

Phytotoxic activity of the zinc oxyde nanoparticles synthesized from different precursors on germination and radicle growth of seeds *lepidium sativum*

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Abstract- In this study, the zinc oxide prepared from three different precursors: zinc sulfate hepta hydrate, zinc nitrate hexahydrate and zinc acetate dihydrate, has been tested on the germination of *Lepidium sativum* seeds. Radicles growth of selected seeds responded differently to the treatment of various oxides synthesized. The results show a very significant inhibitory effect on the germination and the growth of rootlets observed in the case of ZnO synthesized from zinc sulfate heptahydrate, compared to the control and other oxides prepared from zinc nitrate or acetate zinc. We found that, after a week of incubation in the presence of ZnO synthesized from zinc sulfate, 65% inhibition of seed germination, the length of the radicles *Lepidium sativum* decreased almost 90 to 100% and the inhibitory action of this oxide is irreversible after cessation of treatment.

Index Terms- Zinc oxides, precursors, *Lepidium sativum*, germination, index of inhibition.

I. INTRODUCTION

Zinc oxide "ZnO" is a semiconductor widely used in many fields because of its impressive mechanical, chemical and electrical properties [1-3]. It showed considerable interest in the agri-food sector as a preservative [4], in the wastewater treatment for their recycling [5] and in the field hygiene as a bactericidal agent [6-11] and antifungal agent [12, 13].

Reestablished studies of these oxides in relation to plant and animal eukaryotic cells are very limited. However, their biological responses and behavior following treatment with these nanoparticles may be the source of information and novel therapeutic indications. The study of the antimetabolic activity of these molecules can, for example, give us another opportunity in the therapy of cancer diseases. It may also be useful in agriculture for better storage and storing crops seeds and controlling the growth of weeds, because the use of herbicides can result in serious ecological consequences [14].

The objective of this study is to illustrate the inhibitory effect of zinc oxides synthesized from various precursors, the potential for germination and mitotic activity, and the growth of radicles *Lepidium sativum* seeds, using the phytotest method.

II. MATERIAL AND METHODS

The species used in this work is the garden cress (*Lepidium sativum*). It is an annual plant from the family of Brassicaceae. Seeds purchased commercially, belong to the same batch. The tested seeds are carefully selected by removing any damaged or small seed in order to have a uniform size. Seeds are washed with pure distilled water to remove any impurities and are tested at a rate of greater than 95% germination [15-17].

The preparation of zinc oxide was carried out in collaboration with the team of Materials and Applied Catalysis Laboratory "Chemistry Biology Applied to the Environment" of the University of Science of Meknes. From zinc sulfate heptahydrate, zinc nitrate hexahydrate and zinc acetate dihydrate according to the methods described by Zegaoui and al. [6].

Seeds are sown in Petri dishes of 50 mm diameter, coated by a layer of Whatman filter paper type impregnated with 5 ml of aqueous solution of each oxide at a concentration of 1mg / ml. The control Petri dish is impregnated by 5 ml of distilled water and would be the control test. The hydration is done only once at the beginning of the test. All plates are placed in an incubator at 25 ° C in the dark. The process of germination and Radicles elongation of seeds is observed directly in the Petri dishes, every 24 hours for a week. A seed is considered germinated when the protrusion of the root is obvious [18,19]. Three replica control and three replica of each of the oxides are used in Petri dishes each one containing 20 seeds of average size of (20×3) used for each test. Data are expressed as mean elongation Radicles and the results are reported in mm.

Table I: Notation adopted for the oxides prepared from different precursors [6]:

Sample	Notation	Precursor
ZnO(X)	ZnO(S)	ZnSO ₄ , 7H ₂ O
	ZnO(N)	Zn(NO ₃) ₂ , 6H ₂ O

	ZnO(A)	Zn(CH ₃ COO) ₂ · 2H ₂ O
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III. CALCULATION AND EXPRESSION OF RESULTS

III.1. Determining the capacity of germination of the seeds of *Lepidium sativum*:

The percentage of germination of control seeds was 95%, the inhibition observed in the control group is subtracted from the test groups. Also, the percentage inhibition of germination is expressed on all the tested seeds.

