Minufiya J. Agric. Res. Vol.40 No. 5: 1285 – 1303 (2015) "http://www.mujar.net"

EFFECT OF TWO BIOLOGICAL CONTROL AGENTS AND CALCIUM CHLORIDE ON CONTROLLING WHEAT LEAF RUST AND THEIR EFFICACIES ON GRAIN AND FLOUR QUALITIES

Doaa R.M. El-Naggar[(1)], W.M. El-Orabey[(1)], Jehan B. Ali[(2)] and Maisa L. Abd El-Moneim[(3)]

(1) Plant Pathology Res. Inst., ARC, Giza, Egypt
(2) Regional Center for Food and Feed, ARC, Giza, Egypt
(3) Central Lab. of Organic Agric., ARC, Giza, Egypt

(Received: Aug. 11 , 2015)

ABSTRACT: *Leaf rust, caused by Puccinia triticina, has been recognized as the main and widespread disease of wheat in the growing areas of this crop. In the present study, to set up new effective and eco-compatible control methods against the pathogen, the activity of two biological control agents (Bacillus subtilis and Trichoderma harzianum) were tested on two bread wheat cultivars at Al-Nubariya and Kafr El-Hamam Agricultural Research Stations. The two biological control agents were tested alone or in combination with Calciven, as a mineral salt. Greenhouse investigations showed that the most desirable effects on incubation and latent periods, infection type and number of pustules/leaf were improved by treating wheat seedlings with the combinations between biological control agents and Calciven (calcium chloride), with favorability to T. harzianum. Results revealed that Calciven alone or in combination with biological control agents showed the greatest effects on rust severity and area under disease progress curve (AUDPC) values, with a positive impact on yield components. Combination between T. harzianum and Calciven, at Al-Nubariya location, gave the lowest value of rust severity for Gemmiza-7 and Gemmiza-11 with the subsequent lowest value of AUDPC for both studied cultivars. Combination between T. harzianum and Calciven, at Kafr El-Hamam location, gave the highest values of 1000-kernel weight for Gemmiza-7 and Gemmiza-11 with the subsequent highest values of yield/plot for both studied cultivars. The efficiency of the studied treatments on grain and flour characteristics was evaluated on samples which obtained from Kafr El-Haman location. Technological studies were assessed in terms of grain test weight (p/B), percentages of protein content, moisture, ash, flour extraction, wet and dry glutens as well as zeleny (ml), falling number (s). Generally, the studied treatments improved the qualities of wheat grains and flour. The highest percentages of wet and dry glutens were recorded by treating Gemmiza-11 with the combination between T. harzianum and Calciven. The effects of different treatments on the curvilinearity of the relations between rust severity and grain test weight and protein content were performed. The obtained curves indicated that grain test weight as well as grain protein content can be related to rust severity. Relation between rust severity and grain protein content recorded the highest correlation coefficient (0.973), for Gemmiza-7. Collectively and in view of the seriousness of wheat leaf rust, as well as the necessity to improve the quality of wheat, the present study is helpful for further developing integrated biological control approaches for managing leaf rust of wheat.*

Key words: *Wheat, leaf rust, Bacillus subtilis, Trichoderma harzianum, Calciven, grain and flour quality.*

INTRODUCTION

Foliar diseases are among the main factors reducing wheat (*Triticum aestivum* L.) crop yields in many regions around the world (Carreteroa *et al.*, 2011). Wheat leaf rust (*Puccinia triticina* Eriks.) is a serious fungal disease affecting wheat production in large parts of the world (Cawood *et al.*, 2010) causing around 4% mean annual yield reduction (Duveiller *et al.*; 2007).

The main strategy for disease management of wheat rusts depends on early detection of the disease incidence and successful application of effective fungicides on hot spots at the right time with sufficient doses (Karjin, 1996). Several fungicides are effective for disease control, but the future of chemical control is uncertain because of concerns about public health safety from the residue persistence of exposure risks (Moustafa-Mahmoud *et al.*, 1997). Intensive repeated application of fungicides may also cause environmental pollution or development of strains, which could probably be more aggressive if they acquired resistance against the routinely used fungicides (Sallam *et al.*, 2001).

A promising strategy for the management of fungal diseases is based on biological and integrated control schedules which include the application of antagonistic microorganisms (Lima and De Cicco, 2006). Several studies have shown the effectiveness of a large number of plant-beneficial microorganisms, used alone or combined with natural substances, against different plant pathogens are already commercially available (Harman *et al.*, 2004; Haas and Défago, 2005; De Curtis *et al.*, 2007&2012). *Trichoderma* spp. have been known for their abilities to control plant pathogenic fungi. Its mechanisms primarily have included direct effects upon target fungi via competition, mycoparasitism and antibiosis (Abd El-Moity and Shatla, 1981). In addition, these fungi have been shown to directly increase plant growth (Chang *et al.*, 1986). They also have abilities to solubilize plant nutrients and increase plant nutrient uptake (Altomare *et al.*, 1999; Yedidia *et al.*, 2001 and Harman *et al.*, 2004). Moreover, several *Bacillus* spp. including *Bacillus subtilis* have inhibitory effect on a wide range of plant pathogens (Li *et al.*, 2013). *Bacillus* spp. produced at least 66 different antibiotic compounds (Ferreira *et al.*, 1991). Several inorganic salts have been trialed worldwide for their effectiveness to suppress fungal pathogens on a wide range of crops and the majority of published reports indicate a reduction in disease severity (Deliopoulos *et al.*, 2010).Calcium chloride is one of the six identified inorganic chloride salts which have the capacity to suppress fungal disease on a variety of crop species (Williams and Smith, 2001and Melgar *et al.*, 2001).

Traditionally wheat improvement aims at three major aspects viz. disease resistance, grain yield and quality. Several studies identified the wheat quality in grains and flour in terms of grain weight, moisture, protein and ash contents as well as sedimentation of protein (zelney test), falling number and flour extraction. However, there is still ample scope to study its interaction between rust resistance with quality of wheat grains (Oak *et al.*, 2011).

In order to create an approach for a more eco-compatible control of wheat leaf rust as well as the improvement of wheat quality, the main objectives of the present study were to evaluate the effectiveness of *Bacillus subtilis*, *Trichoderma harzianum*, calcium chloride and their combinations in controlling leaf rust and improving grain and flour qualities.

MATERIALS AND METHODS

1. Wheat cultivars

Gemmiza-7 and Gemmiza-11 bread wheat cvs. used in the present study were provided by Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Morroco, *Triticum spelta saharensis* and Thatcher wheat cvs. were provided by Dis. Res. Dept., Plant Pathol.Res. Inst., ARC, Giza, Egypt and were used as a surrounding spreader border, in field experiments.

2. Biological agents and calciven

Two biological agents, *Bacillus subtilis* and *Trichoderma harzianum*, in addition to Calciven (calcium chloride, Merck 15%) were examined against leaf rust disease in wheat. Bioagents were obtained kindly from Central Lab. of Organic Agriculture, ARC, Giza, Egypt. *Bacillus subtilis* was grown on nutrient glucose broth for 48 hours and the bacterial suspension was prepared to contain 30 × 10^6 cfu/ml. *Trichoderma harzianum* was grown in gliotoxin fermentation media developed by Brain and Hemming (1945) for 9 days under complete darkness just to stimulate toxin production (Abd El-Moity and Shatla, 1981) and the fungal suspension was prepared to contain 30×10^6 cfu/ml.

3. Inoculum

Inoculation of the tested wheat cvs., at seedling stage, was carried out using the freshly collected urediospores of *Puccinia triticina* (TTTTR Pathotype). Urediospores were kindly provided by Wheat Dis. Res. Dept., Plant Pathol. Res. Inst., ARC, Giza, Egypt.

