Int. J. Aquat. Biol. (2018) 6(2): 88-94
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

The effects of zinc-enriched *Saccharomyces cerevisiae* on the growth and mineral composition of marine rotifer, *Brachionus plicatilis*

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Abstract: Rotifers are important zooplankton in commercial finfish hatcheries. However, due to the limited variety of food available, zinc content of cultured rotifers in artificial environments may not meet the requirements of fish larval. It has been reported that direct addition of soluble zinc to culture media was not effective on the zinc content of rotifer. Thus, in this study, the effect of zinc-enriched *Saccharomyces cerevisiae* was investigated on the growth and mineral composition of rotifer, *Brachionus plicatilis*. Four different food treatments, including (1) yeast without enrichment (control), (2) yeast containing 21.23 mg g^{-1} of zinc, (3) yeast containing 56.25 mg g^{-1} of zinc, and (4) yeast containing 132.93 mg g^{-1} of zinc, were used to produce rotifer for a period of 10 days. Afterwards, specific growth rate (SGR), the total number of rotifers, total eggs attached to rotifers, and the total number of eggs were measured. Finally, the mineral composition of rotifer in different treatments was analyzed. The findings revealed that yeast enriched with 56.25 mg g^{-1} of zinc significantly improved the growth of rotifers. The maximum number of rotifers (274 ind ml^{-1}), total eggs attached to rotifers (29.3 number ml^{-1}), and the total number of eggs (36 number ml^{-1}) were found in the third treatment. The highest zinc content was observed in the fourth treatment (about 822.5 μg g^{-1} of rotifers). The maximum values of Fe (13.84 μg g^{-1} of rotifers) and Mn (15.22 μg g^{-1} of rotifer) were related to the treatment 4 and control, respectively. However, the amount of Cu did not significantly differ among the treatments. In conclusion, this study found that zinc-enriched yeast improved the growth, reproduction, and body composition of *B. plicatilis*.

Article history:
Received 18 January 2018
Accepted 25 April 2018
Available online 25 April 2018

Keywords:
Saccharomyces cerevisiae
Zinc
Enrichment
Mineral composition

Introduction

Zooplankton such as rotifers are important in the first feeding of fresh and marine fish larvae (Isik et al., 1999). Euryhaline rotifer, *Brachionus plicatilis* is a suitable feed item for the larvae of marine fish and shellfish in terms of their sizes (150-350 μm) and high reproductive rate (Hamre et al., 2008; Lubzens, 1987; Kennari et al., 2008). In marine fish hatcheries, rotifers should be considered not only regarding their density but also their nutritional value, especially mineral composition (Støttrup and McEvoy, 2008). The nutritional value of live feeds affects the growth and survival of fish larvae (Watanabe et al., 1983).

The minerals are responsible for the development of nervous system, growth and survival of aquatic larvae, bone formation, maintenance and adjustment of the colloidal system and acid-base balance of aquatic organisms (Matsumoto et al., 2009). Moreover, minerals play an important role in hormones and enzymes system (Watanabe et al., 1978b). Apines-Amar et al. (2004) indicated that zinc is an important mineral in fish nutrition and plays a vital role in bone health. Although a small amount of zinc in the body of aquatic organisms is present, it is known as an essential element in the fish feed and as a cofactor for over 300 enzymes (Watanabe et al., 1978b).

It has been demonstrated that the levels of zinc in rotifers were lower than levels found in copepods by five folds (Hamre et al., 2008). The cultured rotifers in

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DOI: https://doi.org/10.22034/ijab.v6i2.443

artificial environments have about 80 μg g^{-1} of zinc (Matsumoto et al., 2009), while its value in a planktonic copepods have about 700 μg g^{-1}. The zinc requirement of marine fish is about 15 to 40 mg kg^{-1} (dry mater), and the amount of zinc in cultured rotifers does not meet the needs of marine fish larvae (Matsumoto et al., 2009).

