FINAL REPORT

of the work done on

MAJOR RESEARCH PROJECT

"Standardization of Culture Technology of Duckweed (*Lemna* spp.) and its Utilization as Feed in Carp Poly-Culture System"

(Ref. No.: F. No. 41-70/2012 - SR)

Submitted to

University Grant Commission

New Delhi

Submitted by

Dr.VaneetInder Kaur, Principal Investigator

Dr.Meera D. Ansal, Co-Investigator

Department of Aquaculture, College of Fisheries

Guru Angad Dev Veterinary and Animal Sciences University

Ludhiana – 141004 (Punjab), India

2016

ज्ञान-विज्ञान विमुक्तये

"Standardization of culture technology of duckweed (*Lemna* spp.) and its utilization as feed in carp poly-culture system"

<u>Background of the project</u>

Supplementary feed is one of the key input in fish culture for elevating production, constituting more than 60 % of the input cost. Considering the ever increasing cost of conventional feed ingredients (rice bran, oiled seed cakes, fish meal etc.) and competition with other livestock for the same, it is vital to develop cost effective feeds by substituting the costly conventional fish feed ingredients with some cost effective locally available nutrient rich non-conventional feed resources (NCFR). Among various NCFR, aquatic plants constitute an important resource owing to high nutritive value and digestibility. In the category of aquatic plants, duckweeds has been proved to be the most promising due to their superior nutritive value and exceptionally fast growth rate (Iqbal 1999; Dhawan*et al.,* 2004; Ansal *et al.,* 2008; Ansal and Dhawan 2009; Dhawan and Sharma 2008; Kaur *et al.,*2012, Singh *et al.,* 2012, 2013).

Major duckweed species

Lemna minor

Lemna gibba

Duckweeds are small (1-15 cm) free floating aquatic plants with worldwide distribution. They belong to family Lamnacae and are widely available under five genera i.e. *Lemna, Spirodela, Landoltia, Wolffia* and *Wolfiella* having 37 species (FAO, 2009). Among these four genera, *Lemna* is the largest group of family Lamnaceae and *L. minor* is found to be the most promising one (Mandal *et al.,* 2010). Growth rate of duckweeds is faster than any other higher plant and more closely resemble the exponential growth of unicellular algae. Biomass of duckweeds get doubled in 16 hrs to 2 days under ideal conditions of nutrient availability (NH_3-N = 7-12 mg/l, PO_4-P = 4-8 mg/l), temperature (15 - 30^0C), pH (6.5 to 8.0) and sunlight (Iqbal 1999). Nutrient content in duckweeds vary with the conditions in which they grow (FAO, 2009) and crude protein in duckweeds has been found to vary between 15 - 45 % on dry matter (DM) basis (Ahamad *et al.,* 2003; Effiong *et al.,* 2009). Duckweeds also have better array of essential amino acids than major plant proteins and more closely resembles animal protein (Hillman and Culley, 1978). Further, its amino acid spectrum with regard to lysine (7.5 % of total protein) and methionine (2.6 % of total protein) is much higher as compared to other commonly used plant feed resources (Mishra, 2007), except soybean. Duckweeds also have high levels of vitamin A and pigments particularly beta-carotene and xanthophylls. Duckweeds contain 92-94 % of moisture and harvested biomass can be easily sundried within a period of 24-48 hrs during dry hot summer months. All the duckweeds can be grown on naturally occurring nutrient enriched water (sewage effluents, domestic waste etc.) or manured water, with an average annual DM yield of 10-20 tonnes/ha.

Because of exceptionally fast multiplication rate and excellent nutritional profile of duckweeds, a number of studies have been carried out to produce (FAO, 2009) and exploit duckweed biomass (fresh/dried) as livestock feed, including fish (Leng *et al.,* 1995; Saha *et al.,* 1999, Bairagi *et al.,* 2002, Effiong *et al.,* 2009) in laboratory or field conditions. Being an aquatic species, duckweeds have ample scope of application in aquaculture nutrition. Hence, it is vital to standardize technologies for production of protein rich duckweed biomass for utilization as feed or feed ingredient for fish, under region specific conditions.

In view of the above discussion, the project was undertaken with the aim to standardize the culture technology for two important duckweed species i.e. *Lemna minor* and *Lemna gibba* under local climatic conditions of Punjab and its utilization as one of the feed ingredient in semi-intensive carp polyculture system.

Objectives and targets achieved

Objectives	First year targets	Milestone achieved
• To standardize the culture technique for culture of *L. minor* and *L. gibba*	• Developing package and practice for duckweed (*L. minor* and *L. gibba*) culture • Nutritive value analysis of duckweeds reared under different culture conditions with respect to manuring / fertilization strategies	• Successful culture of two duckweed species *L. minor* and *L. gibba* was carried out with different combinations of organic manures and inorganic fertilizers • Best manure/fertilizer doze worked out with respect to biomass and protein content w. r. t. *L. minor* and *L. gibba* • Out of two duckweed species *L. minor* was found more suitable for culture throughout the year under local climatic conditions w. r. to growth response and productivity
• To find the appropriate incorporation level of duckweed species in supplementary carp feed.	**Second year targets**	
	• Formulation of cost effective nutritionally balanced *Lemna* incorporated diet for carps.	• Both the duckweed species can be incorporated in basal carp diet @ 10 % without compromising fish growth
• To study the economics of duckweed culture and duckweed incorporated diets • To disseminate the technology to farmers for developing cost effectives feeds for carp polyculture system.	**Third year targets**	
	• Formulating cost effective nutritionally balanced (maximum growth and improved flesh composition) *Lemna* incorporated diet for carps in semi-intensive poly culture system • Economics evaluation for duckweed culture and comparative economics of traditional fish feed and *Lemna* incorporated feeds. • Dissemination of culture technology of duckweeds and its incorporation in fish feed through demonstration / lectures to farmers	• *L. minor* incorporated in basal carp diet @ 10 % without compromising fish growth with improved flesh composition in semi-intensive culture system • Sun dried *Lemna* can be incorporated in fingerling diet for higher economic returns in terms of both feed cost reduction from 7 to 27.7% (up to 40% incorporation level) and 20% higher fish growth (at 10% incorporation level). • Demonstration of technology during Pashu Palan Mela of GADVASU during March and September (2014 & 2015) • Technology disseminated through lectures (15), delivered under trainings on fish farming – persons benefited – 190

Detailed Report of the Work Done under the Project

First Year

Objective I

To standardize the culture technique for culture of *L. minor* and *L. gibba*

(September 2012 – January 2014)

Targets

- Developing package and practice for duckweed (*L. minor* and *L. gibba*) culture
- Nutritive value analysis of duckweeds reared under different culture conditions with respect to manuring / fertilization strategies

The above targets were achieved by undertaking following work plan

I – Collection and maintenance of stock of *L. minor* and *L. gibba*

a. Collection of duckweed species (Sept., 2012 – Oct., 2012)

Two species of duckweed (*L. minor* and *L. gibba*) were collected from different natural/manmade aquatic resources (like wetlands, village ponds and road side ditches) from different districts of the State, for maintaining culture stocks for experimental purpose.

Collection of duckweeds from road side ditches/ village ponds

b. Maintenance of stock of *L. minor* and *L. gibba* in earthen pits (Oct. 2012-Dec. 2012)

Methodology

- Stock of *L. minor* and *L. gibba* was maintained in poly sheet lined earthen pits

Construction and maintenance of pits

  - Size of pits- 6 m^2 of 0.3 m depth (3 for each species)
  - Pits were lined with Silpaulin sheet to check the seepage of water and nutrient loss & covered with green net (75% shade) to protect the stock from direct sunlight/extreme high temperature during summers and frost/freezing temperature during winters.

- 2-3 cm thick soil bed was spread over the sheet to hasten the detritus food chain for decomposition of manures.
- Manuring was done with slurry of cow dung (CD) and poultry droppings (PD) (1:1) @ 1 kg/m^2. Slurry was spread evenly over the soil bed and pits were filled with water.
- After 5-7 days of mauring, duckweeds were stocked in the pits @ 125-150 g /m^{2}to cover half of the water surface in the pit.

Preparation of pits for duckweeds culture

Silpaulin lined pit ready for duckweed inoculation

Harvesting of duckweed biomass maintained in earthen pits

- Half of the duckweed biomass was harvested every time it covered the whole water surface in the pit.
- Harvested biomass was sundried and stored in air tight containers.

- Manuring/fertilization was done at 10 days interval with CD:PD slurry (1:1) @ 1 kg/m^2 to provide nutrient for sustained production of duckweeds.
- 25-30% of water in the pits was exchanged every fortnight to check duckweed growth suppression due to excess accumulation of nutrients.

II. Culture of *L. minor* and *L. gibba* in plastic tubs (Feb., 2013-Jan., 2014) to develop package and practice of duckweed culture

Methodology

- Duckweed culture experiments were carried out in 70 litre capacity plastic tubs with surface area – 0.228 m^2, under a green transparent fibre sheet shed
- 24 treatments (in triplicate) for each species **(Table -1)** were selected to assess the efficacy of different organic and inorganic fertilizer w.r.t productivity and nutritive value of duckweeds
  - **Preparation of culture tubs-** Each tub was filled with 50 litres of water after providing a 2 cm soil layer
  - **Pre-stocking mauring/fertilization-** In each treatment, selected organic manure/inorganic fertilizer was added (**Table 1**) to provide the required nutrients for growth of duckweed.
  - **Inoculation of duckweed-** After 1 week of manuring/fertilization, duckweed was inoculated in all the treatments @ 75g (which covered half of the water surface)
  - **Post stocking manuring/fertilization-**It was done at weekly intervals to provide the required nutrients to support sustained growth of duckweeds
  - **Growth of duckweed-** duckweed was allowed to grow in each treatment till it covered the whole water surface

Culture experiment of *Lemna minor* and *Lemna gibba* with different organic and inorganic fertilizers in Plastic tubs

Hravested duckweed biomass

Table 1. Details of treatments* for culture of two duckweed species

L. minor culture			*L. gibba* culture		
Treatment	**Fertilizer**	**Doze (Kg/ha/week)**	**Treatment**	**Fertilizer**	**Doze (Kg/ha/ week)**
T1	CD**	500	**T25**	CD	500
T2	CD	600	**T26**	CD	600
T3	CD	700	**T27**	CD	700
T4	CD	800	**T28**	CD	800
T5	CD	900	**T29**	CD	900
T6	CD	1000	**T30**	CD	1000
T7	PD***	500	**T31**	PD	500
T8	PD	600	**T32**	PD	600
T9	PD	700	**T33**	PD	700
T10	PD	800	**T34**	PD	800
T11	PD	900	**T35**	PD	900
T12	PD	1000	**T36**	PD	1000
T13	CD:PD (1:1)	500	**T37**	CD:PD (1:1)	500
T14	CD:PD (1:1)	600	**T38**	CD:PD (1:1)	600
T15	CD:PD (1:1)	700	**T39**	CD:PD (1:1)	700
T16	CD:PD (1:1)	800	**T40**	CD:PD (1:1)	800
T17	CD:PD (1:1)	900	**T41**	CD:PD (1:1)	900
T18	CD:PD (1:1)	1000	**T42**	CD:PD (1:1)	1000
T19	Urea + TSP****	10 + 2.0	**T43**	Urea + TSP	10 + 2.0
T20	Urea + TSP	12 + 2.4	**T44**	Urea + TSP	12 + 2.4
T21	Urea + TSP	14 + 2.8	**T45**	Urea + TSP	14 + 2.8
T22	Urea + TSP	16 + 3.2	**T46**	Urea + TSP	16 + 3.2
T23	Urea + TSP	18 + 3.6	**T47**	Urea + TSP	18 + 3.6
T24	Urea + TSP	20 + 4.0	**T48**	Urea + TSP	20 4.0

***Number of treatments: 24 for each species of duckweed (*L. minor & L. gibba*)**

No. of replications/treatment: 3

****CD – Cow dung; ***PD – Poultry droppings; **** TSP – Triple Super Phosphate**

Observations Recorded

(i) Water quality analysis

- Water quality in each treatment was analysed w.r.t. temperature, pH, hardness, total alkalinity, ammonical nitrogen, ortho-phosphate and nitrate nitrogen at fortnight intervals, following standard methods of APHA (2005).

(ii) Duckweed biomass -Harvesting & Storage

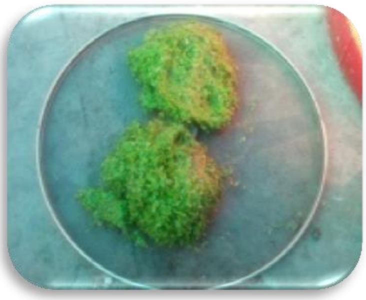
- Half of the duckweed biomass was harvested from each treatment every time it covered the whole water surface.
- The harvested biomass (wet) from all the treatments was weighed and sundried.
- For each treatment, all the harvestings of one month were pooled and stored separately, in airtight poly bags for nutritive value (proximate composition) estimation.

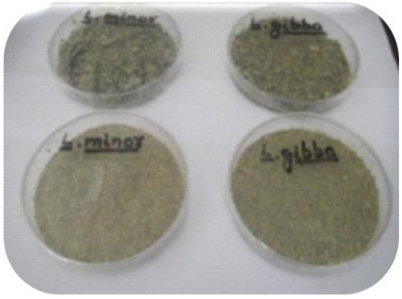
Sun drying of harvested *Lemna* biomass

Sun dried *Lemna* biomass

III. Nutritive value analysis of duckweeds, *L. minor* and *L. gibba* (Nov. 2013 to April 2014)

- ✓ Nutritive value (proximate composition estimation) of duckweed biomass harvested from different treatments (*L. minor* – T1-T24, *L. gibba* - T25- T48) was estimated with respect to crude protein, crude fat (ether extract), crude fibre and ash as per standard methods of AOAC (2000).





Fully grown duckweed in experimental tubs **Fresh *Lemna* biomass** **Sun dried *Lemna* powder for proximate analysis**

<u>Results</u>

Results of duckweed culture (Feb 2013 to Jan. 2014) are presented below

1. **<u>Culture of duckweeds</u>**
   - ✓ Duckweed growth and biomass productions (number of harvestings) varied with
     - Type of manure/fertilizer
     - Dose of manure/fertilizer
     - Season/Month
   - ✓ Higher duckweed biomass production achieved with organic manures as compared to inorganic fertilizers.

2. <u>Number of harvestings/month - (Feb. 2013 to Jan. 2014)</u>

***L. minor* - (Table 2)**

- ✓ During the period of one year, highest number of harvestings (30) was recorded in case of PD treatments followed by CD:PD (29), CD (28) and urea+TSP (16) treatments.
- ✓ Maximum 5 no. of harvestings were recorded in the month of March in all the PD treatments T7-T12.
- ✓ Overall results however revealed higher ***L. minor*** biomass production, in terms of no. of harvestings, during the months of March, May, July, August & September
- ✓ Although growth of ***L. minor*** declined with onset of winters, but the stock survived under low temperature conditions
- ✓ Inorganic fertilizers (urea+TSP) could not support optimum duckweed growth as compared to organic manures (CD, PD & CD:PD)

Table -2 Numbers of harvestings of *L. minor* during different months of the culture period

Treatments	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Total (No.)
	2013											2014	
T1 – T6 **CD/treatments**	2	**3**	1	**3**	1	**4**	**3**	**4**	1	2	1	2	**28**
T7 – T12 **PD/treatments**	2	**5**	2	**4**	1	**3**	**3**	**4**	1	2	1	2	**30**
T13 –T18 **CD:PD/ treatments**	2	**4**	3	**4**	1	**4**	**3**	**4**	1	1	1	1	**29**
T19 – 24 **Urea+TSP/ treatment**	2	0	1	2	1	2	2	**4**	1	1	0	0	**16**

***L. gibba* - (Table-3)**

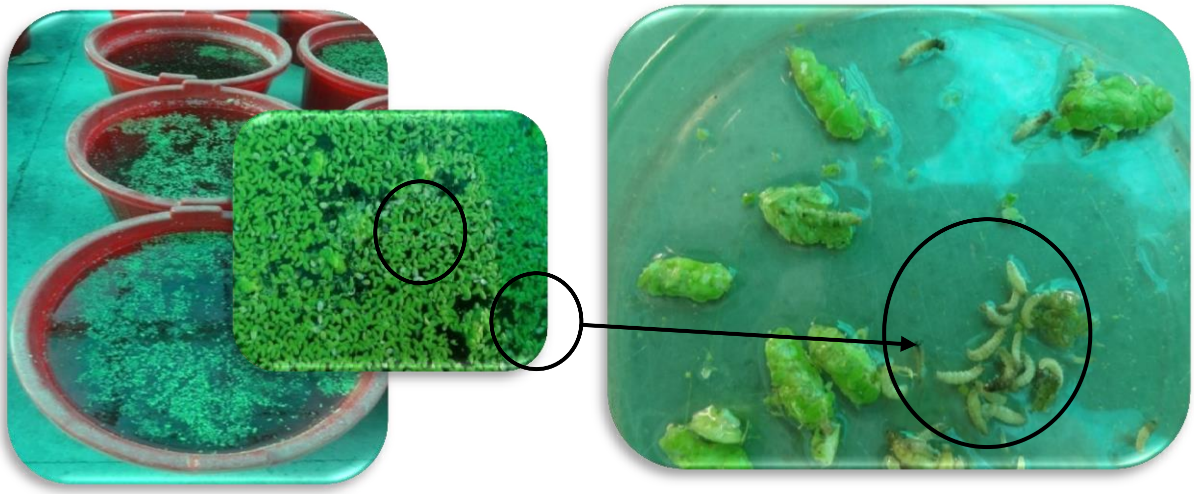
- ✓ During the culture period of one year, highest number of harvestings (17) was recorded in PD treatment followed by CD:PD (16), CD (14) and Urea+TSP (13) treatments.
- ✓ Maximum 5 no. of harvestings were recorded in the month of March in all the PD & CD + PD (1:1) treatments T31-T36 & T37-T42
- ✓ Overall results however revealed higher ***L. gibba*** biomass production, in terms of no. of harvestings, during the months of February, March & July
- ✓ However, *L. gibba* did not grow well in any of the treatments during the post monsoon months and all the stock vanished completely with the onset of winters