4. Experimental design and treatments

Experiments were designed as illustrated in Table (1), including control (untreated) plants.

4.1. Greenhouse experiments

Greenhouse experiments were carried out at the greenhouse of leaf rust (Wheat Dis. Res. Dept., Plant Pathol. Res. Inst., ARC, Giza, Egypt) during 2013/14 growing season. Five seedlings of each cultivar were grown in 7cm diameter plastic pots. Seven days old wheat seedlings were sprayed with three concentrations of *T. harzianum, B. subtilis* and Calciven (at the rate of 1 l/50, 100 and 150 l water) in addition to their combinations, as mentioned in Table (1). Spraying of the different concentrations was done at 24 h before inoculation. Control plants were sprayed with distilled water. Plants were artificially inoculated with urediospores of TTTTR pathotype according to the method of Stakman *et al.* (1962).

Incubation period (number of days between inoculation to the appearance of the first pustule) was measured. Latent period was measured according to (Parlevliet, 1975) by counting the number of visible pustules on marked leaves daily until no more pustules appeared. From these data, time between inoculation and 50% of the pustule just visible was estimated. Number of pustules/leaf (No.p/leaf), number of pustules per unit leaf area cm^2 (2.0×0.5 cm) on the upper side of the leaves were counted as described by (Parlevliet and Kuiper, 1977). Infection types were used according to (Stakmen *et al.* 1962), 0,0;, 1 and 2 (Resistance infection type), 3 and 4 (Susceptible infection type) as shown in Table (2).

Table (1): Abbreviations of different treatments of *Bacillus subtilis, Trichoderma harzianum*,Calciven and their combinations.

Treatment	Construction
Control	Infected plants
T1	Plants sprayed with *Bacillus subtilis* only
T2	Plants sprayed with *Trichoderma harzianum* only
T3	Plants sprayed with Calciven only
T4	Plants sprayed with combination between *Bacillus subtilis* and Calciven
T5	Plants sprayed with combination between *Trichoderma harzianum* and Calciven

Table (2): The infection types of wheat leaf rust reactions adopted by Stakman *et al.* (1962) at seedling stage.

Infection type	Symptoms
0 Low R*	No uredia or other macroscopic sing of infection
0;	No uredia, but hypersensitive necrotic or chlorotic flecks of varying size present
1	Small uredia often surrounded by necrosis
2	Small to medium uredia often surrounded by chlorosis or necrosis
3 High S**	Medium- sized uredia that may be associated with chlorosis or rarely necrosis
4	Large uredia without chlorosis or necrosis

4.2. Field experiments

Field experiments were conducted at Al-Nubariya and Kafr El-Hamam Agricultural Research Stations during 2013/14 growing season. The effects of the high concentration of two bioagents, Calciven and their combinations, as previously mentioned in Table (1), were evaluated on controlling leaf rust on Gemmiza-7 and Gemmiza-11 wheat cvs. Area of experiments were divided into plots (3 × 3.5 m = 10.5 m^2), each plot contained 10 rows and 30 cm between rows. The experiments were planted 15 days after the regular sowing date (the first half of December) to expose the plants to suitable environment of rust incidence and development. Randomized Complete Block design with three replicates was used. Plots were surrounded by a spreader border sown with a mixture of highly susceptible wheat varieties to leaf rust. Artificial inoculation with a mixture of freshly collected urediospores of the most prevalent leaf rust physiologic races (NTTTS, PTTTT, SKPSS, TTTTR and TTSTT) and a talcum powder at a ratio of 1/20 (v/v) was carried out using baby cyclones to assure equal deposition of spores on all spreader plants and generate an epidemic (Tervet and Cassel, 1951).

4.3. Disease assessment

Rust severity was recorded using modified Cobb's scale (Peterson *et al.,* 1948) every 7 days intervals from rust appearance. Also, area under disease progress curve (AUDPC) was assessed for each variety according to the equation adopted by Pandey *et al.* (1989).

$$AUDPC = D\left[\frac{1}{2}(Y_1 + Y_K) + (Y_2 + Y_3 + \ldots Y_{K-1})\right]$$

Where:

D = days between two consecutive recordings (time intervals),

Y_1 + Y_K = sum of the first and the last disease scores and

Y_2 + Y_3 + . . . + Y_{K-1} = sum of all in between scores.

At the harvest stage, yield components in terms of yield/plot (kg) and weight of 1000 kernel (g) were recorded.

4.4. Statistical analysis

Data were statistically analyzed according to Steel and Torrie (1980). Grain protein content and grain test weight were correlated to rust severity using Origin Pro 8.6.0 software Copyright © 1991-2012 OriginLab Corporation.

4.5. Technological studies

All technological studies were carried out at the Wheat and Flour Analysis Lab., Regional Center for Feed and Food, Agric. Res. Center, Giza, Egypt, on samples which obtained from Kafr El-Hamam location. Wheat samples were cleaned mechanically to remove dirt, dockage, imparters and other strange grains by Carter Dockage Tester according to the methods described in USDA (2002). Moisture content percentage was determined by JAC 2100 in Regional Center for Food and Feed AACC (2000). Wheat samples were tempered to 16% moisture and allowed to be rest for 24 hours, and then milled by Laboratory mill CD1 auto Chopin according to the methods described in AACC (2000). The extraction rate of any flour sample was adjusted to recurred rate (72% extraction).

Grain quality assessment

Wheat kernels were subjected to determine test weight pound per bushel according to USDA, (2002). Inframatic 8600 equipment was used to determine percentages of grain protein content dry base, moisture content, ash content. Zelneny Shaker was used to determine sedimentation of protein (Zelney test, ml). Falling Number 1500 Unit was used to determine α-amylase activity (Falling number, s). The aforementioned parameters as well as percentages of flour extraction were determined according to AACC (2000).

Flour quality assessment

Percentages of flour ,moisture content, ash content, wet and dry glutens were determined according to AACC (2000).

RESULTS AND DISCUSSION

Greenhouse experiments:

Data in Table (3) showed the effect of the three different concentrations (1 l/150 l, 1 l/100 l and 1 l/50 l water) of the five constructed treatments on incubation and latent periods, infection type and number of pustules/leaf. Data indicated that the higher concentration of any treatment leads to the most prolonged incubation and latent periods. Furthermore, these high concentrations reduced number of pustules/leaf. The most desirable effects were obtained by treating wheat seedlings with T5 treatment followed by T4 treatment.Gemmiza-11 wheat cv. recorded the most prolonged incubation and latent periods (8.35 and 12.10 day, respectively) when seedlings were treated with the high concentration of T5 treatment, compared with control plants (7.43 and 10.10 day, respectively). The same treatment prolonged the incubation and latent periods of control plants of Gemmiza-7 wheat cv. from 7.11 and 9.50 day, respectively, to 8.11 and 10.90 day, respectively. Generally, significant differences were recorded between cultivars, treatments and the interaction between them, except in incubation period. These effects of *T. harzianum* and *B. subtilis* may be due to that the former can produce a rich mixture of antifungal enzymes which affect the outer layer of urediospores of the pathogen (Harman, 2006) while the later can produce a variety of antibiotics which inhibit plant pathogens (Utkhede, 1984 and Joann *et al.*, 1989) resulting in decreasing the inoculums of the pathogenic spores, infection type and No. of pustules/leaf. Data in Table (3) indicated also that, treating wheat seedlings with Calciven (alone or in combination with bioagents) recorded the lowest values of infection type, for both studied wheat cvs. Data also indicated that, the lowest number of pustules/leaf (4.80) was when Gemmiza-7 wheat cv. sprayed with T5 treatment (at the higher concentration), compared with control (15.33). Meanwhile, it was 9.01 in Gemmeiza-11 wheat cv. when treated with the same treatment and concentration, compared with control treatment (22.66). This might be due to that calcium is responsible for formation of strong cell walls which in turn play a role in disease reduction (El-Gamal *et al.*, 2007). Also, chloride plays several important roles in plants including disease control properties through the osmoregulatory and biochemical functions (Flowers, 1988) and biochemical functions (Homann, 2002). These roles include the serving as key osmotic solute by controlling movement across cell membranes as well as stimulation of important enzymes, such as glutamine-dependent asparagine synthetase (Schnabl & Raschke, 1980 and Rognes, 1980).

