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Potential Use of Eucalyptus Seedling in Recycling of Fish Farming Wastewater in Agriculture

Dalva Paulus^{1*}, Ezequiel Toffoli², Celso Eduardo Pereira Ramos¹ **, Claúdia de Andrade Moura1 , Dislaine Becker3 and Iara Emanoely Francio3**

1 Department of Agronomy, Federal University of Technology – Paraná, Campus Dois Vizinhos, 85660-000, Dois Vizinhos, Paraná, Brazil. ² Federal University of Technology - Paraná, Campus Dois Vizinhos, 85660-000, Dois Vizinhos, Paraná, Brazil. ³ Postgraduate Program in Agroecosistemas, Federal University of Technology – Paraná, Campus Dois Vizinhos, Brazil.

Authors' contributions

This work was carried out in collaboration among all authors. Authors DP and ET designed the study and wrote the first draft of the manuscript. Authors DP, ET, CEPR, CAM, DB and IEF performed the experiments. Authors DP, ET, CEPR, CAM, DB and IEF participated in fieldwork and laboratory analysis. Authors DP and ET managed the analyses of the study. Authors DP, ET, CEPR, CAM, DB and IEF managed the literature searches. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Aims: The reuse of fish wastewater in agricultural activities such as the production of seedlings in commercial nurseries has great potential to minimize production costs and to reduce environmental impacts due to the inappropriate disposal of this waste. The objective of this study was to evaluate the growth, development and quality of *Eucalyptus grandis* W. Hill ex Maiden seedlings produced with different wastewater concentrations from fish farming.

Study Design: The fertigation treatments were using fish farming (Tilapia) wastewater from tanks (FW), daily nutrient solution (DNS), and the combinations of 50% FW + 50% DNS, and 25% DNS + 75% FW, applied daily.

**Corresponding author: E-mail: dalvapaulus@utfpr.edu.br;*

Place and Duration of Study: The experiment was carried out from August to December 2016 at the Federal University of Technology of Paraná, Brazil.

Methodology: The macro and micronutrient contents in leaf tissue, seedling height, stem diameter, the largest root length, leaf area, fresh and dry shoot and root mass and Dickson quality index were all evaluated.

Results: The nutrient contents present in the leaf tissue were adequate for the nutritional demand of the eucalyptus. The seedling quality index (DQI) indicated that the daily nutrient solution (0.21) and 50% FW + 50% DNS (0.20) generated balanced seedlings regarding height and biomass accumulation.

Conclusion: Fertigation of eucalyptus seedlings can be carried out with wastewater from fish farming, but it is necessary to complement the fertilization with other nutrient sources to produce quality seedlings which are suitable for field transplantation.

Keywords: Eucalyptus grandis; effluent; nutrients; tilapia.

1. INTRODUCTION

Water scarcity has become one of the greatest challenges facing the world due to the crisis in its supply, which particularly affects semi-arid regions and is a determining factor for social and economic development [1]. However, the uncontrolled use of water and the prospects of scarcity of water resources, warn of the need to search for techniques to optimize its use, contributing to the reduction of environmental impacts.

Nurseries for the production of forest seedlings are of great importance in meeting the growing demands for seedlings of this sector, due to the increase of areas with forest implantation, recovery of degraded areas and use of wood by the timber industry. However, this production system presents high water consumption, it is necessary to improve the quality of the seedlings and to redefine the water and nutritional balance, so that better quality is obtained and the environmental quality standards are met [2].

Eucalyptus (*Eucalyptus grandis* W. Hill Ex Maiden) was selected for seedling production since it is of great importance in forest plantations due to its high productivity, as well as an important raw material for the cellulose, charcoal and timber industry sectors [3].

The conservation of water resources and environmental sustainability has been a challenge for seedling production systems. The use of effluents as a source of nutrients in agricultural production systems has been a viable option for controlling environmental pollution [4]. Considering this scenario, the reuse of fish wastewater in agricultural activities such as in seedling production in commercial nurseries, has great potential to minimize production costs, mainly with fertilization and water.

Fish farming has become an expanding animal protein production system, with potential for income generation in rural properties. In this sense, Nile tilapia (*Oreochromis niloticus*) has a good level of tolerance to various environmental conditions and high economic value [5], being a fish species that is very used in integrating agricultural activities, such as aquaponics.

