Evaluation of polanga (*Calophyllum inophyllum*) oil cake as a non-conventional ingredient in *Labeo rohita* (Hamilton, 1822) fingerling feed

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ABSTRACT

In the present study, polanga (*Calophyllum inophyllum*) oil cake (POC) was evaluated as a non-conventional ingredient in *Labeo rohita* (Hamilton, 1822) fingerling feed. Proximate composition and fatty acid analysis of POC indicated that it can be a good source of protein (24.2%) and energy (4.4 kcal g⁻¹). Fatty acid analysis indicated that it is dominated by monoenes (39%) followed by saturates (28.42) and polyunsaturated fatty acids (PUFA) n-6 (27.76%). Further, phenol, tannin and flavonoids were detected as prominent secondary metabolites. Five iso-nitrogenous (25-26%, crude protein) feeds incorporated with POC @ 0% (F1), 10% (F2), 20% (F3), 30% (F4) and 40% (F5) level were fed to *L. rohita* fingerlings for 90 days in indoor conditions. At the end of the experiment, it was observed that survival percentage (95-97%) of F1, F2 and F3 groups was statistically similar which was significantly higher ($p < 0.05$) compared to F4 and F5. The gain in weight and specific growth rate (SGR) of F1 and F2 were similar but next higher inclusion levels of POC significantly ($p < 0.05$) reduced the growth in F3, F4 and F5 groups. In addition, feed conversion ratio (FCR) was significantly lower ($p < 0.05$) and similar in F1 and F2. Protein efficiency ratio (PER) and net protein utilisation (NPU) were found significantly higher ($p < 0.05$) in F1 and F2 as compared to the rest of the groups. Haemoglobin, serum protein, albumin and globulin in blood were similar in F1 and F2 ($p > 0.05$), which were significantly higher than the levels in other groups ($p < 0.05$). Serum glutamic-oxaloacetic transaminase (SGOT) and glucose levels increased significantly in F3, F4 and F5 groups compared to F1 and F2. POC was used for the first time in carp feeds as a non-conventional feed ingredient during this study and the results clearly indicated that POC could be incorporated @ 100 g kg⁻¹ in the feed of *L. rohita* fingerlings without any adverse effect on growth, survival and nutrient utilisation.

Keywords: *Calophyllum inophyllum*, Feed, *Labeo rohita*, Polanga oil cake

Introduction

Aquaculture in pond needs suitable supplementary feed for sustainable production. Feed constitutes more than 60% of total operational cost in aquaculture (Mohanty *et al.*, 2011). The traditional ingredients used in aquafeeds are getting costlier and competitive as being used for different animal production systems. Therefore, inclusion of newer non-conventional feed ingredients could help to develop cost effective feeds. Fish need more protein in their diet than land animals and so aquafeed requires protein rich ingredients (Hasan *et al.* 2007). Plant proteins are used as sole dietary protein for carps. Soybean meal and groundnut oil cakes are the major plant proteins used in compounded aquafeeds followed by other oil cakes (Barman and Karim, 2007; Manomaitis, 2009). It is therefore suggested to explore newer non-conventional plant feed resources for formulating cost effective carp feeds (FAO, 2010).

Alexanderian laurel (*Calophyllum inophyllum*), commonly known as Indian doomba or polanga, belongs to

Clusiaceae (or Guttiferae) family. It is an evergreen tree found in the tropical coastal climate and is often planted under the avenue plantation and afforestation programmes. The fruit bears a seed inside a corky shell covering. The oil recovery is around 50% from the seed and rest is cake (Mohanty *et al.*, 2013). Polanga oil cake (POC) is not yet rationally used as feed ingredient. Scanty information is available on the use of this oil cake in the feed for poultry (Behura *et al.*, 1987; Dash *et al.*, 1990; Sahu *et al.*, 1993; Mohapatra and Samal, 2002; Mishra *et al.*, 2005). However, no literature is available on the use of POC as fish feed ingredient. Therefore, the objective of the present study was to generate information on nutritive values, anti-nutritional factors, acceptability and optimum level of incorporation of raw polanga oil cake in carp feed.