The criterion for germination was evaluated by the opening of the seed over the emergence of a rod 3 mm and information relating to the success of germination is noted [16,20] .

The germination capacity of seeds is determined by calculating the level of seed germination percentage [16, 21]:

$$[\text{Number of germinated seeds} / \text{total number of seeds}] \times 100$$

III.2. Determination of inhibition of germination index (GI):

This method is generally developed to determine the phytotoxicity of the solid residues and contaminated soil. In this work, the synthesized oxides are used for doing this testing. Monitoring of seed germination is determined every 24 hours. The number of seeds germinated was noted and the percentage inhibition of germination is calculated as follows:

$$GI\% = (GP_{co} - GP_{tr} / GP_{co}) \times 100$$

GP_{co}: Germination percentage of the control lot

GP_{tr}: Germination percentage of the lot treated by oxide

III.3. Plantlets Vigor of *Lepidium sativum*

After determining the successful germination for seven days for each of the replica, we proceeded to measure the length of the

radical. This value is expressed as mean elongation Radicles and the results are reported in mm.

Plantlets vigor= Percentage of germination x Plantlets length

III.4. Study of the reversibility of the growth of rootlets *Lepidium sativum*

We also examined whether, at a concentration of 1mg/ml oxides prepared, if we would observe an irreversible toxic phenomenon, causing cell damage by inhibition. Reversibility was verified on radicles grown in the presence of oxides for a time of two days. The seeds are transferred to a medium containing distilled water, for periods of 1, 2, 3 and 4 days.

IV. RESULTS AND DISCUSSION

IV.1. Capacity of germination of *Lepidium sativum*:

The results concerning capacity to seed germination of *Lepidium sativum* treated with different zinc oxide prepared from different precursors (Figure 1) show that all the oxides tested exert an inhibitory effect on germination, by comparing them with the control. The shape of the curves in this graph shows an exponential shape, the speed of germination capacity varies with the type of zinc oxide synthesized. ZnO (S) is more effective on the inhibition of germination compared to ZnO (N) and ZnO (A). On the second day of germination, ZnO prepared from zinc sulfate hexahydrate gives only 30% seed germination, compared to 70% in the control, 55% for Zinc oxide prepared from Zinc acetate dihydrate and 50% seed germination in the case of oxide synthesized from zinc nitrate hexahydrate.

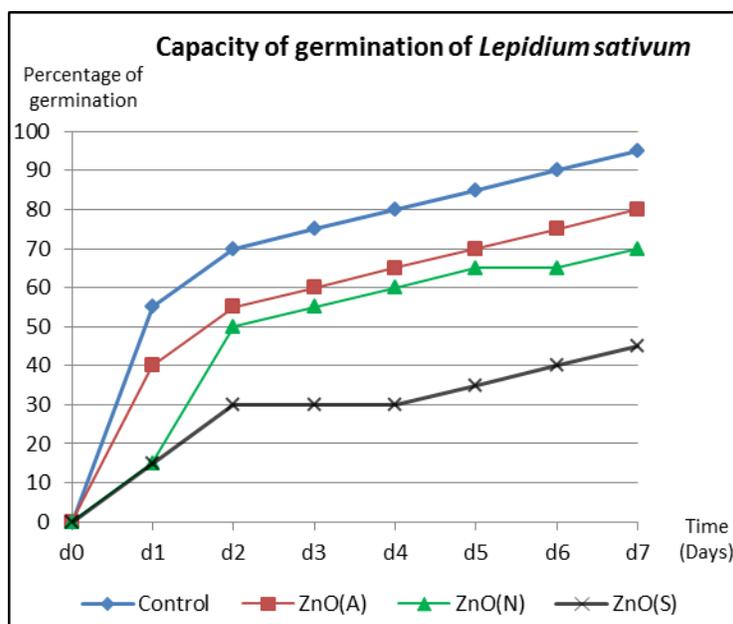


Figure 1: Effect of zinc oxide synthesized from different precursors on the germination of seeds of *Lepidium sativum* versus time. The values are the means of three replicates.

