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Growth promotion efficacy of gold nanoparticle supplementation diet in *Oreochromis mossambicus*

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ABSTRACT

Nutritionally balanced aquafeed is the crucial input for the successful fish production. Currently nanotechnology paves a way to progression of high performing fish. Nanotechnology can be applied for developing high nutrient feeds. Among all metal nanoparticles, gold nanoparticles (AuNPs) have been considered to be highly useful platform for the efficient carrier system due to their well studied synthesis, easy surface functionalization, biocompatibility and less toxicity. The aim of the present study is to investigate the effect of gold nanoparticulated diet on the growth of *Oreochromis mossambicus*. Special attention was given to the effect of the AuNP with different surface chemistries on the aquaculture candidate by synthesizing AuNP in two different routes, chemical (sodium borohydride reduction method-AuNP), and green synthesis (using aqueous extract of *Emblica officinalis* fruit extract-*EO*AuNP). Prior to exposure experiment of AuNP and *EO*AuNP on *Oreochromis mossambicus*, the physicochemical properties of the Au colloids are studied using Ultraviolet-Visible spectroscopy (UV-Vis spectroscopy) and FTIR (Fourier Transform Infrared Spectroscopy) studies. I*n vivo* charecterization of NPs internalized in hepatic tissue of *Oreochromis mossambicus* reared on AuNP and *EO*AuNP supplemented diet were done using TEM studies. The weight gain (WG%), specific growth rate (SGR), feed conversion ratio (FCR) and feed efficiency rate (FER) of the test candidate were assessed. It was observed that low concentrations of added *EO*AuNP to fish feed achieved WG%, SGR, FER more than the conventional *Emblica officinalis* extract (1ml/100 g). Significant reduction ($p<0.05$) in feed conversion ratio (FCR) with the increase in specific growth rate was also observed. Supplementation of AuNP registered retrogressive growth in the test candidate. The results suggest that *EO*AuNP could be used as a promising feed additive in aquaculture.

Key words : *Gold nanoparticles, Green synthesis, Chemical synthesis, Emblica officinalis, Oreochromis mossambicus*

Introduction

Over the last two decades, Indian aquaculture achieved a six and half fold growth with freshwater aquaculture. India is blessed with 2.36 million ha of ponds, 0.19 million ha of canals and rivers and 3.15 million ha of reservoirs. In recent years freshwater aquaculture has increased to about 80% (DADF, 2017). In India the bottle neck in aquaculture development are good quality seed availability, feed availability coupled with land leasing tenure and climate changes. To improve the efficiency of aquaculture production, improvement in feed technology and production of high quality nutrient feed is a prime requisite. (Kaushik, 1995). In intensive aquaculture, supplementary feed formulation of high nutritional quality and their bioavailability is important. Application of phytobiotics, to promote growth enhancement and improve the health status of fishes is now a major focus in fish nutrition research. Nutraceuticals play a crucial role in promoting growth and immunomodulation in fish. Nanotechnology is effective in the delivery of nutraceuticals in fisheries. This novel technology aims to enhance the bio accessibility, bioavailability, and efficacy of the nutrients.

Presently there are many studies on delivery of organic micronutrients and minerals via nanocargo to fishes. Methods of synthesis is crucial to achieve intended quality of NPs. Recently research focusing biogenic NPs are gaining much attention. In biogenic NP synthesis crude extract from microbes, plants, animals and biomolecules are used as reduction and stabilizing agent for inorganic NPs.Such methods avoid hazardous toxic reagents and reaction condition (Vigneshwaran, 2006). Organic and inorganic NPs are attempted for the delivery of micronutrients in aquafeed. The most suitable means of NP deliver is via oral route in feed

Nanotechnology provides sophisticated tools for the aquatic health, fish biotechnology, reproduction of fishes etc. Nanotechnology tools such as nano vaccines, nanomaterials (NMs) nanosensors etc reforms aquaculture. Metal NPs like gold (Au), copper (Cu), and silver (Ag), with interesting properties are widely used in aquaculture industry. AuNPs are less toxic. AuNPs can be conjugated wide variety of organic and biological molecules. In the present investigation, an efficient method for the green synthesis of colloidal gold has been demonstrated using medicinally important *Emblica officinalis* fruit extract. Sodium borohydride reduction method is followed for the chemical synthesis of AuNP. These fully characterized AuNPs was used as an aquafeed supplement on aquatic candidate *Oreochromis mossambicus*.