Table 3 Numbers of harvestings of *L. gibba*during different months of the culture period

Treatments	Feb	Mar	Apr	May*	June	July	Aug	Sept	Oct **	Nov **	Dec **	Jan **	Total (No.)
	2013											2014	
T25 – T30 **CD/treatments**	**4**	**3**	1	0	1	**3**	1	1	0	0	0	0	**14**
T31 – T36 **PD/treatments**	**4**	**5**	2	0	1	**3**	1	1	0	0	0	0	**17**
T37 – 42 **CD:PD/ treatments**	**4**	**5**	1	0	1	**3**	1	1	0	0	0	0	**16**
T43 – 48 **Urea+TSP treatment**	**3**	**3**	1	0	1	**3**	1	1	0	0	0	0	**13**

* Stock vanished due to attack of *Nimphula* insect larvae (Order Lepidoptera, family Pyralidae)

** Stock of *L. gibba* did not grow well during October and vanished completely with the onset of winters in October and could not be revived under local climatic conditions during Nov., Dec., 2013 & Jan. 2014

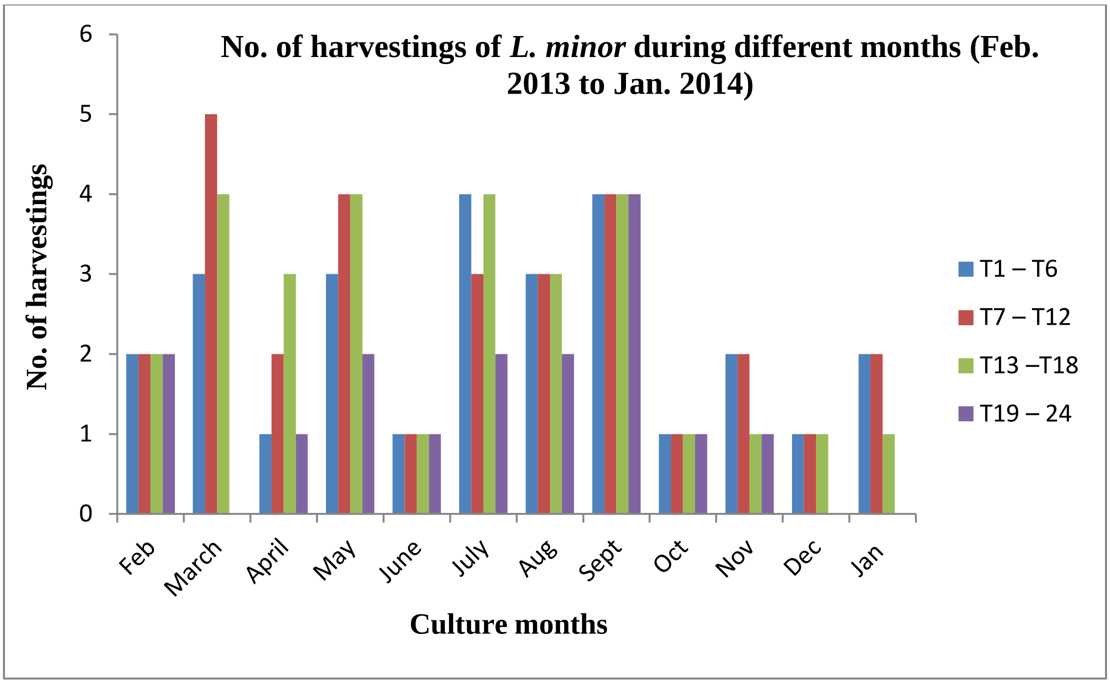


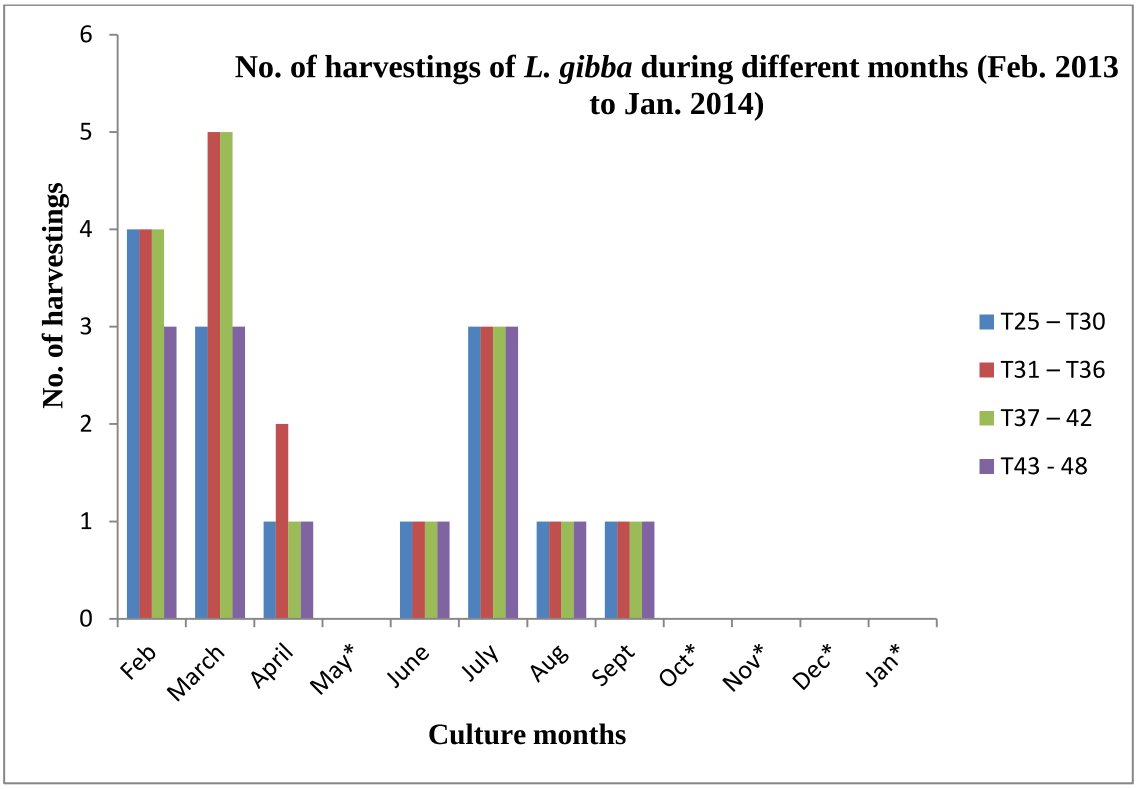
Attack of Nymphula larvae on *Lemna* spp during the month of May & June 2013

. ***L. minor* Vs. *L. gibba***

Parameters	***L. minor***	***L. gibba***
Highest No. of harvestings / Yr.	30	17
Month with maximum harvest	March (05)	March (05)
High yielding months	March, May, July, August, September	February, March, July
Winter tolerance	Yes	No
Best treatment in terms of No. of harvests/Yr.	PD(30)>CD:PD(29)>CD (28)>Urea+TSP (16)	PD(17)>CD:PD(16)>CD (14)>Urea+TSP (13)

Hence, out of two duckweed species ***L. minor*** found **more suitable species** w. r. t. growth response and number of harvestings under local climatic conditions throughout the year.


No. of harvestings of L. minor during different months (Feb. 2013 to Jan. 2014)
No. of harvestings
Culture months
Feb
March
April
May
June
July
Aug
Sept
Oct
Nov
Dec
Jan
T1 – T6
T7 – T12
T13 –T18
T19 – 24


No. of harvestings of L. gibba during different months (Feb. 2013 to Jan. 2014)
No. of harvestings
Culture months
Feb
March
April
May*
June
July
Aug
Sept
Oct*
Nov*
Dec*
Jan*
T25 – T30
T31 – T36
T37 – 42
T43 - 48

***No harvest**

3. Duckweed biomass production- (Feb. 2013 to Jan. 2014)

L. minor – (Table 4 & 6)

Both PD & CD + PD treatments supported higher wet duckweed biomass production as compared to both CD & Urea + TSP treatments

PD

- Productivity range- 13.08 **-15.75** kg/m^2 (131-158 t/ha/yr)
- Highest Biomass productivity recorded in treatment **T8** (PD @ 600 kg/ha/week)

CD + PD (1:1)

- Productivity range- 12.67- **14.25** kg/m^2 (127-143 t/ha/yr)
- Highest Biomass productivity recorded in treatment **T14** (CD:PD @ 600= 300+300 kg/ha/week)

CD

- Productivity range- 9.13–**10.21** kg/m^2 (91.3 – 102.1 t/ha/yr)
- Highest Biomass productivity recorded in treatment **T4** (CD @ 800 kg/ha/week)

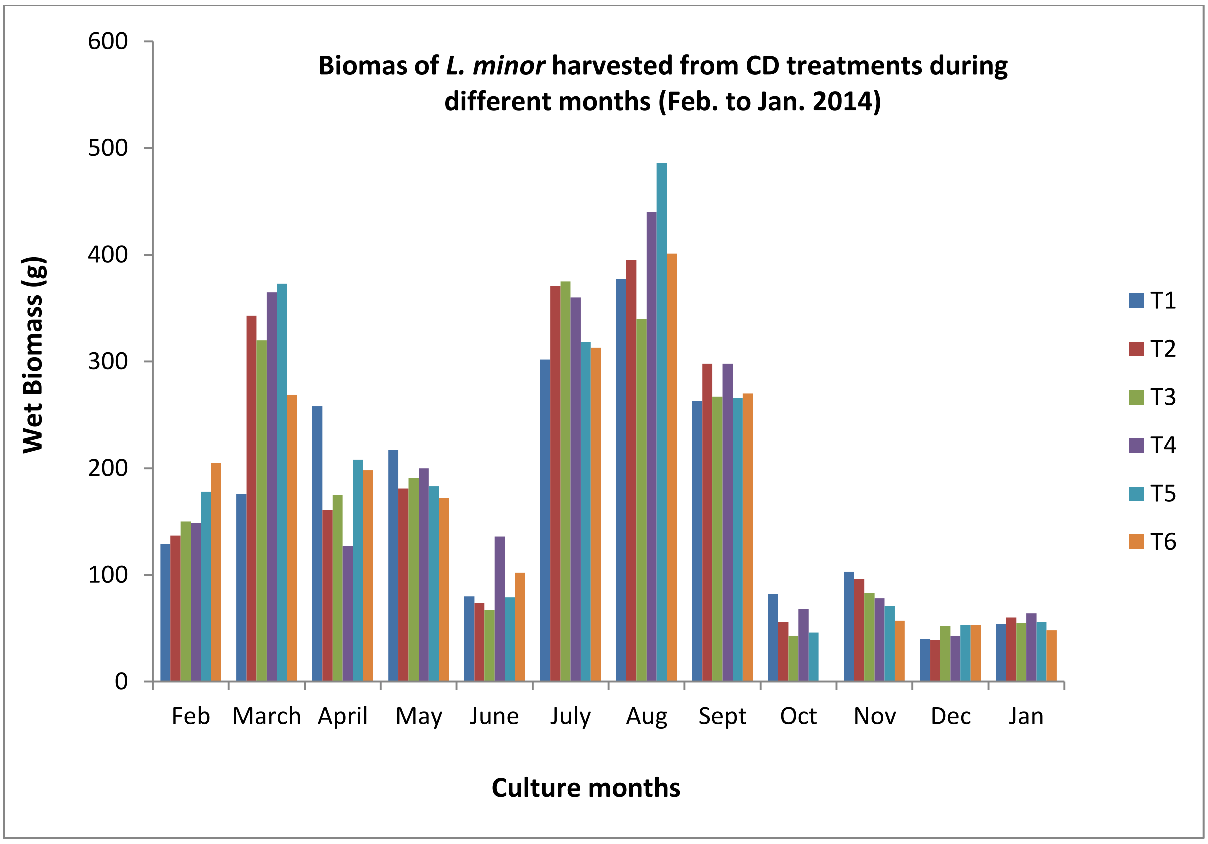
Urea + TSP

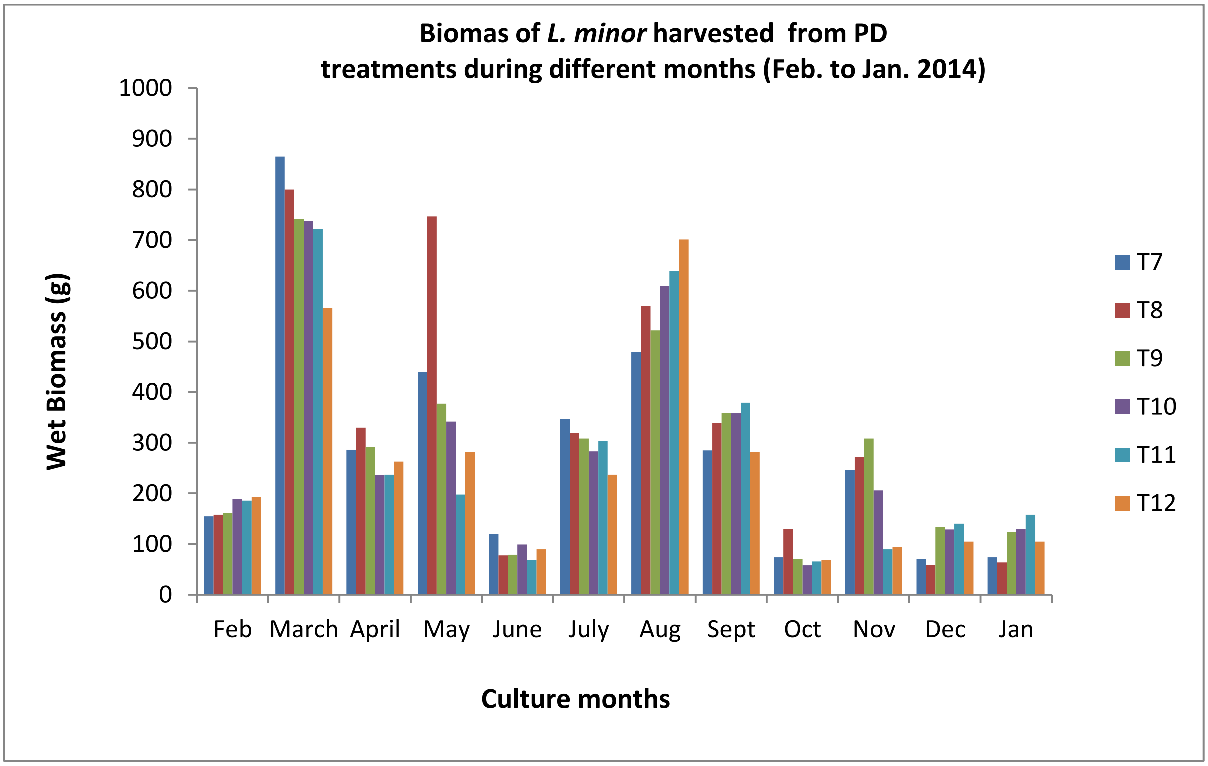
- Productivity range- 5.15 - **5.94** kg/m^2 (51.5 – 59.4 t/ha/yr)
- Highest Biomass productivity recorded in treatment **T24** (Urea+TSP @ 20+4 kg/ha/week)

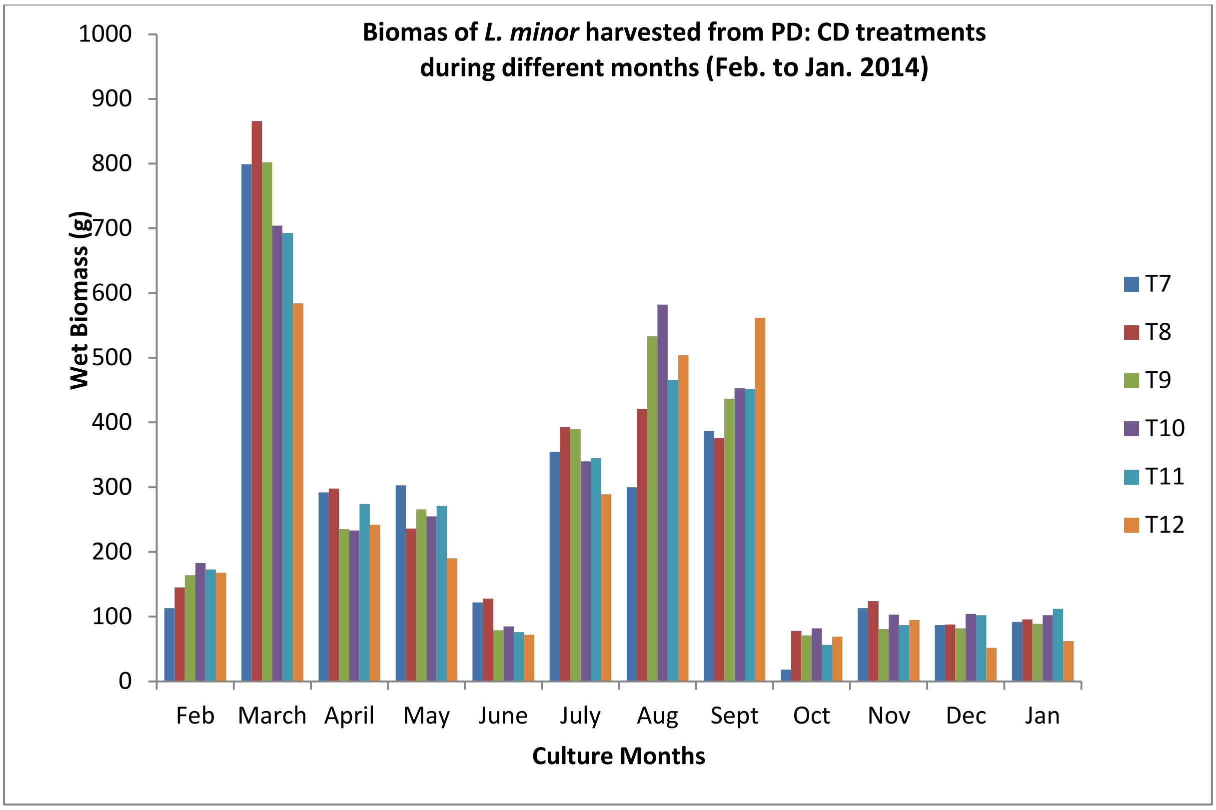
Table 4.Month wise biomass (g) of *L. minor* harvested from different treatments

