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Research Article
Seed Dressing Affect Protein, Antioxidant Enzymes, Ultrastructure, Soil Properties and Microflora of Maize and Wheat Seedlings

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Abstract
Background and Objective: Seed-dressing or coating substances play a role to control underground pests, having ability to kill seed and seedling diseases, promote seedling healthy growth, improve the crop quality and seed germination rate. **Materials and Methods:** This investigation was carried out in order to study protein banding pattern, antioxidant enzymes activity, leaves ultrastructure in maize and wheat coating with fungicides, hattrick (6%) and premis (25%), respectively during three vegetation stages. **Results:** The protein profile of maize and wheat showed the appearance of 6, 4, 23 and 21 monomorphic bands at the 2nd and 3rd stage, respectively. Catalase and peroxidase activities of treated plants were increased. In ultrastructure measurement treatment caused decrease or increase in the measured parameters, with appearance of starch grains in treated maize and disappearance in treated wheat. The soil properties and soil microflora showed marked differences in response to the used treatments. **Conclusion:** From results we can concluded that dressing maize and wheat grains with the prospective dose of hattrick and premis, respectively enhance growth and the response was more pronounced in maize plants.

Key words: Fungicides coating, protein banding, antioxidant enzymes, soil properties and microflora, vegetation, monomorphic bands

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Maize is the Arawak-Carib word from which the name maize is derived. It is also identified as Indian corn and in North America simply as corn[1]. Maize is a plant belonging to the family of grasses (Poaceae). It is cultivated globally, being one of the most important cereal crops worldwide. About 50-55% of maize production is used as food in most developing countries.

Wheat (*Triticum aestivum*) is the principal winter crop and the most important grains crop in the world[2]. It provides an almost 20% of food energy for people in the world as well as in Egypt. Increasing wheat production is the ultimate goal to reduce the wide gap between productivity and consumption[3-5]. Wheat is the most widely grown and consumed food grain of the world[6]. With progressive global climatic change and increasing shortage of water resources and worsening eco-environment, wheat production is influenced greatly[7].

Many studies have developed for crop protection to prevent considerable economic losses, such as pesticide seed-coated technology against crop pests and diseases[8,9]. Seed treatment is the use of pesticide as a cover around the seed where its active substance protects seed from soil borne pathogens and insects[10].

A fungicidal seed treatment is commonly composed of a trace quantity of fungicide evenly distributed among the seeds along with the adhesive substances needed to bind them to the seed surface[11].

A study recognized the function of seed-dressing substances in crop protection[12,13]. These coating substances play a role in control underground pests, kill seed and seedling diseases, promote seedling healthy growth, improve the crop quality and seed germination rate, reduce the use amount of seeds and increase the output and so on[13]. The harm of maize leaf blight, sheath blight, head smut and other harmful organisms have great impact on maize seedling, yield and quality[14,13]. To control the northern maize leaf blight, sheath blight and head smut corn grains are coated by pesticides[13]. Seed coating agent can promote seed germination, prevent and control diseases and insect pests and supply essential nutrients to achieve the purpose of strong seedlings. It can also create a good micro ecological environment[15].

Seed-coating treatments results are usually positive as it reduced the number of pests and improved the quality of the seeds is by conventional treatments[16,17]. However the accumulative toxicity in the soil of these conventional seed-coating agents suggests that they are not the best alternative for the environment. In addition, pests may develop resistance against them, which implies the use of increasingly larger amounts to be efficient.

Seed dressing with insecticides and/or fungicides in addition to protect seeds from pests and diseases, is widely used in conventional agriculture[18]. Meanwhile, fungicide seed dressing should cause negative effects on the component of the soil microflora[19].

So, to evaluate the importance and safety of coating maize grains with hattrick and wheat grains with premis; as a common event that carried out by Ministry of Agriculture in Egypt, this experiment was conducted with an objective to study the effect of dressing the used grains with fungicides on protein banding, antioxidant enzymes and leaves ultrastructure as well as soil properties and its microflora.

MATERIALS AND METHODS

Plant material: This study was carried out at the green house of Faculty of Science, Mansoura University, Egypt from March, 2016 to May, 2016. A homogeneously-sized lot of *Zea mays* (maize) grains (uncoated and coated with hattrick) and *Triticum aestivum* (wheat) grains (uncoated and coated with premis) were selected and surface sterilized by soaking the grains in 0.01% $HgCl_2$ solution for 3 min, then grains washed thoroughly with tap water. The four sets of seeds (uncoated and coated maize and wheat grains) were sown in similar earthenware pots with a diameter of 20 cm filled with equal amounts of soil.

All sets of grains were cultivated and the pots were kept in the greenhouse under a normal day/night conditions and irrigated as usual practice with equal amounts of tap water when required. Super phosphate and urea fertilizers were added to the soil during first week of cultivation. Seedling samples of the used plants were collected after 2, 4 and 8 weeks from sowing and referred to stage 1, 2 and 3, respectively.

