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Effect of Resistance Inducing Chemicals on Wheat Leaf Rust Caused by *Puccinia triticina*

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The efficiency of some inducing resistance chemicals (IRCs) and systemic fungicide were evaluated *in vitro* on suppressing the germination of uredospores of *Puccinia triticina* as well as controlling leaf rust of wheat under greenhouse and field conditions.

Different degrees of inhibition to the germinated uredospores of *P. triticina* were occurred when sprayed the tested IRCs, *i.e.* ascorbic acid, oxalic acid, sodium salicylate, di-basic potassium phosphate, salicylic acid and benzothiadiazole (BTH) and the systemic fungicide, *i.e.* propiconazole (Tilt). Furthermore, the used systemic fungicide was more efficient than IRCs in this regard.

Spraying two wheat varieties (Giza168 and Giza139) with the tested IRCs and fungicides reduced significantly the severity of leaf rust disease compared with control treatment under greenhouse conditions. On the other hand, spraying the two wheat cultivars under field conditions during 2011/2012 growing seasons in two Governorates (El-Sharkiya and Kafr El-Sheikh) with the tested IRCs and fungicides resulted in a significant decrease in the severity of the disease compared with control treatment. In addition, the tested IRCs were low disease efficient comparing to treatments sprayed with the fungicide. All the tested IRCs resulted in a significant increment in yield components, while the fungicide (Tilt) show low efficient in yield components as compared to the control treatment.

Keywords: Chemical control, leaf rust disease, resistance inducing chemicals, systemic fungicides and wheat.

Leaf rust, caused by Puccinia triticina, is the most common rust disease of wheat (*Triticum aestivum* L.). The fungus is an obligate parasite capable of producing infectious uredospores as long as infected leaf tissue remains alive. Uredospores can be wind-disseminated and infect host plants hundreds of kilometres from their source plant, which can result in wheat leaf rust epidemics on a continental scale.

Puccinia triticina was introduced with wheat cultivation in the early 17th century (Chester, 1946), but was often overlooked as an important disease of wheat as it did not appear to affect grain quality as much as other diseases such as stem rust (Leonard and Szabo, 2005) or Fusarium head blight (Goswami and Kistler, 2004).

Yield losses in wheat from *P. triticina* infections are usually the result of decreased numbers of kernels per head and lower kernel weights. *Puccinia triticina* is now recognized as an important pathogen affected wheat production worldwide, causing significant yield losses over large geographical areas (Kolmer, 2005; Marasas *et al*., 2004 and Roelfs *et al*., 1992).

To protect themselves from disease, plants have evolved sophisticated inducible defence mechanisms in which the signal molecules salicylic acid, jasmonic acid and ethylene often play crucial roles. Elucidation of signalling pathways controlling induced disease resistance is a major objective in research on plant-pathogen interactions.

Control of cereal diseases is carried out by fungicides treatments. However, the application of fungicides is limited because of the development of pathogenic strains with fungicide resistance, the action on human health and the environment (Wilson *et al*., 1994). In some cases the use of alternative control like benzothiadiazole (BTH) and other chemical inducers of resistance seem important (Bayoumi and Hafez, 2006 and Hafez *et al*., 2008). Salicylic acid (SA), which exists in many plant organs, is an endogenous signal molecule inducing plant defence response and reducing population of pathogens (Vlot *et al*., 2009). Exogenous applications of SA in non-toxic concentrations were effective in the regulation of biotic and abiotic stresses (Ananieva *et al*., 2004; Eraslan *et al*., 2007; Janda *et al*., 2007 and Xu and Tian, 2008).

