Induction of resistance to root rot disease of wheat grown under field condition

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ABSTRACT

Four compounds namely Fenor (F-760), Strom, salicylic acid (SA) and thiamin (B_1) were tested against root rot disease of wheat under field condition. Wheat grains were soaked in these compounds for 6 h before sowing. Mean disease rating, disease appearance, and distribution of disease were estimated as parameters of disease severity. All tested compounds significantly reduced the root rot of wheat severity during seedling, flowering and ripening stages. Fresh and dry weights were also affected by application of these compounds. Water maintenance capacity in all stages was increased as a result of seed treatments by the above-mentioned compounds. Crop yield and parameters of spikes and grains were significantly improved. These results were discussed and analyzed statistically using *LSD* test.

Keywords: biological control; disease resistance; Fenor; Strom; salicylic acid; thiamin; root rot; wheat

Diseases caused by soil-borne pathogens are responsible for significant economic losses in important crops worldwide (Fraser et al. 1995). Root rot of wheat is considered a serious problem in some regions and causes high reduction in yield (Peining et al. 1976).

The current objective worldwide is to decrease pesticide use. There is a need to develop strategies for disease management in crops that reduce our reliance on agrochemicals, produce fewer residues, and preserve the host resistance in the long term.

Recent studies have been made of the role of natural chemical compounds in defense mechanisms against pathogen attack. Fenor (F-760) is a growth regulator of plant, synthesized by a chemical way. It suppresses a variety of root diseases caused by soilborne pathogens (Schkalikov et al. 1994). It has been also shown that Strom, a derivative of blood proteins, induced root rot tolerance in spring wheat (Schkalikov 1995). Thiamin is a vitamin (B_1) produced by plants that acts as a coenzyme in many physiological processes in plants (Jain 1986). Application of thiamin effectively controls nematode infection of eggplants (Hamada et al. 2001). Salicylic acid (SA) is known to be a signal molecule in acquired resistance to pathogens in several species (Spletzer and Enyedi 1999). It can be used to induce both the synthesis of certain pathogenesis-related (PR) proteins and resistance to pathogen (Spletzer and Enyedi 1999).

Therefore, the present work was undertaken to investigate safe and easy method for the reduction of root rot disease severity as well as the improvement of crop yield and physiological characters of wheat plant by using natural and non-toxic compounds. Fenor, Strom, salicylic acid and thiamin are characterized by simple production, safety for organisms and do not cause any disturbance in the biological balance in nature. These characters give them high value in practice.

MATERIAL AND METHODS

Wheat variety Giza 64 was used in these experiments. It represents one of the famous cultivated wheat in Assiut area, where this research was done. Wheat grains were treated with four compounds separately and each group treated with desired compound was cultivated in designed areas about 2 m^2 in three replicates. In control treatment, grains were treated with distilled water.

The four applied compounds in this work were:

Fenor (F-760) is a quaternary ammonium salt (4-amino-benzoate-2-hydroxypropyl, triethylammonia). The recommended dose is 0.14 g in 10 ml water per 1 kg of seeds (Schkalikov et al. 1994).

Strom is a derivative of blood proteins of fibrinogen, albumin, and globulin with a sodium cellulose glycolic acid. The recommended dose of this compound is 0.2–0.25 g in 10 ml of water per 1kg seeds (Schkalikov et al. 1994).

Thiamin: wheat grains were soaked in 0.3 mM of it before sowing for 6 h.

Salicylic acid: wheat grains were soaked in 0.6 mM of it (in the form of sodium salicylate) before sowing for 6 h.

To detect the root rot disease severity, from each treatment 50 plants were selected randomly three times during the growing season, (seedling, flowering and ripening stages). Mean disease rating (MDR), disease appearance (DA%), and percentage of disease distribution (D%) were determined to indicate the severity of disease.

MDR was calculated according this equation:

$MDR = \Sigma(ab)/a$

where: $\Sigma(ab)$ – the sum of plants
a – the degree of disease
b – the number of plants, which has the same degree of disease
n – is the total number of 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%, 4 = discoloration 51–100%.

