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# CONTENTS

# EDITORIAL

The year 2012 has been observed as the Year of Forest Biodiversity. Forest diversity means, different types of forests that exist in the ecosystem and the species diversity happens to be one of the major component to it. Forest biodiversity is extremely complex, dynamic and varied like no other feature of the earth. Its innumerable plants, animals and microbes physically and chemically unite the atmosphere (the mixture of gases around the earth), geo-sphere (the solid part of the earth), and hydrosphere(the earth's water, ice and water vapour) into one environmental system which makes it possible for millions of species, including humans, to exist.

If we look around the entire earth, there still exists some of the wonderful forests. Some of the best forest biodiversity repositories are in Amazon forests, Africa, Scandinavian forest, Russian Taiga, Cargo basin forest, Africa etc. Also, forests of New Guinea, Bulgeria, Romania, Switzerland and Califonia represent one of the finest floristic resources of the globe. In India, Sundarban, Kajiranga, Shola of Tamilnadu, Western Ghat, northern Himalayas, Arunachal etc. contribute significantly to the rich forest biodiversity of the sub-continent. If we look into Odisha, we have beautiful forest resources in Similipal, Satkosia, Bhitarkanika, Daringibadi, Mahendragiri, Niyamgiri, Deomali, Kuturmali etc.

Amidst benefits that we get from forest which acts as biggest carbon sink, gives food and shelter, holds soil, initiates rainfall etc., the important contribution is that it gives overall cooling effect to earth. According to FAO,2000 and IPCC 2002, 50% of terrestrial carbon stocks reside in forest ecosystems (biomass living and dead, both above and below ground and soil carbon); with much of the remainder in pit lands and wet lands. The significance of the forest carbon reservoir protecting the current stock of carbon in forests and other natural ecosystem, along with deep cut in fossil fuel emissions and reduction in total global anthropogenic emissions are highly necessary to face this dangerous climate change.

The area of land covered by forest is about 30% amounting to just under 40 million $km^2$. Forest area is decreased worldwide by 0.22% per year in the period 1990-2000 and 0.18% per year between 2000- 2005. Forest cover is mainly decreased because of agricultural activities and human settlements. Human activities have dramatically influenced the feature of the earth. The humanbeing as well as the entire living creation of nature will be affected drastically with the rampant anthropogenic pressure on forest habitat especially the biodiversity.

However, the net loss of forest is slowing down as a result of plantation and expansion of natural forests. Proper planning over sustainable forest management should be drawn in time. The forest managers and policy makers should invite a public debate. Forest and biodiversity research activities should be fastened. Participatory forest management has a major role to play in this regard. To enrich the global forest biodiversity, agricultural activities should be curtailed through high yielding crops. Conservation of traditional, endemic and indigenous species should be geared up. Industry, transport and mining activities should be checked at par with the resource utilization. Strict promulgation of wildlife laws against poaching of wildlife and smuggling of timber should be enforced. Forest fire should be checked. Recommendations of climate change, biodiversity and environmental meets should fast be accepted by the developed countries in true spirit. Then only, the celebration of the Year of Forest Biodiversity would be meaningful.

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

# EFFECT OF DRIVERS OF DEFORESTATION AND FOREST DEGRADATION ON FOREST CARBON STOCKS IN COLLABORATIVE FORESTS, NEPAL

**R A Mandal\*, I C Dutta, P K Jha, S B Karmacharya, K K Yadav, B Yadav and S Haque**

Chief of the TU Service Commission, Nepal

*ram.mandal@gmail.com*

## ABSTRACT

There are some key drivers that favor deforestation and forest degradation. Consequently, levels of carbon stock are affected in different parts of same forest types. But the problem lies in exploring the extent of the effects on level of carbon stocking. This paper highlights the variations in levels of carbon stocks in three different collaborative forests of same forest type i.e. tropical Sal (*Shorea robusta*) forest in Mahottari district of the central Terai in Nepal. Three collaborative forests namely Gadhanta-Bardibas, Tuteshwarnath and Banke-Maraha Collaborative Forests (CFMs) were selected for research site. Interviews and workshops were organized with the key informants that include staffs, members and representatives of CFMs to collect the socio-economic data and stratified random sampling was applied to collect the bio-physical data to calculate the carbon stocks. Analysis was carried out using statistical tools. It was found that five major drivers of deforestation and forest degradation namely grazing, fire, logging, growth of invasive species and encroachment. Highest carbon stock was found to be 269.36 t ha$^{-1}$ in Gadhanta- Bardibash CFM. The findings showed that the levels of carbon stocks in the three studied CFMs were different depending on how the drivers and management units influence them.

**Key words :** Collaborative forests, REDD+, deforestation, degradation, carbon stock

## INTRODUCTION

Reducing Emissions from Deforestation and Forest Degradation (REDD+) is considered as the major effective and efficient measure to address the issues of climate change but the clear analysis of drivers of deforestation and forest degradation and adapting the appropriate management options. In fact, avoiding deforestation and forest degradation can reduce 17.4% of Green House Gases (GHGs) emission globally (Anon, 2006).

The global net change in forest area in the period of 2000–2010 was estimated 5.2 million hectares annually (Anon, 2010). Deforestation is estimated to be the cause of 20% of annual greenhouse gas emissions in 1990s (Anon, 2006). The average deforestation rate in South and Southeast Asia was about 1% over the period 2000-2005. The gross deforestation during the 1990s was 2.8 million ha in Asia (Chomitz *et al.* 2007). Reports of tropical deforestation indicate that it occurs in diverse circumstances which obscure underlying patterns of deforestation and it has multiple causes with the particular mix of causes varying from place to place (Rudel and Thomas, 1995). The forest land has been converted into other land use categories, especially shrub-land and agriculture land. It is estimated that about 84,000 hectare of forest land becomes deforested annually between 1991 and 2001(Anon, 2008) in Nepal. Out of this annual deforestation 10000 hectares was in Terai. The annual deforestation rate in the Terai is estimated to be 1.7 %.

The nature of drivers and underlying causes of deforestation and forest degradation are differed even in different collaborative forests. The study undermines, how they are differed, what are the common drivers in these collaborative forests and what are resultant effects on forest carbon stock. Moreover, it is remarkable fact that if effective activities are carried out by the management and protection units it can play a vital role to counteract against the drivers and underlying causes of

deforestation and forest degradation. So, how these organizations are functioning and at what level they are affecting forest carbon stocks have been studied.

## MATERIALS AND METHODS

### Research site

Mahottary district in central part of Nepal was selected for study area. This district is situated in 26° 36' to 28° 10' North and 85° 41' to 85° 57' East. The temperature ranges between 20-25$^0$Celsius and average annual rain fall recorded between 1100-3500 mm. Three collaborative forests namely Banke-Maraha, Tuteshwarnath and Gadhanta- Bardibash collaborative forest(CFM) in Mahottary district were selected for the work.

### Data generation and analysis :

Socio-economic and bio-physical data were collected for the purpose.

### Socio-economic data

The key informants interview and workshops were organized to gather these data. Four workshops were organized to finalize the list of drivers of deforestation and forest degradation with staff of district forest office and three collaborative forest users. Similarly data regarding the forest management and protection units were gathered from informal interaction with local people.

### Bio-physical data

The stratified random sampling was applied to gather the bio-physical data. So, collaborative forests were divided into three main strata namely regeneration, pole and tree strata based on condition of the forest. The pilot sampling was carried out to calculate the required number of sample plot (MacDicken, 1997) measuring 15 sample plots from each stratum of collaborative forest based on co-efficient of variance (Moore *et al.*, 2003). Altogether, 73 samples were collected from collaborative forests. Thirty samples were collected from Banke- Maraha CFM, 21 and 23 sample plots were collected from Tuteshwarnath and Gadhanta –Bardibas CFMs respectively. For this, sample plots were established in the field by navigating the uploaded GPS coordinates. So, the plots were fixed according to the nature of the stratum. If it is tree stratum firstly 20m x 25m sample plot was established and nested plots for poles (10m x 10m), sapling (5m x 5m), seedling (5m x 2m) and litter, herbs and grasses (1m x 1m) were established simultaneously. Similarly, soil sample was fixed in the centre of plot. Height and diameter of sapling, poles and trees were measured in fixed sample plot. Then seedlings, herbs and shrubs were counted and their fresh weights were taken.

Apart from this, soil samples were collected from four different depths 0-10, 10-30, 30-60 cm in order to determine the soil carbon. Bio-physical data were used to determine variation in forest carbon in the collaborative forests due to drivers of deforestation and forest degradation.

The forest biomass was calculated using equation of Chave *et al.* (2005), Above Ground Tree Biomass $AGTB = 0.0509 x \rho D^2 H$, whereas, $\rho$ is wood specific gravity in [g/cm³], D > dbh ( 5cm) and H = height of tree (m). The seedling, sapling (dbh < 5cm), litter, herbs and grasses biomass were calculated by drying the samples in lab. Meanwhile, the root biomass was calculated by using Root Shoot Ratio 12.5% (MacDicken, 1997)

Total Biomass = ABTB + Dry weight of (AGSB + LHS)+ Root Biomass. The biomass was converted into carbon by 0.47% (IPCC, 2006)

Soil Carbon Estimation : Carbon content in the soil was analyzed by Walkley Black Method.

Bulk Density (BD g/cc) = (oven dry weight of soil)/ (volume of soil in the core). It was expressed in tons per ha.

SOC= Organic Carbon Content % x Soil Bulk Density (Kg/cc) x thickens of horizon.

Total Carbon= Total Biomass carbon + Soil carbon

## RESULTS AND DISCUSSION

### Drivers of deforestation and forest degradation

It was found that there were five major drivers and twelve causes are major underlying causes.

## Fire and grazing

These are very common factor which influence to increase the deforestation and forest degradation. It was seen that fire is common in all collaborative forest but grazing was less common in Gadhanta-Bardibash CFM. The underlying causes of the fire is intentional fire and carelessness while the underlying causes of grazing were keeping high number of low productive livestock, limited alternatives for fodder and grasses and open grazing.

## Invasive species

It was observed that where canopy was opened and fire and grazing pressure became high, the invasive species flourished well. So, there was high pressure of this in Tuteshwarnath collaborative forest and it was followed by Banke-Maraha collaborative forest and Gadhanta- Bardibash CFMs.

## Logging

Looking to the underlying causes of illegal logging there are many causes. They are: poverty and lack of livelihood alternatives, limited access to alternatives for fuelwood and timber, inefficient forest fuel wood and timber use, weak law enforcement and impunity due to weak governance, inefficient distribution mechanisms for timber and firewood, high cross border demand for forest products, insufficient technical inputs, greediness of the people (staff and others even police) and increasing unemployment.

Illegal logging has serious effects to increase the rate of deforestation and forest degradation. Indeed, such types of effects are also common in all collaborative forest however intensity of effects was differed. It was found that, there was high logging in Banke Maraha and Tuteshwarnath CFM than Gadhanta-Bardibash CFM.

It was found that logs were generally collected illegally from Tuteshwarnath and Banke- Maraha collaborative forests exported to India. The export of timbers to India was very common in Tuteshwarnath CFM and in Banke-maraha collaborative forest and nil in Gadhanta-Bardibash collaborative forest. There was a bitter fact that the illegal loggers use the students studying in grade 9, 10 and 11 from Khayarmara higher secondary school in illegal logging from Banke- Maraha collaborative forests.

Rate of Timber per cubic feet (NRs. 4000-5000) in India while local price was only NRs 500-800 and government royalty was only 800.

Though Gadhanta- Bardibash collaborative forest is very close to growing small town Bardibash but most of people here used biogas for cooking and some hotels used firewood. Generally this firewood is brought from Sagarnath Plantation Project and Banke Maraha and Tuteshwarnath CFM areas.

Moreover, annually about 5-10 houses were constructed at Bardibash and timbers for these constructions work were supplied from community forests and government managed forests of Churia area. So there is very low pressure of firewood collection on Gadhanta- Bardibash CFM than others.

## Encroachment

This activity was seen common in all collaborative forests however underlying causes were not common. For instance local and temporary market was affecting Tuteshwarnath and Gadhanta-Bardibash CFM but it was absent in Banke-maraha CFM. Temples were made in all collaborative forests while old settlements were more influencing in Banke-Maraha and Bardibash collaborative forests but it was not found in Tuteswarnath CFM. Altogether 10.1 ha forest area was deforested, out of this 2 ha was seen Tuteshwarnath CFM, 5.5 ha in Banke-maraha CFM and 2.5 ha were in Gadhanta-Bardibash CFM. The arms police camp is built at Gadhanta- Bardibash CFM area and temporary arms police post is at Banke- Maraha and it is absent in Tuteshwarnath CFM area. East-west highway and high tension line are common in all CFM.

## Effects of drivers of deforestation and forest degradation on forest carbon :

Drivers of deforestation and forest degradation have high effect on carbon stock of collaborative forests. It was found that loggers steal the logs once/ twice a month and in rainy season from Tuteshwarnath CFM while in case of Banke- Maraha CFM, loggers are targeting to steal the logs when there are festivals and rainy season. In case of Gadhanta- Bardibash CFM, stealing of logs was rare sometimes in Dashain and Tika festivals.

**Effects of protection and management unit**

The Management and Protection organizations are also playing vital positive role to reduce the deforestation and forest degradation. In this context, there are five major institutions namely CFM representatives, Range post, Ilaka, District Forest Office and Security (arm police camp) are functioning to control the illegal logging.

These institutions have not effective equally in protection and management of these collaborative forests. It was found that patrolling was conducted jointly by these institutions to control the illegal logging of these collaborative forests. However, it was most effective in Gadhanta- Bardibash CFM in comparison to others. Though there were range post and representatives in Tuteshwarnath CFM, there was less patrolling work here than other CFM areas. Patrolling work is also irregular in Banke- Maraha CFM, although there are Ilaka, Rangepost, temporary arms post and watcher of CFMs. The loggers are more active during the festival and in rainy season when protection units have difficulties to organize the patrolling in leave to drive the vehicles in rainy seasons.

Other noticeable fact was staff of Tuteshwarnath and Banke- Maraha CFM were very irregular because of less monitoring and evaluation system but it was found reverse in Gadhanta- Bardibash CFM area. The reason of the irregularity was low payment and expensive fare to reach Tuteshwarnath and Banke- Maraha CFM while Gadhanta- Bardibash CFM area is close to Bardibash Market where most of staffs stay. It was heard that sometimes greedy staff also involved in smuggling activities it was more frequent in Banke- Maraha and in Tuteshwarnath CFM area.

Moreover, CFM representatives also play a vital role to control the illegal logging. It was found that representatives of Gadhanta- Bardibash CFM were very active but it was less active in Banke- Maraha CFM and Tuteshwarnath CFM.

**Damage due to different types of drivers**

Grazing and fire, invasive species generally damaged the under growth of the plants while logging affected the pole and tree stage. The former reasons have no significant effect to cause deforestation and forest degradation but logging contributes considerable damage and resulted on the carbon stock of CFMs. It was found frequent logging in Tuteshwarnath and Banke- Maraha CFMs which consequences low carbon stock. In addition, the encroach affect as conversion on forest to other lands.

**Forest Stock Density and Carbon Stocks in Collaborative Forests :**

**Forest Stock Density in CFM (Number of individual per ha)**

It was found that, there was highest number seedlings ha^{-1} of (1822) in Gadhanta- Bardibash CFM and sapling (1525) in Tuteshwarnath CFM while it was lowest number of seedling and sapling in Banke- Maraha CFM that were 450 and 600 respectively. However, there was highest number of poles and trees in Gadhanta- Bardibash CFM 523 and 105 respectively and least individuals in Banke- Maraha CFM.

