

**UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFARMARG, NEW DELHI – 110 002**

FINAL REPORT OF THE WORK DONE ON THE MAJOR RESEARCH PROJECT

1.	Project Report No.	:	Final Report
2.	UGC Reference No.	:	F.No: 42-561/2013 (SR) & Dated 22/03/2013
3.	Period of Report	:	01/04/2013 to 31/03/2016
4.	Title of Research Project	:	Immunomodulatory effect of cow urine in <i>Labeo rohita</i>
5.	(a) Name of the Principal Investigator	:	Dr. S.Venkatalakshmi
	(b) Department	:	Department of Zoology
	(c) College where work has progressed	:	Government College for Women (Autonomous), Kumbakonam – 612 001, Tamil Nadu
6.	Effective date of starting of the project	:	01.06.2013
7.	Grant approved and expenditure incurred during the period of the report		
	(a) Total amount approved Rs.	:	1 st Installment: ₹ 7,85,800/- 2 nd Installment: ₹ 2,82,400/-
	(b) Total expenditure Rs.	:	1 st Installment: ₹ 7,73,569/- 2 nd Installment: ₹ 3,02,308/-
8.	Report of the work done: (Please attach a separate sheet)	:	Enclosed
	(i) Brief objective of the project	:	Annexure – I
	(ii) Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication).	:	Annexure – II

(iii) Has the progress been according to original plan of work and towards achieving the objective, If not, state reasons.	: Yes, the progress has been according to original plan of the work
(iv) Please indicate the difficulties, if any, experienced in implementing the Project.	: The delay in realizing second (final) installment has severely impacted the objectives of the project.
(v) If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate Sheet.	: NA
(vi) If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to University Grants Commission	: Completed Annexure - III
(vii) Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph.D awarded (c) Publication of results (d) other impact, if any	: Annexure - IV

S. Venkatalakshmi
13/02/26

PRINCIPAL INVESTIGATOR
Dr. S. VENKATALAKSHMI,
Principal Investigator,
UGC - Major Research Project,
PG & Research Dept. of Zoology,
Government College for Women (A),
Kumbakonam - 612 001.

B. Balakrishnan
13/2/26

PRINCIPAL
PRINCIPAL
Government College for Women (Autonomous),
KUMBAKONAM.

11. Objectives of the project

- To standardize the protocol for determining the optimal dosage of fresh cow urine (FCU) and distilled cow urine (CUD).
- To identify the most effective route (water additive, immersion, and feed supplement) of administration.
- To examine the effects of cow urine distillate on growth and feed utilization parameters.
- To investigate the impact of cow urine distillate on hematological parameters.
- To analyze the effects of cow urine distillate on immunological parameters.
- To assess the effect of cow urine distillate in challenge studies.
- To study cow urine distillate in the context of *in vitro* leukocyte culture.
- Additionally, to evaluate the chemical composition of fresh urine, cow urine distillate, and residue (gokshar) using gas chromatography-mass spectrometry (GC-MS).

12. Whether objectives of the project achieved (give details)

- **To standardize the protocol for determining the optimal dosage of fresh cow urine (FCU) and distilled cow urine (CUD).**

The primary objective of the study was to develop and standardize a reliable protocol for determining the optimal dosage of fresh and cow urine distillate for enhancing growth and survival rate of rohu fingerlings. This involved evaluating various concentrations of fresh urine and cow urine distillate, assessing the physiological and biochemical responses of the fingerlings, and establishing the dosage that maximizes growth performance without inducing toxicity or adverse effects. Through systematic experimentation and analysis, the objective range of fresh and cow urine distillate concentrations suitable for rohu fingerlings. Fingerlings administered with the standardized dosages showed significant improvements in growth parameters compared to the control group. The optimal dosage was found to maintain high survival rates and normal behavioral and physiological functions, indicating the absence of toxic effects.

