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### Journal of the Andaman Science Association

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### Effect of terracing on Soil and Nutrient Loss from Coconut based Intercropping Systems

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#### Abstract

The natural resources in Andaman and Nicobar Islands are profoundly affected by land degradation as a result of land use change, deforestation, overgrazing and subsistence agriculture. In order to assess the soil loss and evaluate the effect of soil conservation practices, a field experiment was conducted in different coconut based intercropping systems. The results showed higher N loss in the form of nitrate N than Ammoniacal N. The Silt, ammonia and nitrate losses from the experimental plots varied as 1.73-5.38 g/l, 3.11-3.71 mg/l and 3.5-5.17 mg/l, respectively. The measured soil loss from runoff plots was 1.1, 2.7, 2.9, and 3.4 t ha<sup>-1</sup> for pineapple, fodder, sweet potato, and tapioca intercrops, respectively. Similarly, soil loss was predicted from USLE model using rainfall events and other soil, crop, slope and conservation practices data during 2013-14 year. Terracing of sloppy area had significant effect on soil loss (p<0.05) and soil loss was highest under fallow (8.5 t ha<sup>-1</sup> y<sup>-1</sup>) while pine apple intercropping recorded the lowest erosion rate (2.1 t ha<sup>-1</sup> y<sup>-1</sup>). It was observed that USLE predicted the soil loss for all intercrops were in good agreement (R<sup>2</sup> = 0.97) with measured soil loss data by runoff plots.

Keywords: soil and nutrient loss, coconut intercrops, USLE, terracing

#### Introduction

Sustainable management of soil and water resources are very critical for meeting food, fibre and shelter needs of growing population. At the same time, conservation of these resources should form part of production enhancement strategy. Andaman & Nicobar Islands (ANI) despite of having 300 cm annual rainfall, surprisingly experiences water crises both for drinking and irrigation especially during dry season from January to April. The island receives about 75% of its annual rainfall during May-November, of which nearly 50% is lost through runoff carrying 12-15 tonnes soil per ha annually (Singli and Gajja, 1982). Studies have established that runoff is associated with soil loss which increases with slope lengths and steepness. Thus higher intensity runoff from sloppy areas often causes landslides (Rai and Sharma, 1998). According to Sahoo et al. (2013), about 90% of ANI area is affected by various types of erosion with 20-80 t/ha/year erosion rate, which is in excess of tolerance limit (11.2 t/ha/year). National Bureau of Soil Survey and Land Use Planning (NBSS & LUP) reported that out of 0.825 Million ha geographical area, 23% of area is severely eroded due to water erosion (on scale of 1:25000). These

figures exhibit variations probably due to adoptation of different methods, scale and interpretation criterion. But the basic observation of all these studies indicated accelerated erosion due to the removal of forest cover and disturbance to the surface soil in cultivated land.

Soil erosion causes not only loss to productive top soil but also leaches out bases and nutrients from the agricultural lands due to which the soils of these islands became poor in organic matter (Mongia et al. 1989). The studies on soil and nutrient loss from different agricultural land uses in A&N Islands are limited. Pramanik et al. (1998) studied soil loss, nutrient recycling, and water yield under coconut and arecanut based intercropping systems planted in Garacharma watersheds, South Andaman. The results from this study indicated that on an average soil loss under coconut plantation (main crop) and intercrops was 3.4 t/ha during 1992-94. Velmurugan et al. (2008) carried out soil erosion study in Dhanikhari watershed of the South Andaman district using revised Morgan Finney (MMF) model with an aid of remote sensing (RS) and geographical information system (GIS) which indicated accelerated erosion in plantation areas. Shankar and Dharanirajan (2018) studied the drainage morphometry



of Kalpong river watershed, North Andaman using RS & GIS. This study suggests that the Kalpong watershed is susceptible to soil erosion and severe runoff. Due to this, high concentration of nutrient rich sediments was discharged at the mouth of Kalpong River and finally reaching sea.

It can be inferred from these studies showed that in spite of having 85% area under forest cover, AN islands is experiencing soil degradation in the form of water erosion. Majority of agricultural activities in A&N islands include coconut and arecanut based main cropping system along with some intercrops. Due to cultivation of sloppy areas without adequate conservation measures and exposure of agricultural soils during rainy season resulted in loss of large amounts of nutrients to sea through low lying streams. This loss is expected to increase further due to land use change, high pressure for intensive land use and climate change events. This phenomenon not only causes loss of soil fertility but also creates threat to aquatic life by sedimentation of coastal areas. This necessitated quantification of soil and nutrient losses from agricultural lands for creating suitable soil and water conservation mechanism to enhance crop productivity and augment water resources potential. Therefore, a study was conducted to evaluate the effect of terracing and different coconut based intercropping systems on soil erosion by modelling approach whereas nutrient losses were quantified through erosion plot methods.

#### **Materials and Methods**

#### **Study Area**

The present erosion study was conducted at coconut based integrated farming system Unit, Garacharma Farm, Central Islands Agricultural Research Institute (CIARI), South Andaman. The soil of the study site is classified as Entisols with sandy loam texture. The pH varied from 6.1 to 6.8 and EC ranged from 0.20 to 0.35 dS m<sup>-1</sup>. The climate of the study area showed very little annual variation in temperature (25 °C to 30.5 °C), high humidity (79-90%) and wind speeds (4.7-14.8 km/h). On an average the study area receives 3079 mm rainfall with mean monthly variation of 300-500 mm/month during wet season (May-October), 50-230 mm/month during post-wet season (November to January) and <100 mm/ month during dry season (February to April). As the Islands are situated close to equator evaporation rate is fairly high which cumulatively ranges from 97-191 cm per annum.

#### **Erosion study**

The terrain of the study area was hilly and undulated with 10-25% slope with coconut plantation therefore, inward slopping terraces were made to reduce runoff and conserve soil. In order to study the effect of terracing, runoff plots (2 m x 1m x 0.3 m) were established under different coconut based cropping system to assess soil and nutrient losses though runoff. The study included four intercropping systems established in the terrace under coconut main crop viz. pineapple, sweet potato, tapioca and fodder. One runoff plot each was installed under pineapple, sweet potato, tapioca and fodder. One each erosion plots was established at fallow land and natural vegetation as well for comparison. Dikes of runoff plots were made with galvanized metal sheets and the runoff samples were collected from tank installed at end of plot through channel. Runoff volume in each tank was measured for erosive rainfall events and five litres of runoff water was sampled for analysis after thorough stirring. One litre water sample was coagulated by alum, decanted off and dried on a water bath to estimate soil loss (grams). Other portion of sample was used for analysing pH, electrical conductivity and nutrient loss.

#### Soil Erosion Modelling

The Universal Soil Loss Equation (USLE) predicts soil loss for a given site as a product of six major erosion factors (Eq. 1). The values of these erosion factors vary considerably about their means from event to event, but the effects of these fluctuations average out in the long run. Thus, the USLE is suitable for predicting long-term averages, and the soil erosion is estimated as follows (Wischmeier and Smith, 1978):

$$A = R \times K \times LS \times C \times P \qquad \text{eq (1)}$$

Where, A = soil loss per unit area in unit time (t ha<sup>-1</sup> yr <sup>-1</sup>), R = rainfall erosivity factor(MJ mm h<sup>-1</sup> ha<sup>-1</sup> year<sup>-1</sup>)

<sup>1</sup>),K = soil erodibility factor (tons ha<sup>-1</sup> MJ<sup>-1</sup> mm<sup>-1</sup>), L = slope length factor(dimensionless), S = slope steepness factor(dimensionless), C = cover management factor (dimensionless, ranges from zero to one), and P = support practice factor or land management factor (dimensionless, and ranges from zero to one). Each factor is described below:

*Rainfall erosivity factor (R):* It refers to the rainfall erosion index, which expresses the ability of rainfall to erode the soil particles from an unprotected field. From the long field experiments it has been obtained that the extent of soil loss from a barren field is directly proportional to the product of two rainfall characteristics: kinetic energy of the storm and its 30-minute maximum intensity. The product of these two characteristics is termed as  $EI_{30}$  or rainfall erosivity. The erosivity factor, R is the number of rainfall erosion index units ( $EI_{30}$ ) in a given period at the study location. The rainfall erosion index unit ( $EI_{30}$ ) of a storm is estimated as:

$$EI_{30} = \frac{KE \times I_{30}}{100}$$
 eq (2)

Where KE = kinetic energy of storm in metric tonnes/hacm, expressed as:

$$KE = 210.3 + 89 \log I$$
 eq (3)

Where I = rainfall intensity in cm/h, and  $I_{30}$  = maximum 30 minutes rainfall intensity of the storm.

Soil erodibility factor (K): It is a number which reflects the susceptibility of a soil type to erosion, i.e., it is the reciprocal of soil resistance to erosion. It ranges from less than 0.1 for the least erodible soils to close to 1.0 in the worst possible case. The formula used for estimating K is as follows (Foster *et al.*, 1991).

$$K = 2.8 \times 10^{-7} \times M^{1.14} \times (12 - a) + 4.3 \times 10^{-3} \times (b - 2) + 3.3 \times (c - 3) \qquad \text{eq (4)}$$

Where, M = particle size parameter, a =organic matter content (%), b = soil structure code and c = soil permeability class.

*Topographic factor (LS):* Slope length factor (L) is a ratio which compares the soil loss with that from a field of

specified length. Steepness of land slope factor (S) is a ratio which compares the soil loss with that from a field of specified slope. The topographic factor is used to account for the length and steepness of the slope. The longer the slope, the greater is the volume of surface runoff and the steeper the slope, the greater is its velocity. The value of LS can be calculated by using the formula given by Wischmeier and Smith (1978):

$$LS = \left(\frac{\lambda}{22.13}\right)^{m} \left[65.41\sin^{2}\theta + 4.56\sin\theta + 0.065\right] \quad \text{eq (5)}$$

Where  $\lambda$  = field slope length in meters, m = exponent varying from 0.2 to 0.5,  $\theta$  = angle of slope.

*Crop management factor (C):* The C factor is a ratio of the soil loss from a land under a specific crop and management system to the corresponding loss from a continuously fallow and tilled land. The C factor can be determined by selecting the crop type and tillage method. The cover and management factor to account for the effects of vegetative cover and management techniques for reduction of the soil loss would be equal to 1.0 in the worst case. In an ideal case when there is no sediment loss, C would be zero.

*Conservation practice factor (P):* The P factor represents the ratio of soil loss by a support practice to that of straight-row farming up and down the slope. The most commonly used supporting cropland practices are cross slope cultivation, contour farming and strip-cropping. Ideally in an area with full support practice condition, P would be zero meaning there is no sediment loss; whereas in an area without any support practice P = 1.0 indicating maximum possible sediment loss in absence of any soil conservation practice.

The runoff water samples from the constructed plots were collected for various rainfall events during September to October months in 2013. The collected runoff water samples were analysed for soil/silt loss and nutrient losses at laboratory, CIARI following standard procedures (Jackson 1973). The input parameters considered for running USLE soil erosion model from the collected and analysed data are shown in Table 1.

Sl. No.	Factor	Value	Parameters
1	Rainfall erosivity index (R)	505.9	Maximum 30 minutes rainfall intensity;
			Event wise rainfall (mm)
2	Soil erodibility index (K)	0.34	Texture class = Sandy loam; Organic Matter = 0.6%
3	Slope length factor (LS)	0.35	Length of field = $2 \text{ m}$ , Slope = $10\%$
4	Conservation practice factor (P)	0.14	Terracing
5	Crop management factor (C)	0.25	Pineapple
		0.33	Fodder
		0.36	Sweet potato
		0.50	Tapioca

Table 1: Input parameters for predicting soil loss using USLE erosion model

#### **Evaluation of Models' Performance**

The performance evaluation of USLE model was carried in order to examine the effectiveness in estimating soil loss. The performance indices used for evaluation are; root mean squared error (RMSE), mean absolute error (MAE), and coefficient of determination (R<sup>2</sup>). A description of the aforementioned indices is provided below.

*i) Root Mean Squared Error (RMSE)* is a measure of the residual variance and it indicates the overall discrepancy between the target and the output values. A low RMSE indicates good model performance, and vice-versa. A perfect match between the target and the output values would yield RMSE = 0.0. It is expressed as:

RMSE = 
$$\sqrt{\frac{1}{n} \sum_{i=1}^{n} (T_i - O_i)^2}$$
 eq (6)

Where  $T_i$  and  $O_i$  = measured and predicted soil losses, respectively; n = number of data points.

*ii) Mean Absolute Error (MAE)* is a measure of average magnitude errors in a set of predictions, without considering their direction.

Mean Absolute Error (MAE) =  $\frac{1}{n} \sum_{i=1}^{n} |T_i - O_i|$  eq (7)

*iii) Coefficient of Determination (R<sup>2</sup>)* measures the degree to which two variables are linearly related. It is the square of the Pearson's correlation coefficient (r) and describes the proportion of the total variance in the observed data that can be explained by the model. The value of R<sup>2</sup> ranges from 0 to 1, with higher value indicating better agreement between the target and the output values.

$$R^{2} = \frac{\left[\sum_{i=1}^{n} (O_{i} - \overline{O})(T_{i} - \overline{T})\right]^{2}}{\sum_{i=1}^{n} (O_{i} - \overline{O})^{2} \sum_{i=1}^{n} (T_{i} - \overline{T})^{2}} \qquad \text{eq (8)}$$

Where  $\overline{T}$  and  $\overline{O}$  = average of measured and predicted soil losses, respectively.

#### Statistical analysis

The primary data was analyzed using descriptive statistics such as average, percentage, coefficient of variation and standard deviation in Microsoft excel. Statistical significance of terracing and intercropping in coconut garden based on't' test were carried out using SAS package.

#### **Results and Discussion**

Soil erosion is directly related to rainfall and the resultant runoff over the agricultural field. In the island ecosystem, rainfall occurs almost in a continuous patch (rainy days) during the wet season with break in the event. A rainfall event cannot be considered erosive if the corresponding rainfall event depth is lower than 12.7 mm (Wischmeier and Smith, 1978). Accordingly, erosive rainfall events were processed for predicting soil loss using USLE equation and are shown in Fig. 1. We have identified a total of 4 rainfall event which are erosive for which runoff, soil and nutrient data was determined.

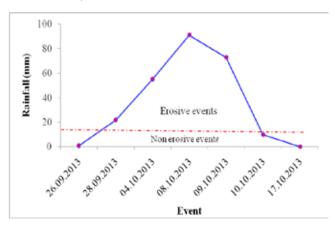


Fig. 1: Erosive and non-erosive events distribution in the study period

Table 2 shows the results of soil nutrient loss in the water samples collected from runoff plots under different intercrops. Nitrogen loss in the form of ammonia and nitrate from the runoff plots varied. 2.95-3.71 mg/l and 3.26-5.17 mg/l, respectively. The results showed that nitrate N loss was higher than Ammoniacal N loss that indicted the aeration status of the soil before the rainfall incidence. Among different cropping systems, N loss was significantly higher for grass followed by pineapple than other systems. There was no significant difference in soil pH and EC. The measured silt loss (g/l) was significantly higher for disturbed fallow land due to direct exposure soil surface to erosive rainfall. Among the intercropping system, tapioca recorded the highest silt loss (5.38 g/l) followed by sweet potato, fodder and pineapple. The measured soil loss in 'g/l' is projected to 't/ha' using measured data of infiltration and soil moisture parameters. From the infiltration experiments conducted in the study area, it was found that around 30-40% of rainfall infiltrated into the soil and maintains saturated moisture conditions at 20-35%.

Sl. No.	Intercropping system	pН	EC (dS m <sup>-1</sup> )	Ammonia (mg/l)	Nitrate (mg/l)	Silt loss (g/l)
			,			
1	Pineapple	5.76	0.02	3.19 <sup>b</sup>	4.32ª	1.73 <sup>a</sup>
2	Sweet potato	5.98	0.03	3.33 <sup>ab</sup>	5.17 <sup>a</sup>	4.56 <sup>b</sup>
3	Tapioca	5.96	0.05	3.11 <sup>b</sup>	3.50 <sup>b</sup>	5.38 <sup>b</sup>
4	Fodder	6.20	0.03	3.71ª	4.15 <sup>a</sup>	4.29 <sup>b</sup>
5	Fallow-disturbed	6.12	0.08	2.95 <sup>b</sup>	3.26 <sup>b</sup>	14.62ª

Table 2: Soil and nutrient losses under different agricultural land uses

The results of soil loss estimated in the present study and the previous study under different agricultural land use practices are shown in Table 3. This was done for comparison and represent more inter cropping systems so to select more suitable system for island condition. The soil loss and nutrients recycling were studied for plantation crops by Pramanik *et al.* (1998) under arecanut and coconut plantations. This study revealed that on an average soil loss under plantation (main crop) with intercrops varied from 0.6 to 9.7 t/ha/year. At the same time the results indicated decrease in soil loss during second year in all the intercropping systems. In general, inclusion of grasses as intercrop under plantation crops significantly reduced the soil loss.

The measured soil loss from the runoff plots was 1.1, 2.7, 2.9, and 3.4 t/ha for Pineapple, Fodder, Sweet potato, and Tapioca, respectively in coconut based main crop system. Among the intercrops, the highest (34-35%) and lowest (11-17%) soil loss was occurred in Tapioca and Pineapple, respectively. Fallow land (without terrace) recorded the highest soil loss among all the system.

SI No		Agricultural land use	Measure	d soil loss (t/	ha/year)
Sl. No	Main crop	Intercrops	1992-93*	1993-94*	2013-14
1	Arecanut	Banana-Cinnamon-Pineapple-Black pepper- Grasses	9.7	4.0	-
		Black pepper-Cinnamon-Grasses	3.5	1.2	-
2	Coconut	Cinnamon-Arecanut-Grasses-Black pepper	6.4	0.6	-
		Pineapple	-	-	1.1°
		Fodder	-	-	2.7 <sup>b</sup>
		Sweep potato	-	-	2.9 <sup>b</sup>
		Таріоса	-	-	3.4 <sup>b</sup>
3	Fallow land	Fallow	-	-	12.5ª

#### Table 3: Observed soil loss based on runoff plots under plantation based intercropping system

\*Adapted from Pramanik et al. (1998)

Soil loss was predicted from USLE model using rainfall events and other soil, crop, slope and conservation practices data during 2013-14 (Table 4). This was carried out for inter comparison and upscaling of the results. The model predicted average annual soil loss indicted significant effect of terracing on soil loss reduction. Accordingly soil loss from intercrops varied between 2.1-4.3 t ha<sup>-1</sup> with terracing as conservation measure for sloppy areas. The estimated soil loss from coconut based intercrops without conservation practice varied between 15.1-30.1 t ha<sup>-1</sup>, which exceeded the tolerance limit of 11.2 t h<sup>-1</sup>. The study revealed that highest soil loss either with or without conservation practice was recorded for fallow land. Thus based on the study results, it is recommended that conservation measure like terracing is highly effective for arresting soil loss in plantation based intercropping system.

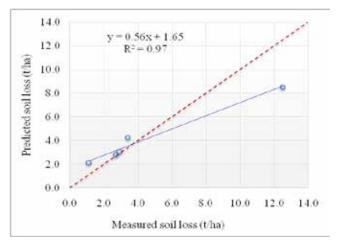
Table 4: Effect of conservation practice on the predicted soil loss using USLE in different coconut based
intercropping system

	With co			ce	Without	conservat	tion prac	tice
Agricultural practice	Soil loss (t ha <sup>-1</sup> )	RMSE	MAE	R <sup>2</sup>	Soil loss (t ha <sup>-1</sup> )	RMSE	MAE	R <sup>2</sup>
Pineapple	2.1*				15.1			
Fodder	$2.8^{*}$				19.9			
Sweet potato	3.1*	1.89	1.24	0.971	21.7	27.69	24.88	0.971
Tapioca	4.3*				30.1			
Fallow land-disturbed	8.5*				60.2			

(\*Significance level p<0.05 between with and without conservation practice)

Comparison results of measured and predicted soil loss under terrace based cropping system resulted low RMSE and MAE of 1.89 and 1.24, respectively (Table 4). Good agreement with coefficient of determination ( $R^2 = 0.97$ ) was found between the measured and predicted soil losses (Fig. 2). This study recommended that the

intercropping of pineapple with coconut plantation in terraced slope significantly reduced the soil loss. Pandey and Chaudhary (2010) also recommended that coconut plantation with crop cover as good vegetative barrier for arresting soil erosion in the Islands.



#### Fig. 2: Comparison of measured and predicted soil loss in coconut based intercropping system with conservation practice

#### Conclusions

This study concludes that plantation crops as main crop and pineapple as intercrop on the terraces can minimise the soil and nutrient losses significantly. This helps in building soil fertility and diversifying coconut plantation areas. Fallow land by virtue of more exposure to erosion factors recorded the highest soil loss. Thus in the island all the coconut garden having more than 10% slope should go for terracing followed by intercropping. Loss of soil not only affects the soil fertility, but negatively impacts the coastal areas by sedimentation. Further, in island ecosystem soils are most precious resource for agricultural production, once lost, cann't be restored back. Thus quantification of soil and nutrient losses under different agricultural land uses would help in monitoring and managing soil and water resources in sustainable manner.

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### The Potential of Integrated Farming System to Supply Feed and Fodder for Dairy Cattles and Evaluation of its Performance

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#### Abstract

In Andaman and Nicobar Islands livestock sector performance is severely limited by fodder and feed supplies especially during dry period (January-April). Consequently livestock are left free to graze in the field. In this context, green fodder cultivation and promotion of selected grasses besides proper utilization of rice straw assumes greater significance to support livestock production in the island. The present study was carried out to, assess the feed and fodder production potential of integrated farming system in coastal lowlands. The study showed that IFS system could supply 2,609 kg rice straw and 12,557 kg green fodder enabled by production of Bajra Napier hybrid + cowpea, fodder sorghum, maize straw (sweet corn) and green foliage of pulses. This would supply 35 kg of green fodder and 17 kg of dry fodder per day. Supplemented with 2.0 - 3.0 kg of concentrate, this would be sufficient for two dairy cattle. This greatly improved the performance of the dairy unit (1750 L/lactation/animal) with the net return of Rs. 1,35,090/-that constitute 48.5% of total farm revenue. Further feeding the animals using the on farm produced feed and fodder resulted in reduced parasitic load and other stresses on the animal. Up scaling this model to the entire farm households would meet the projected demand of the Island production by increased production from the current level.

Keywords: shortage of feed, dairy production, rice straw, crop residues, IFS, Island ecosystem

#### Introduction

Dairying in India is an important livelihood activity and over 80% of milk production in the country is by smallholders. In dairy, feeding accounts for more than 70% of the total cost of milk production and it directly or indirectly affects the entire livestock sector, including animal productivity, health and welfare and the environment (Makkar, 2016). According to an estimate of National Commission of Agriculture, there is a deficiency of concentrates and green fodders in India (Kamra, 1998; DES, 2019). Farmers practicing specialized fodder based production system are limited and most of the grazing lands are public property or forest. In Andaman Islands, dairy and goat are the major livestock reared by the small and marginal land holders. The dairy contribute significantly, both in number as well as in production of milk. The milk production in the Islands decreased from 26,000 tons in 2012 to 16,990 tons in 2018 and per capita milk availability in these Islands is only 110 gm/ day as against the national average of 337 gm/day. The low productivity could be due to poor and imbalanced nutrition, lack of green fodder mainly during dry period

(January to March), infertility and prevalence of parasitic and gastrointestinal parasitic diseases in dairy cattle (Perumal *et al.*2018).

