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Study on the Effect of Wormseed Plants; *Artemisia cina* L. and Chamomile; *Matricaria chamomilla* L. on Growth Parameters and Immune Response of African Catfish, *Clarias gariepinus*

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Abstract: A number of 420 fingerlings of catfish were used to examine the effect of wormseed plants and chamomile on the growth parameters and on non-specific immune response of the African catfish; *Clarias gariepinus*. Both types of herbs were used in rates of 1, 3 and 5% with 3 replicates per each of the 6 treatments. The 7th treatment was kept as a control group. The experimented catfish were fed with the 7 examined diets in the rate of 3% of fish biomass for 1 month. Different growth parameters as well as blood parameters were estimated to evaluate the growth performance and immune response of the experimented catfish. Results revealed that wormseed plants *Artemisia cina* L. in the rate of 3 and 5% and chamomile *Matricaria chamomilla*. L. in the rate of 1% showed the best figures of growth parameters as well as immune response parameters of the examined catfish.

Key words: Fingerlings, *Clarias gariepinus*, *Artemisia*, chamomile, immune reoponse

INTRODUCTION

The African catfish *Clarias gariepinus* is distributed throughout Africa. It is of growing economic value in the African aquaculture industry (Goda *et al*., 2007; Osman *et al*., 2007; Abdelhamid, 2009). However, diseases are major obstacles in aquaculture, especially in the intensive systems. They cause severe economic losses among fish farms (Atallah *et al*., 1999). Wormseed plants have a powerful biological effect against fungi, bacteria and even some harmful insects (Abo-Zeid, 1988). Abdel-Hadi *et al*. (2008) concluded that wormseed plants in the rate of 0.5 mL L^{-1} of 25% solution gave the best estimates of fertility, hatchability and survivability percent among the examined eggs and larvae of carp species and recommended for practical application in carp hatcheries for the control of saprolegniasis and to replace the currently used chemicals; malachite green and formalin with their environmental and public health hazards. Chamomile flowers were used in aquaculture and were studied in the rates of 2 and 5% as feed additives on the artificial feed of overwintered Tilapia fingerlings (*Oreochromis niloticus*) by Bakeer and Mostafa (2006). They stated that dried chamomile flowers in the rate of 2% increased all growth parameters of the experimental fingerlings (BW, WG and SGR) and significantly, increased Feed Conversion Ratio (FCR) and survival rate. They recommended the addition of chamomile flowers (2%) to tilapia feed for overwintering of tilapia fingerlings. Blood analysis is crucial in many fields of ichthyologic research and fish farming and in the area of toxicology and environmental monitoring as possible indicator of physiological or pathological changes in fishery management and diseases investigation (Adedeji *et al*., 2000). Hematological indices are very important parameters for the evaluation of fish physiological status. Their changes depend on fish species, age, the cycle of the sexual maturity of spawners and diseases (Luskova, 1997; Zhitrineva *et al*., 1989). Since, the use of expensive chemotherapeutants for controlling diseases have been widely criticized for their negative impacts (Sahu *et al*., 2008). Therefore, the objectives of the present study were to evaluate effects of graded levels of 2 mediterranean herbs on African catfish concerning their growth performance, feed utilization and blood profile as well as on the non-specific immune response of that fish.

MATERIALS AND METHODS

Fish: A total of 420 fingerlings of catfish from Abbassa were used. Average body weight was 22 g and total length of 12 cm. The fish were acclimatized for 2 weeks in a fiber glass.

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Aquaria: About 21 aquaria were used for carrying out the experiment.

The examined substances: *Artemisia cina* L. or wormseed plants (Shieh Baladi). Matricaria chamomilla or chamomile (Popping flowers).

Feed: A powdered feed (40% protein) was processed to form pellets after mixing with different rates of the herbs under study for fish feeding in the rate of 3% of fish biomass.

Both types of herbs were used in the rates of 1, 3 and 5% with 3 replicates per each of the 6 treatments. The 7th treatment was kept as a control group. Twenty fish were used per aquarium. Their total biomass was detected and the amount of daily feed was estimated according to a feeding rate of 3% of that biomass. The fish were fed on rations with the different 7 treatments for 1 month. The examined fish were sampled and evaluated at the end of the experiment.

