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Evaluation of Two Biologically Active Compounds for Control of Wheat Root Rot and its Causal Pathogens

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The main aim of this study is to evaluate the efficiency of two biologically active compounds (Strom and F-760) in control of wheat root rot disease and its causal organisms. *Fusarium graminearum*, *F. oxysporum*, *F. solani* and *Bipolaris sorokiniana* were used as target organisms. *In vitro*, the two compounds showed fungicidal effect on all investigated pathogens resulted in suppression of radial growth and mycelial dry weight of them. Under greenhouse conditions, treatment of wheat grains with either Strom or F-760 before cultivation significantly reduced the percent of disease distribution as well as the mean disease rating of plants in both seedling and flowering stages. Fresh and dry weights of plants as well as water maintenance capacity were increased as the result of applying these compounds as seed dressing. Also data showed that the membrane stability of plants was injured as a result of infection with all investigated organisms, while this injury was alleviated when F-760 and Strom were applied. The K^+ efflux and the leakage of UV-absorbing metabolites was stimulated with fungal infection. However, F-760 and Storm treatment partially retarded the stimulatory effect on leakage of K^+ and UV-absorbing metabolites of fungal infected plants. On the other side, the fungal infection had inhibitory effects on pigment fractions (chlorophyll a, b, and carotenoids) biosynthesis in wheat leaves. This retarding effect was partially or completely alleviated as the grains were treated with the applied compounds.

KEYWORDS: **Biological control, *Bipolaris sorokiniana*, *Fusarium* spp., K^+ efflux, Membrane stability, UV-absorbing metabolites, Wheat root rot**

Biological control of plant pathogens continues to inspire research and development in many fields. Natural products and chemical compounds discovered as a result of basic research into the molecular mechanisms of pathogenesis and biological control, that have led to the development of "biorational" pesticides.

Research into the mechanism by which plants resist bacterial pathogens (Hutchinson, 1998) led to the discovery of harpin, a protein that is now being used to activate crop defenses prior to pathogen attack. Indeed, a variety of pathogenic and non-pathogenic microorganisms can induce plant defenses and may be useful as biocontrol agents (Van Loon *et al.*, 1998).

Other research on the effects of organic amendments suggests that both chemical and biological components of compost-amended soils can contribute to disease suppression (Abbasi *et al.*, 2002; Bulluck and Ristaino, 2002; Zhang *et al.*, 1998). Lazarovits (2001) concluded that organic amendments containing high nitrogen, such as poultry manure, meat and bone meal, and soymeal, significantly reduced population of a wide spectrum of soil-borne plant pathogens.

Recent studies have been made on the role of natural compounds in defense mechanisms against pathogen attack. Fenor (F-760) is a growth regulator of plant, synthesized chemically. It suppressed a variety of root rot disease caused by soil-borne pathogens (Schkalikov *et al.*, 1994; Hashem and Hamada, 2002). It has been also shown that Strom, a derivative of blood proteins, induced root rot tolerance in spring wheat (Schkalikov, 1995; 1996; Alzoma, 1997). Therefor the purpose of this work is to study the efficacy of F-760 and Strom on resistance of root rot as well as their suppressive role of the main causal pathogens of this disease *in vitro* and *in vivo*.

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Materials and Methods

Fenor (F-760) is a quaternary ammonium salt (4-aminobenzoate-2-hydroxypropyl, triethylammonia). It is a growth regulator of plant, synthesized chemically. The recommended dose is 0.14 kg in 10 liters of water per 1 ton of seeds for controlling root rot disease (Schkalikov *et al.*, 1994).

Strom (natural organic matter) is a derivative of blood proteins of fibrinogen, albumin, and globulin with a sodium cellulose glycolic acid. The recommended dose of this matter is 0.2~0.25 kg in 10 liters of water per 1 ton of seeds for controlling root rot disease (Schkalikov *et al.*, 1994).

Tested Fungi. The main causal agents of wheat root rot *via Fusarium graminearum*, *F. oxysporum*, *F. solani* and *Bipolaris sorokiniana* which were isolated from diseased

plants were used in this study.