The present study evaluates the effect of different doses of *Emblica officinalis* fruit extract and gold colloids with different surface chemistry incorporated diet on the growth and feed utilization of *Oreochromis mossambicus*

Materials and Methods

Synthesis of AuNPs

The chemicals used in this study were mostly dried and were prepared according to the requirements. Auric chloride, trisoduim citrate and sodium borohydride were obtained from Sigma Aldrich. In chemical synthesis, AuNPs were synthesized by Sodium borohydride reduction method. For this purpose, the reduction reaction between 0.3 M tetrachloroauricacid ($HAuCl_4.3H_2O$) and 0.3M Sodium borohydride was used 0.3 M Trisodium citrate was used as the capping agent. Green synthesized AuNPs (*EO*AuNP) was prepared using 1% aqueous extract of *Emblica officinalis* (Ankamwar *et al.,* 2005) and stored under refrigeration for further use.

Experimental techniques used for the characterization of AuNPs

After synthesis of metal nanoparticles, analytical and imaging techniques are employed to analyse physicochemical data of nanoparticles. The characterization was done by measuring the absorption spectrum using UV-Visible spectroscopy using Dynamica Halo DB -20 Spectrophotometer. Structural analysis was done by electron microscopy. Images were taken in Hitachi H-7650. Fourier Transmission Infrared spectroscopy (FTIR) was employed to assess the surface chemistry of the synthesized AuNPs. FTIR spectra of the samples were measured using BRUKER-Alpha-FTIR (ATR). Internalization of nanotized *Emblica officinalis* was confirmed by hepatic tissue TEM studies of the test candidate**.**

Experimental design

Juveniles of *Oreochromis mossambicus* in the range 7±0.55cm length and 5±0.62gm were collected from ADAK, Varkala, quarantined and stocked at 20 fish/1000L tanks and maintained at laboratory conditions. Basal feed was prepared as out lined by Hardy *et al.* (1978). Experimental diets were prepared by incorporating 0.3 M of 10 µl $HAuCl_4$ incorporated green synthesized AuNP and same concentration of synthetic AuNP per 100g of basal feed. Non-treated control diets too were prepared. The experimental schedule was for 60 days and the fishes were fed at 2% of body weight twice daily. The growth parameters WG%, SGR, FCR and FER were assessed in fishes at an interval of 30 days. The randomly selected fishes were divided into five batches, each tank consisting of 30 fishes in triplicate. Group 1 : Control fish fed with the basal feed. (CF), Group 11: Fishes reared on 1% *Emblica officinalis* incorporated diet.(T1), Group 111 : Fishes reared on 2% *Emblica officinalis* incorporated diet. (T2) Group 1V: Fishes reared on *EO*AuNP incorporated diet (T3), Group V : Fishes reared on AuNP incorporated diet (T4). For all the groups, each results are reported in means of three analysis.

Nutritional Index analysis

At the end of experimental regime, the nutritional index parameters such as weight gain (WG), weight gain%(WG%), specific growth rate (SGR), food conversion ratio (FCR), Feed efficiency (FE)were assessed by the method of Tekinay and Davies, 2001.

Weight gain (WG) = Final weight - Initial Weight

Weight gain% (WG%) =

$$\frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

Specific Growth Rate (SGR) =

$$\frac{\text{log of Final weight(g) - log of initial weight(g)}}{\text{No: of days}} \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

Feed Efficiency Rate (FER) =

$$\frac{\text{Body weight gain (g)}}{\text{Feed intake (g)}} \times 100$$

Statistical ananlysis

Data were statistically analysed by Analysis of Variance (ANOVA) along with Duncan's Multiple Range Test (DMRT) (Duncan, 1955) which was applied to find out significant difference between various treatment means and control means for the observed parameters. The growth in terms of total weight of *Oreochromis mossambicus* was expressed as a regression model against experimental days after natural logarithmic (ln) transformation.

