

USE OF ACRYLIC FORMING POLYMERS FILM IN CONTROLLING WHEAT STEM RUST DISEASE

Tohamy, M.R.A.*; M.I. Abou-Zaid*; Faten, K. El-Nashar**;
A.I. Hussain*** and Doaa, R.M. El-Naggar**

* Agric. Bot. and Plant Pathol. Dept., Fac. Agric., Zagazig Univ., Zagazig, Egypt

** Plant Pathol. Res. Inst., Agric. Res. Center, Giza, Egypt

*** National Res. Center, Cairo, Egypt

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ABSTRACT: Two film-forming polymers namely styrene acrylic-emulsion and emulsion wax were used to control stem rust of wheat caused by *Puccinia graminis* f.sp. *tritici* (Eriks) Henn.

All experiments were carried out under greenhouse and field conditions using the most susceptible wheat cultivar (Giza-160). In seedling stage, application of styrene acrylic-emulsion polymer at 24% concentration 24 h before pathogen inoculation led to complete reduction in number of pustules/cm². Adult stage experiments showed significant decreases in number of developed pustules/cm², rust severity and area under disease progress curve (AUDPC), compared with control plants. All treatments under investigation showed an increase in chlorophyll a, b and carotenoids.

Application of film-forming polymers in field experiment during 2001-2002 and 2002-2003 growing seasons led to an increase in yield components. Scanning electron microscope (SEM) studies were carried out to evaluate the mode of action of film-forming polymers in controlling stem rust of wheat.

Scanning electron micrographs showed complete coverage of leaf surface with film forming polymers, sticking groups of ungerminated urediospores, deformation of appressoria as well as unsuccessful germination of urediospores.

Key words: Stem rust, Wheat, Giza-160, Polymers, SEM

INTRODUCTION

Wheat in Egypt is liable to attack by many diseases such as rust, smut, mildew and some other minor diseases. Till now wheat rusts are still the main biotic stress, that limited the productivity of most of wheat cultivars, under the Egyptian environmental conditions.

Stem rust of wheat caused by *Puccinia graminis* f.sp. *tritici* Erikss has been one of the most serious rust diseases in Egypt, particularly in the late sowings. However, it causes a considerable losses in wheat yield under Egyptian environment (El-Daoudy *et al.*, 1994 and Nazim *et al.*, 1983).

Certain passive defense mechanisms against plant pathogens are related to the morphological structure of the plant surface (i.e. the cuticle) providing an effective barrier against direct penetration by parasites (Heath, 1985). The insoluble polymer compounds of the cuticle constitute the main physical obstacle for fungal penetration (Royle, 1975). Such barriers, together with other measures, may be responsible for limiting the host range of particular species of crop pathogens (Heath, 1985).

Various coating polymers such as oils, waxes, polyterpenes, alcohols and silicones were used as artificial barriers over plant surfaces to reduce water losses (Gale and Hagan, 1966) or protect plant organs against invading micro-organisms and prevent the development of certain plant diseases (Gale and Poljakoff-Mayber, 1962 as well as White and Shaffer, 1985). These coating polymers used as protective barriers were non-phytotoxic, permeable to gases, resistant to change environmental conditions, penetration of solar irradiation and biodegradable (Ziv and Frederiksen, 1983).

The objective of the present study was to evaluate the effect of two different film forming polymers namely styrene acrylic-emulsion and emulsion wax applied to wheat plants in both seedling and adult stages on processes associated with the development of stem rust of wheat caused by *Puccinia graminis* f.sp. *tritici*.

MATERIALS AND METHODS

Greenhouse experiments:

Tested cultivar:

The most susceptible wheat cultivar namely Giza-160 was used

in these experiments. Grains of this cultivar were kindly obtained from Crops Research Institute, ARC, Giza, Egypt.

Film forming polymers:

Two different types of polymers *i.e.* styrene acrylic-emulsion and emulsion wax were sprayed on Giza-160 wheat cultivar in all experiments under investigation.

Seedling stage experiments:

Freshly collected urediospores of *Puccinia graminis* f.sp. *tritici* race 11 kindly provided by Wheat Dis. Res. Dept., Pl. Pathol. Res. Inst., ARC (the virulent strain) were used as inoculum.

Seven days old wheat seedlings were divided into two groups. The first group was sprayed with five concentrations of the entire polymers (8%, 12%, 16%, 20% and 24%), 24 hours before inoculation. While, the second group was sprayed with the same treatments 24 hours after inoculation.