Distribution of disease (D%) was detected as:

$D\% = (n/N).100$

where: n – the number of diseased plants

N – the total number of plants (diseased and healthy)

Disease appearance DA% was:

$DA\% = \Sigma(ab).100/NK$

where: $\Sigma(ab)$ is the sum of diseased plant.

N – the general count of both healthy and diseased plants

K – the highest degree of disease

To study the water maintenance capacity of plants in treatments, the uprooted plants were weighed immediately to determine the fresh weight. Plants were subjected to homogenous temperature 37–40°C and weighed every 2 h. The following formula was applied to determine the water lost percentage (%WL):

$\%WL = (W_o - W_t).100/W_o$

where: W_o – the fresh weight of plants at 0 h

W_t – the fresh weight of plants at desired time (2, 4, 6, …, 24 h)

At the end of the experiments, plants were dried in an aerated oven at 70°C until constant dry mass. At the end of the season, plants were harvested and 100 plants from each replicate were selected randomly and kept for further analysis. Length of spike, number of spikelets in spike, weight of spike, weight of 1000 grains, yield in g/m^2 and the percentage of the increase in yield were estimated.

Data obtained in this work were analyzed statistically, and the means were compared using *LSD* test (Mead and Curnow 1983).

RESULTS

Effect of treatments on MDR was demonstrated in Figure 1, which showed that MDR in control was significantly higher than in the other treatments. It was noticed that, MDR gradually increased during the season from seedling to ripening stage. It reached its minimum value in case of SA in seedling stage (1.16) followed by B_1 (1.31) and F-760 (1.34). Whereas, in flowering stage, F-760 and SA caused the same reduction in MDR (1.33). In ripening stage, B_1 reduced MDR in the highest value (1.33).

The data presented in Figure 2 show that reduction in D% due to application of the compounds in seedling and in flowering stages was nearly similar and fluctuated between 54.67–63.27%, but in ripening stage, D% was relatively high (75.66–86.0%). On the other hand, D% in control was very high (80.0–95.0%) at the end of the growing season. Generally, application of these compounds significantly reduced the percentage of disease distribution.

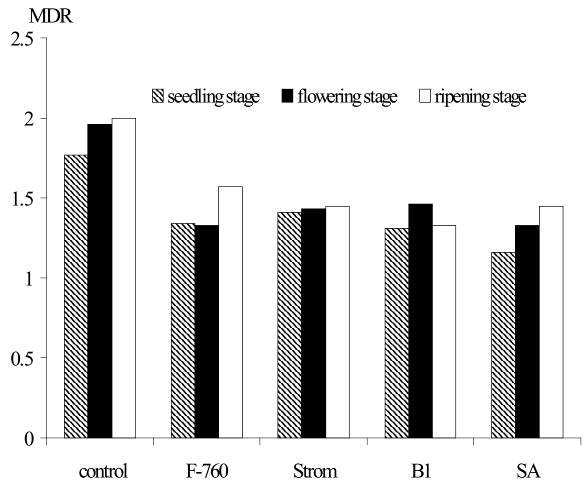

Figure 1. Effect of F-760, Strom, B_1 and SA on mean disease rating of wheat plants

Disease appearance was more reduced by Strom and B_1 than other applications. Generally, all application reduced DA in significant level when compared with control. In seedling stage, it was 27.31% in case of Strom and 27.86% in case of B_1. In flowering stage the lowest value of DA was obtained when Strom was applied (27.11%) followed by SA (33.67%). In ripening stage, DA grew in treatments to reach 60.33% in case of F-760. But in control, DA was higher than other all treatments and fluctuated between 53.54% in seedling and 72.0% in the end of the growing season (Figure 3).

