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EVALUATION OF DIETARY USE OF *AZOLLA PINNATA* ON GROWTH PERFORMANCE AND *AZOLLA MAXICANA* EXTRACT AS DISEASE RESISTANCE IN *LABEO ROHITA* FINGERLINGS

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ABSTRACT

Today's time is the growing awareness for the animal production ,here the fish culture also taking the spotlight as it's the best source of nutrients but some time problem arising due to low nutritional level of supplementary food and in other hand major loss occurred due to Fish diseases. There is need to focused upon aquaculture to develop microbial control strategies and best nutritive supplement. In present study we were studied the fish microbial residents and their potential impact on fish culture. All the fishes were put in portable plastic pools each portable plastic pools stacked with 10 fish those were fed with azolla pinnata.best results were obtaining in diet containing 300 and 350 g per kilo gram of basal feed.also its shows the greter inhibitory activity against fish pathogens *Vibrio, e-Coli.treted by Azolla Maxicana.*extract.

Keywords: *Labeo Rohita, Growth,* Antagonistic Activity, *Azolla Pinnata, Azolla Maxicana,Vibrio, E-Coli.*

I. INTRODUCTION

Now a days the fish culture in small scale farm level increased .and hence need to produce out nutritional rich and well grown fishes for food purpose. Hence to achieve these goals many farmers using herbal base of feed, for this many protein basis's used with their potential as alternatives to fishmeal in fish culture. Also these diets are without affecting the growth performance of fish [1]

many research attempts have been focused upon to use the plant based feed suppliant so use of Azolla also very potential and taking consideration because it have higher crude protein content which ranges from 19-30 % .so that used in fish farm [2] experimental data shows that azolla plant contain protein level up to is 23% - 30% out of which 55% protein are of amino acid [3]

Azolla is a kind of water fern which have tiny branches which floats on water surface, roots remain deep in water. The most extraordinary feature of azolla, it shows symbiosis with nitrogen fixing blue green algae. [4]

So that in present investigation we were used Dry *Azolla pinnata* powder with soybean based diet.

Azolla maxicana extract against various fish pathogens. The major aim of investigation to find growth promoting factor and immune modulation in *labeo rohita* fingerlings. Here we had chosen *labeo rohita* fingerling as test animal so as this formula can be used for wide variety of fresh water fishes having demand in food market.

II. MATERIAL AND METHODS

1. Preparation of Azolla rich diet: and antibacterial extract preparation

The fish feed was prepared out were shown in table 1 here we were used locally available fish feed ingredients along with autoclaved soybean to reduce amount of anti- nutritional factors , rice bran, wheat and vitamin premix also used with groundnut oil cake. *Azolla pinnata* were sun dryed first and used in powdery form in fish diet. Here we made small pellets so as to became easy to consume fish fingerlings. The macronutrient analysis of *Azolla pinnata were done by* using standard method (AOAC, 2005) the The *Azolla pinnata* powder shows following macronutrients level. Shown in figure 1.further azolla maxicana samples were prepared by using Soxhlet were used for antibacterial study.

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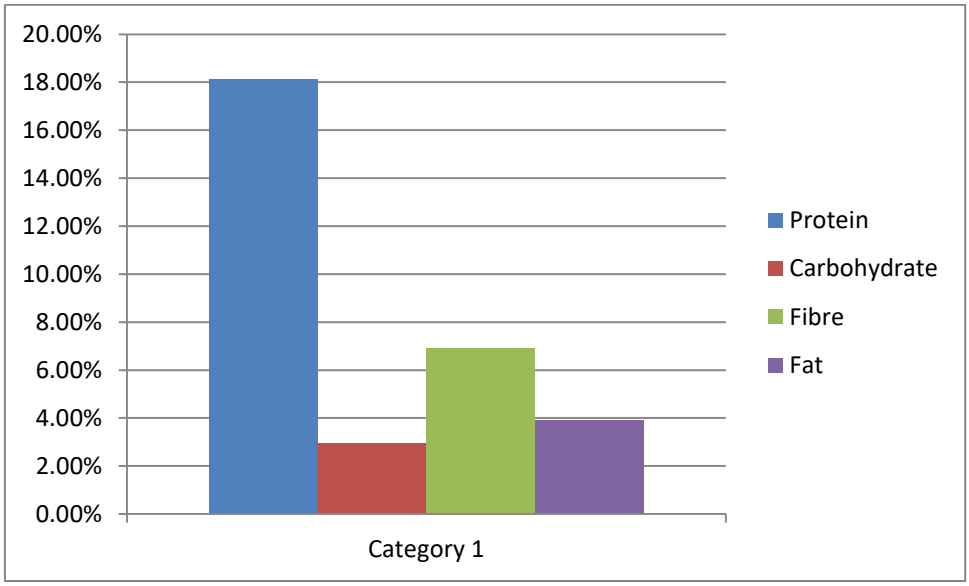