The nutrients required by larvae of fish can be increased in rotifers through direct (short-term enriching) and indirect (through rotifer diets) methods. In the direct one, enrichment materials (including minerals) can be added to culture media of rotifers, but it has been reported that the direct addition of soluble materials such as zinc to the culture media was not effective on zinc content of rotifer (Matsumoto et al., 2009). In the indirect method, the minerals are stabilized in rotifer body through the diet such as algae or yeast (Dhert et al., 2001). Since many years ago, different kinds of yeasts have been used in rotifers nutrition along with algae. Yeast has been used as an intermediary to deliver nutrients to rotifers. The use of omega-3-rich oil along with baker's yeast makes rotifers rich in omega-3 fatty acids (Penglase et al., 2011). Rotifers fed on yeast are able to synthesize unsaturated fatty acids and to elongate the chain (Lubzens et al., 1985). Moreover, yeast enrichment with zinc can be a practical method for increasing the zinc content of rotifer. Therefore, given the need for sufficient levels of minerals in live foods such as rotifers, in the present study, the effects of zinc-enriched baker's yeast *Saccharomyces cerevisiae* were investigated on the growth and the mineral content of rotifers.

Materials and Methods

Zinc-enriched yeast preparation: Zinc sulfate ($ZnSO_4.7H_2O$) was purchased from Sigma-Aldrich, USA. Baker's yeast was provided by *Artemia* and Aquaculture Research Institute at Urmia University, Urmia, Iran. The growth media (YEPD) contained yeast extract, peptone, and glucose, which was obtained from the Jahan Kimia Company in Urmia.

In this study, the method described by Wang et al. (2012) was adopted to enrich yeast in non-growth phase with zinc sulfate. Briefly, 1 g of *S. cerevisiae* was initially cultured in 200 ml of YEPD medium at 27°C, pH=5.8 and 160 rpm for 24 hrs on a shaker incubator (N-Biotech, NB-205V, South Korea). Then, zinc sulfate was added into the medium at the concentrations of 0.5, 1 and 1.5 g per 200 ml of medium. The incubation of yeast cell was performed for 24 hrs under the same conditions. Next, the yeast cells were centrifuged (3000 rpm for 1 min) and washed with normal saline to remove the additional zinc sulfate. Following enrichment, the amount of zinc in the yeast was found to be 21.23, 56.25 and 132.93 mg g^{-1} of the yeast. Finally, the zinc-enriched yeast cells were used for rotifer feeding.

Treatments and test methods: *Brachionus plicatilis* was obtained from the Iran Shrimp Research Center (Bushehr, Iran) and up-scaled to mass density in a wet-lab using *Nannochloropsis oculata*. The rotifers receiving yeast in four groups, including (1) yeast without enrichment (control group), (2) yeast containing 21.23 mg g^{-1} of zinc, (3) yeast containing 56.25 mg g^{-1} of zinc, and (4) yeast containing 132.93 mg g^{-1} of zinc. Each group had three replicates, and all groups were cultured in 2L flask for a period of 10 days.

After disinfection of the flasks, the rotifers with a density of 50 ind ml^{-1} were added. They were fed with 1×10^6 cell ml^{-1} of algae and a 0.5 g of yeast per million rotifers on a daily basis. Water quality parameters, including salinity (30±1 ppt), pH (7.6-8.3) and temperature (24±1°C) were regularly checked. Gentle and continuous aeration was performed to meet the needs of rotifers. The maximum number of rotifers, total eggs attached to rotifers, and total egg numbers were measured on a daily basis. Through the following equation, specific growth rate (SGR) was calculated based on Krebs (1995).

$$SGR=(LnN_t-LnN_0)/t,$$

Where N_0 and N_t are respectively for the initial and final population of rotifer, and t stands for experiment period (days). SGR value was calculated in the exponential phase of population. At the end of the feeding period, the biometric factors in each treatment were measured using a microscope equipped with

Table 1. Mean numbers of rotifers fed with zinc-enriched yeast with different contents (21.23, 56.25 and 132.93 mg g^{-1} of zinc in yeast) for 10 days (mean±SD, n=3).