Fresh weight of treated plants was increased significantly when compared with the control in seedling, flowering and ripening stages (Table 1). SA was responsible for the highest increase in fresh weight in seedling stage and gave 0.85 g/plant. In flowering and ripening stages, the highest increase was due to application of B_1 (6.6 and

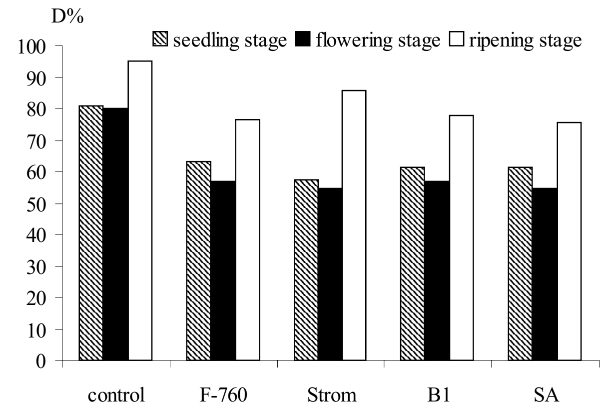

Figure 2. Effect of F-760, Strom, B_1 and SA on distribution of disease percent (D%) in wheat plants

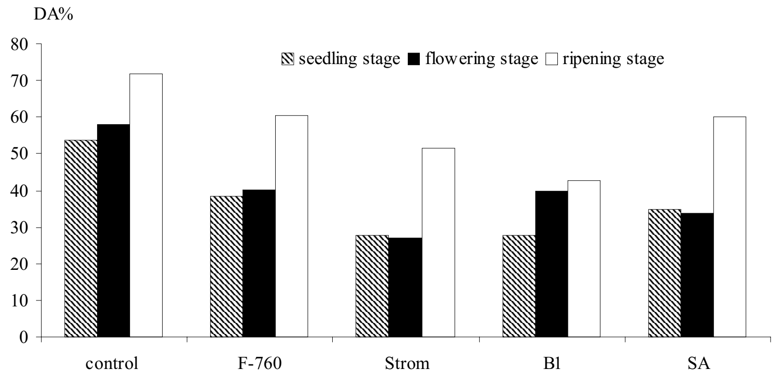

Figure 3. Effect of F-760, Strom, B_1 and SA on disease appearance percent (DA%) in wheat plants

6.88 g/plant respectively). The lowest increase in fresh weight was achieved with F-760 during the three periods.

Dry weight of treated plants was affected insignificantly in seedling and flowering stages and significantly in ripening stage. The highest value of dry weight was obtained when plants were treated by Strom (2.99 g/plant) followed by B_1 (2.69 g/plant).

Effect of treatments of plants with the used compounds on the water maintenance was expressed as percentage of water lost capacity in Table 2. Data showed that all compounds reduced the water lost capacity of plants in significant degree in seedling and flowering stages. However, in ripening stage water lost capacity was not affected significantly after 6 and 24 h.

In seedling stage, Strom seemed to be the most effective compound and reduced the water lost capacity from 38.22% at 6 h to 18.31% at 24 h of control. F-760 occupied the second order in this respect, especially at 6 and 8 h.

In flowering stage, the highest reduction in water lost capacity was distributed among the four tested compounds and fluctuated between 55.19% of control at 2 h by F-760 and 19.75% at 24 h by SA. SA appeared as the highest reducing agent in water lost capacity in ripening stage especially at 4–8 h.

The result in Table 3 showed that all tested compounds improved the crop yield as well as the characters of grains. Strom increased the number of spikelets in spike (19.56%), weight of spike (2.48 g) and weight of 1000 grains (40.56 g) in significant level compared with the control. However, the highest increase in length of spike was achieved by application of SA. All compounds caused increase in crop yield, but F-760 occupied the first order in this parameter.

