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Logo Description : It symbolizes an elephant within an ecological frame of peace and harmony moving towards prosperity and posterity. **Cover photo** (Anticlockwise from top) ; 1. Tabanus sp. flies trapped by canopy 2. King Mackerel, *Scomberomorus commerson* of Kasimedu Harbour 3. Implantation of BM-MSCs on wound bed 4. *In vitro* identification of virulent and non-virulent *A. hydrophila* isolates through hemolytic activity **Cover background photo** : Sunken glory; an ancient temple partly submerged under the waters of Hirakud reservoir(By Manoj V.Nayyar).

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EDITORIAL

Earlier we had discussed about the role of green house gases such as carbon dioxide, methane and nitrous oxide on rise of global temperature inviting climate change. Changes in climate would also increase the risk of unexpected changes in nature and environment. The production of CO_2, CH_4 and N_2O and their negative implications over the environment can be discussed. As per a recent estimation, the total CO_2 entering into environment can be divided as follows i.e. 56% coming from fossil fuel like petrol, diesel etc., 16% coming from loss of forests and 14.3% coming from volcanic eruptions and the rest from miscellaneous sources. Methane source is mostly from volcanic eruption, agriculture, animal husbandry and human activities. Nitrous oxide, which is 289 times more potential than carbon dioxide mostly comes from fertilizer, burning of petrol and diesel in vehicle.

The life span of these green house gases have been estimated to be; methane 72 years, CO_2 100 years and N_2O 289 years. It has been reported that methane is 72 times more potential than CO_2 in causing global worming. India is one of the largest country having vast cattle population. The impression that methane coming from cowdung has been the major share can not be true. A large chunk of people in India belong to poorer section and do not keep stall-fed cattle. In Indian condition, most of cows are free ranging and remain in open pasture land. With the treatment of sun rays and in aerobic condition, production of methane becomes very less. Fact remains, methane is produced in anaerobic condition from the cowdung when it is fermented. Apart from that, due to huge requirement of fire wood and stringent forest regulation people prefer dry cowdung in form of patties in walls and ground and under such aerobic condition methane production is very negligible. More often cowdung has been blamed for methane production, one of causes of concern for climate change. But this methane gas produced from cowdung can be trapped for its use as fuel for domestic use, vehicle and to create electricity. Just like CNG, methane gas can be used to run automobile engines in place of petrol.

India has the largest livestock population of 250 million, which produces close to 125 million tonnes of cowdung. One cow gives enough cowdung in a year to produce methane equivalent to 225 lts of petrol in energy terms. Hence, cow can produce enough methane gas to entirely replace LPG and kerosene in cooking. The entire LPG and kerosene requirements of our 120 crore population can be met by methane gas cylinders produced from the cowdung of 75 million cows. It is reported that the cowdung will replace whole of India's LPG under compressed methane gas(CMG) technology. The advantage is that after extracting methan gas, the manure can be used in agriculture and forestry. Hence, the production of methane in India can be rationalized and finally, methane entering into environment would be very negligible.

Once the methane mantras will work out, in terms of requirement, it would surpass milk. It has been calculated that rearing of one cow can save six plants upto five years. Hence, keeping cows can increase forest resources as well.

What can be the source which can check all carbons that come from soil to sky ? The only machine is plant. So, the slogan should have been; “Have plantation and keep cows”.

(Dr. R. K. Samantaray)
Editor-in-Chief

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PHENOL REMOVAL FROM WATER WITH MODIFIED CARBONS PREPARED FROM BAGASSE ASH

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ABSTRACT

Phenols are classified as priority pollutants because of their potential harm to human health. The discharge limit of phenol in waste waters is low (0.1 mg l-1, as per USEPA). Various industries like paints, adhesives, pharmaceuticals, dye stuffs and intermediates, leather chemicals, pesticides etc. generate waste water with phenols. Adsorption is a common process for phenol removal and various low cost adsorbents have been tested. These include different types of agro-wastes e.g. rice husk, wood charcoal etc. The present study examines the potential of unburnt carbon fraction present in bagasse ash as an adsorbent for phenol. Bagasse, the solid residue obtained after milling sugarcane, is burnt as a fuel in sugar factories. Due to incomplete combustion, the resulting bagasse ash can have up to 30% unburnt carbon. The unburnt carbon separated from bagasse ash was modified to increase its adsorptive capacity. The methods included (i) deashing by HCl and HF (ii) oxidation by H_2O_2 or HNO_3 (iii) deashing followed by oxidation with H_2O_2 or HNO_3. The modified carbons were characterized for composition and surface area. Adsorptive removal was investigated for phenol concentrations between 10 to 2000 mg l^{-1} and carbon dosages between 0.1 to 1 g 100 ml^{-1}. The modified carbons showed high removal (80-100%) for low phenol concentrations (10-100 mg l^{-1}) while a maximum removal of 60% was obtained at higher concentrations (1000-2000 mg l^{-1}). The adsorption follows Langmuir isotherm.

Key words : Phenol, wastewater, unburnt carbon, modified carbons, adsorption

INTRODUCTION

Industrial effluents are one of the major sources of environmental pollution causing damage to aquatic and human life due to mutagenic or carcinogenic effects (Crini *et al.*, 2006). Phenol is one of the basic structural units for a variety of synthetic organic compounds including agricultural chemicals and pesticides. It is 11th on the list of 126 chemicals designated as priority pollutants by the USEPA (Nayak and Singh, 2007). Phenolics are commonly found in wastewater generated from oil, gasoline, coal, paper, petrochemicals, pharmaceuticals, pesticides and dye manufacturing industries (Entezari *et al.*, 2005). USEPA has set a discharge limit of 0.1 mg l-1 of phenol in wastewaters (Dabhade *et al.*, 2009). As per the IEP Rules 1986, the permissible limit of phenol for discharge is 1 mg l-1 into inland surface water and 5.0 mg l-1 in public sewer and marine coastal areas (Gupta *et al.*, 2006).

Various methods have been used to remove phenol from wastewater including microbial degradation, adsorption, chemical oxidation, incineration and solvent extraction. Adsorption is a well-established method for the removal of trace phenolic pollutants from aqueous streams. The process becomes economical when natural or waste materials are used as precursors.

Activated carbon is a highly porous material (surface area 500-3000 m^2g^{-1}), with a combination of mesopores (diameter < 5 nm) and macropores (diameter > 50nm); it is widely used as an adsorbent. It is prepared by carbonization along with physical or chemical activation of high carbon content materials like wood, coconut shells etc. Several carbonaceous precursors, which are readily available in agro-based wastes, have been investigated for the preparation of activated carbon. These include e.g. rice husk (Daffalla *et al.*, 2010), wood charcoal (Mukherjee *et al.*, 2007).

Sugarcane bagasse is yet another important agro-industrial waste, which has been used for the preparation of activated carbon (Boonpoke *et. al.*, 2011). In India, bagasse is widely used as a boiler fuel in sugar factories. Bagasse based boilers operate at an efficiency of 60-70% and the estimated generation of bagasse fly ash is approximately 3 million tons annually i.e. 4% weight of bagasse (Batra *et al.*, 2011). The unburnt carbon content in fly ash is high (16 to 33 %). Therefore, up to ~1 million tons of unburnt carbon is estimated to be annually available from bagasse ash in India.

This study focuses on (i) the physical and chemical properties of unburnt carbon from bagasse fly ash, (ii) modification of unburnt carbon by chemical treatment and characterization of the modified carbons, and (iii) evaluation of the modified carbons for micropollutant (phenol) removal.

MATERIALS AND METHODS

Carbon separation and modifications

The bagasse fly ash was collected from Simbhaoli Sugars Limited, Ghaziabad District, U.P. This ash was separated by gravity separation to obtain the carbon rich fractions. The unburnt carbon fraction thus obtained was dried overnight in an oven at 50°C and analyzed for moisture, ash, volatile matter, methylene blue number and surface area. Five different procedures were followed for modification of the separated unburnt carbon (Table -1). Carbon samples were characterized for moisture content, ash content, volatile matter by American Society of Testing Materials (ASTM), methylene blue number and surface area. Fourier Transform-Infrared (FT-IR) Spectroscopy was done to know the surface functional groups (Perkin Elmer Spectrum1 FT-IR instrument).

Table 1 : Carbon samples and their codes Adsorption studies

Description	Code
Unburnt carbon	UC
Unburnt carbon - ash removed	UC-Ash
Unburnt carbon - Oxidized with H_2O_2	UC-H_2O_2
Unburnt carbon - ash removed and oxidized with H_2O_2	UC-Ash-H_2O_2
Unburnt carbon - Oxidized with HNO_3	UC-HNO_3
Unburnt carbon - ash removed and oxidized with HNO_3	UC-Ash-HNO_3

Batch adsorption studies were carried out in 500 ml shake-flasks (Orbitek Shaker, India) at 150 rpm at 25°C for 6 h. The effect of different carbon dosages was examined using three dosages viz. 0.1 g, 0.5 g and 1.0 g in 100 ml solution. Four different phenol concentrations (10 mgl^{-1}, 100 mgl^{-1}, 1000 mgl^{-1}, 2000 mgl^{-1}) were studied. The supernatant was analyzed for phenol using the 4-aminoantipyrene method.

RESULTS AND DISCUSSION

Table 2 shows the characteristics of the prepared carbons. Low moisture content is preferable and commercial activated carbons have moisture content within 3% to 10%. In this work, the moisture content varied from 5.62 % (UC-Ash) to 11.78% (UC-Ash-HNO_3).

Table 2 : Characterization of prepared carbons

Sample	Moisture (%)	Ash (%)	Volatile matter (%)	Methylene blue number (mg g^{-1})	Surface area (m^2 g^{-1})
UC	6.82	26.39	23.00	17	24
UC-Ash	5.62	10.54	26.64	20	33
UC-H_2O_2	9.67	19.28	24.79	23	66
UC-Ash-H_2O_2	10.92	19.90	34.55	38	140
UC-HNO_3	9.99	3.50	27.13	23	139
UC-Ash-HNO_3	11.78	2.29	35.07	40	197

ture content of commercial activated carbons. High ash content reduces the overall activity of activated carbon. The ash content in UC was rather high at 26.4%. This was subsequently reduced to 10.54%, 3.50% and 2.3% in UC-Ash, UC-Ash H_2O_2, and UC-Ash-HNO3 respectively. Chemical treatment with acids especially HF leads to dissolution of the silica content in the unburnt carbon. The volatile matter in the UC was 23%, which increased upon modification. This may be due to the degradation of the organic compounds in the precursor material, brought about during the process of modification. Adsorption capacity is proportional to surface area (determined by degree of activation). In the present study, it was observed that there was substantial increase in the surface area of the carbon upon modification. The surface area of the starting UC was low (24 m^2 g^{-1}) but it increased especially upon acid treatment (140 m^2 g^{-1}, 139 m^2 g^{-1} and 197 m^2 g^{-1} for UC-HNO_3, UC-Ash-H_2O_2 and UC-Ash-HNO_3 respectively). The surface area of these samples is comparable to Carbon Black N339, a popular additive in the rubber industry which has a surface area of ~96 m^2 g^{-1}. Methylene blue number gives an estimate of the carbon mesopore content (pore size 2 nm-50 nm). The HNO_3 treated carbons (UC-HNO_2 and UC-Ash-HNO_3) have relatively high methylene blue number (~ 40 mg g^{-1}), hence these samples have relatively higher mesopore content. Though methylene blue number increased with increasing surface area, it was not necessarily the same for carbons with similar surface areas. For instance, UC-HNO_3 and UC-Ash H_2O_2 have similar surface areas but the methylene blue numbers are different. Introduction of chemical functional groups was confirmed by FT-IR analysis. Oxidation with HNO_3 or H_2O_2 introduced a variety of oxygen containing functional groups such as -OH, C-H, C=O, C=C, C-C and C-O.

Adsorption studies

Batch adsorption studies with the as-is unburnt carbon shows that it is capable of removing 80- 100% phenol from dilute streams (up to 100 mgl^{-1}). Increasing feed phenol concentration from 10 mgl^{-1} to 1000 mgl^{-1} results in a decrease in phenol removal with all the modified carbons (Fig. 1). At low phenol concentration, a number of vacant sites are available on the adsorbent. These sites get occupied at higher concentrations showing decrease in percentage removal. However, at feed concentration of 2000 mg l^{-1} phenol, there was an increase in phenol removal up to 60%. This is possibly due to the surface polymerization of phenol for the formation of superoxo radical (formed due to the ability of phenol to adsorb oxygen from the solution) (Terzyk, 2003). This phenomenon needs to be further investigated.

Furthermore, it was observed that there was an increase in phenol removal with increasing carbon dosage (Fig. 2). This may be attributed to the increased adsorbent surface area and availability of adsorption sites. In the range studied, complete removal of phenol could be obtained with chemically modified carbons, especially for dilute streams. Treatment with H_2O_2 and HNO_3 after ash removal are both promising. The modified carbons show moderate removal of phenol (up to 60%) in concentrated streams (1000 mg l^{-1} and 2000 mg l^{-1}).

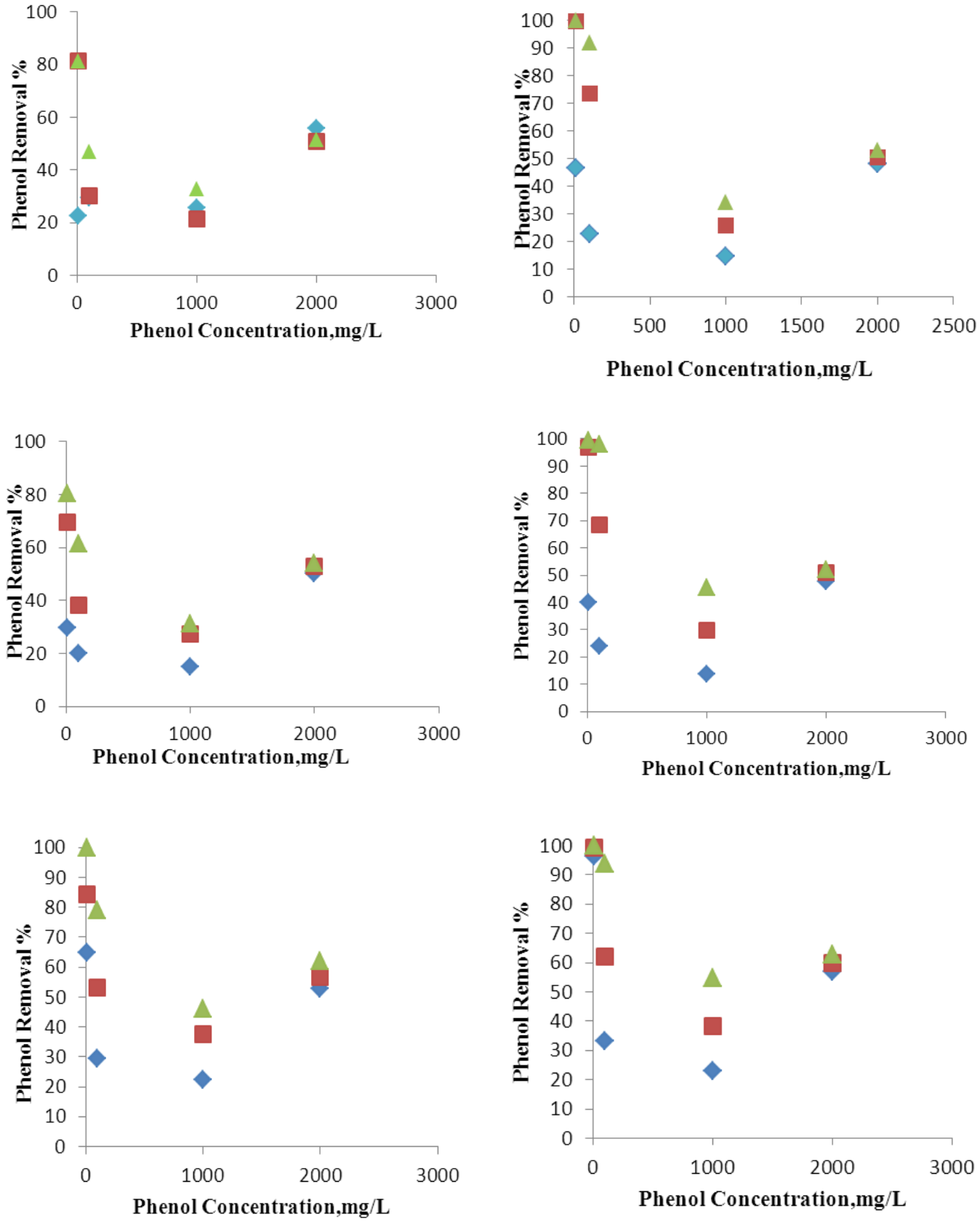

Fig. 1 : Effect of feed concentration on phenol removal for various carbons. Carbon dosage : 0.1 g 100 ml^{-1}, 0.5 g 100 ml^{-1}, 1.0 g 100 ml^{-1}.

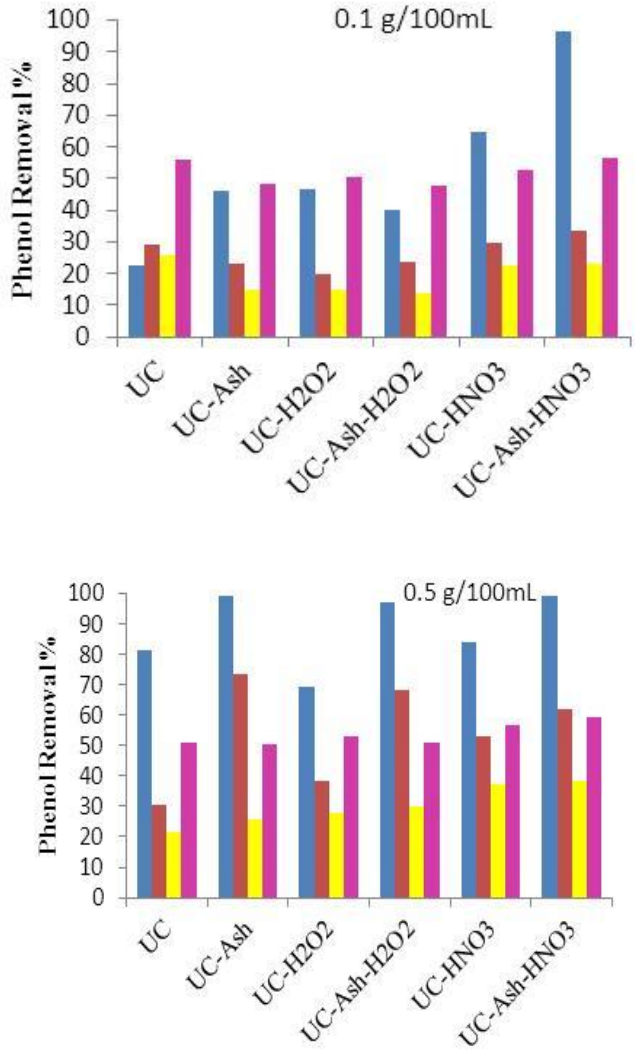

Fig. 2 : Effect of various modifications of carbons on phenol removal at different adsorbent dosages. Initial phenol concentration: 10 mgl^{-1}, 100 mgl^{-1}, 1000 mgl^{-1}, 2000 mgl^{-1}.

Adsorption isotherms relate the equilibrium pollutant concentrations with the amount adsorbed by the carbon. The Langmuir equation is valid for monolayer adsorption onto a surface with a finite number of identical sites and is given by equation (1).