The type of infection was detected using the adopted type scale of Saari and Wilcoxson (1974). Also, number of pustules per unit leaf area

cm² on the upper side of the leaves was counted as described by Parlevliet and Kuiper (1977).

Adult stage experiments:

Artificial inoculation was carried out in booting stage (70 days old) as mentioned by Large, (1954). Plants dusted with urediospores-talcum mixture (1:20) using a baby cyclone (Tervet and Cassel, 1951). Giza-160 wheat cultivar was sprayed with the film forming polymers under investigation in booting stage at two concentrations which considered to be the most effective ones during seedling stage experiments for each particular type of polymers (12% and 24%). All experiments were carried out 24 hours before and after inoculation.

Number of pustules/cm² was counted using the method described above. Rust severity was recorded using modified Cobb's scale (Peterson *et al.*, 1948) during the course of the disease cycle. Also, area under disease progress curve (AUDPC) was calculated using a simple formula adopted by Pandey *et al.*, (1989).

Field experiments:

Inoculation technique used in these experiments was achieved as

mentioned above in adult stage experiments. Field experiments were carried out at Farm of Zagazig (Faculty of Agriculture) in two successive growing seasons (2001-2002 and 2002-2003). Area of each experiment was divided into plots (2 x 1.5 m, 1/1200 feddan) containing 5 rows of 2 m long with 30 cm between rows. Each row was sown by 5 g wheat seeds (Giza 160 susceptible wheat cultivar).

The two polymers (styrene acrylic-emulsion and emulsion wax) were applied at the rate of 12% and 24% for each particular type of polymers. The tested polymers were sprayed before inoculation. Three plots were used as replicates for each one. Untreated plants were sown in three plots and used as infected control plants (diseased plants). However, three infected plots which were treated with Sumi-8 (0.35 ml/l) fungicide were used in this experiment to serve as control treatment (healthy plants).

Rust severity and yield assessment i.e. plant height (cm), spike height (cm), spike weight (g), weight of plot (kg) and weight of 1000 grains (g) were recorded in these experiments.

Determination of photosynthetic pigment contents:

The photosynthetic pigments were extracted from fresh flag leaf of adult infected plants according to the method described by Wettstein (1957) at wave length of 662, 664 and 440 nm for chlorophyll a, b and carotenoids, respectively.

The concentration of the photosynthetic pigments were measured as follows:

$$\text{Chl.a} = 9.784 \times E_{662} - 0.999 \times E_{664} = \text{mg/g.}$$

$$\text{Chl.b} = 21.426 \times E_{644} - 4.65 \times E_{662} = \text{mg/g.}$$

$$\text{Carot.} = 4.695 \times E_{440} - 0.268 (\text{Chl.a} + \text{Chl.b}) = \text{mg/g.}$$

where Chl. a, b and Carot. = concentrations of chlorophyll a, b and carotenoids in mg/g fresh weight.

Scanning electron microscope (SEM) studies:

Specimens of infected leaves were taken at 10 days and treated with 24% concentration for the entire polymers, pre- and post-inoculation. Samples were fixed in 2.5% glutaraldehyde for 24 hours at 4°C then fixed in 1% osmium tetroxide (OSO₄) for one hour, at

room temperature. The specimens were then dehydrated with acetone, critical point dried, and finally sputter coated with gold prior to the examination and photographed in a Jeol T 330A scanning electron microscopy (Harley and Ferguson, 1990).

Characterization of film-forming polymers:

The first polymer is composed of formulation containing acrylic ester (styrene acrylic emulsion) as a binder, water base thickener and coalescing agent. While, the second polymer is composed of formulation containing acrylic ester (styrene acrylic emulsion) as a binder, water base thickener, emulsion wax and coalescing agent.

The entire formulation ingredient imported from, Egyptian Danish Co. for building and construction (Lip Co.).

Statistical analysis procedure:

All data were subjected to statistical analysis according to the procedures "ANOVA" reported by Snedecor and Cochran (1980). Treatment means were compared by the Least Significant Difference test (L.S.D.) at 5% level probability.

RESULTS AND DISCUSSION

I- Greenhouse experiments:

A- Application of film forming polymers before and after pathogen inoculation in seedling stage:

Results indicated that, pre-inoculation treatments of wheat seedlings using film forming styrene acrylic-emulsion polymer yields a significant reduction in infection type, compared with control treatments.

