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The study of imbibition curves in the seeds of corn (*Zea mays*) and red kidney bean (*Phaseolus vulgaris*): effect of nanoparticles and salts

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ABSTRACT
The effect of nanoparticles (Al, ZnO, Fe_2O_3, TiO_2, CuO) applied to *Zea mays* and *Phaseolus vulgaris* seeds was studied to verify their influence on water absorption of the seeds. Water absorption (imbibition) is the first step of seed germination and is the triphasic process. In these work were studied the first two phase of imbibition. Results of these experiments show that nanoparticles may able to diffuse into the seeds, with no effect on the kinetics of imbibition and change the volume of absorbed water by seeds depending on types of nanoparticles. It was identifaied that when the concentration of salts (NaCl) increases, the intake of water in corn and bean seeds decreases. It has been established that when nanoparticles added to the salty solution the absorption process intensify and increase the quantity of absorbed water by seeds relative to the control. These effect depends on the types of nanoparticles and seeds of theplant.
Keywords: *nanoparticles, seed, imbibition, water uptake, salinity.*

1. INTRODUCTION

Water uptake (imbibition) is a first step of seed germination. During imbibition occurs the initiation of biochemical process in the endosperm of seed and that lead to germination completion. The study of the imbibition curve is very important in two aspects, firstly water uptake process is the initial condition for the successful development of seeds germination and knowing the nature of the kinetics of water uptake we can regulate environmental conditions for seed germination, especially in the soils. Secondly for the specially of pregermination techniques aimed at improving seed physiological quality [1,2]. During the soaking of seeds and also food materials, water is progressively absorbed. The extent of water absorption or the amount of water absorbed into the seeds and food materials during soaking depends primarily on the soaking water temperature, initial moisture content of seeds, variety of the seeds, soaking duration, acidity level of the water and physicochemical properties (such as seed structure, size etc) of the food materials [3 -8]. The water absorption by the seeds is the triphasic process. In phase I, known as imbibition, occur a fast diffusion of water from the environment to the seeds under the matrix forces and a large increase in seed moisture content. In phase II, water absorption to the seeds is slows down and reaches saturation. This lag phase of water uptake occurs before the onset of germination, which is spruce fir visible radical. In the start of phase III observed root protrusion and water absorption increases again. The visible germination marks the transition from seed to seedling. The phase of III observed in only viable seeds [9, 10]. The rate of the imbibition depends upon the seed surface area, coat permeability and on the water potential of the external medium that governs the ability of the seed to reach its critical moisture content. The ability of the seed to take up water depend on morphological and chemical properties of the seed coat. An impervious seed coats possess one or more layers of very tightly packed cells, with no pores, intercellular spaces or stomata between them [11, 12]. During seed imbibition absorption of water is differ by the seed parts (seed coat, embryonic axis, cotyledons, and whole seed). The data show that in 72 h imbibition, the embryonic axis was the most hydrated portion of the soybean than any other seed part. Studies show that the embryonic axis hydrates more than the cotyledons [13, 14]. In seeds those that imbibe water quickly may occur imbibition damages to the embryos of germinating. There are some experimental data that seed coat colors as an important external factor contributes in water uptake and early protein synthesis [9,16-19]. For example, experiments of water uptake in seeds showed that absorption in brown seeds was quick in the initial hours than black [15, 20]. The structure and composition of seed coat, morphology and size of seeds have great influence on the ability of seeds water uptake. The water uptake ability of seeds changes during the whole germination process. The initiation of radicle more sensitive to water [21, 23]. This sensitivity or ability of seed to take up water depend on physiological processes [22,24. Under normal conditions, in the period of early seed imbibition develop metabolic processes, synthesize proteins and a new mRNA [25, 26, 27]. The endosperm of seeds allow free movement of water from the environment to the embryo and same time prevents leakage of solutes. There are data that during imbibition occurs leakage of compounds from seeds to the environment in many species [20, 28, 29]. Usually leakage is greater from nonviable or deteriorated seeds than from high quality seeds. It is because that in such seeds lack of compartmentalization of cellular constituents, caused by faulty membranes or cellular rupture. For example, have been shown that nonviable lettuce seeds to have a greater weight and volume increase during imbibition than viable seeds [31]. It is proposed that physical characteristics and seed viability is play important role during imbibition of seeds. In the nonviable embryos leaks more solutes than viable embryos. In the normal condition for seeds an intact endosperm restricts leakage of solutes from the embryo to the environment. Therefore, in nonviable seeds osmotic active molecules accumulate in the extra embryonic fluid. This increase a water potential gradient between the absorbed water and the seed resulting in increased water uptake. In the result nonviable seeds is take additional water [32]. Showing that during the imbibition of cotton seeds, the amount of N-acetylphosphatidylethanolamine molecules, which may be involved in maintaining membrane integrity is increasing. Upon imbibition, in the dry seed rapidly resumes metabolic activity [33]. Within minutes during imbibition the first changes is the resumption of respiratory activity. The initial rapid increase of oxygen consumption, late on declines until the radicle penetrates the surrounding structures. As radicle begins developed in the endosperm another burst of respiratory activity occurs. As radicle begins developed in the endosperm another burst of respiratory activity occurs. During phase I of imbibition the glycolytic and oxidative pentose phosphate pathways both and the Kreb's cycle enzymes become activated [16,34]. Early imbibition of seeds is lethal if lipid reserves in seeds are crystalline. The crystallization of lipids occurs during seed storage in low temperatures. If seeds with crystalline triacylglycerol expose brief heat treatment before imbibition that melts triacylglycerol prevents the loss of cell integrity [36]. In the experiments of Malqorzata and Jolanta (1998) was studied the influence of $Pb(NO_3)_2$ and $Ba(NO_3)_2$ on the water uptake and the germination dynamics in 25 species of plants from 12 families, were tested 34 types of seeds. It was shown that seed coat permeability varied during imbibition of seeds with coats highly permeable to lead and lead did not act by inhibiting water uptake. It was also shown that seed coats were impermeable to lead in the first period of imbibition when water uptake is intense. In the final stages of imbibition, when water uptake is reduced, seed coats became more permeable to lead. The lead that penetrated into the embryos in the final stage of imbibition delayed germination. This shows that seed coats are selectively permeable to lead ions [30, 37].