Evaluation of growth parameters: Different growth parameters (Net weight gain, daily gain, relative growth rate, condition factor and feed conversion ratio) of the experimented catfish were calculated.

Evaluation of immune response using
Survival rate: Will be calculated by deducting the mortal or dead fish out of the initial total number then dividing the number of survived catfish by the total number and multiplied by 100.

Blood parameters: Blood samples were taken from the caudal veins of 2 fish per each aquarium (i.e., total of 6 fish or replicates per treatment were sacrificed) for the determination of total and differential leucocytic counts (Dacie and Lewis, 1995), haematocrit values, Haemoglobin (Hb) (using commercial colorimetric kits; Randox, Germany) and phagocytic activity using Nitro Blue Tetrazolium (NBT) assay where the production of oxygen radicals by macrophages was assayed by the reduction of NBT (NBT; Sigma-Aldrich Chemical, St. Louis, MO, USA) according to Rook *et al*. (1985).

Hepato and spleno/somatic indices: The 6 sacrificed fish from each treatment were dissected. The liver and spleen of each fish were removed, weighed and the hepato/somatic as well as the spleno somatic indices were calculated.

Challenge test (Koch's postulate): Bacterial challenge test was conducted using a virulent strain of *Aeromonas hydrophila*. Ten catfish from each treatment were I/P injected with 0.5 mL of 0.5×10^{6} CFU mL^{-1} of 24 h Tryptic Soy Broth (TSB) culture of *A. hydrophila* (Schaperclaus *et al*., 1992). The fish were kept for 2 weeks, where the clinical signs and daily mortality were recorded.

Statistical analysis: All data were analyzed statistically using Analysis of Variance (ANOVA) test. Significant difference between the treatment means was determined at 5% confidence limit ($p<0.05$) using Duncan's multiple range test (Duncan, 1955). All statistical analyses were done using SPSS program as described by Dytham (1999).

RESULTS AND DISCUSSION

Evaluation of growth parameters of treated catfish fingerlings: Results of Table 1 and Fig. 1 revealed that catfish treated with wormseed plant in the rate of 5% (treatment 3) had significantly the highest biomass and average body length compared with fish in other treatments and in the control group. The next rank was for catfish fed with chamomile in the rate of 1% of the feed (treatment 4) with respect to total body length, followed by treatments 5 and 6, respectively (with no significance difference). Meanwhile, fish in treatments 5 and 6 gave higher body weight than those in treatment 4. Fish in treatment 2 and in the control group had the lowest biomass in this experiment. This could be attributed to the high mortalities among the catfish in these 2 groups (6 and 5 dead fish, respectively). This also accounted for the higher but insignificant average body weight gained by fish of the control group (fewer fish received the same amount of food of other treatments with higher number of fish) rather than fish in other treatments. On the other hand, results of Table 2 and Fig. 2 showed that fish in treatments 3 and 4 gave the highest but insignificant estimates of net and daily weight gains as well as relative growth rate. Fish in the same groups also had the lowest Feed Conversion Ratio (FCR). Thus, they significantly, had the best FCR rather than that of the control fish. On the contrary, fish of the control group gave significantly, the highest condition factor compared with that of fish of treatments 3 and 4. This might be probably because of the same reason of high mortality as mentioned before.

Evaluation of immune response of experimented catfish fingerlings
Survival rate: As shown in Table 1 and Fig. 1, fish in treatment 3 had significantly the highest survival rate compared with that of fish in the control group and treatment 2. Fish in treatments 1 and 4 equally, occupied the second rank followed by fish in treatments 5 and 6, respectively. This could be attributed to the immune stimulant effect induced by wormseed plants and chamomile flowers on the non specific immune response of the catfish under study. These results were supported by Bakeer and Mostafa (2006).

