International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 01-11-2021, Accepted: 17-11-2021, Published: 02-12-2021
Volume 6, Issue 6, 2021, Page No. 647-652

Consequences of changes in photosynthetic photon flux density (PPFD) and manuring practices on the fodder yield of *Azolla pinnata* Brown

Rex Immanuel R, G B Sudhagar Rao, K Boopathi, G Baradhan, S M Suresh Kumar
Department of Agronomy, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India

Abstract
Azolla is one of the most economic and efficient fodder substitutes for livestock, particularly as can be easily digested due to its high protein and low lignin content. However, their continuous supplies throughout the year especially during summer period in the coastal regions are limited. Efficient agronomic strategies may sustain the yield of *Azolla*. A pot culture experiment was conducted at Annamalai University Experimental Farm, Annamalai Nagar during 2016-2017 to formulate a sustainable *Azolla* fodder production technology under resource constraint situation. The experiment was laid out in split plot design with three replications. The treatments comprised of three levels of light penetration in main plots which favored various levels of Photosynthetic Photon Flux Density (PPFD) (M_1-100% light penetration, M_2-75% light penetration and M_3-50% light penetration) and nine nutrient management practice in sub plots S_1-Without manuring (control), S_2- FCM @ 0.2 per cent (v/w basis), S_3- FCM @ 0.4 per cent (v/w basis), S_4-FCM @ 0.6 per cent (v/w basis), S_5- FCM @ 0.8 per cent (v/w basis), S_6-EFYM @ 0.2 per cent (v/w basis), S_7-EFYM @ 0.4 per cent (v/w basis), S_8-EFYM @ 0.6 per cent (v/w basis) and S_9-EFYM @ 0.8 per cent (v/w basis). The interaction effect between levels of light penetration and nutrient management practice was found to be significant. Among the different treatment combinations, cultivation of *Azolla* under 1704.02 µmol/s/m^2 (100 per cent light penetration) with addition of EFYM 0.8% on v/w basis favorably recorded the maximum growth characters *viz.*, frond area (425 mm^{-2} $frond^{-1}$), density (70,147 m^{-2}), number of days taken to cover 1m^2 area (3.98), chlorophyll content (3.94 µg g^{-1}), fresh yield ad dry fodder yield (2,567 and 147 g m^{-2} $week^{-1}$, respectively).

Keywords: *Azolla*, enriched organic manures, green fodder, livestock, milk yield

Introduction

In India, 68 per cent of the population lives in rural areas with agriculture as their main occupation. Livestock support livelihood to more than two - third of the rural households, assuring year-round employment. They provide income and employment to the small holders and other weaker sections of the society including women, migratory tribal, small and marginal farmers. Most often livestock is the only source of cash income for subsistence farms and also serves as insurance in the event of crop failure due to climate change. Livestock plays an important role in Indian economy and support about 20.5 million people for their livelihood and contributed 16 per cent to the income of small farm house holds as against an average of 14 per cent for all rural households. It also provides employment to about 8.8 per cent of the population in India. The gross value added (GVA) of livestock sector is about Rs 962,682 crore at current prices during FY 2019-20 which is about 28.5 per cent of agricultural and allied sector GVA and 5.21 per cent of total GVA (DAHD, 2021) [5].

The increasing human population has created a greater demand for food in general and protein in particular. For the supply of essential amino acids in the diets of human being, supply of about 20-25 per cent of total daily protein requirement has to be made through the incorporation of good quality proteinase foods of animal origin. The area under forest and grasslands is decreasing and the amount of various crop residues available for feeding is also decreasing largely due to the introduction of crops with high yielding dwarf varieties. In addition, the area under food crops is also declining owing to urbanization and industrialization. In India the shortage of dry fodder, green fodder and concentrate has been estimated to 12 to 14, 25 to 35 and 30 to 35 per cent, respectively (Pathak *et al.*, 2015) [9].

Coastal regions are productive areas for cultivation of agricultural crops (Rex Immanuel *et al.*, 2010 [11]; Rex Immanuel and Ganapathy, 2010 [15]; Sudahar Rao *et al.*, 2020 [20]; Rex Immanuel *et al.*, 2021 [16]). These areas provides considerable amount of green or dry fodder. However, these areas are degraded due to several factors and distress the availability of quality fodder (Rex Immanuel and Ganapathy, 2007 [12]; Rex Immanuel and Ganapathy, 2019 [13]). Thus, the search for alternatives to green fodder in coastal region has led to utilize a wonder plant, *Azolla* (green gold) which holds the promise of providing a sustainable feed for livestock.