**Table 1 :** Forest Carbon Stock Density (C t ha^{-1})

| CFM | Seedling | Sapling | Pole | Trees | LHG | Soil | Root | Total |
|---|---|---|---|---|---|---|---|---|
| Banke- Maraha | 6.792 | 7.022 | 23.531 | 79.379 | 4.210 | 61.062 | 15.117 | 197.113 |
| Tuteshwarnath | 3.578 | 6.124 | 48.133 | 81.958 | 3.603 | 61.260 | 17.924 | 222.580 |
| Gadhanta Bardibash | 5.453 | 7.139 | 63.274 | 103.020 | 6.325 | 66.307 | 23.151 | 274.670 |

Carbon stocks in herbs, shrubs, grasses, seedling, sapling and root were very low but there was high contribution of pole, tree and soil carbon. The estimated carbon stock of pole biomass was in Gadhanta- Bardibas CFM (63.274 C t ha$^{-1}$) which was followed by Banke- Maraha CFM (23.531 C t ha$^{-1}$) and Tuteshwarnath CFM (48.133 C t ha$^{-1}$). Generally, there is highest contribution in carbon stock of tree biomass in the forest of these collaborative forests. It showed that there was highest carbon stock in Gadhanta- Bardibash CFM (nearly 103.020 t C ha$^{-1}$) and lowest in Banke- Maraha CFM (almost 79.379 t C ha$^{-1}$). It was found medium C stock in Tuteshwarnath CFM (about 81.958 t C ha$^{-1}$). It was found 66.307, 61.260 and 61.062 t C ha$^{-1}$ in Gadhanta- Bardibash, Tutesharnath and Banke- Maraha CFM respectively.

The estimated total carbon stock 274.670 t ha$^{-1}$ was highest in Gadhanta- Bardibash CFM while it was least 197.113 t C ha$^{-1}$ in Banke- Maraha CFM and the estimated carbon stock of Tuteshwarnath CFM was found about 222.580 t C ha$^{-1}$. The estimated total highest C stock of 550988.02 t found in Banke – Maraha Collaborative Forest having area with 2006 ha while it was least C stock 285813.85 t in Gadhanta- Bardibash was 1450 ha and the total estimated C was 296921.72 t in Tuteshwarnath CFM having area 1334 ha.

**Drivers of Deforestation and forest degradation**

The main drivers of deforestation and forest degradation was illegal logging, grazing, fire, invasive species, encroach in theses collaborative forests and underlying causes are opening crown, intentional fire, market failure, weak governance, increasing population and poverty. This finding was supported by different types of studies. It was found similar types of drivers of deforestation and forest degradation in Readiness Plan Idea Note (R-PIN) (Baral *et al,* 2008) and there are nine major drivers listed by the preliminary report during the preparation of Readiness Preparation Proposal (R-PP) (MoFSC, 2010), Nepal.

**Effects of Forest Management and Protection Unit in Forest Management**

The protection and forest management units have major function to control the illegal logging in the forest. Generally, forestry staffs, executive committee's members of collaborative forests have been taking the help of security forces. So, there are positive impacts on forest conservation. However, some times, more damage in Banke –Maraha and Tuteshwarnath CFM was noticed in comparison to Gadhanta- Bardibash CFM. In fact, intensive and regular patrolling works were organized collectively by these protection and management units to control the deforestation and forest degradation which was observed more effective in Bardibash- Gadhanta CFM than others, so it was highest carbon stock in Gadhanta- Bardibash CFM than others. Hence, collective actions are fruitful to control deforestation and forest degradation and restore the affected forests. This also has impact on the participation of members of collaborative forests and the strength behind its members to have right to collect and trade the forest products. The Agrawal and Ostrom (2001) stated that, the coalition of actors in forest management respecting the local knowledge can play a vital role to add work efficiency in halting the deforestation and forest degradation and storing degraded resource.

**Variation in Carbon Stocks in Forests**

The estimated carbon stock per ha was highest 274.670 t in Gadhanta- Bardibash CFM while it was least 197.113 t C ha$^{-1}$ in Banke- Maraha CFM and the estimated carbon stock of Tuteshwarnath CFM was found about 222.580 t C ha$^{-1}$.

The pilot study done in Kayarkhola watershed in community forest showed that 276.5 t C ha$^{-1}$, the inventory done in 2011 (Rana, 2011) while it was different in studies done in Terai Arc Landscape which was 206.15 t C ha$^{-1}$ in government managed forests, 240 t C ha$^{-1}$ in community forests and 274.58 t C ha$^{-1}$. The inventory was carried out in 2010 (Manadhar, 2010).

**Table 2 :** Statistical analysis; ANOVA

| Groups | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 126.804 | 2 | 63.402 | 7.017 | .001 |
| Within Groups | 840.297 | 93 | 9.035 | | |
| Total | 967.101 | 95 | -- | -- | -- |

Above table showed the calculated value of F< F critical value at 5% confidence interval. Therefore $H_0$ hypothesis is rejected and $H_1$ hypothesis is accepted to prove the effects of drivers of deforestation and forest degradation as well as management and protection units on forest carbon stocks significantly.

## RECOMMENDATION

The smuggling of timbers was common in all these collaborative forests but it had more negative impacts on Banke- Maraha and Tuteshwarnath CFMs because of export of timber to India. Similarly, protection and management units were very effective in Gadhanta – Bardibash CFM but less effective in Banke- Maraha and Tuteshwarnath CFMs.

Therefore, the local drivers and underlying causes of deforestation and forest degradation options should be identified strategically to improve the forest carbon stock of collaborative forests.

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# BREEDING BIOLOGY OF OLIVE RIDLEY SEA TURTLE (*Lepidochelys olivacea*) AND ITS CONSERVATION ALONG RUSHIKULYA ROOKERY IN ODISHA, INDIA

**U K Das\*, A K Nayak and R K Samantaray**

Research Personnel, DGH Sea Turtle Telemetry Project, WII, Dehradun - 248001, Uttarakhand

Former Veterinary Officer, Nandankanan Biological Park

*udayak.dash@gmail.com*

## ABSTRACT

The Odisha coast has played a host for the olive ridley sea turtles since time immemorial and hence facilitated many of their biological processes ranging from mating to nesting. The various activities influencing the sea turtle population along Rushikulya coast have been recorded and analyzed. Rushikulya rookery came to lime light during early 1990s. Under the study 20% sample method was under taken by counting the sample segments and putting minimum human efforts during mass nesting in Rushikulya rookery during 2009. Before mass nesting some of early nests were replaced by constructing artificial hatcheries at different locations to save nests from predation. Studies were made on the development of hatching processes in co-relation with different environmental factors. Also in each nesting, ovi-position and hatching time including other biological data were recorded during rains, it was observed that incubation was delayed. Net barricades were raised along the sea coast to check mortality due to predation and disorientation by illumination. Hence, this studies could give an insight to carry out accurate population estimation, better hatchery management, successful breeding and reduced casualties.

**Keywords :** Rookeries, arribadas, hatcheries, sea patrolling, environmental factors, no-fishing zones, sporadic

## INTRODUCTION

Olive ridleys are circum global in distribution, and are particularly well known for the phenomenon of mass nesting. The Olive ridley sea turtles (*Lepidochelys olivacea*) is the most populous sea turtles in the world. Out of the total population, around 90% of the turtles habited in the Bay of Bengal waters and migrated to the Indian ocean water up to South of Sri Lanka and coasts of Java, Sumatra and Indonesia. This is the only species of sea turtle which have tremendous capacity to migrate and disperse over vast areas and long distances (Bustard, 1974). The olive ridley sea turtle holds the highest level of protection under the Indian wildlife laws since it is protected under Schedule-I of the Wildlife Protection Act, 1972 as well as is listed under Appendix- I to the CITES and also protected under CMS (Convention on Migratory Species) by the South East Asian countries. The other species of marine turtles that occur in the Odisha coast are the Hawksbill sea turtles (*Eretmochelys imbricata*), the Green sea turtle (*Chelonia mydas*). However the presence of the Leatherback sea turtles (*Dermochelys coriacea*) is not confirmed in the Odisha coast ( Dash and Kar, 1990). To initiate the hatchery management and also to aid in the conservation effort, a study was undertaken in Rushikulya rookery during 2000-2004.

The largest known population of the Olive ridley sea turtles occurs along the Odisha coast (Bustard, 1976, Limpus, 1995). Enormous arribadas have been observed over the past decades in Rushkulya region. Rushikulya being the second largest in Odisha with 60,000 turtles nesting in 1996. The sea beach, north of the Rushikulya estuary from the coast of Purunabandha village to village Kantiagada a stretch of 5 km is the place for mass nesting of Olive Ridley. The *L olivacea* population that occurs along the Bay of Bengal has suffered severely over the past decades. Apart from the threats faced while nesting, marine turtles are particularly vulnerable when they aggregate off shore (Richard & Hughes, 1972; Pers. Obs., 2002). The ever-increasing human induced mortality of several thousand breeding turtles along the coast of Odisha has been an alarming concern over the past several years (Pandav *et al.*, 1998). Incidental capture and mortality resulting from such

capture are currently recognized as important threats to sea turtles (Hilllestead *et al.*, 1982). It is believed to account for more deaths than all other human activities combined (Henwood and Stunz, 1987, Robins, 1995). Mortality in the last 10 years along the Ganjam coast had exceeded 5,000 turtles(Pandav and Chowdhury, 1999).

## MATERIALS AND METHODS

Rushikulya rookery falls between Pattasonapur in south to Prayagi in north in Ganjam district. The survey work was undertaken from 14.03.2010 to 02.05.2011. The entire coast of Ganjam district, spreading over 60 kms was surveyed where in intensive study was taken up along Rushikulya Rookery. To support the study, the olive ridley sea turtles (*Lepidochelys olivacea)* mass nesting breadingbiology and mortality pattern were collected from Feb.1998 to April 2011. The study area was surveyed through beach walk and through and through motor ride to collect the specific data on nesting and mortality. The offshore congregation was surveyedin a country boat and also sometimes using trawlers deployed for patrolling duty. The radial distance of congregating and mating pair of turtles from the sea coast or trawler was measured by the help of a hand held Global Positioning System (Garmin Inc.). Hand held Garmin GPS-72 and on board GPS-198 over fishing trawlers were used for collection of congregation data. The congregation was also view ed from the high seas by using an 8 x 48 high magnification Bushnell binoculars. The depth of water at congregation zone was measured by manually and at times through Fish Finder (Garmin Fish finder-250) used in patrolling vessel.

The entire stretch of beach was divided into 100 metre segments. Every 100 meter spots were marked with poles (Bamboo/Casuarina poles) or even cement bags filled with sands and marked with red or black paint. Then the 20 meter width sample sub-segment was marked and noted as 20 mts in each segment. At the beginning of every hours like 6.00 PM, 7.00 PM,…11.00 PM, 1.00 AM, 4.00 AM, … etc. the turtles in oviposition was counted for the nesting turtles.This count was made from the high tide surf zone up to vegetation zone in the nesting beach. The number of turtles in oviposition stage were noted in field note and demarcated as cross mark. The data intimated to the base camp record room over VHF or through cell phones. The turtles counted after each sampling area marked in a specifiedcolours having aX (cross) mark indifferent colours specified for different roockeries in Odisha. This colour is coded and mandatory for a particular rockery (Red for Gahiaramatha, Yellow for Rushikulya and White for Devi rockery regions). This will help in knowing the inter rookery movement during nesting time and multiple nesting in a season.

## RESULTS AND DISCUSSION

On 14.03.2010, biological data was collected at Rushikulya rookery from activities of 10 number of turtles (Table 1). The oviposition time recorded to be highest i.e. 20 minutes in sample no. 7 and lowest i.e. 13 minutes in sample no. 10. During the mass nesting period, the nesting area was measured with the help of GPS and the area was found to be 134 Ha $\pm$ 3.0 Ha.(season 2010-11 for Rushikulya rookery). Analysis was made in 39 segments from evening till early morning (6 PM to 6 AM). In segments 1 to 20 turtles in oviposition was found to be 0 to 1 (almost nil). From early evening till mid-night oviposition figure enhanced and slowly reducedafter late night. Total number of oviposition was highest from 8 to 11 PM. In segement 31 record number of 63 turtles ovipositioned at 9 PM. and also during the perticular night highest no. i.e. 319 turtles ovipositioned in comparision to all other segements.

A total of 2,53,292 number of turtles did their nesting during the arribada of March 3 to March 9 of the year 2011. Along with the mass nesting, there were about 256 sporadic sea turtle nests counted along the Rushikulya rookery from dt 21/2/2011 to 2/3/2011 (Table - 4).

The mass hatchlings of the nests started from 22 April till 2nd May 2011 (11 days). The incubation period for the breeding season 2010-11 year was calculated from the 1st day of mass nesting and the 1st day of mass hatchling process started. It was revealed that the incubation period was 50 days during the nesting season of 2010-2011 in Rushikulya (Table 5).

**Table 1 :** Biological information collected during Massnesting
Name of the beach: Rushikulya rookery Dt 14/3/2010.

| Sl. | Sample Sl No => | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Time of body pit digging | 2.11 | 7.1 | 7.21 | 8.2 | 6.12 | 10.04 | 3 | 9.1 | 11.45 | 3.27 |
| 2 | Time of completion of body pit | 2.18 | 7.6 | 7.28 | 8.28 | 6.19 | 10.1 | 3.08 | 9.17 | 11.51 | 3.34 |
| 3 | Time of digging the nest hole | 2.21 | 7.9 | 7.32 | 8.31 | 6.21 | 10.12 | 3.11 | 6.21 | 11.53 | 3.36 |
| 4 | Time of completion of nest hole | 2.31 | 7.17 | 7.39 | 8.38 | 6.29 | 10.21 | 3.21 | 6.3 | 8.02 | 3.46 |
| 5 | Time of egg laying | 2.32 | 7.19 | 7.41 | 8.42 | 6.32 | 10.25 | 3.22 | 6.32 | 8.04 | 3.49 |
| 6 | Time of completion of egg laying | 2.48 | 7.35 | 7.57 | 8.58 | 6.48 | 10.42 | 3.42 | 6.51 | 8.16 | 4.02 |
| 7 | Time of packing the nest hole | 2.53 | 7.4 | 8.1 | 9.01 | 6.53 | 10.45 | 3.46 | 6.5 | 8.21 | 4.06 |
| 8 | Time of completing packing the nest hole | 2.59 | 7.48 | 8.08 | 9.09 | 7.01 | 10.54 | 3.57 | 6.57 | 8.29 | 4.14 |
| 9 | Oviposition | 16 | 16 | 16 | 16 | 16 | 17 | 20 | 18 | 14 | 13 |

In the above data time is mentioned in minutes and the decimal in seconds.

