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CONTROL OF WHEAT LEAF RUST USING NOVEL EMULSION POLYMERS

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ABSTRACT

The effect of spraying three novel polymeric compounds(Homopolymer,Pure acrylate and Styrene Co-polymer) at three different concentrations (5, 10 and 15%) on the development of wheat leaf rust caused by *Puccinia triticina* Eriks. was assessed on two wheat cultivars (Sakha 93 and Sids 1) at seedling stage. Spraying polymeric compounds before rust inoculation, significantly reduced pustule size and number of pustules/cm^2 and leaf area but not affected incubation period. However, infection type (IT) was significantly affected through some of infection type produced only flecks which were not developed to pustules. These prepared polymers consists of three groups depending on the monomers type of polymers (homo or co-polymer). The efficiency of these polymers depended upon their polymeric types and concentrations. Scanning electron microscope (SEM) examination showed polymeric compounds cause disturbance on leaf surface morphological characters led to alter the topography of the leaf surface. Also, Homopolymer compound inhibit urediospores germination, suppressed germ tube and appressoria formation which led to reduce rust development. Light and transmission electron microscope (LM, TEM) showed that polymeric compounds enhanced and improved the ultra-structure of infected cells compared with untreated infected control. This effect due to increase the unsuccessful pathogen penetration and subsequently reduced the infection efficiency. Conclusively, the obtained results indicated that novel polymeric compounds can be used as a partial effective treatment to control wheat leaf rust disease

Keywords: Polymeric compounds, leaf rust, infection type, urediospores, SEM, LM, TEM.

INTRODUCTION

Rusts of bread wheat plants cause significant losses of wheat production in Egypt and all over the world (Herrera-Foessel *et al.*, 2006). Yeild losses in wheat caused by *Puccinia triticina* Eriks. infection are usually the result of decreased number of kernels per head and lower kernels weights (Kolmer, 2005) Control wheat rusts as obligate parasite, based through genetic breading programs (Statler, 1984; Boulot and El-Sayed, 2001 and Henritte, 2009), induced resistance using biotic and abiotic agents (Sallam, 2001and Sallam *et al.*, 2001 and 2002) and chemical control with fungicides (Shafik *et al.*, 1992 and Jochen, 2009).

Resistant wheat cultivars against rusts may be changed to susceptible due to the development of new aggressive races that capable to infect these resistant cultivars. On the other hand, using fungicides as the most effective chemical control methods, cause some major problems threaten to limit its continued use. Among these problems, rust fungi develop resistant races against the used fungicides, other are not ready to self biodegradable and tend to persist for months and years in environment which affect other benefit organisms than target fungi. (Klinkenberg *et al.*, 1998).

Because of fungicide associated problems, the causal agent of wheat leaf rust (*Puccinia triricina* Eriks.) has developed resistance races to fungicides (Klinkenberg *et al.*, 1998).In this respect, researchers are now trying to use

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environmentally safe alternative methods for controlling wheat rust diseases.

Therefore, there is an argent need to find an alternative mean to control the disease. Coating polymers, such as film-forming anti-transparent have been reported to provide protection against several obligate foliar diseases included wheat rusts (Klinkenberg *et al.*, 1998). Such materials when sprayed on leaves as a pre-inoculation to prevent pathogen from becoming established in plant tissue, seem to be promising alternative to chemical fungicides (Han, 1990).

Application of these materials cause partial resistance in treated plants measured as increasing in incubation period, decreased type of infection value and decreased pustule numbers and size (Sallam, 2001 and Singh *et al.*, 2007). Also, Judith and Eyal (1991) suggested that film-forming compounds alter the topography of the leaf surface, thus interfering with adhesion of the germ tube and recognition of penetration sites .In other way, abnormal appressoria were more frequently observed on mutant or polymeric treated leaves than on naturally waxy plants. Removal and/or change character of epicuticular wax caused disturbance in appressoria formation during urediospore germination of *P. graminis* f. sp. *tritici* on wheat leaves (Andersen *et al.*, 1962).

The present study was conducted to study the effect of three novel polymeric compounds at different concentrations on leaf rust incubation period, infection type, pustule numbers/cm^2 and leaf area and pustule size. Also, the effect of these compounds on the changes of morphological and histopathological fungal structure using scanning electron microscope (SEM), light electron microscope (LM) and transmission electron microscope (TEM) were carried out.

MATERIALS AND METHODS

Wheat Seedlings

Sakha 93 and Sids1, highly susceptible bread wheat cultivars to leaf rust disease, were used in this experiment. Seeds of the two wheat cultivars were kindly obtained from Wheat Breading Research Dept., ARC, Giza, Egypt. Ten wheat seeds were sown in plastic pot (10 cm in diameter) each containing 250 g clay soil. After seven days, resulted seedlings were used in this research work.

Polymeric Materials

Three different emulsion polymeric compounds (Homopolymer, Pure acrylate and Styrene Co-polymer) were prepared by seed emulsion polymerization techniques.

Emulsion polymerization

Vinyl acetate homo polymer (Homopolymer) was prepared by seed emulsion polymerization where carried out in a 500 ml, three-necked round bottom flask equipped with a stirrer, a gas inlet system and a reflux condenser immersed in water bath. The oxygen was removed by purging nitrogen through the mixture for at least 30 minutes. The 60 g distilled water was introduced into the glass reactor with 0.6 g PVA as protective colloid. The mixture was stirred under nitrogen atmosphere at 90°C for one hour then temperature was decreased to 80°C followed by adding 10 g distilled water with 0.4 g NP30 as nonionic surfactant. The pH of the reaction medium was adjusted by adding 0.2 g sodium acetate.

The 6g homopolymer monomer was charged into the reactor. Then, 0.1 potassium persulfate used as an initiator was gradually dropped. The 54 g of homopolymer monomer were injected for 4 h under continuous stirring at 300 rpm and 80 °C. At the end of injected monomers, the temperature was raised from 80 to 85°C for 1 h to complete the polymerization reaction.