Azolla pinnata Brown is an aquatic floating fern that belongs to the family of *Azollaceae*. *Azolla* hosts symbiotic blue green algae, *Anabaena azollae*, which is responsible for the fixation and assimilation of atmospheric nitrogen in the form of proteins. *Azolla*, in turn, provides the carbon source and favorable environment for the growth and development of the algae. It is this unique symbiotic relationship that makes *Azolla* a wonderful plant with high protein content. The rate of nitrogen fixed is at about 25 kg ha^{-1}.

Under green fodder scarce situations, *Azolla* is being promoted as a feed supplement for livestock in many countries including India. Available reports indicated that, *Azolla* is an economic and efficient feed supplement for livestock, containing substantial amounts of protein, essential amino acids as well as fiber, vitamins (vitamin A, vitamin B_{12} and Beta- Carotene), growth promoter intermediaries and minerals like calcium, phosphorous, potassium, ferrous, copper, magnesium *etc* (Semwal Amit *et al*., 2016; [19] Rex Immanuel *et al*., 2019 [17]).

An overall increase of milk yield of about 15 per cent when 1.5 – 2 kg of fresh *Azolla* per day was combined with regular feed and the increase in the quantity of the milk production was higher than could be expected based on the nutrient content of *Azolla* alone. It is not only the nutrients, but also other components, like carotenoids, bio-polymers, probiotics *etc.*, that contributed to overall increase in the production of milk (Pillai *et al*., 2002 [10]).

Azolla has a tremendous significance on the future scenario of fodder market. The animal husbandry and livestock sectors are critical for the rural economy. Dependent communities contribute to their stable income and also their best insurance against any natural disasters. During the critical periods, *Azolla* biomass assures greater importance and served as a form of 'emergency green fodder bank' for livestock. Scientific research and farmer's experiences have demonstrated that protein and other nutrients from *Azolla* biomass during the dry season or off season constitute an essential element in animal diet.

According to 20th livestock census, the bovine population in the country is 304 million and goat population is 149 million (DAHD, 2021 [5]). At least 250 grams of *Azolla* utilized as green fodder for these animals the *Azolla* requirement of our country is more than 110 million kg day^{-1}. *Azolla* production in artificial wetlands does have several absolute agronomic traits that need to be evaluated. The optimum productivity in the right environment, best possible quantity of toxic free nutrients for its maximum production, altering microclimate for their mass production, biological control of pest and diseases, *etc.,* are essential for augmenting the yield and quality of fodder *Azolla* (Rex Immanuel *et al*., 2019 [17]). With this background, the present study was undertaken to find out the effect of different levels of light penetration and organic manuring practices on the growth and yield of *Azolla.*

Materials and Methods

A pot-culture experiment was carried out at Department of Agronomy, Annamalai University Experimental Farm, Annamalai Nagar during February to March, 2016-2017 to formulate the suitable agronomic management strategies for yield maximization of *Azolla* fodder production. The experimental farm is situated at 11°24' N latitude, 79°44' E longitude and at an altitude of +5.79 m above the mean sea level. The weather of Annamalai Nagar is moderately warm with hot summer months. The mean maximum temperature is 33.3°C while the mean minimum temperature is 23.6°C and with a mean relative humidity of 80 per cent. The mean annual rainfall is 1500 mm of which 1000 mm is received during North East monsoon, 400 mm during South West monsoon and 100 mm as summer showers.

Azolla pinnata Brown was used as a test species. The experiment was laid out in split plot design with three replications. The main plot consisted of three levels of light penetration treatments (M_1-100 % light penetration, M_2-75 % light penetration, M_3-50 % light penetration) and the sub-plot consisted of nine nutrient management treatments (S_1 - Without manuring (control), S_2 - FCM @ 0.2 per cent (v/w basis), S_3 - FCM @ 0.4 per cent (v/w basis), S_4 - FCM @ 0.6 per cent (v/w basis), S_5 - FCM @ 0.8 per cent (v/w basis), S_6 - EFYM @ 0.2 per cent (v/w basis), S_7 - EFYM @ 0.4 per cent (v/w basis), S_8 - EFYM @ 0.6 per cent (v/w basis) and S_9 - EFYM @ 0.8 per cent (v/w basis)).