The collected plant samples were used for assessment of some antioxidant enzymes activities during the three vegetative stages. In addition, protein banding pattern and leaf ultrastructure changes were also determined but only at stages 2 and 3. It should be mentioned that, triplicate samples were analyzed for different metabolic activities, but only the mean values are presented in the respective tables. Meanwhile only one sample was used for protein banding and ultrastructure. The soil properties and soil microflora were also carried out but at stage 1 only.

The full data of the treated groups were statistically analyzed and comparison among means was carried out by computer programming method[20] (stat graphic-vers-4-2-Display ANOVA).

Analytical methods

Protein banding patterns: The SDS-polyacrylamide gel electrophoresis[21] was performed in 12% acrylamide slab gels to identify their protein profiles.

Estimation of antioxidant enzymes: The extraction of the peroxidase was carried out according to Khatun *et al.*[22] and its activity was estimated following the method of Mahadevan and Sridhar[23]. The extraction and estimation of the catalase was carried out[24].

Transmission electron microscopy (TEM): Tiny sections (4×4 mm^2) from mature leaves of plants were used for electron microscopy. Stained sections were examined with a JEM-JEOL 2100/Japan Transmission Electron Microscope at the Electron Microscopy Unit/Mansoura University.

Soil sampling and analysis: Soil samples were collected from different pots (5-10 cm depth) representing the treatment and untreated after planting. These samples were then brought to the laboratory in closed plastic bags after collection. The samples were spread over sheets of paper; air dried, thoroughly mixed, passed through a 2 mm sieve to remove gravel and debris and then packed in plastic bags to be ready for physical and chemical analyses.

Physical characteristics: The heavy textured soil samples were determined by using Bouyoucos hydrometer method[25]. While the coarse textured soil samples were determined by dry sieving method (mechanical analysis). Field capacity value (FCV) and permanent wilting point (PWP) were determined[26]. Meanwhile the available water (AW) determined according to Kirkham[27] as the difference between FCV and PWP by the equation:

$$AW = FCV\text{-}PWP$$

Chemical characteristics: The chemical variables estimated in the present study included, calcium carbonate, organic carbon, pH, electrical conductivity, chlorides, sulphates, carbonates, bicarbonates, total nitrogen, total dissolved phosphorous and extractable cations (K^+, Ca^{++} and Mg^{++}). The estimation of calcium carbonate and organic matter were carried out using air dry soil samples, while other variables were determined using soil water extract (1:5).

Soluble cations and anions in the soil paste extract were determined according to methods described by Jackson[28]. Calcium and magnesium were determined by titrating with versenate (EDTA) using murexide as an indicator for calcium, eriochrome black T as an indicator for Ca^{2+} and Mg^{2+}. Potassium was determined by flame photometer. Carbonate and bicarbonate were determined by titration with HCl using phenol phthalein as an indicator for the former and methyl orange as an indicator for the latter. Chloride was determined using Mohr's method. Sulphate was calculated by subtracting the total soluble anions from the total soluble cations.

Soil pH was determined in the saturated soil paste using a Gallenkamp pH meter (Model pH Tester 2™) and total soluble salts were determined by measuring the electrical conductivity (EC) both according to Richards[29].

Organic matter content was determined using Walkely's rapid titration method[30]. Calcium carbonate was determined using Collin's calcimeter[31].

Soil microflora: For isolation of bacteria, nutrient agar containing 0.015% (w/v) nystatin (to inhibit fungi growth) was prepared. Ten grams of rhizospheric dry soil sample was taken for serial dilution series up to 10^{-9} by using saline water (0.85%)[32]. The bacteria were originally isolated by direct technique on TSA (Trypticase soy agar) and incubated for 24 h at 37°C. The developed colonies were purified by streaking on nutrient agar for bacterial identification according to colony and cellular characterization[33].

For isolation of fungi, PDA (Potato dextrose agar) to which 0.05% (w/v) chloramphenicol has been added, to inhibit bacteria growth was prepared. Ten grams of rhizospheres' soil sample was taken for serial dilution series up to10^{-9} by sterile distilled water. Fungi were isolated by inoculating 0.1 mL of the dilutions of the rhizosphere soil samples on PDA plates and incubated for 4 days at 28°C, then as in case of bacteria the appeared colonies were picked and identified[33].

RESULTS

Change in protein banding patterns of leaves: As mentioned previously, the protein profile, in this study was carried out in the leaves of *Zea mays* and wheat treated and untreated plants during stages 2 and 3 only (Table 1, 2, Fig. 1). At stage 2, the treatment of *Zea mays* has no change comparing with the control bands. Whereas stage 3 showed four bands only with disappearance of two bands at 39.433 and 31.24 kDa at both control and treated plants (Table 1). Regarding wheat plant, treated plants have the same bands as untreated plants (23 bands for each), at stage 2. At stage 3, there were 21 monomorphic bands with absence of 2 bands at 167.076 and 78.38 kDa as compared with stage 2 for treated and untreated plants (Table 2).

