain quality characters with significant differences as compared with other foliar spraying treatments in the two growing seasons. In this regard, foliar spraying with SA increased grain yield by 0.57, 1.24, 3.66 and 4.33%, straw yield by 1.44, 2.82, 6.95 and 8.02%, carbohydrates by 0.24, 0.50, 1.24 and 1.43%, crude protein by 0.37, 1.25, 3.83 and 4.28%, phosphorus by 1.03, 2.80, 8.69 and 10.32% and potassium by 0.52, 1.64, 3.07 and 4.02% compared with foliar spraying with AA, foliar spraying with CA, foliar spraying with water and without foliar spraying over both seasons. Generally, it was observed that the foliar spraying treatments sorted as follows; foliar spraying with SA>foliar spraying with AA>foliar spraying with CA>foliar spraying with water > control treatment in both seasons.

This improvement in wheat yields and grains quality characters due to seed soaking and/or foliar spraying with antioxidants (SA, CA and AA) may be due to the role of antioxidants (SA, CA and AA) in maintaining balanced plant growth as previously mentioned and increasing yield attributes, consequently enhancing wheat yields and grain quality. Furthermore, the role of SA in stimulate tolerance plants to many biotic and abiotic stresses as fungi, bacteria, viruses, chilling, salinity, drought and heat (Khan *et al*., 2010). In addition, ascorbic acid contributed in protection the photosynthetic machinery from the damaging effects of salt stress (Khan *et al*., 2006). These results are in coincidence with those reported by Khodary (2004), Sheteawi (2007), Fercha *et al*. (2011), Erdal *et al*. (2013), Bakry *et al*. (2013) and Ghafiyehsanj *et al*. (2013).

Results in Table 9 illustrate that wheat yields and grains quality characters were significantly increased by increasing NPK levels in both seasons. From obtained data, each increase in NPK associated with significant increase in wheat yields and grains quality characters, this observation was true in the two growing seasons. Generally, the highest values of grain yield (20.22 and 20.29 ardab/fed), straw yield (4.490 and 4.514 t/fed), carbohydrates (71.99 and 72.06%), crude protein (13.37 and 13.41%), phosphorus (0.290 and 0.293%) and potassium (1.344 and 1.355%) in grains were obtained as a result of increasing NPK fertilization level up to 100% of the recommended dose (80.0 kg N+22.5 kg P_2O_5+24.0 K_2O/fed) in the first and second seasons, respectively. The increases due to increasing NPK levels from 60% of the recommended dose (48.0 kg N+13.5 kg P_2O_5+14.4 K_2O/fed) to 80% of the recommended dose (64.0 kg N+18.0 kg P_2O_5+19.2 K_2O/fed) and from 60% of the recommended dose to 100% of the recommended dose reached about 1.83 and 2.97% in grain yield/fed, 3.51 and 5.99% in straw yield/fed, 1.75 and 2.27% in carbohydrates, 2.84 and 4.08% in crude protein, 4.43 and 7.56% in phosphorus and 1.14 and 2.16% in potassium percentages by over both growing seasons.

This improvement in wheat yields and grain quality parameters due to increasing NPK levels may be reflected to its effective roles in improving growth, yield attributes and dry matter accumulation in grains which led to increasing the uptake of most nutrients and enhancing yields