**Table 2 :** Number of turtles in oviposition in different sample sgements on 15.03.2010 (6 PM to 6 AM)

| Sample segments | Seg 1-16 | Seg 17 | Seg 18 | Seg 19 | Seg 20 | Seg 21 | Seg 22 | Seg 23 | Seg 24 | Seg 25 | Seg 26 | Seg 27 | Seg 28 | Seg 29 | Seg 30 | Seg 31 | Seg 32 | Seg 33 | Seg 34 | Seg 35 | Seg 36 | Seg 37 | Seg 38 | Seg 39 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6:00 PM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 6 | 2 | 14 | 0 | 25 | 12 | 0 | 23 | 8 | 0 | 25 | 132 |
| 7:00 PM | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 10 | 3 | 6 | 17 | 12 | 60 | 17 | 45 | 19 | 17 | 11 | 28 | 270 |
| 8:00 PM | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 0 | 6 | 0 | 25 | 6 | 38 | 37 | 16 | 25 | 9 | 20 | 23 | 26 | 37 | 32 | 338 |
| 9:00 PM | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 13 | 9 | 0 | 7 | 1 | 30 | 4 | 43 | 63 | 23 | 23 | 14 | 17 | 24 | 23 | 23 | 30 | 364 |
| 10:00 PM | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 5 | 14 | 0 | 14 | 2 | 15 | 11 | 30 | 46 | 24 | 29 | 29 | 16 | 18 | 28 | 22 | 15 | 343 |
| 11:00 PM | 0 | 1 | 1 | 0 | 0 | 0 | 8 | 4 | 22 | 0 | 17 | 7 | 12 | 16 | 52 | 26 | 17 | 28 | 25 | 14 | 20 | 23 | 23 | 17 | 349 |
| 12:00 AM | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 6 | 0 | 12 | 10 | 22 | 8 | 30 | 33 | 16 | 17 | 31 | 12 | 14 | 30 | 19 | 12 | 293 |
| 1:00 AM | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 0 | 8 | 22 | 14 | 12 | 42 | 32 | 14 | 21 | 29 | 12 | 12 | 17 | 32 | 10 | 296 |
| 2:00 AM | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 3 | 5 | 0 | 9 | 18 | 15 | 32 | 28 | 39 | 10 | 16 | 18 | 10 | 12 | 19 | 25 | 7 | 278 |
| 3:00 AM | 0 | 0 | 0 | 0 | 0 |  | 1 | 1 | 2 | 3 | 10 | 21 | 10 | 18 | 23 | 21 | 18 | 5 | 25 | 10 | 8 | 16 | 18 | 5 | 220 |
| 4:00 AM | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 5 | 2 | 1 | 9 | 23 | 12 | 12 | 15 | 23 | 15 | 12 | 15 | 8 | 10 | 15 | 10 | 7 | 202 |
| 5:00 AM | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 1 | 3 | 24 | 25 | 32 | 12 | 23 | 17 | 13 | 17 | 11 | 8 | 13 | 17 | 5 | 233 |
| 6:00 AM | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 32 | 10 | 42 | 29 | 24 | 30 | 12 | 10 | 9 | 12 | 10 | 10 | 3 | 242 |
| Total | 0 | 1 | 1 | 1 | 1 | 4 | 24 | 42 | 67 | 6 | 96 | 160 | 202 | 202 | 350 | 398 | 212 | 286 | 251 | 184 | 203 | 245 | 247 | 196 | 3560 |

**Table 3 :** Information on nesting, hatching and mortality in Rushikulya rookery (1993-2011)

| Sl No | Breeding season | Mass nesting period | No of Turtles nested | Period of hatching | Mortality |
|---|---|---|---|---|---|
| 1 | 2010-2011 | Dt 03/03/2011 to Dt 09/03/2011 | 2,53,292 | Dt 22-4-11 to Dt 1-5-2011 | 108 |
| 2 | 2009-2010 | Dt 14/03/2010 to Dt 19/03/2010 | 1,56,087 | Dt 4-4.10 to Dt 9-4-10 | 90 |
| 3 | 2008-2009 | Dt 14/02/2009 to Dt 18/02/2009 | 2,60,698 | Dt 4.4.09 to Dt 9.4.09 | 94 |
| 4 | 2007-2008 | Dt 04/03/2008 to Dt 10/03/2008 | 1,80,426 | Dt 24.4.08 to Dt 30.4.08 | 335 |
| 5 | 2006-2007 | No Mass Nesting | 74 (Sporadic) | — | 106 |
| 6 (I) | 2005-2006 | Dt 16/02/2006 to Dt 23/02/2006 - 1st Spell | 1,21,556 9(1st) | Dt 9.4.06 to Dt 17.4.06 1st Phase of hatching | |
| 6 (II) | 2005-2006 | Dt 04/04/2006 to Dt 07/04/2006 - 2nd Spell | 78,238 (2 nd) Total-1,98,794 | Dt 22.5.06 to Dt 27.5.06 2nd Phase hatching | 87 |
| 7 | 2004-2005 | Dt 16/02/2005 to Dt 20/02/2005 | 89,311 | Dt 6.4.05 to Dt 11.4.05 | 217 |
| 8 | 2003-2004 | No Mass Nesting | <250 (Sporadic) | — | — |
| 9 | 2002-2003 | Dt 10/03/03 to 15/03/03 | 2,00,202 | — | — |
| 10 | 2001-2002 | Mass Nesting | 4,744 | — | — |
| 11 | 2000-2001 | Mass Nesting | 1,59,000 | — | — |
| 12 | 1999-2000 | No mass Nesting | 150 (Sporadic) | — | — |
| 13 | 1998-1999 | No mass Nesting | 347 (Sporadic) | — | — |
| 14 | 1997-1998 | Mass Nesting | 8,500 | — | — |
| 15 | 1996-1997 | Mass Nesting | 25,000 | — | — |
| 16 | 1995-1996 | Mass Nesting | 1,18,000 | — | — |
| 17 | 1994-1995 | Mass Nesting | 60,000 | — | — |
| 18 | 1993-1994 | Mass Nesting | 2,00,000 | — | — |

(Source : O/o PCCF (WL) Odisha, Prakriti Bhavan, Nilakantha Nagar, Bhubaneswar.

**Table 4 :** Nesting status and mortality of olive ridley sea turtles in Rushilulya rookery

| Sl.No. | Breeding season (Year) | Nesting total number | No. of death turtles |
|---|---|---|---|
| 1 | 1993-94 | 2,00,000 | 5282 |
| 2 | 1994-95 | 60,000 | 3662 |
| 3 | 1995-96 | 1,18,000 | 4644 |
| 4 | 1996-97 | 25,000 | 6454 |
| 5 | 1997-98 | 85,000 | 13,575 |
| 6 | 1998-99 | Nil | 13,671 |
| 7 | 1999-2000 | Nil | 15,732 |
| 8 | 2000-01 | 1,59,000 | 5,483 |
| 9 | 2001-02 | 35,000 | 12,977 |
| 10 | 2002-03 | 2,00,202 | 10,086 |
| 11 | 2003-04 | 2,01,000 | – |
| 12 | 2004-05 | 89,311 | – |
| 13 | 2005-06 | 1,98,794 | 3,242 |
| 14 | 2006-07 | Nil | 4,046 |
| 15 | 2007-08 | 1,80,426 | 5,763 |
| 16 | 2008-09 | 2,60,698 | 5,680 |
| 17 | 2009-10 | 1,56,087 | – |
| 18 | 2010-11 | 2,53,292 | – |

**Table 5 :** Success rate of hatchlings in a hatchery set up at the Gokharkuda sea beach from 22.4.2012 to 02.05.2010

| Sl No Nests | Clutch size | Hatchlings emmergerd out of the nests | Hatchling live in the nest (out of the egg envelope) | Hatchling died in the nest (out of the egg envelope) | Hatchling live in the nest (within the egg envelope) | Hatchling dead in the nest (within the egg envelope) | Half developed live embryos in the nest | Half developed dead embryos in the nest | Putrefied eggs | Unfertilized eggs |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 128 | 78 | 12 | 8 | 8 | 7 | 5 | 2 | 4 | 4 |
| 2 | 116 | 60 | 16 | 6 | 3 | 6 | 6 | 8 | 3 | 2 |
| 3 | 85 | 35 | 5 | 4 | 6 | 8 | 12 | 6 | 5 | 4 |
| 4 | 112 | 84 | 16 | 3 | 4 | 0 | 0 | 2 | 2 | 1 |
| 5 | 68 | 44 | 12 | 2 | 11 | 30 | 46 | 24 | 29 | 29 |
| 6 | 152 | 100 | 14 | 10 | 6 | 4 | 11 | 4 | 6 | 7 |
| 7 | 79 | 34 | 13 | 3 | 4 | 2 | 3 | 2 | 5 | 3 |
| 8 | 89 | 65 | 5 | 8 | 2 | 2 | 3 | 0 | 3 | 1 |
| 9 | 71 | 41 | 14 | 3 | 0 | 2 | 0 | 2 | 6 | 3 |
| 10 | 134 | 80 | 21 | 4 | 6 | 3 | 3 | 1 | 10 | 6 |

## CONCLUSION

The 10 km radius of the offshore waters from the river mouth region is declared as the buffer zone for the Sea turtle breeding purposes. The coastal water of Rushikulya river mouth from north of Aryapalli to south of Prayagi (Longitude 80 º to 85 º 12´ and Latitude 19 º to 19 º 28´) is declared as protected area from 1st November to 31st May for a period of 7 months each year. The Rushikulya nesting ground have the status to be categorized as CRZ-I (Coastal regulation Zone-I). All the rules and regulations of CRZ Act will be put in this sensitive area and have the legal status to conserve the natural resources and biodiversity of this area.

Despite the ecological and population richness of these sea turtles, there are so many environmental, anthropogenic and ecological stress over the existing sea turtle population in Indian Ocean. The coastal and marine areas have not received adequate

protection, and the coastal ecosystem are fragile and rapidly changing. Rapid urban-industrialization, maritime transport, marine fishing, tourism and coastal aquaculture have led to a significant impact over coastal ecology in the Rushikylya region. These interventions put severe stress over nesting grounds and offshore congregation of sea turtles in coastal waters of Odisha. Such rapid depletion and degradation of coastal ecosystem, unless arrested and kept unchecked, will impact the breeding status of olive ridley sea turtles in particular and coastal bio-diversity in general of the region.

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**Fig.1 :** The view of the Rushikulya beach



**Fig.2 :** A mother olive ridley in oviposition (egg laying)



**Fig.3 :** Mating in Coastal Offshore water.
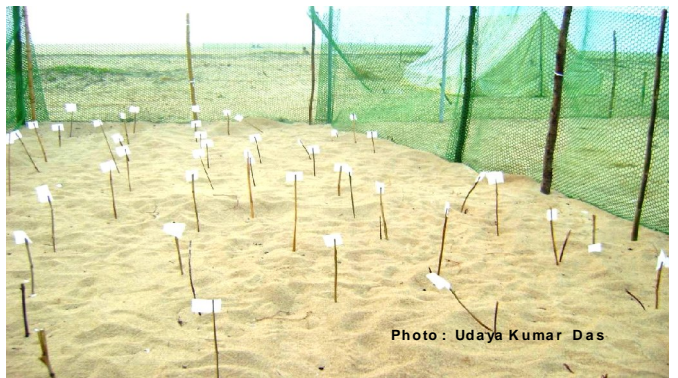


**Fig. 4 :** Hatchery management at Rushikulya



**Fig. 5 :** The configuration of a nest and Egg laying



**Fig.6 :** Baby turtles ready to disperse in sea water and congregate over the nest



**Fig.7 :** Scientific management of nesting beach in Rushikulya rookery
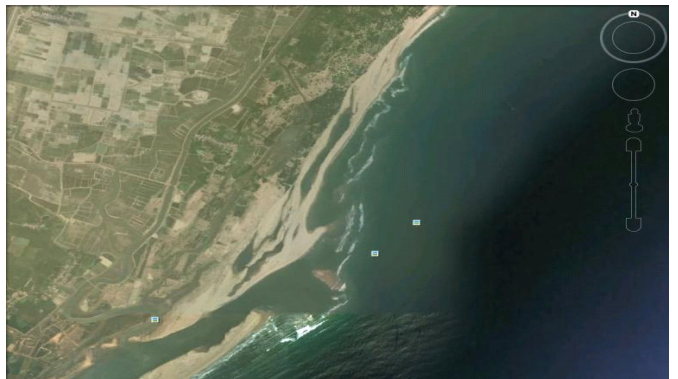


**Fig.8 :** Satellite immagery of Rushikulya rookery

# EVALUATION OF NEEM BIOPESTICIDE TOXICITY IN SPAWN, FRY AND FINGERLINGS OF *Labeo rohita* (HAMILTON)

**A K Swain\*, Swagatika Sahu, Tamasi Senapati and B K Das**

Fish Health Management Division, Central Institute of Freshwater Aquaculture,

Kausalyaganga, Bhubaneswar-751002, Odisha

*anilswain1@rediffmail.com*

## ABSTRACT

Neem biopesticides used in the agricultural field for control of plant diseases are less toxic to other organisms and environment. *Labeo rohita,* the common culturable Indian major carp is the maximum produceable freshwater aquaculture species. Neem biopesticides(Multineem) was taken for toxicity test. Range finding test was conducted at the different stages(spawn, fry and fingerling) of *L. rohita* before toxicity test. The $LC_{50}$ value at 24h, 48h, 72h, 96h exposure period of *L. rohita* spawn was 4.43ppm, 3.12ppm, 2.48ppm, 2.23ppm respectively, for fry 9.39ppm, 8.24ppm, 6.82ppm, 5.95ppm respectively and for fingerling 18.17, 13.59, 11.94, 11.02ppm respectively. The fiducial limits varied from 2.0-4.66 ppm; 5.12-10.05 ppm and 9.75-19.72 ppm in case of spawn, fry and fingerlings respectively. The toxicity vary from spawn to fingerlings of *L. rohita.*

**Keywords :** Biopesticides, multineem, toxicity, *L.rohita*

## INTRODUCTION

Pesticides used in agricultural fields for control of different pests were mostly organic in nature, toxic and create pollution to environment. Biopesticides are pesticides derived from natural products that control pests in agriculture by nontoxic mechanisms. Neem biopesticide is a plant derivative pesticides consists of azadirahtin to control pests in crops.

Plant products were used in aquaculture to control bacterial infections in fish (Behera, 1994; Sahu *et al.,* 1996; Das *et al*., 1999; Das *et al*., 2002). Neem extract is considered of low toxicity towards non-target aquatic life (Martinez, 2002). It has been reported that neem pesticides are target specific and comparatively less toxic, but that long exposure to low concentration of the crude extract of neem delayed the growth of redbelly *Tilapia zilli* (Omoregie and Okpanachi 1997). Studies showed that the recommended dose (5%) of neem seed kernel extract was toxic to Nile tilapia *Oreochromis niloticus*, but neem oil (50% EC at 3 ml/liter) did not appear to harm the fish (Fernandez *et al* 1992). It is found that no Java tilapia *Oreochromis mossambicus* died when exposed at and below 0.01 % neem seed

kernel extract or neem oil (50% EC) (Jayaraj 1992). Neem based pesticide Achook was toxic to zebra fish (Ansari and Sharma 2009). An acute toxicity $(LD_{50})$ of Margosan-O a neem product occurred in rainbow trout *Salmo gairdneri* within 96h in 8.8ml/liter of water and in blue-gill sun-fish *Lepomis macrochirus* within 96h in 37ml/liter of water (Larson, 1987). The $LC_{50}$ of neem oil for carp *Cyprinus carpio* was 302.7ppm at 24h after treatment (Fernandez *et al* 1992).

In the present study, the toxicity of commercially available neem biopesticides (Multineem) in different stages (e.g. spawn, fry, fingerling) of rohu (*L. rohita*) as this biopesticides is commonly used in the agricultural purposes for control of diseases in crop and without any harmful effect to environment and can have some beneficial effect to aquatic environment including carp culture.

## MATERIALS AND METHODS

### Experimental fish

The fish species used in the experiments were Rohu, *Labeo rohita.* Different stages of rohu like spawn (3days old), fry (18days old, avg wt. 3.5±2g) and

fingerlings (75 days old, avg wt. 15±5gm) were used for the total experiments. The fishes were collected from the hatchery and nursery farm of Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, India. Spawn transported in a oxygen filled polythene bags to the laboratory and retained in a glass aquarium of 100l water holding capacity with aeration till acclimatization. After acclimatization in the aquarium live spawns were transferred to experimental glass jars of 1 litre capacity. Fry were transported in oxygen filled polythene bags to the laboratory and kept in a big aquarium of 100l capacity with aeration till acclimatization then transferred to small aquariums of 20l capacity. Fingerlings were transported in open top containers to the field laboratory for the experiment and retained for acclimatization in circular Fiber Reinforced Plastic (FRP) tanks of 300l capacity in the wet laboratory of CIFA. Round the clock aeration was provided to each tank containing experimental fish. The basic physico-chemical parameters of water such as temperature, pH, dissolved oxygen, total alkalinity, total hardness and ammonia etc. were measured systematically to maintain its optimal level (APHA, 1985). The water temperature during the experimental period varied from 24-30°C. Each tank was maintained with 20 numbers of fishes (spawn, fry, and fingerling). During the acclimatization period spawn fed with plankton, fry with powdered feed and fingerling with commercial feed twice daily. For total experiment healthy fishes were transferred to the experimental containers.

## Experimental biopesticide

A commercially available neem biopesticides (neem oil based) which was popularly used by the local farmers "Multineem"(M/S Multiplex Agricare Pvt. Ltd., Karnataka) was purchased from the local market containing Azadirachtin 0.03% E.C. (300 ppm). Various dilutions were prepared separately for the spawn, fry and fingerling from the stock solution and kept carefully for the experiment.

## Experimental containers

All the glass aquariums, jars, plastic pools, air stones, air pipes, were disinfected with 5 ppm potassium permanganate solutions. Spawn were acclimatized

in glass aquarium of 100l in the laboratory condition for one day. During that day the dead spawns were removed from the aquarium. Glass jar of 1l capacity were used for the spawn experiment. Healthy and free swimming spawns were transferred to the glass jar. For fry experiment aquarium of 100l was utilized for the acclimatization to the laboratory conditions. After acclimatization for three day fry were transferred to 20l capacity glass aquarium for the experiment. Fingerlings were acclimatized in 300l capacity FRP tanks in the wet laboratory for 15 days before the experiments in the same tank.

