Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

Plant Pathology Journal 14 (4): 182-188, 2015
ISSN 1812-5387

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/ppj.2015.182.188

Combined Effects of *Streptomyces viridosporus* and *Trichoderma harzianum* on Controlling Wheat Leaf Rust Caused by *Puccinia triticina*

[1]H.H.A. El-Sharkawy, [2]Somaya Tohamey and [3]Amal A. Khalil
[1]Department of Mycology Research and Plant Disease Survey, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt
[2]Department of Wheat Disease Research, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt
[3]Department of Seed Pathology Research, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

ARTICLE INFO
Article History:
Received: June 18, 2015
Accepted: July 31, 2015

Corresponding Author:
H.H.A. El-Sharkawy
Department of Mycology Research and Plant Disease Survey,
Plant Pathology Research Institute,
Agricultural Research Center,
Giza, Egypt

ABSTRACT
This study investigates the ability of two bioagents namely *Trichoderma harzianum* and *Streptomyces viridosporus* to induce elevated levels of resistance in two susceptible genotypes Morocco and Sids-1 against leaf rust disease of wheat. Bioagents were tested at seedling and adult stage of two susceptible wheat genotypes (Morocco and Sids-1) under greenhouse and open field conditions in 2013/2014 growing season. It was indicated that all bioagents and combined effect between them, increased both Incubation Period (IP) and Latent Period (LP) of the disease and decreased the number of pustule cm^{-2}, than the control. The results revealed that the amounts of photosynthetic pigments, chlorophyll a, b were lower in the infected plants than in healthy ones. It was, also indicated that spraying the plants of both the genotypes with the tested bioagents decreased leaf rust severity (%), increased spike weight (g), grains weight/spike (g) and 1000 kernel weight (g) significantly, as compared with control.

Key words: Biological control, leaf rust, *Streptomyces viridosporus*, *Trichoderma harzianum*, wheat

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important cereal crop in all over the world. Wheat is considered the first strategic food crop in Egypt. Rust diseases are among the most widespread and economically important diseases of cereal crops worldwide. Three distinct diseases viz., leaf rust, stripe rust and stem rust, occur annually on wheat in Egypt. Leaf rust caused by *Puccinia triticina*, is the most common rust disease of wheat. Chemical control measures are effective in controlling wheat rust diseases but fungicides are not desirable means of disease control and they are cost expensive and causes serious environmental pollution and may induce pathogen resistance too (Ikediobi, 1985). Biocontrol is a potential, alternative and ecofriendly way to control the disease. Biocontrol agent with a well-known ability to produce antibiotics, parasitizes pathogenic fungi and induces systemic resistance in plants. Even though a plant-mediated response has been confirmed as a component of bioprotection by *Trichoderma* spp. (Djonovic *et al*., 2006). Antagonistic fungi and streptomyces play a major role in regulating many interactions between plants and parasitic microorganisms (Jeffries, 1997). Abd El-Ghany *et al*. (2009) found that *Trichoderma harzianum* and *Saccharomyces cerevisiae* gave reasonable control against leaf rust severity of willow plants with infected leaves percentages of 10.69 and 19.18%, respectively compared with control (Plant inoculated with pathogen uredinio spores without treatment). Kuberan *et al*.

(2012) used of *Trichoderma* against brown blight pathogen caused by *Glomerella cingulata* in tea and revealed that, *Trichoderma* was highly effective to control all isolates of *G. cingulata*. Streptomyces are one of the most attractive sources of biologically active substances such as vitamins, alkaloids, plant growth factors, enzymes and enzyme inhibitors, antibiotics (Omura, 1986; Bonjar *et al*., 2004). *Streptomyces* and other actinomycetes are major contributors to biological buffering of soils and have roles in organic matter decomposition conducive to crop production (Dhingra and Sinclair, 1995). Haddad *et al*. (2009) evaluated seven bacterial isolates (six of *Bacillus* spp. viz., B10, B25, B157, B175, B205 and B281and one of *Pseudomonas* sp. viz., P286 to control coffee rust under field condition and copper hydroxide as fungicide and found that *Bacillus* sp. isolate B157 reduced rust intensity as effectively as copper hydroxide. Roatti *et al*. (2013) found that *Trichoderma harzianum* T39-Induced resistance to Downy Mildew in Grapevine. The objective of the present study was to investigate the effect of *T. harzianum* (T) and *S. viridosporus* and combined effect for controlling the wheat leaf rust disease under greenhouse and field condition.

