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STUDIES ON THE *PISTIA STRATIOTES* REDUCTION OF ACUTE TOXICITY OF MALATHION ON GROWTH, PROXIMATE COMPOSITION AND NUCLEIC ACID IN FRESH WATER FISH *LABEO ROHITA*.

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Abstract

This study investigated the protective effect of *Pistia stratiotes* supplemented diets on growth performance and biochemical constituents of malathion exposed *Labeo rohita*. Healthy fish were acclimatized under laboratory conditions and fed diets containing different levels of *P.stratiotes*. Growth, feed utilization, proximate composition, and nucleic acid contents were evaluated. Malathion exposure significantly reduced growth, feed intake, tissue protein, lipid, energy, ash, DNA, and RNA contents. However, dietary supplementation with *P.stratiotes*, particularly at 30% inclusion, significantly ($P < 0.01$) improved body weight, body length, weight gain, feed utilization, tissue protein, lipid, energy, ash, and nucleic acid contents compared with malathion-treated fish. Two-way ANOVA revealed that dietary *P. stratiotes* levels significantly influenced most growth and biochemical parameters ($P < 0.01$). The results demonstrate that 30% *P.stratiotes* supplementation effectively mitigates malathion induced toxicity and enhances growth performance and nutritional status in *L. rohita*, suggesting its potential as a natural feed additive in freshwater aquaculture.

INTRODUCTION

The term pollution broadly refers to any undesirable change in the natural quality of environment brought about by physical, chemical or biological factors. Water pollution is usually caused by various human sources, industrial facilities and agrochemcials especially in aquatic ecosystem. It has dangerous environmental problem in now a days. In aquaculture, water quality is one of the most important one. But those particles and industrial discharges may carried away effectively by air, wind, rivers and floods into the large water bodies and change their physiochemical properties with high toxicity. It causes damages to aquatic life, especially to fishes. The accumulation of pesticides produces some physiological, growth, biochemical and as well as morphological changes in fresh water fauna (Ramamurthy *et al.,* 1987). However, one of the main factors causing pollution of the environment includes irrational use of organophosphorus insecticides. Malathion is an ***organophosphorus*** insecticide widely used in agriculture to prevent disease-causing pests and parasites and it may cause problems in fishes. The toxic substance in aquatic systems and organisms is reduced by using some acceptable chemical compounds. The chemical substance is reducing toxic metabolites in aquatic environment ponds (Chien, 1992; Briggs and Smith, 1996). Hence, the present investigation was taken up to find out the impacts if *Pistia stratiotes* leaf meal on the reduction of malathion toxicity in fish and improvement of growth, feed utilization and biochemical parameters in freshwater fish *Labeo rohita.*

MATERIALS AND METHODS

The experimental animal

L. rohita were collected from Sabari fish farm, Tirunelveli (Dist) Tamilnadu, India. And then were immediately transported to the laboratory conditions. These fishes were acclimatized to the lab condition for a month. During acclimatization, the animals were fed with dried pellets of 20% protein diet.

Experimental diet

For the experimental supplementary feed, *P. stratiotes* young leaves were added along with chosen ingredients. The fresh colonies of *P. stratiotes* were collected from the local pond, Tuticorin district, Tamilnadu, India. And thoroughly washed to remove dirt. Then dried at room temperature for 1 week. After that, these samples were powdered. Six dry diets were prepared in which fishmeal was replaced with *P. stratiotes* at 0%, 10%, 20%, 30%, 40% and 50% and named as P0, P10, P20, P30, P40 and P50 respectively. Feed preparation Feed formulation was done according to (Hardy 1980) and 35% protein diet was prepared for experimental use.

The fresh water healthy fish, *L. rohita* (having weight of 2.97± 0.10g) were selected for the experiment and were selected from the acclimation tank and starved for 24 hrs prior to the commencement of the experiment. Fishes were screened for any pathogenic infections. The fishes were divided into 7 groups of 25 individuals each and the duration of the experiment was conducted for 80 days. Group 1 served as control and reared in fresh water and fed with control diet. Test animals belonging to 2nd to 7th groups were exposed to 0.9 µl (50% sublethal concentration of malathion). Among the malathion exposed groups, 2nd group was fed with control diet, however 3rd, 4th, 5th, 6th and 7th groups were fed with 10, 20, 30, 40 and 50% *P.stratiotes* leaf diets respectively. The experiment groups 1, 2, 3, 4, 5, 6 and 7 are designated as for the convenience of expression C, C+M, M+P1, M+P2, M+P3, M+P4, and M+P5 respectively. Triplicates were maintained for each group. All the experiments were conducted in epoxy coated cement tanks (0.6 x 0.6 m; capacity 110*l*) containing 100*l* of water. The test media were changed once in two ways. The hydro biological parameters like temperature 28.8 ± 0.5˚C, dissolved oxygen 4.20 ± 0.15ml O_2l^{-1} , pH7.9 ± 0.1, salinity 0.31 ± 0.03 ppt and hardness of water 75 ± 3.30 $mgCaCO_3l^{-1}$ respectively.