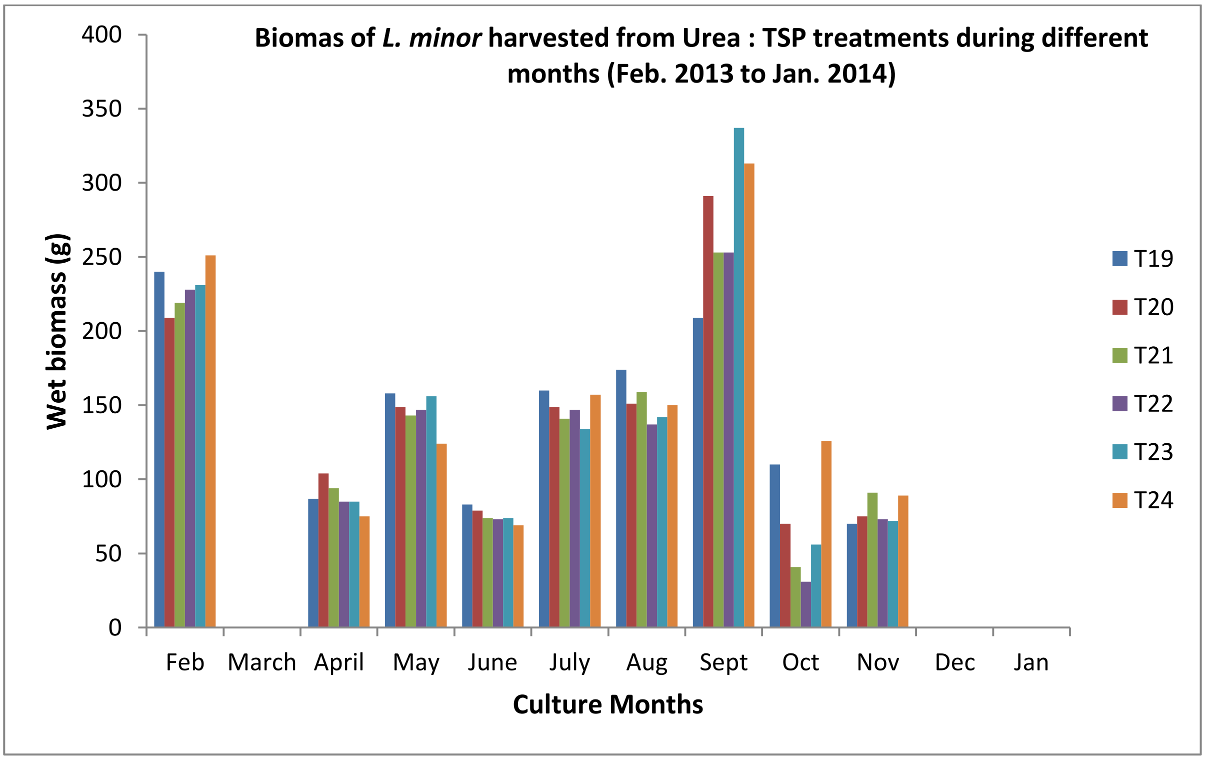
Treat-ment	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Total*
	2013											**2014**	
	CD – treatments												
T1	129	176	258	217	80	302	377	263	82	103	40	54	**2081**
T2	137	343	161	181	74	371	395	298	56	96	39	60	2211
T3	150	320	175	191	67	375	340	267	43	83	52	58	2121
T4	149	365	127	200	136	360	440	298	68	78	43	64	**2328**
T5	178	373	208	183	79	318	486	266	46	71	53	56	2317
T6	205	269	198	172	102	313	401	270	--**	57	53	48	2088
	PD – treatments												
T7	155	865	286	440	120	347	479	285	74	246	70	74	3441
T8	158	800	330	747	78	319	570	339	130	272	59	64	**3593**
T9	162	742	291	377	79	308	522	359	70	308	133	124	3475
T10	189	738	236	342	99	283	609	358	58	206	129	130	3377
T11	186	722	237	198	69	303	639	379	66	90	140	158	3187
T12	193	566	263	282	90	237	701	282	68	94	105	103	**2984**
	CD:PD (1 : 1) – treatments												
T13	113	799	292	303	122	355	300	387	18	113	87	92	2981
T14	145	866	298	236	128	393	421	376	78	124	88	96	**3249**
T15	164	802	235	266	79	390	533	437	71	81	82	89	3229
T16	183	704	233	255	85	340	582	453	82	103	104	102	3226
T17	173	693	274	271	76	345	466	452	56	87	102	112	3107
T18	168	584	242	190	72	289	504	562	69	95	52	62	**2889**
	Urea + TSP- treatments												
T19	240	**--****	87	158	83	160	174	209	110	70	**--****	**--****	1291
T20	209	--	104	149	79	149	151	291	70	75	--	--	1277
T21	219	--	94	143	74	141	159	253	41	91	--	--	1215
T22	228	--	85	147	73	147	137	253	31	73	--	--	**1174**
T23	231	--	85	156	74	134	142	337	56	72	--	--	1287
T24	251	--	75	124	69	157	150	313	126	89	--	--	**1354**

*** Biomass harvested from experimental tubs with surface area 0.228 m^2**
****No harvest**


Biomas of *L. minor* harvested from CD treatments during different months (Feb. to Jan. 2014)
Wet Biomass (g)
600
500
400
300
200
100
0
Feb
March
April
May
June
July
Aug
Sept
Oct
Nov
Dec
Jan
Culture months
T1
T2
T3
T4
T5
T6


Biomas of *L. minor* harvested from PD treatments during different months (Feb. to Jan. 2014)
Wet Biomass (g)
1000
900
800
700
600
500
400
300
200
100
0
Feb
March
April
May
June
July
Aug
Sept
Oct
Nov
Dec
Jan
Culture months
T7
T8
T9
T10
T11
T12


Biomas of L. minor harvested from PD: CD treatments
during different months (Feb. to Jan. 2014)
Wet Biomass (g)
0
100
200
300
400
500
600
700
800
900
1000
Feb
March
April
May
June
July
Aug
Sept
Oct
Nov
Dec
Jan
Culture Months
T7
T8
T9
T10
T11
T12


Biomas of L. minor harvested from Urea : TSP treatments during different
months (Feb. 2013 to Jan. 2014)
Wet biomass (g)
0
50
100
150
200
250
300
350
400
Feb
March
April
May
June
July
Aug
Sept
Oct
Nov
Dec
Jan
Culture Months
T19
T20
T21
T22
T23
T24

L. gibba- (Table 5& 6)

Both PD & CD + PD treatments supported higher wet duckweed biomass production as compared to both CD & Urea + TSP treatments

PD

- Productivity range- 6.32-**8.17** kg/m^2 (63.2-81.70 t/ha/yr)
- Highest Biomass productivity recorded in treatment **T31** (PD @ 500 kg/ha/week)

CD + PD (1:1)

- Productivity range- 6.45- **7.66** kg/m^2(64.5-76.6 t/ha/yr)
- Highest Biomass productivity recorded in treatment **T38** (CD:PD @ 600 = 300+300 kg/ha/week)

CD

- Productivity range- 5.51 – **6.42** kg/m^2 (55.1-64.2 t/ha/yr)
- Highest Biomass productivity recorded in treatment **T26** (CD @ 600 kg/ha/week)

Urea + TSP

- Productivity range- 3.82 – **4.88** kg/m^2 (38.2-48.8 t/ha/yr)
- Highest Biomass productivity recorded in treatment **T43** (Urea+TSP @ 10+2 kg/ha/week)

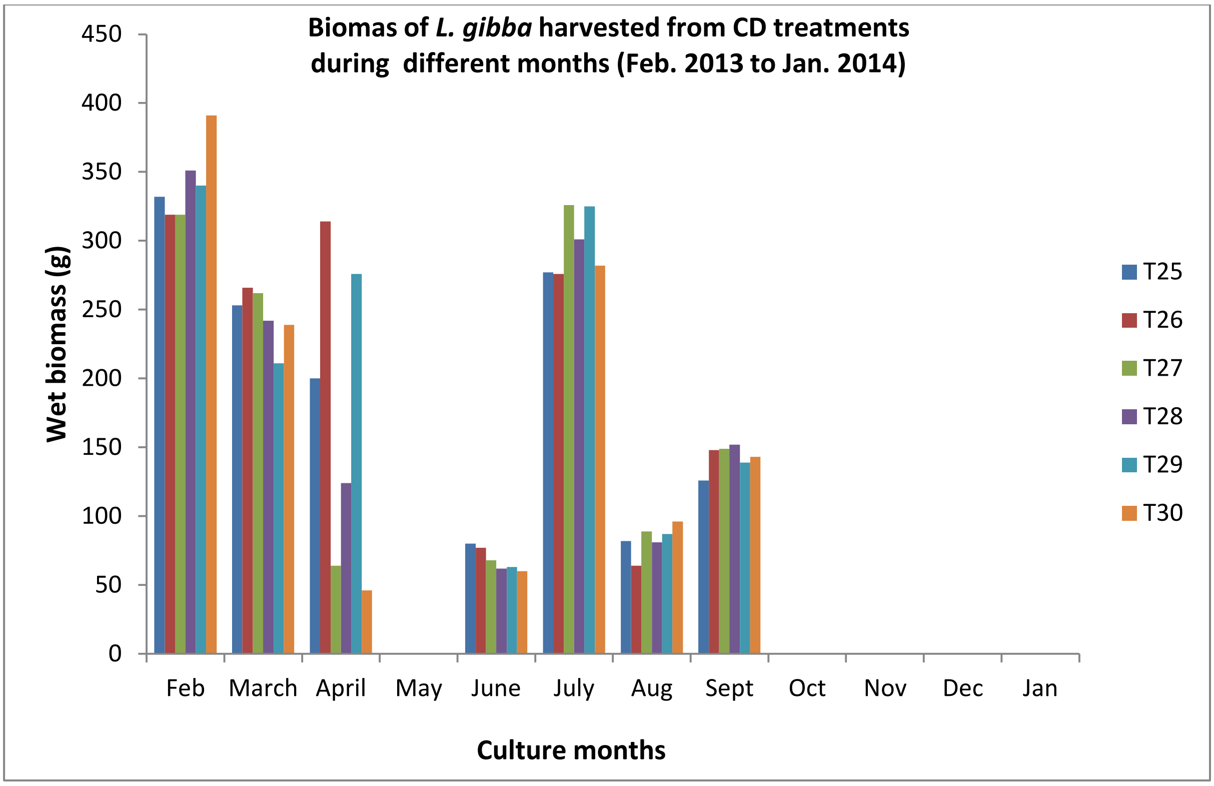
Table 5. Biomass (g) of *L. gibba* harvested from different treatments

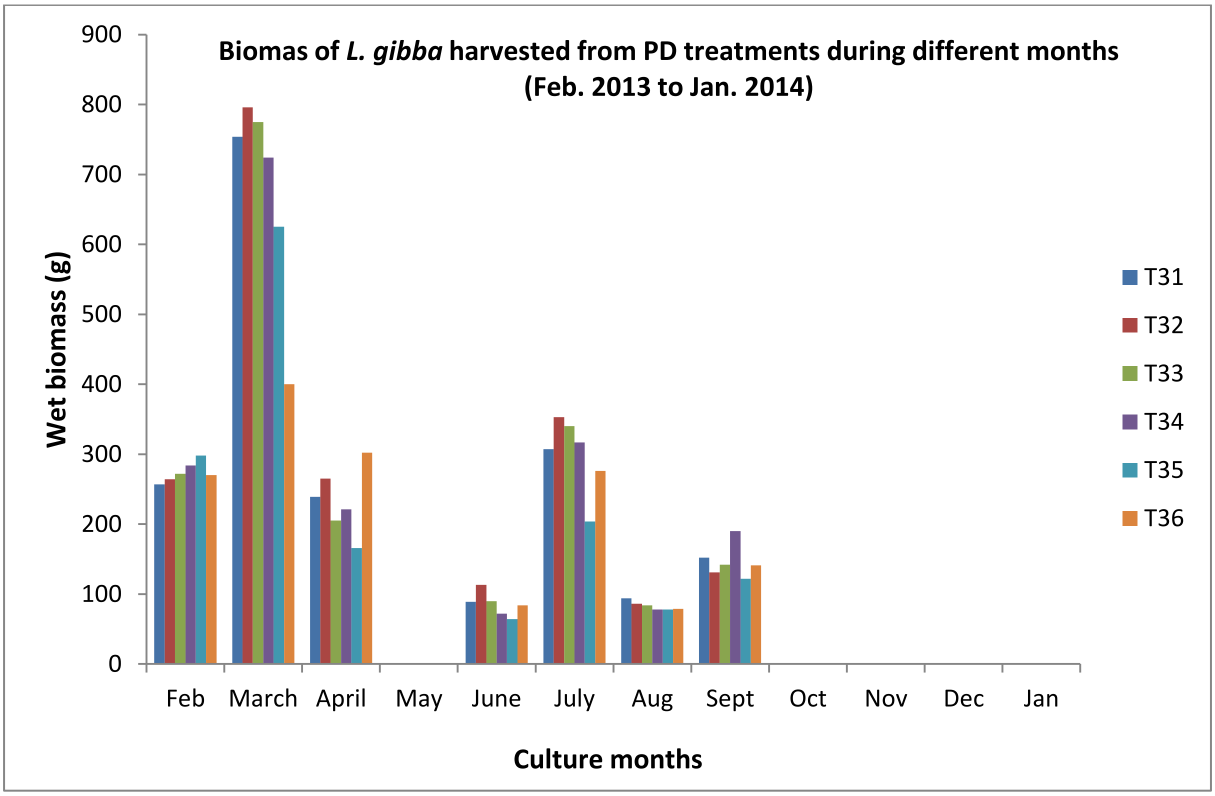
Treat--ment	Feb	March	April	May*	June	July	Aug	Sept	Oct, Nov. & Dec**	Jan **	Total ***
	2013									**2014**	
	CD–Treatments										
T25	332	253	200	-	80	277	82	126	-	-	1350
T26	319	266	314	-	77	276	64	148	-	-	**1464**
T27	319	262	64	-	68	326	89	149	-	-	1277
T28	351	242	124	-	62	301	81	152	-	-	1313
T29	340	211	276	-	63	325	87	139	-	-	1441
T30	391	239	46	-	60	282	96	143	-	-	**1257**
	PD–Treatments										
T31	257	754	239	-	89	307	94	152	-	-	**1862**
T32	264	**796**	265	-	113	353	86	131	-	-	1707
T33	272	775	205	-	90	340	84	142	-	-	1531
T34	284	724	221	-	72	317	78	190	-	-	1506
T35	298	625	166	-	64	204	78	122	-	-	1463
T36	270	400	302	-	84	276	79	141	-	-	**1440**
	CD:PD –Treatments										
T37	261	650	199	-	67	277	101	125	-	-	1677
T38	271	608	179	-	108	324	100	156	-	-	**1746**
T39	266	574	63	-	113	289	108	143	-	-	1556
T40	297	475	64	-	85	317	109	146	-	-	1518
T41	280	503	95	-	79	306	106	144	-	-	1513
T42	282	477	77	-	102	282	113	137	-	-	**1470**
	Urea + TSP–Treatments										
T43	177	271	81	-	97	286	91	109	-	-	**1112**
T44	216	263	83	-	104	253	88	**73**	-	-	1080
T45	196	188	74	-	78	216	106	98	-	-	956
T46	208	143	80	-	78	237	126	97	-	-	963
T47	184	179	75	-	57	203	106	67	-	-	871
T48	189	169	74	-	51	175	107	106	-	-	**871**

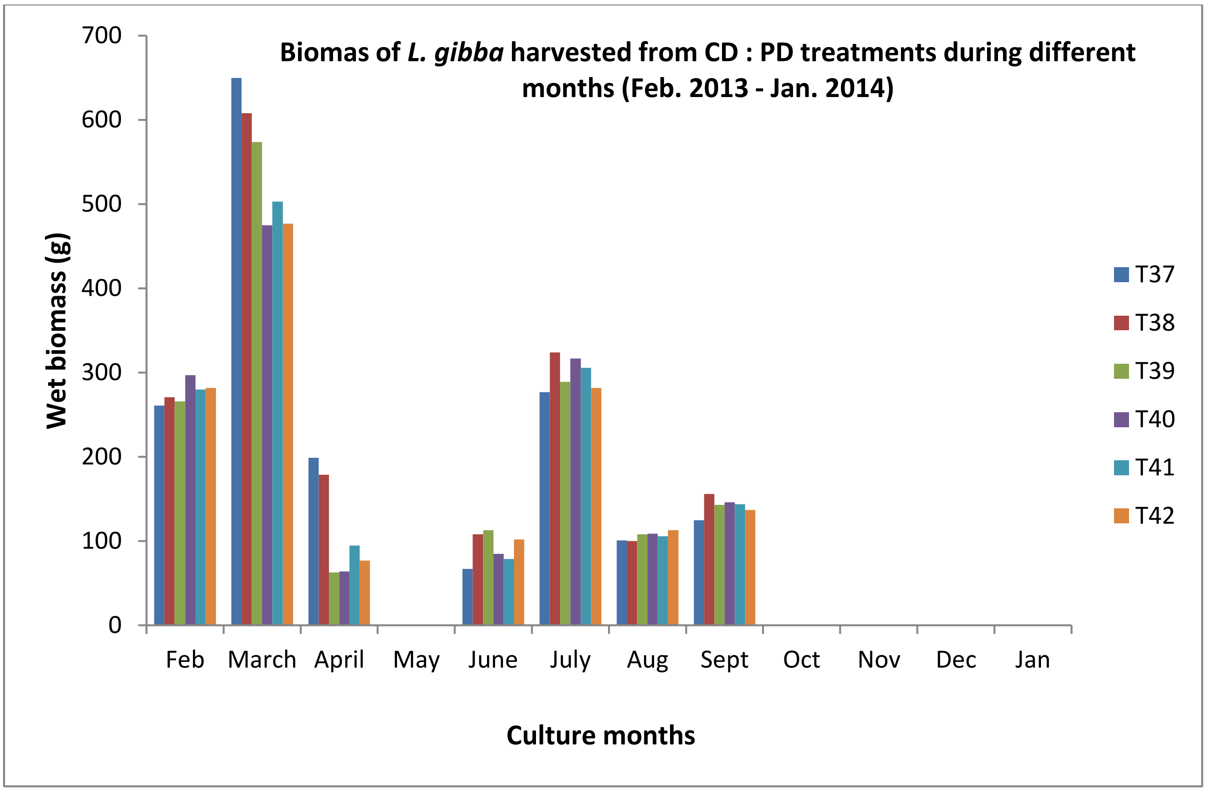
*Stock vanished due to attack of *Nimphula* insect larvae (Order Lepidoptera, family - Pyralidae)