Application of styrene acrylic-emulsion and emulsion wax polymers at concentrations of 12% and 24% yields significant differences in infection type, compared with control plants.

Data presented in Table (1) revealed that, application of film forming compounds namely styrene acrylic-emulsion and emulsion wax reduced the number of pustules/cm² in both pre- and post-inoculation treatments, compared to control treatments. This might be due to the fact that the film forming polymers may exclude the fungus by preventing physical contact between the invading pathogen and the host tissue leading to reduction of pustules/cm² (Judith and Eyal, 1991).

Table (1): Effect of styrene acrylic-emulsion and emulsion wax polymers application, before and after pathogen inoculation, on infection type, number of pustules/cm² and % reduction, in seedling stage.

Polymer tested	Infection type			No. of pustules/cm ²			Reduction %		
	Pre-inoc.	Post-inoc.	Mean	Pre-inoc.	Post-inoc.	Mean	Pre-inoc.	Post-inoc.	Mean
Control	3.67	4.0	3.83a	84.66	63.91	74.29a	0.0	0.0	0.0 f
Styrene acrylic-emulsion									
8 %	3.0	2.0	2.66 c	17.0	45.0	31.0 dc	79.92	29.57	54.75abc
12 %	1.0	3.0	2.0 d	0.667	46.0	23.33 f	99.2	27.99	63.6 a
16 %	3.0	2.0	2.5 c	3.83	46.66	25.25cf	95.47	26.97	61.22ab
20 %	3.0	2.0	2.5 c	8.17	57.33	32.75 cd	90.36	10.26	58.32bcd
24 %	0.0	2.0	1.0 e	0.0	49.33	24.67 Ef	100.0	22.78	61.39ab
Mean	2.0	2.2		5.93	48.86		92.99	23.51	
Emulsion wax									
8 %	3.0	3.0	3.0 b	35.0	52.4	43.7 b	58.43	17.09	37.76 e
12 %	3.0	2.0	2.5 c	31.0	34.0	32.5 cd	63.14	48.11	55.62ab
16 %	3.0	3.0	3.0 b	41.0	36.0	38.5 bc	51.49	33.75	42.62de
20 %	3.0	3.0	3.0 b	35.0	43.5	39.58 b	57.54	30.91	44.2cde
24 %	2.0	3.0	2.5 c	31.0	20.17	25.58 ef	63.0	66.72	64.86 a
Mean	2.8	2.8		34.6	37.21		58.72	39.32	
Pol. Mean	2.4	2.5		20.27	43.04		75.86	31.42	
Total mean	2.52	2.67		26.18	44.94		68.96	28.56	

L.S.D. at 0.05

A =	0.14	2.95	4.91
B =	0.323	6.808	11.33
A x B	0.457	9.63	16.02

A = between pre- and post-inoculation

B = Treatments

Also, data in Table (1) concluded that styrene acrylic-emulsion polymer at concentration of 24% completely reduced the number of pustules/cm² at pre-inoculation infection type followed by 12% concentration where the obtained percentage of reduction were 100% and 99.2%, respectively.

These findings may be related to the thickness of the uniformity of the coat which increased by increasing polymer concentration and consequently increase the impermeability of the coated surface (Judith and Eyal, 1991). However, application of film forming emulsion wax polymer leads to

lower percentage of reduction than styrene acrylic-emulsion polymer where the highest reduction percentage were obtained at concentrations of 12% (63.14%) and 24% (63.0%).

It was reported by Yirgou and Caldwell (1968), Lewis and Day (1972) and Kamp (1985) that the outer layer of plant tissue provides physical and/or chemical barriers against the establishment of plant pathogens. Film coating polymers have been reported to provide additional protection against various foliar pathogens.

B-Application of film forming polymers pre- and post- pathogen inoculation in adult stage:

Data given in Table (2) show the effect of pre- and post application of film forming styrene acrylic-emulsion and emulsion wax polymers for controlling stem rust of wheat in adult stage. It can be concluded that, application of styrene acrylic-emulsion and emulsion wax polymers at 12% concentration in pre-inoculation with stem rust urediospores reduced the number of pustules/cm², rust severity and area under disease progress curve (AUDPC), compared with control treatment. This may be attributed to the protective effect of sprayed polymeric materials, which

provide additional protection against foliar pathogens (Ziv and Frederiksen, 1987) through increasing the impermeability character of leaf surface. However, in post-inoculation treatments the application of styrene acrylic-emulsion polymer at concentration of 24% was the most effective one for reducing the rust severity and AUDPC. Also, application of emulsion wax polymer at concentration of 12% reduced the rust severity and AUDPC, compared with control ones. Differences in the obtained results in different stages of plant tested (seedling, adult and field experiments) might be due to environmental conditions during experiment and the plant age.