The intensification of fish production generates effluents such as manure and wastewater, and when misappropriated can become an environmental problem in water resources [6]. In order to further expand aquaculture activities, it is necessary to develop and apply new technologies in which water and nutrients can be recovered during the cultivation process in order to reduce the impact on the environment [7].

The use of effluents in seedling production has become a sustainable alternative. Irrigation with wastewater offers socio-economic and environmental benefits, mainly in reducing the discharge of effluents into rivers and water sources and recovering nutrients [8]. Discharge of tank effluents in aquaculture contains dissolved nutrients such as nitrogen and phosphorus, specific organic and inorganic compounds, and total suspended solids. These constituents mainly originate from unconsumed feed and metabolic fish residues [9], which present significant amounts of nutrients for agricultural crops [10].

Some studies in the literature indicate the possibility of using fish farming wastewater in cultivating Roman lettuce (*Lactuca sativa*) [11]

This study is important because it integrates suitable agricultural activities for small-scale agriculture, allowing to reduce the amount of fertilizers applied during cultivation and to diminish the environmental impact of the effluents from fish production. In this sense, the objective was to evaluate different doses of tilapia wastewater on the growth, development and quality of eucalyptus seedlings.

2. MATERIALS AND METHODOLOGY

2.1 Plant Material and Growing Conditions

The experiment was conducted from August to December 2016 in the experimental area of the Federal University of Technology of Paraná (25º42'52" S and 53º03'94" W, altitude 530 m), in a model-in-arc protected environment covered with plastic film of 150 microns.

For production of the *Eucalyptus grandis* W. Hill Ex Maiden seedlings, 125 cm^3 polypropylene tubes filled with Carolina Soil® commercial substrate were used. Six commercial eucalyptus seeds were sown per commercial tube with F1 degree of improvement, LCFA004 cultivar, obtained in 2016 from the Institute of Research and Forest Studies (IPEF), produced in the clonal orchard of seeds (PCS) in Piracicaba, São Paulo Brazil. Thinning of the seedlings was carried out at 25 days after sowing (DAS), in which the best seedling per seed lot tube was selected.

The fish wastewater used in the study came from the Tilapia species (*Oreochromis niloticus*) in an intensive rearing system, with a density of 4.5 kg $m⁻³$ of water. The fish were kept in a excavated nursery model tank, with capacity of 1500 liters of water, coated with double-sided plastic canvas in direct contact with the soil, and wood was used on the edges of the tank to support it. The tank water was renewed daily in percentage of 50% of the volume, with motor pump of 1CV.

The oxygen levels were in the 27.4 mg L^{-1} range of the nursery water and were maintained with submersed motor pumps, which performed the water movement at intervals of 15 minutes. The oxygen concentration was monitored with a MO-900-Instrutherm® portable oximeter three times a week at four points in the tank.

The initial biometry of the fish was 3.2 kg, quantified every 20 days, with a final value of 8.5 kg at 111 DAS. The adjusted feed quantity, considering the supply of 3% of the live fish mass [13], was divided into a supply of Anhambi® brand commercial feed in the morning and another at the end of the day.

The fertigation treatments on the seedlings began at 35 DAS, with daily frequency maintaining a 1 mm day¹ irrigation stream volume using a watering can for better irrigation uniformity. The remaining water demand of the eucalyptus plants corresponded to 5 mm day⁻¹ volume [14] with irrigation by sprinkler.

The electrical conductivity and pH of the treatments were monitored every two days with a pHgameter and portable conductivity meter (HI 98130 Hanna®).

The physico-chemical analyzes of the evaluated water treatments were performed according to the criteria of the Standard Methods for the Examination of Water and Wastewater [15], determined at the Applied Ecology Laboratory of the "Luiz de Queiroz" College of Agriculture of the Universidade de São Paulo - Brazil (Table 1).

The meteorological data (temperature, relative air humidity, and solar radiation) were obtained every 15 minutes using AK 172 Akso® dataloggers installed in meteorological shelters, located in the center of the protected environment.

