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Full Length Article

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

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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 $kg^{-1}$ ($P < .05$). The use of *A. mossambicensis* leaf powder at a dose of 4.0 g $kg^{-1}$ 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 abortificient [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 ± 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 $m^3$ 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 6.0–7.8 mg $L^{-1}$, 8.0–8.4 and 26.7–27.2 °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 crude protein g $kg^{-1}$) was formulated using Pearson's square by including 300 g $kg^{-1}$ fishmeal (sardines) and 700 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 is 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 rations 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].

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

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

| Composition (g $kg^{-1}$) | Control diet | *Aspilia mossambicensis* | *Azadirachta indica* |
|---|---|---|---|
| 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 |

$$\text{DWG (g day}^{-1}) = \left(\frac{\text{Final weight} - \text{Initial weight}}{\text{Time (days)}}\right) \quad (2)$$

$$\text{SGR (\% day}^{-1}) = \left(\frac{\ell\text{n Final weight} - \ell\text{n Initial weight}}{\text{Time (days)}}\right) \times 100 \quad (3)$$

$$\text{FCR} = \frac{\text{Total amount of dry feed fed(g)}}{\text{Total wet weight gain of fish(g)}} \quad (4)$$

$$\text{FCE} = \frac{\text{Total wet weight gain of fish(g)}}{\text{Total amount of dry feed fed(g)}} \quad (5)$$

$$\text{Percentage survival (\%)} = \left(\frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100\right) \quad (6)$$

### 2.7. Fish haematological parameters

Blood was collected from caudal vein and then directly transferred into the ethylenediaminetetraacetic acid (EDTA) coated bottles. Packed cell volume (PCV) was determined by micro-haematocrit reader (Sigma 201-M) after centrifuging at 3000 r.p.m. Red blood cells (RBC), white blood cells (WBC) and haemoglobin concentration (Hb) were determined following standard procedures as described by Ispır *et al.* [33] and Gabriel *et al.* [34]. The Hb, PCV and RBC values were used to calculate MCV, MCHC and MCHC indices using the following formulae:

$$\text{The mean corpuscular volume (MCV, fl)} = \frac{\text{PCV}\times 10}{\text{RBC}} \quad (7)$$

$$\text{The mean corpuscular haemoglobin (MCH, pg cell}^{-1}) = \frac{\text{Hb}\times 10}{\text{RBC}} \quad (8)$$

$$\text{The mean corpuscular haemoglobin concentration (MCHC, g dl}^{-1}) = \frac{\text{Hb}\times 100}{\text{PCV}} \quad (9)$$

Differential count of leucocytes (neutrophils, lymphocytes, eosinophils, monocytes, basophils) were determined under the light microscope (Olympus BH-2)) at 100× magnification [33,35].

### 2.8. Statistical analysis

Results are presented as means ± standard deviation and data were tested for normality and homogeneity of variances using Kolmogorov-Sminorv and Levene's tests, respectively. Thereafter, one-way analysis of variance (ANOVA) was used to test for significant differences in the growth performance, feed utilization, survival and haematological parameters measured among the different diets for each medicinal plant. When significant differences were detected, Tukey's post hoc test was performed to determine specific differences among treatments. Pearson correlation was used to establish the relationship between hatchlings production and plant doses and haematological parameters and plants doses. All statistical analyses were performed using SPSS version 20 (IBM, Armonk, NY, USA) for Windows. Results with $P \leq 0.05$ were considered statistically significant for all tests except some Pearson correlations results which used $P \leq 0.010$.

## 3. Results

### 3.1. Fish hatchlings production

The results on hatchlings production of *O. niloticus* post-exposure to various doses of *A. mossambicensis* and *A. indica* indicated variations between the two plants and among doses (Table 2). Both *A. mossambicensis* and *A. indica* leaf powders reduced significantly hatchlings production of *O. niloticus* when compared to the control ($P < .05$; Table 2). The *O. niloticus* fed on *A. indica* (2.0–8.0 g kg$^{-1}$) had significantly reduced hatchlings production than those fed on *A. mossambicensis* (2.0–8.0 g kg$^{-1}$) ($P < .05$). The *O. niloticus* fed *A. indica* at higher dose (8.0 g kg$^{-1}$) had significantly less values of hatchlings production than those fed the lower dose (1.0 g kg$^{-1}$). In contrast, *O. niloticus* fed the lower and higher doses of *A. mossambicensis* leaf powder had similar hatchlings production ($P > .05$). The lowest values of hatchlings production (62.50), was found in *O. niloticus* fed 8.0 g kg$^{-1}$ *A. indica*. The hatchlings production decreased significantly as the doses increased for *O. niloticus* supplemented with *A. indica* ($r = -0.566$, $P < .05$) while they decreased insignificantly for those fed on *A. mossambicensis* ($r = -0.389$, $P > .05$).

