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Assessment of non-adapted, micronutrients loaded adapted-*Saccharomyces cerevisiae* and their culture filtrates as bio-fertilizers for increasing Faba bean growth and productivity.

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ABSTRACT

The effect of Cu^{2+} on the growth and biosorption properties of non-adapted (parental) and Cu^{2+}-adapted *S. cerevisiae* was studied. The level of Cu^{2+} uptake and cells growth was dependent on initial Cu^{2+} concentration. The 50% growth inhibition of non-adapted *S. cerevisiae* was at 82 ppm of Cu^{2+} , while the 50% growth inhibition of Cu^{2+}- adapted *S. cerevisiae* was at 136.7of Cu^{2+} .The results indicated that Cu^{2+}- adapted *S. cerevisiae* was able to grow in the presence of 246 ppm of Cu^{2+} amended glucose-peptone liquid medium. No growth was observed when non-adapted strain grown on 191.33 to 246 ppm of Cu^{2+} amended medium. On the other hand, Cu^{2+}- adapted *S. cerevisiae* significantly tolerated elevated levels of Fe^{3+}, Mn^{2+}, and Zn^{2+} than non-adapted one. It is to be noted that the Cu^{2+}- adapted *S. cerevisiae* can able to uptake different considerable proportion of Fe^{3+}, Mn^{2+}, and Zn^{2+} in addition to Cu^{2+} when was cultured in their presence. The response of yield and its components of three Faba bean cultivars were studied after spraying by micronutrients loaded, non-loaded *S. cerevisiae* suspensions or their culture filtrates. The results indicated that there were significant differences between Faba bean varieties in yield and its components (except, number of seeds/ pod, seed yield/ plant and protein percentage. Foliar application with culture filtrate of Cu^{2+}-adapted *S. cerevisiae* (FT) containing Cu^{2+},Fe^{3+},$Mn^{2+}$and Zn^{2+} to Faba bean plants was the most favorable treatment to increase yield and its components compared with control treatment. With respect to the interaction between Faba bean varieties and the different treatments of micronutrients loaded , non-loaded yeasts or their culture filtrates , the effect on yield and its components was significant (except, plant height, number of branches/plant, number of seeds/pod, seed yield/plant and seed yield/feddan).

Keywords: *Saccharomyces cerevisiae*, metal ions uptake, culture filtrates, biofertilizer, Faba bean.

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INTRODUCTION

Yeasts are an inexpensive, readily available source of biomass. Yeast cells are capable of accumulation of abroad rang of heavy metals. Furthermore, they retain their ability to accumulate heavy metals under a wide range of external conditions. Yeast cells have been shown to accumulate heavy metal ions, such as Cu^{+2}, Co^{+2}, Cd^{+2}, Ni^{+2}, Mn^{+2}, Zn^{+2}, Pb^{+2}, Ag^{+2} and Fe^{+3}[1-4]. *Saccharomyces cerevisiae* is an excellent model microbe in biological research, and has been used for metal accumulation [5-8]. The mechanisms of heavy metal ions biosorption may be involve in many processes, such as sorption by transport, biosorption to cell walls and entrapment in extracellular capsules, precipitation, and oxidation-reduction reactions [9-12]. More important for metal biosorption, the cell surface is vital in controlling bio-interfacial interactions such as molecular recognitions, cell adhesion and flocculation [13]. The fungal cell walls are rigid and provide structural support and shape, mainly composed of 80-90% polysaccharide, with proteins, lipids, polyphosphates and inorganic ions, making up the wall-cementing matrix. The cell walls contain periplasmic enzymes and limit the size and type of substances entering into cells due to their porosity [13]. Yeasts synthesize antimicrobial and other useful substances required for plant growth from amino acids and sugars secreted by bacteria, organic matter and plant roots [14]. *Saccharomyces cerevisiae* is considered a new promising plant growth promoting yeast for different crops. It became in the last few decade a positive alternative to chemical fertilizers safely used for human, animal and environment [15]. Yeast as a natural stimulator is also, characterized by its richness in proteins 4%, carbohydrates 33%, nucleic acids 8%, lipids 4% and minerals 8% such as Na, Fe, Mg, K,P,S, Zn, Mn, Cu, Ni, Si, Cr, Va and Li in addition to thiamin, riboflavin, pyridoxine, hormones and other growth regulating substances, biotin, B12 and folic acid [16]. Plant derived foods provide an important source of proteins and dietary minerals. This is especially true in developing countries where plant foods are a predominant portion of the diet. Hence, there is a need to improve mineral concentrations of seed crops such as rice, wheat, maize, as well as common bean and other legumes. Biofortification is a recent approach aimed at increasing the bioavailable nutrients, such as Fe and Zn, in these staple crops [17] rather than using fortificants or supplements. During the last decades, foliar feeding of nutrients has become an established procedure to increase yield and improve the quality of crop product**s** [18]**.** Also, in previous studies [19-21] have shown that the application of essential micronutrients such as Zink, Iron and Magnesium could improve the yield and yield components of crops. Foliar fertilization of nutrients due to the quick translocation of these nutrients to different parts of the plant is better method than the soil application [22]. Meanwhile role of micronutrients should not be ignored. Iron, zinc and manganese have several important roles in the plant, including protein synthesis, photosynthesis, chlorophyll synthesis, carbohydrate transport and metabolism, growth hormones regulation (auxin) pollen and flower formation [23]; functioning as an activator or cofactor of at least 35 enzymes.

Microbial adaptation is defined as the ability of a microbial population to adjust itself to a changing environment. Fungi isolated from metal-contaminated needles of pine saplings situated near a smelter emitting cadmium, lead and zinc exhibited greater tolerance to these metals than isolated from no contaminated plants [24]. Microbial tolerance to heavy metals could be developed in vitro by experimental manipulation [25-27]. Faba bean (*Vicia faba* L.) is an important winter legume crops and a good source of protein for human and animal feeding.

Varietal differences in yield and yield components of Faba bean crop may assist plant breeders to select for most promising combiners in their breeding programs. Many investigators had reported high variability among Faba bean varieties for yield and its components [28-36].

The purpose of the present study was to investigate the possible use of non- adapted and metal ions loaded adapted *Saccharomyces cerevisiae* and their culture filtrates to improve Faba bean crop.

MATERIAL AND METHODS

Organism and culture conditions

Saccharomyces cerevisiae was obtained from Egyptian Sugar and Integrated Industries Company (ESIIC), Hawamdia, Giza, Egypt. The organism was maintained on YPG solid medium and sub cultured every one week.

Media

YPG medium: contained (g/l) glucose, 20; peptone 10; yeast extract, 3 with or without agar, 20. Glucose-peptone medium [(Its low metal binding affinity [37]: contained (g/l) glucose, 20; peptone, 10, with or without agar, 20.

Metals

Analar-grade metal salts obtained from Merck or BDH were used throughout this work. These salts are: $CuCl_2$, $ZnCl_2.2H_2O$, $MnCl_2.4H_2O$ and $FeCl_3$.