2.2 Treatments and Experimental Design

The experimental design was randomized blocks with four treatments and five replications, in which the treatments were composed of fish wastewater (FW); and daily nutrient solution (DNS) as proposed by Gonçalves and Benedetti [16], composed of 0.45 g L^{-1} calcium nitrate fertilizer sources, 0.30 g L^{-1} ammonium nitrate, 0.25 g L^{-1} mono ammonium phosphate, 0.30 g L^{-1} 100 potassium nitrate, 0.25 g L⁻¹ magnesium sulfate, 0.25 g L⁻¹ ammonium sulfate, and 0.50 g L^{-1} boric acid; and the combinations 50% DNS + 50% FW and 25% DNS + 75% FW. Each experimental unit was composed of 100 seedlings.

Parameter	Unity	FW	DNS	50% FW + 50% DNS 75% FW + 25% SND	
рH	H ₂ O	6.7	5.6	5.9	6.4
Electric conductivity	$mS m-1$	0.4	2.6	1.39	0.73
Chloride (CI ⁻)	mg L^{-1}	2.0	4.0	2.0	1.0
Sulfate $(SO42)$	mg L^{-1}	5.2	312.1	264.5	56.2
Phosphorus (P)	mg L^{-1}	1.0	79.1	48.5	0.3
Ammoniacal nitrogen (N-NH ₃)	mg L^{-1}	1.8	20.0	30.9	13.3
Potassium (K^+)	mg L^{-1}	0.7	132.0	66.0	
Calcium (Ca^{2+})	mg L^{-1}	3.3	77.3	41.5	
Magnesium (Mg^{2+})	mg L^{-1}	0.4	14.2	9.7	
Iron (Fe)	mg L^{-1}	0.0	0.0	0.0	
Copper (Cu)	mg L^{-1}	0.0	0.1	0.1	
Manganese (Mn)	mg L^{-1}	0.0	0.1	0.1	
Boron (B)	mg L^{-1}	0.7	10.6	7.5	
Sodium (Na)	mg L^{-1}	10.0	15.0	19	16
Zinc(Zn)	mgL^{-1}	0.0	0.0	0.0	

Table 1. Chemical analysis of the water used in the fertirrigation of eucalyptus seedlings

Notes (-) element not present in the sample

2.3 Evaluated Parameters

The biometric measurements were shoot height (cm) obtained with a millimeter ruler, from the stem base to the apex of the plant; stem diameter (mm) measured at the stem height with a digital caliper. The biomass accumulation of the seedlings was determined by destructive method with periodicity of 15 days, in which the shoot fresh mass (leaves and stems) and root fresh mass were measured on a precision scale (0.0001 g). Then the roots and shoot were dried in a forced air circulation oven at a temperature of 55 \pm 3°C until reaching constant mass to obtain the shoot and root dry biomass. Eight central plants were used per experimental unit for each evaluation in the periods of 50; 65; 80; 95 and 110 days after sowing.

The leaf area was determined at 111 DAS with a leaf area meter (CID Bio-Science, model CI-202, with photometric cell). The length of the largest root was evaluated with a millimeter ruler, measuring from the stem collar to the end of the longest root; the Dickson Quality Score was calculated based on the equation of Dickson et al. [17].

Completely expanded leaves were collected from the middle third of the plant [18] at 110 DAS in order to determine the macronutrient and micronutrient content of the leaf tissue. The leaf tissue samples were analyzed by the Laboratory of Applied Ecology of the "Luiz de Queiroz"

School of Agriculture of the Universidade de São Paulo – Brazil.

2.4 Statistical Analysis

The variances of the treatments were tested for homogeneity by the Bartlett test and the data normality by the Shapiro Wilk test. The data were submitted to analysis of variance (F-Test) and the means were compared by the Scott Knott test (*P=0.05*), using "SAS Studio" [19].

3. RESULTS AND DISCUSSION

The meteorological conditions during the experiment conduction had an average temperature of 19.8°C, relative average air humidity of 71.1°C, and average solar radiation of 1214.5 $kJm⁻²$ (Table 2). The meteorological conditions during the cultivation were within the proper ranges for eucalyptus seedling growth [20].

