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Application of some Epidermal Coating Antitranspirant Products for Controlling Wheat Leaf Rust Caused by *Puccinia triticina*

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This study aimed to evaluate the effect of spraying three epidermal coating antitranspirant products namely kaolin (Ka), magnesium carbonate ($MgCO_3$) and fulvic acid (FA) at different concentrations before rust inoculation on controlling wheat leaf rust disease. Results in seedling stage indicated that higher concentration of kaolin (6%) followed by fulvic acid (0.06) increased incubation period of cv. Sakha 93 compared with control treatment. However, opposite result was observed in case of cv. Sids 1, where $MgCO_3$ (6%) was the best one. The higher three concentrations of tested products decreased the number of pustules/cm^2 or seedling leaf area. Fulvic acid was the most effective one compared with kaolin and $MgCO_3$. In case of pustule size, kaolin reduced the pustule size followed by $MgCO_3$, while fulvic acid was the lowest one comparing with infected control. Scanning electron micrographs of wheat seedling leaves sprayed 24 h before inoculation showed deformed uredospore shape, abnormal germ tube and appressoria. Also, alter topography of wheat leaf surface compared with untreated leaves. Cross section in sprayed fulvic acid treatment showed increase in the thickness of spongy cell walls comparing with control treatment. Results obtained on wheat cv. Sids 1 adult plants sprayed 3, 7 and 10 days before inoculation (dbi) indicated that none of the tested products used significantly increased incubation period or latent period. On the other hand, concentrations and days before inoculation significantly affected these two parameters. Data obtained also indicated that area under disease progress curve (AUDPC) was decreased by application of these products and their concentrations, while number of pustules/cm^2 significantly affected by the tested product, dbi and the interaction between them. Also, these products changed the infection type from susceptible to moderately susceptible, moderately resistant or resistant according to the product used, concentration and dbi. These results were confirmed by decreasing rust severity and average coefficient of infection in different degrees according to the product used, concentration and dbi. Antitranspirant increased the plant pigments in treated plants compared to untreated infected ones.

Keywords: Antitranspirant, fulvic acid, kaolin, leaf rust, magnesium carbonate, *Puccinia triticina* and wheat.

Wheat (*Triticum aestivum* L.) is liable to attack by many fungal diseases including rusts, smuts and powdery mildew (Schafer, 1987). Wheat rusts till now are still the main diseases, which limit the productivity of most cultivars (Saari and Prescott, 1985 and Anikster *et al*., 1997). The disease caused by *Puccinia triticina* Eriks. has been considered as one of the most serious rust diseases particularly when the pathogen infects wheat plants at the end of milk stage and before dough stage development.

Control of wheat rusts, based through genetic breeding programs (Marasas *et al.*, 2004; Anikster *et al.*, 2005; Goyeau *et al.*, 2006 and Youssef, 2012), induced resistance using biotic and abiotic agents (Sallam, 2001; Sallam *et al*., 2001 and 2002 and Sallam and Hussain, 2012) and chemical control with fungicides (Jochen, 2009). Chemical fungicides are one of the ways used to control rust diseases, but they increase production costs, incidence of health problems among workers and the risk that the fungi develop resistant races against the used fungicides. Therefore, researchers are now trying to use safe environmentally natural products as alternative methods for controlling plant pests. In this respect, using natural compounds have the advantage as antimicrobial effect being less harmful to the ecosystem and biodegraded *in situ* by the microflora and converted into non-toxic compounds (Sanchez Rodriguez *et al*., 2002). The search for new naturally-derived and environmentally friendly products to control diseases as an important part of sustainable agriculture was also carried out (Sanchez Rodriguez *et al.*, 2002). Application of natural products cause partial resistance in treated plants measured as improvement plant growth and decreasing disease incidence in different ways. Several research workers used these compounds in controlling fungal diseases. Zekaria *et al*. (1991) used film-forming compounds to control leaf rust on wheat seedlings. Ziv and Zittes (1992) controlled cucurbit foliar diseases by bicarbonate and film forming polymers. Walter (1992) controlled powdery mildew infection of barley seedlings using film forming polymers with and without a polyamine biosynthesis inhibitor. Marco *et al.* (1994) controlled powdery mildew in squash by application of whitewash, clay and antitranspirant materials. Nasraoui *et al.* (1999) applied antitranspirants Folicate to control *Botrytis cinerea* on bean. Haggag (2002) used antitranspirants film kaolin, Nu film, Bio-film, Folicate and Polyacrylamide Anti-stress 550 in controlling cucumber downy mildew. Tohamy *et al*. (2005) used acrylic forming polymers film in controlling wheat stem rust. Escobar and Castano (2005) used fulvic acid for the management of diseases caused by *Mycosphaerella* spp. Kaolin also used to improve yield and quality of tomato (Contore *et al*., 2009).

The objective of the present study was designed to evaluate the effect of three antitranspirant products (kaolin, magnesium carbonate and fulvic acid) at different concentrations on leaf rust incidence, incubation period, infection type and pustule number/cm^2 as well as leaf area and pustule size in seedling stage. Also, the effect of these products on fungal morphological change using scanning electron microscope was studied and photographed. At adult stage, latent period, disease severity, type of infection, average coefficient of infection, area under disease progress curve and the photosynthetic pigments were determined.

