

1 **EFFECT OF *Foeniculum vulgare* MINGLED**
2 **DIET UPON GROWTH, REPRODUCTION**
3 **AND SPAWNING PERFORMANCE OF**
4 **GUPPY FISH**

5
6
7
8
9
10 **ABSTRACT**
11

Naturally breeding fish cannot meet the demand of humans. So induced breeding is essential to fulfill this demand. Induced breeding ensures a timely supply of fish seed having desired characteristics, and different techniques can be used for this purpose. In this perspective synthetic hormones, extract of pituitary hormones, Gonadotropin releasing hormones, and sometimes Human chronic gonadotropin are used as a spawning inducer for fishes. Plant extracts can also be used as it is a new technique which could be used in aquaculture. The basic aim of our study was to enhance the growth as well as to check the effect of traditional herb *Foeniculum vulgare* on the reproductive performance of *Poeciliareticulata*.

Samples were collected from a local fish shop in Sargodha. Ethanolic extract of *Foeniculum vulgare* seeds was prepared and mixed with different commercial feed. This feed was given to fish for 90 days to test the effect of *Foeniculum vulgare* seed extract on the growth and reproduction performance. Water quality parameters were also maintained and recorded on daily basis. Fecundity and Gonad somatic index were measured after dissection by removing the ovaries. Fecundity was calculated by counting the number of larvae and eggs seen with the naked eye. The hatching rate was measured by counting the number of hatchings in each spawning.

According to the growth results, different levels of fennel seed extract has a significant effect on weight gain, length gain and survival rates of the fish in

different groups. Statistical analysis cleared that T₂ showed the highest weight and length gain, and the Feed Conversion Ratio decreased significantly. The results for reproductive parameters showed that the highest absolute fecundity (9.4 ± 0.19), GSI (8.69 ± 0.2) and spawning increased in T₂ followed by T₁, T₃, and control respectively. The observation of present research show that *Foeniculum vulgare* seed extract can act as an estrogenic compound and growth promoter in commercial aquaculture.

12
13 *Keywords: Foeniculum vulgare, Fecundity, spawning, Gas Chromatography Mass*
14 *Spectroscopy, Reproductive performance.*

15 **1. INTRODUCTION**

16
17 The fisheries division is playing a pivotal role in nourishment,
18 dietary security, profit earning, and to enhance the national
19 economy. Among fishes, some are edible and some are ornamental and
20 might be perceived as decorative fishes.

Comment [MM1]: Please delete all hanging from the manuscript

21 They are likewise called “living jewels” due to their friendly
22 behavior, beautiful color, and shape (Nasser and Rajkumar, 2001).
23 All over the world, the trade of aquatic animals and their
24 products has been increased in the last few years.

25 Ornamental fish keeping is considered an interesting, easy, and
26 relaxant hobby throughout the world. The worldwide exchange of
27 aquatic organisms has been proposed as the wellspring business.
28 Ornamental fishes are very attractive aquatic species in the
29 world.

30 Ornamental fish is a key element of the aquatic environment and
31 considered an ecosystem maintainer in the aquatic environment

32 (Rodriguez, 2006). *Poeciliareticulata* is native species from
33 North Brazil and South America (Ojanguren *et al.*, 2005).

34 *Poeciliareticulata* known as “Guppy fish” belongs to the family
35 Poeciliidae, it is a small ornamental fish also called
36 livebearer, inhibited in a freshwater environment.
37 *Poeciliareticulata* is a commercially important fish due to its
38 attractive colors and variation in the tail that makes it the
39 most trading ornamental aquarium fish in the world (Egset *et*
40 *al.*, 2011).

41 *Poeciliareticulata* start to breed from February till while its
42 peak in July. Female *Poeciliareticulata* are livebearers and
43 give birth to live young. The rearing period of *Poeciliareticulata* in the
44 Southeastern U. S. starts in May and finishes in September and October
45 (Hidebrand, 1921).

46 At the age of 10–12 weeks, females become mature while males
47 mature at the age of 7 weeks. Female *Poeciliareticulata* show
48 polyandry, female can mate with multiple males. Females can
49 give birth 20–200 young in a single spawn. The gestation period
50 of *Poeciliareticulata* is about 4–6 weeks (Coad, 2017).

51 Seeds availability is only possible by the reproduction. Natural
52 reproduction is not enough to meet the seed availability
53 according to demand. Induce breeding is the best way to enhance
54 the reproduction, to produce the seeds.