Table 1: Effect of coating grains with fungicide on protein banding pattern of *Zea mays* leaves

		Zea mays leaves				
		Stage 2		Stage 3		
RF	MW (KD)	Control	Treated	Control	Treated	Frequency
0.352	70.219	+	+	+	+	1.0
0.522	45.229	+	+	+	+	1.0
0.575	39.433	+	+	_	_	0.5
0.665	31.240	+	+	_	_	0.5
0.757	24.622	+	+	+	+	1.0
0.98	13.827	+	+	+	+	1.0
Total bands		6	6	4	4	
Monomorphic (%)		100		100		
Polymorphic (%)		0		0		
Unique (%)		0		0		

MW: Molecular weight, from table of present or absent protein bands showed that, total number of bands = 6, Maximum MW = 70.219 , Minimum MW = 13.827, Mean of band frequency = 0.458

Table 2: Effect of coating grains with pesticide on protein banding pattern of *Triticum aestivum* leaves

		Wheat leaves				
		Stage 2		Stage 3		
RF	MW (KD)	Control	Treated	Control	Treated	Frequency
0.017	167.076	+	+	-	-	0.5
0.044	155.802	+	+	+	+	1.0
0.124	126.670	+	+	+	+	1.0
0.142	120.905	+	+	+	+	1.0
0.162	114.807	+	+	+	+	1.0
0.176	110.723	+	+	+	+	1.0
0.188	107.337	+	+	+	+	1.0
0.204	102.984	+	+	+	+	1.0
0.229	96.533	+	+	+	+	1.0
0.31	78.280	+	+	-	-	1.0
0.33	74.332	+	+	+	+	1.0
0.364	68.072	+	+	+	+	1.0
0.423	58.434	+	+	+	+	1.0
0.449	54.632	+	+	+	+	1.0
0.477	50.814	+	+	+	+	1.0
0.509	46.776	+	+	+	+	1.0
0.54	43.170	+	+	+	+	1.0
0.632	34.025	+	+	+	+	1.0
0.65	32.477	+	+	+	+	1.0
0.703	28.315	+	+	+	+	1.0
0.773	23.624	+	+	+	+	1.0
0.926	15.901	+	+	+	+	1.0
0.977	13.935	+	+	+	+	1.0
Total bands		23	23	21	21	
Monomorphic (%)		100		100		
Polymorphic (%)		0		0		
Unique (%)		0		0		

MW: Molecular weight

Changes in antioxidant enzyme activities: As shown from the recorded data herein (Table 3) the activities of the determined antioxidant enzymes (catalase and peroxidase) of treated *Zea mays* and wheat were increased significantly during the three vegetative stages (stage 1, 2 and 3) as compared with control values.

Change in ultrastructure of the leaves: Of interest to mention that the ultrastructure, in this study was carried out in the leaves of *Zea mays* and wheat treated and untreated plants during 2 and 3 stages only (Table 4, Fig. 2, 3).

According to the ultrastructure measurements of *Zea mays* leaves, cell and vacuole volume and chloroplast number increased and decreased at stage 2 and 3, respectively.

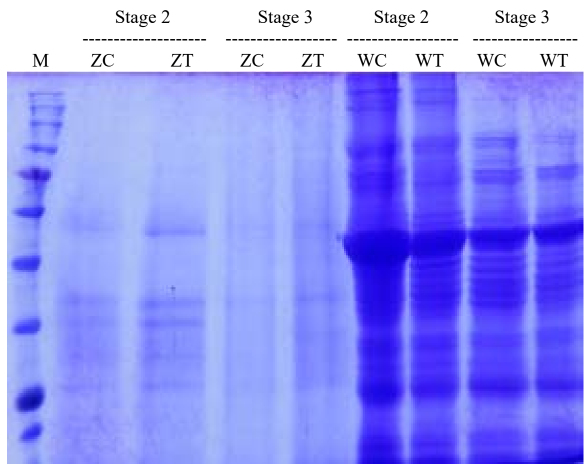

Fig. 1: Effect of coating grains with fungicide on protein banding pattern of *Zea mays* and *Triticum aestivum* leaves

M: Marker, ZC: Control *Zea mays* plants, ZT: Treated *Zea mays* plants, WC: Control wheat plants, WT: Treated wheat plants

Table 3: Effect of coating grains with fungicide on antioxidant enzymes activity of *Zea mays* and *Triticum aestivum* plants

		Zea mays		*Triticum aestivum*	
Parameters	Treatments	Catalase	Peroxidase	Catalase	Peroxidase
Stage 1	Control	4.916	0.377	6.098	0.587
	Treated	11.798*	0.495*	14.645*	0.809*
Stage 2	Control	3.896	0.341	4.883	0.516
	Treated	9.345*	0.438*	11.708*	0.714*
Stage 3	Control	2.735	0.326	3.379	0.425
	Treated	8.197*	0.417*	10.125*	0.583*

*Significant increase or decrease at 0.05 LSD

Total volume of cytoplasm, nucleus and chloroplast decreased at the two stages. The other parameters decreased and increased at stage 2 and 3, respectively. The starch granules non detected in the control while they recorded 8 and 3 in number and 0.243 and 0.065 volume at stage 2 and 3, respectively in the treated sample (Fig. 2).

