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Adsorption and incorporation of the zinc oxide nanoparticles in seeds of corn: germination performance and antimicrobial protection

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Abstract–*The treatments of the seeds are important procedures applied by the agronomical area to improve the culture yield. From these procedures the micronutrients are available for the seeds before and during the germination stages. One high challenge is make efficient these treatment processes and to ensure the adsorption and the incorporation of these micronutrients in the seeds and to improve its performance in the germination phase. In this work studies explored the optimization of the incorporation process and the characteristics of the zinc oxide clusters adsorbed on the surface of the seed. The results were associated with the agronomic responses during the germinations stages of the seeds of corn. The seeds were treated in suspensions containing different concentrations of nanoparticles of zinc oxide and during different treatment times. The adsorptions in the corn surface and the absorption of the nanoparticles for the inner of the seeds were studied together with its antibacterial characteristics and correlated with the germinations indicators. The results showed that is possible to incorporate nanoparticles of zinc oxide in inner of the seeds of corn and improve the germinations indicators. Antibacterial protection was aggregated on the seeds of corn. It´s possible to incorporate 0.280 mg of zinc oxide nanoparticle per seed mass in inner of seeds with the optimal treatment conditions with nanoparticle concentration of 50 mg/L in the suspension and with treatment time of 180 minutes. With the optimal treatment concentration the normal plant percentage increase of 2.70% in relationship to the seeds not treated.*

Keyword—Zinc oxide nanoparticles, treatment of seeds of corn, treatment with nanoparticles, improve of germinations indicators.

I. INTRODUCTION

Technological innovations for corn culture in the last years are largely associated with the decrease of the cost production and for the increase of the culture yields.Despite the accumulated technological information by the agronomic sector the nanotechnology is a new science that can bring excellent opportunities for this field, specifically for the treatment of seeds and for the foliar treatments.

The nanotechnology is very applied by many segments for the development of drugs [1-4], biosensors [5-9], electronic devices [10-14] and others. But, in the agronomic experiments this science is few applied for improve the yield of corn culture, for example.

Some agronomic practices need to be further studied and refined and can be improved with the use of nanotechnology. As a factor of great importance associated with the production, fertilization methods need to be balanced, not only macronutrients, but also with micronutrients, and these may be in the form of nanoparticles [15,16]. In particular, nanoparticlesof manganese (Mn) [17], molybdenum (Mo) [18] and zinc (Zn) [19]may be employed as micronutrients for corn seeds.

The zinc element, for example, has great importance in all stages of the development of the corn plant. According to Barbosa Filho et al. (1990), one of the consequences of zinc deficiency in the plants is the shortening of its internodes, which results in the reduction of the plant growth rate and in the lower production of new leaves and of the yield of the grains. These effects are due to the fact that zinc is required for the tryptophan production, which is a precursor of the indole acetic acid, a growth hormone promoter of the plant [20].

In the traditional processes of corn seed treatment are employed zinc salts such as zinc hydroxide and zinc

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nitrate. When the seed is placed in the soil, which contains water, the salts are dissociated and the zinc ions are leached into the soil. These conditions do not favor the supply of zinc species for the seed and reduces the efficiency of the treatment process.

The addition of zinc oxide nanoparticles may be an interesting alternative to improve the treatment process yield of the seeds. The zinc oxide nanoparticles not are dissociated in water and havea very small dimension. When added to the seed the zinc oxide nanoparticles remains stable and may migrate to the inner of seed and to participate of the metabolic processes. Seed treatment with zinc oxide nanoparticles can be more efficient and to lead to significant improvements of the indicators of plant development.

Recently studies applied metallic nanoparticles in agronomic experiments and obtained promising results, but also negative responses were obtained for the same conditions of applications and for the same type of nanoparticle. For example, studies showed that after long exposition time in suspensions containing 500 mg/L of cerium oxide nanoparticle, in hydroponic systems, the defense mechanisms of the plant is prejudiced [21]. But, others works made the proteomics analysis of roots of soy treated with aluminum oxide nanoparticles and obtained positive responses for the growth performance of the soy in inundation stress conditions [22].

The zinc oxide nanoparticle has been an interesting theme and has been studied for the treatment of seeds, in special for the treatment of seeds of corn. Boonyanitiponget al., (2011) and Lin and Xing (2007) studied the impact of the application of zinc oxide nanoparticles on some plants and reported that the nanoparticles can be affect their developments when applied with higher concentrations than the critical concentrations, but not affect their performance during the germination processes [23,24]. For example, when applied in the treatment of pea (*Pisumsativum*) the zinc oxide nanoparticles not promote negative effects for the germinations indicators, but the treatment promote a decrease of the length of the roots [25].

Pokhrel, L.R. et al. (2013)evaluated the phytotoxic potential of the zinc oxide nanoparticle in the corn culture (*Zea mays L*) and in the cabbage plants (*Brassica oleracea var. Capitata L.*). In the germination indicators and in the length of the roots the nanoparticles presented small toxicity in comparison with the compounds containing free ions of zinc[26]. The authors not founded significant signal of lipid peroxidation processes or the leakage of the ions and verified that the physiologic measures (transpiration, photosynthesis and stomatal conductance) not were affected. In the same studies the authors identified the increase of the activity of the catalase enzyme and of the ascorbate enzyme and a positive regulation of the thermal shock.

The zincoxides nanoparticles can contribute with an antimicrobial protection of the seeds beyond of the benefits in the supplying micronutrient mechanisms for the plants due increase the mobility in the transport mechanisms of the micronutrients. Recently studies showed that the nanoparticles of zinc oxide have antibacterial effect [27-31]. Therefore, the zinc oxide nanoparticles can inhibit or deaththe microorganism undesirable during the germination of the plants, with an antibacterial protection of the seeds.

Many studies have been realized about agronomical and physiological aspects involving the treatment of the seeds by nanoparticles [32-33]. But, there is not evaluation about of the nanoparticle storage in corn seeds after their treatment with different nanoparticle concentration in theZnO nanoparticles suspension and different treatment times. Similarly, there are not correlations between the treatment conditions of the seeds with the real protection capacity of microbial agents. This information is important and interesting for the agronomical area, considering that these treatments can promote a positive effect or a negative effect, depending of the nanoparticles concentrations in suspension or of the treatment time.

In this work was studied the simultaneous treatment of the seeds of corn with different concentration of zinc oxide nanoparticle and different treatment times. The studies explored the optimization of the incorporation process and the characteristics of the zinc oxide clusters adsorbed on the surface of the seed. The results were associated with the agronomic responses during the germinations stages of the seeds of corn. The adsorption characteristics of the nanoparticles on the seed surface (pericarp) were evaluated and the amount of zinc oxide nanoparticles incorporated in the inner of the seed of corn was determined. These information were correlated with the germination indicators of the seeds. The antibacterial properties aggregated on the seeds due the adsorption of the zinc oxide nanoparticle were studied too.

II. EXPERIMENTAL PROCEDURES

2.1 Seeds of corn and nanoparticles

In this work were utilized seeds of a hybrid corn of the type ADV 9275 PRO® courtesy by AdvantaSementes® of the group UPL (United Phosphorus Limited). The seeds were classified as the simple hybrid, with grain semi-hard and with yellow-orange coloration, of precocious cycle and of high technology and with a plant population of 6000/ha. The seeds of corn were treated according the commercial procedures adopted by the company and with the chemical solution formulated according the Table 1.

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Table 1: Suspension adopted for the industrial treatment of the seeds of corn by the companies for the hybridic corn type ADV 9275 PRO®

Chemicalcompound	Active compound	Dose for the treatment of 100kg of seeds
Maxim XL	Fludioxonil 2,5% + Metalacyl – M 1,0%	150 mL
K-Obial 25CE	Deltametrine 2,5%	6 mL
Actellic 500 EC	Pirimifois - Methyl 50%	3 mL
Agrawal	Sulfate ofdodecilbenzenesulfonic	1 mL
Dye	Notapplicable	27 mL

Sourcee: Advanta Sementes® (2014).

The zinc oxide nanoparticles utilized in this work were courtesy by Kher Chemical Research and have a cylindrical stick format with average length of 100 nm and average diameter of 20 nm. The purity grade is 99.5%.

2.2 Treatment of the seeds of corn with different concentration of zinc oxide nanoparticles in the ZnO nanoparticles suspensions and different treatment time

The treatments of the seeds of corn were realized in an open glass reactor containing an aqueous suspension at room temperature. The suspensions with different concentrations of zinc oxide nanoparticles were prepared in 2000 ml of ultrapure water and containing 100 g of seeds immersed in this medium. The agitations were realized by a magnetic system and with different treatment times. All experimental conditions for the seeds treatment are presented in the Table 2.

