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Research Article

EFFICACY OF COW URINE EXTRACT OF OCIMUM SANCTUM ON GROWTH AND SURVIVAL IN OREOCHROMIS MOSSAMBICUS

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ABSTRACT

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Key Words:

Aquaculture, Gir, *Bos indicus*, Indian Cow breed, Cow Urine, Tilapia, *O. sanctum*, Phytochemical, Phytobiotics, Growth, Survival The application of herbal immunostimulants in fish farming for the prevention of diseases is a promising field. A 30-day trial was conducted in a static indoor rearing glass aquaria to investigate the possibility of *Ocimum sanctum* cow urine decoction by distillation (OCUDD) and by fermentation (OCUDF) in enhancing the growth of *Oreochromis mossambicus*. Preliminary studies were conducted with various concentrations (0.01%, 0.1%, 0.25%, 0.5% and 1%) of *O. sanctum* decoction in cow urine and 0.01% (v/v) was selected for the present study. Experimental groups T1-Distillate OCUDD, T2-Ferment OCUDF, T3- *O. sanctum* extract, T4-Cow urine distillate (CUD), T5-Cow urine (CU) were maintained along with a control. Each group has 10 fish maintained in 25L aquaria. Triplicates were maintained. The feeding ration was adjusted every 10th day through sampling of fish. Growth, growth rate, average daily growth, percentage increase in body weight, specific growth rate, feeding rate, feed conversion rate protein efficiency ratio and survival rate was examined in the present study. The results were recorded and discussed. The results indicate an enhancement of growth and survival in the treated groups. The study thus will be a milestone in eco-safe and integrated nutritional aquaculture practices and there by healthy fishes.

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INTRODUCTION

Aquaculture is the fastest growing food-producing sector in the world (Bostock et al., 2010). Aquaculture has the potential to make a significant contribution to the increasing demand for aquatic food in many regions of the world (Biswas 1993; Brooks et al 1997; Choubert and Blanc 1993; Cumaratunga and Mallika 1991). During recent decades there is a significant progress in the aquaculture sector characterized by intensified production and new species. The increased intensification of aquaculture has led to a high number of pathogens, the major cause for the outbreak of diseases in fishes. So far, conventional approaches such as the use of disinfectants and antimicrobial drugs have had limited success in the prevention or cure of fish diseases. The massive use of antimicrobials for disease control and growth promotion in fish increases the selective pressure exerted on the microbial world and encourages the natural emergence of bacterial resistance. The application of vaccines in fish farming to prevent bacterial diseases is effective (Siwicki et al 1998), but protection is species (pathogens) specific and expensive (Robertson 1999). Immunostimulants are natural or synthetic substances that are able to activate non-specific and specific immune responses

(Anderson 1992). The use of immunostimulants for the prevention of fish diseases progress and several preparations have become more promising (Jeney and Jeney 2002). At present natural replacement is given more importance in fish farming. Herbal drugs are known to nossess immunomodulatory properties and generally act by stimulating both specific and non-specific immune responses and thereby increasing the possibilities of growth. Components such as polysaccharides, lectins, proteins, peptides, present in plants have found to stimulate the immune system (Bafna and Mishra 2005).

Ocimum tenuiflorum, previously known as Ocimum sanctum (Tulsi, vernacular), "queen of herbs" is one of the indigenous plants used extensively in India to treat various ailments. Pharmacological studies conducted on the plant have shown that its leaves and seeds possess many medicinal properties like hypoglycemic, adaptogenic, antifertilty, antimicrobial and hepatoprotection (Satyavati and Gupta 1987). It has been implicated that different mechanisms like free radical scavenging, metal chelation, GSH stimulation as well as immunomodulation might act at different levels individually or in combination for adaptogenic, radioprotective and cancer