Concerning wheat, a general decrease in cell and vacuole volume and an increase in cell wall thickness and nucleus volume were detected during the 2 stages. The other parameters decreased and increased at stage 2 and 3, respectively. Starch granules absent at stage 2 but, at stage 3, two granules detected only in the control leaves with volume of 0.411 (Fig. 3).

Soil analysis: As mentioned above the soil properties and soil microflora were carried out at stage 1 for the soil cultivated with treated and untreated maize and wheat grains.

Table 4: Means of cellular and sub cellular measurements of *Zea mays* and *Triticum aestivum* plants in response to treatments as well as control samples

							Chloroplast			Starch/Chloroplast		Mitochondria		
Treatments	Parameters	Cell volume	Cell wall thickness	Cytoplasm volume	Vacuole volume	Nucleus Volume	Number	Volume	Total volume	Number	Volume	Number	Volume	Total volume
Zea mays														
Stage 2	Control	191.783	0.033	93.360	98.423	42.310	3	10.750	32.250	-	-	3	0.448	1.344
	Treated	207.231*	0.009	64.951*	142.280	25.726	5*	4.065	20.325*	8	0.243	2	0.203	0.406
Stage 3	Control	323.922	0.009	152.954	170.968	20.718	8	5.801	46.408	-	-	3	0.115	0.345
	Treated	213.169	0.016	64.381*	148.788	12.196	3*	10.013	30.039*	3	0.065	4	0.262	1.048*
Triticum aestivum														
Stage 2	Control	213.145	0.014	99.046	114.099	23.067	6	8.122	48.732	-	-	6	0.478	2.868
	Treated	204.323	0.025	67.930	136.393	34.844	6	4.186	25.116*	-	-	2	0.328	0.656*
Stage 3	Control	187.777	0.013	73.762	114.015	43.236	6	5.927	35.562	2	0.411	1	0.426	0.426
	Treated	155.452	0.022	107.047*	48.405	48.570	6	12.931	77.586*	-	-	4*	0.613*	2.452*

*Significant increase or decrease at 0.05 LSD

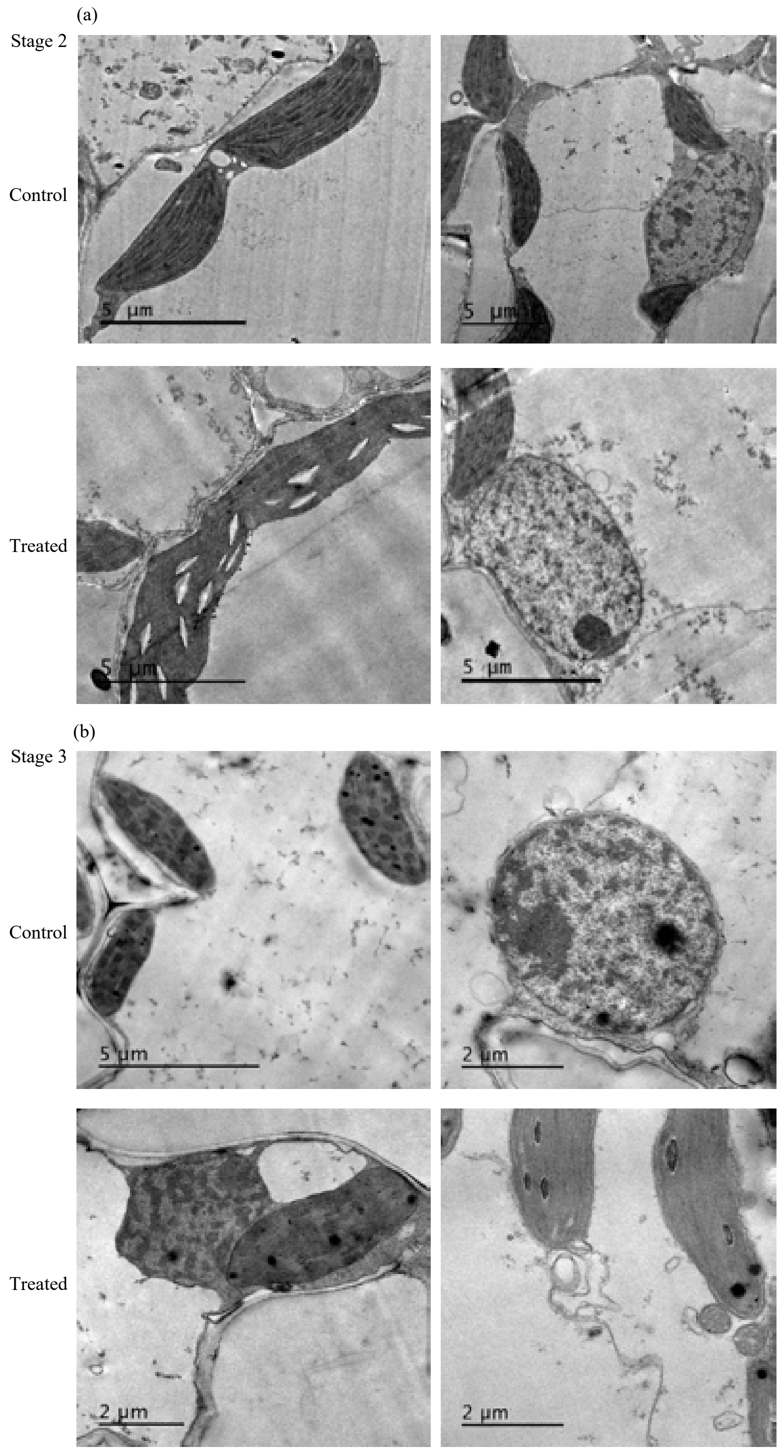

Fig. 2(a-b): Ultrastructure of mesophyll cell of *Zea mays* leaf in response to treatments as well as control samples of, (a) Stage 2 and (b) Stage 3