Fig 1: level of macronutrients *Azolla pinnata* dry powder

Table 1. Ingredient content of experimental diet with *Azolla pinnata*

Ingredients (IN %)	**Experimental Feeds**				
Ingredients	**control**	**DAZ-1**	**DAZ-2**	**DAZ-3**	**DAZ-4**
Soybean	55	55	55	55	55
Wheat	15	15	15	15	15
Rice bran	05	05	05	05	05
Ground nut oil cake	20	20	20	20	20
Azolla pinnata Powder	00	200*	250*	300*	350
Vitamin Premix	05	05	05	05	05
* gram per 1 kg of basal diet					

2. Study Of Growth Parameters :

Here we had noted the SGR (specific growth rate) and FCR (feed conversion ratio) survival were calculated using the following formulas

I. **Body weight gain:** Final fish weight (g) - Initial fish weight (g) [5]

II. **SGR** = 100 × [natural log of final weight –natural log of initial weight] / total duration of the experiment [6]

III. **FCR** = feed given (dry weight) / weight gain (g) [7]

3. Isolation and Biochemical characterization of pathogenic bacteria from *labeo rohita*

infected fish were collected and sample were taken from skin lesions on fins and gills further it spread on culture media like De Man, Rogosa and Sharpe agar,MRS agar, MRS broth, Thiosulfate-citrate-bile salts-sucrose agar TCBS agar and Salmonella Serotyping Assay (SSA). these agar plates and broth were incubated at 37°c up to 24 to 48 hours after that all bacterial growth on culture plated were observed next to it the sub-culture were done for further characterization and identification.

The record put in terms of morphology of colony gram staining reaction and motility tests were done. The bacterial isolates were also analyzed for various biochemical tests *viz.* Catalase Test, Oxidase Tests, Motility Test, Indole Producing Test, and carbohydrates fermentation (arabinose, fructose, galactose, lactose, mannitol, salicin, sucrose and trehalose). These characteristics are considered for the species level identification of bacterial isolates.

4. Feeding Experimental Setup: Collected fish *labeo rohita* were stocked for acclimation in 60 liter portable plastic pools for one week. Fishes were fed two times at 08:00h and at 14:00h. the feeding rate was at 5 % body

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weight day^{-1} for the whole rearing period of 30 days, and the amount of feed was adjusted every tenth day following a bulk weighing of each group of fish.

5. Antagonistic activity: Each soxlet methanol extract was tested at variable grading (10%, 20%, 30%, 50% & 70%). Further these methonolic extract used upon pathogens in vitro on culture media. 5 sets of experiment, and were conducted for 4 week For each bacterium 20 conical flasks (100 ml) have culture medium containing pure strain of bacterial fish pathogens.

III. RESULTS

1. Growth parameters:

Initial-final weight, weight gain, specific growth rate of *Labeo rohita* fingerlings were noted . The highest weight gain and specific growth rate values, at the end of the experiment. FCR and SGR values were also recorded in fish fed control (no *Azolla Pinnata*).

Fish fed with *Azolla pinnata* were shown significant differences. It clearly indicates that from 200,,250.300,350 gram per kilo gram work better.

Best growth shown in DAZ-3 and DAZ-4 which were contained *Azolla Pinnata* powder 300 and 350 gram per kilo gram respectively. The body weight gain, specific growth rates, condition factor, and survival rate were significantly observed to be higher ($p < 0.05$) in fishes fed with basal diet supplemented with *Azolla Pinnata.* And SGR of fish has improved as *Azolla Pinnata* feed augmented.

2. Identification and characterization of microorganisms

Fish pathogens, such as *e. coli, vibrio* sp were isolated from the region of infected fishes. these two pathogens were morphologically characterized as., *e. coli,* and *vibrio sp* they were physically gram negative, motile and rod shape. among the various biochemical assays shown in table 3 .

