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Effect of covering water surface with azolla (*Azolla filiculoides* Lam.) on water quality, growth and production of nile tilapia fed practical azolla-diets in earthen ponds

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Abstract

Although common in Africa's rural areas, covering fish pond with *Azolla* is an extensive aquaculture practice that generates lower fish growth performances. To improve fish yield, a 90-days experiment was implemented to evaluate the effects of several *Azolla* cover (AC) levels on water nutrient, plankton dynamic, growth and production of Nile tilapia supplementary fed in ponds. Six triplicate groups of ponds were covered with one of the following AC extensions: 0%, 15%, 30%, 45%, 60% and 75% of the surface. Fingerlings were fed with a practical diet containing 20% of *Azolla* meal (AM). Except for temperature, all the other physicochemical parameters, and Chlorophyll a concentration and zooplankton abundance were affected by AC ($P < 0.05$). The SGR strongly correlate with the AC level [SGR (%/day) = - 0.0003 $(AC)^2$ + 0.0135 (AC) + 2.2045; $R^2 = 0.96$; $P < 0.001$, N = 18)], and values ranged from 1.50 %/day to 2.24%/day ($P < 0.05$). Yield and fish production ranged from 710 kg/ha to 1940 kg/ha for yield, and from 2900 kg/ha/year to 7870 kg/ha/year for fish production. The best values were obtained with 30% AC ($P < 0.05$), whereas performances with 0, 15 and 45% AC was similar. It appears that fish growth is a result of an interaction between the negative effects of AC on phytoplankton biomass and the positive effects of the food supplied. The study recommends applying a maximum AC level of 30% in ponds for Nile tilapia rearing in tropical areas.

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Introduction

Azolla is a small leaf free-floating fern commonly found in tropical and sub-tropical regions. This fern develops a symbiotic relationship with a cyanobacteria named *Anabaena azollae* Strasburger, an atmospheric nitrogen-fixing organism. Due to its presence in *Azolla* frond cavities, A. *azollae* enable the fern to supply nitrogen to freshwater ecosystems, with a significant agricultural interest in rice-field cultures where *Azolla* fern are known as a biofertilizer (Carrapiço et al., 2000). This fern, naturally rich in protein (Micha, 2000) (15-35 % of dry matter, Detimmerman and Petry 1988) possesses an interesting amino-acids profile (Sanginga and Van Hove, 1989; Leonard, 1997) and is also rich in minerals (Liu, 1988; Li Zhuoxin et al., 1991). The use of *Azolla* as an ingredient in feed for poultry, broiler, hen and chickens (Khatun et al., 1999; Basak et al., 2002; Alalade and Iyayi, 2006) has been tested, with favourable results. Due to the high productivity in natural conditions, nutritive value and fish appetency (Liu, 1988), *Azolla* has been used as a potential ingredient in omnivorous and phytophagous fish feed (Leonard, 1997; Kanangire, 2001; Shiomi and Kitoh, 2001; Micha and Leonard, 2001-2; Fiogbé et al., 2004; Abou et al., 2007). Nowadays, another way of valorising the fern in Asia and African rural areas is through fish farming under an *Azolla* mat in a section of ponds. Indeed, under optimal conditions of temperature, pH and light intensity, *Azolla* populations grow and cover the water surface within a few days. When covering a fraction of pond surface, this fern induces the development of two sub-ecosystems, differing in their ecological characteristics: under *Azolla*, and in *Azolla*-free waters. Research under laboratory conditions has underlined the effects of *Azolla* cover (AC) on physicochemical characteristics of the water underneath. An AC reduces water temperature about 0.3-2 °C (Kröck, 1987; Villegas and Valentin, 1989). About 90 % of light intensity is retained by a full *Azolla* covered surface at a density of 800 g fresh matter m^{-2} (Kröck et al., 1988; Lejeune et al., 1999). As a consequence, phytoplankton productivity decreases. The resulting reduction of dissolved oxygen is considerable, reaching 50% at full (100%) AC. By reducing water pH, an *Azolla* mat protects rice-field from ammonia volatilization (Vlek et al., 1995; Gilmont, 1997; de Macale et al., 2002). Published research on the effects of AC on dynamic of pond organisms, especially on fish growth and production, is lacking. Recently, Abdel-Tawwab (2006) has studied its effects on Nile tilapia and common carp polycultured in fertilized ponds, and did not find any significant difference between fish reared at control (*Azolla*-free ponds) and 25 % AC.