II- Field experiments: Disease severity and yield components:

Data given in Table (3) represent the effect of applying protective film forming polymers i.e. styrene acrylic-emulsion and emulsion wax on rust severity and yield components including spike length (cm), spike weight (g), number of grains/spike, weight of plot (kg) as well as weight of 10³ grains (g) at 2001-2002 and 2002-2003 growing seasons, compared with untreated and fungicidal treated wheat plants.

Table (2): Effect of film forming styrene acrylic-emulsion and emulsion wax polymers application, before and after pathogen inoculation, on rust severity, number of pustules/cm² and area under disease progress curve (AUDPC), in adult stage.

Polymer tested	Rust severity			No. of pustules/cm ²			AUDPC		
	Pre-inoc.	Post-inoc.	Mean	Pre-inoc.	Post-inoc.	Mean	Pre-inoc.	Post-inoc.	Mean
Control	30.0	30.0	30.0 a	18.5	18.5	18.5 a	491.76	491.76	491.76 a
<u>Styrene acrylic-emulsion</u>									
12 %	3.0	10.0	6.5 b	1.0	3.33	2.16 c	47.33	175.0	111.17 b
24 %	3.5	5.0	4.25 b	2.0	3.33	2.67 c	66.5	81.33	73.92 b
Mean	3.25	7.5		1.5	3.33		56.92	128.17	
<u>Emulsion wax</u>									
12 %	6.67	13.0	10.0 b	7.33	10.33	8.85 b	93.33	250.83	172.1 b
24 %	12.33	8.0	10.33 b	8.33	6.0	7.17 dc	194.17	133.0	163.58 b
Mean	9.5	10.5		7.83	8.17		143.75	191.92	
Pol. mean	6.38	9.0		4.66	5.75		100.33	166.04	
Total mean	14.25	19.5		11.58	12.13		296.05	328.90	

L.S.D. at 0.05

A =	n.s.	n.s.	n.s.
B =	5.177	6.957	137.7
A x B	n.s.	n.s.	n.s.
A = between pre- and post-inoculation			
B = Treatments			

Table (3): Effect of applying protective film forming styrene acrylic-emulsion and emulsion wax polymers on rust severity and yield components at 2001-2002 and 2002-2003 growing seasons.

Treatments	Rust severity			Spike length (cm)			Spike weight (g)			No. grains/spike			Weight of plot (kg)			1000 grains wt. (g)		
	2001 /02	2002 /03	Mean	2001 /02	2002 /03	Mean	2001 /02	2002 /03	Mean	2001 /02	2002 /03	Mean	2001 /02	2002 /03	Mean	2001 /02	2002 /03	Mean
Control	36.67	21.66	29.16 a	9.68	9.60	9.64	1.58	1.62	1.59	44.56	46.66	45.61 b	0.41	0.93	0.462 c	17.66	24.66	21.16 c
Fungicide	13.33	6.66	9.99 b	9.17	10.20	9.69	1.67	1.59	1.63	41.67	50.00	45.83 b	0.491	0.65	0.528 bc	21.33	29.23	25.28 b
Styrene acrylic-emulsion																		
12%	16.60	3.67	10.13 b	11.58	10.10	10.84	2.79	1.97	2.38	64.53	52.00	58.26 a	0.525	0.96	0.531 bc	25.16	30.96	28.06 a
24%	12.50	3.33	7.91 b	9.67	10.90	8.78	2.55	2.57	2.56	41.47	54.53	48.00 b	0.663	0.67	0.667 a	26.57	30.40	28.48 a
Mean	14.55	3.5	10.63	10.5	10.63	2.65	2.27	2.46	53.0	53.27	53.13	0.594	0.84	0.600	25.87	30.68	28.27	
Emulsion wax																		
12%	22.50	4.66	13.58 b	9.46	10.10	8.28	1.23	2.28	1.75	43.03	50.30	46.66 b	0.478	0.62	0.545 bc	20.29	30.89	25.39 b
24%	20.0	4.67	12.33 b	9.15	9.90	9.53	1.97	1.88	1.92	45.06	51.20	48.13 b	0.535	0.67	0.611 ab	22.96	30.59	26.77 ab
Mean	21.25	4.67	12.96	9.31	10.0	8.91	1.60	2.08	1.84	44.05	50.75	47.40	0.607	0.64	0.578	21.63	30.74	26.58
Pol. mean	17.9	4.09	10.79	10.25	10.25	2.13	2.18	2.15	54.03	52.01	53.02	0.551	0.87	0.604	23.75	30.71	27.23	
Total mean	20.27	7.43	11.72	9.29	10.10	9.69	1.96	1.97	2.17	46.72	50.78	48.45	0.515	0.80	0.600	22.25	29.46	25.86
L.S.D. at 5%																		
A =	3.297			n.s.			n.s.			3.387			0.052			1.361		
B =	5.292			n.s.			n.s.			5.99			0.093			2.428		
A x B =	n.s.			n.s.			n.s.			8.279			n.s.			n.s.		

