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Application of Vermiwash as Juvenile Fish Feed: A Test Case using Black Mollies (*Poecilia sphenops*)

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ABSTRACT

360 black mollies (*Poecilia sphenops*) were cultured in a glass aquarium (10 L) in laboratory conditions. Live plankton (LP), vermiwash (VW-I, VW-II) and market available feed (MAF) were supplied to assess the growth as well as survival rate of those fishes. Vermiwash proved to be best feed for juvenile fish as the growth of cultured fish was significantly ($P < 0.05$, 250–210%) higher than LP fed fish fed with MF. Maximum survival rate (98%) was shown by juveniles fed vermiwash (VW-I or VW-II) followed by those fed LP (80%) and MAF (75%). Diseases (gill rot or tail and fin rot, eye disease, or scale loss) were not encountered in fish cultured in the vermiwash treatment.

Keywords: aquarium, growth, micronutrients, physico-chemical characteristics

INTRODUCTION

Market available feed (MAF) are not always useful for juvenile fish and feeding of new-born or juvenile fish remains a problem for pisciculturists. Mortality and manifestation of poor health is noticed among fish juvenile fed with conventional feeds (dried and pelleted fed) available on the market. Although live plankton, tubitex and protein powders have shown promising results (Lim *et al.* 1997; Lim *et al.* 2002) there is still a need to develop a low-cost juvenile fish feed. Vermiculture farms have started to use a product called vermiwash (VW), which is different from vermimanure; it is a liquid extract which is prepared from earthworms without harming them. VW might have enzymes and secretions of earthworms which stimulate the growth and yield of crops and even develop resistance in crops receiving this spray (Sivasubramanian and Ganeshkumar 2004). Such a preparation would certainly have soluble nutrients apart from some organic acids and the mucus of earthworms and microbes (Kale 1998; Gupta *et al.* 2007). So far, however, there is no experimental evidence to quantify the effect of such a preparation in fish culture. In this study we tried to assess the potential of VW as juvenile fish feed and to test it as potential baby fish feed to meet the growing demand of low-cost ornamental fish feed throughout the globe as ornamental fish production has gained momentum (Andrews 1990; Chapman *et al.* 1997; Larkin and Degner 2001; Sankar and Ramachandran 2001; Tripathi *et al.* 2002; Gupta 2003; Gajalakshmi and Abbasi 2004b).

MATERIALS AND METHODS

360 black mollies [7 day old laboratory bred (weight: 0.5 ± 0.04 g; length: 1.1 ± 0.02 cm)] were stocked in a plastic tub (100 L); then 30 fish were released into each aquarium (15 L capacity). In all four different treatments were employed to test the efficacy of VW as juvenile fish feeds other conventional feed.

Preparation and application of feed

VW-I was prepared by the method standardized at the Krishnagar Govt. College (2008). A plastic tub (**Fig. 1**) 0.3 m in diameter and 0.5 m in height was fitted with a plastic gate-valve to facilitate drainage of eluates. The tub was filled to a height of 5 cm with gravel (5 mm dia) above which a layer of coarse sand (5 cm) and garden soil (10 cm) was placed. Above the soil, a layer (5 cm) of shade-dried and powdered cow dung along with dried leaves of *Azadirachta indica* was added. Withered neem leaves were settled and sun-dried. The neem leaves were soaked in water for 5 days to retain the moisture for degradation into compost. A change in the color of the leaves from pale to dark brown after 7–9 days showed complete decomposition (Jameson and Venkataramanujam 2002).