The objectives of the present work were to determine the influence of different nanoparticles and osmotic potentials (different concentration of NaCl) on the imbibition curve time of Red kidney bean (*Phaseolus vulgaris)* and corn (*Zea mays*) seeds.

2. MATERIALS AND METHODS

(For the study of imbibition curves the Red Kidney bean (*Phaseolus vulgaris) and* corn (*Zea mays*) seeds were used. Seeds were selected, counted, treated, and dry weighted. Amount of absorbed water by seeds was studied by means of weight method. To this end, each seed were weighted and was numbered before insertion they in solutions. Then, for control option 5 seeds were kept in distilled water and 5 seeds were kept in dispersion solution of 1 mg/ml nanoparticles dissolved in distilled water or 0,6M NaCl. During the experiment, the seeds were weighted one by one every hours. The seeds were removed from the water, drained, superficially blotted with absorbent paper, weighed and returned to the water. Changes in weight due to imbibition were expressed as the amount of water absorbed by seed. The measurements lasted 25-36 hours. The measurement was replicated at least 3-4 times. Was identified the kinetics of absorbed water (mg) versus time of exposure. The amount of absorbed water were measured by the formula

$$\Delta M = M_s - M_d \quad (1)$$

where ΔM is the absorbed water (mg) in given time, M_s is the weight of swollen seeds (mg) and M_d is the weight of dry seeds (mg).

In experiments, different kinds of nanoparticles were used, such as TiO_2 (Rutile, 10-30 nm), Fe_2O_3 (20-40 nm), CuO (30-50 nm), ZnO (10-30 nm) and Al_2O_3 (18 nm) were obtained from SkySpring Nanomaterials, Inc. (Houston, TX, US). The dispersed solution of nanoparticles is prepared in a concentration of 1 mg/ml. In order to obtain properly dispersed and stable nanoparticles suspensions, the nanoparticle suspensions were sonicated for 15 minutes prior use. Pure distilled water was used and accounted for control study in each nanoparticle treatments.

3. RESULTS SECTION

The interactions of nanoparticles with plant seeds have not been studied well yet. There are evidences that nanoparticles are adsorbed on the surface of seeds and can diffuse into them [35, 38]. The study of Khodakovskaya et al. shown, that multiwall carbon nanotubes can penetrate tomato seed and increase the germination rate by rising water uptake [39]. Hence, in this study, we investigated the impact of nanoparticles on the seed imbibition processes of the mono- and dicotyledonous crop plants: corn, and bean. In the primary experiments, we studied the imbibition curves for beans and maize seeds in distilled water. The results of these experiments were given in Table 1 and 2, also in fig.1. The measurement was replicated 4 times. The average dry weight of one seed of *Zea mays* (from 80 seeds) and Red kidney seeds (from 75 seeds) were 327 mg and 552 mg respectively. The absorbed water after 25 hours per mg dry weight for corn seed was 0,39 mg and for bean 1,14 mg. Absorbed water 3 times more in bean then in corn. As it can be seen from the Figure 1 the imbibition curve of corn seeds slightly differences from the bean. The maximum of absorbed water occurs in 20-25 hours in corn seeds, but in bean seeds, it is takes place in 10-11 hours. This suggests that the water uptake in bean seeds occurs quickly than in corn seeds.