Materials and Methods

Wheat seedlings and adult plants:

Two leaf rust susceptible wheat cultivars namely Sakha 93 and Sids 1 were used in these experiments. Grains of the two cultivars were kindly obtained from Wheat Breeding Res. Dept., Crops Res. Inst., ARC. Ten wheat grains were sown in each plastic pot (10-cm-diam.) each contains about 250 g clay soil. After seven days, resulted seedlings were used in seedling experiment. In case of adult plant experiment, 15 grains were sown in each 25 cm in diameter plastic pot contained 2 kg clay soil. After 70 days, approximately juveniles' wheat plants were used in adult experiment.

Natural products used:

Tested antitranspirant products, *i.e*. kaolin (Ka) at 2, 4 and 6%, magnesium carbonate ($MgCO_3$) at 2, 4 and 6% and organic fertilizer product fulvic acid (FA) at 0.02, 0.04 and 0.06%, were sprayed on wheat seedlings or adult plants. Kaolin (Aluminium silicate, $H_2Al_2Si_2O_8.H_2O$ purchased from Application Studies Centre, Alexandria) Pure Lab. Chemical assay magnesium carbonate hydrated basic light ($MgCO_3$, 40-45%, purchased from El-Nasr Pharmaceutical Chemical Co.) and fulvic acid (pH_7, 70% active ingredient, exported by Agricultural Experimental Agency, Sharkiya Governorate, Egypt).

Leaf rust uredospores:

Fresh mixture of aggressive pathotypes in race groups (TT---, TS---, TK---, ST---) of *Puccinia triticina* uredospores were collected from infected adult wheat plants in greenhouse of Wheat Dis. Res. Dept., Plant Pathol. Res. Inst., ARC. Uredospores were mixed with talc powder (1:20 v/v) in baby cyclone and used in case of artificial inoculation.

Natural product application and rust inoculation

Seven days old seedlings (cvs. Sakha 93 and Sids 1) were sprayed over leaf surfaces with any of the previously mentioned different concentrations of antitranspirant products. Seedlings sprayed with water act as infected control. While, seedlings protected by Sumi-8 fungicide (0.35 ml/l water) act as healthy control. After 24 h of spraying antitranspirant products prepared uredospores in baby cyclone were used according to Tervet and Cassel (1951) to dust treated and control seedlings. Three pots, each contained 10 seedlings, were used as replicates for each particular treatment. In case of adult plants, artificial inoculation was carried out in booting stage as mentioned by Large (1954). Wheat plants (cvs. Sakha 93 and Sids 1) at booting stage were sprayed with the antitranspirant products at the previously mentioned concentrations, and then artificially inoculated after 3, 7 and 10 days.

Disease assessment in seedling stage:

Incubation period (IP):

Time from inoculation to commencement of sporulation was recorded according to Katsuya and Green (1967).

Infection type (IT):

After commencement of sporulation, seedling leaves were prepared for scoring of infection type, that were recorded as either high (IT 3-4) or low (IT 0-2) according to Long and Kolmer (1989) and Long *et al*. (1998, 2000 and 2002).

Pustule numbers:

Rust pustules No./cm^2 and leaf area on the upper side of leaves was counted as described by Parlevliet and Kuiper (1977). Total pustule reduction (%) was also calculated.

Pustule size:

Pustule size was estimated according to Broers (1989). Leaf samples of all treatments and controls were taken 10 days post inoculation (dpi). Samples were boiled in lacto phenol and ethanol solution (1:2 v/v) for three minutes. Length (L) and width (W) of 20 randomly chosen pustules were microscopically measured for three leaves of each treatment. The pustule size (µm) was calculated according to the following formula:

$$\text{Pustule size} = 1/4\ \pi\ L\ W$$

Whereas, π=22/7, L= length of pustule and W= width of pustule**.**

Scanning electron microscope (SEM) study:

Segments of wheat (cv. Sids 1) infected leaves treated with the three antitranspirant products at higher concentrations and untreated infected leaves as control were taken 24 h after inoculation. Samples (2 x 5 mm) were fixed in 2.5% glutarldehyde for 24 h at 4^oC, and then fixed in 1% Osmium tetroxide (OSC_4) for one hour, at room temperature. The segments were then dehydrated with acetone, critical point dried and finally sputter coated with gold prior to the examination and photographed in a JEOL JXA-S10A electron microscope of National Research Centre (Harley and Fergusen, 1990). Changes in the morphological fungal structures between treated and untreated were examined and photographed.

Disease assessment in adult stage:

The assessment was carried out on wheat cv. Sids 1. Number of pustules/cm^2 and incubation period (IP) was determined as previously mentioned in seedling experiment. Latent period (LP) estimated when 50% of the development pustules were erupted according to Parlevliet (1975). Disease severity (DS) was estimated and recorded using the modified Cobbs scale adopted by Peterson *et al.* (1948). The type of infection was detected using the adopted type scale of Saari and Wilcoxson (1974) as follows: Immune (O)= 0.0, Resistant (R)= 0.2, Moderately resistant (MR)= 0.4, Mixture types (X)= 0.6, Moderately susceptible (MS)=0.8 and Susceptible (S)=1.0. Difference in rust severity and infection type was photographed. Average coefficient of infection (ACI) was calculated by multiply percentage of disease severity with the previously mentioned type of infection using scale of Saari and Wilcoxson (1974). Area under disease progress curve (AUDPC) was calculated using a simple formula adopted by Pandey *et al.* (1989) as follows:

$$AUDPC= D\ [{}^1/_2\,(y_1 + y_k)+(y_2 + y_3 + \ldots\ldots\ldots\ldots y_{k-1})]$$

Whereas, D= time intervals, y_1+y_k = Sum of the first and last disease scores and $y_2+y_3+\ldots\ldots\ldots\ldots.y_{k-1}$= Sum of all in between disease score.