Training of *Saccharomyces cerevisiae* to copper (II)

Saccharomyces cerevisiae was trained on peptone glucose liquid medium amended with a copper concentration (as $CuCl_2$)that inhibit the growth by approximately50% to tolerate high concentration of Cu^{2+}.For this purpose *S. cerevisiae* was exposed to copper (II) for growing in or to establish the copper (II) sensitivity of yeast. Achievement of yeast culture tolerant to $Cu^{2+}$was obtained during serial subcultures in glucose-peptone liquid medium amended with a copper concentration that inhibit yeast growth by approximately 50% for 7 days (7 transfers). The adaptation process was also carried out at 28°C with shaking at 150 rpm in 250 ml flasks containing 45 ml of copper amended medium. The copper tolerant isolate was maintained on glucose peptone solid medium containing 10 ppm of Cu^{2+} and stored at 4°C.

Inoculums

Non-adapted (parental) and copper-adapted *S. cerevisiae* were grown in flasks (250 ml) each contained 45ml of glucose peptone liquid medium unamended or amended with 10 ppm of Cu^{2+}. The cultures were incubated shaken (150 rpm) at 28°C for 24h.

Metal ions toxicity in non-adapted (parental) and copper-adapted *S. cerevisiae*

Five ml of inoculums were added to each flask (250ml) contained 45ml of glucose peptone liquid medium amended with graded concentrations of Cu^{2+}, Zn^{2+}, Mn^{2+} or Fe^{3+}. The cultures were incubated shaken (150 rpm) at 28°C for 24h. The growth was determined by using spectrophotometer (Agilent UV/VIS Cary-100) at OD_{600}.The cells were harvested by centrifugation (3000 rpm for 10 min.) and dried at 80°C, the supernatants were analyzed for metal contents. Amount of metal taken up by the cells was calculated by difference in initial and final concentration in the solution. The metal ion sorption (%) was calculated as follows:

Unsorbed (residual) metal %= Ax100/B
Sorbed metal%= 100- Ax100/B

A=Metal ion concentration in supernatant (the post-contact solution)
B= Initial metal ion concentration (control).

Analytical methods

Cu, Zn, Mn, and Fe were determined by atomic absorption spectrophotometer FS240 Agilen.

Elemental analysis

Carbon, hydrogen, sulphur, and nitrogen in all samples of yeast were determined by using Vario El Elementar.

Field experiments

Two field experiments were carried out; at new land in the Researches and Production Station of the National Research Centre (NRC) at Al Nubaria district, El-Behaira Governorate, Egypt during winter seasons of 2014/2015 and 2015/2016.The experiments were carried out to study the effect of foliar spray by micronutrients loaded, non-loaded *S. cerevisiae* suspensions and their culture filtrates on three Faba bean cultivars.

The experiments were laid out in split plot design with four replications where Faba bean varieties (Sakha-3,Misr -2 and Nubaria -1) were randomly assigned in main plots, meanwhile six combinations of foliar spraying by micronutrients loaded , non-loaded *S. cerevisiae* suspensions and their culture filtrates were distributed randomly in sub-plots as follows:

1. Control (Tap water).
2. CT: Biomass of Cu^{2+} - adapted *S. cerevisiae* in Tap water.
3. FT: Culture filtrate of Cu^{2+} - adapted *S. cerevisiae* containing Cu (18.17ppm), Fe (24.52ppm), Mn (15.87ppm) and Zn (12.56 ppm).
4. FC0: Culture filtrate of non-adapted *S. cerevisiae*.
5. C0: Biomass of non-adapted *S. cerevisiae* in Tap water.
6. M.M.S (control): Mixed metals solution containing Cu (18.17ppm), Fe (24.52ppm), Mn (15.87ppm) and Zn (12.56 ppm).

The experimental unit area was 10.5 m^2 = (1/400 fed., one feddan =4200 m^2) 3 m long and 3.5 m width which formed of 14 rows from 20 (cm) between rows. Seeds of Faba bean varieties were planted in 1st December in growing seasons. Irrigation was carried out using the sprinklers according zoon system. During seed bed preparation P_2O_5 and K_2O fertilizers were added at the rate of 31.0 and 24.0 (kg/fed), respectively, while nitrogen fertilizer as ammonium nitrate (33.5%) was added at the rate of 33.5 kg N/fed. Foliar spraying by micronutrients loaded and non-loaded yeasts and their culture filtrates were spraying on plant foliage during Faba bean plant growth period at 60 days after sowing.

At harvest the following characters were recorded on a random sample of ten guarded plants from each plot:

1. Plant height (cm).
2. Number of branches /plant.
3. Number of pods /plant.
4. Pods yield /plant (g).
5. Number of seeds /pod.
6. 100 seeds weight (g).
7. Seed yield /plant (g).

The whole plot was harvested to determine seed and biological yields/feddan. Protein% was calculated by multiplying N content by 6.25 according to Chapman and Pratt [38].

Statistical analysis

The data are expressed as the arithmetic mean ± standard error of the means (x± SEM). The Data of field experiments were subjected to statistical analysis according to procedure outlined by Gomez and Gomez [39], whereas, treatment means were compared to L.S.D test. Combined analysis was made for the two growing seasons as results followed similar trend.

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RESULTS AND DISCUSSION

Effect of inoculums size on *S. cerevisiae* biomass production

This experiment was designed to establish the effect of inoculums size obtained from preinoculum medium (YPG) on growth of *S. cerevisiae,* therefore, conical flasks (250 ml) each containing 49 ml, 48 ml, 47 ml, 46 ml or 45 ml of glucose, peptone liquid medium inoculated with 1 ml, 2 ml, 3ml, 4 ml or 5 ml of inoculums. the cultures were incubated shaken (150 rpm) at 28°C for 24 h. At the end of incubation period, the growth was determined at OD_{600}. the results showed that, by increasing inoculums size, the growth of *S. cerevisiae* increased until reached to the maximum at inoculums size 5 ml recorded OD_{600}=9.69 (Table, 1).

Table 1: Effect of inoculums size on biomass production.