Due to an ever-increasing demand for animal proteins in Africa where the practice was commonly observed, it is imperative to additionally feed fish in order to increase the yields. There is no data on growth and production performances of Nile tilapia supplementary fed with artificial food in ponds partially covered with *Azolla*. However, this fern could serve as food for fish hence influencing directly the growth, and its mat on the water surface could have indirect effect on growth through their negative impact on the phytoplankton. The aims of this study are to investigate the effects of AC, on the variation of water nutrient, plankton dynamic and growth in Nile tilapia fed *Azolla*-diet in semi-intensive system. The practical objectives are to analyse growth and production in such fish farming systems, to optimize the use of *Azolla* and to provide farmers with a maximum pond surface area to cover with *Azolla* in tilapia farming.

Material and methods

Study site, Azolla mass production and experimental pond design

The experiment was carried out for 90 days at the rural demonstration site (6°29'15.12"N 2°37'6.42"E, and at 13 m above mean sea level) of the Research Unit on Wetlands of Abomey-Calavi University, in Porto-Novo suburb, Benin (West Africa). The fern *Azolla* (A. *filiculoides* Lam.) was mass cultivated in two ponds of 150 m^2 each to initially fill the experimental ponds. During the experimentation, effort was permanently made to maintain the fern in experimental ponds to their linear growth phase, i.e.

the biomass is about 1-2 kg fresh matter/m² (Van Hove 1989). In the present experimental conditions (water temperature: 24-27 °C; air relative humidity: 68-94%; average insolation: 71%; mean pH: 6.60-7.00; mean ammonium level: 0.2-0.6 mg/L; mean soluble reactive phosphate concentration: 0.2-0.4 mg/L), this phase was attained in about 10 days, with an inoculums of 600 g. Thereafter, the supplement biomass was periodically harvested to maintain the appropriate biomass and for producing the experimental *Azolla*-diets.

Eighteen stagnant earthen ponds of 30 m² each (10 m x 3 m x 1 m) were randomly assigned to six treatments T1, T2, T3, T4, T5 and T6, to represent six levels of pond surface covered by *Azolla*, namely 0% (control), 15%, 30%, 45%, 60% and 75%, respectively. The suitable surface areas were covered with dense mats of *Azolla* one week prior to the experiment. The fern was set in one corner (the same for all experimental ponds) of the ponds to mimic the method generally followed in rural areas, and the mats were constrained to these locations by *Bambusa* sticks planted into the banks.

Fish, artificial feed and feeding

The fish used in the study were male O. *niloticus* (initial mean weight = 15.8 ± 0.2 g) obtained by maleness hormone treatment and provided by Lassissi Fish Farming Center, at Porto-Novo (Benin). They were stocked in ponds at 2 fish/m². All experimental fish were fed for 90 days with a practical cost-effective diet (crude protein: 29.2%; gross energy: 16.9 kJ/g) recommended by Abou et al. (2007) and that contained 20% of the meal of the aquatic fern *Azolla* (Table 1).

Fish were fed every day with a ration calculated on the basis of optimal daily ration rate estimation, according to Melard (1986). Daily rations were divided into two parts, each hand-distributed at 8:00 and 16:00 h, respectively. They were adjusted every two weeks according to fish biomass in each pond.

Estimates of growth and fish production

Once every fortnight, at least 40% of the fish in stock were sampled with a dip net, without entering the pond, and weighed to calculate the actual individual mean weight. At the end of the experiment, the final mean weight, the weight gain, the specific growth rate (SGR), the survival rate, the net yield and the annual fish production were estimated as described in Abou et al. (2007) as follow:

Final mean weight (g) = FB / N

Weight gain (%) = $100 (W_f - W_i)/W_i$

Specific growth rate (SGR, %/day) = $100*[\ln(W_f) - \ln(W_i)]/\text{day}$

Survival rate: N/n

Net yield (kg/ha) = (FB – IB) / *S*,

Annual production (kg/ha/year) = Yield x 365 / Time (days)

where FB and IB are final and initial fish biomass per pond in (g); N and n are final and initial number of fish per pond; W_f and W_i are final and initial mean wet weight in (g); S the pond surface area in (ha).