Where qm and KL are Langmuir parameters related to maximum adsorption capacity and free energy of adsorption, respectively, Ce is the equilibrium concentration in the aqueous solution and qe is the equilibrium adsorption capacity of adsorbent.

Langmuir isotherm plots (not shown here) indicated a good fit for the adsorption data (R2 > 0.95). A comparison of the adsorption capacity at two adsorbent dosages of 0.5 g 100 ml^{-1} and 1 g 100 ml^{-1} is summarized in Table 3. It is observed that all the modified carbons have a higher adsorption capacity than the as-is unburnt carbon and the adsorption capacity decreases with increasing adsorbent concentration.

Table 3 : Summary of adsorption capacity (Langmuir Isotherm)

Carbon	Adsorption capacity (mg phenol/ g carbon)	
	0.5 (g carbon/ 100 ml)	1.0(g carbon /100 ml)
UC	17	8
UC-Ash	33	20
UC-HNO_3	34	19
UC-H_2O_2	27	22
UC-Ash-HNO_3	31	23
UC-Ash-H_2O_2	34	31

Table 3 shows that the adsorption capacity of UC-HNO_3, UC-Ash-HNO_3, and UC-Ash-H_2O_2 increased to 34 mg g^{-1} as compared to 17 mg g^{-1} for UC. This may be due to increased surface area of these modified carbons (Table 2). There was an increase in the adsorption capacity of UC-Ash carbon even though it has lower surface area and lower methylene blue number than the other modified carbons. This may be due to the high pore volume and the incorporation of oxygen containing functional groups during the deashing step. However, there was decrease in phenol adsorption capacity with increasing adsorbent concentration. This may be due to aggregation of adsorbent particles that leads to a decrease in effective surface area of adsorbent available for adsorption.

CONCLUSION

Chemical modification of the unburnt carbon from bagasse fly ash can be done by treatment with acids and hydrogen peroxide. The modification reduces the ash content and increases the surface area. The as-is unburnt carbon can remove phenol from dilute streams (up to 100 mg l^{-1}) and the performance can be improved by chemical modification. At high phenol concentration (2000 mgl^{-1}), surface polymerization of phenol is suspected and needs to be further investigated.

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METEOROLOGICAL DROUGHT OCCURRENCES IN TURA, MEGHALAYA, INDIA

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ABSTRACT

Rainfall always plays a deciding role in sustainable agricultural production. Meghalaya is one of the highest rainfall receiving states of India having rice based mono-cropping system. Tura (West Garo Hills district of Meghalaya) is the major rice producing belt of this state. Rainfed rice cultivation is mostly practised in Meghalaya. The rainfall analysis was done based on fourteen years (1984-1997) daily rainfall data to study monthly, seasonal and yearly drought of Tura based on India Meteorological Department (IMD) protocols. The average annual rainfall of Tura is 4,851.5 mm. During the fourteen years period no extreme and moderate drought years were experienced, but there were mild drought occurrences in nine years. The frequency of drought recorded during month of January, February, November and December was 10, 8, 9 and 11 respectively out of 14 years of record.

Key words : Meteorological drought; rainfed rice; drought year; rainfall analysis

INTRODUCTION

More than 60% of the cultivable rice area in India is rainfed. Rainfall availability is not well assured at all places and time. Temporal and spatial variability of rainfall distribution is observed in India (Dhar *et al.* 1979). In our country nearly 75% of the rainfall is occurring during June to September. Extreme conditions of rainfall are also observed in certain years. Floods and droughts are the two extremes of rainfall distribution. Deficiency of rainfall is the basic cause of drought. As such no general method is available which can be applied for the drought prediction (Salas, 1986). Depending on the climate, drought varies from place to place. Several workers have done meteorological analyses based on rainfall data (Satapathy *et al.* 1998; 1999; Sharma *et al.* 1979, 1987a. and 1987b) analysed the rainfall using the definition of drought month as a month in which the actual rainfall is less than 50% of the average monthly rainfall. Drought year is the year receiving rainfall less than or equal to the average rainfall minus twice standard deviation of the series. Shrivastava *et al.* (2008) used this definition to assess meteorological droughts in North Lakhimpur district of Assam. Sinha (1986) and Ray *et al.* (1987) used this definition to study the drought at Gopalpur, Odisha. Kumar and Kumar (1989) Dabral (1996) analysed the weekly, monthly, seasonal and yearly rainfall of Panthnagar, Ranchi and Barapani respectively for drought; as per the procedure followed by Ramdas and Malick (1948), Sharma *et al.* (1979) and Dhar *et al.*(1979). Tiwari *et al.* (2007) characterize the meteorological drought indices using the data of Hazaribagh station. Similar analysis has been done by various researchers for meteorological drought analysis at various places in India. In the present paper an attempt has been made to study the frequency of drought occurance at Tura in terms of rainfall deficiency. Tura is the major rice contributing region of Meghalaya. The net culturable area is 94,481 Ha with cropping intensity of 126%.

The net irrigated is 11,159 Ha and 2,036 Ha of area are irrigated more than once in a year. Plantation crop is dominated by cashew nut and arecanut. Pine apple, banana and citrus based fruit crops are predominant in this region.

MATERIALS AND METHODS

The study area, Tura is located at 89° 40' to 90° 30` East Longitude and 25° 20` to 26° North Latitude with an altitude of 625 m above mean sea level. The behavioral pattern of rainfall with reference to the amount of rainfall and number of rainy days at Tura were analysed using Weibull approach from historic daily rainfall records (1984-1997). Generally, areas with low rainfall are having high rainfall variability, the North East Hilly (NEH) region, by virtue of receipt of heavy rainfall, comes under low rainfall variability category and it ranges from 8-15%. For Tura, the normal annual rainfall ranges from 750 - 340 cm.

The monthly rainfall, seasonal rainfall (i.e., June to September - monsoon; October to December - post monsoon; and January to May - pre monsoon) and yearly rainfall were analysed. The average monthly, seasonal and yearly rainfall values were worked out. The variation of rainfall for each month, season and year from the mean was determined and the mean deviation for the seasons was calculated. Total numbers of drought months, seasons and year were determined using the following definition:

Drought month: if the actual rainfall is less than 50% of the average monthly rainfall (Sharma *et al.*1979). Drought season: if the annual rainfall is deficient by more than twice the mean deviation of the season (Marathe *et al.* 2001). Drought year: if the annual rainfall is deficient by 20-60% of the average yearly rainfall and if the deficient is more than 60% of the average yearly rainfall it is known as scanty drought year (Dhar et al.1979). The yearly intensity of drought was also determined using the criteria suggested by IMD (1971) which is based on the percentage deviation of rainfall from its long term mean and it is given by (Eq.1).

$$Di = \frac{\text{Ann. Rainfall} - \text{Mean}}{\text{Mean Rainfall}} \times 100 \quad \ldots. (1)$$

Where Di is the percentage deviation from the long-term mean, Pi is the annual rainfall, mm and μ is the long term mean of the annual rainfall, mm Drought codification based on percentage departure of rainfall from normal is presented in Table-1. The percentage of deviation (Di) is then used to categorise the drought.

RESULTS AND DISCUSSION

Descriptive statistical analysis of monthly rainfall was done for Tura station. The co-efficient of variation is more than 100% for the month of January, February, November and December. Standard deviation was maximum for the month of June and minimum for the month of November. The monthly rainfall analysis for drought is presented in Table-2. The highest normal rainfall of 1116.6 mm was observed in the month of July and the lowest 19.91 mm occured in the month of November. The frequency of drought was observed to be the highest at a magnitude of 11 times in 14 years in December; while it is 10, 9, 8 and 6 times in 14 years during January, November, February and March month respectively (Table 3). It indicates that, there is a need for assured irrigation in the above months. From the seasonal analysis of the rainfall, it is evident that 19.8% of rainfall was received during pre-monsoon, 73% during monsoon and 7.2% during post monsoon session (Table 4). So for growing winter season crops during post monsoon season assured irrigation facilities need to be provided, simultaneously ample emphasis may be given to rainwater harvesting during the monsoon season as a high quantum of runoff is anticipated during this seasons. No drought was observed during monsoon, pre-monsoon and post monsoon period. The yearly intensity of drought for Tura, Meghalaya is presented in Table 5. The years are codified according to IMD specification as described in the Table-1. It is found from the Table-5 that, there was no severe drought occurance. However, for the year 1984-1986; 1990-1992; and 1997 there was mild drought occourance. The monthly distribution of rainfall and rainy days is presented in Fig-1.

The average annual rainfall of Tura is 4,851.5 mm with a maximum of 7,584.5 mm corresponding to the year 1984 and a minimum of 3,454.8 mm corresponding to the year 1997. The average monthly rainfall of the place is 240.45, 611.44, 1068.47, 1116.58, 729.12, 626.41 and 295.31 mm for the month of April, May, June, July, August, September and October respectively. The maximum average rainfall is received during the month of July to a tune of 1116.58 mm and the minimum average rainfall is received during the month of November to a tune of 19.91mm.

CONCLUSION

The drought analysis of Tura made according to deficiency of rainfall shows that out of fourteen years there was no severe drought occurance in this region. However, for the year 1984-1986; 1990-1992; and 1997 there was mild drought occourance. For growing rainfed rice during monsoon farmers of this region may depend on monsoon as there was hardly any drought occurance. Since the post monsoon seasonal rainfall is very less, for growing winter season crops arrangement may be done for assured irrigation with proper rainwater harvesting methods.

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Table 1 : Drought codification based on percentage departure of rainfall from normal value

% departure of rainfall from normal	Intensity of drought	Code
0.0 or above	No drought	Mo
0.0 to -25.0	Mild drought	M1
-25.0 to - 50.0	Moderate drought	M2
-50.0 to -75.0	Severe drought	M3
-75.0 or less	Extreme drought	M4

Table 2 : Monthly Normal and Extreme Rainfall along with SD, CV and Percentage contribution at Tura

Month		Extreme Value		Standard Deviation	Coefficient of Variation (%)	Percentage contribution(%)
	Normal (mm) (mm)	Minimum (mm)	Maximum (mm)			
January	24.43	0	124.8	42.78	175.12	0.50
February	23.54	0	99.9	33.05	140.42	0.49
March	62.05	0	152.1	57.47	92.61	1.28
April	240.45	0	623.4	175.17	72.85	4.96
May	611.44	0	1183.6	283.58	46.38	12.60
June	1068.47	0	2701.7	562.18	52.62	22.02
July	1116.58	0	1959.8	489.23	43.82	23.02
August	729.12	0	1552	370.67	50.84	15.03
September	626.41	0	1045.9	197.00	31.45	12.91
October	295.31	0	664.8	171.09	57.94	6.09
November	19.91	0	85.8	29.30	147.14	0.41
December	33.77	0	291.8	79.06	234.10	0.70

Table 3 : Analysis of monthly, rainfall for drought for Tura Station

Month/ Season	Name of month/ season	Average rainfall, mm	Half of the mm average rainfall, mm	No. of drought, month/season/ year	Percentage of drought months
	Jan	24.43	12.21	10	3.64
	Feb	23.54	11.77	8	5.45
	Mar	62.05	31.03	6	7.27
	Apr	240.45	120.23	4	9.09
	May	611.44	305.72	2	10.91
	Jun	1068.47	534.24	0	12.73
Month	Jul	1116.58	558.29	2	10.91
	Aug	729.12	364.56	3	10.00
	Sep	626.41	313.20	1	11.82
	Oct	295.31	147.66	2	10.91
	Nov	19.91	9.96	9	4.55
	Dec	33.77	16.89	11	2.73

Table 4 : Analysis of seasonal rainfall for drought for Tura Station

Month/ Season	Name of month season	Average rainfall, mm	Twice the main dev value mm ,	No. of drought, season
	Pre monsoon	961.91	834.31	0
Season	Monsoon	3540.58	2275.81	0
	Post monsoon	349.00	349.85	0

Table 5 : Yearly intensity of drought for Tura

Year	Annual rainfall, mm	Main rainfall, mm	% deviation	Category	Intensity of Drought
1984	4094.6	4851.49	-15.62	M1	Mild drought
1985	4049	4851.49	-16.56	M1	Mild drought
1986	4276.3	4851.49	-11.88	M1	Mild drought
1987	4421.7	4851.49	-8.88	M1	Mild drought
1988	6514.4	4851.49	34.26	Mo	No drought
1989	5037.7	4851.49	3.82	Mo	No drought
1990	4077.9	4851.49	-15.97	M1	Mild drought
1991	4826.3	4851.49	-0.54	M1	Mild drought
1992	4593.2	4851.49	-5.34	M1	Mild drought
1993	5154.2	4851.49	6.22	Mo	No drought
1994	4214.1	4851.49	-13.16	M1	Mild drought
1995	7584.5	4851.49	56.31	Mo	No drought
1996	5319.1	4851.49	9.62	Mo	No drought
1997	3757.8	4851.49	-22.56	M1	Mild drought

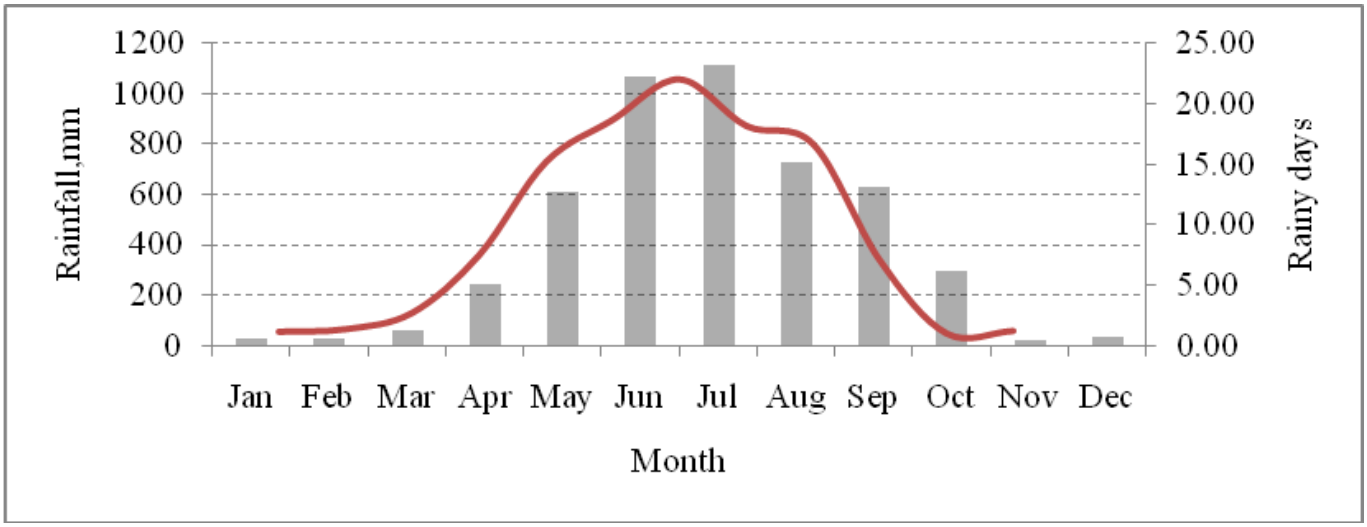

Fig. 1 : Monthly distribution of rainfall at Tura, Meghalaya

TARGETING CHRONIC ULCERATIVE WOUND AT THIGH REGION IN A BULL CALF BY AUTOLOGOUS STEM CELL THERAPY

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ABSTRACT

The regenerative medicine (RM) is an evolving speciality in medicine which is gradually moving forward towards clinical application in many requirements of living beings. Taking this beneficial effect of RM one clinical trial was made for therapeutic application in a chronic nonhealing and ulcerative wound in a male calf of 1.6 years of age. The bone marrow (BM) was collected and then culture and growth was done in stem cell laboratory, CIFA, Rangeilunda, Bhubaneswar. These harvested BM derived mesenchymal stem cells (BM-MSCs) which were implanted at the wound bed. After implantation, faster wound healing was observed and within 21 days complete healing was achieved. So the therapeutic efficacy of stem cells using BM-MSCs found successful in treating chronic non-healing cutaneous wound in bovines without any adverse effect.

Keywords : BM-MSCs, Bovine, Chronic ulcerative wound, RM, bone marrow

INTRODUCTION

Skin is the largest (Slatter, 2003) and a highly complex organ (Johnston, 1981) of body composed of epidermis, dermis and associated adnexa (Fossum *et al.,* 1997). The thickness of skin varies in different species and on different parts of body of same animal and also differs with breed, age and sex (Sisson and Grossman, 1953; Ghosh, 2009). Wound is a break in the continuity of skin while ulcer is an inflammatory lesion of skin and mucous membrane with loss of epithelium (Venugopalan, 2004) which fails to undergo the natural process of healing (Frank, 2002). Wound treatment always pose a great challenge (Dash *et al.,* 2009) to the veterinarians owing to the nature of habit and habitat of different animals. Regarding wound management different types of therapeutic regimens have been evolved. Owing to the beneficial effect of cell based therapy (BM-MSCs) one clinical trial was made targeting one chronic non-healing ulcerative wound at right thigh region above hock of a 1.6 years old bull calf since 6 months and achieved desired outcome within a short period.

MATERIALS AND METHODS

With the informed consent of the owner, the clinical application was designed targeting the chronic wound. The wound dimension was measured (Fig. 1) and bacteriological swab was collected for culture and sensitivity test**.** Biopsy was done from wound for histopathological and histochemical study. Blood samples were taken for haemato-biochemical studies.

Wound was washed, cleaned and dressed with fly repellent spray. The animal was kept fasting for 24 hrs and was sedated with inj. Xylazine @ 0.03 mg/ kg body weight intramuscularly. Under peronial nerve block the proposed site i.e proximal antero-medial aspect of tibia was prepared aseptically for bone marrow collection. A 0.5 cm long skin incision was made at the antero-medial aspect of tibia and bone drilling was done by electrically operated orthopaedic drill with 2 mm drill bit**.** 10 ml of bone marrow was aspirated in a sterile syringe primed with EDTA (Himedia) @ 1mg/ml**.** The aspiration of the BM was made as per the method of Crow and Walshaw (1997) with some modification. The bone marrow derived nucleated cells (BMNCs) were collected from aspirate by volume reduction protocol as per method of Kasten *et al.* (2007). The EDTA mixed bone marrow was dispatched keeping inside ice packed thermo cool to the stem cell laboratory of CIFA, Kausalyaganga, Bhubaneswar within 30 minutes of collection for culture and growth.

Conditioned media was prepared for culture with commercially available basic ingredients as per Table 1. The collected bone marrow was mixed with Histopaque (Sigma, Aldrich, USA), used for isolation of mononuclear cells and was centrifuged at 2000 rpm for 20 minutes. The monocytes remain in buffy layer were seeded on to a 0·1% gelatin-coated six well plate (Tarson, India) and 100 µl of cells were poured into 6 well plate containing 2 ml of composed media at 37^0C and 5% CO_2 in the CO_2 incubator (Contherm Scientiûc Ltd, USA). The medium was changed on regular interval and cell morphology was examined under a Nikon phase contrast microscope (Fig 2,3,4). After complete colony formation and attaining confluence BM-MSCs were taken for therapeutic application.

Stem cell therapy requires complete sterile medium, so the ulcerated wound site was prepared aseptically for application. The prepared BM-MSCs were diluted with NSS at 2:1 ratio and implanted intradermally in circular manner at the wound margin and then at different points on wound bed (Fig 5). It was kept as such for 20 min for better adhering to the site and bandaged with paraffin wet bandage. Outwardly fly repellent spray was sprinkled.

