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**Impact of the exploitation of winter period for cultivation of Egyptian Clover (*Trifolium alexandrinum*) on the total yield of Nile Tilapia (*Oreochromis niloticus*) farms and prevention of some diseases**

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**Abstract**

This experiment was designed to determine the impact of alternative culture for *Oreochromis niloticus* with Egyptian clover (*Trifolium alexandrinum*) during winter season in semi-intensive earthen fish ponds. Two treatments included four earthen ponds (two replicates for each treatment) of one feddan each were stocked by *O. niloticus*. The first treatment (control) without Egyptian clover cultivation, while the second treatment (T1) in which fish were cultured after Egyptian clover cultivation. Results revealed that cultivation of Egyptian clover in fish ponds improves water quality parameters; growth performance and fish health as well as it increases economic return. However, proximate chemical composition of the whole fish body of *O. niloticus* did not significantly affected. In conclusion, cultivation of Egyptian clover farming during winter season in earthen fish semi-intensive ponds helps getting better fish production and improves water quality and fish health. Moreover, using Egyptian clover farming was a truly effective preventive measure for improving of growth performance and facing fish diseases.

**Key words:** Egyptian clover, Nile tilapia, total yield.

**Introduction**

Crop-fish culture is not widely practiced around the world. Most information comes from Asian countries, particularly Philippines, Indonesia and Japan. Where traditional crop farming methods have been refined over centuries. Field Crops are the economically important crop. Fish may provide supplemental income and/or protein, but is not normally the primary crop. The addition of crop production to fish culture is an additional management consideration for farmers. In many cases, there may not be sufficient economic justification for this extra management (Verma, 2013). Integrated farming (or integrated agriculture) is a commonly and broadly used word to explain a more integrated approach to farming as compared to existing monoculture approaches. It refers to agricultural systems that integrate livestock and crop production (Abdel-Hakim *et al.*, 1999).

The inclusion of aquaculture especially fish farming, agro-industries and biogas production have been stressed by Olele *et al.* (1999) and Chan (2006) as make-ups for complete integration.

For centuries, traditional agricultural systems have contributed to food and livelihood security throughout the world. Recognizing the ecological legacy in the traditional agricultural systems may help us develop novel sustainable agriculture. We examine how crop-fish co culture (Tilman *et al.*, 2012), which has been designated a “globally important agricultural heritage system,” has been maintained for over 1,200 y in south China. A field survey demonstrated that although crop yield and crop-yield stability are similar in and crop monoculture, requires 68% less pesticide and 24% less chemical fertilizer than unitary agriculture. A field experiment confirmed this result (Herrero, 2010).

A complementary use of nitrogen (N) between crop and fish, resulting in low N fertilizer application and low N release into the environment. These findings provide unique insights into how positive interactions and complementary use of resource between species

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generate emergent ecosystem properties and how modern agricultural systems might be improved by exploiting synergies between species (Garg *et al.*, 2007 and Tester and Langridge, 2010).

The Egyptian clover (*Trifolium alexandrinum* L.) is an annual forage species, diploid, allogamous, entomophil, originating in the Mediterranean region or in the Near East (Syria, Egypt) (Duke, 2011 and Knight, 2013). Till now, it is cultivated in the sub-tropical region and up to the humid boreal region, being tolerant to moderate-salinity soils (Graves, 2007) and well-adapted to regions with mild winters and without excessive heat during summer. This clover species is appreciated due to the following qualities: it has a good yielding potential, utilization in different forms (fresh forage, hay, silage, pasture, and green fertilizer), high protein content (28-30%), 2-5 harvests during the vegetation period (Graves, 2007 and Zamfir, 2010). This study aimed to illustrate healthy and profitable alternative culture method for *Oreochromis niloticus* with Egyptian clover (*Trifolium alexandrinum*). Moreover the most economic use of water is in this study as we use agriculture drainage water which is considered waste water; meeting the countries policy for careful use of our share of water. Therefore, the study is a demonstration of an applied field trial for increasing both quality and quantity of fish production moreover healthy status for Nile tilapia.

### Material and Methods

#### Experimental Design:

The study had been done in a private farm at Tollumbat No. 7 (using agriculture drainage waste water) in Riyad area, Kafr El-Sheikh governorate, Egypt. Water and fish samples were collected from four shallow (1 fed each and 1.25 m depth) earthen ponds represented two treatments (two replicates). The first treatment (control) was under normal fish culture conditions without Egyptian clover, while the second treatment (T1) represented alternative aquaculture between fish and Egyptian clover.

