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Full Length Article

## *Aspilia mossambicensis* and *Azadirachta indica* medicinal leaf powders modulate physiological parameters of Nile tilapia (*Oreochromis niloticus*)<sup>☆</sup>,

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## ABSTRACT

Growing mixed-sex Nile tilapia, *Oreochromis niloticus* in earthen ponds to table size is a major challenge due to its early maturity and prolific breeding. This study determined the effects of two medicinal plants; *Aspilia* plant, *Aspilia mossambicensis* and Neem tree, *Azadirachta indica* on hatchlings production, growth performance, feed utilization, survival and haematology of *O. niloticus*. Experimental diets were prepared by adding 1.0, 2.0, 4.0 and 8.0 g of either *A. mossambicensis* or *A. indica* leaf powders into a kg of the control diet subsequently administered daily to twenty triplicates of *O. niloticus* for three months. Both *A. mossambicensis* and *A. indica* leaf powder at the used doses, reduced significantly hatchlings production of *O. niloticus* when compared to the control ( $P < .05$ ). The lowest value of hatchlings count was found in *A. indica* dose 8.0 g kg<sup>-1</sup> ( $P < .05$ ). The use of *A. mossambicensis* leaf powder at a dose of 4.0 g kg<sup>-1</sup> improved significantly growth performance and feed utilization ( $P < .05$ ). In contrast, survival rate was not affected significantly by the two plants ( $P > .05$ ). Both plants differentially increased significantly haematological parameters such as Hb concentration, packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), monocyte and lymphocytes while reduced significantly neutrophils and eosinophils ( $P < .05$ ). In conclusion, *A. mossambicensis* and *A. indica* leaf powders control prolific breeding of *O. niloticus*, modulate its growth performance and feed utilization. The two plants also modulate haematological parameters of *O. niloticus* indicating immunological response towards stress or intoxication, however, the values obtained were not beyond the recommended range for healthy fish.

## 1. Introduction

Nile tilapia, *Oreochromis niloticus* is one of the most popular freshwater fish species for aquaculture worldwide. Its suitability for culture is attributed by its neutral taste, ability to tolerate a wide range of environmental conditions and utilization of food from the lowest trophic level [1]. However, growing mixed-sex *O. niloticus* in ponds to table size is a major challenge due to its early maturity and prolific breeding [2–4]. Consequently, ponds become overpopulated with *O. niloticus* of varying sizes which makes management aspects such as feeding and water quality difficult to perform because of size-dependent

requirements. Accordingly, water quality deteriorates, competition for food and space increases and *O. niloticus* diverts energy towards reproduction causing slow growth [5,6]. Synthetic hormones have been used as the popular and favoured techniques in order to overcome its early maturity and prolific breeding [5]. However, their higher cost in addition to their environmental and human health concerns, limit their use [7,8]. A number of medicinal plants have been explored as natural remedy, safe and affordable alternatives to control prolific breeding of *O. niloticus* [7,9,10].

*Aspilia mossambicensis* also known as Wild sunflower is a medicinal plant which belongs to the family Compositae (Asteraceae) within the

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genus *Aspilia*. It is widespread in central and Eastern tropical Africa from Ethiopia, through East Africa, the Congo, Zambia, Zimbabwe, Malawi, Mozambique and Transvaal to Natal [11]. In Tanzania, the plant is found along Lake Victoria [12], Kigoma and Tanga Regions [13]. Based on its medicinal properties, *A. mossambicensis* is used by herbalists and local people to treat several ailments including malaria, bacterial infection and human immunodeficiency virus (HIV) [12,14,15]. The plant is also known to alleviate menstrual cramps as well as uterotonic agent capable of inducing uterine contraction and labour in pregnant women [12,16].

On the other hand, *Azadirachta indica* popularly known as “Neem tree” is a member of the mahogany family, called Meliaceae, which is a broad-leaved evergreen plant that grows up to 30 m tall and 2.5 m girth [17]. It is native to Burma, Nigeria, India and Pakistan, growing in tropical and semi-tropical regions [18]. In East Africa it is also known as ‘the plant of the 40’ because it has been suggested to treat at least 40 different diseases [18]. *A. indica* is known to have medicinal properties such as antimicrobial, anti-inflammatory, antipyretic, spermicidal effect, immuno-contraceptive, anti-fertility activity and abortifacient [19,20]. Based on their medicinal properties, the two plants have the potential to control prolific breeding of *O. niloticus*.

Studies conducted on *A. mossambicensis* are limited to domestic animals such cattle and goats [21] where it has been shown to stimulate growth. Furthermore, *A. mossambicensis* was reported to improve survival, weight gain and immunological parameters in HIV patients [14]. To date, the ability of *A. mossambicensis* in controlling prolific breeding and its effects on growth performance and haematological parameters of fish are unknown. On the other hand, *A. indica* have been subjected to extensive research in various animal species based on its medicinal properties. However, most studies on *A. indica* used extracts which require technical know-how during their preparation beyond the reach of most fish farmers at large scale production [22–25]. It is known that, medicinal plants modulate physiological functioning of fish in a positive or negative way depending on the type of the plant and dose administered [26]. Higher growth performance, survival rate of cultured animals and feed utilization are primary goals of fish farmers. Moreover, haematological evaluation is useful in monitoring the health status of fish [27].