With projected increase in population and development of tourism in the Islands, the productivity of the livestock needs to be doubled to cater the demand of meat, milk and other livestock products. Any increase in the productivity should come by effectively utilizing the available resources but without negative impact on the environment (Kundu et al. 2006). In the small holder farming systems prevalent in these Islands, the production of forage and fodder is often a sidelined activity and not being practiced as integral part of farming. The animals are allowed for grazing in the open grasslands, or the animals are fed with grasses and legumes which grow voluntarily on the field bunds or in waste or fallow lands. Fodder shrubs and trees may or may not be present on field bunds. The locally available feed resources are the major driving force for improving productivity of animals in developing countries like India. To maximize profitability from the dairying, one need to ensure that the dairy animals receive required quantity of protein,

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energy, minerals and vitamins in a cost effective way, preferably from locally available feed resources such as local grasses, natural forages and green fodder (Hossain *et al.* 2017).

In the coastal and lowlands of this island the availability of feed and fodder is severely constrained particularly during dry season. In these areas integrated farming system (IFS) provide the means for addressing the issues of livestock farming in the islands by providing opportunity for production of feed and fodder and efficient waste recycling for increasing the farm productivity. Thus in this study, we made an assessment of feed and fodder production potential of IFS based on long-term field experiment with the aim to enhance the production of dairy animals in smallholder farms under island conditions. Further an attempt was made to qualitatively assess the impact of providing on-farm produced feed and fodder to the dairy animals on production and animal health.

#### **Materials and Methods**

The study was conducted during 2014 to 2018 in an area of 0.75 ha at Field Crops Research Farm, Bloomsdale of ICAR- Central Island Agricultural Research Institute, Port Blair. The crop components were allocated an area of 0.65ha with 0.35ha used for raising different rice based cropping systems viz., rice (*Oryza sativa L.*) – maize (*Zea mays L.*), rice (*Oryza sativa L.*)- green gram (*Vigna radiata*), rice (*Oryza sativa L.*) – sorghum (*Sorghum bicolor*) and rice (*Oryza sativa L.*) – vegetables (okra/brinjal/cowpea). In 0.30ha vegetables were grown on the beds and rice-fish in the sunken furrows of BBF. Fodder crops (Bajra-Napier hybrid and cowpea) were grown in an area of 500 m<sup>2</sup> on a bed, field bunds and on the slopes of the beds in BBF system.

The livestock component comprised of two Crossbred Holstein Friesian (HF) cows integrated with other components since April 2014. The green fodder requirement of the animals was met from fodder crops grown on the beds, fodder sorghum from rice-sorghum rotation, and crop residues such as rice straw, maize stover and other crop residues. On an average 25-30 kg green fodder was given to each animal every day, besides some concentrate feed to maintain the nutritional status of the dairy unit. The lactating animals were fed with 1 kg concentrate for every 2.5 to 3.0 l of milk yield along with 2 kg dry fodder while calves and heifers were fed with 1 kg feed. The animals were allowed for restricted grazing within the farm on rotational basis in different field units in the morning after milking to keep the animals active. Proper weeding of unwanted plants and cleaning of field bunds were carried out periodically. Standard livestock management practices were followed to provide healthy and hygienic conditions for animals. During the experimental period the animals were routinely observed for health status and the details were recorded.

#### **Results and discussion**

#### **Fodder production**

Fodder and feed are the most valuable and cheapest sources of food for livestock. They are the rich source of energy, mineral nutrition, carbohydrates and protein. In India, the three major sources of fodder supply are crop residues, cultivated fodder and fodder from common property resources like forests, permanent pastures and grazing lands. In the Islands, the cultivated fodder production is very limited, accounted only 10ha in 2006 (Kundu et al. 2006). As the constant supply of good quality forage in sufficient quantities is a basic necessity in livestock farming, fodder requirement of dairy animals without affecting the production of major crops such as rice, vegetables and other food crops can be achieved by proper planning and crop allocation through farming systems approach. The study showed that on an average IFS system could produce 2,609 kg rice straw and 12,557 kg green fodder besides other crop waste i.e 583 kg of chaffy grains in a year (Table 1).

In addition to rice straw, green fodder produced in IFS includes the Bajra Napier hybrid, cowpea, fodder sorghum, maize straw (sweet corn), green foliage of pulses, vegetables (cow pea, French beans, cauliflower, okra, brinjal) and fodder from the slopes of the beds of BBF system. BN hybrid is available throughout the year besides seasonal availability of crop wastes. After rice, sorghum (TN local) was grown as a fodder crop during January – March in area of 1500 m<sup>2</sup> with average

production of 38.4 t ha<sup>-1</sup>. By adjusting the sowing date and staggered planting, the green fodder availability can be extended even during dry months of April – May with one or two irrigations. In normal rainfall years, the ratoon crop of sorghum can be harvested till May. At times fodder was obtained from subabul (*L. leucocephala*) and other MPT located on the boundary. Besides, there are other crop residues like sweet corn straw, pulses and vegetables which are available during February to March at the time of harvesting of these crops.

SI.	T4	On farm product			
No.	Item	2015-16	2016-17	2017-18	Mean (kg)
1	Rice straw	2,185	2,705	2,938	2,609
2	Green fodder	10,390	12,077	15,205	12,557
3	Chaffy grains	750	555	445	583
4	Agro-forestry	350	450	380	390

#### Table 1: Amount of feed and fodder produced in IFS system (0.75ha)

#### **Composition of rice straw**

Rice straw is the only dry fodder produced in the system and it is available after harvest of rice in November. In the present study medium and long duration rice varieties were grown during July to November. With proper drying and storage the rice straw was fed to the animals throughout the year and it was sufficient to supplement the green fodder requirement of a herd of 3 adults. The chemical composition of paddy straw varies between varieties, growing seasons and agronomic management. In general higher nitrogen and cellulose contents are found in early season rice compared to others. Paddy straw contains 25-45% cellulose, 21-32% hemi-cellulose, 2.0-5.8% lignin and 3.5 - 4.7% crude protein with low nitrogen, vitamins and minerals (Table 2). Feeding cattle with high quantities of silica hinder the nutrient availability to rumen microbes and eventually limits the necessary nutrient uptake for a satisfactory performance of the animals (Van Soest PJ (2006). Such materials should be nutritionally improved by further processing of the straw (silage / hay making) or mixing with other fodder / concentrates. Presence of anti-nutritional factors like silicates and oxalates in rice straw with low nutritive value, poor palatability and limited ruminal degradation (as in-vitro dry matter digestibility) render paddy straw insufficient to support nutrient requirement of the animals without addition of nutritional supplement (Sharma et al., 2001). In corroboration with the previous studies, rice straw harvested from the system was of required quality and can be safely fed to the ruminants supplemented with concentrate feed and green fodder.

Sl.No.	Chemical composition	Values (range)
1	Dry matter (g kg <sup>-1</sup> FM)	865 - 930
2	Mineral matter (g kg <sup>-1</sup> DM)	112 - 252
3	Hemicellulose (g kg <sup>-1</sup> DM)	210 - 325
4	Cellulose (g kg <sup>-1</sup> DM)	250 - 456
5	Lignin-sa (g kg <sup>-1</sup> DM)	20.0 - 58.5
6	Crude protein (g kg <sup>-1</sup> DM)	35.6 - 47.5
7	C (nitrogen fraction) (g kg <sup>-1</sup> CP)	125 - 230

SI.No.	Chemical composition	Values (range)
	Nutrient composition (%)	
8	Ν	0.4 - 0.7
9	$P_2O_5$	0.15 - 0.29
10	K <sub>2</sub> O	0.95 - 2.1
11	Ca	0.63 - 2.21
12	Mg	0.82 - 2.35
13	Fe	0.15 - 0.56
14	Mn	0.08 - 0.14
15	Zn	0.04 - 0.12
16	Cu	0.05 - 0.12

#### Assessment of feed and fodder availability

Given the average nutritional composition of the rice fodder samples evaluated, it is suggested that their use for feeding cross breed or high yielding animals, as the only food source, is not sufficient to meet the maintenance requirements of the animals (Sarnklong *et al.*, 2010). However, this feed presents relevant potential for strategic use as part of the diet of animal categories with lower nutrient requirements in times of food shortage, as well as to preserve the body condition of the animals, intensify the production system, and still allow a better quality of postpartum nutrition. The variation in the nutritional value of rice straw evaluated in this study was explained by the effects of rice varieties, manuring, and other agronomic practices. Previous research has also identified variations in the nutritional value of rice straw due to difference in rice genotypes and crop development cycle (Santos, 2010) and grain production (Vadiveloo, 2003).

	Availability (kg)		Per day	Per day availability		ment per o animal			
Month	Green fodder	Dry fodder	Green fodder	Dry fodder	Green fodder	Dry fodder	Concentrate	- Remarks	
January	1046	521	35	17	30-50	10-12	3.0 – 6.0 (5 -6.0 kg during lactation)	All are sufficient	
February	1046	521	35	17	30-50	10-12	3.0 - 6.0	All are sufficient	
March	1046	521	35	17	30-50	10-12	3.0 - 6.0	All are sufficient	
April	1046	521	35	17	30-50	10-12	3.0 - 6.0	All are sufficient	
May	1046	521	35	17	30-50	10-12	3.0 - 6.0	All are sufficient	
June	1046	-	35	-	30-50	10-12	3.0 - 6.0	Sufficient GF Preserved / silage dry fodder along with others	

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July	1046	-	35	-	30-50	10-12	3.0-6.0	Sufficient GF Preserved / silage dry fodder along with others
August	1046	-	35	-	30-50	10-12	3.0 - 6.0	Preserved / silage dry fodder along with others
September	1046	-	35	-	30-50	10-12	3.0 - 6.0	Deficit of dry fodder, met by more concentrate
October	1046	-	35	-	30-50	10-12	3.0 - 6.0	Deficit of dry fodder, met by more concentrate
November	1046	-	35	-	30-50	10-12	3.0 - 6.0	Deficit of dry fodder, met by more concentrate
December	1046	-	35	17	30-50	10-12	3.0 - 6.0	All are sufficient
Total production	12,557	2607						Feed, fodder, concentrate requirement is met

\* 1 kg paddy straw can replace 4-5 kg of green fodder on dry matter basis, Concentrate, and green and dry fodder requirements are based on National Dairy Development Board recommendation for adults

As indicated in the table 3, when the animals are supplemented with rice straw (2 kg/animal/day), the green fodder requirement is substantially reduced from 27,375 kg to 16,425 kg year<sup>1</sup>. This deficit can be further reduced by supplementing with excess dry fodder (400 kg) available in the system or further augmented by planting one or two fodder trees (Sesbania sesban, S. grandiflora) on field boundaries or near the sheds. Such an increased fodder production was achieved earlier by growing short duration forages in the gap period of the prevalent crop sequence which is a standard practice in irrigated areas. For example in the wheat-sorghum-maizebaira sequence, forage crop mixtures like maize +cowpea, sorghum + cowpea or bajra + cowpea were grown in the gap period that exists between April and June which with an yield of 35-40 tonnes/ha, without affecting main crop (Lal and Tripathi 1987).

#### Performance of dairy unit under IFS

The clinical observations of the dairy animals fed with feed and fodder produced in the IFS as against

the free ranching animals are presented in table 4. The productivity of the animal is negatively affected by the combination of abiotic and biotic stresses. It was observed that most of the parasitic infections are acquired during grazing and intermingling with other animals. In contrast, dairy animals reared in the IFS, are fed with suitable mixture of feed/fodder were less infected with these parasites. Similarly plant poisoning and aflatoxicosis are more prevalent among the freely grazing animals. Further, walking stress and bloat are more prevalent in freely ranching animals. Thus on-farm production and feeding of animal with dry and green fodder are less prone to these stresses as compared to the free ranching animals. This provides more scope of increasing the livestock production relying on the good health of animals that also improves the stability of IFS production.



		Stress	level			
Sl.No.	Biotic (disease and pest) and Abiotic stresses	Dairy animals in IFS	Free ranching animals	Remarks		
1	Helmintic parasite	1	3	Animals graze in open place or nearby water bodies (as snail is the intermediate host) suffer more helmintic infestation.		
2	Leptospirosis	1	2	Freely grazing animal in paddy field and other places suffer leptospirosis and spread by rat		
3	Hump sore	0	2	Animals reared in pasture system have suffered more than in backyard system		
4	Tick infestation	0	3	Tick infestation spreads through contact mostly while grazing		
5	Mastitis	1	3	It leads to loss of productivity which occurs due to improper personal & udder hygiene and care, injuries, insect bites, fly bite, sitting in contaminated water, during grazing in the jungles or pasture land		
6	Foot and mouth diseases	0	2	It is contagiuos viral disease which spread from one animal to another in grazing field.		
7	Calf Pneumonia	0	2	Free ranching animal suffer more		
8	Metabolic disorders			Occurs due to deficiency of mineral elements. Thus suitable feed / fodder and mixture of specific minerals needs to be fed		
9	Mycotoxin in Feedstuffs & Aflatoxicosis	0	2	Mycotoxin causes serious problem leading to heavy mortality and morbidity		
10	Walking stress	0	3	Animals walking longer distance and more time to receive sufficient feed/ fodder suffer from reduced growth & reproduction rate, increased inter-calving interval, reduced milk production and vulnerable to diseases		
11	Tympany or bloat	0	2	Proper mix of feed and fodder is essential. It is difficult to ensure proper feed mix for freely grazing animals		

#### Table 4: Effect of IFS on animal health (based on qualitative observation)

(Severity incidence scale: 0 - very low/nil; 1 - slight / occasional; 2 - moderate; 3 - severe)

The total milk production in 2018 was 16.99 thousand liters with mean per day productivity of 1.38 L per animal. It showed that the animal productivity (milk) is very low in the islands probably due to poor and imbalanced nutrition, lack of green fodder mainly during

dry period (January to March), infertility and prevalence of parasitic and gastrointestinal parasitic diseases in dairy cattle (Perumal 2018). Though majority of the farmers rear the animals in mixed farming system, lack of feed and fodder poses the major limitation in increasing the productivity of the animals. This requires suitable modification of the existing farming system by proper allocation of resources for different enterprises. In the present study rice based cropping systems was modified by inclusion of short duration fodder crops like sorghum, cowpea, maize in integrated farming systems approach that increased the fodder availability especially during dry months. In the IFS, the average milk production per lactation was around 1750 L with per day productivity of 6.0 L which far exceeds the average productivity of the Islands. The resource recycling between the enterprises further enhances the productivity of the each component enterprises and the total farm productivity. The results revealed that on an average 48.5 % of total net income is accounted by dairy component alone and ensured income flow all through the year.

		Rearing	method	
Sl.No	Items	Dairy animals in IFS	Free ranching animals <sup>*</sup>	
1	Average milk production per lactation (L/animal)	1750	500-800	
2	Mean lactation period (days)	295	270-290	
3	Mean daily milk production (L/day/animal)	6.0	1.85 - 2.75	
4	No. of calving	2 in 3 years	1 to2 in 3 years	
5	Mean annual production from dairy unit (L/year)	3250	1150	
6	Mean gross return (Rs.) from dairy unit	2,61,570	80,500	
7	Mean total cost (Rs)	1,26,480	45,250	
8	Mean net return (Rs)	1,35,090	59,250	
9	Employment generation (man days year-1)	195	110	
10	Total cost of external purchase	54,750	15,000	
11	Imputed value of family labour	53,625	30,250	
12	Imputed value of recycled products	18,105	-	
13	B:C ratio	2.07	1.78	
14	Contribution to the total farm income	48.5 %	35-40%	
15	Upscaling to 15,000 ha (milk production = 3250 /ha x 15000 ha) t per year	48750	-	
	Potential milk production (Lakh litres per day)	1.33	-	

#### Table 5. Performance of dairy component under IFS and free ranching

\* Based on Kundu et al., 2009; DES, 2018 (AN Administration) and personal interaction with two dairy farmers

Based on the above findings, it is postulated that by bringing 15,000 ha annual cropped area under IFS, potentially 1.33 lakh L of milk per day can be produced in the island. This could meet the projected demand of 43,254 t per annum milk (Kundu *et al.* 2006) required in 2031. Thus on farm production of feed and fodder in farming systems approach will not only meet the fodder requirement of milch animals but also has the potential to enhance the milk production and improve the per capita milk availability in the Islands in future.

Other than feed and fodder for milk production, waste management is very important task in livestock farming. In the IFS unit, nearly 10,360 kg of cow dung and 8790 L of urine & shed washing were produced. The waste water



emerging from the cattle shed was used for irrigating the fodder crop or used for fish production in ponds or in rice-fish system. The solid waste (cow dung) was used for composting of farm waste and helped in recycling of nutrients. In free ranching system of animal rearing, this valuable manure is lost.

 Table 6: A glimpse of waste generation in dairy unit and its potential use under IFS

Item	Mean	Remarks
Cow dung (kg)	10,360	Used in compost preparation.
Urine & shed washings (L)	8,790	Used for irrigating vegetables and fodder cultivated in the beds of BBF system and also used for rice-fish system in the furrows.
Shed waste (kg)	3,620	Recycled into compost preparation

#### Conclusions

The shortage of feed and fodder is hampering the development of dairy units in the island though the demand is kept increasing. The shortage can be met from on-form production of green fodder, use of rice straw and crop residues produced in IFS units. Due to the fact that in these areas paddy straw is abundantly available from cultivating paddy, farmers can use rice straw as the main roughage source to their animals but supplement it with green fodder and concentrates. For further use during wet season the excess straw can be converted into hay /silage by suitable treatment. Most importantly, integration of fodder in the existing cropping pattern (intercropping in the coconut and areca nut) should be encouraged for improving fodder production. At the same time every animal needs to be supplemented with required concentrate feed (1.5 to 2.0 kg /day). The role of agroforestry systems in augmenting the supply of green forage needs to be emphasized to farmers. Although forage based feeding systems help lower feed costs, the scope for such systems is limited in India because of the need to give priority to food crops.

The use of poor quality fodder or grasses leads to more energy loss as the animal utilizes more energy for grazing, mastication, digestion and other activities related to that feed or fodder. Therefore, the crossbred cows requires a suitable grazing/walking and feeding policy or strategy to prevent or minimise the deleterious effect stimulated by walking and dry season to the cows of Andaman and Nicobar Islands. And an ideal methodology needs to form for supplementation of suitable feed, fodder, electrolytes & antioxidants to minimise the stresses.

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#### **Scope for Microbial Amelioration of Salinity Stress in Plants**

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#### Abstract

Presence of higher amount of soluble salts in soil and irrigation water is a major limiting factor for crop productivity in tropical and semi-arid regions of the world. Though physiological insights about the mechanisms of salt tolerance in plants have been gained, translation of such information to aid in crop improvement has been limited. The identification and exploitation of soil rhizosphere bacteria and mycorrhizal fungi for alleviating salinity stress opens new alternatives for salinity management. The organisms offer several beneficial effects to plants such as improved nutrient and water uptake, growth promotion, and alteration of plant metabolism under salinity stress. This review aims to evaluate the beneficial effects of soil rhizosphere bacteria and mycorrhizal fungi on the plant response to saline stress, with the possible application to improve the crop production under varying salinity conditions.

Key words: salinity stress, ion homeostasis, mycorrhizae, rhizobacteria, phytohormones

#### Introduction

Salinity is a key factor hampering crop productivity and a major cause of the abandonment of lands, particularly in the coastal areas for agricultural purposes. Over the years developing salt-tolerant crops has been given prominence and few major-determinant genetic traits of salt tolerance have been identified (Flowers, 2004; Munns and Tester, 2008). But these efforts could not produce desired results at field leaving lots of issues to address for adequately managing salinity stress on plants. Several recent studies have demonstrated that local adaptation of plants to their environment is driven by genetic differentiation in closely associated microbes (Rodriguez and Redman, 2008). Thus, an alternative strategy to improve crop salt tolerance lies at harnessing the salt-tolerant microbes that enhance crop growth. Enhancement of crop growth in a wide range of salinities by utilizing salinity tolerant soil microbes are discussed below, particularly this approach may succeed where it has proved difficult to develop salttolerant germplasm.

Though there are wide range of microbes having beneficial effect on crop plant under saline environment, much focus in this review is given on symbiotic relationships such as arbuscular mycorrhizal fungi (AMF), whose hyphal networks ramify throughout the soil and within the plant cells, and root-associated plant growth-promoting rhizobacteria (PGPR). These soil dwelling microbes in the rhizosphere region have evolved several mechanisms to protect themselves to survive in these adverse conditions and also trigger consequential changes in plants leading to crop adaptation to abiotic stresses. Further, in an effort to survive and prevail at the rhizosphere, some microbes possessing the cellulase enzyme capable of dissolving the cellulose cell wall of plant roots gain entrance into the apoplast of plants, the cell wall interior as well as the vascular bundle where they live and undergo normal metabolic activities (Khan *et al.* 2016). They also provide the plant with useful metabolites that are very essential for the plant to overcome abiotic stress.

Although changes in ion uptake by plant root occur within minutes of exposure to salinity (Davenport, 2007), ion (e.g. Na<sup>+</sup>, Cl<sup>-</sup>) accumulation to toxic levels in photosynthetically active mature leaves takes time providing scope for its amelioration. Thus understanding soil microbial role in alteration of ion homeostasis and improve plant nutrition in salinized crops is very important. As plant meristems are actively growing tissues where cell division and further expansion governs sink strength and affects plant carbohydrate status, microbial impacts on plant energetics are also briefly discussed. These mechanisms do not work in isolation but rather in an integrated manner to finally affect the major physiological processes limiting growth under salinity. In general, studies proved that these microbes induce salinity tolerance in crops by production of phytohormones, volatile compounds, exo-polysaccharides, ACC deaminase and osmolyte, and triggering antioxidant activities (Wang *et al.*, 2003; Glick 2012). Thus this review seeks to evaluate microbial effects within the context of physiological and agronomic responses of plants to salinity (Munns, 1993) according to temporal changes in both osmotic and ionic stresses.

### 2. Soil-plant-microbial interactions and salinity stress

Several studies have shown that plant-microbe interactions not only depend on the plant and microbe alone but the surrounding environment as well. Soil and the soil microclimate are the two most important factors affecting the interactions among other factors (Fig. 1). Review of relevant literatures showed that soil properties influencing the microbial distribution and the plantmicrobes interactions are soil type, moisture condition, organic matter content, fertility level, presence of toxic compounds, soil temperature etc. These properties are the key factors in deciding the microbial diversity and distribution, the interaction comes next only to this (Wagg et al. 2014). In managed ecosystem land use is important drivers of microbial distribution. It is important consideration in plant-microbe interaction because microbial diversity and composition are widely recognized as key factors in driving ecological functions (Vivant 2013).

Globally, in terms of environmental stresses, saline stress is considered the most severe stress that effect not only the soil and plant growth but also the living organisms. Salinity reduces plant growth chiefly in three ways, (i) osmotically induced water stress, (ii) specific ion toxicity due to high concentration of sodium and chloride ion and (iii) nutrient imbalances which hampers the uptake of water by plants (Greenway and Munns 1980). Ultimately photosynthetic rate decreases not only because of reduced leaf area and lesser gas exchange but also due to feedback inhibition of unused photosynthates, after exposure to salinity (Gayathri and Smith 2017).