Field experiments

Data given in Tables (4 & 5) represents the effect of different bioagents (*T. harzianum* and *B. subtilis*), Calciven and their combinations on leaf rust disease parameters, at two locations (Al-Nubariya and Kafr El-Hamam) during 2013/14 growing season. Data indicated that spraying Calciven alone or in combination with any of the studied bioagents led to the greatest effects on rust severity and AUDPC values. Non significant differences between locations were recorded, for both disease parameters.

At Al-Nubariya location, spraying the studied wheat cvs. with T5 treatment gave identical lowest values of rust severity (3.0) and AUDPC (42.0). T4 treatment gave the subsequent identical impact on rust severity (3.67) and AUDPC (44.33), for both studies wheat cvs.

At Kafr El-Hamam location, spraying Gemmiza-7 and Gemmiza-11 wheat cvs. with the T5 treatment gave the lowest values of rust severity (6.67 and 8.33, respectively) and AUDPC (59.50 and 70.0, respectively). T4 treatment gave the subsequent impact on rust severity (8.33 and 11.67, respectively) and AUDPC (70.0 and 95.50, respectively). These results are in harmony with those obtained by Abd El-Moneim *et al.* (2011) and may be due to the aforementioned antifungal mechanisms as well as the dehydrating effect of $CaCl_2$ solution (Kwang *et al.*, 2003).

Statistical analysis of the obtained rust severity results and AUDPC recorded the significant differences between the applied treatments, alone or interacted with cultivars.

Table (3): Effect of spraying different concentrations of bioagents (*Bacillus subtilis* and *Trichoderma harzianum*), Calciven and their combinations, 24 hours before inoculation of wheat seedlings of Gemmeiza-7 and Gemmeiza-11 with *Puccinia triticina* on the incubation period, latent period, infection type and number of pustules/leaf.

Treatment	Conc.	Incubation period		Latent period		Infection type		No. of pustules/leaf	
		Gem-7	Gem-11	Gem-7	Gem-11	Gem-7	Gem-11	Gem-7	Gem-11
Control (untreated plants)		7.11	7.43	9.50	10.10	4	4	15.33	22.66
T1 *B. subtilis*	1 l/150 l water	7.00	7.50	9.57	10.23	4	4	15.01	20.02
	1 l/100.l water	7.17	7.51	9.64	10.23	4	4	14.92	19.91
	1 l/50 l water	7.33	7.60	9.70	10.40	3	3	14.11	18.66
	Mean	7.17	7.54	9.64	10.29			14.68	19.53
T2 *T. harzianum*	1 l/150 l water	7.20	7.50	9.70	10.30	4	4	13.33	19.10
	1 l/100 l water	7.29	7.51	9.70	10.81	4	4	12.50	18.33
	1 l/50 l water	7.90	8.13	10.30	11.20	3	3	10.90	15.96
	Mean	7.46	7.71	9.90	10.77			12.24	17.80
T3 Calciven	1 l/150 l water	7.53	7.71	10.33	10.64	2	2	10.41	15.57
	1 l/100 l water	7.82	7.87	10.42	10.83	2	2	8.58	12.91
	1 l/50 l water	8.04	8.19	10.70	11.80	1	2	5.06	9.2
	Mean	7.79	7.92	10.48	11.09			8.02	12.56
T4 *B. subtilis* + Calciven	1 l/150 l water	7.66	7.88	10.43	10.56	2	2	10.11	16.98
	1 l/100 l water	7.83	7.96	10.61	10.98	1	2	8.28	12.46
	1 l/50 l water	8.03	8.22	10.73	11.90	1	1	5.03	9.16
	Mean	7.84	8.02	10.59	11.15			7.81	12.87
T5 *T. harzianum* + Claciven	1 l/150 l water	7.53	7.67	10.42	10.98	2	2	9.81	14.01
	1 l/100 l water	7.88	7.93	10.42	11.21	1	2	7.50	12.45
	1 l/50 l water	8.11	8.35	10.90	12.10	1	1	4.80	9.01
	Mean	7.84	7.98	10.58	11.43			7.37	11.82
L.S.D. at 5% of:									
Cultivar (C)		0.10		0.11		---		0.24	
Treatments (T)		0.28		0.31		---		0.68	
C × T		NS		0.44		---		0.97	

Low = 1 liter/150 liter water, medium= 1 liter/100 liter water and high= 1 liter/50 liter water.
NS= Non-significant at P = 0.05

Table (4): Effect of spraying *Bacillus subtilis*, *Trichoderma harzianum* and Calciven or their combinations on rust severity of Gemmiza-7 and Gemmiza-11 wheat cvs., under field conditions of Al-Nubariya and Kafr El-Hamam locations during 2013/14 growing season.

Treatment	Nubariya		Mean	Kafr El-Hamam		Mean	General mean
	Gemmeiza-7	Gemmeiza-11		Gemmeiza-7	Gemmeiza-11		
Control (infected plants)	46.67	33.33	40.00	53.33	43.33	48.33	44.17
T1 (*B. subtilis*)	26.67	16.67	21.67	33.33	36.67	35.00	28.34
T2 (*T. harzianum*)	23.33	10.00	16.67	26.67	16.67	21.67	19.17
T3 (Calciven)	4.33	6.00	5.17	8.66	13.33	11.00	8.08
T4 (*B. subtilis* + Calciven)	3.67	3.67	3.67	8.33	11.67	10.00	6.84
T5 (*T. harzianum* + Claciven)	3.00	3.00	3.00	6.67	8.33	7.50	5.25
Cultivar mean	17.95	12.11	15.03	22.83	21.67	22.25	18.64
Location mean	15.03			22.25			

LSD at 5 % of:

Cultivars (C)	NS	Location (L)	NS	C × L	NS
Treatments (T)	3.91	C × T	5.53	C × T × L	NS

Table (5): Effect of spraying *Bacillus subtilis*, *Trichoderma harzianum* and Calciven or their combinations on area under disease progress curve (AUDPC) of Gemmiza-7 and Gemmiza-11 wheat cvs., under field conditions of Al-Nubariya and Kafr El-Hamam locations during 2013/14 growing season.