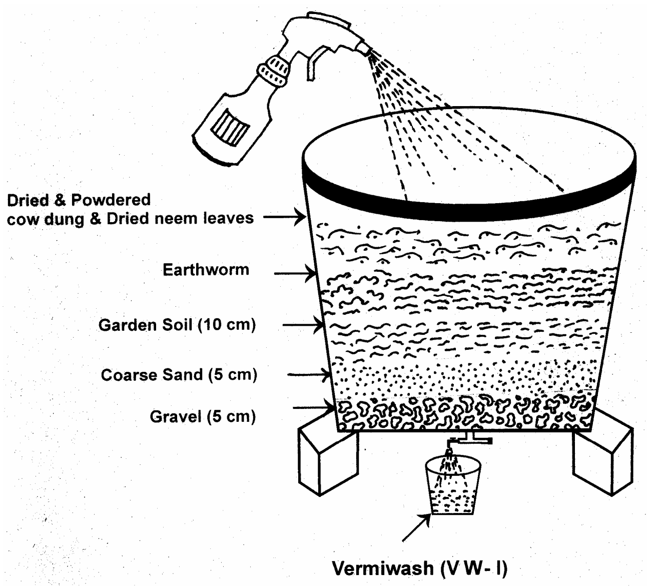

Fig. 1 Illustration of preparing Vermiwash (VW-I) various layers in the container are presented, following the method of Ismail (1997a).

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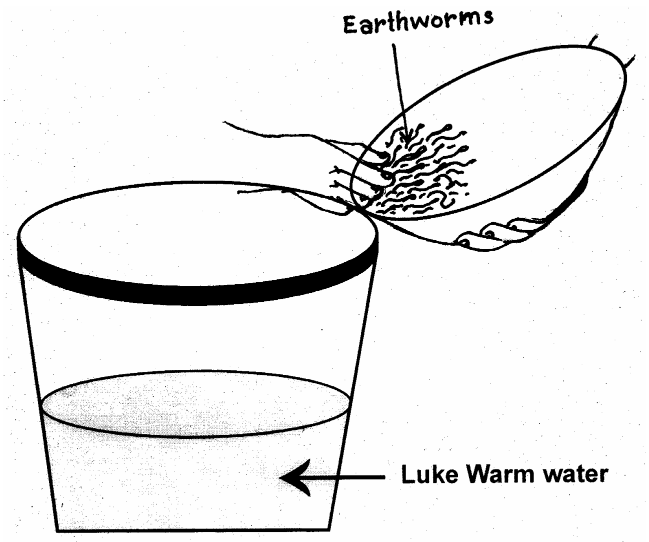

Fig. 2 Earthworms were placed in luke warm water, then washed and removed from water. The water was used as vermiwash (VW-II), following the method of Kale (1998).

The mixture of cowdung and leaves of *A. indica* is a very good substrate for culturing earthworms (Gajalakshmi and Abbasi 2004b). This was gently moistened with tap water. The unit was moistened daily (75% relative moisture level was maintained). Adult earthworms were collected from our laboratory culture and 250 adult earthworms (*Eudrillus euginae*) ranging in length and weight from 14–29 cm (mean 21.5 ± 6.8 cm) to 1.6–3.0 g (mean 2.55 ± 1.1 g), respectively were released. After 10 days, eluates were taken out daily by slowly sprinkling 2 l of tap water from the top (Ismail 1997a). The water spiraled and slowly percolated through the compost and worms via drilospheres.

VW-II was prepared to get the benefits of the secretions of body fluids of the earthworm (**Fig. 2**), following the method of Kale (1998). 600 g of adult earthworms (approximately numbering 200-250 worms) of the same species (*E. euginae*)were produced and poured into a container filled with 500 ml of lukewarm distilled water (37–40°C) and agitated for 2 min then released back into the tanks. The agitation in lukewarm water made the earthworms release quantities of mucus and body fluids. This is actually the coelomic fluid which contains enzymes of earthworms secreted during agitation (Gupta 2005).