**Table 2**
Hatchlings production for *Oreochromis niloticus* fed *Asipilia mossambicensis* (AM) and *Azadirachta indica* (AI) at different doses.

| Treatment | Hatchlings production (number) |
|---|---|
| Control | 259.4 ± 36.6$^{a}$ |
| AM1 (1 g/kg) | 149.4 ± 52.6$^{b}$ |
| AI1 (1 g/kg) | 100.3 ± 22.0$^{b}$ |
| AM2 (2 g/kg) | 121.2 ± 51.6$^{b}$ |
| AI2 (2 g/kg) | 69.7 ± 9.6$^{c}$ |
| AM4 (4 g/kg) | 94.2 ± 14.4$^{b}$ |
| AI4 (4 g/kg) | 76.1 ± 12.9$^{c}$ |
| AM8 (8 g/kg) | 101.9 ± 19.7$^{b}$ |
| AI8 (8 g/kg) | 62.5 ± 6.2$^{c}$ |

Values in the same column sharing the same superscript are not significantly different. AM = *Asipilia mossambicensis* and AI = *Azadirachta indica*.

### 3.2. Fish growth performance

The results on growth performance of *O. niloticus* post-exposure to various doses of *A. mossambicensis* and *A. indica* indicated variations between the two plants and doses as depicted in Fig. 1(a–c). Feeding *A. mossambicensis* and *A. indica* at the lower (1.0 g kg$^{-1}$) and higher (8 g kg$^{-1}$) doses reduced significantly WG, SGR, DWG of treated *O. niloticus* than the control ($P < .05$). Significantly higher WG, DWG and SGR (46.8 g, 0.56 gday$^{-1}$ and 0.84 %day$^{-1}$, respectively) of *O. niloticus* were obtained at a dose of 4.0 g kg$^{-1}$ *A. mossambicensis* when compared to the remaining groups fed both plants and the control ($P < .05$). Moreover, *O. niloticus* fed on *A. indica* diet containing 2 g kg$^{-1}$ (AI2) had significantly higher growth performance compared to those fed on AI1 and AI8 ($P < .05$).

### 3.3. Feed utilization

Results for FCR and FCE of *O. niloticus* administered various doses of *A. mossambicensis* and *A. indica* indicated plant and dose specific effects as shown in Fig. 2(a and b). Inclusion of *A. mossambicensis* and *A. indica* at 1.0 g kg$^{-1}$ and 8.0 g kg$^{-1}$ doses, respectively, increased significantly FCR of *O. niloticus* compared to control ($P < .05$). *A. mossambicensis* at an inclusion level of 4.0 g kg$^{-1}$ reduced significantly FCR (2.11) value when compared to > 2.50 obtained from AM1, AI1, AI4, AM8 and AI8 ($P < .05$). In contrast, *A. mossambicensis* at an inclusion level of 4.0 g kg$^{-1}$ revealed the highest FCE (0.47) of all the other doses for both plants and the control group.

### 3.4. Survival (%)

Similarly, plant and dose specific effects were obtained on percentage survival (Fig. 3). Both plants did not significantly affect the percentage survival when compared to control ($P > .05$). However significant differences were obtained among different doses in the treated groups. Significantly higher percentage survivals were found at higher doses (8.0 g kg$^{-1}$) for *A. mossambicensis* (86.7%) and *A. indica* (85%) when compared to the lower survival (58.3%) obtained from fish fed *A. mossambicensis* at a lower dose of 2.0 g kg$^{-1}$ ($P < .05$).

