Evaluating the Efficacy of Oregano (*Origanum vulgare*) and Natrum Muriaticum to Enhance the growth and Induced Breeding of Tilapia (*Oreochromis mossambicus*)

Abstract: Monitoring fish health in a repeatable and accurate manner can contribute to the profitability and sustainability of aquaculture. There is a growing interest in fishery industry to replace synthetic chemicals by natural products for induced breeding. This study is aimed to find out the efficacy of oregano (*Origanum vulgare*) and natrum muriaticum (homeopathy preparation) in edible fish tilapia (*Oreochromis mossambicus*). The present study showed biochemical compounds like protein levels in muscle and ovary during the breeding time in tilapia, induced by *Origanum vulgare* and homeopathy preparation Natrum muriaticum 30c potency. Both caused anincrease in protein level at the end of the experiment in tilapia. In protein profile study the variation in their number and staining intensities of different fractions may reflect their different type of metabolic activity, reproductive age, and their environmental conditions. Comparing haematological parameters between treatments within the same experimental design can be a reliable monitoring tool. It is believed that this parameter can better reflect the health of fish than any of the other parameters separately. The increased concentration of Hb in the blood of the *Origanum vulgare* treated groups may have increased oxygen delivery to the tissues, and, as a result, their ovarian tissue growth was improved.We have found oregano (*Origanum vulgare*) extract to be remarkably effective on fish growth and the fish immune system, enabling us to reach 3rd goal of SDGs approach (Ensure healthy lives and promote well-being for all at all ages).

Key words:Oregano,*Origanum vulgare*, Natrum muriaticum,Tilapia,*Oreochromis mossambicus*.

Introduction: Aquaculture is the breeding, rearing and harvesting of fish [21] or farming of aquatic organisms and a kind of agriculture in water [2]. Aquaculture is a growing applied science with lots of potential for innovation [24]. Application of controlled reproduction, an important factor in aquaculture, has considerably increased the commercial fish production [6]. Particularly, in the past 25 years, aquaculture has expanded rapidly and led to a giant multi-billion-dollar industry in many parts of the world [27]. Induced breeding is one of the most effective methods for mass production in aquaculture [14]. There is a growing interest in industry to replace synthetic chemicals by natural products with bioactive properties. Aromatic plants are excellent sources of bioactive compounds. One such aromatic plant is oregano; studies are lacking addressing the effect of its bioactivity in edible fishes. This

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study is aimed to find out the efficacy of oregano (*Origanum vulgare*) in edible fish tilapia (*Oreochromis mossambicus*). There has been extensive research addressing the positive effects of medicinal plant extracts using food fish as animal models. The present research was an attempt to assess the efficacy of *Origanum vulgare* and Natrum muriaticum 30c, considering the 3rd goal of SDGs, as an animal model study.

Materials and Methods: *Oreochromis mossambicus* (Tilapia), an omnivorous edible fish was selected for the present study as the experimental animal. It inhabits freshwaters and successfully establishes in a variety of environmental conditions. It can grow up to a maximum length of 40cm. Females producing as many as 200-300 eggs per spawn, every 30 days. *Oreochromis mossambicus*was selected for the present study, because of its commercial importance, easy availability and faster growth. The fishes were collected from a fishery farm at KPK fish farm Archampatti, Trichy. *Oreochromis mossambicus* having a length of 20-25 cm approximately were selected for the study. The female fishes of 150-180 grams of weight were chosen for the study.

The naturmmuraticum solution of dilution namely 30 centesimal (30C) are brought from Trichy Homeo Medicals. The above-mentioned dilution was preferred as it is known as the typical potency with peculiar potenzing effect. From natrum muriaticum 30 centesimal potency 0.02% dilution was prepared.1 ml of natrum muriaticum 30 c potency was diluted to 0.02% by adding 5 litre of water. The fish were grouped into four sets consisting of 8 each. The first and second set was with 16 individuals, one in each tub and treated as control. The third and fourth set was with 16 individuals one in each tub and treated as experimental (Natrummuriaticum and *Origanum vulgare*). Experimental fishes were left in each tub of 5 litre capacity containing the solution prepared with n. muriaticum of 0.02% and *Origanum vulgare* of 0.02% (set two and three respectively). The medium in which the fishes left was fed with pellet diet at the same time. The experimentwas carried out for 4 days (0, 1st, 2nd, 3rd days) and the medium was changed morning at 8 am and evening at 4 pm. Evening only water was used. On the day before medication (0 day) and 5th day of the experiment fish were autopsied, the ovary was removed and analysed for the total protein content by using Lowry et al., method (1951), SDS polyacrylamide gel electrophoresis (Laemmli, 1970) was done to study the protein profile in the ovary tissue.Haematological study was done using haematological auto analyzer-Mindray cell counter.