Table.2: Experimental planning matrix showing the experimental conditions for the treatment of 100g of seeds of corn with different concentrations of zinc oxide nanoparticles and different treatment times

Treatment time (minute)	69	180	450	720	830
Concentration of zinc oxide nanoparticles (mg/L)	6.95	50.00	155.00	260.00	303.05

The experimental conditions were defined utilizing a factorial experimental planning with the concentrations of zinc oxide nanoparticle (C) and with the treatment time (t) being the experimental planning factors (variables). The range adopted for the treatment times was 69 min to 830 min and the range for the concentrations of the zinc oxide nanoparticle was 6.95 mg/L to 303.05 mg/L, respectively. As experimental responses were evaluated the concentration of zinc oxide nanoparticles incorporated in the inner of the seeds of corn.

After each treatment the seeds of corn were extracted of the glass reactor and dried at room temperature and accommodated in paper bags and identified according with the respectively treatments. The paper bags containing the seeds of corn were stored in a dry chamber with controlled temperature of 20ºC and humidity of 40%.

According the methodology of experimental planning were realized 11 experimental treatments and with triplicate. Table 3 presents the experimental matrix with all treatment conditions applied for the seeds of corn.

Table.3: Experiments adopted according experimental planning for the treatment of the seeds of corn

Experiments	Concentration of zinc oxide nanoparticles -----mg/L-----	Treatment time -----minute-----
1	260.00 (+1)	180 (-1)
2	50.00 (-1)	180 (-1)
3	260.00 (+1)	720 (+1)
4	50.00 (-1)	720 (+1)
5	155.00 (0)	450 (0)
6	155.00 (0)	450 (0)
7	155.00 (0)	450 (0)
8	155.00 (0)	69 ($-2^{1\backslash 2}$)
9	155.00 (0)	830 ($+2^{1\backslash 2}$)
10	6.95 ($-2^{1\backslash 2}$)	450 (0)
11	303.05 ($+2^{1\backslash 2}$)	450 (0)

The seeds of corn also were treated with concentration of 1000 mg/L of zinc oxide nanoparticles. The objective was to evidence the adsorption effects of the zinc oxide nanoparticles on the surface of the seeds.

To statistical analysis of the effect of the factors (variables) in the incorporation of the nanoparticle in the inner of seeds and in the adsorption characteristics on the surface of the seed were applied statistical tests type t, F and P, together with variance analysis and of estimated effects.

2.3 Quantification of the concentration of zinc oxide nanoparticle in the inner of seeds of corn

For all experimental treatment conditions was determined the concentration of zinc oxide nanoparticle incorporated in the inner of the seeds of corn and on the surface of the seed by atomic absorption spectrometric technique (AAS).

For AAS analysis 2g of seeds of corn treated and shelled was diluted by acid digestion procedures in a solution of 5ml of concentrated nitric acid (Merck - 65%) on the digester block (Model TE-040/25 Tecnal)during one hour at 90ºC and by 2 hours at 180ºC.

The digestion solutions were diluted with 10ml of deionized water (18.2 MOhm.cm – Milli-Q) before all AAS analysis for the determination of the total concentration of zinc oxide nanoparticles in the inner of the seeds or on the inner surface. Thus, was possible to correlate the amount of the zinc oxide nanoparticle incorporated in the inner of seed with the different concentration of zinc oxide nanoparticle in the suspension and with different treatment times applied in the treatments. Similar experiments were realized with the seeds in shell and shelled seeds.

2.4 Chemical and physical characterization of the seeds of corn

After the treatments, according Table 2, the seeds of corn were submitted to analysis by the absorption atomic spectrometry (AAS) to determine the percentage of the zinc oxide nanoparticle adsorbed on its surface and the percentage incorporated in the inner of the seed. In these experiments the seeds with its shell were analyzedand the total of the zinc specimens was determined by the AAS experiments with a system Model 800 Analyst AAS - Perkin Elmer®, with wavelength 213.9 specifically for detection of zinc component,and realized in quadruplicate for each treatment condition.

The scanning electron microscopy (SEM) was applied to evaluate the characteristics of the morphology and of the distribution of the nanoparticles adsorbed on the surface of the seeds. The SEM also was applied to evaluate the integrity of the cellules of the seeds of corn. The energy dispersive spectrometry (EDS) was simultaneously applied for the chemical identification of the zinc oxide nanoparticles and to obtain the chemical distribution of the nanoparticles on the cellular structures. The system FEG Model Tescan Mira3 and EDS Model Oxford X-Max 50 were applied for these analyses. All samples were coated with a thin film of gold to analysis.

2.5 Germination tests and evaluation of the vigor of the seeds of corn treated with zinc oxide nanoparticles

The germination tests and the vigor information's of the seeds of corn treated with different concentration of the zinc oxide nanoparticles in suspension and with different treatment times were realized during April and May of 2015.

Amounts of the seed of corn were treated only with water, without zinc oxide nanoparticles, but with equal times utilized for the treatments in suspensions containing nanoparticles. These seed of corn were considered as the standard seed of corn.

Thirty experiments were established as a results of the combinations between the treatments times and concentrations of zinc oxide nanoparticle employed in the treatments. The experimental planning utilized was the completely randomized (D.I.C.), organized with the factorial scheme 5x6 (time x concentration) and with four repetitions for each experimental condition. The respective values for the factors were: t1: 69 minutes; t2: 180 minutes; t3: 450 minutes; t4: 720 minutes e t5: 830 minutes e C1: 0 mg/L (standard seed of corn); C2: 6.95 mg/L; C3: 50.00 mg/L; C4: 155.00 mg/L; C5: 626.00 mg/L e C6: 303.05 mg/L.

The germination tests were realized in substrates of paper towel (Germitest) hydrated with a volume of distilled water equivalent to the three times of the seed mass. The vigor of the seeds was evaluated with first germination counting and with the determination of the germination velocity.

The germination tests were realized with four sub-samples of 50 seeds for each treatment, according the criteria established by the Rules for Seed Analysis in Brazil [31]. The rules containing the seeds were put up in a germination camera type B.O.D. (Model MA 415) and maintained at 25±2 ºC.

The evaluation of the first germination counting was realized on the fourth day after the installation of the tests. The final germination counting (second counting), obtained by the sum with first germination counting, was realized on the seventh day after the installation of the tests. The dates were converted for percentage values of normal plants and for the not normal plants [34].

The germination velocities were determined employing the equation of Edmond e Drapala (1958), equation (1):

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$$V.G.= [(D_1 \times P_1) + (D_2 \times P_2)] / (P_1 + P_2) \quad (1)$$

where: V.G. is the germination velocity expressed as the medium number of days for the germination; D1 and D2 a number of days between the sowing and the first and the second germination counting; P1 and P2 is the number of normal plants or abnormal plants in the first and in the second germination counting.

The collected dates from the germination tests were analyzed with the variance analysis methods applying the F test and the regression analysis ($p \leq 0.05$) and utilizing the computational applicative SISVAR, a variance analysis systems for the balanced dates.

2.6 Microbiological characterization of the seeds of corn

The antimicrobial activity tests of the seeds of corn treated with zinc oxide nanoparticles were realized according the Japanese Industrial Standards Methods (JIS Z2801:2010) with some modifications. The tests were realized with triplicate with the *Staphylococcus aureus* (ATCC 25923) and with the bacteria *Escherichia coli* (ATCC 11229). The microbiological tests were realized with the seeds of corn treated by 180 minutes with the suspensions containing 6.95, 50.00 and 303.05 mg/L of zinc oxide nanoparticle and with seeds of corn treated only ultrapure water (considered the standard samples). The microbiological tests were realized with the objective to analyses the presence of the antibacterial activity and if is dependent of the concentration of zinc oxide nanoparticles in the treatment suspensions. The microbiological tests were realized with three suspensions of treatment containing a low concentration, a medium concentration and a high concentration of the zinc oxide nanoparticles.

The bacteria were reconstituted in sterile distilled water and seeded in brain-heart infusion broth (MERCK) and incubated in a microbiological oven at 35ºC by 24 hours. The inoculum was peaked in a nutrient agar (MERCK) and incubated during 24 hours in a microbiological oven at 35ºC. After these procedures the bacteria were removed and diluted in test tube containing 4.0 ml of saline solution of 0.8% (MERCK). The suspension was adjusted to McFarland standard solution. These procedures were realized for all microorganisms.

Aliquots of 0.2 ml of the bacteria suspensions' were distributed (spreader) on the surface of the seeds of corn (~0.43 cm^2) and deposited on the Petri dishes. The dishes were placed in the microbiological oven at 35ºC to incubate the bacteria during different times, 0, 2, 4, 6, 10 and 24 hours.

For each incubation time were quantified the viable bacteria utilizing the Pour Plate Technical, with the homogenation of the seeds of corn in 10 mL of Luria Bertani broth. The volume 0.1 ml each dilution was placed on the Petri dishes and placed 7.0 mL of standard agar for the growth and count of the microorganisms (PCA). The samples were incubated in a bacteriological oven at 35ºC by 48 hours.

The dilution that presented an amount of bacterial colonies between 30 and 300 was submitted to the counts and the number of viable bacteria per square centimeters was determined according equation (2):

$$n = (c.d.v)/A \quad (2)$$

where nis the number of viable bacteria per cm^2, c is the colonies counted, d is volume (ml) of the broth for washing andA is the surface area (cm^2).