Table 1. Formulation and proximate composition (on dry matter basis) of supplementary-fed diets.

Diets	**%**
Ingredients (g/100 g diet)	
Fish meal£	20
Azolla meal	20
Cottonseed meal	30
Maize bran	16
Brewery draff	12
Cassava starch*	1
Salt (NaCl)	1
Proximate composition	
Moisture (%)	9.9
Crude protein (%)	29.2
Crude lipid (%)	9.4
Crude ash (%)	12.5
Crude fibre§ (%)	10.2
NFE† (%)	38.7
Gross energy‡ (kJ/g)	16.9

£Locally made of grounded *Sardinella aurita* fingerlings

*Binder

§Calculated according to Ovograin Feeds Depot, Abomey-Calavi, Benin and Leonard (1997) for ingredients and *Azolla filiculoides*, respectively.

†Nitrogen-Free Extract, calculated as: 100-(%protein + %lipid + %ash + %crude fibre)

‡ Calculated from energetic values of nutrients (kJ/g): protein 23.4, lipid 39.8, carbohydrates 17.2 (Tacon, 1990).

Table 2. Effect of Azolla cover on water quality in ponds with Nile tilapia fed with a diet containing 20% of Azolla meal.

Treatments	T1: 0% AC	T2: 15% AC	T3: 30% AC	T4: 45% AC	T5: 60% AC	T6: 75% AC
Transparency (cm)	17.8 ± 1.0[a]	18.6 ± 1.3[a]	20.8 ± 0.8[b]	26.9 ± 1.0[c]	30.0 ± 1.4[d]	30.3 ± 1.7[d]
Temperature (C)	30.0 ± 0.5	30.0 ± 0.8	29.9 ± 0.6	29.9 ± 0.6	29.8 ± 0.8	29.8 ± 0.5
pH	7.77 ± 0.15[a]	7.66 ± 0.09[ab]	7.65 ± 0.06[ab]	7.44 ± 0.14[bc]	7.36 ± 0.16[c]	7.26 ± 0.06[c]
Dissolved oxygen (mg/L)	7.25 ± 0.37[a]	6.95 ± 0.71[a]	6.81 ± 0.19[a]	4.32 ± 0.27[b]	4.16 ± 0.39[bc]	3.54 ± 0.31[c]
Nitrate (mg/L)	0.33 ± 0.05[a]	0.30 ± 0.00[a]	0.29 ± 0.01[a]	0.21 ± 0.03[b]	0.18 ± 0.03[b]	0.16 ± 0.04[b]
Ammonium (mg/L)	0.66 ± 0.04[a]	0.59 ± 0.06[b]	0.49 ± 0.01[c]	0.34 ± 0.06[d]	0.27 ± 0.03[de]	0.21 ± 0.03[e]
Soluble reactive phosphate (mg/L)	0.34 ± 0.08[a]	0.30 ± 0.01[ab]	0.29 ± 0.03[ab]	0.24 ± 0.01[b]	0.16 ± 0.02[c]	0.13 ± 0.03[c]

[a,b,c,d,e]In each line, means with no letters or with the same letters as superscripts are not significantly different ($P > 0.05$). Values are means ± S.D. of three replicates. AC means *Azolla* cover.

Table 3. Growth and annual fish production (means ± S.D.) of Nile tilapia reared under Azolla cover in ponds and fed with a diet containing 20% of Azolla meal.