Treatment	Nubariya		Mean	Kafr El-Hamam		Mean	General mean
	Gemmeiza-7	Gemmeiza-11		Gemmeiza-7	Gemmeiza-11		
Control (infected plants)	490.00	338.33	414.17	536.66	455.00	495.83	455.00
T1 (*B. subtilis*)	192.33	131.50	161.92	280.00	315.00	297.50	229.71
T2 (*T. harzianum*)	174.66	80.50	127.58	192.33	131.50	161.92	144.75
T3 (Calciven)	46.66	57.16	51.91	116.00	106.00	111.00	81.46
T4 (*B. subtilis* + Calciven)	44.33	44.33	44.33	70.00	95.50	82.75	63.54
T5 (*T. harzianum* + Claciven)	42.00	42.00	42.00	59.50	70.00	64.75	53.38
Cultivar mean	165.00	115.64	140.32	209.08	195.50	202.29	171.31
Location mean	140.32			202.29			

LSD at 5 % of:

Cultivars (C)	NS	Location (L)	NS	C × L	NS
Treatments (T)	46.57	C × T	NS	C × T × L	NS

Yield components

Data in Table (6 & 7) indicated the effect of spraying *Bacillus subtilis*, *Trichoderma harzianum* and Calciven or their combinations on yield components (weight of 1000-kernel (g) and yield/plot (kg)) of Gemmiza-7 and Gemmiza-11 wheat cvs., under field conditions of Al-Nubariya and Kafr El-Hamam locations during 2013/14 growing season. Results indicated that all treatments increased yield components, compared with control plants. Spraying adult plants of both cvs. with *T. harzianum* alone or in combination with Calciven gave the highest values of yield components, at the two studied locations. This effect could be attributing to the growth regulators which can be produced by these antagonistic microorganisms as mentioned by Abd El-Moity and Shatla (1981); Sankar and Jeyarajan (1996) and Reguchender *et al.* (1997). In addition, these bioagents have been shown to directly increase plant growth (Chang *et al.*, 1986). They also have abilities to solubilize plant nutrients and increase plant nutrient uptake (Altomare *et al.*, 1999; Yedidia *et al.*, 2001 and Harman *et al.*, 2004). *Trichoderma* spp. is beginning to be used in reasonably large quantities in plant agriculture, both for disease control and yield increase (Harman, 2006). Statistical analysis results of weight of 1000-kernel (g) recorded significant differences between treatments and the interaction of cultivars and locations. While, cultivars, treatments and their interaction recorded significant differences in yield/plot (kg).

Grain quality assessment

Test weight and grain protein content are two of the most important tools in wheat grading system. Data given in Table (8) indicate the effect of different treatments on rust severity and their impact on grain test weight (P/B) and percentage of grain protein content.

Table (6): Effect of spraying *Bacillus subtilis*, *Trichoderma harzianum* and Calciven or their combinations on weight of 1000-kernel (g) of Gemmiza-7 and Gemmiza-11 wheat cvs., under field conditions of Al-Nubariya and Kafr El-Hamam locations during 2013/14 growing season.

Treatment	Nubariya		Mean	Kafr El-Hamam		Mean	General mean
	Gemmeiza-7	Gemmeiza-11		Gemmeiza-7	Gemmeiza-11		
Control (infected plants)	33.80	34.10	33.95	38.40	41.33	39.87	36.91
T1 (*B. subtilis*)	34.23	34.83	34.53	38.66	41.66	40.16	37.35
T2 (*T. harzianum*)	36.88	38.10	37.49	40.94	44.70	42.82	40.16
T3 (Calciven)	35.43	35.30	35.37	39.66	42.53	41.10	38.23
T4 (*B. subtilis* + Calciven)	35.75	35.75	35.75	40.33	43.31	41.82	38.79
T5 (*T. harzianum* + Claciven)	37.80	39.13	38.47	41.70	44.96	43.33	40.90
Variety mean	35.65	36.20	35.93	39.95	43.08	41.52	38.72
Location mean	35.92			41.51			

LSD at 5 % of:

Cultivars (C)	NS	Location (L)	NS	C × L	0.38
Treatments (T)	0.47	C × T	NS	C × T × L	NS

Table (7): Effect of spraying *Bacillus subtilis, Trichoderma harzianum* and Calciven or their combinations on yield/plot (kg) of Gemmiza-7 and Gemmiza-11 wheat cvs., under field conditions of Al-Nubariya and Kafr El-Hamam locations during 2013/14 growing season.

Treatment	Nubariya		Mean	Kafr El-Hamam		Mean	General mean
	Gemmeiza-7	Gemmeiza-11		Gemmeiza-7	Gemmeiza-11		
Control (infected plants)	3.86	4.63	4.25	4.48	5.42	4.95	4.60
T1 (*B. subtilis*)	4.12	4.93	4.53	4.58	5.72	5.15	4.84
T2 (*T. harzianum*)	4.66	5.82	5.24	5.19	6.15	5.67	5.46
T3 (Calciven)	4.24	4.96	4.60	4.76	5.88	5.32	4.96
T4 (*B. subtilis* + Calciven)	4.28	5.23	4.76	4.77	5.89	5.33	5.04
T5 (*T. harzianum* + Claciven)	4.69	5.96	5.33	5.21	6.42	5.82	5.57
Variety mean	4.31	5.26	4.79	4.83	5.91	5.37	5.08
Location mean	4.78			5.37			

LSD at 5 % of:

Cultivars (C)	0.13	Location (L)	NS	C × L	NS
Treatments (T)	0.16	C × T	0.51	C × T × L	NS

Table (8): Effect of spraying *Bacillus subtilis, Trichoderma harzianum* and Calciven or their combinations on grain test weight (p/B), percentage of grain protein content and their relations to rust severity of Gemmiza-7 and Gemmiza-11 wheat cvs.

Treatment	Rust severity		Test weight p/B		Grain protein content (%)	
	Gemmiza-7	Gemmiza-11	Gemmiza-7	Gemmiza-11	Gemmiza-7	Gemmiza-11
Control (untreated plants)	53.33	43.33	59.04	57.31	10.39	11.10
T1 *B. subtilis*	33.33	36.67	60.39	58.00	10.42	11.33
T2 *T. harzianum*	26.67	16.67	60.13	57.83	10.43	11.63
T3 Calciven	8.66	13.33	60.00	57.76	10.67	11.80
T4 *B. subtilis* + Calciven	8.33	11.67	60.65	58.24	10.65	11.83
T5 *T. harzianum* + Claciven	6.67	8.33	60.61	58.17	10.63	11.90
L.S.D. at 5% of :						
Cultivars (C)			0.24		0.19	
Treatments (T)			0.41		0.33	
C × T			NS		NS	

Rust severity versus grain test weight

Data given in Table (8) indicated that all treatments increased the grain test weight for both studied cultivars, compared with control. The obtained test weight values ranged from 59.04 to 60.65 for Gemmiza-7 and from 57.31 to 58.24 for Gemmiza-11. The highest values of test weight were recorded in T4 for Gemmiza-7 and Gemmiza-11 (60.65 and 58.24, respectively). Generally, the measured test weights were in acceptable range according to USDA (2002). Statistically, significant differences were recorded between cultivars as well as between treatments. While, non significant differences were recorded when cultivars were interacted with treatments. Figure (1) showed the effect of different treatments on the curvilinearity of the relation between rust severity and grain test weight. This figure indicated that grain test weight (*y*) of Gemmiza-7 can be related to rust severity (*x*) by the following formula with a correlation coefficient (R^2) of 0.539:

$$y = 60.72 - 0.025\,x$$

While, the grain test weight of Gemmiza-11 can be related to rust severity by the following formula with a correlation coefficient (R^2) of 0.328:

$$y = 58.23 - 0.016\,x$$

Rust severity versus grain protein content

Data given in Table (8) indicated that all treatments increased the grain protein content (%) for both studied cultivars, compared with control. The obtained percentages of grain protein content were ranged from 10.39 to 10.67% for Gemmiza-7 and from 11.10 to 11.90% for Gemmiza-11. The highest GPC for Gemmiza-7 (10.67%) was obtained in T3 treatment. While, for Gemmiza-11 wheat cv., spraying wheat plants with T5 treatment gave the lowest rust severity (8.33) which accompanied by highest GPC (11.90). Statistically, significant differences were recorded between cultivars as well as between treatments. While, non significant differences were recorded when cultivars were interacted with treatments. This may be due to the fact that diseases-like leaf rust that attack leaves or other vegetative parts were reported to damage grain quality by reducing the composition of assimilates available for grain development (Drijepondt *et al.*, 1991). Also, Oak *et al.*, (2011) reported that high disease resistance (low rust severity) leads to improved grain protein content in rust resistant cvs. Figure (2) showed the effect of different treatments on the curvilinearity of the relation between rust severity and GPC (%). This figure indicated that GPC (*y*, %) of Gemmiza-7 can be related to rust severity (*x*) by the following formula:

$$y = 12.07 - 0.022\,x$$

with a correlation coefficient (R^2) of 0.973. While, the GPC (%) of Gemmiza-11 can be related to rust severity by the following formula:

$$y = 10.68 - 0.006\,x$$

with a correlation coefficient (R^2) of 0.799.