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preventive action (Uma Devi 2001). *O. tenuiflorum* leaf and seed extracts were found to exhibit antiviral activity against bovine herpes virus 1 *in vitro* (Shynu *et al* 2006). Leaf powder was found to have enhancing effect on humoral and cell mediated immune response in immuno-suppressed poultry (Sadekar *et al* 1998a, 1998b). Butanolic extract of leaves had an immuno-restorative effect in immuno-suppressed mice (Khajuria *et al* 1998). In humans, *O. sanctum* is found to be neuroproective and anticholinergic and also acts as an immunomodultor with potential antioxidant activity (Vijaysree *et al* 2015). *O. sanctum* has been used to enhance the specific and non-specific immune system (Venkatalakshmi and Michael 2001, Logambal *et al* 2000).

From the ancient period in India, cow urine has been used for several medicinal purposes and the description on its use has been in several classical ayurveda texts like Charaka samhita and Shushruta samhita. Cow is believed to be a sacred animal in India and its urine is known to cure several diseases. In Veda, cow urine is compared with the nectar (Krishnamurthi et al 2004; Gururaja et al 2011). In India, drinking of cow urine has been practiced for thousands of years. Cow urine is found to be effective against reversal of certain cardiac and kidney diseases indigestion and stomach ache, oedema, skin diseases etc (Sadasivam et al 2010). One of the forms of cow urine with popular use nowadays is cow urine distillate (CUD), a transparent liquid obtained when cow urine is subjected to distillation. The distillate is having more acceptability than crude cow urine and shows activities of crude cow urine and its effects were proven in aquaculture (Padmapriya and Venkatlakshmi 2014; Priya and Venkatalakshmi 2016; Sattanathan and Venkatalakshmi 2015; Durga et al 2015). Bioenhancers are substances, which do not possess drug activity of their own but promote and augment the bioactivity or bioavailability or the uptake of drugs in combination therapy. Such bioenhancers have been earlier isolated only from plant sources. Several investigations have reported synergistic effect (Logambal 1995), and antibacterial effect of plant extracts, with significant reduction in the minimum inhibitory concentration of the antibiotic (Betoni, et al., 2006), against many pathogenic bacteria. It has been found that cow urine and its distillate also possess bioenhancing ability and help to reduce the dose of antibiotics. They also increase the efficacy of antibiotics against infectious agents (Jain et al 2010). Bioenhancing or synergistic property of CU Decoction with herbal extracts has not been much investigated in aquaculture. The literatures reviewed showed that no work has been reported on the combined effects of O. sanctum and cow urine. Hence, the aim of the present study is to determine the potency of O. sanctum extract in cow urine on growth and survival as it has exhibited positive effects on the haematological analysis (Logambal and Venkatalakshmi 2017; in pub) and immunological studies (Logambal and Venkatalakshmi 2017; inprogress).

MATERIALS AND METHODS

Fish and their maintenance

Fingerlings of *Oreochromis mossambicus* weighing $(0.814\pm0.2 \text{ g})$ and length $(1.02\pm0.2 \text{ cm})$ of both sexes were procured from S.M fisheries, Swamimalai, Kumbakonam. Fishes were brought to the wet laboratory and acclimatized for one week

prior to experimentation. Chlorine free water was used throughout the course of the experiment. Glass aquaria were washed and then sundried to avoid fungal contamination. Water quality parameters such as pH, temperature, dissolved oxygen, and ammonia, are maintained within acceptable ranges. Healthy fishes were then transfered to glass aquaria (Vol 25 L). For each treatment three replicates were used and in each replicate 10 fingerlings were stocked. They were regularly fed with formulated feed and the medium was changed daily to remove faeces and food remnants.

Water quality parameters

Water quality was analyzed weekly for a number of different parameters like temperature, pH, dissolved oxygen, turbidity, salinity, TDS, and ammonia was recorded and maintained the throughout the experiment. These parameters were analyzed by using Systronics 371 Model water quality analyzer portable fit.