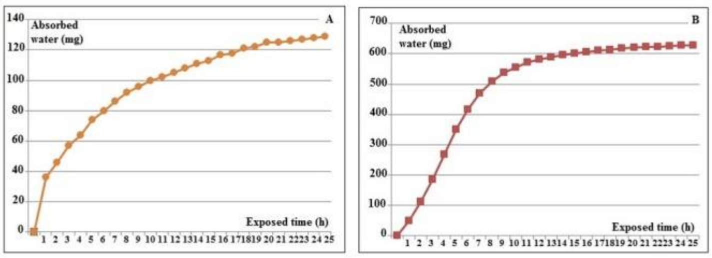

Figure 1. Imbibition curves of Zea mays (A) and Red kidney bean (B) seeds in distilled water.

Table 1. Absorbed water by seeds of red kidney bean.

Exposed time (hours)	Average absorbed water (mg) from 5 seeds	Average absorbed water (mg) from 5 seeds	Average absorbed water (mg) from 5 seeds	Average absorbed water (mg) from 5 seeds	Average of four repetitions (mg)
1	073	41	03	080	049
2	170	95	14	170	112
3	244	189	42	260	184
4	301	267	124	383	269
5	387	322	196	495	350
6	431	397	273	566	417
7	478	425	356	619	469
8	500	451	416	669	509
9	545	471	465	670	538
10	561	485	494	679	555
11	584	497	510	702	573
12	594	508	523	704	582
13	608	509	535	707	590
14	614	512	543	714	596
15	622	515	548	723	602
16	628	517	556	726	607
17	633	521	559	728	610
18	635	527	562	729	613
19	637	530	570	730	617
20	645	531	571	731	620
21	646	533	575	732	622
22	649	535	576	733	623
23	650	536	581	734	625
24	654	537	582	734	627
25	655	538	584	734	628

Table 2. Absorbed water by seeds of *Zea mays*.

Exposed time (hours)	Average absorbed water (mg) from 5 seeds	Average absorbed water (mg) from 5 seeds	Average absorbed water (mg) from 5 seeds	Average absorbed water (mg) from 5 seeds	Average of four repetitions (mg)
1	41	50	15	36	36
2	49	62	22	49	46
3	58	75	31	64	57
4	66	85	39	68	64
5	72	95	48	81	74
6	78	104	53	86	80
7	82	111	56	94	86
8	87	115	60	107	92
9	90	120	64	109	96
10	95	125	66	113	100
11	98	128	69	115	102
12	101	130	73	117	105
13	103	131	76	121	108
14	107	134	77	124	111
15	110	135	78	128	113
16	111	137	80	139	117
17	113	138	83	140	118
18	116	142	85	141	121
19	119	141	87	142	122
20	122	143	87	143	125
21	122	144	89	144	125
22	125	144	89	145	126
23	127	145	90	146	127
24	128	146	92	147	128
25	130	147	93	147	129

The main purpose of the study was to investigate the effects of nanoparticles on the water absorption process of seeds and in subsequent experiments depending on the duration of seed expiration, were investigated the absorption curves of metal-based nanoparticles in suspension solution, such as Fe_2O_3, TiO_2, ZnO, Al_2O_3 and CuO. As shown in the method, 5 seeds were selected and each seed was also placed in distilled water and nanoparticle solution. The seeds were removed from the solution hourly, dried with filter paper, and weighed on an analytical scale and placed into the solution again. All seeds were dried and weighed (5 controls, 5 tests) at 3-5 minutes intervals. Figure 2 shows the imbibition curves of *Zea mays* in the suspension solution of nanoparticles and in the distilled water. As seen in Figure 2, nanoparticles in *Zea mays* seed do not affect the nature of the absorption process. In the nanoparticle solution, the seeds absorb water intensively in the first 4-8 hours, after 9-10 hours the water absorption is slower and after 25 hours reach the maximum value. From the results of the experiments, it is clear that Fe_2O_3 nanoparticles reduce the amount of water absorbed by *Zea mays* seeds, a decrease is also observed in CuO and Al_2O_3 nanoparticles in the first 15-16 hours. However, ZnO nanoparticles increase the amount of adsorbed water, while the TiO_2 nanoparticles increase relatively. Similar experiments were also performed on Red kidney bean seeds. The results of these experiments are given in Figure 3. In the case of red kidney bean seeds, nanoparticles do not have a significant effect on the nature of the absorption process, and imbibition curves maintain their shape. However, in some nanoparticles, for example, Fe_2O_3 and TiO_2 nanoparticles are observed lag phase within first 2 hours, and this nanoparticles decreases the amount of absorbed water during all exposed time. The amount of absorbed water during all exposed time in the solution of ZnO was more than in distilled water and does not effect on the kinetics of imbibition curves. Nanoparticles of ZnO and Al_2O_3 does not effect on the kinetics of imbibition curves of Red kidney bean.