Table 1: Total biomass, average body weight, average total length and survival rate of catfish fed with wormseed plants and chamomile for 1 month

Serial no.	Treatment (T)	Total biomass (g)	No. of fish	Total body weight (g)	Total body length (cm)	Mortality	Survival rate (%)
1	1	301	19	15.8	13.4	1	95
2		302	17	17.8	15.1	3	85
3		302.4	18	16.8	14.4	2	90
Mean±SE		301.8±0.003^{c}	18±0.004	16.8±0.004^{c}	14.3±0.004^{b}	2±0.004	90±0.021^{ab}
4	2	222	14	15.9	14.3	6	70
5		281.4	14	20.1	16	6	70
6		252	14	18.0	15.2	6	70
Mean±SE		251.8±0.126^{d}	14±0	18±0.009^{bc}	15.2±0.004^{ab}	6±0	70±0^{d}
7	3	358	19	18.8	15.5	1	95
8		377.4	19	19.9	15.1	1	95
9		368.6	19	19.4	15.3	1	95
Mean±SE		368±0.041^{a}	19±0	19.4±0.002^{ab}	15.3±0.001^{a}	1±0	95±0^{a}
10	4	314	16	19.6	15.7	4	80
11		343	20	17.2	14.3	0	100
12		331.2	18	18.4	15.0	2	90
Mean±SE		329.4±0.062^{bc}	18±0.009	18.4±0.005^{abc}	15±0.003^{ab}	2±0.009	90±0.042^{ab}
13	5	351	18	19.5	14.5	2	90
14		321	16	20.1	15.1	4	80
15		336.6	17	19.8	14.8	3	85
Mean±SE		336.2±0.064^{b}	17±0.004	19.8±0.001^{ab}	14.8±0.001^{ab}	3±0.004	85±0.021^{abc}
16	6	313	18	17.4	14.3	2	90
17		331	15	22.1	15	5	75
18		336.6	17	19.8	14.7	3	85
Mean±SE		326.9±0.052^{bc}	16.7±0.007	19.8±0.010^{ab}	14.7±0.002^{ab}	3.3±0.007	83.3±0.032^{bc}
19	7	329	16	20.6	14.5	4	80
20		307.2	15	20.5	14.9	5	75
21		285.6	14	20.4	14.7	6	70
Mean±SE		307.3±0.092^{bc}	15±0.004	20.5±0.0004^{a}	14.7±0.001^{ab}	5±0.004	75±0.021^{cd}

Means having the same superscript letters in the same column are not significantly different at $p<0.05$

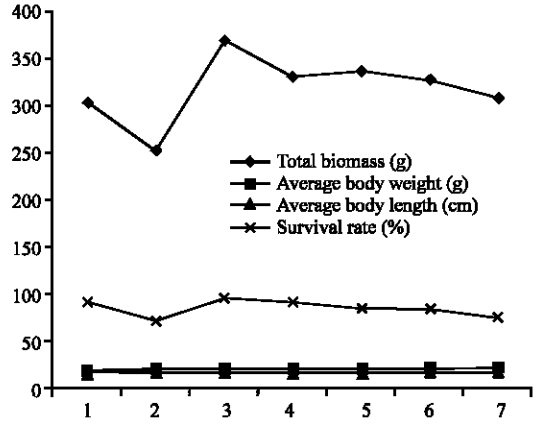

Fig. 1: Average total biomass, body weight, body length and survival rate of the experimented catfish in the 7 treatments

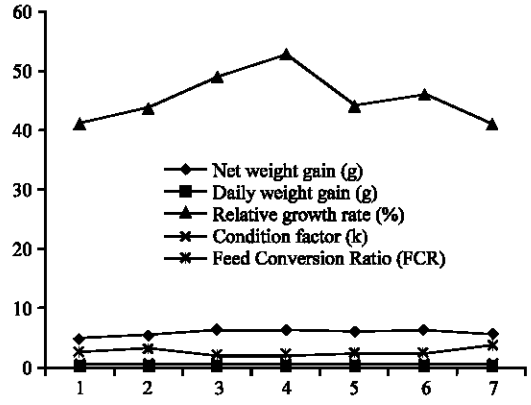

Fig. 2: Average net and daily weight gain, relative growth rate, condition factor and feed conversion rate of the experimented catfish in different treatments