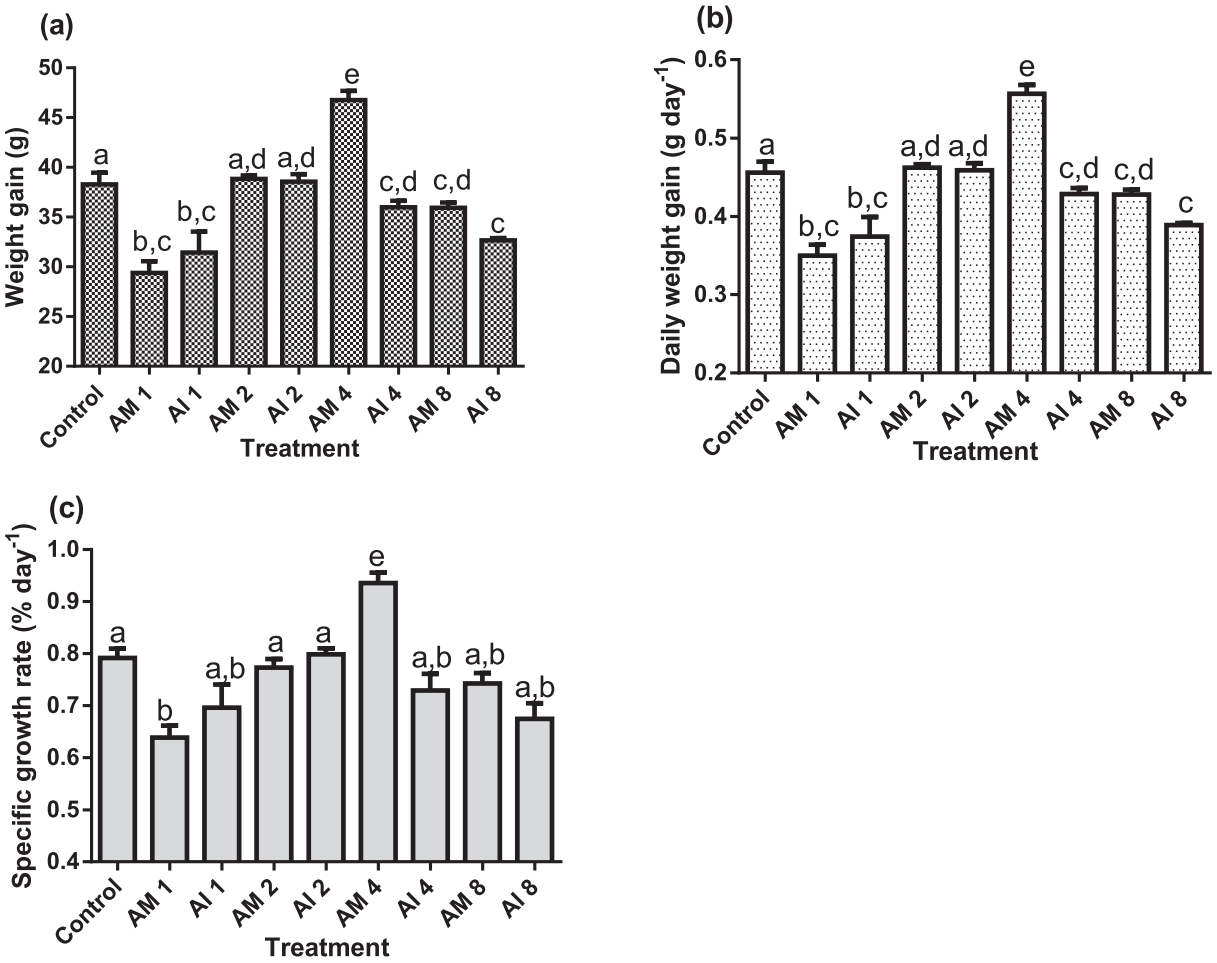



**Fig. 1.** Growth performance of *Oreochromis niloticus* fed on diets supplemented with *Aspilia mossambicensis* (AM) and *Azadirachta indica* (AI) at different doses (0.0, 1.0, 2.0, 4.0 and 8.0 g/kg). (a) Weight gain (g), (b) Daily weight gain (gday$^{-1}$), (c) Specific growth rate (%day$^{-1}$). Different letters above error bars indicate significant differences ($P < .05$).

### 3.5. Haematological parameters

The results on haematological parameters of *O. niloticus* fed various doses of *A. mossambicensis* and *A. indica* indicated immunological modulation (Table 3). The Hb concentration, PCV, MCH, MCHC and WBC of *O. niloticus* fed *A. mossambicensis* and *A. indica* were significantly higher than control ($P < .05$). The *O. niloticus* fed the diets supplemented with both medicinal plants containing doses ranging from 2.0 g kg$^{-1}$ to 8.0 g kg$^{-1}$ recorded the highest levels of PCV, MCH, MCHC and WBC. In addition, the *O. niloticus* fed *A. mossambicensis* at