## Experimental design

Initially part of the experiment was range finding test rohu spawn, fry and fingerling with the neem biopesticides. After the range finding bioassay tests 20 nos of spawn, fry and fingerlings were released in the respective experimental containers containing emulsified neem biopesticides to determine median lethal concentrations along with their triplicates and control sets (APHA-AWWA WPCF, 1975). Fish mortalities occurred during the experimental period was noted. The change in behavior, movement, clinical signs and symptoms in each of the experiments at different times were noted and recorded during the experimental period of 96 hours. Neem biopesticide dilution for the spawn, fry and fingerlings experiments were prepared and tested for 96 hours. All the experimental containers were provided with aeration. In each case the control was maintained away from the bio-assay tanks. The percentage of fish mortality in each of the tank and each experiments were recorded. Dead fishes were removed from the tank during the observation. Reservoir available in the CIFA campus water was utilized for the experiments. The water quality parameter such as temperatures, pH, dissolved oxygen, total hardness, total alkalinity and ammonia of experimental water of control tank as well as bio-assay tanks were recorded.

## Experimental analysis

Lethal concentration ($LC_{50}$) were calculated from the fish mortality data. The mortality percentage were converted to probit values and biopesticide

concentration were transformed to log values (Reish and Oshida, 1987; Newman, 1995). Linear regression was derived and from the transformed data the $LC_{50}$ was calculated. As per Reish and Oshida (1987) method the slope function, 95% confidence limit and 95% fiducial limit were calculated. Cumulative mortalities were calculated across treatment duration for each of the three experiment and a one-way ANOVA was done using NCSS 6.0 statistical system Windows (Hintze, 1995).

## RESULTS

### Bioassay test for *L. rohita* spawn

Biassay test for *L. rohita* spawn was conducted after getting the range finding. The experiment was conducted between 2.1ppm to 5.4ppm with 0.3ppm

difference. Both in range finding and bioassay test in control there was no mortality of spawn during the exposure periods. Initial mortality 5% started at 24h at 3.0ppm and 100% mortality occurred at 96h of 2.7ppm(Table-1). The regression equation (y) at 24h, 48h, 72h, 96h exposure period is -165.55+144.22x, -70.55+106.53x, -182.98+254.70x, -71.12+149.77x respectively with slope function response 1.25, 1.34, 1.16, 1.29 respectively. The confidence limit during 24h, 48h, 72h, 96h is 1.05ppm, 1.08ppm, 1.06ppm and 1.11ppm respectively. The fiducial upper limit, lower limit, $LC_{16}$, $LC_{84}$ at different exposure period is presented at Table 2. The $LC_{50}$ value at 24h, 48h, 72h, 96h exposure period of *L. rohita* spawn is 4.43ppm, 3.12ppm, 2.48ppm, 2.23ppm respectively (Fig.1).

**Table 1 :** Cumulative percentage mortality of *L. rohita* spawn exposed to different concentration of Multineem biopesticide at different hours duration.spawn released

| Concen-trations (ppm) | No. of *L. rohita* spawn released | Cumulative percentage mortality | | | | Response | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 24h | 48h | 72h | 96h | 24h | 48h | 72h | 96h |
| 2.1 | 20 | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 0.4 |
| 2.4 | 20 | 0 | 10 | 40 | 60 | 0 | 0.1 | 0.4 | 0.6 |
| 2.7 | 20 | 0 | 35 | 70 | 100 | 0 | 0.35 | 0.7 | 1.0 |
| 3.0 | 20 | 5 | 60 | 100 | 100 | 0.05 | 0.6 | 1.0 | 1.0 |
| 3.3 | 20 | 10 | 60 | 100 | 100 | 0.1 | 0.6 | 1.0 | 1.0 |
| 3.6 | 20 | 10 | 70 | 100 | 100 | 0.1 | 0.7 | 1.0 | 1.0 |
| 3.9 | 20 | 25 | 75 | 100 | 100 | 0.25 | 0.75 | 1.0 | 1.0 |
| 4.2 | 20 | 30 | 85 | 100 | 100 | 0.3 | 0.85 | 1.0 | 1.0 |
| 4.5 | 20 | 50 | 85 | 100 | 100 | 0.5 | 0.85 | 1.0 | 1.0 |
| 4.8 | 20 | 55 | 90 | 100 | 100 | 0.55 | 0.9 | 1.0 | 1.0 |
| 5.1 | 20 | 80 | 100 | 100 | 100 | 0.8 | 1.0 | 1.0 | 1.0 |
| 5.4 | 20 | 85 | 100 | 100 | 100 | 0.85 | 1.0 | 1.0 | 1.0 |

**Table 2 :** Acute toxicity of Multineem biopesticide to *L. rohita* spawn

| Exposure time (h) | Regression equation(y) | LC$_{50}$ (ppm) | Slope function response(S) | Confidence limit (ppm) | Fiducial upper limit (ppm) | Fiducial lower limit (ppm) | LC$_{16}$ (ppm) | LC$_{84}$ (ppm) |
|---|---|---|---|---|---|---|---|---|
| 24 | -165.55+144.22x | 4.43 | 1.25 | 1.05 | 4.66 | 4.21 | 3.52 | 5.52 |
| 48 | -70.55+106.53x | 3.12 | 1.34 | 1.08 | 3.36 | 2.88 | 2.31 | 4.17 |
| 72 | -182.98+254.70x | 2.48 | 1.16 | 1.06 | 2.64 | 2.33 | 2.12 | 2.88 |
| 96 | -71.12+149.77x | 2.23 | 1.29 | 1.11 | 2.48 | 2.00 | 1.73 | 2.88 |
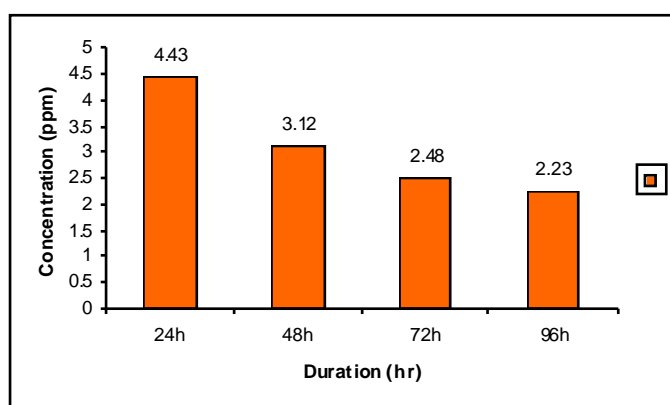


**Fig. 1 :** LC$_{50}$ of neembiopesticides to *L. rohita* spawn.

**Bioassay test for *L. rohita* fry**

Biassay test for *L. rohita* fry was conducted after getting the range finding. The experiment was conducted between 4.5ppm, 6ppm, 7.5ppm, 9ppm, 12ppm, 15ppm. Both in range finding and bioassay test in control there was no mortality of fry during the exposure periods. Initial mortality 5% started at 24h at 6.0ppm and 100% mortality occurred at 48h of 15ppm (Table-3). The regression equation (y) at 24h, 48h, 72h, 96h exposure period is -153.23+80.93x, -211.17+122.47x, -132.79+94.72x, -131.56+102.05x respectively with slope function response 1.17, 1.33, 1.39, 1.41 respectively. The confidence limit during 24h, 48h, 72h, 96h is 1.07ppm, 1.13ppm, 1.12ppm and 1.16ppm respectively. The fiducial upper limit, lower limit, LC$_{16}$, LC$_{84}$ at different exposure period is presented at Table 4. The LC$_{50}$ value at 24h, 48h, 72h, 96h exposure period of *L. rohita* fry is 9.39ppm, 8.24ppm, 6.82ppm, 5.95ppm respectively (Fig.2).

Table 3 : Cumulative percentage mortality of *L. rohita* fry exposed to different concentration of Multineem biopesticide at different hours duration.

| Concen-trations (ppm) | No. of *L. rohita* fry released | Cumulative percentage mortality | | | | Response | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 24h | 48h | 72h | 96h | 24h | 48h | 72h | 96h |
| 4.5 | 20 | 0 | 0 | 10 | 15 | 0 | 0 | 0.1 | 0.15 |
| 6 | 20 | 5 | 5 | 40 | 65 | 0.05 | 0.05 | 0.4 | 0.65 |
| 7.5 | 20 | 10 | 30 | 50 | 70 | 0.1 | 0.3 | 0.5 | 0.7 |
| 9 | 20 | 15 | 75 | 80 | 90 | 0.15 | 0.75 | 0.8 | 0.9 |
| 12 | 20 | 15 | 85 | 100 | 100 | 0.15 | 0.85 | 1.0 | 1.0 |
| 15 | 20 | 95 | 100 | 100 | 100 | 0.95 | 1.0 | 1.0 | 1.0 |

**Table 4 :** Acute toxicity of Multineem biopesticide to *L. rohita* fry.

| Exposure time (h) | Regression equation(y) | LC$_{50}$ (ppm) | Slope function response(S) | Confidence limit (ppm) | Fiducial upper limit (ppm) | Fiducial lower limit (ppm) | LC$_{16}$ (ppm) | LC$_{84}$ (ppm) |
|---|---|---|---|---|---|---|---|---|
| 24 | -153.23+80.93x | 9.39 | 1.17 | 1.07 | 10.05 | 8.77 | 7.80 | 10.8 |
| 48 | -211.17+122.47x | 8.24 | 1.33 | 1.13 | 9.31 | 7.29 | 6.17 | 11.02 |
| 72 | -132.79+94.72x | 6.82 | 1.39 | 1.12 | 7.65 | 6.08 | 4.90 | 9.48 |
| 96 | -131.56+102.05x | 5.95 | 1.41 | 1.16 | 6.89 | 5.12 | 4.22 | 8.41 |
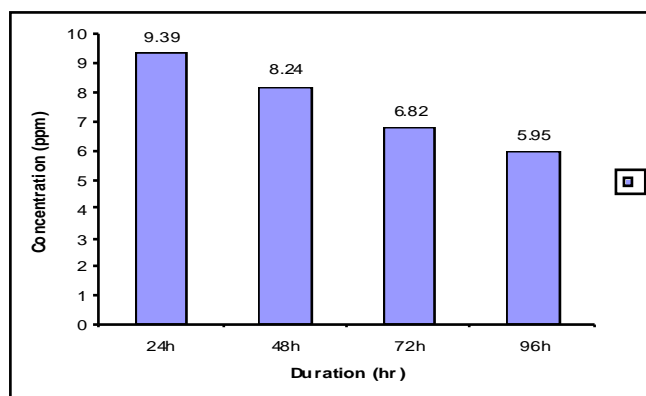
**Fig.2 :** LC$_{50}$ of neem biopesticides to *L. rohita* fry.

**Bioassay test for *L.rohita* fingerling**

Biassay test for *L. rohita* fingerling w as conducted after getting the range finding. The experiment w as conducted betw een 9ppm, 12ppm, 15ppm, 18ppm,

21ppm, 24ppm. Both in range finding and bioassay test in control there w as no mortality of fry during the exposure periods. Initial mortality 15% started at 24h at 15.0ppm and 100% mortality occurred at 48h of 24ppm (Table-5). The regression equation (y) at 24h, 48h, 72h, 96h exposure period is -418.40+161.03x,-201.90+97.89x, -273.02+130.06x, -329.10+156.60x respectively w ith slope function response 1.21, 1.35, 1.26, 1.23 respectively. The confidence limit during 24h, 48h, 72h, 96h is 1.08ppm, 1.11ppm, 1.15ppm and 1.13ppm respectively. The fiducial upper limit, low er limit, LC$_{16}$, LC$_{84}$ at different exposure period is presented at Table 6. The LC$_{50}$ value at 24h, 48h, 72h, 96h exposure period of *L. rohita* fingerling is 18.17ppm, 13.59ppm, 11.94ppm, 11.02ppm respectively (Fig.3).

**Table 5 :** Cumulative percentage mortality of *L. rohita* fingerling exposed to different concentration of Multineem biopesticide at different hours duration.

| Concen-trations (ppm) | No. of *L. rohita* fingerling released | Cumulative percentage mortality | | | | Response | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 24h | 48h | 72h | 96h | 24h | 48h | 72h | 96h |
| 9 | 20 | 0 | 5 | 10 | 15 | 0 | 0.05 | 0.1 | 0.15 |
| 12 | 20 | 0 | 50 | 50 | 60 | 0 | 0.5 | 0.5 | 0.6 |
| 15 | 20 | 15 | 70 | 90 | 95 | 0.15 | 0.7 | 0.9 | 0.95 |
| 18 | 20 | 50 | 80 | 95 | 100 | 0.5 | 0.8 | 0.95 | 1.0 |
| 21 | 20 | 75 | 90 | 100 | 100 | 0.75 | 0.9 | 1.0 | 1.0 |
| 24 | 20 | 90 | 100 | 100 | 100 | 0.9 | 1.0 | 1.0 | 1.0 |

**Table 6 :** Acute toxicity of Multineem biopesticide to *L. rohita* fingerling

| Exposure time (h) | Regression equation(y) | LC$_{50}$ (ppm) | Slope function response(S) | Confidence limit (ppm) | Fiducial upper limit (ppm) | Fiducial lower limit (ppm) | LC$_{16}$ (ppm) | LC$_{84}$ (ppm) |
|---|---|---|---|---|---|---|---|---|
| 24 | y =-418.40+161.03x | 18.17 | 1.21 | 1.08 | 19.72 | 16.82 | 14.8 | 21.97 |
| 48 | y =-201.90+97.89x | 13.59 | 1.35 | 1.11 | 15.12 | 12.24 | 10.07 | 18.35 |
| 72 | y =-273.02+130.06x | 11.94 | 1.26 | 1.15 | 13.74 | 10.38 | 9.39 | 15.10 |
| 96 | y =-329.10+156.60x | 11.02 | 1.23 | 1.13 | 12.50 | 9.75 | 9.11 | 13.32 |
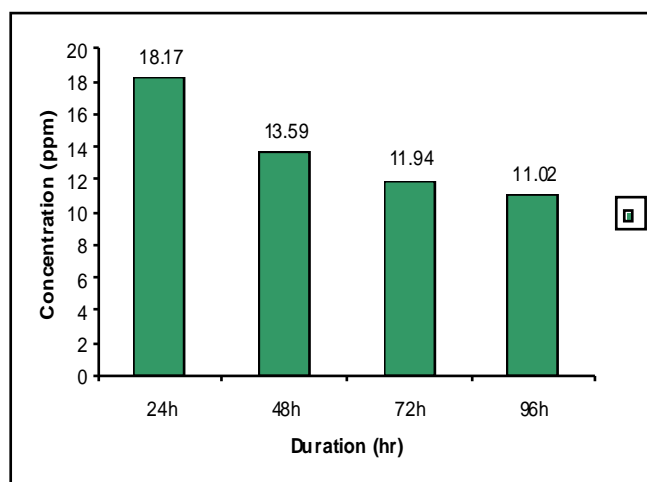
**Fig.3 :** $LC_{50}$ of neem biopesticides to *L. rohita* fingerling.

## DISCUSSION

Traditional medicinal plants are environmental friendly containing a wide range of biological bioactive molecules having antimicrobial properties. Sensitivity of fishes to neem vary in a wide range as the amount of active compounds in a given weight of neem varies widely with the part of the plant, its place of origin or even the individual tree (Winkler *et al,* 2007; Mousa *et al,* 2008).

In the present study, an attempt has been made to study the toxicity of the neem biopesticide to spawn, fry and fingerlings of *L. rohita* by estimation of the lethal concentration $LC_{50}$. Acute toxicity tests are the foremost step towards understanding the immediate toxic effect of a particular substance on biological systems. Precisely, the objective of this test is to find out the concentration of a particular biopesticide that influences the physiological functions of a living organism lending to patho-physiological conditions and even death in relatively a short time. In the present observation, the 96h $LC_{50}$ values of neem biopesticide were determined as 2.23, 5.95 and 11.02 ppm for spawn, fry and fingerlings of rohu respectively.