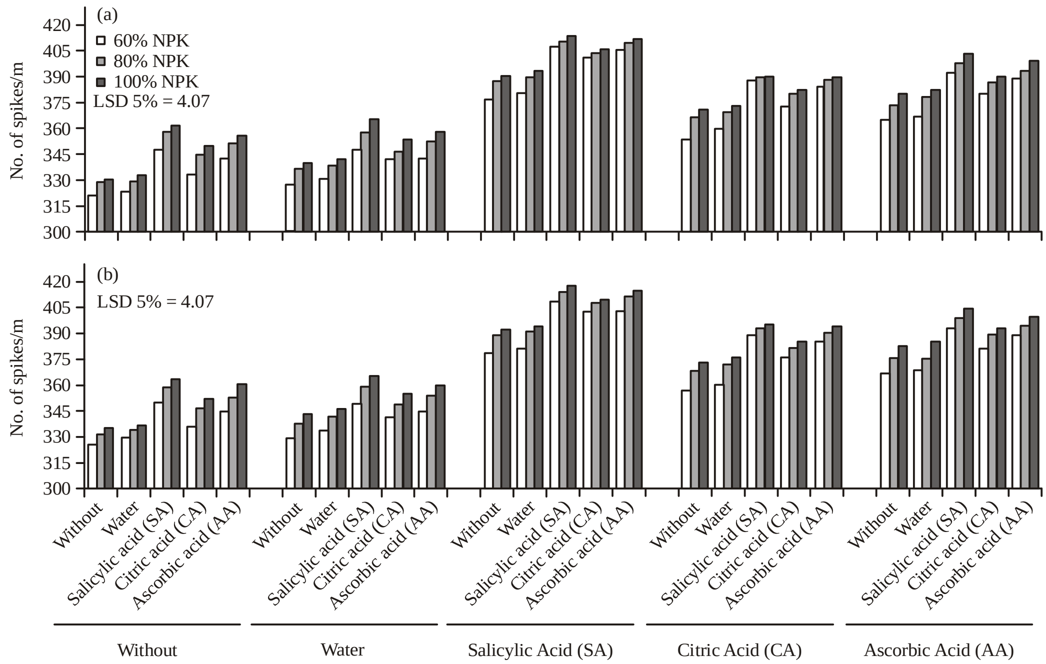

Fig. 1(a-b): Number of spikes/m^2 as affected by the interaction among seed soaking and foliar application treatments of antioxidants and NPK fertilization levels during (a) 2011/2012 and (b) 2012/2013 seasons

and grain quality. The results achieved in this work are partially compatible with those obtained by Meena *et al*. (2013), Youssef *et al*. (2013), Haileselassie *et al*. (2014), Khan *et al*. (2014) and Seadh and Abido (2014).

Interactions: There are many significant effects of the interactions among studied factors on studied characters. We present only the significant three way interaction among seed soaking and foliar spraying treatments with antioxidants and NPK levels on grain yield and its components (number of spikes m^{-2}, grains weight/spike and 1000-grain weight) in both seasons as presented in Table 8 and 9.

As shown from results graphically illustrated, the best interaction treatment which produced the highest values of number of spikes m^{-2} (Fig. 1), grains weight/spike (Fig. 2), 1000-grain weight (Fig. 3), grain yield/fed (Fig. 4) was soaking wheat seeds before sowing in Salicylic Acid (SA) at the level of 300 ppm for 12 h and spraying plants with SA twice after 40 and 55 DFS at the level of 150 ppm of each one beside fertilization with 100% of the recommended dose (80.0 kg N+22.5 kg P_2O_5+24.0 K_2O/fed). This interaction treatment followed by soaking wheat seeds in SA and spraying plants with AA in addition fertilization with 100% of the recommended dose of NPK and then soaking wheat seeds in SA and spraying plants with SA besides fertilization with 80% of the recommended dose of NPK. Notably, soaking wheat seeds in SA and spraying plants with SA plus fertilization with 80% of the recommended dose of NPK significantly increased grain yield and its components compared with control treatment (untreated seeds before sowing and without foliar spraying with antioxidants and mineral fertilization with 100% of the recommended dose of NPK fertilizers).