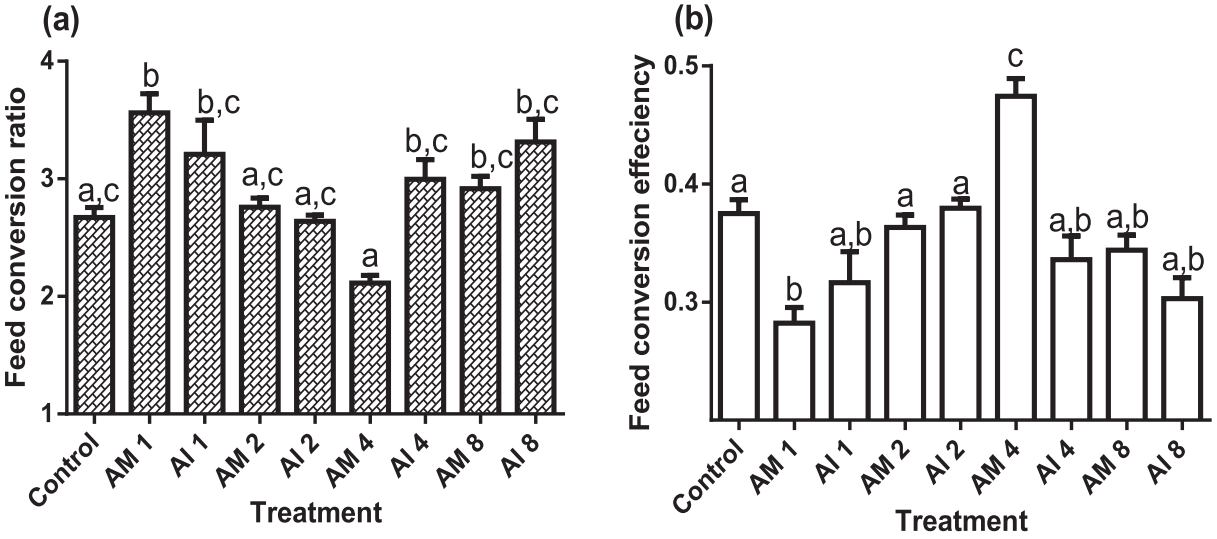



**Fig. 2.** Feed utilization of *Oreochromis niloticus* fed on diets supplemented with *Aspilia mossambicensis* (AM) and *Azadirachta indica* (AI) at different doses (0.0, 1.0, 2.0, 4.0 and 8.0 g/kg). (a) Feed conversion ratio and (b) Feed conversion efficiency. Different letters above error bars indicate significant differences ($P < .05$).

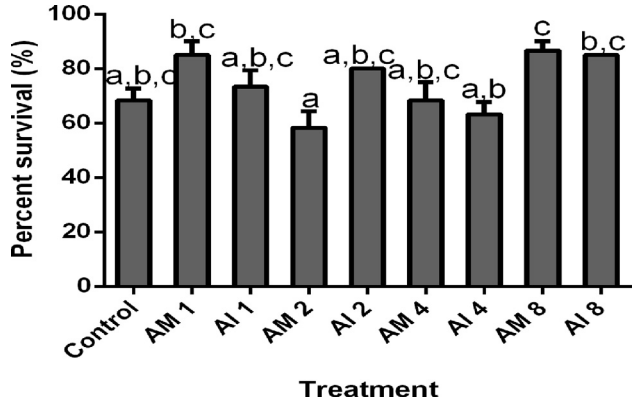



**Fig. 3.** The percentage survival of *Oreochromis niloticus* fed on diets supplemented with *Aspilia mossambicensis* (AM) and *Azadirachta indica* (AI) at different doses (0.0, 1.0, 2.0, 4.0 and 8.0 g/kg) during the study period. Different letters above error bars indicate significant differences ($P < .05$).

4.0 g kg$^{-1}$ (AM4) and 8.0 g kg$^{-1}$ (AM8) inclusion levels had significantly higher MCV values than control ($P < .05$). Moreover, Hb, PCV, MCV and MCH of *O. niloticus* fed *A. mossambicensis* had slightly higher values than *A. indica* supplemented ones at similar doses. The RBC values were generally statistically similar among all *O. niloticus* fed the two plants and the control group ($P > .05$).

Differential leukocyte count for *O. niloticus* fed *A. mossambicensis* and *A. indica* doses revealed significant increase in the percentage of monocytes when compared to the control ($P < .05$). However, neutrophils for both plants and eosinophils of *A. indica* decreased significantly when compared to the control ($P < .05$). The highest monocyte values (17.5 and 16.5%) were found in *O. niloticus* fed 4.0 g kg$^{-1}$ *A. indica* and *A. mossambicensis,* respectively. In contrast, the highest value (31%) for neutrophils was noticed in *O. niloticus* fed the control diet whereas the lowest values (18.5 and 16.5%) were obtained in *O. niloticus* fed the higher doses (8.0 g kg$^{-1}$) for both *A. indica* and *A. mossambicensis*, respectively. Conversely, eosinophils values were generally low in *O. niloticus* fed the control diet (1.5%) and those fed on diets supplemented with 1.0 and 4.0 g kg$^{-1}$ *A. mossambicensis* (1.0%). Eosinophils were below detection level in any of *O. niloticus* fed the *A. indica* diets. The results indicated higher levels of lymphocytes (62–68%) followed by neutrophils (16.5–22.5%), monocytes (11.5–17.5%) and eosinophils (1.0–1.5%).