During the experimental period, test animals were fed *ad libitum* with chosen experimental diets to respective exposures twice a day at 07.00 and 18.00 for 1hr each. Unconsumed feed was removed after 1hr of feeding. Feeding, growth, biochemical parameters and nucleic acid were measured at 20 days intervals.

Sample collection and Analyses

At the beginning and end of the feeding trial, all fish from each replicate were weighed and counted for calculation of weight gain, specific growth rate, feed conversion ratio. At the end of the feeding period five fishes from each replicate were sacrificed for growth , biochemical and Nucleic acids . The muscle samples devoid of bones and analyzed for proximate composition. Crude protein was measured by (Lowry *et al*., 1951) crude lipid was estimated by the (Bradgon method 1951) ash content performed by (Paine 1964) and energy was determined by (Karzinkin and Tarkovskaya 1964) method.

RESULTS

The mean body weight and length of L.*rohita* were enhanced in malathion exposed fish fed on *P.stratiotes* diets. The feeding and growth parameters were enhanced in malathion exposed fish fed on *P.stratiotes* diets (Table 1). However, the weight gain, feed intake and feeding rate of C + M Vs M + P3 treatments of *L. rohita* were significantly enhanced to t 7.6 P < 0.01 t 19.33 P < 0.01 t 41.20 P<0.01 on day 80. But it did not differ significantly (P>0.05) in conversion rate, cross conversion efficiency and feed conversion ratio. But there were significantly (P<0.01) differences between M + P2 Vs M + P3 treatments. But cross conversion ratio did not differ (P < 0.05)(See Table 1). Malathion exposed *L.rohita* received 30% of *P.stratiotes* elicited high rate of feed utilization and growth parameters as compared to other *P.stratiotes* diets. Two way ANOVA test showed that the both growth and feed utilization parameters hold significant (P<0.01) impact in relation to different concentration of *P.stratiotes* and rearing period of malathion exposed in *L rohita*. (Table 1.1).

The tissue protein content was significantly (P <0.01) improved in malathion exposed fish received the *P.stratiotes* diet. Among the *P.stratiotes* diets, malathion exposed *L.rohita* fed 30% *P.stratiotes* diet (M + P30)of treatment showed that elicited the improvement of tissue protein as compared to other diet except control diet (Table 2). A significant (P <0.01) positive correlation was obtained between tissue protein contents and malathion exposed fish received *P.stratiotes* diets . It proves that *P.stratiotes* diet reduced the malathion toxicity in fish *L.rohita* which in turn improved the protein content. Two-way ANOVA test showed that protein content was significantly (P < 0.01) related to levels of *P.stratiotes* diets (Table 2.1). Muscle protein registered the high quantum of protein followed by liver. The regression equation obtained for muscle and liver protein was Y=0.045 + 23.36 and Y=0.032+4.05 in malathion exposed *L.rohita* received 30% *P.stratiotes* diet. Among the tested tissues protein content was reduced by five times in liver and three times in muscle as compared to control fish. A significant (P<0.01) and positive correlation was obtained for the relationship between protein (of both muscle and liver) and the levels of *P.stratiotes* diets. Similar results were obtained for the lipid content of the muscle and liver of L. rohita (Table 2). The two-way ANOVA result showed that the lipid contents of both muscle and liver was also significantly (P<0.01) related to different levels. Similarly, energy and ash contents were reduced in malathion exposed *L.rohita* as compared to control fish. Even though malathion exposed in *L.rohita* received the *P.stratiotes* diets, the energy and ash contents were significantly (P<0.01) improved in malathion exposed fish received the experimental diets up to 30% . The two-way ANOVA revealed that the energy, ash and nitrogen free extract contents was significantly (P<0.01) related to levels of experimental diets; however, rearing period not hold significant (P>0.05) in ash and energy contents. The similar trend was also obtained in ash (Table 4). The regression equation obtained for the ash was 0.0449 in malathion exposed *L.rohita*, the 'b' value obtained for the ash in *L.rohita* was 18.562. And nitrogen free extract showed decreased when *P.stratiotes* level increased up to 30%. It indicates the opposite trend up to fish treated with M + P30 diet (Table 4). Like C+M Vs M+P3 and M+P2 Vs M+P3 treatments was also obtained significant (P< 0.01) results on the 80.

Table 1. Effect of *Pistia stratiotes* diets on growth and feeding parameters in malathion exposed carp, *Labeo rohita* as a function of time. Each value is the mean $(\bar{X} \pm SD)$ of three estimations.

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Rates are exposed as mg g^{-1} live fish day^{-1} and efficiencies as %.