Materials and methods

Feed, fish and experimental design

POC from the local oil mill was collected and analysed for its proximate composition in National Fish Feed Testing Laboratory at ICAR-Central Institute of Freshwater

Aquaculture (ICAR-CIFA), Bhubaneswar, India. In addition, the POC was subjected to qualitative detection of secondary metabolites *viz.*, tannin, phenol, alkaloids, flavonoids, saponins and glycosides. Five isonitrogenous (25-26% CP) feeds (F1-F5) were formulated incorporating raw POC meal at 0% (F1), 10% (F2), 20% (F3), 30% (F4) and 40% (F5) levels with groundnut oil cake, rice bran, wheat flour and vitamin and mineral premix (Supplevite-M, Jeco Vet Chem Pvt. Ltd, India) as co-ingredients (Table 1). Feed pellets of 2 mm dia were made with portable mechanical pelletiser and oven dried at 60°C. Pellets were crumbled to 0.5 mm size and stored in air tight plastic containers.

Proximate composition and fatty acid analysis

Proximate composition of POC, experimental diets and whole body carcass of the fish was determined according to AOAC (1990). Fiber content of the feed was determined using fiber tech and gross energy was determined by bomb calorimeter (IKA Calorimeter system, C5000 control). In addition, fatty acid composition was analysed for POC, feeds and fish whole body carcass samples. Lipids from the sample were extracted by adding (2:1v/v) chloroform-methanol mixture containing 0.01% butylated hydroxytoluene (BHT) (Folch *et al.*, 1957). The weight of lipids was determined

Table 1. Chemical composition of polanga (*Calophyllum inophyllum*) oil cake (% dry matter, DM basis)

Nutrients					
Moisture	Crude protein	Crude lipid	Crude fiber	Ash	Nitrogen free extract (NFE)
70	24.2	9.3	7.6	6.0	52.7
Phytochemicals					
Tannin	Phenol	Alkaloid	Flavonoid	Saponin	Glycoside
+++	+++	+	++	+	+
Fatty acids (%)					
ΣSFA	ΣMUFA	ΣPUFA n-6	ΣPUFA n-3	Total	
28.427	39.357	27.7675	0.3895	95.941	

+ detected, ++ moderately detected, +++ adequately detected

The experiment was conducted at the wet laboratory of the ICAR-CIFA during November 2013 February 2014. Fifteen flow-through cement tanks (300 l), with a flow rate of 0.5 l min⁻¹, were used for rearing *Labeo rohita* (Hamilton, 1822) fingerlings (average 5 g) in triplicates. Each tank was stocked with 22 fingerlings and the experiment was carried out for 90 days. Fish were fed *ad libitum*, twice daily at 09 00 and 16 00 hrs with the test feeds. The leftover feed was siphoned out after 2 h of feeding, dried and weighed to calculate the daily feed intake. Fish were weighed at monthly intervals and routine water quality parameters, *viz.*, temperature, transparency, total alkalinity, hardness un-ionised ammonia, dissolved oxygen and pH were monitored twice a week as per APHA (1989). Proximate composition and fatty acid analysis of POC, test feeds, initial and final carcass were determined. At the end of the experiment, blood was drawn by caudal vein puncture from ten fish sampled from each group. A part of blood was collected in heparinised vial for whole blood haemoglobin assay and rest was allowed to clot in normal vial for serum collection. The serum was separated by centrifugation (5000 rpm, 5 min). Haemoglobin was determined immediately whereas, serum samples were pooled treatment-wise and kept at -20°C for further analysis.

gravimetrically after evaporation of the solvent. Fatty acid methyl esters (FAME) were prepared by acid-catalysed transesterification of total lipids according to the method of Christie (1982). Fatty acid methyl esters were separated by a gas chromatograph equipped with a flame-ionisation detector (Shimadzu GC-2010, Kyoto, Japan) on a DB- 25 capillary column (20 m × 0.10 mm I.D., 0.10 µm J and W Scientific, Santa Clara, CA, USA). The fatty acids were identified using fatty acid methyl ester (FAME) standards. Area percentage normalised values for the fatty acids were taken as weight percentage.

Qualitative analysis of phytochemicals

Qualitative estimation of phytochemicals (alkaloids, sterol, flavanol, flavonoids, phenol, tannin, glycoside and saponin) was done. Extract was prepared with n-hexane, ethyl acetate and methanol and analysed using the standard protocol as described by Harbone (1998) and Evans (2000).