Thus, it is essential to better understand the causes and controls of soil microbial distribution, effect of salinity on plants and the changes in plant system brought as a result of microbial-plant interaction under saline environment so as to harness its potential for amelioration of salinity stress in plants.

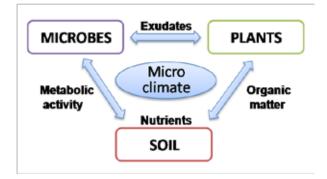


Fig.1 Soil-plant-microbe interactions

#### Soil microbes in alleviating salinity stress

Review of literatures indicated that many PGPR and MR have been investigated for their role in improving plant-water relations, ion homeostasis and photosynthetic efficiency in plants under salt stress. However, their amelioration mechanisms are intricate and often not completely understood in different crops under varying conditions. These mechanisms are regulated by a complex network of signaling events occurring during the plant-microbe interaction and consequently ensuing stress alleviation (Smith et al., 2017). The classes of microbes belonging to these group/ genera are Micrococcaceae, Bradyrhizobium, Bacillus, Microbacterium. Pseudomonas. Curtobacterium. Variovorax, Paenibacillus, Pantoea, and many others (Kasim et al. 2016). Here we discuss the beneficial effect of some of the PGPR and MRF on plant against salt induced stress.

#### Water homeostasis and osmolyte accumulation

Maintaining water homeostasis and the functioning of photosynthetic structures are essential for alleviating the impact of salinity on plant growth and crop yield, even if salinity-induced losses in turgor may be transient, owing to plant uptake of ions from the soil allowing foliar osmotic adjustment (Munns and Tester, 2008). Studies showed that under stress, plants inoculated with mycorrhiza (Auge', 2001) or PGPR (Creus *et al.*, 2004) often show enhanced osmotic adjustment. A schematic representation of salt stress alleviation by mycorrhizal fungi and plant growth-promoting rhizobacteria is represented in Fig. 2.

Root colonization by AMFs can induce major changes in the relative abundance of the major groups of organic solutes (Sheng et al., 2011), such as modifying the composition of carbohydrates and inducing accumulation of specific osmolytes such as proline, thus facilitating osmotic adjustment. Nevertheless, the concentration of proline under saline conditions may not always indicate significant osmotic adjustment, unless subcellular compartmentation is considered (Pe'rez-Alfocea et al., 1993). However, better growth of AM-inoculated Jatropha curcas compared with non-inoculated plants when exposed to salinity (1.7-8.5 dS m 1 NaCl for 60 d) resulted from increased soluble sugars and proline in the leaves of inoculated plants, allowing maintenance of leaf water status (Kumar et al., 2010). Similarly the introduction of a proBA gene derived from Bacillus subtilis into Arabidopsis thaliana increased production of free proline, which was associated with increased salt tolerance in the transgenic plants (Chen et al., 2007).

Further, improved exploitation of soil water due to the mycorrhizal hyphal contribution to water uptake and induced changes in root morphology also reported. Indeed, the mycorrhizal maintenance of root turgor during drought was apparently not related to osmotic adjustment, despite fungal alteration of concentrations of several key solutes, but to change apoplastic/symplastic water partitioning (Auge' and Stodola, 1990). While these local changes contribute to root growth maintenance, soil microbes also affect the ability of the roots to take up water under both drought and saline stresses.

#### Source-sink relations and energetic metabolism

In addition to osmoregulation, stimulation of carbohydrate transport and metabolism between source and sink tissues has also been proposed as a mechanism to alleviate metabolic feedback inhibitions of photosynthesis, thus avoiding photo-inhibition during the osmotic phase of salinity when carbohydrates usually accumulate (Munns,

1993). Symbiotic microorganisms are known to directly modulate these source-sink relations by enhancing sink activity through increased exchange of carbohydrates and mineral nutrients. When colonized by AMF plant roots become a strong sink for carbohydrates, as these fungi can consume up to 20% of the host photosynthate (Heinemeyer et al., 2006). It is also proposed that sugar accumulation may be due to the hydrolysis of starch in inoculated seedlings, as mycelium growth requirements mobilize carbon reserves, which could help decrease salinity-induced starch accumulation as a consequence of the inhibition of sink activity in growing tissues (Balibrea et al., 2000). Thus maintenance of an active carbohydrate sink in symbiotic roots, when assimilate transport and use in other sink tissues is impaired, could help maintain the source activity of mature leaves for longer, thereby improving salt tolerance (Pe'rez-Alfocea et al., 2010).

#### Ion homeostasis

Salt tolerance in glycophyte species is mostly related to the exclusion of toxic ions present in saline soil from the leaves thereby avoiding or delaying toxic effects (Munns and Tester, 2008). Microbes can alter root uptake of toxic ions and nutrients by altering host physiology and modifying physical barriers around the roots, or by directly reducing foliar accumulation of toxic ions (Na<sup>+</sup>, Cl<sup>-</sup>) while improving the nutritional status of both macro-(N, P, K) and micronutrients (Zn, Fe, Cu, Mn) mostly through selective uptake along with enhanced water flow into the root. Further, nutrients may also become more accessible to the plant due to microbial-induced changes in rhizosphere pH (organic acid excretion) and/or chelation with organic molecules (siderophores) exuded by microbes. Particular importance has been attached to microbial enhancement of K<sup>+</sup>/Na<sup>+</sup> ratios in plants (Giri et al., 2007). Hence, any contribution of the soil biota towards maintaining the homeostasis of toxic ions must benefit plant growth under salinity (Fig. 2).

It has been demonstrated that the AMF *Glomus intraradices* can selectively take up elements such as  $K^+$ ,  $Mg^{2+}$ , and  $Ca^{2+}$  while avoiding  $Na^+$  uptake to keep internal  $K^+/Na^+$  and  $Ca^{2+}/Na^+$  ratios within narrow limits, despite changes in concentration of the ions in the soil

environment. Concentrations and distributions of Na<sup>+</sup> and Cl<sup>-</sup> within the fungal tissue suggest that this AMF acts to exclude Na<sup>+</sup> but include Cl<sup>-</sup>. These selective mechanisms for ion uptake could partially alleviate salinity stress in host plants by improving their nutrition (Hammer *et al.*, 2011). If a significant proportion of the elemental uptake in plants occurs via the mycorrhizal fungi, this could explain the often higher K<sup>+</sup>/Na<sup>+</sup> ratios in mycorrhizal plants (Giri *et al.*, 2007).

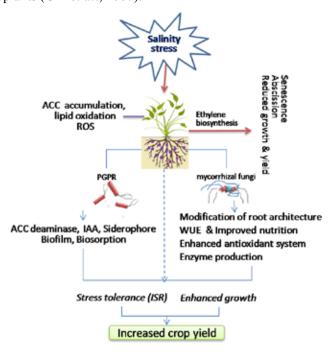


Fig. 2. A schematic representation of salt stress alleviation by mycorrhizal fungi (MF) and plant growth-promoting rhizobacteria (PGPR)

#### Stress tolerance through PGPR

Salinity impairs plant growth by causing osmotic imbalance and ion toxicity. Several studies have revealed that PGPRs and MRF are capable of maintaining optimum biological functions in cereals, legumes and vegetables grown under stress conditions. Some selected examples of growth promotion with inoculation of these rhizobacteria under stressful environments are included in Table 1.

One of the common hypotheses employed in most of the studies conducted under salinity stress was the lowering of ethylene level by the ACC-deaminase activities of PGPR. These studies conducted under both controlled and natural environments in greenhouse showed that inoculation with PGPR containing ACCdeaminase significantly increased plant growth and yield compared to that of un-inoculated control. In addition to regulating plant nutrition by enhancing K<sup>+</sup> uptake over Na<sup>+</sup> in plants under salt stress conditions (Nadeem et al., 2009) inoculation with PGPR also enhances the uptake of other major nutrients as well as improves the water content of stressed plants. The inoculation with Pseudomonas spp. improved the eggplant growth by depressing the uptake of Na<sup>+</sup> and increasing the activities of antioxidant enzymes under salinity stress conditions. According to them, regulation of mineral uptake and increase in the antioxidant enzyme activities may be the two key mechanisms involved in alleviation of salt stress.

PGPR strains have been reported to be equally effective when applied with other microbial populations. For example, Figueiredo *et al.* (2008) evaluated the effect of co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici* on growth, nitrogen content and nodulation of common bean (*Phaseolus vulgaris* L.) under abiotic stress environment in a greenhouse. The results showed that co-inoculation enhanced the plant growth, nitrogen content and nodulation of bean under drought stress compared to uninoculated control.

The PGPR strains are effective not only for improving plant growth under salinity stress but are also helpful for enhancing plant growth and development under heavy metals, flooding and drought stress (Glick *et al.*, 2007). Plant growth promoting rhizobacteria have been shown as effective biocontrol agents against a number of plant pathogens. The above discussion clearly indicates that PGPR strains are very helpful to enhance plant growth not only under salinity stress but also other stresses such as drought, flooding, heavy metals, pathogen attack, etc.

Сгор	Bacterial strain	Effect on plants	Reference
Tomato ( <i>Solanum</i> <i>lycopersicum</i> )	Achromobacter piechaudii	ARV8 Inoculation increased fresh and dry weight as well as water use efficiency of tomato by decreasing the ethylene production under stress.	Mayak <i>et al.</i> (2004a)
	Pseudomonas fluorescens, P. aeruginosa, P. stutzeri	All PGPR strains enhanced the root and shoot growth of tomato. Sodium contents (Na) were low in plants inoculated with P. Stutzeri and showed relatively better grwoth compared to other two strains.	Tank and Saraf (2010)
Cotton ( <i>Gossypium</i> hirsutum)	Klebsiella oxytoca	In addition to significant increase in height and dry weight of cotton plants, inoculation with PGPR uptake ofmajor nutrients like N, P, K, and Ca increased while Na deceased.	Yue et al. (2007)
Groundnut ( <i>Arachis hypogae</i> a)	P. fluorescens TDK1, P. fluorescens PF2 and P. fluorescens RMD1	Bacterial strains proved useful for increasing salt tolerance of groundnut. The impact of strainswas variable and P. fluorescens TDK1 provedmost effective than other ones.	Saravanakumar and Samiyappan (2007)
Maize (Zea mays)	Pseudomonas spp., Enterobacter aerogenes, Flavobactrium ferrugineum	PGPR enhanced the growth of maize under salinity but with variable efficacy. Overall, high chlorophyll content, relative water content and K+/ Na+ ratio was observed in inoculated plant than uninoculated control.	Nadeem <i>et al.</i> (2009)
Canola ( <i>Brassica napus</i> L.)	Pseudomonas spp.Rate of seed germination and seedling growth was significantly higher. ACCdeaminase producing Pseudomonas spp. enhanced canola tolerance against salinity stress.		Jalili <i>et al</i> . (2009)
Black gram	Ochrobactrum pseudogrignonense	Osmoregulation, ACCd, ROS	Saikia <i>et al.</i> , 2018
Mung bean (Vigna radiata)	Pseudomonas syringae, Pseudomonas fluorescens,	IAA production, siderophore activity nitrogenase activity, P- solubilization	Ahmad <i>et al.</i> (2011)
V. faba	R. leguminosarum	Osmoregulation and ACCd	Cordovilla <i>et al.</i> , 1999

#### Table 1: A summary of beneficial effect of soil microbes on plants under salinity stress

#### **Commercial prospects**

The role of PGPR and MRF based bio-formulations has shown great potential toward sustainable agriculture and seen as an alternative to chemicals. Successful commercial application of microbial inoculants to improve crop growth and yield in saline soil implies that the inoculants are also salt tolerant, which highlights the potential of using microorganisms from saline habitats. Certain PGPR, whose ability to colonize the root system is undiminished by salinity (Paul and Nair, 2008), offer considerable potential as inoculants. Although some mycorrhizal species (e.g. Scutellospora calospora) reach maximum spore germination under high-salt conditions, spore germination of some AMF was delayed and the specific rate of hyphal extension was reduced in the presence of NaCl (Juniper and Abbott, 2006). The extent to which spore germination was inhibited was not the same for all species investigated but was similar for isolates of the same species, and was independent of the salinity of the environment of origin. However, the evidence that propagules from root pieces have a higher capacity to germinate under high salinity than the spores of the same species suggests that specific technologies can be developed to optimize fungal viability under saline conditions in order to optimize colonization of the host plant. One interesting proposition is to pre-treat mycorrhizal hyphae with salt prior to inoculation into saline environments (Sharifi et al., 2007), which almost doubled root colonization and stimulated root and shoot growth of soybean plants. Thus, parallel programmes of independently increasing both microbial and plant salt tolerance may assist the productivity of crops grown in saline environments.

#### Conclusions

Significant improvement in plant tolerance to abiotic stress will improve yield and production of crops to feed humans and livestock. This can be achieved by two approaches. One approach is via development of salinity tolerant plant species. The other method is the use of PGPR and MRF which will go a long way in supporting the plant to develop both intrinsic and extrinsic ability to tolerate stressful conditions and sustain yield. However, a major problem in rainfed agroecosystems is predominance of abiotic stresses like high temperature, salinity, and drought where the survival of bioinoculants is a problematic issue. The variations in results from laboratory to field are more compounded due to various abiotic stresses that prevail under field conditions for microbial inoculants to establish and to show the desired effect. Such problems can be overcome by sound screening programme for efficient stress tolerant PGPRs for effective deployment of these strains to draw one or more beneficial effects. Use of soil microbes for salinity alleviation will form the core of the future adaptation strategy to climate change as the salinity is projected to increase due to uncertainty in rainfall and sea water intrusion in the coastal areas.

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## A Comparison of Growth and Colouration of *Carassius auratus* (Linnaeus, 1758) under Different Culture Systems

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#### Abstract

The present experiment was conducted for 60 days to compare growth and colouration of *Carassius auratus* (Linnaeus, 1758) among three culture systems such as aquaria (AQ), cement tanks (CT) and cages (CG) suspended in pond. Fry of gold fish with mean length  $4.1 \pm 0.19$  cm and mean weight  $2.26 \pm 0.24g$  were stocked with 0.2 nos/l and fed with marigold (*Tagetes erecta*) enriched diet at 5% of body weight. Highest (P<0.05) growth was showed in cages (8.14±0.29 cm and 7.96±0.70 g.) followed by cement tanks (7.13±0.17 cm and .46 ±0.22 g) and aquaria (6.51±0.13 cm and  $5.65\pm0.14g$ ). Survival was highest in aquaria (68%) followed by cages (60%) and cement tank (59%). Fish skin colour parameters reacted differently according to culture systems. After 60 days of rearing, lightness (L\*) and whiteness (W\*) values decreased in all three systems and highest (P<0.05) was found in aquaria (L\*, 49.0. ±1.18; W\* 23.5±1.43) followed by cement tanks (L\*, 45.2. ±1.01; W\* 17.9±0.59) and cages (L\*, 41.54. ±1.31; W\* 11.50±1.04). Redness (a\*), Yellowness (b\*) and saturation (C\*) values increased in all three systems and highest (P<0.05) were in cages (a\*  $36.3\pm2.9$ ; b\*  $55.48\pm1.33$ ; c\*  $66.46\pm1.01$ ) followed by cement tanks (a\*  $31.3\pm1.11$ ; b\*  $52.40\pm1.22$ ; c\*  $61.05\pm0.87$ ) and Aquaria (a\*  $22.6\pm1.08$ ; b\*  $45.70\pm1.8$ ; c\*  $57.8\pm0.48$ ). Muscle carotenoid content were highest (P<0.05) in caged fish ( $24.80\pm1.44 \mu g/g$ ) followed by cement tanks ( $19.26\pm0.63 \mu g/g$ ) and aquaria ( $12.26\pm1.13 \mu g/g$ ). At the end of experiment, there were significant differences between aquaria, cement tanks and cages in final mean length, mean weight, percent weight gain, SGR, FCR, FER, PER, colouration and muscle carotenoid content of gold fish.

Keywords: Aquaria, Cement tanks, Cages, Gold fish, Redness, whiteness, saturation

#### Introduction

At the dawn of 21<sup>st</sup> century, fish keeping is reflected in ubiquitous aquaria that feature as an integral part of modern interior decoration (Oliver, 2001). Ornamental fish is regarded as the star product of pet market in the world today. It is a multi-million-dollar industry that supports thousands of the rural people in developing countries. Ornamental fish production is slowly gaining momentum in India and efforts are being made to increase the country's share in the global market. To develop India into a leading exporter, it is necessary to produce high quality fishes in bulk quantity, which is major requirement in the international trade. It is well known that, in addition to body shape and fin shape, attractive size and skin pigmentation are the most important quality criteria that determine the commercial value of gold fish (Paripatananont et.al 1999, Gouveia et.al 2003). Gold fish is one of the most popular ornamental fish and have a high

market value in ornamental fish trade. (Lee & Newman, 1977). Therefore culture of gold fish (*Carassius auratus*) envisages not only production but also improvement of their colour and aesthetic beauty. Fish are not able to perform de novo synthesis of carotenoids (Goodwin, 1984) and depends on dietary carotenoid content for colouration. Hence, a direct relationship between dietary carotenoid and skin pigmentation exists in them (Halten *et.al.* 1997). Besides dietary carotenoid pigments, availability of natural fish food organism, water quality developmental status of animal also influences the skin pigmentation.

Survival and growth of organisms in aquatic environment are determined to a large extent by the physical, chemical and biological properties of the water (Rogan and Cross, 1996; Diana *et.al.* 1997). Generally, ornamental fish ponds and tanks of India are subjected to a wide range of management practices, from application of organic manure (Jha *et.al.* 2004) to supplementary feeding with imported pelleted feed (Sinha and Das, 2004) and introduction of exogenous zooplankton (Jha & Bharat 2005a), for increasing yield. Good colour qualities in fish can be achieved by feeding them a high quality diet and maintain optimum water quality. Any culture system and management protocol would have a different effect on the interaction of water quality, phytoplankton & Zooplankton. This could lead to difference in survival, growth and colouration of gold fish produced in different aquaculture systems. In the present experiment, we attempted to compare growth and colouration of gold fish *Carassius auratus* reared in glass aquaria, cemented tanks and net cages suspended in pond.

#### **Material and Methods**

Experimental work was undertaken in four glass aquaria (0.45 mx 0.3m x 0.3 m; 40 l capacity), four cemented tanks (1.5m x 1.25m x 1m; 1875 l capacity) and four net cages (1m x 1m x 1m; 1000 l capacity) placed in cemented pond at the Central Institute of Fisheries Education (CIFE), Mumbai, India. Gold fish fry were collected from local ornamental fish market (Kurla, Mumbai) and transferred to ornamental fish rearing unit of CIFE, where they wee acclimated in an aerated FRP tank for 15 days prior to the study. Fish (mean length 4.1±0.19 cm and mean weight  $2.26 \pm 0.24$  g) were randomly assigned to each culture units with 0.2 nos/l stocking density, as optimized in an earlier experiment and were reared for 60 days. Feed was prepared using marigold (Tagetes erecta) petal meal as carotenoid source. The Cyanotech (2002) method was adopted to estimate the total carotenoids in marigold petal meal. Proximate composition of feed was analysed according to methods described by AOAC (1990). During the experiment, goldfish were fed twice a day (at 10 am and 5 pm ) with 5% of their body weight. Uneaten feed and faecal matter were siphoned once in two days. Dead fish were removed daily, they were not replaced during the course of study.

Water samples were collected weekly at 9 A.M. and routine water quality parameters (Temperature, dissolved oxygen, pH, total alkalinity, total hardness, ammonia, nitrite, nitrate, salinity) were estimated according to J. Andaman Sci. Assoc. 24 (2):2019

methods as described by APHA (2005). Samples of plankton were collected with plankton net made of standard bolting silk cloth (No. 21 with 77 mesh/ cm<sup>2</sup>). Collected plankton samples were concentrated to 20 ml and preserved in 4% formalin. Enumerations of plankton were performed under a stereoscopic microscope using Sedgwick Rafter Counting Cell. Primary productivity (Gross and Net) of each culture systems were measured by Light and Dark bottle method. Fortnight sampling was done for growth (length and weight) and skin colour of gold fish. The Olson (1979) method was adopted to estimate the total amount of carotenoid present in fish muscle tissue, at the beginning and end of the experiment.

Fish skin colour was measured using Lab Scan XE Colorimeter having a wavelength range from 400 - 700nm and equipped with EasyMatch @QC software to collect, display, analyze and store colour data (Made in USA), with measurements standardized with respect to the white calibration plate. The value of L\* represents lightness (0 for black and 100 for white), the a\* value represents the red/ green dimension with positive value for red, negative ones for green and 0 is neutral and the value of b\* represents the yellow/ blue dimension with positive values for yellow and negative ones for blue and 0 is neutral. A standard white tile with reflectance value of  $L^* = 95.91$ ,  $a^* = +0.09$  and  $b^* = +0.2,02$  was used as the reference. The sample is illuminated by a xenon flash lamp and reflected light is collected by a 15 - station fibre optic ring. For maximum stability, the instrument is automatically standardized to an internal reference tile whenever measurements are made. Colorimetric values of skin colour were performed on both lateral sides of each fish body. Whiteness (W\*) and saturation (C\*) values were obtained after calculation, using L\* a\* b\* values (Han et.al. 2005).

#### **Statistical Analysis**

The Statistical Analysis was carried out using PC-SAS programme for windows, release v6.12 (SAS Institute, Cary, NC, UK). Comparison among all the culture systems was done by one way ANOVA. Duncan's multiple range test (Duncan, 1955) was used to detect the significance of differences of mean between groups. The

**asa** 

level of significance employed was 0.05. The results were expressed as mean  $\pm$  standards error (Mean  $\pm$  S.E.) for the respective effect.

#### Results

#### Water quality

The physico -chemical parameters of water monitored during the experimental period of 60 days. Water temperature was observed to be in the range of 20 to 28° C. Dissolved oxygen was in the range of 3.2 to 6.5 mg l<sup>-1</sup> throughout the experiment, while pH was in the range of 7 to 8.5. Free carbon dioxide was 0 - 3 mg l<sup>-1</sup>, total hardness was in the range of 200 to 320 mg l<sup>-1</sup>. Total alkalinity was 50 to 130 mg l<sup>-1</sup>, while ammonia concentration was in the range of 0.1 to 0.35 mg l<sup>-1</sup> Nitrite and Nitrate were in the range of to and to respectively.

#### **Primary productivity**

Gross primary productivity (GPP) and Net Primary productivity (NPP) was recorded at fortnightly intervals.

Highest GPP (1560 to 2371.2 mg C/ $m^3$ /day) was observed in cages at every sampling followed by cement tanks. Aquaria showed negative NPP.

#### **Plankton analysis**

The abundance of plankton differed greatly among the three culture systems. In general, the plankton community was represented largely by Cladocerans (*Daphnia carinata*, *Moinamicrura*), copepods (*Cyclops*) and rotifers (*Brachionus spp. Keratellaspp*) and phytoplankton (*Chorella sp.*, *Microcystis sp.*, *Pediastrum sp.*,). Average plankton abundance was highest in cage followed by tanks, but it was absent in aquaria throughout the experiment.

#### Chemical analysis of feed

Eight parameters (crude protein, crude fat, total carbohydrate, crude fibre, crude ash, moisture, nitrogen free extract and gross energy) were estimated to find out the composition of the feed given to the experimental fishes. Table 1.