Results and Discussion

The present study deals with the effect of Natrum muriaticum and *Origanum vulgare* on the biochemical, haematological and histological, protein level of muscle, blood and ovary. The results of this study revealed the levels of muscle and ovary protein in control fish on 0-day, 4th day and treated on 4th day (Table 1 and Figure 1). The level increased both in control and treated on 4th day of the experiment.The observed metabolic changes during breeding reflects a shift from regulation of oogenesis to the regulation of breeding.

The ovary tissue samples of tilapia fish treated with natrum muriaticum and *Origanum vulgare* exhibited different protein fractions on SDS-PAGE analysis.Detailed electrophoretic analysis under denaturing conditions of the proteins during the reproductive cycle has clearly shown that there are striking similarities between the protein fractions of ovary tissues. The analysis of polypeptides of tilapia fish by SDS-PAGE revealed the presence of ten fractions with molecular weight ranging from (Figure-2)20 kDa to 200 kDa on 0 day. There is no difference in 0 day and 4th day of control. In the ovary nearly two polypeptides were with high molecular weight (above 100kDa) and the rest were with low molecular weight polypeptides. Similarly, in the experimental fish the presence of ten fractions with molecular weight ranging from 20kDa to 200kDa was obvious. Among this, two of them were with high molecular weight (above 100kDa) and the remaining were with low molecular weight. Three bands in all the fish were more prominent with molecular weight of 29 kDa, 36 kDa and 45 kDa and 66kDa and 97kDa. These are considered as specific bands for reproductive functions of the fish on the observations made in tilapia fish.

From the electrophoretic study it may be understood that there are nearly seven fractions present in all the samples. Almost all the samples showed more or less similarities in the molecular weight of their protein fractions. There is a difference among the control and experimental groups on 4th day (*Origanum vulgare* treated) not in natrum muriaticum treated i.e there is no specific bands with molecular weight of 116KDa and 200KDa (Lane D). Fish vitellogenin displays a high variability in their protein size and subunit number in general [4]. Fish vitellogenins are quite unstable. Vitellogenin molecular weight 176 kDa was identified in female rainbow trout [19,26,4]. Vitellogenin in teleosts are ranging from 300 to 600 kDa indifferent species [9]. Vitellogenin isolated from African catfish *Clarias gariepinus*is520 kDa [17] and for trout *Salmo gairdneri*is 470 kDa [5], 440 kDa for sea trout *Salmo trutta*[21].

Vitellogenin for sea bass *Dicentrarchuslabrax*is 445 kDa [16], 500 kDa for viviparous blenny *Zoarces viviparous* [11], 490 kDa for common carp *Cyprinus carpio*[8]. According to [10], SDS-PAGE of the oocyte extract of Tilapia, *O.mossambicus* showed minor bands at 26

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and 24 and 23kDa, a major protein band at 106 and very faint bands at 83and 175kDa.The variation in their number and staining intensities of different fractions may reflect their different type of metabolic activity, reproductive age, and their environmental conditions. The result showed that the synthesis of proteins during Natrum muriaticum and *Origanum vulgare* exposure in tilapia fish signifies that and these proteins are female specific proteins. These proteins are involved in vitellogenesis. However, further experiments are needed to sequence the whole vitellogenin at gene level.

The use of haematological and blood biochemistry parameters has proven to be effective and repeatable ways to monitor fish health. Testing these parameters is becoming more common in aquaculture studies. However, interestingly, blood performance can be much more reliable and accurate for monitoring fish health and growth.