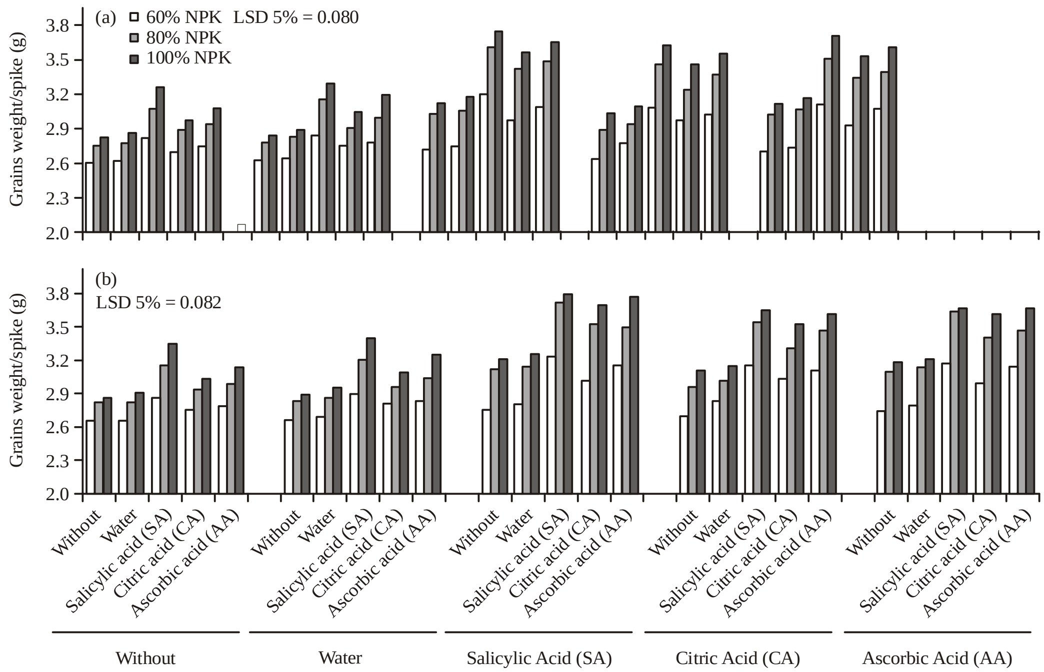

Fig. 2(a-b): Grains weight/spike as affected by the interaction among seed soaking and foliar application treatments of antioxidants and NPK fertilization levels during (a) 2011/2012 and (b) 2012/2013 seasons