The other two emulsion polymer latexes based on methyl methacrylate, BuA, AA, (MMA) and Styrene, BuA, AA are shown sin tables I and II and were prepared by semi-batch emulsion polymerization technique with solid content of 50%. Typically, the copolymers synthesis has been carried in two steps; (1) pre-emulsion step and (2) seeded emulsion polymerization step. In the pre-emulsion step, 30% of distilled water, SDBS emulsifier, and the monomers (MMA and BuA) or (Styrene and BuA) were added into glass beaker and mixed for 30 min using high speed homogenizer. The acid monomer (AA) was added during continuous homogenization. In the seeded emulsion polymerization step, distilled water,

NP30 emulsifier, $NaHCO_3$, 50% of the initiator, and 10% of the pre-emulsion were added into four necked flask equipped with continuous stirring under reflux.

The seed polymerization reaction was carried out at 80°C using thermo stated water bath for 30 min under inert N_2 atmosphere. The remaining pre-emulsion (90%) and initiator were step wise added into the reactor within 3 h. The recipe used for preparation of the emulsion polymer latexes is given in Table I.

Table II represent physical, chemical stability and mechanical and characteristics of the prepared emulsions polymers property.

Leaf Rust Urediospores

Mixture of aggressive fresh urediospore races of *Puccinia triticina* Eriks. were collected from infected adult wheat plants in greenhouse of Wheat Disease Research Dept., Plant Pathol. Res. Instit., ARC. These urediospores were mixed with talc powder (1:20 v/v) in baby cyclone and used in case of artificial inoculation.

Polymeric Application and Rust Inoculation

Seven days old seedlings of Sakha 93 and Sids 1 were sprayed over leaf surfaces with the previously mentioned different concentrations of polymeric compounds. Seedlings sprayed with water act as infected control. While seedlings protected by sumi-eight fungicide (0.35 ml /L water) act as healthy control. After 24 h of spraying polymeric compounds prepared urediospores in baby cyclone were used to dust treated and control seedlings. Three pots each contain 10 seedlings were used as replicates for each particular treatment.

Disease Assessment

Incubation period (IP)

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

Type of infection (TI)

After commencement of sporulation seedling leaves were prepared for scoring of infection type, that were recorded as either high (IT3-4) or low (IT0-2) according to Long and Kolmer (1989) and Long *et al.* (1998, 2000 and 2002).

Pustule numbers

Number of rust pustules per cm^2 and leaf area on the upper side of leaves was counted as described by Parlevliet and Kuiper (1977). Percentage of 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 ethanol (1:2 v/v) solution for three minutes. Length (L) and width (W) of 20 randomly chosen pustules were measured for three leaves of each treatment. The pustule size (μm) was calculated according to the following formula:

Pustule size = 1/4 Л L W

Whereas Л=22/7, L = length and W = width

Statistical Analysis

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

Microscopic Studies

Scanning electron microscope

Segments of treated polymeric infected leaves with homopolymer at 10% and untreated infected leaves as control treatment were taken 24 hrs (dpi). Samples (2 x 5mm) were fixed in 2.5% glutarldehyde for 24 hrs at 4°C, 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 Center (Harley and Fergusen, 1990). Changes in the morphological fungal structures between treated and untreated were examined and photographed.

Light microscope (LM)

Samples of healthy and infected wheat leaves were collected after five days from inoculation, the semi thin section made by Transmission Electron Microscope Unit of Fac. Agric., Cairo Univ. were investigated by light microscope and photographed.

Transmission electron microscope (TEM)

Similar samples as previously mentioned in SEM were taken but at 5 dpi. Samples were fixed overnight in cold 2.5% glutraldehyde prepared in 0.1 potassium phosphate buffer (pH 7.4) and post fixed in 1% Osmium tetroxide (OSO_4) in the same buffer for 3 hrs.

After staining overnight in 1% uranyl acetate, the leaf segments were dehydrated in the ethanol acetone. Series and imbedded in squrr's medium (Spurr,1969). Ultra thin sections were cut with glass Knife on LKB ultra microtome, mounted on copper grids and stained for 10 minutes, with mixture of an equal volume of saturated uranyl acetate and acetone followed by lead citrate. The stained sections were examined and photographed by JEOL JEM.1400 electron microscope in Electron Microscope Unit, Faculty of Agriculture, Cairo University. Histopathological changes between treated and untreated control were examined and photographed.

RESULTS

Results referred to the incubation period indicated that none of the used polymeric compounds or their concentrations significantly increased the incubation period. It is worthy to mention that incubation period increased in protected seedling with sumi-8 fungicide, to 9 days in Sids 1 cultivar compared to control or other treatment which were ranged between 8 and 8.6 days (Table 1).

Tables 2 and 3 recorded the effect of spraying polymeric compounds at different concentrations on number of pustules per cm^2 and leaf area. Results revealed that both of tested polymeric compounds and their concentrations significantly reduced the number of pustules for each of cm^2 and seedling leaf area. Also number of pustules decreased in case of interaction between tested concentrations and tested cultivars. On the other hand, non-significant differences were detected between tested cultivars.

Table I. Recipe of Methyl Methacrylate/Butyl Acrylate (MMA) or Styrene /Butyl acrylate Emulsion Polymers

	(MMA)	(ST)
Distilled water	51	51
Methyl methacrylate (MMA)	20	--
Styrene (ST)	--	22
Butyl acrylate (BuA)	30	28
Acrylic acid (AA)	1.5	1.5
Sodium dedocyl benzene sulfonate (SDBS)	1.8	2
Sodim Bicarbonate	0.1	0.1
Potassium persulfate	0.1	0.1

All the used chemicals were fine chemicals imported from Sigma-Aldrich Germany.