55 Availability of seeds can be possible by reproduction as well as
56 artificially induced breeding in hatchery (Sudha and Gokula,
57 2015). Due to the lack of proper environmental condition,

58 reproduction and growth of fishes is disturbed, and it is
59 difficult to meet the demand of fish seed by natural resources.
60 So, to fulfill this demand synthetic stimulators are used for
61 fish breeding (Ojima and Iwata, 2009).

62 For egg development and spawning, female need appropriate amount
63 of lipid and protein. Diet plays a significant role for
64 development and reproduction of ornamental fish, and various
65 feeds have been utilized for its raising.

66 Supplement feed, hormones, anti-infection agents, and numerous
67 herbal products are used for enhancements for fecundity and
68 gametes maturation of ornamental fish (Ghosh *et al.*, 2007).

69 There are many ways to stimulate the reproduction of fish.
70 Reproduction of fish can be provoked by using one of these
71 products in ornamental fish, Ovaprim a liquid form injection
72 which is a mixture of GnRH and domperidone in the liquid of
73 propylene glycol.

74 Ovaprim used as a spawning abet to induce evolution (Yanong *et al.*,
75 2010). Gonadosomatic index (GSI) is the ratio between body weight and
76 gonads weight of fish.

77 Gonadosomatic index involves to identify the spawning season of
78 fish. While fecundity is the relation between numbers of eggs
79 produced in each spawn and body weight of fish. It is most
80 important parameters used in the study of reproduction of fish (Hunter and
81 Macewicz, 2001).

82 Gonadosomatic index and fecundity measures are helpful in measuring population
83 dynamics, population carrying capacity and productivity (Shankar and
84 Kulkarni, 2005).

85 It evaluates the reproductive system of a fish by calculating the total body size. It
86 is considered as the most important parameter of fish reproductive biology,
87 provides us a comprehensive view about the fish breeding and the
88 reproductive status of related species, especially in finding the fish breeding
89 seasons. Fertility and effectiveness are estimated by Gonadosomatic Index and
90 fecundity (Chavan and Muley, 2014).

91 To check the effect of traditional herb *Foeniculum vulgare* on growth rate,
92 reproductive performance of *Poeciliareticulata*.

93 To evaluate the influence of *Foeniculum vulgare* on spawning of female
94 *Poeciliareticulata*.

95

96

97 **2. MATERIAL AND METHODS**

98

99 Complete Random Design (CRD) was used for sampling purposes to
100 evaluate the variable of growth (weight gain, length gain,
101 FCR, and survival rate) and Reproduction (Fecundity, GSI,
102 Hatching rate, and spawning).

103 The present study was conducted in the Laboratory, Department
104 of Zoology, The University of Lahore, Sargodha Campus.
105 *Poeciliareticulata* stock of 120 was kept in the 12-glass
106 aquarium of size of 12×12×18. Juvenile
107 *Poeciliareticulata* having size range of 0.19 ±0.02 were divided
108 into four experimental groups. Each glass aquarium for
109 experimental treatment contains 10 juvenile guppies (30 in
110 triplicate). Fish feed used during the experiment was
111 commercial.

112 **Sample Size**

113 Present experiment was performed for 3 months (90 days) after
114 being acclimatized in laboratory conditions for two
115 weeks. Initial stocks of 120 juvenile *Poeciliareticulata*
116 having an average weight of 0.19 ± 0.007 were purchased from
117 a commercial fish shop in Sargodha.

118 Fish were identified by following the Coad (2017). Guppies were
119 kept in the four aquariums to make them acclimatized the
120 laboratory condition for two weeks before the research.
121 After two weeks, fingerlings were divided into four Aquarium
122 manually @ ten fish per aquarium.

123 During this period juveniles were observed and diseased
124 juveniles were separated from the healthy fishes, then
125 remaining stocks were divided into four treatments having an
126 average weight of 0.18 ± 0.01 g (T_1), 0.19 ± 0.01 g (T_2),
127 0.19 ± 0.01 g (T_3), and 0.19 ± 0.01 g (T_4).

128 *Poeciliareticulata* were provided with a feed named "INCH GOLD"
129 a commercial feed of 3% of body weight twice a day i.e.,
130 8:00 am and 6:00 pm. Aquarium water was changed weekly to
131 maintain a healthy and clean water environment.

132 ***Foeniculum vulgare* SEEDS EXTRACT PREPARATION**

133 *Foeniculum vulgare* seeds were purchased from the herbal shop in
134 Sargodha, Pakistan. *Foeniculum vulgare* seeds were identified
135 by the Department of Botany, Lahore University, Sargodha
136 Campus. *Foeniculum vulgare* seeds were grinded with a blender
137 to get a fine powder.