Days*	Yeast zinc content (mg g^{-1})			
	21.23	56.25	132.93	un-enriched yeast
1	72.6±3.51 [a]	72.6±3.79 [a]	75.0±3.61 [a]	75.0±1.00[a]
2	87.0±4.36 [a]	78.6±8.33 [b]	89.3±4.04 [a]	78.6±4.04 [b]
3	89.6±3.06 [ab]	76.0±8.89 [b]	92.0±4.58 [a]	77.0±4.58 [b]
4	99.3±5.51 [a]	83.3±6.81 [a]	97.3±7.02 [a]	90.3±11.24 [a]
5	110±3.79 [a]	107 ±6.51 [a]	99±10.5 [a]	104±4.51 [a]
6	127±7.0 [a]	122±10.8 [a]	91.6±12.1 [a]	121±11.3 [a]
7	165±5.03 [a]	173±4.00 [a]	153±6.81 [a]	153±6.56 [a]
8	189±3.06 [ab]	197±11.2 [a]	181±8.08[ab]	172±3.61 [b]
9	214±9.87 [ab]	223±16.0 [a]	201±2.65 [ab]	190±2.52 [b]
10	257±10.9 [ab]	274±33.2 [a]	271±10.5 [a]	216±9.53 [b]

*Data was analyzed in each day separately.

Different letters in each row have a significant difference (P<0.05).

Table 2. The SGR of rotifers fed with zinc-enriched yeast in different contents (21.23, 56.25 and 132.93 mg g-1 of zinc in yeast) after 10 days (mean±SD, n=3).

Treatments	SGR
Yeast containing 21.23 mg g^{-1} of zinc	0.1265±0.008[a]
Yeast containing 56.25 mg g^{-1} of zinc	0.1317±0.003[a]
Yeast containing 132.93 mg g^{-1} of zinc	0.1295±0.012[a]
Un-enriched yeast	0.1071±0.013[b]

Different letters in each row have a significant difference (P<0.05).

micrometer lenses.

Analysis of minerals: After centrifuging the yeast cells and rotifers (2000 rpm for 20 min) and discarding the supernatant, the precipitant was used for the analysis of minerals. Through the use of MLS-1200 MEGA Microwave, the samples were digested with nitric acid under cold water for 30 minutes. Using an atomic absorption (Nov AA 400, Analytic Jena, Germany), the mineral concentrations (Zn, Mn, Cu and Fe) of digested sample were measured (Lowry and Lopez, 1946).

Statistical analysis: SPSS statistical software, version 21 was used to run the analyses. Using Levene's and Shapiro-Wilk test, the data were analyzed for homogeneity of variances and normality, respectively (P<0.05). One way ANOVA was used for the analysis of groups and was followed by Duncan honest significant difference test. Differences among the means were considered significant at P<0.05. The data are displayed with mean±SD.

Results

After the eighth day till the end of the culture period, the total numbers of rotifers in the treatment groups were higher than that in the control group (Table 1). However, no significant differences were observed among the groups receiving zinc-enriched yeast. At the 10th day, the maximum total number of rotifers was related to those fed with the yeast containing 56.25 mg g^{-1} zinc.

The findings revealed that the enriched-yeast improved SGR of the rotifer compared to the control group (P<0.05). No significant differences were found in SGR of the groups fed with zinc-enriched yeast. However, the rotifers fed with enriched-yeast containing 56.25 mg g^{-1} zinc showed higher SGR than other treatments (Table 2).

The numbers of total eggs in rotifers fed with the enriched-yeast for 10 days are given in Table 3. The maximum number was found in the group containing 56.25 mg zinc. In addition, the numbers of these factors in the rotifers fed with the enriched yeast were higher than the control group. Based on the findings, the maximum total egg numbers attached to the rotifers were found in the treatment of 56.25 mg zinc which was significantly different compared to the other groups at the 8th day (P<0.05) (Table 4).