Benzothiadiazole (BTH) is a chemical inducer of resistance and a functional analogue to SA (Görlach *et al*., 1996). It induces systemic acquired resistance (SAR) during the activation of signal transduction pathway, while it has no anti-microbial properties (Görlach *et al*., 1996). Investigators showed that BTH and other chemical inducers protected several plant species against viral, bacterial and fungal pathogens (Bán *et al*., 2004; Hafez and Király, 2004; Bayoumi and Hafez 2006 and Körösi *et al*., 2009). It was shown that BTH suppressed the grey mould disease caused by *B. cinerea* in strawberry (Terry and Joyce, 2000), induced resistance against *Penicillium expansum* in peach (Liu *et al*., 2005) and pear fruits (Cao *et al*., 2005) during postharvest storage. Benzothiadiazole (BTH) protected rock melons, hami melons and passion fruits (Huang *et al*., 2000 and Willingham *et al*., 2002). It also was protected white pepper fruits from *Botrytis cinerea* (Hafez, 2010).

Some chemical compounds, *i.e.* salicylic acid (SA); di-basic potassium phosphate (K2HPO4); oxalic acid, Ascorbic acid, Sodium salicylate and benzothiadiazole (BTH) have been shown to induce resistance in plants (Ata *et al*., 2008 and Ragab *et al*., 2009).

Materials and Methods

Effect of some inducing resistance chemicals (IRCs) and fungicide on urediospores germination:

Tested inducing resistance chemicals (IRCs), *i.e.* ascorbic acid, oxalic acid, sodium salicylate, di-basic potassium phosphate, salicylic acid and BTH and the systemic fungicide propiconazole (Tilt), were evaluated for their inhibitory effect on the *in vitro* germination of *Puccinia triticina* uredospores (the casual organism of wheat leaf rust).

In this regard, stock solution was prepared from the tested IRCs (depending on their molecular weight) and fungicide (depending on its active ingredient). Two concentrations (250 and 200 ppm) of IRCs as well as (80 and 200 ppm) of BTH were prepared and individually sprayed using atomizer on clean glass slides and left to air dry. Glass slides were put on glass rods in Petri-dishes containing distilled water (90% relative humidity). Uredospores of *P. triticina* obtained from Cereal Dis. Dept., Plant Pathol. Res. Inst., ARC. Mixed spores were prepared by adding 1g of uredospores to 20g of Talc powder. Tested uredospores suspension was prepared under aseptic conditions, by adding 1g of uredospores plus Talc powder to 20ml of distilled water, and adjusted using a haemocytometer slide to concentration of 105 spores/ml. One drop of cotton blue stain (0.5%) was added to the spore suspension in order to clear germ tube during count of germination percentage of uredospores. One drop of the stained spore suspension was put on each glass slide and two slides were put in each a Petri-dish containing sterilized distilled water over U shaped glass rod. Five Petri-dishes were prepared for each treatment, and then incubated in dark at 22±2°C. The averages percentages of germinated uredospores were assayed using light microscope 3, 6, 12 and 24 h after incubation (Reeser *et al.,* 1983).

Greenhouse experiments:

Four IRCs, *i.e.* ascorbic acid, oxalic acid, sodium salicylate, di-basic potassium phosphate, salicylic acid and BTH and the systemic fungicide, *i.e.* propiconazole (Tilt), were individually tested for their effects on the severity of wheat leaf rust caused by artificial inoculation by *P. triticina* uredospores under greenhouse conditions, in order to choose the most efficient IRCs and/or fungicide.

Ten grains of wheat, vars. Giza 139 (highly susceptible) and Giza 168 (resistant), were sown under greenhouse conditions in plastic pots (25-cm-diam.) at the rate of 10 seeds/pot in the beginning of December when received normal irrigation and fertilization. Three pots were used as replicates for each particular treatment. One week old seedlings, of each tested variety, were separately sprayed with each of the tested chemical inducers, then after 24h plants were uniformly artificially inoculated with fresh uredospores and then the pots were kept under conditions of high humidity for 24 hours. Disease severity was assessed weekly and the average was recorded. These experiments were conducted in the Cereal Dis. Dept., Plant Pathol. Res. Inst., ARC, Giza.