138 To get *Foeniculum vulgare* seed extract, *Foeniculum vulgare* seed
139 powder (50 g) was taken and 96% ethanol mixed with a ratio
140 of 1: 1. The extract was kept at room temperature for 24
141 hours with continuous stirring.

142 What-man filter paper No.1 was used to filter the extract. The
143 obtained extract was diluted with distilled water to obtain
144 the final solution (Nazari and Roozbehani, 2015).

145 **Treatment Feed Preparation**

146 Feed was prepared by followings (Haghighi *et al*, 2014). Commercial
147 feed which is “Inch gold” used in feed and mixed with
148 *Foeniculum vulgare* seeds extract in Petri dish. Four treatments
149 diets with different *Foeniculum vulgare* seeds extract
150 concentration were prepared i.e. 50, 100, 150µl/g while fourth
151 diet was control having 0µl *Foeniculum vulgare* extract.

152 These diets were termed as D₀, D₁, D₂, and D₃. Feed pellets were dry
153 at room temperature. The pellets were air dried and stored in
154 air tight container at room temperature until fed. This feed
155 was fed to fishes, twice a day, at the 3 % of body weight, for
156 90 days. From all groups required parameter evaluation was
157 checked after fifteen days.

158 **Seed Analysis**

159 *Foeniculum vulgare* seed extract was analyzed through gas
160 chromatography-mass spectroscopy system (Hammouda *et al.*,
161 2014).

162 GC-MS system contains an auto-sampler unit, injector use, and a
163 gas chromatograph with a mass spectrometer. With a ratio of
164 1: 100, approximately 2µl samples were inserted into split
165 mode. Helium gas was used as the carrier with a pressure of
166 (65.2 kPa).

167 The ionization energy used to identify the compounds by GC-MS
168 was 70 eV. The temperature in the Column oven was 80°C to
169 220°C. The flow rate of helium gas was used as 1.5 l ml/min
170 as a carrier gas.

171 The average temperature range for the injector and MS transfer
172 line was 220°C to 290°C. Total time required for separation
173 was 65 minutes. The MS capillary column was extracted the
174 *Foeniculum vulgare* seed extract compounds (Alam, 2019).

175 ***Foeniculum vulgare* seed extract composition (%) by GC-MS**

Chemical compound	%
4-Hexen-2-one	0.20
3-Hydroxytetrahydropyran	0.65
Acetic acid	0.10
Fenchone	11.68
Delta-3-carene	0.52
Camphor	Trace
Tetradecane	0.90
Benzaldehyde-4-methoxy	10.01
L-Histidine	Trace

Benzene -1- methoxy-4- (2-propene)	2. 52
Fenchyl acetate	0. 11
Cyclohexane	0. 41
1-Methyl-2-methylene-4-isopropyl	Trace
Trans-anethole	64. 49
1-Tetradecene	0. 74
1-Methoxy-3-(ethenylcarbonyl)B	0. 81
Hexatriacontane	1. 54
10-Nonadecanone	3. 17

176

177 shows the chemical component of fennel seed extract. With the aid
 178 of GC -MS, 18 compounds were described. Trans-anethole (64.49
 179 percent), Fenchone (11.68 percent), and Benzaldehyde-4-methoxy
 180 (10.01) were the main constituents of the ethanolic extract of
 181 fennel seed. Furthermore, the fennel seed contained significant
 182 amounts of different minor constituents, with a contribution of
 183 less than 10%.

184

185 3. RESULTS AND DISCUSSION

186

187 GROWTH PARAMETERS

188

188 4.4.1 Weight gain and length gain

189

189 Table 1: Growth data of *Poeciliareticulata* record after 14 days

190

Trail.

Difference between Initial reading and after 14 days reading of samples
--

Comment [MM2]: The table is required to initiate with serial number 1, meaning that the first entry in the table should be designated as '1' in the serial number column.

							$L_f - L_i$
1	T ₀	0.17	0.31	0.12	1.93	3.07	1.41
2	T ₁	0.19	0.38	0.19	1.95	3.73	1.78
3	T ₂	0.19	0.44	0.25	1.97	4.09	2.12
4	T ₃	0.18	0.39	0.21	1.92	3.66	1.74

193

194 Calculated results for weight gain and length gain were presented
 195 in Table 4.5. Statistical analysis shows that maximum weight
 196 gain was observed in T₂ while minimum growth rate was recorded
 197 in T₀ (Control group) respectively.