DISCUSSION

Data obtained from this study demonstrate that all treatments (F-760, Strom, B_1 or SA) have a significant reductive effect on MDR, D% and DA% of root rot. SA caused minimum degree of MDR in seedling stage (1.16). The lowest value of D% was achieved by application of Strom (57.36%) in seedling stage. Disease appearance (DA%) was also affected by Strom and reached to its minimum value (27.31%) in seedling stage. In other periods, the three parameters were also affected significantly compared with control. Generally, the effect of compounds on the three measured parameters was slightly decreased from seedling to ripening stage. Russian researchers studied reduction of root rot disease of many plants by F-760 and Strom and their results confirmed our data (Schkalikov et al. 1994, Schkalikov 1995). They suggested that these compounds induce the immunity system against pathogens and stimulate growth of plant. The recent studies indicate that plant treatment with some vitamins results in pathogen resistance. In this context, foliar application of riboflavin (B_2) effectively controls several diseases of tobacco (Dong et al. 1995), and it reduces powdery mildew of strawberry plants in combination with methionine, metal ions, and surfactant (Wang and Tzeng 1998). In addition, ascorbic acid (C), thiamin (B_1) and pyridoxine (B_6) treatments were effec-

Table 1. Effect of F-760, Strom, B_1 and SA on fresh and dry weight of wheat plants in different growing stages (g/plant)

Treatments	Seedling stage		Flowering stage		Ripening stage	
	fresh weight	dry weight	fresh weight	dry weight	fresh weight	dry weight
Control	0.60	0.073	4.8	1.05	4.83	1.83
F-760	0.67	0.083	5.27	0.96	5.55	2.11
Strom	0.72	0.083	6.54	1.04	6.56	2.79
B_1	0.73	0.077	6.6	1.03	6.88	2.62
SA	0.85	0.087	6.55	1.07	6.85	2.59
$LSD_{0.05}$	0.601	N.S	0.401	N.S	0.449	0.259

Table 2. Effect of F-760, Strom, B_1 and SA on maintenance of water of wheat plants under field conditions

Treatments	Percentage of water lost related to fresh weight at 37–40°C after intervals in hours					
	2	4	6	8	10	24
Seedling stage						
Control	19.82	24.19	37.18	45.34	51.35	72.73
F-760	13.53	18.61	20.86	28.81	36.08	64.21
Strom	13.29	18.35	22.97	29.28	35.81	59.41
B_1	15.76	23.05	30.29	37.08	43.11	69.05
SA	15.95	24.47	31.32	39.02	43.46	59.77
$LSD_{0.05}$	2.24	2.79	3.16	3.16	3.53	5.75
Flowering stage						
Control	2.7	3.65	6.45	7.85	9.26	14.48
F-760	1.21	3.19	5.56	7.10	9.23	13.03
Strom	1.78	2.61	4.87	6.13	8.16	12.84
B_1	1.57	2.64	4.35	6.27	7.87	12.75
SA	2.11	3.62	5.97	6.80	7.47	11.62
$LSD_{0.05}$	0.446	0.734	1.25	0.798	0.754	1.048
Ripening stage						
Control	3.27	4.5	5.29	6.94	8.0	11.46
F-760	2.47	3.88	4.43	7.03	8.17	11.20
Strom	2.27	3.73	4.53	5.93	8.45	11.64
B_1	1.8	3.67	4.45	5.90	7.04	11.59
SA	2.19	3.44	4.30	5.73	8.20	11.43
$LSD_{0.05}$	0.453	0.516	N.S	0.702	0.735	N.S

tive in inhibiting nematode infection of eggplants (Hamada et al. 2001). SA is the only plant-derived compound shown to induce defense response known as systematic acquired resistance (SAR) (Enkerli et al. 1993). It is well established that the induction of SAR and the enhancement of disease resistance result from elevated endogenous tissue levels of SA (Enyedi et al. 1992). Also, Spletzer and Enyedi (1999) concluded that root feeding with 200 μM SA to tomato plants can (i) significantly elevate foliar SA levels (ii) induce pathogenesis-related (PR)-IB gene expression, and (iii) activate SAR that is effective against *Alternaria solani*.