Table II. Characteristics of the Prepared Emulsions Polymers Property Value

	MMA	ST
pH	8.2	8.1
Solids by weight (%)	48.7	48.9
Polymer conversion (%)	99.0	98.9
Particle size (nm)	99.0	89
Brookfield viscosity RV no. 50 rpm, (cPs)	40,000	30,000
Chemical stability	Excellent	Excellent
Stability against $CaCl_2$	Pass	Pass
Freeze thaw at 15°C, (cycles)	4	4
Hardness (pencil) test	2B	3B
Adhesion to PVC sheet	Gt_o	Gt_o

Three concentrations (5, 10 and 15%) were prepared from the original compound using water.

Table 1. Effect of different concentrations of three polymeric compounds on mean incubation period (days) of infected wheat cultivars by leaf rust pathogen (*Puccinia triticina*)

Treatment	Poly. Conc. %	Cultivars Sakha 93	Sids 1	Mean	Mean
	5	8	8	8	
Homopolymer	10	8,6	8.3	8.45	
	15	8.6	8.3	8.45	
	Mean	8.4	8.2		8.30
	5	8	8	8	
Pure acrylate	10	8	8	8	
	15	8.6	8.6	8.6	
	Mean	8.2	8.20		8.20
	5	8	8	8	
Styrene Co-polymer	10	8	8.6	8.3	
	15	8	8.6	8.3	
	Mean	8	8.4		8.20
Infected		8	8	8	
Sumi -8		8.6	9	8.8	
Mean		8.24	8.4		
Mean of concentration	5% 8.0				
	10% 8.28				
	15% 8.50				

LSD at 5.0% all of treatment and their interactions non- significant

Table 2. Effect of different concentrations of three polymeric compounds on mean number of pustules /cm^2 area and percentage reduction of infected wheat cultivars by leaf rust pathogen (*Puccinia triticina*)

Treatment	Poly. Conc. %	Cultivars Sakha 93		Sids 1		Mean	Mean
		Sakha 93	Red.%	Sids 1	Red.%		
	5	26.66	63.8	24.66	79.78	25.66	
	10	4.66	93.67	8.33	93.17	6.49	
Homopolymer	15	12.66	82.81	13.33	89.07	12.99	
	Mean	14.66		15.44			15.05
	5	25.66	65.16	20.33	83.33	22.99	
Pure acrylate	10	19.66	73.3	23.66	89.6	21.66	
	15	11.66	84.17	22.33	81.69	16.99	
	Mean	18.99		22.1			20.54
	5	33.33	54.75	28	77.04	30.66	
Styrene Co-olymer	10	19.66	73.3	19.33	84.15	19.49	
	15	18	75.56	19.66	83.89	18.83	
	Mean	23.66		22.33			22.99
Infected cont.		73.66	0	122	0	97.83	
Sumi-8		4	94.56	6	95	5.00	
Mean		26.99		37.57			
Mean of concentration	5%	26.67					
	10%	15.89					
	15%	16.28					

LSD at 5% for			
Cultivars (A)	NS	AB	NS
Polymers (B)	3.557	AC	8.085
Concentrations (C)	5.719	CB	NS
		ABC	NS

Table 3. Effect of different concentrations of three polymeric compounds on mean number of pustules/leaf area and percentage reduction of infected wheat cultivars by leaf rust pathogen (*Puccinia triticina*).

Treatment	Poly. Conc. %	Cultivars				Mean	Mean
		Sakha 93		Sids 1			
		Sakha 93	Red.%	Sids 1	Red.%		
	5	29.66	89.27	69.66	76.78	49.66	
Homopolymer	10	7	97.46	11	96.33	9	
	15	13	95.3	28.33	90.55	20.66	
	Mean	16.55		36.33			26.44
	5	82.33	70.24	75.66	74.78	78.99	
Pure acrylate	10	61	77.95	67	77.66	64	
	15	35.65	87.11	52.66	82.44	44.16	
	Mean	59.66		65			62.38
	5	52.53	81.01	90.33	69.89	71.43	
Styrene Co-polymer	10	36.33	86.86	62.66	79.11	49.49	
	15	49.66	82.85	59	80.33	54.33	
	Mean	46.17		70.66			58.41
Infected cont.		276.66	0	300	0	288.33	
Sumi-8		3	98.91	2.66	99.11	2.83	
Mean		80.41		94.96			

Mean of concentration	5%	6.70	
	10%	40.83	
	15%	39.72	
LSD at 5%for			
Cultivars (A)	NS	AB	NS
Polymers (B)	8.455	AC	32.376
Concentrations (C)	45.971	CB	NS
		ABC	NS

As far as the effect of different concentration of three polymeric compounds on infection type (IT), data obtained indicated that tested concentrations significantly reduced infection type compared with infected control. While, all other tested factors including tested cultivars and polymeric compounds and their interaction were non-significant on infection type (Table, 4).

Data obtained in Table 5 indicated that spraying polymeric compounds at different concentrations on Sakha 93 and Sids 1 wheat seedlings reduced pustules size .This effect was clearly detected between tested cultivars and concentrations. With the exception of significant differences between tested cultivars and concentrations as well as polymeric compounds and tested concentrations in all other combination were non-significant.

In general, increasing concentrations of used polymeric compounds from 5 to 15% in pre inoculation application had a corresponding effect in reducing number of pustules per cm^2 and leaf area, infection type and pustule size.

Scanning Electron Micrographs

Scanning electron micrographs of wheat seedling leaves treated before inoculation with the tested homopolymer compound showed coverage of leaf surface with sprayed compound compared with healthy untreated leaves .Also, uncovered leaf hair and stoma were clearly observed in control treatment. Epicuticular characters of polymeric treated leaf surface showed that, disturbance in morphological structures of leaf (leaf hair, epidermal cells, veins and stomata) comparison with healthy uninfected treatment. Also, polymeric material covered all leaf surface (Fig.1).

Spraying leaves of wheat seedlings with homopolymer compound pre-inoculation cause shrinking of all treated urediospores and inhibit their germination comparing with control treatment (Fig. 2). In control treatment, spherical uridiospor germinate and develop germ tube orient to the stoma and infect leaf through appressoria present on the leaf stoma.

