# The Beneficial Effect of The Yeast Enriched with Nano Zinc Particles on Mineral Status of Grape Seedlings

**Rasha, S. Abdel-Hak[1], Shimaa, R. Hamed[2], Saleh, M.M.S.[1], Merwad, M.A.[1]**

[1]*Pomology Dept., Biological and Agricultural Research Institute, National Research Centre El-Buhouth St., Dokki, Cairo, Egypt*

[2]*Microbial Biotechnology Dept., Biotechnology Research Institute, National Research Centre El-Buhouth St., Dokki, Cairo, Egypt*

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**Abstract:** This investigation included two integrated experiments, the first one is the microbiological experiment that carried out in Microbial Biotechnology Department, NRC, Cairo, Egypt, where multiple incorporation strategies were used for production zinc oxide Nanoparticles by enrichment of yeast with zinc including; (1): In the first procedure, zinc was added to the liquid medium just after the yeast was inoculated (growth phase), (2): For integration, it was added after 24 h of incubation (non-growth phase) in the second procedure. The obtained results showed that the first method (growth phase) was adopted in order to reduce the chance of medium contamination. Zinc sulphate has no passive effect on yeast cell biomass. The second experiment is horticultural one which was done in National Research Centre greenhouse on Flame seedless grape seedlings which included five treatments as follows: control (spraying with water only), frozen yeast enriched with zinc (as foliar spray), active yeast enriched with zinc (as foliar spray), frozen yeast enriched with zinc (as soil application) and active yeast enriched with zinc (as soil application). When zinc content in the grape leaves as ppm was examine after 15 days, the results showed that active fresh yeast enriched with zinc that treated as foliar spray recorded the highest rate of zinc in grape leaves (180 ppm), followed by active fresh yeast enriched with zinc as soil application which recorded 170.8 ppm. On the other hand, the untreated plants (control) showed the lowest value of zinc in the leaves (91.6 ppm) when compared with the other treatments.

**Keywords:** Grape, Yeast, ZnO NPs, Bio-fertilizer, foliar spray, soil application

## I. Introduction

Grape (*Vitis vinifera* L.*)* is one of the most significant commercial fruit crops grown in temperate and tropical areas of the world. It is primarily consumed as fresh fruit and ranks as Egypt's second-largest fruit crop. The farmed area has developed fast in the last two decades due to its strong net return, reaching 172.533 feddans that producing 1586342 tones **(FAO, 2020).**

The ground water is impacted by excessive fertilizer use, which also causes eutrophication in aquatic habitats. Recently more natural, risk-free, and affordable additives have received a lot of attention. Yeast has become a popular issue in academia as it is harmless, nourishing, and easy to use. It is a type of fresh yeast with high biological activity or brewer's yeast-based soluble paste or powder.

Low-molecular-weight organic matter, amino acids, nucleotides, peptides, nitrogen, phosphorus, and trace elements are just a few of the powerful components found in yeast extract. Moreover, it is free of chemically synthesized hormones and toxic ingredients (**Vieira *et al.*, 2016 and Xi *et al.*, 2019).** Yeast extract could improve the yield and quality of needle mushrooms **(Abo EL-Fadl *et al.*, 2017).** The application of yeast significantly increased the vegetative growth, yield, and quality of vegetables **(El-Tohamy *et al*., 2008; Fawzy, 2010; Ahmed *et al.*, 2011; Shehata *et al.*, 2012; Shafeek *et al.*, 2015)** and also led to an increase in elemental content, such as N, P, K, Fe, and Zn, in vegetables **(Dawood *et al.*, 2013).**

Grape production and quality are influenced by a number of variables, such as the climate, management required to the vine yard, nutrient intake, irrigation, etc. Crop productivity can be increased by integrated nutrient management. Particularly the role of secondary and micronutrients which are very important for vine growth and productivity.