***In vitro* tests.** From each fungal species, mycelial disc (5-mm in diameter) of 4 days old culture was inoculated onto PDA medium containing the recommended dose of the compounds, separately in Petri dishes and incubated at 28±1°C for 7 days. Then the radial growth of these organisms was estimated. In another experiment these fungi were inoculated (1 m*l* of homogenous spore suspension per conical) into 250 m*l*-conical flasks containing 50 m*l* of PD broth amended with the recommended dose of the compounds, separately and incubated at 28±1°C. The dry weight of mycelia was estimated as an indicator for the vegetative growth 10 days after incubation. All treatments were in 3 replicates and repeated twice.

Pot experiments. Each fungal species was grown on barley grain medium in 500 m*l*-conical flask for 15 days. Inoculum of each pathogen was mixed thoroughly with clay soil at the rate 3% (w/w) and placed in pots (20-cm in diameter). Pots were watered for 7 days before sowing. Wheat grains (cv. Giza 164) were treated with suspension of either 1.4% (w/v) of F-760 or 2.5% (w/v) of Strom before sowing. Ten treated grains were sown in pots which were infested with the pathogens. Also treated grains were sown in another groups of pots without addition of pathogens. Non-treated grains were planted in non-infested pots to serve as a control. All treatments were in 3 replicates and arranged in complete randomized design.

Measurements. After 2 weeks from planting, the percent of survival plants were estimated. From 30-days old seedlings, 5 plants of each treatment were up-rooted and examined for detection the mean disease rating (MDR) and the distribution of disease (% D) as the following:

Mean disease rating (MDR):
MDR = Σ (ab)/n, where
Σ (ab) is the total number of plants.
a is the degree of disease.
b is the number of plants has the same degree of disease.
n is the total number of the diseased plants.

To detect the different degrees of disease, plants were classified into 5 categories: 0 = healthy plants; 1 = root just yellow or discoloration less than 10%; 2 = discoloration (11~25%); 3 = discoloration (26~50%) and 4 = discoloration (51~100%).

Percent of disease distribution (% D) was calculated as:
% D = n/N×100 where
n is the number of diseased plants and N is the total number of plants (diseased and healthy).

At the flowering stage, the remaining plants were collected and examined to detect the MDR and % D.

To study the water maintenance capacity of plants in treatments, the plants were up-rooted and weighed immediately for determination of the fresh weight. Plants were subjected to homogenous temperature 37~40°C and weighed every 2 hours. The following formula was applied to determine the water lost percentage (% WL).

$\%\ WL = (W_o - Wt) \times 100/W_o$, where
W_o is the fresh weight of plants at 0 hours.
W_t is the fresh weight of plants at desired time (2, 4, 6, 8, 10 and 24 hours).

At the end of the experiments, plants were dried in an aerated oven at 70°C until constant dry mass for estimation of the dry weight.

Data obtained in the above mentioned tests were analyzed statistically, and the means were compared using LSD test (Mead and Curnow, 1983).

Cell membrane stability was determined according to the method of Blum and Ebercon (1981). Leaf discs (number of 10, each 1cm diameter) were rinsed three times with deionized water and placed in 30 cm^3 deionized distilled water for 24 h at 10°C. The electrical conductivity (conductimeter, YSI Model 35 Yellow Springs, OH, USA) of the bathing solution was measured at 25°C. Following the measurements, leaf discs were autoclaved for 15 min, cooled to 25°C, and the electrical conductivity of the bathing solution was measured for the second time. The degree of injury was calculated according to the equation:

$$\text{Percentage injury } (\%) = 1 - (T_1/T_2)(1 - C_1/C_2).\ 100$$

Where T_1 and T_2 are the first (before autoclaving) and second (after autoclaving) conductivity measurements of the treatment, respectively, C_1 and C_2 are the first and second conductivity measurements of the control. The flame photometric method (Williams and Twine, 1960) using Carl Zeiss flame photometer was used for the determination of potassium. The leakage of UV absorbing metabolites according to Navari-Izzo *et al.* (1989) was also used as a criterion for evaluating cell membrane integrity of leaf discs. The data were then expressed as A_{280} (10 cm^3)/g fresh matter after 24 h leakage in distilled water. The contents of chlorophylls a, b and carotenoids were determined spectrophotometrically (Metzner *et al.*, 1965).