MATERIALS AND METHODS

Fungal inoculum of leaf rust: Fresh mixture of aggressive urediospores of *Puccinia triticina* Eriks. Urediospores were collected from infected adult wheat plants in greenhouse of Department of Wheat Disease Research, Plant Pathology Research Institute, Agricultural Research Center, Egypt. Dry preparation of urediospores were mixed with talc powder (1:20 v/v) in baby cyclone and used in case of artificial inoculation.

Bioagents strains: *Trichoderma harzianum* was isolated from wheat plant rhizosphere located in El-Sharkia governorate and identified at the Plant Pathology Research Institute, Agricultural Research Center, Egypt. *Streptomyces viridosporus* was obtained from dr Huda Husein Badr, Department of Bacterial Diseases Research, Plant Pathology Research Institute, Agricultural Research Center, Egypt. Inoculum of *T. harzianum* was prepared with conc. (10^6 spores mL^{-1}), *Streptomyces viridosporus* (10^8 CFU mL^{-1}) as described by El-Naggar *et al*. (2012).

Experiments: Two isolates bioagent were tested to evaluate their capabilities to induce resistance against leaf rust infection (*P. triticina*). These bioagent were *T. harzianum* (T) and *S. viridosporus* (S) and T+S. In addition to the use tilt fungicide (2.5 mL^{-1}). The active ingredient in tilt fungicide is propiconazole 41.8 and 58.2% other ingredients, tilt was obtained from Syngenta Co, Egypt. The experiments were carried out by seedling and adult plant stages and field experiments using the two susceptible cvs. (Morocco and Sids-1). Wheat grains were obtained from Department of Wheat Research, Crops Research Institute, Agriculture Research Centre, Giza. Recommended dose of chemical fertilizers NPK (75-31-48) kg/feddan. Recommended dose of chemical fertilizers NPK (0.0006-0.000248-0.000384) kg pot^{-1}.

Feddan = 4200 m^2

Seedling stage experiment: Ten wheat grains of two susceptible genotypes; Morocco and Sids-1 were grown in plastic pots (7 cm diameter) containing clay soil and received normal irrigation and fertilization in pots. Three pots were used for each particular treatment. The method of inoculation was carried out as described by Stakman *et al*. (1962). Seedlings (7 days old) were sprayed separately over leaf surfaces with mentioned different bioagents. Seedlings sprayed with water act as control. While, seedlings protected by tilt fungicide (2.5 mL^{-1}). After 72 h of spraying bioagents prepared urediospores in baby cyclone were used to dust treated and untreated control seedlings and then sprayed in the inoculation chambers with distilled water, then inoculated by shaking and brushing rusted materials over the plants and sprayed gently again with water in order to induce initial "dew" on the plants. Finally, the inoculated plants were kept in moist chambers (22-24°C) for 14 h to allow the rust spores to germinate and cause infection (Marshall, 1992). Three pots were used as replicates for each particular treatment. Artificially inoculated planted were examined to estimate the following disease parameters:

- Number of pustules/cm^2, per unit leaf area (2.0×0.5 cm) on the upper side of the leaves, was counted as described by Parlevliet and Kuiper (1977)
- Infection type were (0, 0^0, 1, 2) low infection and (3, 4) high infection

Adult stage experiment: Ten wheat grains of two susceptible genotypes; Morocco and Sids-1 were grown in plastic pots (25 cm diameter) after 60 day from sowing, plants sprayed with bioagents and after 72 h inoculated with urediospores of *P. triticina*. Inoculated Plants were immediately transferred to dew chamber pre-conditioned to an air temperature of 15-17°C and incubated for 48 h. The plants were then transferred to the greenhouse bench and grown under the conditions described above. Artificially inoculated plants were examined to estimate the following disease parameters:

- **Incubation period:** Time (day) between inoculation and appearance of the first flecks.
- **Latent period:** Time (day) between inoculation and 50% of pustule just visible on erupted (Parlevliet, 1975).
- **Estimation of chlorophyll content:** Pigments were extracted by methanol for 24 h at laboratory temperature after adding a trace of sodium carbonate (Robinson and Britz, 2000), then photosynthetic pigments were determined spectrophotometrically. The quantity of photosynthetic pigments in leaves was determined by the equation introduced by Mackinney (1941):

- Chlorophyll a (mg g^{-1}) = (16.5×E665-8.3×E650)/5
- Chlorophyll b (mg g^{-1}) = (33.8×E650-12.5×E665)/5
- Total chlorophyll (mg g^{-1}) = (25.5×E650+4×E665)/5
- Carotenoids (mg g^{-1}) = (4.2×E452.5-0.0264×Chl. A-0.496 ×Chl.b)/5

These experiments were carried out in Department of Wheat Disease Research, Plant Pathology Research Institute, Agricultural Research Center, Egypt.