In this research, the following parameters like white blood cells (WBC), Lymph, granulocytes(GRAN), red blood cells(RBC) and Hemoglobin (HGB) ,Hematocrit(HCT), Mean corpuscular volume(MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration(MCHC),Red cell distribution width-coefficient of variation(RDW-CV), Red cell distribution width-standard deviation(RDW-SD),platelet count test (PLT),Mean platelet volume(MPV), Platelet distribution width (PDW),Plateletcrit (PCT),Platelet large cell coefficient(P-LCC),Platelet large cell ratio(P-LCR)(Table-2) are measured using haematological auto analyzer. However, comparing haematology data as biological indicators between fish species or even the same species in different experimental conditions is extremely difficult, if not impossible. Despite these limitations, comparing haematological parameters between treatments or within the same experimental design can be a reliable monitoring tool. It is believed that these parameters can better reflect the health of fish than any of the other parameters separately. The increased concentration of Hb in the blood of the *Origanum vulgare* treated groups may have increased oxygen delivery to the tissues, and as a result, their ovarian tissue growth was improved.

Ovarian histology studies after the administration of Natrum muriaticum and *Origanum vulgare* in tilapia showed significant changes. In tilapia, at 0 day of the control fish ovary (Figure 3.) growth phase (Stage I-primary oocytes), cortical alveolus phase (Stage II) and vitellogenic stage (III) were observed. In growth phase many primary oocytes were seen. In cortical alveolus phase follicle increase in size, nucleus enlarged and vitelline envelope begin to form. At 4^{th} day of the control fish shows (Figure 4) growth phase (Stage-I), cortical

alveolus phase (Stage II), vitellogenic stages (Stage III) and matured oocytes (Stage IV). Experimental (Natrum muriaticum treated) fish at 4th day show (Figure 5) cortical alveolus phase (Stage II), vitellogenic stages (Stage III) and matured oocytes (Stage IV). Experimental (*Origanum vulgare* treated) fish at 4th day (Figure 6) shows vitellogenic stage (Stage III) and matured oocytes (Stage IV). At this stage, number and size of the yolk vesicles increased.

The histology of teleosts fish has been studied by [29]. He also observed the development of ovarian eggs in Flounders,*Liopsetta obscura*. [3] examined the seasonal changes in the ovary of *Ophiocephalus punctatus* while [13] reported the histological changes of the ovarian cycle in carp *Cirrhinusmrigala*. According to the pattern of the oocyte development, the ovaries of the fish have been classified into three types by [25]. [18] stated that target organs of toxicity and mechanism of action was identified by histopathology. Fish are diversified vertebrate group, adapted to huge range of aquatic environments. It is also reflected in gonadal morphology and reproductive strategies, highlighting the need for comparative studies among different fish groups [20].

Increasing Haemoglobin levels in studies after feeding fish with barberry root (*Berberis vulgaris*) [23] and garlic (*Allium sativum*) [7] were observed. Other studies showed a similar trend in HG fish when barramundi (*Latescalcarifer*) was fed on butyric acid[1] in red hybrid tilapia (*Oreochromis mossambicus* × *O. niloticus*) on exopolysaccharide derived from reishi mushroom (*Ganoderma lucidum*) [28]. Hence, natrum muriaticum and *Origanum vulgare* can be used for induced breeding of tilapia fish. By using this preparation production of edible tilapia fish can be increased and in turn offer good opportunities for entrepreneurs.

Conclusion: Fish and Health Benefits of Fisheries and Aquaculture have been considered as an important means of poverty elevation and food security besides promoting health and wellbeing. The present research was an attempt to assess the efficacy of *Origanum vulgare* and Natrum muriaticum 30c, considering the 3rd goal of SDGs, as an animal model study. We have found oregano (*Origanum vulgare*) extract to be remarkably effective on fish growth and the fish immune system and sustainable fishery management of *Oreochromis mossambicus*. The study is important in addressing the use of synthetic chemicals in fisheries and aquaculture. Natural compounds or plant materials are very important in addressing the negative effects of synthetic hormones. Moreover, as the recent advances in fisheries and aquaculture, it is noted that the use of eco-friendly materials is very important in attaining the sustainable development goals. Therefore, this study is vital in fisheries and aquaculture as it involves natural compounds in addressing issues in fish health.