The $LC_{50}$ of aquaneem to rohu was reported to be 2.36 ppm (Das *et al* 2002). Similarly $LC_{50}$ of neem pesticide Triologus was reported to be 112 ppm to *Ctenopharyngodon idella* (Hamdy *et al.,* 2008) and neem leaf (96h $LC_{50}$) to nile tilapia and cat fish was 1.8 and 4 g/l respectively (Mousa *et al,* 2008).

Similarly, the toxicity of neem pesticides and different neem extracts on other non target aquatic organisms

has been estimated by several investigator (Singh *et al,* 1996; Dunkel and Richardsi, 1998; El-Shazly and El-Sharnoubi, 2000; Scott and Kaushek, 2000; Das *et al*, 2002). The variation of $LC_{50}$ varies due to age of the fish and their tolerance. The fingerlings have tolerated to higher range of toxicity than the spawn. Synthetic pesticides e.g. organosulphate, organochlorine, carbamate and synthetic pyrethroids as compared to the neem based pesticides is more toxic to fish (Winkaler *et al.,* 2007).

Slope functions is the target of the response curve for 24, 48, 72 and 96h respectively which can be used effectively for determining the 95% confidence limit (Das *et al.,* 2002). In the present study, this generally reflect the threshold limit of neem pesticide in the response curve. The mean slope functions at stages of spawn, fry and fingerlings provides greater degree of accuracy while interpreting the toxicity data. The confidence limit is calculated for estimating 95% fiducial limit to determine the upper limit and lower bound lethality and for executing the mode of application of the particular substance (Das 1998, Das *et al.,* 2002). In the present set of experiments, fiducial limits varied from 2.0-4.66 ppm; 5.12-10.05 ppm and 9.75-19.72 ppm in case of spawn, fry and fingerlings respectively. This indicates the tolerance limit of the rohu at various stages of development.

Microbial infections caused by bacteria, virus, fungi can significantly affects the survival of carps in composite fish culture. Ecofriendly substances are gaining importance in place of antibiotics due to its residual and antibiotic resistance genes developed in the cultured species which have direct health hazards in human being. Plant products e.g. neem has been used as bactericidal and fungicidal agents in aquaculture for controlling pathogens (Das *et al,* 1999, 2002). In the present study neem based biopesticides has been evaluated for its toxicity to early stages of rohu as we found its potentiality for reducing *A. hydrophila* & *Edwardsiella tarda* loads in *invitro* condition (Swain *et al.,* 2011).

The present study is extremely useful to take into consideration the acute lethal dose of a substance in order to protect the ecosystem and living organism before its application into any system which could be detrimental and cause environmental and physiological disruption. Further, the cumulative lethal burden in any dose beyond the tolerable limit might cause damage to the host.

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# BIODIVERSITY OF SACRED GROVES OF KOLIPOSH RANGE IN BONAI DIVISION, ORISSA, INDIA

**H S Palei*[1], A K Mishra[2], P K Dash[3] and P K Mohapatra[1]**

*[1]Centre for Biodiversity Conservation M-71, Housing Board, Colony, Baramunda, Bhubaneswar-751003

[2]Divisional Forest Officer Bonai, Sundargarh

[3]Vasundhara, Bhubaneswar

*wolfsnakes@gmail.com*

## ABSTRACT

Sacred groves are remnant patches of forest being conserved by various communities. These are considered as repository of germplasm. Sacred groves are connected with religious beliefs and taboos of the tribals and local people which helps in biodiversity conservation. The study aims at assessment of ecological role of sacred groves and formulating strategies for their long term conservation. Altogether 25 numbers of sacred groves have been studied in Kuliposhi range of Bonai forest division. Many rare and endangered species of flora and fauna have been documented during the study within the sacred groves. Many cultural and religious values are associated with biodiversity conservation were studied. Various threats for the degradation of sacred groves were also discussed. The richness of species composition and size of the sacred groves indicate the health of the sacred groves.

**Key words :** Sacred groves, germplasm, biodiversity, endangered, richness.

## INTRODUCTION

Sacred groves have been documented from different parts of Asia, Africa, Europe, Australia and America (Khumbongmayum, 2005). Early documentation of sacred groves in India was done by Brandis (1897) and afterwards a number of works have been carried out on different aspects of sacred groves (Gadgil and Vartak 1975; Barman 1992; Tripathi 2001 and Ramakrishan 1996). Studies on socio-cultural practices (Gadgile and Vartak 1975, 1976; Boojh and Ramakrishan 1983; Khiewtam and Ramakrishnan 1989; King *et al.,* 1997; Tiwari *et al.* 1998; Sinha and Maikhuri, 1998) and floral diversity of the sacred groves (Hajra, 1975; Balasubramanyan and Induchoodan 1996; Khan *et al.* 1997; Boraiah *et al.,* 2003; Kumar and Swamy 2003; Mohanta *et al.,* 2009) have also been carried out in different parts of the country.

Sacred groves can be considered as *in situ* germplasm conservation centres maintained or conserved by the local tribes/communities in a religious faith. These act as natural gene pool preserve and serve as an example of habitat preservation through community participation (Gadgil and Vartak, 1975). According to Hindu mythology, Siddhapeeth is a holy place where all our wishes get fulfilled. These Siddhapeeths have their own forests called sacred forests. The sacredness, religious beliefs and taboos play a significant role in promoting sustainable utilization and conservation of flora and fauna of the sacred groves. However, with the passage of time, considerable changes have taken place in the extent of the sacred groves, in their vegetation structure, peoples' perception towards the sacred sites and the religious beliefs and taboos. Therefore, a holistic understanding of the current status, structure and function of sacred grove is essential for assessing their ecological role and formulating strategies for their conservation. Hence the present study was undertaken to document the same in Bonai forest division.

## MATERIALS AND METHODS

The study was undertaken in Bonaigarh and Lahunipada block of Koliposh range in Bonai division of Sundargarh district of Orissa. There are 25 identified sacred groves, of various sizes, in this

range. The groves consist of dry deciduous types of forest. The climate is very hot and humid with maximum and minimum temperatures ranging from 42°C and 10°C respectively with an average annual rainfall of 1500mm.
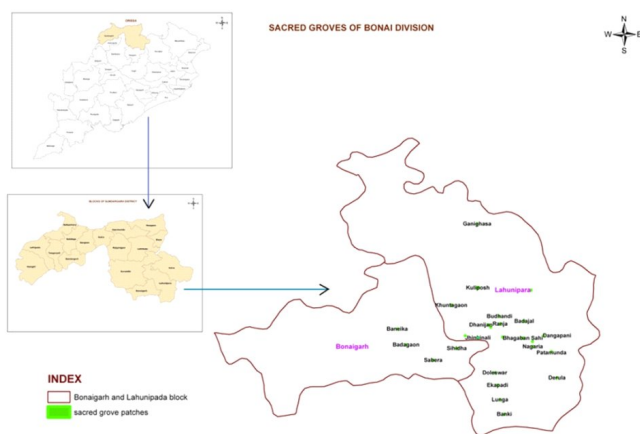


The study was carried out during January to March 2011. Before the field visit a consultative meeting of forest staff and officials and local villagers was organized in Koliposh range of Bonai division. Basic information on the ecological, socio-cultural and Status of different of sacred groves were gathered through semi-structured interviews with the local villagers using the questionnaire format. For detailed investigation, sacred groves were randomly selected to know the status of floral and faunal wealth, ethno-cultural values and existing management status of these groves.

During the study, plant species belonging to various life forms were recorded, and specimens of flowering plants were Photographed, collected and identified taxonomically with the help of different floras field key (Flora of Orissa, Flora of British India etc.). Each of the plant material was assigned in a field note book and documented as to Binomials with family, local name, part used and therapeutic uses. During the field visits, the various uses of plants were gathered from the local people in around the grove. In addition, various literatures have been used to known the values of each species.

Mammalian species were recorded by direct sightings or from the indirect evidences like the foot markings, call and scats of the animals. Birds

encountered were observed by Nikon Monarch 8X42 binoculars, photographed using Lumix FZ100 (24X) digital camera and identified by using Grimmet *et al.*, 2006. The checklist was prepared using the standardized common and scientific names of the birds of the Indian subcontinent by Manakadan and Pittie (2001). Reptiles were surveyed by active search methods by turning logs, stones and searching bushes and trees. Basking reptiles were found along the edges of foot path and on rocks. Amphibians were surveyed in leaf litter and were also searched during night time by using powerful search lights. Herpetofaunal species were identified by using field guide (Dutta *et al.* 2009).

## RESULTS AND DISCUSSION

In the present study 25 SGs have been documented within Koliposh Range of Bonai Division out of the identified list of 81 sacred groves. The size of sacred groves as per this study is quite variable. It ranges from 0.058 Acre to 7.004 Acre. The total area covered by 25 SGs is approximately 17.406 Acre. The linkages between sacred groves and villages differ from place to place and most of the villages have their own sacred groves. The geographical location of sacred groves in study area is also quite variable, as few of the sacred groves are located on near reserved forest and (Bhagban sahi, Patamunda, Lunga, Doleswar and Jhinknali), some are near the streams and river side (Banki, Khuntagaon & Sulabdihi, while others are on Village and human habitation areas. The observation on the canopy structure reveals that the groves with large area have good canopy and smaller groves have often open canopy due to various stages of degradation because of biotic pressures like livestock grazing.

### Floral diversity

A total of 102 species of plants under 46 families and 84 genera including 64 species of trees, 06 species of herbs and 10 species of shrubs, 15 species of climbers, 06 species of epiphytes and zone grass were identified. Out of the 102 species, 20 species are rare, 12 are occasional and 70 species are common to the sacred groves (Table 1).

### Faunal diversity

During the study period, a total of 10 species of mammals (Table 3), 58 species of birds belonging to 32 families (Table 2) and 20 species of reptiles

(Table 4) and 8 species of amphibians (Table-5) were recorded from different SGs of Koliposh Range.

## Cultural and religious values

Many of the sacred groves are associated with certain deities, and known by different names, such as, *Gramashree, Pitabali, Kuanri, Rambhadevi*. In such groves annual rituals and ceremonies are performed to propitiate the deity. During these rituals sacrifices of animals (such as fowl, goat, ship etc.) are made. In other sanskritized groves offerings of vegetable items (Banana, Coconut, Orange, Guava, Mango and other fruit as well as flower and leaf) are made. These rituals are performed for the well-being of the people, animals, crops, disease, well protect for the villagers etc. SGs have important socio-cultural functions, in addition to the religious functions. Several festivals are performed at SGs. It is report that, among the tribal communities of Orissa, social gatherings take place in these groves on the occasion of Salai, Karama, Maghe, Bodam, Jahira pooja as well as wedding ceremonies.

Each village normally has one or two *"Dehuri or Kala"* and they worship their Gods in their respective sacred groves. Tribes recognize and worship a number of Gods symbolized in the form of old trees (*Shorea robusta* and other sp.), wild animals (i.e. *Durga maa* (Tiger), *Laxmi maa* (Elephant), Nagdevta (Snake) and ancestral spirits (*Thakurani*). Wherever, a new settlement is initiated, the nearest small patch of forest is recognized and worshipped as sacred grove, which is locally known as "*Thakuranisal*". The worship is organized by the villagers of the respective sacred groves normally every year, mainly during harvest seasons (Sal and Mahul flower inf season; Mango fruiting season and rice harvesting season). At the time of worships sacrifice of domestic animals, such as domestic fowl (black coloured individuals are preferred), goat, ship etc. are common practices. Prayers are offered for rains, protection from animals and diseases as well as the overall well being of the village. They also believe that the constellation of stars has power and these represent the spirits, hence, animals must be scarified to these spirits.

## Existing threats

Of the total 25 surveyed sacred groves, many harbor good vegetation but major factors responsible for the degradation of those groves due to livestock grazing and forest fire.

## SUGGESTION AND RECOMMENDATION

The number and type of species of a habitat supports indicate its health. SGs of this range support a rich biodiversity evidenced by this study. Thus, there is ample scope for further research on plant diversity, community attributes, and natural regeneration. Detailed ethnobotanical surveys, biodiversity explorations, and research and pooling data from such investigations could be helpful in developing suitable measures for conserving precious plant wealth. The findings of these studies will help in the development of improved plantation technology using appropriate species for plantation. The reorientation of local indigenous communities towards restoration of traditional knowledge through environmental awareness programmes by the Government or NGOs will be an effective strategy for conservation of plant resources to a desired extent.

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**Table 1 :** List of plants recorded in different sacred groves.

| Sl. No. | Local Name | Botanical Name | Family | Habit | Status |
|---|---|---|---|---|---|
| 1 | | *Eranthemum purpurascens* | Acanthaceae | Herb | Common |
| 2 | | *Trema orientalis* | Ulmaceae | Tree | Common |
| 3 | | *Vitis adnata* | Vitaceae | Climber | Common |
| 4 | Akanabindi | *Cissampelos pareira* | Menispermaceae | Climber | Rare |
| 5 | Amba | *Mangifera indica* | Anacardiaceae | Tree | Common |
| 6 | Ambada | *Spondia pinnata* | Anacardiaceae | Tree | Common |
| 7 | Anantamula | *Hemidesmus indicus* | Asclepiadaceae | Climber | Common |
| 8 | Anchuu | *Morinda pubescence* | Rubiaceae | Tree | Common |
| 9 | Ankala | *Alangium salvifolium* | Cornaceae | Tree | Common |
| 10 | Asadhua | *Capparis zeylanica* | Caparidaceae | Climber | Rare |
| 11 | Asana | *Terminalia tomentosa* | Combretaceae | Tree | Common |
| 12 | Ata/ Badhiala | *Annona squamosa* | Annonaceae | Tree | Common |
| 13 | Atundi | *Combretum albidum* | Combretaceae | Climber | Common |
| 14 | Babul | *Acasia nilotica* | Mimosaceae | Tree | Common |
| 15 | Bahada | *Terminalia belerica* | Combretaceae | Tree | Occasional |
| 16 | Bamboo | *Bambusa bamboo* | Poaceae | Grass | Common |

| 17 | Bandhana | *Ougeinia oogenesis* | Leguminosae | Tree | Occasional |
|----|----------|---------------------|-------------|------|------------|
| 18 | Bandu | *Dodonaea viscosa* | Sapindaceae | Shrub | Rare |
| 19 | Bara | *Ficus bengalensis* | Moraceae | Tree | Common |
| 20 | Baruna | *Crataeva magna* | Capparidaceae | Tree | Rare |
| 21 | Baula | *Mimusops elengi* | Sapotaceae | Tree | Common |
| 22 | Bela | *Aegle marmeolus* | Rutaceae | Tree | Common |
| 23 | Bhadabhadalia | *Olax scandens* | Olacaceae | Shrub | Occasional |
| 24 | Bhalia | *Semecarpus anacardium* | Anacardiaceae | Tree | Common |
| 25 | Bhaludimiri | *Ficus hispida* | Moraceae | Tree | Common |
| 26 | Bhuincha | *Flacourtia indica* | Flacourtiaceae | Shrub | Occasional |
| 27 | Bhuinkurma | *Rauwolfia serpentina* | Apocynaceae | Herb | Rare |
| 28 | Chara | *Buchnania lanzan* | Anacardiaceae | Tree | Common |
| 29 | Chaunri | *Bauhinia semla* | Fabaceae | Tree | Occasional |
| 30 | Dhadura | *Anogeissus latifolia* | Combretaceae | Tree | Common |
| 31 | Dhala | *Anogeisus acuminata* | Combretaceae | Tree | Common |
| 32 | Dhamabhurudu | *Gardenia turgida* | Rubiaceae | Shrub | Occasional |
| 33 | Dhatundi | *Combretum roxburghii* | Combretaceae | Climber | Common |
| 34 | Dhobani | *Dalbergia paniculata* | Fabaceae | Tree | Occasional |
| 35 | Dimiri | *Ficus recimosa* | Moraceae | Tree | Common |
| 36 | Gambhari | *Gmelina arborea* | Verbenaceae | Tree | Common |
| 37 | Gandhapalasa | *Butea superba* | Papilionaceae | Climber | Common |
| 38 | Gangasiuli | *Nyctanthes arbor tristis* | Oleaceae | Shrub | Common |
| 39 | Garuda | *Radermachera xylocarpa* | Bignoneaceae | Tree | Rare |
| 40 | Giridhini | *Scindaspus officinalis* | Araceae | Climber | Rare |
| 41 | Gudamari | Gymnema sylvestri | Asclepiadaceae | Herb | Rare |
| 42 | Gurudu | Gardenia gummifera | Rubiaceae | Tree | Rare |
| 43 | Hadjodi | *Cissus quadrangularis* | Vitaceae | Climber | Rare |
| 44 | Harida | *Terminalia chebula* | Combretaceae | Tree | Common |
| 45 | Jadi | *Ficus religiosa* | Moraceae | Tree | Common |
| 46 | Jamun | *Syzygium cumini* | Myrtaceae | Tree | Common |
| 47 | Kadamba | *Anthocephalus cadamba* | Rubiaceae | Tree | Common |
| 48 | Kalama | *Mallotus philippensis* | Euphorbiaceae | Tree | Common |
| 49 | Kalikendu | *Diospyrous malabarica* | Ebenaceae | Tree | Common |
| 50 | Kanchana | *Bauhinia varigata* | Fabaceae | Tree | Common |
| 51 | Kaniara | *Cochlospermum religiosum* | Bixaceae | Tree | Rare |
| 52 | Kantakoli | *Ziziphus oenoplia* | Rhamnaceae | Shrub | Common |
| 53 | Karada | *Cleistanthus collinus* | Euphorbiaceae | Tree | Common |
| 54 | Karanja | *Pongamia glabra* | Leguminaceae | Tree | Common |
| 55 | Kasi | *Bridelia retusa* | Euphorbiaceae | Tree | Common |
| 56 | Kathachampa | *Plumeria rubra* | Apocyanaceae | Tree | Common |
| 57 | Kathasiali | *Xylia xylocarpa* | Fabaceae | Tree | Rare |
| 58 | Kendu | *Diospyros melanoxylon* | Ebenaceae | Tree | Common |
| 59 | Kew | *Cheilocostus speciosus* | Costaceae | Herb | Rare |