The maximum zinc content (about 822.5 µg g^{-1}

Table 3. Total number of eggs in rotifers fed with zinc-enriched yeast in different contents (21.23, 56.25 and 132.93 mg g-1 of zinc in yeast) for 10 days (mean±SD, n=3).

	Yeast zinc contents (mg g^{-1})			
Days*	21.23	56.25	132.93	Un-enriched yeast
1	12.6±2.89[a]	13.0±6.08 [a]	12.3±3.21 [a]	13.3±6.11 [a]
2	11.6±4.73 [a]	12.3±3.06 [a]	17±6.08 [a]	7.67±2.52 [b]
3	17.0±7.00 [a]	14.0±6.25 [a]	11.3±3.51 [a]	17.6±4.62 [a]
4	10.6±2.08 [a]	12.6±3.21 [a]	16.6±4.04 [a]	13.0±3.46 [a]
5	20.6±2.08 [a]	19.33±10.41 [a]	23.0±6.08 [a]	13.0±7.21 [ab]
6	19.0±5.20 [a]	24.3±4.16 [a]	22.0±4.36 [a]	14.6±3.21 [a]
7	27.3±1.53 [a]	30.6±11.72 [a]	20.6±5.69 [a]	18.6±11.06[ab]
8	34.6±3.21 [ab]	36.6±3.21 [a]	18.3±8.08 [cb]	16.3±5.69 [c]
9	31.0±8.19 [a]	31.0±11.14 [a]	34.6±4.16 [a]	23.6±7.51 [b]
10	31.0±6.56 [a]	35.0±5.29 [a]	30.3±1.15 [a]	28±12.70 [a]

*Data was analyzed in each day separately.
Different letters in each row have a significant difference (P<0.05).

Table 4. Total number of attached eggs in rotifers fed with zinc-enriched yeast in different contents (21.23, 56.25 and 132.93 mg g-1 of zinc in yeast) (mean±SD, n=3).

Days*	Yeast zinc contents (mg g^{-1})			
	21.23	56.25	132.93	Un-enriched yeast
1	7.0±1.00 [a]	10.0±6.56 [a]	6.3±1.53 [a]	10.6±3.79 [a]
2	5.3±3.06 [b]	13.0±6.25 [a]	10.6±2.52 [a]	14.6±4.93 [a]
3	15.3±7.09 [a]	12.0±6.00 [a]	7.3±5.03 [b]	13.6±4.51 [a]
4	9.3±1.53 [a]	10.0±5.29 [a]	11.6±5.51 [a]	9.0±2.65 [a]
5	16.0±4.35 [a]	14.3±9.50 [a]	14.6±6.66 [a]	8.6±6.51 [b]
6	12.6±3.21 [a]	17.3±3.06 [a]	15.3±6.66 [a]	9.3±0.58 [a]
7	18.6±1.53 [a]	25.0±10.82 [a]	14.6±7.02 [a]	13.6±7.02 [a]
8	21.0±2.65 [ab]	24.6±4.73 [a]	9.6±7.64[b]	10.6±5.69 [b]
9	20.3±8.50 [a]	22±1.45 [a]	21.6±5.51 [a]	16.3±7.64 [a]
10	21.6±9.07 [a]	29.3±5.69 [a]	23.3±1.53 [a]	22.3±11.8 [a]

*Data was analyzed in each day separately.
Different letters in each row have a significant difference (P<0.05).

rotifer) was obtained at the highest zinc supplementation level. The maximum Fe content (13.84 μg g^{-1} of rotifer) and Mn (15.22 μg g^{-1} of rotifer) were obtained in treatment 4 and control, respectively, but Cu content did not differ among the treatments (Fig. 1).