Field experiments: The field experiments were carried out at experimental farm of Etay El-Barood (El-Behera Governorate) and Kafr El-Hamam (El-Sharkia Governorate) stations during 2013/2014 growing season. The experimental design was completely randomized block with three replicates. The plot size was 10.5 m^2 containing 6 rows with 20 cm row to row spacing. Each row was sown by 5 g wheat grains. Artificial inoculation using mixtures of *P. triticina* was carried out at booting stage (approximately 92 days after planting). The tested bioagent were sprayed, 3 days before inoculation, to determine the effect of different bioagent on the disease parameters i.e., leaf rust severity (%) according to Long *et al.* (1992), disease parameters were expressed by five types, immune = (0), resistance = (R), Moderately Resistance (MR), Moderately Susceptible = (MS) and Susceptible = (S) as described by Roelfs *et al.* (1992) and yield parameters i.e., spike weight (g), grain weight/spike (g), 1000 grain weight (g) and test wheat.

Statical analysis: Data was statistically analyzed according to Snedecor and Cochran (1990). The combined analysis was conducted for the data of two seasons. The Least Significant Differences (LSD at 5%) used to compare the treatments means.

RESULTS AND DISCUSSION

Greenhouse experiments

Seedling stage

Effect of spraying with bio agent on component Infection type and number of pustules/cm^2: Data in Table 1 showed that, the rust incidence measured as infection type and number of pustules/cm^2 of the two genotypes Morocco and Sids-1, was significantly reduced than the control, as a result of spraying with *T. harzianum* and *S. viridosporus* and combination. The Infection Type (IT), was significantly decreased than the control in both the cultivars. The highest decrease in the number of pustules/cm^2 was obtained by the pretreatment with T+S followed by *S. viridosporus*, while, the lowest effect was found with *T. harzianum*.

Adult stage

Effect of spraying the two susceptible wheat genotypes (Morocco and Sids-1) with *T. harzianum* and *S. viridosporus* and combination (T+S), under greenhouse condition on Incubation Period (IP) and Latent Period (LP): Incubation Period (IP) was significantly increased than the control in both cvs. (Morocco and Sids-1) as a result of spraying *T. harzianum* and *S. viridosporus* and combination under evaluation (Table 2). However, this increment reached to 16 day of *T. harzianum*+*S. viridosporus*, followed by S. *viridosporus* (14 day), while the lowest effect was found with *T. harzianum* (12 day). Spraying with *T. harzianum* and *S. viridosporus* and combination, significantly increased the Latent Period (LP) of *P. triticina*, estimated in seedling stage of both than the control. The highest increase in latent period was obtained with *T. harzianum*+*S. viridosporus* (20 and 21 day) followed by *S. viridosporus* (18 and 18 day), while, the lowest increase in LP was obtained by spraying with *T. harzianum* (16 and 17 day) of Morocco and Sids-1, respectively. Spraying with *T. harzianum* and *S. viridosporus* and combination, significantly resulted in the reduction of rust severity of *P. triticina*, estimated in seedling stage of both than the control. The highest reduction of rust severity with obtained by spraying with *T. harzianum* +*S. viridosporus* (46.7 and 42.9%) followed by *S. viridosporus* (41.3 and 31.4 %), while, the lowest reduction was obtained by spraying with *T. harzianum* of Morocco and Sids-1, respectively.

On chlorophyll a, b and carotenoids mg g^{-1} at adult stage: Data in Table 3 indicated that, the amounts of photosynthetic pigments (chlorophyll a, b) were lower in the inoculated leaves than those in the healthy (un-inoculated) ones. In general, the highest content of these pigments was reported in tilt treated plants and bio inducer under study. The leaves of

Table 1: Effect of spraying the two susceptible wheat genotypes (Morocco and Sids-1) with *Trichoderma harzianum* and *Streptomyces viridosporus* and combination (T+S), under greenhouse condition at seedling stage

	Wheat genotypes							
	Morocco				Sids-1			
Treatments	IT	Reduction (%)	No.	Reduction (%)	IT	Reduction (%)	No.	Reduction (%)
Trichoderma harzianum	3^{b}	25.0	32.2^{c}	33.2	3^{b}	00.0	32.1^{c}	34.6
Streptomyces viridosporus	2^{c}	50.0	25.5^{d}	43.6	2^{c}	33.3	30.2^{d}	38.5
T+S	1^{d}	75.0	23.1^{f}	48.9	2^{c}	33.3	25.2^{d}	48.7
Tilt	0^{e}	100.0	00.0^{g}	100	0^{e}	100	00.0^{g}	100.0
Control	4^{a}	00.0	45.2^{b}	00.0	3^{b}	00.0	49.1^{a}	00.0