** Stock of *L. gibba* did not grow well during October and vanished completely with the onset of winters in November and could not be revived under local climatic conditions during Nov., Dec., 2013 & Jan. 2014

*** Biomass harvested from experimental tubs with surface area 0.228 m^2


Biomas of L. gibba harvested from CD treatments during different months (Feb. 2013 to Jan. 2014)
Wet biomass (g)
450
400
350
300
250
200
150
100
50
0
Feb
March
April
May
June
July
Aug
Sept
Oct
Nov
Dec
Jan
Culture months
T25
T26
T27
T28
T29
T30


Biomas of L. gibba harvested from PD treatments during different months (Feb. 2013 to Jan. 2014)
Wet biomass (g)
900
800
700
600
500
400
300
200
100
0
Feb
March
April
May
June
July
Aug
Sept
Oct
Nov
Dec
Jan
Culture months
T31
T32
T33
T34
T35
T36


Biomas of L. gibba harvested from CD : PD treatments during different months (Feb. 2013 - Jan. 2014)
Wet biomass (g)
0
100
200
300
400
500
600
700
Feb
March
April
May
June
July
Aug
Sept
Oct
Nov
Dec
Jan
Culture months
T37
T38
T39
T40
T41
T42

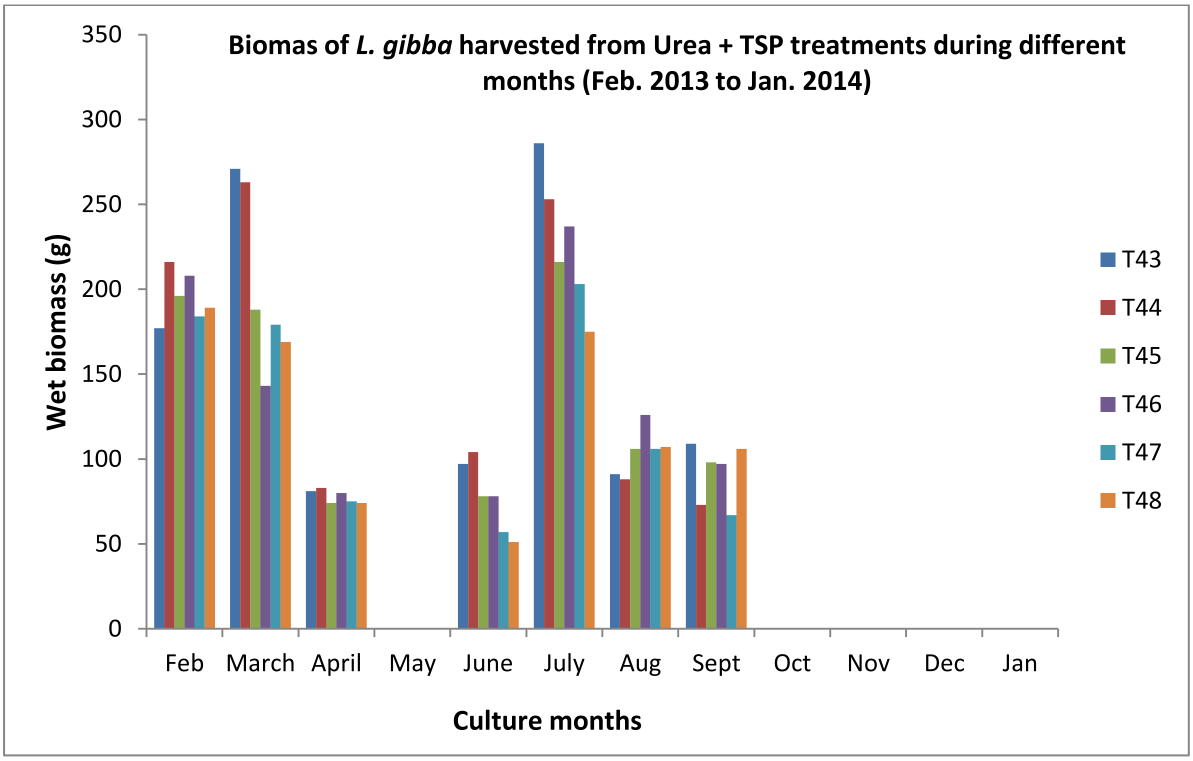

Biomas of L. gibba harvested from Urea + TSP treatments during different months (Feb. 2013 to Jan. 2014)
Wet biomass (g)
0
50
100
150
200
250
300
350
Feb
March
April
May
June
July
Aug
Sept
Oct
Nov
Dec
Jan
Culture months
T43
T44
T45
T46
T47
T48

Table 6. Comparative maximum wet biomass of *L. minor* and *L. gibba* harvested during culture period (Feb. 2013 to Jan. 2014)

<table>
<tr><th rowspan="2">Particulars</th><th colspan="2">CD</th><th colspan="2">PD</th><th colspan="2">CD:PD</th><th colspan="2">Urea : TSP</th></tr>
<tr><th>Biomass/ production/ productivity</th><th>Dose (kg/ha/wk)</th><th>Biomass (kg)</th><th>Dose (kg/ha/wk)</th><th>Biomass (kg)</th><th>Dose (kg/ha/wk)</th><th>Biomass (kg)</th><th>Dose (kg/ha/wk)</th></tr>
<tr><td colspan="9">L. minor</td></tr>
<tr><td>Kg/tub ($0.228m^2$)</td><td>2.33</td><td rowspan="3">800</td><td>3.59</td><td rowspan="3">600</td><td>3.25</td><td rowspan="3">600
300:300</td><td>1.35</td><td rowspan="3">20+4</td></tr>
<tr><td>Kg/m^2</td><td>10.21</td><td>15.75</td><td>14.25</td><td>5.94</td></tr>
<tr><td>t/ha/yr</td><td>102.1</td><td>158</td><td>143</td><td>59.40</td></tr>
<tr><td colspan="9">L. gibba</td></tr>
<tr><td>Kg/tub ($0.228m^2$)</td><td>1.46</td><td>600</td><td>1.86</td><td>500</td><td>1.74</td><td>600
300:300</td><td>1.11</td><td>10+2.0</td></tr>
<tr><td>Kg/m^2</td><td>6.42</td><td></td><td>8.17</td><td></td><td>7.66</td><td></td><td>4.88</td><td></td></tr>
<tr><td>t/ha/yr</td><td>64.20</td><td></td><td>81.70</td><td></td><td>76.6</td><td></td><td>48.8</td><td></td></tr>
</table>

Note: The values (Table 4-6) are the mean of the triplicate.

Hence, **among four manures, PD and CD:PD were found to be the suitable manures** in terms of maximum productivity of both duckweed species viz. ***L. minor* (158 t/ha/yr, 143 t/ha/yr) and *L. gibba* (81.70 t/ha/yr, 76.6 t/ha/yr).** However, out of two duckweed species, *L. minor* performed better than *L. gibba* in terms of production with all the manures.

4. <u>Nutritive Value analysis (Proximate composition) of duckweeds</u>

Results for nutritional value analysis of both duckweed species viz. *L. minor* (Table 7A – 7C) and *L. gibba* (Table 8A – 8C) are presented with respect to

a. **Manure types**
b. **Manure dozes and**
c. **Mnaure type and season**

Species 1- *L. minor* [Table 7A – 7C(1-2)]

Nutritional value (% DM) w. r. to manure type throughout the culture period (Table 7A)

- Significantly higher crude protein in CD:PD (22.86%), followed by CD (21.92%), PD (21.86%) and Urea+TSP (19.52%), respectively (CD:PD=CD=PD>Urea+TSP).
- Significantly higher ether extract in PD (1.94%), followed by CD (1.84%), CD:PD (1.76%) and Urea+TSP (1.31%), respectively (PD=CD=CD:PD>Urea+TSP).
- Significantly higher crude fibre in CD:PD (8.51%), followed by PD (8.18%), Urea+TSP (8.17%) and CD (7.25%), respectively (CD:PD=PD=Urea+TSP>CD).
- Highest ash in Urea+TSP (31.17 %), followed by PD (31.15 %), CD:PD (30.19 %) and CD (30.11 %) respectively, but the differences were non-significant.

Table 7A.Comparative nutritional value of *L. minor* (%DM basis) cultured on different manures throughout the culture period

Manure Type	Parameters (% DM)*			
	Crude Protein	Ether extract	Crude fibre	Ash
CD	21.92[a] ± 0.42	1.84[a] ± 0.11	7.25[b] ± 0.24	30.11 [a] ±0.69
PD	21.86[a] ± 0.48	**1.94[a]± 0.13**	8.18[a] ± 0.16	31.15 [a] ±0.45
CD:PD	**22.86[a] ± 0.57**	1.76[a] ± 0.08	**8.51[a] ± 0.24**	30.19 [a] ±0.53
Urea+TSP	19.52[b] ± 0.49	1.31[b] ± 0.05	8.17[a]± 0.23	**31.17 [a] ±0.60**

***Values are means of 06 dozes of individual manures ± S.E. (p<0.05)**

Among all manures, **CD:PD** resulted in **significantly higher crude protein (22.86%), hence, CD:PD can be recommended as the best manure to harvest *L. minor* in terms of protein rich biomass.**

Nutritional value w. r. to manure doses (Table 7B)

- Crude protein (%) content in harvested *L. minor* varied between 21.32 to 23.56, 20.74 to 23.83, 21.34 to 26.27 and 18.97 to 20.82 in CD, PD, CD:PD and Urea+TSP treatments, respectively.
- Ether extract (%) in harvested *L. minor* varied between 1.68 to 2.14, 1.62 to 2.10, 1.42 to 2.25 and 1.13 to 1.54 in CD, PD, CD:PD and Urea+TSP treatments, respectively.
- Crude fibre (%) content in harvested *L. minor* varied between 6.46 to 8.87, 7.47 to 8.81, 7.26 to 9.86 and 7.68 to 8.59 in CD, PD, CD:PD and Urea+TSP treatments, respectively.
- Ash (%) content in harvested *L. minor* varied between 27.88 to 33.04, 29.38 to 33.16, 29.53 to 30.58 and 29.70 to 32.40 in CD, PD, CD:PD and Urea+TSP treatments, respectively.

Table 7B: Comparative nutritional value of *L. minor* (% DM basis) harvested from different treatments

Manure	Treatment	Parameters			
CD		Crude Protein*	Ether Extract*	Crude fibre*	Ash*
	T1	21.32^{bcdef} ±1.18	1.87^{abcde} ± 0.55	7.13^{bcd}± 0.72	28.84^{ab} ±1.32
	T2	21.97^{bcdef} ±1.18	2.14^{ab} ± 0.66	6.52^{d}± 0.34	27.88^{b}±1.05
	T3	21.88^{bcdef} ± 0.86	1.68^{abcde} ± 0.61	6.94^{cd}± 0.62	29.42^{ab} ±1.73
	T4	23.56^{abcd} ±1.14	2.16^{abc} ± 0.69	6.46^{d}± 0.53	29.23^{ab} ±1.72
	T5	23.21^{abcde}± 0.96	1.78^{abcde} ± 0.67	7.51^{bcd}± 0.56	**33.04^{a}±2.06**
	T6	22.39^{abcdef} ± 0.81	1.69^{abcde} ± 0.83	8.87^{ab}± 0.50	32.24^{ab} ±1.86
PD	T7	20.74^{cdef} ± 0.71	1.84^{abcde} ± 0.52	8.33^{abc}± 0.47	29.38^{ab} ±0.90
	T8	21.76^{bcdef} ±1.32	2.10^{ab} ± 0.65	7.94^{bcd}± 0.53	31.10^{ab} ±0.89
	T9	22.71^{abcdef} ±1.10	1.62^{abcde} ± 0.66	8.81^{ab}± 0.42	31.94^{ab} ±1.12
	T10	23.83^{abc} ± 0.91	2.06^{abc} ± 0.72	8.38^{abc}± 0.23	29.89^{ab} ±0.65
	T11	22.39^{abcdef} ±1.45	1.75^{abcde} ± 0.64	8.08^{abcd}± 0.34	31.39^{ab} ±1.14
	T12	23.03^{abcdef} ±1.52	1.65^{abcde} ± 0.85	7.47^{bcd}± 0.34	33.16a±1.61
CD:PD	T13	24.92^{ab} ±1.43	1.94^{abcd} ± 0.61	8.93^{ab}± 0 .34	30.13^{ab} ±1.78
	T14	**26.27^{a} ±1.48**	1.67^{abcde} ± 0.57	7.89^{bcd}± 0.33	30.63^{ab} ±1.47
	T15	22.59^{bcdef} ±1.66	**2.25^{a} ± 0.68**	7.26^{bcd}± 0.45	29.77^{ab} ±1.57
	T16	21.34^{bcdef} ±1.23	1.75^{abcde} ± 0.44	8.54^{abc}± 0.68	29.53^{ab} ±0.65
	T17	22.29^{abcdef} ± 0.81	1.42^{bcde} ± 0.43	**9.86^{a}**± 0.85	30.58^{ab} ±1.28
	T18	23.04^{abcdef} ±1.14	1.58^{abcde} ± 0.45	8.68^{abc}± 0.51	30.48^{ab} ±1.01
Urea+TSP	T19	20.34^{cdef} ±2.03	1.28^{cde} ± 0.34	8.38^{abc}± 0.27	29.70^{ab} ±1.41
	T20	19.55^{def} ±1.34	1.54^{abcde} ± 0.56	7.68^{bcd}± 0.49	32.40^{ab} ±2.42
	T21	20.82^{bcdef} ± 0.45	1.44^{bcde} ± 0.37	8.17^{abcd}± 0.52	30.61^{ab} ±1.56
	T22	20.78^{bcdef} ± 0.98	1.17^{de} ± 0.17	8.11^{abcd}± 0.41	31.02^{ab} ±1.42
	T23	18.97^{f} ± 0.89	1.20^{de} ± 0.13	8.09^{abcd}± 0 .66	32.14^{ab} ±0.89
	T24	19.32^{ef} ±1.52	1.13^{e} ± 0.12	8.59^{abc}± 0.95	31.07^{ab} ±0.91

*** Values w.r.t. pooled samples from each treatment**

Among all manures dozes (each manure having 6 dozes), **CD:PD @ 600 kg/ha/wk resulted in significantly higher crude protein (26.27%), hence, CD:PD can be recommended @ 600 kg/ha/wk as the best manure to harvest *L. minor* in terms of protein rich biomass.**

<u>Nutritional value of *L. minor* w. r. to different manures (Table 7C- 1) & different seasons (Table 7C-2)</u>

- During different seasons, crude protein (%) content in harvested *L. minor* varied between 21.34 to 24.31, 21.88 to 23.07, 19.42 to 26.65 and 17.03 to 21.98 in CD, PD, CD:PD and Urea+TSP treatments, respectively.

- During different seasons, ether extract (%) in harvested *L. minor* varied between 1.39 to 3.01, 1.35 to 2.55, 1.32 to 1.95 and 1.20 to 1.82 in CD, PD, CD:PD and Urea+TSP treatments, respectively.

- During different seasons, crude fibre (%) content in harvested *L. minor* varied between 6.37 to 8.59, 7.89 to 8.43, 6.77 to 9.73 and 5.76 to 8.66 in CD, PD, CD:PD and Urea+TSP treatments, respectively.

- During different seasons, ash (%) content in harvested *L. minor* varied between 27.56 to 33.31, 29.76 to 31.27, 29.13 to 31.73 and 29.33 to 32.77 in CD, PD, CD:PD and Urea+TSP treatments, respectively.