A = Between seasons

B = Treatments

Results indicate that applying protective polymers on rust severity during the two growing seasons were significant. Rust severity in 2001-2002 growing season was 12.50 while in 2002-2003 growing season was 3.33, compared with control (36.67 and 21.66, respectively). These might be due to that the coated surface created a low water potential at infection sites because of its hydrophobicity (Gale and Hagan, 1966). Also, untreated plants were susceptible to infection by the pathogen resulting higher rust severity than protective ones.

Also, data in Table (3) reveal that spike length and spike weight (g) were significantly affected in the two growing seasons. Accordingly, spike length was higher at 2002-2003 growing season than 2001-2002. Number of grains/spike and weight of 10^3 grains were also affected by the two growing seasons. The aforementioned criteria increased at 2002-2003 growing season than 2001-2002 one. This might be due to the fact that changing in the environmental conditions might affect rust severity and consequently yield components.

It is worthy to mention that, applying film forming styrene acrylic-emulsion and emulsion wax polymers at concentrations of 12% and 24% caused an increase in number of grains/spike, weight of plot (kg) and weight of 10^3 grains (g), compared with either untreated control and even protected wheat plants. In this respect, film-coating polymers suppressed the stem rust pustules formation through increasing the resistance in wheat cells to enzymatic degradation by the pathogen (Judith and Eyal, 1991).

Photosynthetic pigment contents:

Results in Table (4) reveal that all tested concentrations of film forming styrene acrylic-emulsion polymer were better than those of emulsion wax polymer for increasing the photosynthetic pigment contents (mg/g fresh weight), compared with un-treated control plants. Pre-inoculation application of 12% concentration of styrene acrylic-emulsion polymer led to increase chl.a+b content (8.49 mg/g), compared with control plants (6.21 mg/g). This effect might be due to that the tested polymeric materials can be considered as solar energy

adsorbent and consequently favour the photosynthesis i.e. increasing photosynthetic pigment contents (Goodman *et al.*, 1986). However, post-inoculation application of the tested polymer led to an increase in chlorophyll a, b and carotenoid contents comparing with the control rather than those of pre-inoculation one.

Table (4): Application effect of film forming styrene acrylic-emulsion and emulsion wax polymers in pre- and post-pathogen inoculation on photosynthetic pigment content as mg/g fresh weight of susceptible Giza-160 wheat variety.

Treatments	Pre-inoculation				Post-inoculation			
	Chl. a	Chl. b	a + b	Carot.	Chl.a	Chl. b	a + b	Carot.
Control	4.27	1.94	6.21	1.81	4.27	1.94	6.21	1.81
Styrene acrylic-emulsion								
12%	5.64	2.85	8.49	1.88	5.92	3.49	9.41	1.95
24%	5.50	2.29	7.79	1.87	6.26	3.18	9.44	2.10
Mean	11.14	2.57	8.14	1.88	6.09	3.34	9.43	2.03
Emulsion wax								
12%	3.58	1.30	4.88	1.0	6.25	6.39	12.64	0.15
24%	3.40	1.94	5.70	0.50	3.87	4.38	8.25	0.26
Mean	3.49	1.62	5.29	0.75	5.06	5.39	10.45	0.21
Pol. mean	7.32	2.09	6.72	1.32	5.58	4.36	9.94	1.12
Total mean	5.79	2.02	6.46	1.57	4.93	3.15	8.07	1.46

III-Scanning Electron Microscope examination (SEM):

SEM micrographs gave an overview on the effect of spraying wheat seedlings leaves with film forming polymers to control infection with *P. graminis* f.sp. *tritici*. The treatments had affected urediospores germination, germ tube and appressoria formation.