Treatments	T1: 0% AC	T2: 15% AC	T3: 30% AC	T4: 45% AC	T5: 60% AC	T6: 75% AC
Initial weight (g)	15.9 ± 0.3	15.8 ± 0.3	15.7 ± 0.1	15.8 ± 0.3	15.7 ± 0.2	15.8 ± 0.2
Final weight (g)	119.6 ± 5.3[a]	119.7 ± 4.4[a]	129.2 ± 1.7[b]	123.0 ± 2.8[ab]	82.6 ± 0.8[c]	60.7 ± 2.3[d]
Weight gain (%)	653.9 ± 22.7 [a]	659.2 ±25.9 [a,]	722.9 ± 8.4 [b]	676.8 ± 12.0 [ab]	427.5 ± 10.2 [c]	285.1 ± 17.7 [d]
SGR (%/day)	2.24 ± 0.03[a]	2.25 ± 0.04[a]	2.34 ± 0.01[b]	2.28 ± 0.02[ab]	1.85 ± 0.02[c]	1.50 ± 0.05[d]
Survival rate (%)	86.7 ± 5.0	86.1 ± 3.8	87.2 ± 1.9	84.4 ± 1.9	87.8 ± 5.1	85.0 ± 6.0
Net yield (kg/ha)	1750 ± 40[a]	1740 ± 30[a]	1940 ± 60[b]	1760 ± 10[a]	1140 ± 90[c]	710 ± 30[d]
Annual production (kg/ha/year)	7160 ± 160[a]	7070 ± 110[a]	7870 ± 240[b]	7140 ± 40[a]	4610 ± 37[c]	2900 ± 140[d]

[a,b,c,d]In each line, means with no letters or with the same letters as superscripts are not significantly different ($P > 0.05$). Values are means of three replicates. AC means Azolla cover.

Water quality assessment

Fortnightly in the *Azolla*-free area of each experimental pond, physicochemical parameters such as water transparency, temperature, dissolved oxygen and pH were measured at 10 cm deft at the following times: 8:00, 11:00, 14:00 and 17:00 h, using a Secchi disk, an oxythermometer (WTW Oxi 197, precision: ± 0.01 °C and ± 0.01 mg/L) and a pH meter (WTW pH 330, precision: ± 0.01), respectively. Nitrate, ammonium and soluble reactive phosphate levels were determined by cadmium reduction, phenate and ascorbic acid methods respectively, according to APHA (1992).

Phytoplankton biomass, measured as chlorophyll *a* concentration, was estimate using a mixter of methanol and acetone extraction method according to Péchar (1987). To estimate the zooplankton abundance, 20 L of water was filtered through a 55-µm plankton net to obtain a concentrated samples. Sub-samples were taken with a Hansen-Stempel pipette and organisms were analyzed using a 1-mL Sedgwick-Rafter counting cell under a binocular microscope. The absolute abundance was calculated according to APHA (1992) as follow:

$$A_b = (n / v_1) * (v_2 / v_3)$$

Where A_b is the absolute abundance; n is the number of organism countered; v_1 the analyzed sample volume; v_2 the concentrated volume; v_3 the total water volume filtered.

Statistical analysis

Results for water quality, growth and production parameters were analyzed using one-way analysis of variance (Anova). Survival data were log-transformed prior to analysis. A posteriori comparisons of the means were performed using Tukey's test to identify differences between treatments at $P = 0.05$. All statistical analyses were performed with SPSS version 17.0 (SPSS, Chicago, Illinois, USA).

Results and discussion

Effect of Azolla cover on water quality

Mean values of water quality parameters are summarized in Table 2. Except for temperature (range: 29.8-30.0 °C), significant variations were found in all parameters. Water transparency (range: 17.8-30.3 cm) increased when increasing the fraction of the surface covered by *Azolla* ($P < 0.05$), whereas pH (range: 7.26-7.77), dissolved oxygen (range: 3.54-

7.25 mg/L), nitrate (range: 0.16-0.33 mg/L), ammonium (range: 0.21-0.66 mg/L) and soluble reactive phosphate (0.13-0.34 mg/L) decreased as AC level increased (P < 0.05).

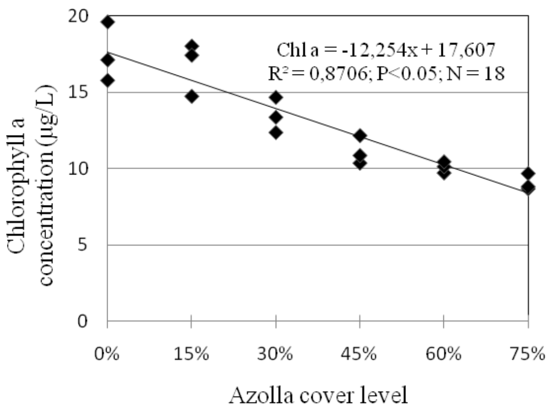

Fig. 1. Variation of Chlorophyll a concentration in Azolla-free water surface of Nile tilapia ponds covered with the fern Azolla.