Crude Protein(%)	Crude Fat(%)	Total Carbohyd rate (%)	Crude fibre (%)	Crude ash (%)	Moisture (%)	NFE (%)	Energy (Kcal/kg)
32.375	6.2	54.26	10.75	7.16	6	43.515	4.16
31.5	6.2	53.34	9.85	8.96	7	43.49	4.17
33.25	5.2	51.7	10.2	9.85	7	41.5	4.15
32.37±0.50*	5.86±0.33*	53.10±0.74*	10.26±0.26*	8.65±0.79*	6.6±0.33*	42.83±0.66*	4.16±0.00*

#### Table 1: Chemical analysis of feed used during the experiment

N.B. \* indicates (Mean  $\pm$ S.E.)

#### **Growth parameters**

Mean length was  $6.51 \pm 0.13$  cm in aquaria,  $7.13\pm0.17$  cm in cement tanks and  $8.14\pm0.29$  cm in cages (Table 5, Fig. 1) & Mean weight was  $5.65 \pm 0.14g$  in aquaria  $6.46 \pm 0.22$  g, in cement tanks and  $7.96 \pm 0.70$  g in cage. Highest average daily growth (ADG) was recorded in cages ( $0.094\pm 0.002$  g) followed by cement tank ( $0.074\pm0.002$  g). Lowest ADG was observed in aquaria ( $0.056\pm0.003$  g). Highest percent weight gain (PWG) was recorded in cages ( $278.17 \pm 2.54$ ) followed by cement

tanks (203.93±0.35) and aquaria (159.12±0.07). Highest specific growth rate (SGR) was recorded in cage (21.60 ±0.37), followed by cement tanks (18.34 ± 0.18 and aquaria (15.55 ± 0.22). Feed Conversion Ratio (FCR) of *Carassius auratus* in different culture systems ranged from 3.1 ±0.05 (aquaria) to  $2.33 \pm 0.02$  (cages). Moreover FCR of cement tanks (2.73± 0.06 is acceptable as per the commercial culture aspects. The feed efficiency ratio (FER) values of *Carassius auratus* in different culture system lies between 0.30 ± 0.00(aquaria) to 0.41 ± 0.00 (cages). However FER of fishes of cement tanks was

 $0.35\pm 0.02$ . FER of Cage ( $0.17\pm 0.01$ ) culture system was aquaria ( $0.17\pm 0.01$ ). All the growth parameters are given in Table 2.

Culture system	ADG	PWG	SGR	FCR	FER	PER
AQ	0.056±0.003ª	159.12±0.07ª	15.55±0.22ª	3.1±0.05ª	0.30±0.00ª	0.10±0.00ª
СТ	$0.074{\pm}0.002^{b}$	203.93±0.35 <sup>b</sup>	18.34±0.18 <sup>b</sup>	$2.73 \pm 0.00^{b}$	$0.35 {\pm} 0.02^{b}$	0.13±0.02 <sup>b</sup>
CG	0.094±0.002°	278.17±2.54°	21.60±0.37°	2.33±0.02°	0.41±0.00°	0.17±0.01°

#### **Table 2: Growth parameters**

Means with different superscripts in the same column are significantly different (P < 0.05).

#### Survival rate

During the course of experiment, survival rate of *Carassius auratus* was observed and at the end of 60 days survival percentage was calculated. Highest survival was achieved in aquaria (68%) followed by cages (60%) and cement tanks (59%).

#### **Colour analysis**

#### Lightness (L\*)

The value of skin colour of *Carassius auratus* was obtained from the instrument directly and the result are shown in the Table 3.The better L\* was found in cages  $(41.54\pm1.3)$  followed by cement tanks  $(45.21\pm1.01)$  and aquaria  $(49.0\pm1.18)$ . There was significant difference of L\* value among culture systems.

#### Redness (a\*)

The Redness value of experimental fish obtained from the instrument for three production system is shown in the Table 3. The highest a\* value was found in cages  $(36.29\pm1.07)$  and lowest in aquaria  $(22.6\pm1.08)$ .. There was significant difference of a\* value among the three culture systems.

#### Yellowness (b\*)

The Yellowness value of skin colour of experimental fish species was obtained from the instrument directly and is given in the Table 3. Fishes of cages showed maximum yellowness ( $55.48\pm1.33$ ) in contrast to minimum seen from aquaria ( $45.7\pm1.8$ ). There was significant difference of b\* value among culture systems.

### Table 3. Fortnight colour analysis of Carassius auratus in aquaria, Cement tank and cages for60 days (Mean ±S.E.)

Samp- ling		L* (Lightnes	ss)		a* (Redness	))	ł	o*(Yellowne	ss)		* (Whitenes (100-L*) <sup>2</sup> + :		(Sat	C* ıration = √(a	* <sup>2+</sup> a* <sup>2</sup> )
	AQ	СТ	CG	AQ	СТ	CG	AQ	СТ	CG	AQ	СТ	CG	AQ	СТ	CG
1	55.1±0.79	55.1±0.79	55.1±0.79	18.2±0.68	18.2±0.68	18.2±0.68	37.6±0.83	37.6±0.83	37.6±0.83	41.4±0.76	41.4±0.76	41.4±0.76	41.3±0.76	41.3±0.76	41.3±0.76
15	54.0±1.00ª	54.0±1.89 <sup>b</sup>	52.5±1.56°	20.8±1.23ª	22.8±1.08 <sup>b</sup>	25.10±1.00°	43.0±1.58ª	43.9±2.33b	45.79±1.51°	35.7±1.00ª	32.1±0.95 <sup>b</sup>	29.50±0.59°	47.7±1.00ª	49.6±2.54 <sup>b</sup>	59.0±2.34°
30	52.3±0.61ª	51.3±0.87 <sup>b</sup>	50.1±0.82°	22.7±0.6ª	26.9±8.86 <sup>b</sup>	28.18±0.77°	45.0±0.86ª	47.8±0.8 <sup>b</sup>	48.88±1.03°	30.5±0.12ª	26.6±0.77 <sup>b</sup>	24.68±0.60°	50.4±2.22ª	54.8±0.86 <sup>b</sup>	56.41±0.56°
45	49.3±1.11ª	47.7±0.64 <sup>b</sup>	46.69±0.54°	25.2±0.74ª	28.4±0.96 <sup>b</sup>	31.94±0.9°	46.1±1.14ª	49.7±0.8 <sup>b</sup>	52.69±0.84°	26.9±0.58ª	22.4±0.12 <sup>b</sup>	18.52±0.58°	52.6±1.11ª	57.24±0.56 <sup>b</sup>	61.61±0.41°
60	49.0±1.18ª	45.2±1.01 <sup>b</sup>	41.54±1.31°	22.6±1.08ª	31.3±1.11 <sup>b</sup>	36.29±1.07°	45.7±1.8ª	52.4±1.22b	55.48±1.33°	23.5±1.43ª	17.9±0.59 <sup>b</sup>	11.50±1.09°	57.8±0.48ª	61.05±0.87 <sup>b</sup>	66.46±1.01°

#### Whiteness (W\*)

Whiteness value of *Carassius auratus* obtained after calculation are shown in the Table 3.W\* value

found in aquaria  $(23.54\pm1.43)$  was higher than cement tanks $(17.96\pm0.59)$  and cages  $(11.59\pm1.09)$  and significant difference was found among culture systems.

The saturation value of skin colour of experimental fishes was obtained by using formula are given in the Table 3. The Saturation value of redness and yellowness constituted the leqast in aquaria ( $57.80\pm0.08$ ), medium in cement tanks( $61.05\pm0.87$ ) and highest in cage culture system ( $66.46\pm1.01$ .

#### Carotenoid content of Marigold and fish muscle

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Carotenoid content of Marigold was  $3.34 \pm 0.45$ . Initial carotenoid content of gold fish muscle was  $2.13 \pm 0.35 \ \mu g/g$ . After 60 days of rearing it was  $12.26\pm1.13 \ \mu g/g$  in aquaria reared fish,  $19.26\pm0.63 \ \mu g/g$  in cement tanks reared fish and  $24.80\pm1.44 \ \mu g/g$  in caged fish. The carotenoid values obtained are given in Table No. 4

Culture sustance	Mariaald (0/)	Fish muscle (μg/g)				
Culture systems	Marigold (%)	Initial (0 days)	Final (60 days)			
AQ		2.13±0.35	12.26±1.13ª			
СТ	3.34±0.45	2.13±0.35	19.26±0.63 <sup>b</sup>			
CG		2.13±0.35	24.80±1.44°			

#### Table 4: Carotenoid content ( $\mu$ g/g) of marigold petal and fish muscle

Means with different superscripts in the same column are significantly different (p<0.05)

#### Discussion

Nothing influences health and well being of fish as much as water quality. The explanation for this is sample, fish metabolism and biological functions are directly linked to the physical, chemical and biological properties of water. Ornamental fish are highly adaptable to culture conditions and are capable of living under a wider range of environmental condition (Chapman, 2000). Throughout the present experiment, water quality parameters of all culture systems were within the optimum level of gold fish production (Jhingram, 1991), which indicated that water quality did not produce any stress to the fish during the experiment.

In the present study, pond-based cage culture system showed higher length increment and weight gain of *Carassius auratus* compared to cement tank and glass aquaria confirming that different culture systems affect the growth and survival of fish. In the beginning of the experiment, all the culture systems had more or less similar mean length and weight. After 15 days of rearing, all the three systems recorded length increment and weight gain, this continued till end of the experiment. The higher growth rate has been observed in cages, possibly due to natural feed and supplementary feed. Studies with other fish species have yielded similar results; for example, significantly lower growth rate of channel catfish, *Ictalurus punctatus* were obtained in concrete pools compared to earthen ponds (Shell, 1996). Even in cases of walleye, *Stizostedionvitreum*, younger fry (upto 6.5 cm) are usually cultured in ponds as the growth rate is better compared to tanks (Summerfelt *et.al.* 1996). Other studies also revealed that live food alone or live food mixed with artificial food improves fish production (production (Dabrowski*et.al* 1983; Lubzens*et.al*, 1984; Abi-Ayad and Kestemont, 1994). Mitra & Mahapatra (1056) and Jhingran& Pullin (1985) have attested the importance of zooplankton in carp nursery ponds of India.

Growth observed in aquaria and cemented tanks may be due to growth stimulatory effect of Marigold. The addition of carotenoid rich micro algae *Haematococcus pluvialis* has been found to enhance the growth of rainbow trout, *Onchorhynchus mykiss* (Sommer et.al. 1992). A higher growth rate was achieved in red swordtail by feeding with 8% Spirulina in the diet (James et.al. 2006). Further, there are also reports (Torrissen, 1984) that link carotenoids to growth enhancement in Atlantic salmon fry (*Salmo salar*), or to improvement of survival rate in kuruma prawn, *P. japonicas* (Chien & Jeng, 1992).

The present study resulted nearly similar rates of survival of *Carassius auratus*in cemented tanks (59%0

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and cages (60%) as compared to aquaria (68%). In this case, mortality in tanks and cages were probably associated to handling during harvesting. High survival in aquaria may be due to good maintenance in controlled condition.

Lightness (L\*) and Whiteness (W\*) values of gold fish (C. auratus) were decreasing in fishes of each culture systems after every sampling. At the end of rearing period, these values were significantly (p<0.05) decreased and the lowest value was observed in fish reared in cages. As the time progressed, redness (a\*), yellowness (b\*) and saturation (C\*) values of fishes were significantly increased in all the culture systems. The highest value was found in cages followed by cement tanks and aquaria. This is supported by findings of Yanar et.al. (2010) for gold fish and electric yellow cichlid. They found that a higher red (a\*) and yellow (b\*) colouration and a lower lightness (L\*) were obtained on skin of gold fish reared in fibre glass aquarium compared to those in glass one. During rearing period, the fish may obtain additional red and vellow pigment from consuming natural foods present in the pond water and cement tanks. The aquaria water was devoid of natural food, this may be the reason for low skin pigmentation aquaria reared fish. The pigmentation obtained in aquaria may be due to carotenoids of feed only. Feeding diets containing natural carotenoid sources such as paprika, marigold, Spirulina, shrimp shell meal, micro-algae and china rose was reported to improve skin pigmentation of several fish species including Koi carp, gold fish, red porgy (Gouveia et.al. 2003; Hancz et.al. 2003; Kalinowski et.al. 2007; Sinha and Asimi, 2007).

Analysis of carotenoid content in Marigold (*Tagetes erecta*) petal meal showed  $3.34 \pm 0.45$  % in dry weight. Ramamoorty *et.al.* (2010) reported that the carotenoid pigment in marigold was 2.30% in his experiment. These differences might be due to the origin of the marigold flower since differences in pigment composition have been observed even among closely related species cultivated in conditions absolutely identical.

Muscle carotenoid content of gold fish was increased in three culture systems after 60 days of rearing. Highest carotenoid was found in cages followed by cement tanks (less live food density) and aquaria (devoid of live food). The result in cages may be due to live food present in pond water (where cage was installed and carotenoid content of marigold. The result in aquaria and cement tanks may be dur to marigold only. Ezhil *et.al.* (2008) studied the total carotenoids in red swordtail colour enhancer at cheaper price. Sommer et. al. (1992) observed that the pigment deposition in muscle of rainbow trout, *Oncorhynchus mykiss* increased with increase in concentration of green algae, *Haematococcus pluvialis* in the feed. The total carotenoid content in the muscle of red sword tail, Xiphophorus helleri increased when fed with 8% Spirulina in their diet (James *et.al.* 2006).

#### Conclusion

From the experimental results, cage culture system appeared to be better alternative to cement tank and glass aquarium due to their higher assimilatory capacity and greater abundance of plankton in natural environment. Further research in this aspect might bring still better improvement in survival rate, growth and colouration.

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## Groupers Resources (Actinopterygii:Perciformes: Epinephelidae) of Andaman and Nicobar Islands, With Two New Records

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#### Abstract

Revision of the grouper fishes from the Andaman and Nicobar Islands is presented in this paper based on the collections housed in the Zoological Survey of India, Port Blair. Groupers belong to the family Epinephelidae comprising of about 163 species in 16 genera in the world, of which 58 species and 8 genera are known from the Andaman and Nicobar Islands. The family Epinehelidae comprises 8 genera including *Aethaloperca, Anyperodon, Cephalopholis, Cromileptes, Epinephelus, Hyporthodus, Plectropomus* and *Variola*. Grouper species are identified by their colour pattern as well as morpho-meristic characters such as size of the fins, shape and relative size of the head, number of fin rays, scales, gill rakers and body shape. In addition, the pyloric caeca counts and pattern have also been used for identification of species. The use of molecular methods such as DNA fingerprinting and more recently bar-coding has played a major role in resolving taxonomic ambiguity in groupers. The four species *Cromileptes altivelis, Epinephelus lanceolatus, Plectropomus laevis* and *Plectropomus areolatus* are currently classified as Vulnerable according to the IUCN Red List and only bony fish, the giant grouper *Epinephelus lanceolatus*, is protected under the Indian Wildlife Act of 1972. The present study provides two new record *Epinephelus polyspila* Randall and Satapoomin, *Epinephelus poecilonotus* (Temminck and Schlegel) and essential background information for the formulation of a Grouper Management Plan, because there is concern that grouper stocks are being overfished.

Key words: Review, Groupers, conservation, management, Andaman and Nicobar

#### Introduction

The Andaman and Nicobar Islands are situated in the Bay of Bengal between  $6^{\circ}45' \cdot 13^{\circ} 45'$  N and  $92^{\circ}10' - 94^{\circ}15'E$ , and consist of 352 islands 220 islets and rock. Theycover a distance of almost 470 square kilometres over North South, with a coastline of 1962 km, and represent 600,000 km<sup>2</sup> of India's Exclusive Economic Zone (EEZ). The coast is under the influence of a diverse set of oceanographic and ecological conditions. The shelf topography of these islands contains frequent rises supporting coral reefs, which are characterized as fringing reefs on the eastern side and barrier reefs off west; the depressions are known as passages and straits. Besides coral reefs, the shore is composed of rocky and sandy areas and vast stretches of Mangrove swamps with a few freshwater rivers and streams.

The family Epinephelidae currently comprises about 163 species in 16 genera in the world (Eschmeyer and Fong, 2020). They are commonly known as groupers, rockcods and hinds (Heemstra and Randall, 1993). The Epinephelidaeare further classified into 4 sub families, namely Diploprioninae, Epinephelinae, Liopropominae and Grammistinae (Craig, M.T., Y. Sadovy de Mitcheson & P.C. Heemstra 2011). Representatives of 8 genera comprising of 58 species Aethaloperca rogaa (Forsskål), Anyperodon leucogrammicus (Valenciennes), Cephalopholis argus Schneider, Cephalopholis aurantia (Valenciennes), Cephalopholis boenak (Bloch), Cephalopholi scyanostigma (Valenciennes), Cephalopholis formosa (Shaw & Nodder), Cephalopholis leopardus (Lacepède), Cephalopholis microprion (Bleeker), Cephalopholis miniata (Forsskål), Cephalopholis nigripinnis (Valenciennes), Cephalopholissex maculata (Rüppell), Cephalopholis sonnerati (Valenciennes), Cromileptes altivelis (Valenciennes), Epinephelus amblycephalus (Bleeker), Epinephelus areolatus (Forsskål), Epinephelus bleekeri (Vaillant), Epinephelus caeruleopunctatus (Bloch), Epinephelus chlorostigma (Valenciennes), Epinephelus coioides (Hamilton), Epinephelus corallicola (Valenciennes), *Epinephelus epistictus* (Temminck and Schlegel), Epinephelus erythrurus (Valenciennes), Epinephelus fasciatus (Forsskål), Epinephelus faveatus (Valenciennes), Epinephelus flavocaeruleus (Lacepède), Epinephelus fuscoguttatus (Forsskål), Epinephelus heniochus Fowler, Epinephelus hexagonatus (Bloch & Schneider), Epinephelus lanceolatus (Bloch), Epinephelus longispinis (Kner), Epinephelus macrospilos (Bleeker), Epinephelus malabaricus (Bloch & Schneider). Epinephelus melanostigma Schultz, Epinephelus merra Bloch, Epinephelus morrhua (Valenciennes) Epinephelus miliaris (Valenciennes), Epinephelus ongus (Bloch), Epinephelus poecilonotus (Temminck and Schlegel), Epinephelus polyphekadion (Bleeker), Epinephelus polyspilaRandallandSatapoomin,Epinepheluspolystigma quovanus (Bleeker), Epinephelus (Valenciennes), Epinephelus radiatus (Day), Epinephelus retouti Bleeker 1868, Epinephelussex fasciatus (Valenciennes), Epinephelus spilotoceps Schultz, Epinephelus tauvina (Forsskål), Epinephelus tukula Morgans, Epinephelus undulosus (Quoy&Gaimard), Hyporthodus octofasciatus (Griffin), Gracila albomarginata (Fowler & Bean), Plectropomus areolatus (Rüppell), Plectropomus laevis (Lacepède), Plectropomus maculatus (Bloch), Hyporthodus octofasciatus (Griffen 1926), Plectropomus pessuliferus (Fowler), Variola albimarginata Baissac and Variolalouti (Forsskål) are reported to be present in Andaman and Nicobar Islands (Rajan, 2002, 2003 and 2015, Rajan et al. 2013). Groupers are generally identified using a combination of their morphological features and meristic characters as reported in the FAO catalogue on groupers (Heemstra and Randall, 1991, 1993 and Matthew Craig 2011). This is because several species of groupers resemble each other in colour pattern and appearance, which often leads to misidentification.

Some species occur in depths of 100 to 200 m (occasionally to 500 m), however the majority inhabit depths of less than 100 m, and juveniles are often found in tide pools. Most groupers are ambush predators, hiding amongst the coral and rocks. Groupers are popular marine food fish of high market value in many parts of the world and many are currently being exported in whole

frozen form. Groupers constitute the main focus of the commercial fisheries and an important component of the catch in Andaman and Nicobar Islands. The buying price of a fish varies with size and species. The price of per kilogram may vary from Rupees 800 to 1000 depending upon the buying category used by the buyer. A common practice is to divide the groupers into red and black categories with [red/black] being more highly valued. The fresh chilled fish are packed in thermo cool boxes with ice and are exported daily by air. Chennai is the main grouper export market. Lack of data makes it nearly impossible to compare the catch from different years and to show the true trend in the grouper fishery.

There is a dedicated fishery for "perches" along the coast of Andaman and Nicobar Islands using hook and line, gill nets and traps which includes a targeted fishery for groupers. This fishery targets habitats that are generally hard, covered with dense growth of coral, rocks and sea fans, and rich in groupers.

#### Materials and methods

Groupers were collected from the fish landing centre of Andaman and Nicobar Islands as well as in the field with the help of SCUBA. The fresh fish samples were photographed. Besides the morphological features like colour pattern, shape and relative size of the head and body, data such as the total length, standard length, total body weight, body depth, and number of spines and rays were also recorded.

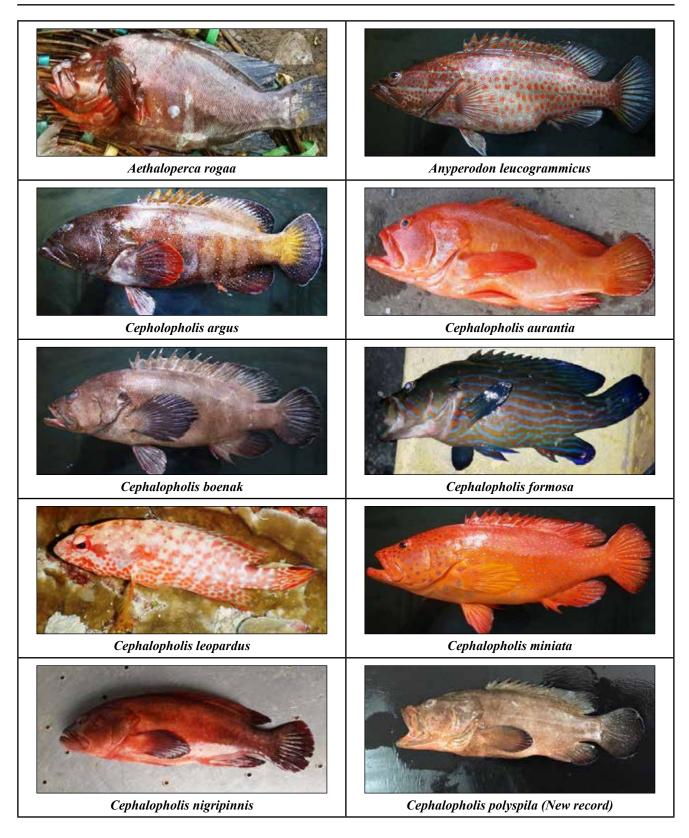
#### **Results and discussion**

Traditional approaches to species identification in groupers is often difficult due to the presence of several colour morphs within a single species and a wide variation in the colour pattern between juveniles and adults and live and dead individuals of the same species. Under mentioned species are often confused but can be identified reliably from external morphological characters such as *Epinephelus coioides, E. tauvina* and *E. malabaricus* (Heemstra and Randall, 1993). Supportive techniques are needed to confirm the taxonomic status of groupers, which are very important both from fisheries and aquaculture points of view.