Inoculums size (ml)	Growth $O.D_{600}$
1	8.18
2	8.25
3	8.69
4	9.49
5	9.69

Adaptation of *S. cerevisiae* to copper (II) tolerance in liquid medium

There are a few reports of copper (II) biosorption by growing yeast cells. Copper (Cu) is an essential element required by all living organisms. Thus, the growth of the original *S. cerevisiae* was determined on liquid medium amended with copper (0 to 246 ppm as copper chloride). This was done to determine the copper concentration that reduced the biomass growth by approximately 50%. Results presented in Fig.1 show that 82 ppm of copper (II) reduced the cells growth by 44.12%. The yeast was adapted to copper (II) by serial transfers (7transfers) on glucose, peptone liquid medium containing 82 ppm copper (II) for 7 days. Lopez and Vazquez [37]established peptone as being responsible for this, rather than any other nitrogen-containing organic substrates, because of its comparatively low metal binding affinity. Data presented in Table 2 showed that during the adaptation process, the growth of yeast was increased until reached to the maximum at the 7^{th} transfer (from OD_{600}= 0.62 to OD_{600}= 7.17). This may be because, during 7 transfers, the culture rapidly adapts and can tolerate high copper concentration (82 ppm). On the other hand, the broth and the cells become reddish orange in color initially at the 4^{th} transfer, this is also appear when *S. cerevisiae* grows in solid medium containing copper (II) then the cells becomes reddish orange in color (Fig.2A). This may be due to some pigments or compound responsible for copper tolerance resistance in *S. cerevisiae* or as a result of oxidation-reduction reactions. Yap *et al*., [40] reported that at the Cu exposure of 200-600 mg/l, deep bluish mycelia of tolerant strain of *Trichoderma atroviride* were clearly visible which was indicative of the presence of Cu ions. Some workers [41-43] suggested that with active biomass, metals are concentrated through a combination of surface reactions, intra- and extracellular precipitation, and intra- and extracellular complexation reactions. After 7 transfers on liquid medium containing 82 ppm of copper (II), the growth of adapted yeast was determined on copper (II) amended medium (0-246 ppm), after 24h of incubation, the 50% growth inhibition was approximately 136.7 ppm of Cu^{2+}, (Fig., 1). As shown in Fig.1, the increasing of Cu^{2+}concentration in the growth medium caused inhibition on the growth of two kinds of yeasts (non-adapted and copper-adapted), but Cu^{2+}-adapted strain was exhibited 100% of growth at Cu^{2+}concentrations of 27.33 ppm and 54.66 ppm .These results were agree with the results of Sun *et al.,* [44], they found that the growth of the yeast was slower as the initial copper concentration increased. On the other hand, the growth of Cu^{2+}-adapted *S. cerevisiae* is higher than that of non-adapted one when grown on liquid media containing 0-246 ppm of Cu^{2+}.No growth was observed when non-adapted *S. cerevisiae* grown on 191.33-246 ppm of Cu^{2+}amended medium. [45 and 46] reported that high Cu^{2+} levels are toxic, mainly because Cu^{2+}catalyzes the synthesis of reactive oxygen species that cause cellular damage. Whereas ,Cu^{2+}-adapted *S. cerevisiae* was exhibited growth (62.9-21.5%)at the same concentrations of Cu^{2+}, this due to high metal resistance could be attributed to metallothionins (MTs) and/or other metal-induced small protein quantities in *S. cerevisiae* [47 and 48].These results were agree with the results of Donmez and Aksu [49].

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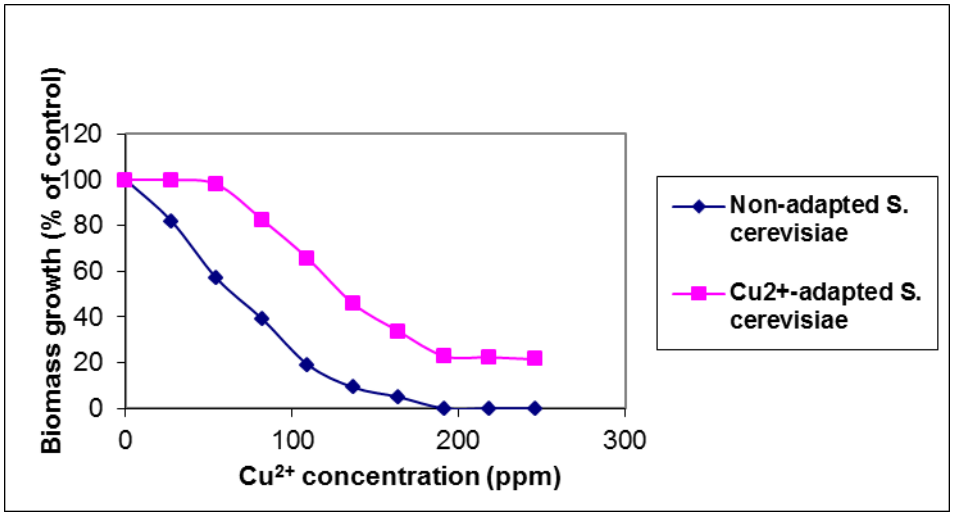

Figure 1: Cu^{2+} toxicity in non- adapted (parental) and Cu^{2+}adapted *Saccharomyces cerevisiae*.

Table 2: Training of *Saccharomyces cerevisiae* to a Cu^{2+} concentration that inhibit the growth by about 50%.

Transfer No.	Final pH	Growth OD_{600}
0	5.10	3.08
1	5.15	0.62
2	4.63	0.98
3	4.99	1.12
4	5.08	1.46
5	4.93	1.58
6	4.85	4.28
7	4.97	7.17

Initial pH, 6.02

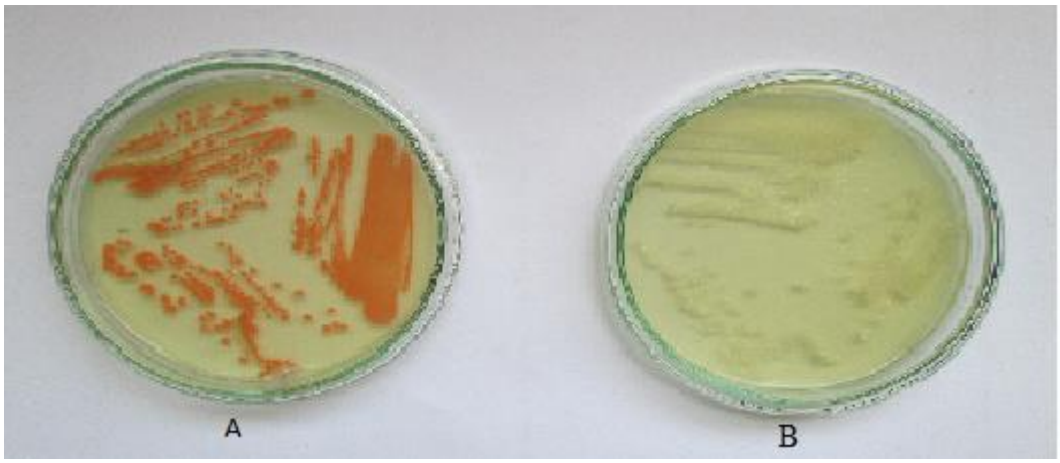

Figure 2: Showed the difference between $Cu2^{+}$- adapted and non-adapted *S. cerevisiae* in color. Fig.(2A) appears the cells of Cu^{2+}- adapted *S. cerevisiae* become reddish orange in color during the training process to copper, while, Fig.(2B) appears no color formation in case of non-adapted *S. cerevisiae* grown on a medium free Cu^{2+}.

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The elemental analysis of dried non-adapted and Cu^{2+}- adapted *S. cerevisiae* biomass obtained from glucose-peptone media unamended or amended with 27.33 ppm of Cu^{2+} have differential ratios of C, H, N and S (Table 3). These differential ratios were due to the exposure of cells with Cu^{2+} in the growth media, but this requires further in-depth study.

Table 3: Carbon, hydrogen, sulphur, and nitrogen content in non-adapted and Cu^{2+}- adapted *S. cerevisiae*.