The ponds were stocked with monosex males of Nile tilapia, *O. niloticus* fingerlings at an average initial length of about 17.56 and 17.18 cm and an average initial weight of 37.24 and 36.31 g for control and T1, respectively. Each pond was stocked with 12600 fish/feddan (3 fish/m<sup>2</sup>). Nile tilapia fingerlings were fed on a commercial diet containing 30% crude protein six days/week at a daily feeding rate of 3% of an average fish-body weight twice at 9.0 am and 3.0pm during the experimental period. The trial started on the 13<sup>th</sup> of May and harvested on 23<sup>rd</sup> of September 2015 (22 weeks). At the end of the experiment, ponds were drained and fish were harvested, counted and the individual weight and length were measured.

#### Water sampling:

Water samples were monthly collected from different places at each site by a PVC tube column sampler at depth of half meter from the water surface. The samples at each site were mixed in a plastic bucket and a sample of 1 liter was placed in a polyethylene bottle, kept refrigerated and transferred cold to the laboratory for analysis. Nile tilapia fish were collected and transported to the laboratory in an ice box, where tissue samples were taken.

#### Egyptian clover farming:

Just after harvesting fish in the beginning of October planting of Egyptian clover seeds began, after conducting different services to the Egyptian clover crop. Egyptian clover was cultured without fertilization. Flooded the seeds in water for 24 hours and then evenly distributed using stores tractors because of wet ponds of fish production. And for equal distribution of seeds in the field 60KG of seed germination were used, the irrigation depended on rainwater as recommended by **Baotong (1984) and Salah, (2003)**.

#### Water management:

Hydrogen ion concentration (pH) was measured with pH meter (Model 25, Fisher Scientific). Total dissolved solids (TDS as g/l) were determined using a salinity-conductivity meter (model, YSI EC 300). Temperature and dissolved oxygen were measured by using a digital oxygen meter (Model YSI 55). The concentration of total hardness (mg/l as Ca CO<sub>3</sub>), total ammonia (NH<sub>4</sub>-N+NH<sub>3</sub>-N), unionized ammonia (NH<sub>3</sub>-N), nitrite (NO<sub>2</sub>-N) and nitrate (NO<sub>3</sub>-N) were measured by methods described in Boyd and Tucker (1992). Transparency (cm) was measured by using a Secchi Disc of 20 cm diameter.

#### Growth parameters:

Random samples (30 fish from each pond-60 fish for each treatment) were taken biweekly during the experimental period. Body measurements (body weight in g and body length in cm) at biweekly intervals throughout the whole experimental period.

i) Fish condition factor (K) was calculated as  $K = (Wt/L^3) \times 100$ ; where Wt is the total gutted weight of the fish (g), and L is the total length (cm) according to Schreck and Moyle (1990).

ii) Specific growth rate (SGR) was calculated according to Jauncey and Rose (1982) as  $SGR = (\ln W_2 - \ln W_1 \times 100) / t$ , where w<sub>1</sub> = first fish weight in grams, w<sub>2</sub> = final fish weight in grams, t= period in day.

iii) Daily weight gain (DWG) was calculated using the formula:

$DWG = [\text{Average } W2 \text{ (g)} - \text{Average } W1 \text{ (g)}] / t$ , where  $W1$  and  $W2$  = the initial and final fish weight, while  $t$  is the experimental period in days.

#### Chemical composition:

At the end of the experiment, five fish from each pond exposed to the chemical composition (moisture, protein, fat and ash content) of the whole fish body according to AOAC (2000).

#### Fish Samples for clinical examination

A total of 200 live fishes of 240-350 gram weight with disease signs were collected and transported to the laboratory within few hours four:

5.1. Full clinical and post mortem examination (Schaper-clause, 1992).

5.2. Bacteriological examination: Swabs taken under aseptic condition from skin lesion and internal organs namely liver, kidney and spleen were inoculated on tryptic soy broth Oxoid (at 25°C for 24 hrs then streaked into MacConkey and T.S.A. agar) Oxoid (Selective colonies were restreaked onto Aeromonas base media) Oxoid (and an TCBS media and Ordal S media. Pure colonies were streaked onto soft agar to be used for further studies. Bacterial isolates were identified by colonial morphology, growth characters on specific media and microscopically appearance as well as phenotypic characteristics (Buller 2004).

#### Mycological examination:

Isolation of fungi was carried out from naturally infected fish, samples were taken from fish showing skin lesions, eye, fins, gills, mouth, spleen, liver and kidney lesions were collected and inoculated onto Sabouraud dextrose agar (SDA) medium plates and incubated at 20±2°C for 3-4 days, subculture on the same media was done for purification. All positive cultures were examined for colonial growth, morphological features and microscopical characteristics. The morphological features include appearance of the cultures, rate of growth, texture of the surface colonies, colonies color according to Refai and Al Doory, (1986).