Control: Inoculated and free from treatment, Tilt: Fungicide, IT: Infection type , No: No. of pustule/cm^2, values of each column followed by same letter are not significantly different according Duncan multiple range test (p = 0.05)

Table 2: Effect of spraying the two susceptible wheat genotypes, Morocco and Sids-1 with *Trichoderma harzianum* and *Streptomyces viridosporus* and combination (T+S), under greenhouse condition at adult stage

	Wheat genotypes							
	Morocco				Sids-1			
Treatment	IP	LP	RS	Reduction (%)	IP	LP	RS	Reduction (%)
Trichoderma harzianum	12^{e}	16^{e}	48^{d}	36.0	13^{d}	17^{d}	52^{c}	25.7
Streptomyces viridosporus	14^{c}	18^{c}	44^{e}	41.3	14^{c}	18^{c}	48^{d}	31.4
T+S	16^{b}	20^{b}	40^{f}	46.7	18^{a}	21^{a}	40^{f}	42.9
Tilt	00^{h}	00^{h}	00^{g}	100.0	00^{h}	00^{h}	00^{g}	100.0
Control	10^{g}	14^{g}	75^{a}	00.0	11^{f}	15^{f}	70^{b}	00.0

Control: Inoculated and free from treatment, RS: Rust severity (%), IP: Incubation period day LP: Latent period day, values of each column followed by the same letter are not significantly different according to Duncan multiple range test (p = 0.05)

Table 3: Effect of spraying the two susceptible wheat genotypes, Morocco and Sids-1 with *Trichoderma harzianum* and *Streptomyces viridosporus* and combination (T+S) on chlorophyll a, b and carotenoids fresh weight under greenhouse condition at adult stage

	Morocco						Sids-1					
	Un-inoculated (mg g^{-1})			Inoculated (mg g^{-1})			Un-inoculated (mg g^{-1})			Inoculated (mg g^{-1})		
Treatment	a	b	c	a	b	c	a	b	c	a	b	c
Trichoderma harzianum (T)	5.9	2.2	1.6	1.7	1.8	3.0	2.1	3.2	0.6	1.9	1.4	2.6
Streptomyces viridosporus (S)	5.9	2.2	1.8	1.9	2.0	2.7	6.2	2.2	2.2	2.5	2.2	2.7
T+S	5.6	2.4	2.0	2.0	2.2	2.8	6.8	1.9	0.9	0.8	1.0	2.6
Tilt	6.1	1.6	2.5	2.7	3.8	2.2	3.7	3.2	1.4	2.6	1.0	3.0
Control	2.3	4.7	1.3	1.2	1.3	3.8	4.8	2.6	0.9	2.0	1.3	2.8

a: Chlorophylla a, b: Chlorophylla b, c: Carotenoids

the two genotypes (Morocco and Sids-1), was significantly increased after treated with Tilt and bio inducers compare with the inoculated by *P. triticina* only. Data also, revealed that, chlorophyll a content in the leaves of the two genotypes, significant increased in the un-inoculated and inoculated plants with *P. tritici* and treated with bio agents (*S. viridosporus*, *T. harzianum* and *T. harzianum* +*S. viridosporus)*. Chlorophyll a content of Morocco genotype was (5.6, 2.0), (5.9, 1.9) and (5.9, 1.7). Un-inoculated and inoculated plants, respectively, Sids-1 was (6.8, 0.8), (6.2, 2.3) and (2.1, 1.9), un-inoculated and inoculated plants. Chlorophyll b content in the leaves of the two genotypes, significant increased in the un-inoculated with *P. tritici* than the inoculated plants and treated with bio agents (*S. viridosporus*, *T. harzianum* and *T. harzianum*+*S. viridosporus*). Chlorophyll b content of Morocco genotype was (2.5, 2.2), (3.2, 1.4) and (1.9, 1.0), un-inoculated and inoculated, respectively, Sids-1 was (1.9, 1.0), (2.2, 2.5) and (3.2, 1.4), un-inoculated and inoculated plants. Carotenoids content in the leaves of the two genotypes, significantly increased in the inoculated with *P. tritici* than the un-inoculated plants and treated with bio agents (*S. viridosporus*, *T. harzianum* and *T. harzianum*+ *S. viridosporus*). Carotenoids content of Morocco genotype was (2.0, 2.8), (1.8, 2.7) and (1.6, 3.0), un-inoculated and inoculated, respectively, Sids-1 was (0.9, 2.6), (2.2, 2.7) and (0.6, 2.6), un-inoculated and inoculated plants, respectively. Berghaus and Reisener (1985) found that chlorophyll content decreased in wheat plants infected with stem rust pathogen *Puccinia graminis* f. sp. *tritici* in all host-pathogen interaction. Somaya and El-Sharkawy (2014**)**, found that chlorophyll content decreased in wheat plants infected with leaf rust pathogen *Puccinia tritici.*, in all host-pathogen interaction. In addition, the chlorophyll content was most markedly decreased in cultivars of infection type. All the induced materials used in wheat leaf rust disease. Plants have evolved a complex and varied defense mechanisms to protect themselves against pathogen attack. These mechanisms may be constitutive or induced but can fail when a plant is infected by a virulent pathogen, as the pathogen avoids triggering resistance reactions (Pieterse and Van Loon, 1999).