Determination of photosynthetic pigments:

The flag leaves of the adult plants of wheat (cv. Sids 1) inoculated 3 days after treating with kaolin, magnesium carbonate and fulvic acid at 6, 6 and 0.06% concentrations, respectively were used. Samples separately collected 20 days after inoculation to determine the photosynthetic pigments (chlorophyll a, b and carotenoids). A half gram of fresh leaves was cut into small pieces and grounded in absolute acetone. The homogenate centrifuged at 3000 rpm for 15 minutes, then supernatant completed to 15 ml with acetone. The colour intensity was measured at wavelength of 661.6, 644.8 and 470 nm for chlorophyll a and b as well as carotenoids, respectively, using Milton Roy Spectronic 1202. Equations were applied for calculating the chlorophyll (Chl.) and carotenoids (Car.) pigments as mg/g fresh weight according to Boyer (1993) as follows:

Chl. "a"= $(11.24xE_{661.6})$-$(2.04xE_{644.8})$, Chl. "b"= $(20.13xE_{644.8})$-$(4.19xE_{661.2})$, Chl. "a+b"= $7.05xE_{661.6}+18.09xE_{644.8}$ and Car.= $(1000E_{470}$-1.90a - 63.14b)/214. Whereas: E= absorbance wave length.

Statistical analysis:

Statistical analysis was conducted to calculate least significant differences (LSD) according to Gomez and Gomez (1984).

Results

Results in Table (1) indicated that non-significant differences in the mean of incubation period were detected between cultivar tested and the three antitranspirant products used. On the other hand, significant differences were observed in tested concentrations, which prolonged the incubation period compared with the control treatment. This effect was observed with FA followed by $MgCO_3$ then Ka in case of cv. Sakha 93. Opposite was observed in case of cv. Sids 1 where magnesium carbonate was the best followed by kaolin then fulvic acid. It is worthy to mention that, significant differences were detected between the interaction of cultivars and products as well as the tested products and their concentrations.

Table 1. Effect of spraying different concentrations of three antitranspirant products on incubation period (days) of infected two wheat cultivar seedlings by leaf rust pathogen

Treatment	Concentration (%)	Cultivar			General mean
		Sakha 93	Sids1	Mean	
Kaolin	2	7.33	7.66	7.49	
	4	8.00	7.66	7.83	
	6	8.00	7.66	7.83	
	Mean	7.77	7.66		7.71
Magnesium carbonate	2	8.00	8.00	8.00	
	4	8.00	7.66	7.83	
	6	8.00	8.00	8.00	
	Mean	8.00	7.88		7.94
Fulvic acid	0.02	8.00	7.00	7.50	
	0.04	8.33	7.33	7.83	
	0.06	8.33	7.33	7.83	
	Mean	8.22	7.22		7.72
Control (infected)		7.66	7.00	7.33	
Sumi-8 (healthy)		8.66	9.00	8.83	
General mean		8.06	7.75		
LSD at 5% for: Cultivar (A)= n.s; Product (B)= n.s; Concentration (C)= 0.2; A x B= 0.315; B x C= n.s; A x C= 0.343; A x B x C= n.s					

Data in Tables (2 and 3) summarized the influence of the used products on number of pustules/cm^2 and leaf area at seedling stage of the two tested wheat cultivars (Sakha 93 and Sids 1). In this respect, higher tested concentrations in the three tested products significantly reduced the number of pustules/cm^2 and/or leaf area. Significant differences were recorded among the three antitranspirant products and their concentrations on the number of pustules/leaf area compared with the infected control (Table 3). In this respect, fulvic acid was the best one followed by magnesium carbonate then kaolin in case of mean number of pustules/leaf area. On the other hand, fulvic acid has more effect in reducing number of pustules/cm^2 compared with both of kaolin and magnesium carbonate.

The inhibition effect of the three tested products in reducing disease components extended to infection type and pustule size (Tables 4 and 5). Only significant effect was observed between higher and lower concentration used as well as untreated infected control (Table 4). However, significant differences were observed among the tested three antitranspirant products. Kaolin resulted in the highest pustules size followed by magnesium carbonate. Fulvic acid was the highest affected one on pustule size comparing with untreated infected control. Also, significant differences were calculated among tested concentrations. The higher concentration was more effective than the lower one.