Field experiments:

Wheat grains (vars. Giza 139 and Giza 168) were sown under field conditions, in plots (2×2.5m) with the rate of 40 g/plot, in the beginning of December and received normal irrigation and fertilization. After 70 days, growing plants were uniformly inoculated by injecting leg with the prepared spore suspension and also by dusting with the mixed leaf rust spores. After 7 days of inoculation, wheat plants were separately sprayed with each of the tested chemical inducers and the fungicide. These treatments were repeated every 15 days for 3 times.

Disease assessment:

Both artificially and naturally inoculated plants were carefully examined to estimate the severity of infection by leaf rust depending on the devised and modified scale (0-4) in seedling stage also plant reaction (Infection type) expressed in five types in adult stage, *i.e.* Immune = 0, Resistant = (R), Moderately resistant = (MR), Moderately susceptible = (MS) and Susceptible = (S) as described by Stakman *et al*. (1962).

Effect of the tested chemical inducers on yield components:

At the end of May, formed grains were harvested and weighed for each individual plot.

Statistical analysis:

Obtained data were statistically analyzed using the standard procedures for split designs as mentioned by Snedecor and Cochran (1972).

Results

Effect of some inducing resistance chemicals (IRCs) and fungicide on urediospores germination:

All the tested IRCs and fungicide resulted in different degrees of suppression to the uredospores germination of the causal fungus compared with control treatment. This reduction was gradually increased by increasing the used concentration (Table 1). However, the fungicide (Tilt) was more efficient than IRCs in this respect, where no one of the tested IRCs caused complete inhibition to the germinated urediospores even at 250 ppm. On the other hand, BTH at 200 ppm recorded the highest effective value on the IRCs caused more than 50% inhibition of urediospore germination after 24 h.

Table 1. Effect of different concentrations of inducing resistance chemicals (IRCs) and fungicide (Tilt) on uredospores germination of *P. triticina*

Chemical inducer	Concentration	Germination (%) of *Puccinia triticina* uredospores after incubation at 22±2°C for;			
		3 h	6 h	12 h	24 h
Ascorbic acid	200 ppm	31.0	38.2	51.0	58.6
	250 ppm	28.0	37.5	50.7	57.5
Oxalic acid	200 ppm	30.0	38.0	49.4	54.5
	250 ppm	28.0	37.0	48.6	54.0
Sodium salicylate	200 ppm	27.5	35.6	46.8	52.6
	250 ppm	26.0	34.2	46.5	50.5
Di-basic potassium phosphate	200 ppm	41.3	50.9	61.2	69.3
	250 ppm	40.1	50.7	60.5	68.0
Salicylic acid	200 ppm	32.6	41.2	53.8	59.4
	250 ppm	33.0	40.0	53.0	58.8
Benzothiadiazole (BTH)	80 ppm	24.3	33.4	46.4	48.5
	200 ppm	21.6	32.0	44.6	47.5
Tilt (fungicide)	25ml/100 l	0.0	0.0	0.0	0.0
Control		52.0	72.7	87.2	93.0
L.S.D. at 5% for:	Treatment (T)	.000	.000	.000	.000
	Concentration (C)	.061	.033	021	.007
	(T×C)	.010	.417	.036	.117

Greenhouse experiments:

The tested IRCs and fungicide resulted in significant reduction to the severity of leaf rust on both tested wheat varieties (Giza168 and Giza139) (Table 2). In addition, the fungicide (Tilt) was more efficient in disease severity, than IRCs on both varieties. BTH at 200 ppm and oxalic acid at 250 ppm show high significant reduction of the disease severity as compared with the control on both varieties, while di-basic potassium phosphate showed the lowest reduction of disease severity as compared with the control on both varieties.