III. RESULTS AND DISCUSSIONS

3.1 Treatment of the seeds of corn with zinc oxide nanoparticles

Adsorption of the zinc oxide nanoparticles on the surface of the seed of corn

The seeds of corn were treated in suspension containing different percentage of zinc oxide nanoparticle. Fig. 1 show images obtained by the SEM of the surface of the seeds of corn treated with zinc oxide nanoparticle during 180 minutes in suspensions containing 50 mg/L, treated in suspension containing 1000 mg/L and for seeds treated with water without nanoparticles (standard seeds).

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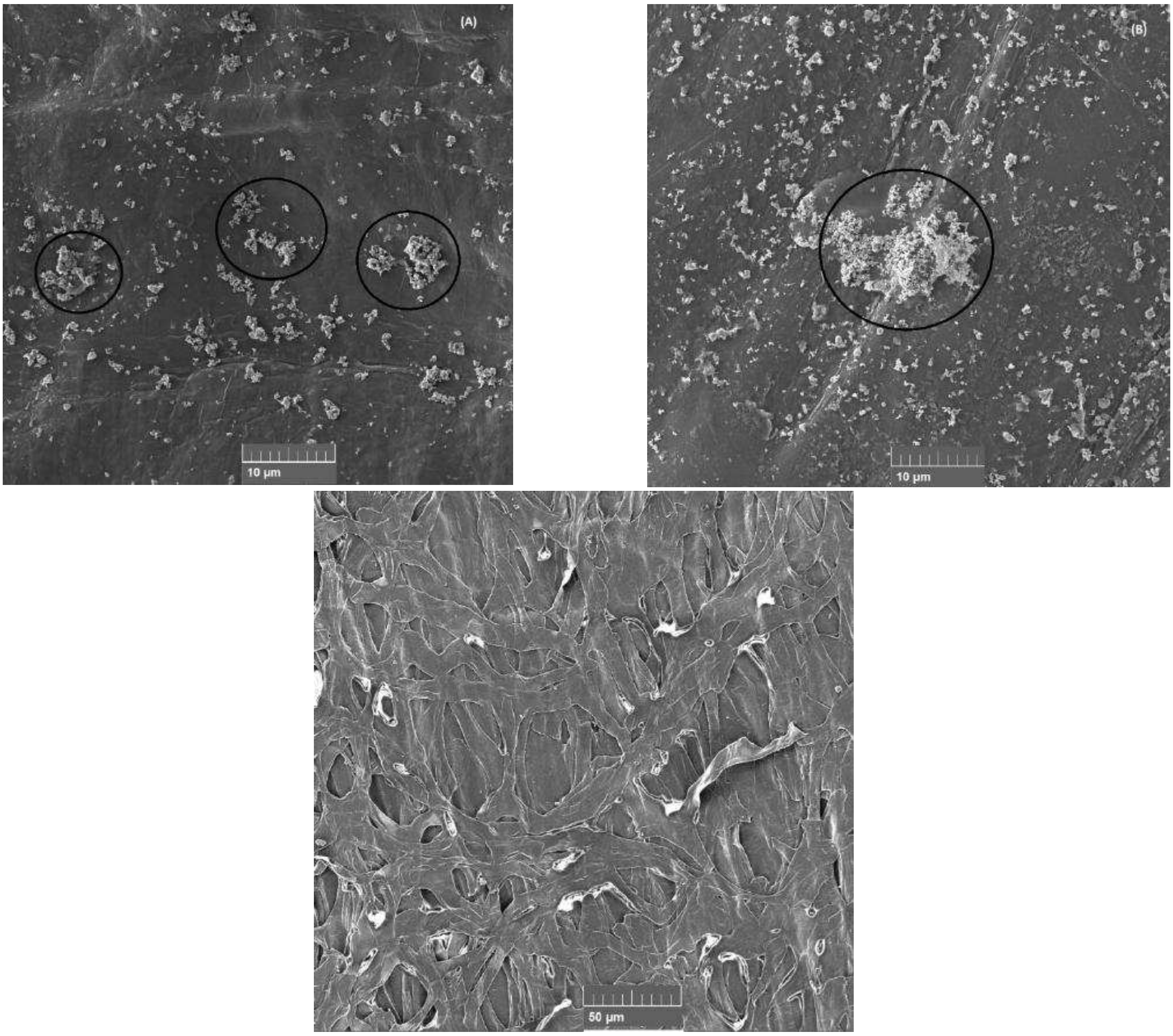

Fig. 1: Images obtained with electron microscopy of the surface of seeds of corn treated by 180 minutes with suspension containing different concentrations of zinc oxide nanoparticles: (a) 1000 mg/L, (b) 50 mg/L and (c) standard (without zinc oxide nanoparticles in suspension).

The images revels that surface of the seeds of corn treated only water not present nanoparticles adsorbed on the surface, Fig. 1(c). But, others images show clusters formed by zinc oxide nanoparticles distributed on the surface of the seeds of corn treated in the suspensions containing the nanoparticles. With the treatment in the suspension containing 50 mg/L areformed clusters on the surface of the seeds with sizes between 1 and 2 micrometers and casually the formation of the higher clusters. With the treatment in the suspensioncontaining 1000 mg/L a larger amount of higher cluster are formed on the surface of the seeds and with sizes between 5 and 10 micrometers. The growth and shape of the clusters on the surfaces are typical of agglomerates formed during aphysical adsorption phenomenon. There are the adsorptions of the first zinc oxide nanoparticles in specific regions of the seed and after this time there are the growths of the clusters to long time.

The surfaces of the seeds of corn are formed by fibrous cells (pericarp) that are interlaced. These structure generate cavities (micro-cavities) that are preferential paths for the migration of the mineral compounds that are adsorbed on the surface of the seeds for the inner of seeds. The Fig. 2(a) shows details of the pericarp morphology and its interlacedstructure.

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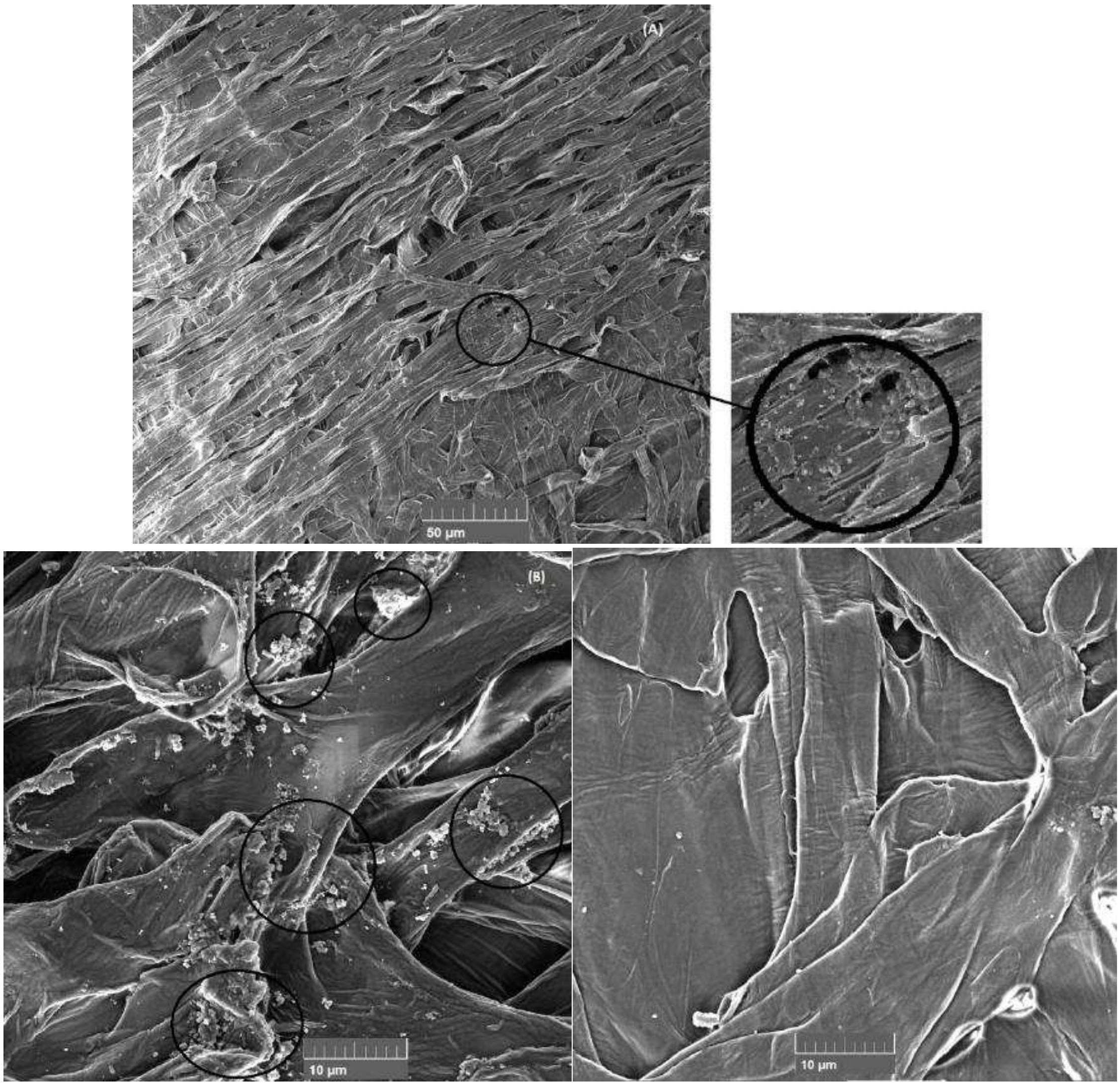

Fig. 2: Images obtained by electron microscopy showing details of the cell fibrous distribution (pericarp cells) on the surface of the seed of corn (a) and the formation and distribution of the clusters constituted by zinc oxide nanoparticles (b) Seeds treated with 180 minutes and in suspension containing 1000 mg/L and (c) standard (without zinc oxide nanoparticles in suspension).