| 60 | Khakada | *Casearia elliptica* | Flacourtiaceae | Tree | Common |
|----|---------|-----------------------|----------------|------|--------|
| 61 | Kumbhi | *Careya arborea* | Lecythidaceae | Tree | Common |
| 62 | Kurchi/ Kurein | *Holarrhena pubescens* | Apocynaceae | Tree | Common |
| 63 | Kurma | *Haldinia cordifolia* | Rubiceae | Tree | Common |
| 64 | Kusuma | *Schleichera oliosa* | Sapindaceae | Tree | Common |
| 65 | Lembu | *Citrus medica* | Rutaceae | Shrub | Common |
| 66 | Mahi | *Garuga pinnata* | Burseraceae | Tree | Common |
| 67 | Mahula | *Madhuca indica* | Sapotaceae | Tree | Common |
| 68 | Mayurachulia | *Elephantopus scaber* | Asteraceae | Herb | Common |
| 69 | Mundi | *Mitragyna parvifolia* | Rubiaceae | Tree | Common |
| 70 | Musakani | *Cocculus hirsutus* | Menispermaceae | Climber | Rare |
| 71 | Nagapheni | *Opuntia stricta var dillenii* | Euphorbiaceae | Shrub | Occasional |
| 72 | Nima | *Azadirachta indica* | Meliaceae | Tree | Common |
| 73 | Palasa | *Butea monosperma* | Fabaceae | Tree | Common |
| 74 | Panasa | *Artocarpus heterophyllus* | Moraceae | Tree | Common |
| 75 | Panchaangulia | Gloriosa superba | Liliaceae | Climber | Rare |
| 76 | Papendaru | *Oroxylum indicum* | Bignoniaceae | Tree | Rare |
| 77 | Piasala / Bija | *Pterocarpus marsupium* | Fabaceae | Tree | Rare |
| 78 | Pijuli | *Psidium guajava* | Myrtaceae | Tree | Common |
| 79 | Ping | *Celastrus paniculatus* | Celastraceae | Climber | Rare |
| 80 | Pippali | *Piper longum* | Piperaceae | Climber | Rare |
| 81 | pittamari | *Cipadessa baccifera* | Meliaceae | Shrub | Occasional |
| 82 | Rasna | *Vanda teslata* | Orchidaceae | Epiphyte | Common |
| 83 | Rasna | *Vanda testacea* | Orchidaceae | Epiphyte | Common |
| 84 | Rasna | *Acampe rigida* | Orchidaceae | Epiphyte | Rare |
| 85 | Rasna | *Acampe praemorsa* | Orchidaceae | Epiphyte | Common |
| 86 | Rasna | *Aerides odorata* | Orchidaceae | Epiphyte | Occasional |
| 87 | Rasna | *Rhynchostylis retusa* | Orchidaceae | Epiphyte | Common |
| 88 | Rimuli | *Protium serratum* | Burseraceae | Tree | Occasional |
| 89 | Rohini | *Soymida febrifuga* | Meliaceae | Tree | Common |
| 90 | Sahada | *Streblus asper* | Moraceae | Tree | Common |
| 91 | Sala / sargi | *Shorea robusta* | Dipterocarpaceae | Tree | Common |
| 92 | Salara | *Flemingia bracteata* | Leguminosae | Herb | Common |
| 93 | Salei | *Boswellia serrata* | Burseraceae | Tree | Occasional |
| 94 | Satabari | *Asparagus recemosus* | Liliaceae | Climber | Common |
| 95 | Senha/Sidha | *Lagerostromia parviflora* | Lythraceae | Tree | Common |
| 96 | Siali | *Bauhinia vahlii* | Fabaceae | Climber | Common |
| 97 | Sija | *Euphorbia sp* | Euphorbiaceae | Shrub | Common |
| 98 | Simili | *Bombax ciba* | Bombacaceae | Tree | Common |
| 99 | Sisu | *Dalbergia sisoo* | Fabaceae | Tree | Common |
| 100 | Sunari | *Casia fistula* | Caesalpineaceae | Tree | Common |
| 101 | Tala | *Borassius flaviliflora* | Arecaceae | Tree | Common |
| 102 | Tentuli | *Tamarindus indica* | Caesalpineaceae | Tree | Common |

**Table 2 :** List of mammals recorded during survey

| SL. No. | Common Name | Scientific Name | Evidences | Resident/occasionally visited |
|---|---|---|---|---|
| 1 | Jackal | *Canis aureus* | Scat | Occasionally visited |
| 2 | Jungle cat | *Felis chaos* | Direct sighting, scat | Occasionally visited |
| 3 | Sloth bear | *Melursus ursinus* | Digging, dropping | Occasionally visited |
| 4 | Wild pig | *Sus scrofa* | Digging | Occasionally visited |
| 5 | Hanuman langur | *Semnopithecus entellus* | Direct sighting and dropping | Occasionally visited |
| 6 | Indian Hare | *Lepus nigricollis* | Dropping | Resident |
| 7 | Porcupine | *Hystrix indica* | Caves, burrows, dropping | Resident |
| 8 | Little Indian Field Mice | *Mus booduga* | Direct sighting | Resident |
| 9 | Five-striped squirrels | *Funambulus pennantii* | Direct sighting | Resident |
| 10 | Indian Pipistrelle | *Pipistrellus coromandra* | Direct sighting | Resident |

**Table 3 :** List of birds recorded during survey

| SL. No. | Common Name | Scientific Name |
|---|---|---|
| **I.** | **Ardeidae** | |
| 1 | Cattle Egret | *Bubulcus ibis* (Linnaeus, 1758) |
| 2 | Indian Pond-Heron | *Ardeola grayii* (Sykes, 1832) |
| **II.** | **Accipitridae** | |
| 3 | Black-shouldered Kite | *Elanus caeruleus* (Desfontaines, 1789) |
| 4 | Black Kite | *Milvus migrans* (Boddaert, 1783) |
| 5 | Crested Serpent-Eagle | *Spilornis cheela* (Latham, 1790) |
| **III.** | **Phasianidae** | |
| 6 | Red Jungle fowl | *Gallus gallus* (Linnaeus, 1758) |
| **IV.** | **Turnicidae** | |
| 7 | Small Buttonquail | *Turnix sylvatica* (Desfontaines, 1789) |
| 8 | Jungle Bush quail | *Perdicula asiatica* (Latham, 1790) |
| **V.** | **Charadriidae** | |
| 9 | Yellow-wattled Lapwing | *Vanellus malabaricus* (Boddaert, 1783) |
| 10 | Red-wattled Lapwing | *Vanellus indicus* (Boddaert, 1783) |
| **VI.** | **Scolopacidae** | |
| 11 | Common Sandpiper | *Actitis hypoleucos* Linnaeus, 1758 |
| **VII.** | **Columbidae** | |
| 12 | Blue Rock Pigeon | *Columba livia* Gmelin, 1789 |
| 13 | Spotted Dove | *Streptopelia chinensis* (Scopoli, 1786) |
| 14 | Eurasian Collared-Dove | *Streptopelia decaocto* (Frivaldszky, 1838) |
| 15 | Yellow-legged Green-Pigeon | *Treron phoenicoptera* (Latham, 1790) |
| **VIII.** | **Psittacidae** | |
| 16 | Rose-ringed Parakeet | *Psittacula krameri* (Scopoli, 1769) |
| **IX.** | **Cuculidae** | |
| 17 | Indian Cuckoo | *Cuculus micropterus* Gould, 1838 |
| 18 | Asian Koel | *Eudynamys scolopacea* (Linnaeus, 1758) |
| 19 | Greater Coucal | *Centropus sinensis* (Stephens, 1815) |
| **X.** | **Strigidae** | |
| 20 | Spotted Owlet | *Athene brama* (Temminck, 1821) |
| **XI.** | **Caprimulgidae** | |
| 21 | Large-tailed Nightjar | *Caprimulgus macrurus* Horsfield, 1821 |
| 22 | Common Indian Nightjar | *Caprimulgus asiaticus* Latham, 1790 |

| | | |
|---|---|---|
| **XII.** | **Apodidae** | |
| 23 | Asian Palm-Swift | *Cypsiurus balasiensis* (J.E. Gray, 1829) |
| 24 | House Swift | *Apus affinis* (J.E. Gray, 1830) |
| **XIII.** | **Alcedinidae** | |
| 25 | Small Blue Kingfisher | *Alcedo atthis* (Linnaeus, 1758) |
| 26 | White-breasted Kingfisher | *Halcyon smyrnensis* (Linnaeus, 1758) |
| **XIV.** | **Meropidae** | |
| 27 | Small Bee-eater | *Merops orientalis* Latham, 1801 |
| **XV.** | **Coraciidae** | |
| 28 | Indian Roller | *Coracias benghalensis* (Linnaeus, 1758) |
| **XVI.** | **Capitonidae** | |
| 29 | Brown-headed Barbet | *Megalaima zeylanica* (Gmelin, 1788) |
| 30 | Coppersmith Barbet | *Megalaima haemacephala* (Müller, 1776) |
| **XVII.** | **Motacillidae** | |
| 31 | White Wagtail | *Motacilla alba* Linnaeus, 1758 |
| 32 | Grey Wagtail | *Motacilla cinerea* Tunstall, 1771 |
| 33 | Paddyfield Pipit | *Anthus rufulus* Vieillot, 1818 |
| **XVIII.** | **Campephagidae** | |
| 34 | Large Cuckoo-Shrike | *Coracina macei* (Lesson, 1830) |
| **XIX.** | **Pycnonotidae** | |
| 35 | Red-whiskered Bulbul | *Pycnonotus jocosus* (Linnaeus, 1758) |
| 36 | Red-vented Bulbul | *Pycnonotus cafer* (Linnaeus, 1766) |
| **XX.** | **Irenidae** | |
| 37 | Common Iora | *Aegithina tiphia* (Linnaeus, 1758) |
| **XXI.** | **Laniidae** | |
| 38 | Brown Shrike | *Lanius cristatus* Linnaeus, 1758 |
| **XXII.** | **Turdinae** | |
| 39. | Oriental Magpie-Robin | *Copsychus saularis* (Linnaeus, 1758) |
| 40. | Indian Robin | *Saxicoloides fulicata* (Linnaeus, 1776) |
| **XXIII.** | **Timaliinae** | |
| 41 | Jungle Babbler | *Turdoides striatus* (Dumont, 1823) |
| **XXIV.** | **Sylviinae** | |
| 42 | Common Tailorbird | *Orthotomus sutorius* (Pennant, 1769) |
| 43 | Ashy Prinia | *Prinia socialis* Sykes, 1832 |
| 44 | Common Chiffchaff | *Phylloscopus collybita* (Vieillot, 1817) |
| **XXIV.** | **Nectariniidae** | |
| 45 | Purple Sunbird | *Nectarinia asiatica* (Latham, 1790) |
| **XXV.** | **Dicaeidae** | |
| 46 | Thick-billed Flowerpecker | *Dicaeum agile* (Tickell, 1833) |
| **XXVI.** | **Zosteropidae** | |
| 47 | Oriental White-eye | *Zosterops palpebrosus* (Temminck, 1824) |
| **XXVII.** | **Estrildidae** | |
| 48 | White-throated Munia | *Lonchura malabarica* (Linnaeus, 1758) |
| 49 | Spotted Munia | *Lonchura punctulata* (Linnaeus, 1758) |
| 50 | Black-headed Munia | *Lonchura malacca* (Linnaeus, 1766) |
| **XXVIII.** | **Passerinae** | |
| 51 | House Sparrow | *Passer domesticus* (Linnaeus, 1758) |
| **XXIX.** | **Sturnidae** | |
| 52 | Asian Pied Starling | *Sturnus contra* Linnaeus, 1758 |
| 53 | Common Myna | *Acridotheres tristis* (Linnaeus, 1766) |
| **XXX.** | **Oriolidae** | |
| 54 | Black-hooded Oriole | *Oriolus xanthornus* (Linnaeus, 1758) |
| **XXXI.** | **Dicruridae** | |
| 55 | Black Drongo | *Dicrurus macrocercus* Vieillot, 1817 |
| **XXXII.** | **Corvidae** | |
| 56 | Indian Treepie | *Dendrocitta vagabunda* (Latham, 1790) |
| 57 | House Crow | *Corvus splendens* Vieillot, 1817 |
| 58 | Jungle Crow | *Corvus macrorhynchos* Wagler, 1827 |

**Table 4 :** List of reptile species recorded in the sacred groves

| SL. No. | Common Name | Scientific Name | Habitat |
|---|---|---|---|
| 1 | Indian Garden Lizard | *Calotes versicolor* | Bushes, trees |
| 2 | Indian Rock Lizard | *Psammophilus blanfordanus* | Rocks, trees |
| 3 | Bark gecko | *Hemidactylus leschenaultii* | Trees, rocks |
| 4 | Spotted Indian House gecko | *Hemidactylus brookii* | Bushes, below rock boulders |
| 5 | Smooth House gecko | *Hemidactylus frenatus* | Rocks, trees |
| 6 | Clouded ground gecko | *Geckoella nebulosus* | Below rock boulders, leaf litters |
| 7 | Common Indian Skink | *Eutropis carinata* | Rocks, leaf litter |
| 8 | Eastern Bronze Skink | *Eutropis macularia* | Rocks, leaf litter |
| 9 | Common Snake Skink | *Lygosoma punctata* | Below rock boulders, logs and leaf litter |
| 10 | White spotted Supple Skink | *Riopa albopunctata* | Below rock boulders, logs and leaf litter |
| 11 | Common Indian Monitor | *Varanus bengalensis* | On forest floor, on trees |
| 12 | Common Sand Boa | *Gongylophis conicus* | Sighted on forest floor |
| 13 | Buff Striped Keelback | *Amphiesma stolata* | Below rock boulder also sighted on forest floor |
| 14 | Common Vine Snake | *Ahaetulla nasuta* | On bushes |
| 15 | Common Indian Trinket Snake | *Coelognathus helena* | Sighted on forest floor near rock boulders |
| 16 | Common Indian Bronze-back | *Dendrelaphis tristis* | Sighted on forest floor, also on trees |
| 17 | Common Indian Rat Snake | *Ptyas mucosus* | Sighted on forest floor, also inside degraded termite mound |
| 18 | Checkered Keelback Water Snake | *Xenochrophis piscator* | On forest floor |
| 19 | Common Indian Krait | *Bungarus caeruleus* | Sighted below a rotten log, also inside degraded termite mound |
| 20 | Binocellate Cobra | *Naja naja* | On forest floor |