Sample	C%	H%	S%	N%
Non-adapted *S. cerevisiae* (original)	46.14	11.547	0.711	6.683
Cu^{2+}-adapted *S. cerevisiae*	47.57	13.125	0.192	2.425
Original *S. cerevisiae* grown in the presence 27.333- Cu^{2+}	45.56	13.680	0.414	7.063
Cu^{2+}-adapted *S. cerevisiae* grown in the presence 27.333- Cu^{2+}	46.61	8.235	0.259	5.550
Cu^{2+}-adapted *S. cerevisiae* grown in the presence of mixed metals (Cu, Fe, Mn, and Zn)	42.46	6.648	0.454	7.369

Metal tolerance

The sensitivity of the non-adapted (parental) and Cu^{2+}- adapted *S. cerevisiae* to Fe^{3+}, Zn^{2+}or Mn^{2+} were determined. *Saccharomyces cerevisiae* was grown on glucose- peptone liquid medium containing increasing concentrations of each individual tested metal and the biomass growth was determined. Data shown in Figs 3 and 4 indicate that copper-adapted *S. cerevisiae* significantly tolerated elevated levels of Fe^{3+}(0-258.5 ppm) and Mn^{2+}(0-208 ppm)than non-adapted one. Also, the adapted strain could tolerate up to 51.7 ppm of Zn^{2+} than non-adapted one then the growth was dropped at Zn^{2+}concentrations from 68.9- 155.0 ppm (Fig., 5). This co-tolerance to heavy metals indicated that the mechanism(s) conferring tolerance to copper was not unique for that metal. Although metal- tolerant microorganisms isolated from natural environments polluted with metals often exhibit tolerance to multiple metals, each of the metal was usually present at elevated concentrations in these environment. Consequently, co-tolerance in these instances, in contrast to the development to each individual metal, cannot to confirm. Co tolerance to metals has been demonstrated in vitro, copper- trained *Rhizopus stolonifer* and *Cunninghamella blakesleeana* acquired a tolerance to elevated levels of Cd, Co, Ni and Pb [25].Romero *et al.,* [50] reported that *Talaromyces helicus* was trained with high copper levels, and became co-tolerant to cobalt, lead and cadmium when was cultured in their presence. In addition to the Cu- adaptation was the result of physiological mechanisms, and the activated biochemical processes conferred resistance to Cu^{2+} as well as to other heavy metals.

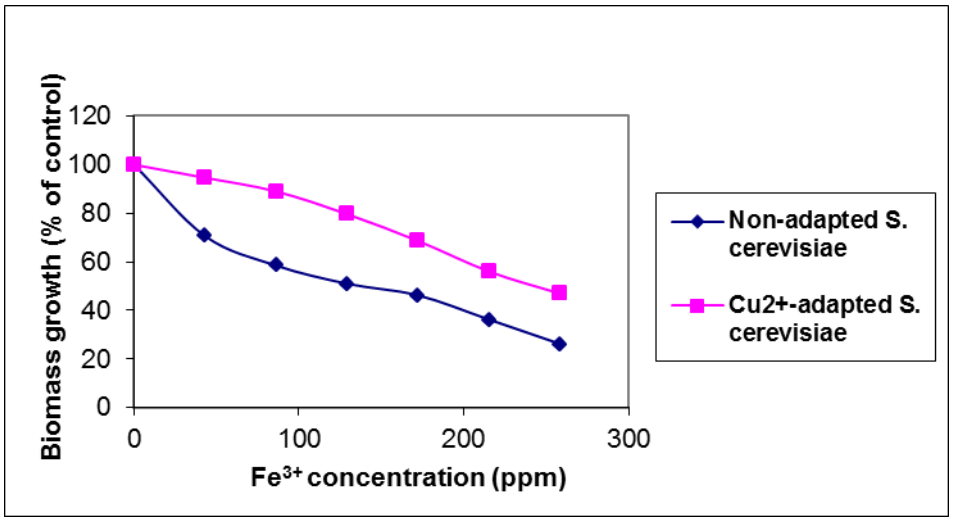

Figure 3: Fe^{3+} toxicity in non- adapted (parental) and Cu^{2+}-adapted *Saccharomyces cerevisiae*.

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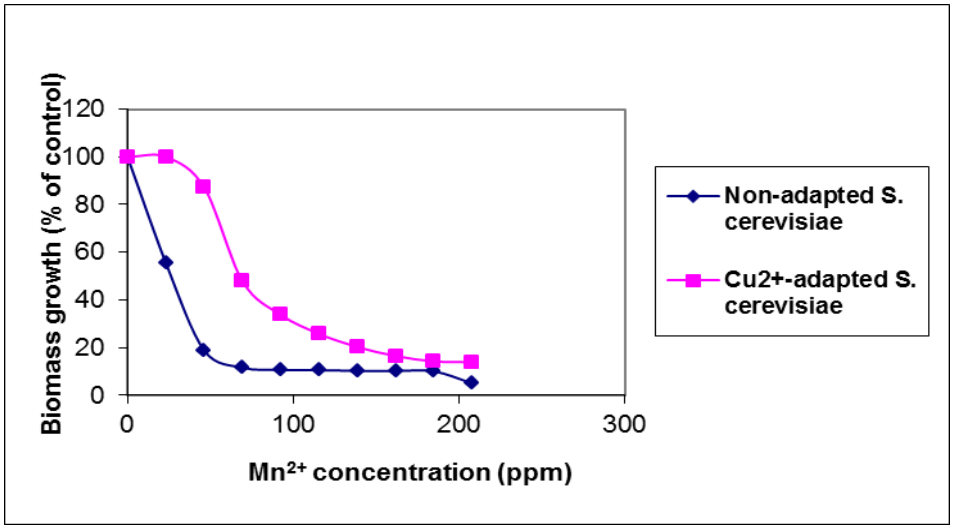

Figure 4: Mn^{2+} toxicity in non- adapted (parental) and Cu^{2+}adapted *Saccharomyces cerevisiae*.

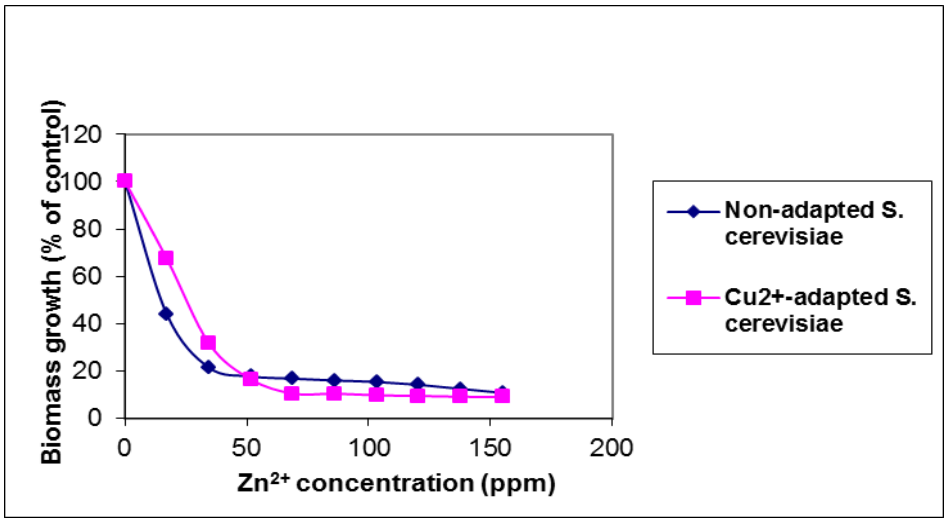

Figure 5:Zn^{2+} Toxicity in non- adapted (parental) and Cu^{2+}adapted *Saccharomyces cerevisiae*.