198 The increase in length was also observed during the experiment to
 199 check the effect of *Foeniculum vulgare* extract on total length
 200 gain. Length increase is presented in Table 4.5. Analysis of
 201 increase in length presented in graph (Fig 1) shows that the
 202 maximum length was attaining by Treatment T₂ and T₀ achieved
 203 minimum length.

204 Growth performance results showed that with a concentration of
 205 100µl of *Foeniculum vulgare* seed extract containing has a
 206 positive effect (P<.0.05) on the growth parameters of
 207 *Poeciliareticulata*.

208

209 **Figure 1: Increase growth rate observation fed with *Foeniculum***
 210 ***vulgare* concentrations.**

Comment [MM3]: Figure missing. The figure is required to initiate with serial number 1, meaning that the first entry in the figure should be designated as '1' in the serial number.

211 Weight gain, height increase, survival rate, and feed conversion
 212 ratio were recorded in (Table 4.6). Growth parameters were
 213 recorded after fifteen days. The statistically analyzed data
 214 showed that as the concentration of *Foeniculum vulgare*
 215 increases the weight and length were also increases
 216 significantly ($p < 0.05$).

217 **Table 4.6: Growth parameters of *Poeciliareticulata* fed diets**
 218 **containing different concentrations of *Foeniculum vulgare* for**
 219 **90 days.**

Growth parameters	T ₀ (0 µl)	T ₁ (50 µl)	T ₂ (100 µl)	T ₃ (150 µl)
Initial weight (g)	0.17 ± 0.00 8 ^a	0.18 ± 0.01 a	0.19 ± 0.00 8 ^a	0.19 ± 0.00 7 ^a
Final weight (g)	0.31 ± 0.03 ^c	0.38 ± 0.01 b	0.44 ± 0.03 ^a	0.39 ± 0.03 ^b
Weight gain (g)	0.12 ^c	0.21 ^b	0.25 ^a	0.19 ^b
Initial Length (cm)	1.93 ± 0.13 ^a	1.95 ± 0.16 a	1.97 ± 0.1 ^a	1.92 ± 0.14 ^a
Final	3.07 ± 0.1 ^c	3.66 ± 0.2 ^b	4.10 ± 0.3 ^a	3.73 ± 0.2 ^b

Length (cm)				
Net length gain	1.14 ^c	1.71 ^b	2.12 ^a	1.81 ^b
Feed conver sion ratio	3.11±0.8 ^a	2.52±0.4 ^b	2.24±0.1 ^c	2.93±0.2 ^{ab}
Survival rate (%)	100 ± 0.00 ^a	100 ± 0.00 ^a	100 ± 0.00 ^a	100 ± 0.00 ^a

220 Note: Values given are mean ± standard error. Means with the same
 221 letter in the same column are not statistically significantly
 222 (P <.05) different

223 4.4.2 Feed conversion ratio

224 According to the results, data is presented in Table 4.6,
 225 different concentrations of fennel extract has a positive
 226 effect on the FCR of feed fed by *Poeciliareticulata* in
 227 experimental treatments. FCR values were recorded as in T₀
 228 (3.11±0.8), T₁ (2.52±0.4) T₂ (2.24±0.1), and T₃ (2.93±0.2),
 229 respectively. A significant decrease in the FCR value of T₂ is
 230 observed when compared with other groups in the experiment.
 231 Fennel seed extract contains anethole which is a digestive
 232 stimulator, a reason for the decrease in FCR. Graphical

233 presentation (Fig 4.2) of Feed Conversion Ratio shows that
234 highest in T₀ while it was lowest in T₂. The results indicate
235 that T₂ has significantly differed from T₁, T₃, and T₀. The
236 results regarding FCR showed that *Foeniculum vulgare* has the
237 effect of decreasing the Feed conversion ratio.

238

239 **Figure 4.2. Mean values of feed conversion ratio under varying**
240 **stocking concentration of fennel extract on *Poeciliareticulata***

Comment [MM4]: Figure missing

241 4.4.3 Survival rate

242 Graphical presentation of survival rate (Fig 4.3) showed that
243 fennel extract did not negatively affect the Survival rate of
244 *Poeciliareticulata* in every experimental group. The survival
245 rate in all experimental and control were consistently high
246 (P>0.05). The survival rate of all treatments is recorded as
247 100%. It is inferred that *F. vulgare* seed extract has no
248 adverse effect on the Survival rate of *Poeciliareticulata*.