### 3.6. Relationship between haematological parameters and the plants doses

The results on correlation between haematological parameters and the plant doses are shown in Table 4. The *O. niloticus* Hb concentration (r = 0.684 and r = 0.834), PCV (r = 0.727 and r = 0.752) and monocyte (r = 0.923 and r = 0.737) increased positively as the doses of the plants increased for *A. indica* and *A. mossambicensis*, respectively ($P < .05$), while only WBC (r = 0.548), MCV (r = 0.609) and lymphocytes (r = 0.686) were positively correlated for *O. niloticus* supplemented with *A. mossambicensis* ($P < .010$ except WBC, $P < 0.05$). On contrary, neutrophils (r = −0.735 and r = −0.775) and eosinophils (r = −0.663 and r = −0.629) decreased negatively as the doses increased for *O. niloticus* supplemented with *A. indica* and *A. mossambicensis* ($P < .05$), respectively.

## 4. Discussion

The present study intended to determine the effects of *A. mossambicensis* and *A. indica* leaf powders on some physiological parameters of *O. niloticus* when used to control its prolific breeding. The two plants were able to control prolific breeding partially by reducing significantly hatchlings production of *O. niloticus*. Similar reduction in the number of

**Table 3**
Haematological parameters of *Oreochromis niloticus* fed on *Aspilia mossambicensis* and *Azadirachta indica* doses during the study period.

| Treatment | RBC ($10^6$ Ml$^{-1}$) | Hb (g dL$^{-1}$) | PCV (%) | MCV (fl) | MCH (pg cell$^{-1}$) | MCHC (g dL$^{-1}$) | WBC ($10^3$ µL$^{-1}$) | Lymphocytes (%) | Neutrophils (%) | Monocytes (%) | Eosinophils (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 2.0 ± 0.5$^a$ | 5.1 ± 0.3$^a$ | 21.0 ± 4.6$^a$ | 104.3 ± 1.4$^a$ | 26.1 ± 4.4$^a$ | 25.1 ± 3.8$^a$ | 6.8 ± 1.4$^a$ | 58.5 ± 2.9$^a$ | 31.0 ± 4.6$^a$ | 9.0 ± 1.2$^a$ | 1.5 ± 0.6$^a$ |
| AM1 (1 g/kg) | 2.1 ± 0.6$^a$ | 5.9 ± 0.1$^a$ | 21.0 ± 2.3$^a$ | 102.2 ± 6.1$^a$ | 28.9 ± 1.0$^a$ | 28.4 ± 2.3$^{ab}$ | 6.3 ± 0.6$^a$ | 64.0 ± 2.3$^c$ | 22.0 ± 2.3$^b$ | 13.0 ± 1.2$^b$ | 1.0 ± 1.2$^a$ |
| AI1 1 g/kg | 2.1 ± 0.3$^a$ | 5.8 ± 1.2$^a$ | 19.5 ± 0.6$^a$ | 95.9 ± 11.2$^a$ | 29.2 ± 12.2$^a$ | 29.9 ± 6.9$^{ab}$ | 8.9 ± 0.5$^b$ | 66.0 ± 2.3$^b$ | 22.5 ± 4.0$^b$ | 11.5 ± 1.7$^{ab}$ | 0.0 ± 0.0$^b$ |
| AM2 2 g/kg | 2.0 ± 0.7$^a$ | 8.4 ± 1.2$^d$ | 23.0 ± 1.2$^{ab}$ | 131.0 ± 54.0$^{abc}$ | 49.2 ± 23.8$^{abc}$ | 36.6 ± 3.2$^d$ | 7.3 ± 1.5$^{ab}$ | 66.5 ± 4.0$^{bc}$ | 18.5 ± 4.0$^{bc}$ | 14.0 ± 1.2$^b$ | 1.0 ± 1.2$^a$ |
| AI2 2 g/kg | 1.9 ± 0.4$^a$ | 7.0 ± 0.0$^b$ | 20.5 ± 2.9$^a$ | 115.7 ± 41.2$^{ac}$ | 38.5 ± 10.6$^{bc}$ | 34.6 ± 4.8$^b$ | 7.3 ± 1.5$^{ab}$ | 68.0 ± 2.3$^b$ | 19.5 ± 1.7$^{bc}$ | 14.0 ± 0.0$^b$ | 0.0 ± 0.0$^b$ |
| AM4 4 g/kg | 2.0 ± 0.0$^a$ | 9.6 ± 0.6$^d$ | 29.0 ± 4.6$^c$ | 144.5 ± 24.6$^c$ | 47.9 ± 3.3$^c$ | 33.6 ± 3.4$^{bd}$ | 6.9 ± 0.3$^a$ | 64.0 ± 2.3$^c$ | 19.5 ± 1.7$^{bc}$ | 16.5 ± 0.6$^c$ | 0.0 ± 0.0$^b$ |
| AI4 4 g/kg | 1.9 ± 0.6$^a$ | 7.5 ± 0.3$^b$ | 24.0 ± 0.0$^b$ | 132.8 ± 46.3$^{ac}$ | 41.8 ± 13.6$^b$ | 31.2 ± 1.3$^b$ | 7.3 ± 0.9$^a$ | 62.5 ± 1.7$^{ac}$ | 20.0 ± 2.3$^{bc}$ | 17.5 ± 0.6$^c$ | 0.0 ± 0.0$^b$ |
| AM8 8 g/kg | 2.1 ± 0.3$^a$ | 9.2 ± 1.0$^d$ | 34.0 ± 6.9$^c$ | 173.5 ± 59.2$^c$ | 44.8 ± 3.2$^c$ | 28.8 ± 11.7$^{abcd}$ | 8.9 ± 0.8$^b$ | 68.5 ± 1.7$^b$ | 16.5 ± 1.7$^c$ | 15.0 ± 3.5$^{bc}$ | 0.0 ± 0.0$^b$ |
| AI8 8 g/kg | 2.5 ± 0.6$^a$ | 6.5 ± 0.1$^c$ | 30.0 ± 1.2$^c$ | 123.8 ± 22.1$^{ac}$ | 27.0 ± 5.3$^{ab}$ | 21.8 ± 0.4$^c$ | 7.9 ± 1.8$^{ab}$ | 64.0 ± 1.2$^c$ | 18.5 ± 1.7$^{bc}$ | 17.0 ± 1.2$^c$ | 0.0 ± 0.0$^b$ |