Table 2. Effect of spraying different concentrations of three antitranspirant products on number of pustules/cm^2 area and reduction (%) of infected two wheat cultivar seedlings by leaf rust pathogen

Treatment	Concentration (%)	Cultivar					General mean
		Sakha 93		Sids1		Mean	
		Pustule (µm)	Reduc. (%)	Pustule (µm)	Reduc. (%)		
Kaolin	2	8.00	83.12	35.00	71.31	26.50	
	4	4.00	96.25	19.67	83.87	11.83	
	6	1.00	99.06	9.67	92.07	5.33	
	Mean	7.66		21.44			
Magnesium carbonate	2	22.33	79.06	22.00	81.96	22.16	
	4	14.33	86.56	20.00	83.60	17.16	
	6	11.67	89.05	3.33	97.27	7.50	
	Mean	16.11		15.11			
Fulvic acid	0.02	12.33	88.44	30.67	74.84	21.50	
	0.04	1.33	98.75	18.33	94.97	9.83	
	0.06	1.00	99.06	8.33	93.17	4.66	
	Mean	4.88		19.11			
Control (infected)		106.67	0.0	122	0.0	114.33	
Sumi-8 (healthy)		4.00	96.25	6.00	95.08	5.00	
General mean		27.86		36.73			

LSD at 5% for: Cultivar (A)= n.s Product (B)= n.s Concentration (C)= 4.66
A x B= n.s B x C= n.s A x C= 8.14 A x B x C= n.s

Table 3. Effect of spraying different concentrations of three antitranspirant products on number of pustules/leaf area and reduction (%) of infected two wheat cultivar seedlings by rust pathogen

Treatment	Concentration (%)	Cultivar					
		Sakha 93		Sids 1		Mean	General mean
		Pustule (µm)	Reduc. (%)	Pustule (µm)	Reduc. (%)		
Kaolin	2	51.67	81.43	153	49	102.33	
	4	37	86.7	102.67	65.77	69.83	
	6	29.33	89.46	57	81	43.16	
	mean	39.33		104.22			71.77
Magnesium carbonate	2	53.67	80.71	64	78.66	58.83	
	4	39.33	85.86	37.33	87.55	38.33	
	6	18	93.53	37.67	87.44	27.83	
	mean	37		46.33			41.66
Fulvic acid	0.02	55.33	80.12	19	93.66	37.16	
	0.04	32.67	88.26	17.67	94.11	25.17	
	0.06	24.33	91.25	19	93.66	21.66	
	mean	37.44		18.55			27.99
Control (infected)		278.3	0	300	0	289.15	
Sumi-8 (healthy)		3	98.92	2	99.33	2.5	
General mean		79.01		94.22			
LSD at 5% for: Cultivar (A)= n.s Product (B)= 1.43 Concentration (C)= 15.5 A x B= n.s B x C= n.s A x C= n.s A x B x C= n.s							

Table 4. Effect of spraying different concentrations of three antitranspirant products on infection type of leaf rust pathogen on two wheat cultivar seedlings

Treatment	Concentration (%)	Cultivar			General mean
		Sakha 93	Sids 1	Mean	
Kaolin	2	2.83	2.67	2.75	
	4	2.50	2.33	2.41	
	6	2.50	2.00	2.25	
	Mean	2.61	2.33		2.47
Magnesium carbonate	2	2.67	3	2.83	
	4	2.67	2.67	2.67	
	6	1.67	1.67	1.67	
	Mean	2.33	2.44		2.39
Fulvic acid	0.02	2.83	2.67	2.75	
	0.04	2.67	2.33	2.50	
	0.06	1.33	2.00	1.66	
	Mean	2.27	2.33		2.30
Control (infected)		3.67	3.67	3.67	
Sumi-8 (healthy)		1.67	1.33	1.50	
General mean		2.51	2.42		
LSD at 5% for: Cultivar (A)= n.s Product (B)= n.s Concentration (C)= 0.99 A x B= n.s B x C= n.s A x C= n.s A x B x C= n.s					

Table 5. Effect of spraying concentrations of three antitranspirant products on pustule size (µm) and reduction (%) of two infected wheat cultivar seedlings by leaf rust pathogen

Treatment	Concentration (%)	Cultivar					
		Sakha 93		Sids 1		Mean	General mean
		Pustule (µm)	Reduc. (%)	Pustule (µm)	Reduc. (%)		
Kaolin	2	348.97	57.59	359.70	44.50	354.33	
	4	157.00	80.92	256.43	60.44	206.71	
	6	86.35	89.50	109.38	83.12	97.86	
	mean	197.44		241.83			219.63
Magnesium carbonate	2	332.00	59.65	523.33	19.28	427.66	
	4	130.83	84.10	244.60	62.27	187.71	
	6	83.87	89.80	189.71	70.73	136.79	
	mean	182.23		319.21			250.72
Fulvic acid	0.02	624.50	22.89	444.83	31.38	539.66	
	0.04	633.23	23.04	368.96	43.09	501.14	
	0.06	109.90	86.64	181.60	71.98	145.75	
	mean	459.21		331.79			395.51
Control (infected)		822.94	0.0	648.33	0.0	735.63	
Sumi-8 (healthy)		118.67	85.57	147.00	77.32	132.83	
General mean		356.09		337.63			
LSD at 5% for: Cultivar (A)= n.s Product (B)= 12.34 Concentration (C)= 73.2 A x B= n.s B x C= 90.05 A x C= 114.29 A x B x C= n.s							

Scanning electron micrographs of wheat seedling leaves, sprayed 24 hours before inoculation with the three antitranspirant products (kaolin at 6%, $MgCO_3$ at 6% and fulvic acid at 0.06%), showed deformed uredospore shape, abnormal germ tube and appressoria. Alter topography of wheat leaf surface especially when kaolin and $MgCO_3$ sprayed before inoculation (Fig. 1). In case of scanning electron microscope cross section, fulvic acid increased thickness of cell walls of spongy tissue surrounded vascular bundles comparing with control treatment. Fungal structure and uredospores completely substitute the spongy cells surrounded vascular bundles (Fig. 2).