Zinc (Zn) is an important component for plants because of its role in a variety of vital cellular processes, such as ion homeostasis, enzyme activation, and metabolic and physiological procedures **(Yang *et al.*, 2020; Alsafran *et al.*, 2022).** Zn is a crucial nutrient for plants that is involved in numerous bio-physicochemical processes **(Noman *et al.*, 2019; Zaheer *et al.*, 2022).** Zinc, which is essential to the activity of more than 300 enzymes from all six enzyme classes, is present in all of them. Only this nutrient is found in all six enzyme groups (lyases, transferases, hydrolases, isomerases, oxidoreductases, and ligases). Zn impacts the activity, structural integrity, and folding of different proteins as a fundamental or catalytic enzyme **(Castillo-González *et al.*, 2018; Zaheer *et al.*, 2020b)**. In addition to its critical role in ribosome structural integrity, Zn plays a variety of other important bio-physicochemical activities in plants, including gene regulation and activation, protein synthesis, glucose metabolism, and morphological and anatomical participation in bio-membranes **(Hafeez *et al.*, 2013; Zaheer *et al.*, 2020a)** .This element is necessary for seed growth and boosts cytochrome synthesis **(Karthika *et al.*, 2018; Alatawi *et al.*, 2022)**. Moreover, Zn is crucial for a variety of physiological processes in plants, including hormone regulation (such as the synthesis of tryptophan, a precursor to indole acetic acid **(Bhantana *et al.*, 2021)**. According to **Gupta *et al.* (2016),** among the physiological reactions that depend on Zn availability in plants are the control of auxin, recovery of photosystem II, stabilization of $CO_2$ quantity in the mesophyll, and other processes. In order to ensure appropriate plant function, it is important for plants to effectively absorb, transport, and distribute Zn in their tissues, cells, and intracellular sites **(Zlobin, 2021).**

Despite its value, Zn deficiency interferes with plant metabolism's fundamental processes, resulting in growth slowdown and leaf chlorosis, which can reduce nutrient uptake and eventually lead to Zn deficit in the human diet **(Li *et al.*, 2013)**. Nevertheless, Zn toxicity inhibits the absorption of essential elements, promotes the production of reactive oxygen species, and leads to heavy metal toxicity **(Zhang *et al.*, 2020).**

The expression "nanoparticle," which describes a particle with a diameter of less than 100 nm, is the cornerstone of nanotechnology. A somewhat greater surface area to volume ratio, which increases their level of interactivity and physicochemical flexibility, is the primary characteristic that sets nanoparticles apart from other materials **(Mauter *et al.*, 2018).** Nanotechnology is employed as nano-fertilizers in agriculture because of their special characteristics (highly porous ratio, controlled-release kinetics at strategic location). To control and limit the delivery of one or more nutrients and meet the essential nutritional needs of a plant, nano-fertilizers may have been encapsulated or bound with nanomaterial **(Saleem *et al.*, 2020a; Ahmed *et al.*, 2021).** Zinc oxide (ZnO) nanoparticles, among the several forms of nanoparticles, were shown to significantly increase plant growth and productivity. Zn was a critical nutrient for regulating essential plant activities that was made available by the ZnO nanoparticles **(Saleem *et al.*, 2020b; Liu *et al.*, 2022).** Due to their nano size and high surface area size ratio, Zn nanoparticles can be applied topically or ingested by plants. Both application methods (foliar and soil) efficiently transport the element **(Czyzowska and Barbasz, 2022).** According to recent research **(Subbaiah *et al.*, 2016),** spraying 25 nm ZnO nanoparticles on maize leaves had a positive effect on plant growth, yield, and Zn content in the grain. Surprisingly, plants were sprayed with 100 ppm of ZnO nanoparticles, and 36 ppm of Zn was discovered in the grains of those plants. ZnO nanoparticles can therefore be employed as a productive nano-fertilizer to increase agricultural productivity, facilitate plants' uptake of micronutrients, and support plant growth and development.

It has been developed for the biogenesis production of metal/metal oxide NPs to use a variety of biotic resources, including algae, fungi, bacteria, and plant extracts, etc. Among all the available eco-friendly approaches for M/MO NPs manufacturing, the use of plant extract is a relatively simple and uncomplicated way to create NPs in large quantities when compared to bacteria and yeast assisted techniques. As effective resources for the production of M/MO NPs, many kinds of natural bio-extracts (i.e., bio-components like plants, fungus, bacteria, and yeast) have been used. For the synthesis of regulated M/MO NPs, bio-extract of plant has been found to have the highest efficacy as stabilizing, capping, and reducing agents. Techniques for biologic synthesis are highly helpful for controlling all aspects, including shapes, sizes, structures, and other unique qualities **(Chouke *et al.*, 2022).**

The aim of this study is to investigate the effect of yeast enriched with zinc as Nano particles either as foliar or soil applications on the behavior of grape seedlings.