Field experiments

Effect of spraying wheat plants with *Trichoderma harzianum* and *Streptomyces viridosporus* and combination (T+S) on leaf rust severity (%): Data in Table 4 indicated that spraying the two wheat cultivars with the tested bio inducers decreased rust severity (%) at the two locations under this study. The highest reduction of disease severity (%) was obtained by spraying with T+S followed by *S. viridosporus*, while the spraying with *T. harzianum* gave the lowest reduction on the Morocco and Sids-1, respectively. The highest significantly reduction in disease severity (%) in both cultivars was recorded with the spraying with *S. viridosporus*+*T. harzianum*, S. *viridosporus* and *T. harzianum* and combination (T+S), was (40, 45%), (55, 50%) and (60, 60%) in both genotypes Morocco and Sids-1, respectively. The rust severity in plants treated with tilt fungicide was (5, 10%), in both cultivar and was (90, 90%) in untreated plants in, Morocco and Sids-1, respectively at Etay

Table 4: Effect of spraying the two susceptible wheat genotypes, Morocco and Sids-a1 with *Trichoderma harzianum* and *Streptomyces viridosporus* and combination (T+S) on disease parameters at two locations (Kafr Elhamam and Etay El Barood) in 2013-2014 growing seasons

	Kafr Elhamam						Etay El Barood					
	Morocco			Sids-1			Morocco			Sids-1		
Treatment	DS	IT	ACI	DS	IT	ACI	DS	IT	ACI	DS	IT	ACI
Trichoderma harzianum	60^{d}	MS	48^{d}	60^{d}	MS	48^{d}	60^{d}	MS	48^{d}	60^{d}	MS	48^{d}
Streptomyces viridosporus	50^{f}	MS	40^{f}	50^{f}	MS	40^{f}	55^{e}	MS	44^{e}	50^{f}	MS	40^{f}
T+S	40^{h}	MR	16^{h}	35^{i}	MR	14^{i}	40^{h}	MR	16^{h}	45^{g}	MR	18^{g}
Tilt	5^{k}	R	1^{k}	10^{j}	R	2^{j}	5^{k}	R	1^{k}	10^{j}	R	2^{j}
Control	85^{b}	S	85^{b}	80^{c}	S	80^{c}	90^{a}	S	90^{a}	90^{a}	S	90^{a}

DS: Disease severity, IT: Infection Type, ACI: Average of collection infection, S: 1 R: 0.2 MS: 0.8 MR: 0.4, values of each column followed by the same letter are not significantly different according to Duncan multiple range test (p = 0.05)

Table 5: Effect of spraying the two susceptible wheat genotypes, Morocco and Sids-1 with *Trichoderma harzianum* and *Streptomyces viridosporus* and combination (T+S) on some yield parameters at two locations (Kafr Elhamam and Etay El Barood) in 2013 -2014 growing seasons