Metal uptake by growing *S. cerevisiae*

The non-adapted and Cu^{2+}-adapted *S. cerevisiae* were exposed to different concentrations of metal ions i.e. Cu^{2+}, Zn^{2+}, Mn^{2+}, or Fe^{3+} for growing in order to establish the metal uptake by yeast. Results presented in Tables (4-7) show that the uptake of all metals from each culture by Cu^{2+}-adapted *S. cerevisiae* was significantly higher than the non-adapted one. As shown in Table (4) when the initial Cu^{2+} concentration was increased from 27.33 to 191.33 ppm, the Cu^{2+} uptake capacity of Cu^{2+}-adapted strain increased from 13.9 to 38.84ppm, while the Cu^{2+} uptake capacity of non-adapted *S. cerevisiae* was decreased from 12.566 to 0.0 (no growth). At Cu^{2+} concentrations of 27.33 and 191.33 ppm, growing adapted *S. cerevisiae* biomass took up 50.88 and 20.30% of the total amount of copper (II) within the 24h, respectively. While non-adapted *S. cerevisiae* took up 45.98 and 0.0% (no growth) of the total amount of copper (II) under the same conditions. On the other hand, the pH values were decreased from 5.62 to 5.0 by increasing of Cu^{2+} concentrations in all cultures. In waste waters of pH 3-5, yeast biomass could previous an effective bio accumulator for removal of copper (II) [49].*S. cerevisiae* can be able to uptake of Cu^{2+} as well as Zn^{2+}, Mn^{2+}, and Fe^{3+}. Ferric ions uptake was increased with increasing Fe^{3+} concentration up to 344.63 ppm by non-adapted and Cu^{2+}-adapted *S. cerevisiae*, while the adapted strain took up Fe^{3+} higher than non-adapted one (Table, 5). The pH values were decreased with increasing Fe^{3+} concentration from pH 5.34 to 3.60. The results showed that the uptake of Mn^{2+} and Zn^{2+} by *S. cerevisiae* appeared to be low compared with Cu^{2+} and Fe^{3+}. On the other hand, the uptake of Mn^{2+} and

Zn^{2+} by Cu^{2+}-adapted *S. cerevisiae* was higher than non-adapted one (Tables, 6 and7). The pH values were changed with increasing of metals concentrations at the end of incubation (24h.). At 23.11 ppm initial Mn^{2+} concentration, the Mn^{2+} uptake by non-adapted and Cu^{2+} adapted *S. cerevisiae* was 13.4% (3.09 ppm) and 22.37% (5.17 ppm), respectively. Whereas, at Mn^{2+} concentration of 138.66 ppm, Mn^{2+} took up by non-adapted and Cu^{2+} adapted was 4.45 ppm and 7.515 ppm, respectively. At 17.23 ppm initial Zn^{2+} concentration, the non-adapted and Cu^{2+} adapted *S. cerevisiae* took up 5.49 ppm and6.91 ppm, respectively. Whereas, at Zn^{2+} concentration of 103.38 ppm, Zn^{2+} took up by non-adapted and Cu^{2+} adapted *S. cerevisiae* took up 5.29 ppm and6.96 ppm, respectively, Tables (6-7). [49] reported that heavy metal adapted cells of yeasts are able to bio accumulate heavy metal cations in high concentrations and to remove them from the cultivation suspension. The biomass of non-adapted and Cu^{2+} adapted *S. cerevisiae* accumulate considerable amounts of Cu^{2+}, Zn^{2+}, Mn^{2+}, or Fe^{3+}. These results indicate that biosorption of metals by biomass of yeast is a relatively non-specific process with each metal binding site being able to be used by any number of metal species depending on their relative concentrations and chemical properties, the nature of the ligands and external physiochemical factors [51]. Some workers [52 and 53] reported that metal ion uptake in yeasts is known to involve an initial rapid binding of metal ions to negatively charged sites on the cell wall which is a multi-laminate, microfibrillar structure consisting of up to 90% polysaccharides followed by a slower, energy-dependent entry. The outer mannan-protein layer of the yeast cell wall as well as the inner glucan-chitin layer are important heavy metal accumulation.

Table 4: Cu^{2+} uptake by growing non-adapted and Cu^{2+} adapted *S. cerevisiae*.

Cu^{2+} concentration (ppm)	Final pH	Cu^{2+} uptake (%)by non-adapted	Final pH	Cu^{2+}uptake (%)by Adapted
0.0	5.10	0.0	5.00	0.0
27.33	5.10	45.98	5.20	50.88
54.67	5.10	33.72	5.50	43.03
81.99	5.20	30.76	5.00	42.93
109.33	5.20	14.95	5.20	36.82
136.67	5.00	8.23	5.20	26.72
163.99	5.00	4.44	5.10	22.40
191.33	5.00	0.00	5.00	20.30

Initial pH in the presence of Cu^{2+} =5.62
Initial pH at 0.0 Cu^{2+} =6.02

Table 5: Fe^{3+} uptake by growing non-adapted and Cu^{2+} adapted *S. cerevisiae*.

Fe^{3+} concentration (ppm)	Final pH	Fe^{3+} uptake (%)by non-adapted	Final pH	Fe^{3+} uptake (%)by adapted
0.0	4.28	0.0	5.0	0.0
43.079	5.00	53.973	5.26	55.656
86.158	4.84	44.196	4.97	53.830
129.237	4.63	33.846	4.72	47.611
172.316	4.48	30.570	4.45	37.245
215.395	4.27	28.261	4.29	32.411
258.474	4.02	14.741	4.10	18.650
301.553	3.90	8.232	3.88	10.285
344.632	3.70	6.620	3.69	8.620
387.711	3.62	3.912	3.60	5.111

Initial pH in the presence of Fe^{3+} =5.43

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Table 6: Mn^{2+} uptake by growing non-adapted and Cu^{2+} adapted *S. cerevisiae*.

Mn^{2+} concentration (ppm)	Final pH	Mn^{2+} uptake (%)by non-adapted	Final pH	Mn^{2+}uptake (%)by adapted
0.0	4.39	0.0	4.90	0.0
23.11	5.15	13.40	5.58	22.37
46.22	5.60	10.69	5.82	18.70
69.33	5.65	8.43	5.78	10.32
92.44	5.69	6.52	5.76	8.65
115.55	5.68	4.99	5.80	6.73
138.66	5.75	3.21	5.75	5.42
161.77	5.75	2.48	5.71	3.64
184.88	5.74	1.10	5.84	2.21
207.99	5.74	0.20	5.80	1.80

Initial pH in the presence of Mn^{2+} =6.1
Initial pH at 0.0 Mn^{2+} =6.02

Table 7: Zn^{2+} uptake by growing non-adapted and Cu^{2+} adapted *S. cerevisiae*.