Table 2. Effect of spraying different concentrations of inducing resistance chemicals (IRCs) and fungicide (Tilt) on the severity of wheat leaf rust on two wheat varieties under greenhouse conditions

Treatment	Concentration	Disease severity	
		Giza139	Giza168
Ascorbic acid	200 ppm	3	2
	250 ppm	2	1
Oxalic acid	200 ppm	2	1
	250 ppm	1	0
Sodium salicylate	200 ppm	3	2
	250 ppm	2	2
Di-basic potassium phosphate	200 ppm	4	3
	250 ppm	3	3
Salicylic acid	200 ppm	3	2
	250 ppm	2	2
Benzothiadiazole (BTH)	80 ppm	1	0
	200 ppm	1	0
Tilt (fungicide)	25ml/100 l	0	0
Control		4	4

Field experiments:

Effect of some inducing resistance chemicals (IRCs) and fungicide on disease severity and disease incidence under field conditions:

Spraying the fungicide (Tilt) on wheat plants was more efficient in reducing the natural infection by leaf rust on both varieties (Giza168 and Giza139) and both location (El-Sharkiya and Kafr El-Sheikh) (Table 3). In addition, the sprayed IRCs, *i.e.* BTH at 200 ppm and ascorbic acid at 250 ppm recorded high efficiency in reducing disease severity and disease incidence on Giza139 and BTH at 200 ppm and sodium salicylate at 250 ppm on Giza168 in El-Sharkiya, while BTH at 200 ppm and sodium salicylate at 250 ppm recorded high efficiency in reducing disease severity and disease incidence on the both tested wheat varieties in Kafr El-Sheikh. On the other hand, di-basic potassium phosphate at 250 and 200 ppm recorded low efficiency in reducing disease severity and disease incidence on both tested wheat varieties and both locations. In addition, significant differences were detected between the values of disease severity and disease incidence due to the effect of the location.

Table 3. Effect of spraying different concentrations of inducing resistance chemicals (IRCs) and fungicide (Tilt) on the severity of leaf rust under field conditions in two Governorates during 2011/12 growing season

Treatment	Concentration	Disease severity and diseases incidence			
		El-Sharkiya Governorate		Kafr El-Sheikh Governorate	
		Giza139	Giza168	Giza139	Giza 168
Ascorbic acid	200 ppm	5Mr *	5Mr	15Mr	10Mr
	250 ppm	Tr-Mr	5Mr	10Mr	5Mr
Oxalic acid	200 ppm	Tr-MS	5Mr	5MS	10Mr
	250 ppm	5Mr	Tr-Mr	10Mr	5Mr
Sodium Salicylate	200 ppm	10Mr	5R	10Mr	5Mr
	250 ppm	5Mr	5R	5Mr	10R
Di-basic potassium phosphate	200 ppm	10S	Tr-MS	20S	20MS
	250 ppm	10S	Tr-MS	20S	20MS
Salicylic acid	200 ppm	15MS	10Mr	20MS	20Mr
	250 ppm	5MS	5Mr	5MS	10Mr
BTH	80 ppm	5Mr	Tr-R	5Mr	10R
	200 ppm	Tr-Mr	Tr-R	5Mr	5R
Tilt fungicide)	25ml/100 l	0	0	0	0
Control		50S	5MS	70S	10MS

*Immune = 0, Resistant = (R), Moderately resistant = (MR), Moderately susceptible = (MS) and Susceptible = (S)

Effect of some inducing resistance chemicals (IRCs) and fungicide on field yield components.

The effect of spraying plant with chemical inducers at different concentrations on yield components (weight of grains per plot) was studied. Data presented in Table (4) show that all concentrations of the tested compounds significantly increased weight grains per plot than the control in both varieties but not in-between particular compound(s) and others. All yield components were always significantly increased in most cases by increasing concentration of each compound in both varieties in El-Sharkiya and Kafr El-Sheikh. Benzothiadiazole (BTH) (80ppm and 200ppm) and Sodium salicylate (200ppm and 250ppm) gave the highest yield components than the others on the both varieties and both locations. In contract, the least effective compounds that increased yield components were the Tilt (fungicide) and di-basic potassium phosphate (250ppm and 200ppm).