Discussion

Minerals such as zinc are present in aquatic environments and in addition to providing the needs of living organisms, they can be accumulated in aquatic organisms. In artificial environments (namely marine fish hatcheries), the growth of rotifers, due to the limited variety of food available, may result in inadequate concentrations of minerals, especially zinc. Access to trace minerals for fish larvae is vital since they have important roles in immunity enhancer, stress releaser, cellular metabolism, formation of skeletal structures, disease resistance and other physiological functions, as well as, serve as cofactors and/or activators of a variety of enzymes (Failla, 2003; Satoh, 2003; Antony Jesu Prabhu et al., 2016).

Due to the solubility of zinc, its enrichment is not the same as fat-soluble materials. Therefore, in this study, initially, the baker's yeast was exposed to various concentrations of zinc and in this way, the amount of zinc increased in yeast after 24 hours. The results of the first step demonstrated that it is possible to enrich baker's yeast. The enriched yeast was used as a feed to increase the zinc content of rotifers. This method of enrichment has already been used to increase zinc levels in *Chlorella* by Matsumoto et al. (2009) and selenium enriched yeast in rotifer culture by Penglase et al. (2011). These two studies found that indirect enrichment of rotifers with minerals had positive effects on the growth rate of rotifers than

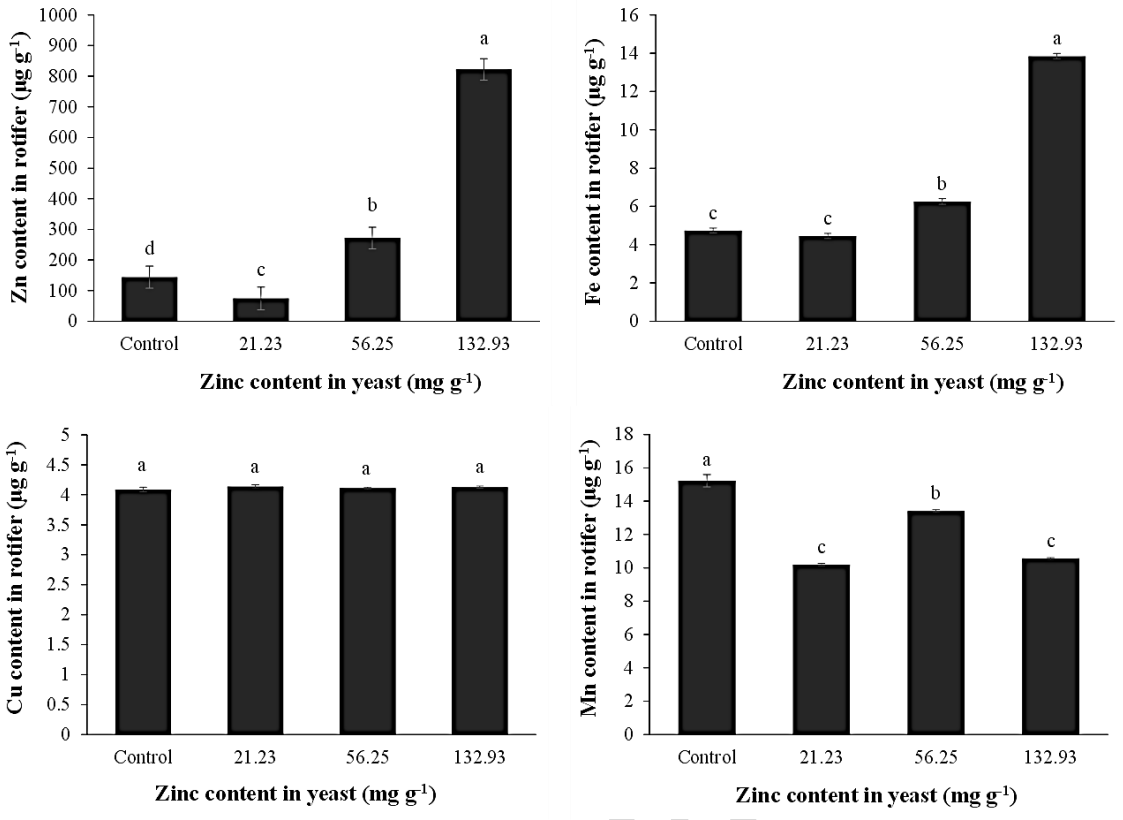

Figure 1. The amounts of zinc, Fe, Cu and Mn of the rotifers fed with zinc-enriched yeast. Different letters above the bars mean significant difference. Data are displayed in mean±SD, n=3.