Nutrient solutions significantly influenced nutrient content in leaf tissue. The highest levels of nitrogen, phosphorus and potassium in leaf tissue were determined under the 50% FW + 50% DNS treatment (Table 3). It was verified that nitrogen was the mineral element that was extracted in greater quantity by eucalyptus seedlings, followed by potassium, calcium, magnesium, phosphorus and sulfur. For the micronutrients, the descending order of extraction were manganese, iron, boron, zinc, copper.

Results of nutrient contents of leaf tissue were compared with data from the literature. The nutrient content bands in the eucalyptus leaf tissue considered adequate by Gonçalves [21] are: 13.5-18.0 g kg⁻¹ nitrogen (N); 0.9-1.3 g kg⁻¹ phosphorus (P) ; 9-13 g kg⁻¹ potassium (K); 6-10 g kg $^{-1}$ calcium (Ca); 3.5-5.0 g kg $^{-1}$ magnesium (Mg); 1.5-2 g kg⁻¹ sulphur (S); 400-600 mg kg⁻¹ manganese (Mn); 35-50 mg kg $^{-1}$ zinc (Zn); 30-50 mg kg $^{-1}$ boron (B); 7-10 mg kg $^{-1}$ copper (Cu); and 150-200 mg kg^{-1} iron (Fe).

The nitrogen macronutrient present in the leaf tissue produced with fish wastewater was below the range recommended by Gonçalves [21] for eucalyptus seedling production. The other treatments obtained N, K, Ca, Mg and S values in the range recommended by the author. However, all treatments presented values above the recommended range for P. The high phosphorus concentration can be possibly explained by the ability of this cultivar to absorb the nutrient, distribute it and use it to produce biomass. The genetic materials of eucalyptus present different capacities of phosphorus absorption, translocation and use [22].

Regarding the micronutrients, B, Cu, Mn and Zn mineral elements resulted in levels within the recommended range for all treatments. Fe was within or above the appropriate range.

It can be observed that the wastewater concentration had adequate nutrient contents for the seedling growth, with the exception of nitrogen, and that the use of this waste could represent an important possibility for intensive fish farming associated with seedling production in nurseries as a way of using waste (fish excrement and food leftovers), aiming to make seedling production more sustainable by integrating the production of two activities, which results in a greater diversity of products and use of non-exploited resources.

Regarding the visual aspect of the seedlings, no signs of nutritional deficiency were observed in any of the evaluated treatments.

The presence of nutrients in wastewater is very interesting from the agronomic point of view, since these are important to improve the fertility of the growing substrate, resulting in growth and increase in crop productivity [8]. In the literature [10] verified that the use of tilapia wastewater in a water recirculation system, met the nutritional demand for the growth of butter head lettuce.

In relation to height, higher seedling growth was observed when fertigated with 50% FW + 50% DNS, with a height increase of 21.1 cm at 110 DAS (Table 4), being 21.74% higher than the treatment with daily nutrient solution. The lowest growth in height was obtained in the treatment with wastewater. Considering that N is an essential nutrient for the development of meristematic cells [23], its low concentration may have influenced the lower development of plant height in this treatment.

The minimum height parameter of eucalyptus seedlings for field transplantation is 15 cm [24]. Taking into account this height parameter, all treatments (except for the fish farming wastewater) met the appropriate height conditions for transplantation. According to Reis et al. [25], height is an important parameter for classifying and selecting seedlings in the nursery, as seedlings with heights within the recommended standards by the literature have higher survival rates in the field.

It can be stated that the 50% FW + 50% DNS seedling treatment could be transplanted at 95 DAS, according to the [24] height parameter, which means a reduction of 15 days in the nursery. This time reduction can reduce the cost of the seedlings, resulting in greater productive efficiency of the nursery.