Results and Discussion

Synthesis of AuNPs

An immediate change in the solution color from yellow to purple was found in both the reaction mixtures produced through chemical and green synthesis methods (Fig. 1, 2). The appearance of violet color indicates the formation of AuNPs in the reaction mixture and the efficient reduction of Au^{3+} to Au^{0}The formation of colloidal AuNPs was investigated using UV -Vis absorption .

Fig. 1.

Fig. 2.

UV absorption spectra of AuNPs

The value absorption spectrum of *EO*AuNP was in agreement with the expected plasmon absorbance of the AuNPs (Fig. 3). For the chemically synthesized NPs (AuNP), the peak is found to be smaller around 520 nm (Fig. 4). In both the reaction mixtures, the observed intensity of SPR peak suggests the complete reduction of AuNPs.

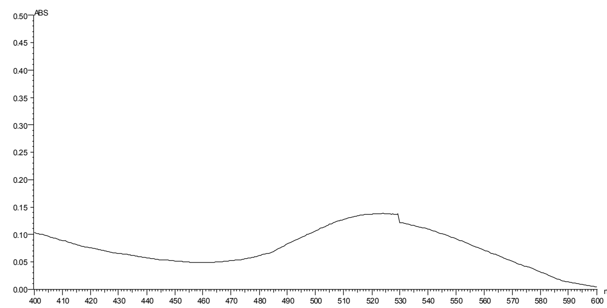

Fig. 3. UV spectrum of *EO*AuNP

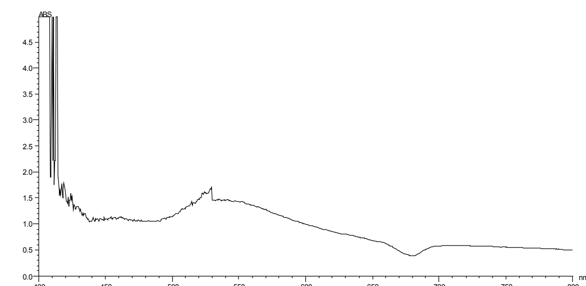

Fig. 4. UV spectrum of chemically synthesized AuNP

Fourier Transmission Infrared Spectroscopy

FTIR spectroscopy is conducted to identify the functional groups present on NPs. The high similarity between extract and colloidal solutions indicated that the same compounds existed in both media (Fig. 5, 6). Despite the similarity between extract and colloidal solution spectra, some absorption peak exhibited marked shifts in their position. It was assumed that shifts in peak position were related to

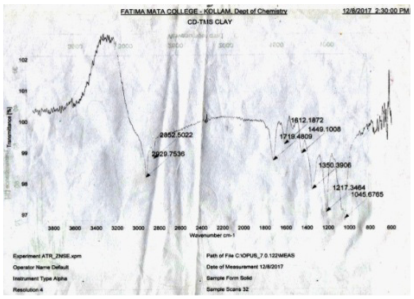

Fig. 5. FTIR of *Emblica officinalis*

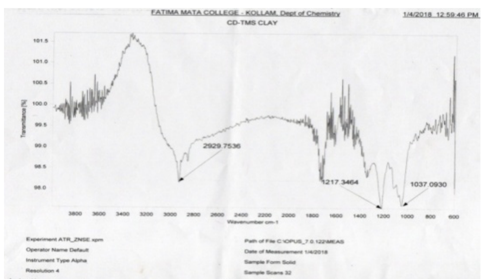

Fig. 6. FTIR of *EO*AuNP fruit extract

adsorption of extract constituents on to the AuNP surfaces. The absorption peak that exhibited major shifts and their possible interpretations are presented in Table 1.

FTIR Measurements

The FTIR measurements reported in Table 1 were carried out in an attempt to obtain information about the nature of the organic protection layer that surrounds the AuNPs. The vibrational frequency found with the AuNP solution correspond to the C-O bond. Based on similar analysis by Guan *et al.,* (2007), the large shifts of some peaks in the spectra following AuNP formation are due to the influence of the nearby metal surface. Therefore, the shifted peak, listed in Table 1, are assumed to be associated with the organic matter around the AuNPs formed during their synthesis. It is obvious from the spectrum of biomass and AuNPs that the C-O- bond possibly from the ascorbic acid lead to the bioreduction. Ascorbic acid could adhere to the metal nanoparticles by the interaction through C-O- bond. Surface functionalization of AuNPs has a huge impact on NP behaviour. Ahamad *et al.,* (2010) reported in *Ocimum basilium*, transformation of rosmarinic acid and luteolin to the keto form from their enol form cause Ag ion reduction to Ag nanoparticles. Noruzi *et al.,* (2001) reported the functional groups NH_2,OH and C-O groups as the stabilizing agents for AuNPs from rose petals (*Rosa hybrida*)