Cement pots of 0. 5 m^2 area were chosen and durable plastic sheet (silpauline, a polythene tarpaulin) was spread in the pond, all the sides have secured properly by placing bricks over the side walls. Sieved fertile soil of about 15 kg was spread uniformly in the pot. About one kilogram of fresh *Azolla* culture is applied uniformly in the pond. The depth of water should be 20 cm maintained for cultivation of *Azolla*, it will ensure excellent and faster growth of *Azolla*. The irrigation water was found to be slightly saline in reaction (pH - 7.6) with medium level of soluble salts (EC - 1.58 dSm^{-1}). As a whole, the irrigation water was found to be good for irrigation.

The microclimate of the artificial pool area was also monitored. The light intensity was measured at canopy level of *Azolla* at every day at 8 AM, 12 Noon and 4 PM using Lux meter (Model no: LX-1102) and the average was recorded in 100 foot candle. Canopy and water temperature was measured by using Digital thermometer (Model no: HTC™ DT-2) and recorded at every day at 8 AM, 12 Noon and 4 PM and average was recorded in °C. Water temperature of *Azolla* pond was measured by using Digital thermometer (model no: HTC ™ DT-2) and recorded at every day at 8 AM, 12 Noon and 4 PM and average was recorded in °C. The measured lux units were converted into photosynthetic photon flux density (PPFD) using the procedure suggested by Thimijan and Heins (1982) [21].

Regularly after each irrigation, fresh cow dung manure and enriched farm yard manure (EFYM) is worked out based on the weight and volume basis and the required quantity of manure was added in each pond as per the treatment schedule. The EFYM used for the experiment was prepared by thoroughly mixing two percent of rock phosphate with well decomposed FYM and incubated for 21 days under anaerobic condition (CN ratio 17: 1, N 0.98%, P_2O_5 1.8% and K_2O 0.5%). The ponds are completely filled with water after that the *Azolla* are inoculated in the pond. Whenever the water in the pond is lost by any means, water was added to the pond up to the reference point (up to 20 cm).

Plant sample from individual plots were collected at weekly interval and the outline of the *Azolla* frond was plotted over a graph sheet and the total area was calculated and recorded in mm^{-2}. The number of *Azolla* plants in 1/10th square metre area were counted using quadrate at weekly interval and recorded in m^{-2}. The resprouting potential of *Azolla* was assesed between harvesting and average days taken to cover 1 m^2 area were recorded. The chlorophyll content of the treated and untreated *Azolla* plant was estimated at 7 days interval by using chlorophyll meter (Model no: CCM-200 plus) as suggested by Arnon (1949) [2].

Harvesting of *Azolla* was done separately according to treatment, to such an extent that small open spaces remain in the pond water. The first crop was harvested after the full growth *i.e* on 15th day after inoculation and the subsequent harvest was done at seven days interval. Plastic sieves are used to harvest the biomass from the pond's surface.

The plants were harvested at the specific period interval and the moisture was removed from the surface with blotting paper. The fresh weight was determined by using electronic balance and expressed as g m^{-2} $week^{-1}$. The average weight of *Azolla* was also calculated based on the weekly harvested data and expressed as g m^{-2} $week^{-1}$. Weighed masses of *Azolla* were dried in a hot air oven at 60˚C ± 5˚C and the dry weight was determined and recorded in g m^{-2} $week^{-1}$. *Azolla* was fed to the livestock in fresh form. Harvested *Azolla* was well washed with water before feeding to livestock. It was given to the animals mixed with concentrates at the ratio of 1: 1. The daily milk yields of tagged cow's were recorded in litter.

The data on various parameters studied during the investigation was statistically analyzed as per the procedures suggested by Gomez and Gomez (1984) [6]. The critical differences were also worked out at 5 per cent probability level for comparison.

Results and Discussion

Artificial shade effects on the microclimate of *Azolla* culture area

The effects of levels of light penetration and nutrient management on air at canopy level and water temperature of artificial pool are presented in Table 1. Among the different treatments, the maximum photosynthetic photon flux density (PPDF) of 1693.45 µmol/s/m^2 was registered under 100 per cent light penetration (M_1). However, the maximum PPDF reduction of -61.41 per cent was noticed under 50 per cent light penetration (M_3).