	Kafr Elhamam								Etay El Barood							
	Morocco				Sids-1				Morocco				Sids-1			
Treatment	S	G	K	T	S	G	K	T	S	G	K	T	S	G	K	T
Trichoderma harzianum	2.5^{j}	2.0^{i}	40^{g}	3.1^{g}	2.51^{i}	2.0^{i}	45^{k}	3.2^{k}	2.6^{h}	2.1^{h}	40^{g}	2.9^{i}	2.7^{g}	2.2^{g}	45^{k}	2.9^{i}
Streptomyces viridosporus	2.7^{g}	2.1^{h}	45^{f}	3.5^{e}	2.76^{k}	2.3^{k}	50^{e}	3.7^{d}	2.7^{g}	2.3^{k}	45^{k}	3.0^{h}	2.9^{e}	2.5^{e}	50^{e}	3.2^{k}
T+S	3.2^{c}	2.5^{e}	55^{d}	3.7^{d}	3.5^{b}	2.9^{b}	55^{d}	3.9^{c}	2.9^{e}	2.5^{e}	60^{c}	3.2^{k}	3.0^{d}	2.7^{d}	60^{c}	3.5^{e}
Tilt	3.5^{b}	2.7^{d}	62^{b}	4.0^{b}	3.7^{a}	3.0^{a}	65^{a}	4.3^{a}	3.2^{c}	2.8^{c}	65^{a}	3.5^{e}	3.5^{b}	2.9^{b}	60^{c}	3.7^{d}
Control	2.0^{i}	1.5^{k}	35^{i}	2.7^{j}	2.3^{k}	1.7^{j}	39^{h}	2.9^{i}	2.0^{i}	1.5^{k}	35^{i}	2.1^{k}	2.7^{g}	2.0^{i}	40^{g}	2.7^{j}

S: Spike weight, G: Grain spike Weight, K: 1000 Kernel weight, T: Test wheat**,** values of each column followed by the same letter are not significantly different according to Duncan multiple range test (p = 0.05)

Elbarood. However, data of Kafr El Hamam, showed that, the highest significantly reduction in disease severity (%) in both cultivars was recorded with the spraying with *S. viridosporus+ T. harzianum*, *S. viridosporus* and *T. harzianum* and combination (T+S), were (40, 35 %), (50, 50%) and (60, 60%) in both cultivars Morocco and Sids-1, respectively. The rust severity in plants treated with tilt fungicide was (5, 10%), in both genotypes and was 85 and 80% in untreated plants in, Morocco and Sids-1, respectively. The inhibition of rust severity by the antagonistic species when they were applied to leaves may be due to inhibitory substances produced by these bioagents or due to competition for nutrients and space or induced the plant to produced defense chemical compounds against their pathogen. These results are in a good agreement with those obtained by Biles and Hill (1988), who found that *T. harzianum*, was effective in reducing sporulation capacity of fungus *Cochliobolus sativus* on excised wheat seedling leaves. Also, Eldoksch *et al.* (2001) stated that under greenhouse condition of plant guard (*Trichoderma harzianum*) and yeast (*Saccharomyces cerevisiae*) gave reasonable control of leaf rust severity with disease reduction percentages of 64.29 and 19.14 %, respectively. Verma *et al*. (2007) indicated that several *Trichoderma* spp., have proved to be effective mycoparasites. Bochow and Fritzche (1991**)** reported that the effectiveness of *Streptomyces* isolates against *Phytophthora infestans* was due to the induction of host resistance by the antagonists. *Streptomyces* spp. has been implicated as effective biological control agents (El-Abyad *et al*., 1993; Etebarian *et al*., 2003). Ningthoujam *et al*. (2009) screened several actinomycetes against some major rice fungal pathogens such as *Curvularia oryzae*, *Pyricularia oryzae*, *Bipolaris oryzae* and *Fusarium oxysporum* and showed potent antagonistic activities in dual culture assay. El-Naggar *et al*. (2012) studied the effect of *Trichoderma harzianum*, *S. plicatus* and *Pseudomonas fluorescence* to induce elevated levels of resistance in grape against *Plasmopara viticola* the causal organism of downy mildew of grape and they found that the greatest reduction for disease severity was observed in response to treatment with *T. harzianum* followed by *S. plicatus*. El-Banoby *et al.* (2013) found that the highest reduction of *Botrytis fabae* growth area on faba bean was obtained with *Trichderma harzianum*, *Bacillus subtilis* and *Ampelomyces qusisqualis* which reduced the growth area by 79.3, 62.6 and 60.3%, respectively. Nourozian *et al.* (2006) evaluated *Bacillus subtilis*, *Pseudomonas fluorescens* and *Streptomyces* sp., as potential biological agents for control of Fusarium Head Blight (FHB) caused by *Fusarium graminearum* and found that *Streptomyces* sp., reduced disease severity of FHB 21 day after inoculation, also the yield of wheat from plants treated with *Streptomyces* sp. and *F. graminearum* was significantly greater than controls inoculated with the pathogen alone, treatments with *Streptomyces* spp., alone increased the yield of wheat compared to the un-inoculated controls. Zarandi *et al*. (2009) found that *Streptomyces sindeneusis* have antagonistic activity against *Magnaporthe oryzae* the causal agent of rice blast.