Table 4. Effect of spraying different concentrations of inducing resistance chemicals (IRCs) and fungicide (Tilt) on yield components under field conditions in two Governorates during 2011/12 growing season

Treatment	Concentration	Yield components (Kg/plot)			
		El-Sharkiya Governorate		Kafr El-Sheikh Governorate	
		Giza 139	Giza 168	Giza 139	Giza 168
Ascorbic acid	200 ppm	2.34	2.73	2.2	2.52
	250 ppm	2.38	2.77	2.24	2.58
Oxalic acid	200 ppm	2.02	2.67	1.88	2.44
	250 ppm	2.04	2.69	1.94	2.5
Sodium Salicylate	200 ppm	2.43	2.93	2.24	2.68
	250 ppm	2.47	2.94	2.32	2.7
Di-basic potassium phosphate	250 ppm	1.94	2.32	1.82	2.2
	200 ppm	1.92	2.3	1.78	2.3
Salicylic acid	200 ppm	2.29	2.82	2.12	2.52
	250 ppm	2.32	2.83	2.2	2.48
BTH	80 ppm	2.52	2.97	2.32	2.54
	200 ppm	2.54	2.98	2.28	2.66
Tilt (fungicide)	25ml/100 l	1.86	2.13	1.56	1.96
Control		1.35	1.94	1.25	1.76
L.S.D. at 5%		.000		.000	

Discussion

During the last decades, the world is suffering great pollution by many pollutants including agrochemicals including pesticides and fungicides. Therefore, the current strategy of pest management depends on using alternative methods other than pesticides, fungicides and/or using these chemicals at the first periods of plant growth prior to fruit maturity. Hence, this work aimed to using IRC_s (as safe chemicals) in alternation with systemic fungicides, in which the fungicides spray at the first period of infection (before green pods harvesting) to minimize the infection to low level for a period of about 45 days (the time of flowering and green pods formation until pre-maturity) then spraying IRCs just before and during harvesting the green pods in order to obtain green pods of permitted ratio and/or free from fungicides residue.

All the tested IRCs and fungicides caused different degrees of suppression to the germinated urediospores of the causal fungus compared with control treatment. This suppression was gradually increased by increasing the tested concentration. However, fungicides were more efficient than IRCs in this respect, where none of the tested IRCs caused complete inhibition to the germinated uredospores even at 250 ppm.

The obtained data of pot experiment in greenhouse experiment showed that the tested IRC_s, *i.e.* BTH, ascorbic acid and oxalic acid caused significant reduction to the disease in comparison with the control treatment and fungicides were more efficient than IRCs. It is well known that fungicides, especially systemic ones, are more efficient in management of many fungal diseases (Mersha *et al*., 2012 and Richardson, 2006). Also, IRCs were reported as alternative and/ or safe management of many diseases (Sharma *et al.,* 2011, Abada *et al.,* 2008 and Abdel-Monaim *et al.,* 2012).

It has been found from field experiment that spraying wheat plants three times with Tilt as fungicide or sprays three times with any of BTH, ascorbic acid or oxalic acid as IRCs, resulted in a significant reduction to the disease as compared with unsprayed (control) plants. However, the IRCs treatments were low efficiency in the diseases severity when compared with the tested fungicides. On the other hand, spraying wheat plants with any of BTH, ascorbic acid or oxalic acid as IRCs, resulted a significant increasing of yield components as compared with unsprayed (control) plants. However spraying wheat plants with the tested IRCs showed significant increasing of yield components as compared with Tilt fungicide. Moreover the produced new shoots are of low fungicides residue, which the long period after the latter fungicides spray is capable to cause metabolic changes to be another safe compounds or became unpoisoned.

The reduction in wheat leaf rust may be due to the effect of the tested fungicides and RICs. In addition, the role of fungicides in reducing the disease is well known (Richardson, 2006) and the role of RICs is explained by many hypothesis, where acquired resistance induced by restricted infection is not due to a specific component of the pathogen, but rather to gradual appearance and persistence of a level of metabolic disturbance leading to stress on the host.