Soil physical and chemical properties: Regarding the physical and chemical analysis of the planting soil where the treated and untreated grains of *Zea mays* were grow. It was observed that, silt and sand percent increase but clay percent decrease. Meanwhile in case of *Triticum aestivum* although sand percent increase, both of silt and clay decreased and consequently the texture of the soil of the treated grains turned to sandy loam for both soils where treated *Zea mays*

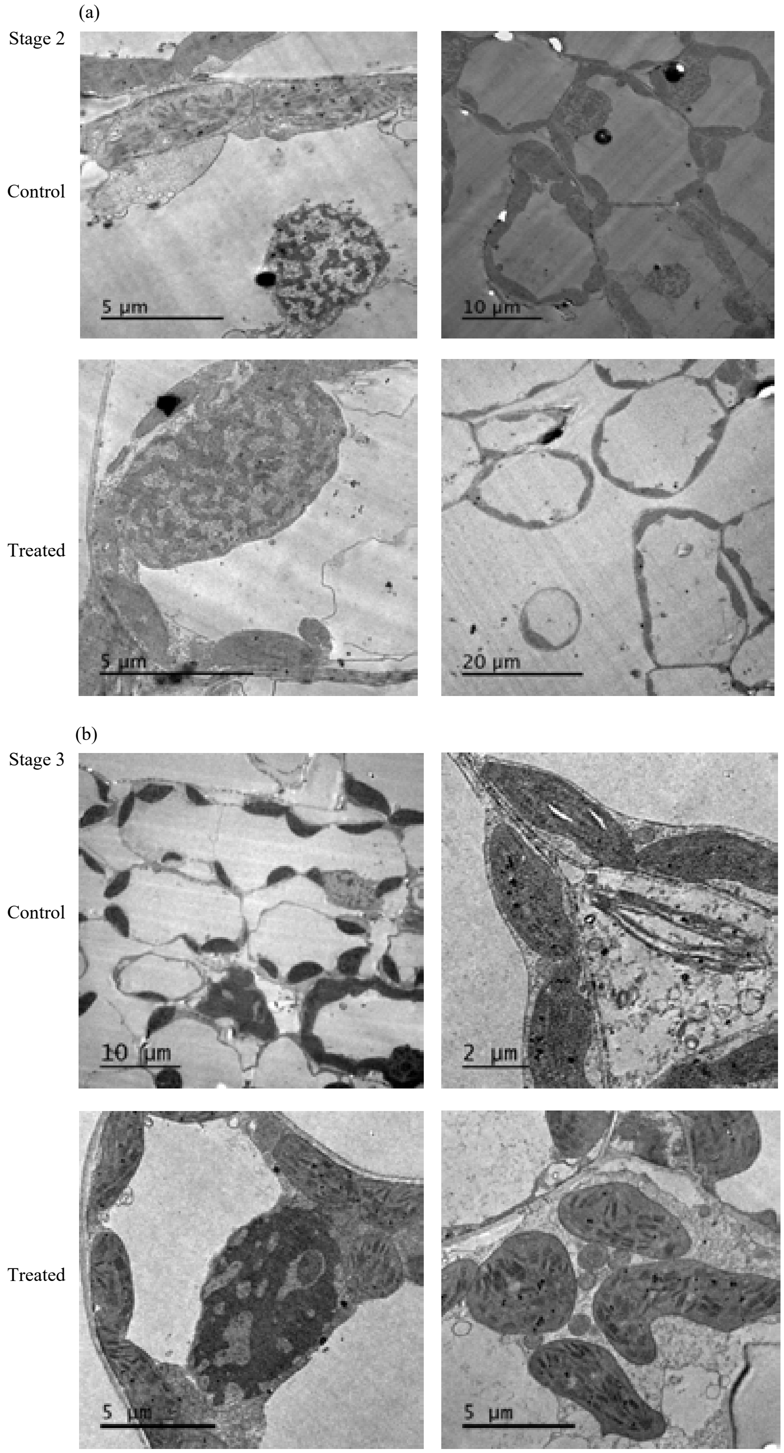

Fig. 3(a-b): Ultrastructure of mesophyll cell of *Triticum aestivum* leaf in response to treatments as well as control samples of (a) Stage 2 and (b) Stage 3

and wheat cultivated (Table 5). Concerning the PWP and FCV a clear decline in their percent were detected. Soil pH and EC values either increased in soil of treated *Zea mays* or have Adding or decrease after no change plants as compared with the control values. In most cases the response of soil physical and chemical properties to grain-dressing with pesticide was more pronouncing in case of *Zea mays* plant.