Mode of feeding

Each of the growth treatment was fed with formulated feed of 2% total body weight (Venkatalakshmi 2006). The fish were fed twice a day for an hour between 9.00 am to 10.00 am and 4.00 pm to 5.00 pm (Amit Jana *et al* 2014). Fish tanks were cleaned daily by siphoning out residual feed and faecal matter. The unfed was collected and dried (60° C) in a hot air oven and weighed. The faeces were also collected separately dried and weighed.

Collection of Cow urine

Urine was collected from six disease free tagged Gir cows throughout the study. The early morning (4.00-5.00am) first urine of Gir, was collected from Goshala, Sri Vittal Rukmini Samsthan, Govindhapuram, near Kumbakonam. The urine of was pooled, and transported to laboratory in sterile airtight containers (Sattanathan and Venkatalakshmi 2015).

Collection of Ocimum sanctum leaves

Matured leaves of *O. sanctum* were collected from local market, Kumbakonam, Thanjavur. This was authenticated by Department of Botany, Government College for Women (A), Kumbakonam, Tamilnadu and the specimen samples were deposited in the laboratory herbarium. Fresh leaves were used for the study.

Preparation of O. sanctum Cow Urine Decoction by distillation (OCUDD)

100 gram of *O. sanctum* fresh leaves were surface sterilized with sterile distilled water thrice. These leaves were cut into small pieces and added to 200 ml of fresh Gir cow urine. This was distilled at $50-60^{\circ}$ C using by glass multiple distillation apparatus for 4-5 hrs (Edwin Jarald *et al* 2008).

Preparation of O. sanctum Cow Urine Decoction by fermentation (OCUDF)

100 gram of *O. sanctum* fresh leaves were cut into small pieces and placed in an earthen pot containing 200 ml of fresh Gir cow urine. The pot was incubated over night at room temperature. After 24 h of incubation 50 ml of crude extract was centrifuged at 2000 rpm for 10 minutes and supernatant was collected in a sterile container and stored in a refrigerator for further analysis.

Preparation of Cow urine distillate (CUD)

Gir Cow urine was distilled at 50-60°C using glass distillation apparatus for 3-4 hours (Sattanathan and Venkatalakshmi 2015).

Preparation of O. sanctum extract

100 gram of *O. sanctum* fresh leaves were surface sterilized with sterile distilled water thrice. These leaves were cut into small pieces and added to 200 ml of distilled water. This was distilled at 50-60° C using by glass multiple distillation apparatus for 4-5 hrs (Kokarte *et al* 2005).

Experimental setup

After two weeks of acclimatization five groups of fish were treated, each separately with T1 -OCUDD, T2 - OCUDF, T3 - *O. sanctum* extract (OSE), T4 - CUD, T5 - Cow urine (CU) at 0.01% concentration respectively. A control group was maintained separately without any treatment for seven days.

Morphological analysis

The length and weight of the fishes were measured individually. The fishes were weighed by digital electronic balance of mg sensitivity (Systronics, India). Ruler was used to measure the total length from head to tip of the caudal fin. The fingerlings were released in water immediately after body measurements (Monjural Islam 2014).

Growth and Food utilization parameters

The weight and length of individual fish were recorded at the initiation of experiment and then at the interval of 10 days. The growth parameters were calculated by using the following formulae (Petursewiez and Macfutyen 1970).

Protein efficiency ratio = -----Crude protein fed

Survival rate

Survival rate is calculated by following formulae:

Statistical analysis

The results of the present studies were expressed as mean \pm SE. The significance of sample mean between control and CUD treated fish was tested using one way ANOVA. Differences were considered significant at p < 0.05.