Table 4. Effect of different concentrations of three polymeric compounds on Infection type of wheat cultivars by leaf rust pathogen (*Puccinia triticina*)

Treatment	Poly. Conc. %	Cultivars Sakha 93	Sids 1	Mean	Mean
	5	3	2	2.5	
	10	1.66	1.33	1.49	
Homopolymer	15	2	1.66	1.83	
	Mean	2.22	1.66		1.94
	5	2.66	1	1.83	
Pure acrylate	10	2	2.33	1.16	
	15	2	2	2	
	Mean	2.22	1.77		1.99
	5	3	1	2	
Styrene Co-polymer	10	2.66	2.33	2.49	
	15	2	2	2	
	Mean	2.55	1.77		2.16
Infected cont.		4	4	4	
Sumi-8		1	1	1	
Mean		2.4	2.16		

Mean of concentration	5%	2.56	
	10%	1.94	
	15%	1.89	
LSD at 5%for			
Cultivars (A)	NS	AB	NS
Polymers (B)	NS	AC	NS
Concentrations (C)	0.274	CB	NS
		ABC	NS

Table 5. Effect of different concentrations of three polymeric compounds on pustule (µm) size and percentage reduction of wheat cultivars by leaf rust pathogen (*Puccinia triticina*)

Treatment	Poly. Conc. %	Cultivars Sakha 93		Sids 1		Mean	Mean
		Sakha 93	Red.%	Sids 1	Red.%		
	5	480.4	41.62	347.26	46.16	413.83	
Homopolymer	10	118.5	85.6	162.33	74.85	140.36	
	15	179.3	78.21	264.26	59.03	221.78	
	Mean	259.4		257.91			258.76
	5	231.3	71	333.6	48.28	282.45	
Pure acrylate	10	227.7	72.33	235.5	63.49	231.6	
	15	219.7	73.3	163.5	74.65	191.6	
	Mean	226.23		244.2			309.65
	5	342.76	58.34	315.26	51.13	329.01	
Styrene Co-polymer	10	260.36	68.36	254.36	60.57	257.36	
S	15	162.23	80.28	205.13	68.2	183.68	
	Mean	255.11		258.25			322.58
Infected cont.		822.93	0	645.1	0	734.01	
Sumi-8		71.13	91.35	146.5	77.29	108.81	
Mean		326.96		310.4			

Mean of concentration	5%	341.77	
	10%	209.78	
	15%	215.61	
LSD at 5.0 % for		AB	NS
Cultivars (A)	4.66	AC	66.073
Polymers (B)	N.S	BC	80.923
Concentrations (C)	46.721	ABC	NS

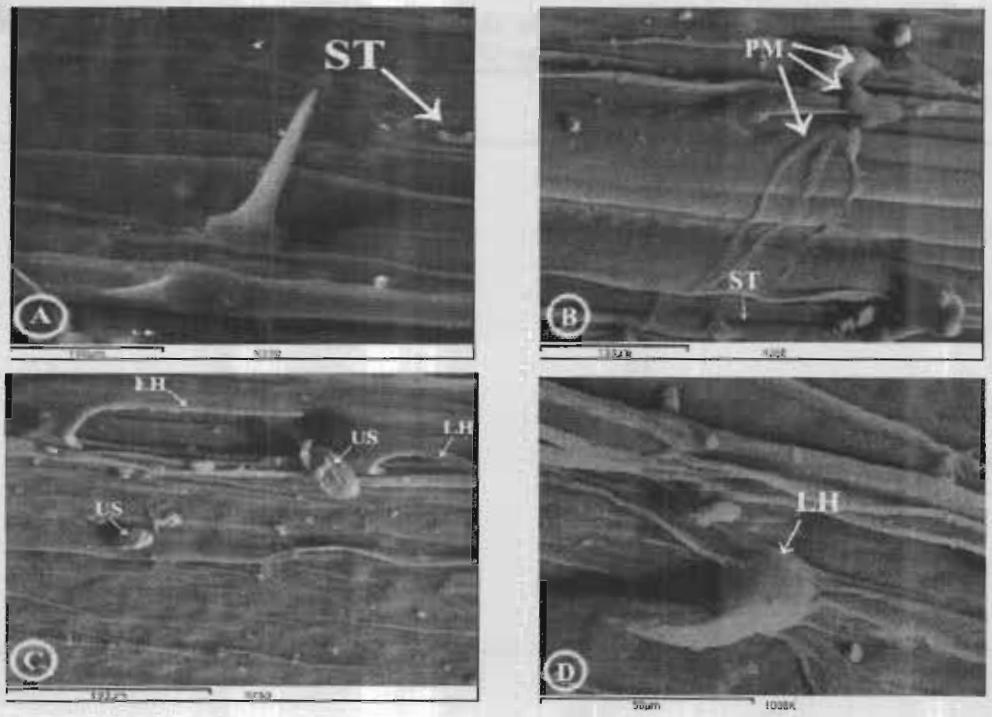

Fig. 1. Scanning electron micrographs showing leaf surface of healthy untreated control (Fig. A) and treated polymeric material (PM) in infected leaf seedlings (Figs. B, C and D). In untreated healthy control notice the clear surface of healthy leaf and stoma (ST).Figs. B, C and D showing polymeric material covering leaf surface, ungerminated urediospores (US), curved leaf hair (LH) and invisible stoma covered with the polymeric materials