Table 5: Determined soil physical properties

Treatments	Parameters	Sand (%)	Silt (%)	Clay (%)	Soil texture	PWP (%)	FCV (%)	AW (%)
Zea mays	Control	50.35	22.76	26.89	Sandy clay loam	17	27	10
	Treated	60.56	25.68	13.76	Sandy loam	8	18	10
	Control	55.92	22.52	21.56	Sandy clay loam	8	18	10
Triticum aestivum	Treated	73.96	16.4	9.64	Sandy loam	5	12	7

Table 6: Determined soil chemical properties

	Zea mays		*Triticum aestivum*	
Parameters	Control	Treated	Control	Treated
Soluble cations (cmol kg^{-1} soil)				
K^+	0.264	0.392	0.23	0.301
Ca^{2+}	3.40	4.50	2.30	2.50
Mg^{2+}	1.20	0.80	2.80	1.30
Soluble anions (cmol kg^{-1} soil)				
CO_3^{2-}	-	-	-	-
HCO_{3-}	0.90	0.70	0.60	0.40
Cl^-	0.50	1.00	1.01	0.70
SO_4^{2-}	8.63	10.06	9.80	8.19
Element concentration (ppm)				
N	27.10	18.33	56.01	16.19
P	5.23	3.41	6.62	9.69
K	13.312	9.74	21.76	10.75
Easily oxidized carbon (%)	2.27	0.65	1.35	1.62
Total carbon (%)	3.33	0.84	1.76	2.11
Organic matter (%)	5.75	1.46	3.03	3.63
$CaCo_3$ (%)	0.45	1.30	2.90	1.20
pH	8.21	8.33	8.20	8.20
EC (dSm^{-1})	2.046	2.352	2.28	1.86

Table 7: Effect of coating *Zea mays* and *Triticum aestivum* grains with fungicide on the microflora of soil

	Zea mays			*Triticum aestivum*		
Parameters	Fungi	Bacteria	Total count/g soil of bacteria	Fungi	Bacteria	Total count/g soil of bacteria
Control	*Trichoderma* sp.	*Staphylococcus* sp.	370×10^6 cells	*Penecillium* sp.	*Staphylococcus* sp. *Streptococcus* sp.	298×10^6 cells
	Penecillium sp.	*Streptococcus* sp.			*Bacillus* sp. *Pseudomanus* sp.	
Treated	*Trichoderma* sp.	*Staphylococcus* sp. *Streptococcus* sp.	30×10^6 cells	*Rhizopus* sp.	*Bacillus* sp.	23×10^4 cells

In soils of treated *Zea mays* and wheat plants, the concentration of Ca^{2+} and K^+ cations were increase whereas, the concentration of Mg^{2+} cation was decrease as compared with that of control values. In soil planted with treated Zea and wheat plants there was an increase and decrease, respectively in the concentration of sulfates and chlorides and general decrease in the concentration of bicarbonates. In general, the concentrations of different elements such as N, P and K were decreased in soils of Zea and wheat treated plants as compared with control values. The easily oxidized carbon, total carbon and organic matter were generally decreased and increased, respectively in soils of treated Zea plants and wheat plants respectively as compared with control values (Table 6).

Changes in the soil microflora: The analysis of the soil sample showed that, the soil where the untreated *Zea* cultivated has *Trichoderma* and *Penecillium* as well as *Staphylococcus* and *Streptococcus* (with 370×10^6 cells account) where the soil of the treated *Zea mays* contains *Trichoderma* only while *Penecillium* disappeared. While the used treatment has no effect on the types of bacteria the number decreases, as it record 30×10^6 cells only. The soil of the untreated and treated wheat contains *Penecillium* and *Rhizopus*, respectively. Regarding bacteria, *Streptococcus*, *Staphylococcus*, *Bacillus* and *Pseudomanus* spp. (with 298×10^6 cells account) were detected in the untreated soil and only *Bacillus* (23×10^4 cells) was detected in the treated one (Table 7, Fig. 4).