Scanning electron micrographs of wheat leaves treated before and

after inoculation showed coverage of the leaves with the polymeric materials (Fig. 1). These results are in harmony with those obtained by Sallam, Minaas (2001) who used some plant extracts such as *halfa* barr and chamomile as well as some bioagents from *Trichoderma* spp. to control wheat leaf rust disease.

Scanning electron micrographs 24 hr before inoculation show that, both of orientation of the germinating urediospores towards the stomata and

the formation of appressoria were affected (Fig. 2-B). It is thought that the leaf surface provides certain physical stimuli that orient the germinating urediospores toward the leaf stomata (Dickinson, 1979 and Lewis and Day, 1972). The stimulus can be associated in part with chemical factors originating in the stomata (Edwards and Bowling,

1986). It is suggested that, the distribution of appressoria over the coated leaf surface are associated with distribution of the mechanism(s) coupled with orientation of the germinating urediospores towards the stomata and formation of appressoria. Also, shrinkage of germ tube and deformation of appressoria were also observed (Fig. 2-C).

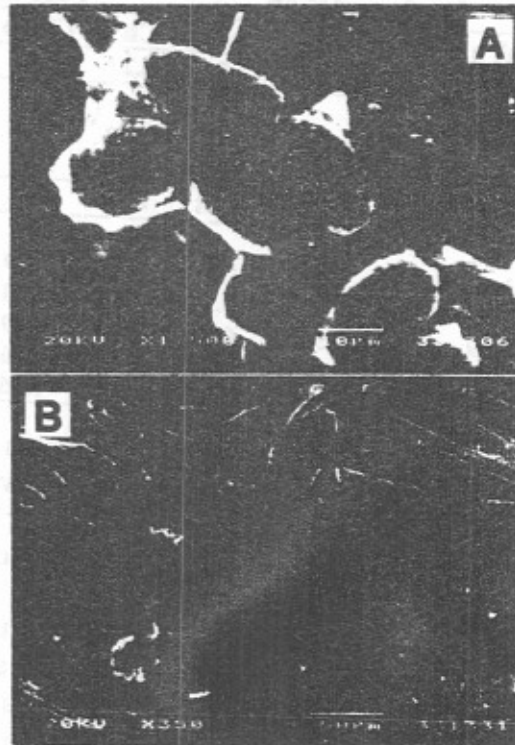


Fig. (1): Scanning electron micrographs showing the leaf surface of ten days old wheat seedlings: A) post-inoculation with application of emulsion wax polymer [notice: sticking groups of un-germinated urediospores (us) with polymer material] and B) spraying of emulsion wax polymer [notice: complete coverage of leaf surface].