Variation (%) of canopy temperature was observed due to different treatment combinations, the maximum average temperature of 31.97°C in fodder *Azolla* canopy was observed with the treatment M_3 (100% light penetration) which was 0.65 per cent higher than the mean canopy temperature (32.18°C). Whereas, the temperature reduction of -0.46 and -0.24 per cent over mean was observed with 75 and 50 per cent light penetration, respectively. Likewise, the maximum average temperature of 37.54°C in fodder *Azolla* pond water was observed with the treatment M_3 (100% light penetration) which was 16.87 per cent higher than the mean pond water temperature (32.12°C). Whereas, the temperature reduction of -3.5 and -15.44 per cent over mean was observed with 75 and 50 per cent light penetration, respectively.

Table 1: Microclimate under different artificial shades during *Azolla* growth period

Treatment	PPDF (µmol/s/m^2)			Canopy air temperature (°C)			Water temperature (°C)		
	M_1	M_2	M_3	M_1	M_2	M_3	M_1	M_2	M_3
S_1	1677.85	961.26	403.37	31.78	31.86	32.10	37.81	31.23	28.22
S_2	1678.95	962.00	403.58	32.08	32.30	32.11	37.78	31.22	28.19
S_3	1692.78	962.04	403.95	32.14	32.35	32.21	37.77	31.14	28.13
S_4	1693.44	962.16	404.39	32.16	32.38	32.23	37.73	31.12	28.11
S_5	1693.65	962.39	404.39	32.34	32.40	32.29	37.72	31.01	27.96
S_6	1693.67	962.46	404.55	32.38	32.41	32.32	37.50	30.93	27.86
S_7	1703.52	962.48	404.66	32.41	32.42	32.35	37.46	30.91	27.85
S_8	1703.17	962.73	404.64	32.49	32.43	32.36	37.17	30.88	27.76
S_9	1704.02	962.87	404.73	32.50	32.44	32.39	36.97	30.48	27.54
Mean	1693.45	962.27	404.25	31.97	32.33	32.26	37.54	30.99	27.96

Growth characters

The growth characters such as average individual frond area, density, number of days taken to cover 1 m^2 of *Azolla* varied significantly due to the different levels of light penetration (Table 2 and 3). The treatment M_1 (100% light penetration) recorded the maximum average frond area of 391 mm^{-2} $frond^{-1}$, density of 66,698 m^{-2} and the minimum number of days taken to cover 1m^2 of 6.53 day. The minimum frond area of 226 mm^{-2} frond-1, density of 49,581 m^{-2} and maximum number of days taken to cover 1m^2 of 8.08 day were recorded in M_3 (50 % light penetration) treatment.

Nutrient management practices exerted significant influence on average frond area of fodder *Azolla*. The maximum average frond area of 340 mm^{-2} $frond^{-1}$, density of 61,630 m^{-2} and minimum number of 5.16 days taken to cover 1m^2 area were observed in addition of EFYM @ 0.8 per cent on v/w basis (S_9). This was followed by addition of EFYM @ 0.6 per cent on v/w basis (S_8) and FCM @ 0.8 per cent on v/w basis (S_5) and they were on par with themselves. The minimum frond area of 272 mm^{-2} $frond^{-1}$, density of 54,410 m^{-2} and maximum number of 9.86 days taken to cover 1m^2 area were recorded under without manuring (control) treatment (S_1)

The interaction effect between the levels of light penetration and nutrient management were also found to be significant. Among the treatment combinations, 100 per cent light penetration and addition of EFYM @ 0.8 per cent on v/w basis (M_1S_9) registered the maximum average frond area of 425 mm^{-2} $frond^{-1}$, density of 70,147 m^{-2} and minimum number of 3.98 days taken to cover 1m^2 area. This was followed by M_1S_8 and M_1S_5 and on par with each other. The minimum average frond area of 194 mm^{-2} $frond^{-1}$, 45,821 m^{-2} and maximum number of 9.25 days taken to cover 1m^2 area were noticed with 50 per cent light penetration and without manuring (M_3S_1).

Table 2: Effect of PPDF and nutrient management on average frond area (mm^{-2} $frond^{-1}$) and density (m^2) of *Azolla*

Treatments	Average frond area (mm^{-2} $frond^{-1}$)				Density (m^2)			
	M_1	M_2	M_3	MEAN	M_1	M_2	M_3	MEAN
S_1	353	269	194	272	62995	54414	45821	54410
S_2	366	280	204	283	64260	55723	47117	55700
S_3	378	294	216	296	65408	57019	48426	56951
S_4	394	308	229	311	67004	58618	49801	58474
S_5	411	324	243	326	68607	60119	51445	60057
S_6	381	297	219	299	65662	57298	48715	57225
S_7	398	311	231	313	67305	58883	50121	58770