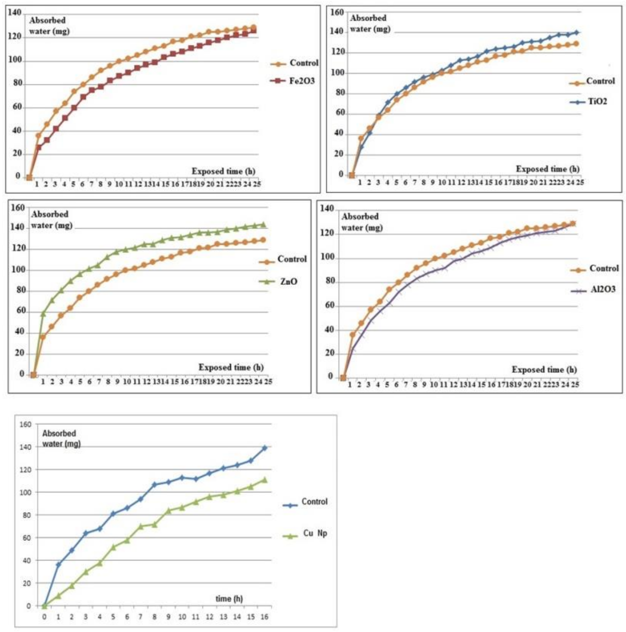

Figure 2. Imbibition curves of *Zea mays* seeds in distilled and dispersion water of nanoparticles.

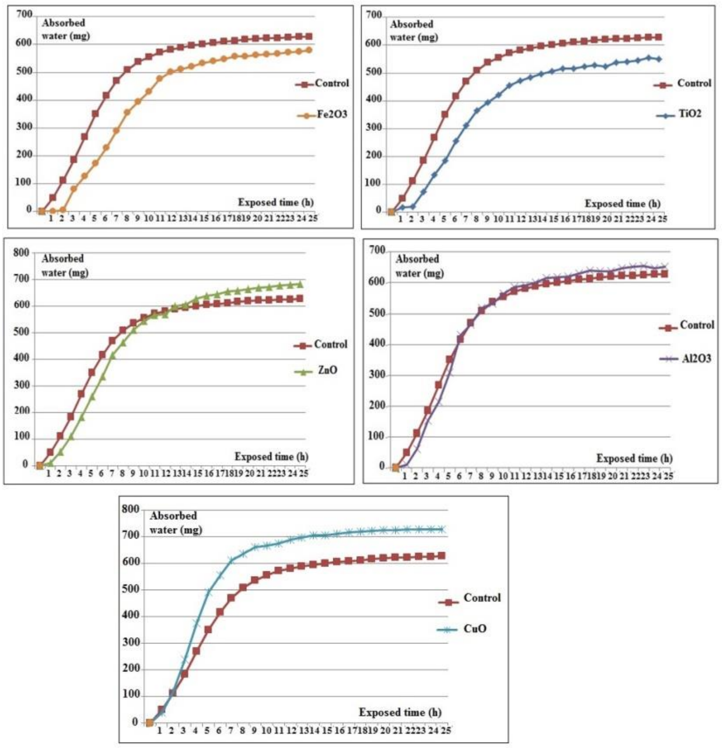

Figure 3. Imbibition curves of Red kidney bean seeds in distilled and dispersion water of nanoparticles.