Grain moisture, ash, zeleny, falling number and flour extraction

Mean values of some properties of Gemmiza-7 and -11 wheat cvs. grains, as affected by different foliar treatments, were given in Table (9). Percentage of moisture content among all treatments of Gemmiza-7 and Gemmiza-11 were ranged from 10.00 to 10.8% and from 9.7 to 10.3, respectively. The lowest two moisture content values (10.00 and 9.70%) were recorded in T4 treatment, for Gemmiza-7 and -11, respectively.

Percentage of ash content for all treatments was found quite close to each other (Table 9). For Gimmiza-7, all treatments led to increase ash content values. As a result of milling process, nutrient level of high extraction rate flour increases directly with increase in flour ash values (Matthews and Workman, 1977). However, the effect of different treatments on the percentage of ash content of Gemmiza-11 was varied from one treatment to another. The highest ash content (1.30 %) was observed in Gemmiza-7 with T3 treatment.

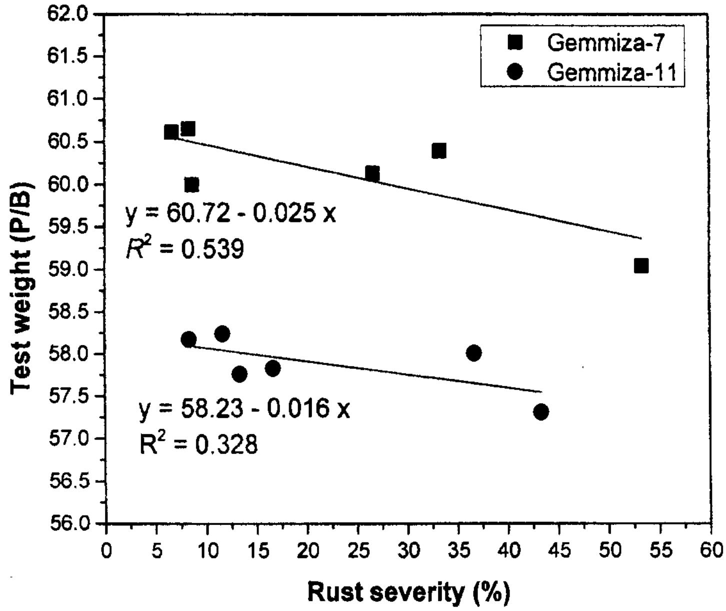

Fig. (1): Correlation coefficient between percentage of leaf rust severity and grain test weight (P/B) of Gemmiza-7 and Gemmiza-11 wheat cvs. as affected by spraying with *Bacillus subtilis*, *Trichoderma harzianum* and Calciven or their combinations, combined with control untreated plants.

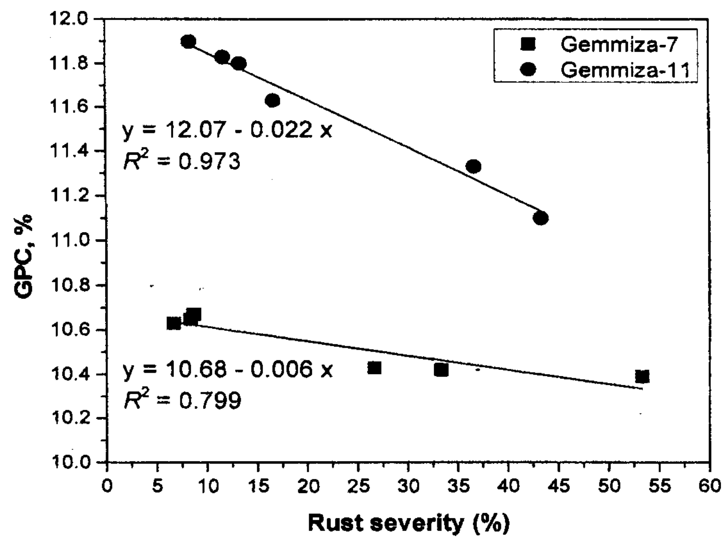

Fig. (2): Correlation coefficient between percentage of leaf rust severity and percentage of grain protein content of Gemmiza-7 and Gemmiza-11 wheat cvs. as affected by spraying with *Bacillus subtilis*, *Trichoderma harzianum* and Calciven or their combinations, combined with control untreated plants.

Table (9): Effect of spraying *Bacillus subtilis*, *Trichoderma harzianum* and Calciven or their combinations on some grain quality parameters (grain moisture (%), ash (%), zeleny (ml) and flour extraction (%) as well as falling number (s)) of Gemmiza-7 and Gemmiza-11 wheat cvs.

Treatment	Moisture %		Ash %		Zeleny (ml)		Falling number(s)		Flour extraction %	
	Gem-7	Gem-11	Gem-7	Gem-11	Gem-7	Gem-11	Gem-7	Gem-11	Gem-7	Gem-11
Control	10.80	10.30	1.21	1.09	37.67	39.33	414.00	382.50	68.30	69.20
T1 (*B. subtilis*)	10.08	10.00	1.25	1.10	40.67	39.33	387.67	395.00	67.90	71.90
T2 (*T. harzianum*)	10.20	10.10	1.27	1.08	39.00	39.33	382.00	397.67	66.10	69.70
T3 (Calciven)	10.40	10.20	1.30	1.09	40.00	34.67	408.00	371.50	69.50	69.60
T4 (*B. subtilis* + Calciven)	10.00	9.70	1.26	1.08	38.67	33.33	404.00	367.50	66.60	72.10
T5 (*T. harzianum* + Claciven)	10.06	9.80	1.26	1.12	42.00	44.00	410.33	396.50	68.10	66.00
L.S.D. at 5% of :										
Cultivars (C)	0.14		0.04		2.72		20.67		2.55	
Treatments (T)	0.24		0.05		4.17		35.69		4.42	
C × T	NS		NS		NS		NS		NS	

Regarding the sedimentation of protein (zeleny test, ml), T5 treatment recorded the highest percentages (42.00 and 44.00 ml) for Gemmiza-7 and Gemmiza-11, respectively, compared to control plants (37.67 and 39.33 ml, respectively). Generally, all treatments raised the sedimentation of protein of Gemmiza-7, compared to control plants. While for Gemmiza-11, the effect of foliar spraying of the studied treatments was varied.

Falling number is an internationally recognized measure, which indirectly assesses α-amylase activity in flour (Hagberg, 1961 and Perten, 1964), and is used to test if wheat is suitable for bread-making or not. Data given in Table (9) revealed that the falling numbers were differed from cultivar to another. These results were in harmony with those obtained by Farrell and Kettlwell (2009) where they stated that cultivar type is an important factor that affects the falling number. Also, fungal infection may affect the falling number with a reciprocal relation (Svensson, 1990). This is what evidenced in Table (9) where control infected Gemmiza-7 wheat cv. recorded the highest falling number (414.00 s). Furthermore, all treatments applied onto Gemmiza-7 wheat cv. reduced the falling number. The highest reduction was recorded by T2 treatment (382.00 s) followed by T1 treatment (387.67 s). Such effects of *T. harzianum* and *B. subtilis* were in accordance with those obtained by Sallam (1997). However for Gemmiza-11, the effect of foliar spraying of the studied treatments on falling number was varied. Generally, flours with a low falling number (< 250 s) produce sticky bread crumbs (Mustătea *et al.*, 2006), while those with a high falling number (> 400 s) produce dry bread crumbs.