Effect of spraying the wheat plants with *Trichoderma harzianum* and *Streptomyces viridosporus* and combination (T+S) on Yield parameters: Spraying wheat plants of the two genotypes with *S. viridosporus+T. harzianum*, *S. viridosporus* and *T. harzianum*, significantly increased both spike weight (g), grain spike weight/g and 1000 kernel weight g), compared with non sprayed (control) plants

(Table 5) in both genotypes, in Etay El-Barood and Kafr El-Hamam. *Trichoderma* and *Streptomyces* isolates significantly increased the yield of wheat, perhaps due to the production of growth regulators such as gibberellin-like substance and auxins (Katznelson and Cole, 1965). Studies even show that use of *Streptomyces* enhances growth of the crop plants (Brown, 1974). Haggag *et al.* (2014) found that *Pseudomonas putida*, methyl jasmonate and chitosan showed a significantly greater decrease the diseases of wheat to some foliar diseases as leaf blotches, powdery mildew and leaf rust incidence and increased of total phenols, peroxidase, chitinase and total soluble protein in wheat as well as plant growth and yield, also showed significant increase in grain yield of wheat was observed with the application of different soil and foliar products management practices.

CONCLUSION

The results of the study concluded that the application of bioinducers could be used to overcome the negative effect against wheat leaf rust disease. In this connection, these bioinducers treatments have best beneficial effects as compared to chemical fungicide. Application of bioinducers is applicable, safe and cost effective method for controlling rust wheat diseases. Also, the use of bioinducers in agriculture could be a suitable alternative for integration in disease control systems and do not leave a toxic residue in the product.

REFERENCES

Abd El-Ghany, T.M., M.E.T. Eman and H.H. El-Sheikh, 2009. Efficacy of fungal rust disease on willow plant in Egypt. Aust. J. Basic Appl. Sci., 3: 1527-1539.

Berghaus, R. and H.J. Reisener, 1985. Changes in photosynthesis of wheat plants infected with wheat stem rust (*Puccinia graminis* f.Sp. *tritici*). J. Phytopathol., 112: 165-172.

Biles, C.L. and J.P. Hill, 1988. Effect of *Trichoderma harzianum* on sporulation of *Cochliobolus sativus* on excised wheat seedling leaves. Phytopathology, 78: 656-659.

Bochow, H. and S. Fritzche, 1991. Induction of phytoalexin biosynthesis by culture filtrates of bacterial antagonists. Bulletin-SROP, 14: 158-161.

Bonjar, G.H.S., M.H. Fooladi, M.J. Mahdavi and A. Shahghasi, 2004. Broadspectrim, a novel antibacterial from *Streptomyces* sp. Biotechnology, 3: 126-130.

Brown, M.E., 1974. Seed and root bacterization. Annu. Rev. Phytopathol., 12: 181-197.

Dhingra, O.D. and J.B. Sinclair, 1995. Basic Plant Pathology Methods. CRC Press, USA., pp: 287-296, 390-391.

Djonovic, S., M. Pozo, L.J. Dangott, C.R. Howell and C.M. Kenerley, 2006. Sm1, a proteinaceous elicitor secreted by the biocontrol fungus *Trichoderma virens* induces plant defense responses and systemic resistance. Mol. Plant Microbe Interact., 19: 838-853.

El-Abyad, M.S., M.A. El-Sayed, A.R. El-Shanshoury and N.H. El-Batanouny, 1993. Inhibitory effects of UV mutants of *Streptomyces corchorusii* and *Streptomyces spiroverticillatus* on bean and banana wilt pathogens. Can. J. Bot., 71: 1080-1086.

El-Banoby, F.E., M.A. Abd-Alla, I.H. Tolba, A.A. Morsy, G. El-Gamal-Nadia and M.S.A. Khalil, 2013. Biological control of chocolate spot disease of Faba Bean using some bioagents unde field conditions. J. Applied Sci. Res., 9: 4021-4029.

El-Naggar, M.A., M.D.F. Alkahtani, M.A. Yassin and K.M. Morsy, 2012. New approach to acquired resistance enhancement against *Plasmopara viticola* using different biotic inducers. J. Plant Sci., 7: 67-77.

Eldoksch, H.A., M.F. Atteia and S.M.H. Abdel-Moity, 2001. Management of brown leaf rust, *Puccinia recondita* of wheat using natural products and biocontrol agents. Pak. J. Biol. Sci., 4: 550-553.

Etebarian, H.R., E.S. Scott and T.J. Wicks, 2003. Evaluation of streptomyces strains as potential biological control agents of *Phytophthora erythroseptica*. Iran. J. Plant Pathol., 39: 173-174.