Test for alkaloids: About 0.2 g of dry extracts of the sample was boiled with 5 ml of 2% HCl on a steam bath for 10 min. The mixture was filtered and 1 ml of the filtrate measured into a test tube. Each 1 ml filtrate was treated with 2 drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water). A reddish brown precipitate indicated the presence of alkaloids.

Test for flavonoids: About 0.2 g each of the dry extracts was heated with 10 ml of ethyl acetate in boiling water for 3 min. The mixture was filtered consecutively and the filtrates used for aluminum chloride test, for which 4 ml of the filtrates was shaken with 1 ml of 1% aluminum chloride solution. A yellow precipitate indicated the presence of flavonoids.

Test for glycosides: Legal's test was carried out for detection of glycosides. The extract (0.1 g) was treated with NaOH solution and formation of a pink to red colour solution indicated the presence of glycosides.

Test for saponin: Saponins were determined using the frothing test. About 1 ml of the extract was diluted with 4 ml of distilled water and the mixture was shaken vigorously and then observed on standing for a stable froth.

Test for phenol: About 2 ml aqueous extract was stirred with two drops of solution A [10% ferric chloride (FeCl_3) and 1% ferric cyanide (FeCN_6)] and two drops of solution B [1% ferric chloride and 1% potassium ferric cyanide ($\text{K}_3\text{Fe}_3\text{CN}_6$)] at 110°C . Change in colour of the extract to blue indicated presence of phenol.

Test for tannin: About 2 ml of distilled water and a few drops of FeCl_3 solution were added to the extract. The formation of a green precipitate indicated the presence of tannins.

Test for sterols: Sterols were determined using sulfuric acid test. The fraction of extract was treated with ethanol and H_2SO_4 . Formation of violet blue or green colour indicated the presence of sterols.

Haematological analysis

Blood samples were analysed for haemoglobin (Van Kampen and Zijlstra, 1961). Serum glucose was estimated as per method of Kaplan (1984). The sera samples were also used for determining total protein (Flack and Woollen, 1984), albumin (Doumas, 1971). Globulin was determined by subtracting albumin from total serum protein. In addition, sera samples were also analysed for triglycerides (McGowan *et al.*, 1983), cholesterol (Allain, 1974), high density lipoprotein (HDL) and low density lipoprotein (LDL) (Burtis and Ashwood, 1994). Very low density lipoprotein (VLDL) was calculated by dividing the triglyceride value with 5 and expressed as mg dl^{-1} . Serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) activities were determined using Greiner diagnostic kits (Bahlingen, Germany) (Bergmeyer, 1974).

Data collection and analysis

Fish were weighed at monthly intervals to ascertain the growth parameters. The weight gain (%), specific growth rate

(SGR), food conversion ratio (FCR), protein efficiency ratio (PER), net protein utilisation (NPU) and survival rate (%) were calculated as detailed below:

$$\text{Weight gain (\%)} = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}} \times 100$$

$$\text{SGR} = \frac{\ln \text{Final weight} - \ln \text{Initial weight}}{\text{Days of experiment}} \times 100$$

$$\text{FCR} = \frac{\text{Feed consumed (dry weight)}}{\text{Live weight gain (wet weight)}}$$

$$\text{PER} = \frac{\text{Live weight gain}}{\text{Protein consumed}}$$

$$\text{NPU (\%)} = \frac{\text{Protein gain in carcass}}{\text{Protein intake in food}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Initial no. of larvae} - \text{Final no. of fry}}{\text{Initial no. of larvae}} \times 100$$

Data were analysed using one way ANOVA (Snedecor and Cochran, 1967) and difference between means were tested using Duncan's multiple range test.

Results

The water quality parameters recorded in the experimental tanks were as follows: temperature, $25\text{--}27^\circ\text{C}$; transparency, 25-28 cm; total alkalinity (CaCO_3), $90\text{--}110\text{ mg l}^{-1}$; hardness, $80\text{--}96\text{ mg l}^{-1}$; un-ionised ammonia, $<0.1\text{ mg l}^{-1}$; dissolved oxygen, $5\text{--}7\text{ mg l}^{-1}$ and pH, $7.5\text{--}8.5$. The chemical composition of POC is presented in Table 1. It contained 24% CP and 4.4 kcal g^{-1} energy. Table 2 presents the chemical composition of all the five test feeds (F1-F5).