The physiological parameters like body temperature (rectal ^{0}C), heart rate (beats/min), respiration rate (breaths/min) and status of visible mucous membrane were recorded on the day of presentation, BM collection, BM-MSCs implantation and 21 days after healing. The size of the wound was measured during healing period and also after healing. Biochemical parameters on day of presentation and after 21 days of healing were estimated (Table- 2). Histopathology with Haematoxylin and Eosin stain and Masson-Trichrome stain for study of collagen content were carried out. Quantitative estimation of collagen was also done with Sircol™ assay kit method with the help of spectrophotometer at 555 nm wave length.

RESULTS AND DISCUSSION

The physiological, haematological and biochemical parameters though varied but remained within the normal range. As per culture and sensitivity test, inj. Ceftriaxone sodium @ 10 mg /kg body weight was

administered parentrally twice daily for 5 days before stem cell implantation. The photographs of the wound at different stages up to healing were evaluated by three surgeons and showed good healing at 3 weeks. The result supports the findings of comparison of photographs by Borena *et al.,* (2009). Histopathology showed sprouting of capillaries with neovascularisation, formation of granulation tissue (Fig 6), fibroblasts, hair follicles and epithelialisation which supports the progression of healing process. Histochemical study showed formation of more collagen content after stem cell therapy. The quantitative estimation of collagen content with Sircol TM method on zero day, 14th day and 18th day were 12.38 µg/mg, 24.16 µg/mg and 28.41 µg/mg which supports the findings of Ghani *et al.* (1981), Pascoe (1985), Curtis (1993) and Singh and Singh (1993) regarding wound healing. The pain free walking distance which was studied during the healing period showed gradual increase towards end of complete healing (Dash *et al*., 2009). As per the study of Mc Farlin *et al*. (2006) the BM MSCs accelerate the wound healing process in cutaneous wound in case of Sprague-Dawley rat. Supporting this Vojtassak *et al*. (2006) reported the healing of a 25 year old open wound in human being affected with diabetes within 4 weeks following BM-MSCs application. In the present study the wound was healed within 21 days of BM-MSCs application (Fig. 7).

Hence, the present clinical study showed the autologous implantation of BM derived MSCs safe, effective and simple procedure for therapeutic efficacy of chronic nonhealing and ulcerative wounds within 3-4 weeks in bovines.

Table 1 : Constituents of the composed media.

Constituents of media	Amount
FBS (Fetal Bovine Serum) 10% , Lonza	5 ml
Sodium Pyruvate 0.1 % Himedia	0.5 ml
NEA (Non essential amino acids) 0.1%, Himedia	0.5 ml
DMEM (Dulbecco-modified Eagle medium) (MP pharmaceuticals	18.5 ml
Streptomycin (Sigma, Aldrich)	0.5 ml
L 15 (Livosys 15) washing media	25 ml
Total	50 ml

Table 2 : Biochemical parameters measured before and after implantation.

Parameters	on the day of presentation	Healing after therapy
Blood glucose (mg/dL)	61.27	77.48
Serum urea (mmol/L)	7.59	8.35
Serum Creatinine (mg/dL)	1.52	1.21
Serum AST (IU/L)	85.03	102.04
Serum ALT (IU/L)	31.43	23.29
Serum ALP (IU/L)	112.23	101.35
SerumCholesterol (mg/dL)	62.35	70.03
Triglyceride (mg/dL)	7.0	11.04

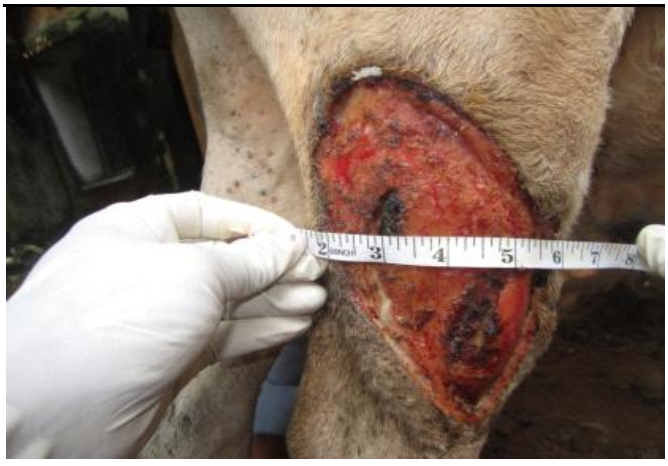

Fig 1. Measurement of the wound.

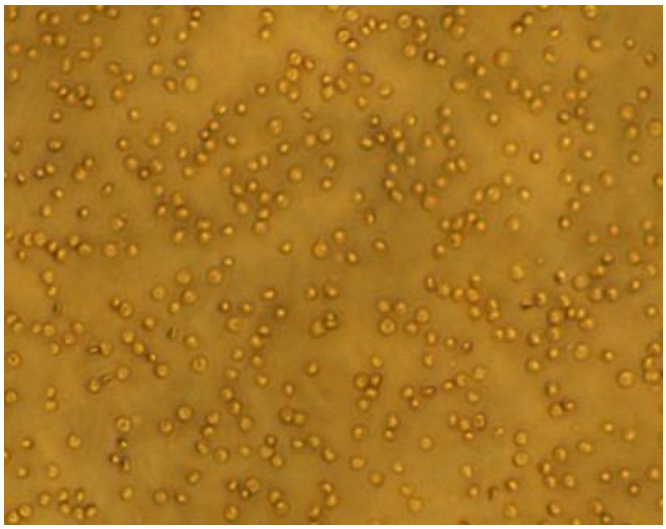

Fig. 2: Photomicrograph of cultured BM cells on day-1.10X

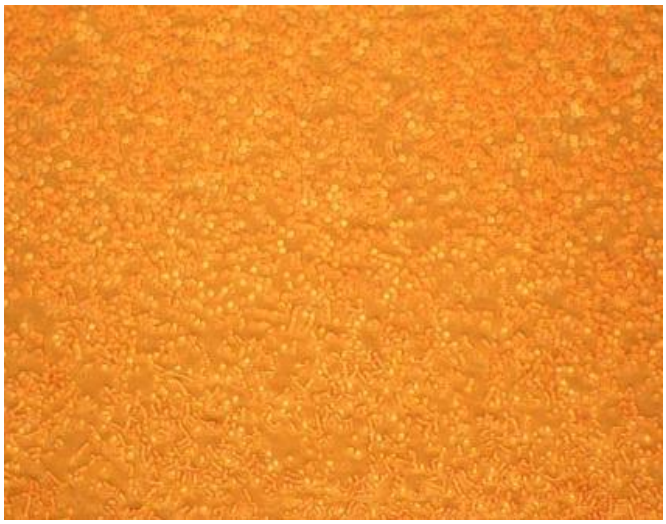

Fig. 3: Photomicrograph of cultured BM cells on day-3. 10X

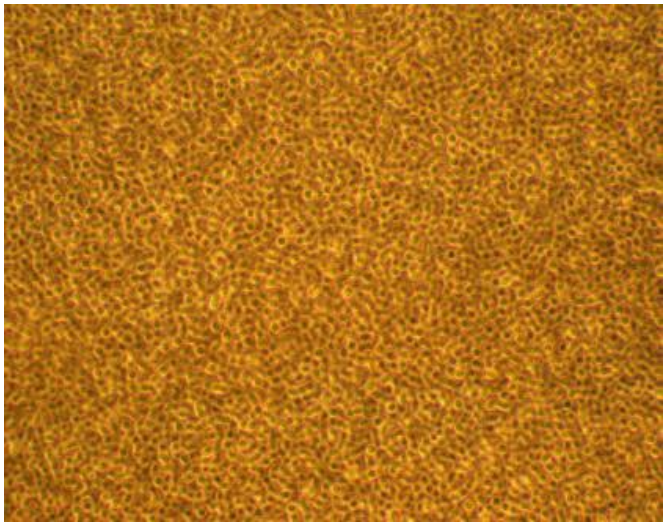

Fig. 4: Photomicrograph of cultured BM cells on day-5. 10X

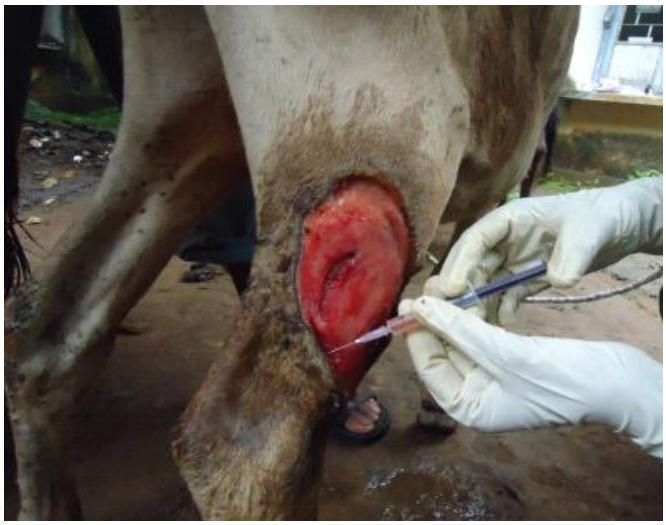

Fig. 5: Implantation of BM-MSCs on wound bed

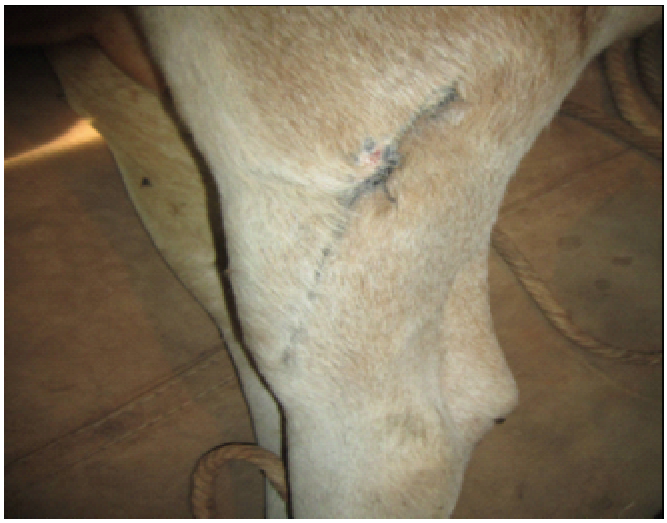

Fig. 6: Neovasculirization after BM-MSCs application

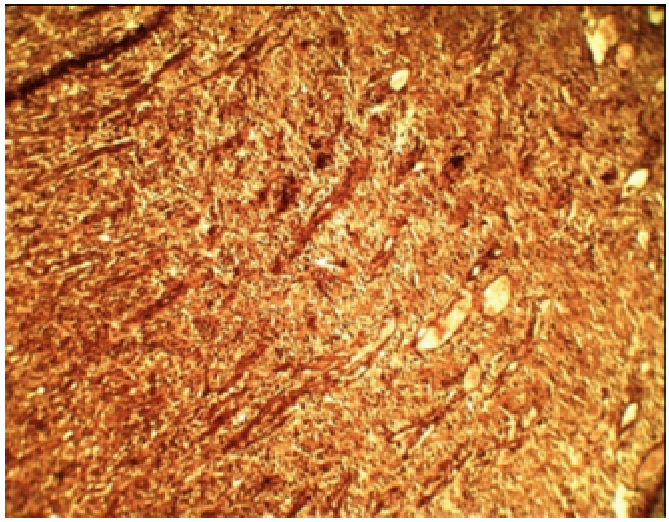

Fig. 7: Healing of wound after 21 days of stem cell therapy

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IN VITRO ANTIBACTERIAL ACTIVITY OF AQUATIC WEED *Spirodella polyrrhiza* TO HUMAN BACTERIAL PATHOGENS

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ABSTRACT

Extracts of Spirodella (*Spirodella polyrrhiza*) have been investigated for antibacterial activity using well diffusion assays against various strains of human bacterial pathogens e.g. *Vibrio cholerae 01(ogawa) Environmental* [BDW(3)], *Vibrio cholerae O1(ogawa)*Clinical (Polymixin B resistance) [BD(3)], *Vibrio choleraeO1(ogawa)*Clinical (Polymixin B sensitive) [BM(4)], *Vibrio cholerae O1(inaba)* EL Tor, *Vibrio choleraeO-139* [JP-22], *Enteropathogenic E.coli* [11044] NICED, *Enterotoxigenic E.coli* [B132, NICED], *Aeromonas hydrophila* [NICED], *Salmonella paratyphi* subtype(A) [C6915], *Shigella dysentreriae* Type(1) [HO15]. Ethyl acetate: ethanolic extract showed comparatively higher mean antibacterial activity than that of ethanolic, methanolic and ethyl acetate extract of *Spirodella polyrrhiza*. Most of the extracts showed inhibition to all the tested bacterial pathogens. When the sensitivity was compared to the antibiotics e.g. Ciprofloxacin (Cf) 5 mcg, Norfloxacin (Nx)10 mcg, Tetracyclin (T) 30 mcg, Gentamycin (G)120 mcg and Azithromycin (At)15 mcg, the zones of inhibition were found to be comparable to the extracts of Spirodella. The study suggested that *Spirodella polyrrhiza* has got the potentiality as an antimicrobial agent of pharmaceutical interest.

Key words : *Spirodella polyrrhiza*, antibacterial, *Aeromonas hydrophila*, *Vibrio* spp., *E. coli, Shigella* spp.

INTRODUCTION

Aquatic weeds are diverse group of photosynthetic plants found in the freshwater environment. Aquatic plants are believed to be source of medicines and research are going on exploring the medicine from the wealth of sea in a large scale. Although aquatic weeds are detrimental to the water bodies by its rooted vegetation, developing the bioactive compounds from plant is highly useful. According to WHO estimates around 80% of the world's population depends in the traditional medicines for their primary health care, majority of which use plant or their active principles (Gias Uddin, 1998). Aquatic plants are able to produce a wide range of biologically active substances with antibacterial, antiviral, antifungal, enzyme inhibiting, immunostimulant, cytotoxic and antiplasmodial activities (Ghasemi *et al.,* 2004). Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry (Boowitzka, 1995; Fables *et al*., 1995; Fly *et al*., 2004; Tuney *et al*., 2006). Treatment of microbial diseases became a growing global concern due to multiple drug resistance developed by pathogenic microbes through continuous use of commercial antimicrobial drugs (Service, 1995). Isolation of microbial agents less susceptible to regular antibiotics and recovery of increasing resistant isolates during antibacterial therapy is rising throughout the world, which highlights the need for new principles (Shahidi and Karimi 2004). It is generally considered that natural compounds are biodegradable and so more environmentally acceptable. Commercial applications of aquatic weed derived compounds have, as yet, received no attention in the area of pharmaceuticals, antibiotics and other biologically active compounds. Various aquatic weeds are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial and antifungal activity (Dash 2009).

Spirodella is a genus of aquatic plant, commonly called duckweed. They were formerly members of the Lemnaceae. *Spirodella* species are free-floating thalii; 2-5 plants may remain connected to each other. Plants are green, but may have a red or brown

underside. Multiple roots (7 to 12 numbers) emerge from each thallus. Spirodella is larger (10 mm) than *Landoltia* (usually 3-6 mm with 2-3 roots per thallus) and *Lemna* (2-5 mm, one root per thallus) (Armstrong 2005). In general, isolation of bioactive compounds from aquatic weed is done with two objectives. The first is to discover new compounds for pharmaceutical or biocontrol applications. The other is to better understand the interactions of individual organism within their natural communities. For each of these purposes there is a need to screen new cultivable organisms (Faleh et al., 995; Kulik, 1995; Moore, 1961; Patt erson et al., 1994, 1995 & Izzo et al., 1995). The aim of the study reported here was to investigate the antimicrobial activity of various extracts of *Spirodella polyrrhiza* against some human bacterial pathogens for the first time.

MATERIALS AND METHODS

Aquatic plants were collected from various ponds of Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, Odisha, India. After collection the aquatic plants were washed properly with tap water followed by distilled water and dried in the shade for 7-8 days depending upon the moisture contents. Then they were made into pieces and grinded by Philips mixture grinder. After grinding, respective materials were sieved individually and collected for extraction.

Preparation of the extracts

Dry *Spiodella* powder was submitted to lipid soluble polar solvents (hexane, ethanol, methanol, ethyl acetate) as well as aqueous medium for extraction, using a Rotavapor (Büchli rotary evaporator-11) at 55- 60^{0}C. Subsequently the residual extracts were suspended in the respective solvents to final concentration of 10 mg/ ml. All of the extracts were preserved at 4 °C.

Test organisms

Taking into account of the prevalence and economic importance of bacterial diseases, ten gram –negative human pathogens *Vibrio cholerae 01(ogawa) Environmental* [BDW(3)], *Vibrio cholerae O1(ogawa)*Clinical (Polymixin B resistance) [BD(3)], *Vibrio choleraeO1(ogawa)*Clinical (Polymixin B sensitive) [BM(4)], *Vibrio cholerae O1(inaba)* EL Tor, *Vibrio choleraeO-139* [JP-22], *Enteropathogenic E.coli* [11044] NICED, *Enterotoxigenic E.coli* [B132, NICED], *Aeromonas hydrophilla* [NICED], *Salmonella paratyphi* subtype(A) [C6915], *Shigella dysentreriae* Type(1) [HO15] were selected to assess susceptibility patterns against the extracts prepared in the present study. All strains were collected from Regional Medical Research centre, Bhubaneswar, India.

The bacterial pathogens used in this work were collected from Regional Medical Research centre, Bhubaneswar, India. Pure cultures of different bacterial strains inoculated in brain heart infusion (BHI) broth (Hi-media India) and incubated at 37^{0}C for 18 h and subsequently used for antimicrobial assay.

Inhibitory effect by well diffusion method

Antibacterial sensitive test of crude extracts of *Spirodella* was done by well diffusion method. Wells were punctured in to the Nutrient Agar plates using a 6 mm diameter sterile puncture prepared locally. The wells were shield by molten agar rose in order to prevent leakage of products from the well. The plates were swabbed with 100 µl of test pathogen corresponding to 10^6 CFU/ml and uniformly spread in the plate. After half an hour of incubation the wells were charged with 70 µl(100mg) of the product. The plates were incubated for 24 h at 37^0 C in a BOD incubator. After the incubation period is over zone of inhibition was measured using a slide caliper. Wells with solvent (70 ml) were taken as control. Activity of the aquatic weed extracts against bacterial pathogens was determined after 24 h. at 37^0 C by measuring the diameter of the halo around the wells (average of three experiments) [16]. The antibacterial activities of plant extracts were compared with inhibition zones around five commercial antibacterial discs, i.e. Ciprofloxacin (Cf) 5mcg, Norfloxacin (Nx)10mcg, Tetracyclin (T) 30mcg, Gentamycin (G)120mcg, Azithromycin (At)15mcg.

RESULTS AND DISCUSSION

Antibacterial activity of various extracts of Spirodella was tested against different strains of bacterial

pathogens. After initial screening of *A. hydrophila*, the extracts representing ethyl acetate (B), ethyl acetate**:**ethanol (C), ethanol (D) and methanol (E) were screened against few human bacterial pathogens. The detailed of the antibacterial sensitivity of *S. polyrrhiza* extracts are represented in the Table-1.