Values in the same column sharing the same superscript are not significantly different ($P \geq .05$). AM = *Aspilia mossambicensis*, AI = *Azadirachta indica*, WBC = white blood cells, RBC = red blood cells, Hb = hemoglobin, PCV = packed cell volume, MCV = mean corpuscular volume, MCV = mean corpuscular hemoglobin and MCHC = mean corpuscular hemoglobin concentration.

**Table 4**
Correlation between *Aspilia mossambicensis* and *Azadirachta indica* doses and haematological parameters of *Oreochromis niloticus*.

| Parameters | *Aspilia mossambicensis* | | *Azadirachta indica* | |
|---|---|---|---|---|
| | $r^2$ | *P* value | $r^2$ | *P* value |
| Hb (g $dL^{-1}$) | 0.834 | 0.000* | 0.648 | 0.002* |
| PCV (%) | 0.752 | 0.000* | 0.726 | 0.000* |
| Neutrophils (%) | −0.775 | 0.000* | −0.735 | 0.000* |
| Monocytes (%) | 0.737 | 0.000* | 0.923 | 0.000* |
| Eosinophils (%) | −0.629 | 0.003* | −0.663 | 0.001* |

Values with an asterisk (*) are significantly different at $P = .010$, r = correlation coefficient.

hatchings has also been obtained after feeding *O. niloticus* with *A. indica* ethanol extracts [10] and *A. indica* saponin [36]. Contrary to the findings obtained from this study, the former and the latter noticed completely inhibition of hatchlings production using doses between 1.0 g $kg^{-1}$ and 4.0 g $kg^{-1}$ of *A. indica* leaf extract and *A. indica* saponins, respectively. In this study, hatchlings production was not completely inhibited even at the highest dose (8.0 g $kg^{-1}$). This deviation can be explained by the differences in the forms of *A. indica* leaves experimented. In this study, leaf powder was used without any further purification whereas in previous studies extracts and pure phytocompound (saponin) were used. Reduction of hatchlings is possibly because of antifertility phytocompounds such as saponins, flavonoids and alkaloids present in these plants [12,37]. The results also revealed that *A. indica* was more effective in controlling prolific breeding of *O. niloticus,* presumably due to its spermicidal effect [20,38] which has lead the plant to be used for contraceptive purposes in variety forms including pills, vaginal foam or creams [39]. In general, farmers can use the two plants to partially control prolific breeding depending on availability. However, *A. indica* controls prolific breeding more effective than *A. mossambicensis* at similar doses.