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Table.1-Changes in the protein (mg%) in the muscle and ovary during the period of induced breeding in *O.mossambicus*

Type of treatment	Days of Sampling	Mean±S.D (Ovary protein)	Mean±S.D (Muscle protein)
Control	0 day	2.75±0.08	3.2±0.14
Control	4th day	2.93±0.11	3.48±0.17
Experimental (H)	4th day	3.19±0.15	4.32±0.21
Experimental (A)	4th day	3.31±0.13	4.5±0.17

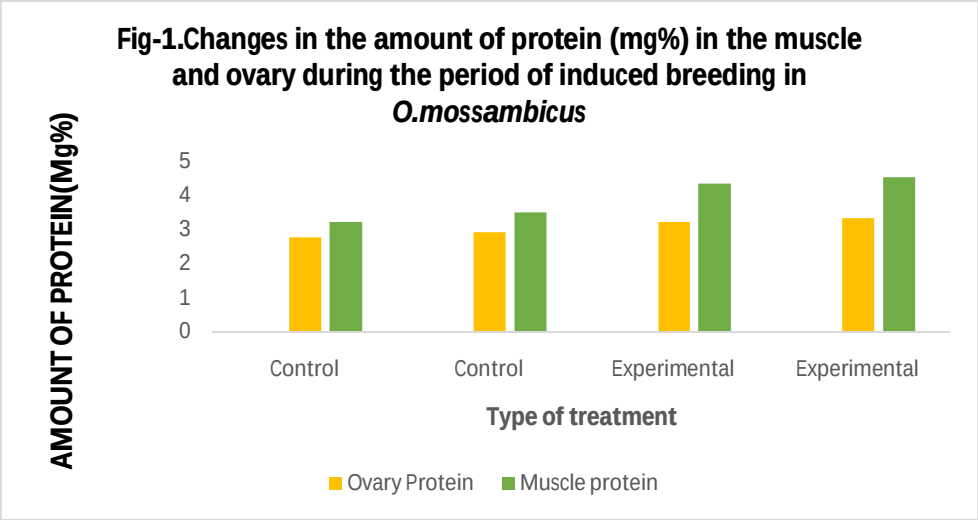

Figure-2. Electrophoresis of ovary protein extracts from previtellogenic or vitellogenic oviparous females –protein profile. M-Protein ladder; Lane-A -0-day; Lane-B-4th day Control and Lane-C- 4th day Experimental(H) Lane-D-Experimental(A). The molecular weight of the standard proteins is indicated on the left: myosin (200kDa), b-**galactosidase (116.25 kDa), phosphorylase-**b **(97 kDa), BSA (66 kDa) and ovalbumin (45 kDa).**

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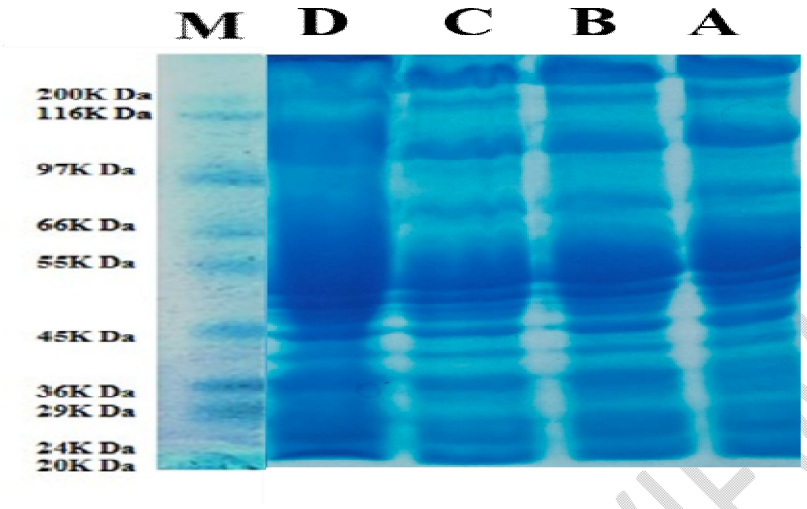

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Table-2.Hematological parameters of Tilapia (Sample1-Control 0-day, Sample 2-

S. No	Modules	Samples				
		1(0 day-C)	2(4th day-C)	3(4th day-E (H)	4(4th day-E(A)	Unit
1.	**WBC**	151	152	162	**165**	10^ 3/µl
2.	**Lymph**	3.0	3.0	3.4	3.2	10^ 3/µl
3.	**Lymph%**	92.5	92	91.3	93.1	%
4.	**RBC**	2.3	2.4	2.5	**2.7**	10^ 6/µl
5.	**HGB**	12.3	12.5	12.4	**13.7**	g/dL
6.	**HCT**	31.2	31.5	32.9	32.2	%