Out of ten human bacterial pathogens screened against *S. polyrrhiza*, ethyl acetate extract showed most sensitive to *V. Ch O1 (I)* EL TOR (23.33 mm) followed by *S. dysenteriae* Type-1 (22.33 mm) and *V. Ch O1 (O)* Env (21 mm) and other *Vibrio cholerae* isolates. *A. hydrophila* did not show any activity to the ethyl acetate extract. Similarly, ethyl acetate**:**ethanol extract showed highest activity to *S. dysenteriae* Type-1 (avg. zone of inhibition, 23.33 mm) followed by *V. Ch O1 (O)* Env and *A. hydrophila* (avg. 19mm each). The merit of sensitivity of ethanol extract of *S. polyrrhiza* was in the order of *V. Ch O-139* (avg. zone 22.66 mm), *V. Ch O1 (O)* Env. (avg. zone 21 mm) and *S. dysenteriae* Type-1 (avg. zone 19.33 mm). The methanol extract showed most sensitive to *S. dysenteriae* Type-1 (avg. zone 20.66 mm) followed by *V. Ch O1 (O)* Env. (avg. zone 19 mm). The sensitivity of the human pathogens to the commercial antibiotics is enumerated in the Table 2.

Drug development from medicinal plants is the recent advances as for the antibiotic resistance has been developed among most of the microbial community. Use of plant derivatives is one of the common forms of therapy available to vast of the world population. Ayurved, Sidhya and Unani form of medicines are widely used in India. Drug from sea is continuing from last two decades. Sea weeds, marine algae and other forms of marine natural products are taken top most priorities for development various types of medicines. However freshwater aquatic plants are not taken momentum so far. The present study was directed to develop some antibacterial bioactive compounds from aquatic plant, *S. polyrrhiza.* The aquatic plant was processed from raw, dried and powdered and finally extracted using different solvents as per the polarity using rotary evaporator and resultant products were tested against a wide group of human bacterial pathogens. The bacterial pathogens tested are important from human health point. These pathogens are gram positive and gram negative bacteria.

The results obtained from the present study concerning the biological antimicrobial activity of antimicrobial agents produced by *Spirodella* were recorded. It is clear from the above result that all the four extracts (ethyl acetate, ethyacetate and ethanolic extract in combination; methanolic and ethanolic extracts) of *Spirodella* are sensitive to ten pathogens tested here except ethyl acetate extract to *A. hydrophila*. Ethyl acetate, ethyacetate and ethanolic extract in combination are comparatively more effective against most of the pathogens tested in the present experiment. *V.ch O1 (O)Env.* BDW-3 was sensitive to all the crude extracts of *Spirodella* and the sensitivity was quite similar to most of the antibiotics tested here except Azithromycin. *V.ch O1 (O) Clin.PB-R.* BD-3 and *V.ch O1 (O) Clin.PB-S.* BM-4 strains sensitivity pattern is similar to the antibiotics Ciprofloxacin. Sensitivity of ethyl acetate extracts of Spirodella to *A. hydrophila* NICRD strain was above the Gentamycin. From the above study it is clear that crude extracts are showing promising results against the human bacterial pathogens and those are comparable to the current trend of antibiotics. Screening efforts aimed to identify antimicrobial agents in aquatic plants have revealed that several promising lead compounds will be purified. A variety of solvents with different polarities were used for the extraction of the bioactive materials. No antimicrobial activity was detected in the other solvents and aqueous extracts. This probably was because of polar nature of the active components.

In conclusion *Spirodella* extracts possess certain constituents with antibacterial properties that can be used as antimicrobial agents for the therapy of microbial infectious diseases. The extracts showed maximum activity against pathogenic microbes subjected to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation after further purification.

Table 1 : Antimicrobial sensitivity of extracts of *Spirodella polyrrhiza*

Sl No.	Bacteria	Extracts			
		1B	1C	1D	1E
		Zone of Inhibition (mm)			
1	V.ch O1 (O)Env. BDW-3	21.0±1.00	19.0±2.0	21.0±1.00	19.0±1.00
2	V.ch O1 (O)Clin.PB-R. BD-3	20.0±1.00	17.66±0.58	14.0±1.00	11.33±0.58
3	V.ch O1 (O)Clin.PB-S. BM-4	18.0±1.00	10.66±1.52	14.66±0.58	9.66±1.15
4	EPEC. 11044NICED	12.0±1.00	16.66±2.09	12.0±1.00	18.0±1.00
5	ETEC. B132NICED	14.0±1.00	16.66±1.15	16.66±1.52	13.0±1.00
6	A.hydrophillaNICED	0	19.0±2.00	10.66±0.58	8.66±1.15
7	S.DYSENTERIAEType-1. HO15	22.33±1.52	23.33±1.52	19.33±1.52	20.66±1.52
8	S.pratyphiSubtype- (A). C6915	18.33±1.52	17.33±1.52	10.0±1.00	13.66±1.15
9	V.ch O1 (I)EL Tor	23.33±1.52	10.66±1.52	12.33±0.58	10.0±1.00
10	V.ch O-139JP-22	9.0±1.00	14.66±0.58	22.66±1.52	14.66±0.58

Data are represented mean +- Standard error, 1B: ethyl acetate and ethanol extract; 1C: ethy acetate extract; 1D: ethanol extract; 1D: methanol extract

Table 2 : Antibacterial sensitivity of bacterial pathogens to antibiotics

Bacteria	Zone of inhibition (mm)				
	CIPROFLOXACIN (20) 5mcg	NORFLOXACIN (17) 10mcg	TETRACYCLIN (19) 30mcg	GENTAMYCIN (15) 120mcg	AZITHROMYCIN (18) 15mcg
V.ch O1 (O)Env. BDW-3	21	21	17	20	25
V.ch O1 (O) Clin.PB-R. BD-3	20	19	18	21	30
V.ch O1 (O) Clin.PB-S. BM-4	18	16	13	17	22
EPEC 11044NICED	20	18	19	15	19
ETEC B132NICED	21	19	21	17	20
A.hydrophillaNICED	28	23	21	15	21
S.dysenteriaeType-1. HO15	24	23	19	16	19
S.pratyphiSubtype- (A) C6915	20	18	20	17	18
V.ch O1 (I)EL Tor	21	19	24	16	19
V.ch O-139JP-22	33	20	19	25	25

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PREVALENCE OF TRYPANOSOMOSIS AND VECTORS AMONG CATTLE AND BUFFALOES OF ODISHA

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ABSTRACT

A field survey on sero-prevalence of trypanosomosis among cattle and buffaloes was carried out through enzyme linked immunosorbent assay (ELISA) in six agro-climatic zones of Odisha. A total of 439 blood samples from 334 cattle and 105 buffaloes were randomly collected from Keonjhar, Biridi, Purusottampur, Bhawanipatna, Sambalpur and Niali blocks of which, 31 cattle and 10 buffaloes were found positive to surra indicating a prevalence of 9.28 and 9.52 %, respectively. The overall prevalence in the state was 9.33 %. Tabanus speciesof flies, known to be the major vector of surra, were identified on the basis of morphological features after their collection in the specially designed traps used near cattle and buffalo sheds in selected areas.

Key words : Trypanosomosis, Tabanus, ELISA, agro climatic zones

INTRODUCTION

Trypanosoma evansi (Synonym : *T. equinum*), the first pathogenic trypanosome, was identified in 1880 in India from the blood of infected camels and horses. The disease trypanosomosis due to *T. evansi* is named variously in different parts of the world. 'Surra' is the most widely used common name of trypanosomosis in India. Life cycle of *T. evansi* is direct i.e., without involvement of intermediate host. The transmission of the disease is purely mechanical where hematophagous insect passes the blood from an infected animal to another during the course of interrupted feeding. Vectors capable of infecting susceptible hosts are either flies (*Tabanid*, *Stomoxys, Lyperosia, Atylotus* and *Hematopota* spp.) or ticks (*Ornithodorus* sp.).

MATERIALS AND METHODS

A field survey on prevalence of trypanosomosis among cattle and buffaloes of Odisha was carried out through enzyme linked immunosorbent assay (ELISA). Animals were randomly selected irrespective of sex, age and breeds.

Hand-made devices were used to collect flies near the shed or resting places of cattle and buffaloes included in the study**.** A mosquito net was fitted to a circular iron ring of about one foot diameter in one side and closed on other side in a conical shape. It was moved over the animal's body in order to trap flies while flying. Alternatively, this job was accomplished with the help of a canopy specially designed and installed near the animal shed where the flies were collected in container fitted at the top (Fig.1). Flies trapped in either of the procedures were examined with the help of a magnifying lens and identified on the basis of their morphological features as described by Soulsby (1982).

RESULTS AND DISCUSSION

A total of 439 blood samples, 334 cattle and 105 buffaloes were randomly collected from Keonjhar, Biridi, Purusottampur, Bhawanipatna, Sambalpur and Niali blocks of which 31 cattle and 10 buffaloes were found positive to surra indicating a prevalence

Zone II	-	North Central Plateu
Zone IV	-	East & South-eastern coastal plain
Zone V	-	North eastern ghat
Zone VIII	-	Western undulating zone
Zone IX	-	Western central table land
Zone X	-	Mid central table land

Fig.1 : Canopy with a container atop installed near cattle shed to collect flies.

of 9.28 and 9.52 % respectively. The overall prevalence in the state was 9.33 %(Table-1). Highest (12.76%) and lowest (5.26%) degree of sero-prevalence was recorded in Niali block of mid central table land and Purusottampur block of north-eastern ghat respectively.

Table 1 : Sero-prevalence of bovine trypanosomosis in Odisha during 2011.

Serial no.	Blocks Selected	No. of bovine blood samples examined	No. of samples sero-positive to surra through Ab ELISA	Preval-ence (%)
1	Keonjhar	62	05	8.06
2	Biridi	80	09	11.76
3	Purusottampur	57	03	5.26
4	Bhawanipatna	79	07	11.25
5	Sambalpur	67	05	7.46
6	Niali	94	12	12.76
Total		439	41	9.33

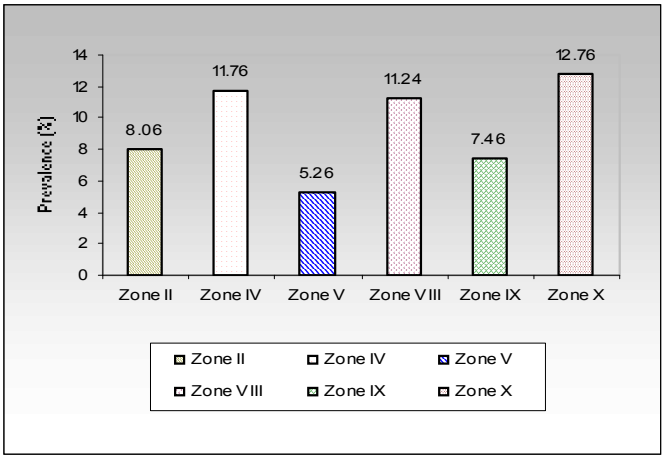

Fig 2 : Sero-prevalence of bovine trypanosomosis in six agroclimatic zones of Odisha.

Flies collected in the specially designed fly traps near cattle and buffalo sheds in selected areas were identified morphologically as *Tabanus, Stomoxys* and *Musca sp.*

Tabanus spp. flies were robust with powerful wings and large eyes. The eyes were almost contiguous in males and separated by a narrow space in the females and projected posterior beyond the lateral margin of the thorax. The antennae had two short basal segments and a third which was large and usually ringed having four annulations and had a tooth like projection on its basal part. There was branching of the fourth longitudinal vein. The proboscis was relatively short, soft and hangs down. The flies were brown with longitudinal stripes on the abdomen (Fig.3-4).

The proboscis of *Stomoxys sp*. flies were prominent and directed horizontally in forward direction and had small labella. The wing veins were gently forward in nature and ending at or behind the apex of the wing. The thorax was grey and had four longitudinal dark stripes, of which the lateral pair was narrow. The abdomen was shorter and broader and had three dark spots on each of the second and third segments.

Fig. 3 : *Tabanus spp*. flies trapped in the canopy near cattle shed in Niali block.

The adults of *Musca sp*. were 8–12 mm long. Their thorax was gray, with four longitudinal dark lines on the back. The whole body was covered with hair-like projections. The females are slightly larger than the males, and have a much larger space between their red compound eyes. Flies had only one pair of wings; the hind pair was reduced to small halters. Characteristically, the medial vein showed a sharp upward bend.

Fig.4 : Magnified view of tabanus fly.

T. evansi is normally transmitted from the infected animals to healthy group by mechanical means. Vectors capable of infecting susceptible hosts are flies (*Tabanid, Stomoxys, Lyperosia* and *Hematopota sp.)* or *ticks (Ornithodorus sp.*). However, Tabanids, commonly known as horse flies or deer flies, are the most potential vector of the transmission of surra in India (Evans, 1880). Adult female tabanids are blood feeders, whereas both male and females of muscoid flies do so. About 13 species of tabanid flies are incriminated in the transmission of animal trypanosomosis in Indian sub-continent (Veer *et al*., 2002). Evidence has been seen of transmission through sexual intercourse, through consuming infected meat, and also through vampire bats in geographical areas where they are found. Hematophagous bat may play the role of a natural reservoir maintaining the multiplicative cycle of *T. evansi* in their blood and tissues (Heitor *et al*., 2005). Bats may succumb to the infection or survive and recover (Gill, 1977).

The flies passively transfer the parasites from the infected animal by sucking the blood and regurgitating into another healthy animal. Development of parasite does not take place in the body of the vector. Because the trypanosomes remain alive in the food canal of the flies for 4-8 hours, the said flies can feed more than one time, thus increasing the chance of successful transmission. In this context the role of Tabanid flies is very important because its mouthpart can trap about 10 µl of blood.

As per the meteorological data for the agro-climatic zone –IV (Niali), the average maximum and minimum temperature remains between 23.3^{0}C and 32.3^{0}C during July to October. This temperature favours the propagation of *Tabanus* flies (Burnet and Hays, 1974). Such information could be well correlated to the predominance of surra during pre-monsoon and monsoon seasons due to active breeding of the vectors.

Tabanus flies were recovered and identified near the cattle and buffalo sheds in all the study areas. Effective methods to control flies could be of some help to combat the loss. Spraying of insecticides may be used to control flies, but large scale adoption of such procedure is not free from environmental hazards. Clearing of the bushes around the cattle sheds may be of some help. Use of fly proof nets for affected animals would be another measure to prevent its spread and such practice was also noticed in some of the rural areas included in the present study. Presence of Tabanus sp. of flies, the major known vector of surra, helped to correlate the prevalence of this extracellular blood protozoan disease among cattle and buffaloes. The *Stomoxys* and *Musca* sp. of flies having less potentiality of disease transmission were also collected and identified during such process.

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IN VITRO DIFFERENTIATION ABILITY OF POST-THAWED BLASTOMERES DERIVED FROM EARLY BLASTULA STAGE EMBRYOS OF INDIAN MAJOR CARP, *Chirrhinus mrigala* (HAM.)

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ABSTRACT

Blastomeres derived from early blastula stage embryos (64-cell stage) of *Cirrhinus mrigala* cryo-preserved along with 10 % DMSO (Dimethyl sulfoxide) can effiiciently differentiated into different cell types representative of three embryonic germ layers after thawing from cryogenic preservation. Post-thawed blastomeres were cultured in composed medium for development of mrigal embryonic stem (ES) cell like-colonies. After sub-culture of these colonies at a seeding density of $2x10^7$ cells/ml and 6.4×10^6 cells/ml in presence of 5 ng/ml b-FGF and at lower densities 5×10^4 cells/ml in presence of dexamethasone (1 ìM) along with 2 % DMSO along with other necessary supplements. Under these conditions they directly differentiated into melanocyte-like cells, chondrocyte-like cells and hepatocyte-like cells respectively.

Key words : Differentiation, post-thawed, blastomeres, melanocyte-like cells, chondrocyte-like cells, hepatocyte-like cells

INTRODUCTION

The cryopreservation of germ cells, gametes and embryos is considered as a useful technology for conservation of genetic resources (Routray *et al.*, 2009). This promising technique has a great role in aquaculture sector. In teleosts, cryopresevation of sperms has been possible in large number of species (Zhang and Rawson,1995). The cryopreservation of blastomeres in liquid nitrogen (LN_2) is reported in few species (Chao and Liao, 2001), viz. zebra fish, *Brachydanio rerio* (Harvy,1983); Rainbow trout, O*ncorhycous mykiss* (Nilsson and Cloud,1998), Chum salmon, *O. keto* (Kusuda *et al.,* 2002); Whiting, *Shilago japonicas*; Perjerry, *Odonstesthes bonariensis* and Medaka, *Oryzias latipes (*Strüssmann *et al.,*1999). Fish blastomeres at early embryonic stage before attainment of early gastrula have diploid karyotype and retain pluripotency for further development. Kusuda *et al.,* (2004) checked pluripotency of cryopreserved blastomeres by transplanting them into blastula stage of gold fish (*Carassius auratus*) *in vivo.*

In this study, isolated blastomeres derived from early blastula stage embryos from Indian major carp, *Cirrhinus mrigala* (Hamilton, 1822) were successfully cryopreserved along with 10% DMSO, After freezing and thawing these post-thawed blastomeres were cultured in composed medium for development of ES-like colonies. These mrigal ES-like colonies were sub-cultured along with appropriate inducers to check their potential to develop into different cell types.

MATERIALS AND METHODS

Brood stock management and induced breeding of *Cirrhinus mrigala*

The brood fish of Indian major carp, *Cirrhinus mrigala* (Ham. 1822), mrigal, used in this study were reared in earthen ponds (0·2 ha) of the farm facility of the Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, Odisha, India. For induced breeding, two to four males and females (1:1) were injected intra-peritoneally with ovaprim (Salmon GnRH + Domperidone, Syndel Laboratories) at a rate of 0.2ml

and 0·4 ml/Kg body mass to males and females respectively. After 5 to 6 h following hormone administration male and females were stripped for milt and eggs. Fertilization was done by mixing semen and ova over an enamel tray with addition of freshwater to it. Embryos were collected in sterilized petridishes.

Isolation of blastomeres from mrigal embryos

The viable embryos were incubated for 1.5-2 hours in water at room temperature for achieving the early blastula stage. After 1.5-2 hours of incubation, approximately 200-300 embryos of early blastula (64-cell stage) were taken for isolation of blastomeres. The embryos were first sterilized with 70 % ethanol and then washed with sterile 1× D-PBS (pH 7·4). Embryos were then pipetted four to five times with a wide mouth sterilized pipettes to rupture the chorion and release the mass of aggregated blastomeres. These aggregated blastomeres became single cell blastomeres by centrifugation for 5 min at 500 g in a table-top centrifuge.

Cryopreservation of isolated blastomeres

The isolated blastomeres thus obtained were suspended in the pre-cooled (4°C) freezing medium containing Dulbecco's modification of Eagle's medium (DMEM, Sigma), 10 % fetal bovine serum (FBS, Sigma) and 10 % DMSO (Sigma). One ml of blastomeres with a density of $5x10^7$ cells/ml were kept in a 2 ml labeled cryovial (Nunc, Denmark) and cryopreserved following a slow freezing protocol (Dash *et al.,* 2008).

Thawing protocol

Thawing procedure was mainly carried out according to (Routray et al., 2009). Thawing was performed in a rapid warming phase. The cryovials (containing blastomered) from LN_2 after 3 month of cryogenic storage were thawed in a water bath at 28 ºC for 60 seconds, with 10 volumes in a five-step procedure, the first four times for 5 minutes each in fresh DMEM without FBS to remove excess cryoprotectants and the fifth time in medium with FBS to maintain better viability. The viability of blastomeres was determined by haemocytometer.

% of Live cells = Unstained cells / (unstained cells + stained cells) x100.