Table 7C (1): Comparative nutritional value of *L. minor* among manures during different seasons

Season	CD	PD	CD:PD	Urea+TSP
	Crude protein (%)			
Winter (Dec- Feb)	21.34^{a}±0.93	**21.88^{a}±1.38**	19.42^{ab}±1.39	17.03^{b}±1.28
Pre-monsoon (March – May)	**24.31^{a}±0.60**	23.07^{ab}±0.78	23.55^{ab}±0.77	21.98^{b}±0.67
Monsoon (June-August)	21.69^{b}±0.65	22.57^{b}±0.76	**26.65^{a}±1.24**	18.83^{c}±0.82
Post-monsoon (Sept – Nov.)	21.97^{ab}±0.85	22.04^{ab}±0.89	**23.21^{a2}±0.75**	19.77^{b}±0.99
	Crude Fat (%)			
Winter (Dec- Feb)	1.39^{a}±0.09	1.35^{a}±0.12	1.32^{a}±0.02	1.20^{a}±0.06
Pre-monsoon (March – May)	1.72^{a}±0.13	1.80^{a}±0.08	**1.95^{a}±0.17**	1.32^{b}±0.06
Monsoon (June-August)	2.21^{a}±0.17	2.25^{a2}±0.12	1.84^{a}±0.12	1.31^{b}±0.09
Post-monsoon (Sept – Nov.)	**3.01^{a}±0.43**	3.15^{a}±0.32	1.73^{b}±0.11	1.82^{b}±0.52
	Crude Fibre (%)			
Winter (Dec- Feb)	6.47^{b}±0.42	**7.89^{a}±0.34**	6.77^{ab}±0.47	5.76^{b}±0.54
Pre-monsoon (March – May)	**8.59^{a}±0.46**	8.27^{a}±0.29	8.34^{a}±0.47	8.66^{a}±0.39
Monsoon (June-August)	6.37^{c}±0.42	8.43^{b}±0.51	**9.73^{a}±0.46**	8.31^{b}±0.33
Post-monsoon (Sept – Nov.)	6.98^{b}±0.40	8.10^{a}±0.25	**8.81^{a}±0.27**	8.41^{a}±0.31
	Ash (%)			
Winter (Dec- Feb)	28.53^{b}±0.85	29.76^{b}±0.72	29.22^{b}±0.71	**32.77^{a}±0.95**
Pre-monsoon (March – May)	27.56^{a}±1.64	**31.27^{a}±1.07**	29.13^{a}±1.59	29.33^{a}±1.61
Monsoon (June-August)	**33.31^{a}±1.33**	32.62^{a}±1.26	31.73^{a}±0.57	32.63^{a}±0.56
Post-monsoon (Sept – Nov.)	**31.57^{a}±0.86**	30.96^{a}±0.43	30.79^{a}±0.42	31.51^{a}±0.40

Values are mean ± S. E.
Values with same superscript in row do not differ significantly ($P \leq 0.05$)

Table 7C (2) : Comparative nutritional value of *L. minor* in different manures with respect to seasons

Season	CD	PD	CD:PD	Urea+TSP
	Crude protein (%)			
Winter (Dec- Feb)	21.34^{b}±0.93	21.88^{a}±1.38	19.42^{c}±1.39	17.03^{b}±1.28
Pre-monsoon (March – May)	**24.31^{a}±0.60**	**23.07^{a}±0.78**	23.55^{b}±0.77	**21.98^{a}±0.67**
Monsoon (June-August)	21.69^{b}±0.65	22.57^{a}±0.76	**26.65^{a}±1.24**	18.83^{b}±0.82
Post-monsoon (Sept – Nov.)	21.97^{b}±0.85	22.04^{a}±0.89	23.21^{b}±0.75	19.77^{ab}±0.99
	Crude Fat (%)			
Winter (Dec- Feb)	1.39^{c}±0.09	1.35^{c}±0.12	1.32^{b}±0.02	1.20^{b}±0.06
Pre-monsoon (March – May)	1.72^{bc}±0.13	1.80^{bc}±0.08	**1.95^{a}±0.17**	1.32^{b}±0.06
Monsoon (June-August)	2.21^{b}±0.17	2.25^{b}±0.12	1.84^{a}±0.12	1.31^{b}±0.09
Post-monsoon (Sept – Nov.)	**3.01^{a}±0.43**	**3.15^{a}±0.32**	1.73^{ab}±0.11	**1.82^{a}±0.52**
	Crude Fibre (%)			
Winter (Dec- Feb)	6.47^{b}±0.42	7.89^{a}±0.34	6.77^{c}±0.47	5.76^{b}±0.54
Pre-monsoon (March – May)	**8.59^{a}±0.46**	8.27^{a}±0.29	8.34^{b}±0.47	**8.66^{a}±0.39**
Monsoon (June-August)	6.37^{b}±0.42	**8.43^{a}±0.51**	**9.73^{a}±0.46**	8.31^{a}±0.33
Post-monsoon (Sept – Nov.)	6.98^{b}±0.40	8.10^{a}±0.25	8.81^{ab}±0.27	8.41^{a}±0.31
	Ash (%)			
Winter (Dec- Feb)	28.53^{bc}±0.85	29.76^{b}±0.72	29.22^{a}±0.71	**32.77^{a}±0.95**
Pre-monsoon (March – May)	27.56^{c}±1.64	31.27^{ab}±1.07	29.13^{a}±1.59	29.33^{a}±1.61
Monsoon (June-August)	**33.31^{a}±1.33**	**32.62^{a}±1.26**	**31.73^{a}±0.57**	32.63^{a}±0.56
Post-monsoon (Sept – Nov.)	31.57^{ab}±0.86	30.96^{ab}±0.43	30.79^{a}±0.42	31.51^{a}±0.40

Values are mean + S. E.
Values with same superscript in column do not differ significantly ($P \leq 0.05$)

<u>Overall results</u> – *L. minor*

Among manures - Significantly highest crude protein (%) in **PD (21.88) during winters**, in **CD (24.31) during pre-monsoon**, in **CD:PD during monsoon (26.65) and pre-monsoon (23.21**), respectively.

Among seasons – Best seasons are - Pre-monsoon (March-may) and monsoon (June-August) for harvesting protein rich *L. minor* biomass from CD:PD (26.65).

Species 2- *L. gibba* [Table 8A – 8C (1-2)]

<u>Nutritional value (% DM) w. r. t. manure type (Table 8A)</u>

- Significantly higher crude protein (%) in CD:PD (27.15), followed by Urea+TSP (26.48) PD (24.93) and CD (23.55) respectively (CD:PD=Urea+TSP≥PD≥CD)
- Significantly higher ether extract (%) in CD:PD (2.42), followed by Urea+TSP (1.98), PD (1.65) and CD (1.61) and respectively (CD:PD≥Urea+TSP≥PD=CD).
- Significantly higher crude fibre (%) in Urea+TSP (9.03), followed by CD (8.83), CD:PD (8.63) and PD (8.57) and respectively (Urea+TSP≥CD=CD:PD≥PD).
- Significantly higher ash (%) in Urea+TSP (34.77), followed by CD:PD (27.44), CD (24.78) and PD (24.70) and respectively (Urea+TSP>CD:PD>CD=PD).

Table 8A.Comparative nutritional value of *L. gibba* (%DM basis) cultured on different manures throughout culture period

Manure Type	Parameters (% DM)			
	Crude Protein	Ether extract	Crude fibre	Ash
CD	$23.55^{b} \pm 0.84$	$1.61^{b} \pm 0.07$	$8.83^{ab} \pm 0.15$	$24.78^{c}\pm0.59$
PD	$24.93^{ab} \pm 1.16$	$1.65^{b} \pm 0.18$	$8.57^{b} \pm 0.19$	$24.70^{c}\pm0.65$
CD:PD	$\mathbf{27.15^{a} \pm 0.58}$	$\mathbf{2.42^{a} \pm 0.15}$	$8.63^{ab} \pm 0.13$	$27.44^{b}\pm0.46$
Urea+TSP	$26.48^{a} \pm 0.74$	$1.98^{ab} \pm 0.21$	$\mathbf{9.03^{a} \pm 0.07}$	$\mathbf{34.77^{a}\pm0.94}$

Nutritional value w. r. to manure doses (Table - 8B)

- Crude protein (%) content in harvested *L. gibba* varied between 21.53 to 25.68, 23.51 to 26.15, 25.86 to 28.29 and 26.96 to 27.97 in CD, PD, CD:PD and Urea+TSP treatments respectively.

- Ether extract (%) in harvested *L. minor* varied between 1.27 to 2.05, 0.66 to 2.79, 1.70 to 3.49 and 0.74 to 2.60 in CD, PD, CD:PD and Urea+TSP treatments respectively.

- Crude fibre (%) content in harvested *L. minor* varied between 8.16 to 9.35, 7.78 to 9.09, 8.10 to 9.29 and 8.76 to 9.20 in CD, PD, CD:PD and Urea+TSP treatments respectively.

- Ash (%) content in harvested *L. minor* varied between 23.50 to 26.22, 22.28 to 27.16, 26.20 to 29.58 and 31.49 to 41.37 in CD, PD, CD:PD and Urea+TSP treatments respectively.

- Among all the dozes of four manures, highest crude protein (28.29%), ether extract (3.49%), crude fibre (9.35%) and ash (41.37%) were recorded in T15 (CD:PD @ 700 kg/ha/wk), T14 (CD:PD @ 600 kg/ha/wk), T3 (CD @ 700 kg/ha/wk) and T23 (Urea + TSP @ 900 kg/ha/wk), respectively.

Table 8B: Comparative nutritional value of *L. gibba* (% DM basis) harvested from different treatments

Manure	Treatment	Parameters (% DM)			
CD		**Crude Protein***	**Ether Extract***	**Crude fibre***	**Ash***
	T1	22.23^{a} ± 2.52	2.01^{cdefgh}± 0.06	9.06^{abc}±0.27	26.22^{ef}±1.34
	T2	23.46^{a} ± 2.37	1.66^{defgh}± 0.14	8.93^{abcd}±0.19	24.64^{ef}±1.81
	T3	21.53a ± 2.55	1.27^{hijk}± 0.10	**9.35^{a}±0.53**	25.76^{ef}±1.01
	T4	23.78^{a} ± 2.71	1.29^{hijk}± 0.17	8.16^{bcd}±0.21	23.50^{f}±2.10
	T5	24.62^{a} ± 1.21	2.05^{cdefgh}± 0.02	8.47^{abcd}±0.26	24.78^{ef}±0.94
	T6	25.68^{a} ± 0.81	1.54^{fghijk}± 0.05	9.01^{abc}±0.43	23.79^{f}±1.50
PD	T7	25.67^{a} ± 2.87	0.79^{ijk}± 0.21	8.14^{bcd}±0.52	22.28^{f}±2.30
	T8	24.49^{a} ± 2.71	0.66^{k}± 0.08	7.78^{d}±0.60	25.35^{ef}±1.72
	T9	25.14^{a} ± 2.67	1.60^{efghij}± 0.16	8.89^{abcd}±0.63	24.22^{ef}±1.43
	T10	23.51^{a} ± 3.60	1.70^{defgh}± 0.28	8.97^{abc}±0.16	24.36^{ef}±0.41
	T11	26.15^{a} ± 3.47	2.33^{bcdefg}± 0.16	8.51^{abcd}±0.22	27.16^{cd}e±1.51
	T12	24.50^{a} ± 2.99	2.79^{abc}± 0.19	9.09^{abc}±0.44	24.86^{ef}±1.49
CD:PD	T13	27.49^{a} ± 0.72	3.21^{ab}± 0.15	8.73^{abcd}±0.16	29.58^{bcd}±1.06
	T14	25.86a ± 1.67	**3.49^{a}± 0.38**	8.43^{abcd}±0.20	27.48^{cde}±0.41
	T15	**28.29^{a} ± 1.96**	2.55^{bcde}± 0.21	9.29^{ab}±0.40	26.20^{ef}±2.21
	T16	28.01^{a} ± 0.81	1.81^{defgh}± 0.04	8.10^{cd}±0.38	26.87^{cde}±0.66
	T17	26.82^{a} ± 1.33	1.78^{defgh}± 0.06	8.91^{abcd}±0.36	27.07^{cde}±0.57
	T18	26.53^{a} ± 1.79	1.70^{defgh}± 0.08	8.32^{abcd}±0.19	27.42^{cde}±0.78
Urea+TSP	T19	27.09^{a} ± 1.93	0.74^{jk}± 0.09	8.94^{abc}±0.21	32.60^{bc}±2.56
	T20	27.16^{a} ± 1.51	1.37^{ghijk}± 0.14	9.18^{abc}±0.05	35.04^{b}±2.39
	T21	24.91^{a} ± 2.12	2.14^{cdefgh}± 057	9.08^{abc} ±021	33.92^{bc}±1.43
	T22	24.90^{a} ± 2.25	2.43^{bcdef}± 0.55	9.03^{abc}±0.23	34.21^{bc}±1.39
	T23	27.97^{a} ± 0.26	2.31^{bcdefg}± 0.30	8.76^{abcd}±0.26	**41.37 ^{a}±2.69**
	T24	26.96^{a} ±0.09	2.60^{bcd}± 0.55	9.20^{abc}±0.08	31.49^{bcd}±0.42

*** Values w.r.t. pooled samples from each treatment**

Nutritional value of *L. gibba* w. r. t. different season and manures (Table 8C 1-2)

- During different seasons, crude protein (%) content in harvested *L. gibba* varied between 17.23 to 26.73, 16.94 to 33.97, 22.11 to 29.01 and 19.96 to 29.95 in CD, PD, CD:PD and Urea+TSP treatments respectively.

- During different seasons, ether extract (%) in harvested *L. gibba* varied between 1.56 to 1.71, 1.54 to 1.80, 2.29 to 2.63 and 1.48 to 3.18 in CD, PD, CD:PD and Urea+TSP treatments respectively.

- During different seasons, crude fibre (%) content in harvested *L. gibba* varied between 8.14 to 9.11, 8.10 to 9.29, 8.24 to 9.12 and 8.97 to 9.18 in CD, PD, CD:PD and Urea+TSP treatments respectively.

- During different seasons, ash (%) content in harvested *L. gibba* varied between 22.79 to 26.58, 23.41 to 26.58, 26.45 to 29.16 and 32.58 to 40.58 in CD, PD, CD:PD and Urea+TSP treatments respectively.

- Significantly higher crude protein (%) in CD:PD (33.97) during winter, ether extract in CD (3.18) during winter, crude fibre in CD:PD (9.29) during monsoon and ash in CD (40.58) during winter season respectively.

Table 8C (1) : Comparative nutritional value of *L. gibba* (%DM) in different manures

Season*	CD	PD	CD:PD	Urea+TSP
	Crude protein (%)			
Winter (Feb)	26.73^{bc}±0.59	**33.97^{a}±0.76**	29.01^{b}±1.33	24.92^{c}±0.70
Pre-monsoon (March – April)	25.81^{bc}±0.46	25.23^{c}±0.93	27.47^{ab}±0.46	**28.24^{a}±0.68**
Monsoon (June-August)	17.23^{b}±1.79	16.94^{b}±0.64	28.01^{a}±0.68	**29.95^{a}±0.54**
Post-monsoon (Sept)	22.17^{a}±1.90	**22.93^{a}±1.53**	22.11^{a}±1.69	19.96^{a}±1.12
	Crude Fat (%)			
Winter (Feb)	1.67^{a}±0.15	1.80^{a}±0.46	2.63^{a}±0.46	**3.18^{a}±0.69**
Pre-monsoon (March – April)	1.57^{b}±0.11	1.56^{b}±0.22	2.29^{a}±0.22	1.48^{b}±0.12
Monsoon (June-August)	1.56^{a}±0.21	1.76^{a}±0.62	**2.61^{a}±0.36**	1.83^{a}±0.33
Post-monsoon (Sept)	1.71^{a}±0.23	1.54^{a}±0.56	**2.29^{a}±0.28**	1.82^{a}±0.39
	Crude Fibre (%)			
Winter (Feb)	8.14^{a}±0.24	8.10^{a}±0.54	8.24^{a}±0.27	**9.03^{a}±0.31**
Pre-monsoon (March – April)	8.94^{a}±0.24	8.42^{a}±0.30	8.44^{a}±0.20	**8.97^{a}±0.09**
Monsoon (June-August)	9.01^{a}±0.38	**9.29^{a}±0.22**	9.12^{a}±0.26	8.99^{a}±0.08
Post-monsoon (Sept)	9.11^{a}±0.23	8.59^{a}±0.44	8.92^{a}±0.29	**9.18^{a}±0.10**
	Ash (%)			
Winter (Feb)	25.52^{bc}±1.05	24.11^{c}±1.39	29.16^{b}±1.05	**40.58^{a}±2.29**
Pre-monsoon (March – April)	22.79^{c}±1.11	23.41^{bc}±1.25	26.45^{b}±0.86	**32.58^{a}±1.06**
Monsoon (June-August)	26.21^{b}±0.46	26.58^{b}±0.90	27.44^{b}±0.68	**33.45^{a}±1.65**
Post-monsoon (Sept)	26.58^{b}±0.77	26.02^{b}±0.83	27.69^{b}±0.54	**34.67^{a}±1.98**

*** No harvesting of *L. gibba* during May 2013 &October 2013 – January 2014 (Nil growth)**
Values are mean ± S. E.
Values with same superscript in row do not differ significantly (P≤ 0.05)

Table 8C (2): Comparative nutritional value of *L. gibba* (%DM) in different seasons

Season*	CD	PD	CD:PD	Urea+TSP
	Crude protein (%)			
Winter (Feb)	**26.73^{a}±0.59**	**33.97^{a}±0.76**	**29.01^{a}±1.33**	24.92^{b}±0.70
Pre-monsoon (March – April)	25.81^{a}±0.46	25.23^{b}±0.93	27.47^{a}±0.46	28.24^{a}±0.68
Monsoon (June-August)	17.23^{c}±1.79	16.94^{c}±0.64	28.01^{a}±0.68	**29.95^{a}±0.54**
Post-monsoon (Sept)	22.17^{b}±1.90	22.93^{b}±1.53	22.11^{b}±1.69	19.96^{c}±1.12
	Crude Fat (%)			
Winter (Feb)	1.67^{a}±0.15	**1.80^{a}±0.46**	2.63^{a}±0.46	**3.18^{a}±0.69**
Pre-monsoon (March – April)	1.57^{b}±0.11	1.56^{b}±0.22	2.29^{a}±0.22	1.48^{b}±0.12
Monsoon (June-August)	1.56^{a}±0.21	1.76^{a}±0.62	2.61^{a}±0.36	1.83^{b}±0.33
Post-monsoon (Sept)	**1.71^{a}±0.23**	1.54^{a}±0.56	2.29^{a}±0.28	1.82^{b}±0.39
	Crude Fibre (%)			
Winter (Feb)	8.14^{b}±0.24	8.10^{a}±0.54	8.24^{b}±0.27	9.03^{a}±0.31
Pre-monsoon (March – April)	8.94^{ab}±0.24	8.42^{a}±0.30	8.44^{ab}±0.20	8.97^{a}±0.09
Monsoon (June-August)	9.01^{ab}±0.38	9.29^{a}±0.22	**9.12^{a}±0.26**	8.99^{a}±0.08
Post-monsoon (Sept)	**9.11^{a}±0.23**	8.59^{a}±0.44	8.92^{ab}±0.29	9.18^{a}±0.10
	Ash (%)			
Winter (Feb)	25.52^{ab}±1.05	24.11^{a}±1.39	29.16^{a}±1.05	**40.58^{a}±2.29**
Pre-monsoon (March – April)	22.79^{b}±1.11	23.41^{a}±1.25	26.45^{a}±0.86	32.58^{b}±1.06
Monsoon (June-August)	**26.21^{a}±0.46**	26.58^{a}±0.90	27.44^{a}±0.68	33.45^{b}±1.65
Post-monsoon (Sept)	26.58^{a}±0.77	26.02^{a}±0.83	27.69^{a}±0.54	34.67^{b}±1.98

*** No harvesting of *L. gibba* during May 2013 &October 2013 – January 2014 (Nil growth)**
Values are mean ± S. E.
Values with same superscript in column do not differ significantly (P≤ 0.05)

<u>Overall results</u> (*L. gibba*)

Among manures - Significantly highest crude protein (%) in **PD (33.97)** during **winters**, in **Urea+TSP** during **pre-monsoon (28.24)** and **monsoon (29.95)** and in **PD (23.21**) during post-monsoon, respectively.