S_8	413	328	246	329	68893	60417	51713	60341
S_9	425	338	256	340	70147	61675	53067	61630
MEAN	391	305	226		66698	58241	49581	
	SEm±		CD (p=0.05)		SEm±		CD (p=0.05)	
Main	0.98		3.38		106.70		545.32	
Sub	1.46		5.76		428.67		1061.22	
M x S	3.12		9.95		749.28		1810.82	

Table 3: Effect of PPDF and nutrient management on average number of days taken to cover 1 m^2 area and chlorophyll content (µg g^{-1}) of *Azolla*

Treatments	Average number of days taken to cover 1 m^2 area				Chlorophyll content (µg g^{-1})			
	M_1	M_2	M_3	MEAN	M_1	M_2	M_3	MEAN
S_1	9.25	9.54	10.81	9.86	3.33	2.44	1.40	2.39
S_2	7.65	8.21	10.45	8.77	3.44	2.65	1.43	2.51
S_3	7.11	7.96	8.60	7.89	3.61	2.82	1.59	2.67
S_4	6.86	7.71	8.33	7.63	3.66	2.97	1.66	2.76
S_5	5.62	7.32	7.76	6.90	3.82	3.15	1.84	2.94
S_6	6.32	6.83	7.32	6.82	3.64	2.83	1.65	2.71
S_7	6.27	6.29	6.97	6.51	3.79	3.03	1.81	2.88
S_8	5.48	5.75	6.43	5.88	3.84	3.19	1.86	2.96
S_9	3.98	5.47	6.05	5.16	3.94	3.26	2.09	3.10
MEAN	6.53	7.23	8.08		3.67	2.93	1.70	
	SEm±		CD (p=0.05)		SEm±		CD (p=0.05)	
Main	0.12		0.39		0.01		0.04	
Sub	0.13		0.48		0.01		0.05	
M x S	0.17		0.52		0.03		0.09	

Physiological attributes

The chlorophyll content is varied significantly due to the different levels of light penetration. The treatment M_1 (100% light penetration) recorded the maximum chlorophyll content of 3.69 µg g^{-1}. The minimum chlorophyll content of 1.70 µg g^{-1} was recorded in M_3 (50% light penetration) treatment. The maximum chlorophyll content of 3.10 µg g^{-1} was observed under adding EFYM @ 0.8 per cent on v/w basis (S_9).

This was followed by EFYM @ 0.6 per cent (S_8) and FCM @ 0.8 per cent (S_5) and on par with themselves. The minimum chlorophyll content of 2.39 µg g^{-1} was recorded in without manuring (S_1) treatment.

The interaction effect between the levels of light penetration and nutrient management were also found to be significant. Among the treatment combinations, 100 per cent light penetration and addition of EFYM @ 0.8 per cent on v/w basis (M_1S_9) registered the maximum chlorophyll content of 3.94 µg g^{-1}.

This was followed by M_1S_8 and M_1S_5 and on par with each other. The minimum chlorophyll content of 1.40 µg g^{-1} was noticed with 50 per cent light penetration and without manuring (M_3S_1).

***Azolla* fodder yield**

The fresh and dry fodder yield of *Azolla* are varied significantly due to the different levels of light penetration. The treatment M_1 (100% light penetration) recorded the maximum fresh and dry fodder yield of 2,249 and 126 g m^{-2} $week^{-1}$, respectively. The minimum fresh and dry fodder yield of 1,083 and 53 g m^{-2} $week^{-1}$ were recorded with 50 per cent light penetration treatment (M_3). The maximum fresh and dry fodder yield of 1,885 and 103 g m^{-2} $week^{-1}$ was observed with the addition of EFYM @ 0.8 per cent on v/w basis (S_9). This was followed by addition of EFYM @ 0.6 per cent on v/w basis (S_8) and FCM @ 0.8 per cent on v/w basis (S_5) and on par with themselves. The minimum fresh and dry fodder yield of 1,352 and 71 g m^{-2} $week^{-1}$ was recorded under without manuring (control) treatment (S_1). Among the treatment combinations, 100 per cent light penetration and addition of EFYM @ 0.8 per cent on v/w basis (M_1S_9) registered the maximum fresh and dry fodder yield of 2,567 and 147 g m^{-2} $week^{-1}$. This was followed by M_1S_8 and M_1S_5 and on par with each other. The minimum fresh and dry fodder yield of 855 and 41 g m^{-2} $week^{-1}$ was noticed with 50 per cent light penetration and without manuring (control) (M_3S_1).