***In vitro* culture of post-thaw blastomeres for development of mrigal ES- like colonies**

The post-thaw blastomeres were cultured in complete growth medium composed of Leibovitz-15 (L-15), Dulbecco's modified Eagle's medium with 4·5 g /L glucose (DMEM) and Ham's F12 (LDF) in 50: 35:15 ratio, 10 % FBS, additional components supplemented were 15 mM HEPES [4-(2-hydroxyethyl)-1-piperazine-ethane-sulphonic acid], 8 nM sodium selenite, 100µM ß-mercaptoetanol, 1mM sodium pyruvate, 1mM non-essential amino acid (NEAA), 100 IU/ml penicillin, 0.1mg/ ml streptomycin, 10 ng/ml basic human fibroblast growth factor (bFGF), 10 ng/ ml human leukemia inhibitory factor (hLIF) (All from Sigma, USA), 1 % FS and 1 % FEE. The procedure followed here was adopted from Routray et al. (2009).

The cultured cells were incubated at 28 ºC with 5 % CO_2 (Contherm, Hult City, New Zealand). They were regularly observed for morphological intactness under inverted microscope (Hund Wetzlar, Germany). The medium was changed at every 3-day interval. When cells attained 80 % confluence the sub-cultured was done at a splitting ratio (1:2) in 4-5 days interval.

Fish serum used here was collected from caudal vein of adult mrigal and allowed to clot for 30 min at room temperature. Then kept overnight at 4 ºC, serum separated by centrifugation (200g for 10 min), sterilized through 0.4 µm syringe filter, heat inactivated at 56 ºC for 30 min and finally stored at -20 ºC in aliquots for further use.

Preparation of fish embryo extract (FEE)

Approximately 300 embryos of mrigal, rohu and catla (2-h post fertilization) were collected for preparation of fish embryo extract (FEE). The main aim of using

from rohu and catla along with mrigal here for preparation FEE was closely related species is more mitogenic than same species (Hong *et al.,* 2000). Embryos washed thoroughly with 70 % ethanol and rinsed several times with PBS-A (pH 7.4) to avoid contamination. The washed embryos were dechorionated manually and homogenized with 2-5 ml of PBS-A (pH 7.4) in a tissue homogenizer (Braun). The resulting homogenate was centrifuged for 10 min at 15,000×g at 4 °C (Heraeus centrifuge, Thermo Scientific, Germany). The supernatant was diluted 1:1 with PBS-A (pH 7.4), passed through the 0.4µm and 0.22µm syringe filter. Protein concentration was determined by using the method described by Bradford, 1976. The solution was made to a concentration of 10 mg/ ml and stored at –20 ºC following a standard protocol (Fan *et al.,* 2004).

Differentiation of mrigal-ES cells into melanocyte-like cells (ectodermal lineage)

After trypsinization the isolated undifferentiated mrigal-ES like cells were seeded at $2x10^7$ cells/ml on 0.1 % gelatin coated 35 mM petridish along with medium containing DMEM with 10 % fetal bovine serum, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 0.1 mM 2-mercaptoethanol, 50 U/ml penicillin, and 50 ìg/ml streptomycin. The media was changed in every 2 days interval. Regularly microscopic observation was performed to detect the generation of differentiated cells in the populations.

Differentiation of mrigal-ES cells into chondrocyte-like cells (mesodermal lineage)

The undifferentiated mrigal ES-like cells were harvested by trypsinization and seeded in 6.4×10^6 cells on 1 mg/ml collagen coated 35 mm petridish cultured along with differentiating medium containing DMEM with 10 % fetal bovine serum, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 0.1 mM 2-mercaptoethanol, 50 U/ml penicillin, and 50 ìg/ml streptomycin along with 5 ng/ml bFGF. The media was change in every 2 days interval cultured for 21 days. Regularly microscopic observation was performed to detect the generation of differentiated cells in the populations.

Differentiation of mrigal-ES cells into hepatocyte-like cells (endodermal lineage)

After trypsinization the isolated undifferentiated mrigal-ES like cells were seeded at 5×10^4 cells/ml into 1 mg/ml collagen coated six-well plates along with differentiating medium containing DMEM with 10 % fetal bovine serum, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 0.1 mM 2-mercaptoethanol, 50 U/ml penicillin, and 50 ìg/ml streptomycin and dexamethasone (1 ìM) along with 2 % DMSO.

RESULTS AND DISCUSSION

The methodology followed here was schematically represented in figure-1. The survival rate of post-thawed blastomeres (Fig. 2.a) after 3 months of cryogenic storage along with 10 % DMSO was almost 70 %.These blastomeres had round, polygonal morphology having 20-50 ìmi diameter. Post– thawed blastomeres upon culture in composed complete growth medium developed into mrigal ES-like colonies within 4-5 days (Fig.2 b). These colonies were characterized by smooth appearance, tightly packed cells and uniform distinct sharp boundaries. Upon sub-culture these dissociated undifferentiated cells at appropriate seeding densities in the presence of inducers differentiated into cell types of 3 embryonic germ layers.

The mrigal ES-like cells upon seeding at higher seeding density (2×10^7 cells ml^{-1}) after 7 days of culture developed into pigmented melanocytes. These cells appeared black due to heavy deposition of melanin granules having flat morphology (Fig.2 c). The dissociated undifferentiated mrigal ES-like cells obtained after sub-culturing and seeding at higher density (6.4×10^6 cells ml^{-1}) developed into chondrocytes like-cells in the presence of bFGF showing a peculiar morphology having mineralized nodules at center after 21 days of culture (Fig. 2.d).

Primary hepatocytes (Fig. 2 e) started to differentiate from mrigal-ES like cells grown on collagen coated petridishes supplemented with dexamethasone and DMSO. These cells were characterized by their polygonal or cuboidal morphology present in homogenous distribution.

Mrigal blastomeres cryopreserved in liquid nitrogen for three months showed approximately 60-70 % survival rate. These values are almost same as that of pererrey blastomeres, 20-67 % (Strüssmann *et al.,*1999) but lower than that of rohu (*Labeo rohita*) (Dash *et al,* 2008) and Leopard danio (*Brachydanio frankei*) (Routray *et al.,* 2009). The viability of post-thawed cells varies among species due to several species-specific differences (Routray *et al.*, 2009). These post-thawed blastomeres were obtained from early blastula embryos of mrigal successfully and used for development of ES-like cells in complete growth medium. These colonies have similar morphology as reported in other teleosts (Alvarez *et al.,* 2007). ES cells also offers a unique opportunity to reveal the mechanism of differentiation paradigm without formation of EBs. Here, we have shown differentiation of mrigal ES-like cells into cell types of 3 embryonic germ layers melanocyte-like cells, chondrocite-like cells and hepatocyte-like cells.

Melanocytes or their precursors melanoblasts are cells derived during neurulation stage of embryonic development. These are generated from the neural crest. After extensive migration, they reside in the skin and inner ear as highly dendritic, heavily pigmented cells and are generally located in the epidermal basal cell layer of these areas; including hair follicle development. Melanocytes from ES cells have been reported in mammalian system by induction of dexamethasone (Yamane *et al.,* 1999). Generation of melanocytes in mammals and teleoest required different gene expression and mechanism (Mellgren and Johnson, 2004). However, here melanocyte differentiation was observed from mrigal ES-like cells upon seeding at higher seeding density. The reason may be formation of melanocytes required tight cell-cell interaction which is only accomplished by seeding at higher seeding density. Similar observation has been made in medaka ES cells (Hong *et al.,* 1996).

Among the differentiated phenotypes from ES cells the formation of mesodermal originated chondocytes has less chance. In this study, ES cells cultivated at higher seeding density along with bFGF on collagen coated matrix differentiated chondrocyte-like cells were observed. The reason for this may be chondrogenic differentiation is generally initiated by the inductive action of specific growth factors and depends on intimate cell-cell interactions (Denker *et al.,* 1998). The bFGF has been known as an important factor in modulating chondrocyte mitogenesis and synthesis of cartilage matrix. Administration of exogenous source of bFGF support articular cartilage repair in humans in case of cartilage defects. In monolayer culture, bFGF act as mitogen to increase the proliferation of human septal and auricular chondrocytes (Chua *et al.,* 2007). Higher seeding density increase cell-cell interaction which is prime requisite for chondrocyte formation.

The liver is the largest organ in vertebrates and it serves a variety of important functions. Hepatocytes, the primary cells of the liver, perform various functions, including metabolizing diverse dietary molecules, detoxifying compounds and storing glycogen. Dexamethasone is a synthetic glucocorticoid hormone promotes the differentiation of different tissues *in vivo*. Dimethylsulfoxide (DMSO) is a commonly used inducer of differentiation (Baribault and Marceau, 1986). Following, Soto-Gutie´rrez *et al.* (2007) here, primary hepatocytes were observed due to supplementation of these two chemical factors into *in vitro* culture.

This study infer about the retention of pluripotency of post-thawed blastomeres through process of differentiation. This study has the potential application in modern and basic biological research.

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ISOLATION AND CHARACTERIZATION OF VIRULENT *Aeromonas hydrophila* ISOLATES ASSOCIATED WITH DISEASES OF ORNAMENTAL FISHES BELONGING TO FAMILY *Cyprinidae* and *Poecilidae*

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ABSTRACT

Ornamental fishes belonging to family *Cyprinidae* and *Poecilidae* were affected by disease conditions such as dropsy, ulcer, fin rot, tail rot, gill rot, septicemia and pop eye. *Aeromonas hydrophila* was found to be associated with these conditions. Most of these bacterial isolates were found to be virulent upon checking them through phenotypic, molecular, biochemical as well as *in vivo* pathogenesis tests.

Key words : *Aeromonas hydrophila*, Aerolysin, Bacteria, Ornamental fish, Pathogenesis, virulence.

INTRODUCTION

In the global scene, ornamental fish keeping is considered as the second largest hobby next only to photography and popular for their aesthetic beauty. Ornamental fish keeping was initiated as a small time hobby using gold fish during the 18th century. In 20th century; aquarium keeping and rearing of ornamental fish got worldwide recognition. At present this technique has developed in to an extensive and global component of international trade worth millions of dollars. Since 1985, the international trade in ornamental export has increased on an average of 14 % per year. Besides this it also provides employment and revenue generation to the country.

In India, a variety of fresh water and marine ornamental fishes are available. About 600 species have been identified as potential fishes with ornamental value. The trade in India earn about Rs. 50 crores /year. However, the success of ornamental fish culture or breeding depends on the health status of the candidate species (Hossen, 2008). Several diseases of economic importance are recorded. The incidence of microbial pathogens, especially those of bacterial origin is one of the most significant factors affecting ornamental fish culture. *A. hydrophila* is among the most common bacteria in freshwater habitats throughout the world (Larsen and Jensen, 1977; Paniagua *et al*., 1990). Several disease conditions such as septicemia, tail rot, fin rot, ulcers, dropsy, and abnormal distension etc. of a wide variety of freshwater ornamental fishes worldwide have been reported to be due to *A. hydrophila* (Musa *et al*., 2008).

Although a lot of work has been done on disease conditions of other fish affected by *A. hydrophila*, reports on association of this bacterium in diseases of ornamental fishes are very limited. Therefore, this study attempted to isolate, identify and characterize naturally collected different isolates of *A. hydrophila* associated with different disease conditions of ornamental fishes collected from different parts of India over a period of two years.

MATERIALS AND METHODS

Areas of sampling

Suspected ornamental fish samples were collected from hatchery, farms and pet shops of different states in Odisha, West Bengal, Tamil Nadu and Andhra Pradesh. The number of suspected ornamental fish samples belonging family Cyprinidae and Poecilidae were collected which includes both exotic and indigenous fishes.

Isolation and characterization of bacteria

The samples were processed for bacteriological studies, *i.e*. bacterial isolation and identification by

microbiological procedures as per the method described by (Cruickshank *et al*, 1975).

Phenotypic determination of virulence factors

Congo red dye uptake

The ability to take up Congo red dye was determined following the method described by Paniagua *et al*., 1990. Briefly, agar plates were supplemented with 50 ìg /ml of Congo red dye. Five micro liter of bacterial suspension was streaked onto the plates and incubated at 37 ^{0}C for 24 h. Plates showing orange colonies were identified as positive (virulent) whereas non-virulent showed white colonies.

Hemolytic activity

The strains were tested for â-hemolysin activity on nutrient agar base (Hi Media, India) supplemented with 5% rabbit erythrocyte. The bacterial suspensions were streaked on to the plates and incubated at 37 ^{0}C for 24h. A clear and colorless zone around the colony conform the â-hemolytic activity (Gerhardt *et al*. (1981).

Proteolytic activity

Gelatin was assayed by a radial diffusion method described by (Gudmundsdottir, 1996). Briefly, 10 µl of each bacterial suspension was placed in 4 mm diameter well cut in 1 % agarose gel plate supplemented with 3 % gelatin and incubated at 22 ^{0} C for 20 h. Then the plates were immersed with saturated ammonium sulphate solution at 70 ^{0} C to precipitate un-hydrolyzed gelatin. The presence of transparent zone around the colonies indicates proteolytic activity.

Molecular identification

Bacterial genome was isolated following the method described by Figureas *et al*. (2000) and PCR reaction was carried out for amplification of the aerolysin gene by using a published set of primer Pollard *et al*. (1990).

***In vivo* pathogenesis test**

The bacteria tested for pathogenic virulent characters are further subjected to *in-vivo* pathogenesis tests by using fish, rohu (*Labeo rohita*); average body weight ranging from 25-30 g. Fishes were divided into five groups, for virulence study of *A. hydrophila*. Selected bacterial isolates were grown in BHI at 37 ºC for 24 h, centrifuged at 5000 X g for 10 min, washed in sterile saline and the final pellet was suspended in sterile PBS solution. The fishes were injected intra-peritoneally with 0.1ml (10^4 –10^8 CFU ml^{-1}) of bacterial suspension using six fish per each dilution. Control fish received the same dose of non-virulent bacterial isolate identified through phenotypic and molecular means. Clinical signs and mortalities were recorded up to 7 days. The end point was calculated using the method of Reed and Muench, 1938.

RESULTS AND DISCUSSION

Dropsy, ulcer, fin rot, tail rot, gill rot, septicemia and pop eye diseases were found to be important diseases affecting the ornamental fish such as guppy (*Poecilia reticulata*), molly *(Poecilia sphenops*), sword tail (*Xiphophorus helleri)*, gold fish (*Carassius auratus*), koi carp (*Cyprinous carpio* Var. Koi) and Indian rosy barb (*Puntius conchonius*) from which *A. hydrophila* was isolated. However, in case of disease conditions of fish such as dropsy bacteria other than *A. hydrophila* were also found to be associated, but probably as secondary pathogen. Earlier studies on disease conditions of ornamental fishes reveal that *A. hydrophila* plays a major role in dropsy, fin rot and ulcerated disease of fish (Ventura and Grizzle, 1998; Kumar *et al*, 1986; Kumar *et al*, 1991) and from the gold fish (Bandyopadhyay *et al*., 2003). The two important spp. of A*eromonas* i.e. *A. hydrophila* and *A. sobria* causing diseases in warm water aquarium fishes have been reported by McGarey *et al*. (19991). Potential sources for transmission of *Aeromonas spp.* could be poor water and feed quality leading to stress and disease conditions to the aquarium fishes (Daskalvo, 2006; Kuhn *et al*., 1997). The biochemical properties of *A. hydrophila* isolated from various disease conditions are presented in Table 1. Out of 297 suspected fish samples studied, 9.09% of ornamental fish species belonging to family Cyprinidae and Poecillidae were found to be affected by *A. hydrophila.* (Table-2).

Table 1 : Biochemical characteristics of *A. hydrophila* isolated / associated with different disease condition.

Characters	*A. hydrophila*	Characters	*A. hydrophila*	Characters	*A. hydrophila*
Gram's staining	-	H_2S production	+	Sucrose	+
Methyl red	+	Gas production	_	Mannose	+
Vp	+	Sugar Mellibiose (Mb)	+	Arabinose	+
Manitol fermentation	+	Dextrose	+	Inulin	+/-
Manitol mortility	+	Maltose	+	Fructose	-
Indole production	_	Lactose	+	Adinitol	_
Oxidation	+	Salicin	_	Sorbitol	+
Catalase	+	Inositol	_	Gelatinase	+
Citrate utilization	+	Galactose	+	xylose	_
Glucose	+	Dulicitol	_	Casein	+
Sucrose	+	Mannitol	+		
Lactose	+/-	Rhamnose	+		

Table 2 : Disease conditions of ornamental fishes of family *Poecilidae* and *Cyprinidae* associated with *A. hydrophila*

Diseases condition	Targeted organ	Causative agents	Affected Fish
Dropsy	Abdomen /intestine/ kidney	*A. hydrophila* (8)	Rosy barb, melon barbs, gold fish, guppy, molly.
Ulcer (surface and internal)	Near dorsal fin/intestine/ ulcer tissue	*A. hydrophila* (3)	Melon barb, guppy, gold fish.
Gill rot	Gills	*A. hydrophila (2)*	Sword tail, molly
Fin rot	Fin	*A. hydrophila (2)*	Rosy barb, molly
Tail rot	Tail	*A. hydrophila* (4)	Gold fish, koi carp, guppy, molly.
Pop eye diseases / corneal opacity	Eye/ eye cavity/near by tissue of eye	*A. hydrophila* (4)	Fancy gold fish, sword tail, molly, rosy barb.
Septicemia	Mouth / entire body/ septic flesh	*A. hydrophila, (4)*	Melon barb, Sword tail, guppy, gold fish

The virulence of *Aeromonas hydrophila* is multifactorial including adhesions, S-layer, lipopolisaccharides, siderophores, and an array of exoenzymes and endoenzymes such as aerolysin/ hemolysin, lipase and protease activity which contribute to the pathogenesis of *A. hydrophila* infection (Stelema *et al*., 1986; Pemberton *et al*., 1997; Wong *et al*., 1998; Zhang *et al*., 2000; Sha *et al*., 2002). To identify the virulent from non-virulent *A. hydrophila* isolates*;* phenotypic, molecular characterizations as well as *in vivo* pathogenesis tests were done. Upon screening of the 27 isolates of *A. hydrophila* in Congo red dye uptake method, it was found that four isolates showed the orange colony whereas remaining others formed white colony (Fig.1, A). It has been reported that uptake of Congo red in case of *A. hydrophila* is through the sideropore (Santos *et al*., 1999). Several researchers have also found the ability of virulent strains of *A. hydrophila* for binding the Congo red (Statner and George, 1987; Palumbo *et al*., 1989). Apart from *A. hydrophilla*, other virulent enterogenic bacteria such as *E. tarda* (Ling *et al*., 2000), *Yersinia entercolitica* (Farmer *et al*., 1992) and *Vibrio cholera* (Nataro and Kaper, 1998) also uptake Congo red with appearance of orange colony. For further confirmation, these four virulent isolates of *A. hydrophila* were tested for hemolytic activity. It is known that pathogenic isolates of *A. hydrophila* secret aerolysin toxin that causes the lyses of the

RBCs and results in hemorrhagic signs on the skin and internal organs of fish. In this study, hemolytic activity of aerolysin toxin was observed on blood agar medium (Fig.1, B) which could be due to the hemolysin gene that shows the â-hemolytic activity in virulent strain of *A. hydrophila* (Kingombe *et al*., 1999). Proteolytic activity is another way to identify virulent and non-virulent isolates by determining the gradation of gelatinase production. The virulent isolates showed a clear zone in gelatin agar plate (Fig.2) where as no zone was found around the inoculums of non-virulent ones. Such proteolytic activity of *A. hydrophila* has also been reported by Austin and Austin, 1999 which enhances the pathogenicity of bacteria in fish. These virulent isolates were also used for molecular identification by amplifying the aerolysin gene and were found to be positive showing the amplified band of length 209 bp (Fig.3) where as no such band was found in the non virulent strains. From this, it was found out that the strains that showed the aerolysin gene activity also contain the gene encoding for â-hemolysin (Baloda *et al*., 1995) also isolated *A. hydrophila* and *A. sobria* from drinking water, fish and food products and identified the aerolysin gene of amplified band 209 bp. In challenge study, virulent isolates showed LD_{50} value ranged from $10^{5.5}$ to $10^{6.5}$ CFU ml^{-1} where as no mortality was recorded in control group injected with the same dose of non-virulent bacterial isolates during the said period.