Blood parameters: Results of Table 3 and Fig. 3 revealed that fish in all treatments of chamomile had the highest total leucocytic count (treatments 6, 5 and 4, respectively) especially, fish in treatment 6 which were significantly higher than those of other treatments and the control group regarding their total leucocytic counts. These results might indicate a more positive effect of chamomile on cellular immune response. These figures of total leucocytic counts in catfish of the control group disagreed with those recorded by Abdelhamid *et al.* (2009) and Adedeji *et al.* (2009) who recorded higher estimates of total leucocytic count. On the other hand, fish in all treatments of wormseed plants showed the highest figures of blood haemoglobin, Packed Cell Value, (PCV) and NBT (treatments 2, 1 and 3, respectively) especially, fish in treatment 2 which gave significantly higher estimates than those of other treatments and the control group. Fish in treatment 4 occupied the next

Table 2: Initial, final body weight, net weight gain, daily gain, relative growth rate, condition factor and FCR of catfish fed with wormseed plants and chamomile for 1 month

Serial no. of aquaria (replicates)	T	Initial Body weight (g)	Final Body weight (g)	Net wt gain (g)	Daily gain (g)	Relative growth rate (%)	Condition factor (k)	Feed Conversion Ratio (FCR)
1	1	12.1	15.8	3.7	0.123	30.6	0.66	3.1
2		11.7	17.8	6.1	0.203	52.1	0.52	2.0
3		11.9	16.8	4.9	0.163	41.2	0.56	2.6
Mean±SE		11.9±0.0009	16.8±0.004	4.9±0.005^{a}	0.163±0.0002^{a}	41.3±0.046^{a}	0.58±.0003^{abc}	2.6±0.002^{ab}
4	2	12.1	15.9	3.8	0.127	31.4	0.54	4.1
5		12.9	20.1	7.2	0.240	55.8	0.49	2.3
6		12.5	18.0	5.5	0.184	44.0	0.51	3.2
Mean±SE		12.5±0.002	18±0.009	5.5±0.007^{a}	0.184±.0002^{a}	43.7±0.052^{a}	0.51±0.0001^{c}	3.2±0.004^{ab}
7	3	12.8	18.8	6.0	0.200	46.9	0.51	2.0
8		13.2	19.9	6.7	0.223	50.8	0.58	1.9
9		13.0	19.4	6.4	0.212	49.2	0.54	2.0
Mean±SE		13±0.001	19.4±0.002	6.4±0.002^{a}	0.213±4.871^{a}	49.0±0.008^{a}	0.54±0.0002^{bc}	1.97±0.0003^{b}
10	4	12.3	19.6	7.3	0.243	59.4	0.51	1.9
11		11.8	17.2	5.4	0.180	45.8	0.59	2.0
12		12.0	18.4	6.4	0.212	53.3	0.55	2.0
Mean±SE		12.03±0.001	18.4±0.005	6.4±0.004^{a}	0.213±0.0001^{a}	52.8±0.029^{a}	0.55±0.0002^{bc}	1.97±0.0003^{b}
13	5	14.3	19.5	5.2	0.173	36.4	0.64	2.8
14		13.2	20.1	6.9	0.230	52.3	0.58	2.2
15		13.8	19.8	6.0	0.202	43.5	0.61	2.5
Mean±SE		13.8±0.002	19.8±0.001	6.03±0.004^{a}	0.202±0.0001^{a}	44.1±0.034^{a}	0.61±0.0001^{ab}	2.5±0.001^{ab}
16	6	12.4	17.4	5.0	0.167	40.3	0.57	2.5
17		14.6	22.1	7.5	0.250	51.4	0.66	2.3
18		13.5	19.8	6.3	0.209	46.7	0.62	2.4
Mean±SE		13.5±0.005	19.8±0.010	6.3±0.005^{a}	0.209±0.0002^{a}	46.1±0.024^{a}	0.62±0.0002^{ab}	2.4±0.0004^{ab}
19	7	12.8	20.6	7.8	0.260	60.9	0.68	1.6
20		16.7	20.5	3.8	0.127	22.8	0.62	5.8
21		14.6	20.4	5.8	0.194	39.7	0.64	3.7
Mean±SE		14.7±0.008	20.5±0.0004	5.8±0.009^{a}	0.194±0.0003^{a}	41.1±0.081^{a}	0.65±0.0001^{a}	3.7±0.009^{a}