- **To identify the most effective route (water additive, immersion, and feed supplement) of administration.**

The objective of the study was to identify the most effective route water additive, immersion and feed supplement for administering cow urine distillate in rohu fingerlings. The objectives were successfully achieved, as the study demonstrated that water additive route significantly enhanced growth, survival and feed utilization compared to other routes. The findings provide a clear recommendations for the optimal mode of cow urine distillate application in aquaculture practices.

- **To examine the effects of cow urine distillate on growth and feed utilization parameters.**

The study aimed to examine the effects of cow urine distillate on growth and feed utilization parameters in rohu fingerlings. The objectives were achieved, as Gir cow urine distillate significantly improved weight gain, feed conversion rate, daily growth rate, and specific growth rate compared to control. The results confirm the positive role of gir cow urine distillate in enhancing growth and feed efficiency in rohu fingerlings.

- **To investigate the impact of cow urine distillate on hematological parameters.**

The study aimed to investigate the impact of cow urine distillate from Gir, Haryana and HF exotic breed on hematological parameters in rohu. The objectives were successfully achieved, as administration of Gir breed cow urine distillate showed significant variations in hematological parameters in hemoglobin, red blood cell count, white blood cell count, and hematocrit levels. The results identified the most effective Gir cow urine distillate in improving the blood health and physiological status of rohu.

- **To analyze the effects of cow urine distillate on immunological parameters.**

The study aimed to investigate the impact of Gir cow urine distillate on immunological parameters in rohu fingerlings. The objectives were successfully achieved, as administration of Gir cow urine distillate significantly enhanced key immunological responses, including increased levels of lysozyme activity, respiratory burst activity compared to control. The current results indicating improved overall health status in the rohu fingerlings.

➤ **To assess the effect of cow urine distillate in challenge studies.**

The study aimed to assess the effect of Gir cow urine distillate on disease resistance in rohu fingerlings against *Aeromonas hydrophila*. The objectives were successfully achieved, as water additive route showed significant differences in enhancing resistance to infection, with Gir cow urine distillate providing the highest protection by improving survival rates, immune responses and overall health of rohu fingerlings.

➤ **To study cow urine distillate in the context of *in vitro* leukocyte culture.**

The study aimed to assess the effect of gir cow urine distillate on *in vitro* peripheral leucocyte culture compared with standard drugs. The objectives were successfully achieved, as 2.5µl/mL of gir cow urine distillate significantly enhanced immune response activity like ROS, RNS, and myeloperoxidase activity, and phagocytic activity, indicating its potential as an immunostimulant in rohu peripheral leucocyte.

➤ **Additionally, to evaluate the chemical composition of fresh urine, cow urine distillate, and residue (gokshar) using gas chromatography-mass spectrometry (GC-MS).**

The study aimed to evaluate the chemical composition of gir fresh, distillate, and residue using GC-MS analysis. The objectives were successfully achieved, as GC-MS profiling identified and quantified key bioactive compounds, including phenolics, flavonoids, and volatile constituents, in each form of gir cow urine. The results provided a comprehensive chemical characterization, forming a scientific basis for its observed biological and immunostimulatory effects.

13. Achievements from the project

a) Demonstrated immunostimulatory effect of cow urine distillate.

The study established that cow urine distillate, particularly from the Gir breed distillate acts as potent immunostimulant in *Labeo rohita* fingerlings.

b) Breed specific superiority

Among the tested breeds (Gir, Haryana and HF cross bred) Gir cow urine distillate showed the highest efficacy, confirming its superiority in enhancing fish health and immunity

c) Optimal route administration identified.