Among seasons – February was found best for harvesting protein rice *L. gibba* biomass from PD (33.97).

5. Water quality analysis

Water quality parameters in terms of temperature, pH, hardness, total alkalinity, ammonical nitrogen, ortho-phosphate, nitrate nitrogen, recorded in different treatments (at fortnightly intervals), are presented in Table 9 &10. Water quality parameters did not vary significantly throughout the culture period.

Table -9 *L. minor* culture- Water quality parameters in different treatments during one year culture period (Feb. 2013 to Jan. 2014)

Treatments	Parameters						
	Temperature (^{0}C)	pH	Total Alkalinity (mgl^{-1})	Hardness (mgl^{-1})	Ortho Phosphates (mgl^{-1})	Ammonia-nitrogen (mgl^{-1})	Nitrate-nitrogen (mgl^{-1})
T1	21.73^a±0.54	8.32^a±0.64	379.64^a±0.67	409.09^a±0.78	1.139^a±0.98	0.059^a±0.87	0.478^a±0.69
T2	21.65^a±0.65	8.67^a±0.76	406.28^a±0.78	403.40^a±0.55	1.218^a±0.47	0.051^a±0.79	0.433^a±0.89
T3	21.12^a±0.45	8.63^a±0.56	405.35^a±0.64	406.85^a±0.86	1.176^a±0.76	0.082^a±0.65	0.446^a±0.87
T4	21.85^a±0.62	8.61^a±0.66	410.57^a±0.59	410.37^a±0.62	1.372^a±0.58	0.076^a±0.76	0.411^a±0.55
T5	21.78^a±0.70	8.65^a±0.57	418.35^a±0.68	412.71^a±0.59	1.250^a±0.54	0.007^a±0.69	0.526^a±0.78
T6	21.95^a±0.62	9.32^a±0.62	403.35^a±0.71	382.28^a±0.57	1.212^a±0.65	0.078^a±0.84	0.487^a±0.88
T7	21.97^a±0.53	8.60^a±0.59	414.07^a±0.39	412.28^a±0.86	1.375^a±0.87	0.098^a±0.69	0.539^a±0.74
T8	22.62^a±0.47	8.56^a±0.76	408.00^a±0.59	418.85^a±0.71	1.314^a±0.69	0.097^a±0.68	0.565^a±0.68
T9	23.08^a±0.52	8.23^a±0.78	418.78^a±0.89	397.71^a±0.79	1.200^a±0.76	0.107^a±0.78	0.524^a±0.76
T10	22.19^a±0.61	8.56^a±0.57	433.00^a±0.73	407.42^a±0.57	1.303^a±0.83	0.099^a±0.86	0.586^a±0.69
T11	22.23^a±0.60	8.58^a±0.89	423.71^a±0.59	413.85^a±0.59	1.216^a±0.69	0.109^a±0.82	0.542^a±0.75
T12	22.21^a±0.55	9.26^a±0.87	420.14^a±0.79	414.21^a±0.49	1.226^a±0.71	0.092^a±0.71	0.602^a±0.59
T13	22.36^a±0.54	8.45^a±0.69	414.57^a±0.59	404.70^a±0.61	1.411^a±0.69	0.063^a±0.59	0.413^a±0.82
T14	22.94^a±0.59	8.48^a±0.59	431.00^a±0.69	390.00^a±0.62	1.435^a±0.67	0.075^a±0.69	0.428^a±0.88
T15	22.20^a±0.62	8.56^a±0.59	434.57^a±0.78	378.30^a±0.85	1.278^a±0.88	0.079^a±0.71	0.499^a±0.67
T16	22.32^a±0.73	8.41^a±0.69	438.14^a±0.89	409.69^a±0.70	1.413^a±0.72	0.085^a±0.67	0.479^a±0.71
T17	22.24^a±0.87	8.44^a±0.84	434.57^a±0.81	423.69^a±0.67	1.367^a±0.75	0.052^a±0.88	0.469^a±0.84
T18	22.30^a±0.62	8.45^a±0.71	421.00^a±0.69	408.15^a±0.62	1.360^a±0.87	0.055^a±0.59	0.459^a±0.79
T19	21.47^a±0.61	8.30^a±0.74	373.07^a±0.68	389.80^a±0.71	1.313^a±0.86	0.055^a±0.73	0.506^a±0.60
T20	20.99^a±0.89	8.60^a±0.70	363.23^a±0.71	386.90^a±0.59	1.422^a±0.76	0.065^a±0.77	0.556^a±0.58
T21	21.61^a±0.76	8.58^a±0.69	365.23^a±0.69	396.00^a±0.68	1.311^a±0.88	0.049^a±0.54	0.627^a±0.70
T22	21.64^a±0.72	8.93^a±0.81	398.16^a±0.75	381.81^a±0.66	1.325^a±0.58	0.054^a±0.65	0.574^a±0.60
T23	23.08^a±0.69	8.51^a±0.56	402.08^a±0.69	392.90^a±0.79	1.274^a±0.67	0.063^a±0.68	0.503^a±0.78
T24	21.75^a±0.68	8.11^a±0.67	395.41^a±0.63	414.00^a±0.82	1.251^a±0.66	0.202^a±-.78	0.554^a±0.82

Table 10. *L. gibba* culture- Water quality parameters in different treatments during one year culture period (Feb. 2013 to Jan. 2014)

Treatments	Parameters						
	Temperature (^{0}C)	pH	Total Alkalinity (mgl^{-1})	Hardness (mgl^{-1})	Ortho-Phosphates (mgl^{-1})	Ammonical nitrogen (mgl^{-1})	Nitrate nitrogen (mgl^{-1})
T25	20.15 ^{a}±0.89	8.42 ^{a}±0.98	375.81 ^{a}±0.88	406.72 ^{a}±1.07	1.102 ^{a}±1.05	0.172 ^{a}±0.76	0.487 ^{a}±0.76
T26	19.48 ^{a}±1.04	8.52 ^{a}±0.77	374.16 ^{a}±0.67	392.00 ^{a}±0.96	1.175 ^{a}±0.89	0.196 ^{a}±0.55	0.497 ^{a}±0.55
T27	20.06 ^{a}±0.78	8.58 ^{a}±0.87	377.58 ^{a}±0.78	387.00 ^{a}±0.68	1.197 ^{a}±0.95	0.164 ^{a}±0.76	0.477 ^{a}±0.68
T28	19.82 ^{a}±1.05	8.47 ^{a}±0.68	415.54 ^{a}±0.89	415.81 ^{a}±0.89	1.021 ^{a}±0.78	0.176 ^{a}±0.78	0.492 ^{a}±0.61
T29	19.75 ^{a}±1.03	8.59 ^{a}±0.69	409.45 ^{a}±0.65	403.27 ^{a}±0.58	1.046 ^{a}±0.76	0.141 ^{a}±0.69	0.479 ^{a}±0.73
T30	21.85 ^{a}±0.95	8.63 ^{a}±0.78	338.09 ^{a}±0.57	401.00 ^{a}±0.73	1.057 ^{a}±0.88	0.189 ^{a}±0.72	0.439 ^{a}±0.84
T31	19.91 ^{a}±0.78	8.52 ^{a}±0.79	356.36 ^{a}±0.79	400.72 ^{a}±0.64	1.905 ^{a}±0.68	0.176 ^{a}±0.54	0.491 ^{a}±0.57
T32	20.04 ^{a}±0.82	8.63 ^{a}±0.77	365.00 ^{a}±0.89	395.45 ^{a}±0.57	1.036 ^{a}±0.72	0.189 ^{a}±1.05	0.458 ^{a}±0.75
T33	20.14 ^{a}±0.91	8.63 ^{a}±0.69	356.81 ^{a}±0.59	386.90 ^{a}±0.86	1.973 ^{a}±0.66	0.195 ^{a}±0.87	0.453 ^{a}±0.86
T34	20.79 ^{a}±0.87	8.57 ^{a}±0.87	345.09 ^{a}±0.81	398.09 ^{a}±0.78	1.869 ^{a}±0.79	0.150 ^{a}±0.67	0.461 ^{a}±0.63
T35	20.12 ^{a}±0.89	8.70 ^{a}±0.59	336.00 ^{a}±0.85	395.63 ^{a}±1.05	1.904 ^{a}±0.54	0.153 ^{a}±0.55	0.497 ^{a}±0.56
T36	21.10 ^{a}±0.76	8.69 ^{a}±0.67	345.63 ^{a}±0.78	392.18 ^{a}±0.67	1.890 ^{a}±0.72	0.103 ^{a}±0.67	0.452 ^{a}±0.84
T37	20.07 ^{a}±0.84	8.52 ^{a}±0.59	351.10 ^{a}±0.92	310.72 ^{a}±0.88	0.920 ^{a}±0.65	0.193 ^{a}±0.59	0.498 ^{a}±0.66
T38	20.39 ^{a}±0.82	8.73 ^{a}±0.78	356.60 ^{a}±0.67	389.45 ^{a}±0.54	0.892 ^{a}±0.67	0.203 ^{a}±0.68	0.468 ^{a}±0.61
T39	20.94 ^{a}±0.79	8.74 ^{a}±0.81	359.22 ^{a}±0.78	398.90 ^{a}±0.68	0.945 ^{a}±0.68	0.210 ^{a}±0.88	0.443 ^{a}±0.74
T40	20.72 ^{a}±0.78	8.77 ^{a}±0.74	350.00 ^{a}±0.89	356.18 ^{a}±0.67	0.809 ^{a}±0.78	0.189 ^{a}±0.58	0.435 ^{a}±0.73
T41	19.74 ^{a}±0.88	8.67 ^{a}±0.68	348.44 ^{a}±0.69	362.18 ^{a}±0.71	0.842 ^{a}±0.71	0.197 ^{a}±0.68	0.493 ^{a}±0.67
T42	20.44 ^{a}±0.79	8.73 ^{a}±0.71	347.11 ^{a}±0.89	386.45 ^{a}±0.63	0.933 ^{a}±1.08	0.164 ^{a}±0.61	0.448 ^{a}±0.84
T43	21.03 ^{a}±0.77	8.78 ^{a}±0.80	320.25 ^{a}±0.58	356.72 ^{a}±1.04	0.942 ^{a}±0.76	0.184 ^{a}±0.74	0.474 ^{a}±0.61
T44	21.03 ^{a}±0.83	8.69 ^{a}±0.68	329.75 ^{a}±0.69	344.54 ^{a}±0.89	0.807 ^{a}±0.89	0.160 ^{a}±0.58	0.442 ^{a}±0.59
T45	21.01 ^{a}±0.69	8.68 ^{a}±0.79	328.25 ^{a}±0.58	358.18 ^{a}±0.65	0.922 ^{a}±0.57	0.171 ^{a}±0.78	0.473 ^{a}±0.74
T46	21.14 ^{a}±0.78	8.67 ^{a}±0.89	313.33 ^{a}±0.68	353.72 ^{a}±0.83	0.878 ^{a}±0.76	0.162 ^{a}±0.67	0.449 ^{a}±0.64
T47	21.10 ^{a}±0.69	8.67 ^{a}±0.69	321.87 ^{a}±0.48	366.27 ^{a}±0.59	0.802 ^{a}±0.78	0.169 ^{a}±0.76	0.457 ^{a}±0.76
T48	21.12 ^{a}±0.59	8.65 ^{a}±0.85	333.87 ^{a}±0.78	357.63 ^{a}±0.57	0.857 ^{a}±0.57	0.195 ^{a}±0.54	0.472 ^{a}±0.88

Objective II (1st & 2nd Yr)

To find the optimum incorporation level of duckweeds (*L. minor* and *L. gibba*) in supplementary carp feed

Target

- Formulating cost effective nutritionally balanced *Lemna* incorporated diet for carps

Work plan to achieve the target

- Preparation of diets by inclusion of *L. minor* and *L. gibba* at different incorporation levels
- Proximate composition of feed ingredients and prepared diets.
- Feeding experiments of carp with different diets.
- Study of growth and water quality parameters (fortnightly intervals).

Experiment 1. Efficacy of *L. minor* and *L. gibba* incorporated supplementary diets in carps fingerlings (Nov. 2013- April 2014)

Methodology

Experimental setup

Experiment was carried out in FRP pools (5'3" x 3'8" x 2'5")

Duration of experiment	: 6 months (Nov. 2013 to April 2014)
No. of Treatments	: 11 - Ten *Lemna* based diets (5 for each *Lemna spp.*) & one control diet
No. of Replicates	: 03
Fish Species	: Common Carp (***Cyprinus carpio*** Linn.)

Culture of Duckweeds

L. minor and *L. gibba* stock were maintained in poly sheet (silpaulin) lined earthen pits (4 m^2) in net house. Soil layer (2 – 3 cm) was spread at the bottom of pits and manuring was done with slurry of 1 kg cow dung (CD) and 1 kg poultry droppings (PD), which was spread over the soil layer and water was filled up to 1.5' level. One kg fresh inoculum of duckweed was added after 1 week of manuring. Half of duckweed was harvested every time it covered the whole surface. Re-manuring was done with 1 kg of CD and 1 kg of PD slurry every fortnight. Harvested *Lemna* was sun dried and powdered for incorporation in different experimental diets.

Preparation of experimental diets

- Ten experimental supplementary diets were formulated by replacing basal diet (Control diet - C) at five different levels (10 - 50 %) with *L. minor* (D1, D2, D3, D4, D5) and *L. gibba* (D6, D7, D8, D9, D10) as given below

Table 11. Percent Composition of experimental diets

Ingredients	Control diet(C)	*L. minor* incorporated diets					*L. gibba* incorporated diets				
		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Rice bran*	49	44	39	34	29	24	44	39	34	29	24
Mustard meal*	49	44	39	34	29	24	44	39	34	29	24
L. minor*	-	10	20	30	40	50	-	-	-	-	-
L. gibba*	-	-	-	-	-	-	10	20	30	40	50
Vitamin Mineral mixture	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Common salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

* Deoiled, ** Sundried

- The proximate composition of feed ingredients, *L. minor, L. gibba* and prepared diets (Table 12 & 13) was carried out following the standard method of AOAC (2000).

Table 12. Proximate Composition (%) of feed ingredients (on DM basis)

Parameter	Feed Ingredients			
	Rice bran	Mustard meal	*L. minor*	*L. gibba*
Crude Protein	17.00	39.49	24.90	26.81
Ether Extract	1.45	1.25	1.94	1.87
Crude Fibre	17.75	11.85	10.36	8.90
Nitrogen Free Extract	51.90	40.24	32.05	37.12
Ash	11.90	7.17	30.75	25.30

Table 13. Proximate Composition (%) of experimental duckweed incorporated supplementary diets (on DM basis)

Parameters	Control diet(C)	*L. minor* incorporated diets					*L. gibba* incorporated diets				
		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Crude Protein	27.68	27.34	27.01	26.67	26.34	26.00	27.53	27.39	27.24	27.10	26.95
Ether Extract	1.32	1.38	1.42	1.49	1.55	1.61	1.37	1.42	1.47	1.52	1.57
Crude Fibre	14.50	14.05	13.61	13.16	12.72	12.28	13.91	13.32	12.73	12.14	11.55
Nitrogen Free Extract	47.16	45.77	44.38	42.96	41.56	40.17	46.27	45.38	44.48	43.59	42.71
Ash	9.34	11.46	13.58	15.72	17.83	19.94	10.92	12.49	14.08	15.65	17.22

Preparation of experimental pools

- A Soil base (1-2 inch) was provided at the bottom of experimental pools.
- Pools were filled with bore well water one week before the start of the experiment.
- Manuring of pools was done with cow dung @ 20,000 kg $ha^{-1}yr^{-1}$ (2.81 kg $pool^{-1}yr^{-1}$). One fourth of the manure (0.70 kg $pool^{-1}$) was applied one week prior to stocking of fish and rest in equal fortnightly intervals (0.09 kg $pool^{-1}$)

Fish stocking

- Each pool was stocked with common carp fingerlings @ 10/pool.
  Av. total body length 8.10 – 8.51cm
  Av. body weight 8.25 – 8.50 g

Feeding of fish

- Fish in each treatment was fed once a day during morning hours @ 2 % FBW.

Water Level

- Water level in culture pools was maintained up to 1.5".

Fish Growth estimation

- Fish sampling was done at fortnightly intervals to record total body length and weight. Net weight gain (NWG), percent net weight gain (%NWG), specific growth rate

(SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) for every treatment were calculated as per standard formulae given below (Table – 14 & 15)

NWG = Average final body wt. (g) - Average initial body wt. (g)

%NWG=Final body weight (g) - initial body weight (g) /initial body weight (g) x 100

$$\textbf{SGR (\% increase in weight /day)} = \frac{\text{ln final body wt - ln initial body wt.}}{\text{Culture days}} \times 100$$

ln = natural logrithum

$$\textbf{FCR} = \frac{\text{Feed given (g)}}{\text{Weight gain (g)}}$$

$$\textbf{PER} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

Water quality estimation

- Water quality parameters w.r.t temperature, pH, dissolved oxygen, total alkalinity, hardness, ortho-phosthate and ammonical nitrogen was carried out at fortnightly intervals (Table -16), following standard methods (APHA, 2005)

Statistical analysis

The data was statistically analysed using Statgraphic statistical package SPSS-16. One way ANOVA and Duncan's multiple range test was applied to work out the effect of different diets on water quality and growth of experimental fish to determine differences among the treatments at 5% significance level ($P \leq 0.05$).