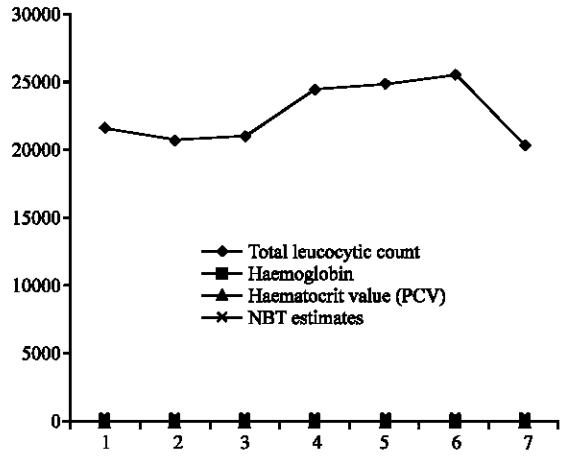

Fig. 3: Average total leucocytic count, haemoglobin content, haematocrit value and NBT estimates of the experimented catfish in different treatments

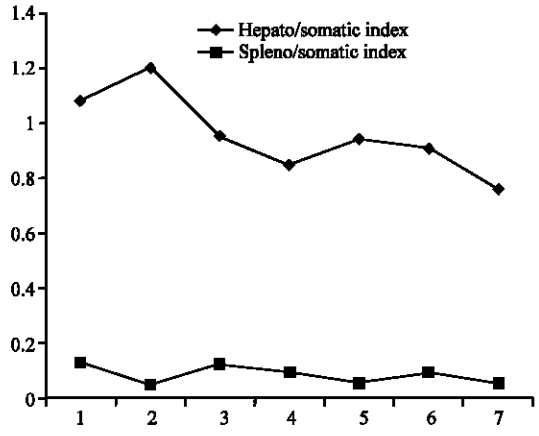

Fig. 4: Average hepato/somatic and spleno/somatic indices of the experimented catfish in different treatments

rank after wormseed plants in this regard. Meanwhile, treatments 5, control and 6 had the lowest figures, respectively with no significant differences amongst them. These results might indicate a more positive effect of wormseed plants on humoral immune response of experimental catfish. Similar results and figures of haemoglobin in catfish of the control group were reported by Abdelhamid *et al.* (2009). On the contrary, estimates of haematocrit value in catfish of the control group disagreed with those recorded by Adedeji *et al.* (2009) who recorded higher figures of PCV.

Hepato/somatic and spleno/somatic indices: Fish in all treatments of wormseed plants had the highest hepato/somatic indices (treatments 2, 1 and 3,

Table 3: Total and differential leucocytic counts, haematocrit values, haemoglobin and NBT estimates of catfish fed with wormseed plants and chamomile for 1 month