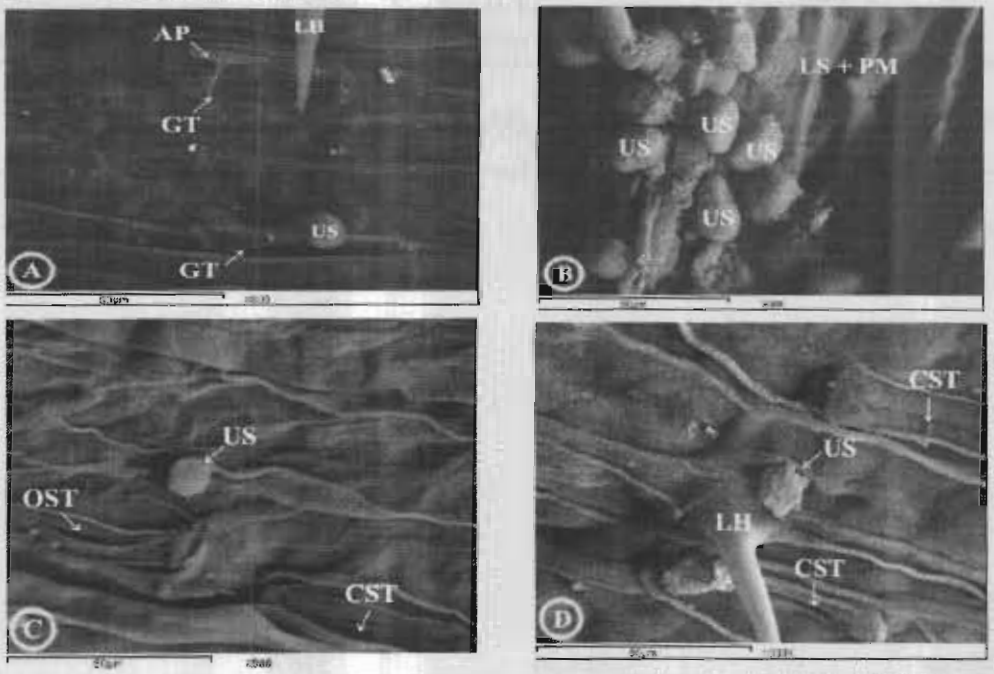

Fig. 2. Scanning electron micrographs showing spherical standard shape of urediospor(US), germ tube (GT) and ideal appressoria (AP) in control treatment (Fig. A). In Figs B, C and D infected and treated leaves with polymeric material. Notice leaf surface(LS)covered with polymeric material(PM), shrinking un-germinated uridiospore (US), closed stoma (CST), opened stoma (OST) and curved leaf hair (LH)

In treated infected leaves urediospores elongated and became oval in its shape, other became shrink and ungerminate. Most of observed stomata covered by the polymer and became closed. Spraying polymeric compound (Homopolymer) on leaf surfaces of wheat seedlings before inoculation inhibit urediospores germination as previously mentioned then this compound dry and contain cracked particles of polymeric compound (Fig. 3).

Light microscope

In Fig. 4 semi thin section of healthy artificially infected and the treated polymeric infected Sids 1 wheat cultivar leaves were investigated by light microscope. Samples were chosen after 5 days post inoculation (dpi) to shed some overview light on the effect of spraying polymeric compound (Homopolymer) on wheat leaf surface to control rust disease. Healthy treatments indicate normal morphological mesophyell cells and chloroplast organelles distributed beside the plasma lemma. Also, transfer sections of infected leaves. In Fig.4 B and C) showed that fungal hyphae penetrate the intercellular spaces of mesophyll cells. Disturbance of chloroplast distribution in infected cells was observed .Also, mature pustules were observed through the upper leaf surfaces. The fungal invasion structures extended near to the vascular bundles. Overview of leaves treated with tested compounds revealed that, normal cell morphology, normal distribution of chloroplast in cells beside the cell plasma lemma compared to the infected control. Absent of fungal hyphae in intercellular spaces of mesophyll tissue was also observed. Polymeric compound covered the upper epidermis and stoma.

Transmission Electron Microscope (TEM)

Cytopathological changes were detected by transmission electron microscope (TEM). These changes were extended to most of the cell organelles, i.e. cell wall, plasma lemma, cytoplasm, chloroplasts, mitochondria and nuclei comparing with healthy and infected pre treated wheat seedlings with Homopolymer compound. Fig. 5 indicates the structure of healthy wheat cells including the previously mentioned organelles. Photographs of healthy wheat cells indicated that all organelles and their distribution in the cells were normal without any changes in their structures. Plasmodesmata were clearly observed between healthy cells. Fig.6 indicate the changes in infected cells .This figure show that fungus completely lyses cell contents including irregular, thickened and twisted cell wall. Alternation were observed in chloroplasts and mitochondria in different way including swollen, increasing starch grains and complete deterioration of the chloroplast envelope, with an obvious cells. Nuclei of the infected cells showed irregularity in shape and the dense area of chromatin became light comparing with control treatment. Fungal structures were observed in inter and intra cellular spaces.

In case of polymeric treated cells, Fig.7 indicate improvement in cell structures compared with infected control Also ,less disorganized of chloroplast, cell wall etc.

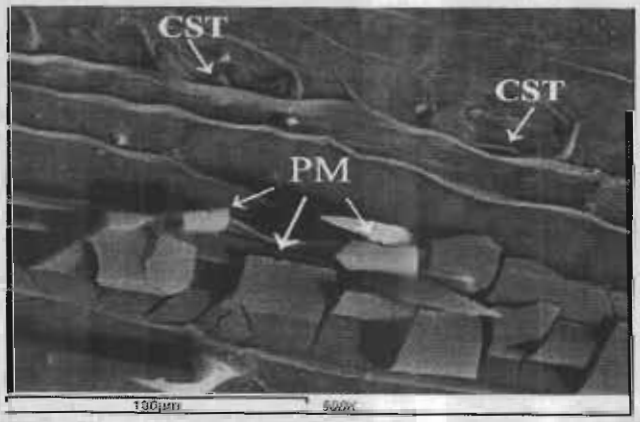

Fig. 3. Scanning electron micrograph showing dried and plated polymeric material (PM) changed to crack parts. Closed stoma (CST) on leaf surface were observed after removing polymeric material from this parts of leaf surface .