In subsequent experiments, the process of water absorption of seeds was first studied in solutions of various concentrated NaCl salts. Imbibition curves were obtained in 3 different concentrations of NaCl salt, both in *Zea mays* and Red kidney bean seeds. The results of the experiments are given in Figure 4. Obviously, the form of imbibition curves in the saline solution (the process of water absorption of seeds) does not undergo serious changes. On the other hand, the concentration of the NaCl solution does not play a significant role. When the concentration of the solution increases threefold from 0.2 M, the amount of water absorbed in both *Zea mays* and Red kidney bean seeds is reduced by 1.5 times. What is interesting about the ability of the seeds to absorb water in salt water with the presence of nanoparticles? Thus, the experiments with nanoparticles were performed in NaCl solution at the same time. For this purpose, the dispersion solution of nanoparticles was prepared in 0.6 M NaCl solution. The dispersion solutions of nanoparticles were processed on the ultrasonic device for 15 minutes prior to the exposure of seeds. Water absorption ability of the seeds was monitored for 25 hours and imbibition curves were obtained. The results of the experiments are given in Figure 5. Clearly, the absorption amount of *Zea mays* seeds in the given nanoparticles (Fe_2O_3, TiO_2, Al_2O_3, ZnO) in the saline water are close to absorption in the distilled water and eliminate the effect of NaCl salt, and even increase the amount of water absorbed by ZnO nanoparticles relative to control.

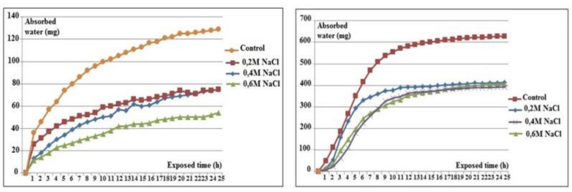

Figure 4. Effect of NaCl on the imbibition curves of *Zea mays* (A) and Red kidney bean (B) seeds.

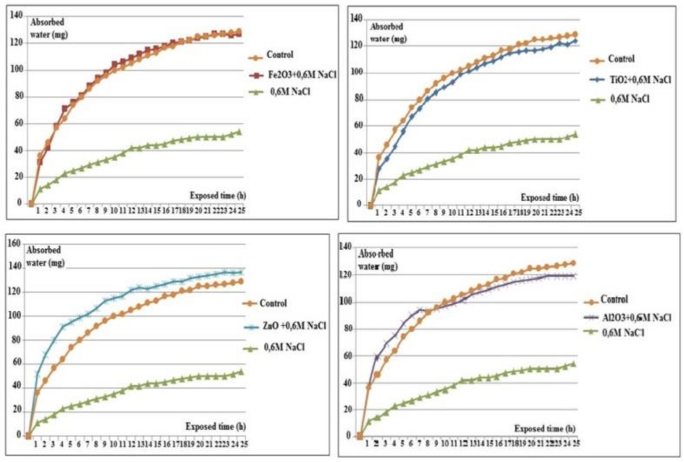

Figure 5. Effect of nanoparticles in NaCl solution on the imbibition curves of *Zea mays*.

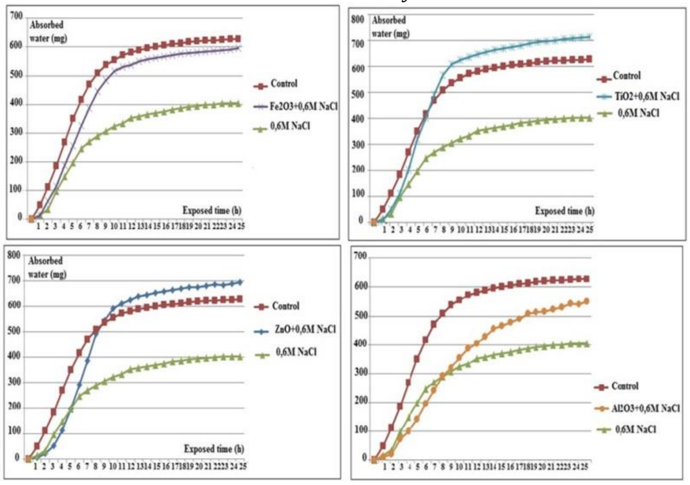

Figure 6. Effect of nanoparticles in NaCl solution on the imbibition curves of *Zea mays*.

The amount of water absorbed in the Red Bean seeds with Fe_2O_3 nanoparticles has been found to be close to absorption in distilled water. Even TiO_2 and ZnO nanoparticles increase slightly, while the Al_2O_3 particles increase slightly to the control level. Different results have also been observed in the nature of the imbibition curves. Thus, the intensity of absorbed water the presence of nanoparticles in the first hours has increased. But in the subsequent hours, the absorption of water was the same as in distilled water. Thus, the results of experiments have shown that given metal based nanoparticles seriously alter the absorption process of salting water in seeds and the amount of absorbed water is close to the amount of water absorbed in distilled water.