Table 3. Nutrient contents of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) of leaves of fertigated eucalyptus seedlings with different concentrations of fish wastewater (FW) and daily nutrient solution (DNS)

	N	P		Cа	Mg			Cu	Fe	Mn Zn	
------mg kg ----------------------------g kg -------------------											
FW	$12.50c*$.45 _b	12.85 a	8.85 a	4.84a	.57 _b	50.20a	10.05a	262.40a	477.52 b	40.04 _b
DNS	15.60 h	.39 _b	12.70 a	9.63 a	5.01a	.52 _b	38.60 _b	7.05 _b	189.30 b	535.80 a	48.30 a
50% FW + 50% DNS	18.00a	.82 a	$12.95a$ 6.14 b		3.60 _b	1.94 a	50.10a	7.23 _b	198.10 b	401.76 c	35.10c
75% FW + 25% DNS	16.50 _b	.36 b	$10.87 h$ 9.10 a		4.96 a	∴88 a	41.20 b	9.80a	255.30a	420.32 c	39.75 b
Mean	15.65	.51	12.34	8.43	4.60	1.73	45.29	8.53	226.28	458.85	40.80
$C.V.$ $(\%)$	4.20	8.10	3.50	4.20	6.30	5.50	2.50	22.15	15.13	3.40	4.10

*Note. *Means followed by distinct letters in the column differ from the Scott Knott test at P=0.05; C.V.: Coefficient of variance*

Table 4. Height of fertigated eucalyptus seedlings with different concentrations of fish wastewater (FW) and daily nutrient solution (DNS) in the period of 50, 65, 80, 95 and 110 days after sowing

*Note. *Means followed by distinct letters in the column differ by the Scott Knott test at P=0.05; C.V.: Coefficient of variance*

For the stem diameter, it was verified that the fertigation with 50% FW + 50% DNS presented the best results in relation to the other treatments (Table 5). It is possible to note that 50% FW + 50% DNS treatments and daily nutrient solution reached diameter patterns higher than 2 mm, which correspond to adequate standards for eucalyptus seedlings [24]. Larger diameter seedlings present higher survival in the field due to their capacity to form and grow new roots [26].

The length of the largest root resulted in significant differences between treatments. The largest root length was observed in the 75% FW + 25% DNS treatment (23.1 cm) (Table 6).

The daily nutrient solution resulted in a larger leaf area (161.30 cm²), differing significantly from the other treatments. Leaf area is important in plant production, as it is related to photo assimilate production. On the other hand, the greater leaf area results in greater transpiration in the transplant of the seedlings in the field, meaning stress for the plant in this initial phase [27].

The increase of 43.33% in the leaf area of the plants irrigated with daily nutrition solution may be related to the greater absorption of potassium, nitrogen and phosphorus from fertilizer sources, which are important to be available in the initial stages of development of eucalyptus seedlings, as they contribute to the cellular elongation and increase in the leaf area [28].

In analyzing the DQI results, thedaily nutrient solution treatments (0.21) and 50% FW + 50% DNS (0.20) did not differ for this variable (Table 6). The combination of 50% FW + 50% DNS enabled the formation of quality seedlings, balanced for height and biomass accumulation. According to Gomes et al. [29], the minimum DQI value must be greater than 0.20 as a parameter of seedling quality. In this sense, the daily nutrient solution and 50% FW + 50% DNS presented DQI values within the appropriate range reported in the literature.

The treatments with higher shoot fresh mass gains were the daily nutrition solution(4.69 g plant⁻¹) and 50% FW + 50% DNS (4.65 g plant⁻¹) (Table 7). The treatment with the least biomass accumulation was the wastewater. This result may be related to the low nitrogen availability presented by the solution and determined by the analysis of macro and micronutrients contents in the leaf tissue (Table 3), being that the nitrogen concentration (12.50 g kg^{-1}) was below the range for eucalyptus [21]. Biomass accumulation is an important feature related to plant growth; the higher its value, the better the seedling quality [30].

*Note. *Means followed by distinct letters in the column differ by the Scott Knott test at P=0.05; CV: Coefficient of variance*

*Note. *Means followed by distinct letters in the column differ from the Scott Knott test at P=0.05; C.V.: Coefficient of variance*

*Note. *Means followed by distinct letters in the column differ from the Scott Knott test at P=0.05; C.V.: Coefficient of variance*

Table 8. Shoot dry matter (S.D.M.) of fertigated eucalyptus seedlings with different concentrations of wastewater of the fish (F.W.) and daily nutrient solution, in the periods of 50, 65, 80, 95 and 110 days after sowing

*Note. *Means followed by distinct letters in the column differ from the Scott Knott test at P=0.05; C.V.: Coefficient of variance*