Results

In vitro test results, F-760 had a higher fungicidal effect than Strom on all investigated organisms. F-760 inhibited the radial growth of *F. oxysporum* to 61.1% and *B. sorokiniana* to 51.17% of the controls (Fig. 1). Strom showed an inhibitory effect which fluctuated between 15.89% in case of *F. graminearum* and 31.11% with regard to *F. solani*.

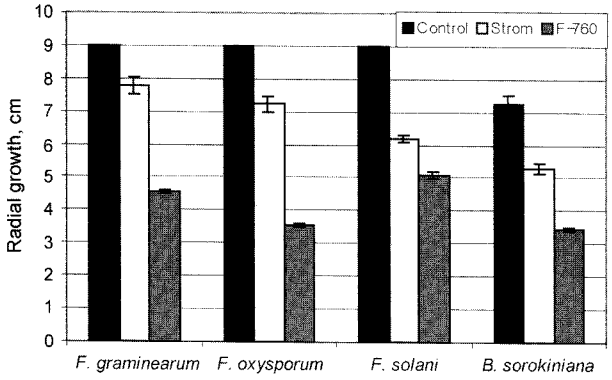

Fig. 1. Effect of Strom and F-760 used the recommended dose on radial growth of mycelia of the tested fungi.

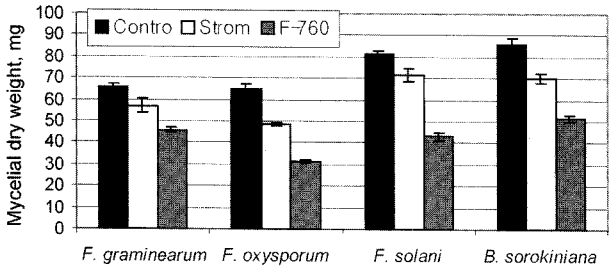

Fig. 2. Effect of Strom and F-760 used the recommended dose on mycelial dry weight of the tested fungi.

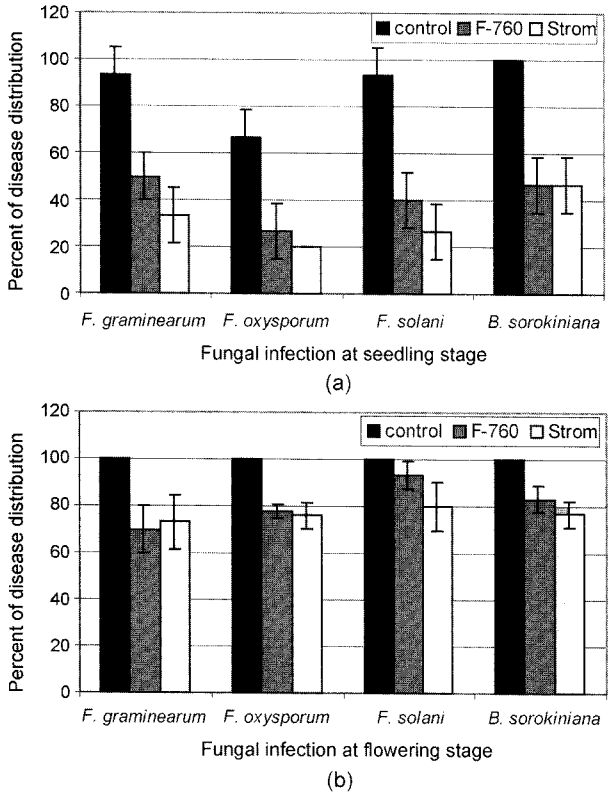

Fig. 3. Effect of treatment with Strom and F-760 on the percent of disease distribution (% D) of wheat plants infected with the main casual pathogens of root rot (a) in seedling stage and (b) in flowering stage.

The mycelial growth of the tested fungi was also inhibited significantly as a result of application of both Strom and F-760. The highest reduction of mycelial dry weight was obtained in case of *F. oxysporum* (51.36% of control) when F-760 was applied while, *F. graminearum* showed the lowest inhibition (30.07% of control). Strom inhibited the organisms in relatively low level which ranged between 11.40% and 25.17% of controls, (Fig. 2).