Fig. 2(b) shows the clusters of zinc oxide nanoparticles adsorbed preferentially on the edges of the pericarp cells. The higher clusters are adsorbed on the edge of the pericarp while few small clusters are adsorbed on the surface of the pericarp cell. This is a strong indicative that the adsorption processes of the zinc oxide nanoparticle on the surface of the seeds of corn occurs preferentially on the edge of the pericarp cells and around of the micro-cavities which favor the migration of micronutrient for the inner of seeds. Fig. 2(b) show details for the pericarp cells of the seed of corn treated only water, suspension without nanoparticles. The images revels the absence of the nanoparticles and cluster on the surface or on the edges of the cells.

Fig. 3(a) shows details of the clusters adsorbed on the edge of the pericarp cells for the seeds treated in suspension containing 1000 mg/L and during 180 minutes. The results prove that the clusters are preferentially formed on the edge of the pericarp cells. The clusters are constituted by nanostructures with dimension between 50 and 250 nanometers of zinc oxide. These nanostructures are very lower than the dimensions of the micro-cavities formed by the interlacing of the pericarp cells, which have dimensions of micrometers. These characteristics suggests that the clusters of the zinc oxide nanoparticles can be considered a zinc micronutrient reserves and are easily transported from the surface of the corn seed for its inner. Fig. 3(c) revel the absence of nanostructures or of clusters on the edge of pericarp cells of the seed treated only water (standard seed).

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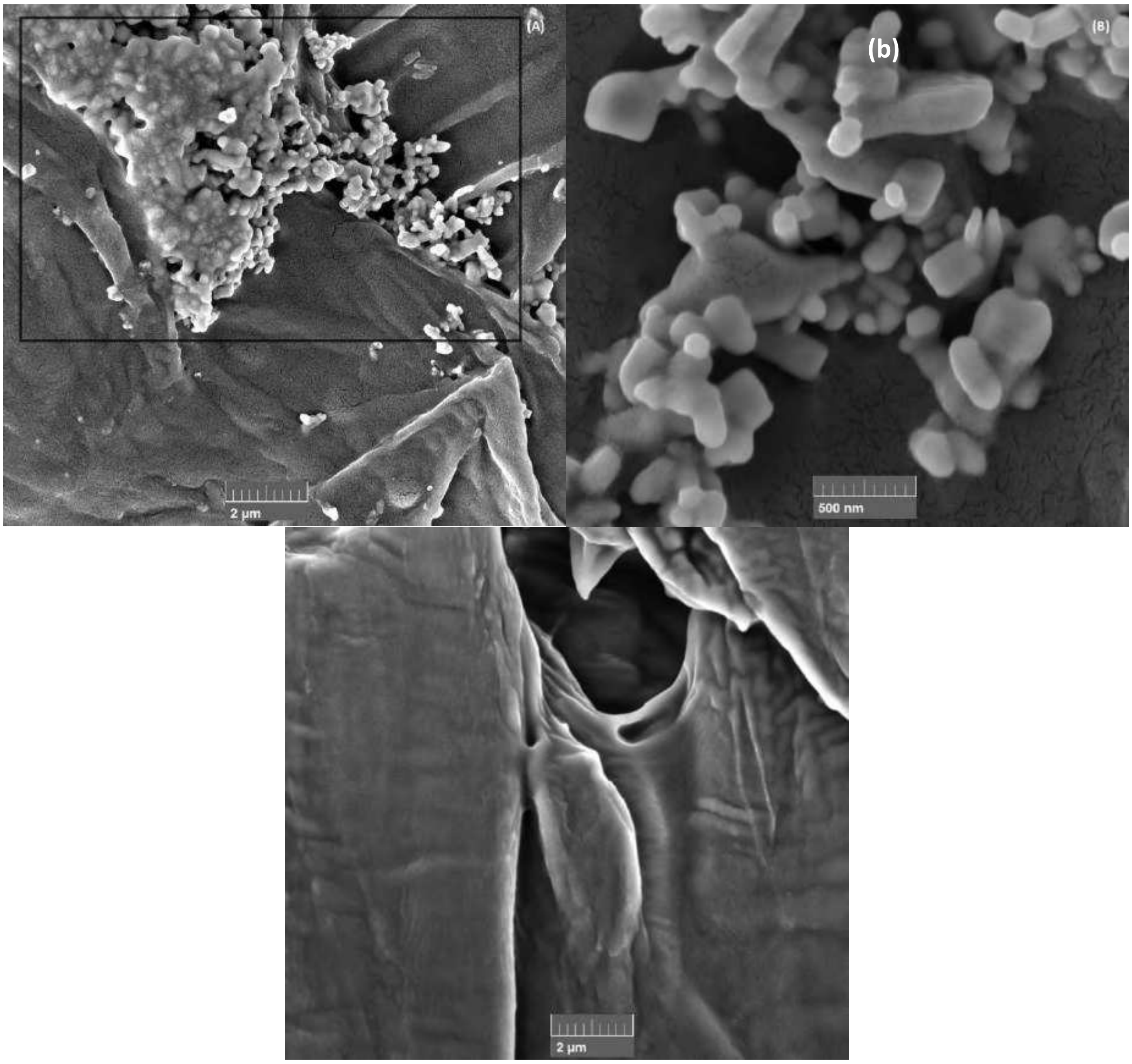

Fig. 3: Images obtained with electron microscopy showing details of the clusters constituted by zinc oxide nanoparticles and adsorbed on the edge of the pericarp cells (a) and the details of the nanoparticles (b). Seeds of corn treated with 1000 mg/L and time 180 minutes and (c) standard (without zinc oxide nanoparticles in suspension).

Details of the surface of a seed treated with 1000 mg/L are showed in Fig. 4(a). The images evidence the formation of clusters on the edge of the pericarp cell. Some regions were highlighted and studied by EDS and named by the Spectrum2 and Spectrum3. Fig. 4(b) shows the EDS results obtained from the region Spectrum2 and proves the presence of zinc oxide. The EDS result presented in Fig. 3(c) proved the absence of the zinc oxide nanoparticle in the region Spetrum3, which is a region on surface of the seed of corn. These results demonstrate that the clusters formed or the nanostructures observed on the surface of the seed of corn are constituted by zinc oxide.