Parameters	Control (NC)	T1 (OCUDD)	T2 (OCUDF)	T3 (OSE)	T4 (CUD)	T5 (CU)
Initial Weight W1 (g)	0.79±0.016	0.81±0.007	0.82±0.013	0.83±0.019	0.81±0.013	0.82±0.015
Final Weight W2 (g)	1.231±0.009	2.358±0.028	1.846±0.013	1.831±0.014	1.631±0.017	1.479±0.013
Increase in total weight (g)	0.441	1.548	1.026	1.001	0.821	0.659
Initial Length (cm)	1.5±0.141	1.12±0.158	1.14±0.116	1.14±0.152	1.17±0.081	1.15±0.160
Final Length (cm)	1.533±0.121	1.89±0.174	1.73±0.207	1.68±0.157	1.53±0.150	1.41±0.172
Increase in total length (cm)	0.033	0.772	0.596	0.543	0.365	0.262

Table 1 Effect of Cow Urine Decoction on the body weight and body length of O. mossambicus

NC- Negative control; OCUDD-Ocimum sanctum cow urine distillate decoction; OCUDF-Ocimum sanctum cow urine ferment decoction; OSE-Ocimum sanctum extract; CUD-Cow urine distillate; CU-Cow urine.

Growth Growth Rate	= Final weight - Initial weight (g) Weight gain = (mg day ⁻¹)
Olowill Kale	No. of days x initial weight
Specific Growth	Ln (Final weight)-Ln (Initial weight) n rate (%) = X 100
Specific Glown	Number of days
Demonstrate CL	Final weight - Initial weight
Body weight (%	herease in=X 100 (6) Initial weight
Average Daily	Final body weight - Initial body weight Growth= (mg day ⁻¹) No. of feeding days
Food utilization	parameters were calculated as follows:
Feeding rate = -	Total dry food consumed (g body wt ⁻¹ day ⁻¹) o. of days x initial live wt. of fish

Growth performance and survival

The growth parameters like growth, growth rate, average daily growth, specific growth rate, were observed at an interval of 10 days for 30 days (Table 1). Growth was found to be higher, 2.358 g and 1.846 g in OCUDD and OCUDF respectively compared to control 1.231 g (p<0.05; Fig 1). So is the pattern in average live weight gain OCUDD > OCUDF > OSE > CUD > CU > Control. The average length of the fish proportionately increases with the growth. OCUDD shows a significantly higher increase in length (p<0.05). The least average daily growth is in control (0.0147 mg/day) and maximum is in OCUDD (0.0516 mg/day). Specific growth rate is higher in OCUDD compared to the control and other treated groups (Fig 2).

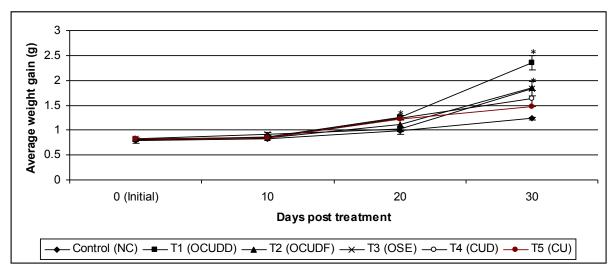
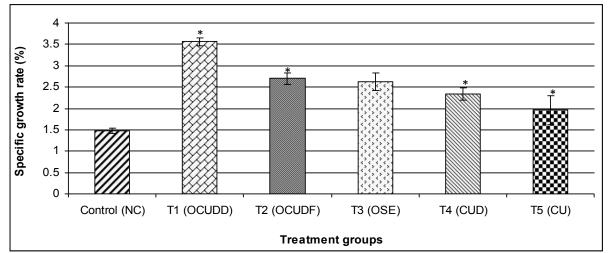
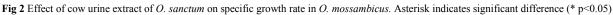


Fig 1 Effect of cow urine extract of O. sanctum on average weight gain in O. mossambicus. Asterisk indicates significant difference (* p<0.05)