Results revealed significant dose-specific effects of the two plants on growth performance, feed utilization and feed conversion efficiency. Low WG, SGR, DWG, FCE and high FCR values which is an indication of slow growth and low feed utilization were noticed in *O. niloticus* fed the lower and higher doses of *A. mossambicensis* and *A. indica*. These results are in agreement with those obtained by Obaroh and Nzeh [24] which revealed negative effect of *A. indica* (crude extract) on *O. niloticus* WG, SGR and FCR at higher doses (4.0 and 8.0 g $kg^{-1}$). Similar findings were also reported by Jegede and Fagbenro [40] in *Tilapia zilii* after exposure to *A. indica* leaf powder doses (1.0–2.0 g $kg^{-1}$). Furthermore, dose dependent retardation of growth in *T. zilii* was shown by Omoregie and Okpanachi [41] following exposure to variety doses of *A. indica* (0.78 and 1.56 mg $L^{-1}$) bark crude extract. In contrast, saponin extracted from *A. indica* did not show any negative effect on *O. niloticus* growth performance and feed utilization even at higher doses of 4.0 and 8.0 g $kg^{-1}$ [5].

Findings from this study demonstrate that the two plants influence growth performance differently depending on the type of the plant and dose used. Better growth performance and feed utilization were obtained in *O. niloticus* supplemented with *A. mossambicensis* compared to *A. indica* groups. Previous studies conducted on *A. mossambicensis* are restricted to domestic animals and human being [14,21]. The use of *A. mossambicensis* has been shown to influence positively growth performance in terrestrial animals such as cattle and goats [21]. Based on its growth enhancement effects and medicinal properties, farmers from Kenya preferred using it as a fodder crop for livestock. On the contrary, it has been documented that *A. indica* caused depressed growth performance in cattle [20]. The differences might be contributed by variations in protein content between the two plants. Proximate analysis of the plants in the present study showed that *A. mossambicensis* has higher crude protein compared to *A. indica* (Table 1). Furthermore, *A. mossambicensis* prepared by herbalists in Tanzania against HIV patients was found to improve survival and weight gain in the treated patients [14]. These results imply that *O. niloticus* farmers interested in controlling prolific breeding using the two plants should use dosages below 4 g $kg^{-1}$ and 2 g $kg^{-1}$ for *A. mossambicensis* and *A. indica*, respectively.

The two plants increased significantly haematological values for Hb, PCV, MCH, MCHC, WBC, lymphocytes and monocytes, but did not affect RBC. The significant increase in Hb and PCV obtained in the present study is in agreement with the findings reported by Obaroh *et al.* [42] after feeding *O. niloticus* with diets containing *Mangifera indica* doses (0.5–8 mg $kg^{-1}$). Similarly, Gabriel *et al.* [34] found no significant effect of *Aloe vera* supplemented diets on *O. niloticus* RBC. However, the results obtained in the present study are contrary to those obtained by Fafioye [23] on *O. niloticus* fed on *A. indica* doses (0.1–0.5 g $L^{-1}$) which revealed significant decrease in Hb concentration, RBC, PCV and MCH values. Similarly, Saravanan *et al.* [22] reported significant decrease in Hb, PCV, MCV, MCH and MCHC values from *C. mrigala* after exposure to *A. indica* (1.0 g $L^{-1}$). In addition, sharp decrease in PCV was noticed in *O. niloticus* subjected to higher doses (> 1.2 g $L^{-1}$) of *A. indica* water extract [43].

Haematological parameters such as Hb, PCV, MCV, MCH and MCHC are particularly known to indicate erythrocyte status and oxygen carrying capability in fish [34]. Therefore, the increased levels in these parameters indicate the stimulation of erythropoiesis, hence increasing the capacity of oxygen transport and strengthening the defense mechanisms against physiological stress [34]. Generally, these results imply that feeding *O. niloticus* with diets containing *A. mossambicensis* and *A. indica* at the dosage used improve immune system because most values of haematological parameters obtained from this study are within the ranges for healthy *O. niloticus* cultured under semi intensive system as described by Bittencourt *et al.* [44]. The ranges described by Bitterncourt *et al.* [44] are 0.7–28 × $10^6$ $Ml^{-1}mm^{-3}$, 6.58–15.98 g $dL^{-1}$ and 15–45% for RBC, Hb and PCV, respectively. In addition, Clauss *et al.* [45] also showed that, the accepted range of PCV for fish is between 20 and 45%. Fish with PCV values above 45% and below 20% are considered to have polycythaemia as a result of dehydration and anaemia, respectively. Most of the PCV values in the present study were above 20% and below 45% indicating the fish were in good health. However, the surprising increase in most RBC indices while RBC values were the same among treatments suggest the possibility of regenerative anaemia. This condition occurs when demand for RBC is higher such that reticulocytes are prematurely released from the bone marrow into circulation [46].