Table 4: Effect of PPDF and nutrient management on the average fresh and dry yield of *Azolla* (g m^{-2} $week^{-1}$)

Treatments	*Azolla* fresh fodder yield				*Azolla* dry fodder yield			
	M_1	M_2	M_3	MEAN	M_1	M_2	M_3	MEAN
S_1	1922	1280	855	1352	106	66	41	71
S_2	2030	1320	964	1438	112	68	46	75
S_3	2141	1428	970	1513	119	75	47	80
S_4	2270	1566	1077	1638	127	83	53	87
S_5	2407	1695	1213	1772	136	91	60	95
S_6	2157	1447	1068	1557	120	76	52	82
S_7	2300	1592	1085	1659	129	85	53	89
S_8	2443	1708	1235	1795	139	92	62	97
S_9	2567	1810	1278	1885	147	98	65	103
MEAN	2249	1538	1083		126	81	53	
	SEm±		CD (p=0.05)		SEm±		CD (p=0.05)	
Main	40.27		109.60		3.67		11.18	
Sub	32.86		81.43		1.97		4.72	
M x S	57.43		154.69		1.25		3.03	

Effect of *Azolla* fodder on milk yield of lactating cow

Among the feed combinations provided to the dairy cow, feeding with 500g CF + 500 g fresh *Azolla* (1: 1 ratio) twice day^{-1} recorded an average higher milk yield of about 7.00 litre day^{-1} was observed. The increase in milk yield ranges from 17. 92 to 26.71 per cent over control. The lower average milk yield of about 4.75 litre was recorded when provided with 1000g CF twice day^{-1}.

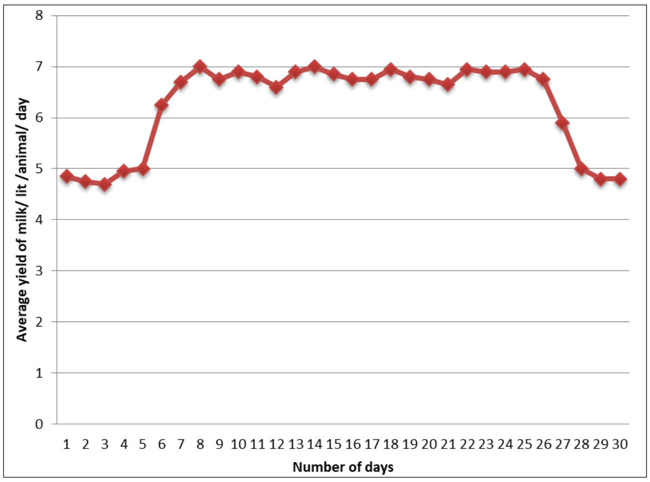

Fig 1: Milking pattern of cow as influenced by fresh *Azolla* feeding

Discussion

Cultivation of *Azolla* under 100 per cent light penetration with highest photosynthetic photon flux density (PPFD) significantly registered the maximum growth values of fodder *Azolla viz.*, frond area, density, number of days taken to cover 1 m^2 area and chlorophyll content. This is because of the fact that even distribution of light and avoiding mutual shading effects helped solid open architecture and efficient use of the resources per unit area leading to manifestation of higher growth values of *Azolla*. The optimum light penetration to *Azolla* pond led favourable ecological conditions exhibits more vigorous growth under the treatment 100 per cent light penetration.

Azolla that received high PPFD *i.e* 100 per cent light penetration was dense and produce maximum of green and dry biomass. It also allowed the plants to multiplied thick mate of *Azolla* in short time of period. High PPFD (1704.02 $\mu mol/s/m^2$) and temperature (>30° C) during summer months appeared to be responsible for high productivity of the *Azolla*. Shading reduced the nitrogenase activity in all *Azolla* species. Abdul Aziz (2012) [1] reported that the performance of *Azolla* decreased per unit increase of shading and the anthocyanin production was also low. In this present investigation a linear decrease in plant density, frond surface area, fresh weight and dry weight were observed when the light penetration was gradually reduced. The possible reason that in high shading or low light intensity plants are unable to perceive the developmental signal in leaves that induced incompetence in food synthesis. The present results are in agreement with the findings of Liu *et al*., (2008) [7] and Sadeghi *et al*. (2013) [18].