All tested compounds increased fresh weight of plants significantly in the three periods of growing season. SA gave the best result in seedling stage. On the other hand, dry weight was affected significantly in ripening stage only. This may be due to the increase in weight of spike and grains. It is evident that the retentiveness of metabolic balance in plant tissues is reflected in normal growth. Accordingly, growth declines in plants stressed by root rot, may be consequence of the blockage of anyone of the phases leading to synthesis of material upon which the living cells depend for division, growth and maintenance. In this respect, Schkalikov et al. (1994) and Schkalikov (1995) mentioned that the application of F-760 and Strom stimulated the fresh weight of plants as well as the crop yield and spike's qualities. In addition, Hamada and Al-Hakimi (2001) reported that soaking seeds in B_1 or SA increased the fresh and dry weights of shoots and roots of wheat plants. The mitigative effects of grain soaking in F-760, Strom, B_1 or SA on growth of the root rot stressed plants may be one aspect of the role of these

Table 3. Effect of F-760, Strom, B_1 and SA on the crop yield and quality of grains

Treatments	Length of spike	Number of sipkelets in spike	Weight of spikes	Weight of 1000 grains	Yield (g/m^2)	Increase (%)
Control	7.87	15.59	1.58	36.74	433.59	100
F-760	9.75	18.04	2.14	40.22	691.93	159.58
Strom	10.03	18.64	2.48	40.56	655.03	151.07
B_1	9.78	18.31	2.35	39.99	684.70	157.91
SA	10.14	18.43	2.31	39.28	650.57	150.04
$LSD_{0.05}$	0.537	1.240	0.294	1.440	33.019	N.S.

compounds in hairy root growth, which should be considered helpful in water and ion uptake and concomitant increase in growth via cell division and cell enlargement.

Application of compounds increased significantly the water maintenance capacity. This was conspicuous in reduction of the water lost percentage especially in seedling stage. This indicates that these compounds encourage the capability of plant to resist drought (Schkalikov et al. 1994). In the other hand, Rapacz et al. (2000) concluded that resistance of barley and meadow fescue to fungal pathogen (*Bipolaris sorokiniana*) was closely associated with a high water content of leaves.

Length and weight of spike, number of spikelets in spike, weight of 1000 grain and yield (g/m^2) were significantly increased by application of these compounds.

Finally, we can conclude that root rot disease may be generated by the superoxide radical (O_2) in invaded plant tissue that is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase. In this context, Albrecht et al. (1998) demonstrated that the *F. solani* toxin dihydrofusarubin interacted with the electron transport chain of illuminated spinach chloroplasts, resulting in the formation of superoxide radicals generated by photosystem I. Also, they concluded that isomarticin toxins from *Fusarium decemcellulare* Brick reacted with the coenzyme thiamin pyrophosphate, inhibiting anaerobic decarboxylation of pyruvate and oxidative decarboxylation of α-ketoglutarate, and inhibited glutamine synthesis. Whereas, Mednestev et al. (1988) showed that naphthazarin toxins from *Fusarium decemcellulare* Brick served as electron acceptors from redox systems of microflora and reduced oxygen, resulting in the formation of reactive oxygen species. These toxins are also known to enhance membrane permeability, which might be the main reason for the accumulation of stress-related compounds in xylem fluid of blight-diseased citrus (Nemec 1995).

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ABSTRAKT

Indukce rezistence k onemocnění kořenovou hnilobou u pšenice pěstované v polních podmínkách

V polních podmínkách jsme testovali čtyři látky proti onemocnění kořenovou hnilobou u pšenice: Fenor (F-760), Strom, kyselinu salicylovou (SA) a thiamin (B_1). Před kultivací byla zrna pšenice namáčena v těchto látkách po dobu 6 h. Průměrný přírůstek choroby, incidence a rozšíření choroby byly využity jako parametry stanovení intenzity choroby. Všechny testované látky významně redukovaly intenzitu kořenové hniloby po vzejití rostlin a v době kvetení a zrání. Aplikace těchto látek rovněž ovlivnila čerstvou hmotu i sušinu. Výsledkem ošetření osiva uvedenými látkami byla vyšší schopnost zadržovat vodu ve všech růstových fázích. Výnos a výnosové parametry klasů a zrn byly výrazně zlepšeny. Výsledky byly statisticky vyhodnoceny s využitím *LSD* testu.

Klíčová slova: biologická kontrola; rezistence k chorobám; Fenor; Strom; kyselina salicylová; thiamin; kořenová hniloba; pšenice

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