Live plankton (LP) was sieved from a local pond and concentrated to 10,000 individuals/l in a conical flask with the help of a plankton net (No. 48 Nybolt cloth made) and then 100 ml of that concentrate was applied to the aquarium. "Akfeed", a locally produced fish feed was purchased from the market, mixed with 100 ml of water, and applied in the aquaria directly. The treatments were designated as follows:

T1 – VW-I (100 ml wash, so that solid material in dry weight remained 50 mg aquaria^{-1} day^{-1})

T2 – VW-II (100 ml wash, solid material remained 50 mg aquaria^{-1} day^{-1})

T3 – LP (100 ml concentrate, so that solid material remained 50 mg aquaria^{-1} day^{-1})

T4 – MAF solution in distilled water (100 ml solution made from ground market available feed so that solid material remained 50 mg aquaria^{-1} day^{-1}).

Water supply

The water in the aquarium was completely changed regularly, i.e. every 7 days. Fresh water was supplied from the same source (aerated ground water, DO = 6 mg l^{-1}, pH = 7.10, hardness = 140 mg l^{-1}, TDS = 210 mg l^{-1}). The water of aquarium was analyzed after 7 days for various physico-chemical parameters following Standard Methods (2002). Estimation of weight was done using a standard electronic weighing balance and length was measured using a standard measuring scale.

Checking for disease

Individual fish was tested externally everyday for occurrence of any disease by a trained fish pathologist (Department of Fisheries, Govt. of West Bengal).

Statistical analysis

The results obtained in this study were evaluated statistically. Kruskal-Wallis one-way analysis of variance by ranks was applied to determine the significant differences among treatments. Duncan's Multiple Range Test (Duncan 1955) was also performed to test the significance of difference between every possible pair of treatment (SPSS Software).

The Specific Growth Rate (SGR) was estimated as follows (Brown 1946):

$$SGR = [(\log wf - \log wi) / t] \times 100$$

where log wf and log wi are the logarithm of final weight and initial weight respectively and t is the experimental period in days.

RESULTS

Physico-chemical properties of the water of the MF, LP, VW-I and VW-II treated aquaria were analyzed weekly. We observed the lowest values (3 mg l^{-1}) of dissolved oxygen (DO) concentration of water in the aquaria administered with MF. The concentration of free CO_2 (1.4 mg l^{-1}) and BOD (5 mg l^{-1}) showed highest values in these aquaria. This indicates a stressful condition for the cultured fish in those aquaria. The aquaria treated with LP also showed a similar trend of values.

However, the physico-chemical qualities of the water treated with VW-I and VW-II remained good throughout the experimental period as the values of DO (6 mg l^{-1}), free CO_2 (0.8 mg l^{-1}) and BOD (1 mg l^{-1}) were favourable for fish growth.

The values of TDS, hardness and pH were higher in the MF- and LP-treated aquaria (**Table 1**) than VW-I- and VW-II-treated aquaria.

There was gradual and steady increase in the growth (length and weight) (**Fig. 3A, 3B**) of cultured fish in four feed test series during the experimental period. Fish, which were fed VW, had the highest average weight gain (> 2.05 g) followed by those fed live plankton (1.0 g) and MF (0.95 g). Among various treatments maximum length (**Fig. 3B**)

Table 1 Physico-chemical values of the water in various treatments (average of 45 days).

Parameters	VW-I	VW-II	LP	MF	Initial water values
Temp (°C)	29 ± 0.51	29 ± 0.54	29 ± 0.56	29 ± 0.69	29 ± 0.67
pH	7.4 ± 0.14	7.4 ± 0.20	7.1 ± 0.17	6.9 ± 0.15	7.0 ± 0.14
Conductance (μS.cm^{-1})	220 ± 14.6	220 ± 15.8	240 ± 14.5	390 ± 19.5	160 ± 11.6
Co_2 (mg.ℓ$^{-1}$)	0.8 ± 0.09	0.8 ± 0.06	1.0 ± 0.11	1.4 ± 0.16	0.6 ± 0.13
Dissolved Oxygen (mg.ℓ$^{-1}$)	6.00 ± 0.57	6.00 ± 0.53	4.00 ± 0.38	3.00 ± 0.29	8.00 ± 0.69
Biological Oxygen Demand (mg.ℓ$^{-1}$)	1.00 ± 0.07	1.00 ± 0.09	2.00 ± 0.44	5.00 ± 0.56	–
Total Hardness (mg.ℓ$^{-1}$)	120 ± 14.8	120 ± 13.5	140 ± 13.6	140 ± 14.2	110 ± 10.5
Total Dissolved Solids (mg.ℓ$^{-1}$)	230 ± 19.3	230 ± 16.8	230 ± 18.6	400 ± 20.5	180 ± 17.9
Specific Growth Rate (SGR)	1.72	1.76	0.84	0.76	–