Incubation period (IP), Latent period (LP), Number of pustules/cm^2, Area under disease progress curve (AUDPC), Infection type (IT), Rust severity % (RS) and Average coefficient of infection (ACI) were evaluated on Sids 1 wheat adult plants sprayed with the three tested products at different concentrations and infected after 3,7 and 10 days from spraying .

Results in Table (6) referred to the incubation period and latent period indicated that none of the tested products used significantly increased IP or LP. On the other hand, used concentrations and days before inoculation (dbi) significantly affected the two parameters. In this respect, the two parameters were significantly increased compared with untreated infected control. Also, the interaction among product, concentration and dbi were significantly affected in case of LP. However, only the interaction between products and concentrations was significantly observed.

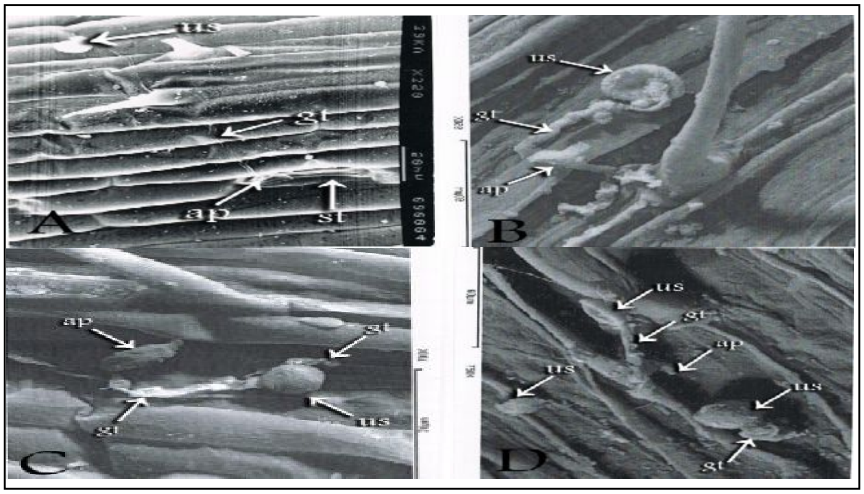

Fig. 1. Scanning electron micrographs showing the effect of spraying three antitranspirant products (kaolin at 6%, $MgCO_3$ at 6% and fulvic acid at 0.06%) 24 hours before wheat seedling inoculation. A) Wheat leaves sprayed with water as infected control treatment, notice the normal leaf topography surface, normal shape of uredospores (us), development of germ tube (gt) and appressoria (ap) on stoma (st). B) Kaolin treatment, notice the deformed shape of uredospore, abnormal germ tube and appressoria. B) Abnormalities of leaf surface covered by kaolin particles. C) Magnesium carbonate treatment, notice the alter topography of leaf surface and ungerminated uredospore. D) fulvic acid treatment, notice normal leaf surface with spherical or mumified uredospore but disorganized germ tube and appressoria.

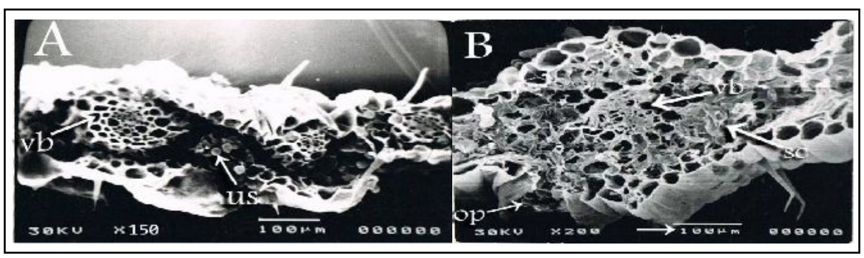

Fig. 2. Scanning electron microscope cross section in infected control and fulvic acid sprayed wheat cv. Sids 1 leaves before inoculation. A) Untreated infected control section showing fungal structure and uredospores substitute spongy tissue surrounded the vascular bundles. B) Fulvic acid treatment showing normal structure of main vascular bundle (vb), thickness of cell walls (sc) of spongy tissue and open pustule (op) on leaf stoma.

Table 6. Effect of spraying wheat adult plants of cv. Sids 1 with different concentrations of three antitranspirant products sprayed 3, 7 and 10 days before inoculation (dbi) with *Puccinia triticina* uredospores on incubation period (IP) and latent period (LP) under greenhouse conditions

Treatment	Concen. (%)	IP (days) 3	7	10	Mean	LP (days) 3	7	10	Mean
Kaolin	2	12.0	11.33	11.00	11.44	19	17	13.66	16.55
	4	12.33	11.66	11.33	11.77	20	17.66	15	17.55
	6	12	11.33	11.33	11.55	20	17.66	15.66	17.77
	Mean	12.11	11.44	11.22	11.59	19.66	17.44	14.77	17.29
Magnesium carbonate	2	12.33	11.00	11.00	11.44	16	16.33	17	16.44
	4	12.66	11.66	11.33	11.88	16	17.66	18.33	17.33
	6	12.66	11.33	11	11.66	16	18	19	17.66
	Mean	12.55	11.33	11.11	11.66	16	17.33	18.11	17.14
Fulvic acid	0.02	11	11.66	12.33	11.66	15.33	15.33	16.66	15.77
	0.04	11.33	11.33	12.66	11.77	15.33	17.33	21.00	17.88
	0.06	11.33	11.66	12	11.66	16	18	20.66	18.22
	Mean	11.22	11.55	12.33	11.70	15.55	16.88	19.44	17.29
Control (infected)					10.33				15.33
Sumi-8 (healthy)					11.66				17.66
General mean		11.96	11.44	11.55		17.07	17.21	17.44	7