Rajan

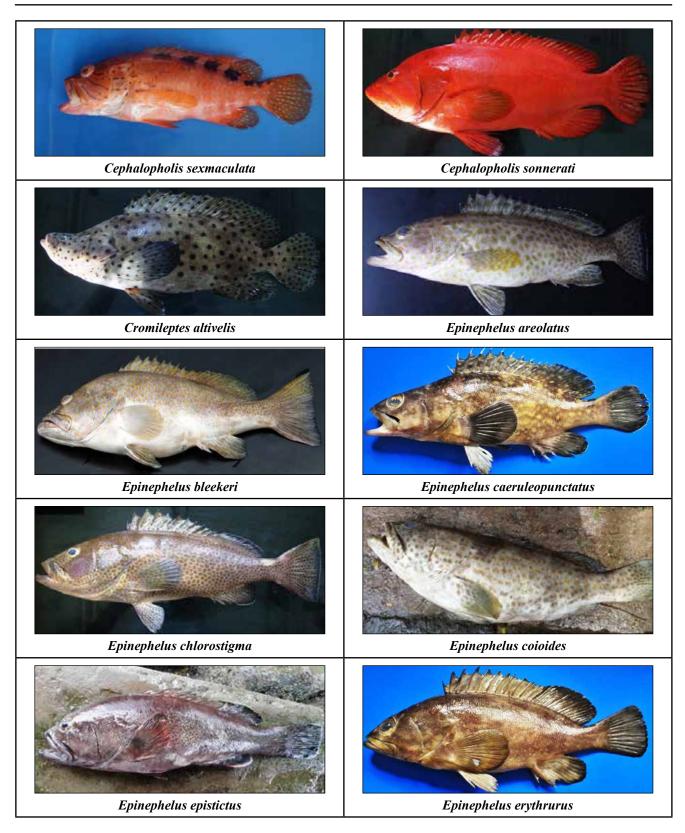
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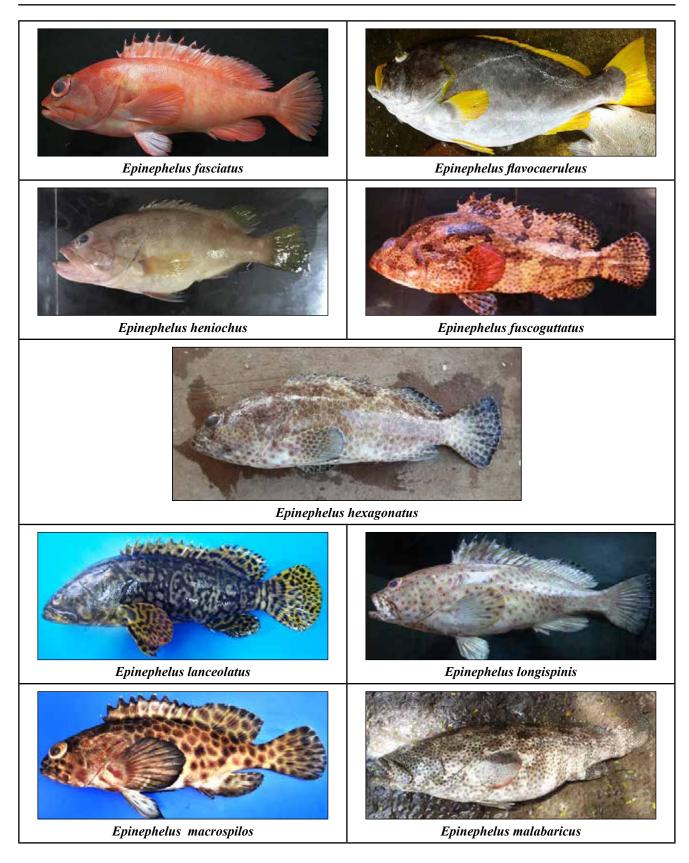


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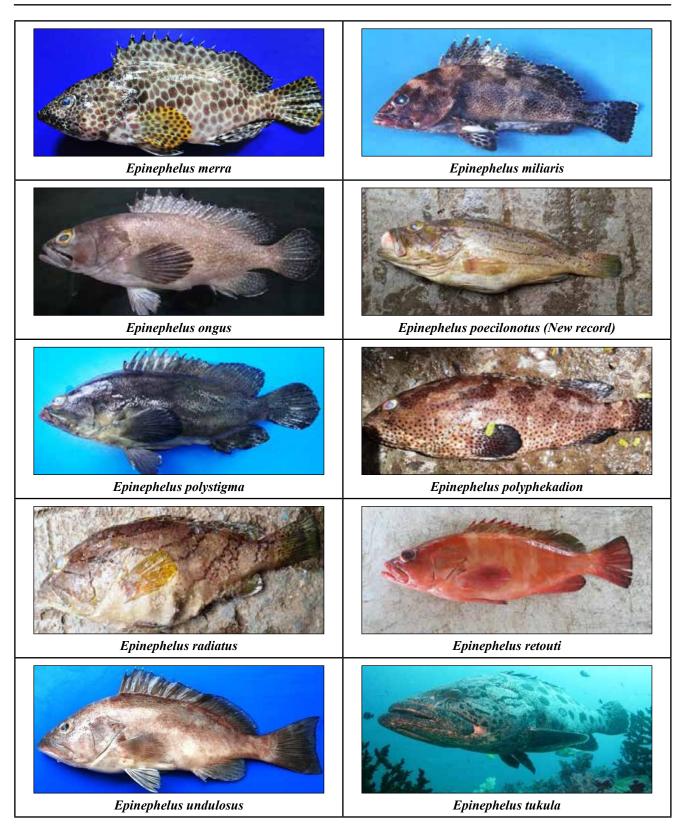


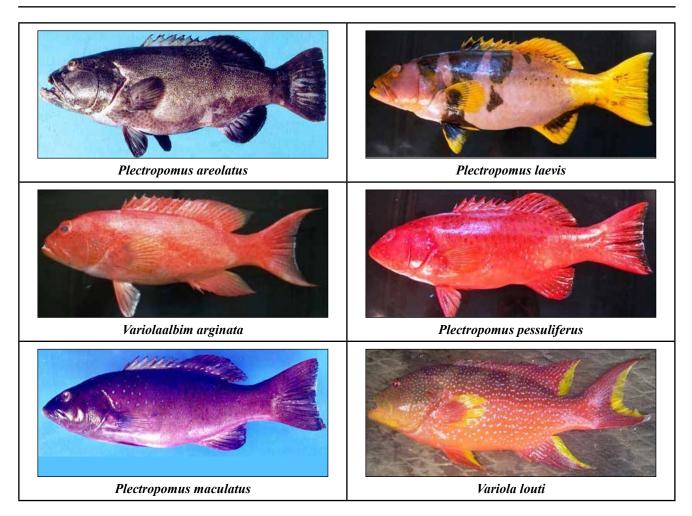




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All the species of the genus Epinephelus and Hypothrodus have 10-11 spines and 14 - 18 rays on the dorsal fin. The anal fin has 3 spines and 7 - 10 rays; genus Cephalopholis has 9 spines and 13 - 17 rays on the dorsal fin (Table1). Epinephelus malabaricus has been confused with E. coioides. These two groupers are best differentiated by colour. E. malabaricus has wellspaced small black spots on the head, body and fins and scattered larger whitish spots and blotches on the head and body, whereas E. coioides has larger orange -brown spots and lacks whitish spots. Faded museum specimens of the two species may be difficult to differentiate. E. malabaricus occurs on protected reefs and adjacent mangrove habitats, common in the catches of these islands. Plectrpomus laevis has two colour phases, one whitish or pale yellowish with 5 dark brown to black saddle-like bars with scattered small dark-edged blue

spots. The second phase is brown, olivaceous, red with pale bars and numerous small dark-edged blue spots.

The large sized commercially important groupers, *Epinephelus fuscoguttatus, E. malabaricus* and *E. coioides* were present in these islands. The smaller species like the reticulated groupers *E. macrospilos* and *E. merra* were abundant in the catch. The size range of the species collected indicated that mostly juveniles were collected in the case of the larger species whereas almost all size classes of the smaller species of groupers have been reported from Andaman and Nicobar Islands, of which *E. areolatus, E. malabaricus* and *Plectropomus spp.* were dominant and the same species were also dominant in this study. Many species of groupers resemble each other morphologically and in certain species even the meristic characters have been found to overlap. This makes identification a difficult

task. Groupers have been misidentified in early literature (Heemstra and Randall, 1993). Total dependence on the colour pattern for identification could be the major reason for this misidentification. This could be the reason for the mentioning species like *E. tauvina*in earlier records. The species were identified using their colour pattern as well as meristic characters as suggested by Heemstra and Randall (1993). It was found that 58 species of groupers belonging to the 8 genera *Aethaloperca, Anyperodon, Cephalopholis, Cromileptes, Epinephelus, Hyporthodus, Plectropomus* and *Variola*were available in these waters, *E. malabaricus, E. coioides, E. polyphekadion, E. merra, E. longispinis, E. coeruleopunctatus* d. *formosa. E. malabaricus* were found to be the dominant species off the coast of Andaman and Nicobar Islands.

#### **Conservation and Management**

Export of grouper fishery in Andaman Islands started in the year 1996 and it was initially started in Havelock Islands, with realization of the income potential the fishery expanded throughout Andaman and Nicobar Islands. At present the grouper fishery is mainly carried out by the fishermen of Gutapara, Wandoor of South Andaman, now it is expanded to middle and north Andaman Islands. In general these fishermen visit almost all areas of Andaman Islands for grouper fishing. Catches of groupers rose from 202t in 1997 to 750t in 2015. Ready cash for the sale of grouper provide an incentive to fishermen, which promoted and encouraged fishing without much care about the overfishing and stock collapse. This site specificity and the relatively slow growth rate of groupers make them particularly vulnerable to over-fishing. In addition, some groupers use localized spawning sites to which they migrate from distances of several kilometres; and these sites are often exploited by local fishermen who catch large numbers of fishes during the brief spawning period of 1 or 2 weeks.

This removal of a considerable number of reproductively active fish from the population may be detrimental to sustained yields of the fishery. No regulation in catch is responsible for serious depletion of populations of this grouper species particularly *Epinephelus lanceolatus*. In recognition of this problem the collection

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of this species is totally banned and is protected under the Indian Wildlife Act, 1972. Species such *Cromileptes altivelis, Epinephelus fuscoguttatus, E.lanceolatus, Plectropomus laevis, P.areolatus* and *P.pessuliferus* have always been favourites due to their high market value. Unfortunately these are species which are listed in the IUCN Red List as either "*Near threatened*" or "*Vulnerable*" thus stressing the vulnerability of groupers to intense fishing effort. When a fishery targets a single species, it is possible to overfish the species quickly in the locality where the fishery has started first.

An extremely important factor affecting the success of a size limit in increasing yields is the degree of survival of undersized fish that are taken and released. For commercial use, the removal of groupers less than average maturity length of 12 inches from the medium size grouper and 16 inches from the large size grouper can be prohibited. The current level of catch and effort data collection is not adequate for monitoring and assessment requirements of the resource. Exporters and buyers could be encouraged to submit their daily purchase sheets on monthly basis. The most commonly caught species are Aethaloperca rogaa, Anyperodon leucogrammicus, Cephalopholis argus C. miniata C. sonnerati, Epinephelus areolatus, E. coioides, E. flavocaeruleus, E. longispinis, Е. malabaricus, E. polyphekadion, Plectropomus areolatus, P. laevis, P. maculatus, P. pessuliferus, Variola albimarginata and V.Louti, it is suggested that these species be used as the indicator species. However, species such as E. fuscoguttatus, V. loutiand C. argus were seen to contribute more towards the overall catch composition. On the other hand, P. pessuliferus, which now has a higher value are caught in greater quantities and contribute highest to the current catch composition. Cromileptes altivelis is very rare species and it should be protected. More comprehensive surveying is needed to provide a more accurate estimate. However, these results do provide a good benchmark for management.

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# Phytochemical, Antioxidant and Antibacterial Activity of *Chromolaena odorata* from Andaman Islands, India

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#### Abstract

The present study was aimed to evaluate the phytochemical, antioxidant and antibacterial activity of *Chromolaena* odorata (Eupatorium odoratum). In-vitro phytochemical and antioxidant properties of methanolic extract of *C. odorata* exhibited the maximum amount of total phenol ( $39.72 \pm 5.25 \text{ mg GAE/g}$ ), flavonoids ( $100.75 \pm 1.75 \text{ mg RE/g}$ ), DPPH radical scavenging activity (82.83 %), ABTS (23.18 mg/g) and total antioxidant activity (75.42 mg/g). Besides, the methanol and DMSO dissolved extracts of *C. odorata* revealed the considerable amount of *in-vitro* antibacterial activity against almost all the seven tested fish and human pathogenic bacteria. The highest amount of antibacterial activity with zone of inhibition of 21 mm was recorded against *Aeromonas hydrophila* and *Pseudomonas fluorescens*. The present study showed that the presence of potential active constituents in *C. odorata* may assist in combating the free radicals and also inhibits the growth of microbes.

Keywords: Chromolaena odorata, Eupatorium odoratum, phytochemical, antioxidant, antibacterial, Andaman Islands

#### Introduction

Chromolaena odorata (synonyms to Eupatorium odoratum) is a well known traditional medicinal herb belonging to the Family Asteraceae. Commonly called as siam weed, devil weed, French weed, Christmas bush, bitter bush, baby tea, Santa Maria and common floss flower (Prawiradiputra, 2007; Patel et al. 2010; Chakraborty et al. 2011; Vaisakh and Pandey, 2012). Even though it is an invasive species, possess various useful attributes such as antibacterial (Lavanya and Brahmaprakash, 2011; Ravikumar et al. 2011), anti-inflammatory (Owoyele et al. 2005; Avyanar and Ignacimuthu, 2009; Vaisakh and Pandey, 2012), anti-malarial (Pisutthanan et al. 2005; Doss et al. 2011), anti-viral (Pisutthanan et al. 2005), antihepatotoxic (Alisi et al. 2011; Asomugha et al. 2014), anthelmintic (Vital and Rivera, 2009) and antioxidant (Venkata Raman et al. 2012) properties. It is also reported that the bioactive compounds present in C. odorata has the ability treat a variety of diseases (Vital and Rivera, 2009; Harlina et al. 2013).

In Andaman and Nicobar Islands, C. odorata is abundant and also used to cure various ailments by the

local population including the indigenous tribes. The leaves of C. odorata are widely used by the indigenous tribes such as Nicobarese and Onges and they call it as 'Ureh bes' in Nicobari language which is used to treat against skin injuries, body ache, piles, centipede and snake bites (Chander et al. 2014; Chander and Vijayachari, 2018). Karen tribes call it as 'Sawpokwela' in their language and uses the leaves to cure fever, cough, skin injuries and breathing problems (Chander et al. 2015). In the wake of emerging antibiotic resistance and its subsequent transmission into food chain, the use of broad spectrum natural bioactive compounds without harmful side effects are highly encouraged for human and animal health management in the recent years. Studies on the phytochemical, antioxidant and antibacterial activity of C. odorata from Andaman and Nicobar Islands are scanty except the preliminary reports on its antimicrobial effect (Jai Sunder et al. 2012; Natheer et al. 2012). With this background, the present study was aimed at evaluation of phytochemical, antioxidant and antibacterial activity of C. odorata against fish and human pathogenic bacteria.

#### Materials and methods

#### (A). In-vitro phytochemical and antioxidant activity

#### Collection and preparation of sample

Fresh leaf samples of *C. odorata* were collected from ICAR-Central Island Agricultural Research Institute, South Andaman and shade dried at room temperature for 3-4 days. The dried leaves were homogenized with pestle and mortar and stored at 4 °C for further analysis.

#### Solvent extraction

One gram of *C. odorata* leaf powder was dissolved in 50 ml of 80 % methanol and incubated for 24 hours at room temperature. After incubation, the sample was centrifuged for 10 minutes and the supernatant was filtered into a fresh tube using Whattman no. 40 filter paper. The final crude methanol extract was used for phytochemical and antioxidant analysis.

#### **Total phenolic content**

Total phenolic content of the crude methanolic extract was determined using Folin-Ciocalteau (FC) assay as described by Singleton and Rossi (1965) with minor modification and the final results were expressed as Gallic acid equivalents (GAE)/ g of sample.

#### **Total flavonoids**

Total flavonoids content of the crude methanol extract was determined by spectrophotometric method of Zishen *et al.* (1999) with slight modification and the flavonoid content was expressed as mg Rutin equivalents per gram of dried extract (mg RE/ g).

#### Antioxidant assays

Crude methanolic extract of *C. odorata* was tested for its antioxidant activity. Different concentration of the extracted test samples (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ ml) were used for the assay. Standards were also taken in their respective concentrations.

## DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity

The scavenging effect of crude methanol extract was determined according to the method of Farasat *et al.* (2013) with minor modification. The half-maximal inhibitory concentration (IC50) was calculated by the linear regression analysis and expressed as mean of determinations. Ascorbic acid was used as positive control.

## ABTS (2, 2-azinobiz-3-ethylbenthiazoline-6sulfonic acid) radical cation scavenging assay

Free radical scavenging activity of the methanolic extract was determined by following the method of Seenivasan *et al.* (2013) with minor modification. Trolox was used as standard.

#### Total antioxidant activity (TAA)

Total antioxidant activity of the crude methanolic extract obtained from *C. odorata* was determined according to Prieto *et al.* (1999) with slight modification. Total antioxidant activity was expressed as the number of equivalents of ascorbic acid in milligram per gram of extract.

#### (B). In-vitro antibacterial activity

#### **Preparation of extracts**

In order to investigate *in-vitro* antibacterial activity, one gram of dried leaf powder of *C. odorata* was dissolved in 25 ml of 80 % methanol and kept for overnight incubation in an orbital shaking incubator. The sample was centrifuged at 4500 rpm for 10 minutes and then the supernatant was filtered with Whattman no. 1 filter paper. The filtrate was evaporated at 50 °C in hot air oven for complete evaporation of the solvent and based on the yield; the extract was diluted in 80 % methanol and DMSO separately, to obtain a concentration of 10 mg/ ml. The final solution was tested for its antibacterial activity against the selected bacteria.

## Preparation of inoculum and plates for *in-vitro* antibacterial activity

Pure cultures of Aeromonas hydrophilla (ATCC 35654). Staphylococcus aureus (ATCC 25923). Escherichia coli (ATCC 4157), Streptococcus pneumonia (ATCC 49619), Vibrio alginolyticus (ATCC 17749), Edwardsiella tarda (ATCC 15947) and Pseudomonas fluorescens (ATCC 13525) were procured from Microbiologics, USA. The *in-vitro* antibacterial activity was evaluated by agar well diffusion method (Holder and Boyce, 1994). Bacterial strains were inoculated in nutrient broth and incubated at 37° C for 18-24 hours. Mueller Hinton agar plates were prepared and 50 µl of respective bacterial culture (107 cfu/ml) was evenly spread throughout the plate using sterile glass L-rod spreader. Wells were made in each agar plate by using a sterile cork borer (diameter of 5.5 mm). Both methanol and DMSO dissolved extracts (50 µl) were loaded separately into the wells in duplicate. Fifty µl of streptomycin (10 mg/ ml) was used as positive control and the respective solvent alone of same volume was used as negative control. The plates were incubated at 37 °C and the zone of inhibition was measured after 24 hrs of incubation.

#### Statistical analysis

All the data were expressed as mean  $\pm$  standard error in triplicates and the statistical analysis was executed using SPSS 16.0 software. One-way ANOVA and Duncan's multiple range tests were applied to check the significant difference among the mean and P value < 0.05 was regarded as significant.

#### **Results and discussion**

Andaman and Nicobar Islands are known for considerable amount of floral diversity and *Chromolaena odorata* is one among the species with ethno medicinal value and widely used to cure various ailments by the indigenous tribes (Chander and Vijayachari, 2018). In the present study, the leaves of *C. odorata* was selected for the investigation as it provides significantly higher percentage of yields than its other plant parts (Hanphakphoom *et al.* 2016). Methanol was employed for extraction as it is a high polar organic solvent reported to provide high extraction yield and high content of phytochemicals from the leaves of *C. odorata* (Stanley *et al.* 2014; Hanphakphoom *et al.* 2016).

The in-vitro phytochemical and antioxidant properties of C. odorata are provided in table 1, which depicts the total phenol  $(39.72 \pm 5.25 \text{ mg GAE/g})$ , flavonoids (100.75 mg GAE/g) $\pm$  1.75 mg RE/ g), DPPH radical scavenging activity (82.83 %), ABTS (23.18 mg/g) and total antioxidant activity (75.42 mg/g). The total phenol and flavonoid content obtained in the present study is much higher than the earlier study where the phenol content was reported to be in the range of 1.54 to 3.42 mg GAE/ g and flavonoid content to be 2.52 to 9.85 mg RE/ g for the leaf extract of C. odorata by using various solvents (Hanphakphoom et al. 2016). On the other hand, various studies revealed the presence of total phenolic contents and few studies could not detect the flavonoid contents and vice versa for C. odorata leaf extracts (Anyasor et al. 2011; Mondal et al. 2012; Danlami et al. 2013). But the present study revealed the higher amount of flavonoid than total phenol content as reported earlier (Hanphakphoom et al. 2016).

Table 1: In-vitro phytochemical and antioxidant activity of Chromolaena odorata.
Data are represented as mean $\pm$ SE (n= 3).

Phytochemical & antioxidant activity	Results
Total Phenol	$39.72 \pm 5.25$ mg GAE/ g
Flavonoid	$100.75\pm1.75$ mg RE/ g
DPPH	82.83 %
TAA	75.42 mg/ g
ABTS	23.18 mg/ g

As far as DPPH radical scavenging activity of *C. odorata* is concerned, the result obtained in this present study is comparable with the earlier reports where it was found in the range of 5.65-87.93 % (Amatya and Tuladhar, 2011) and 24.68-61.78 % (Parameswari and Suriyavathana, 2012) for the ethanolic leaf extracts. Likewise, DPPH radical inhibition was observed to be 59.10 % and 52.13 % for the ethanolic and methanolic leaf extracts, respectively (Bhargava *et al.* 2013). Besides, the ABTS scavenging activity of ethanolic leaf extract of *C. odorata* was recorded as 29.92-63.34 % by Parameswari and Suriyavathana, (2012) and the total antioxidant activity of 10.39  $\mu$ g/ ml ascorbic acid equivalent (AAE) was observed for the methanolic leaf extracts of *C. odorata* (Krishanti *et al.* 2010).

The differences in the phytochemical contents and its biological activity might be due to the type of extraction methods used, efficiency of the solvent used for extraction, ratio between the plant sample to solvent, different parts of the plant taken for analysis and the differences in their geographic location, climate, season and its growth phase (Maji *et al.* 2010; Kothari *et al.* 2012).

The fish pathogens viz. Aeromonas hydrophila causes haemorrhagic septicaemia and red pest disease; Edwardsiella tarda causes edwardsiellosis and emphysematous putrefactive disease; Pseudomonas fluorescens causes abdominal dropsy and fin rot disease and Vibrio alginolyticus causes vibriosis in fishes (Noga, 2010; Roberts, 2012). The human pathogenic bacteria such as Escherichia coli causes neonatal meningitis, enteric and systemic infections; Staphylococcus aureus causes endocarditis and staphylococcal food borne diseases and Streptococcus pneumonia causes pneumonia and meningitis in humans (Mitchell and Mitchell, 2010; Bachir and Abouni, 2015; Tong et al. 2015). All these pathogens are belonging to the gram negative group except, Staphylococcus aureus and Streptococcus pneumonia which falls under the gram positive category. In the recent past, ethno-medicine based treatment measures are getting prominence to control the above mentioned fish and human pathogenic infections in the light of emerging antimicrobial resistance.