Treatments of wheat grains with both biological active compounds (Strom and F-760) before cultivation significantly reduced the percent of disease distribution in seedling and flowering stages (Fig. 3a and b) in soil infested with the tested causal agents of root rot of wheat. The highest reduction in percent of disease distribution in seedling stage was achieved as a results of treatment of seeds with Strom in case of *F. oxysporum* (20%) and *F. solani* (26.70%). F-760 occupied the second order in this respect which reduced the disease percent to 26.7% in case of *F. oxysporum* and to 40% in case of *F. solani* in seedling stage.

The MDR of plants was reduced significantly as a result of application of the tested compounds (Strom and F-760) comparing with the control. In control treatment (with the pathogen only) MDR ranged from 1.33 in case

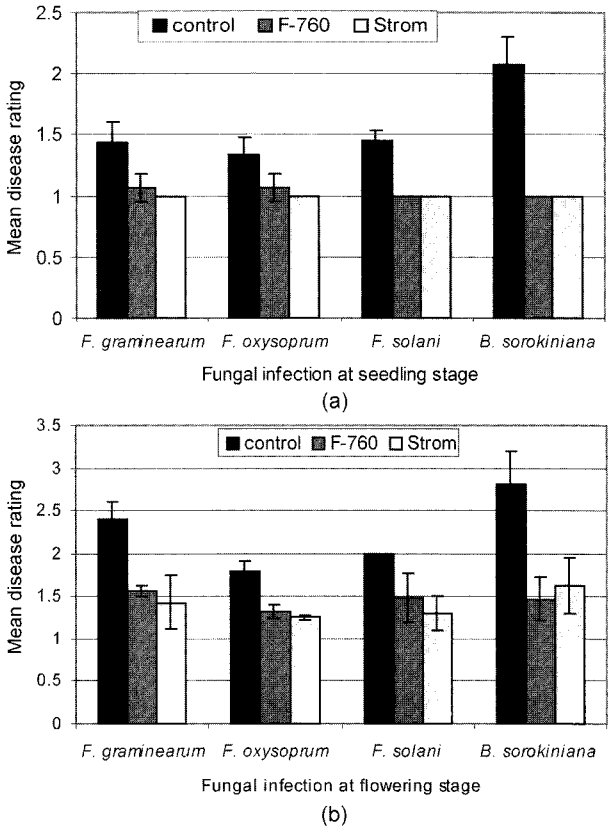

Fig. 4. Effect of treatment with Strom and F-760 on the mean disease rating (MDR) of wheat plants infected with the main casual pathogens of root rot (a) in seedling stage, (b) in flowering stage.

of *F. oxysporum* and 2.8 in case of *B. sorokiniana* in seedling stage. Treatments when biological active compounds were applied, it was reduced to 1.0 or 1.06 in seedling stage (Fig. 4a). In flowering stage it was relatively higher even in control treatment (1.8~2.8). Application of Strom or F-760 reduced it to 1.26 or 1.32 respectively in case of *F. oxysporum* as a highest rate of reduction (Fig. 4b).

Generally, MDR in seedling stage was less than that obtained in the flowering stage even in control treatment.

Also fresh and dry weights and the water lost (water maintenance capacity) of the tested plants during 24 h. were measured as indicators of effect Strom and F-760.

Data represented in Table (1 and 2) revealed the positive effect of these treatments on the fresh weight of wheat plants especially in seedling stage. All treatments increased the fresh and dry weight of plants in seedling and flowering stages except in three cases. In seedling stage, dry weight of plants infected with *F. graminearum* and *B. sorokiniana* did not affect significantly. Also fresh weight of plants under treatment with *F. solani* in flowering stage has less effect. Generally, Strom stimulated the increase of fresh and dry weight of seedling of wheat, but in flowering stage this effect was fluctuated between Strom and F- 760.