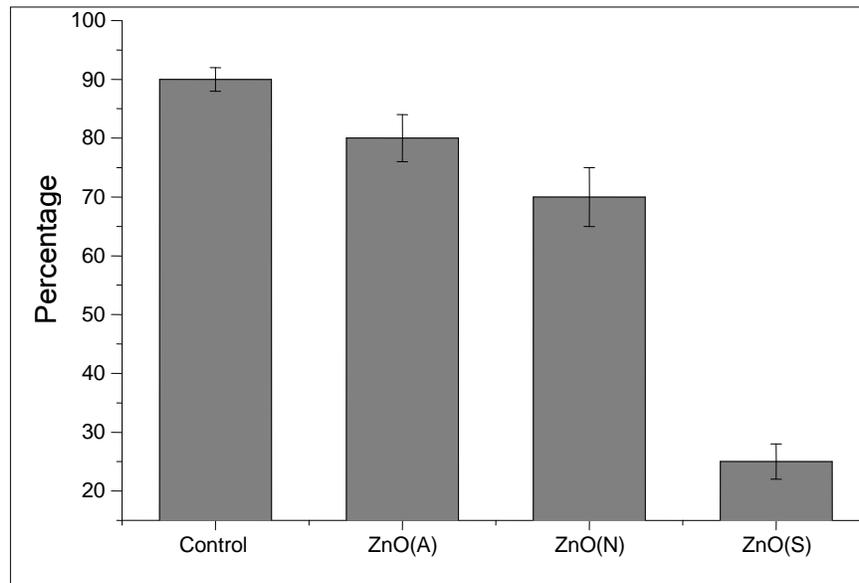


Figure 2: Effect of zinc oxide synthesized from different precursors on rate of germination of *Lepidium sativum* after 7 days of treatment. The values are the means \pm SD for three replicates.

Figure 2 summarizes the ability of seed germination after 7 days of incubation and therefore confirms the inhibitory effect of the three oxides which ZnO(S) is the most effective followed by ZnO(N) and ZnO(A). The germination rate of seeds was tested is 40%, 70% and 80% for ZnO (S), ZnO (N) and ZnO (A) respectively, and is 95% for the control. Comparing the growth inhibiting activity observed in this study, in eukaryotic vegetal cells radicles seeds of *Lepidium sativum*, with the bacterial prokaryotic cells [6], we found a similarity concerning the behavior of the two types of cells. Indeed, ZnO (S) is effective as a bactericide agent on both Gram positive bacteria (*Staphylococcus aureus*) and gram negative (*Escherichia coli*).

ZnO(N) and ZnO (A) have an effect more or less moderate on these bacteria studied [6].

IV.2. Inhibition of Germination Index(GI):

The GI is an indicator parameter of toxicity of the product tested on seed germination. Figure 3 shows the magnitude of inhibition index of the germination of each ZnO studied by expressing it as percentage inhibition of germination. Treatment with ZnO (S) shows a significant inhibitory effect on the germination of *Lepidium sativum*, this inhibition reached 50% after seven days of treatment. For the others oxides such inhibition reached 20% for ZnO (N) and 10% for ZnO (A).