Leaf chlorophyll content is well established as a common reference system when physiological reactions are quantified. The results showed significant decreases of chlorophyll content in 25 and 50 per cent shading. Shading conditions may seriously impair or partially active for chlorophyll synthesis. The anthocyanin to total chlorophyll ratio significantly increased with increased light quantity was reported by Abdul Aziz (2012) [1]. The fronts growing at the top received higher light tended to have more chlorophyll a and b as well as total chlorophyll in comparison with lower parts. Further, increasing shading environment promoted ethylene production and decreased the metabolic activity due to low sugar supply and early ethylene production and consequently reduced the carbohydrate generation.

The enhanced growth of *Azolla* due to application of EFYM @ 0.8 per cent on v/w basis could be ascribed to the higher urea hydrolysis as higher urea activity in the water might have released more nutrients and organic acids into water solutions for the utilization of *Azolla* which in turn in growth improvement. The organic manure with lower C: N ratio recorded higher mineralization rate than others with higher C: N ratio. On other hand addition of enriched farmyard manure ensured continuous supply of essential nutrients especially phosphorous due to slow release from the organic source. It facilitate the nutrient absorption pattern of the *Azolla* and reduced the rate of transformation and their by minimizing loss of applied nutrients particularly under standing water condition.

Moreover, additions of enriched farm yard manure increase the solubility of Ca - P compounds that ultimately responsible for maintaining ideal water quality and favoured for enhancing the growth of fodder *Azolla*. *Azolla anabaena* also fixes atmospheric nitrogen at faster rate that favoured for maintaining an optimum nutrient content which might promoted the *Azolla* growth in terms of higher surface area, density and chlorophyll content which in term greater photosynthetic accumulation leading to higher yield of fodder *Azolla*. The findings of present results are in line with reports of Rex Immanuel *et al*. (2019) [14].

The treatment combination of 100 per cent light penetration with the addition of EFYM @ 0.8 per cent on v/w basis (M_1S_9) recorded significantly higher growth attributes, yield and quality of fodder *Azolla*. This might be due to synergetic and cumulative effect of light penetration and use of organic manures. Higher level of PPFD offered the plants to achieve the maximum photosynthetic area which favoured for effective utilization of available resources without any competition. Application of EFYM could be ascribed to higher urea hydrolysis which might have released more NH_4-N into water solution and continuously increased the balanced availability of nutrients that in turn better growth of fodder *Azolla*. Similar findings were also reported by Pillai *et al.* (2002) [10].

The significant yield increment might be due to synergetic effects of higher PPFD for maintaining dense plant population which effectively utilized both above and below of ground resources which facilitated conducive environment for plant growth. Application of well EFYM constantly maintained nutrient availability in the water resulted in better source to sink conservation which in turn enhanced production of higher yields and quality of *Azolla*. The sustained and enhanced availability of light and nutrients till *Azolla* would have enhanced better source and sink relationship which contributed to higher nutrient content of *Azolla*. The findings are in accordance with the result by Ben and Bullockb (2007) [3].

Striking improvement in yield of *Azolla* with the addition of EFYM @ 0.8 per cent on v/w basis markedly increased the green and dry fodder yield. Steady and continuous supply of nutrients throughout the entire crop growth period due to gradual transformations and mineralization of organics, solubulization water insoluble P components by organic acids released during decomposition of organics resulting in greater P availability to fodder *Azolla* coupled with higher K availability might have played a key role in ensuring larger photosynthesis surface area would have helped in enhanced in supply of assimilates resulting in production of superior yield (Rex Immanuel *et al*., 2019) [14].

The increase in milk yield might be due to feeding the animal with most nutritive aquatic plant, owing to its high protein and carotenoid contents and of generally good amino acid profile which improved digestibility of major nutrients and resulted milk gain in cow. This is in line with the report of Chatterjee *et al*. (2013) [4], Semwal Amit *et al*. (2016) [19] and Manpreet Kour *et al*. (2020) [8].

Conclusion

Azolla is the most potential aquatic plant for livestock feed due to its eco-friendly and ease of cultivation, productivity and nutritive value. Based on the results of the present investigation it can be concluded that cultivation of fodder *Azolla* under 100 per cent light penetration with the application of enriched farm yard manure @ 0.8 on w/v basis pave ways for enhancing the productivity and sustained yield of *Azolla* fodder.