VW-I – Vermiwash-I; VW-II – Vermiwash-II; LP – Live plankton; MF – Market-available feed; "±" – standard deviation of the mean; Dash – Zero

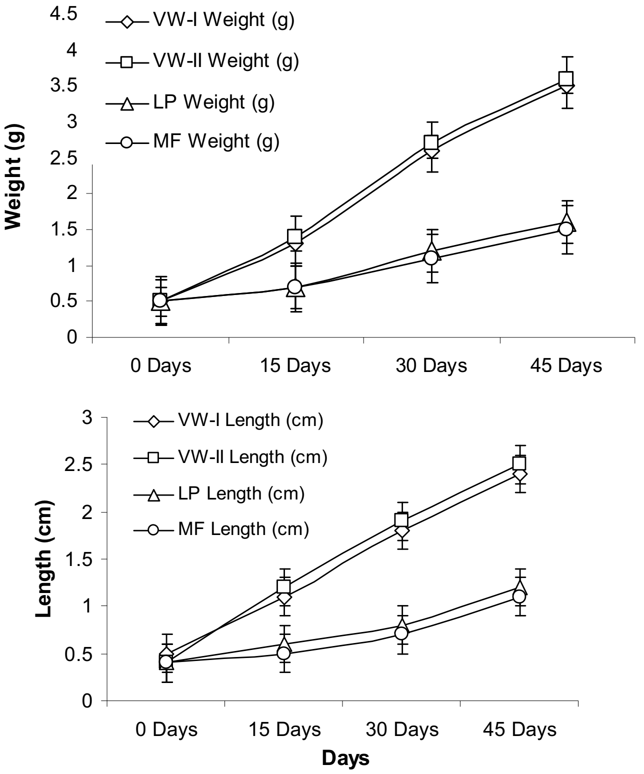

Fig. 3 Changes in weight and length of cultured fishes among various treatments.

Table 2 Results of Duncan's Multiple Range Test.

Days of treatment	Values of H (df=9)	Comparison		
0	Nil	Nil		
15	19.83	MF * LP	**	VW-I * VW-II
		10.14		19.83
30	20.44	MF * LP	**	VW-I * VW-II
		14.24		20.44
45	21.32	MF * LP	**	VW-I * VW-II
		16.16		21.33

" * = P > 0.01, ** = P < 0.01 " – used to denotes level of significance

Table 3 Occurrence of diseases and loss of scales among test fish in various treatments.

Disease	Feed used	0 Days	15 Days	30 Days	45 Days
Tail and fin rot	VW-I	–	–	–	–
	VW-II	–	–	–	–
	LP	–	2	2	1
	MF	–	3	3	3
Gill rot	VW-I	–	–	–	–
	VW-II	–	–	1	1
	LP	–	1	1	1
	MF	–	1	1	1
Eye disease	VW-I	–	–	–	–
	VW-II	–	–	–	–
	LP	–	–	1	1
	MF	–	–	1	1
Scale loss	VW-I	–	–	–	–
	VW-II	–	2	1	1
	LP	–	3	3	4
	MF	–	–	–	–