LSD at 5% for:	Incubation	Latent		Incubation	Latent
Product (A)=	n.s	n.s	AxB =	n.s	n.s
Concentration (B)=	0.08	0.86	AxC =	0.79	1.08
dbi (C)=	0.54	0.62	BxC =	n.s	2.53
AxBxC=	n.s	n.s	AxBxC =	3.69	n.s

As far as the effect of different concentrations of the three tested products used on number of pustules/cm^2 and AUDPC, obtained data indicate that AUDPC decreased as the result of the affected product, concentration and dbi. On the other hand, number of pustules/cm^2 only significantly affected by the tested product, dbi and the interaction among them (Table 7). Data in Table (8) and Fig. (3) indicate that spraying the three natural products and their concentrations affected the infection type of treated wheat plants. These products change the infection type (IT) in treated infected plants to moderately susceptible (Ms), moderately resistant (Mr) in 3 and 7 dbi. However, this changed to Mr or R in case of 10 dbi. Rust severity (RS) also decreased when the tested products were sprayed before inoculation compared with untreated infected plants. Results obtained from multiply percentage of disease severity by type of infection reveal that all tested products reduced the average coefficient of infection (ACI), in different degrees according to the product used, concentration and dbi compared with control. All of the three tested concentrations and dbi significantly decreased the ACI value. Also, interaction between product and dbi significantly decreased the ACI compared with the control treatment.

Table 7. Effect of spraying wheat adult plants of cv. Sids 1 with different concentrations of three antitranspirant products 3, 7 and 10 days before inoculation (dbi) with *Puccinia triticina* uredospores on number of pustules/cm^2 and area under disease progress curve (AUDPC) under greenhouse conditions

Treatment	Concentration (%)	Days before inoculation									
		No. of pustules/cm2					AUDPC				
		3	7	10	Mean	Reduc. (%)	3	7	10	Mean	Reduc. (%)
Kaolin	2	38.0	40.0	65.3	47.8	28.4	158.8	194.5	282.5	212.1	74.8
	4	27.6	35.6	43.1	35.4	91.4	116.0	165.5	173.7	151.7	81.4
	6	25.6	24.9	24.8	25.1	93.9	116.2	126.7	155.0	132.6	84.3
	Mean	30.4	33.5	44.4	36.1	71.2	130.3	162.3	203.7	165.5	80.2
Magnesium carbonate	2	19.7	19.8	65.1	34.9	91.6	52.2	76.3	225.5	118.0	86.0
	4	14.0	15.1	58.3	29.3	92.9	47.2	71.0	403.9	174.0	79.3
	6	6.6	13.3	39.6	19.8	95.2	42.1	67.5	127.0	78.9	90.6
	Mean	13.5	16.1	54.3	27.9	93.2	47.1	71.6	252.1	123.6	85.3
Fulvic acid	0.02	21.5	39.5	76.1	45.7	88.9	379.7	173.0	65.2	205.9	75.5
	0.04	15.8	29.2	59.6	34.9	91.8	257.3	105.3	54.0	138.9	83.5
	0.06	3.3	13.7	48.4	21.8	94.7	236.5	49.3	11.4	99.1	88.2
	Mean	13.6	27.5	61.4	34.1	91.8	291.2	109.2	43.5	148.1	82.4
Control (infected)	412.0					0.0				442.0	0.0
Sumi-8 (healthy)	15.0					96.4				68.2	91.9
General Mean		19.1	25.7	53.4			82.9	102.3	246.5		

LSD at 5% for:	No. of pustules	AUDPC
Product (A)=	11.5	20.5
Concentration (B)=	n.s	9.6
dbi (C)=	96.3	84.4
AxB =	n.s	n.s
AxC =	9.7	10.2
BxC =	n.s	13.3
AxBxC=	n.s	n.s

Table 8. Effect of spraying wheat adult plants of cv. Sids 1 with different concentrations of three antitranspirant products 3, 7 and 10 days before inoculation (dbi) with *Puccinia triticina* uredospores on infection type, rust severity and average coefficient of infection (ACI) under greenhouse conditions