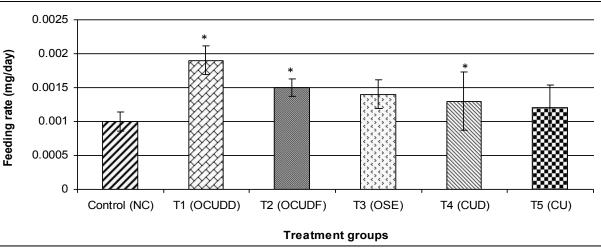


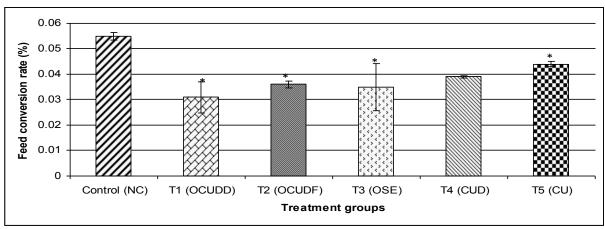
Fig 3 Effect of cow urine extract of O. sanctum on feeding rate in O. mossambicus. Asterisk indicates significant difference (* p<0.05)

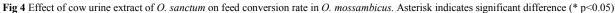
The highest survival rate of 100% was recorded in OCUDD, OCUDF treated groups and least survival rate of 80% was recorded in control (Table 2).

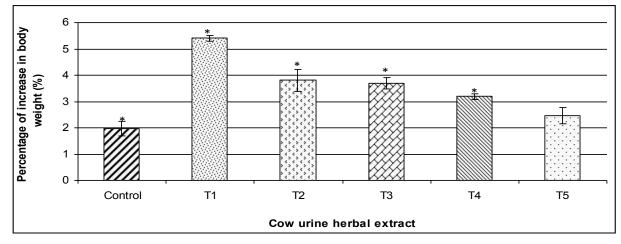
Food utilization parameters

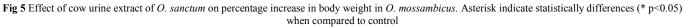
Feeding rate decreases as the days increases i.e feeding rate decreases as the weight increases.

There is a similar pattern for the all the treated groups and control. On 30^{th} day the feeding rate is high (0.0019 mg/day) compared to other groups (Fig 3; Table 3). Feed conversion rate is maximum in control and minimum in OCUDD. All the treated groups have less FCR compared to control (p<0.05; Table 3).









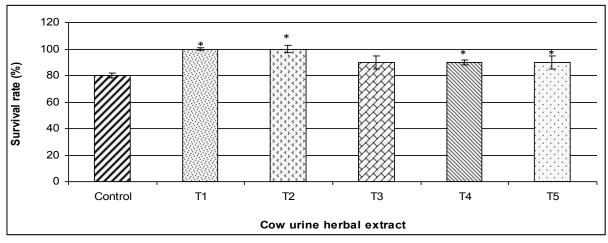


Fig 6 Effect of cow urine extract of O. sanctum on survival rate in O. mossambicus. Asterisk indicate statistically differences (* p<0.05) when compared to control

Parameters	Control (NC)	T1 (OCUDD)	T2 (OCUDF)	T3 (OSE)	T4 (CUD)	T5 (CU)
Growth (average weight gain) W2-W1 (g)	0.441	1.548	1.026	1.001	0.821	0.659
Growth rate (mg/day)	0.0186	0.0637	0.0417	0.0402	0.0337	0.0267
Average Daily Growth (mg/day)	0.0147	0.0516	0.0342	0.0333	0.0273	0.0219
Percentage of increase in body weight (%)	55.82	191.11	125.12	120.60	101.35	80.36
Specific Growth rate (%)	1.478	3.561	2.704	2.637	2.333	1.966
Survival rate (%)	80	100	100	90	90	90

Table 2 Effect of Cow Urine Decoction on the Growth parameters of O. mossambicus

NC- Negative control; OCUDD-Ocimum sanctum cow urine distillate decoction; OCUDF-Ocimum sanctum cow urine ferment decoction; OSE-Ocimum sanctum extract; CUD-Cow urine distillate; CU-Cow urine.