**Table 5 :** List of amphibian species recorded in the sacred groves

| SL. No. | Common Name | Scientific Name | Habitat |
|---|---|---|---|
| 1 | Common Asian toad | *Duttaphrynus melanostictus* | Leaf litter and among rock boulders |
| 2 | Syhadra Cricket frog | *Fejervarya syhadrensis* | Leaf litter and damp places |
| 3 | Indian Bull frog | *Hoplobatrachus tigerinus* | On forest floor |
| 4 | Short-headed Burrowing frog | *Sphaerotheca breviceps* | On forest floor |
| 5 | Indian burrowing frog | *Sphaerotheca rolandae* | Below rock boulders and on forest floor |
| 6 | Painted balloon frog | *Kaloula taprobanica* | Inside tree hole |
| 7 | Ornate Narrow-mouthed frog | *Microhyla ornata* | Below rock boulders |
| 8 | Common Indian Treefrog | *Polypedates maculatus* | On forest floor and also on trees |

**Figs. :**  1. *Celastrus paniculatus*, 2. *Gardenia gummifera*, 3. *Cocculus hirsutus*, 4. *Gymnema sylvestre*,
5. *Oroxylum indicum*, 6. *Pterocarpus marsupium*, 7. *Rauvolfia serpentina*, 8. *Scindaspus officinalis*,
9. *Radenmanchera xylocarpa*, 10. *Piper longum*, 11. *Costus speciosus*, 12. *Gloriosa superba*

**Fig. 13 :** Sacred groove of Koliposh Range, Bonai

**Fig. 14 :** Goddess being worshiped at Sacred groove

**Fig. 15 :** Indian Roller [*Coracias benghalensis* (Linnaeus, 1758)]

**Fig. 16 :** Black-headed Oriole [*Oriolus xanthornus* (Linnaeus, 1758)]

**Fig. 17 :** Jackal [*Canis aureus* (Linnaeus, 1758)]



**Fig. 18 :** Spotted Indian House Gecko [*Hemidactylus brookii* (Gray, 1845)]



**Fig. 19 :** Indian Rock Lizard [*Psammophilus blanfordanus* (Stoliczka)]



**Fig. 20 :** Eastern Bronze Skink [*Eutropis macularia* (Blyth, 1853)]



**Fig.21 :** Common Indian Trinket Snake [*Coelognathus helena helena* (Daudin, 1803)]



**Fig.22 :** Common Indian Bronzeback [*Dendrelaphis tristis* (Daudin, 1803)]



**Fig.23 :** Indian Burrowing frog [*Sphaerotheca rolandae* (Dubois, 1983)]



**Fig. 24 :** Indian Bull frog [*Hoplobatrachus tigerinus* (Daudin. 1802)]

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# USE OF RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) FOR TYPING OF *Nitrosomonas* SPECIES ISOLATED FROM FRESHWATER FISH PONDS

**Dipti Mohanta, B K Das\* and J K Jena**

*\*Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-751002, Odisha*

*basantadas@yahoo.com*

## ABSTRACT

Autotrophic oxidation of ammonia to nitrite, the first stage in nitrification, is of major importance in the global cycling of nitrogen in aquatic ecosystems. Furthermore, molecular techniques are considered to provide a comprehensive use of species diversity of natural microbial populations. In freshwater environments, *Nitrosomonas* bacteria are generally the predominant Ammonium Oxidizing Bacteria (AOB) group, as evidenced from the bacterial genera present in the freshwater fish pond. *Nitrosomonas* species isolated from fish pond was subjected to the DNA fingerprinting studies using Random Amplified Polymorphic DNA (RAPD) analysis. RAPD technique is a powerful tool for genetic studies and is a method of producing a genotyping fingerprint of a particular species. This technology is proving to be quite useful in typing strains of bacteria involved in biological treatment as well as environmental monitoring and to be considered the most economically feasible approach. Decamer random primers of OPC series (20 primers) were used for screening from which 13 primers showed amplification of the genomic DNA of *Nitrosomonas* species. Decamer random primers of OPC series (20 primers) were used for screening from which 17 primers showed amplification of the genomic DNA of *Nitrosomonas* species. DNA fingerprinting pattern of *Nitrosomonas* showed 3-7 prominent polymorphic bands and there were 1-4 unique bands of each primers amplified while their molecular weight ranged from 352-4878 bp. There were common bands found in a series of primers i.e. fragment size of 528 bp in OPC-1, OPC-2 and OPC-8; 1209 bp in OPC-4, OPC-6, OPC-7, OPC-9, OPC-10 and OPC-19; 1.286 k in OPC-11 and OPC-13; 1743 bp in OPC-1, OPC-10, OPC-13, OPC-14, OPC-15 and OPC-19; 1763 bp in OPC-13 and OPC-19; 2097 bp in OPC-2, OPC-6, OPC-7, OPC-9, OPC-10, OPC-11 and OPC-17. This RAPD profile study of *Nitrosomonas* (DN-1) strain will be useful for environmental monitoring of this genus for further nitrification studies.

**Keywords :** Nitrosomonas, RAPD, primer, identification, genetic relationship

## INTRODUCTION

The global growth of aquaculture has brought an increase in negative environmental impacts through the discharge of substantial amounts of polluting effluents containing uneaten feed and feces (Read and Fernandes, 2003; Crooker and Contreras, 2010). This organic enrichment causes environmental deterioration of both the receiving water bodies and sediments (Crab *et al.*, 2007). In aquaculture, ammonia wastes originate from animal excreta and decomposing organic solids such as uneaten feed (Stewart *et al.*, 2006). Nitrification, the microbially mediated oxidation of ammonia ($NH_3$) to nitrite ($NO_2^-$) and nitrate ($NO_3^-$), represents a key process in the global cycling of nitrogen and plays a critical role in

facilitating the removal of nitrogen. Two distinct groups of mostly lithoautotrophic bacteria perform these two stages: ammonia-oxidizing bacteria such as the genus *Nitrosomonas* and nitrite-oxidizing bacteria such as the genus *Nitrobacter*, respectively (Bock and Koops, 1992; Head *et al.*, 1993; Teske *et al.*, 1994). All characterized freshwater ammonia oxidizers belong to a coherent group within the â subclass of the class *Proteobacteria*, comprising the genera *Nitrosomonas* and *Nitrosospira* (Voytek and Ward, 1995). Medium selectivity and the ability of some *Nitrosomonas* strains to outcompete other ammonia oxidizers in liquid cultures (Belser, 1979).

Nitrifcation is of fundamental importance in aquatic systems because it links organic matter

decomposition (ammonification) and elimination of fixed nitrogen from the ecosystem (denitrifcation) (Jantti *et al.*, 2011). This process is, therefore, involved in the self-purification of aquatic systems because it prevents accumulation of potentially toxic or dangerous forms of nitrogen (non-ionized ammonia, nitrite) (Féray and Montuelle, 2002). Nevertheless, it produces nitrate, which may be involved in eutrophication (Karydis, 2009). Fish in terms of processes directly linked to their activities and their effect on water quality. Usually in a Recirculating Aquaculture System (RAS), autotrophic nitrifying bacteria (AB) remove ammonia at a sufficient rate to maintain water quality at a level adequate to prevent ammonia toxicity to the fish (Zhu and Chen, 1999). Little information is available on the bacterial communities present within aquaculture systems, novel microbiological techniques offers new opportunities for the study of these communities. The application of known and new technologies can capture inorganic nitrogen from water and reduce organic enrichment of sediments. Biological methods, including Integrated Multi-trophic Aquaculture are now gaining interest for increasing in situ removal of nitrogen (Crooker and Contreras, 2010).

Identification of *Nitrosomonas* strain, still remain a difficult task although many different techniques have been developed. The introduction of molecular techniques into microbial ecology has enabled the detection and reliable quantiûcation of natural populations of nitriûers. In last decade, various techniques that rely on different nucleic acid patterns and therefore discriminate at genetic level have been developed to gain information about genetic diversity and genetic relationship between different organisms (Head *et al.*, 1993; Teske *et al.*, 1994). It has been demonstrated that RAPD-PCR may be useful for determination of taxonomic identity, establishment of systematic relationships and assessment of genetic differentiation of plants and animals including mammals (Hadrys *et al.*, 1992). Though reproducibility with RAPD markers is somewhat questionable, they are quite useful due to their simplicity, low cost

and throughput capabilities (Waugh and Powell, 1992). RAPD analysis of *Nitrosomonas* species is based on the ability of a single primer of RAPD nucleotide sequence to generate polymorphic amplification product for any genome in question. RAPD is a rapid method for characterize genetic differences and has been used to fingerprint variety of bacterial species (Mohanraj *et al.*, 2011; Ripabelli *et al.*, 2003; Bhowmick *et al.*, 2011; Mahmud *et al.*, 2007). Due to its technical simplicity and speed, RAPD methodology has been used for diversity analyses (Iqbal *et al.*, 2007).

## MATERIALS AND METHODS

### Sample collection

The water samples and sediments were collected from four fish ponds of assorted size complex CIFA, Kausalyaganga, Bhubaneswar and these samples were used as source for isolation and cultivation of *Nitrosomonas sp.* (Ammonium oxidizing bacteria).

### Isolation and Identification

The samples were inoculated in selective media Ammonium Oxidizing Bacterial medium (AOB medium) at 28°C in dark for a period of 45 days (Schneider and Rheinheirmer, 1988). Growth and development was indicated by change in color of AOB of medium (pink and orange). The conversion of ammonia into nitrite was indicated by the production of acid, which turned the colour of the indicator from orange to yellow. To be precise, this was evident from the change in colour of phenol red indicator pre-added into the medium

### Extraction and purification of genomic DNA

The genomic DNA isolation was done according to the standard SIGMA-Aldrich kit (Gen*Elute Bacterial Genomic DNA Kit). An overnight bacterial broth culture of 3ml in volume was centrifuged for 5 minutes at 10,000 rpm at 4°C. The pellet obtained after centrifugation was resuspended thoroughly in 180µl of Lysis Solution T(B 6678). The RNA-free genomic DNA was obtained by adding 20µl of Rnase

A SolutionT (R6148). It was mixed and incubated for 2 minutes at room temperature. Cell lysis was done by adding 20µl of Proteinase – K solution to the sample for 30 minutes at 55° C. 200µl of Lysis Solution C (B 8803) was added to the sample and for homogenous mixing, it was vortexed thoroughly for about 15 seconds and incubated at 55Ú C for 10 minutes. Column preparation was done by adding 500 µl of the Column Preparation Solution (C2112) to each pre assembled GenElute Miniprep Binding Column and placed in a 2ml of collection tube. The material was centrifuged at 10,000 rpm for 1 minute. 200µl of ethanol (95-100%) was added to the lysate and mixed thoroughly by vortexing for 10 seconds. The entire content of the tube was transferred into binding column. A wide bore pipette tip was used to reduce shearing of the DNA when transferring contents into the column. It was then centrifuged at 10,000 rpm for 1 minute. Collection tube containing the flow-through liquid was discarded and the column was placed in a new 2ml collection tube. First wash was made by adding 500µl of Wash Solution O (W 0263) was added into the column and centrifuged for 1 minute at 10,000 rpm. Then the collection tube was discarded which contained the flow-through liquid and the column was placed in a new 2ml collection tube. Second wash was prepared by Wash solution concentrate (B 6553) diluted with ethanol. 500µl of it was added into the column and then centrifuged for about 3 minutes at 10,000 rpm to dry the column. Collection tube containing the flow-through liquid was discarded and the column was placed in a new 2 ml collection tube. Finally 200 µl of the elution solution (B6803) was pipetted out and placed directly at the center of the column. The material was centrifuged for a minute at 10,000 rpm to elute the DNA. The elute, containing pure genomic DNA was subsequently stored at 4ºC for further analysis.

The genomic DNA samples was also examined on an 0.8% (w/v) agarose gel to check that, the genomic DNA had not become excessively fragmented and stored at -20ºC for further use. Purity of the genomic DNA sample was checked by measuring the ratio of OD260 nm/OD$_{280}$ nm with help of UV- spectro-photometer (Biorad, SmartSpec 3000). The concentration of the nucleic acid in the sample was quantified by measuring absorbance at 260 nm and calculated by using the formula as given below :

$$\text{Total DNA (µg /mL)} = OD_{260} \times 50 \times \text{Dilution factor}$$

## RAPD Assay

### Primers

Random primers 10-mer oligonucleotide primers (Operon Technologies, INC., Alameda, USA) were used for generating RAPD fingerprints. These primers had G+C content of 60 to 70% and that they have no self complementary ends. In the RAPD-PCR analysis, 20 arbitary primers (OPC-01 to OPC-20) were screened using genomic DNA of isolate Nitrosomonas sp (DN-1). The sequences of all primers were mentioned in Table 1.

### PCR

Prior to amplification, the genomic DNA samples were diluted to a concentration of 25ng/µl. Each PCR reaction (25 µl) consisted of 20.25 µl dH$_2$O, 2.5 µl 10X PCR buffer (Genei, India), 1 ìl 100 µM dNTPs (Genei, India), 0.5 µl (5 p mol) of each forward and reverse primer (Operon Technologies, INC., Alameda, USA), followed by 0.25µl (0.75 U) Taq DNA polymerase (Genei, India) and 1 µl genomic DNA. The amplification profile was: 94°C for 5 min followed by 45 cycles of denaturation for 1 min at 94°C, at appropriate annealing temperature (55$^0$ C) for 1 min and extension at 72°C for 1 min 30 sec, followed by a final extension for 10 min at 72°C. Then the PCR product was stored at 4$^0$C until further use. The amplification products were analyzed by electrophoresis in 1.2 % agarose gels and detected by staining with ethidium bromide.

### Analysis of RAPD finger print patterns

Estimation of molecular weight of the PCR band in comparison to molecular weight standards was made from a minimum of two samples using AlphaEase®FC Imaging Software (Alpha Innotech

Corp., USA). The comparision of the band was done by using the equation $S_{AB} = 2N_{AB}/(N_A + N_B)$ (Where, $S_{AB}$ = Shared DNA bands between two species A and B/ genetic similarity between A and B, $N_{AB}$ = Number of DNA bands shared in common between A and B, $N_A$ and $N_B$ = Total number of bands possessed by the species A and B, respectively).

**RESULTS AND DISCUSSION**

In the RAPD-PCR analysis, 20 arbitrary primers (OPC-01 to OPC-20) were screened using genomic DNA of isolate *Nitrosomonas sp* (DN-1). Out of these 20 primers, 17 primers (OPC-1, OPC-2, OPC-3, OPC-4, OPC-6, OPC-7, OPC-8, OPC-9, OPC-10, OPC-11, OPC-12, OPC-13, OPC-14, OPC-15, OPC-17, OPC-19 and OPC-20) were taken based on their ability to produce consistent and distinguishable fragment pattern (fig1 and fig. 2). Various primers showed varying degree of polymorphism and 85% primers (17 out of 20 used primers) were amplified in the current study. Total 76 numbers of bands were observed in OPC-01 to OPC-20. DNA fingerprinting pattern of *Nitrosomonas* showed 3-7 prominent polymorphic bands and there were 1-4 unique bands of each primers amplified while their molecular weight ranged from 0.352-4.878 kb.

OPC-3, OPC-8 and OPC-19 produced 3 bands and the molecular weights were ranged from 1.036-3.357 kb 0.528-1.614 kb and 1.209-1.743 kb respectively. Similarly, 4 bands were observed in case of OPC-6 (1.209-2.097 kb), OPC-10 (0.838-2.097 kb), OPC-11 (1.286-4.413 kb), OPC-12 (1.519-4.878 kb), OPC-13 (1.286-3.928 kb), OPC-14 (1.743-4.016 kb), OPC-17 (0.793- 3.163 kb) and OPC-20 (1.545- 2.722 kb). In case of OPC-1 and OPC- 15, 5 distinct bands were observed and its molecular weight of amplified fragments ranged from 0.528 to 3.048 kb for OPC1 and 0.717 to 3.270 kb for OPC 15. Six bands were observed in case of OPC-2 (0.352- 2.097 kb), OPC-4 (0.366- 2.373 kb) and OPC-7 (0.665–2.097 kb), while seven bands were observed only in case of OPC-9 (0.653- 2.097 kb). There were common

bands found in a series of primers i.e. fragment size of 0. 528 kb in OPC-1, OPC-2 and OPC-8; 1.209 kb in OPC-4, OPC-6, OPC-7, OPC-9, OPC-10 and OPC-19; 1.286 kb in OPC-11 and OPC-13; 1.743 kb in OPC-1, OPC-10, OPC-13, OPC-14, OPC-15 and OPC-19; 1.763 kb in OPC-13 and OPC-19; 2.097 kb in OPC-2, OPC-6, OPC-7, OPC-9, OPC-10, OPC-11 and OPC-17. The summary of number of bands amplified and their molecular weight of each amplified bands are presented in table-2 and table-3.