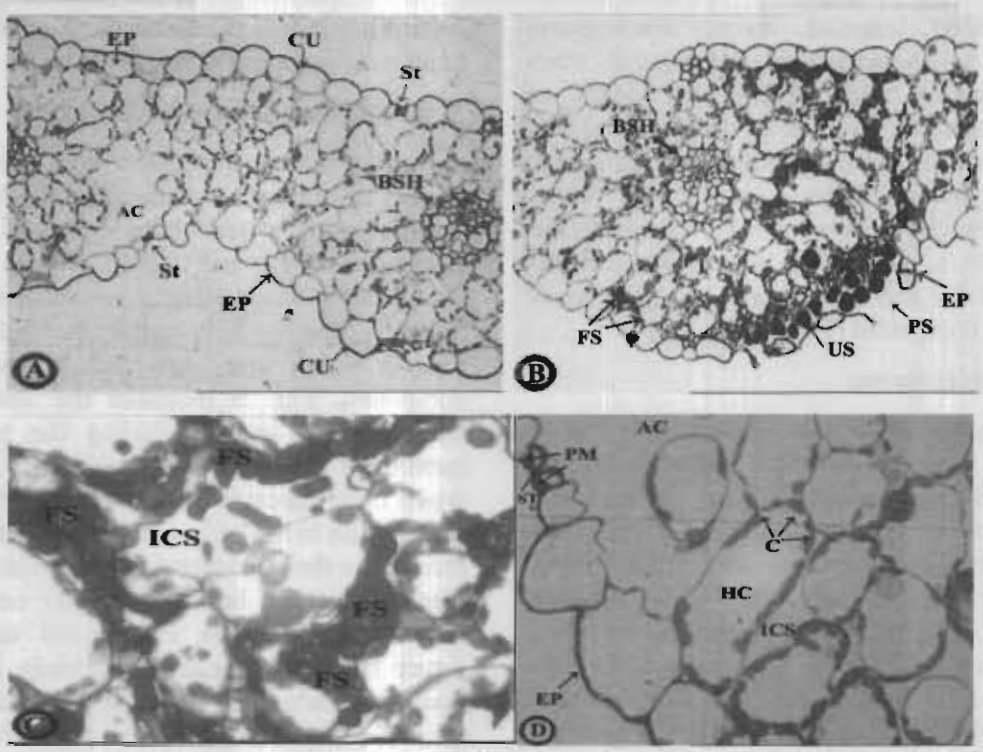

Fig. 4. Light microscope cross section of healthy (A-160 X) infected (B- 160X and C-460X) and treated polymeric material (D-460X) 24 hrs pretreated Sids 1 wheat cultivar leaves. In Fig. A, normal morphological mesophyll cells and chloroplast organelles distribute beside the plasma lemma. Notice normal bundle sheath (BSH), stoma (ST), sub-stomal air chamber (AC), upper and lower epidermis (EP) covered with cuticle(CU) .Fig. B, indicate infected cells showing disturbance of cell wall (CW), cell morphology and unsymmetrical chloroplast distribution in addition to rupture epidermis (EP),fungal structure(FS)with mature pustule (PS) contain urediospores (US). Fig.C, show the infected leaf cells, intercellular spaces(ICS) filled with fungal structures with disturbance in cell contents. Fig. D, show the infected and polymer pretreated leaf. Notice polymeric material covered the epidermis (EP) and stoma (ST) indicated by arrows with normal healthy cells (HC) and chloroplast symmetrical distributed.

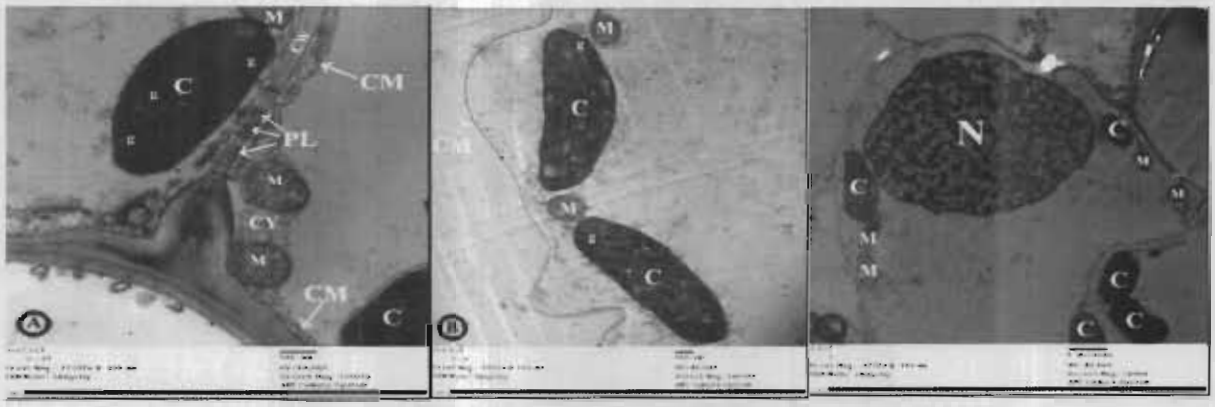

Fig. 5. Transmission electron micrograph showing ultra structure of healthy Sids 1 wheat leaf cells. Figs. A and B, showing symmetrical structure of cell wall (CW), cytoplasm (CY), mitochondria (M), chloroplast (C) with obvious grana (g) and thylakoids. Also, plasmodesmata (PL) run through the cell wall between cells. Fig. C, show nucleus (N) with highly dense chromatin giving nucleus the appearance of black and white net