Microscopical examination was done for wet preparations of the skin lesions and mycelia cultured on (SDA) to detect septation of hyphae according to Dvorak and Otcerasek (1969).

**Parasitological examination:** all fishes were grossly examined for detection of any visible cysts, larvae or whole parasites according to (Paperna, 1996). The identification of the detected parasites was carried out according to Lom and Dykova (1992) and Woo (1995).

#### Statistical analysis

Statistical analysis: One-way ANOVA and Duncan's multiple range tests were used to evaluate the significant difference of the data which were computed by applying the computer program (SAS, 1996). Significant differences are stated at  $P < 0.05$ . Data were statistically analyzed according to Bailey (1981).

### Results and Discussion

#### 1. Water Quality parameters:

##### 1.1. Physico-Chemical Parameters:

Water quality parameters are presented in Table 1. Water temperature did not significantly varied among treatments throughout the experimental period and ranged from 25.6°C in T1 to 25.9°C in control. Application of clover farming caused a significant decrease ( $P < 0.05$ ) of water pH (7.63 in T1) compared with control ponds (8.14). Dissolved oxygen (DO) was not affected significantly by treatments ( $P > 0.05$ ) and it was higher (4.9 mg/l) in T1 followed by control (4.6 mg/l). DO attained lower value in control. The higher values of DO in T1 may be attributed to good water quality conditions caused by clover farming. Boyd (1998) reported that waters with a pH range of 6.5 – 9 are the most suitable for fish production. However, DO was still lower than that recommended by Boyd (1998), who advised 5.0 mg/l to satisfy needs of fish welfare.

Secchi disc readings were positively affected by treatments ( $P < 0.05$ ). The average values of transparency were generally higher in T1 (23.70 cm) compared with lower secchi disc reading of control ponds (20.70 cm). This resulted from the role of clover farming for Water purification.

Cultivation of clover in fish ponds significantly decreased all the inorganic dissolved nitrogen, namely total ammonia ( $\text{NH}_3\text{-N} + \text{NH}_4\text{-N}$ ), nitrite ( $\text{NO}_2\text{-N}$ ) and nitrate ( $\text{NO}_3\text{-N}$ ) content in the water. The levels of these nutrients in the two treatments were significantly ( $P < 0.05$ ) different with higher values in control ponds (0.32, 0.41 and 0.14 mg/l, respectively). Significant higher concentrations of nitrogen nutrients in T1 (0.24, 0.35 and 0.10 mg/l, respectively) might have been principally due to clover farming as it attracted nitrate ions and improve nutrient retention from soil and water (Mumpton, 1999). The values of the total alkalinity ranged between 412.20 and 388.70 mg/l, for two treatments. The obtained results showed that all parameters of water quality were in the suitable range required for Nile tilapia (Boyd, 1979 and Abdel-Hakim et al., 2000).

**Table 1: Some water quality parameters of earthen ponds.**

Variable	NO.	Control	T1
Tem.(C°)	6	25.9±0.6	25.6±0.5
DO oxygen	6	4.60±0.4	4.90±0.4
PH	6	7.63±0.4	8.14±0.5
S. disk (cm)	6	23.7±0.85	20.7±0.70
NH3 mg/l	6	0.24±0.01	0.32±0.01
NO2 mg/l	6	0.035±0.01	0.041±0.01
NO3 mg/l	6	0.10±0.02	0.14±0.02
T. alk. (mg/l)	6	388.7±9.4	412.2±9.4

**2. Growth parameters:**

**2.1. Body weight and length:**

Growth and survivability which together determine the ultimate yield are influenced by a number of biological parameters such as genetic materials and managerial practices, including water and food quality, energy content of the food and stocking density (El-Sayed 1999 and Ashagrie et al., 2008).

As described in Table (2), the initial body weight (37.24 and 36.31g) and body length (17.56 and 17.18 cm) for Nile tilapia reared at the beginning of the experiment. The differences in initial BW and BL among the different treatments were insignificant indicating the random distribution of fish around the different experimental treatments. At the end of the experimental period the averages of BW were 256.57 and 278.68g and BL found to be 29.30 and 29.60cm for Nile tilapia for the two treatments control and T1, respectively and the differences among treatments were significant (P<0.05). This result may be attributed to Egyptian clover farming was increased of nitrogen fixation in fish pond.