It has been mentioned that inducing acquired resistance is persistent and generally is pathogen nonspecific (Doubrava *et al.,* 1988). Moreover, Larcke (1981) reported that unlike elicitors of phytoalexins accumulations, which are elicited at the site of application, may be responsible for localized protection and induces systemic acquired resistance that sensitizes the plant response rapidly after infection. These responses inducing phytoalexins accumulation and lignifications and induce enhance activities of chitinase and β-glucanase (Metranx and Boller, 1986, Abd El-Kareem *et al.,* 2001). Kessmann *et al.* (1994) reported that the mechanism of systemic acquired resistance is apparently multifaceted, likely resulting in stable broad spectrum disease control and they could be used preventatively to bolster general plant health, resulting in long lasting protection. In addition, Vernooij *et al.* (1994) mentioned also that salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance to plant pathogens, but is required in signal transduction. So, resistance might be correlated with the production of oxidative enzymes in the treated healthy and diseased plant tissues (Wen *et al.,* 2005). In this respect, Melo *et al.* (2006) mentioned that polyphenoloxidase and peroxidase are enzymes of broad spectrum among plants catalyze the hydroxylation of monophenols to O-diphenols and their oxidation to O-diquinones. They added that quinines are highly reactive molecules that can spontaneously complex various types of molecules into large types

The use of RIC_s was previously used as alternative method for controlling many rust diseases including leaf rust of wheat (Sillero *et al*., 2012; Cawood *et al*., 2010 and Abada *et al*., 2008).

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تأثير بعض الكيماويات المستحثة لمقاومة مرض صدأ الاوراق فى القمح المتسبب عن الفطر *Puccinia triticina*

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تم اختبار فعالية بعض الكيماويات المستحثة للمقاومة وكذلك أحد المبيدات الفطرية الجهازية (التلت) علي تثبيط إنبات الجراثيم اليوريدية لمكافحة مرض صدأ الأوراق فى القمح المتسبب عن فطر بكسينيا تريتيسينا في تجارب أجريت بالمعمل والصوبة والحقل.

أحدثت كل الكيماويات المستحثة للمقاومة وهى حامض اسكوربيك ، حامض الاوكساليك ، فوسفات البوتاسيوم الثنائية القاعدية، حامض السالسيلك ، بنزوثيديازول بالاضافة إلى احد المبيدات الفطرية الجهازية (التلت) درجات مختلفة من التثبيط لإنبات الجراثيم اليوريدية. بالإضافة لذلك ، فقد كان المبيد الفطرى أكثر فعالية في التأثير على انبات الجراثيم عن الكيماويات المستحثة للمقاومة.

أوضحت نتائج تجربة الصوبة أن رش نباتات صنفي القمح (جيزة ١٣٩ وجيزة ١٦٨) بالكيماويات المستحثة للمقاومة والمبيد الفطرى (التلت) تسببت في إحداث انخفاض معنوي للمرض مقارنة بنباتات المقارنة.

أوضحت تجارب الحقل خلال موسم ٢٠١١، ٢٠١٢ التى أجريت فى كل من محافظتي (كفر الشيخ والشرقية) أن رش نباتات صنفى القمح (جيزة ١٣٩ وجيزة ١٦٨) بالكيماويات المستحثة للمقاومة والمبيد الفطرى (التلت) تسببت في إحداث انخفاض معنوي لشدة الإصابة بمرض صدأ الاوراق مقارنة بنباتات المقارنة بالاضافة إلى أن جميع الكيماويات المستحثة المستخدمة أظهرت انخفاض معنوي قليل لشدة الإصابة بمرض صدأ الأوراق مقارنة بالنباتات المعاملة بالمبيد الفطرى (التلت).

أدت المعاملة بالكيماويات المستحثة والمبيد الفطرى (التلت) زيادة معنوية فى انتاج المحصول (الحبوب) مقارنة بنباتات الكنترول بالإضافة إلى أن النباتات المعاملة بالمبيد الفطرى (التلت) أدت زيادة معنوية قليلة فى انتاج المحصول (الحبوب) مقارنة بالنباتات المعاملة بالكيماويات المستحثة.