Table 3 Effect of Cow Urine Decoction on the food utilization parameters of O. mossambici	Table 3 Effect of Cow	Urine Decoction on t	he food utilization	parameters of O. mossambicu.
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Parameters	Control (NC)	T1 (OCUDD)	T2 (OCUDF)	T3 (OSE)	T4 (CUD)	T5 (CU)
Feeding rate (mg/day)	0.0010	0.0019	0.0015	0.0014	0.0013	0.0012
Food absorbed (mg/day)	0.0236	0.0459	0.0353	0.0350	0.0312	0.0281
Absorption rate (mg/day)	0.0009	0.0018	0.0014	0.0014	0.0012	0.0011
Absorption efficiency (%)	34.77	74.95	54.69	58.09	38.54	59.19
Feed Conversion rate (%)	0.055	0.031	0.036	0.035	0.039	0.044
Protein efficiency ratio (%)	0.012	0.027	0.028	0.043	0.022	0.018

NC- Negative control; OCUDD-Ocimum sanctum cow urine distillate decoction; OCUDF-Ocimum sanctum cow urine ferment decoction; OSE-Ocimum sanctum extract; CUD-Cow urine distillate; CU-Cow urine.

On the 30th day experiment the fish exposure with OSE had highest feeding rate (0.0074 mg/day), followed by OCUDD (0.0054 mg/day) > OCUDF and CU (0.0047 mg/day) > CUD (0.0045 mg/day) > control (0.0037 mg/day) (Fig 7). Fig 8-10 demonstrate highest food absorbed, absorption efficiency, absorption rate feed conversion efficiency in T3 treated groups. The maximum gross conversion efficiency (33.50 %), net conversion efficiency 34.07 %), was efficient in OCUDD, compared to other treated groups (Fig 11 and 13).

DISCUSSION

Exponential growth of world population and the growing demand for the nutritious and healthy food will increase the demand of fisheries products whose productivity is already in great stress by excessive fishing pressure, intensive culture, organic pollution, toxic contamination, habitat degradation and climate change. Fish feed is the most expensive input in aquaculture operation.

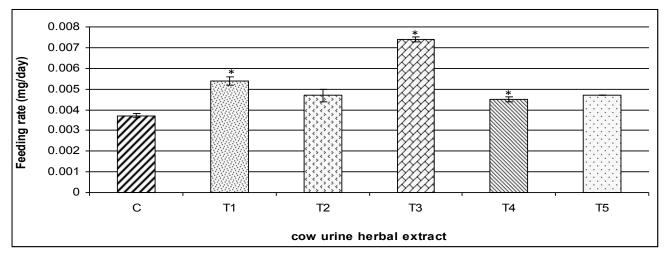


Fig 7 Effect of cow urine extract of *O. sanctum* on feeding rate in *O. mossambicus*, Asterisk indicate statistically differences (* p<0.05) when compared to control

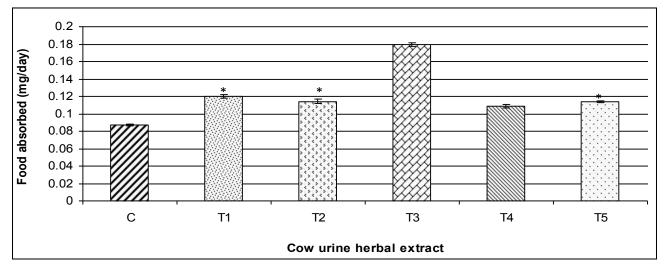


Fig 8 Effect of cow urine extract of O. sanctum on food absorbed in O. mossambicus. Asterisk indicate statistically differences (* p<0.05) when compared to control

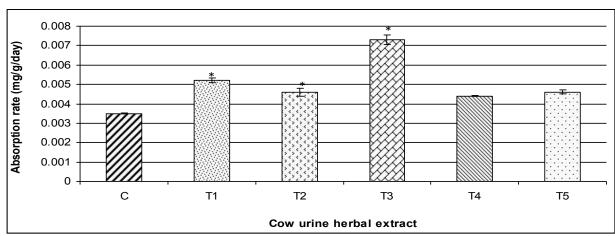


Fig 9 Effect of cow urine extract of O. sanctum on absorption rate in O. mossambicus. Asterisk indicate statistically differences (* p<0.05) when compared to control