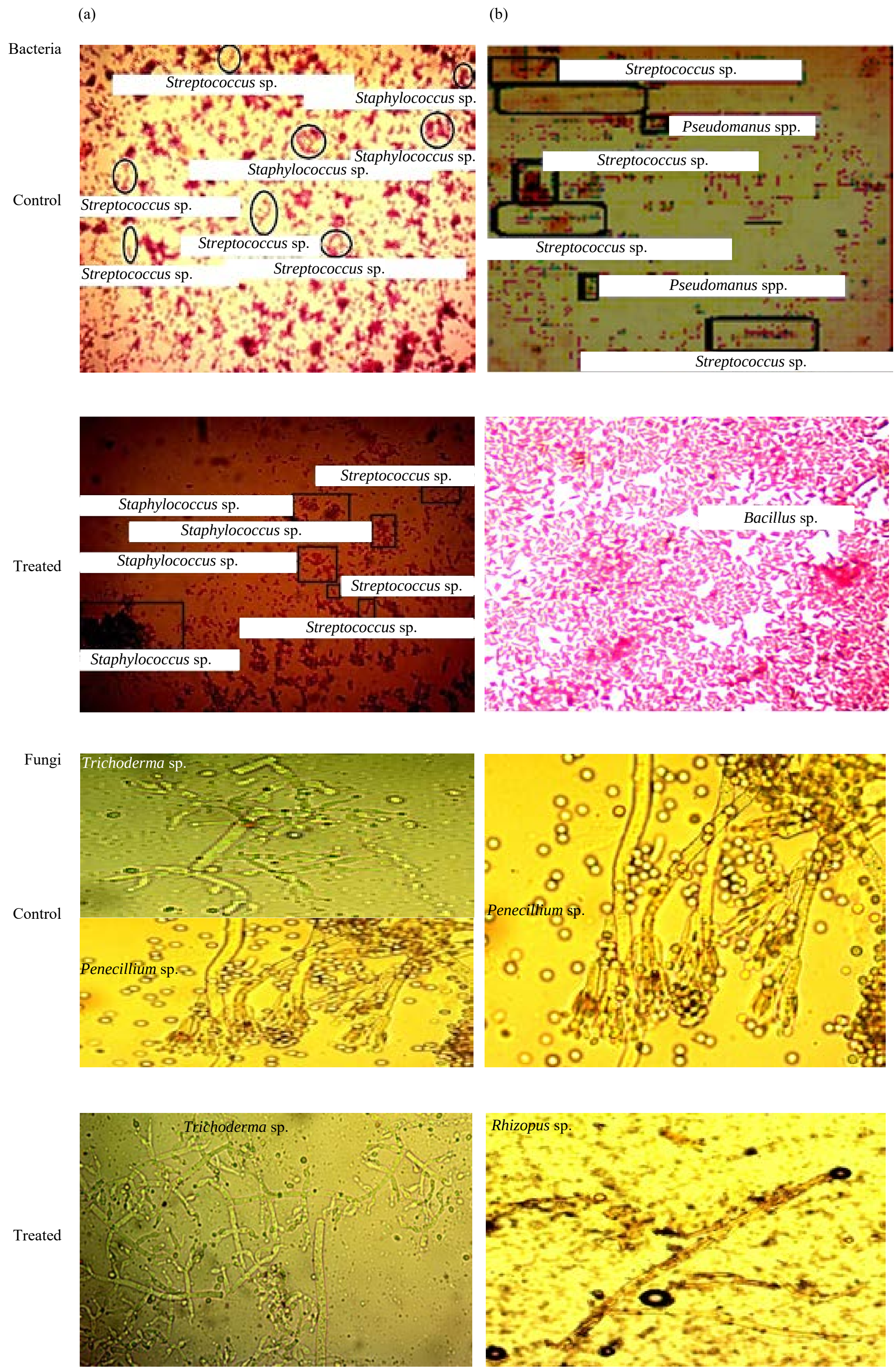

Fig. 4(a-b): Effect of coating (a) *Zea mays* and (b) *Triticum aestivum* grains with fungicide on the microflora of soil

DISCUSSION

The determined protein profile in *Zea mays* leaves cleared that 6 bands only were detected ranging from molecular weights of 70.219-13.827 kDa. With respect to wheat leaves, 23 monomorphic protein bands were detected ranging from 167.076-13.935 kDa.

The disappearance of protein at 39.433 and 31.24 kDa in zea leaves or at 167.076 and 78.28 kDa of wheat leaves may be due to degradation of these proteins to supply the growing seedling with the soluble compounds. In coincide with the present results a decrease in protein content of fungicides treated wheat was recorded[34].

In this study, the same protein band which expressed in both treated and untreated *Zea mays* and *Triticum aestivum* are supported by a study which detected that protection of seed against pathogens and pests should not come at the expense of seed quality[35]. On the other hand, the accumulation of some functional substances, such as compatible solutes and protective proteins, is an important element of the physiological and biochemical response to stressful conditions[36].

Catalase and peroxidase activities of treated plants were increased. The magnitude of increase was more pronounced in treated wheat plants than those of treated *Zea mays* plants. In support, the activity of antioxidant enzymes increased with increasing the concentration of used pesticides[37,38].

Various enzymes which act as defense mechanism in plants are synthesized to protect the plant if necessary. The primary enzyme that is actively included in the functioning of the defense mechanism in all of them is peroxidase. Peroxidase [EC 1.11.1.7] has a role in the plant defense reactions against potential pathogens[39]. It plays a significant role in protecting the plants against pathogenic attacks[40].

According to the ultrastructure measurements of *Zea mays* leaves, cell and vacuole volume and chloroplast number increased and decreased at stage 2 and 3, respectively. Total volume of cytoplasm, nucleus and chloroplast decreased at the 2 stages. The other parameters decreased and increased at stage 2 and 3, respectively. Concerning wheat, a decrease in cell and vacuole volume and an increase in cell wall thickness and nucleus volume were detected during the 2 stages. The other parameters decreased and increased at stage 2 and 3, respectively.