Respecting the flour extraction, data presented in Table (9) indicated that T3 and T2 treatments for Gemmiza-7 had highest and lowest flour yields (69.5 and 66.1%, respectively) while T4 and T5 treatments for Gemmiza-11 had highest and lowest flour yields (72.1 and 66.0 %, respectively). D'Appolonia and Emeritus (1996) reported that milling separates the bran and germ fractions from the endosperm, which is used to make flour, and reduces endosperm particles to the correct size. A series of separation and sizing steps converts one hundred pounds of wheat into about seventy-five pounds of various flour types. Patent flour is made from the purest clear flour which is made from less pure fractions and has higher protein and bran content. Straight flour contains all the flour fractions and has a protein and bran content that falls in between the other two. Also, the flour extraction rate affects the protein content, farinographic water absorption and gluten strength (Orth and Mander, 1975). With an increase in exaction rate, the protein content, fiber, sugar, lipids and mineral matter increase, whereas the starch decreases (Kent and Amos, 1967). Statistically, significant differences were recorded between cultivars as well as between treatments, in all studied parameters.

Flour quality assessment

Quality assessment of different wheat flours was given in Table (10) in terms of percentages of moisture, ash, wet and dry gluten contents. Moisture contents for Gemmiza-7 were ranged from 15.07 (in control plants) to 15.70% (in T3 treatment). While for Gemmiza- 11, T1 treatment recorded the highest moisture content (15.93 %) while T5 treatment recorded the lowest moisture content (14.80%). On the other hand, T5 treatment of Gemmiza-7 flour recorded the lowest weight in ash (0.44%) and the highest ash content was recorded with T2 and T3 treatments (0.51%). For Gemmiza-11, T5 treatment recorded the highest ash content (0.52%) while the lowest ash content (0.45%) was recorded in control plants. In this respect D'Appolonia and Emeritus, (1996) reported that ash is the mineral residue remaining after organic matter has been incinerated. Wheat bran contains more minerals than does endosperm, so ash content roughly correlates with flour type. However, nutrient level increases directly with flour ash values and nutritional quality of flours produced worldwide is lower than the wheat as a result of milling process (Barret *et al.*, 1980). Additionally data in Table (10) showed that wet and dry gluten from wheat meal of Gemmiza-7 were ranged from 25.17 to 28.40% and from 17.67 to 20.03%, receptivity. The highest percentages of wet and dry gluten in Gemmiza-7 were recorded in T1 treatment (28.40 and 20.03%, respectively), whereas lowest percentages were recorded in T4 treatment (25.17 and 17.67 %, respectively). Results also showed that wet and dry gluten from wheat meal of Gemmiza-11 were ranged from 28.40 to 33.97% and from 20.27 to 24.07%, receptivity. The highest percentages wet and dry gluten in Gemmiza-11 were recorded in T5 treatment (33.97 and 24.07%, respectively), whereas the lowest percentages were recorded in T4 treatment (28.40 and 20.27 %, respectively). Wheat is unique among the edible grains because only wheat flour has the protein complex called "gluten" that can be formed into dough with the rheological properties required for the production of leavened bread. Gluten protein is the basis of man's attraction to wheat because wheat gluten alone can sustain human desire for leavened bread products. The rheological properties of gluten are needed not only for bread production, but also in the wider range of foods that can only be made from wheat, Viz., noodles, pasta, pocket breads, pastries, cookies, and other products. Statistically, significant differences were recorded between cultivars as well as between treatments, in all studied parameters.

Table (10): Effect of spraying *Bacillus subtilis*, *Trichoderma harzianum* and Calciven or their combinations on percentages of some flour quality parameters (percentages of moisture, ash, wet and dry glutens) of Gemmiza-7 and Gemmiza-11 wheat cvs.

Treatment	Moisture %		Ash flour %		Wet gluten %		Dry gluten %	
	Gem-7	Gem-11	Gem-7	Gem-11	Gem-7	Gem-11	Gem-7	Gem-11
Control	15.07	15.10	0.45	0.45	26.80	29.87	18.87	21.33
T1 (*B. subtilis*)	15.25	15.93	0.47	0.50	28.40	31.53	20.03	22.40
T2 (*T. harzianum*)	15.35	15.20	0.51	0.49	27.20	31.60	19.30	22.80
T3 (Calciven)	15.70	15.35	0.51	0.46	25.60	29.90	17.97	21.33
T4 (*B. subtilis* + Calciven)	15.30	15.30	0.48	0.46	25.17	28.40	17.67	20.27
T5 (*T. harzianum*+ Claciven)	15.30	14.80	0.44	0.52	27.90	33.97	19.93	24.07
L.S.D. at 5% of :								
Cultivars (C)	0.20		0.06		1.04		0.63	
Treatments (T)	0.63		0.07		1.80		1.09	
C × T	NS		NS		NS		NS	

REFERENCES

AACC (2000). AACC Method 46-30, reapproved: 1999. In: Approved Methods of the American Association of Cereal Chemists, 10th ed. AACC International, St. Paul, MN.

Abd El-Moity, T.H. and M.N. Shatla (1981). Biological control of white rot disease of onion (*Sclerotium cepivorum*) by *Trichoderma harzianum*. Phytopathology Z., 100:29-35.

Abd El-Moneim, L. Maisa, Doaa R.M. El-Naggar and Gamalat A. Hermas (2011). Biological control of stem rust by using two bioagents and garlic extract. Egypt. J. of Appl. Sci., 26 (7): 259-272.

Altomare, C., W.A. Norvell, T. Bjorkman and G.E. Harmony (1999). Solubilization of phosphates and micronutrients by the plant growth promoting and biocontrol fungus *Trichoderma harziaanum* Rifai. Appl. Environ. Microbial., 65: 2926-2933.

Barret, F.F., R.J. Lowewr and K. Kulp (1980). Nutrient levels in internationally milled flours. Cereal Chem., 57: 361-366.

Brain, P.W. and H.G. Hemming (1945). Gliotoxin a fungistatic metabolic product of *Trichoderma viride*. Ann. Appl. Biol., 32: 214- 220.

Carreteroa, R., M.O. Bancal and D.J. Miralles (2011). Effect of leaf rust (*Puccinia triticina*) on photosynthesis and related processes of leaves in wheat crops grown at two contrasting sites and with different nitrogen levels. Eur. J. Agronomy, 35: 237-246.

Cawood, M.E., J.C. Pretorius, A.J. van der Westhuizen and Z.A. Pretorius (2010). Disease development and PR-protein activity in wheat (*Triticum aestivum*) seedlings treated with plant extracts prior to leaf rust (*Puccinia triticina*) infection. Crop Protection, 29: 1311-1319.

Chang, Y.C., R. Baker, O. Kleifeld and I. Chet (1986). Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. Plant Dis. 70: 145-148.

D'Appolonia, B. L. and Emeritus (1996). How Flour Affects Bread Quality. Department of Cereal Science, North Dakota State University,Fargo, ND58105, Lallemand Baking update, No. 17.

De Curtis, F., V. De Cicco and G. Lima (2012). Efficacy of biocontrol yeasts combined with calcium silicate or sulphur for controlling durum wheat powdery mildew and increasing grain yield components. Field Crops Research, 134: 36-46

De Curtis, F., A.M. Spina, D. Piedimonte, G. Lima and V. De Cicco (2007). Biological and integrated control of wheat powdery mildew. J. Plant Pathol., 89: 38.