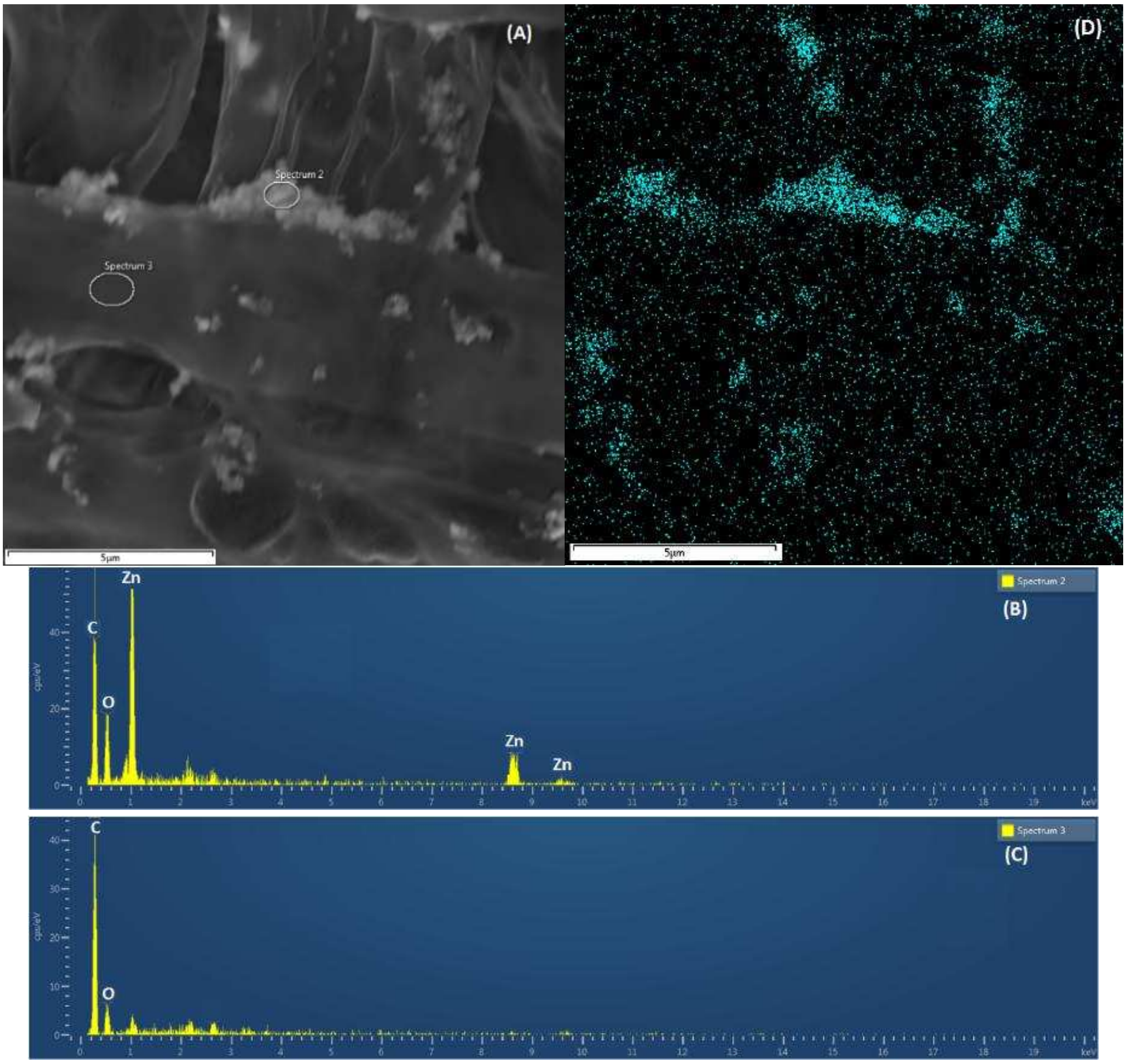

Fig. 4: (a) Images obtained by electron microscopy with details of the fixed nanoparticle cluster on the interfaces of the fiber cells on the surface of the seed of corn, (b) chemical distribution of the zinc oxide nanoparticles obtained by EDS, (c) chemical analysis by EDS in region defined as "Spectrum 2" proving the presence of zinc oxide in a cluster and (d) chemical analysis by EDS in the region defined as "Spectrum 3" proving the absence of zinc in the fiber cell of the pericarp of the seed of corn.

Fig. 4(d) shows the correspondent chemical distribution of oxide zinc compound on the complete surface analyzed by EDS and proves that the nanoparticles and clusters observed are constituted by zinc oxide and are fixed majority on the edge of the pericarp cells.

The characteristics of the clusters on the pericarp cells of the seeds of corn are important aspects for understand the transport mechanisms of the zinc elements for the inner of the seed of corn, which associated with the development of the seeds during its germination stages, for example. The preliminary results of this work show that the zinc oxide nanoparticles tend to form clusters after the treatment of the seeds of corn, which grows and fixated mostly in the interface regions between pericarp cells. In these interfaces are formed interlaced structures constituted by pericarp cells and formed an inter-diffusion path (micro-channels into the interior) that favor the transport of the minerals components from the clusters fixed on the surface of the seed for its inner. In this case the transport of the zinc oxide nanoparticles for the inner of the seeds of corn.

The clusters are formed preferentially on the interface regions and can be considered as minerals reservoirs of the zinc oxide nanoparticles for the seed. The clusters are constituted by nanoparticles with lower dimensions than these micro-channels and the nanoparticles released can be easily transported for the inner of the seed along of the times and to participate of the important metabolic germination mechanisms of the seeds of the corn.

The clusters are formed by the adsorptions of the zinc oxide nanoparticles in specifically sites on the surface of the seeds of corn. In the physical adsorption phenomenon

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the characteristics of the cluster depends of the specimen concentration in the suspension medium. The increase of the specimen concentration in the suspension medium promotes the increase of the amount of clusters and the increase of its sizes. But the growth rate of the cluster decreases with the increase of the time because the saturation of the adsorption area is expected [35].

Fig. 5(a) shows the relationship between the mass of zinc oxide nanoparticles per seed adsorbed on the surface of the seed of corn and the concentration of zinc oxide nanoparticles in the treatment suspensions, obtained by atomic absorption techniques. The results confirm that the mass of zinc oxide nanoparticles on the surface increase with the increase of the concentration in the suspensions. Utilizing the suspensions more concentrated is possible to obtain higher mass of the oxide zinc per seed adsorbed on the surface. The amount of zinc oxide mass is directly associated with the concentration of nanoparticle in the ZnO nanoparticles suspensions. With higher concentration is possible to obtain higher mass of zinc oxide nanoparticles on the surface of seed, but with lower mass increase the adsorption rate. The amount of adsorbed mass of zinc oxide nanoparticles on the seed surface can be related with nanoparticle mass in the ZnO nanoparticles suspension utilizing the equations 3 obtained from the mathematical adjusting of the diagram presented in the Fig. 5:

$$\mathbf{M\ (mg_{nano}/g_{seed}) = A.c^{n}}, \qquad \mathbf{n = 0.44} \text{ and } \mathbf{A = 4.37} \qquad (3)$$

where M is the zinc oxide mass adsorbed on the seed surface per seed mass, c is the zinc oxide mass in the suspension medium and A is an adjust constant.

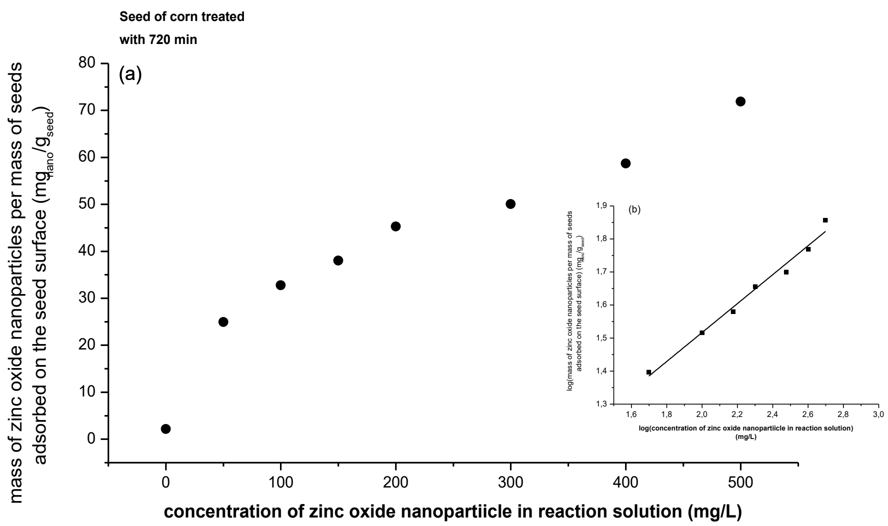

Fig. 5: (a) Relationship between the mass of zinc oxide nanoparticle per seed mass adsorbed on the surface of the seed of corn and the concentration of nanoparticles in ZnO nanoparticles suspensions and (b) linear relationship between the mass of zinc oxide per seed mass and the concentration of the nanoparticles in suspension. All treatments realized with 720 minute

Fig. 5(b) confirm that the relationship ($R^2 = 0.98$) between the mass of zinc oxide nanoparticle adsorbed on the seed and the concentration of zinc oxide nanoparticles in suspension medium is typical of a physical adsorption process.

The results suggest that the conditions of the treatment processes influence the adsorption of zinc oxide nanoparticles on the seeds of corn.So, the conditions of the treatment processes can be influences the amount of the zinc oxide nanoparticle incorporated in inner of the seeds, being that the clusters on the surface can be considered the reservoirs of the nanoparticles.

Incorporation of the zinc oxide nanoparticles to inner of the seed of corn

The atomic absorption technique was utilized to available the incorporation capacity of the zinc oxide nanoparticles to inner of the seeds of corn when treated with suspensions containing different concentrations of nanoparticles and with different times. Table 4 shows the values for the mass of zinc oxide nanoparticle per seed mass determined for the seeds of corn after the treatment with different ZnO nanoparticles suspensions.

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Table 4: Values for the mass of zinc oxide nanoparticle incorporated in the inner of the seed of corn per seed mass. Experiments realized with different concentrations of nanoparticle in suspension and different treatments times. Mass determined by atomic absorption technique

Experiment	Concentration of nanoparticles in the reaction suspension (mg_{nano}/L)	Treatment time (min)	Mass of zinc oxide nanoparticle incorporated in inner of seeds of corn per seed mass (mg_{nano}/g_{seed})
1	260.00	180.0	0.200
2	50.00	180.0	0.100
3	260.00	720.0	0.200
4	50.00	720.0	0.100
5	155.00	450.0	0.200
6	155.00	450.0	0.200
7	155.00	93.0	0.100
8	155.00	831.0	0.300
9	6.95	450.0	0.000
10	303.5	450.0	0.200
No treated (standard)	-	-	0.000

The seeds treated only water solution not presented significant values for the zinc oxide nanoparticle mass incorporated. The atomic absorption technique utilized has a detection limit of one part per million (ppm), so for these studies, the values for the mass of zinc specimens in the inner of the standard seeds were considered null. The results show that the treatment processes employed is efficient to incorporate the zinc oxide nanoparticles to inner of the seeds of corn. Utilizing the treatment process with the suspension containing higher concentrations of nanoparticles is observed a tendency of increase of the amount of the mass of zinc oxide nanoparticles in inner of seeds.But, the effect of the treatment time not is evident in these processes.