## II. Material and Methods

### 2.1. The microbiological experiment

### 2.1.1 Microbial sample

The Egyptian sample of *Saccharomyces cerevisiae* was used in this study which was isolated from the Egyptian soil (local isolate), purified and identified in microbial biotechnology laboratory, National Research Centre, Egypt. Using local isolate was preferred from our research team to be appropriate with environmental conditions in Egypt. Yeast extract, peptone, and glucose were used as a growth medium (YEPD).

### 2.1.2 Synthesis of ZnO NPS

Synthesis of Zn NPs by the enrichment of yeast with zinc, multiple incorporation strategies were used in this study including; (1): In the first procedure, zinc sulphate was added to the liquid medium just after the yeast was inoculated (growth phase). (2): For integration, it was added after 24 h of incubation (non-growth phase) in the second procedure **(Ponce *et al.*, 2002).**

### 2.1.3 ZnO NPs characterization

**Visual characterization**

Visual change in the reaction mixture color from white to yellowish was the preliminary characterization of the biosynthesis of ZnO NPs.

### UV–visible spectroscopy

The colloidal solution of the biosynthesized ZnO NPs was monitored by using a beam UV–visible spectrophotometer (Carry 100 Ultraviolet–visible spectrophotometer, Agilent, USA) at a resolution of 1 nm in a wavelength range between 300 and 700 nm to determine their surfaceplasmon resonance peaks.

### HRTEM

The morphology, size, and the selected area electron diffraction pattern (SAED) of the biosynthesized ZnO NPs were investigated using HRTEM (JEOL JEM-1200, Japan) with an operating voltage 200 keV. A drop of colloidal ZnO NPs was applied on a carbon-coated copper grid and dried in air before being investigated by HRTEM.

### 2.2. Horticultural experiment

Experiment was done in National Research Centre greenhouse on Flame seedless grape seedlings. The experiment was established in a completely randomized design with five replicates. Each seedling was considered as an experimental unit, which included five treatments as follows:

1- Control (spraying with water only).

2- Frozen yeast enriched with zinc (as foliar spray).

3- Active yeast enriched with zinc (as foliar spray).

4- Frozen yeast enriched with zinc (as soil application).

5- Active yeast enriched with zinc (as soil application).

Treatments were added on 15/4/2020. The rate of yeast was 0.6 cm$^3$ alleviated to 100cm$^3$. The concentration of zinc in the yeast extract was 88 in active yeast and 107.6 in the frozen yeast. Samples of grape leaves were taken to analysis after 15 days of addition (sample 1) and after 30 days of addition (sample2).

## III. Results

### 1. Microbial experiment

### Synthesis and characterization of ZnO NPs

ZnO NPs powder had a fluffy appearance with white to yellowish color. Transmission electron microscope images of the as-prepared ZnO NPs as shown in Fig. 1. The as-prepared sample (Fig. 4a) exhibited relatively small spherical particles (15–30 nm) which were coagulated in large clusters.