Deliopoulos, T., P.S. Kettlewell and M.C. Hare (2010): Fungal disease suppression by inorganic salts: A review. Crop Protection. 29: 1059-1075.

Dowson, W.J. (1957). Plant disease due to bacteria Second Ed., Cambridge the University Press, London, pp. 23.

Drijepondt, S.C., Z.A. Pretorius, D. Van Lill and F.H.J. Rijkenberg (1991). Effect of Lr34 resistance on leaf rust development, grain yield and baking quality in wheat. Plant Breeding, 105: 62-68.

Duveiller, E., R. Singh and J. Nicol (2007). The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics. Euphytica, 157: 417-430.

El-Gamal, Nadia G., F. Abd El-Kareem, Y.O. Fatouh and Nehal S. Al-Mougy (2007). Induction of systemic resistance in potato plants against late and early blight disease using chemical inducer under greenhouse and field conditions. Res. J. Agric. Biological Sci., 3 (2): 73-81.

Farrell, A.D. and P.S. Kettlewell (2009). The relationship between grain weight and alpha-amylase in winter wheat: varietal comparison from UK field experiments. Euphytica 168: 395-402.

Ferreira, J.HS., F.N. Matthee and A.C. Thomas (1991). Biological control of *Eutypa lota* on grapevine by an antagonistic strain *Bacillus subtilis*. Phytopathology, 81: 283-287.

Flowers, T.J. (1988). Chloride as a nutrient and as an osmoticum. In: Tinker, P.B., Laechi, A. (Eds.), Advances in Plant

Nutrition, Vol. 3. Praeger, New York, NY, pp. 55-78.

Haas, D. and G. Défago (2005). Biological control of soil-borne pathogens by fluorescent pseudomonas. Nat. Rev. Microbiol., 1129: 1-10.

Hagberg, S. (1961). Simplified method for determining α-amylase activity. Cereal Chem. 38: 202-203.

Harman, G.E. (2006). Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology, 96: 190-194.

Harman, G.E., R. Petzoldt, A. Comis and J. Chen (2004). Interactions between *Trichoderma harzianum* strain T22 and maize intred line M017 and effects of these interactions on diseases caused by *Pythium ultimum* and *Colletotrichum graminicola*. Phytopathology, 94:147-153.

Homann, P.H. (2002). Chloride and calcium in photosystem II: from effects to enigma. Photosyn. Res., 73: 169-175.

Joann, L.R., F.L. Lukezic, R. Craig and G.W. Moorman (1989). Biological control of Geranium rust by *Bacillus subtilis*. Dis. Control and Pest Management, 79(3): 367-370.

Karjin, H. (1996). Effectiveness of new fungicides against stem rust and leaf rust of wheat. Proceeding of the 9th European Powder Mildews, Conference. 2-6 September (Lunteren, The Netherlands, p. 4-14).

Kent, D.W. and A.J.E. Amos (1967). Modern Cereal Chemistry, 6th Ed. London: Food, Trade Press Ltd. pp. 2-10.

Kwang, H.U., A. Pyung, K. Soonok and L.H. Yong (2003). Calcium/calmodulin-dependent signaling for prepenetration development in *Colletotrichum gloeosporioides*. American Phytopathol. Soc., 93(1): 82-87.

Li, H., J. Zhao, H. Feng, L. Huang and Z. Kang (2013). Biological control of wheat stripe rust by an endophytic *Bacillus subtilis* strain E1R-j in greenhouse and field trials. Crop Protection, 43: 201-206.

Lima, G. and V. De Cicco (2006). Integrated strategies to enhance biological control of postharvest diseases. In: Berkeblia, N., Shiomi, N. (Eds.), Advances in Postharvest Technologies for Horticultural Crops, Research Signpost, Kerala, India, Chapter 8: 173-194.

Matthews, R.H. and M.Y. Workman (1977). Nutrient composition of selected wheat products. Cereal Chem., 54: 1115-1123.

Melgar, R., H. Magen, M.E. Camozzi and J. Lavandera (2001). Potassium chloride application in wheat in the Pampean region, Argentina. Response to potassium or to chloride? In: Proceedings of the World Fertilizer Congress, 3-9 August 2001, Beijing, China.

Moustafa-Mahmoud, S.M., Hoda Z. Mohamed and Afaf M. Amr (1997). The role of vesicular-arbuscular mycorhiza (VAM) and rate of phosphorus fertilizer in controlling cotton seedling disease caused by *Rhizoctonia solani* (AG-4). 1st International Conf. Plant Pathology Res. Inst., Giza, Egypt, September 15-18. (abstract)

Mustătea, P., N.N. Săulescu, G. Ittu, C. Tican, I. Lobonţiu and G. Bunta (2006). Pre-harvest amylase activity and sprouting in Romanian wheat cultivars. Roman. Agric. Res., 23: 1-6.

Oak, M.D., R.M. Patil, D.N. Bankar, J.H. Bagwan, A.L. Bipinraj, S.A. Tamhankar, B.K. Honrao and S.C. Misra (2011). Performance of durum wheat lines for quality and rust resistance. J. Wheat Res., 3(1): 71-72.

Orth, R.A. and K.C. Mander (1975). Effect of milling yield on lour composition and bread making quality. Cereal Chem., 62: 441-446.

Pandey, H.N., T.C.M. Menon and M.V. Rao (1989). A simple formula for calculating area under disease progress curve. Rachis, 8(2): 38-39.

Parlevliet, J.E. (1975). Partial resistance of barley to leaf rust (*Puccinia hordei*). I- Effect of cultivars and development stage on latent period. Euphytica, 24:21-27.

Parlevliet, J.E. and H.J. Kuiper (1977). Partial resistance of barley to leaf rust *Puccinia hordei*. IV. Effect of cultivar and development stage on infection frequency. Euphytica 26: 249–255.

Perten, H. (1964). Application of the falling number method for evaluating α-amylase activity. Cereal Chem. 41: 127-140.

Peterson, R.F., A.B. Campbell and A.E. Hanna (1948). A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Can. J. Res., 26:496-500.

Reguchender, T., K. Kajappan and R. Samiappan (1997). Evaluating methods of application of bio control agent in control of mung bean root rot. Indian Phytopathology, 50(2): 229- 234.

Rognes, S.E. (1980). Anion regulation of lupin asparagine synthetase:chloride activation of the glutamine-utilizing reactions. Phytochemistry, 19: 2287-2293.

Sallam, Minass, E.A. (1997). Studies on leaf rust of wheat in Egypt. Ph.D. Thesis, Agric. Bot. Dept., Fac. Agric., Zagazig Univ., 84.

Sallam, Minaas, E.A., Hoda M.M. Diab, S.M. Moustafa and Doa'a R.M. El-Nagar (2001). Induced resistance in wheat against stem rust disease by salicylic acid, cobalt ions and growth regulators. Egypt. J. Appl. Sci., 16(6): 1-12.

Sankar, P. and R. Jeyarajan (1996). Biological control of sesames root rot by seed treatment with *Trichoderma* spp. and *Bacillus subtilis*. Indian Journal of Mycology and Plant Pathology, 26(2): 217- 220 (c.f. CAB Abstract 1996- 1998).

Schnabl, H. and K. Raschke (1980). Potassium chloride as stomatal osmoticum in *Allium cepa* L., a species devoid of starch in guard cells. Plant Physiol., 65: 88-93.

Stakman, E.C., D.M. Stewart and W.Q. Loedering (1962). Identification of physiologic races of *Puccinia graminis* f.sp. *tritici*. A.R.S. USA Agric. Res. Service Bull. E., 617:p. 53.

Steel, R.G.D. and J.H. Torrie (1980). Principles and Procedures of Statistics. McGraw-Hill Book Company, New York. pp. 187-188.