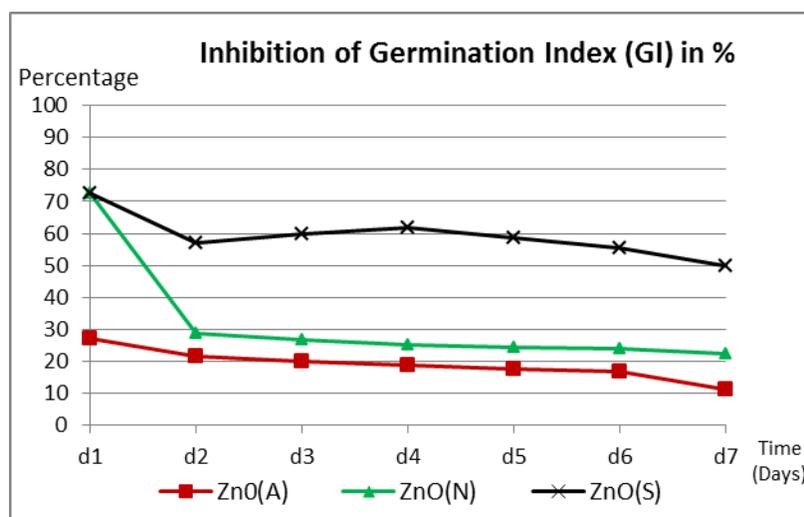


Figure 3: Inhibition Index of seed germination of *Lepidium sativum* in% for three Zinc oxides synthesized from different precursors. The values are the means of three replicates.

IV.3. Plantlets Vigor of *Lepidium sativum*

The plantlets vigor of *Lepidium sativum* informs us about the mitotic capacity of radicles, the greater their length, the more speed of growth and multiplication of cells are increased and vice

versa. Figure 4 shows that the length of the radicle *Lepidium sativum* after 7 days incubation is decreased nearly half in the presence of ZnO (A), 75% for ZnO (N) and 90 to 100% for the ZnO (S) compared to the control.