The project compared three routes (water additive, feed additive and immersion). It was concluded that water additive is the most effective method for delivering cow urine distillate to rohu fingerlings.

d) Growth and utilization improvement

Addition of 0.1% Gir CUD in water additive significantly enhanced growth performance and feed utilization in rohu fingerlings indicating its potential as natural growth promoter.

e) Enhanced hematological and immunological parameters

Gir CUD additive improved hematological and immunological indices and activated leucocytes showing clear immunostimulatory effects both *invivo* and *invitro*.

f) Increased disease resistance

Fish treated with Gir CUD exhibited enhanced resistance against *A. hydrophila* pathogenic infections, highlighting its role in disease management and sustainable aquaculture.

g) Identification of bioactive compounds:

GC-MS analysis of Gir CUD identified several bioactive compounds responsible for its immunostimulatory, growth promoting and health enhancing properties.

i) Practical implications for aquaculture

The findings provide a cost effective, natural, and eco-friendly approach for improving fish health and productivity, particularly in rohu culture system.

14. Summary of the findings

Cow urine has many beneficial properties particularly in the area of agriculture and therapeutics. It has also been observed in scientific research that the urine of Indian cows is highly effective as compare to the urine of other species. It is a good biopesticide and also effective against many diseases including cancer. It is a very potent immunoenhancer. In '*Sushrut Samhita*' and '*Ashtanga Sangraha*' cow urine has been described as the most effective substance/secretion of animal origin with innumerable therapeutic values. Urine of cow contains all the beneficial elements so it is natural and universal medicine that fulfills the deficiency of the elements in the body. Cow urine contains 24 types of salts and the medicines made from cow urine are capable of curing even the most incurable diseases. Cow urine contains 95% water, 2.5% urea, and 2.5% minerals, salts, hormones and enzymes. It contains iron, calcium, phosphorus, salts, carbonic acid,

potash, nitrogen, ammonia, manganese, sulphur, phosphate, potassium, urea, uric acid, amino acids, enzymes, cytokines, lactose etc. Cytokines and amino acids many play a role in immunoenhancement. Most of the medicines are made by distilling urine and collecting vapours known as ark or distillate. A number of ailments could be treated and this is being used even for the most threatening diseases like cancer, AIDS, diabetes and skin problems. Accordingly, Cow urine distillate was selected for this study and succeeded with the following results.

1. Cow urine distillate has been proved for its immunostimulant activity in animals by activating the macrophages and augmenting their engulfment power as well as antibacterial activity. Hence, for the present investigation cow urine distillate has been targeted as the immunomodulator to be studied in the economically important fish species *Labeo rohita*.
2. Cow urine distillate of three different breeds like, Gir, Haryana (Indigenous breed), HF cross bred (Exotic breed) were compared for their efficiency for modulation on growth, food utilization, haematological and immunological parameters in vitro cell cultures and utility on disease resistance at 0.1% (v/v) concentration.
3. Among the routes studied the water additive method exhibited the maximum efficacy in terms of augmented growth, haematological parameters (total erythrocyte count, haemoglobin, total leukocyte and differential leukocyte counts such as lymphocytes, monocytes and neutrophils), immunological parameters (neutrophil activity, lysozyme activity) which ultimately endeavor the maximum disease resistance and relative percent survival in fish *L. rohita* against *Aeromonas hydrophila* infection.
4. The studies on the immunostimulant properties of CUD was proved by its stimulatory effect on the in vitro cell cultures of peripheral blood leukocytes at 100 µl concentration at 72 hours post stimulation in terms of increased viable cell counts, Reactive oxygen species activity, Reactive oxygen species activity, and myeloperoxidase activity.
5. Hence, it could be concluded that Gir cow urine distillate at 0.1% concentration could be administered as water additive in aquaculture and could be advised to aquaculture farmers for their profitable income in an eco-safe, ecofriendly and organic manure.