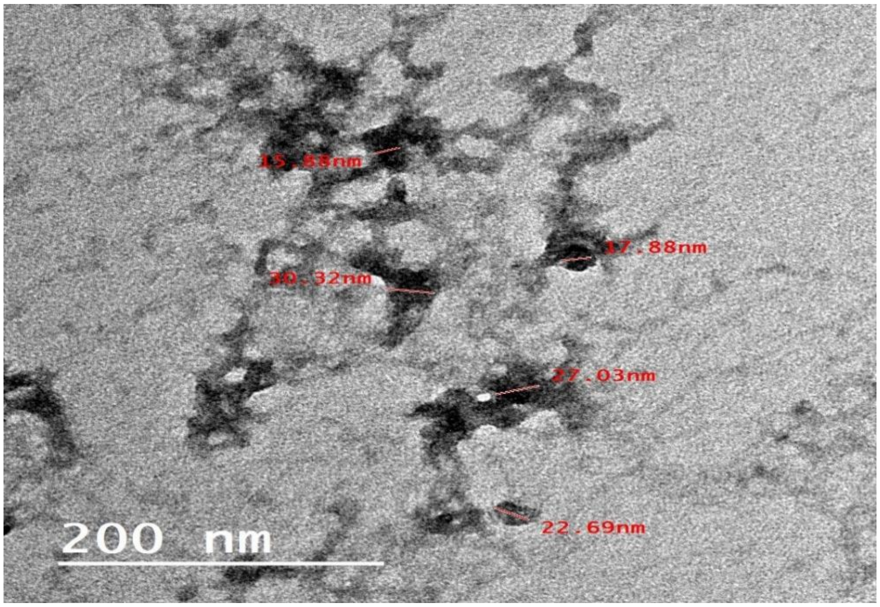

Figure 1. SEM image of ZnO NPs synthesized by green technology

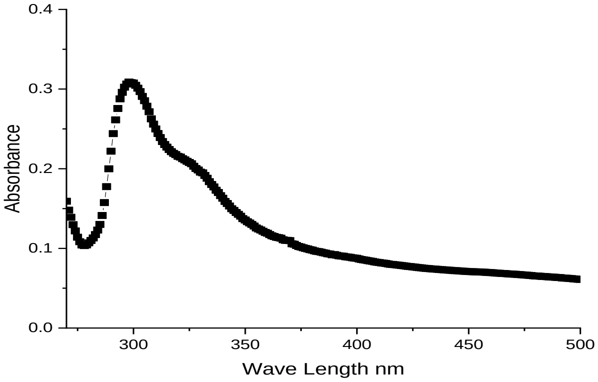

Figure 2. UV image of ZnO NPs synthesized by green technology

The spectrum of ZnO NP is characterized by a strong absorption in an UV band extending considerably into the UV-range, with a local absorption peak at 300-325 nm. Peak absorbance of sample was measured to be 0.34 (Fig. 2).

## 2. Horticultural experiment

This experiment displayed two types of yeast extract enriched with Zn which applied as foliar or soil application. The first type is the active fresh yeast form, and the second is the frozen form of the yeast. However, it is clear from the results in table (1) and figure (3) when the zinc content in the grape leaves as ppm was examine after 15 days after application (sample 1), that active fresh yeast enriched with zinc that treated as foliar spray recorded the highest rate of zinc in grape leaves (180ppm), followed by active fresh yeast enriched with zinc as soil application which recorded 170.8 ppm. Meanwhile, the frozen form of yeast enriched with zinc that sprayed on the leaves was ranked the third by recording 159.3ppm, followed by the frozen form of the yeast that added as soil application (120.0ppm). On the other hand, the untreated plants (control) showed the lowest value of zinc in the leaves (91.6 ppm) when compared with the other treatments.

Table (1): Zinc concentration on grape leaves as affected by application of yeast enriched with zinc after 15 days (sample 1):

| **Treatments** | **Zn ppm** |
|---|---|
| 1. Control (applied with water only). | 91.6 |
| 2. Frozen yeast enriched with zinc (foliar spraying.). | 159.3 |
| 3. Active yeast enriched with zinc (foliar spraying.). | 180.0 |
| 4. Frozen yeast enriched with zinc (soil application.). | 120.6 |
| 5. Active yeast enriched with zinc (soil application.). | 170.8 |

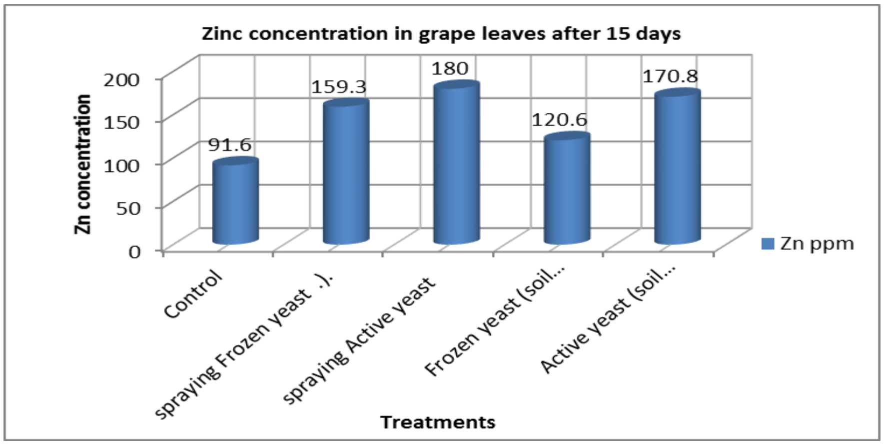

Figure 3. Zinc concentration in grape leaves after 15 days.

The results presented in table (2) and figure (4)show that zinc content as ppm in the grape leaves after 30 days of application (sample 2), since the control treatment gave the highest values of Zn (90ppm), followed closely by the foliar application with frozen yeast (82ppm). Meanwhile, the lowest values of zinc content in the leaves (50, 53ppm) were obtained respectively from the active yeast enriched with zinc as foliar spray and soil application, respectively, follows in an ascending order by the soil application with the frozen yeast (65ppm).