249

250 **Figure 4.3: Mean values of survival rate under varying stocking**
251 **concentrations of fennel extract on *Poeciliareticulata***

Comment [MM5]: Figure missing

252

253 4.5 REPRODUCTION PARAMETERS

254 4.5.1 Gonadosomatic index

255 Table 4.7: Age, fish, and gonad weight, Gonadosomatic index (GSI)
256 of *P. reticulata*

Sr.	Treatme	T ₀	T ₁	T ₂	T ₃
-----	---------	----------------	----------------	----------------	----------------

no	parameters				
1	Age in days	180-192	180-198	180-210	180-195
2	Fish weight	0.79±0.008 ^c	0.93±0.008 ^b	1.04±0.01 ^a	0.93 ±0.008 ^b
3	Gonads weight	0.023±0.002 ^d	0.041±0.004 ^c	0.065±0.001 ^a	0.053±0.003 ^b
4	GSI	6.07 ±0.11 ^c	7.03 ±0.04 ^b	8.69 ±0.15 ^a	7.54 ±0.05 ^{ab}

257 Note: Values (mean ±SEM) of GSI are superscripted by alphabets
258 within the same line are different significantly (P<0.05).

259 Final data of reproduction parameters are described in Tables 4.7.

260 The gonadosomatic index was measured by the weight of the fish
261 and the gonad's weight of the fish. Gonad's weight gain at
262 maturity is shown in Fig 4.4. The highest Gonadosomatic index
263 was observed in T₂ followed by T₁, T₃, and T₀ separately. The
264 groups that contain *F. vulgare* seed extract, the Gonadosomatic
265 index (GSI) is significantly high (P < 0.05). The highest GSI
266 was observed in T₂ (8.69±0.2) and lowest GSI was found in T₀
267 (6.07±0.2) respectively.

268

269 Figure 4.4: Effect of *Foeniculum vulgare* mingle diet containing
 270 different concentrations on Gonadosomatic index (Mean \pm SD)
 271 of *Poeciliareticulata*

272 4.5.2 Fecundity

273 Table 4.8: The impact of different concentrations of *Foeniculum*
 274 *vulgare* extract on reproductive parameters of
 275 *Poeciliareticulata*(♀) for 90 days

Sr	Parameter	Treatments			
		T ₀	T ₁	T ₂	T ₃
1	Fish weight	0.79 \pm 0.008 ^c	0.93 \pm 0.008 ^b	1.04 \pm 0.01 ^a	0.93 \pm 0.008 ^b
2	Fish length	3.9 \pm 0.02 ^d	4.4 \pm 0.12 ^c	5.6 \pm 0.08 ^a	5.1 \pm 0.04 ^b
3	Absolute fecundity	5.7 \pm 0.	7.9 \pm 0.12 ^c	9.4 \pm	8.3 \pm 0.15 ^b

		12 d		0. 19 a	
4	Relative fecundity	46.8 ± 1. 20 c	53.5 ± 0.98 ^b	62.3 ± 1. 87 a	57.1 ± 1.71 ^{ab}
5	Gonadosomatic index	6.07 ± 0. 11 d	7.03 ± 0.04 c	8.69 ± 0. 15 a	7.54 ± 0.05 ^b
6	Hatching (%)	62.3 ± 1. 69 c	81.2 ± 0.08 ^b	91.3 ± 4. 1 ^a	85.5 ± 2.3 ^b

276 Note: Values (mean ± SEM) superscripted by various letter sets
277 inside a similar line are differ essentially (P < 0.05).

278 Findings of reproductive parameters are presented in Table 4.8 and
279 Fig 4.5. Significant differences were found among treatments in
280 Gonadosomatic index (P<0.05); although absolute fecundity and
281 relative fecundity of guppy fish significantly increased
282 (P<0.05) in T₂ fed with 100 µl of *Foeniculumvulgare* extract.

283 As the gonads weight increase, relative fecundity and absolute
284 fecundity also increase. The numbers of eggs were highest count
285 in T₂. This showed that fennel seed extract with 100µl
286 concentration in diet has significant results (p<0.05) on the
287 Gonads initial maturity and fecundity.

288 The present study reported that, the gestation period of
289 *Poeciliareticulata* was 25-35 days with an average of 28 days.

290 In *Poeciliareticulata*, treatment is interior and happens through
291 mating of couples demonstrating explicit mating conduct. Male
292 play out S-formed stance known as 'sigmoid display' and
293 orientates himself before the females toward the start of
294 courtship (Krumholz, 1948).