Zn^{2+} concentration (ppm)	Final pH	Zn^{2+} uptake (%)by non-adapted	Final pH	Zn^{2+}uptake (%)by adapted
0.0	4.77	0.0	5.09	0.0
17.23	4.75	31.89	5.23	40.11
34.46	4.95	23.82	5.58	25.65
51.69	5.15	15.76	5.64	17.33
68.92	5.37	9.80	5.75	12.21
86.15	5.45	7.00	5.75	10.62
103.38	5.50	5.10	5.71	6.73
120.61	5.50	3.20	5.75	4.33
137.84	5.54	2.30	5.70	3.21
155.07	5.56	1.80	5.76	2.10

Initial pH in the presence of Zn^{2+} =6.1
Initial pH at 0.0 Zn^{2+} =6.02

Effect of various heavy metal ions on the uptake of copper (II)

The effect of various sorbates associated with copper (II) in glucose - peptone liquid medium was studied. The sorbates included Zn^{2+},17.23 ppm; Mn^{2+}, 23.11 ppm ; Fe^{3+},43.088 ppm and Cu^{2+} ,27.33 ppm .The results in Table(8) showed that the copper (II) uptake by Cu^{2+} adapted *S. cerevisiae* was decreased from 50.88% to 33.53% in the presence of tested heavy metal ions. It is to be noted that the biomass of *S. cerevisiae* grown on mixed metal ions amended medium removed different considerable proportion of Fe^{3+}, 43.08%, Mn^{2+},31.33% and Zn^{2+}, 26.53% in addition to Cu^{2+} 33.53%with biomass growth as OD_{600}=8.8,whereas, when Cu^{2+} adapted *S. cerevisiae* grown in the presence of a half concentration of the previous mixed metal, the uptake of Cu^{2+}, Zn^{2+}, Mn^{2+}, and Fe^{3+}were 14.97, 48.39, 29.26 and 33.22%, respectively. From data in Table (8) it appears that Cu^{2+} adapted *S. cerevisiae* is capable of accumulating considerable amounts of Cu^{2+} in the

presence of other heavy metal ions with only a limited decrease 17.35% in the level of accumulation at the higher metal ions concentrations (Zn^{2+}, Mn^{2+}, and Fe^{3+})with biomass growth as OD_{600}=7.7. This indicates that yeast cells are selective in their uptake of metal cations and that the mechanisms of heavy metal uptake are not over whelmingly inhibited by elevated ionic strength. These results were agreed with the results obtained by [2 and 50].

Table 8: Uptake of mixed heavy metal ions by growing Cu^{2+} -adapted *S. cerevisiae.*

Metal ion	Metal ion concentrations (ppm)	Residual	Adsorbed	Metal ion uptake (%)
Cu^{2+}	13.67	11.62	2.05	14.97
	27.33	18.17	9.16	33.53
Fe^{3+}	21.54	11.12	10.42	48.39
	43.08	24.52	18.56	43.08
Mn^{2+}	11.56	8.18	3.38	29.26
	23.11	15.87	7.24	31.33
Zn^{2+}	8.62	5.76	2.86	33.22
	17.23	12.56	4.58	26.58

Initial pH in the presence of metals= 5.24

Varietal differences

The results reported in Table (9) indicate clearly that there were significant differences between Faba bean varieties in plant height, number of branches/ plant, number of pods/ plant, 100 seed weight (seed index),seed yield/ feddan and biological yield/ feddan, except in number of seeds/ pod, seed yield/ plant and protein percentage. Moreover, Sakha-3 variety had the highest mean values from plant height, number of branches/ plant and protein percentage, while Nubaria-1 variety had the highest values for number of pods/ plant, number of seeds/ pod, 100 seed weight and seed yield/ plant, as well as, Misr-2 variety had the highest values for seed yield/ feddan and biological yield/ feddan.

The differences among Faba bean cultivars in yield attributes may be due to the differences in number of nodules formed by each cultivar and consequently growth of each cultivar depend mainly on nitrogen fixation [54]. Also, [28] obtained varietal differences in partition and migration of dry matter. They added that Faba bean cultivars differed in carbon equivalent of each of vegetative components, seeds and straw, number of glucose "g" required to from 1 "g" of vegetative matter, seeds and straw, yield energy per plant as well as per feddan and above ground biomass energy per feddan. Also coefficient of energy of crop index, harvest index and migration coefficient varied among the Faba bean cultivars studied.

It could be concluded that the results of the cultivars differences, herein, are confirmed with those obtained by [28-36].

Effect of micronutrients loaded adapted, non-loaded non-adapted *S. cerevisiae* or their culture filtrates on Faba bean properties

It is clear from Table (9) that spraying Faba bean plants with micronutrients loaded , non-loaded *S. cerevisiae* or their culture filtrates significantly increased plant height, , number of branches/ plant, number of pods/ plant, number of seeds/ pod , 100 seed weight (seed index), seed yield/ plant ,seed yield/ feddan , biological yield/ feddan and protein percentage.

It could be concluded that application with FT treatment to Faba bean plants was the most favorable treatment to increase yield and its components compared with control treatment followed by FC0 and C0 treatments. Yeasts synthesize antimicrobial and other useful substances required for plant growth from amino acids and sugars secreted by bacteria, organic matter and plant roots [14]**.** Meanwhile role of micronutrients

should not be ignored. Iron, zinc and manganese have several important roles in the plant, including protein synthesis, photosynthesis, chlorophyll synthesis, carbohydrate transport and metabolism, growth hormones regulation (auxin) pollen and flower formation [23]; functioning as an activator or cofactor of at least 35 enzymes. These results are in harmony with those obtained by [18-22].

Table 9: Effect of cultivar differences and micronutrients loaded adapted, non-loaded non-adapted *S. cerevisiae* or their culture filtrates on yield and its components of Faba bean (Average of 2014/2015 and 2015/ 2016 seasons).

Characters Treatments	Plant height "cm"	No. of branches /plant	No. of pods /plant	No. of seeds /pod	100 seed weight "g"	Seed yield "g/plant"	Seed yield "kg/fed"	Biological yield "ton/fed"	Protein %
Cultivars									
Sakha- 3	81.83	2.94	7.67	2.61	79.89	9.23	410.06	1.89	22.06
Misr-2	80.94	2.61	8.17	2.56	75.33	10.11	425.17	2.26	21.72
Nubaria-1	79.50	2.17	8.39	2.72	92.00	10.18	419.28	2.05	21.89
L.S.D at 5%	1.19	0.20	0.45	n.s	1.09	n.s	10.35	0.12	n.s
Micronutrients loaded adapted, non-loaded non-adapted *S. cerevisiae* or their culture filtrates.									
1- Control	70.56	1.67	3.00	2.00	71.89	4.64	329.78	1.60	21.54
2- CT	76.78	2.11	6.44	2.44	81.56	10.42	357.89	1.95	21.76
3- FT	90.33	3.78	11.22	3.22	91.11	12.61	565.56	2.51	22.96
4- FC0	85.44	3.11	10.00	2.89	84.78	11.04	429.44	2.21	21.98
5- C0	82.11	2.44	9.11	2.67	82.89	10.44	426.56	2.14	21.77
6-M.M.S	79.33	2.33	8.67	2.56	82.22	9.88	399.78	2.00	21.33
L.S.D. at 5%	2.53	0.60	0.76	0.42	2.85	0.68	21.92	0.11	0.60

1-Control (Tap water).
2-CT: Biomass of Cu^{2+} - adapted *S. cerevisiae* in Tap water.
3-FT: Culture filtrate of Cu^{2+} - adapted *S. cerevisiae* containing Cu (18.17ppm), Fe (24.52 ppm), Mn (15.87ppm) and Zn (12.56 ppm).
4-FC0: Culture Filtrate of non-adapted *S. cerevisiae*.
5-C0: Biomass of non-adapted *S. cerevisiae* in Tap water.
6-M.M.S: Mixed metals solution containing Cu (18.17ppm), Fe (24.52ppm), Mn (15.87ppm) and Zn (12.56 ppm).