Table -14. Growth parameters (g) of *C. carpio* fed with *L. minor* and *L. gibba* incorporated supplementary diets

Parameters	C (Control)	D1	D2	D3	D4	D5	C (Control)	D6	D7	D8	D9	D10
		***L. minor* based diets**						***L. gibba* based diets**				
Av. Initial length	7.77 [a] ±0.36	8.54 [a] ±0.20	8.52 [a] ±0.25	8.46[a] ±0.26	8.12 [a] ±0.21	8.14 [a] ±0.23	7.77[a] ±0.36	8.18 [a] ±0.21	8.10 [a] ±0.22	8.39 [a] ±0.23	7.84[a] ±0.18	8.64 [a] ±0.21
Av. Final length	9.72 [a] ±0.28	9.77 [a] ±0.31	9.78 [a] ±0.20	9.52 [a] ±0.26	9.42 [a] ±0.35	9.59 [a] ±0.25	9.72[ab] ±0.28	8.98 [cd] ±0.21	9.00[cd] ±0.22	10.09 [a] ±0.17	8.67[d] ±0.14	9.37[bc] ±0.22
Av. Initial weight	8.53 [a] ±0.47	8.25 [a] ±0.48	8.64 [a] ±0.51	8.38 [a] ±0.64	8.25 [a] ±0.66	8.50 [a] ±0.59	8.53[a] ±0.47	8.25 [a] ±0.54	8.25 [a] ±0.54	8.25 [a] ±0.54	8.25 [a] ±0.54	8.42 [a] ±0.54
Av. Final weight	13.90 [b] ±0.71	16.70 [a] ±1.28	13.80 [b] ±0.65	14.00 [b] ±0.49	12.90[bc] ±1.22	11.00 [c] ±0.65	13.90[a] ±0.71	14.50 [a] ±0.62	12.70[a] ±0.99	10.70[b] ±0.56	10.10[b] ±0.41	9.70[b] ±0.50
% NWG	62.95	102.42	59.72	67.06	56.36	29.41	62.95	75.75	53.93	29.69	22.42	15.20
SGR	0.27	0.39	0.26	0.28	0.25	0.14	0.27	0.31	0.24	0.14	0.11	0.08
PER	1.58	1.93	1.56	1.72	1.54	1.50	1.58	1.69	1.36	1.23	1.18	1.04
FCR	2.29	1.89	2.36	2.17	2.45	2.56	2.29	2.14	2.69	2.98	3.12	3.54

Values are mean ± S. E.($P<0.05$); Values with same superscript in row do not differ significantly ($P\leq 0.05$)
Control was same for both *L. minor* and *L. gibba* incorporated diets, but was compared separately (C and D1-D5 & C and D6-D10) to know the significant differences of both the species separately from control

Table 15. Water quality parameters in different treatments – *L. minor* & *L. gibba* incorporated supplementary diets

Parameter	Control (C)	D1	D2	D3	D4	D5	Control (C)	D6	D7	D8	D9	D10
pH	7.89[a] ±0.11	8.00[a] ±0.00	7.89[a] ±0.11	8.00[a] ±0.00	7.89[a] ±0.11	7.89[a] ±0.11	7.88[a] ±0.11	7.88[a] ±0.11	8.00[a] ±0.00	7.88[a] ±0.11	8.00[a] ±0.00	7.88[a] ±0.11
Temperature (^{0}C)	14.03[a] ±1.12	14.12[a] ±1.14	14.16[a] ±1.14	14.31[a] ±1.12	14.27[a] ±1.12	14.35[a] ±0.44	14.03[a] ±1.12	14.03[a] ±1.12	14.12[a] ±1.14	14.15[a] ±1.14	14.31[a] ±1.11	14.26[a] ±1.11
D.O.(mgl^{-1})	5.66[a] ±0.91	5.66[a] ±0.94	5.44[a] ±1.00	4.77[a] ±1.00	5.00[a] ±0.68	4.88[a] ±0.91	5.67[a] ±0.91	5.67[a] ±0.91	5.67[a] ±0.94	5.44[a] ±1.06	4.78[a] ±1.09	5.00[a] ±0.68
Total alkalinity (mgl^{-1})	428[a] ±7.24	436[a] ±9.42	419[a] ±8.39	428[a] ±8.91	433[a] ±7.30	427[a] ±8.39	428[a] ±7.24	428[a] ±7.24	436[a] ±9.42	419[a] ±8.39	428[a] ±8.91	433[a] ±7.30
Total hardness (mgl^{-1})	406[b] ±11.94	413[ab] ±12.38	433[ab] ±13.43	436[ab] ±10.97	445[a] ±10.89	433[ab] ±10.06	406[b] ±1.19	406[b] ±1.19	413[ab] ±1.23	433[ab] ±1.34	436[ab] ±1.09	445[a] ±1.08
Orthophosphate (mgl^{-1})	1.56[a] ±0.11	0.79[b] ±0.04	0.82[b] ±0.10	1.00[b] ±0.10	0.76[b] ±0.08	0.80[b] ±0.14	1.48[a] ±0.16	1.21[a] ±0.13	1.23[a] ±0.21	1.69[a] ±0.10	1.28[a] ±0.24	1.30[a] ±0.17
Ammonical-N (mgl^{-1})	0.10[a] ±0.02	0.11[a] ±0.01	0.11[a] ±0.27	0.10[a] ±0.03	0.11[a] ±0.03	0.03[a] ±0.00	0.04[a] ±0.00	0.04[a] ±0.01	0.02[a] ±0.01	0.02[a] ±0.01	0.03[a] ±0.00	0.03[a] ±0.01

Values are mean ± S. E.; Values with same superscript in row do not differ significantly ($P \leq 0.05$)

Control was same for both *L. minor* and *L. gibba* incorporated diets, but was compared separately (C and D1-D5 & C and D6-D10) to know the significant differences of both the species separately from control

Results

Water quality

Optimum water quality is required for optimum growth of fish under controlled conditions. In the experimental present study, the water temperature (14.03-14.35^{0}C), pH (7.88-8.00), dissolved oxygen (4.77-5.67 mgl^{-1}), total alkalinity (419-436 mgl^{-1}), total hardness (406-445 mgl^{-1}), orthophosphate (0.76-1.69 mgl^{-1}) and ammonical nitrogen (0.02-0.11 mgl^{-1}) were well within the recommended range (Boyd and Tucker, 1998) in all the treatments for supporting optimum growth in carps throughout the culture period and the differences among treatments were insignificant ($p<0.05$).

Fish Survival -At the termination of the experiment, 100 % survival of *C. Carpio* was recorded in all the treatments and control showing equal acceptability of *Lemna* incorporated diets to that of control diet.

Fish Growth

i. *L. minor* incorporated supplementary diets

The final body weight (g) in different treatments increased from 8.53 to 13.90 in C, 8.25 to 16.70 in D1, 8.64 to 13.80 in D2, 8.38 to 14.00 in D3, 8.25 to 12.90 in D4 and 8.50 to 11.00 in D5. At the termination of the experiment, average final body weight (g) of fish was maximum in D1 (16.70), followed by D3 (14.00), C (13.90), D2 (13.80), D4 (12.90) and D5 (11.00) respectively and the differences were significant (D1>D3=C=D2≥D4≥D5). %NWG, SGR and PER was maximum in D1 (102.42, 0.39 and 1.93) and minimum in D5 (29.41, 0.14, 1.50). Likewise FCR was minimum in D1 (1.89) and maximum in D5 (2.56) showing maximum feed efficiency of diet D1. Overall results revealed that sundried *L. minor* can be incorporated in carp diet up to 40 % level without having any negative impact on fish growth, however, best results in terms of fish growth were recorded at 10 % incorporation level, which resulted in 20.14 % higher growth in terms of body weight.

ii. *L. gibba* incorporated supplementary diets

The final body weight (g) in different treatments increased from 8.53 to 13.90 in C, 8.25 to 14.50 in D6, 8.25 to 12.70 in D7, 8.25 to 10.70 in D8, 8.25 to 10.10 in D9 and 8.42 to 9.70 in D10. At the termination of the experiment, average final body weight (g) of fish was maximum in D6 (14.50), followed by C (13.90), D7 (12.70), D8 (10.70), D9 (10.10) and D10 (9.70) respectively and the differences were significant (D6=C=D7>D8=D9=D10). %NWG, SGR and PER was maximum in D6 (75.75, 0.31 and 1.69) and minimum in D10 (15.20, 0.08, 1.04). Likewise FCR was minimum in D6 (2.14) and maximum in D10 (3.54) showing maximum feed efficiency of diet D6. Overall results revealed that sun dried *L. gibba* can be incorporated in carp diet up to 20 % level without having any negative impact on fish growth, however, best results in terms of fish growth were recorded at 10 % incorporation level, which resulted in 4.32 % higher growth in terms of body weight.

L. minor* Vs. *L. gibba

Of the two duckweed species tested, sun dried ***L. minor*** was found to have **more potential** for utilization as non-conventional feed resource in carp feed **(up to 40 % incorporation level) with additional dual benefit in terms of feed cost reduction and fish growth enhancement** at 10 % incorporation level. In contrast, sun dried *L. gibba* was found suitable for incorporation in carp diet up to 20 % level only, with additional dual benefit in terms of feed cost reduction and fish growth enhancement at 10 % level.

<u>Objectives III-V (3rd Year)</u>

- To find the optimum incorporation level of duckweeds in supplementary carp feed
- To study the economics of culture
- To disseminate the technology to farmers for developing cost effectives feeds for carp polyculture system

Targets

- Formulating cost effective nutritionally balanced *L. minor* incorporated diet for carps in semi-intensive culture system
- Comparative economic analysis

Work plan to achieve the target

- Feeding experiments of carps with *L. minor* incorporated diets in cemented tanks
- Study of water quality parameters
- Biochemical analysis of fish flesh (nutritive value estimation)
- Dissemination of technology through demonstration and trainings to farmers

<u>Work Done</u>

Experiment - Efficacy of *L. minor* incorporated supplementary diets for carps in semi-intensive culture system

Methodology

Experimental setup

Experiment was carried out in cemented tanks 20 m^2

Duration of experiment	: 06 months
	Growth studies - January to June 2015 (06 months)
	Biochemical studies – July 2015 to September 2015
No. of Treatments	: 06 (*L. minor* based diets & 1 control diet)
No. of Replicates	: 03
Fish Species	: Rohu (***Labeo rohita***) & Common Carp (***Cyprinus carpio)***

Culture of Duckweeds

L. minor stock was maintained in poly sheet (silpaulin) lined earthen pits (4 m^2) in net house. Soil layer (2 – 3 cm) was spread at the bottom of pits and manuring was done with slurry of 1 kg cow dung (CD) and 1 kg poultry droppings (PD), which was spread was over the soil layer and water was filled up to 1 m level. One kg fresh inoculum of duckweed was added after 1 week of manuring. Half of duckweed was harvested every time it covered the whole surface. Re-manuring was done with 1 kg of CD and 1 kg of PD slurry every fortnight. Harvested *Lemna* was sun dried and powdered for incorporation in different experimental diets.

Preparation of experimental diets

- Five experimental supplementary diets were formulated by replacing basal diet (Control diet-C) at five different levels (10 - 50 %) with *L. minor* (D1, D2, D3, D4, D5) as given below

Table 18. Composition (%) of experimental diets

Ingredients	**Control diet (C)**	***L. minor* incorporated diets**				
		D1	**D2**	**D3**	**D4**	**D5**
Rice bran*	49	44	39	34	29	24
Mustard meal*	49	44	39	34	29	24
Sun-dried *L. minor*	-	10	20	30	40	50
Vitamin Mineral mixture	1.5	1.5	1.5	1.5	1.5	1.5
Common salt	0.5	0.5	0.5	0.5	0.5	0.5

*Deoiled

- The proximate composition of feed ingredients, *L. minor* and prepared diets (Table 19 & 20) was carried out following the standard method of AOAC (2000).

Table 19. Proximate Composition (%) of feed ingredients (on DM basis)

Parameter	**Rice Bran**	**Mustard meal**	***L. minor***
Crude Protein	17.00	39.49	24.90
Ether Extract	1.45	1.25	1.94
Crude Fibre	17.75	11.85	10.36
Nitrogen Free Extract	51.90	40.24	32.05
Ash	11.90	7.17	30.75

Table 20. Proximate Composition (%) of experimental duckweed incorporated supplementary diets (on DM basis)

Parameters	Control diet (C)	*L. minor* incorporated diets				
		D1	D2	D3	D4	D5
Crude Protein	27.68	27.34	27.01	26.67	26.34	26.00
Ether Extract	1.32	1.38	1.42	1.49	1.55	1.61
Crude Fibre	14.50	14.05	13.61	13.16	12.72	12.28
Nitrogen Free Extract	47.16	45.77	44.38	42.96	41.56	40.17
Ash	9.34	11.46	13.58	15.72	17.83	19.94

Preparation of experimental tanks

- A Soil base (1-2 inch) was provided at the bottom of experimental tanks.
- Tanks were filled with bore well water one week before the start of the experiment.
- Manuring of all the tanks was done with cow dung @ 20,000 kg ha^{-1} yr^{-1} (40 kg $tank^{-1}$ yr^{-1}). One fourth of the manure (10 kg $tank^{-1}$) was applied 15 days prior to stocking of fish and rest in equal fortnight instalments (2.5 kg $tank^{-1}$).

Fish stocking

- Fry of rohu, *L. rohita* (Ham.) and common carp, *C. carpio* (Linn.) were stocked @ 10,000 ha^{-1} (20 fish $tank^{-1}$ viz. rohu – 10, common carp – 10).
- Stocking size of rohu
  - Av. total body length 4.21-4.30 cm
  - Av. body weight 1.10 – 1.12 g
- Stocking size of common carp
  - Av. total body length 4.61-4.71 cm
  - Av. body weight 1.41-1.54 g

Feeding of fish

- Fish were fed with different diets @ 5 % fish body weight (FBW) for the first two months and 2 % FBW for following four months.

Water Level

- Water level in experimental tanks was maintained up to 1.5”

Water quality estimation

Water quality parameters w.r.t temperature, pH, dissolved oxygen, total alkalinity, hardness, ortho-phosthate, ammonical nitrogen and nitrate nitrogen analyzed at fortnightly intervals (Table -21), following standard methods (APHA, 2005).

Fish Growth and flesh quality estimation

Growth parameters - Fish sampling was done at fortnightly intervals to record total body length and weight. Net weight gain (NWG), percent net weight gain (%NWG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) for every treatment were calculated as per standard formulas (Table – 22)

Biochemical analysis - Flesh samples of both the fish species were collected from each treatment at the end of the experiment and flesh quality in terms of total protein (Lowery *et al.*, 1951), total lipids (Folch *et al*., 1957), total carbohydrates (Dubois *et al.*, 1965), moisture and ash contents were estimated (Table – 22).

Statistical analysis

The data was statistically analysed using Statgraphic statistical package SPSS-16. One way ANOVA and Duncan's multiple range test was applied to work out the effect of different diets on water quality and growth of experimental fish to determine differences among the treatments at 5% significance level ($P \leq 0.05$).

Table 21. Water quality parameters in different treatments during the experimental period

Parameter	C	D1	D2	D3	D4	D5
Temperature (^{0}C)	29.39^a±0.71	29.26^a±0.70	29.15^a±0.69	29.24^a±0.74	29.07^a±0.75	29.17^a±0.71
pH	8.60^b±0.04	8.69^{ab}±0.05	8.65^{ab}±0.03	8.69^{ab}±0.03	8.73^a±0.05	8.67^{ab}±0.04
D.O. (mgl^{-1})	7.28^a±0.17	7.00^a±0.19	7.37^a±0.26	6.97^a±0.20	7.38^a±0.23	7.49^a±0.30
Total alkalinity (mgl^{-1})	167.08^a±0.63	174.77^a±0.28	184.62^a±0.68	172.62^a±0.23	171.69^a±0.51	184.15^a±0.25
Total hardness (mgl^{-1})	174.46^a±0.93	183.39^a±0.95	181.08^a±0.94	186.31^a±0.68	198.92^a±0.67	200.92^a±0.46
Orthophosphate (mgl^{-1})	0.203^a±0.01	0.216^a±0.02	0.185^a±0.1	0.212^a±0.02	0.200^a±0.03	0.208^a±0.02
Ammonical-N (mgl^{-1})	0.099^a±0.01	0.119^a±0.02	0.122^a±0.01	0.012^a±0.01	0.013^a±0.01	0.111^a±0.01
Nitrate-N (mgl^{-1})	0.159^a±0.02	0.148^a±0.01	0.134^a±0.02	0.146^a±0.02	0.148^a±0.02	0.153^a±0.02

Values are Mean ±S. E.
Values with same superscript in row do not differ significantly ($P \leq 0.05$)

Table.22. Changes in growth parameters and flesh composition (g/100g on wet weight basis) of *L. rohita* and *C. carpio* fed on experimental diets