In vivo charecterization of AuNPs internalized in hepatic tissue of _Oreochromis mossambicus_ reared on AuNP and EOAuNP supplemented diet based on TEM studies

Electron microscopy observation exhibited a clear images of the AuNPs present in the liver tissues of *Oreochromis mossambicus* fed with AuNP and *EO*AuNP supplemented diet.TEM images were presented in Figure 7 and 8 for green synthesized and

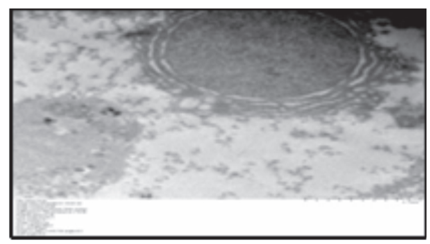

Fig. 7. TEM micrographs of hepatic tissue of *Oreochromis mossambicus* reared on *EOAuNP* supplemented diet.

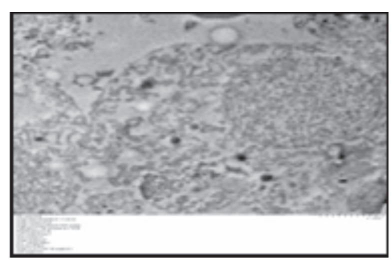

Fig. 8. TEM micrograph of hepatic tissue of *Oreochromis mossambicus* reared on AuNP supplemented diet.

Table 1. Peaks with shift in their positions in the extract compared to the *EO*AuNP; shift values are given as the subtraction of the peak position in the *EO*AuNP solution from the position in the extract

Extract source	Peak position in extract (cm^{-1})	Peaks position in *EO*AuNP solution (cm^{-1}).	Shift in position (cm^{-1})	Types of Chemical bond.
Emblica officinalis	1045.6765	1037.0930	8.5835	C-O- bond

chemically synthesized AuNPs respectively. The shape of AuNPs were found to be spherical in shape. No aggregation or localized accumulation of NPs was found in either of the liver samples.

Dobrovolskaia and McNeil (2007) reported particles less than 500 nm is internalized by phagocytosis and smaller particles entre by receptor mediated endocytosis. Nativo *et al., (*2008) in TEM studies showed AuNP trapped in endosomes. Hillyer and Albrecht (2001) reported AuNPs after oral administration cross the gastrointestinal wall readily.Scown (2001) reported significant uptake of CeO2 NP that is administrated via oral route in the liver of Zebra fish.

Growth

T1 fortified diet showed intermediate growth performance than those that fed on control and T3 supplemented diet. Inverse relationship between the fish growth and increasing dose of *EO* (T2) was found with the experimental animals. Growth rate of fish fed on T3 diet surpassed all other experimental groups and control at the end of the experiment. The fishes reared on (T4) diet did not gain weight compared to control and other treatment groups (Figure 9, 10, Table 2, 3)

Regression Analysis

The growth in terms of weight of *Oreochromis mossambicus* was expressed as a regression model against days of observation after natural logarithmic transformation. The regression graph and regression

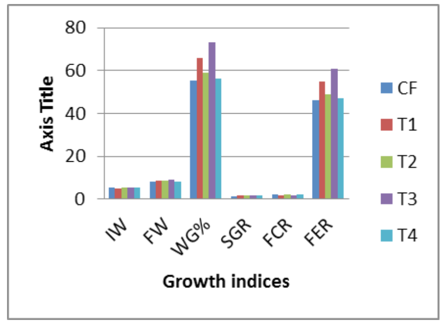

Fig. 9. Growth indices of *Oreochromis mossambicus* reared on experimental diet for 30 days