direct methods. Thus, minerals bound to an ingestible particle can be absorbed very efficiently (Penglase et al., 2011). The findings of the present study corroborate this conclusion. Also, Nguyen et al. (2008) reported that the direct addition of zinc had no effect on the zinc content of rotifers, but when enriched *Chlorella* was used, significant differences were found among the experimental groups. The effectiveness of using insoluble zinc as zinc-yeast in aquatic organisms is very important because the soluble zinc is rapidly released into the environment (Li and Robinson, 1996).

In this study, zinc-yeast had a positive effect on the population growth of rotifer, *B. plicatilis,* in the first three groups, whereas it had an adverse effect on the fourth group. Although positive effects of zinc-enriched *Chlorella* on the growth rate of the rotifer *B. plicatilis* have already been reported in the literature (Matsumoto et al., 2009), they found no change in the lorica length of zinc-enriched rotifers. Zinc raises enzyme activities and immune system function and affects the composition of rotifers (Watanabe et al., 1978a). Minerals in natural environments such as aquatic ones have a significant effect on the growth and composition of all microorganisms (Nguyen et al., 2008 ; Fujita, 1972). In our research, the maximum density of rotifers was obtained in the day 10^{th} in the 56.25 mg g^{-1} of zinc group and SGR and reproductive factors were improved in the rotifers fed with this amount of zinc-yeast. This finding is consistent with those reported by Penglase et al. (2011). They also found that the growth of rotifers is affected by minerals such as zinc, selenium, and iodine. Sarma and Tamborenea (1991) reported that SGR of *B. plicatilis* is within the range of 0.1-2, but most species show SGR of less than 0.5 of the days. Our findings revealed that rotifers fed with enriched-yeast possessed greater SGR compared to that of the control group. Improvements in growth could be attributed to better metabolism, enzyme activities, and immune system resistance (Nordgreen et al., 2013). In particular, the SGR of rotifers depends on the amount of food mineral (Watanabe et al., 1978a; Hamre-Srivastava et al., 2008).

In the present study, the highest amount of zinc content (822.5 μg g^{-1} of zinc in rotifer) was observed in the group fed with enriched yeast containing 132.93 mg g^{-1} zinc. In line with the present results, the zinc content of *Daphnia* has been investigated in different concentrations of zinc in media culture (Muyssen et al., 2002). In that study, a high level of zinc increased the *Daphnia* zinc content, while its low amount had no effect on *Daphnia*. The zinc content of enriched rotifers fulfils the larvae requirements of marine fish [15-40 mg kg^{-1} of the food (NRC, 1993)]. Watanabe et al. (1978a) reported that proximate composition of rotifers varied in different food sources such as yeast and algae, but their mineral composition remained constant. Moreover, they reported that zinc content of rotifers without enrichment was approximately 80 μg g^{-1} dry weight. On the contrary, others have reported positive effects of minerals through the enrichment of yeast or algae (Matsumoto et al., 2009; Fujita, 1972; Takahashi et al., 2005).

The amounts of Fe in treatments corresponded to the rises in zinc level, but the trend of Mn content was in reverse to the Fe and zinc contents. This finding is in line with that reported by Matsumoto et al. (2009). They found that Mn and Cu levels decreased with an increase in zinc levels of rotifers. These effects of minerals in rotifers may indicate an antagonistic interaction to keep mineral homeostasis. In addition, presumably, the Mn content of rotifers is affected to a great extent by a host of nutrients or materials. Additional studies are warranted to elucidate on this finding. Likewise, Nguyen et al. (2008) found an antagonistic effect of zinc and Mn on *Artemia*. They reported that, in enriched *Artemia* nauplii with both zinc and Mn, the level of Mn reduced, but in the enrichment solely with Mn, the Mn content of *Artemia* nauplii was more than previous one.