The feeds were iso-nitrogenous with 25-26% protein. The incorporation of POC in the experimental feeds replaced the groundnut oil cake at 4, 7, 10 and 13 g and rice bran at 6, 13, 20 and 27 g per 100 g of F2, F3, F4 and F5 feeds, respectively. Survival, growth, nutrient utilisation, weight gain and whole body composition of *L. rohita* fingerlings under different test feeds are presented in Tables 3 and 4, respectively. At 0, 10 and 20% inclusion levels of POC (F1- F3), survival rate was significantly ($p<0.05$) higher (95-97%) than that of 30-40% inclusion (F4-F5), where the survival was $<85\%$. The SGR (% growth day^{-1}) was statistically similar in F1 and F2 and significantly higher ($p<0.05$) than other groups. Weight gain (%) was significantly higher ($p<0.05$) in fingerlings fed with F1 and F2 feeds than the other test feeds. FCR was significantly lower ($p<0.05$) in F1 and F2 than rest of the feeds. PER and NPU were also significantly higher ($p<0.05$) in F1 and F2 compared to all other test feeds. The final whole body protein content was alike ($p>0.05$) up to 10% POC inclusion level (F1 to F2) and thereafter, it decreased significantly ($p<0.05$) in F3 to F5 (Table 3).

Table 2. Composition of the test feeds of *Labeo rohita* fingerlings incorporating polanga oil cake at different levels as non-conventional ingredient (% DM basis)

Ingredients	Test feeds of <i>L. rohita</i>				
	F1	F2	F3	F4	F5
POC	0	10	20	30	40
GNOC	37	33	30	27	24
RB	59	53	46	39	32
Vitamin-mineral pre-mix*	2	2	2	2	2
Wheat flour	2	2	2	2	2
<i>Proximate composition</i>					
Crude protein	26.12	25.83	25.79	25.73	25.78
Ether extract	6.67	6.09	6.12	6.33	6.56
Crude fibre	8.43	7.8	8.09	8.5	8.12
Ash	7.86	7.7	7.13	7.45	7.49
Nitrogen free extract	50.92	52.58	52.87	51.99	52.05
<i>Fatty acid composition (%)</i>					
ΣSFA	22.68	23.24	24.95	25.113	25.84
ΣMUFA	49.17	47.41	48.131	47.26	48.62
ΣPUFA n-6	23.96	24.82	24.92	24.86	23.96
ΣPUFA n-3	0.38	0.40	0.55	0.44	0.42
Total	96.20	95.87	98.55	97.66	98.85

*Supplevite - M (Jeco Vet Chem Pvt. Ltd, Mumbai, India) Each 1 kg of Supplevite - M contains: Vitamin A: 200000 IU, Vitamin D₃: 40000 IU, Vitamin B₂: 0.8 g, Vitamin E: 300 IU, Vitamin K: 400 g, Calcium panthionate: 1 g, Nicotinamide: 4 g, Vitamin B₁₂: 2.4 mg, Choline chloride: 60 g, Calcium: 300 g, Manganese: 11 g, Iodine: 0.4 g, Iron: 3. g, Zinc: 6 g, Copper: 0.8 g, Cobalt: 0.18 g.

The data are presented as mean ± SE, Means bearing different superscripts in a row differ significantly (p<0.05)

Table 3. Carcass composition of *Labeo rohita* fingerlings fed different test feeds containing polanga oil cake for 90 days (% DM basis)

Parameters	Initial	Test feeds of <i>L. rohita</i>				
		F1	F2	F3	F4	F5
Proximate composition						
Moisture	79.69±0.07	77.7±0.08 ^{ab}	77.1±0.17 ^a	78.8±0.2 ^{bc}	79.3±0.8 ^c	79.9.0±0.9 ^c
CP	64.86±0.21	66.13±0.03 ^c	67.33±0.61 ^c	66.24±0.21 ^b	66.09±0.11 ^{ab}	65.47±0.03 ^a
EE	9.40±0.06	14.28±0.09 ^c	14.56±0.05 ^c	12.86±0.06 ^b	11.39±0.0 ^a	10.56±0.05 ^a
Ash	23.42±0.18	16.45±0.02 ^{ab}	15.24±0.15 ^a	17.93±0.05 ^b	19.66±0.01 ^c	21.23±0.01 ^d
Fatty acid						
ΣSFA	27.71	26.25	24.33	24.61	27.25	28.58
ΣMUFA	43.70	44.92	43.12	42.38	41.97	40.37
ΣPUFA n-6	15.44	18.12	18.44	18.21	18.04	18.05
ΣPUFA n-3	2.82	4.08	4.40	4.64	5.46	6.94
Total	89.67	93.37	90.29	89.84	92.72	93.93