Serial no. of Ex. fish	T	Total Leucocytic count	Differential Leucocytic count			Hemoglobin (Hb) (PCV)	Hematocrit Value	NBT estimates (mg mL^{-1})
			Neutrophils	Monocytes	Lymphocytes			
1		24000	5	2	93	7.14	22.3	0.54
2		21500	6	1	93	7.20	22.5	0.35
3		20300	5	1	94	7.08	22.24	0.70
4		21100	6	2	92	7.14	22.1	0.27
5		20700	4	3	93	7.56	23.7	0.34
6		21800	5	3	92	5.88	18.9	0.32
Mean±SE	1	21566.7±5.5^{abcd}	5.2±0.003	*2±0.004*	92.8±0.003	7±0.002^{ab}	21.96±0.66^{ab}	0.42±0.001^{a}
7		22100	5	2	93	7.14	22.2	0.34
8		18900	4	2	94	7.14	23.1	0.43
9		22000	6	1	93	7.14	21.9	0.40
10		19100	6	1	93	8.40	26.1	0.47
11		23000	6	3	91	8.80	27.4	0.49
12		18700	5	3	92	8.40	26.8	0.36
Mean±SE	2	20633.3±8.2^{cd}	5.3±0.004	2±0.004	92.7±0.004	7.8±0.003^{a}	24.58±1.004^{a}	0.42±0.0003^{a}
13		21900	5	2	93	7.90	24.0	0.35
14		22900	6	2	92	5.88	17.6	0.63
15		19100	6	2	92	7.56	22.9	0.32
16		20100	8	2	90	6.70	21.0	0.24
17		19600	7	3	90	6.30	19.2	0.19
18		22100	8	3	89	5.88	17.6	0.27
Mean±SE	3	20950±6.6^{bcd}	6.7±0.005	2.3±0.002	91±0.007	6.7±0.004^{bc}	20.38±1..11^{b}	0.3±0.001^{ab}
19		21900	7	2	91	7.56	24.1	0.25
20		26400	7	3	90	6.30	18.8	0.24
21		23400	6	2	92	7.56	23.4	0.20
22		28100	9	2	89	5.88	16.9	0.28
23		18600	9	1	90	5.88	17.0	0.30
24		28100	6	3	93	7.14	22.1	0.27
Mean±SE	4	24416.7±16.1^{abc}	7.3±0.006	2.2±0.003	90.8±0.006	6.7±0.003^{abc}	20.38±1.32b	0.26±0.0002^{bc}
25		23100	9	1	91	4.62	12.9	0.12
26		27300	5	1	94	5.88	17.64	0.39
27		28000	6	2	92	7.56	25.0	0.21
28		24200	5	2	93	4.62	13.9	0.07
29		23500	8	2	90	6.70	21.2	0.27
30		22900	4	1	95	5.66	18.8	0.20
Mean±SE	5	24833.3±9.5^{ab}	6.2±0.008	1.5±0.002	92.5±0.008	5.8±0.005^{c}	18.24±1.85b	0.21±0.001^{c}
31		30100	6	2	92	5.46	17.2	0.25
32		28200	5	2	93	4.20	11.9	0.27
33		21100	5	2	93	6.70	22.0	0.13
34		24400	5	5	90	7.56	23.5	0.17
35		23000	7	2	91	7.14	21.4	0.23
36		26000	6	2	93	7.56	24.0	0.25
Mean±SE	6	25466.7±14.1^{a}	5.7±0.004	2.5±0.005	92±0.005	6.5±0.006^{bc}	20±1.89b	0.22±0.0002^{bc}
37		12000	4	2	94	6.70	21.1	0.22
38		22200	1	5	94	6.30	19.0	0.27
39		28500	7	4	89	7.14	22.9	0.26
40		24100	4	3	93	5.88	17.7	0.26
41		23100	6	2	92	4.24	13.2	0.26
42		11900	12	4	84	6.70	20.7	0.31
Mean±SE	7	20300±28.9^{d}	5.7±0.016	3.3±0.005	91±0.017	6.2±0.004^{bc}	19.1±$^{1.39}$b	0.26±0.0001^{bc}

respectively) than the rest of treatments especially, fish in treatment 2 which had significantly higher indices than those of fish in treatments 4 and 6 as well as in the control group.

On the contrary, fish in treatment 2 showed the lowest spleno/somatic index compared with all other treatments and even the control group.

However, fish in other treatments of wormseed plants (1 and 3) had the highest spleno/somatic indices followed by these of fish in treatments of chamomile (4, 6 and 5, respectively) without a significant difference and then succeeded by those of fish in the control group and treatment 2 with a significant difference (Table 4 and Fig. 4). Thus, wormseed plants and chamomile enhanced the development of liver and spleen; the main blood forming organs in fish (in addition to the fore-kidney) and as a consequence, stimulated the immune response of catfish.