Treatment	Concen. (%)	Infection type (days)			Rust severity (%)days					ACI (days)				
		3	7	10	3	7	10	Mean	Reduc. (%)	3	7	10	Mean	Reduc. (%)
Kaolin	2	Ms-S	Ms-Mr	R	43.3	7.5	5.0	18.6	78.5	39.0	5.6	1.4	15.3	81.3
	4	Mr-Ms	Ms	Mr	15.3	4.8	2.0	7.4	91.8	9.0	3.8	0.80	4.5	94.4
	6	Ms	S	Mr-R	15.2	3.3	2.5	7.0	91.9	15.5	3.3	0.73	6.5	92.0
	Mean				24.6	5.2	3.2	10.9		21.2	4.2	0.96	8.8	
Magnesium carbonate	2	Ms	Ms	Mr-R	21.3	3.8	3.4	9.5	88.9	17.0	3.1	1.06	7.1	91.3
	4	Ms	Ms	Mr-Ms	25.8	6.1	2.6	11.5	86.6	20.7	4.9	1.56	9.0	88.9
	6	Mr-Ms	Mr-R	Mr-Ms	14.0	4.2	3.9	7.4	91.8	8.4	1.3	2.35	4.0	95.1
	Mean				20.4	4.7	3.3	9.5		15.4	3.1	1.65	6.7	
Fulvic acid	0.02	Mr-Ms	Ms	Mr	30.0	4.5	3.0	12.5	85.5	18.0	3.6	1.20	7.6	90.7
	0.04	Mr	Mr	R-Mr	16.6	2.2	2.5	7.4	91.8	6.6	0.9	0.80	2.7	96.7
	0.06	R	Mr	R-Mr	32.5	2.3	2.9	12.6	85.4	6.5	0.9	0.86	2.8	96.6
	Mean				26.3	3.0	2.8	10.7		10.4	1.8	0.95	4.4	
Control (infected)				S				86.7					81.7	
Sumi-8 (healthy)				Mr				6.7					3.3	

LSD at 5% for ACI: Product (A)= n.s Concentration (B)= 1.36 dbi (C)= 20.15
AxB= n.s AxC= 2.01 BxC= 3.15 AxBxC= n.s

Fig. 3. The effect of spraying three antitranspirant products (kaolin and Mg CO_3 at 2, 4 and 6% and fulvic acid at 0.02, 0.04 and 0.06%. Letter (C) indicates infected control treatment. Numbers 1, 2 and 3 indicate minimum, medium and higher concentration, respectively. Notice that lower concentration showing disease severity and infection type similar to that in control treatment, while these parameters decreased with the other higher tested concentrations.

Pigment contents (Chlorophyll a and b) and carotenoids differed according to the product used. Data in Table (9) indicate that chlorophyll content (a + b) in infected plants was lower (0.9417 mg/g fresh weight) compared with healthy uninfected plants treated with Sumi-8 fungicide (4.9570 mg/g fresh weight). All other plants sprayed with antitranspirant products used increased chlorophyll a and b compared with infected control (2.9602, 3.9359 and 3.3114 for kaolin, $MgCO_3$ and fulvic acid, respectively). In other word, chlorophyll content (a + b) increased in all infected plants treated with these products .Similar effect was also obtained in case of carotenoids.

Table 9. Effect of spraying wheat adult plants of cv. Sids 1 with the higher concentration of the three antitranspirant products 3 days before inoculation (dbi) with *Puccinia triticina* uredospores on chlorophyll A, B, A+B and carotenoid as mg/g fresh weight, 20 days after inoculation

Treatment	Concentration (%)	Chl. A	Chl. B	Chl. A+B	Carotenoid
Kaolin	0.6	2.1334	0.8269	2.9602	0.4380
Magnesium carbonate	0.6	2.8357	1.1002	3.9359	0.6140
Fulvic acid	0.06	2.3495	0.9618	3.3114	0.4897
Control (infected)	0.00	0.7332	0.2085	0.9417	0.1534
Sumi-8 (healthy)	0.035	3.7560	1.2010	4.9570	0.7310

Discussion

Results obtained in the present research work showed that the three antitranspirant products (kaolin, $MgCO_3$ and fulvic acid) and their concentrations used as foliar spray serve as an alternative mean to protect wheat plants against *Puccinia triticina*. The best results on wheat seedlings was recorded through increasing incubation period, decreased number of pustules/cm^2 and leaf area, change the infection type and reduced pustule size. Also, this effect was extended to the adult wheat plants when sprayed with these products at 3, 7 and 10 days before inculcation (dbi). The treatments increased incubation period (IP) and latent period (LP) but decreased number of pustules/cm^2, area under disease progress curve (AUDPC), rust severity (RS), infection type (IT) and average coefficient of infection (ACI). In untreated control infected plants, uredospores imbibe water, swell and develop a germ tube after coming into contact with a film of moisture such as dew or light rain on the leaf surface. Germination occurs after 4-8 h at 20^oC under 100% humidity (Zhang *et al*., 2003). Germ tube growth is controlled by the thigmotrophic response to the topography of the leaf surface, with growth oriented perpendicular to the long axis of the epidermal cells. Germ tubes continue to grow on the leaf surface until endogenous spore reserves are depleted or until a stoma is encountered (Dickinson, 1969). At the stoma, the germ tube stops elongating and protoplasm flows towards the tip to form an appressoria occurs with 24 h after inoculation and germ tubes that have not found stoma by this stage do not survive (Zhang *et al*., 2003). In case of treated wheat plants with antitranspirant products, the mode of infection action is different according to the used products. Obtained results indicated that inoculation with the pathogen following treatment with these products fails to cause disease, or the degree of disease severity is reduced. This is due to that pre-emptive application of hydrophobic and hydrophilic kaolin particles which reduced the wheat leaf rust incidence depending on creating a low water potential at infection sites. The previously explanation was in agreement with the result obtained by many researchers (Zekaria *et al*., 1991; Hsieh and Huang, 1997 and 1999; Glenn *et al*., 2001; Haggag, 2002; Gaballah and Moursy, 2004; Rosati *et al*., 2006 and Wesam *et al*., 2009).