Fath El Bab et al., (2014) studied the effect of exploitation of fish in ponds previously cultivated by wheat in the winter season on growth performance and total yield of *O. niloticus*, found a decrease in body weights of Nile tilapia. They emphasized that by considering wheat crop stressful for soil of fish ponds. Moreover, Chakraborty (2007) noticed that alternation of fish culture with wheat showed the lowest of body length. In addition to that, Othman (2006) summarized the integrated farming systems to include the economic benefits in terms of increased food production, where it led to an increase in tiller length of crops and increased also the length of Nile tilapia. Ugwumba and Orji (2006) added to that the integrated farming system increased both weight gain and length of Nile tilapia.

**Table 2: Growth parameters of Nile Tilapia under both culture systems.**

Variable	No.	control	T1
initial weight	60	37.24±0.92a	36.31±0.92a
final weight	60	256.57±1.29b	278.68±1.29a
initial length	60	17.56±0.09a	17.18±0.09b
final length	60	29.60±0.78b	29.30±0.78a
initial K	60	0.66±0.04a	0.75±0.04a
final K	60	1.04±0.18a	1.05±0.18a
DWG, g/fish	60	1.57±0.07b	1.80±0.07a
SGR, %/day	60	1.38±0.12b	1.48±0.12a
Survival rate%		89.85%	96.11%
Total yield Kg/feddan		2766.34	3329.40

Means with the same letter in the same raw are not significantly different at P≥0.05.

**2.2. Condition factor:**

Condition factor of fish is essentially a measure of relative muscle to bone growth and the differing growth responses of these tissues to diet treatment may be reflected by changes in condition factor (Ibrahim et al., 2000; Soltan et al., 2006). It is frequently assumed to reflect not only characteristics of fish such as health, reproductive state and growth but also characteristics of the environment such as habitat, water quality and prey availability (Liao et al. 1995). As described in table (2), the averages of initial (K) for Nile tilapia were 0.69 and 0.72. While at the end of this experiment became 1.02 and 1.11 for control and T1, respectively.

The differences between treatments were significant ( $P < 0.05$ ). These results are some what in agreement with those of **Baotong, (1984)** who reported that, Condition factor (K) increased when alternation of fish culture with planting Clover while alternation by wheat culture decreased the Condition factor. Concerning studies of **Garg et al. (2007)** on alternative culture on growth of crops and fish and farmer income, they found an increased soil nitrogen, improved condition factor and the fish grew well when food supply was adequate.

### 2.3. Daily weight gain (DWG):

The averages of (DWG) of *O. niloticus* were 1.57 and 1.80g/fish for the two treatments; control and T1, respectively (table 2). Analysis of variance of data indicates that, the differences among treatments were significant ( $P < 0.05$ ). The obtained results indicate that, the (DWG) form alternation was higher than that obtained from the control. Same remark was recorded by **Bwanika (2007)** who noticed that the average daily gain of alternative culture was better than the unitary ponds. Similar results by **Igbinosa and Okporie (2013)** were obtained and emphasized that weight gain improved by increasing of nitrogen in earthen ponds due to the integrated farming system. Moreover, **Jian et al. (2011)** found that, farmed fish with field crops has led to positive interactions; integrated use of resources between species generate appropriate ecosystem and all modern agricultural systems characteristics have been improved by exploiting synergies between species, where alternative culture causes increasing the weights of both crops and fish, because the feces of fish reduced the fertilizers which used to produce crops and nitrogen found in soil reduced the fertilization which used for farmed fish and increased fish weights and total yield.

### 2.4. Specific growth rate (SGR):

The averages of (SGR) of *O. niloticus* were 1.38 and 1.48%/day for the two treatment, respectively (Table 2). These results indicate that, the (SGR) for first treatment was higher than obtained in control. The analysis of variance of these results indicates that, the differences between treatments were significant ( $P < 0.05$ ). **Rafael (2010)** studied the physiological condition of the halophyte *Salicornia bigelovii*: Aptential food and biofuel crop for integrated aquaculture agriculture systems, he found that, each species has a specific and unique needs of food and pond fertilization success is critical to a successful education institution aquaculture, were fertilization increases the phytoplankton, zooplankton and other aquatic organisms that fish get them easily, which increased the growth rate.

### 2.5. Survival rate and Total yield:

Table (2) shows that fish culture after Egyptian clover farming in fish ponds showed the highest survival rate compared to the fish culture without Egyptian clover cultivation in fish ponds. This may be due to farmed fish with field crops has led to positive interactions and integrated use of resources between species generate appropriate ecosystem and all modern agricultural systems characteristics have been improved by exploiting synergies between species where alternative culture causes increasing the weights of both crops and fish.