The use of pesticides in agriculture is applied worldwide, where the problem lies in the lack of information regarding the risks and hazards of pesticide use[41]. Thus, there is a demand for a safer and more ecofriendly alternative[42,43].

In the current study, the absence of the starch grains in the chloroplast of wheat leaves is in coincide with the decrease in polysaccharide response to grain treated with fungicide; as detected by the authors in another study. On the other hand, investigation into the ultrastructure of leaves exhibiting whip-tail indicated that chloroplasts near the lesions became bulbous and enlarged with spherical protrusions bounded by chloroplast and tonoplast membranes[44].

In both soils where treated *Zea mays* and wheat cultivated, clay decreased and sand increased and consequently the texture of the soil of the treated grains turned to sandy loam. In this respect, the soil sand content was increased while silt and clay content decreased[45]. Concerning the PWP and FCV a clear decline in their percent were detected.

In this connection, it was reported that insecticides treatments affected both soil physical and chemical characteristics as soil nitrogen and phosphorus contents decreased by 36 and 20%, respectively as well as soil pH and EC were adding in soil of treated Zea plants[46].

In intensive agriculture, seed coating is a technique of applying several compounds, such as pesticides, fertilizers and biostimulant substances, to the seed surface so they can start to act on the seedlings during germination and/or at the seed-soil interface immediately after sowing[47].

Soil microflora in this study, showed marked differences in response to the used treatments. Higher concentration of pesticides showed phytotoxic effect on some useful soil microorganisms[48,49]. In this concern, pesticides are designed to react with living cells. Though a wide range of such chemicals is directed to protect plants from pathogenic organisms, many of pesticides adversely influence the symbiotic relationships between the host plants and the microorganisms[50].

Regarding the appearance of *Rhizopus* in the soil of the treated wheat seeds in this study, this may be due to using of pesticides as a source of energy by some microbes, in other cases pesticide could be toxic to other organisms. Also, some pesticides' residues could be carbon or energy source to microorganisms and are degraded and assimilated by microorganisms. Although many reports exhibit their deleterious effects on soil microorganisms as well, different groups of pesticides exhibit manifold variations in toxicity[51].

Pesticides application may also inhibit or kill certain group of microorganisms and outnumber other groups by releasing them from the competition[52]. By repeated and extensive application of pesticides, it ultimately reaches the plant body and soil, which in turn may interact with plant growth and with soil organism and their metabolic activities[53].

Concerning the disappearance of *Penecillium* in the soil cultivated with the treated zea seeds in this study, it was reported that fungicides applications killed or inhibited the activity of certain fungi which led to a rapid flush of bacterial activity[54].

In the current study the correlated changes observed in both soil properties and soil microflora are in harmony with those which stated that pesticides application decreases the amount of organic matter in soil, thus effect on the diversity of the microbial ora and fauna[55].

Soil microorganisms have the ability to carry out biochemical transformations of various elements like nitrogen (N), phosphorus (P), sulfur (S) and carbon (C). Pesticides may activate or deactivate specific soil microorganisms and/or enzymes so they effect on mineralization of organic matter, nitrogen fixation, nitrification, denitrification, and ammonification[56-58]. According to dosage, soil properties and many environmental aspects, pesticide effect on microorganisms will vary[59]. Because these microbes are involved in various element-recycling and -transformation processes, any change in their number or ratio could potentially prohibit/enhance one or other of the reaction chains important for soil fertility.

Although pesticides are important, their effects on non-target organisms are of great concern because this poses a risk to the entire ecological system[59] and as stated before, the usual practice of the application of pesticides and fertilizers could affect some groups of organisms in the soil, but the overall effect on the soil community would be small[60]. Seed dressing substance can stimulate seed germination, prevent diseases and supply essential nutrients to achieve the purpose of strong seedlings. It can also create a good micro ecological environment[15].

In this study the decrease of bacterial number from 370×10^6 cells to 30×10^6 cells and from 298×10^6 cells to 23×10^4 in the soil cultivated with treated grains of maize and wheat respectively are in accord with a study which reported the reduction of microbes population in all soil samples taken from elds under a rice–wheat cropping system[61].

Micro- and meso-fauna were already influenced after a single seed dressing application[62]. Microorganisms and soil fauna contribute to the decomposition of plant residues in agricultural fields, the mineralization of plant residues and the recycling of plant nutrients[63,64].

CONCLUSION

It could be concluded that usage of hattrick and premis at the recommended dose may be helpful in stimulating the growth of *Zea mays* and *Triticum aestivum*, respectively in addition protect grains. The magnitude of response of the determined fractions in relation to the grain-dressing was more pronounced in maize plants than that in wheat plants, at the most cases and this could be attributed to the natural susceptibility of *Zea mays* to disease infection and hence, increasing its resistance by coating its grains. In consequence up to now there is a demand for a safer and more ecofriendly alternative.

SIGNIFICANCE STATEMENT

This study confirmed that hattrick and premis at the recommended dose may be helpful in stimulating the growth of *Zea mays* and *Triticum aestivum*, respectively in addition protect grains. Thus, although this study stated the efficacy of coating the seeds, up to now there is a demand for a safer and more ecofriendly alternative.

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