In-vitro antibacterial activity of methanol and DMSO dissolved extracts of C. odorata was presented in table 2. Antibacterial activity of C. odorata was observed against all tested gram negative and gram positive bacteria except for the DMSO dissolved extract against Escherichia coli. Likewise, the highest amount of antibacterial activity with 21 mm of zone of inhibition was recorded against both Aeromonas hydrophila and Pseudomonas fluorescens. The zone of inhibition (mm) obtained in this present study against E. coli, P. fluorescens, S. aureus and S. pneumonia is much higher than the inhibition recorded in the earlier study for the same bacteria (Natheer et al. 2012). It was also reported that the ethanol and methanolic extracts of Eupatorium odoratum revealed antibacterial and anti-fungal activities against various tested organisms (Jai Sunder et al. 2012). Further, excellent antibacterial activity was exhibited by the extracts of E. odoratum against ten fish bacterial pathogens (particularly Vibrio sp.) isolated from the diseased ornamental fishes (Ravikumar et al. 2011). Likewise, methanolic extract of C. odorata showed the highest antibacterial activity against V. harvevi and also the artificial diet incorporated with bioactive compounds of C. odorata improves the survival rate of tiger prawn, Penaeus monodon (Harlina et al. 2015 & 2019). The reason behind the in-vitro growth inhibition of these microbes might be due to the presence of active constituents in the extracts which may act as alone or in combination (Natheer et al. 2012).

Free radicals (reactive oxygen species) are considered to be involved in many of the life threatening ailments. To combat the free radicals, plant based bioactive compounds (phyto-antioxidants) are considered as safe when compared to the synthetic antioxidants (Krishanti *et al.* 2010). Further, it is well documented that the antimicrobial compounds present in *C. odorata* such as flavonoids are helpful to inhibit the biosynthesis of cell wall of pathogens which in turn restrict its growth (Anyasor *et al.* 2011; Lavanya and Brahmaprakash, 2011). The above mentioned bioactive compounds present in *C. odorata* may be responsible for its curing activity against various ailments as evident by the earlier reports (Akinmoladun *et al.* 2007). As Andaman Islands



are known for its rich marine biodiversity, the present study elucidated that the terrestrial herbs present in these Islands such as *C. odorata* also possess the potentially considerable amount of phytochemical, antioxidant and antibacterial activity when compared to the earlier reports on seaweeds and mangroves (Sivaramakrishnan *et al.* 2017a,b & 2019). The present study revealed the potential of *C. odorata* to be used as a therapeutic against various ailments of humans as well as animals. However, further studies and in depth analysis on various compounds and its properties need to be elucidated towards its potential industrial application on a large scale for the benefit of animal and human mankind.

Table 2: In-vitro antibacterial activity	of methanol and DMSO dissolved extracts of Chromolaena odorata

	Zone of inhibition (mm)					
Bacteria	Methanol extract (50 μl) 10 mg/ ml	Positive control (50 μl) (Streptomycin) 10 mg/ ml	Negative control (50 μl) (Methanol)	DMSO extract (50 µl) 10 mg/ ml	Positive control (50 μl) (Streptomycin) 10 mg/ ml	Negative control (50 μl) (DMSO)
Aeromonas hydrophila	18	42	0	21	30	0
Escherichia coli	15	24	0	0	21	0
Vibrio alginolyticus	6	24	0	14	27	0
Edwardsiella tarda	14.5	26	0	15.5	24	0
Pseudomonas fluorescens	21	26	0	9.5	23	0
Staphylococcus aureus	11.5	18	0	10	18	0
Streptococcus pneumonia	10	29	0	14.5	27	0

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## Woody Pepper: A Potential Spice Crop for Intercropping in Arecanut Gardens

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#### Abstract

Arecanut is one of the important plantation crops grown commercially in the Andaman and Nicobar Islands. However, mono-cropping is prevalent in most of the plantations, thereby resulting in underutilization of already scarce land resource. Intercropping using various annual and perennial crops could not only increase the income of farmers, but would also help in better utilization of available resources. Woody pepper is a novel spice of Piperaceae family, which grows taking support of perennial trees. The species is a potential candidate for commercial cultivation in the islands and present article highlights some important field observations, qualitative phytochemical analysis, model for arecanut based planting system and future prospects for exploitation of woody pepper as a novel intercrop for arecanut plantations of these islands.

Keywords: Bay islands, cropping model, plantation crop, underutilized spice

#### Introduction

Arecanut is one of the important plantation crops grown in many states such as Karnataka, Kerala, Tamil Nadu, North eastern parts of India and Andaman and Nicobar Islands. It is grown in about 3,500 ha in the Andaman and Nicobar islands. Due to low maintenance requirements, assured demand and better prices for fresh and dried nuts, cultivation of arecanut is popular among island farmers. Profitable intercrops for arecanut gardens of Andaman islands have been identified (Waman et al., 2019). In Andaman and Nicobar islands, though a few farmers cultivate crops such as clove, ginger, black pepper, cinnamon, banana, pineapple etc. as intercrops in arecanut plantations (Waman, 2019), a large number of plantations are mono-cropped. It has been well established that growing intercrops in arecanut gardens with appropriate technological interventions such as green manuring, irrigation, mulching etc. would increase the yield of arecanut as well the system productivity (Bhatet al., 1999, Bhat and Sujatha, 2007; Hussain et al., 2008; Sujatha et al., 2011).

The choice of intercrops is an important factor which depends on the local availability and requirement of resources. Considering scarcity of land in the islands and increasing competition from non-agricultural sectors, adoption of high value intercrops is a key step to make island agriculture profitable. While searching for newer intercrops in a location, the local diversity and ethnic use of plants need to be considered for better adoption and ease of cultivation. Woody pepper (*Piper pendulispicum* C. DC.) is one such underutilized species in Andaman and Nicobar Islands, which is locally used by islanders as a spice for culinary purposes (Waman *et al.*, 2018).

Present article aims at identifying potential of this spice as a novel intercrop in arecanut gardens of these islands. Surveys were also conducted in Andaman islands to gather information about the species and results have also been presented. A model for arecanut- woody pepper based cropping system has been proposed. Further, the qualitative phytochemical analysis has also been presented hereunder.

#### **Materials and Methods**

Field surveys were carried out during 2016 to 2019 in North and Middle Andaman and Little Andaman islands to identify home gardens growing woody pepper as backyard crop. Standards used for growing the species were documented. Interviews were conducted with the farmers to know about the cultivation and marketing practices followed by them. A model was prepared for utilizing the spice as intercrop in the arecanut plantations. Samples of stem were procured from a farmer's field in Little Andaman Island and brought to author's laboratory to conduct qualitative phytochemical analysis.

Stem pieces were scraped off to remove the outer bark layer. Stem was made into smaller pieces, dried using hot air oven at 50 °C and powdered. For analysis of phytochemical components, aqueous, ethanolic (95%, v/v), methanolic (95%, v/v) and acetone (95%, v/v) extracts were prepared. For this, 2.5g of powder was extracted with 25ml of the said solvents by cold percolation for 72 h. Extracts were then filtered through Whatman's filter paper and filtrate was collected in air tight container. Qualitative analysis was carried out using standard procedures. Various tests were conducted to detect the presence of phytochemicals viz. carbohydrates (Molish, Fehling's and Benedict's tests), glycosides (Keller-Kiliani test), tannins (catechin test), alkaloids (Wagner' test), amino acids (Ninhydrin test), phenolic compounds (ferric chloride test and gelatine test), proteins (Millon's test), volatile oils (Sudan III test), saponins (foam test) and flavonoids (alkaline reagent test).

#### **Results and discussion**

Woody pepper is commonly known as Choi Jhaal and is known to be used by settler Bengali community of the islands. Unlike black pepper, wherein berries are used as spice, stem pieces of this vine are used to impart pungency in culinary preparations. Earlier, the produce was known to be harvested destructively from the forests, thereby causing damage to natural populations. However, recently cultivation of woody pepper has been reportedly taken up by some farmers in the backyards to meet the local demand (Waman et al., 2018). Promotion of this spice as a crop would not only assure income to the growers but would also reduce burden on the natural stocks, thereby assisting its conservation. In order to explore the possibility of promoting this species as a potential new crop, systematic surveys were undertaken during 2016 to 2019 in home gardens and local markets of Andaman Islands.

Surveys carried out in Little Andaman, Middle Andaman and North Andaman Islands resulted in

identification of ten locations of cultivation, all of which were in fields of Bengali farmers. A total of 67 vines were observed in these gardens (CIARI, 2019), of which 36% were trained on arecanut as standard followed by mango (27%), jackfruit (18%), forest trees (12%), coconut (4%) and drumstick (3%) (Fig. 1). The perception of farmers on cultivation practices, harvesting, utilization and marketing was documented. The choice of standard for growing the vine varied among the farms and mango tree standards were observed in six farms followed by forest trees (five farms), arecanut and coconut (two farms each), jackfruit and drumstick (one farm each). Number of vines per farm varied between 1 and 47. In local markets, the produce (i.e. the stem pieces) was sold at Rs. 300-400/- per kilogram in North and Middle Andaman, while the price was as high as Rs. 600/- to 1,000/- in Little Andaman (Fig. 2).

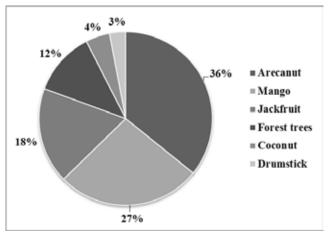


Fig. 1: Percentage share of different species used as standard for woody pepper



Fig. 2: Stem pieces of woody pepper sold in local market of Andaman Islands

Sufficiently thickened stems are one of the criteria for fetching higher rates and the vines are said to attain the desired thickness in about 5-6 years from planting. Subsequent harvesting could be done at 4-5 years intervals. Yield of fresh stem pieces of 8 to 10 kg/ harvest could be obtained, whereas yield of 16 kg from a vine of 5 years age was observed at a farmer's field in Middle Andaman. Realizing the profitability of its cultivation, many other arecanut farmers are showing interest to plant woody pepper in the surveyed areas.

Arecanut gardens of the islands could be used for undertaking large scale cultivation of this unique spice. For this, a hypothetical model with five year harvest cycle was proposed for an acre of arecanut garden (Table 1). In such gardens, woody pepper could be planted in staggered manner at the rate of 110 vines per year for five years, covering the entire plantation. Considering yield of 10 kg stems/ vine and selling price of Rs. 250/- per kg, island farmers could get additional income of Rs. 2,75,000/- per year by cultivation of this newer spice. Ratooning of the crop will ensure regular supply of income to the island farmers and sufficient produce will be available to meet the demands from local islanders. This model is now being developed in an arecanut garden at ICAR-CIARI, Port Blair on experimental basis to test the feasibility. The prospects of the crop could be further increased if it is promoted by the tourism sector in sale as well as use in unique food preparations which will increase the consumption levels and demand. In a nutshell, woody pepper could be promoted as a profitable crop for the backyard and commercial scale cultivation in the arecanut plantations of the islands.

Area proposed for expansion	Arecanut gardens of hilly uplands
No. of plants per acre	550
System of planting the vines	Staggered planting of 110 vines/ year (for 5 years)
Expected yield per vine (5 years old)	10 kg
Projected yield/ acre/ year (after 5 years)	1.1 t
Selling price of produce (fresh)	Rs. 250/- per kg
Additional income per acre/ year	Rs. 2,75,000/-

Table 1: Proposed model for systematic cultivation of woody pepper as an intercrop in arecanut

Qualitative phytochemical analysis of the stem powder suggested presence of tested phytochemicals in variable proportions depending on the solvent employed (Table 2). High proportions of carbohydrates were observed in ethanolic extract using Molish and Benedict's tests, while very high levels in Methanolic extract using Benedict's test. Using Fehling's test, low carbohydrates were detected in all the extracts studied. No carbohydrates were detected in aqueous extract when tested using Molish test. Presence of glycosides in tested extracts was of the order: aqueous > acetone >ethanol > methanol. Low levels of tannins and phenolic compounds were detected in all the extracts using catechin and ferric chloride tests, respectively. Very high proportions of alkaloids and phenolic compounds (gelatin test) were noticed in the aqueous extracts. Piperine is the dominant alkaloid in most of *Piper* species and hence, stem of this species could also contain this compound, which is known to impart the spicy taste. Flavonoids were in high proportion in ethanolic extract, while acetone extract did not show any traces of it. Irrespective of the solvents used, amino acids, proteins and saponins were not detected in the sample. *Piper* species are known to have considerable medicinal and antimicrobial properties owing to presence of bioactive compounds (Salehi *et al.*, 2019; Chinthamani *et al.*, 2020). Volatile oil in higher proportions was detected in acetone extracts. As acetone is a common solvent for recovery of oleoresins from spices (Singh *et al.*, 2007),

such results are justified. This preliminary information could help the researchers to carry out detailed studies in future.

Standardization of efficient propagation techniques, suitability of different soil types, growth and development under different canopy levels, water and nutrient requirements, occurrence of pests and diseases, harvesting and storage studies, biochemical estimation of the economic products and quality aspects are suggested as the future line of works for full scale commercial exploitation of this vine crop under plantation based cropping systems of islands. Some of these works are in progress at ICAR- Central Island Agricultural research Institute, Port Blair. Simultaneous feasibility studies in other arecanut growing areas would help in hastening the process of information generation and further promotion of this spice as a suitable intercrop in more areas.

Constituents	Test performed	Aqueous	Ethanol	Methanol	Acetone
Carbohydrates	Molish test	-	+++	++	+
	Fehling's test	+	+	+	+
	Benedict's test	+	+++	++++	++
Glycosides	Keller-Kiliani test	++++	++	+	+++
Tannins	Catechin test	+	+	+	+
Alkaloids	Wagner's test	++++	++	+	+++
Amino acids	Ninhydrin test	-	-	-	-
Phenolic compounds	Ferric chloride test	+	+	+	+
	Gelatine test	++++	-	+++	-
Proteins	Million's test	-	-	-	-
Volatile oil	Sudan III test	-	++	+	+++
Saponins	Foam test	-	-	-	-
Flavonoids	Alkaline reagent test	+	+++	++	-

Table 2: Qualitative phytochemical analysis in dehydrated stem powder of woody pepper				
extracted using different solvents				

-: Nil, +: low, ++: medium, +++: high, ++++: very high

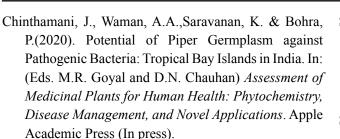
It could be concluded from these findings that, woody pepper could be promoted as a potential intercrop in the existing arecanut gardens of Andaman and Nicobar Islands.

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## Humpsore: Stephanofilariasis With Reference to Andaman and Nicobar Islands

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## Abstract

Humpsore or stephanofilariasis is caused by *Stephanofilaria assamensis*, transmitted by fly *Musca conducens* in different animal species particularly in bovine and bubaline species in eastern region of India including Andaman and Nicobar Islands. It causes pruritus, damage in the hair follicles, skin glands leads to alopecia and severe ulcerative nodular dermatitis, exudation, granulation, ulceration and incrustation. It causes delayed puberty in heifers, reduced milk production, increased inter-calving interval and reduced fertility rate in milch cows. Various therapeutic measures include levamisole hydrochloride, organophosphorous compounds, Ivermectin, petroleum jelly, tobacco ointment, etc. are used in alone or combination of two or more for effective control of stephanofilariasis. Control and eradication of stephanofilariasis may be successfully achieved by targeting life cycle of *M. conducens*. Success of the treatment is depending upon the control fly infestation and microfilaria in the blood of the affected animals. Simultaneously, other managemental procedure such as blanket treatment of the affected animals, improvement of the general cleanliness and hygiene has reduced the fly population and prompt treatment of fresh injuries or cuts and intermediate host free environment are to reduce the prevalence of humpsore.

Key words: Stephanofilariasis, prevalence, clinical symptoms, pathophysiology, treatment, control measures

## Introduction

Livestock enterprise constitutes an important part in wealth of a country as it provides draft power, leather, manure, milk and meat to the vast majority of the people throughout the world. Humpsore is caused by S. assamensis, which widely affects the bovine, bubaline and caprine species, elephant, black rhinoceros and Nilgi in India, common in eastern region of India particularly in Assam, Tripura, West Bengal, Odisha and Andaman and Nicobar Islands and also in other states such as Andhra Pradesh, Telangana, Bihar and Gujarat, besides Southeast Asian countries (Rai et al., 2010). Stephanofilariasis causes pruritus, damage to the hair follicles and skin glands and alopecia. It also causes ulcerative nodular dermatitis, exudation, granulation, ulceration and incrustation, depending on the stage. It causes unclean or unhygienic milk production. Stephanofilariasis is not only affecting the hump but also affects other parts of the body, inner canthus of the eye, scrotum, udder and sternal area (Watrelot-Virieux and Pin, 2006). Size of the humpsore varies from a few cm to more than 30 cm and

this is endemic to Andaman and Nicobar Islands (Rai *et al.*, 1990; Rai *et al.*, 1992) as these islands have typically hot and humid climate throughout the year and rainfall is extending more than 8 months with higher relative humidity (average 80%), which favour the un-interrupted growth of parasites, flies, insets, lice, etc.

Stephanofilaria can be characterized as the agent of a disease commonly observed in exuberant high occupancy pastures with large quantities of wet faeces, principally in hot and rainy seasons (Sutherst *et al.*, 2006). Five species of the genus Stephanofilaria have been described as bovine parasites in a number of regions of the world; their intermediate hosts are *Haematobia irritans*, *Musca conducens*, *Musca planiceps* and *Musca autumnalis* (Riviera and Aycardi, 1985). Stephanofilariasis is transmitted biologically by *M. conducens* in Andaman and Nicobar Islands (Rai *et al.*, 1995). This disease affects both male and female animals. The affected male is unsuitable for draught and plough purposes and affected females lose their productivity, growth rate and fertility rate. Stephanofilariasis causes delayed

puberty in heifers and diminished milk yield, increased inter-calving interval in milch cows. Thus, the farmers suffer severe economic losses. It is also considered as a zoonotic disease; however, its occurrence in humans is rare (Novaes et al., 2006). It was also reported that stephanofilariasis has showing higher prevalence rate in exotic and its crossbreds (20.17%) than in zebu (16.14%) cattle (Singh et al., 2002). Further, Johnson and Toleman (1988) reported that the prevalence rate was lower in light coloured than in dark coloured cattle in zebu type. Various workers attempted to eradicate the disease and variation in success rate both in India and in other parts of the world is due to indirect life cycle of the parasite and non-availability of effective and economical treatment protocol for this disease. This review explains about the incidence, pathophysiology, treatment and control of humpsore in cattle and buffaloes with special reference to Andaman and Nicobar Islands.

#### **Etiological factor**

A breach in the continuity of epidermis of the skin is the pre-requisite condition for the subsequent development of humpsore, continuous ocular discharge from the inner canthus of the eye and feeding of the discharge by flies, injuries in the base of the horn and base of the ear, abdominal wall or other regions favour for development of the humpsore. Breach is the origin of the lesion in the skin which becomes enlarged by secondary bacterial infections (Staphylococcus aureous, Staphylococcus albus, etc.). Severity of the lesions varies with different predilection sites. It is observed that the humpsore lesions mostly on or around the hump and also observed on the back, ventral surface of the body, anterior and posterior to the navel and on the abdomen. Topographical studies suggest that S. zaheeri is a well adapted and widespread parasite whereas S. assamensis is confined mainly to the eastern parts of India. A humid tropical climate with thick vegetation appears to be ideal environment for Stephanofilaria species in general and for S. assamensis in particular. Therefore the stephanofilariasis is more prevalent in cattle and buffaloes of Andaman and Nicobar group of Islands.

#### Economic importance of stephanofilariasis

The disease is located mainly on or near the regions of the hump and neck and it causes extensive skin damage. The value of the animals is reduced due to the ugly looking lesion leads to huge economic loss to the farmers (Ibrahim et al., 2013). Severe infections can cause considerable stress to the affected animals which inturn affects the health and wellbeing. Humpsore can be particularly annoying for dairy cattle in endemic regions; it considerably hampers the manual or mechanical milking and makes it impossible to comply with hygienic measures for milking. This disease causes loss of milk production, reduced working capacity and damage in the hide. Damaged hides can be downgraded and even rejected at slaughter. Affected females, particularly milch cows/buffaloes exhibit poor growth rate, reduced milk production and fertility failure. Thus, farmers suffer heavy economic losses due to humpsore.

#### **Clinical examination**

The disease can be diagnosed clinically by direct visual method and report from the disease register of the farm. The sore size is varied from a few cm to more than 30 cm. Most important clinical signs is intense pruritus, which is characterised by rubbing of the sore with wall, pillar, trees or fencing which causes a central ulcer or excoriation of the parts, which is complicated by screw worm fly and secondary bacterial infections. In initial stages, the skin is covered by gravish white crust which favours for stephanofilarial infection. The non-ulcerated lesions frequently become excoriated favouring further deposition of stephanofilarial larvae and gradually obtain the characters of ulcerated lesions resulting into partial or completely devoid of hairs. The ulcerated lesions have central ulcers of various sizes and shapes surrounded by larger peripheral crusty areas in most of the lesions with irregular boundary. The discharge of the ulcers is pureblood, serum or blood mixed serum. The surface of the ulcers is frequently hemorrhagic and moist but dry surface covered by thin blackish or brownish crusts and a number of flies are seen feeding on the discharges. The colour of the crusty/scabby part is found to vary from gravish white to blackish and this part is raised from the

ulcerated part or normal skin surface. The thickness of the crusty part of the lesions is up to 2.3 cm according to character and degree of crust formation, compared to the normal skin thickness of about 2.5 mm. The shape of the lesions is characteristically circular although roughly rectangular, triangular or irregular shaped lesions are common (Rahman and Khaleque, 1974). Close contact of the animals and negligent management of cutaneous abrasions could be the cause of higher incidence of humpsore under farm conditions. It is also observed that the humpsore lesions mostly on or around the hump and also on the back, ventral surface of the body, anterior and posterior to the navel and on the abdomen (Dewan, 1971).



Fig 1: Cattle with Humpsore

Clinically two types of lesions are considered for descriptive and diagnostic purposes. Early lesions are smaller in size (about 3 to 4 cm in diameter) characterized by formation of thin, mildly granular crusts in most lesions and rarely laminated. The old lesions, on the other hand, are larger in size and had coarsely granular crusts with many cracks and crevices with or without centrally located ulcer. Both types are partially or completely hairless. These lesions are again classified according to severity depending on amount of crusts, exudation, size of lesion and ulceration into 3 types such as mild, moderate and severe.

#### **Pathological Examination**

Microfilaria of *S. assamensis* is observed in peripheral blood smears of cattle (Das, 1955) and Sen *et al.* (1956) recovered microfilariae from humpsore lesions. The

disease is transmitted by M. conducens, the biological vector and distributed throughout the world (Rai et al., 2010). These flies become infected with microfilariae when they bite or feed on the wounds that cause the worms in the skin of cattle and other final hosts. Microfilariae develop into infective larvae (L3) inside the flies in about 3 weeks. Such flies re-infect their hosts while feeding. The fly also carries the microfilariae from infected animals in proboscis and inoculates the microfilariae to healthy animals by biting. Patnaik (1970) found that the larva of S. assamensis develops in M. conducens and become infectivity after 23-25 days at 25°C. Poor condition and high rainfall are the predisposing factors of humpsore especially in north-eastern part of India including Andaman and Nicobar islands. Gross morbid lesions of the humpsore are to be examined systematically. Clinical signs coupled with skin biopsies provide the best means of establishing a definitive diagnosis.