Exposure period(days)	Control (C)	C + M	M + P1	M + P2	M + P3	M + P4	M + P5
Whole Fish Weight (g wet weight)							
20	57.45±0.04	57.45±0.05	57.56±0.04	57.45±0.08	57.45±0.08	57.48±0.09	57.44±0.04
40	60.34±0.03	47.82 ±0.52	51.78±0.49	53.95±0.10	54.58±0.37	51.89±0.56	50.04±0.07
60	65.55±0.32	39.47±0.58	54.93±0.13	57.92±0.28	61.02±0.18	54.91±0.25	52.11±0.11
80	72.19±0.15	37.20±0.19	58.83±0.16	63.30±0.40	67.53±0.45	58.64±0.20	54.39±0.26
Weight Gain (g wet weight)							
20	2.89±0.03	-9.63±0.10	-5.78±0.12	-4.66±0.11	-2.9±0.06	-5.59±0.22	-7.4±0.72
40	5.21±0.13	-9.35±0.21	3.15±0.12	4.97±0.17	6.47±0.31	3.02±0.03	2.07±0.07
60	6.64±0.15	-12.27±0.18	3.90±0.73	5.38±0.40	6.5±0.08	3.73±0.09	2.28±0.05
80	8.10±0.09	-11.74± 0.1	3.94±0.12	7.16±0.03	10.98±0.47	5.80±0.18	3.29±0.27
Feed Intake (g dry matter)							
20	30.66±1.69	19±0.81	24±1.63	26.66±1.25	27.80±0.17	24.66±0.47	21.33±1.25
40	35.33±2.05	15.66±1.25	26.33±2.62	30±2.62	31.33±2.05	27.66±1.69	23±2.44
60	39±0.82	12±3.26	28±4.54	33.66±2.62	37.33±2.05	32.38±2.66	25.94±1.93
80	45.11±1.69	10.65±1.41	31.23±2.78	35.85±2.30	43.32±1.96	36.76±1.31	27.10±1.17
Feeding Rate (mg g-1 live fish day-1)							
20	28.89±0.93	17.79±0.52	20.04±0.66	22.86±0.20	23.84±0.27	22.09±0.34	18.83±0.48
40	30.93±0.40	15.37±0.45	21.90±0.38	25.19±0.74	27.65±0.49	24.22±0.66	20.68±0.64
60	34.93±0.74	10.53±1.17	23.27±1.42	25.48±0.96	32.21±0.64	26.07±1.35	21.54±0.82
80	36.79±1.21	7.69± 0.43	26.08±1.63	29.39±2.28	35.30±0.86	30.23±1.65	24.1±1.63
Conversion Rate (mg g-1 live fish day-1)							
20	2.53±0.08	-8.31±067	-5.17±0.16	-4.19±0.25	-2.53±0.34	-4.66±0.29	-6.46±0.03
40	4.51±0.31	-8.23±0.15	2.69±0.18	4.33±0.22	5.55±0.30	2.67±0.18	1.92±0.08
60	5.61±0.32	-10.6±0.15	3.50±0.13	4.70±0.06	5.61±0.22	4.17±0.05	2.03±0.03
80	7.12±0.66	-10.32± 0.06	3.54±0.09	6.46±0.20	9.59±0.40	5.09±0.04	2.81±0.18
Cross Conversion Efficiency (%)							
20	8.55±0.47	-48.14±0.80	-27.15±2.13	-17.39±0.80	-10.78±1.01	-22.54±1.21	-34.84±1.22
40	14.65±0.50	-54.33±1.69	12.52±0.44	17.53±0.42	20.58±0.49	11.53±0.28	10±0.82
60	17.33±0.26	-101.31±0.88	14.65±0.26	17.53±0.33	18.41±0.25	12.19±0.13	9.23±0.24
80	18.75±0.22	-130.22±0.74	13.47±0.29	21.25±0.22	26.53±0.18	16.67±0.24	11.84±0.08
Feed Conversion Ratio							
20	11.23±0.15	-2.14±0.05	-3.40±0.28	-5.71±0.18	-9.37±0.14	-4.57±0.08	-2.79±0.16
40	6.75±0.16	-1.90±0.08	7.99±0.05	5.82±0.13	9.02±0.09	9.02±0.09	11.52±0.32
60	5.88±0.14	-1.02±0.06	6.90±0.12	5.53±0.30	8.55±0.10	5.66±0.10	10.86±0.22
80	5.40±0.16	-0.86± 0.07	7.65±0.18	4.74±0.10	6.24±0.23	3.88±0.11	8.71±0.19

Table 1.1.Two- way ANOVA for effect of *Pistia stratiotes* leaf meal diets on growth and feed utilization in malathion-exposed carp *Labeo rohita* as a function of time.