The data are presented as mean ± SE

Means bearing different superscripts in a row differ significantly (p<0.05)

There was a linear increase in final whole body moisture content in the groups fed with POC containing feeds from 20-40 % incorporation. Reverse trend was observed in case of ether extract contents. The final whole body ash content differed significantly (p<0.05) in all the groups with an increasing trend having increased level of POC incorporation.

Phenol, tannin, flavonoids, alkaloids, glycoside and saponin were detected as phytochemicals in TOC. Among all these phytochemicals, tannin, phenol and flavonoids were found to be more (based on visual estimation) compared to other phytochemicals tested in the present study (Table 1). The fatty acid profile of POC and test feed is given in Table 1 and 2.

Table 4. Survival, growth and nutritional indices of *Labeo rohita* fingerlings fed different levels of polanga oil cake incorporated diets

Parameters	Test feeds of <i>L. rohita</i>				
	F1 (0% POC)	F2 (10% POC)	F3 (20% POC)	F4 (30% POC)	F5 (40% POC)
Survival (%)	95.45±2.62 ^b	96.97±1.52 ^b	95.45±2.62 ^b	84.85±1.52 ^a	77.27±5.25 ^a
Av. int. wt. (g)	5.76	5.53	5.45	5.15	5.23
Wt. gain (g)	1.91±0.18 ^b	2.04±0.14 ^b	0.78±0.14 ^a	0.56±0.10 ^a	0.57±0.24 ^a
Wt. gain (%)	33.40±4.02 ^b	37.12±3.41 ^b	14.32±1.33 ^a	11.07±1.09 ^a	10.88±1.60 ^a
SGR (% day ⁻¹)	0.48±0.05 ^b	0.53±0.04 ^b	0.22±0.04 ^a	0.17±0.03 ^a	0.17±0.07 ^a
FCR	3.62±0.27	3.24±0.19	9.09± 0.84	12.56±1.69	13.80±1.85
PER	1.12±0.08 ^b	1.20±0.05 ^b	0.48±0.08 ^a	0.36±0.06 ^a	0.42±0.16 ^a
NPU (%)	16.7±0.09 ^c	17.8±0.07 ^c	7.53±0.04 ^b	5.15±0.05 ^a	5.28±0.07 ^a

The data are presented as mean ± SE

Means bearing different superscripts in a row differ significantly (p<0.05).

SGR: Specific growth Rate; FCR: Feed conversion ratio; PER: Protein efficiency ratio, NPU: Net protein utilisation

The Σ MUFA and Σ PUFA n-6 content of POC was recorded as 39 and 28%, respectively. Some blood parameters viz., haemoglobin, glucose, serum protein, serum albumin, serum globulin, triglyceride, cholesterol, LDL, HDL, VLDL and serum enzymes like GOT and GPT of the fish grown in all the feeding group were studied. Haemoglobin, serum protein and albumin, cholesterol HDL and LDL content were similar in F1 and F2 but gradually decreased with increasing levels of POC in feed (F3-F5). Triglycerides and VLDL did not show significant variation (p>0.05) among all the feeding groups. In serum GOT and GPT, an increasing trend was observed (p<0.05) with increasing level of POC in the feed (F3-F5). Similar trend was observed for blood glucose level (Table 5).

Discussion

The nutritive value of POC and its varied use has been studied by a few workers (Lim, 2012; Chavan *et al.*, 2013).