Parameters	Diets						Diets					
	L. rohita						*C. carpio*					
	C	D1	D2	D3	D4	D5	C	D1	D2	D3	D4	D5
Av. Initial length (cm)	4.3 ^{a} ± 0.072	4.25^{a} ±0.088	4.21 ^{a} ±0.091	4.29 ^{a} ±0.065	4.25 ^{a} ±0.064	4.28 ^{a} ±0.065	4.71^{a} ±0.079	4.67^{a} ±0.106	4.61^{a} ±0.225	4.61^{a} ±0.116	4.62^{a} ±0.109	4.67^{a} ±0.095
Av. Final length (cm)	14.00 ^{a} ±0.105	14.22^{a} ±0.478	13.43^{b} ±0.487	13.47^{b} ±0.481	14.17^{a} ±0.472	13.94^{a} ±0.372	15.00^{b} ±0.247	15.8^{a} ±0.451	13.22^{d} ±0.239	13.97 ^{cd} ±0.218	14.17 ^{c} ±0.183	14.25^{bc} ±0.239
Av. Initial weight (g)	1.12^{a} ±0.045	1.09^{a} ±0.046	1.13^{a} ±0.069	1.11^{a} ±0.06	1.11^{a} ±0.068	1.13^{a} ±0.062	1.54^{a} ±0.084	1.53^{a} ±0.068	1.48^{a} ±0.110	1.41^{a} ±^{a}0.118	1.51^{a} ±^{a}0.094	1.51^{a} ±0.082
Av. Final weight (g)	35.70^{a} ±1.086	36.40^{a} ±0.718	30.70^{b} ±0.857	29.00^{b} ±0.650	26.75^{cd} ±0.891	26.20^{cd} ±0.879	55.00^{a} ±3.187	56.70^{a} ±3.246	38.90^{b} ±1.853	38.80^{b} ±2.275	40.60^{b} ±1.634	41.30^{b} ±1.967
% NWG	3087.5	3239.45	2616.68	2512.61	2309.91	2218.58	3471.43	3605.88	2528.37	2651.77	2588.74	2635.10
SGR	1.92	1.95	1.84	1.81	1.77	1.74	1.98	2.01	1.81	1.84	1.82	1.83
PER	1.87	1.96	1.72	1.80	1.80	1.81	1.91	2.09	1.72	1.78	1.82	1.77
FCR	1.93	1.87	2.15	2.08	2.11	2.12	1.89	1.75	2.15	2.11	2.09	2.17
Flesh Composition												
Total Protein	14.07^{a} ±0.43	15.53^{a} ±0.55	15.37^{a} ±0.48	15.43 ^{a} ±0.54	14.93^{a} ±0.26	14.47^{a} ±0.26	13.87^{a} ±0.35	14.45^{a} ±0.34	14.45^{a} ±0.36	14.12^{a} ±0.31	14.11^{a} ±0.28	13.89^{a} ±0.45
Total lipid	2.10 ^{ab} ±0.24	2.41 ^{a} ±0.13	2.69^{a} ±0.06	2.69^{a} ±0.03	2.32 ^{ab} ±0.02	2.25 ^{ab} ±0.11	2.78^{ab} ±0.21	3.43^{a} ±0.23	3.10^{a} ±0.16	3.12^{a} ±0.13	3.23^{a} ±0.12	3.15^{a} ±0.21
Total carbohydrates	2.97^{ab} ±0.06	2.10^{b} ±0.21	2.19^{b} ±0.05	2.14^{b} ±0.37	3.80 ^{a} ±0.04	3.37 ^{a} ±0.46	2.57^{a} ±0.16	2.43^{a} ±0.18	2.12^{b} ±0.15	2.17^{b} ±0.27	2.56^{a} ±0.14	2.18^{b} ±0.26
Ash	1.35^{ab} ±0.23	1.06^{b} ±0.17	1.78^{a} ±0.25	1.57^{a} ±0.18	1.57^{a} ±0.17	1.09^{b} ±0.21	1.29^{b} ±0.26	1.06^{b} ±0.21	2.01^{ab} ±0.35	2.61^{a} ±0.28	2.60^{a} ±0.27	2.52^{a} ±0.15
Moisture	79.51^{a} ±0.62	78.90 ^{a} ±0.53	77.97 ^{a} ±0.28	78.17^{a} ±0.08	77.38 ^{a}±0.13	78.82 ^{a} ±0.46	79.25^{a} ±0.34	78.63^{a} ±0.25	78.32^{a} ±0.31	77.98^{a} ±0.17	77.50^{a} ±0.28	77.98 ^{a} ±0.51

Values are mean ± S.E; Values with same superscript in row do not differ significantly (P≤ 0.05) Control was same for both *L. minor* and *L. gibba* incorporated diets, but was compared separately (C and D1-D5 & C and D6-D10) to know the significant differences of both the species separately from control

Results

Water quality

Optimum water quality is required for optimum growth of fish under controlled conditions. In the experimental present study, the water temperature (29.07-29.39^{0}C), pH (8.60-8.73), dissolved oxygen (6.97-7.49 mgl^{-1}), total alkalinity (167.08-184.62 mgl^{-1}), total hardness (174.46-200.92 mgl^{-1}), orthophosphate (0.185-0.216 mgl^{-1}), ammonical nitrogen (0.012-0.122 mgl^{-1}) and nitrate nitrogen (0.134-0.159 mgl^{-1}) were well within the recommended range (Boyd, 1992; Boyd and Tucker, 1998) in all the treatments for supporting optimum growth in carps throughout the culture period and the differences among treatments were insignificant.

Fish Survival -At the termination of the experiment, 100 % survival of *L. rohita* and *C. carpio* was recorded in all the treatments and control showing equal acceptability of *Lemna* incorporated diets to that of control diet.

Fish Growth

Labeo rohita

The final body weight (g) in different treatments increased from 1.12 to 35.70 in C, 1.09 to 36.40 in D1, 1.13 to 30.70 in D2, 1.11 to 29.00 in D3, 1.11 to 26.75 in D4 and 1.13 to 26.20 in D5. At the termination of the experiment, average final body weight (g) of fish was maximum in D1 (36.40), followed by C (35.70), D2 (30.70), D3 (29.00), D4 (26.75) and D5 (26.20) respectively and the differences were significant (D1=C>D2=D3≥D4=D5). %NWG, SGR ad PER was maximum in D1 (3248.67, 1.95 and 1.96) and minimum in D5 (2218.58, 1.74 and 1.81). Likewise FCR was minimum in D1 (1.87) and maximum in D5 (2.12) showing maximum feed efficiency of diet D1. Overall growth results showed 10 % incorporation level of *L. minor* as best among all other treatments and control.

C. carpio

The final body weight (g) in different treatments increased from 1.54 to 55.00 in C, 1.53 to 56.70 in D1, 1.48 to 38.90 in D2, 1.41 to 38.80 in D3, 1.51 to 40.60 in D4 and 1.51 to 41.30 in D5. At the termination of the experiment, average final body weight (g) of fish was maximum in D1 (56.70), followed by C (55.00), D5 (41.30), D4 (40.60), D2 (38.90) and D3 (38.80) respectively and the differences were significant (D1=C>D2=D3=D4=D5). %NWG, SGR ad PER was maximum in D1 (3605.88, 2.01 and 2.09) and minimum in D2 (2218.58, 1.81 and 1.72). Likewise FCR was minimum in D1 (1.75) and maximum in D5 (2.17) showing maximum feed efficiency of diet D1. Overall growth results showed 10 % incorporation level of *L. minor* as best among all other treatments and control.

Significantly higher growth in terms of final body weight, NWG %, SGR and PER along with minimum FCR was recorded in both the fish species with *L. minor* supplemented diet (D1) up to 10 % incorporation level. Fish growth declined with further incorporation of *L. minor.*

Flesh Quality

L. rohita

The flesh protein content (%) was maximum (15.53) in D1 and minimum (14.07) in C, but the protein content in different treatments did not differ significantly. Maximum total lipid (%) was recorded in D2 and D3 (2.69) and minimum in C (2.10) and the lipid content among treatments did not differ significantly. The total carbohydrate content (%) was maximum (3.80) in D4 and minimum (2.10) in D1 and the difference among the treatments were not significant. Ash content (%) was maximum (1.78) in D2 and minimum (1.06) in D1 and the difference among treatments were significant (D2=D3=D4≥C≥D5=D1). The flesh moisture content (%) was maximum (79.51) in C and minimum (77.38) in D4 but the moisture content in different treatments did not differ significantly.

C. carpio

The flesh protein content (%) was maximum (14.45) in D1 and D2, and minimum (13.87) in C, but the protein content in different treatments did not differ significantly. Maximum total lipid (%) was recorded in D1 (3.43) and minimum in C (2.78) and the lipid content among treatments did not differ significantly. The total carbohydrate content (%) was maximum (2.57) in C and minimum (2.12) in D2 and the difference among the treatments were significant (C=D4=D1>D5=D3=D2). Ash content (%) was maximum (2.61) in D3 and minimum (1.29) in C and the difference among treatments were significant (D3=D4=D5≥D2≥C=D1). The flesh moisture content (%) was maximum (79.25) in C and minimum (77.50) in D4 but the moisture content in different treatments did not differ significantly.

Results revealed that sun dried *L. minor* can be incorporated in carp diet up to up to 10 % incorporation level, without compromising the fish growth. *Lemna* incorporation at higher level (> 10 %) resulted in reduced fish growth, due to decreased apparent protein digestibility (Hassan *et al*., 1990) of plant protein sources at higher levels. Decline in fish growth after certain inclusion level of any ingredient can also be attributed to the presence of anti-nutritional factors, which could directly or indirectly (through their metabolic products) interfere with food utilization, and hence affects health and production of animals (Fasakin *et al.,* 2001). Further, the ash content of diets increased with *Lemna* inclusion level from 10-50 %, showing depressing effect of higher concentration of minerals on fish growth after 10 % inclusion level. Flesh quality of rohu and common carp in terms of total protein and lipids did not vary significantly among *Lemna* incorporated diets (10-50 %) indicating that although amino acid profile of *Lemna* is superior among various plant protein sources, but it has not affected the flesh composition of fish.

Overall results of the experimental studies revealed that dried *Lemna* powder can be incorporated in carp diet @ 10 % by replacing conventional feed ingredients without compromising fish growth and flesh quality. Hence, *L. minor* can be incorporated in carp diet @ 10 % level by replacing basal diet ingredients.

Conclusions

S. No.	Results	Conclusion/Recommendations
1.	Suitable duckweed species w. r. t. growth response under culture conditions throughout the year, no. of harvestings, biomass productivity, winter tolerance and nutritive value	***L. minor***
2.	Best manure w. r. t. productivity	Poultry droppings
3.	Best dose of poultry droppings w. r. t. Productivity	600 kg/ha/wk
4.	Best manure w. r. t. nutritive value	Cow Dung : Poultry droppings (1:1)
5.	Best dose of Cow Dung : Poultry droppings (1:1) w. r. t. nutritive value	600 kg/ha/wk
6.	Best months w. r. t. productivity	March, May, July, August & September
8.	Best duckweed species for incorporation in carp feed	***L. minor***
9.	Sun dried *L. minor* incorporation level in carp fingerling diet	Up to 40 % level with additional dual benefit at 10 % incorporation level with 7 % reduction in feed cost and over 20.4 % enhancement in fish growth
10.	Sun dried *L. minor* incorporation level in carp fry diet	Up to 10 % incorporation level

- ***L. minor*** culture can be taken up under local climatic conditions of Punjab by using organic manures like **Poultry droppings** (@ 600 kg/ha/wk) and combination (1:1) of **cow dung and poultry droppings** (@ 600kg/ha/wk) during **pre-monsoon and monsoon months**.
- Although, the harvested biomass can be utilized as feed resource in carp feed i.e., **up to 40% in fingerling diet** (27.7% feed cost reduction) and up to **10% in fry diet** (7% feed cost reduction), but it is **recommended for incorporation in fingerling diet** for higher economic returns in terms of both feed cost reduction from 7 to 27.7% (up to 40% incorporation level) and 20% higher fish growth (at 10% incorporation level).

ACHIEVEMENTS FROM THE PROJECT

- Package of practice for culture of duckweed species, *L. minor*
- Low cost *Lemna* based carp diets

CONTRIBUTION TO THE SOCIETY

Duckweed culture technology has ample scope of application at framer as well as community level. e.g.

- Unutilized or underutilized nutrient rich water resources such as village ponds can be utilized for duckweed production, which will not only bio-remediate the eutrophic village pond but also help in yielding protein rich biomass in the form of duckweed.
- Enhanced aquaculture productivity of bio-remediated village ponds
- Harvested duckweed holds immense potential for utilization as fodder/feed resource (both in fresh and dried form) in livestock feeding, including fish.
- Duckweed culture can also be taken as a backyard activity in livestock shed waste water pits, which will not only help in addressing environmental issues w.r.t. waste disposal but also recover waste water nutrients for recycling in aquaculture in the form of a protein rich duckweed biomass.
- Duckweed culture can be integrated with fish framing activity through utilizing the dyke space without much labour and input cost.

References

Ahamad, M.U., Swapon, M.R.S., Yeasmion, T.U., Rahman, M.S., Ali and M.S., 2003.Replacement of sesame oil cake by Duckweed (*Lemna minor*) in broiler diet. *Pakistan Journal of Biological Sciences*. **6**: 1450-1453.

Ansal, M. D. and Dhawan, A. 2009. Efficacy of Duckweed - *Spirodela* for low cost carp feed formulation. *Indian Journal of Animal Nutrition.* **26:** 378-383.

Ansal, M. D., Dhawan, A and Kaur, V.I. 2008. Efficacy of duckweed-*Spirodela* in carp diet.*Indian Journal of Ecology*. 35: 139-142.

AOAC. 2000. Official Methods of Analysis. 17th ed., AOAC International, Gaithersburg, MD

APHA. 2005. Standard Methods for the Examination of Water and Wastewater. 21st Edition.*American Public Health Association*. Washington, D.C.

Bairagi, A., Ghosh, K.S., Sen, S.K. and Ray, A.K. 2002. Duckweed (*Lemna polyrrhiza*) leaf meal as a source of feedstuff in formulated diets for rohu (*Labeo rohita* Ham.) fingerlings after fermentation with a fish intestinal bacterium. *Bioresource Technology*. **85:** 17-24.

Boyd, C. E. and Tucker, C. S. 1998. Pond Aquaculture water quality management. Kluwer Academic Publishers, Boston, London.

Dhawan, A. and Sharma, B. 2008.Efficacy of *Azolla* in carp diet. In: I.S.B. Singh, A. Mohandas, V. Joseph, S. S. Pai and L. Paul (eds.) Books of abstracts – 8th*Indian Fisheries Forum,* Kolkata. 22nd Nov to 26th Nov. 2008. P. 144.

Dhawan, A., Kaur, V. I. and Virk, P. 2004. Non- conventional fish feed resources: An overview. *Fishing Chimes.* **23:** 66-72.

Dubois, M., Gilles, K. A., Hamilton, J. K., Roberts, P. A. and Smith, F. 1956.Calorimetric method for the determination of sugars and related substances.*Alen.Chemistry*. **28:** 350-356.

Effiong, B.N., Sanni, A. and Fakunle, J.O. 2009.Effect of partial replacement of fishmeal with duckweed (*Lemna pauciscostata*) meal on the growth performance of *Heterobranchus longifilis* fingerlings. *Report and Opinion*.**1**: 76-81.

FAO. 2009. *Use of algae and aquatic macrophytes as feed in small scale aquaculture - A review*. FAO Fisheries and Aquaculture Technical Paper 531.FAO Publications, Rome, Italy.

Fasakin, E. A., Balogun, A. M. and Fasaru, B. E. 2001. Use of duckweed, *Spirodela polyrrhiza* L. Schleiden, as a protein feed stuff in practical diets of tilapia, *Oreochromis niloticus* L. *aquaculture Research.* **30** (5):8. 313-18.

Folch, J., Lees, M. and Salone-Stanley, G. H. 1957. A simple methods for the isolation and purification of the total lipids from animal tissues. *Journal of Biological Chemistry.* **226:** 497-509.

Hassan, M. R., Moniruzzaman, M. and Omar Farooque, A. M. 1990.Evalution of leucaena and water hyacinth leaf meal as a dietary protein sources for the fry of Indian major carp *Labeo rohita* (Hamilton).*In*: The second Asian Fisheries Forum (eds. Hirano R and Hanyu). Asian Fisheries Society, Manila, Philippines. pp. 275-282.

Hillman, W.S. and Culley, D.D. 1978. The uses of duckweeds.*American Scientist***. 66:** 442-451.

Iqbal, S. 1999. Duckweed aquaculture. Potentials, possibilities and limitations for combined waste water treatment and animal feed production in developing countries. SANDEC report no. 6, Duebenderf, Switzerland. pp.91.

Leng, R.A., Stambolie, J.H. and Bell, R. 1995. Duckweed: A potential high protein feed resource for domestic animal and fish. AAAP Conference. Proc. Bali., pp. 103-114.

Lowery, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. 1951.Protein measurements with Follin phenol reagent.*Journal of Biological Chemistry.* **193:** 265-75.

Mandal, R. N., Datta, A. K., Sarangi, N. and Mukhopadhyay, K. 2010.Diversity of aquatic macrophytes as food and feed components to herbivorous fish – a review. *Indian Journal of Fisheries* **57** (3): 65-73.

Mishra, B. 2007.On Duckweed, the multi-use Aquatic Plant. *Fishing Chimes*.**27**: 11-13.

Saha, J.K., Rahmatullah, S.M. and Mazid, M.A. 1999. Optimization of stocking density of duckweed, *Wolfiaarhiza* (Linn.) and *Lemna* sp. *Bangladesh Journal of Fisheries Research.***7:** 161-168.

Singh, T. P., Ansal, M. D. and Kaur, V. I. 2013. Efficacy of *Spirodela* incorporated diets in semi-intensive carp polyculture system – Growth and flesh quality of Indian major carps. *Indian Journal of Animal Nutrition.* **30** (1): 72-79.

Publications

Kaur, V. I., Ansal, M. D. and Dhawan, A. 2012. Effect of feeding duckweed (*Lemna minor*) based diets on the growth performance of rohu, *Labeo rohita* (Ham.). *Indian Journal of Animal Nutrition.* **29** (4): 406-409.

Kaur, V. I., Ansal, M. D. and Jassal, G. 2016. Effect of Duckweed (*Lemna minor*) Incorporated Diets on Growth Performance and Flesh Quality of Carps in Semi-Intensive Culture System. Extended abstract In: Indian Ecological Society International Conference –2016 Natural Resource Management: Ecological Perspective (Sher-e-Kashmir University of Agricultural Sciences & technology of Jammu, February 18-20, 2016.

Kaur, V.I., Ansal M D and Jassal, G. 2016. Effect of Duckweed (*Lemna minor*) Incorporated Diets on Growth Performance and Flesh Quality of Carps in Semi-Intensive Culture System. *Indian Journal of Ecology*. 43 (special issue 2): 692-696