Source of Variation	SS	Df	MS	F	P-value
Whole fish weight (g wet weight)					
Between Rearing period	144.39295	3	48.1309845	1.88598564	$P > 0.05$
Between *Pistia* diets	803.43592	6	133.905987	5.24703102	$P < 0.01$
Error	459.36602	18	25.5203345		
Total	1407.1949	27			

Weight gain (g wet weight)					
Between Rearing period	311.86809	3	103.956029	16.2416894	$P < 0.01$
Between *Pistia* diets	738.97759	6	123.162931	19.2425018	$P < 0.01$
Error	115.21021	18	6.40056746		
Total	1166.0559	27			
Feed intake (g dry matter)					
Between Rearing period	250.57987	3	83.5266226	6.64037345	$P < 0.01$
Between *Pistia* diets	1419.8991	6	236.649857	18.8136834	$P < 0.01$
Error	226.41486	18	12.5786032		
Total	1896.8939	27			
Feeding rate (mg g^{-1} live fish day^{-1})					
Between Rearing period	93.936211	3	31.3120702	3.20462837	
Between *Pistia* diets	998.58127	6	166.430212	17.033271	$P < 0.01$
Error	175.87601	18	9.77088968		
Total	1268.3935	27			
Conversion rate (mg g^{-1} live fish day^{-1})					
Between Rearing period	240.22597	3	80.0753238	15.7436824	$P < 0.01$
Between *Pistia* diets	561.37194	6	93.5619893	18.395308	$P < 0.01$
Error	91.551379	18	5.0861877		
Total	893.14929	27			
Gross conversion efficiency (%)					
Between Rearing period	2710.2864	3	903.428785	2.15582042	$P < 0.05$
Between *Pistia* diets	29182.643	6	4863.77378	11.6062528	$P < 0.01$
Error	7543.1692	18	419.064954		
Total	39436.098	27			
Feed conversion ratio					
Between Rearing period	382.34087	3	127.446956	8.01198753	$P < 0.01$
Between *Pistia* diets	213.67299	6	35.6121655	2.23876846	$P < 0.05$
Error	286.32661	18	15.9070337		
Total	882.34047	27			

Table 2. Effect of *Pistia stratiotes* diets on protein content (mg g^{-1} wet tissue) in malathion exposed carp, *Labeo rohita* as a function of time. Each value is the mean$(\bar{X} \pm SD)$ of three estimations.

Exposure period(days)	Control (C)	C + M	M + P1	M + P2	M + P3	M + P4	M + P5
Muscle							
0	20.45±1.0	20.45±1.0	20.45±1.0	20.45±1.0	20.45±1.0	20.45±1.0	20.45±1.0
20	22.10±1.17	18.78±1.30	29.35±1.17	19.05±0.95	29.98±1.03	20.78±1.07	19.03±1.28
40	26.73±1.25	13.32±2.35	27.39±0.27	22.19±0.78	22.92±1.95	23.15±2.30	20.60±1.36
60	28.79±1.25	10.48±1.38	19.25±1.22	24.65±1.40	25.02±1.10	26.42±139	22.10±1.19
80	35.42±0.95	7.31± 2.34	10.58±0.09	26.01±2.35	27.43±0.09	32.63±1.53	24.02±1.19

	Liver						
0	4.93±0.15	4.93±0.15	4.93±0.15	4.93±0.15	4.93±0.15	4.93±0.15	4.93±0.15
20	5.40±0.19	3.03±0.15	3.93±0.27	4.02±0.09	4.14±0.49	4.09±0.20	3.85±0.17
40	6.88±0.25	2.95±0.32	3.99±0.17	4.09±0.31	4.60±0.52	4.28±0.05	3.95±0.18
60	8.16±0.15	2.10±0.51	4.68±0.45	4.86±0.32	5.69±0.25	5.18±0.38	4.18±0.52
80	10.05±0.27	1.85±0.13	6.02±0.52	6.85±0.09	7.42±0.23	6.19±0.28	5.56±0.35

Table 2.1. Two- way ANOVA for effect of *Pistia stratiotes* leaf meal diets on protein (mg g^{-1} wet tissue) in malathion exposed carp *Labeo rohita* as a function of time.

Source of Variation	SS	Df	MS	F	P-value
Muscle					
Between Rearing period	32.7894457	4	8.19736143	0.2997379	P > 0.05
Between *Pistia* diets	514.580789	6	85.7634648	3.13595553	P < 0.01
Error	656.362354	24	27.3484314		
Total	1203.73259	34			
Liver					
Between Rearing period	19.97934	4	4.994836	5.216081	P < 0.01
Between *Pistia* diets	44.46927	6	7.411545	7.739837	P < 0.01
Error	22.98202	24	0.957584		
Total	87.43063	34			

Table 3. Effect of *Pistia stratiotes* diets on lipid content (mg g^{-1} wet tissue) in malathion exposed carp, *Labeo rohita* as a function of time. Each value is the mean$(\bar{X} \pm SD)$ of three estimations.