295

296 **Figure 4.5: Comparison of absolute fecundity and relative**
297 **fecundity changes in test groups and control during**
298 **experiment.**

299 4.5.3 Hatching

300 Results for hatching rate (%) were presented in Table
301 4.8. According to graph (Fig 4.6) highest value was observed in
302 T₂ (p< 0.05) which show that increased significantly followed
303 by T₁, T₃ and T₀ respectively. Results were best in T₂ which
304 contains 100 µl extract of *Foeniculum vulgare*. Highest and
305 lowest hatchling rate were respectively in T₂ and control
306 group. According to result *Foeniculum vulgare* has a positive
307 effect towards increase in hatching percentage.

308

309 Figure 4.6: Number of young ones released by control and
 310 experimental groups with varying concentrations of *Foeniculum*
 311 *vulgare*.

312

313 4.5.4 Spawning performance

314 Table 4.9: Evaluation of spawning response of *Poeciliareticulata*

Parameters	Treatments			
	T ₀	T ₁	T ₂	T ₃
Survival				
rate	100 ±	100 ±	100 ±	100 ±
percentage	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
e				
Number of larva	45 ± 3.5 ^d	74 ± 1.4 ^c	91 ± 0.81 ^a	82 ± 1.6 ^b
Number of ova	34 ± 2.4 ^d	68 ± 2.9 ^c	81 ± 2.1 ^a	75 ± 2.4 ^b
Spawning				
time (hours)	88 ± 0.81 ^a	65 ± 0.81 ^c	39 ± 2.94 ^d	76 ± 1.63 ^b

315 Note: Values (mean SEM) superscripted by alphabets are
 316 significantly different (P < 0.05).

317 To evaluate the influence of *Foeniculum vulgare* on the spawning
 318 performance of Guppy fish results were presented in the Table
 319 4.8. Graphical presentation (Fig 4.7) presented that guppy fish
 320 laid more egg when treated with Ethanolic extract of *Foeniculum*

321 *vulgare* with 100µl concentration. Treatment (T₂) when treated
322 with 100µl *Foeniculum vulgare* resulted a significantly positive
323 effect (p<0.05) 91±2.1 on no of young ones produced by
324 *Poeciliareticulata*. Present findings indicated that herbal
325 extract of *Foeniculum vulgare* is a positively effective
326 stimulator for spawning of *Poeciliareticulata*.

327

328 **Figure 4.7: graphical comparisons of different doses of *Foeniculum***
329 ***vulgare* mingle diet on spawning performance in *Reticulata*.**

330

331

332 4. DISCUSSION

333 The success of aquaculture depends on a number of parameters, the
334 most important is a suitable diet that contains the complete
335 balanced nutritional diet for the average growth of fish
336 (Salehi, 2008).

337 The aim of this research was to investigate the effect of *F.*
338 *vulgare* extract mingles diet on the growth and reproductive
339 performance of *Poeciliareticulata*. According to results of the
340 present study, FCR decreased in T₂ as compared to other
341 treatments. *Foeniculum vulgare* seed extract contains the
342 Anethole and Estragole perform the function as a digestive
343 stimulant may be a reason for the decrease in FCR (Frankicet
344 *al.*, 2009).

345 More than this growth of *Poeciliareticulata* significantly (p
346 <0.05) positive increase in T₂. The growth parameter, the

347 survival rate was uniformly the same in all treatments which
348 are about 100% in all treatments.

349 This justified that *Foeniculum vulgare* has no ($p > 0.05$) effect on
350 the survival rate. There are similarities between the current
351 study and those investigated by Sotoudeh & Yeganeh (2017). The
352 finding of the research is also co-related with the work of
353 Yilmaz *et al.* (2012). His research investigated that dietary
354 thyme improved the growth performance of *Dicentrarchus labrax*.

355 Water parameters recorded during the experiment were temperature,
356 alkalinity, dissolved oxygen, TDS, TSS, and EC. Water
357 temperature recorded during the experiment was range from 24°C
358 to 30°C.

359 Environmental parameter, the temperature was accordingly to the
360 work of Dawes, (1991). The results of this study are congruous
361 with the finding of Yilmaz *et al.*, (2012). They evaluated that a
362 dietary cumin level of 1% provides the best survival rate for
363 tilapia, *Oreochromis mossambicus*, with no inauspicious effect on
364 growth performance simultaneously.