VW-I – Vermiwash-I; VW-II – Vermiwash-II; LP – Live plankton; MF – Market-available feed
Dash – Zero

and total weight (**Fig. 3A**) gains were recorded with VW-II-treated series. There was no significant ($P < 0.05$) difference between VW-I- and VW-II-treated aquaria, but there was a significant ($P < 0.05$) difference in growth among the rest of the treatments with VW-I and VW-II-treated series. In growth analysis Kruskal-Wallis one-way analysis of variance by ranks revealed a significant difference ($H \geq 19.83$; $P < 0.01$) among some treatments during the entire period of investigation. In statistical analysis of growth Duncan's Multiple Range test (DMRT) further revealed significant differences between all pairs of means except for VW-I- and VW-II- and LP- and MF-applied treatments (**Table 2**). The findings were similar for total length of the cultured fish (**Fig. 3B**). The occurrence of various diseases was highest in fish cultured with MF, followed by those fed LP. The most important finding of this study is that the fish cultured in VW-I and VW-II were free of any disease (**Table 3**).

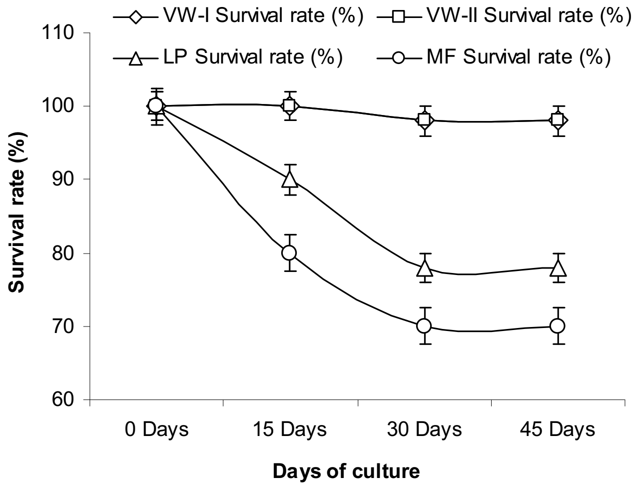

Fig. 4 Survival rates of cultured fishes.

DISCUSSION

The growth and survival percentage of fish were significantly higher in the VW-treated series (**Fig. 4**) possibly because of the presence of several micronutrients, metabolites, vitamins (Pro vitamin D and B complex) and some free amino acids in vermiwash (Springett and Syers 1979; Kale 1998; Ping and Boland 2004). The juvenile fish were able to consume very small sized food particles present in VW. Though there were plenty of food particles in LP- and MF-treated aquaria, the food particles were much larger (> 3 times when observed under an ocular microscope) than juvenile fish can consume.

VW contains macro- and micronutrients along with cocoons, small worms, debris of body parts (mainly in edible forms), and is reported to contain growth hormones, antibiotics and vitamins (Atlavinyte and Daciulyte 1969; Lee 1985; Ismail 1997b), which are beneficial for growth of the fish (Chakrabarty *et al.* 2009). Probably these substances helped the fish to remain disease-free. On the other hand, the fish in the remaining treatment series were ill fed as the juvenile fish were not able to consume the larger food particles present in these treatment series and were susceptible to various diseases.

The physico-chemical qualities of the water of the aquaria receiving VW-I and VW-II showed tolerable conditions for aquatic life even after 7 days. The color and odour of the water remained good probably due to presence of a huge beneficial anaerobic bacterial load in VW-I- and VW-II- (Gupta *et al.* 2007; Ansari 2008)-treated aquaria. This may prevent other harmful oxygen demanding bacterial growth (Chakrabarty 2008) in the aquaria. The poor growth rate observed in fish fed with MF or live plankton suggests that these feed were not suitable for the juvenile fish. This finding suggests that juvenile fish prefer VW as feed due to its better palatability and consumption than others. In conclusion, VW is very useful not only in agriculture, but also very effective in aquaculture and can be used as nutritive baby fish feed at a very low cost.

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