Effect of interaction between Faba bean cultivars and micronutrients loaded adapted, non-loaded non-adapted *S. cerevisiae* or their culture filtrate

Data in Table (10) indicated that number of pods/ plant, 100 seed weight, biological yield/ feddan and protein percentage significantly responded to the interaction between Faba bean cultivars and the different treatments of micronutrients loaded, non-loaded *S. cerevisiae* or their culture filtrates. However, plant height, number of branches/ plant, number of seeds/ pod, seed yield/ plant and seed yield/ feddan under study showed no significant response to the interaction (Table 10). It is noteworthy to mention that foliar application of Nubaria-1 cultivar with FT treatment (Culture Filtrate of Cu^{2+} - adapted *S. cerevisiae* containing Cu, Fe, Mn and Zn) was the most favorable treatment for each one of number of pods/ plant, number of seeds/ pod , 100 seed weight, seed treatment for each one of number of pods/ plant, number of seeds/ pod , 100 seed weight, seed yield/ plant, seed yield/ feddan and biological yield/ feddan. On the other hand, Sakha-3 cultivar with FT treatment (Culture Filtrate of Cu^{2+} - adapted *S. cerevisiae* containing Cu, Fe, Mn

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and Zn) produced the highest values of plant height and number of branches/ plant. With respect to the protein percentage the most effective treatment was spraying Misr-2 cultivar with FT treatment (Table 10).Metal toxicity can be markedly affected , either reduced or increased , by binding with organic substances. These metal binding processes may occur either extracellularly or intracellularly. Binding or chelation of a metal by released metabolites has been advanced as a mechanism of detoxification. Citric acid, oxalic acid, exopolysaccharides and enzymes which can be produced by many yeasts and fungi, can readily chelate metal ions such as cu^{2+}, pb^{2+}, Zn^{2+}, Fe^{2+} and other metals in form organic metals and may control metal availability in the growth medium[55-57].

Table10: Effect of interaction between cultivars and micronutrients loaded adapted, non-loaded non - adapted *S. cerevisiae* or their culture filtrates on yield and its components of Faba bean (Average of 2014/ 2015 and 2015/ 2016 seasons).

Treatments		Plant height "cm"	No. of branches /plant	No. of pods /plant	No. of seeds /pod	100 seed weight "g"	Seed yield "g/plant"	Seed yield "kg/fed"	Biological yield "ton/fed"	Protein %
Cultivars	micronutrients loaded adapted, non-loaded non-adapted *S. cerevisiae* or their culture filtrates									
Sakha- 3	1-Control	70.67	1.67	3.00	2.00	69.67	4.00	330.00	1.26	21.77
	2- CT	76.67	2.33	6.00	2.33	80.67	9.80	345.00	1.65	22.10
	3- FT	94.00	4.33	10.67	3.00	88.00	11.80	546.67	2.10	22.10
	4- FC0	87.67	3.67	9.33	3.00	80.67	10.73	428.33	2.13	23.03
	5- C0	82.67	3.00	8.67	2.67	78.33	9.63	420.33	2.30	21.93
	6- M.M.S	79.33	2.67	8.33	2.67	82.00	9.40	390.00	1.90	21.43
Misr-2	1-Control	70.67	2.00	2.67	2.00	69.33	5.00	355.00	2.13	21.77
	2- CT	80.00	2.00	6.67	2.33	76.00	11.10	360.00	2.40	21.00
	3- FT	86.67	3.67	9.67	3.00	78.00	12.43	560.00	2.63	23.47
	4- FC0	84.33	3.00	10.33	2.67	73.00	11.23	443.33	2.30	21.73
	5- C0	83.33	2.33	10.00	3.00	76.33	10.77	426.00	2.07	21.37
	6- M.M.S	80.67	2.67	9.67	2.33	79.33	10.13	406.67	2.03	20.97
Nubaria-1	1-Control	70.33	1.33	3.33	2.00	76.67	4.93	304.33	1.40	21.10
	2- CT	73.67	2.00	6.67	2.67	88.00	10.37	368.67	1.79	22.17
	3- FT	90.33	3.33	13.33	3.67	107.33	13.60	590.00	2.80	23.30
	4- FC0	84.33	2.67	10.33	3.00	100.67	11.17	416.67	2.18	21.17
	5- C0	80.33	2.00	8.67	2.33	94.00	10.93	433.33	2.06	22.00
	6- M.M.S	78.00	1.67	8.00	2.67	85.33	10.10	402.67	2.07	21.60
L.S.D. at 5%		n.s	n.s	1.3	n.s	4.93	n.s	n.s	0.19	1.04

For experimental conditions, see footnote in Table, 9.

CONCLUSION

From the present study, it was found that that Cu^{2+}- adapted *S. cerevisiae* was able to grow in the presence of 246 ppm of Cu^{2+} amended glucose-peptone liquid medium. When compared with non-adapted *S. cerevisiae* (parental), it was clear that Cu^{2+}concentration that inhibits the growth of parental strain was initially 191.33 ppm. On the other hand, Cu^{2+}- adapted *S. cerevisiae* significantly tolerated elevated levels of Fe^{3+}, Mn^{2+}, and Zn^{2+} than non-adapted one. The high tolerance to Cu^{2+}observed inCu^{2+}- adapted *S. cerevisiae* could be attributed to metallothionins and/ or other metal- induced small protein quantities in *S. cerevisiae* (Birch and Walker, 2000; and Carman and Han, 2009). The uptake of Fe^{3+}, Mn^{2+}, Zn^{2+} or Cu^{2+} by growing Cu^{2+}- adapted *S. cerevisiae* significantly higher than non-adapted one. Living yeast adsorption can be divided into extracellular and intracellular adsorption. It is to be noted that the growing Cu^{2+}- adapted *S. cerevisiae* can able to uptake different considerable proportion of Fe^{3+}, Mn^{2+}, Zn^{2+} in addition to Cu^{2+} when was cultured in their presence. It is noteworthy to mention that foliar application of Nubaria-1 cultivar with FT treatment (Culture Filtrate of Cu^{2+} - adapted *S. cerevisiae* containing Cu, Fe, Mn and Zn) was the most favorable treatment for each one of number of pods/ plant, number of seeds/ pod, 100 seed weight, seed yield/ plant, seed yield/ feddan and biological yield/ feddan. On the other hand, Sakha-3 cultivar with FT treatment (Culture Filtrate of Cu^{2+} - adapted *S. cerevisiae* containing Cu, Fe, Mn and Zn) produced the highest values of plant height and number of branches/ plant. With respect to the protein percentage the most effective treatment was spraying Misr-2 cultivar with FT treatment