Table 9. Root fresh mass (R.F.M.) of fertirrigated eucalyptus seedlings with different concentrations of fish wastewater (FW) and daily nutrient solution (DNS), in the periods of 50, 65, 80, 95 and 110 days after sowing

*Note. *Means followed by distinct letters in the column differ from the Scott Knott test at P=0.05; C.V.: Coefficient of variance*

Table 10. Root dry mass (R.D.M.) of fertigated eucalyptus seedlings with different concentrations of fish wastewater (FW) and daily nutrient solution (DNS), in the periods of 50, 65, 80, 95 and 110 days after sowing

*Note. *Means followed by distinct letters in the column differ from the Scott Knott test at P=0.05; C.V.: Coefficient of variance*

Fertigation with the daily nutrient solution and 50% FW + 50% DNS yielded higher average shoot dry matter compared to the other treatments (Table 8). The shoot dry mass is related to the increase in the stem diameter and quantity of leaves. We can relate this increase in SFM and SDM to the photosynthesis performed by the leaves, which enabled greater photoassimilate storage by the plant shoot [31], and also by the higher LRL, RFM and RDM which provided greater nutrient uptake for plants.

The lowest biomass accumulation in the shoot occurred in the fertigation of the seedlings with only wastewater. Similarly, [12] report that the irrigation of ornamental sunflowers can be carried out with effluents from fish farming when there is another source of nutrients in order for the crop to produce flowers that meet the quality standards.

On the other hand, [32] verified that the use of clonal minijardim effluent in fertilizing eucalyptus seedlings resulted in seedlings within the quality standards, therefore constituing a reuse alternative for this effluent.

According to Rocha et al. [33] Eucalyptus sp. seedlings with higher leaf area values provide higher photosynthetic rates and consequently higher dry matter accumulations. The daily nutrient solution and 50% FW + 50% DNS, which resulted in higher leaf area values, also resulted in greater biomass accumulation.

Besides indicating rusticity, the shoot dry mass directly correlates with the initial performance of the seedlings and their survival in the field [29]. The seedlings of the daily nutrition solution treatments and 50% FW + 50% DNS presented greater accumulation of assimilates and rusticity, constituting an important parameter for quality of seedlings that will be transplanted to the field.

The fresh root mass resulted in significant differences between treatments, with the best results (2.53 g plant⁻¹) being found in the 50% FW + 50% DNS solution (Table 9). Fertigation with 50% FW + 50% DNS contributed to forming uniform roots and without tillering, an important quality parameter for rooting seedlings in the field.

Fertigations with 50% FW + 50% DNS and daily nutrition solution provided higher root dry mass gains (Table 10). The lower dry biomass accumulation of roots occurred in the fertigation with the fish wastewater, possibly due to the smaller availability of nutrients, mainly nitrogen.

In the literature, [34] reported that the application of wastewater fertigation to *Croton floribundus* Spreng. and *Copaifera angsdorffii* Desf. forest species seedlings in nurseries favored dry mass accumulation, both for the shoot and the root system.

The root dry matter has been known as an important parameter for estimating the survival and growth of seedlings in the field, meaning that the more abundant and vigorous the root system, the greater the chances of survival in the field [35]. The significant effect of the 50% FW + 50% DNS treatment on seedling growth for both shoots and roots was possibly due to the concentration of available macronutrients, such as nitrogen and phosphorus, which enabled the formation of abundant roots and quality seedlings.

4. CONCLUSION

The fertigation with 50% FW $+$ 50 DNS and daily nutrient solution presented the best growth results in height, diameter, quality index, accumulation of fresh and dry biomasses, resulting in quality seedlings, balanced in height and accumulation of biomass. Fertilization with only residual water from tilapia production resulted in seedlings with lower growth and development.

Fertirrigation of eucalyptus seedlings can be carried out with effluent from fish farming, but it is necessary to complement the fertilization with other nutrient sources to produce quality seedlings which are suitable for transplantation in the field.

It is suggested that growers use nursery water reservoirs in tilapia farming (Oreochromis niloticus), where the water contributes nutrients and integrates agricultural activities, benefiting both activities.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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