The statistical analysis (Variance Analysis) of the results presented in the Table 5 showed with the certainty of 86 % ($p < 0.14$) that the treatment time and the concentration of the zinc oxide nanoparticles in the suspensions are significant factors to define the amount of the mass of nanoparticles incorporated in inner of the seeds of corn.

Table 5 : Variance analysis ($p < 0.14$) for the results of amount of mass of zinc oxide nanoparticles incorporated in ineer of the seed of corn per seed mass. Dates presented in the Table

	SS	dF	MS	F	P
$[ZnO]_n$ (L)*	0.029235	1	0.029235	10.43938	0.031945
$[ZnO]_n$ (Q)*	0.010973	1	0.010973	3.91825	0.118875
Time (L)*	0.009536	1	0.009536	3.40517	0.138741
Time (Q)	0.000026	1	0.000026	0.00936	0.927579
Interaction $[ZnO]_n$ and Time	0.000000	1	0.000000	0.00000	1.000000
Erro	0.011202	4	0.002800		
Total SS	0.064000	9			

*significant variables - $[ZnO]_n$: concentration of zinc oxide nanoparticles in the suspension - Time: treatment time of seeds of corn in ZnO nanoparticles suspension.

A quadratic model ($R^2 = 0.82$) was adopted to relationship the mass of zinc oxide nanoparticles incorporated in the seeds with the variables of concentration of nanoparticle in suspensions and with treatment times, Fig. 6(a). The model shows a linear dependence between the values of mass incorporated in inner of the seeds with the treatment time variable, indicating that higher treatment times promote higher amounts of the nanoparticles in inner of the seeds of corn.

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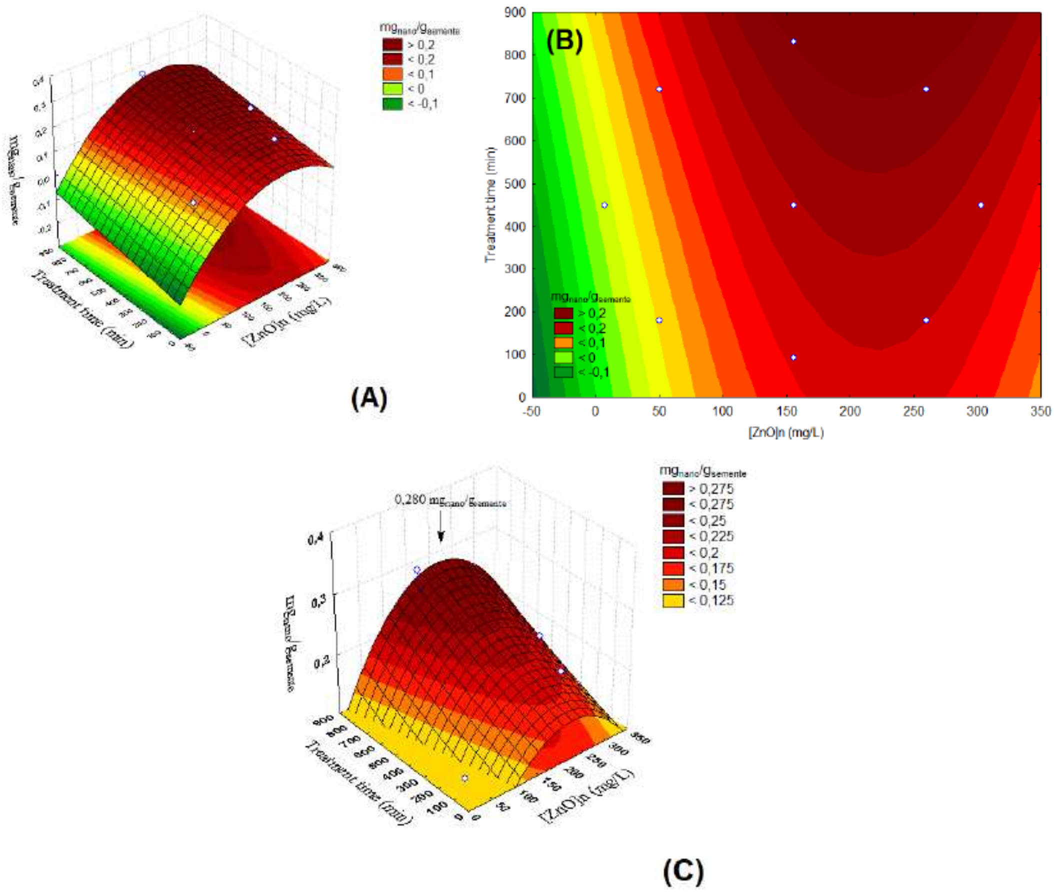

Fig. 6: Relationship between the mass of zinc oxide nanoparticles incorporated in seeds of corn per seed mass (mg_{nano}/g_{seed}) with the concentration of nanoparticles in the suspension ([ZnO]n – mg/L) and different treatment times (Time-min). Results obtained from a quadratic model ($R^2 = 0.82$) obtained with a experimental design with confiability of 86 %. (a) Response surface from quadratic model, (b) level curve from quadratic model and (c) response surface with highlight for optimal condition for incorporate the maximum amount of nanoparticles in inner of seeds of corn.

The concentration variable is a positive factor too, but influence with a quadratic factor. With higher concentrations of the zinc oxide nanoparticles in suspension medium is possible to incorporate higher amount of the mass of nanoparticles in inner of the seeds of corn. The increase of the mass in inner of the seeds not is linear but increase until a maximum value, which occur proximally to the concentration of the zinc oxide nanoparticles of 225 mg/L in the suspension medium, Fig. 6(b). Fig. 6(c) evidences the maximum conditions to incorporate the maximum amount of mass of zinc oxide nanoparticle in inner of the seeds of corn.

So, is possible to establishes that the best condition for the treatment processes to incorporate the maximum amount of mass of zinc oxide nanoparticle in inner of seed of corn is with the maximum values for the treatment time and with the concentration of 225 mg/L in suspension medium. With this condition is possible to incorporate 0.280 mg_{nano}/g_{seed} in inner of the seed of corn.

From the EDS analysis not was possible to detect zinc oxide nanoparticles in inner of the seeds of corn. But the atomic absorption technique showed that is possible to incorporate the nanoparticles with low amounts in inner of the seeds. The amount of the mass of the nanoparticles incorporated in inner of seeds is very low and should not have toxically character.

Fig. 7 show the images obtained with electronic microscopy detailing the inner cells of the seeds of corn treated by 180 minutes with the ZnO nanoparticles suspension containing low concentration of the nanoparticles (50 mg/L) and with the suspension containing high concentration of nanoparticles (1000 mg/L). The results reveal cells with absolutely integrity. The inner cells not were affected by the zinc oxide nanoparticle incorporated in seed.