This study was therefore conducted to determine the effect of various doses of *A. mossambicensis* and *A. indica* leaf powders on hatchlings production, growth performance, feed utilization, survival rate and haematological parameters of *O. niloticus*.

## 2. Materials and methods

### 2.1. Ethical statement

The study was carried out in accordance with the Tanzanian laws and Sokoine University of Agriculture guidelines for the care of experimental animals. All procedures of the current work were approved by the Committee of the College of Agriculture of the Sokoine University of Agriculture (SUA).

### 2.2. Experimental fish and their management

Juvenile *O. niloticus* males and females weighing between 30 and 50 g (mean weight  $41.5 \pm 3.1$  g) were collected from SUA ponds located in Morogoro region, Tanzania. The fish were acclimatized for two weeks before the start of the experiment. After the acclimatization period, three replicates of 20 fish (10 females and 10 males) were stocked and raised in  $3.6 \text{ m}^3$  experimental tanks for three months. Each culture tank was supplied with 2700 L clean water with optimum quality of dissolved oxygen, pH and water temperature recommended for *O. niloticus* farming [28]. Water quality parameters were monitored on a daily basis and in each tank, a complete replacement of water was done once every week. Dissolved oxygen, pH and temperature during

the entire study ranged from  $6.0\text{--}7.8 \text{ mg L}^{-1}$ ,  $8.0\text{--}8.4$  and  $26.7\text{--}27.2 \text{ }^\circ\text{C}$ , respectively.

### 2.3. Plants collection and preparations

The plant leaves were collected based on ethno-botanical knowledge using available literature, visual observations and identification by a botanist according to guidelines by Smith [13] and Styles and White [29]. The leaves of *A. mossambicensis* were collected from Magamba village located at Lushoto district in Tanga region whereas *A. indica* leaves were collected from Morogoro municipal. The collected leaves were thoroughly washed and shade dried in a dry room at room temperature for two weeks. The dried leaves were ground into fine powders by using a Lab Mill (Serial number 19911, Christy Hunt Engineering, LTD, England) fitted with 1.0 mm screen. The powders were then kept in dry containers and stored at room temperature pending feeds formulations.

### 2.4. Feed formulation and feeding regimes

The control diet ( $250 \text{ crude protein g kg}^{-1}$ ) was formulated using Pearson's square by including  $300 \text{ g kg}^{-1}$  fishmeal (sardines) and  $700 \text{ g kg}^{-1}$  maize bran. Eight experimental diets were formulated by adding 1.0, 2.0, 4.0 and 8.0 g of either *A. mossambicensis* (AM1, AM2, AM4 & AM8, respectively) or *A. indica* (AI1, AI2, AI4, and AI8, respectively) to a kilogram of the control diet. Proximate composition of the control diet and plants used in the present study are given in Table 1. The diets prepared were fed to fish twice a day (10.00 and 17.00 h) at a rate of 3% body weight per day for three months.

### 2.5. Hatchlings count

After every two weeks, number of hatchlings produced by *O. niloticus* was counted from each experimental tank and hatchlings count (HC) was recorded as described before [30].

### 2.6. Fish growth performance, feed utilization and percentage survival

All *O. niloticus* were weighed and their individual initial weights (g) recorded to the nearest 0.01 g by using a sensitive weighing balance before stocking in the tanks. Subsequent weighing of *O. niloticus* individuals was conducted every 14 days by scooping out the fish using a scoop net and their weights determined as described before. Feed ratios were adjusted based on fish body weight obtained after every two weeks. After 90 days of culture, all *O. niloticus* were removed, counted for final mean body weight (FMW) and percentage survival determination. Growth performance (specific growth rate; SGR, weight gain; WG, and daily weight gain; DWG), feed utilization (feed conversion ratio; FCR and feed conversion efficiency; FCE) and percentage survival (Sr) were calculated at the end of the experiment using the following formulae according to Hopkins [31] and Silva and Anderson [32].

$$\text{WG (g)} = \text{Final weight(g)} - \text{Initial weight(g)} \quad (1)$$

**Table 1**

Proximate composition (dry weight basis) of control diet, *Aspilia mossambicensis* and *Azadirachta indica* ( $\text{g kg}^{-1}$ ).

Composition ( $\text{g kg}^{-1}$ )	Control diet	<i>Aspilia mossambicensis</i>	<i>Azadirachta indica</i>
Moisture	88	91	83
Crude protein	250	216	147
Crude fat	101	24	15
Crude fiber	85	196	176
Ash	78	204	110
Carbohydrate	398	269	469

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