Magnesium carbonate and other salts of potassium were used in research to improve yield production and control plant disease. In this respect, El-Khaly and Gaballah (2005) and El-Khaly *et al*. (2005) used $MgCO_3$ to reduce losses in wheat grain depending on relative humidity that showed a positive correlation with wheat yield and its component. Also, Glynn (2010) used potassium salts to inhibit germination of apple scale conidia.

Fulvic acid (FA) produced by the biodegradation of organic materials. The activity of FA depending on the organic substance used. Fulvic acid antitranspirant product showed the highest values for reducing disease incidence, possibly due to the high potassium content in the used solution (Escobar and Castano, 2005 and Li *et al*., 2005). Fulvic acid contains high potassium content which makes the leaf cellular walls more resistant and as a result, the germination of spores became more difficult. Also, FA increased the nitrate reductase activity free proline content, chlorophyll content and water content of winter wheat leaves, thus drought stress can be mitigated. Li *et al.*, (2005) indicated that FA increased photosynthesis, reduced stomatal opening status and transpiration rate, thus led to growth stimulation and water loss reduction. The previously used three products appear to have a beneficial effect on plants, which reduced disease incidence as manifested by a significantly higher chlorophyll content of the older leaves. Photographs obtained from SEM indicated that all products used inhibited germination of leaf rust uredospores, subsequent formation of appressorium or collapsed of uredospores and germ tubes and reduced leaf rust severity. Also, FA increased cell wall thickness of spongy tissue due to increase photosynthesis in wheat leaves resulted from increasing chlorophyll a and b in leaf epidermal cells. These results are in agreement with those obtained in wheat leaf rust and other different plant diseases (Ziv and Frederiksen, 1986; Zekaria *et al*., 1991; Marco *et al*., 1994; Haggag, 2002 and Escobar and Castano, 2005). According to the obtained data, its worthy to mention that, reduction of wheat leaf rust by treatment with kaolin, $MgCO_3$ and FA are inexpensive, none toxic, environmentally safe and applicable in organic agriculture.

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معاملة أسطح أوراق القمح ببعض مواد مضـادات النتح لمقاومة صـدأ الأوراق المتسبب عن فطـر *Puccinia triticina*

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الهدف من هذه الدراسة هو تقييم فعالية رش ثلاث مواد من مضادات النتح (كاولين وكربونات المغنسيوم وحمض الفولفيك) بتركيزات مختلفة قبل العدوى على مقاومة مرض صدأ أوراق القمح وأجريت العدوى بإستخدام خليط من جراثيم سلالات الفطر الأكثر شدة في الإصابة وهي الجراثيم اليوريدية لفطر *Puccinia triticina* Eriks. المسبب لمرض صدأ الأوراق. أوضحت نتائج معاملة البادرات رشا قبل العدوى بـ ٢٤ ساعة أن التركيز الأعلى من الكاولين (٦%) متبوعاً بحمض الفولفيك (٠,٠٦%) سبب زيادة فترة الحضانة في صنف القمح سخا ٩٣ مقارنة بالنباتات غير المعاملة (الكونترول) لوحظ العكس في حالة الصنف سدس ١حيث كان التركيز الأعلى من كربونات المغنسيوم (٦%) هو الأفضل في زيادة فترة الحضانة وانخفض عدد البثرات/سم٢ أو للمساحة الكلية لأوراق البادرات خاصة مع حمض الفولفيك مقارنة مع الكاولين وكربونات المغنسيوم. إنخفض حجم البثرات معنويا عند إستخدام حمض الفولفيك رشا على أوراق النباتات متبوعاً بكربونات المغنسيوم في حين أن كان الكاولين الأقل تأثيراً على حجم البثرة مقارنة بالنباتات غير المعاملة .

أوضحت نتائج الفحص بالميكروسكوب الإلكتروني الماسح لأوراق البادرات المرشوشة قبل العدوى ب ٢٤ ساعة تشوه الجراثيم اليوريدية وأنابيب الأنبات وأعضاء الألتصاق مع حدوث تحول في طبوغرافية سطح الأوراق المعاملة. أوضح القطاع العرضي للأوراق المعاملة بحمض الفولفيك زيادة سمك جدر خلايا النسيج الإسفنجي المحيط للحزم الوعائية مقارنة بالأوراق المصابة وغير المعاملة .

أظهرت نتائج رش هذه المواد على أوراق نباتات القمح البالغة قبل العدوى ب ٣ ,٧ , ١٠ أيام عدم تأثيرها معنوياً على فترة الحضانة أو الزمن اللازم لتكشف ٥٠% من البثرات في حين أن استخدام التركيزات المرتفعة رشا قبل العدوى بثلاثة أيام أثر معنوياً على كلا القرائتين السابقتين. أيضاً انخفضت المساحة تحت المنحنى وشدة الإصابة ومعامل الإصابة باستخدام التركيزات المختلفة من هذه المواد كما تغيرت طرز الإصابة وتحولت من القابلية للإصابة إلى متوسطة القابلية أو متوسط المقاومة أو المقاومة طبقا لنوع المادة المستخدمة وتركيزها والفترة الزمنية قبل العدوى. أدى إستخدام هذه المواد إلى زيادة الصبغات النباتية في النباتات المعاملة .