Table 2: Effect of zinc concentration on grape leaves after 30 days (sample 2):

| Treatments | Zn ppm |
|---|---|
| 1. Control (spraying with water only). | 90 |
| 2. Frozen yeast enriched with zinc (foliar spraying). | 82 |
| 3. Active yeast enriched with zinc (foliar spraying). | 50 |
| 4. Frozen yeast enriched with zinc (soil application). | 65 |
| 5. Active yeast enriched with zinc (soil application). | 53 |

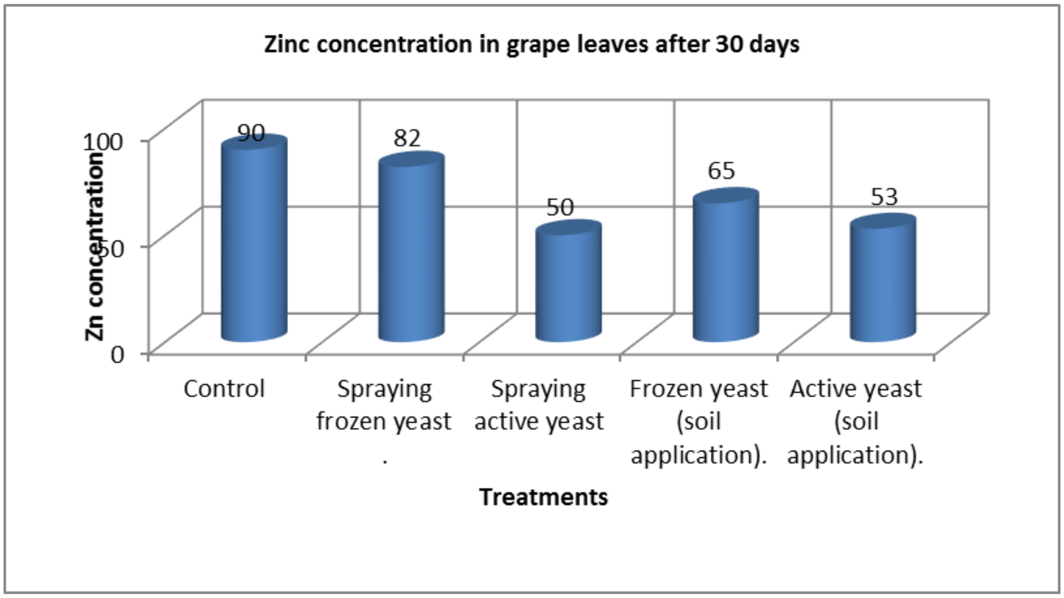

Figure 4. Zinc concentration in grape leaves after 30 days.

## IV. Discussion

The ability to create highly ionic metal oxide Nano particulates of any size and form, one of the most recent developments in nanotechnology, may help establish new approaches for the creation of nano bio-fertilizers. Regarding the microbiological experiment, because of their capacity to integrate metals into their cells and their high protein concentration, yeasts have been utilized as a delivery system for mineral supplements. The ability of yeast to collect metal ions from aqueous solutions through various physicochemical interactions is well recognized **(Mowll and Gadd, 1983).**

According to the obtained results, zinc sulphate has not any passive effect on yeast cell biomass, however, **According to Anil *et al.* (2011),** a rise in the medium's zinc concentration has an impact on the proliferation of yeast cells. For the two distinct enrichment techniques used, the absorbance decreased with the change in Zn concentration by 10% and 21%, respectively. The amount of zinc given to the medium determines how much yeast growth is slowed down. This is most likely caused by less effective cell division. Earlier studies using yeast revealed that some metals, like copper, caused yeast cells to proliferate quickly for 13–20 hours while oxygen use outpaced oxygen supply **(Guo *et al.*, 2022).** Thus, it may be concluded that the growth of yeast in the presence of trace elements depends mainly on the composition of the medium, which helps in continuous supply for the yeast cells with oxygen and increases the growth and the ability of the yeast to incorporate the trace elements from the medium in high concentrations.

Regarding the field experiment, it is clear from the obtained results that the highest effect for all treatments either as foliar or soil application, also active or frozen yeast compared with the control was clearly observed after 15 days of application, which considered as higher active period for transforming zinc nutrient in the plant metabolism compared to 30 days after application. This may explain by the high concentrations of zinc in the leaves after 15 days of application than after 30 days. So, the level of zinc in the leaves was decreased in all treatments after 30 days comparing with the untreated plants.