Exposure period(days)	Control (C)	C + M	M + P1	M + P2	M + P3	M + P4	M + P5
Muscle							
0	1.15±0.04	1.15±0.04	1.15±0.04	1.15±0.04	1.15±0.04	1.15±0.04	1.15±0.04
20	1.23±0.02	0.80±0.05	0.82±0.07	0.88±0.08	0.95±0.04	0.90±0.03	0.80±0.05
40	1.50±0.02	0.68±0.07	0.85±0.03	1.07±0.05	1.10±0.06	1.02±0.08	0.94±0.03
60	1.98±0.04	0.43±0.05	0.98±0.04	1.20±0.09	1.23±0.05	1.14±0.08	1.08±0.04
80	2.14±0.07	0.29±0.05	1.17±0.07	1.29±0.05	1.82±0.02	1.25±0.05	1.18±0.07
Liver							
0	0.25±0.07	0.25±0.07	0.25±0.07	0.25±0.07	0.25±0.07	0.25±0.07	0.25±0.07
20	0.28±0.07	0.19±0.07	0.21±0.05	0.21±0.02	0.22±0.08	0.20±0.05	0.20±0.05
40	0.37±0.07	0.15±0.08	0.23±0.07	0.24±0.08	0.26±0.05	0.23±0.08	0.21±0.05
60	0.58±0.07	0.10±0.04	0.29±0.06	0.36±0.05	0.40±0.08	0.30±0.05	0.26±0.04
80	0.85±0.07	0.05±0.02	0.41±0.05	0.56±0.08	0.61±0.05	0.53±0.08	0.38±0.02

Table 3.1. Two- way ANOVA for effect of *Pistia stratiotes* diets on lipid content (mg g^{-1} wet tissue) in malathion exposed carp, *Labeo rohita* as a function of time.

Source of Variation	SS	df	MS	F	P-value
Muscle					
Between Rearing period	0.618731	4.000000	0.154683	2.578498	P < 0.05
Between *Pistia* diets	2.367537	6.000000	0.394590	6.577641	P < 0.01
Error	1.439749	24.000000	0.059990		
Total	4.426017	34.000000			
Liver					
Between Rearing period	0.333646	4.000000	0.083411	8.167106	P < 0.01
Between *Pistia* diets	0.277657	6.000000	0.046276	4.531064	P < 0.01
Error	0.245114	24.000000	0.010213		
Total	0.856417	34.000000			

Table 4. Effect of *Pistia stratiotes* diets on energy (KJg^{-1} wet tissue), ash and nitrogen free extract ($mg\ g^{-1}$ dry matter) in *Labeo rohita* as a function of time. Each value is the mean $(\bar{X} \pm SD)$ of three estimations.

Exposure period (days)	Control (C)	C + M	M + P1	M + P2	M + P3	M + P4	M + P5
Energy							
0	1.60±0.03	1.60±0.03	1.60±0.03	1.60±0.03	1.60±0.03	1.60±0.03	1.60±0.03
20	1.70±0.008	1.45±0.005	1.48±0.002	1.50±0.02	1.50±0.02	1.52±0.02	1.46±0.02
40	2.08±0.05	1.31±0.008	1.53±0.06	1.61±0.01	1.61±0.01	1.66±0.003	1.50±0.003
60	2.24±0.008	1.20±0.004	1.60±0.05	1.70±0.003	1.70±0.003	1.88±0.04	1.58±0.001
80	2.48±0.01	0.81±0.003	1.65±0.02	1.83±0.003	1.83±0.003	2.15±0.05	1.63±0.001
Ash							
0	20.40±0.09	20.40±0.09	20.40±0.09	20.40±0.09	20.40±0.09	20.40±0.09	20.40±0.09
20	21.12±0.05	16.18±0.05	16.90±0.02	17.10±0.09	17.98±0.05	17.02±0.09	16.506±0.03
40	22.05±0.03	13.72±0.10	17.50±0.09	18.02±0.05	18.73±0.09	18.35±0.02	17.010±0.02
60	24.10±0.02	10.74±0.008	20.05±0.08	20.96±0.003	21.60±0.09	19.73±0.05	18.65±0.02
80	24.88±0.03	6.10±0.02	21.10±0.05	22.40±0.003	23.08±0.10	20.54±0.001	20.20±0.10
Nitrogen Free Extract							
0	52.82±0.10	52.82±0.10	52.82±0.10	52.82±0.10	52.82±0.10	52.82±0.10	52.82±0.10
20	49.87±0.17	61.02±0.11	59.09±0.35	57.81±0.43	56.15±0.09	58.23±0.04	59.62±0.13
40	42.47±0.45	69.18±0.02	55.24±0.11	53.66±0.43	52.03±0.95	54.82±0.09	55.79±0.37
60	36.39±0.09	76.75±0.48	49.35±0.37	47.60±0.85	44.66±0.09	50.49±0.08	53.73±0.37
80	31.16±0.43	84.40±0.11	45.29±0.17	41.47±0.08	37.94±0.37	46.11±0.10	48.66±0.57

Table 4.1 Two-way ANOVA for effect of *Pistia stratiotes* leaf meal diets on energy (KJG^{-1}), ash and nitrogen free extract ($mg\ g^{-1}$ wet tissue)in malathion exposed carp, *Labeo rohita* as a function of time.