365 *Tribulus Terrestris* showed the same results with a significant
366 difference ($P < 0.05$) in weight gain (Yeganeh *et al.*, 2017). The
367 present investigation showed similar findings as *Thymus*
368 *vulgaris*, *Rosmarinus officinalis*, and *Trigonella foenum graecum*
369 had a positive response towards the growth performance of
370 *Dicentrarchus labrax* (Yilmaz *et al.*, 2010).

371 After the chemical analysis of *Foeniculum vulgare* seed extract
372 resulted that *Foeniculum vulgare* extract contains the trans-

373 anethole (64.49%) which is similar in structure to 17-beta-
374 estradiol.

375 The presence of trans-anethole may cause a significant increase in
376 estrogenic hormones; lead to an increase in reproductive
377 performance and fecundity (Albert-Puleo, 1980). An increase in
378 the reproduction activities of *Poeciliareticulata* fed feed
379 containing *F. vulgare* seed extract, a reason for increasing the
380 level of estrogenic hormones lead to increase the reproductive
381 performance. A similar result was reported by Nazari and
382 Roozbehani, (2015). Their finding showed that the fertility
383 rate of *Poeciliareticulata* enhanced when used *Foeniculum vulgare*
384 extract in the diet.

385 Among dietary treatments used in this research, T₂ show positive
386 increase in GSI (8.69 ± 0.2), Fecundity (9.4 ± 0.19), and
387 hatching rate. Dada and Adeparusi (2012) intimate that diets
388 with *Sesamum indicum* supplement and seed powder of *Croton*
389 *zambesicus* were improved female *C. gariepinus* GSI.

390 Dada and Ajilore (2009) distinguished an increase in egg diameter
391 and fecundity of *C. gariepinus* when treated with *Garcinia* seeds
392 extract. Sadeghpour *et al.* (2015) commented that increase in
393 serum level estrogen in mice female when injected with extracts
394 of *Foeniculum vulgare*.

395 The findings of another research are also consistent with this
396 current research, fecundity of guppy was directly proportional
397 to the bodyweight of the fish, which means that the fecundity
398 increased with the increase in body weight (Shahjahan *et al.*,

Comment [MM6]: Reference missing; Please
check all references

399 2013). The use of injection of ovaprim shown the highest
400 fecundity of *Pterophyllus scalare* suggested by Chatterjee et al.,
401 (2013).

402 Ghosh et al. (2007) reported that incorporation of probiotics in
403 feed influenced the reproductive performance of livebearers in
404 terms of high fecundity, high Gonadosomatic index, high fry
405 survival rate, reduction in fry mortality and deformity, and
406 higher average weight and length gain of fish fry.

407 Nielsen and Baatrup, (2006) treated *Poeciliareticulata* with
408 estrogens that enter into the aquatic system, results of this
409 research showed that no significant difference was seen in the
410 Gonadosomatic index (GSI).

411 A similar observation was found with *Cyprinus carpio* when treated
412 with *Ferula coskunii* (Yilmaz et al., 2006). Current research
413 finding indicated that *Poeciliareticulata* treated with
414 *Foeniculum vulgare* seed extract, increased number of young's
415 ones produced by guppy fish in T₂ were 91±2.1 while the control
416 group produced 45±2.5. Dada (2012) investigated that feed
417 supplement with *G. kola* seed powder improved hatchability and
418 fecundity of *C. gariepinus*.

419 Successful spawning of *C. punctatus* at 0.3 and 0.5 ml/kg and
420 3000 IU/kg weight of HCG was noted by Kather and Sridhar (2002).
421 For *H. fossilis* the utilization of ovaprim came about greatest
422 Successful body spawning (Chatterjee et al., 2013). Effective
423 spawning of *C. punctatus* is when treated with HCG. *Natrum*

424 *huriaticum* showed a positive effect on the spawning performance
425 of *Poeciliareticulata*(Sudha and Gokula, 2018).

426 *The findings* of previous research are similar to the results of
427 the current investigation. There are also similarities between
428 the work of (Sudha and Gokula, 2015) when *Puntiusconchoni*
429 treated with similar herbal medicine *Natrum muriaticum* on
430 spawning response.

431

432

433 4. CONCLUSION

434

435 • The present study investigated that *F. vulgare* seeds
436 extract revealing a significant influence on the growth
437 performance of *Poeciliareticulata*.

438 • Under the report of the current investigation, *F. vulgare*
439 seed extract demonstrates a significant beneficial
440 impact on reproductive parameters.

441 • Increasing the level of estrogen hormones in fish fed with
442 *F. vulgare* ethanol extract, due to the presence of trans-
443 anethole, reproduction activities and fecundity of the
444 fish was increased.