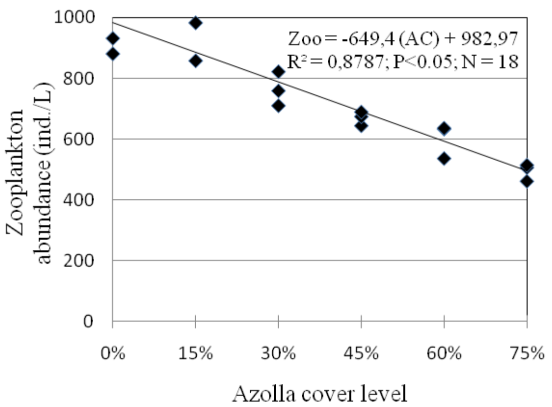

Fig. 2. Variation of zooplankton abundance in Azolla-free water surface of Nile tilapia ponds covered with the fern Azolla.

As presented in Figs 1 and 2, the Chlorophyll a concentration (range: 9.08-17.51 µg/L) and the abundance of zooplankton (range: 495-941 individual/L) are strongly negatively correlated with the AC level; both the biological parameters decreased as AC level increased (P < 0.05).

In this study, covering the fish ponds with *Azolla* inversely affects pH, dissolved oxygen, nitrogen compounds and soluble reactive phosphate. Water temperature seems unaffected. Similar results had been reported under laboratory conditions (Kröck, 1987; Vlek et al., 1995; de Macale et al., 2002). Our results are also in agreement with data from pond experiments in which drastic changes in water chemistry were found (Janes et al., 1996; Abdel-Tawwab, 2006). Water transparency increased whereas chlorophyll a and zooplankton abundance progressively decreased as AC levels increased. Different biological mechanisms, especially out-competition with phytoplankton, are associated with the presence of free-floating macrophytes on the water surface (Abdel-Rahman et al., 2002; Meerhoff et al., 2003; Abdel Tawwab, 2006). In the present experiment, AC competes with phytoplankton for light when shading of the pond surface increased, and also for nutrients assimilation. This could reduce phytoplankton abundance and therefore chlorophyll a concentration, as seen in ponds with AC higher than 15%. The reduction of dissolved oxygen in ponds at 45 to 75% AC levels might result from a reduction of the planktonic photosynthesis process in those ponds, resulting from the reduction in phytoplankton abundance. This could also explain the decreasing trend of pH and the increase in water transparency when AC level increases, as these parameters are positively and inversely correlated with phytoplankton biomass (Tang et al., 2002; Håkanson and Boulion, 2003). Despite these negative effects of AC on water quality, values obtained are within the range required for optimal growth of tilapias (Boyd, 1990).

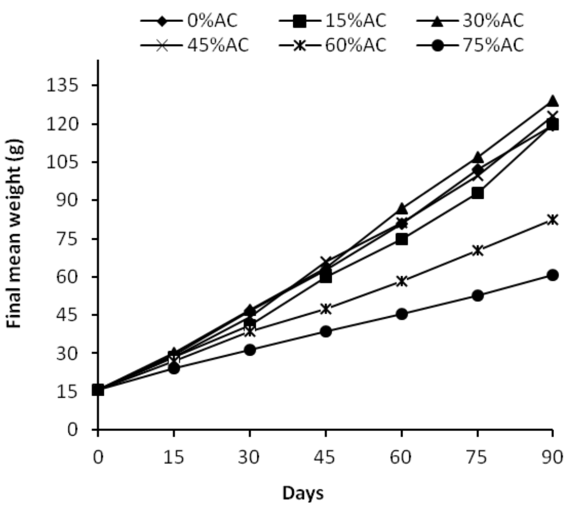

Fig. 3. variation in growth of Nile tilapia supplementary fed Azolla-diet under Azolla cover systems. AC means Azolla cover.