Source of Variation	SS	Df	MS	F	P-value
Energy					
Between Rearing period	0.26486857	4	0.06621714	1.50252842	P > 0.05
Between *Pistia* diets	1.52693714	6	0.25448952	5.77460345	P < 0.01
Error	1.05769143	24	0.04407048		

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Total	2.84949714	34			
Ash					
Between Rearing period	42.0168921	4	10.504223	1.55864469	P >0.05
Between *Pistia* diets	230.462707	6	38.4104511	5.6994454	P < 0.01
Error	161.743953	24	6.73933136		
Total	434.223552	34			
Nitrogen Free Extract					
Between Rearing period	361.268846	4	90.3172114	1.77229207	P >0.05
Between *Pistia* diets	1927.64759	6	321.274598	6.30436229	P < 0.01
Error	1223.05635	24	50.9606814		
Total	3511.97279	34			

Nucleic Acids

The nucleic acid (DNA and RNA) were high in control fish and it declined in malathion exposed fish. But the tissue DNA and RNA were enhanced in malathion exposed fish fed on *P.stratiotes* supplemented diets (Table 5). The C+M Vs M+P3 treatment of DNA and RNA was significantly (P<0.01) improved in *L.rohita*. Similar results was also obtained in M+P2 Vs M+P3 treatment groups on day 80. But the DNA content was not significant (P<0.01) in malathion exposed in *L.rohita* received 20 and 30 % *P.stratiotes* diets. An increase of DNA and RNA contents in malathion exposed *L.rohita* were related to the increasing of *P.stratiotes* levels in the diet and exposure period. The two way ANOVA elicited that DNA and RNA contents were significantly (P< 0.01) related to the different levels of *P.stratiotes* diets. However the rearing period of RNA did not show the significant (Table 5.1; Fig 1).

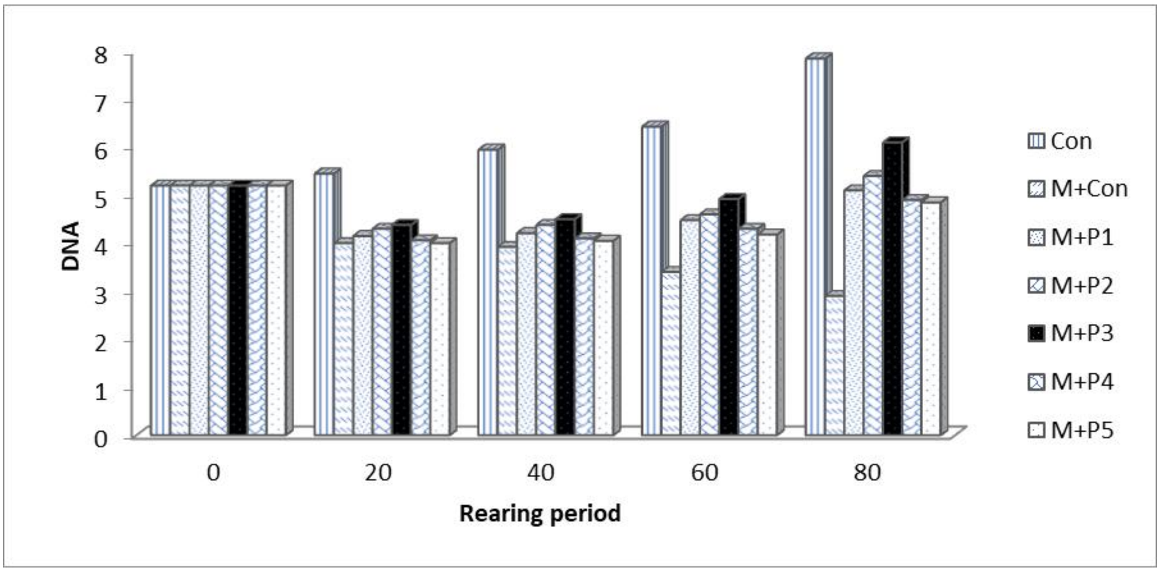
Table 5. Effect of *Pistia stratiotes* diets on nucleic acids in malathion exposed carp, *Labeo rohita* as a function of time. Each value is the mean $(\bar{X} \pm SD)$ of three estimations.