All matters applied in this research significantly stimulated the capacity of plants to maintain water and reduced

Table 1. Effect of Strom and F-760 on percent of water lost and fresh and dry weights of plants infected with the main root rot pathogens in seedling stage

Treatments	Water lost (%) related to fresh weight after intervals in hours						Weights in g/plant	
	2	4	6	8	10	24	fresh	dry
Control	8.68	17.65	23.58	32.10	41.21	64.02	1.40	0.24
Strom	6.94	12.78	18.96	28.17	35.06	63.33	1.47	0.25
F-760	5.75	12.68	16.64	26.72	36.66	65.43	1.48	0.25
Fusarium graminearum	9.58	17.93	24.77	31.18	41.27	68.58	1.05	0.18
F. graminearum + Strom	7.62	12.27	18.71	29.48	39.40	71.44	1.31	0.17
F. graminearum + F-760	6.44	12.25	19.99	27.83	38.49	69.94	1.24	0.17
F. oxysporum	12.03	20.19	31.74	41.87	50.86	71.09	1.11	0.19
F. oxysporum + Strom	10.06	18.47	28.90	37.47	46.37	75.29	1.35	0.22
F. oxysporum + F-760	9.34	17.45	26.57	35.37	45.64	71.74	1.26	0.22
F. solani	11.16	20.44	27.69	35.60	42.22	70.77	1.03	0.20
F. solani + Strom	9.66	17.44	24.91	33.18	41.83	65.38	1.65	0.25
F. solani + F-760	7.10	16.23	23.21	31.48	37.17	62.92	1.38	0.21
Bipolaris sorokiniana	12.76	20.64	29.84	38.47	42.85	71.36	1.25	0.20
B. sorokiniana + Strom	9.14	17.00	24.22	35.25	41.21	71.79	1.50	0.21
B. sorokiniana + F-760	10.67	17.30	25.52	35.51	40.18	71.28	1.42	0.20
LSD (P = 0.05)	0.881	1.383	1.999	1.483	2.033	2.895	0.0931	0.0163

Table 2. Effect of Strom and F-760 on percent of water lost and fresh and dry weights of plants infected with the main root rot pathogens in flowering stage

Treatments	Water lost % related to fresh weight after intervals in hours						Weights in g/plant	
	2	4	6	8	10	24	fresh	dry
Control	6.93	11.91	17.17	20.94	26.57	37.46	1.85	0.91
Strom	5.40	10.45	15.94	19.44	22.30	30.53	1.92	0.95
F-760	5.80	9.87	12.87	17.05	21.64	29.13	1.87	0.92
Fusarium graminearum	7.03	10.11	14.12	18.16	20.52	30.53	1.15	0.68
F. graminearum + Strom	5.69	8.89	11.52	16.13	19.31	25.80	1.55	0.84
F. graminearum + F-760	4.72	5.94	9.17	14.88	17.75	25.60	1.40	0.81
F. oxysporum	7.88	11.13	14.37	18.04	21.88	29.22	1.66	0.81
F. oxysporum + Strom	5.26	7.66	10.32	17.08	18.53	28.13	1.85	0.92
F. oxysporum + F-760	6.05	7.99	10.76	15.47	18.84	26.60	1.89	0.95
F. solani	8.08	10.45	13.02	14.92	18.99	27.02	1.80	0.83
F. solani + Strom	5.75	8.77	11.95	15.10	18.37	26.94	1.87	0.93
F. solani + F-760	6.23	8.79	11.14	13.68	18.18	25.87	1.97	0.91
Bipolaris sorokiniana	8.04	12.16	15.78	19.24	24.04	33.15	1.48	0.85
B. sorokiniana + Strom	5.40	8.13	10.82	14.38	20.64	29.97	1.99	0.96
B. sorokiniana + F-760	5.93	8.13	11.55	15.40	20.75	26.25	1.97	0.97
LSD (P = 0.05)	0.611	0.804	0.962	1.410	1.885	1.782	0.0788	0.0375

Table 3. Effect of application of Strom and F-760 on the membrane stability, K^+ efflux, UV absorbing metabolites and pigments contents (chlorophyll a, chlorophyll b and carotenoids) of plants infected with the main root rot pathogens