References

1. Abdul Aziz. New Method of Large-scale Production of *Azolla pinnata* var. *pinnata* R. Brown: A Multipurpose Crop. International Journal of Applied Agricultural Research,2012:7(1):1-9.
2. Arnon DI. Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*. Plant Physiology,1949:24:1-15.
3. Ben C, Bullockb S. Shedding light on plant competition: Modelling the influence of plant morphology on light capture (and vice versa). Journal of Theoretical Biology,2007:244:208-217.
4. Chatterjee A, Sharma P, Ghosh MK, Mandal, Roy MPK. Utilisation of *Azolla microphylla* as feed supplement for crossbred cattle. International Journal of Agriculture and Food Science Technology,2013:4:207-214.
5. DAHD. Annual Report, 2021. Department of Animal Husbandry and Dairying, Govt of India, 2021, 237.
6. Gomez KA, Gomez AA. Statistical procedures for agricultural research. II Ed. John Wiley and Sons, New York, 1984, 68.
7. Liu X, Min C, Xia-shi, L, Chungchu, L. Research on some functions of *Azolla* in CELSS system. Acta Astronautica,2008:63:1061-1066.
8. Manpreet Kour, Nazam Khan, Rajeev Singh, Vikas Mahajan, Suraj A Amrutkar, Dhirendra Kumar. Effect of *Azolla* (*Azolla pinnata*) Supplementation on Milk Yield, Composition and Economics in Crossbred HF Cows. International Journal of Current Microbiology and Applied Sciences,2020:9(10):2661-2666.
9. Pathak AK, Singh S, Sharma RK, Khan M. Effect of tanniferous leaf meal based multi-nutrient blocks on feed intake, hematological profile, immune response and body weight changes in *Haemonchus contortus* infected goats. Veterinary World,2015:8(5):572-579.
10. Pillai KP, Premalatha S, Rajamony S. *Azolla*: A sustainable feed substitute for livestock. LEISA Magazine,2002:4:15-17.
11. Rex Immanuel R, Imayavaramban V, Lyla Elizabeth, Kannan T, Murugan G. Traditional farming knowledge on agroecosystem conservation in Northeast coastal Tamil Nadu. Indian Journal of Traditional Knowledge,2010:9(2):366-374.
12. Rex Immanuel R, Ganapathy M. Growth and physiological attributes of *Ceiba pentandra* (L.) Gaertn. seeds and seedlings under salt stress. Journal of Agricultural and Biological Science,2007:2(6):12-16.
13. Rex Immanuel R. Ganapathy M. Agro-techniques for afforestation of degraded coastal agricultural lands with silk cotton (*Ceiba pentendra* (L.) Gaertn.). Journal of Pharmacognosy and Phytochemistry,2019:8(2):1587-1590.
14. Rex Immanuel R. Seasonal temperature effect on the biomass productivity of fodder *Azolla*, International Journal of Research and Analytical Reviews,2019:6(1):237x-241x.
15. Rex Immanuel R, Ganapathy M, Performance of field crops along with four tree species in coastal Tamil Nadu. Indian Journal of Forestry,2010:33(1):67-70.
16. Rex Immanuel R, Sudahar Rao GB, Senthilkumar N. Effect of irrigation management and organic manuring on the growth and physiological attributes of direct sown rice (*Oryza sativa* L.). Crop Research,2021:56(3&4):75-82.
17. Rex Immanuel R, Thiruppathi M, Sudhagar Rao GB, Vigil Anbiah S, Chitharanjan S. Agronomic effectiveness of enriched farm yard manure (EFYM) on biomass productivity of fodder *Azolla*. International Journal of Research and Analytical Reviews,2019:6(1):464-472.
18. Sadeghi R, Zarkami R, Sabetraftar K, Van Damme P. A review of some ecological factors affecting the growth of Azolla spp. Caspian Journal Environmental Science,2013:11(1):65-76.
19. Semwal Amit, Kunwar Amit, Prasad Anoop, Kumar Ashok. *Azolla*- an environment eco-friendly pteridophytic species. European Journal of Biomedical and Pharm. Sciences,2016:3(6):210-213.
20. Sudahar Rao, Balachandrakaumar V, Rex Immanuel R, Nambi J, Suthin Raj T. Influence of zinc and iron fortified micronutrients on the growth, yield and economics of rice (Oryza sativa L.). Crop Research,2020:55(5&6):202-207.
21. Thimijan, Richard W, Royal Heins D. Photometric, Radiometric and Quantum Light Units of Measure: A Review of Procedures for Interconversion. Hort. Science,1982:18:818-822.