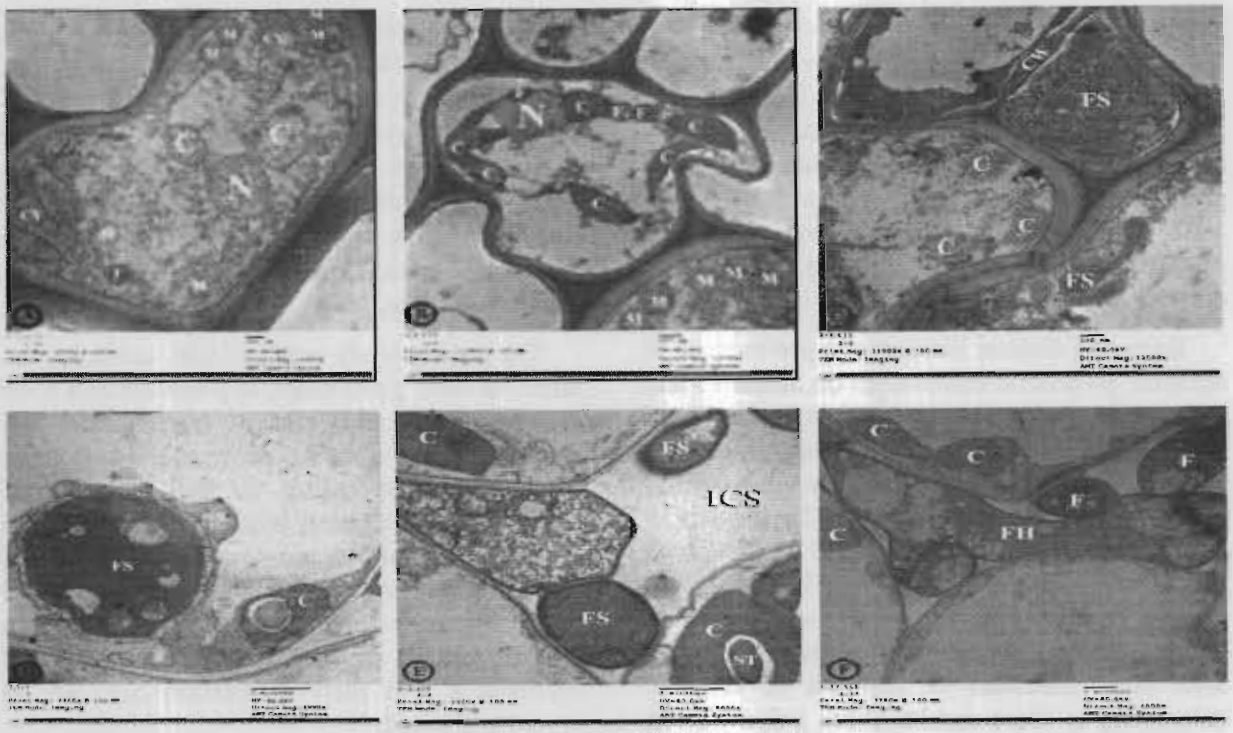

Fig. 6. Transmission electron micrograph showing leaf cell ultra structure of infected Sids 1 wheat seedling 5 days post inoculation (dpi). Fig. A, completely layside, disorganized cell contains, deteriorated chloroplast (C), mitochondria (M) and nucleus (N) Fig. B, disorganized cell shape with abnormalities of cell organelles. Fig. C, show irregular, twisted and thickened cell wall (CW) associated with layside and fungal structures (FS) which were observed in both intra and intercellular spaces (ICS). Fig. D,) fungal structures impeded in cytoplasm (SY). Also, disorganized chloroplast contain big starch granule (ST).Fig. E, fungal structures distributed in inter and intra- cellular spaces(ICS). Fig. F, different fungal structures including fungal haustoria (FH) in intercellular spaces.

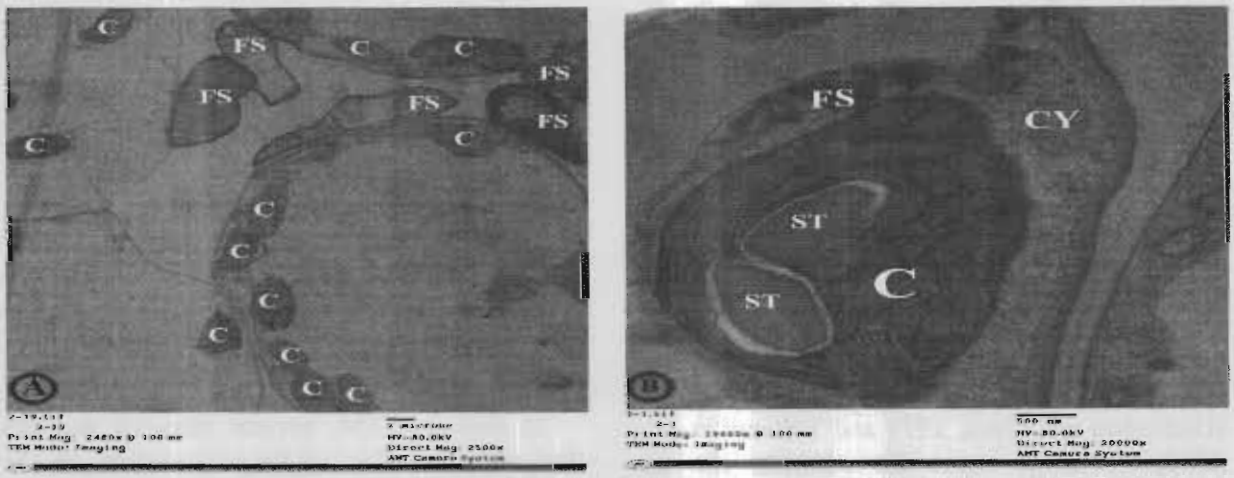

Fig. 7. Histological ultra structure of plant cells in polymeric pretreated leaves of Sids 1 wheat cultivar before fungal inoculation were intermediated between healthy and untreated infected plants. Fig. A, semi arrangement of chloroplast (C), fungal structures (FS) in intercellular spaces. Fig. B, semi disorganized chloroplast with increasing starch granules (ST) associated with dense cytoplasm (CY) and intercellular fungal structures

DISCUSSION

Results obtained in this study indicated that using polymeric compounds reduced leaf rust disease incidence through decreasing the number of pustules /cm^2 and per leaf area as well as infection type and pustule size on infected seedlings of Sakha 93 and Sids 1 wheat cultivars.