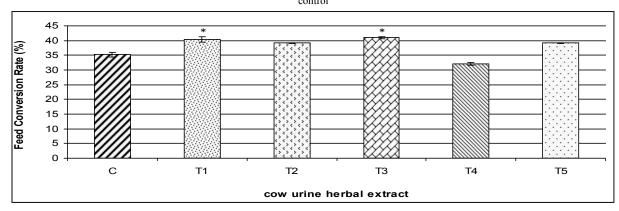
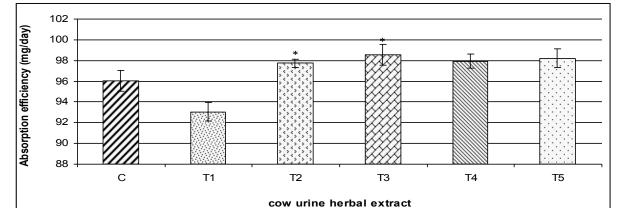
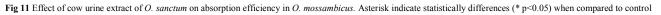
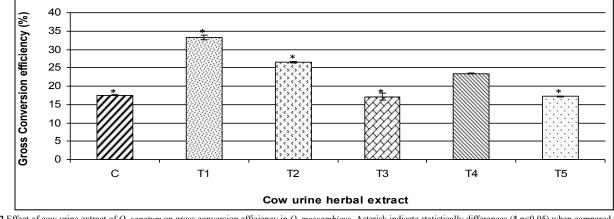
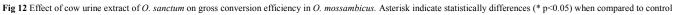


Fig 10 Effect of cow urine extract of O. sanctum on feed conversion rate in O. mossambicus. Asterisk indicate statistically differences (* p<0.05) when compared to control









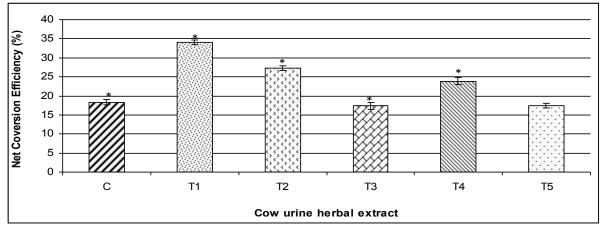


Fig 13 Effect of cow urine extract of O. sanctum on net conversion efficiency in O. mossambicus. Asterisk indicate statistically differences (* p<0.05) when compared to control

To overcome this, it could be economical to use this method of administration of cow urine extract in *O. sanctum* to improve its health and growth thereby augmenting the aquaculture production. This method of administration was found to be most effective in comparison with other routes (Venkatalakshmi and Ebanesar 2012).

The results clearly reveal that the growth of *O. mossambicus* reared in cow urine extract in *O. sanctum* showed significantly (p<0.05) better growth characteristics than control. Numerous plants have shown to improve the growth parameters, length, weight, specific growth rate and survival rate in *O. mossambicus* (Karpagam and Krishnaveni 2014). The growth rate, feeding conversion rate, absorption rate was found to be statistically significant among treated groups and with the control (p<0.05) in the present study.

Initially the growth was slower and then gradually increased with days. A similar pattern is seen in all the experimental groups. The average weight gain and growth rate is maximum in OCUDD treated group. This may be due to the action of the compounds that enhance the growth through various means, must have dissolved more in distillate than in ferment. Overall growth is expressed both in weight as well as in length and this trend is very useful for an aquaculturist in terms of his profit.

Feeding rate and absorption rate is higher in OCUDD. This may be attributed to the compounds extracted in distillation may enhance the production of enzymes or action of enzymes as discussed below.