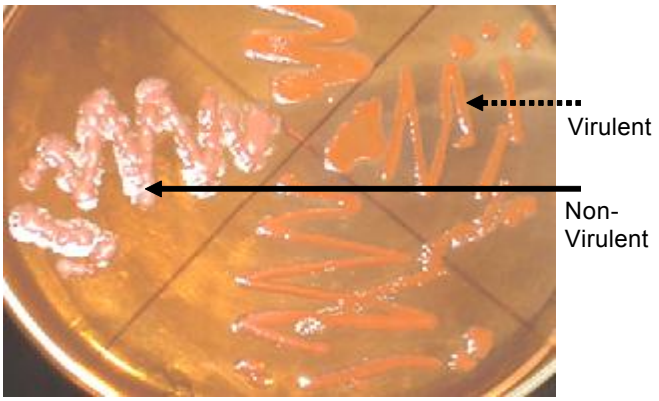

Fig 1 (A) : In vitro identification of virulent and non-virulent *A. hydrophila* isolates through Congo red activity on agar plate. (⋯▸ shows virulent and ⟶ shows non-virulent strain).

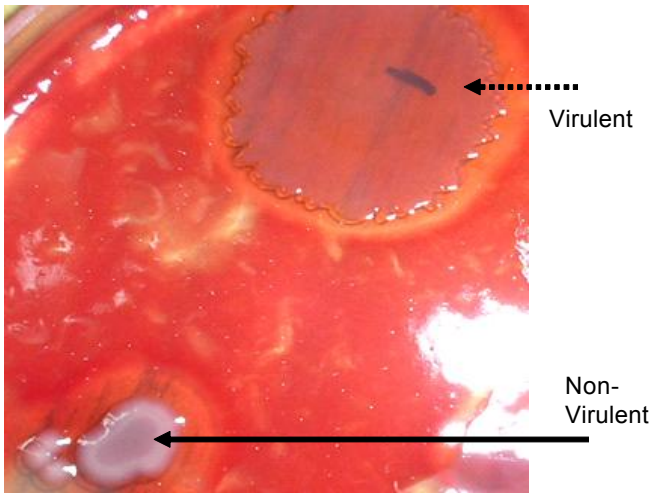

Fig 1 (B) : In vitro identification of virulent and non-virulent *A. hydrophila* isolates through Hemolytic activity on agar plate. (⋯▸ shows virulent and ⟶ shows non-virulent strain).

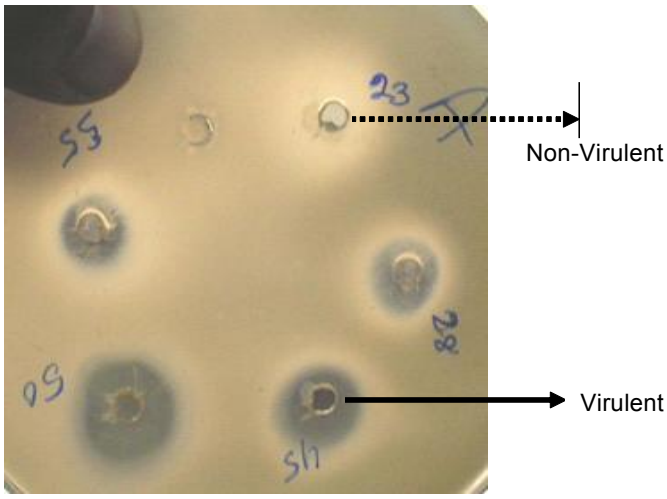

Fig 2 : In vitro identification of virulent and non-virulent *A. hydrophila* isolates through Proteolytic activity on agar plate. (⋯▸ shows virulent and ⟶ shows non-virulent strain)

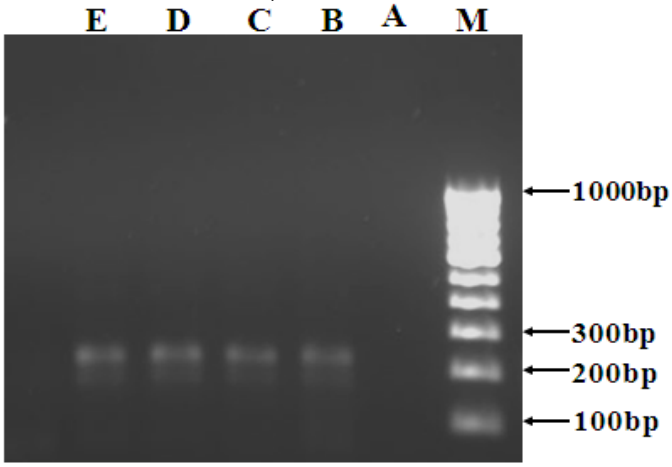

Fig 3 : PCR amplification of 209-bp aerolysin gene of virulent and non-virulent *A. hydrophila* isolates on 1.2 % agar ose gel. Lane M : 100-bp DNA ladder, Lane A : Non – virulent and lane (B, C, D, E) virulent strains.

The present investigation showed that *A. hydrophila* is an important causative agent of disease conditions affected ornamental fishes in India. The presence of several virulence factors and genes in some isolates highlighted the necessary to implementation control/ managemtal measures for diseases practices including additive prophylactics practices.

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OCCURENCE OF PARASITIC COPEPODS, *Lernaeenicus sprattae* ON KING MACKEREL (*Scomberomorus commerson*), ALONG SOUTH-EAST OF INDIA

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ABSTRACT

This study was carried out to investigate the extent of parasitation of copepod pennellidae on King Mackerel (*Scomberomorus commerson*), along South-East coast of India during September 2009.This is the first report from this region and the infection is discussed in relation to biological parameters. Fifty to sixty numbers of parasites were recorded from belly region of King Mackerel. Parasitic copepod identified as *Lernaeenicus sprattae*, belongs to family Pennellidae harms several fish species, which, feed voraciously and kill fry and fingerlings of the many marine fishes. *Lernaeenicus* parasites would stunt the growth of fish through inhibiting reproduction, thus these type of parasitation would lead to severe economic loss in the commercial species of the marine fishes of India.

Keyword : Parasitic copepod; *Lernaeenicus sprattae,* new host record; King Mackerel (*Scomberomorus commerson*) off Chennai Coast.

INTRODUCTION

Seer fishes are pelagic fishes, fast swimmers and predatory in nature belongs to Family-*Scombridae*, Order- *Perciformes*. They are a delicacy in several regions of South India and Sri Lanka. In Tamil Nadu and Andhra Pradesh, this fish is called “Vanjaram” and is usually the costliest variety available. These are commercially important marine fishes of India. Approximately 10,000 species of copepods have been described and about 2,000 of them mainly parasitize the fish. Among 2000, *Pennellidae* are widespread and highly visible gonochoric nature on marine fish. All species of this family are known for larval development, and have a life cycle involving two hosts (dixenic cycle) (Raibaut, 1996). Some penetrate only a short distance into the fish tissues, others burrow deeply into all organs seeking areas with a rich blood supply (Natarajan *et al.*, 1973). Most cause localized changes in adjacent tissues and some result in loss of condition or reduced gonad development (Raibaut, 1996). Gopalakrishnan *et al.,* 2010 reported double parasitism (isopod Cymothoidae and copepod Pennellidae) on the black-barred halfbeak fish, *(Hemiramphus far)* from southeast coast of India. In this study, an interesting Parasitic copepod *Lernaeenicus sprattae,* Pennellidae) is reported on the belly region of King Mackerel (*Scomberomorus commerson*) fish.

MATERIALS AND METHODS

Specimens of King Mackerel (*Scomberomorus commerson*), Phylum-Chordata, Class-Actinopterygii, Order-Perciformes, Family-Scombridae, Genus-*Scomberomorus*, Species-*commerson*, commonly called King Mackerel, were collected with a trawl net from the inshore waters of the Kasimedu Fisheries Harbour, (13°06' 59.50"N 80°17' 38.99"E) Bay of Bengal off Chennai coast, during September 2009. The parasites were identified according to Kirtisinghe (1932) and Gnanamuthu (1953). Collected parasites were fixed in 70% ethanol (w/v). The taxonomic classification of the King Mackerel fish host was carried out by Fischer *et al.,* 1974 and Froese *et al.* 2008.

RESULTS AND DISCUSSION

Lernaeenicus hemiramphi was first described and reported from *Hyporhamphus xanthopterus* (Hemiramphidae) fished from the Ceylon coast by Kirtisinghe (1932). Later, it was redescribed by

Gnanamuthu (1953) and John *et al.* (1973), according to specimens obtained from *H. far* caught along the Madras coast (Table-1). This species seems to be a specific parasitic copepod of Hemiramphidae. However, Shiino (1965) collected *L. hemiramphi* from *Coryphaena* sp. (Coryphaenidae) caught in Hawaiian waters. Fifty to sixty numbers of *Lernaeenicus sprattae* were attached to belly region of host fish (Fig.1 & 2). Recent report of *Lernaeenicus sprattae* (Crustacea: Copepoda) has been made on *Hemiramphus* Far. The total body length of parasite was ranged from 90-120mm (Fig.2 & 3) in King Mackerel.

Fig.1 : King Mackerel, *Scomberomorus commerson* collected from Kasimedu Fisheries Harbour

Fig.2 : Parasitic copepods, *Lernaeenicus sprattae* attached massively on ventral portion of a King Mackerel, *Scomberomorus commerson.*

Fig.3 : Group of parasitic copepods, *Lernaeenicus sprattae* collected from abdomen region of King Mackerel, *Scomberomorus commerson* .

This is the first report on the occurrence of an infection by parasitic copepod; *Lernaeenicus sprattae* in the seer fish from the South-Eastern coast of India, Such mass infestation of pennellid copepod, on belly portion of seer fish is probably uncommon, but not accidental. Parasites in the belly portion are usually associated with atrophy and partly from damage associated with feeding and attachment leads to anaemic condition. This may lead to severe economic loss in the commercial species of the marine fishes of India particularly Seer fishes (Bragoni, *et al.,* 1984).

Table 1 : Occurrences of Parasitic Copepods, *Lernaeenicus sprattae on* different marine fishes

Sl.No.	Host	Copepods	References
1	Flying fish, *Cypselurus* (*Hirundichthys*) *speculiger*	*Pennella sp.*	Daniel *et al.* (1967a)
2	Flying fish, *Cypselurus* (*Hirundichthys*) *speculiger*	*Pennella sp.*	Daniel *et al.* (1967b)
3	Commerson's Anchovy fish,*Stolephorus commersonii*	*Lernaeenicus sprattae*	Rajkumar *et al.* (2006a)
4	Black-barred Halfbeak fish, *Hemiramphus far*	*Lernaeenicus hemiramphi*	Gopalkrishnan *et al.* 2010
5	Black-barred Halfbeak fish, *Hemiramphus far*	*Lernaeenicus sprattae*	Rameshkumar, G. and S. Ravichandran, 2013
6	King Mackerel , *Scomberomorus commerson*	*Lernaeenicus sprattae*	Present study

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BIOCHEMICAL COMPOSITION OF TWO EDIBLE AROIDS

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ABSTRACT

Two edible aroids were selected and the proximate composition of their corms and cormels was estimated. A field experiment was carried out of two important tuber crops viz. taro and elephant foot yam were subjected to study the nutritional value of *Colocasia esculenta* cv. Muktakeshi and *Amorphophallus paeoniifolius* cv.Gajendra. Aroids taken were grown in two different agro-climatic conditions i.e. in G.Udayagiri, Khajuripada block of Kandhamal district and Odapada, Dhenkanal sadar block of Dhenkanal district. Two focused tuber crops *A. Paeoniifolius*, *C.esculenta* were analysed for dry matter, starch, sugar, crude protein, carbohydrate, cholesterol, sodium, potassium, calcium and magnesium content. Analytical study revealed that nutrient content were found more in tubers of Kandhamal than Dhenkanal district. The work has a great potential in presenting the food potential as well as medicinal value of Odisha region where its productivity was well supported by climatic condition.

Key words : Edible aroids, elephant foot yam, taro, corms, cormels, proximate composition

INTRODUCTION

Aroids include the tuber bearing plant belonging to the family Araceae viz.Taro (*Colocasia esculenta*) and Elephant foot yam (*Amorphophallus paeoniifolius*). Taro is mainly cultivated in Asia, Africa and pacific as well as Caribbean Islands. Taro is cultivated in almost all the state of India. It is originated in South East Asia including India (Watt., 1889). The occasionally beautiful and often bizarre combination of spathe and spadix called the inflorescence, sometimes referred to as a flower is a distinguishing feature of all aroids. Tuber crops are the third important food crops after cereals and legumes. World production of the crop is estimated to be 5.5 million tones annually and provides about one third of food intake of more than 400 million people in the tropics. The starch root crops are marginal to poor in protein content, but they contain wide, variety of minerals and trace element, including relatively substantial quantities of iron and calcium, as well as potassium and magnesium (Englberger *et al*., 2003). Taro and elephant foot yam are popular tuber crops in Eastern India especially Odisha. Corms and cormels of elephant foot yam and taro are consumed as vegetable after boiling and baking. Many delicious cuisines are prepared from taro and elephant foot yam. Value added products like taro chips and elephant foot yam pickles are very popular in Eastern India. Aroids are not only source of foods, but also widely used as animal foods and raw material for industry. Mixed cropping of taro is the closest or the most resemble forest in heavily modified rural and forest border landscape (Marjokorpi and Ruokolainen, 2003 ; van Noordwijk *et al*., 2007).

Elephant foot yam too has some useful health benefits like the root acts as carminative, restorative, stomachic and tonic. It is dried and used in the treatment of piles and dysentery, the fresh root acts as an acrid stimulant and expectorant. It is much use in our country in the treatment of acute rheumatism. Elephant foot yam is considered to be a healthy low-fat food and is a rich source of essential fatty acids (Omega-3 fatty acids) which are known to increase the good cholesterol levels in the blood. Eating elephant yam helps in increasing the estrogen levels in women's bodies, thus helping in maintaining the hormonal balance. Elephant foot yam campanulatus is found to be analgesic and used in treatment of piles (Shipi *et al*.,2005).

MATERIALS AND METHODS

Elephant foot yam and taro demonstrations were conducted under National Agricultural Innovation Programme (NAIP), in tribal dominated districts of Kandhamal and Dhenkanal in Odisha during 2010-11. In Kandhamal district, Khajuripada and G.Udhayagiri blocks and in Dhenkanal district Odapada and Dhenkanal Sadar blocks were selected for demonstrations. Gajendra variety of elephant foot yam and Muktakeshi variety of taro was distributed to the farmers. In all the locations same package of practices were followed for elephant foot yam and taro. At harvest corms and cormels of elephant foot yam and taro were sampled for analysis. The samples were analysed for carbohydrate, dry matter, starch, sugar, crude protein, cholesterol, sodium, potassium, calcium and magnesium by following standard procedures.

The source of the materials and nutrient are given in Table-1.

Chemicals

All chemicals and reagents were of analytical grade. NaOH, HCl, $CuSo_4$, petroleum ether, ethanol, sodium hydroxide, sodium carbonate, tannic acid, potassium acetate, phosphate, sodium di-hydrogen, potassium ferri-cyanite (Fisher chemic Ltd.) Cupric sulphate (Universal Laboratory Pvt.Ltd.) and Methanol from Qualigens (Glaxo India Ltd.)

Anonymous (1990) procedures were used for biochemical analysis for estimation of fat, crude protein, crude fiber etc.

Fresh corms were homogenized in 80 % ethanol (8 to 10 hours). Extracts were evaporated to dryness following the method described by Frankova *et al.* (2003). Using anthrone method (Trevelyan and Harrison,1952), total soluble sugar was quantified.

For extraction of starch, Rose *et al.* (1991) method and for estimation Anthrone method (Trevelyan and Harrison, 1952) were followed.

Total energy was calculated using formula,

For minerals (Na, K, Ca, Mg) were estimated using wet ash procedure.

Na and K were estimated using flame photometer as described by Tothet *et al.* (1948). Ca and Mg were estimated using complexiметric titration with ethylene diamminetitra acetic acid (EDTA) using the method described by Ringbomet *et al.* (1958).

RESULTS AND DISCUSSION

The proximate composition of elephant foot yam corms was presented in Table 1. In elephant foot yam corms, higher dry matter (23.0%), starch (13.65%), sugar (1.10%), crude protein (2.16%), fat (0.48%), potassium (40.6 mg 100 g^{-1} dwb), magnesium (1.30 mg 100 g^{-1} dwb) were found from G.Udayagiri block. Calcium (3.23 mg 100 g^{-1} dwb) was rich in Odapada block. Crude fiber (1.68%), sodium (33.5 mg 100 g^{-1} dwb) were higher in Dhenkanal sadar. Lower dry matter (20.0%), starch (10.13%), sugar (0.82%), crude protein (1.12%), fat (0.24%), magnesium (1.08 mg 100 g^{-1} dwb) in Odapada block, potassium (39.81 mg 100 g^{-1} dwb) in Dhenkanal sadar, crude fiber (1.22%) in Khajuripada block, sodium (33.12 mg 100 g^{-1} dwb) and calcium (2.92 mg 100 g^{-1} dwb) in G.Udayagiri block were noticed. The variation in nutrient composition might be due to variation of soil nutrient status.

Proximate composition of taro cormels was presented in the Table 2. Higher protein (2.5%) and sodium (26.71 mg 100 g^{-1} dwb) were noticed in G.Udayagiri block. Fat (1.20%), sugar (1.75%) and magnesium (1.42 mg 100 g^{-1} dwb) were higher in Khajuripada block. Dietary fiber (3.30%), starch (14.5%) and calcium (2.28 mg 100 g^{-1} dwb) were rich in Odapada block. Potassium (26.92 mg 100 g^{-1} dwb) was higher in Dhenkanal sadar. Lower dietary fiber (2.67%) in G.Udayagiri block, potassium (26.58 mg 100 g^{-1} dwb), calcium (2.06 mg 100 g^{-1} dwb) from Khajuripada block, protein (2.28%), fat (0.78%) and sodium (26.16 mg 100 g^{-1} dwb) in Odapada block, starch (14.0%), sugar (1.30%) and magnesium (1.21 mg 100 g^{-1} dwb) in Dhenkanal sadar were observed. The variation in nutrient composition might be due to variation of soil nutrient status.

Table 1 : Nutrient composition of corms of *A.paeonifolius*

Composition	Dhenkanal		Kandhamal	
A.paeonifolius	Dhenkanal Sadar	Odapada	G.Udayagiri	Khajuripada
Dry matter (%)	21.0	20.0	23.0	22.5
Starch (%)	10.50	10.13	13.65	13.11
Sugar (%)	1.06	0.82	1.10	0.98
Crude Protein (%)	1.29	1.12	2.16	1.38
Fat (%)	0.27	0.24	0.48	0.32
Crude fiber (%)	1.68	1.62	1.27	1.22
Na (mg/100gm of dry weight)	33.5	33.26	33.12	32.94
K (mg/100gm of dry weight)	39.81	40.42	40.64	40.60
Ca (mg/100gm of dry weight)	3.12	3.23	2.92	2.98
Mg (mg/100gm of dry weight)	1.16	1.08	1.30	1.28

NB : All data are fresh weight basis except minerals, which are expressed in mg 100 g^{-1} on dry weight basis.