Treatments	Membrane stability	K^+ efflux mg/g (d. m.)	U.V absorbance (%)	Pigments, mg/g (d.m.)		
				Chl.a	Chl.b	Carotenoids
Untreated plants	100.00	0.498	0.0	7.827	4.917	3.657
Strom	90.50	0.752	13.76	6.532	4.112	2.995
F-760	93.32	0.671	11.88	8.051	5.071	3.792
Fusarium graminearum	44.08	5.881	55.51	2.746	0.941	1.775
F. graminearum + Strom	68.50	1.535	36.83	4.951	0.792	2.891
F. graminearum + F-760	87.52	1.595	42.15	5.163	3.517	1.937
F. oxysporum	60.40	2.029	49.11	2.394	0.875	1.522
F. oxysporum + Strom	79.48	1.048	30.59	3.483	3.784	0.746
F. oxysporum + F-760	89.41	1.070	29.22	3.541	2.653	1.231
F. solani	60.88	2.778	55.29	1.485	0.607	0.965
F. solani + Strom	74.37	1.263	34.27	3.288	1.507	1.449
F. solani + F-760	76.28	1.392	36.67	2.756	1.526	1.205
Bipolaris sorokiniana	62.00	2.926	41.50	2.467	1.044	1.159
B. sorokiniana + Strom	77.07	1.455	35.92	4.424	4.281	0.810
B. sorokiniana + F-760	74.32	1.487	20.44	3.215	3.433	0.714
LSD (P = 0.05)	8.078	0.552	8.146	1.216	0.685	0.553

the rate of water lost until 10 hours after uprooting the plants under temperature 37~40°C. At 24 hour, the reduction of water lost was not continuos. In seedling stage F-760 reduced the lost of water during the all experiment time more than Strom in case of *F. oxysporum* and *F. solani*. F-760 occupied the first order in reduction the water lost around all the incubation period in case of *F. oxysporum*, *F. solani* and *F. graminearum* in seedling and flowering stages. On the other hand, plants infested with *B. sorokiniana* were affected by Strom more than other compound to maintain water capacity.

The data presented in Table 3 showed that infection had an inhibitory effect on membrane stability. Furthermore, the data herein obtained clearly demonstrate the effectiveness of F-760 and Strom in alleviating partially the depressive effect of infection on membrane stability of the tested plants.

With respect to the K^+ efflux and the leakage of UV-absorbing metabolites, the results presented in Table 3 revealed that the K^+ efflux and the leakage of UV-absorbing metabolites was stimulated with fungi infection. However F-760 and Storm treatment partially retarded the stimulatory effect on leakage of K^+ and UV-absorbing metabolites of fungi infected plants.

The data herein obtained Table 3 clearly demonstrated that the fungal infection had inhibitory effects on pigment fractions (chlorophyll a, b, and carotenoids) biosynthesis in wheat leaves. This retarding effect of fungal infection was partially or completely alleviated as the grains were treated with the applied compounds (F-760 and Strom).

Discussion

Application of biologically active compounds (Strom and F-760) significantly suppressed the radial growth and the mycelial dry weight of the main wheat root rot causing organisms (*F. graminearum*, *F. oxysporum*, *F. solani* and *B. sorokiniana*) *in vitro*. F-760 has a relatively higher fungicidal effect than Strom on all investigated organisms. *Fusarium oxysporum* was the most sensitive one for the two compounds. This finding was corroborated by the results of Schkalikov *et al.* (1994), Al-Afandi (1995) and Alzoma (1997). They investigated the efficacy of these compounds against the root rot of different Russian strains of wheat and their causal pathogens. They reported that Strom and F-760 are effective compounds against the wheat root rot disease. In this respect Hawke (1994) found that application of blood meal to a sandy soil resulted in the death of microsclerotia of *Verticillium dahliae*.