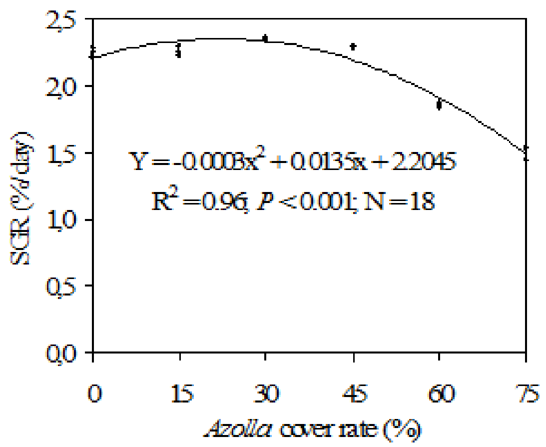

Fig. 4. Variation of specific growth rate (SGR) of Nile tilapia fed with diet containing 20% Azolla, under different Azolla cover level in ponds. Each parameter from the equation is statistically different from 0 ($P < 0.001$).

Growth and production performances

Variation in fortnight mean weight showed growth over the experimental time (Fig. 3). Growth and fish production calculations were summarized in Table 3. The best final mean weight was obtained with 30 % AC-fish, whereas performance with 0, 15 and 45 % AC-fish were similar ($P > 0.05$). The lowest values were obtained with 60 and 75 % AC systems ($P < 0.05$). No differences were found in survival rate ($P > 0.05$). The variation in SGR, weight gain, yield and annual fish production follow the trend in final mean weight. Growth in fish is strongly related to the AC level in ponds. A second order polynomial relationship (Fig. 4) was found between AC level and SGR of fish: [SGR (%/day) = - $0.0003(AC)^2$ + 0.0135(AC) + 2.2045; $R^2 = 0.96$; $P < 0.001$; N = 18]. Fish Yield and production ranged from 710 kg/ha to 1940 kg/ha for yield, and from 2900 kg/ha/year to 7870 kg/ha/year for fish production.

Survival rates were identical in all experimental ponds, suggesting that the fish had adapted to the AC system in ponds, even up to the level of 75%. SGR in fish was similar until 45% AC level, and decreased after this level to 75% AC ponds. The same trends of variation were found in net fish yield and annual production. In fertilized ponds covered at 0, 25, 50 and 75% of the water surface by *Azolla pinnata* for 165 days and without artificial feeding, Abdel-Tawwab (2006) reported reduced values of net fish yield, from de 441 to 701 kg/ha. The values obtained here were higher, from 710 to 1940 kg/ha, although the experimental conditions were different. Those last values were also superior to the data reported by Abou et al. (2007). However, the yield and production obtained here were similar to 1950 kg/ha and 7910 kg/ha/an obtained with the same experimental diet (Abou, 2007) but without covering the ponds, thus justifying that the last system do not automatically impact growth in a supplementary feeding scenario. It seems that the negative effects of AC on phytoplankton do not automatically induce the same effects on fish growth in that system. Nile tilapia is a planktonophagous fish that filtered water in permanence, and many investigations have demonstrated a positive relationship between phytoplankton and fish production (Diana et al., 1991; Abdel-Tawwab et al., 2002). It is well-known that the macrophytes cover in water surface results in a decrease of phytoplankton biomass and zooplankton abundance (Meerhoff et al., 2003; Abdel-Tawwab, 2006; Bicudo et al., 2007; Genkai-Kato, 2007; Cazzanelli et al., 2008). However in open water where fish permanently feed, this system might enhance fish predation on phytoplankton and zooplankton, with a great emphasis on fish growth. Thus, a system with an AC level that balances phytoplankton production and ensures fish grazing will better maintain fish growth.

From these findings, AC in pond reduces nutrient in water and affects phytoplankton biomass and zooplankton abundance in *Azolla*-free water surface. However, these effects do not impact linearly fish growth in a semi-intensive system. It seems that fish growth result from these negative effects and the induced-positive effect of artificial feed; and there could be an AC level that balances these effects. From this study, we recommended a maximum AC of 30 % in the production of Nile tilapia fed with diet containing 20% of AM in earthen ponds.

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