Attempts were made to incorporate POC in the feed of poultry to reduce the feed cost (Dash *et al.*, 1990; Mishra *et al.*, 2005). So far, no information is available on the use of POC as feed ingredient for fish particularly in carps (www.feedipedia.org). Crude protein content of the POC as reported by earlier authors varied from 22-27% (Behura *et al.*, 1987; Mohapatra and Samal, 2002). In the present study, the CP and NFE value of POC were 24% and 53%, respectively and therefore, it could be used as a source of protein and energy in the test feeds. Mohanty *et al.* (2011) mentioned that threonine, lysine and tryptophan requirement of *L. rohita* is 4.28, 5.58 and 1.3% of protein, respectively. The amino acid profile of POC was studied by Mohapatra and Samal (2002). They reported that threonine, lysine and tryptophan content of POC are at 0.89, 1.4 and 0.38% of protein, respectively, whereas, same in groundnut oil cake are reported at 1.42, 1.82 and 1.0% of protein, respectively

Table 5. Blood constituents of *Labeo rohita* fingerlings fed different levels of polanga oil cake

Attributes	Feed-1 (0% POC)	Feed-2 (10% POC)	Feed-3 (20% POC)	Feed-4 (30% POC)	Feed-5 (40% POC)
Haemoglobin (g dl ⁻¹)	6.92±0.13 ^b	7.78±0.66 ^b	5.86±0.19 ^a	5.65±0.09 ^a	4.83±0.07 ^a
Glucose (mg dl ⁻¹)	238.1±1 5.7 ^a	241.0± 6.0 ^a	252.8±8.3 ^b	288.9±12.3 ^c	290.5± 6.0 ^c
Protein (g dl ⁻¹)	4.40±0.04 ^c	4.37±0.05 ^c	4.13±0.01 ^b	4.10±0.02 ^b	3.83±0.01 ^a
Albumin (g dl ⁻¹)	1.47±0.01 ^c	1.48±0.01 ^c	1.38±0.01 ^b	1.40±0.01 ^b	1.27±0.01 ^a
Globulin (g dl ⁻¹)	2.94±0.02 ^c	2.89±0.06 ^c	2.84±0.03 ^{bc}	2.75±0.01 ^b	2.56±0.01 ^a
SGOT (u l ⁻¹)	139.87±2.59 ^a	142.22±1.05 ^a	192.77±0.51 ^b	271.23±0.52 ^c	375.58±2.56 ^d
SGPT (u l ⁻¹)	15.91±0.34 ^a	16.36±1.51 ^a	18.94±1.04 ^{ab}	20.71±1.03 ^b	39.58±0.60 ^c
Triglyceride (mg dl ⁻¹)	228.93±9.52 ^a	249.06±5.24 ^a	247.38±4.67 ^a	249.90±6.05 ^a	249.90±2.22 ^a
Cholesterol (mg dl ⁻¹)	182.13±1.67 ^c	182.02±1.56 ^c	177.78±0.78 ^b	172.08±0.87 ^a	170.18±0.19 ^a
HDL (mg dl ⁻¹)	58.08±0.91 ^c	55.84±1.44 ^c	51.84±0.28 ^b	46.32±1.74 ^a	42.8±0.68 ^a
LDL (mg dl ⁻¹)	128.80±0.24 ^b	129.04±0.83 ^b	127.16±1.33 ^b	127.28±1.04 ^b	121.39±2.76 ^a
VLDL (mg dl ⁻¹)	45.79±1.90 ^a	49.81±1.05 ^a	49.48±0.93 ^a	49.98±1.21 ^a	49.98±0.44 ^a

The data are presented as mean ± SE

Means bearing different superscripts in a row differ significantly (p<0.05). SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase, HDL: High-density lipoprotein LDL: Low-density lipoprotein; VLDL: Very low-density lipoprotein