In all the treated groups feed conversion rate is less than the control; OCUDD exhibits least FCR. The lower the FCR the lower is the amount of feed used to produce 1kg of fish. Therefore the feed which gives lower FCR is often the one that gives the lowest cost production. Hence use of this product (after few experimental field trails) in aquaculture should be economical.

Feed conversion ratio in relation to body weight decrease as the body weight increased as compared to larger fish in similar condition. FCR values that expresses food consumed per unit weight gained by the body has been variously termed as food quotient, food coefficient or growth efficient (Tayyaba Ali and Salim 2004). The value of conversion of rate besides depending upon nutrient content of feed also varies with (i) method of presentation of food (ii) environmental factor such temperature, dissolved oxygen concentration, etc (iii). Size of fish (iv) stocking density (v) stage of sexual maturity. This gave an idea of extending the same study by incorporating in feed.

Although we have reports for O. sanctum to enhance growth in various animals like, Epinephelus tauvina (Sivaram et al 2004), Catla catla (Chitra and Krishnaveni 2011), Cyprinus carpio (Pavaraj et al., 2011) and Labeo rohita (Das et al., 2013), and cow urine to enhance growth in various animals (Khan and Srivastava 2005) including fish, Labeo rohita (Sattanathan and Venkatalakshmi 2015; Priya and Venkatalakshmi 2016), C. mrigala (Padmapriya and Venkatalakshmi 2014; Vasanthi and Venkatalakshmi 2015), O. mossambicus (Durga and Venkatalakshmi 2016), this study authenticates a better performance when administered together. Cow urine is known for bioenhancing ability in mice (Krishnamurthy et al 2004). In the present study synergistic effect is seen. Only when the health status of the fish is good will they grow better and we have literatures to show improved quality of health by O. sanctum or cow urine separately. Cow urine contains iron, calcium, phosphorous, carbonic acid, enzymes, nitrogen, sulphur, phosphate, sodium, manganese, aminoacids, uric acid, potash, silicon, chlorine, magnesium, calcium salt, vitamin ABCDE, lactose, gold acids, copper, (Badhuria 2002; Rajesh 2013) and other nutrients. Thus, it strengthens the fact that cow urine is not toxic as 95% of its content being water, 2.5% urea and the remaining 2.5%, a mixture of minerals, salts (Bhaduria 2002) and other nutrients which may improve the health of the fish. Gang et al (2005) evaluated the effect of distilled cow urine and found to potentiate egg production and good quality eggs in white leghorn layers. The extract used in the present study may improve the quality of the fish eggs produced and thereby enhancing the growth, stamina and survival. Tulsi is reported to have health promoting agents that has wound healing (Udupa et al 2005), anti-inflammatory, anticoagulant, antipyretic, antioxidant (Singh et al., 2004), antimicrobial (Nair, 2009; Benbotovic et al 2013), hepatoprotective (Srinivas 2009), immunomodulatory (Logambal 2000; Venkatalakshmi 2002; Jeba 2011) properties.

Among the possible mode of action of phytogenic bioactive compounds on growth performance could be, effect on the activities of digestive enzymes (Jang *et al* 2010). The increase in enzyme production can result in improvements in digestibility and availability of nutrients from feed stuffs

(Chesson 1987). Advantage of using the product used in present study includes reducing the cost in aquculture production, ecofriendly and economical and easily accessible for fish farmer. Thus, there is a greater chance of *O. sanctum* decoction in cow urine to be used as phytobiotics (added to feed in order to enhance the performance of animals) after few more studies.

CONCLUSION

The results of the present study indicated the beneficial role of the selected plant extract in cow urine as the growth promoter for tilapia, *O. mossambcus*. The study is extended to feed based exposure and the work is in progress. This study states possibilities of using tulsi extracted in cow urine as a rich source of nutrients that enhance the growth of the fish and thereby achieving the goal of augmenting aquaculture production, in an eco-friendly cost effective and sustainable manner.

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