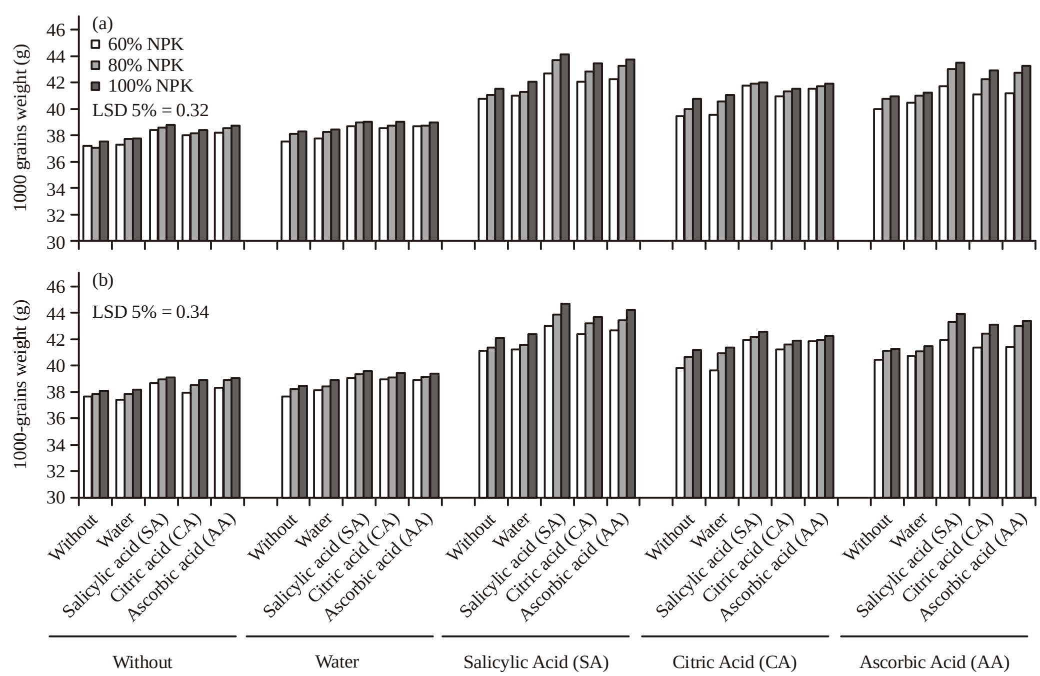

Fig. 3(a-b): 1000-grain weight as affected by the interaction among seed soaking and foliar application treatments of antioxidants and NPK fertilization levels during (a) 2011/2012 and (b) 2012/2013 seasons

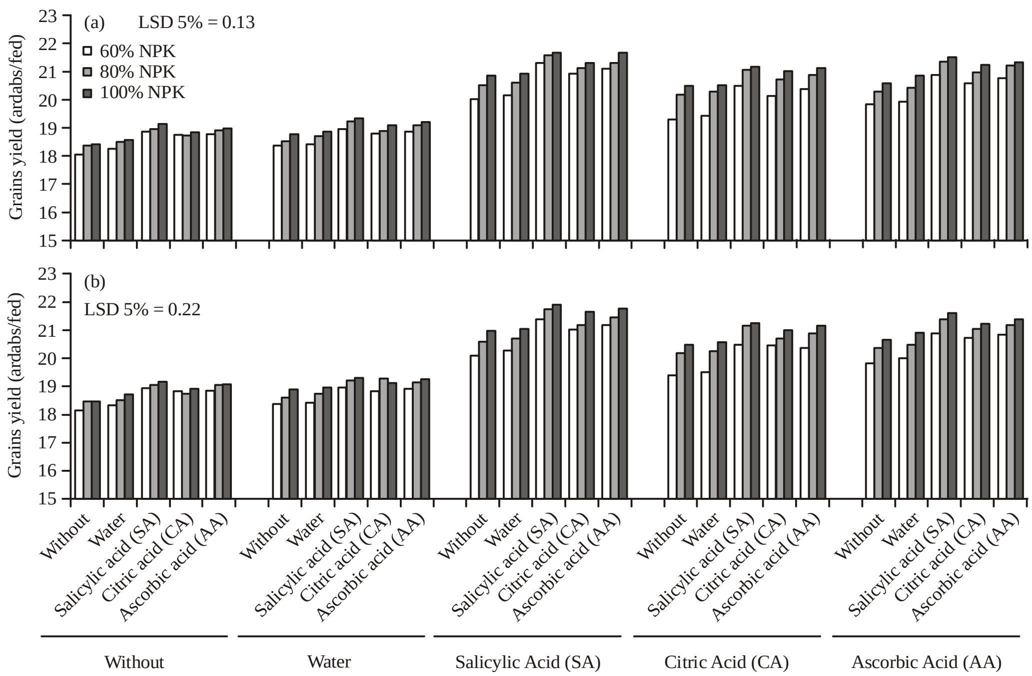

Fig. 4(a-b): Grain yield as affected by the interaction among seed soaking and foliar application treatments of antioxidants and NPK fertilization levels during (a) 2011/2012 and (b) 2012/2013 seasons

CONCLUSION

In general, the obtained results show that soaking wheat seeds before sowing in Salicylic Acid (SA) at the level of 300 ppm and spraying plants with SA twice at the level of 150 ppm beside fertilization with 100% of the recommended dose (80.0 kg N+22.5 kg P_2O_5+24.0 K_2O/fed) maximized wheat growth, yields and its components as well as grain quality. Moreover, it could be recommended that soaking seeds in SA and spraying plants with SA and fertilization with 80% of the recommended dose of NPK which exceeded in grain yield and quality upon the control treatment (untreated seeds before sowing and without foliar spraying with antioxidants and mineral fertilization with 100% of the recommended dose of NPK fertilizers) to reduce soil pollution with mineral fertilizers.

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