Fig. 1 : Biochemical constituent in *A.paeonifolius*

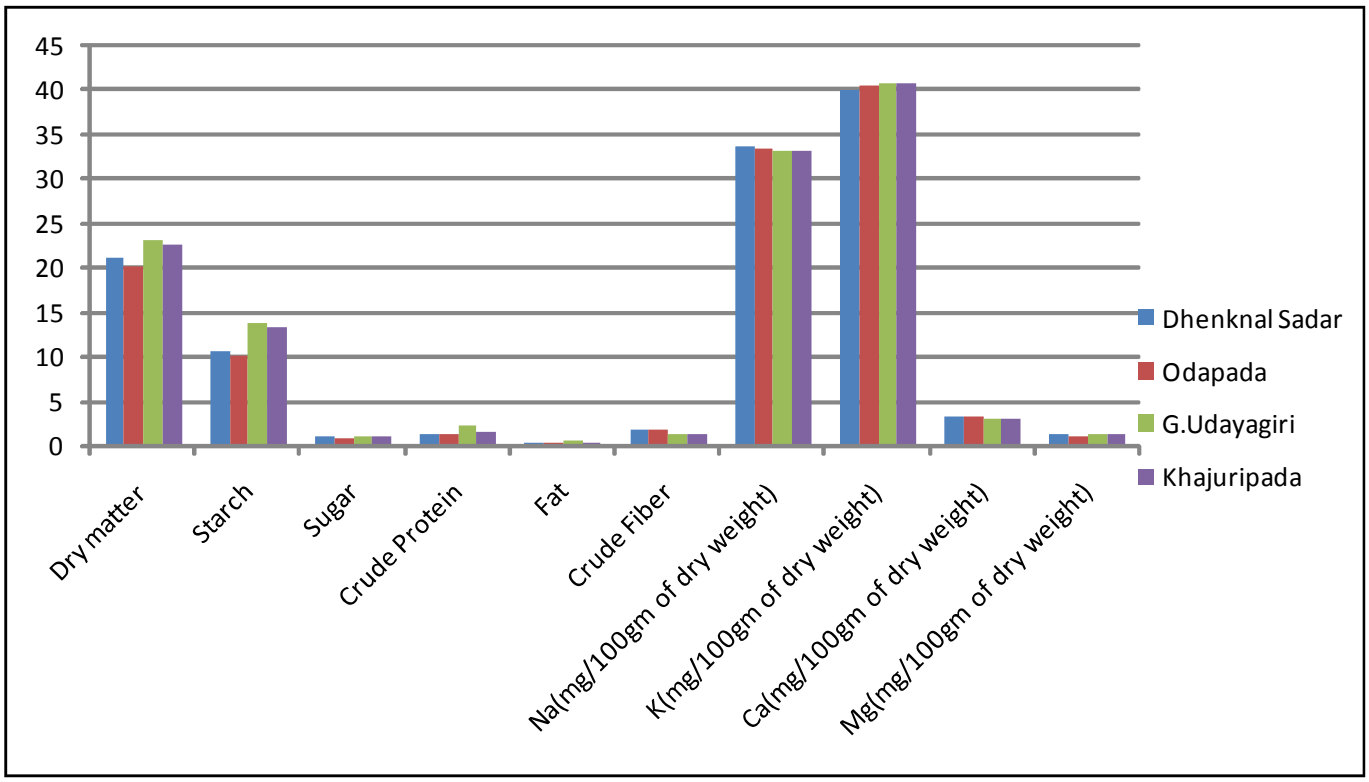

Table 2 : Nutrient composition of corms of *C.esculenta*

Composition	Dhenkanal		Kandhamal	
C.esculenta	Dhenkanal Sadar	Odapada	G.Udayagiri	Khajuripada
Protein (%)	2.30	2.28	2.50	2.39
Fat (%)	0.92	0.78	1.00	1.20
Dietary fiber (%)	3.20	3.30	2.67	2.74
Starch (%)	14.0	14.50	14.12	14.02
Sugar (%)	1.30	1.65	1.70	1.75
Na(mg/100gm of dry weight)	26.22	26.16	26.71	26.58
K(mg/100gm of dry weight)	26.92	26.80	26.71	26.58
Ca(mg/100gm of dry weight)	2.20	2.28	2.10	2.06
Mg(mg/100gm of dry weight)	1.21	1.26	1.40	1.42

NB : All data are fresh weight basis except minerals, which are expressed in mg 100 g^{-1} on dry weight basis.

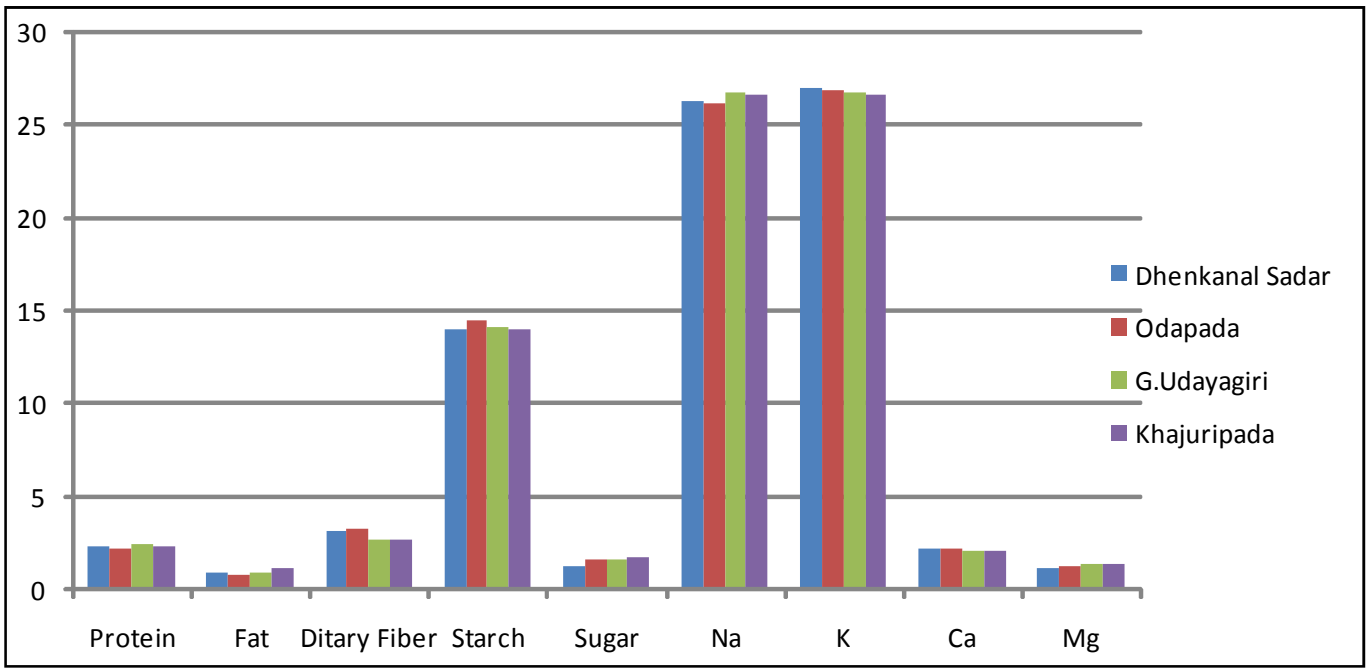

Fig. 2 : Biochemical constituent in *C.esculenta*

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A CASE STUDY OF RESOURCE CONSERVING METHOD OF SYSTEM OF RICE INTENSIFICATION (SRI) IN TRIPURA : AN ECONOMIC ANALYSIS

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ABSTRACT

The study on economics analysis of system of rice intensification (SRI) was conducted in Tripura state. SRI requires lesser amount of critical inputs, which constitutes more than 25 per cent of the total cost. Therefore, cutting on these items has significant bearings for the farmers whose net returns are shrinking day by day. Since, SRI is a labor intensive technology, it has not been widely adopted.

Key words : SRI, resource, conservation, economic and NEH Region.

INTRODUCTION

New agricultural technologies may show great promise in on-station and on-farm trials. Still, the proof of the pudding is in the eating, and the performance of new technologies in farmers' self managed fields is of critical importance for farmer acceptance/adoption and technology uptake/ diffusion. Understanding such performance thereby has great potential feedback value for agricultural research and development (R & D). A number of factors complicate such performance or impact assessment of new technologies in farmers' fields (CIMMYT, 1993; Doss, 2006; Feder *et al*., 1985). For one, the adoption of new technologies is often associated with resource endowment differentials between adopters and non-adopters (CIMMYT, 1993; Feder *et al*., 1985). Such differences typically also imply underlying management and productivity differences between the two adopter categories. This can be offset by focusing on partial adopters of new technologies, i.e. those farmers that apply both the new and conventional technology on the same farm. Yet the extent of adoption is likely associated with inter alia the relative performance of the new technology on the adopters' farm. These differences introduce potential biases in empirical technology comparisons in farmers' fields. Specification effects in terms of how technology options are contrasted can thus introduce bias in impact assessment, with potentially misleading implications for R&D. The North-Eastern India is a chicken-necked region, connected to the mainland with a narrow corridor and surrounded by international boundaries of Bangladesh, Bhutan and China. North-Eastern India consists of seven states namely Assam, Arunachal Pradesh, Meghalaya, Mizoram, Nagaland, Tripura and Sikkim having area of 255.08 million hectares which is about 8 per cent of country's land mass. Area under rice is 885 thousand ha with production of 1804 thousand ton (Anonymous, 2010). It occupies 8 per cent area under rice. Tripura is second largest producer of rice in the North-Eastern region after Assam, the state is still facing deficit of rice by 3.1 lakh ton (Anonymous, 2010). Rice is practiced in all three season *i.e., Aush, Aman* and *Boro*. It is grown in upland as well as in low land. In recent years, several strategies, viz. direct seeded rice/dry seeded rice (DSR), alternate wetting and drying, SRI and wet seeded rice (WSR) have been tried (Tabbal *et* al., 2002). Among all these technologies, SRI is very popular method and most suitable to Tripura and beneficial to the farmers by saving inputs and increasing production and ultimately the income. The system of rice intensification is a package of rice production. It is considered to be a disembodied technological change in rice cultivation. System of

rice intensification involves the use of certain management practices, which together provide better growing conditions for rice plants, particularly in the root zone than those plants grown under traditional practices. SRI, as initially developed by de Laulanie´ (1993) in Madagascar for lowland irrigated rice, is based on the application of the six practices viz, the use of very young, 8- to 12-day-old seedlings in transplantation; transplanting single seedlings per hill quickly, with minimal root disturbance; widely spaced hills, ranging from 20 × 20 up to 50 × 50cm; an alternate wet and dry soil moisture regime (no permanent flooding) to maintain aerobic soil conditions; the use of organic, rather than mineral, fertilizers; frequent weeding, preferably performed using a surface rotary hoe, during early crop development stages so as to control weeds and aerate the soil (Stoop, 2011).This system seems to be promising to overcome the shortage of water in irrigated rice.

MATERIALS AND METHODS

Tripura state of North Eastern Hill Region (NEHR) of India was selected purposively, since state having the largest area under rice in the region after Assam. Two districts (West and Dhalai district) on the basis of highest and lowest area, respectively under system of rice intensification were selected for the study. Two blocks one from each district and four villages, two from each block on the basis of highest area were selected for the study. Study involved secondary and primary data. The primary data pertaining to 2010-11 which has been collected from farmers' fields using random sampling method. Since, the main focus of the study was system of rice intensification (SRI), out of 120 farming households, 60 each for system of rice intensification adopter (hereafter referred to as SRI), and without system of rice intensification (hereafter referred to as non-SRI) farmers were selected randomly, for comparative analysis (Singh *et al*., 2009). Cost concepts (CACP) for comparative economics were adopted. Rate of interest was charged 12 per cent. Different depreciation rates have been taken according to the nature of the fixed assets.

RESULTS AND DISCUSSION

In Tripura, SRI was introduced in 2001 and it was adopted in both the season viz, aman and boro 23 per cent and 10.78 per cent of total area under rice crop during 2007-08 (Table 1). The state Government was giving a package of Rs. 4500 per ha as kind to encourage the farmers to take up SRI. The commitment of field staff, strong policy and political support enabled fast spread of SRI in Tripura involving use of certain management practices which together provide better growing conditions for rice plants, particularly in the root zone, than grown under traditional practices. SRI is also known as resource conserving technology because it saves water up to 25-50 per cent (Prasad, 2010), seed saving 92 per cent (Barah, 2009), 20-40 per cent more yield (Patel et al., 2006) and net income is more than double farmers net income (Sita Devi, 2009).

Economic feasibility of SRI

A comparison between SRI and non-SRI (Table 2) indicated that most of the input requirements were less under SRI method of rice cultivation. The expenditure on seed was reported 0.26 per cent of total cost on SRI farms, whereas, 2.32 per cent expenditure was reported on non-SRI farms, hence, 2.06 per cent cost saving was reported on SRI farms. This was in line with the findings of Anthofer (2004), Yang and Suon (2004) and Devi and Ponnarasi (2009). This may be due to less seed requirement in SRI. No doubt, SRI is labor intensive and it was consumed on SRI farms. All the rice cultivation activities are performed manually in the study area. Maximum farmers of Tripura are marginal and small, and use of machinery in rice cultivation is negligible. Labor cost was higher in SRI. Hence, SRI was found employment generating technology. Expenditure on fertilizers was found lesser on SRI (15.84%) as compared to non-SRI farms (17.52%). This was due to proper absorption and application of fertilizer according to crop need in SRI farm. The same results were reported by Thiyagarajan (2004). The overall cost of irrigation was less in SRI as compared to non-SRI method. This may be due to proper follow up of package of practice under SRI in respect to

irrigation. This finding was in line with that of Barah, (2009) and Mahendra kumar *et al.,* (2010) and estimated 25-40 per cent saving of water by alternate drying and wetting system, which is considered a unique advantage of SRI. Although, this parameter was not covered in the study, nevertheless, from the existing evidences, it may be concluded that adoption of SRI saved the water in the state. Cost of plant protection measures incurred less on SRI farms and it was estimated 1.89 per cent against 4.37 per cent on non-SRI farms (Yang and Suon, 2004). It showed fewer incidences of diseases and pests in SRI. This finding was also supported by Thiyagarajan (2004) and found 55-70 per cent lower rates of disease and pest prevalence with SRI vs. conventionally-grown rice. The total cost of cultivation per hectare i.e. Cost A was lower by about 12.38 per cent in SRI method (₹ 16886.68/ha) than non-SRI (18977.81/ha) method. Cost B, was also found 3.43 per cent less on SRI than the non-SRI farms. Cost C, was estimated 25.07 per cent less on SRI farms than the non-SRI farms. This finding was similar to that of Devi and Ponnarasi (2009) who estimated that total cost of cultivation per hectare was lower by about 10 percent in SRI method. SRI farmers received higher gross returns (49638.40/ha) in comparison to the non-SRI method (42574.64/ha). The SRI farms yield was higher by 610 kg/ha. Hirsch (2000) estimated higher (8 t/ha) yield in System of Rice Intensification than the national average yield (2 t/ha). Stoop *et al.,* (2002) reported up to 15 t/ha in infertile soils under SRI method. Anthofer (2004) found 41 per cent more yield under SRI than the non-SRI method. Many farmers opined that higher yield was due to intensification of plant, more tillering and healthy grain and less crop lodging under SRI method. Input variables *viz;* fertilizer and plant protection were found positively significant in SRI method, whereas, under Non-SRI method fertilizer found positive and seed was negatively significant.

System of Rice Intensification (SRI) yielded up to 15 t/ha of paddy without external inputs resulting from the judicious management of the major crop production (Stoop *et al.* 2002, Hirsch, 2000). It saves water with higher yield (Satyanarayana *et al.*, 2006). Further, it required 46 per cent lesser seed rate, 50 per cent lesser expenditure on chemical fertilizers and 71 per cent lesser labor requirement, while yield increased by 41 – 130 per cent yield against traditional method (Yang and Suon 2004). adoption of SRI technique would help increase rice production without increasing the area under cultivation (Devi

Table 1 : Area production and productivity under SRI in during 2007-08

District	Area (ha)		Production (M.T.)		Productivity(kg/ha)	
	Conventional	SRI	Conventional	SRI	Conventional	SRI
	Aman season					
North	23516 (17.90)	2000 (12.59)	56323(16.40)	5963 (11.41)	2395	2982
Dhalai	12856 (9.79)	1434 (9.03)	33101(9.64)	5412 (10.35)	2575	3774
West	5236 (39.87)	8014 (50.47)	134922(39.30)	25985 (49.73)	2577	3243
South	4258 (32.42)	4428 (27.89)	118941(34.64)	14882 (28.48)	2793	3361
Sub-total	131315	15876	343287	52242	2614	3291
			Boro season			
North	824 (1.68)	715 (4.77)	1795 (1.42)	1592 (3.40)	2179	2226
Dhalai	1571 (3.20)	114 (0.76)	3769 (2.99)	300 (0.64)	2399	2631
West	25303 (51.69)	7494 (50.06)	65650 (52.24)	24832 (53.07)	2595	3314
South	21248 (43.41)	6646 (44.39)	54436 (43.32)	20067 (42.88)	2562	3019
Tripura	48946	14969	125649	46791	2567	3126

Note : Figures in parentheses are percentage to the total

and Ponnarsi, 2009). System of rice intensification not only boost production by 50-100 per cent, and often much more, but also induces greater resistance to pests and diseases and reduce vulnerability to drought, lodging, storm damage *etc*. (Anonymous, 2009). It has economic and ecological benefits and help to increase rice production by 26 per cent, it saves 40 per cent water use due to alternate drying and wetting system, which is considered an unique advantage (Barah, 2009). The adoption of SRI technique would help increase rice production without increasing the area under cultivation (Devi and Ponnarsi, 2009). The area under rice in Tripura is about 241 thousand hectare with production and productivity of 624.66 thousand metric ton and 2.456 t/ha respectively during the year 2007-08 (Anonymous, 2010). To maintain the food security of the country as well as of the state, to produce more from less land with a system of crop intensification. For increasing rice productivity, System of Rice Intensification (SRI) is one of the interventions identified for increasing productivity. SRI is a package of agronomic approaches which together exploit genetic potential of rice plants, create a better environment, enhance soil health and reduce inputs. The results of SRI from many countries have shown an increase of 30-70 per cent in rice yields while using less water and lowering production costs. At the present rate of growth of population in India, we have to produce 130 million ton of rice by 2030 from the present level of 96.43 million ton (2007-08). This can be tackled through introduction of SRI (Renganathan and Ramesh, 2010). Present study is an attempt to look into the economics of System of Rice Intensification in the state.

Undoubtedly, SRI requires lesser amount of critical inputs, which constitutes more than 25 per cent of the total cost. Therefore, cutting on these items has significant bearings for the farmers whose net returns are shrinking day by day. Although, SRI is labor intensive technology and it has not been widely established. For further spread of the technology, it is warranted that the government should undertake initiative to provide the small machinery, so that the labor efficiency may be increased and small and medium farmers could also get benefit through this SRI technology.