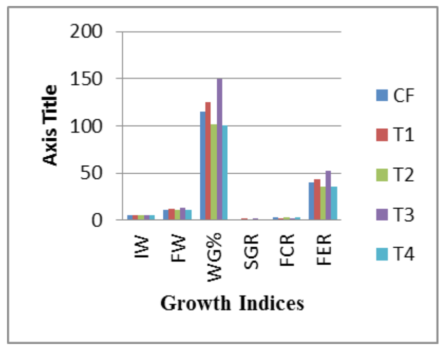

Fig. 10. Growth indices of *Oreochromiss mossambicus* reared on experimental diet for 60 days

Table 2. Growth indices of *Oreochromis mossambicus* reared on experimental diet for 30 days

Growth Indices	C.F	T1	T2	T3	T4
Initial weight (g)	5.24±0.24	5.12±0.13	5.30±0.28	5.34±0.20	5.21±0.28
Final weight (g)	8.14±0.14	8.50±0.08	8.42±0.08	9.24±0.12	8.15±0.15
Weight gain%	55.34^{a}±0.01	66.00^{d}±0.02	58.87^{c}±0.16	73.00^{e}±0.28	56.42^{b}±0.04
SGR	1.46^{a}±0.03	1.70^{b}±0.10	1.53^{a}±0.01	1.83^{c}±0.08	1.50^{a}±0.03
FCR	2.10^{d}±0.04	1.81^{b}±0.04	2.03^{c}±0.02	1.64^{a}±0.01	2.12^{d}±0.02
FER	46.00^{a}±0.08	55.01^{d}±0.02	49.05^{c}±0.03	60.86^{e}±0.04	47.00^{b}±0.03

Values in the same row with different superscript letters vary significantly. (p<0.5). Each value represents the mean ± of three separate experiments

Table 3. Growth indices of *Oreochromis mossambicus* reared on experimental diet for 60 days

Growth Indices	C.F	T1	T2	T3	T4
Initial weight (g)	5.24±0.24	5.12±0.13	5.30±0.28	5.34±0.20	5.21±0.28
Final weight (g)	11.28±0.15	11.52±0.10	10.75±0.08	13.07±0.28	10.47±0.06
Weight gain%	115.26^{c}±0.10	125^{d}±0.04	102.00^{b}±0.01	150.00^{e}±0.20	100.96^{a}±0.05
SGR	1.28^{b}±0.02	1.37^{c} ±0.03	1.20^{a}±0.01	1.53^{d}±0.02	1.16^{a}±0.05
FCR	2.49^{c}±0.02	2.30^{b}±0.05	2.80^{d}±0.02	1.92^{a}±0.02	2.83^{d}±0.04
FER	40.02^{c}±0.04	43.40^{d}±0.06	35.70^{b}±0.08	52.00^{e}±0.05	35.32^{a}±0.03

Values in the same row with different superscript letters vary significantly. (p<0.5). Each value represents the mean ± of three separate experiments equation for weight gain and exposure period are given in Figures 11 to 15. Since each treatment group showed individual growth performance, regression analysis was observed separately for each treatment. High slope value for weight gain and exposure days was exhibited by T3 supplemented group followed by T1 and control. Control group showed better b value than T2 and T4. T4 group showed least b value.

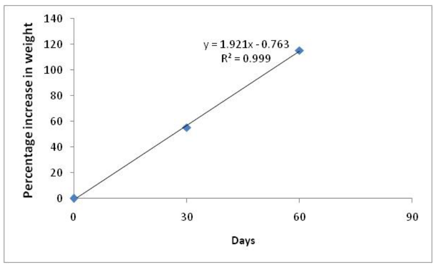

Fig. 11. Regression between weight and growth period of *Oreochromis mossambicus* reared on CF

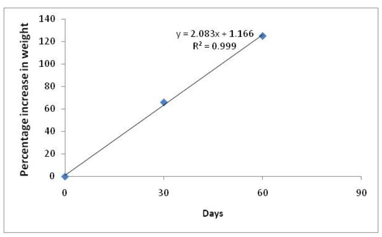

Fig. 12. Regression between weight and growth period of *Oreochromis mossambicus reared* on T1