On the other hand, it is clear from the obtained results that the yeast in active fresh form is more effective than the frozen one either as foliar or soil application, especially when examine after 15 days of application, which considered more effective period after application and the main period to analysis the zinc in the leave comparing with 30 days after application. In this concern, **Shimaa *et al.* (2023)** reported that active fresh yeast extract enriched with Zn at 20 $cm^3/l$ as spraying gave the best results in respect to leaf mineral content of Zn of grape during two seasons.

Via the process of assisted diffusion, which is mediated by membrane potential and transporters, plants predominantly take Zn from soil through their roots **(Marschner, 2012)**. Zn is either stored or transferred to the plant's higher parts (leaves and stems) after being absorbed by the plant roots. Roots contain the majority of zinc, whereas just little amount travels to the leaves and stems **(Sofo *et al.*, 2018; Molnár *et al.*, 2020)**. Zn is mostly transported long distances (from the root to the shoot) in the xylem using the transpiration stream **(Page and Feller, 2015)**. Zn can move through the xylem as a compound or a free cation, although $Zn^{2+}$ ion transport is encouraged by the xylem sap's acidic pH (5.5) **(Alves *et al.*, 2004).** The $Zn^{2+}$ ions pass through the boundary of the casparian strip in the root endodermis and then penetrate symplastically in living cells of the pericycle and xylem parenchyma, bordering the xylem. $Zn^{2+}$ is therefore transported actively from the xylem cells to the apoplastic xylem **(Alves *et al.*, 2004).** Zn is transferred through short- and long-distance pathways from the phloem to various plant components and emerging sinks **(Moreira *et al.*, 2018).** Additionally, Zn transport in phloem is comparatively higher than in xylem due to the higher amount of chelating agents such organic acids in phloem sap **(Verma *et al.*, 2021).** Zn is thought to be transported through phloem tissues as Zn-complexes or in ionic form. A variety of cation diffusion facilitators help Zn get to the vacuole.

Non-glandular trichomes (NGTs) and the general cuticular area (i.e., leaf portions covered by the cuticle without stomata and trichomes) are the two places where Zn is absorbed **(Li *et al.*, 2019).** However, after its absorption, Zn's subsequent mobility (i.e., translocation) is thought to be restricted in sunflower and other plant species **(Zhang and Brown, 1999; Du *et al*., 2015; Li *et al*., 2017).** It is yet unknown how Zn moves throughout the leaf once it has been absorbed, as well as the variables that control its mobility. The proper application of zinc foliar fertilizers and the creation of new, more effective Zn foliar fertilizers are hampered by this lack of understanding The movement of zinc from the apoplast of leaf epidermal cells to the vascular tissues of the leaf (phloem and xylem), long-distance translocation within the vascular tissues, and unloading from the vascular tissues and translocation into the target tissues are the three theoretical steps involved in the translocation of foliarly absorbed zinc. It has been suggested that the weak binding of Zn within the leaf epidermal cell wall **(Du *et al*., 2015),** low mobility within the phloem **(Wu *et al*., 2010; Xue *et al*., 2015),** the use of various Zn fertilizer types with varying properties **(Doolette *et al*., 2018),** or variations in the phenological stages of the plant that affect the translocation of the element **(Fernández and Brown, 2013)**. Also, it has been noted that a zinc deficit encourages the transfer of zinc from old to young leaves **(Xie *et al*., 2019).**

## V. Conclusion

From the abovementioned results, the microbiological experiment shows that the results of multiple incorporation strategies that used for enrichment of yeast with zinc indicated that, the first method (growth phase) was adopted in order to reduce the chance of medium contamination. The concentration of zinc in yeast cells was raised as the concentration of zinc sulphate increased in the medium. With 0.4 g/l zinc concentration in the medium, zinc incorporation in yeast cells was 81.25% higher than with 0.0195 g/l zinc concentration in the medium. Zinc sulphate has no passive effect on yeast cell biomass, however the OD of yeast cell biomass was increased due to zinc concentration in the medium. Regarding the horticultural experiment, it could be deduced that spraying active fresh yeast enriched with Zn followed by active fresh yeast enriched with zinc as soil application were the most effective treatments for improving the rate of zinc in grape leaves. Active fresh yeast especially when used as foliar application decreased the utilization rate of zinc fertilizer to obtain a good production, consequently reduced the pollution resulted from applying the chemical fertilizers.

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