Histopathological study revealed that there is marked hyperkeratosis of stratum conium of epidermis and discontinuity or loss of integrity of the superficial layer of the epidermis. There is severe dermatitis and also the proliferation of fibrous connective tissue and a diffuse infiltration of the dermis by mononuclear inflammatory cells and eosinophils (Johnson et al., 1986) indicate that there is hyperkeratosis and parakeratosis in the epidermis. Cross and longitudinal section of stephanofilarial parasites are seen in tissue sections which are encapsulated with fibrous connective tissue in hair follicle. Microfilariae and eosinophils are found in the dermis. Microfilariae are found enclosed within limiting membranes in the dermis immediately adjacent to the stratum germinativum. Adults are found in cysts at the base of hair follicles or in the base of the rete pegs and are surrounded by zones of inflammatory cells, predominantly mononuclear cells. The deeper layers of the dermis are contained perivascular aggregations of lymphocytes, histiocytes and eosinophils. Probably, these findings are related to the death of the parasite and the consequent sensitization of the host. There is an extensive proliferation of fibrous connective tissue (non-neoplastic) at the reticular area of the dermis.

The concentration of the worms or microfilaria in the lesions revealed in ear-sore that one worm per 1.58 sq cm, 3.89 sq cm and 12.3 sq cm, skin areas of chronic, moderate and typical sore lesions, respectively (Agrawal, 1977) and sex ratio of 1:3 with predominance of female worms in humpsore. It appears that the two sexes may differ in their antigenicity or adaptability, one sex being more vulnerable to host reactions. Sharma Deorani (1967) revealed that the histopathology of humpsore by recognising lesions in four stages, i.e. sub-acute, acute, desquamative and reparative. The worms were found in both the dermis and the epidermis. Microfilariae were observed only in the desquamative and subacute stages. The reparative or healing stage was characterised by the presence of thick contracted scar tissue but the absence of worms

## Stephanofilariasis with reference to Andaman and Nicobar Islands

Andaman and Nicobar Islands has typically tropical humid climate throughout the year and annual rainfall extending more than 8 months with higher relative humidity (average 80%) favours the un-interrupted growth of the parasites, flies, insets, lice, etc. Disease pattern in all the major islands is more or less similar to that of the adjacent regions of Southeast Asian Island countries. The disease treatment protocols, control models, developed in Andaman and Nicobar Islands will largely be applicable to such regions also. However, occurrence of certain diseases may vary, particularly those which spread due to group grazing or prevalent population of intermediate hosts. Prevalence and factors responsible for increasing the incidence of humpsore and its effect on health and wellbeing, production and reproduction of the dairy herds in Andaman and Nicobar islands are depicted in Figure 2.

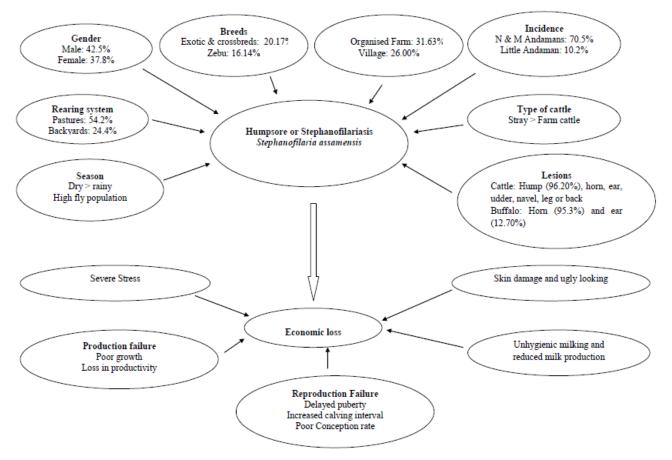


Fig 2: Incidence of Humpsore and its effect on production and reproduction in Andaman and Nicobar Islands (Rai *et al.*, 1994; Rai *et al.*, 1995)



The humpsore parasite becomes adult and starts laying eggs at around 4-6 months in sore (Srivastava and Dutta, 1963). The sore either undergoes reparative stage or become crusty during the month of July (Rai et al., 1995). These eggs ingested by fly (M. conducens) and develop the larval stage I, II and III in inside of the body of the fly within 25-35 days (Srivastava and Dutta, 1963). The infective larva (stage III) reaches in the saliva of the fly and may get deposited in the skin injuries during the feeding of the fly on the wounds. Thus, the infective larva deposited during September may become mature by January and this vicious life cycle continues. The infective larva inside the injury on the host feeds on live cells resulting in gradual increase in the size of the wound and excretion of serum, dead cells etc. in form of oozing from the wound, which attracts the flies further. Repeated deposition of larvae is required to develop a full-fledged sore and if the wound is made fly-proof, the sore may heal/

undergo reparative stage and further spread is prevented (Rai *et al.*, 1995). However, the filariids are known to be long-lived and it is difficult to accept that animals can get rid of the infection in as short a span as 6-8 months. It appears more likely that the dry season causes the latent lesions to flare up into the clinical form due to reinfection and increased numbers of vector bites, thus, apparently raising the proportion of infected animals. Therefore, the treatment should be conducted once in six months or twice in a year in Andaman and Nicobar Islands.

#### Therapeutic approaches

Completely approved treatments for stephanofilariasis are not available for cattle and buffaloes and different treatment protocols have varied success rate (Table 1). The therapeutic approaches were assessed on the basis of skin healing and normal posture of the animal.

Sl. No.	Treatment Protocol	References
1	Surgical removal or cauterizing agents	Mishra (1969)
2	Ointment made from 64 preparations (conch shell ash, lead monoxide, sulphur, tobacco, etc.)	Hassan (1969)
3	Application of Petroleum jelly	Patnaik (1970)
4	Neguvon 6% liniment + Asuntol 6% dusting powder	Patnaik (1970)
5	Antimosan $(s/c) + 1\%$ gentian violet	Ahmed and Ali (1973)
6	Trichloropon applied with 6-10% petroleum jelly or castor oil	Rahman and Khaleque (1974)
7	8% Trichlorophon + 4% sulphonamide ointment (Healed within 20-26 days)	Baki and Dewan (1975)
8	Parentral administration of 8% Trichlorphon (very effective)	Baki and Dewan (1975)
9	Antimony potassium tartrate + Phenothiazine (4-8% ointment)	Dutta and Hazarika (1976)
10	Tobacco ointment (80% of cases cured)	Dutta and Hazarika (1976)
11	External application of Antimony potassium tartrate	Dutta and Hazarika (1976)
12	Trichlorophon (6-10%) in petroleum jelly or castor oil (Cured within 7 days)	Rahman and Khaleque (1974)
13	Supona 20 and Sumithion @ 4% concentration (Toxic signs @ 6%)	Das et al. (1977)
15	Levamisole hydrochloride + blanket treatment	Rai et al. (1994)

Table 1: Available therapeutic measures for Humpsore



Sl. No.	Treatment Protocol	References
16	Levamisole HCl + Zinc oxide ointment (mild and moderate size healed within 5-13 days)	Rai <i>et al.</i> (1994) Rai and Ahlawat (1995)
17	Less than 2 cm diameter healed within 5-6 days	
18	2-5 cm diameter healed with 7-8 days	
19	5-8 cm diameter healed within 10-14 days	
20	8-12 cm diameter healed within 15-18 days	
21	more than 12 cm diameter healed within 17-25 days	
22	Ivermectin + Levamisole + Mastilep ointment	Choudhury and Das (2012)
23	Levamisole (3ml s/c) at lesion+ Dermocept ointment (healed in 11 days)	Phukan <i>et al.</i> (2005)
24	Ivermectin + Topicure spray (healed within 21 days)	Puttalakshmamma et al. (2012)
25	15% Tobacco ointment	Al Masud et al. (2017)
26	Ivermectin + Zinc oxide ointment twice daily	Islam et al. (2018)

#### **Prevention and control**

The control and eradication of stephanofilariasis in bovines can be successfully achieved especially by targeting the life cycle of the intermediate host, *Musca conducens*. Simultaneously, other measures such as blanket treatment of affected animals during active phase of the parasite after cordoning the area, improvement in general cleanliness and hygiene to reduce the fly population, promptly attending to fresh injuries/cuts and concerted efforts to make the intermediate host sterile for parasite may be undertaken (Rai *et al.*, 2010).

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# **Treatment of Post-Partum Uterine Prolapse in A Non-Descript Cow: A Case Report**

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#### Abstract

A three years old non-descriptive cow with post partum uterine prolapse was treated successfully with reduction, re-placement and repositioning in its original place. Uterine prolapse is one of the most common post partum obstetrical problems, which significantly affects the reproductive as well as productive performance of the dairy cattle by prolonging the duration of return to estrus, increasing the calving to conception interval, calving interval and decreasing the conception rate. The affected cow showed protrusion of congested uterine mass through the vulva and vagina after its calving. The animal was active, alert and apparently healthy and diagnosed to suffer with uterine prolapse based on the clinical examination. With use of epidural anaesthesia, proper reduction and proper lubrication of the prolapsed uterine mass, the mass was repositioned in its place followed by application of Buhner's sutures at vulva to prevent recurrence of the prolapse. Eventually the animal had recovered.

Key words: Post-partum uterine prolapse, non-descript cow, treatment, case report

#### Introduction

Uterine prolapse is defined as the protrusion of uterine mass through vulva with exposure of mucosal surface (Gustafsson et al., 2004) and it occurs between 48 and 72 h after parturition (Arthur, 1996, Roberts, 1971) or sometime up to several hours. Post partum uterine prolapse has been seen in third stage of labour in the dairy cow (Joseph et al., 2001). Hypocalcaemia (milk fever), hypoglycemia, prolonged dystocia, fetal oversize, fetal traction, retained fetal membranes, excessive straining, uterine atony or uterine inertia, poor uterine tone, tympany, increased intra-abdominal pressure or hormonal imbalance due to excessive estrogen available in the feed or plants (phyto-estrogen) are the main etiological factors for uterine prolapse (Hanie, 2006, Jackson, 2004). Prevalence of uterine prolapse was reported as 0.3 to 0.5% in cattle (Luktuke and Chaudhary, 1965) and 6.6 % to 12.9 % in buffaloes (Nanda and Sharma, 1982). Hormonal imbalance in the sense that increasing the concentration of estrogen and relaxin causes relaxation of pelvic ligaments and softening of cervical canal, which induce the uterine prolapse in dairy cows. It is considered as a veterinary emergency as without treatment, the affected cow is usually to die within a short period of time (Murphy and

Dobson, 2002, Miesner and Anderson, 2008). The present case report explains successful treatment with reduction, replacement and repositioning of prolapsed uterine mass in the non-descriptive post partum dairy cow.



## Fig. 1: Uterine Prolapse

#### Case history and observation

A three years old non-descriptive cow was attended at the door step of a farmer with history of protrusion

anti-inflammatory analgesic (Injection Meloxicam 0.2 mg/kg B. Wt) and antihistamine-Chlorphenaramine maleate (Injection Avil–12 mL). The prescribed treatment was repeated for five days excluding Calborol. The cow recovered uneventfully without any complications.

#### Discussion

Prolapse of reproductive organs including cervicovaginal prolapse or uterine prolapse are common gestational accident among reproductive disorders in dairy animals. Long myometrial contractions, , violent straining, and hypocalcaemia, hypoglycemia, lack of exercise, relaxed atonic flaccid uterus, extreme laxity of perineum and vulvar lips, excessive estrogen content in the feed, forced traction of the foetus leads are predisposing factors of post-partum uterine prolapse in ruminant species such as cattle and buffalo (Roberts, 1971, Noakes et al., 2001, Kumbhar et al., 2009). Therefore, calcium borogluconate and dextrose saline were given to minimize the impending symptoms of hypocalcaemia and hypoglycemia. Caudal epidural anaesthesia with Lignocaine was administered before the reduction and replacement of the prolapsed uterine mass to reduce straining and desensitization of the perineum (Noakes et al., 2001). Moreover, uterine prolapse is more common in post- partum than in prepartum period mainly due to abrupt release of increased intra-abdominal pressure, loss of muscular tonicity or uterine inertia in ruminant species (Noakes et al., 2001).

In case of absolute fetal oversize and low nutritional status of dam, the animal suffers uterine prolapse. Lower calcium (hypocalcemia), lower phosphorus and higher concentration of serum magnesium were also etiological factors to induce uterine prolapse (Ahmed *et al.*, 2005, Akhtar *et al.*, 2008). In untreated cases of uterine prolapse, the animal suffers hemorrhagic shock, septic metritis, infertility or death. As the uterine prolapse is an emergency veterinary treatment, therefore, it needs immediate and proper treatment of this condition that not only saves the life of dam but also protects the future fertility of the affected cow, otherwise there will be interference in blood flow to the prolapsed mass may leads to into edema, septic condition, cyanosis which

of uterine mass through the vulva since ten hours after parturition. The placenta was expelled out. Cow is parturated with a male calf and the calf was apparently healthy (Fig. 1). The cow was active, alert and apparently healthy with sternal recumbency and severe tenesmus. Physiological parameters were observed within the normal range. The prolapsed mass was highly congested and larger (hanging down up to hocks when the cow was standing). Prolapsed uterine mass and the maternal caruncles were highly oedematous, and covered with faeces, dirt and blood clots.

#### Treatment

A non-descriptive cow of three years of age was attended with post-partum uterine prolapse. Clinical examination confirmed that the uterine prolapse was bilateral. Prolapsed uterine mass was checked for any injury, washed gently with warm water followed by warm saline and then washed with 1% KMnO<sub>4</sub> solution to remove the dirt and debris. Epidural injection was given with Lignocaine (2%; 5ml) at first and second inter-coccygeal space to minimize straining during replacement of prolapsed organ. Oedema and size of the prolapsed uterine mass was reduced with the help of ice water application and saturated sugar solution. Urinary bladder was emptied with lifting of the mass to give way to flow the urine. This procedure was repeated three times in order to reduce the size of both prolapsed horns. The prolapsed uterine mass was kept on a clean and moist piece of cloth. The prolapsed uterine mass was repositioned with the help of fist and palm inside vagina and then pushed gently into the pelvic cavity till complete repositioning of the uterine horns which was later assessed by per-rectal examination. During reposition, liquid paraffin was used as a lubricant. Once the prolapsed mass was reposed inside, oxytetracycline tablets (Terramycin; 4 no) were placed inside the uterus to provide the antibacterial coverage. Buhner's suture was placed in the vulva with sterile cotton thread dipped in betadine solution to retain the uterus in place. The suture was removed after 14 days. The animal was treated with fluid therapy, 5% DNS - 2000 mL/day and Calborol 450 mL slow intravenous, antibiotic (Injection Enrofloxacin 5 mg/ kg B. Wt, injection Streptopenicillin 2.5 g intramuscular),

later on leads to develop into gangrene (Kapadiya *et al.*, 2015). Dung, blood spots and other dirt materials should be removed carefully and KMnO<sub>4</sub> solution was used to prevent the uterine infection (Prakash *et al.*, 2016, Paul *et al.*, 2017). Hemorrhagic shock, thromboembolism, septic metritis, infertility or death is the potential squeal of a prolonged uterine prolapse (Pothiappan *et al.*, 2013). Therefore, administration of broad spectrum suitable antibiotic treatment was followed for at least five days after replacement of the prolapsed mass which will prevent secondary bacterial infection (Borobia- Belsue, 2006).

#### Conclusion

Uterine prolapse occur in peri-parturient or post partum period. Prompt diagnosis and treatment of uterine prolapse is very important task and delayed treatment leads to critical condition like oedema, fibrosis, necrosis, septicemia, toxaemia and death of the animal. Therefore, the farmers and veterinarian act carefully, promptly for early recovery of the condition which will save the cow from life threatening condition. Present case reports the successful correction of uterine prolapse along with its management.

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## Post-partum Uterine Prolapse in Nicobari Sow- A Case Report

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## Abstract

A Nicobari sow was presented with complete uterine prolapse 12-14 hours post farrowing. The prolapsed uterine mass was exposed outside for a longer period as it is occurred during night hours and could be seen in the morning. Attempts were made to reposition the prolapsed uterine mass to its normal anatomical position, however, the sow died during the treatment period.

## Introduction

Uterine prolapse is one of the common complications in ruminant species especially in third stage of labour and less frequently occurred in porcine species. In pig, prolapse is generally partial which involves only one horn whereas in ruminant species, it is complete and involves eversion of the gravid cornua (Noakes et al., 2009). Moreover, rectal and vaginal prolapse is also more common in pigs as compared to uterine prolapse (Supakorn et al., 2017, Roberts, 1971). Hypocalcaemia (milk fever), hypoglycemia, prolonged dystocia, fetal oversize, fetal traction, excessive straining, uterine atony or uterine inertia, poor uterine tone, increased intra-abdominal pressure or hormonal imbalance due to excessive estrogen available in the feed or plants (phyto-estrogen) are the main etiological factors for uterine prolapse (Hanie, 2006, Jackson, 2004). Hormonal imbalance in the sense that increased concentration of estrogen and relaxin causes relaxation of pelvic ligaments and softening of cervical canal, which induce the uterine prolapse in pigs. It is considered a veterinary emergency as the affected pig usually dies within a short period of time without treatment Present case report explains attempts to treat with repositioning of prolapsed uterine mass in the Nicobari sow, but could not be succeeded as sow died during the treatment period. However, the case has sent a message that the prolapsed uterine mass in pigs should be attended on urgent basis.

#### Case history and observation

A Nicobari sow of 2 years age was attended for treatment of uterine prolapse. History revealed that the

farrowing was normal and nothing abnormal was noticed around farrowing. However, it was observed that sow was sitting in the water trough for in the morning. On inspection, it was observed that both the uterine horns were everted exposing the endometrium. Prolapsed uterine masses were oedematous and congested (Fig 1 and Fig 2). Body temperature, pulse and respiration rate were 102°F, 85 beats/minute and 17/minute, respectively.



Fig 1. Nicobari pig with uterine prolapse in standing position



Fig 2. Nicobari pig with uterine prolapse in lateral recumbency position

## Treatment

Prolapsed uterine mass was washed with normal saline and 1% KMnO<sub>4</sub> solution to remove to clean the

dirt. Attempts were made to reposition prolapsed uterine mass with difficulties. The sow was given 2000 mL of 5% DNS by intravenous route. Sow died in the course of treatment and management.

## Discussion

Uterine prolapse is occasionally observed in pigs up to several days after parturition or during farrowing. Excessive abdominal pressure or straining due to mal-position of fetus, fetal/maternal disproportion or inflammation in the birth canal or trauma with swelling are thought to be the main etiological factors to induce uterine prolapse in sow (Zimmerman et al., 2012). Sows affected with uterine prolapse should be treated immediately as extensive prolapsed results into internal haemorrhage, shock and death (Noakes et al., 2009). The uterus should be cleaned avoiding any damage and re-placed back into the genital opening and birth canal by gentle push. Proper repositioning of prolapsed uterus is most important to prevent any damage or injury or trauma (Gowda et. al., 2014) and each uterine horn should be inverted starting with its tip and gradually reduced until the uterine body has been reached in its original place (Zimmerman et al., 2012). Stress and uterine damage will decide the survival and return to normal reproductive performance of sows (Blaes et al., 2001). Complete and correct reposition of the prolapsed uterus and its restoration of tone will prevent its reoccurrence.

In multiparous pigs, repositioning and retention of the uterus is difficult by pushing, therefore it is better to keep the hind quarter well elevated (Arthur *et al.*, 2001). A successful retention of prolapsed mass is possible by pushing the uterine mass (Dewry *et al.*, 2017). The type, duration of the exposure, degree of damage and contamination with dirt and microorganism will decide the success of the treatment (Wachida and Kisani, 2011). Present study clearly sent a message that early and proper management of prolapsed uterus is necessary for restoration of the reproductive status of the pigs.

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# **Community Zonation and Periodical Soil Salinity Incline Mapping of Mangrove Ecosystem**

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## Abstract

Soil salinity plays a key role in forming mangrove community zonation patterns along the coastline. In order to assess the community zonation of mangroves distributed along varying soil salinity gradient in the reserve forests of Pichavaram, Tamil Nadu the present study used spatial modelling approach. Based on the Importance Value Index (IVI), the study concentrated on major mangrove species such as *Avicennia* spp, *Rhizophora* spp, *Excoecaria agallocha, Acanthus ilicifolius, Bruguiera cylindrica, Lumnitzera racemosa.* The average annual soil salinity was recorded between 12 to 35 psu. Spatial modelling clearly indicated that the Pichavaram mangrove community patterns were distributed with euhaline, polyhaline, mesohaline, oligohaline and limnatic zones of soil salinity. Further, spatial modelling showed that mangrove species were site specific according to their soil salinity. The finding will help improve species specific natural conservation and restoration in the near future.

Keywords: Mangroves, salinity gradient, distribution pattern, spatial modelling, importance value of index

## Introduction

Mangrove ecosystems are dynamically influenced by various factors (soil salinity, frequency of tidal inundation, sedimentation, soil chemistry, freshwater inputs and groundwater availability) resulting in complex patterns of mangrove community structure and function (Field, 1998). The amount of freshwater discharge into the wetland determines the overall availability of soil, water salinity and the availability of sediment nutrients for vegetative growth (Woodroffe, 1992). Mangrove soils can be considered halotropic soils and mangroves are salt-tolerant forest ecosystems spreading in the coastal tracts of tropical and subtropical regions (Polidoro et al., 2010; Thornton, 1965). About 73 species are considered true mangroves. Of these, 69 species are reported from the Indian and Pacific regions, (Spalding et al., 2010). True mangroves (e.g., Avicennia spp. and Rhizophora spp.) tolerate higher soil salinity and their tolerance varies among the species more than between non-mangroves (Kathiresan and Thangam, 1990; Kathiresan et al., 1996).

Mangrove cover is spread over an area of 4,740 km<sup>2</sup> in India (about 0.14% of the country's total geographical

area) and Tamil Nadu mangrove cover of 39 km2 area was assessed along the south coastal stretch (FSI, 2015). Since the salinity variations found to be common in the mangrove regions. Community zonation based on salinity and soil parameters has been reported by many authors (Watson 1928; Bunt and Williams 1981; White et al., 1989; Aragones et al., 1998). Currently, remote sensing has been proved to generate information on many components in the coastal environment (Navak et al., 1992, Navalgund & Bahuguna, 1999). The data are useful in mapping the mangrove density-wise (Navak et al., 1992) and also to map dominant communities of mangrove patterns. Habitat maps derived from remote sensing techniques are widely used to assess the status of coastal resources and serve as a basis for coastal planning, conservation, management, and monitoring (Green et al., 2000; Nagendra, 2001). The present study combines the data from the field and from remote sensing, to analyse and identify the mangrove distribution patterns and to fill the knowledge gaps in mangrove zonation pattern and its relationship with soil salinity variations along Pichavaram mangrove ecosystem.

#### Materials and methods

## Study area

Pichavaram mangrove forest formed along the southeast coastal district of Cuddalore between (Lat. 11° 29' N; Long. 79°47' E) Vellar and Coleroon estuaries (fig. 1). The Vellar and Coleroon estuaries and mangroves in Pichavaram cover an area of about 941 ha in 2011



(Gnanappazham, 2014). The fresh water flow comes for non-perennial rivers of vellar and Coleroon rivers in the form of runoff during rainy time. At the same time sea water, continuously flowing diurnal inundation in this region. About 75 - 90% of total rainfall was recorded during the north-eastern monsoon, while low rainfall was recorded during south-west monsoon (April - June) accounting to an annual average rainfall of 914.4 cm (IMD, 2015).