445 • Considering, fennel seed extract within the diet has a
446 significant increase in the Gonad's initial maturity and
447 Gonadosomatic index increase is directly proportional to
448 a female's body size.

449 • As the gonad's weight increases, both relative fecundity
450 and absolute fecundity also increase the number of eggs.

451 • It is suggested that herbal extracts, which contain
452 estrogenic compounds as in *Foeniculum vulgare* can be used as
453 a supplement in fish diets.

454 • In the future, the use of plant extracts in aquaculture will
455 be an effective tool to attain durable and economically safe
456 fish production.

457

458 REFERENCES

459

460 Coad, B. W. (2017). Review of the livebearer fishes of Iran (Family: Poeciliidae).
461 Iranian Journal of Ichthyology, 4(4), 305-330.

462 Dada, A. A. (2012). Effect of Garcinia kola seed meal on egg quality of the North
463 African catfish (*Clarias gariepinus*) (Burchell, 1822) broodstock. African
464 Journal of Food, Agriculture, Nutrition and Development, 12(5), 6447-6459.

465 Egset, C. K., Bolstad, G. H., Rosenqvist, G., Endler, J. A., & Pelabon, C. (2011).
466 Geographical variation in allometry in the guppy (*Poecilia reticulata*). Journal
467 of Evolutionary Biology, 24(12), 2631-2638.

468 Frankic, T., Voljc, M., Salobir, J., & Rezar, V. (2009). Use of herbs and spices and
469 their extracts in animal nutrition. Acta Agric Slov, 94(2), 95-102.

470 Ghosh, S., Sinha, A., & Sahu, C. (2007). Effect of probiotic on reproductive
471 methods by GC/MS. African Journal of Traditional, Complementary
472 and Alternative Medicines, 11(2), 277-279.

473 Nasser, A. K. V. and Rajkumar, U. (2001). Ornamental fish-prospects
474 for culture. Souvenir issued on the Occasion of the
475 inauguration of Visakhapatnam RC of CMFRI, 24-26.

476 Nazari, A., & Roozbehani, S. (2015). Influence of fennel *Foeniculum vulgare*
477 extract on fertility, growth rate and histology of gonads on guppy
478 *Poecilia reticulata* Turkish Journal of Fisheries and Aquatic Sciences, 15(3),
479 457-463.

- 480 Ojima, D., & Iwata, M. (2009). Central administration of growth hormone-
481 releasing hormone triggers downstream movement and schooling behavior of
482 chum salmon (*Oncorhynchus keta*) fry in an artificial stream. *Comparative*
483 *Biochemistry and Physiology Part A: Molecular & Integrative Physiology*,
484 152(3), 293-298.
- 485 Rodríguez, R. D. R. (2006). Ornamental fish: economic, ecologic and health
486 implications of the trade. *JAINA Boletín Informativo*, 16(1), 6-27.
- 487 Salehi, H. (2008). Benefit-cost analysis for fingerling production of
488 kutum *Rutilus frisii kutum* (Kamensky, 1901) in 2005 in Iran. *Aquac. Asia Mag*,
489 13(3), 35.
- 490 Sudha, C., & Gokula, V. (2015). Induced spawning of rosy barb
491 *Puntius conchoni* by *Natrum muriaticum*. *IJAPBC*, 4(4), 801-804
- 492 Yanong, R. P., Martinez, C., & Watson, C. A. (2010). Use of Ovaprim in
493 ornamental fish aquaculture. *EDIS*, 2010(2).
- 494 Yilmaz, E., Genc, M. A., Cek, S., Mazlum, Y., & Genc, E. (2006). Effects of
495 orally administered *Ferula coskunii* (Apiaceae) on growth, body composition
496 and histology of common carp, *Cyprinus carpio*. *Journal of Animal and*
497 *Veterinary Advances*, 5(12), 1236-1238. Yilmaz, S., Ergün, S., & Turk, N.
498 (2012). Effects of cumin-supplemented diets on growth and disease
499 (*Streptococcus iniae*) resistance of tilapia (*Oreochromis mossambicus*). *The*
500 *Israeli Journal of Aquaculture – Bamideg*, 64. p5
- 501 Yılmaz, S., Sebahattin, E., & Celik, E. (2010). Effects of herbal supplements on
502 growth performance of sea bass (*Dicentrarchus labrax*): Change in body
503 composition and some blood parameters. *Energy (kJ/g)*, 1(3), 217-222

504
505
506

APPENDIX

Comment [MM7]: Appendix missing