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REFERENCES

[1] Huang CP, Westman D, Quirk K, Huang JP. Water Sci Technol 1988; 20: 369–376.
[2] Brady D, Duncan JR. Appl Microbiol Biotechnol 1994; 41: 149-154.
[3] Benitez P, Castro R and Barroso, CG. Anal Chem Acta 2002; 458:197-202.
[4] Can C, Wen D, Wang J. Bioresour Technol 2014; 156: 380-383.
[5] Can C, Wang J. Appl Microbiol Biotechnol 2008; 79:293-299.
[6] Can C, Jianlong W. J Environ Eng 2010; 136: 95-102.
[7] Ferreira IMP, Pinho OC, Vieira E, Tavarcla JG. Trends Food Sci Technol 2010; 21:77-84.
[8] Wang J, Can C. Biotechnol. Adv 2006; 24:427-451.
[9] Macaskie L E, Dean A C R.J Gen Microbiol 1984; 130: 53-62.
[10] Lundgren D G, Valkove-Valchanova M, Reed, R. Chemical reactions important in bioleaching and bioaccumulation. In Workshop on Biotechnology for the Mining, Metal Refining and Fossil Fuel Processing Industries. Biotechnology Bioengineering Symposium 16 1986; Eds Ehrlich, H. L. & Holmes, D. S. pp. 7-22. New York: John Wiley and Sons Inc.
[11] Gadd G M. Fungi and yeasts for metal accumulation. In: Microbial Mineral Recovery, eds. H Ehrlich and C L Brierley. McGraw-Hill, New York, 1990a; pp, 249-275.
[12] Gadd G M. Experientia 1990b; 46: 834-839.
[13] Ahimou F, Touhami A, Dufrêne YF. Yeast 2003; 20:25-30.
[14] Boraste A, Vamsi KK, Jhadav A, Khairnar Y,Gupta N,Trivedi S, Patil P, Gupta G,Gupta M, Mujapara AK, Joshi P. Int J Microbiol Res 2009;1:23-31.
[15] Omran YA. Studies on histophysiological effect of hydrogen cyanamide (Dormex) and yeast application on bud fertility, vegetative growth and yield of Roumi Red grape cultivar. Ph.D. Thesis 2000; Fac of Agric Assiut Univ Egypt.

[16] Nagodawithana WT. Yeast Technology. Universal Food Corporation Milwaukee, Wisconsin1991; Published by Van Nostrand Reinhold, New York .p.273.
[17] Pfeiffer WH, McClafferty B. Crop. Sci 2007; 47:88-105.
[18] Röemheld V. El-Fouly MM. Foliar nutrient application: Challenge and limits in crop production. Proc. 2nd International Workshop on "Foliar Fertilization" April 4-10 Bangkok, Thailand 1999; pp. 1-32.
[19] Davis F R. J American Rhododendron Society 1983; 37(1).
[20] Fox TC, Guerinot ML. Annu Rev Plant Physiol Plant Mol Biol 1998; 49, 669-696.
[21] Kobraee S, NoorMohamadi G, HeidariSharifAbad H, DarvishKajori F, Delkhosh B. Annu Rev Res Biol 2013; 3: 83-91.
[22] Neumann PM. J Plant Nutr 1982; 5: 1209-1215.
[23] Hafeez B, Khanif YM, Saleem M. Am J Exp Agric 2013; 3: 374-391.
[24] Bewley RJF. Holarctic Ecol 1981; 4:215-220.
[25] Garcia-Toledo A, Babich H, Stotzky G. Can J Microbiol 1985; 31: 485-492.
[26] Saad AM, Abdel-Hadi MA. Bull NRC Egypt 2000; 25: 297-306.
[27] Saad AM. Eur J Biotechnol Biosci 2015; 3:1-6.
[28] Ahmed MA, Hassanein MS, Zaki NM. Egypt J Appl Sci 1997; 12: 134-154.
[29] Hassanein MS. Annals Agric Sci Moshtohor 2000; 38: 1383-1398.
[30] Hassanein M S, Magda A F Shalaby, Rashad M El-Sh. (2000). Annals Agric Sci Moshtohor2000; 38: 2141-2155.
[31] Zeidan MS, Abd El-Lateef EM. Egypt J Appl Sci 2001; 16:112-127.
[32] El-Abagy HMH, Amin AA, Rashad El-Sh M, Hassanein MS. Egypt J Appl Sci 2003; 18: 563-579.
[33] Al Ghamdi S. African Crop Science Proceedings 2007; 8: 709-714.
[34] Osman AAM, Yagoub SO, Tut OA. Australian J. Basic and Appl. Sci., 2010; 4: 5516-5521.
[35] Ragab AAM, Tantawy EA, Abd El-Rasoul Sh M. Int J Acad Res 2010; 2: 245-253.
[36] Bakry BA, Elewa TA, El karamany MF, Zeidan MS, Tawfik MM. World J Agric Sci 2011; 7: 68-72.
[37] Lopez-Errasquin E, Vazquez C. Chemosphere 2003; 50:137–143.
[38] Chapman HD, Pratt RF. Methods of Analysis for Soils, Plants and Waters. Berkeley: Division of Agricultural Sciences, University of California 1982.
[39] Gomez K A, Gomez AA. Statistical procedure for agricultural research, 2nd edition John Wiley and Sons, Inc. New York.1984.
[40] Yap CK, Yazdani M, Abdullah F, Tan, SG. Sains Malaysiana 2011; 40: 119-124.
[41] Wilde EW and Benemann JR. Biotechnol Adv 1993; 11:781-812.
[42] Simmons P, Singleton I. Appl Microbiol Biotechnol 1996; 45:278–285.
[43] Aksu Z. Wastewater Treatment with Algae, edited by Wong Y-S, Tam NFY © Springer Verlag and Landes Bioscience 1998; pp. 37-53.
[44] Sun X, Liu L, Zhao Y, Ma T, Zhao F, Huang W, Zhan J. Food Chem 2016; 192: 43-52.
[45] Alper H, Moxley J, Nevoigt E, Fink GR. Stephanopoulos G. Science 2006; 314(5805):1565-1568.
[46] Abdulrehman D, Monteiro PT, Teixeira MC, Mira NP, Lourenco AB, Dos Santos SC, Cabrito TR, Francisco AP, Madeira SC, Aires RS, Oliveira AL, Sa- Correia I, Freitas AT. Nucleic Acids Res 2011; 39(Database issue), D136–D140.
[47] Birch RM and Walker GM. Enzyme Microbial Technol 2000; l26*:* 678–687.
[48] Carman GM, Han G-S. J Lipid Res 2009; 50: S69-S73.
[49] Dönmez G, Aksu Z. Process Biochem 1999; 35: 135-142.
[50] Romero MC, Reinoso EH, Urrutia MI, Kiernan AM. Electr J Biotechnol 2006; 9: 221-226.
[51] Gadd G M. Accumulation of metals by microorganisms and algae. In: biotechnology, Vol 6(b), Special Microbial Processes, ed. H.-J. Rehm. VCHV, Wenham, 1988; pp. 401-433.
[52] Huang CP, Huang CP, Morehart, AL. Water Res 1990; 24: 433-439.
[53] Volesky B, May H, Holan ZR. Biotech Bioeng 1993; 41:826-829.
[54] Tawfic MA, Azzazy MA, Mohamed MA. Egypt J Agron 1991; 16:95-105.

[55] Levi MP. The mechanism of action of copper- chrome- arsenate preservatives against wood detroying fungi. Annual Convention British Wood Preserving Association 1969; pp.113-127.
[56] Ross IS. Trans Br Mycol Soc 1975; 64:175-193.
[57] Okpokwasili GC. Biotechnology and Clean Environment. Proc. of the 20th Annu. Conf. of the Biotechnology Society of Nigeria (BSN), 14th – 17th, November, 2007 at the Ebonyi State University, Abakaliki, Nigeria.