Exposure period(days)	Con	M+Con	M+P$_1$	M+P$_2$	M+P$_3$	M+P$_4$	M+P$_5$
DNA (mg g^{-1} wet tissue)							
0	5.20±0.48	5.20±0.48	5.20±0.48	5.20±0.48	5.20±0.48	5.20±0.48	5.20±0.48
20	5.45±0.15	4.00±0.48	4.15±0.25	4.30±0.70	4.38±0.13	4.06±0.73	4.00±0.10
40	5.95±0.95	3.92±0.10	4.21±0.25	4.38±0.11	4.50±0.70	4.10±0.43	4.05±0.65
60	6.43±0.43	3.40±0.20	4.48±0.11	4.60±0.70	4.92±0.53	4.30±0.17	4.18±0.01
80	7.85±0.05	2.90±0.10	5.10±0.30	5.46±0.72	6.10±0.50	4.90±0.10	4.85±0.22
RNA (mg g^{-1} wet tissue)							
0	6.85±1.80	6.85±1.80	6.85±1.80	6.85±1.80	6.85±1.80	6.85±1.80	6.85±1.80
20	7.45±0.10	6.30±1.10	6.42±0.72	6.50±0.15	6.68±0.53	6.43±0.75	6.23±0.10
40	8.10±0.60	4.80±0.72	6.80±0.05	6.92±0.10	6.97±0.25	6.57±0.10	6.43±0.73

60	11.05±1.12	2.30±0.05	6.95±0.70	7.10±0.11	7.35±0.28	7.05±0.08	6.83±0.22
80	11.28±0.05	1.85±1.19	7.02±1.05	7.25±0.25	7.80±0.05	7.20±0.39	7.02±0.11

Table 5.1 Two- way ANOVA for effect of *Pistia stratiotes* leaf meal diets on DNA and RNA (mg g^{-1} wet tissue) in malathion exposed carp, *Labeo rohita* as a function of time.

Source of Variation	SS	df	MS	F	P-value
DNA					
Between Rearing period	5.558989	4	1.389747	4.214861	P < 0.01
Between *Pistia* diets	15.045989	6	2.507665	7.605311	P < 0.01
Error	7.913411	24	0.329725		
Total	28.518389	34			
RNA					
Between Rearing period	1.138857	4	0.284714	0.175417	P >0.05
Between *Pistia* diets	52.043914	6	8.673986	5.344176	P < 0.01
Error	38.953743	24	1.623073		
Total	92.136514	34			



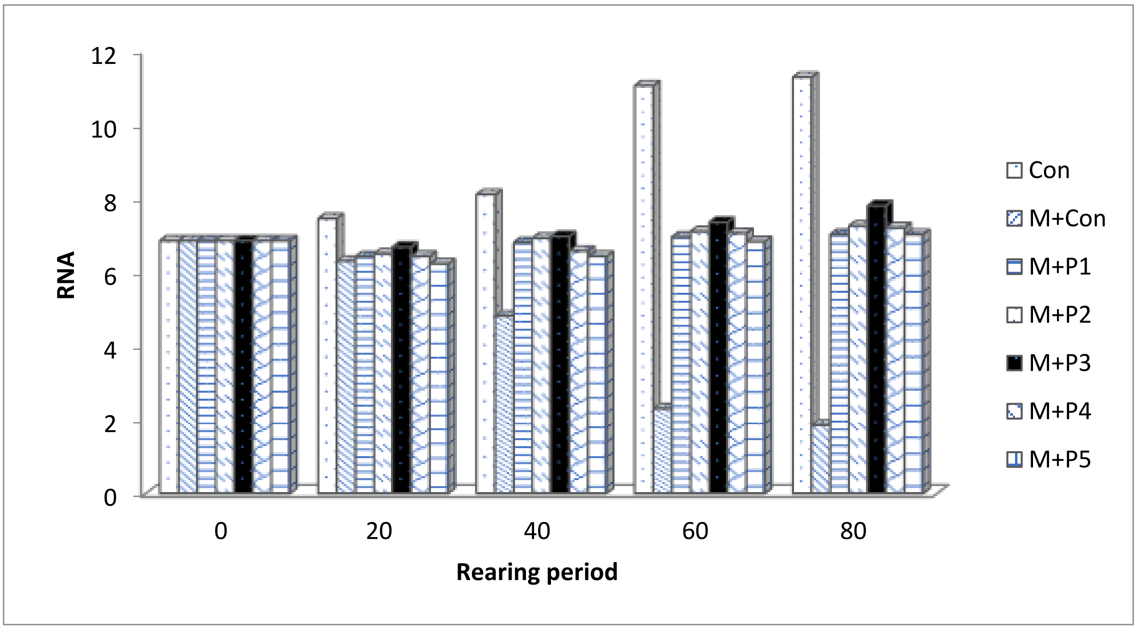


Fig. 1. Effect of different levels of *Pistia stratiotes* on DNA and RNA in malathion exposed *Labeo rohita.*

DISCUSSION

In the present study fresh water fish *L. rohita* exposed to sublethal level of malathion and fed on different concentrations of *P.stratiotes* diet. The pesticides act as a metabolic depressor in the aquatic environment and causes pressure on biologically active molecules. Mathers *et al.,* (1985) found that when a high portion of food energy was utilized for metabolism less energy would be left for growth in *Micropterus salmodius* exposed to toxicants. Several workers have investigated the impacts of various pesticides and heavy metals on growth and biochemical constituents of various aquatic organisms. Therefore, this study observed that, supplementation of *P.stratiotes* in the diets improved feed utilization and biochemical parameters in malathion exposed fish. Sublethal effects of malathion in fish elaborately described in chapter dietary of *P.stratiotes* meal in the diet improved the feed utilizations parameters in malathion exposed fish.