Table 2: Growth study in *Labeo rohita* fingerlings dietary use of *Azolla Pinnata*

Sr No	Test Group	Body Weight Gain (G)		Specific Growth Rate	Feed Conversion Ration
		Weight (Initial)	Weight GAIN		
1.	**Control**	20±0.86	5.22±0.25	0.0026	4.76
2.	**DAZ-1**	20±0.50	11.23±1.25	0.0051	3.71
3	**DAZ-2**	20±0.78	12.32±1.41	0.0062	4.11
4	**DAZ-3**	20±0.79	15.33±1.34	0.089	4.89
5	**DAZ-4**	20±0.80	17.31±1.41	0.0092	4.90

Among the various biochemical assays studied the positive results were observed in all 2 pathogens isolates, such as methyl red, nitrate reduction, catalase, oxidase and maltose, mannitol, rhamnose for sugar assimilation. whereas the negative results were obtained in indole, vogus proskur, citrate utilization, urease and arabinose for sugar assimilation. arabinose, fructose, glucose, lactose were fermented by isolated *vibrio* sp., *e. coli,* (table 3).

Based on the morphological characteristics and biochemical properties, the isolated fish pathogens are identified as *vibrio* sp., and *e. coli.*

Table 3: Basic biochemical test for pathogenic organism isolated from *Labeo rohita*

Vibrio sp	
Hemolysis	Beta Hemolysis
Indole	+ve
Motility	Motile
MR (Methyl Red)	-ve

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E. coli	
Indole	+ve
Motility	Motile
MR (Methyl Red)	+ve
Nitrate Reduction	+ve

3. Antagonistic effect: To evaluate the antagonistic effect of *Azolla maxicana* against the fresh water fish pathogens, *vibrio* sp., *e. coli,* isolates were isolated from the various other deseased fresh water fishes. The antagonistic activity of *Azolla maxicana*. isolate was done against fish pathogens by Cross streak method.

The zone of inhibition showed against, *e. coli (*12.98±.0.4), *vibrio sp (*10.1 ±1.33) at end of day 21 the good result were obtain at concentration 50% & 70% (table 4 A & B). *but here the concetration 70% shows inhibition up to day 28.* The size and weight of the *Labeo rohita* increased when compared to that of control fish without *Azolla pinctata* feed,. it shows that the *Azolla maxicana* effectively can be used as antibacterial agent in Fish culture farms which showed greater growth improvement.

Table 4A: Antibacterial activity of the *Azolla maxicana* extract against fish pathogens isolated from various diseased fishes (50%).

Sr. no	**Isolated pathogens**	**Zone of Inhibition**			
		Day -7	**Day-14**	**Day-21**	**Day-28**
1.	*E. Coli*	8.02±0.1	9.03±1.2	12.98±.0.4	7.01±0.2
2.	*Vibrio*	4.8 ±1.42	6.3 ±1.51	10.1 ±1.33	7.2± 1.60

Table 4B: Antibacterial activity of the *Azolla maxicana* extract against fish pathogens isolated from various diseased fishes (70%).

Sr. no	**Isolated pathogens**	**Zone of Inhibition**			
		Day -7	**Day-14**	**Day-21**	**Day-28**
1.	*E. Coli*	9.01±0.4	9.95±1.4	14.91±.0.2	18.01±0.3
2.	*Vibrio*	5.2 ±1.18	6.98 ±1.45	11.13 ±1.41	10.2± 1.30

IV. CONCLUSION

Filicophyta as the major source of feed during pond culture of macro-phytophagous fish species *Labeo rohita and Cirrihinus mrigala were shown better growth,*Feed acceptance trial was conducted for these seven species using *Azolla imbricata* and *Azolla pinnata* species. Had been recorded higher growth rates fed with azolla [8].

Azolla filiculoides in pond and examined its potential as a fish feed. In a feeding experiment with *Tilapia nilotica*,. Diets C and D exerted the same effect on the growth of Tilapia after 3 weeks of feeding with a 17% decrease of growth compared to the control. Based on the results, it was observed that azolla can replace about 20% of tilapia feed, which indicates the beneficial effect of the use of aquatic plant. [9] similar kind of study using dry azolla meal as a sole protein source for feeding tilapia *Oreochromis niloticus.* [10] The inclusion levels of azolla meal were 0, 15, 20, 30, 40 and 45% on dry weight basis in diet. Comparing growth results, juveniles fed with 15% of azolla meal have exhibited best growth followed by azolla free diet. Considering cost of feed, the study recommended to use 45% azolla incorporated diet for tilapia in a fertilized pond. Another study carried out on Azolla pinnata shows that the feed macronutrients value along with protein, vitamins, and minerals. Which shows the better alternative for the commercial fish food *Barbonymus gonionotus* [11]

It was observed that the survival percentage of *Labeo rohita* fingerlings of different experimental group didn't show any significant difference between the groups. But in contradiction there are reports of higher survival and net production of fish when the pond was treated with azolla extract.

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From the present investigation, we can concluded that the antagonistic effects of azolla extract against the fresh water fish pathogens is effective and safe which helpful in the bacterial disease control in *Labeo rohita*.

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