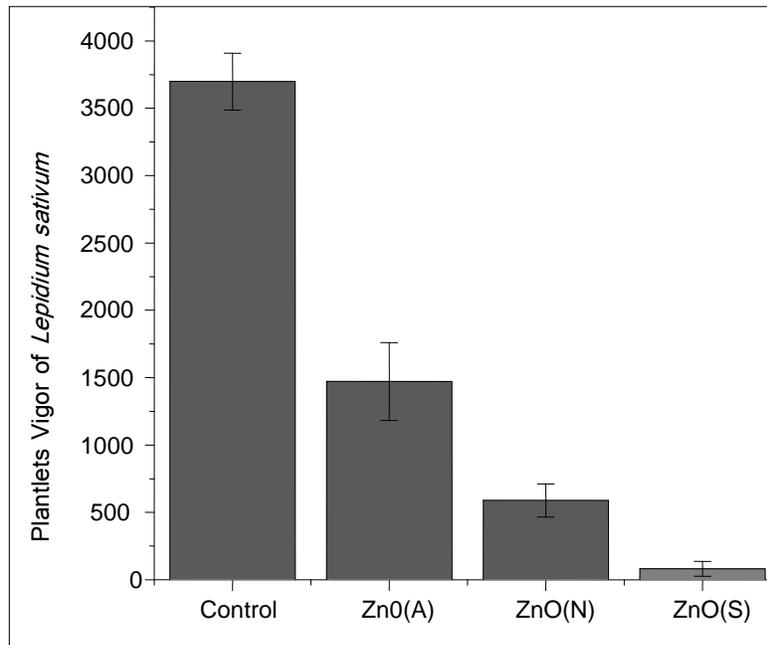


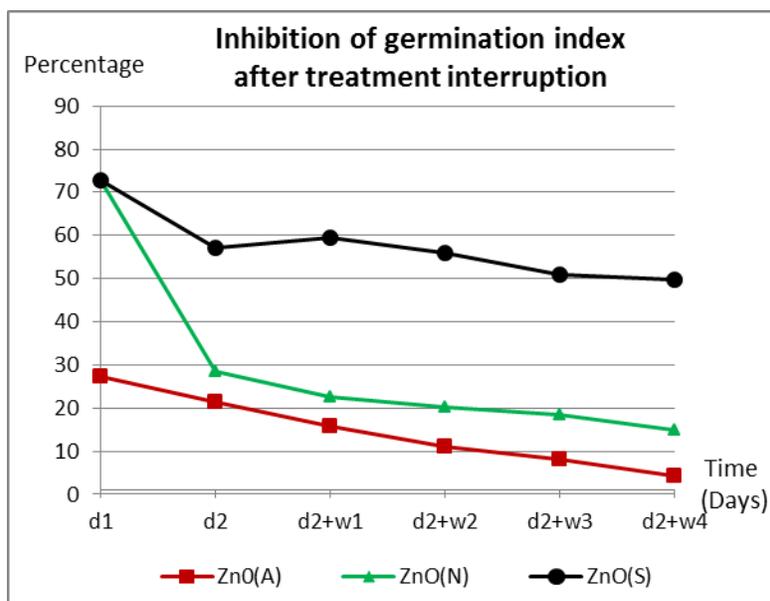
Figure 4: Effect of zinc oxide synthesized from different Precursors against the Plantlets Vigor of *Lepidium sativum*. The values are the means ± SD of three replicates.

IV.4. Reversibility of the growth of rootlets *Lepidium sativum*

The interest provided by this test consists in verifying the durability of the growth inhibitory activity or antimitotic ability of the product studied. The results that emanated may be of considerable importance in the medical and health sector. Indeed, the treatment of multi resistant organisms and invasive cancer cells is the biggest disappointment present in public health.

In this study, the use of zinc oxide as inhibitor of cell growth, and test the reversibility of growth after treatment, allowed us to classify them according to their effectiveness. We found that rehydration of the seeds tested after two days of

incubation shows that the length of the radicles of *Lepidium sativum* has not changed significantly in the case of ZnO (S); the recovery rate of germination is null after two days of hydration and less than 10% after four days of rehydration, figure5. However, the rootlets have continued to grow in a more or less moderate rate in the case of ZnO (N) and more pronounced in the case of ZnO (A). We conclude that the use of zinc oxide synthesized from zinc sulfate heptahydrate definitely inhibits the growth of eukaryotic plant cells as well as those bacterial prokaryotic [6].



w: Distilled water

d1: seeds treated for 1 day

d2: seeds treated for 2 day

d2+w1: Seeds treated for 2 day, then transferred into distilled water for 1 day

d2+w2: Seeds treated for 2 day, then transferred into distilled water for 2 day

d2+w3: Seeds treated for 2 day, then transferred into distilled water for 3 day

d2+w4: Seeds treated for 2 day, then transferred into distilled water for 4 day

Figure 5: Effect of interruption of treatment with zinc oxide synthesized from different precursors on the growth of radicles of *Lepidium sativum*. The values are the means of three replicates.

This particular activity of ZnO (S), compared to the other above-mentioned oxides can be explained by the shape and size of crystalline particles of each. Crystallographic studies and electron microscopy [6] showed that ZnO (S) is formed of heavily agglomerated nanoparticles are in the form of clusters having an average size greater than 100 nm while the nanoparticles of ZnO(A) have a hexagonal crystal form and an mean size of about 40 nm. The ZnO (N) is present in the form of elongated particles with a size between 22 and 85nm. According to the literature, the mechanism of action on living cells involves the hydroxyl radical; regeneration of H₂O₂ molecules appear to be toxic to cells [22-24]. The hydroxide radical is more abundant in the ZnO (S) crystal form [6], it is more likely that these radicals involved in the inhibitory efficiency more marked on the vital activity of prokaryotic and eukaryotic cells.

V. CONCLUSION

The effect of the oxides on the seeds of *Lepidium sativum* varies as a function of the precursor in which they are synthesized. The overall results obtained in this work allowed us to conclude that zinc oxide has an inhibitory effect on the germination and growth of seeds of *Lepidium sativum*. The sprouting inhibitory activity is very significant and irreversible in the presence of zinc oxide synthesized from zinc sulfate heptahydrate.

ZnO (S) appears to be effective in the inhibition of mitotic activity of cells in roots division. This irreversible antimitotic

power, needs to be tested on animal cells that are mitotically active such as cancer cells generating malignant tumors in order to operate in medicine to eradicate their invasion and growth.

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