This study indicated increase in WBC of *O. niloticus* in the two plants for most doses. Significant increase in WBC of *O. niloticus* supplemented with the highest dose of *A. mossambicensis* concurs with the findings obtained by Saravanan *et al.* [22] in *Cirrhinus mrigala* after the exposure to *A. indica* leaf extract at various doses (0.25–1.50 mg $L^{-1}$). The increase in WBC is a defensive mechanism of *O. niloticus* due to inclusion of the medicinal plants in its diets [25,47,48]. The body of *O. niloticus* treated with the medicinal plants stimulated the immune system which reacted by producing disease or foreign particles fighting cells.

The results further showed that, both plants increased the percentage of lymphocytes and monocytes while they decreased neutrophils and eosinophils in a dose-dependent manner. The higher percentage of lymphocytes followed by neutrophils, monocytes and eosinophils obtained from this study concurs with findings reported by Gabriel *et al.* [49] and Martins *et al.* [50] in *O. niloticus*. Increase in monocytes and lymphocytes percentages from *O. niloticus* treated with *A. mossambicensis* and *A. indica* is in agreement with findings obtained by Martins *et al.* [50] after subjecting fish to stressors. In general, this study revealed high percentage of monocytes compared to previous studies on *O. niloticus* while testing variety of medicinal plants [34,51] presumably due to variations in medicinal properties of the different plants used. The monocytes increased significantly as the doses increased for both plants.

The increase in lymphocytes and monocytes and the decrease in neutrophils and eosinophils are due to immunological modulation by the two medicinal plants. In the present study, the levels of neutrophils and eosinophils decreased significantly as the doses increased for both medicinal plants. Differential count of leukocytes is very essential in vertebrates including fishes because each of the five leucocytes (lymphocyte, monocyte, neutrophil, eosinophil and basophil) has a specific immunological function. Neutrophils are primary phagocytes in response to disease, stress or inflammation whereas lymphocyte has immunological functions including the production of immunoglobulin and modulation of immune defense. Eosinophils respond to inflammation process and defense against parasites. Monocytes also known as phagocytic cells are responsible for defense against infections and bacteria [52]. Accordingly, in normal conditions each group exists at a certain percentage, however, they can be altered in response to infection, disease, toxic substances or stress [35]. Ultimately, increase in lymphocytes and monocytes indicates that the cells in the treated groups were protecting the body of *O. niloticus* from introduced foreign particles of the medicinal plants contained in the diets. This was confirmed by the decreased levels of neutrophils and eosinophils which indicate stress, intoxication or over production of certain specific steroids in the body, such as cortisol, due to the use of the two medicinal plants. The results on differential count of leucocytes suggest immunological modulation of *O. niloticus* due to application of the two medicinal plants. However, the two medicinal plants did not alter the immunity beyond the normal healthy ranges. According to Davis *et al.* [52] neutrophils and lymphocytes account for about 80% of total leucocytes whereas monocytes, eosinophil and basophils fall in the remaining 20%. These ranges were similar to the values obtained in this study.

The existence of significant positive correlation in WBC, MCV and lymphocytes for only *O. niloticus* treated with *A. mossambicensis* indicates immunomodulation stimulation differences between the two medicinal plants. Apparently, *A. mossambicensis* has higher immune modulation effects than *A. indica* at similar inclusion levels. Possibly, this might be the reason for the faster growth performance and enhanced feed utilization obtained for *O. niloticus* supplemented with *A. mossambicensis*, an observation which requires further studies.

## 5. Conclusions

The findings from this study indicate that, both *A. mossambicensis* and *A. indica* leaf powders controlled partially prolific breeding of *O. niloticus*. In general, *A. indica* is more effective in controlling prolific breeding when compared to *A. mossambicensis*. The two plants also revealed plant-specific modulation on fish growth performance and feed utilization. Based on the present experimental conditions, for better growth and feed utilization of *O. niloticus*, the dose inclusion limit should be 2.0 g $kg^{-1}$ and 4.0 g $kg^{-1}$ for *A. indica* and *A. mossambicensis*, respectively. Haematological findings indicate that the two medicinal plants improved immune system as a response towards stress, intoxication or over production of certain specific steroids but was within the body cells to counteract. In general, for most measured parameters, higher values were recorded for fish fed on diets supplemented with *A. mossambicensis* compared to those fed on *A. indica* indicating plant-specific effects. To our knowledge, this is the first study reporting the effects of *A. mossambicensis* on growth performance and haematological parameters in fish.

## Competing interests

There is no conflict of interest to declare.

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