This study indicated a net gain in SRI of 32751 as compared to non-SRI. This gain was caused by a reduction in cost of cultivation by 2091.13/ha and

Table 2 : Cost of cultivation of rice under SRI and Non-SRI Methods during 2010-11 (₹/ha)

Particulars	SRI Farms			Non-SRI Farms		
	West	Dhalai	Overall	West	Dhalai	Overall
Seed (2.32)	39.50(0.23)	47.59(0.28)	43.54 (0.26)	163.22(0.88)	717.50(3.66)	440.36*
Hired labor (67.05)	12209.14 (72.31)	12969.36 (76.79)	12589.25 (74.55)	11627.95(63.32)	13822.80 (70.55)	12725.38
Fertilizer (17.52)	3155.88(18.69)	2192.82(12.98)	2674.35* (15.84)	4251(23.15)	2398.50(12.24)	3324.75*
Irrigation (8.74)	1158.37(6.86)	1362.53(8.07)	1260.45 (7.46)	1520.37(8.28)	1796.88(9.17)	1658.625
Plant protection (4.37)	321.51(1.91)	316.63(1.88)	319.07* (1.89)	801.28(4.37)	856.15(4.38)	828.715
Total cost (100)	16884.42(100)	16888.94(100)	16886.68 (100)	18363.82(100)	19591.80(100)	18977.81
Yield (ton)	4.72	3.66	4.19	3.69	3.47	3.58
By-product (ton)	2.93	2.81	2.87	3.12	3.10	3.11
Gross income	56684.20	42592.60	49638.4	45067.97	40081.30	42574.64
Net income	39799.78	25703.65	32751.715	26704.15	20489.50	23596.83
Cost A_1	16884.42	16888.94	16886.68	18363.82	19591.80	18977.81
Cost A_2	16884.42	16888.94	16886.68	18363.82	19591.80	18977.81
Cost B_1	16884.42	16888.94	16886.68	18363.82	19591.80	18977.81
Cost B_2	81848.35	34307.91	58078.13	83988.82	36154.30	60071.56
Cost C_1	18030.84	18145.72	18088.28	32891.46	33204.30	33047.88
Cost C_2	82994.78	35564.68	59279.73	98516.46	49766.80	74141.63
R^2 Value	-	-	0.33	-	-	0.44

Note:Figures in parentheses are percentage to total cost.
*Significant at 5% level of Significance

an increase in yield by 610 kg/ha. The SRI saved 2.06 per cent of seed, 1.68 per cent of fertilizer, 1.28 per cent of irrigation, and 2.48 per cent of plant protection. However, for further spread of the SRI, peoples' participation is needed.

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STUDIES ON THE PRIMARY PRODUCTIVITY OF THE RIVER PANDU, KANPUR, INDIA

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ABSTRACT

Primary productivity of the River Pandu was assessed at its Kanpur frontage during 2010-11. The study was carried out to monitor its productive potential and ambient pollution load. Results of the study pertaining to Gross Primary Productivity(GPP), Net Primary Productivity(NPP), Community Respiration(CR), NPP/CR and NPP/GPP ratio indicated an unhealthy, polluted condition of the river due to anthropogenic waste discharge in to it. Special heed is needed to save the river from further pollution and initiate corrective measures to depollute or alleviate pollution load on it in order to save the river Pandu -as the river is an important tributary of the river Ganga.

Keywords : Primary productivity, GPP, NPP, CR

INTRODUCTION

Rivers, the life-line of millions of living beings, are the most important and main natural resources of fresh water. They not only harvest and store the natural precipitation but also recharge the underground water table. River water is frequently used for diversified purposes some times as a tradition, religion, rituals or as a grave necessity due to unavailability of safe water supply. Hence, the health and well being of millions of living beings invariably depends on water quality. The growing river pollution menace in the form of reduced river flow, river discharge due to excessive water extraction, declining storage capacity due to silting of river bed, and deteriorating water quality and consequently quenching river bio-diversity has made it imperative to continuously monitor the water quality of the rivers.

Productivity is the rate at which inorganic carbon is converted in to organic carbon in an ecosystem. It is the capacity of the aquatic ecosystem to convert radiant energy to chemical energy via primary transducers and its subsequent transfer to higher levels of the ecosystems via primary consumers. Its assessment provides information pertaining to photosynthetic production of organic matter and other energy related functional aspects of an aquifer. It has been a reliable index of pollution assessment (Wetzel and Linkens 1979; Christi *et al.*, 2011)). It is the back bone of all food chains and food-webs of an ecosystem and generates 70% of the world's atmospheric oxygen (Reynolds, 1984). The photosynthetic production by primary transducers can be expressed as Gross Primary Productivity (GPP) or Net Primary Productivity (NPP). The difference between the two basically is that during photosynthesis algae synthesize organic matter, a part of which is simultaneously used up for this work. GPP can not be determined directly but can be computed by adding the metabolic activities of algae to the quantity lost in respiration to NPP. Primary productivity depends on a number of factors such as availability of nutrients, quality, quantity and duration of light, transparency or euphotic zone of the water body and the presence or absence of inhibitors or toxicants in the ambient water.

MATERIALS AND METHODS

River Pandu, a tributary of river Ganga owes its provenance from the pumped storage of lower Ganga canal, approximately 110 kilometers North-West of Kanpur. It flows on the southern outskirt of Kanpur through Panki Industrial Estate (PIE) covering a distance of about 64 kilometers before its confluence with river Ganga in South-West of Kanpur in Fatehpur district. During its course through Kanpur, five major drains namely Thermal Power Plant drain, Panki Municipal drain, Ganda Nala, Halwa Khanda drain and Central Ordinance Depot (COD) drain, carrying sullage water, sewage and industrial effluent discharge their wastes in to it without any pre-treatment. Lean flow of the river does not permit enough dilution of the pollutants to permit natural purification of the river to the desired extent which is likely to affect the phyco-diversity and thus primary productivity of the river.

Table 1 : Details of Five Sampling Sites on River Pandu

Sl. No.	Number and name of site	Location of site
1.	Station 1	Upstream of confluence of Panki Power Plant Drain
2.	Station 2	Downstream Panki municipal Drain
3.	Station 3	Approx. 30 Km downstream station 3
4.	Station 4	Approx. 15Km downstream station 4
5.	Station 5	Approx. 30 Km downstream station 5

Material sampling was done at five sampling stations of the river during 2010-2011(Table - 1). Samples were collected from different zones. Periodic estimations were made round the year but greater details were worked out only during winter and summer because in monsoon period sampling could not be carried out properly and got hampered due to over flow and inapproachability of the river. Sampling stations on the river were Tikra village, Panki industrial area, Mardanpura, Meharbanpurwa and Hamirpur road bridge. Random sampling was done at each station and samples were then compounded to get a composite sample result. Physical and chemical analyses of the samples were done as per standard procedure (Anon, 1998). Primary productivity was estimated by light and dark bottle method (Gaarder and Gran, 1927, Anon 1998) with three hours of *in situ* incubation period (12 noon to 3.0 P.M.) below the water surface. On completion of the incubation period, Dissolved Oxygen(DO) was fixed at the site following Winkler's Azide modification method (Anon, 1998) for further laboratory investigations.

RESULTS AND DISCUSSION

Analysis of Table- 2 clearly indicated that river water was alkaline with temperature in the bio-kinetic range of 10-45^{o}C. Water transparency was quite low and may be attributed to the high concentration of total solids released in the river by murky wastes from different drains in huge quantity. Except at station 1, D.O. was invariably below 4.0 mg/l. High BOD values ranging from 12.6 to 60 mg/l indicated that river carries a heavy load of bio-degradable wastes. Nutrient concentration of the river in the form of chloride, phosphate and ammonia was quite high though the river was poor in nitrate contents. Hardness values exceed the prescribed permissive limits of ISI (Anon, 1982). Sulphates were in the range of 0.78-12.7 mg/l. All these parameters affected the productivity of the river. GPP values in the river ranged from 0.015-0.844 mg C/l/day. The maximum value was noticed at station 5 and minimum at station 3 in the months of April and September respectively. Average GPP values at stations 1-5 were 0.44, 0.22, 0.56.and 0.66 mg C/ l /day respectively.

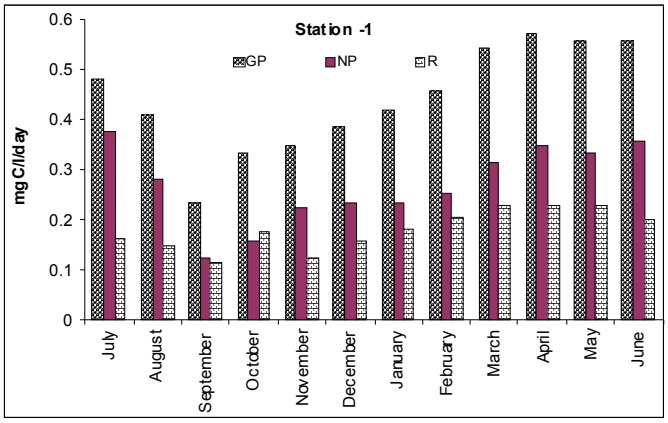
NPP values in the river varied from 0.028 to 0.564 mg C/l/day. Lowest value was noticed twice at station 3 in the months of February and April while maximum at station 5 in April. High total solid concentration, low transparency and high turbidity as a consequence of ash discharged from Panki thermal power plant highly reduced the euphotic zone of the river at station 2. Besides this, heavy metals present in the coal ash also seem to exert a negative pressure on the growth, development and population of primary producers. All these factors probably acted in synergy to reduce the growth and development of primary producers at this station. Albeit sewage- a rich source of plant nutrition (N and P) stimulating the plant growth is also discharged through the drain but despite that primary producer's population was very meek due to factors mentioned above. Primary productivity values obtained at this station were quite insignificant and hence, have not been taken in to account. Only a few periphytic algae could survive at this station. Community respiration, expressed as percent of gross production was invariably below 50% at all stations except station 3 where values invariably were above 50% and reached to a maximum of 91.64% in April. This indicated that at this station respiratory intake of oxygen surpassed the photosynthetic production of oxygen. According to (Ketchen *et al.,*1958) in a healthy aquatic ecosystem, respiration should be 5-10% of the gross production. Thus, it is apparent that values recorded from different stations of the river in general and at station 3 in particular are much higher than those prescribed for a healthy ecosystem (Ketchen *et al.*, 1958). This indicates an unhealthy disturbed ecosystem with low NPP draining more dissolved oxygen than producing it photo-synthetically. NPP/ GPP ratio ranged from 0.10-0.71 at different stations. Minimum value was noticed at station 3 in February and maximum at station 5 in May. (Ketchen *et al.*,1958) opined that NPP

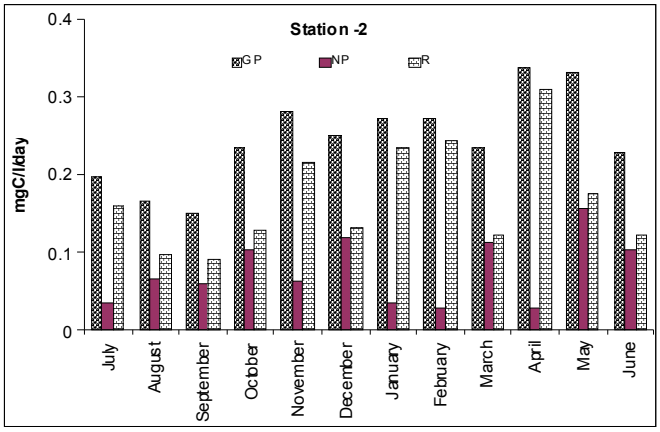
GPP should approach unity in a healthy ecosystem. As per the data obtained in the present study, it may be concluded that water quality of the river slightly improved downstream at station 5 in May. The factors that propped up the growth of primary producers and the improved production include water temperature in the bio-kinetic range, ample nutrients (N and P) and low concentration of toxic materials due to natural dilution - as the river recouped in due course of time. All these factors enhanced the productivity at this station as compared to other stations. NPP/R ratio in the river water ranged from 0.99 to 2.37. Both minimum and maximum values were reported at station 4 in the months of February and May respectively. (Yeragi and Shaik 2003) stated that NPP/ R ratio exceeding one is an indication of gross pollution. Taking this value in to account, it was concluded that the whole river stretch investigated was highly polluted. Weather conditions also affected the productivity. The reported high productivity in summers than in winters or rains confirms the findings of (Patra 1985) who reported co-rrelation between temperature and productivity. At all stations, peak production was reported in April and minimum in September. Low primary productivity in September may be attributed to high current velocity, turbulence and turbidity in the river due to run off water from the catchment areas reducing water transparency and there by euphotic zone of the river leading to reduced light intensity. All these adversely affected the primary producers' population and primary productivity as well. High current velocity constantly washed away the producer organisms. Thus, the already meek plankton community got disturbed and productivity declined significantly during that period. The finding was in confirmity with Ahmed *el al.* (2005); emphasized the effect of phytoplankton population on productivity and reported that phytoplankton abundance and productivity correlate significantly. The maximum productivity in summer as reported in the present investigation was in variance with (Kar *et al.*,1987) who reported maximum production in river in monsoon but was in concordance with (Patra 1985),Venkatesharaju *et al.,* 2010, i.e. the peak production in summer and minimum in rains in the aquatic habitats studied by them. The present study corroborates the findings of these authors. It was thus evident that despite optimum temperature, surplus nutrients and bright sunshine, the productivity of the river was quite low due to anthropogenic stress leading to potential toxic condition resulting from the discharge of huge industrial and municipal wastes in to the river and that too directly sans any pre treatment. River was grossly polluted and required special heed from government and non-governmental organisations so that it can recuperate and survive as a healthy natural ecosystem. It should not be allowed to be converted into a waste depository to carry the anthropogenic wastes beyond its assimilating capacity.

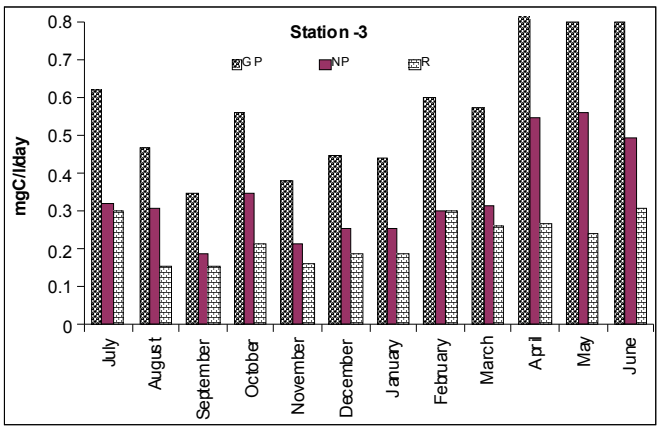
Table 1: River water quality at different stations in light of ISI standards

Parameters	Station 1	Station 2	Station 3	Station 4	Station 5	IS:2296
Colour	-	Murky	Light Murky	Murky	-	-
Odour	-	Org. pungent	Faecal foul	Faecal foul	-	Unobjectionable
Temperature	25.1	27.8	25.7	26.8	25.8	-
Transparency	17.2	3.3	8.4	7.2	14.9	-
pH	7.8	8.2	8.1	8.3	8	6.5-8.5
Total Solids	121	934	587	830	417	500
Dissolved Oxygen	6	2.4	1.77	2.3	3.2	4.0-6.0
B.O.D.5	21.2	39.5	45.2	67	44.4	2.0-3.0
Total Hardness	805	966	557	1087	775	300.0
Chloride	25.5	80.7	62	75	44.8	250.0
Phosphate	0 24	1.72	1.47	2.09	1.39	0.01*
Nitrate N	0.14	0 05	0.44	0.43	0.69	20.0
Ammonia N	0.46	2.6	18.6	9.1	9.1	1.2
Sulphate	0.78	5.3	4.4	12.7	3.1	400.0

All values in mg/l except temperature (oC), transparency (cm) and pH. *As per Vollenwieder(1961).







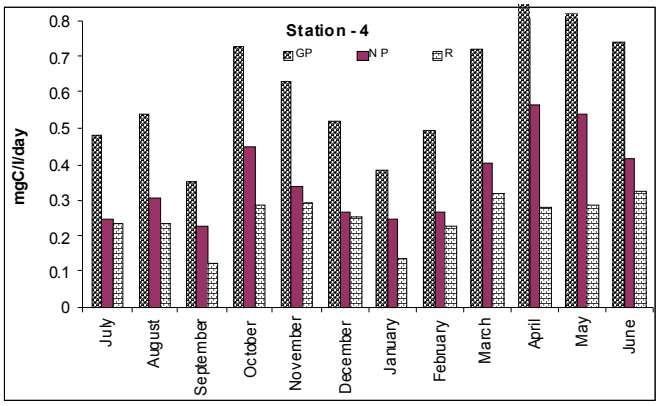


Fig. 1 - 5 : Bar diagram showing GP, NP and R values of 5 different stations

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2. **Preparation of manuscript.** Papers should be written in simple and clear language, strictly following the latest ***e-planet*** journal style not exceeding six printed pages. Avoid footnotes in the text. The complete scientific name (genus, species and authority for the binomial) of all the experimental organisms should be given at the first mention both in the Abstract and Materials and Methods. International System of Units in abbreviated form should be used for all the measurements. Spell out the acronyms in the first instance.

Manuscript should be typed in double-spacing on one side of Bond Paper (A-4). Tables must not exceed 12 vertical columns. Leave liberal margins on both the sides. Arrange the manuscript in the order of title, author(s), address of each author, abstract (approx.200 words), key words introduction, materials and methods, results and discussion, acknowledgement (if any) and references.

2.1. **Title.** A short title of the paper should appear on the top of the article, followed by the long title in capital/bold letters. The short title appears on alternate printed pages of each article.

2.2. **Author(s).** Author(s) name(s) should be typed in bold letters, first initials and then surname. Corresponding author's name should be specified by an asterisk mark and e_mail address should be indicated.

2.3. **Address.** The address of corresponding author should be typed in italics indicating the place where the work was carried out. If the present address is different, it should be given as footnote in the first page.

2.4. **Abstract**. Maximum 200 words convening the objectives, methodology and the most important results.

2.5. **Key words.** Maximum of 5-6 key words should be provided for subject indexing.

2.6. **Introduction**. It should be concise and include the scope of the work in relation to the state of art in the same field along with specific objectives.

2.7. **Materials and Methods.** A full technical description of the methods followed for the experiment(s) should be given, providing enough information. Detailed methodology should be given when the methods are new while for standard methods, only references may be cited.

2.8. **Results and Discussion.** In this section, only significant results of the experiment(s) should be reported. Along with the tables and figures, the discussion should deal with interpretation of results and relate the author's findings with the past work on the same subject. The conclusions drawn should be explicitly listed at the end of this section.

3. **References.** Refer this copy as sample for references. For ex.*:* Thankppan A, Das BK, Barman HK and Samal SK (2008) Genetic fingerprinting of *Aeromonas hydrophila* isolated from diseased fresh water fishes of eastern India; e-planet, 6(2): 01-06.Distinction for the same author and same year be done as e.g. 1969a, 1969b. Unpublished data, and personal communication are not acceptable as references but may be referred to parenthetically in the text.

4. **Tables.** Number the tables consecutively in Arabic numerals. Tables should have comprehensible legends. Conditions specific to a particular experiment should be stated. Zero results must be represented by 0 and not determined by n.d. The dash sign is ambiguous. For values <1, insert a zero before the decimal point.

5. **Illustrations.** All graphs, diagrams and half-tones should be referred to as Figure and should be numbered consecutively in Arabic numerals. The figures should either match with the column width (8.5 cm) or the printing area (17.8 x 22 cm). The legends should be brief and self-explanatory. All graphs, figures should be drawn by MS-EXCEL and submitted in editable format. Define in the footnote or legend any non-standard abbreviations or symbols used in a table or figure. Photographs, which must be kept to a minimum, should be good quality glossy prints.

6. **Reviews.** Full scape Review articles are invited. Very special review articles are also considered.