In the seeds, the moisture content (or the amount of water absorbed) should reach a certain level (critical price) for the germination process to commence, for example, seeds begin to germinate when the moisture is 25-30% in rice seeds [40]. This level is different in different seeds and largely depends on the size of the seeds. Temperature, time, osmotic potential of the environment, physical and chemical factors play an important role in the formation of critical moisture in the seeds. The intake of water absorption of the seeds depends on the initial moisture contained in them, the lower the moisture, the greater the intake of water absorption [41]. The process of water absorption of seeds is characterized by the imbibition curve and by studying the kinetics of these curves, they determine the critical moisture required for germination. The imbibition curves obtained in our experiments are characteristic curves for both corn and bean (Fig.1) and they cover the I and II phases of the imbibition process. Despite the fact that water absorption in the seeds is widely studied, it still remains topical. However, the influence of nanoparticles on this process has lately been of special interest. The results of our experiments show that the nanoparticles change the intensity of the absorption process, the amount of water absorbed in mono- and dicotyledonous seeds (Fig.3,4). ZnO nanoparticles in corn seeds increase the amount of water absorbed but does not change in the seeds of the bean. Fe_2O_3 nanoparticles reduce the absorbed water in both corn and bean seeds. TiO_2 nanoparticles do not change in corn seeds but reduces bean seeds. CuO nanoparticles do not change in corn seeds but increase in bean seeds. The effects of nanoparticles can depend on the structure of the seeds, the morphology of their shells and their nanoparticles. Despite the fact that the seeds are well-watered, nanoparticles are one of the main structures that regulate their ability to absorb water [32].

One of the interesting results of our experiments is the effects of nanoparticles in salty environments. The salt rate and osmotic potential in the water absorption process of the seeds is a serious influence. Alam and his colleagues (2003) studied the absorption process in rice seeds at the concentrations of NaCl and $CaCl_2$ salts and osmotic pressure of 0.22 MPa of polyethylene glycol. Their results show that when the concentration of salts increases, the intake of water in rice seeds decreases and the complete imbibition of seeds occurs after 48 hours at 150 mM concentration and after 72 hours at 250 mM concentration. At this time, the moisture content in the seeds is 25-30%, which provides germination. It has been established that $CaCl_2$ (3-9 mM) is added to the NaCl salt and the absorption of water intensifies [40]. From our experiments, it has become clear that the amount of water absorbed in the 0.2M concentration of NaCl salt has decreased in both corn and bean seeds and this decrease has increased even further when the concentration of the solution increases. However, the nature of the water absorption curves has not changed (Fig.4). The results show that when nanoparticles are added to the NaCl salt, intake of water in both corn and bean seeds intensifies (Fig.5,6). ZnO nanoparticles increase the amount of water absorbed in corn seeds in comparison with control. Both ZnO and TiO_2 in the bean seeds intensify the absorption process and increase the quantity of absorbed water relative to the control. Zheng *et al.and Khot et al.* [43,44] reported that nanosized TiO_2 contributed to water absorption by spinach seeds and promote capsule penetration for intake of water. Other nanoparticles also bring the water absorption of the seeds to the distilled water in salty environments and bring the quantity of absorbed water closer to the amount controlled. This effect of nanoparticles is similar to those obtained during the addition of Ca element in the NaCl salt (Alam et al. 2003). However, the mechanism of nanoparticles intensification of water absorption of seeds in salty water is unclear and requires additional experiments.

4. CONCLUSION

The effect of nanoparticles (Al, ZnO, Fe_2O_3, TiO_2, CuO) applied to *Zea Mays* and *Paseolus vulgaris* seeds was studied to verify their influence on water absorption of the seeds. Water absorption (imbibition) is the first step of seed germination and is the triphasic process. In these work were studied the first two phase of imbibition. Results of these experiments show that nanoparticles may able to diffuse into the seeds, no effect on the kinetics of imbibition and change the volume of absorbed water by seeds depending on types of nanoparticles. The results show that when the concentration of salts (NaCl) increases, the intake of water in corn and bean seeds decreases. It has been established that when nanoparticles added to the salty solution the absorption process intensify and increase the quantity of absorbed water by seeds relative to the control. These effect depends on the types of nanoparticles and seeds of the plant.

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