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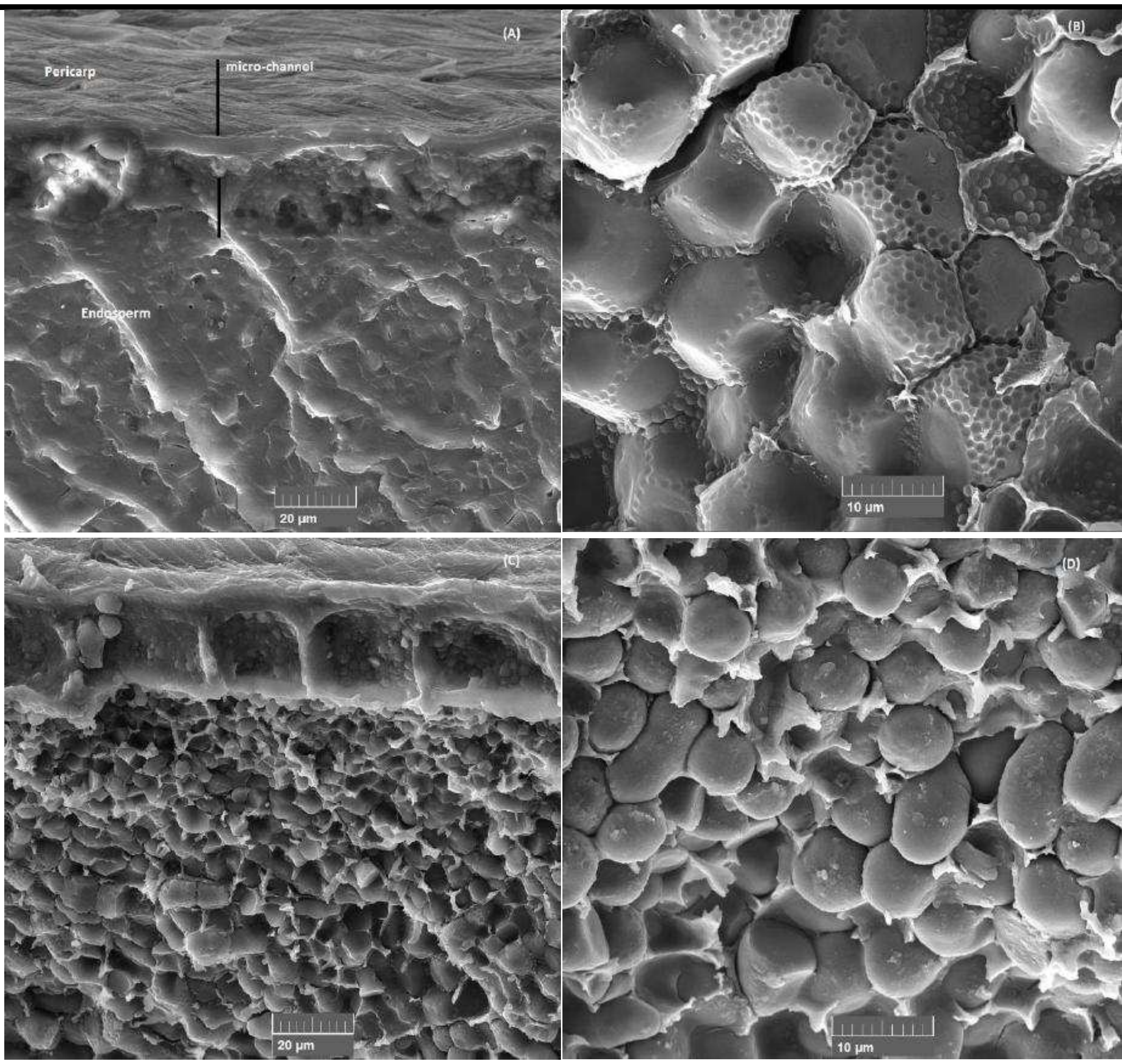

Fig. 7: Images obtained with electronic microscopy for the seeds of corn treated by 180 minutes in a suspension with different concentrations of zinc oxide nanoparticles. (a) and (b) seeds treated with 50 mg/L and (c) and (d) seeds treated with 1000 mg/L.

These results generates the expectancy that the treatment conditions studied not cause a negative effect on the integrity of the seeds of corn and that not will promote a negative effect on the germination of these seeds.

The atomic absorption and electronic micrographs proved that is possible to incorporate the zinc oxide nanoparticles without compromising the structural integrity of the seed cells, i.e., not have significant structural cell damages due the presence of the nanoparticles.

The zinc oxide nanoparticles are adsorbed by fibrous cells constituents of the pericarp structure on the surface of the seeds of corn. The nanoparticles form clusters that are fixed in the interface formed by these cells. These interface form micro-channels that communicate the inner regions with the surface of the seeds and can favor the transport of the nanoparticles from cluster to the inner of the seeds. So, the clusters formed on the surface can be considerate as a zinc mineral reservoir for the seed. By diffusion mechanisms through micro-channels the nanoparticle can be incorporated to inner of the seed and the zinc mineral be provided to the germination mechanisms.

The formation of the zinc oxide nanoparticle cluster is very fast but on the surface and the interdiffusion processes of the nanoparticles to inner of the seed should be very slow, so that the nanoparticle cluster should serve as a zinc reservoir during long time and during the development of the corn plant. Its possible predict that during germination phase zinc oxide nanoparticle will migrate to inner of the seeds considering that the nanoparticles have dimension approximately of 100 nm and the micro-channels have dimensions approximately of some micrometers.

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3.2 Germination Tests

Normal plants and abnormal plants

The germination tests were realized with seeds of corn treated with the experimental conditions showed in the Table 4. The amount of the normal plants and abnormal plants were determined applying the agronomical procedures.

The following results show the percentage values for the normal plants after the germination period of the seeds of corn in function of the different treatment conditions. The results were available by the variance analysis ($p \leq 0.05$) and showed that the both factors are statistically significant to increase the values of the percentage of the normal plants.

Fig. 8 shows a quadratic dependence for the normal plant percentages and the time treatment. The values of the normal plants percentage increases with the increase of the time treatment until optimal value of 180 minutes. With the optimal treatment time the normal plant percentage increase of 2.70% in relationship to the seeds not treated (standard).But, for treatments realized with times higher than 600 minutes the effect is negative for the germination process and the values of the percentage normal plants are lower than the values for the standard seeds.

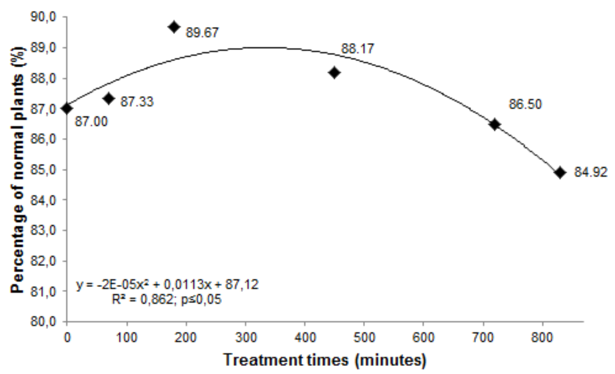

Fig. 8: Relationship between normal plants percentage and the treatment time for the seeds of corn treated in the suspensions containing zinc oxide nanoparticles.

Likewise, Fig. 9 shows a quadratic dependence for the normal plant percentages and the zinc oxide nanoparticles concentrations in the suspensions medium. The values of the normal plants percentage increase with the increase of the concentration of zinc oxide nanoparticles in the suspension medium until optimal value of 50 mg/L. With the optimal treatment concentration the normal plant percentage increase of 2.70% in relationship to the seeds not treated (standard). But, for treatments realized with concentration of zinc oxide nanoparticle higher than 240 mg/L the effect is negative for the germination process and the values of the percentage normal plants are lower than the values for the standard seeds.

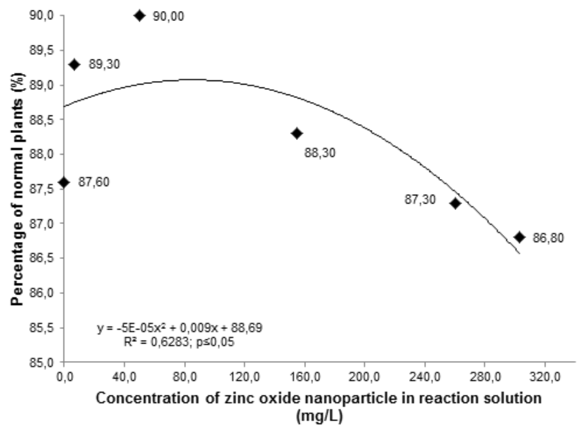

Fig. 9: Relationship between normal plants percentage and the zinc oxide nanoparticles for the seeds of corn treated in the suspensions containing zinc oxide nanoparticles

The increase of the normal seeds percentage is a benefit aggregated by the treatment processes of the seed of corn with the zinc oxide nanoparticles. The results obtained with the atomic absorption technical demonstrated that is possible to incorporate the nanoparticles to the inner of the seeds. The amounts of the nanoparticles in inner of the seeds increase with the treatment time but are limited by the zinc oxide concentration in the suspension medium. To incorporate the maximum amount of the nanoparticles in the seeds the best zinc oxide concentration in the suspension is 225 mg/L, but for the best germination results for the seeds of corn occur with the treatment containing 50 mg/L.So, is possible to conclude that the best germination performance for the seeds of corn is associated with the presence of the zinc oxide nanoparticle in the inner structure, because the seeds not treated presented a worse performance.

With treatment conditions with times higher than 600 minutes and with concentrations higher than 240 mg/L the negative effect is highlighted and promotes the lower values for the normal plants percentage than the values obtained for the standard seeds (no treated). It's quite possible that the negatives effects are associated with the small dangers caused in the cellular compounds, but that not were detected by de electronic microscopy. Taiz and Zeiger (2013) related that high concentrations of the zinc oxide nanoparticles generally promotes oxidative dangers in the vegetable cellular structures by peroxidation of the lipids and promotes the degradation of the some cellular compounds [36].

Fig. 10 and 11 show the values obtained for the abnormal plant percentage and confirm the presence of positive and negative effects of the treatments in function of the

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treatment time and of the zinc oxide nanoparticles, respectively. The results confirm that the best treatment time is the 180 minutes and the best zinc oxide concentrations is the 50 mg/L.

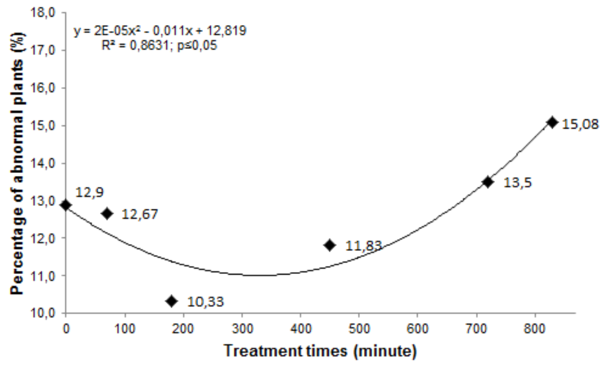

Fig. 10: Relationship between abnormal plants percentage and the treatment time for the seeds of corn treated in the suspensions containing zinc oxide nanoparticles.