(Babiker, 2012). Therefore, POC incorporation in higher percentage in the test diet certainly reduced the contribution of these essential amino acids in the test feeds. Hence, growth retardation due to increase in POC level may be attributed to the above limiting essential amino acids as one of the factor. The fatty acid contribution of POC and POC containing feed is reflected in the carcass composition. Increasing trend of PUFA in the carcass may be influenced by the level of POC incorporation in feed but certainly it did not contribute to overall growth performance of the fish. Ether extract (EE) of POC was predominantly composed of oleic acid (C18:1) 39%, followed by linoleic acid (C18:2) 27%, stearic acid (18:0) 11% and palmitic acid (C16:0) 15%. Crane *et al.* (2005) revealed the presence of the above fatty acids in *Calophyllum inophyllum* oil in the range of 39-50%, 21-31%, 13-14% and 11-13%, respectively. Σ PUFA n-6 and Σ PUFA n-3 of POC in the present study are found as 28 and 0.4%, respectively. Similar result was also reported by Chavan *et al.* (2013). Phytochemicals *viz.*, tannin, saponin, alkaloids, resins and flavonoids, detected in the present study were in agreement with the findings of other workers (Balogun and Ogunniyi, 2012; Indrakumar *et al.*, 2012). Bhushan *et al.* (1975) reported calaustralin, a 4-phenyl-coumarin from seed of *Calophyllum inophyllum* as a non-fatty toxic constituent. Obviously, calaustralin content of the test feed increased with increased level of POC incorporation. This may be another factor for reducing growth and survival in higher POC incorporated feed groups. Mukhopadhyay and Ray (1999) opined that tannin in higher concentrations reduce feed intake and affect protein and carbohydrate digestibility, though beneficial in lower concentration in fish feed. Francis *et al.* (2001) further clarified that tannin at higher concentration affects protein utilisation, because it binds to lysine and makes this essential amino acid unavailable to monogastric animals and fish. The fish feed containing higher level of alkaloids reduces the feed intake due to its anti-palatability property (Glencross *et al.*, 2006). Feed intake, FCR, weight gain% and SGR were similar in 0 and 10% incorporation of POC. However, feed intake and all the growth parameters reduced with increasing levels of POC incorporation in feed. There is no published literature available in fish to compare the present findings but incorporation of POC in poultry feed resulted in growth depression at more than 5% inclusion levels (Behura *et al.*, 1987; Dash *et al.*, 1990). Bhehura *et al.* (1987) reported that inclusion of POC at 10 and 15% level caused growth depression in chicks but it did not have any remarkable changes in haematological and biochemical parameters of blood or any pathological changes observed in any of the vital organs. Mishra *et al.* (2005) observed that inclusion of POC at 5% level in the diet of RIR chicks had no adverse effect on the growth performance and could partially replace the conventional protein sources. In the present study,

the changes in hematological parameters *viz.*, haemoglobin, serum protein, serum albumin, serum globulin, triglycerides and cholesterol were non-significant in F1 and F2 groups ($p>0.05$), but reduced significantly ($p<0.05$) with increased levels of POC in the feeds (20-40%). Lower blood glucose level was observed in F1 and F2 groups which increased significantly at higher incorporation levels of POC in feeds (F3-F5). Das *et al.* (2004) observed that blood glucose level of *L. rohita* fingerlings increased when they were exposed to stress factors. In the present study, the blood glucose level of the fingerlings increased significantly in the groups F3-F5, may be due to the physiological stress. Serum GOT and GPT are two enzymes considered as biological indicators of hepatic health. Hegab *et al.* (1993) opined that in hepatic toxicity, these enzymes are increased and liberated into blood. In the present study, increased levels of serum GOT and GPT recorded in F3-F5 fed groups could be attributed to the effect of higher inclusion of POC in the feeds. Hepatic toxicity and increased levels of GOT and GPT due to chemical pollutants are also reported in carps by Parvathi *et al.* (2011) and Chavan and Muley (2014).

Efforts are being made to replace the conventional protein sources by non-conventional ones in fish diets. Hossain *et al.* (2001) added 10% of *Sesbania aculeata* seed meal to the diet of common carp and tilapia as non-conventional ingredient without compromising growth. Similar result was reported by Aanyu *et al.* (2012) in tilapia with cotton seed cake incorporation at 20% level. Rath *et al.* (2014) replaced groundnut oil cake and rice bran at 150 g each with incorporation of 300 g rain tree pod meal kg^{-1} feed in *L. rohita* larval diet. Presently, inclusion of 100 g of raw POC as non-conventional ingredient could replace 40 g of groundnut oil cake and 60 g of rice bran kg^{-1} feed without compromising the survival and growth of *L. rohita* fingerlings, fed for 90 days. Moreover, POC is considered as a low-priced oil cake than groundnut oil cake and rice bran. However, the present study revealed that there is scope to use 100 g raw POC kg^{-1} feed for *L. rohita* fingerlings without compromising survival and growth. However, more detailed studies are required to fill the gaps in knowledge for the possible detoxification to increase the incorporation rate (Abbeddou and Makkar, 2012).

Raw POC could be used as non-conventional plant protein source up to 10% level in the feed of *L. rohita* fingerlings without any adverse effect on its growth and survival and could partially replace the conventionally used costly groundnut oil cake and rice bran. The incorporation level may further be increased by suitable processing of POC. POC as a non-conventional feed ingredient in carp feed is being reported for first time in this present study.

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