Conclusion

This study found that yeast with 56.25 mg g^{-1} of zinc had an improved effect on the growth and reproductive factors of rotifers. On the other hand, the highest amount of zinc content was obtained in rotifers fed with yeast containing 132.93 mg zinc. Thus, the employment of this indirect method for the enrichment of rotifers was found to have no negative effect on the Fe and Cu contents of rotifers. On the other hand, the amount of Mn decreased compared to the one found in the control group.

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Int. J. Aquat. Biol. (2018) 6(2): 88-94
E-ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

چکیده فارسی

تاثیر مخمر نانوایی غنی شده با روی بر رشد و ترکیب مواد معدنی روتیفر آب شور *Brachionus plicatilis*

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چکیده:
روتیفرها زئوپلانکتون مهم در تغذیه لارو ماهیان در تفریخگاه‌های تجاری ماهیان می‌باشند. مقادیر روی موجود در روتیفرهای پرروشی به‌دلیل محدویت مواد غذایی نیاز لارو ماهیان را تامین نمی‌کند. گزارش شده که افزودن مستقیم روی به محیط کشت روتیفر تاثیری بر میزان روی آن‌ها ندارد، بنابراین در تحقیق حاضر تاثیر مخمر غنی شده با روی بر رشد و ترکیب مواد معدنی روتیفر آب شور *Brachionus plicatilis* مورد بررسی قرار گرفت. برای این منظور ۴ تیمار غذایی شامل (۱) مخمر بدون غنی سازی (گروه شاهد)، (۲) مخمر حاوی ۲۱/۲۳ میلی گرم روی در گرم مخمر، (۳) مخمر حاوی ۵۶/۲۵ میلی گرم روی در گرم مخمرو (۴) مخمر حاوی ۱۲۳/۹۳ میلی‌گرم روی در گرم مخمر و هر کدام با سه تکرار در کشت‌های روتیفر به‌مدت ۱۰ استفاده شدند. در پایان آزمایش در هر کدام از تیمارها نرخ رشد ویژه، تعداد کل روتیفر، تعداد تخم‌ها، تعداد تخم‌های چسبیده به روتیفر مورد ارزیابی قرار گرفت. همچنین مقادیر مواد معدنی روتیفرها با استفاده از جذب اتمی مورد سنجش قرار گرفت. نتایج نشان داد که استفاده از مخمر حاوی ۵۶/۲۵ میلی‌گرم روی در گرم مخمر به‌طور معنی‌داری سبب بهبود شاخص های رشد و تولید مثل گردید. حداکثر تعداد روتیفر، تعداد کل تخم‌های چسبیده به روتیفر و تعداد کل تخم‌ها به ترتیب ۲۷۴ (روتیفر در میلی‌لیتر)، ۲۹/۳ (تعداد در میلی‌لیتر) و ۳۶ (تعداد در میلی‌لیتر) به‌دست آمد. براساس آنالیز ترکیب مواد معدنی بیشترین مقدار روی در تیمار ۴ به مقدار ۸۲۲ میکروگرم بر گرم روتیفر بود. حداکثر مقدار آهن (۱۳/۸۴ میکروگرم بر گرم روتیفر) و منگنز (۱۵/۲۲ میکروگرم بر گرم روتیفر) به‌ترتیب در تیمارهای ۴ و شاهد حاصل شد اما مقدار مس در بین تیمارها تفاوت معنی‌داری را نشان نداد. در جمع بندی می‌توان گفت که مخمر غنی شده با روی باعث بهبود فاکتورهای رشد و تجمع روی در بدن روتیفر می گردد.

کلمات کلیدی: مخمر نانوایی، روی سولفات، مخمر غنی شده، ترکیب مواد معدنی.