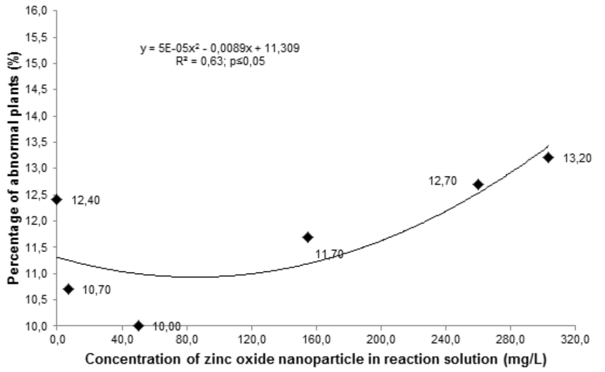

Fig.11: Relationship between abnormal plants percentage and the zinc oxide nanoparticles for the seeds of corn treated in the suspensions containing zinc oxide nanoparticles

Dead seeds and germination rate

The variance analysis with the germinations results not showed statically significance ($p>0.05$) of the treatment time and of the zinc oxide concentration in the suspension medium in the amount of the dead seeds and in the germination rate.

These results are strong indicators that the zinc oxide nanoparticles in inner of the seeds of corn not promote high toxic effects to the seeds. Though there are negative effects to the normal plants percentage the toxic effect of the nanoparticles shall be small enough to prejudice only the cellular physiology and to affect only the normality of the plants, but not to lead to death of the plants.

The presence of the zinc oxide nanoparticles in the seeds of corn not affected the germination rate. This indicator shows that the nanoparticles not prejudice the metabolic mechanisms of the seeds of corn during its germination phase. These results are expected, since that if the toxic effect exists shall be small and not shall compromise the seeds metabolism of significant forms. Likewise, the zinc oxide nanoparticles availability to the corn plant shall occur in future phases and during the growth of the plant, that is, in the vegetative and reproductive phases of corn plants.

3.3Microbiological analysis of the seeds of corn

The clusters formed by zinc oxide nanoparticles on the surface of the seeds of corn, besides serving as micronutrient reservations, can be to contribute for the antimicrobial protection of the seeds, in special with the antibacterial protections. The ionic metals and metallic nanoparticles have excellent antimicrobial properties [37-38]. These characteristics can to aggregate the antibacterial property on the surface of the seeds of corn and protect them the harmful microorganisms and prevent the seeds of future disease produced by some bacteria during the germination phase.

Fig. 12 and 13 show the microbiological results, with death curves, obtained with seeds treated in ZnO nanoparticles suspensions containing different zinc oxide nanoparticles in the suspension during 180 minutes. The bacteria utilized were the *Escherichia* coli and *Staphylococcus* aureus.

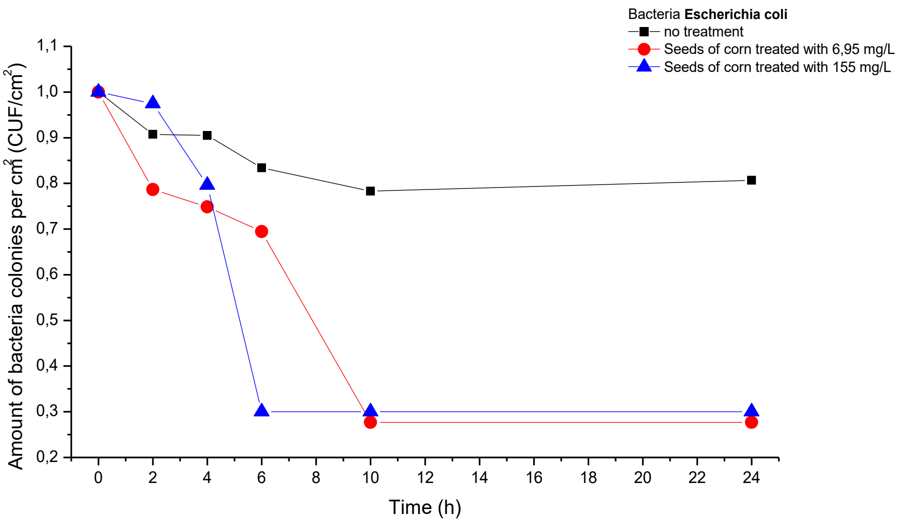

Fig. 12: Microbiological results with death curves test with bacteria Escherichia coli for seeds of corn treated with 6.95 mg/L and 155.00 mg/L of zinc oxide nanoparticles in the suspension.

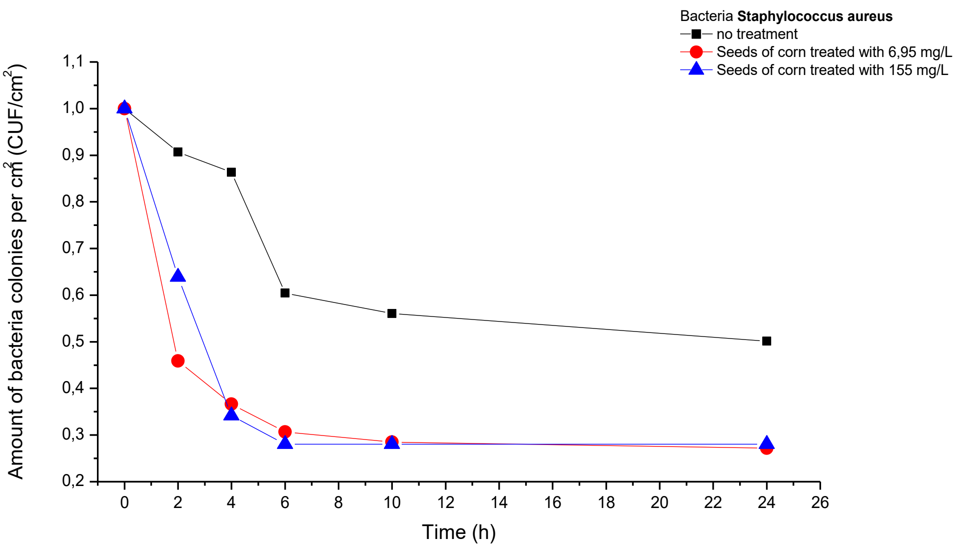

Fig. 13: Microbiological results with death curves test with bacteria Staphylococcus aureus for seeds of corn treated with 6.95 mg/L and 155.00 mg/L of zinc oxide nanoparticles in the suspension.

The microbiological results indicate the reducing of 73.0% of the bacterial colonies for both type of bacteria, while for the standard seeds (no treated) reduced for lower values to 20%. These results are antibacterial protection indicatives and prove that the zinc oxide nanoparticles aggregates the antibacterial properties on the seeds of corn

With the bacteria*Escherichia* coli the microbiological results showed that the seeds treated with 6.95 mg/L eliminate 73.0% of the bacterial colonies in 10 hours, while the seed treated with 155 mg/L eliminates 73.0% in 6 hours. This different between the elimination time is associated with the amount of the nanoparticles adsorbed on the surface of the seeds of corn during its treatment processes.

For the bacteria Staphylococcus aureus the microbiological results showed that the seeds treated with suspension containing 6.95 mg/L and containing 155 mg/L eliminates 73.0% of the bacterial colonies in 6 hours. The same values for the elimination time is expected considering that the gram positive bacteria is more susceptible to antimicrobial agents than the gram negative microorganisms and the antibacterial actions of the zinc oxide nanoparticle are more fast, even with lower concentrations (FIORI, 2009).

IV. CONCLUSIONS

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The results proved that is possible to incorporate and to adsorber zinc oxide nanoparticles in inner of seeds of corn and to improve the germinations indicators. The optimal treatment conditions to incorporate the nanoparticles occur with nanoparticle concentration of 50 mg/L in the suspension and with treatment time of 180 minutes. With these conditions is possible to incorporate 0.280 mg of zinc oxide nanoparticle per seed mass in inner of seeds.

The germinations indicators are influenced by the treatment conditions. The values of the normal plants percentage increase with the increase of the zinc oxide nanoparticles in the suspension until optimal value of 50 mg/L. With the optimal treatment concentration the normal plant percentage increase of 2.70% in relationship to the seeds not treated (standard). But, for treatments realized with concentration of zinc oxide nanoparticle higher than 240 mg/L the effect is negative for the germination process and the values of the percentage normal plants are lower than the values for the standard seeds.

The treatment with zinc oxide nanoparticles aggregated the antibacterial characteristics on the seeds of corn. This new property can be interpreted with a antimicrobial protection of the seeds of gram-negative and gram-positive bacteria and promote the prevention of the seeds of corn of possible diseases generated by these microorganism.

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