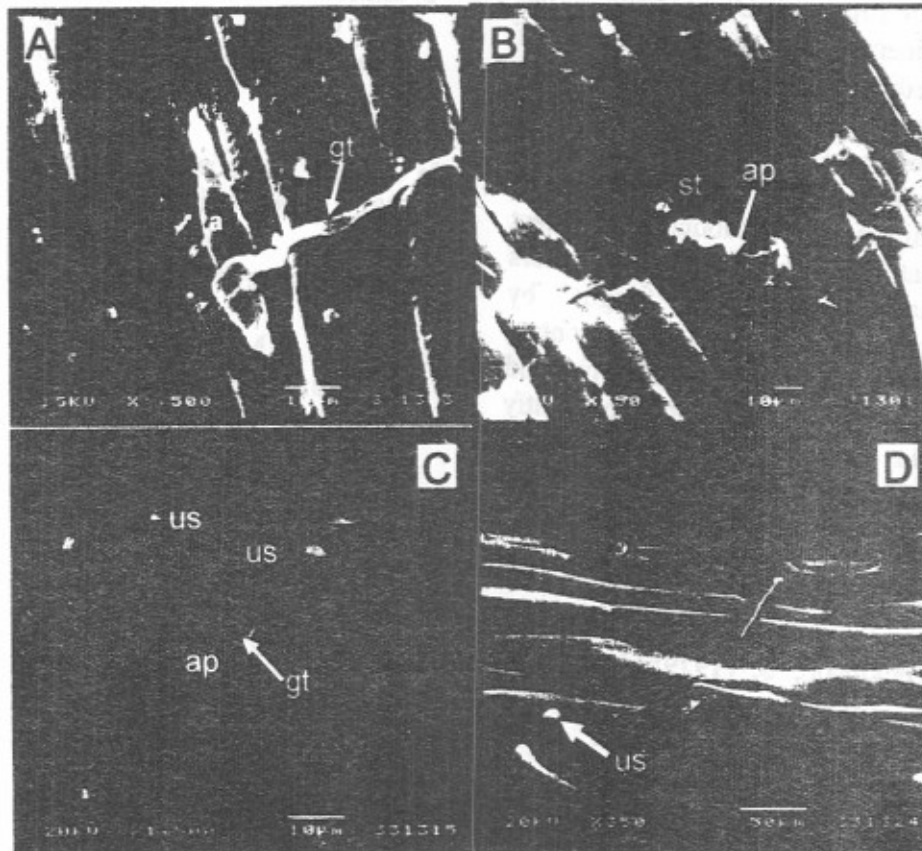


Fig. (2): Scanning electron micrograph showing the germination of urediospores (us) of *P. graminis* f. sp. *tritici*, 24 h after treatment of wheat leaves with film forming Styrene acrylic emulsion polymer: A) untreated control plants [notice: the normal leaf surface coverage; typical germ tube (gt) and typical appressorium (ap)], B) deformation and disorientation of appressorium (ap) present on the stomata (st) C) shrinkage of germ tube (gt) and deformation of appressoria (ap), and D) un-succeeded germination of urediospores (us).

Fig. (2-D) shows the unidentified germination of urediospores where there are no appressoria observed due to the pre-inoculation application of styrene acrylic emulsion polymer. This may be attributed to the fact that, the film forming polymers may exclude the fungus by preventing physical contact between the invading pathogen and the host tissue, which apparently can stimulate the formation of infection structures in unidentified ways (Judith and Eyal, 1991).

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استخدام أغشية بوليمرات الأكريليك في مقاومة مرض صدأ الساق في القمح

محمد رضا أحمد تهامي* - محمد إبراهيم أبو زيد* - فاتن كامل النشار** -

أحمد إسماعيل حسين*** - دعاء راغب محمد النجار**

* قسم النبات الزراعي وأمراض النبات - كلية الزراعة جامعة الزقازيق - الزقازيق - مصر

** معهد بحوث أمراض النبات - مركز البحوث الزراعية - الجيزة - مصر

*** المركز القومي للبحوث - القاهرة - مصر

تم في هذه الدراسة استخدام نوعين من المركبات البوليمرية (أكريليك الاستيرين الغروي والشمع الغروي) لمقاومة فطر بكسينيا جرامينس تحت نوع تريبتساي والمسبب لمرض صدأ الساق في القمح. تم إجراء تجارب الصوبة والحقل على الصنف القابل للإصابة جيزة-١٦٠ وقد لوحظ أن رش البادرات باستخدام أكريليك الاستيرين الغروي قبل العدوى بالمسبب المرضي بـ ٢٤ ساعة وبتركيز ٢٤% أدى إلى الاختزال الكامل لعدد البثرات في وحدة المساحة. أما في طور النباتات البالغة لوحظ اختزالات معنوية في كل من عدد البثرات في وحدة المساحة وشدة الإصابة بالصدأ بالإضافة إلى المساحة الواقعة تحت منحني التقدم المرضي مقارنةً بانبثبات البالغة غير المعاملة. نتيجةً للمعاملة باستخدام المركبات البوليمرية زاد محتوى الأوراق المصابة من صبغات الكلوروفيل (أ، ب) وكذا الكاروتينات في الصنف المختبر. وقد أدت جميع المعاملات إلى زيادة معنوية في محتوى المحصول في الموسمين ٢٠٠١/٢٠٠٢ و ٢٠٠٢/٢٠٠٣. أوضحت دراسات الميكروسكوب الإلكتروني وجود تغطية كاملة لأسطح الأوراق المعاملة مع وجود مجموعات متلاصقة من الجراثيم اليوريدية غير النابتة كما لوحظ تشوه عضو الالتصاق بالإضافة إلى وجود جراثيم يوريدية غير نابتة.