Haddad, F., L.A. Maffia, S.G. Eduardo, E.S.G. Mizubuti and H. Teixeira, 2009. Biological control of coffee rust by antagonistic bacteria under field conditions in Brazil. Biol. Control, 49: 114-119.

Haggag, W.M., M.M. Hussein, M.M. Tawfik and S.F. El Habbasha, 2014. Enhancement of wheat resistant to diseases by elicitors. Int. J. Sci. Res., 3: 1526-1530.

Ikediobi, C.O., 1985. Biochemistry and Physiology of Yam Storage. In: Advances in Yam Research: The Biochemistry and Technology of the Yam Tuber, Osuji, G. (Ed.). Biochemical Society of Nigeria, Nigeria, pp: 109-141.

Jeffries, P., 1997. Mycoparasitism. In: The Mycota: A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research, Wicklow, D.T., K. Esser, P.A. Lemke and B.E. Soderstrom (Eds.). Springer, New York, USA., pp: 149-164.

Katznelson, H. and S.E. Cole, 1965. Production of gibberellin-like substances by bacteria and actinomycetes. Can. J. Microbiol., 11: 733-741.

Kuberan, T., R.S. Vidhyapallavi, A. Balamurugan, P. Nepolean, R. Jayanthi and R. Premkumar, 2012. Isolation and biocontrol potential of phylloplane *Trichoderma* against *Glomerella cingulata* in tea. J. Agric. Technol., 8: 1039-1050.

Long, D.L., A.P. Roelfs and J.J. Roberts, 1992. Virulence of *Puccinia recondita* f. sp. *tritici* in the United States during 1988-1990. Plant Dis., 76: 495-499.

Mackinney, G., 1941. Absorption of light by chlorophyll solutions. J. Biol. Chem., 104: 315-322.

Marshall, D., 1992. Virulence of *Puccinia recondita* in Texas from 1988 to 1990. Plant Dis., 76: 296-299.

Ningthoujam, D.S., S. Sanasam, K. Tamreihao and S. Nimaichand, 2009. Antagonistic activities of local actinomycete isolates against rice fungal pathogens. Afr. J. Microbiol. Res., 3: 737-742.

Nourozian, J., H.R. Etebarian and G. Khodakaramian, 2006. Biological control of *Fusarium graminearum* on wheat by antagonistic bacteria. Songklanakarin J. Sci. Technol., 28: 29-38.

Omura, S., 1986. Philosophy of new drug discovery. Microbiol. Rev., 50: 259-279.

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.

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

Pieterse, C.M.J. and L.C. Van Loon, 1999. Salicylic acid-independent plant defence pathways. Trends Plant Sci., 4: 52-58.

Roatti, B., M. Perazzolli, C. Gessler and I. Pertot, 2013. Abiotic stresses affect *Trichoderma harzianum* T39-induced resistance to downy mildew in grapevine. Phtopathology, 103: 1227-1234.

Robinson, J.M. and S.J. Britz, 2000. Tolerance of a field grown soybean cultivar to elevated ozone level is concurrent with higher leaflet ascorbic acid level, higher ascorbate-dehydroascorbate redox status and long term photosynthetic productivity. Photosynth. Res., 64: 77-87.

Roelfs, A.P., R.P. Singh and E.E. Sari, 1992. Rust Diseases of Wheat: Concepts and Methods of Disease Management. CIMMIYT, Mexico, DF., USA., ISBN: 9686127704, pp: 81.

Snedecor, G.W. and W.G. Cochran, 1990. Statistical Methods. 8th Edn., Iowa State University Press, Ames, Iowa, USA.

Somaya, T. and H.H.A. El-Sharkawy, 2014. Effect of some plant essential oils against wheat leaf rust caused by *Puccinia triticina* f. sp. *tritici*. Egypt. J. Biol. Pest Control, 24: 211-216.

Stakman, E.C., D.M. Stewart and W.O. Loegering, 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici*. United States Department of Agriculture, Agricultural Research Service, No. E617, USA. http://naldc.nal.usda.gov/download/CAT10243018/PDF.

Verma, M., S.K. Brar, R.D. Tyagi, R.Y. Surampalli and J.R. Valero, 2007. Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. Biochem. Eng. J., 37: 1-20.

Zarandi, M.E., G.H.S. Bonjar, F.P. Dehkaei, S.A.A. Moosavi, P.R. Farokhi and S. Aghighi, 2009. Biological control of rice blast (*Magnaporthe oryzae*) by use of *Streptomyces sindeneusis* isolate 263 in greenhouse. Am. J. Applied Sci., 6: 194-199.