Svensson, G. (1990). Linkages between sprouting resistance and some agronomic traits in wheat. In: Ringlund, K., Mosleth, E., Mares, D.J. (Eds.), Fifth International Symposium on Pre-Harvest Sprouting in Cereals. West View Press, Boulder, Co, USA, 227-232.

Tervet, I. and R.C. Cassel (1951). The use of cyclone separation in race identification of cereal rusts. Phytopathology, 41:282-285.

USDA, (2002). United Stats Department of Agriculture. Equipments Handbook. Grain Inspection, Packers and Stockyards Administration,1400 Independence Ave., S.W. Washington, D.C. 20250-3600.

Utkhede, R.S. (1984). Antagonism of isolates of *Bacillus subtilis* to *Phytophthora cactorum*. Canadian J. Bot., 62: 1032-1035.

Williams, J. and S.G. Smith (2001). Correcting potassium deficiency can reduce rice stem diseases. Better Crops, 85: 7-9.

Yedidia, I., A.K. Srivastva, Y. Kapulnik and I. Chet (2001). Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. Plant Soil, 235:235-242.

تأثير استخدام إثنين من كائنات المكافحة البيولوجية وكلوريد الكالسيوم على مقاومة صدأ الأوراق في القمح وجودة الحبوب والدقيق

دعاء راغب محمد النجار[1] ، وليد محمد العرابي[1] ، جيهان بسطامي علي[2] ، مايسة لطفي عبد المنعم[3]

(١) معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزة – مصر

(٢) المعمل المركزي للأغذية والأعلاف – مركز البحوث الزراعية – الجيزة – مصر

(٣) المعمل المركزي للزراعة العضوية – مركز البحوث الزراعية – الجيزة – مصر

الملخص العربي

تُصاب مساحات القمح على مستوى العالم بالعديد من الأمراض ومنها مرض صدأ الأوراق والذي يُحدثهُ الفطر بكسينيا تريتيسينا وهو من الأمراض واسعة الإنتشار. تهدف الدراسة الحالية إلى استخدام طرق مقاومة جديدة صديقة للبيئة وتتميز بالكفاءة العالية من خلال استخدام اثنين من كائنات المكافحة الحيوية (بكتيريا الباسيلس ساتلس وفطر التريكودرما هارزيانم) واختبار قدرتهم الذاتية على مقاومة مرض صدأ الأوراق في صنفين من قمح الخبز (جميزة – ٧ وجميزة–١١) ، ذلك بالإضافة إلى دراسة تأثير اتحادهم مع الكالسفين (ملح كلوريد الكالسيوم) على مقاومة المرض . أجريت الدراسات الحالية تحت ظروف الصوبة وكذلك الظروف الحقلية بموقعي النوبارية وكفر الحمام . أوضحت نتائج الصوبة أن اتحاد الكالسفين مع كائنات المكافحة الحيوية يؤدي إلى أفضل النتائج وكان التركيز العالي (١ لتر/٥٠ لتر ماء مقطر) لاتحاد الكالسفين مع فطر التريكودرما هارزيانم هو الأفضل تأثيراً حيث أدى إلى زيادة فترات ظهور الأمراض لجميزة –٧ وجميزة –١١ لتصل إلى ٨.١١ و ٨.٣٥ (مقارنة بالكنترول الذي سجل ٧.١١ و ٧.٤٣ ، على الترتيب) ، وكذلك إلى زيادة فترات كمون المسبب المرضي لجميزة –٧ وجميزة –١١ لتصل إلى ١٠.٥٨ و ١١.٤٣ (مقارنة بالكنترول الذي سجل ٩.٥٠ و ١٠.١٠ ، على الترتيب) ، بالإضافة إلى خفض عدد البثرات لكل ورقة مع وجود فوارق معنوية بين الأصناف المختلفة وكذلك بين المعاملات المختلفة. أما تجارب الحقل فقد تم فيها دراسة تأثير استخدام التركيز العالي للمعاملات المختلفة على شدة حدوث المرض والمساحة تحت المنحنى المرضي وبعض مكونات المحصول (وزن الألف حبة ومحصول الحوض). أوضحت النتائج الحقلية مدى قدرة الكالسفين (بمفرده أو متحداً مع كائنات المكافحة الحيوية) على تقليل شدة حدوث المرض ومن ثم المساحة تحت المنحنى المرضي والذي انعكس بدوره ايجابياً على مكونات المحصول . كان اتحاد الكالسفين مع فطر التريكودرما هارزيانم هو الأفضل تأثيرا في موقعي الدراسات الحقلية ، وسجل موقع النوبارية أقل قيمة لشدة حدوث المرض لتصل إلى ٣.٠٠ في جميزة–٧ وجميزة–١١ (مقارنة بالكنترول الذي سجل ٤٦.٦٧ و ٣٣.٣٣ ، على الترتيب) مما ترتب عليه تسجيل أقل قيمة للمساحة تحت المنحنى المرضي لتصل إلى ٤٢.٠٠ في كلا الصنفين (مقارنة بالكنترول الذي سجل ٤٩٠.٠٠ و ٣٣٨.٣٣ ، على الترتيب) . أوضحت النتائج أيضاً أن اتحاد الكالسفين مع فطر التريكودرما هارزيانم هو الأفضل تأثيرا في موقعي الدراسة على وزن الألف حبة ، وسجل موقع كفر الحمام

أعلى قيم لوزن الألف حبة (٤١.٧٠ و ٤٤.٩٦ جرام في جميزة-٧ وجميزة-١١ ، على الترتيب) مقارنة للكنترول (٣٨.٤٠ و ٤١.٣٣ جرام في جميزة-٧ وجميزة-١١ ، على الترتيب) ، مما أدى بدوره إلى زيادة محصول الحوض في ذات الموقع ليصل إلى ٥.٢١ و ٦.٤٢ كيلوجرام (في جميزة-٧ وجميزة-١١ ، على الترتيب) مقارنة بالكنترول (٤.٤٨ و ٥.٤٢ كيلوجرام ، على الترتيب). تم أيضاً في هذا البحث دراسة تأثير المعاملات المختلفة على خواص الحبوب والدقيق وذلك بإجراء الدراسات التكنولوجية اللازمة مثل تحديد درجة امتلاء الحبة (بوند/ بوشل) ومحتويات البروتين (%) والرطوبة (%) والرماد (%) والبروتين المترسب (ملي) وتحديد زمن السقوط (ثانية) والنسبة المئوية لإستخلاص الدقيق(%) بالإضافة إلى تحديد محتويات الجلوتين الرطب (%) و الجاف (%) . أوضحت نتائج الدراسات التكنولوجية بشكل عام أن المعاملات محل الدراسة أدت إلى تحسين جودة الحبوب والدقيق ، وفي هذا السياق سجل الصنف جميزة-١١ أعلى نسبة جلوتين رطب أو جاف (٣٣.٩٧ أو ٢٤.٠٧ ، على الترتيب) عند معاملته بالتريكودرما هارزيانم مع الكالسفين . تم أيضا دراسة العلاقات الخطية بين شدة حدوث المرض والوزن النوعي للحبوب ومحتوى البروتين بها ، وسجل الصنف جميزة-٧ أعلى معامل ارتباط (٠.٩٧٣) بين شدة حدوث المرض ومحتوى البروتين في الحبوب . في ضوء خطورة مرض صدأ الأوراق في القمح والاحتياج الدائم لتحسين جودته فقد خلصت النتائج إلى مدى الاستفادة من الدراسة الحالية في تطوير أساليب مقاومة هذا المرض بمنهج علمي تكاملي فعال ذو طبيعة صديقة للبيئة .