Averages of total yield at the end of the experiment are listed in Table (2). As described in this Table *O. niloticus* gained the highest yield (3329.40kg) for cultivated ponds compared with 2766.34kg in control ponds. These results may be attributed to the effect of Egyptian clover cultivation in fish ponds. **Garg et al. (2007)** when studied the effect of alternative culture on growth of crops and fish and farmer income, they found that, the alternative culture increased soil nitrogen, improved body weight and total yield and the fish grow well when the supply of food was adequate. Such improvement may also be attributed to that integration increases the phytoplankton, zooplankton and other aquatic organisms that fish get them easily, which increased the nutritive value of the feed intake giving more haemopoietic stimulation and activating the liver cells increasing the anabolic capacity to produce. However, barseem stimulates the development of bacteria in the soil (probiotics) which not only accelerate the decomposition of organic matter, but also fixes atmospheric nitrogen; essentially needed for the zooplankton, which form the nutritious and preferred food for many aquaculture species. Since fertilizer benefits the growth of bacteria and benthos, thus provides supplementary food for zooplankton and fish improving fish vitality. These remarks came more or less close to those mentioned by **Sophin (2007) and Rafael (2010)**.

### 3. Chemical composition of fish:

The changes in chemical composition during development and in response to different factors are the result of differential growth of tissues. The main tissues involved in the whole-body growth are bones, muscles and adipose tissues. The relative development of these tissues is very important for the conformation of fish and thus its yield in processing (**Soltan et al., 1999**). Proximate analysis shows significant ( $P \leq 0.001$ ) effects in the two treatments. As described in Table (3) fish culture after Egyptian clover farming in fish ponds released the highest values of protein and fat while fish culture without Egyptian clover farming in fish ponds released the highest values of ash.

Jian et al. (2011) reported that, farmed fish with field crops has led to positive interactions and integrated use of resources between species generate appropriate ecosystem and all modern agricultural systems characteristics have been improved by exploiting synergies between species where alternative culture causes increasing the weights of both crops and fish and increasing percentages of protein and fat for Nile tilapia.

**Table 3: Least-square means and tested standard error of the factors affecting on chemical composition % DM basis of Nile tilapia.**

Variable	No.	control	T1
Moisture%	3	65.90±0.07	65.55±0.07
Protein%	3	63.10±0.06b	64.40±0.06a
Fat*	3	18.75±0.05b	19.45±0.05a
Ash%	3	18.15±0.06a	16.15±0.06b

**5. Clinical and parasitological inspection of fish:**

The examined Tilapia in alternative ponds showed no pathognomonic lesions, Plate (1)



Plate (1): Apparently healthy fish

On the other side, some unitary cultured fish suffered showed external mucus secretions (excessive sliminess), enlarged head to total body shape; indicating emaciation, inflammation and haemorrhagic patches under pectoral fins, abdominal distention and some fish gathered near water inlets (gasping atmospheric oxygen) with decreased feed intake (poor appetite) and loss of equilibrium (lethargy) (Plate 2). This may be due to poor water quality and turbidity water temperature (Noga, 1996).



Plate (2): Inflammation and haemorrhagic patches under pectoral fins.

Moreover, postmortem examination showed distended and enlarged gall bladder due to hepatitis (Plate 3).



Plate (3): Distended and enlarged gall bladder due to hepatitis.

In addition to that, the intestine was enlarged, swollen because of parasitic infection (Plate 4) (Lom and Dykova, 1992). In addition to that, more systemic infection showed reddish inflamed testes with losing normal bright creamy white appearance, (plate, 5).



Plate (4): Swollen and enlarged intestine



Plate (5): Inflamated reddish testes

tilapia. *Aeromonas* spp., *Pseudomonas fluorescens*, *Vibrio anguillarum*, *Flavobacterium columnare*, *Edwardsiella tarda*, *Streptococcus* spp. and *Enterococcus* sp. are commonly found in the facilities (Plumb, 1997). Under certain circumstances (as the effect of crowding, pollution and parasitic infestation, exerting much stress on the fish), thus upsetting the balance between bacteria and these commensal bacteria become secondary opportunistic pathogens and invade fish. On the other side, the prevalence of some parasites (*Ascaris*, *Clinostomum*, *Eimeria*, *Hexamita* and *Ichthiophtherius*) in unitary ponds were found. Alternated ponds were found more healthier, which may be attributed to the presence of low load of intermediate hosts in alternative farms. This closely met the findings of Pillay, 1990. These clinical findings are close to what reported by Schaperclaus, (1992).

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