In greenhouse experiments, the collected data indicated the efficiency of Strom and F-760 in reduction of root rot disease of wheat plants in seedling and flowering stages. Mean disease rating and the percent of disease distibution were significantly reduced as a result of application of these biologically active compounds as seed dressing before planting. *F. oxysporum* was affected in the highest level among the tested organisms. Generally, MDR in seedling stage was less than that obtained in the flowering stage even in control treatment. These results were confirmed by findings of many researchers (Schkalikov, 1995; 1996; Schkalikov and Schekhovtcova, 1994; Schkalikov *et al.*, 1994; Al-Afandi, 1995; Alzoma, 1997; Hashem *et al.*, 2000). All of these researchers suggested that the main role of these compounds is through their induction of the resistance system of plants against the casual pathogens of root rot as well as the stimulation of plant growth.

Water maintenance capacity of wheat plants were significantly improved as the result of application of the target compounds either in seedling or flowering stage. This resulted in reduction of the rate of water lost until 10 hours after uprooting of plants. Fresh and dry weights of investigated plants were also increased as a result of application of these compounds. In another work, we found that application of these compounds as seed treatment significantly reduced the rate of water lost of wheat plants until 24 hours in all stages of growth as well as improvement of fresh and dry weights under field conditions (Hashem and Hamada, 2002). Results of Maiceeva (1999) has proved the ability of both Strom and F-760 to increase the water maintenance capacity for 4 hours after uprooting of wheat plants. She pointed out the increase of water maintenance capacity of plants results in induction of surviving them against dryness and improves the physiological processes. This leads in general to improve the health of plants which makes them more resistant against facultative parasites which attack the weak plants in most cases. In other words, The best and acceptable interpretation of the effect of these compounds against plant pathogens is that they induce the resistance of plants against pathogens and stimulate plant growth which recover any disturbance produced from the plant pathogens (Nickell, 1983; Vidhyasekaran, 1990). Also Lazarovits (2001) reported that pathogen control by application of organic compounds was showen to arise from the ammonia and (or) organic acid generated, the concentrations of which are controlled by pH, organic matter content, soil buffering capacity, and nitrification rate.

Membrane stability of the investigated plants was severely deteriorated by fungal infection. The stressed plants exhibited significantly low values of percentage which is based on a relationship between cellular constituents and the fraction which leaked out. These results could explain the findings of Ashor *et al.* (1993) who investigated the effect of *F. solani* nophthazarin toxins dihydrofusarubin and isomarticin on the cytology and ultrastructure of rough lemon seedlings. They concluded that the initial toxic effects of the fungal phytotoxins alone or in combination are on organellar membranes, primarily those of the chloroplasts, plasmalemma and tonoplast. On the other hand, fungal infection showed a higher electrolyte leakage and potassium efflux than the control due to loss of semipermeablility properties and membrane integrity. Such leakage of ionic solutes and potassium efflux (Palta *et al.*, 1977) as well as leakage of cellular metabolites (Navari-Izzo *et al.*, 1993) are frequently used to asses membrane integrity. In this respect, Nemec (1995) concluded that fungal phytotoxins enhanced membrane permeability. F-760 or Strom ameliorated the phytotoxic effect due to fungal infection and the membrane became more stabilized and exhibited higher values of percentage. The soaking of grains in F-760 or Strom could enhance fungal infection tolerance of several crop species (Schkalikov *et al.*, 1994; Schkalikov, 1995). They suggested that these compounds induce the resistance system against pathogens and stimulate growth of plants.

The contents of photosynthetic pigments (chlorophyll a, chlorophyll b or carotenoids) were variably affected by the different fungal infection. The results clearly show that fungal infection decreased content of photosynthetic pigments. These results are agreement with Albrecht *et al.* (1998) who showed that the *F. solani* toxin dihydrofusarubin caused degradation of leaf pigments close to the veins of tobacco leaves treated with the toxin in the light, thus indicating that dihydrofusarubin acts as a non-host specific toxin. Also, they demonstrated that dihydrofusrubin interacted with the electron transport chain of illuminated spinach chloroplasts, resulting in the formation of superoxide radicals generated by photosynthesis I.

The effectiveness of the applied compounds varied with the applied fungi. F-760 or Strom treatment exerted a stimulatory action on pigment biosynthesis. The exact relations between the ameliorating effects of F-760 or Strom on retarding effects of fungal infection stress and pigment biosynthesis can even be a more metabolic adaptation to infection stress *via* activated or regenerated key enzymes, a prospect which deserves further consideration.

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