The weight gain, feed intake, feeding rate, conversion rate and cross conversion efficiency of *L.rohita* fish belonging to M+P3 (30% diet) were close to the control fish and FCR value of fish was low as compared to other diets. *P.stratiotes* leaves contain steroids and ascorbic acid (Vitamin C). Lanno *et al*., (1985) suggested that high level of dietary ascorbic acid (10 g kg^{-1} diet) improved the body weight gain in copper exposed rainbow trout as compared to fish fed on low levels of ascorbic acid (0.1-9 g kg^{-1} diet). James *et al.,* (2009) studied that, 6% supplementary diet of *Spirulina* created the reduction of copper uptake in tissues and elimination of more copper through the fish feces which in turn to improve the food utilization in *Cirrhinus mrigala*. Similarly Sunisa *et al.,* (2012) found that, *Moringa oleifora* diet reduced the (Pb) lead toxicity in *Puntius altus* and which in turn to improve the degenerated kidney,liver and gills of lead exposed fish.

The present study also revealed that biochemical parameters were improved in malathion exposed *L.rohita* fed on *P.esculenta* supplemented diets. The biochemical parameters either increase or decrease in the metabolic rate depending on the site of action. Magar and Afsar Shaikh (2012) reported that, proteins are the building blocks of the animal's body and it is most fundamental and biochemical substances to mountain the blood glucose and energy source during the stress period.

Protein reduction might be due to several pathological processes including damage of renal system, decrease in liver protein synthesis and even protein elimination in the urine. Natarajan *et al.,* (1992) reported that the fresh water prawns *Macrobranchium rosenbergii* of protein, glycogen and free amino acid were significantly decreased when actual toxicity of pesticides at different concentrations. Similarly, Thenmozhi *et al.,* (2011) worked on *L.rohita*, reduction on macro- and micro-molecules are directly proportional to the concentration of malathion and exposure periods and also depletion of protein, glycogen and free amino acids were evaluated during various periods of exposure. And also, Tilak *et al.,* (2003) observed the reduction of protein content in brain, liver, and ovary of fish *Channa punctatus* exposed to fenvalerate.

Sunisa *et al.,* (2015) also found that 20%*Moringa oleifora* diet significantly reduced the lead (Pb) toxicity and in that way enhanced the growth performance and protein contents without any adverse effect in fish, *Puntius altus.*

Like P.stratiotes diet (30%) also enhanced the nucleic acids (DNA and RNA) digestive enzymes (protease, lipase and amylase) and respiratory enzymes (SDH and GDH) in malathion exposed *L.rohita.* In the present study the DNA and RNA contents in muscle decreased in malathion treated fish and slightly improved in fish fed with *P.stratiotes* diet. According to Das and Mukherjee (2000) worked on Indian major carp *L.rohita* tissues of DNA were elevated when exposed to quinalphos for 15, 30 and 45 days, the DNA levels are disturbanced in the normal synthesis and thereby turnover rate of DNA besides the degenerative changes. Asifa Parveen and Vasanta (1986) found that DNA and RNA contents were significantly decreased in fish *Claria batrachus* exposed to endosulfan. Similarly, Maruthanayagan and Sharmila (2004) revealed that the effect of monocrotophos on *Cyprinus carpio* toxicant on the nucleic acids and it proved that the pesticides lead to several changes in biochemical contents like DNA and

RNA which may due to the increased activity of the enzyme DNAase and inhibition of RNA polymerase function. But during recovery period the DNA and RNA levels increased progressively. Malla Reddy and Bashamohideen (1988) observed that nucleic acids of DNA/RNA and protein/DNA ration were used as indices of protein synthesis and size of cell are considered to be important and form treatment with the pesticides.

Nucleic acid content is considered as an index of capacity of an organism for protein synthesis. Several studies effect of toxicants on the nucleic acid content in fishes has been reported. Hence the protein synthesis increased in fish when the fish is fed with protein-rich feed and then only nucleic acid enhanced in pesticide treated aquatic organisms. The present investigation was noticed that the 30% *P.stratiotes* diet improved the nucleic acid contents as close value to control.

CONCLUSIONS

With the obtained results, it could be concluded that aquatic weed (*P.stratiotes*) can be supplemented to freshwater carp *L. rohita* diet up to 30% to improve growth performance, biochemical, and nucleic acid contents activity. And above parameters also reduced or enhanced in malathion exposed *L.rohita* by *P.stratiotes* diets.

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