Reducing disease incidence in pre-inoculated sprayed polymeric compounds on Sakha 93 and Sids 1 bread wheat cultivars might be attributed to their physical and /or chemical barriers against the establishment of plant pathogen as shown by Lewis and Day (1972), Yang and Elhugpboe (1972), Royal (1975) Ziv and Fredriksen (1983 and 1987) and Tohamy *et al.* (2005). In our conclusion, these compounds may be provide an impenetrable surface associated with their thickness or resistant to enzyme. According to these results, these polymers can be applied as protected parries over the leaf surfaces to protect tissues against invading by leaf rust pathogen.

It is worthy to mention that, stomata present on the both surface of leaves, usually consisting of two guard cells with opening space between them. Opened and closed stomata controlling evaporation of water from the leaf ,alternative gasses (CO_2 & O_2) into leaf as well as considered as microorganisms entrance way to infect their hosts especially in case of obligate pathogens .Leaves of wheat surface covered with substances composed of a verity of organic compounds and polymers ,many of which are derived from lipids .In some resistant cultivars wax covering leaf surface help to reduce water evaporation from leaves, reflect light and protect plant against pathogen. In susceptible wheat cultivars, leaf surface provides certain physical stimulating factors affect urediospore germination that orient the germ tube toward the leaf stoma casing the disease incidence (Dickinson and Gson, 1979 and Tohamy *et al.*, 2005). In some cases the stimulus can be associated in part with chemical factors originating in the stomata (Edwards and Bowling 1986). In case of treated wheat leaves with polymeric compounds, epicuticular leaf surface structural change and this effect could be partially responsible for the differences in plant susceptibility. This change might be due to that polymeric compounds exclude the fungus by preventing physical contact and eliminate chemical factors originating in stomata between the invading pathogen and host tissue, which apparently help in formation of infection structures in unidentified way (Judith and Eyal, 1991 and Sallam, 2001).

The present study revealed that, urediospores failed to form germ tubes, appressoria and their distribution on the coated leaf surface are associated with spraying polymeric compounds which cause disruption in the infection of mechanisms associated with orientation of the germinating urediospores towards the stoma and formation of appressoria. However, when the coating layer is incomplete on leaf surface in case of disease development, the formation of appressoria and their orientation are not as severely affected.

The three polymeric compounds used here represent three types of polymers. The first one (Homopolymer) is hard and brittle, the second (Pure acrylate) is tacky and has good adhesion on leaf surface, the last one (Styrene Co-polymer) has good adhesion and high surface tension. According to their properties cracked particles were observed in SEM study when the first compound was used due to its hardness and brittle properties. These particles removed from leaf surface by the air, wind and /or agriculture practices, fall down in soil and self biodegradable.

Results obtained in cross section of light microscope indicated that *P.triticina* infect Sids 1 wheat seedlings causing disease incidence and showed the disturbance of cell organelles. Similar results using light microscope were obtained by Brzezicka *et al.* (1990) who studied the cytological change in mesophyll tissue of infected wheat plants with leaf rust pathogen.

In case of TEM, several research workers deal with the effect of fungal infection on the ultra structure of infected plant cells. In this respect, abnormalities of cell wall and plasma-lemma were observed by Jackobs *et al.* (1993) on leaf rust. Obtained results were in agreement with his results and the result of Harder *et al.*

(1978) on the abnormalities of plasma-lemma when wheat plants infected by stem rust pathogen. Changes in chloroplast observed in this research work were in harmony with those obtained by Coffey (1975) and Heath *et al.* (1997). They observed that degradation of chloroplast was the direct result of continuity between the envelopes of chloroplast and the fungal infection. Also, Kang (1996) and Heath *et al.* (1997) studied in details the effectiveness of rust fungi on the nucleus deterioration which were similar in results obtained in this research work.

The results of this study suggested that using polymers in coating plant leaves help in controlling foliage disease is a positive application methods due to their non-phytotoxic, permeable to gases, resistant to changing environmental conditions penetration of solar irradiation and self biodegradable.

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مقـاومة مـرض صـدأ أوراق القمح باسـتخدام أغشـية مستحلـب البوليمـــرات

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تم تقييم تأثير ثلاثة مركبات من البوليمرات (Homopolymer,Pure acrylate and Styrene Co-polymer) بتركيزات مختلفة (٥ ، ١٠ ، ١٥%) على تكشف مرض صدأ الأوراق المتسبب عن فطر بكسينيا تريتيسينا على صنفين من أصناف القمح عالية القابلية للإصابة بالمرض وهما سخا ٩٣وسدس ١. أوضحت النتائج أن رش هذه المركبات بتركيزاتها الثلاثة على أوراق بادرات القمح قبل العدوى بالمسبب المرضى بـ ٢٤ ساعة أدى إلى إنخفاض معنوى في عدد البثرات فى وحدة المساحة (سم٢ أو الورقة) مقارنة بالكونترول المصاب إلا أنها لم تؤثر معنويا في زيادة فترة حضانة المسبب وأوضحت أيضا أن شدة المرض مقدرة باستخدام طرز الإصابة تغيرت إلى المقاومة لأن بعض مناطق الإصابة أظهرت فقط فلكسات ولم تتكشف إلى بثرات وكذا إنخفض حجم البثرة معنويا كما أظهرت النتائج أن تأثير هذه المركبات يتوقف على التركيز المستخدم. هذا وقد أوضح الفحص المجهرى بإستخدام الميكروسكوب الإلكترونى الماسح تغطية أسطح الأوراق بمركبات البوليمرات مما سبب تغيرا طبوغرافيا علي سطح الأوراق مع حدوث تثبيط لإنبات الجراثيم وأنابيب إنباتها وأعضاء إلتصاقها على سطح الأوراق. أوضحت نتائج الفحص بإستخدام الميكروسكوب الضوئى والإلكترونى النافذ تغيرطفيف فى التركيب الداخلى لخلايا وأنسجة أوراق البادرات المعاملة بمركبات البوليمرات مقارنة بالبادرات غير المعاملة (كونترول) ويفسر ذلك بقدرة هذه المركبات على تثبيط إنبات جراثيم الفطرأوإعاقة تكوين أنابيب الإنبات وأعضاء الإلتصاق للمسبب المرضى.