Table 4: Total body, spleen and liver weights as well as spleno/somatic & hepato/somatic indices of catfish fed with wormseed plants &chamomile for 1 month

Serial no. of Ex. fish	Treatment	Total body weight (g)	Liver weight (g)	Spleen weight (g)	Hepato/ somatic index	Spleno/ somatic index
1		18.0	0.20	0.04	1.111	0.222
2		15.6	0.18	0.03	1.154	0.192
3		11.0	0.10	0.01	0.909	0.091
4		18.5	0.21	0.02	1.135	0.108
5		19.8	0.20	0.02	1.010	0.101
6		28.3	0.33	0.01	1.166	0.035
Mean±SE	1	18.5±0.024	0.20±0.0003	0.022±4.95	1.081±0.0004^{ab}	0.125±0.0003^{a}
7		15.8	0.23	0.01	1.456	0.063
8		20.6	0.25	0.01	1.214	0.049
9		18.6	0.23	0.01	1.237	0.054
10		18.0	0.24	0.01	1.333	0.056
11		29.1	0.23	0.01	0.790	0.034
12		29.8	0.28	0.01	0.940	0.034
Mean±SE	2	21.98±0.025	0.24±8.335	0.01±0.00	1.2±0.001^{a}	0.048±5.08^{b}
13		18.6	0.24	0.01	1.290	0.054
14		17.5	0.16	0.03	0.914	0.171
15		15.7	0.14	0.01	0.892	0.064
16		15.0	0.10	0.01	0.667	0.067
17		12.0	0.12	0.03	1.000	0.250
18		15.8	0.15	0.02	0.949	0.127
Mean±SE	3	15.8±0.010	0.15±0.0002	0.018±4.17	0.952±0.0009^{abc}	0.122±0.0003^{a}
19		20.0	0.19	0.02	0.950	0.100
20		15.7	0.16	0.02	1.019	0.127
21		16.9	0.14	0.01	0.828	0.059
22		18.2	0.16	0.02	0.879	0.110
23		23.2	0.19	0.02	0.819	0.086
24		13.4	0.08	0.01	0.597	0.075
Mean±SE	4	17.9±0.015	0.15±0.0002	0.017±2.19	0.847±0.0006^{bc}	0.093±0.0001^{ab}
25		43.9	0.34	0.02	0.775	0.046
26		24.6	0.28	0.01	1.138	0.041
27		15.0	0.16	0.01	1.067	0.067
28		34.2	0.40	0.03	1.170	0.088
29		20.0	0.17	0.01	0.850	0.050
30		24.7	0.16	0.01	0.648	0.041
Mean±SE	5	23.7±0.030	0.25±0.0004	0.015±3.55	0.941±0.0009^{abc}	0.056±7.881^{b}
31		25.0	0.19	0.01	0.760	0.040
32		21.0	0.17	0.01	0.810	0.048
33		19.2	0.12	0.01	0.625	0.052
34		20.9	0.22	0.03	1.053	0.144
35		27.4	0.27	0.03	0.985	0.110
36		19.0	0.23	0.03	1.211	0.158
Mean±SE	6	22.1±0.014	0.2±0.0002	0.02±4.64	0.907±0.001^{bc}	0.092±0.0002^{ab}
37		23.0	0.12	0.01	0.522	0.044
38		23.1	0.11	0.02	0.476	0.087
39		19.0	0.14	0.01	0.737	0.053
40		24.6	0.20	0.01	0.813	0.041
41		24.2	0.24	0.01	0.992	0.041
42		18.0	0.18	0.01	1.000	0.056
Mean±SE	7	21.98±0.012	0.17±0.0002	0.012±1.73	0.757±0.001^{c}	0.054±7.425^{b}

Challenge results: Neither mortalities nor abnormal clinical signs were noticed among the injected catfish in all treatments. This could be attributed to the high natural or genetic tolerance of the African catfish.

CONCLUSION

An overall evaluation, fish in treatment 2 and 3 of wormseed plants, alternatively had the best figures rather than fish in all other treatments. On the other hand, fish in treatment 4 of chamomile showed the best results compared to other rates of the same herb with respect to growth and immune response parameters.

Thus, it's recommended to add *Artemisia cina* L. (wormseed plants) in the rate of 3 and 5% as well as *Matricaria chamomilla* L. (chamomile) in the rate of 1% to the artificial feeds of the African catfish *Clarias gariepinus*.

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