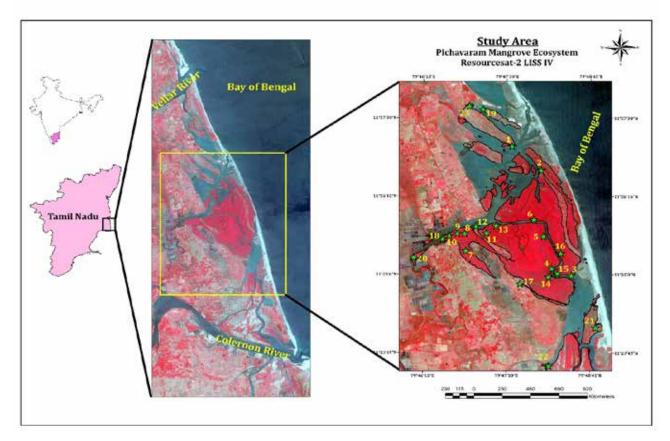


Fig. 1. Satellite imagery showing study area with mangrove forest (dark red patches)

## Mangrove survey

Pichavaram mangrove vegetation survey was carried out with selected 23 sampling sites in the period of January to December 2015. In this study, all the 23 sampling were 20×20 m in dimension and 10 m away from the creek (i.e. Which approximate occupies more than 15 pixels (approximately 87 m) in a LISS IV data Importance Value Index (IVI) was calculated by applying the principles of Misra (1968) and Muller- Dombois and Ellenberg (1974).

## **Importance value Index (IVI)**

= Relative frequency  $(RF_i)$  % +

Relative dominance  $(RD_{a})$  % +

Relative density  $(RD_i)$  % (1)

## Soil sampling

Soil sampling was conducted based on the mangrove vegetation survey. Selected sites of 23 quadrats with

three different depths (10, 15, 30 cm). Soil samples were collected and instantly measured (EC and pH) by using field instrument WTW pH 310. Soil salinity derived from the electrical conductivity of the soil by following standard protocol (Misra, 1968).

(2)

Where, EC = Salinity (psu) =  $0.062 \times EC \times \frac{\% \text{ of water in soil}}{100} \times 10$ Electrical conductivity (m mho cm<sup>-1</sup> or dS m<sup>-1</sup> or mS cm<sup>-1</sup>)

#### Image processing and geographical model

Habitat type classification of Pichavaram mangrove forest was performed using Geocoded Resourcesat-2 LISS IV dataset (15 July 2015, and Path-102; Row-65) with less than 5% cloud cover was acquired from National Remote Sensing Agency (NRSA) data centre (NDC) Hyderabad, India. The Classification was performed using a visual interpretation of colour composite (FCC) with bands 4, 3, 2 displayed at the scale of 1:25,000 followed by Ajai *et al.*, 2012. Data processing of spatial mapping illustrated in the below Fig. 2. Satellite image processing was done by using ERDAS 9.1 and ArcGIS 10.2 version. Soil salinity value of 23 sample points were interpolated in the study area boundary (excluding sandy area) to prepare a rasterized soil salinity maps classification of soil salinity gradient mapping in ArcGIS (Banerjee and Ananda Rao, 1989).

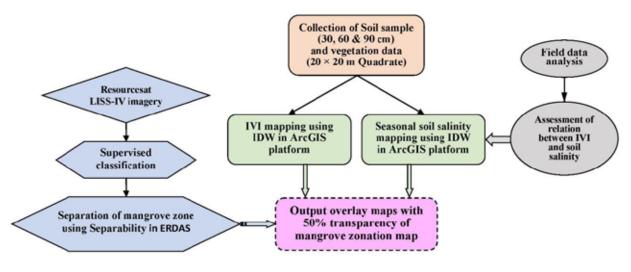


Fig. 2. Short schematic representation of methodology

The study identified *Avicennia* spp dominated forest but the species level distribution of the *Avicennia* spp was not identified. Generally, two *Avicennia* spp are found in this place – *Avicennia marina* and *Avicennia officinalis* as back mangroves while *Rhizophora* spp. Patches were found in waterfronts and *Excoecaria agallocha* and mixed vegetation (i.e. Salt marshes, other true mangroves, and some terrestrial plants) in the landward sides. This has been done to retrieve the distribution of these species within the *Avicennia* spp. dominated community, *Rhizophora* spp. community patches and other mixed vegetation patches. A soil salinity map was prepared by interpolation technique from the field data in GIS environment. IVI maps of the concerned species are prepared from the soil salinity map using the developed relation between them. Then using these IVI maps species priority map was prepared and finally distribution of the two *Avicennia* spp., *Rhizophora* spp. community patches and other mixed vegetation patches were extracted from the community zonation map and priority maps.

An IWD (Inverse weighted distance) method of interpolation was adopted in ArcGIS environment to interpolate the soil salinity from 23 known points with the areas of island having same soil type.



Fig. 3. Mangrove zonation map of Pichavaram.

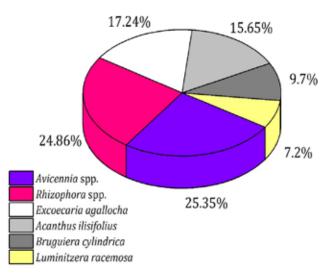
Supervised classification of the present study area mapping clearly indicating the distribution of mangroves in Pichavaram (Fig. 3)

## Results

### **Distribution pattern**

Out of 11 true species in Pichavaram mangrove ecosystem, with an exception of *Acanthus ilicifolius* (herb), all remain tree within 23 quadrats sampling sites.

Avicennia marina and Avicennia officinalis were the two Avicennia spp. found and also the considerable density of Avicennia marina and Rhizophora spp. were observed in the whole region of Pichavaram. Along the river bed of the study area, Rhizophora spp. was found as dominant species and the soil salinity was recorded as moderate. Six taxa of true mangroves were studied for Importance Value Index (Fig. 4). The higher IVI was recorded for the species of Avicennia spp. (99.59) at site 10, followed by Rhizophora spp. (98.27) at site 8 and Acanthus ilisifolius (99.58), whereas the lower IVI was recorded at site 20. Based on the analysis, Avicennia spp. and Rhizophora spp. occupied 55% area of Pichavaram mangrove ecosystem.



## Fig. 4. Percentage of Importance Value Index (IVI) for six mangrove species

Based on the mangrove survey, three land cover categories were identified in Pichavaram, 1. Avicennia forest dominated principally by Avicennia marina and A. officinalis; 2. Rhizophora forest dominated by R. apiculate and R. mucronata; 3. Mixed forest dominated by a mixture of mangrove species in Pichavaram like A. marina, A. officinalis, R. apiculata, Excoecaria agallocha, Bruguiera cylindrica, Aegiceras corniculatum, Luminitzera racemose, Ceriops decandra and acanthus ilicifolius.

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S. No.	Mangrove species / sampling sites	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1.	Acanthus ilisifolius	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2.	Aegiceras corniculatum	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
3.	Avicennia marina	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+
4.	Avicennia officinalis	-	-	+	-	+	+	-	-	+	+	-	-	+	+	+	+	-	+	+	-	+	+	+
5.	Bruguiera cylindrica	-	-	-	-	+	+	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	+	-
6.	Excoecaria agallocha	-	-	+	+	+	-	-	-	+	-	+	-	-	+	-	-	+	+	-	-	-	-	-
7.	Luminitzera racemose	-	-	-	+	-	+	-	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-
8.	Rhizophora apiculata	-	+	-	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	+	+	+
9.	Rhizophora mucronata	-	+	+	+	+	+	+	+	-	-	-	+	-	+	+	-	-	-	+	-	+	+	+
10.	Rhizophora annamalayana	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
11.	Ceriops decandra	-	-	-	+	+	-	-	-	+	-	-	-	+	+	-	+	-	+	-	-	-	-	-

## Table. 1. Distribution of major mangrove species in quadrats

+ Present - Absent

## Mapping based on IVI

Among these mangroves, Importance Value Index (IVI) was applied to generate the spatial maps for Pichavaram and the results showed the gradients of IVI for six different mangrove taxa. In the central part of the Pichavaram and some of the western part, IVI was found to be high due to naturally rehabilitation process and nonanthropogenic activity restricted by forest department compared to opposite direction of the region. It might also be due to the proper plantation and afforestation management activities are proceeding in this region.

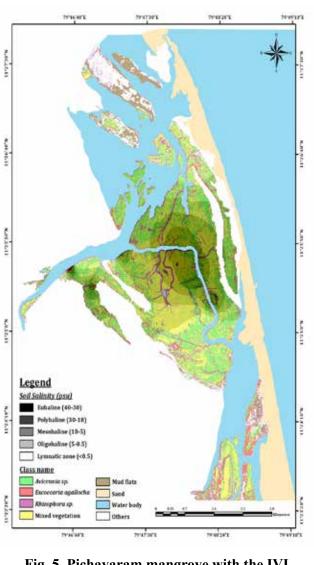


Fig. 5. Pichavaram mangrove with the IVI mapping

## Seasonal soil salinity mapping

Spatial maps of soil salinity with varying seasons were shown in the fig (5a, 5b, 5c, 5d). The highest soil

salinity was recorded during the summer season (35,64 psu) while the lowest was observed during monsoon season (12.09 psu). This might be highly influenced by the vellar, coleroon and Chinnavaikal water resources.

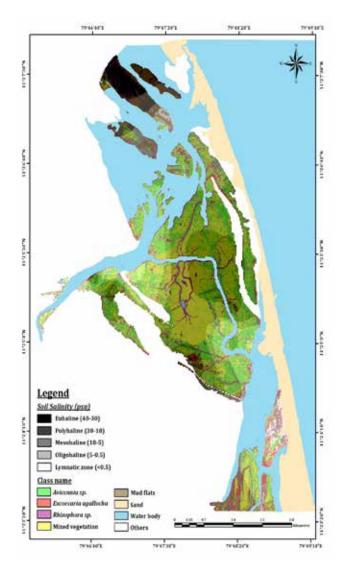


Fig. 5a. Soil salinity (*psu*) distribution pattern in the Pichavaram area during Post-monsoon season

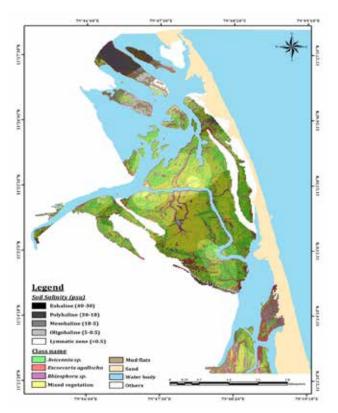


Fig. 5b. Soil salinity (*psu*) distribution pattern in the Pichavaram area during Summer season.

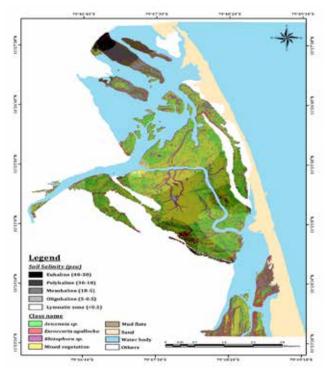


Fig. 5c. Soil salinity (*psu*) distribution pattern in the Pichavaram area during Premonsoon season.

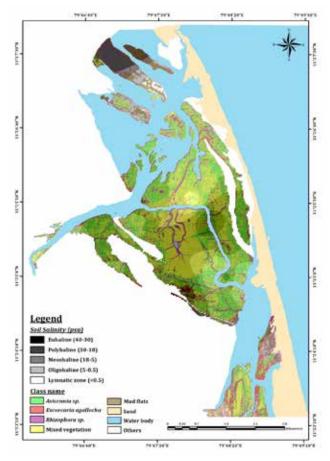


Fig. 5d. Soil salinity (*psu*) distribution pattern in the Pichavaram area during Monsoon season

## Discussion

Avicennia marina and Avicennia officinalis were the two Avicennia spp. found in Pichavaram mangrove ecosystem, of which the former species was found to be salinity tolerant and dominated than the latter species. The brackish water that accumulates in the bowl-shaped mangrove soil substratum during monsoon makes the system hypersaline during summer. This situation is evident in degraded areas at Pichavaram where the soil salinity goes up to 100 psu (MSSRF, 1998). Two distinct zones of *Rhizophora* and *Avicennia* spp. can be identified in the study area, where *Rhizophora* zone occurs along the tidal creeks and channels with a width ranged between 5 and 12 m and height of 4-7 m. The zone pattern of *Avicennia* starts behind the *Rhizophora* zone showed the width ranged between 20 and with a pure community accounting for more than 9.54 ha of the area. Along the river bed of the study area, *Rhizophora* spp. was found dominant and the soil salinity recorded as moderate. *Avicennia marina* also found to be dominant in those areas, but the soil salinity was recorded as moderate to high. Considerably, Importance Value Index (IVI) were highly recorded for *Avicennia* spp. (99.59) followed by *Rhizophora* spp. (98.27). Other species occupied 55% in the Pichavaram mangrove ecosystem were recorded, of which *Excoecaria agallocha* (15.65) showed low IVI.

The area of Avicennia spp. occupied 9.54 ha and Rhizophora spp. 3.31 ha were calculated through remote sensing and GIS. The wetland has monospecific domination of Avicennia marina (Forsk.) vierh., while other species like Rhizophora mucronata, Rhizophora apiculata and Excoecaria agallocha showed their presence but in low abundances. (Kathiresan, 2000; Selvam et al., 2002). The dominance of Avicennia spp. in the inwards side might be due to monoculture practice of afforestation taken place in the past. Large mudflat formation found along the Pichavaram mangrove wetland and Vellar estuary showed contrary to beach formation from direct interaction with the Bay of Bengal. Similarly, tidal water inflow to the wetland is reduced due to the formation of new sand spit in the mouth of river Coleroon (Kathiresan, 2000). From field observation, intertidal mudflats are found between mangroves and hence high tidal mudflat was observed on the northern and eastern part of Pichavaram.

Soil salinity range in sundarbans (17.3 psu and 23.8 psu) correlates with optical salinity and high species diversity (Matilal *et al.*, 1986; Pal *et al.*, 1996). Cintron et al. (1978) reported that salt tolerant species like *Avicennia* spp could be adapted up to 90 psu soil salinity, but their growth is very less. Higher soil salinity was recorded in summer (April-June) of 35.64 psu showing the seawater influence from the mouth of Vellar, Coleroon and Chinnavaikal, whereas it is lowered (12.09 psu) during monsoon season (October-December). In Pichavaram mangrove ecosystem, an exclusive patch of *Rhizophora apiculata* and *Rhizophora mucronata* was found in

sheltered areas especially along the creeks and main canal, where the tidal currents have an impact with 3.31 ha area. *R. apiculata* and *R. mucronata* were found dominating the outer part of the mangrove island facing the seaward area. This indicates that mangroves can survive in a terrestrial environment with slightly higher soil salinity. The seasonal salinity maps have not showed any drastic variations in Pichavaram mangrove ecosystem, unlike in the past.

Spatially interpolated seasonal soil salinity map shown the gradient of soil salinity and 50% overlay of mangrove zonation map. The visualization of the mangrove zonation with soil salinity gradient can be easily understood in GIS platform. In Premonsoon and Postmonsoon seasons, the maps were overlayed and there was no much drastic variation in soil salinity were observed when compared to Monsoon and Summer. Importance Value Index map (i.e. Relative frequency, Relative dominance and Relative density) also showed the growth of mangroves in Pichavaram mangrove ecosystem. From the central part of Pichavaram mangrove ecosystem, rich vegetation was found and moved towards the seaward side which decreased mangrove vegetation gradually. Preferably, the zone of a pattern of Pichavaram mangrove ecosystem expressed the whole region and based on the literature study there were no evidence of GIS-based soil salinity gradient along vegetation pattern in the present study area.

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# A note on Performance of *Annona* Hybrid 'Arka Sahan' under South Andaman Condition

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## Abstract

To evaluate performance of *Annona* hybrid 'Arka Sahan' under island condition, grafts of this hybrid were introduced in Andaman islands during 2015. Studies suggested that, during initial period, the grafts established well without exhibiting any mortality. Vigorous vegetative growth was observed and partial fruit set was noticed even without manual pollination. Fruit physicochemical parameters were also studied in this communication. However, plants suffered due to dry stem rot and an unidentified fruit and stem borer pest, causing significant mortality in the plants. This report would serve as a reference for planning any research in future on this hybrid under island condition.

Keywords: Bay islands; fruits; fruit quality; natural pollination

## Introduction

Annona hybrid 'Arka Sahan' is the first tri-specific hybrid developed at ICAR-Indian Institute of Horticultural Research Institute, Hessarghatta, Karnataka. The hybrid is characterized by large sized fruits, less number of seeds per fruit and better shelf life. Further, the hybrid requires assisted pollination to facilitate fruit set and development (Jalikop and Kumar 2007). The hybrid is getting popular in a number of states in mainland India. Being a hybrid, its performance in newer areas needs to be evaluated before it could be promoted on large scale.

Soil and climatic conditions of Andaman and Nicobar Islands have been reported to favour growth and development of species of Annonaceae family and *A. squamosa*, *A. reticulata*, *A. glabra* and *A. muricata* are being grown in these islands (Singh *et al.* 2016, Bohra *et al.* 2019). These species are found in a number of backyard gardens in the islands, while species such as *A. reticulata* are commonly noticed in places such as Long island. Considering these, an attempt was made to introduce the hybrid to evaluate its performance under island condition.

## Materials and methods

Grafted plants of *Annona* hybrid 'Arka Sahan' were introduced from the ICAR-IIHR, Hessarghatta and hardened for one year. Experiment on performance

evaluation was initiated during 2015 at two spacing (5.0 m  $\times$  2.5 m and 5.0 m  $\times$  5.0 m). Two years old grafts were planted in pits of size 60 cm  $\times$  60 cm  $\times$  60 cm filled with soil and well rotten farmyard manure (20 kg). Grafts were grown under rainfed condition and only protective irrigation was given during dry period. After three years of planting, growth parameters such as mean plant height (m), mean number of branches per plant, mean collar girth (cm) and mean canopy spread (m) were measured following standard procedures.

No hand pollination was carried out and fruits were allowed to set during 2018 and fruit related parameters were recorded in the set fruits. Fruit weight (g) was recorded using electronic balance; while fruit polar length (cm), polar circumference (cm), equatorial length (cm) and equatorial circumference (cm) were measured using scale. Fruits were cut opened, rind was removed and its thickness was measured using vernier caliper. Pulp weight (g), seed weight (g) and mean seeds per 100 g pulp were determined. Total soluble solids content (°B) of pulp was determined using hand held refractometer. Results obtained during present study were compared with the report published from ICAR-IIHR, Bengaluru. Symptoms of a fungal disease were observed during the experimentation. Pure cultures of pathogen were isolated on potato dextrose agar medium and samples were identified from National Centre for Fungal Taxonomy, New Delhi.

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Spacing (m <sup>2</sup> )	Mean plant height (m)	Mean number of branches per plant	Mean collar girth (cm)	Mean canopy spread (m)
5.0 × 2.5	$1.8 \pm 0.08$	$5.9 \pm 0.70$	$17.8 \pm 0.35$	$2.3\pm0.09$
5.0 × 5.0	$1.6 \pm 0.21$	$7.8\pm0.73$	$18.6 \pm 1.20$	$2.1 \pm 0.19$

## Table 1: Field performance of Annona hybrid 'Arka Sahan' under island condition after three years of planting

Parameters	Andaman#	Bengaluru*	West Bengal**
Fruit weight (g)	233	285-296	162
Fruit polar length (cm)	7.4	-	8.7
Fruit polar circumference (cm)	24.6	-	-
Fruit equatorial length (cm)	8.4	-	7.6
Fruit equatorial circumference (cm)	25.6	-	-
Total soluble solids (°B)	25	26-32	24.8
No. of seeds/ 100 g fruit weight	8.2	5.9-6.9	-
Mean Seed weight (g)	0.25	0.29-0.36	-
Pulp weight (g)	171.6	-	-
Pulp (%)	73.7	-	52.0
Skin thickness (cm)	0.35	0.16-0.17	-

# naturally pollinated condition, \*Jalikop and Kumar (2007), \*\*Nandi et al. 2018

## **Results and discussion**

During initial period of establishment, grafts grew well and vigorous growth was noticed. Only two plants were found to die during establishment and gap filling was done, both of which survived. After three years of planting, plants attained an average height of 1.6-1.8 m. At this stage, about 5.9- 7.8 branches were produced in each plant. As the plants during this phase were small and their canopies did not overlap, variations observed in growth of plants could not be attributed to spacing. Collar thickness of the plants varied between 17.8 and 18.6 cm, while canopy spread of 2.1 to 2.3 m was recorded.

Fruiting was observed to certain extent in plants without manual pollination. Fruits (Fig. 1) were comparatively smaller (233.0 g) than that reported

earlier (Jalikop and Kumar 2007) from crop grown under Bengaluru condition (285-296 g), probably due to absence of manual pollination. Island grown fruits had polar length of 7.4 cm, polar circumference of 24.6 cm, equatorial length of 8.4 cm and equatorial circumference of 25.6 cm. Fruits grown in the island had less TSS (25.0 °B) than that grown in Bengaluru (26-32 °B). Fruits had more number of seeds per 100 g fruit weight (8.2), than those reported from Bengaluru (5.9-6.9). However, seed size was smaller in island grown fruits as mean seed weight of 0.25 g was noticed in these fruits, when compared to 0.29 to 0.36 g in those grown in mainland India. Each fruit had about 171.6 g pulp, which contributed to 73.7% of fruit weight. Under West Bengal condition (Nandi et al., 2018), fruits of 'Arka Sahan' were much smaller (162 g) with less pulp content (52.0%) than that reported in the islands. Varied performance of cultivars in new



areas is due to climatic variations and tree physiological factors (Rymbai *et al.* 2014) and hence, fruit parameters of 'Arka Sahan' considerably varied in areas tested. Thicker fruits provide advantage in distant transportation of fruits and hence, the thicker skinned fruits (0.35 cm) observed in islands could be of advantage.



## Fig. 1: Fully developed fruits of *Annona* hybrid 'Arka Sahan' under island condition

However, plants and fruits were found to be infested with unidentified pest, causing considerable damage. The larvae of the said pest bored into fruits and stem, which was noticed in the form of exudation of frass from the damaged area. Further, stems in some plants exhibited dry rot symptoms, thereby causing mortality of plants. Branches started gradually drying up due to both pest and disease. The pathogen was identified as Phomopsis annonacearum, which was not reported earlier from the islands. As no chemical insecticides/ fungicides were available for control in the islands, within a period of few months the pest and pathogen could spread in the complete orchard thereby killing all the plants. Hence, further investigations could not be carried out regarding identification of the pest or developing management strategies for it. Under West Bengal condition, bacterial wilt has been reported in plants of 'Arka Sahan' with plant mortality to the tune of 20% (Nandi et al. 2018).

## Conclusion

From the present investigation, it could be concluded that, growth and performance of the hybrid was

satisfactory in the initial period under island condition. Further, fruit set was also noticed with acceptable fruit qualities in island grown plants. Manual pollination could help in improving fruit parameters in future. However, considering the pest and pathogen issues, the hybrid is not recommended for cultivation in the islands in the absence of suitable plant protection package.

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