Control 4th day, Sample 3-N.mur treated 4th day, Sample 4-O.vul treated 4th day)

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7.	**MCV**	42.5	42.5	40.9	43.9	fL
8.	**MCH**	45.8	45	43.4	45.4	Pg
9.	**MCHC**	31.9	32	32.8	32.1	g/Dl
10.	**RDW-CV**	12.8	12.8	12.5	13.0	%
11.	**RDW-SD**	31.0	31.0	32.5	30.1	fL
12.	**PLT**	45	45	37	42	10^ 3/µl
13.	**MPV**	5.3	5.3	5.4	5.7	Fl
14.	**PDW**	15.9	15.9	15.1	15.9	-
15.	**PCT**	0.327	0.328	0.335	0.341	%
16.	**P-LCC**	6	6	5	5	10^ 3/µl
17.	**P-LCR**	5.5	5.5	5.2	5.2	%

Fig 3. Oocytes-0 Day Control-4X

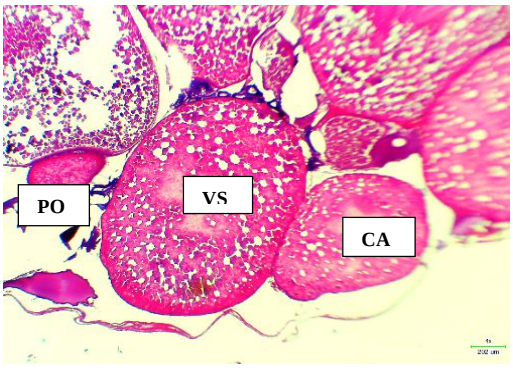
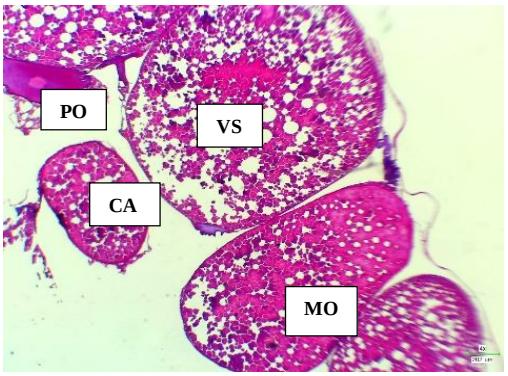


Fig 4-Oocytes-4thDay Control-4X



PO-primary oocyte; CA-Cortical alveolus stage; VS-Vitellogenic stage, MO-Mature Oocytes.

Fig 5 Oocytes-4th Day (N. m treated)

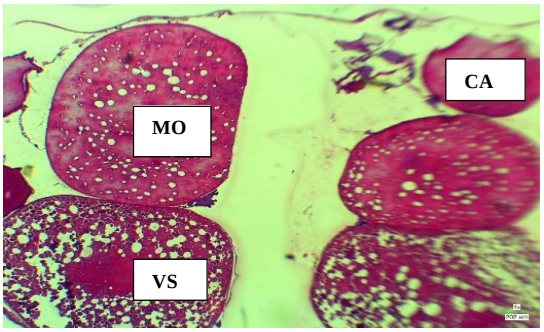
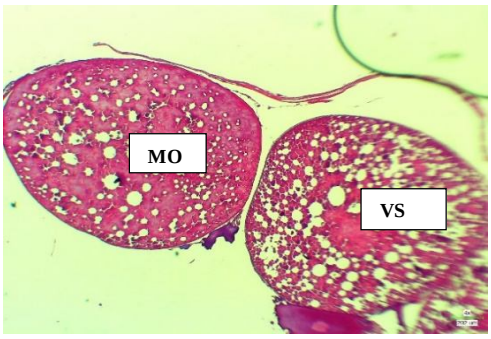


Fig 6Oocytes-4th Day(O.v treated)-4X



CA-Cortical alveolus stage; VS-Vitellogenic stage, MO-Mature Oocytes.

Declarations: I declare that the research article has been composed by myself and that the work has not been submitted to any other publication.

Ethical Approval: Not applicable.

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