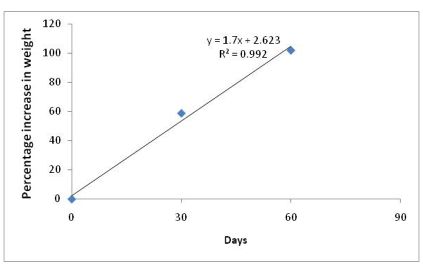

Fig. 13. Regression between weight and growth period of *Oreochromis mossambicus* reared on T2

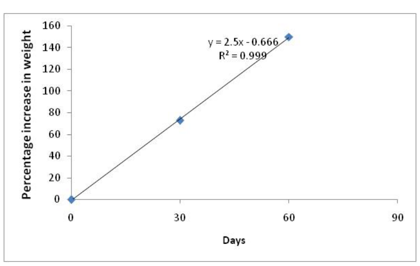

Fig. 14. Regression between weight and growth period of *Oreochromis mossambicus* reared on T3

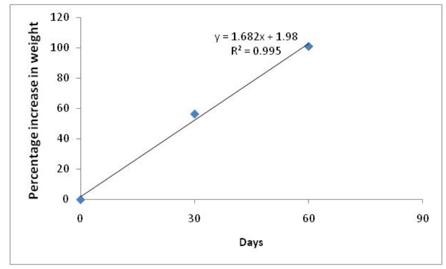

Fig. 15. Regression between weight and growth period of *Oreochromis mossambicus* reared on T4

Regression between weight and growth period of *Oreochromis mossambicus* in different treatments

It was observed that low concentrations of added nanotized *Emblica officinalis* (*EO*AuNP) to fish feed achieved SGR, FER, WG% more than the conventional *Emblica officinalis* extract (1ml/100 g). According to *Rather et al.,* (2011), this may be due to the change in their properties due to the reduction of the herbal extract from their macromolecule state to nanoscale. In the present study retention of *EO*AuNP in blood stream for prolonged period might have enhanced its bioavailability. Enhanced growth with T1 diet could be due to phytochemicals in fish diets which boosted monogastric organisms, essential vitamins and nutrients with improvement in digestion of feed. Soltan and El-Laithy, (2008) reported improved FCR, WG in Nile Tilapia fed on dietary *Foeniculum vulgare* powder. Comparison of growth performance of fishes fed with 1% and 2% *EO* incorporated diets suggests that not all herbal doses can improve fish growth. Gouveia *et al.*, (1993) reported better growth performance in rain bow trout with 20% inclusion of the plant protein in diet but retrogressive growth with 45% inclusion. According to Kroghdahl, (1989); Masumoto *et al.*, (1996) anti nutritional factors (ANF), low digestibility of plant proteins, and poor palatability causes reduced growth in fishes fed on high level of plant protein fortified diet.

Significant reduction ($p<0.05$) in feed conversion ratio (FCR) with the increase in specific growth rate in fishes reared on *EO*AuNP indicates the superior quality as a feed additive. Enhanced feed utilization in fishes fed with diet containing T3 suggests that nano form of *Emblica officinalis* improved protein and diet digestibility, which resulted in better growth and feed efficiency.In a comparative study of *Cyprinus carpio* reared on nanoginger and ginger extract, improved growth and immunity is found with nanoform than the traditional ginger Korni and Khalil (2017). There was retrogressive growth in the growth of fish with the dietary inclusion of AuNP (T4) in fish diet. Increased absorption of some compounds lead to bioaccumulation, reduces excretion and cause toxic effects. In T4 supplemented diet FCR found to increase. This reflects impairment of growth performance and feed utilization by the AuNPs synthesized through chemical method. Akin results are observed by Nasrin Aktar *et al.,* (2018) in channel cat fish fed on Fe NPs.

Conclusion

Growth and metabolic performances are associated with homeostatic processes that mirror the efficiency of nutrients incorporated in feed. Present results clearly indicates nanotized *Emblica officinalis* diet elevated growth and stimulated appetite and proved to be an excellent feed additive. Green nanotechnology can be applied in aquaculture to improve animal production in pond culture. Here application of nanotechnology is updated for the enhancement of growth parameters in *O.mossambicus* using nanotized *Emblica officinalis* fortified diet.

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Competing Interests

Authors have declared that no competing interests exist.

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