15. Contribution to the society

The current research examining the use of cow urine distillate (CUD) as an immunostimulant in fish offers valuable insights into sustainable aquaculture, food security, environmental conservation, and public health issues. The aquaculture sector is experiencing rapid growth in food production; however, it encounters significant economic challenges due to disease outbreaks, particularly those caused by *Aeromonas hydrophila*, which also increases reliance on antibiotics. This study suggests that cow urine distillate can improve fish growth performance, enhance hematological parameters, strengthen immune responses, and increase disease resistance, positioning it as a natural and cost-effective alternative to synthetic pharmaceuticals and antibiotics. Additionally, by lowering antibiotic residues in fish, the research contributes to the provision of safer and healthier food for consumers. The use of cow urine distillate, regarded as a traditional and accessible bio-resource, aligns with environmentally friendly methodologies and indigenous knowledge-based approaches. This method is particularly beneficial for small-scale and rural fish farmers, as it lowers production costs, improves survival rates, and enhances profitability while preserving environmental integrity. Environmentally, the findings advocate for sustainable aquaculture practices by reducing chemical inputs that could lead to water contamination and disrupt aquatic ecosystems. The improved health and disease resilience of fish facilitate better stock management and reduced mortality rates. However, its application in aquaculture remains limited. Based on the results of this study, it is concluded that

- i. Cow urine distillate is effective for promotion of growth of *L. rohita* at 0.1% concentrations.
- ii. The Indian cow breed's CUD are more potential than the CUD of exotic breeds.
- iii. To get maximum benefits, it would be better if CUD is applied through feed or immersion route.
- iv. Cow urine distillate enhances the health parameters of *L. rohita* interms of RBC, WBC counts and Hb level.
- v. CUD acts a good immune potentiator by enhancing the immune responses of neutrophils, lymphocytes interms of Myeloperoxidase activity, Reactive Nitrogen Species, Reactive Nitrogen Species, Lysozyme activity and Disease resistance to *Aeromonas hydrophila*.

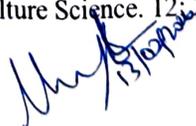
improving survival rates. Additionally, the developed protocols have the potential for application in field settings, which could broaden the market for novel, stress-free therapeutics.

16. Whether any Ph.D enrolled/produced out of the project

The project follow who has registered for Ph.D degree (Xerox copy is enclosed) in Bharathidasan University (Tiruchirappalli Tamil Nadu) has been conferred the Ph.D degree is shortly submitting the synopsis of his Ph.D. thesis on April, 07, 2017.

17. no of publications out of the project : 05

1. Sattanathan. G and S. Venkatalakshmi., 2015. The Dose dependent effect of Gir Go-Ark on the Growth and Food Utilization of *Labeo rohita* fingerlings. Life Sciences International Research Journal. 2 (1): 70-75.
2. Sattanathan. G and S. Venkatalakshmi., 2015. Efficacy of different breeds of cow urine distillate on growth and food utilization Of Indian Major Carp, *Labeo Rohita* (Hamilton) Fingerlings. *Species*, 2015, 14(46), 169-185.
3. Sattanathan. G and S. Venkatalakshmi, 2016. Application of Cow Urine Distillate for Enhancement of Nutrient Value and Water Quality of Indian Major Carp, *Labeo rohita* (Hamilton) Fingerlings. LS: International Journal of Life Sciences. 5 (1): 1-8.
4. Sattanathan. G and S. Venkatalakshmi, 2016. Growth, food utilizations responses and survival rate of Indian major carp, *Labeo rohita* fingerlings under exposure of Cow urine distillate. Innovare journal of Life Sciences, 4(3): 9-13.
5. Sattanathan. G and S. Venkatalakshmi, 2017. Cow urine distillate as an ecosafe and economical feed additive for enhancing growth, food utilization and survival rate in rohu, *Labeo rohita* (Hamilton), Journal of Fish Aquaculture Science. 12: 241-252.


13/07/2017

PRINCIPAL INVESTIGATOR
Dr. S. VENKATALAKSHMI,
Principal Investigator,
UGC - Major Research Project,
PG & Research Dept. of Zoology,
Government College for Women (A),
Kumbakonam - 612 001.