Identification of *Nitrosomonas* strain, still remain a formidable task although many different molecular techniques into microbial ecology has enabled the detection and reliable quantiûcation of natural populations of nitriûers. The RAPD analysis is quite useful due to their simplicity, low cost and throughput capabilities (Iqbal *et al.*, 2007; Waugh and Powell, 1992). RAPD analysis of *Nitrosomonas* species is based on the ability of a single primer of RAPD nucleotide sequence to generate polymorphic amplification product for any genome in question. RAPD assay has mostly been used for interspecies discrimination, in epidemiological studies and is one of the most promising methods for distinguishing individual bacterial strains and estimating the nucleotide sequence diversity. RAPD typing is fast to perform, especially in cases where fingerprinting can be performed directly on single colonies growing on an agar plate. In the present study, RAPD assay was chosen to type one isolates of *Nitrosomonas* using OPC primer. Seventeen selected primers were able to generate distinct RAPD band profiles. The amplified bands varied from 2 to 6 and there are 2-3 unique prominent bands in the isolates for each primers amplified. These specific amplified bands will definitely aid in studying the distribution of the isolates. Moreover, these bands could be cloned and sequenced in future for designing suitable primer(s) to be used in PCR based diagnosis. This RAPD profile study of *Nitrosomas* strain will be useful for environmental monitoring of this genus for further nitrification studies.

**Table 1 :** Sequence (5'-3') of primers OPC (1-20)

| Sl. No | Name of primers | Sequence (5'-3') |
|--------|-----------------|------------------|
| 01 | OPC-01 | TTCGAGCCAG |
| 02 | OPC-02 | GTGAGGCGTC |
| 03 | OPC-03 | GGGGGTCTTT |
| 04 | OPC-04 | CCGCATCTAC |
| 05 | OPC-05 | GATGACCGCC |
| 06 | OPC-06 | GAACGGACTC |
| 07 | OPC-07 | GTCCCGACGA |
| 08 | OPC-08 | TGGACCGGTG |
| 09 | OPC-09 | CTCACCGTCC |
| 10 | OPC-10 | TGTCTGGGTG |
| 11 | OPC-11 | AAAGCTGCGG |
| 12 | OPC-12 | TGTCATCCCC |
| 13 | OPC-13 | AAGCCTCGTC |
| 14 | OPC-14 | TGCGTGCTTG |
| 15 | OPC-15 | GACGGATCAG |
| 16 | OPC-16 | CACACTCCAG |
| 17 | OPC-17 | TTCCCCCCAG |
| 18 | OPC-18 | TGAGTGGGTG |
| 19 | OPC-19 | GTTGCCAGCC |
| 20 | OPC-20 | ACTTCGCCAC |

**Table 2 :** Molecular weight of *Nitrosomonas* sp. (DN-1) in kb using different OPC primers (from OPC-1 to OPC-10)

| Primer | OPC1 | OPC2 | OPC3 | OPC4 | OPC5 | OPC6 | OPC7 | OPC8 | OPC9 | OPC10 |
|--------|------|------|------|------|------|------|------|------|------|-------|
| Total No of Bands | 5 | 6 | 3 | 6 | - | 4 | 6 | 3 | 7 | 4 |
| Molecular | 3.048 | 2.097 | 3.356 | 2.373 | - | 2.097 | 2.097 | 1.614 | 2.097 | 2.097 |
| weight | 1.743 | 1.583 | 1.257 | 1.438 | | 1.553 | 1.883 | 0.855 | 1.812 | 1.743 |
| in kb | 0.923 | 1.331 | 1.036 | 1.209 | | 1.384 | 1.357 | 0.528 | 1.494 | 1.209 |
| | 0.691 | 0.838 | | 0.997 | | 1.209 | 1.209 | | 1.209 | 0.838 |
| | 0.528 | 0.528 | | 0.665 | | | 0.978 | | 1.141 | |
| | | 0.352 | | 0.366 | | | 0.665 | | 0.941 | |
| | | | | | | | | | 0.653 | |

**Table 3 :** Molecular weight of *Nitrosomonas* sp. (DN-1) in kb using different OPC primers (from OPC-11 to OPC-20)

| Primer | OPC11 | OPC12 | OPC13 | OPC14 | OPC15 | OPC16 | OPC17 | OPC18 | OPC19 | OPC20 |
|---|---|---|---|---|---|---|---|---|---|---|
| Total No of Bands | 4 | 4 | 4 | 4 | 5 | 0 | 4 | 0 | 3 | 4 |
| Molecular weight in kb | 4.413 | 4.878 | 3.928 | 4.016 | 3.270 | - | 3.163 | - | 1.743 | 2.722 |
| | 3.495 | 2.862 | 2.862 | 3.437 | 1.743 | | 2.097 | | 1.445 | 2.382 |
| | 2.097 | 2.463 | 1.743 | 2.633 | 0.968 | | 1.374 | | 1.209 | 1.679 |
| | 1.286 | 1.519 | 1.286 | 1.743 | 0.780 | | 0.793 | | | 1.545 |
| | | | | | 0.717 | | | | | |

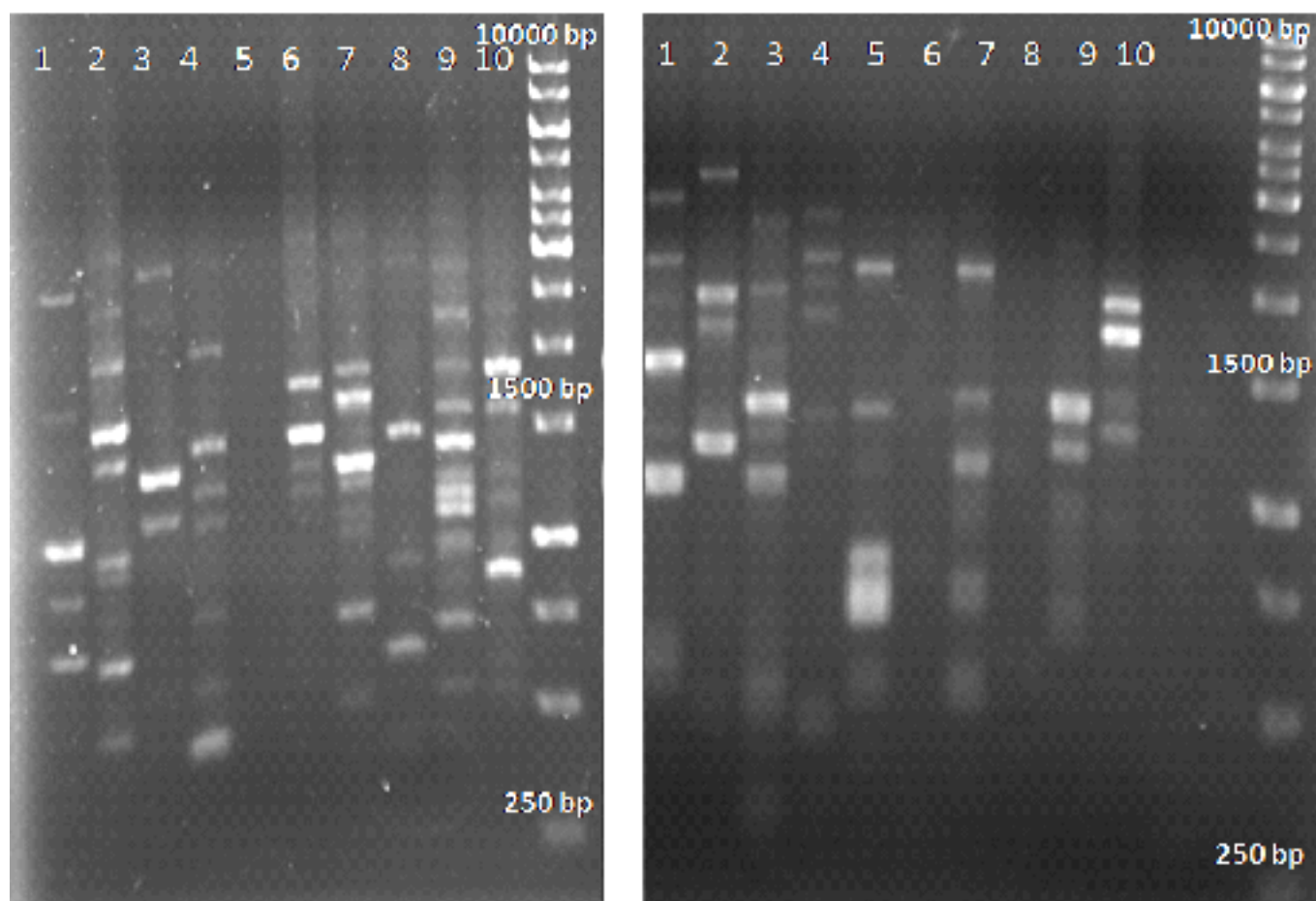

**Fig. 1 & 2 :** RAPD amplification of *Nitrosomonas* sp. with variable primers (Fig 1. Lanes: 1. OPC-1, 2. OPC-2, 3. OPC-3, 4.OPC-4, 5. OPC-5, 6. OPC-6, 7. OPC-7, 8. OPC-8, 9. OPC-9, 10. OPC-10 and Fig 2. Lanes: 1. OPC-11, 2. OPC-12, 3. OPC-13, 4.OPC-14, 5. OPC-15, 6. OPC-16, 7. OPC-17, 8. OPC-18, 9. OPC-19, 10. OPC-20). In both figures molecular weight marker of range 250-10000 bp was used.

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# NEST BUILDING TO FLEDGLING STAGE IN RED VENTED BULBUL: A CASE STUDY

**Puspanjali Parida***

*PG Dept. of Zoology, North Orissa University, Baripada -757 003 (Odisha)

*p_parida2000@yahoo.co.in*

## ABSTRACT

Nest building to fledgling stage in Red Vented Bulbul (*Pycnonotus cafer*) was studied in two pair of birds. It was found that both sexes take part in nest (cup shaped) building. The time required for the nest construction was 48 hours. The incubation period for egg was 10 days. The nestling phase lasted about 10-11 days. In comparison to the adult, the fledglings were smaller in size; tail feather and pinnacle on head were not well developed. The total time required for nest building to fledgling stage was 29-30 days. It was found that in both the cases the hatching success was 100%, nesting success was 100% nesting success was 100% and fledgling success was 100%.

**Keywords :** *Pycnonotus cafer*, Bulbul, nest, nestling, fledgling.

## INTRODUCTION

Red Vented Bulbul (*Pycnonotus cafer*) is found in different parts of Odisha. Scatterd notes on some of the features of the breeding biology of *P. cafer* were given by Ali (1930), McCann (1931), Dutt (1932), Baker (1932) and Lamba (1968). Breeding of a pair of *P. cafer* in human settlements was recorded by Dixit (1963) and Prajapati (2006). Prajapati *et al.* (2011) studied the hatching success, nestling success and nesting success of *P. cafer* by observing 35 nests in a sanctuary. Detail study of nest building to fledgling stage with photographs was not studied earlier. In the present study, two pair of Bulbuls were observed very closely in residential area and the important events were recorded by the help of camera and also recorded the hatching success, nestling success and nestling success.

## METERIALS AND METHODS

Nest building to fledgling stage in Red Vented Bulbul was observed closely using a binocular and the important events were recorded and photographed. Observations were made for 2-3 hours in the day time and 5-6 hours at night during and months period.

The diameter of nest was measured by the help of scale. The egg size could not be measured accurately because handling the eggs might have affected their incubation.

## RESULTS AND DISCUSSION

### Nest building

*P. cafer* prepared a nest on 28th and 29th April 2009 on the top of a 3 feet height plant, planted on a pot (1.2 meter from the ground) in a residential house. The nest was placed at the junction of bifurcated branches to get firm support from the bottom and made in wire like dried plant materials (about 4–5 cm. long and up to 0.5 to 1.5 mm thick dry twigs). Both male and female birds were involved in the preparation of the nest. The materials were brought by their beaks and the diameter of nest was about 4 inches. The time required to complete the nest was 48 hours (two days) (Fig 1, Fig 2, Fig 3 and Fig 4).

### Egg - laying and incubation

On 3rd May (after three days from the nest completion) at morning (between 7am to 7.30am) the female laid one spherical egg with brown patches (diameter is about 1.5 cm) inside the nest. Similarly, on 4th and 5th May around same time, the female

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laid one egg. Hence, the clutch size of the bird was three and the egg was laid consecutively for three days (Fig.5) with interval of 24 hours. The female started incubating the eggs by sitting on it after the last egg was laid. She incubated the eggs throughout the night. The role of male bird was to bring food for his mate and guard the nest while the female takes breaks and feeds. Actually, it was difficult to distinguish male and female bird from their external appearance. The incubation was continued for 10 days.

## Hatching of eggs or nestlings

On 15th May (after 10 days), at morning (around 7 am), one egg was hatched (Fig 6). The other two eggs were hatched at afternoon and evening on the same day. After the hatching, parents had no rest and were constantly collecting food for the newly hatched ones (nestlings). Young nestlings used to sleep most of the time, begging for food as and when required parents used to feed them regularly. So the nestlings are altrical (entirely depending parents for food).

## Feeding

Though they could not see, newly hatched ones would beg if stimulated by sounds, touch or nest movement. All these could mean that either of the parents would have arrived with food. Beaks are large with fleshy edge. They raise their heads on wobbly neck and wave their tiny wings (Fig. 11 and Fig. 12). The eyes were opened on 22nd May (8th day) (Fig. 13). During the entire period, from hatching of eggs till fledging, both the parents regularly visited the nest with food (grass, grain, insect etc.), visibly held in their beak.

## Feather development

The nestings were completely naked with closed eyes (Fig. 7). The nestlings do not have feathers for first few days (5 days of nestling stage)) (Fig 8) and were brooded by the mother throughout the day and night. When their body was covered with feather (from 6th day of nestling stage), the brooding was stopped (Fig. 9 and Fig. 10). The nestling phase continued from 10-11 days.

Body was fully covered with brown colour feathers on 23rd May (9th day of nestling stage) (Fig. 14). Next day white colour feathers were appeared on the ventral side (Fig. 15), 25th days (11th day of nestling stage) the feather on the head was well developed with small pinnacle (Fig. 17). From 25th morning, there was a heavy rain and continued upto next day afternoon. On 26th evening, all the three nestlings came out of the nest and were known as fledgling (Fig. 16,17,18).

## Fledgling

In comparison to the adult, the fledglings were smaller in size. Tail feather and pinnacle on head were not well developed. Body was covered with blackish brown and white colour feathers but had no red feathers (Fig. 18). The fledgling uttered low volume chirps. The fledglings took flight but were clumsy and unsteady on the wing but tried effortlessly. Both parents did post-fledging feeding.

On 26th night one of the fledgling (escorted by both the parents) was completely successful to leave the nest (Fig. 17 and Fig. 18). Next day (27th May), other two fledglings were flied upto 10 ft height and sit on a electric wire. Both the parents came and guided them to fly one by one by showing the path (by flying a small distance, about 1-2 meter) or destination (Fig. 18). Observing the fledglings after they took refuge in thick bushes was difficult. Empty nest did not contain any remnant of egg shell, suspected to be eaten out by the mother.

## Nest building to fledgling

The empty nest was removed manually from the top of plant after the fledglings left the nest. But surprisingly on 7th June during morning time (after a gap of 10 days), again a pair of *P. cafer* (It was difficult

to say whether the same pair or different) came to the same place (same plant) where eggs were laid. They prepared the nest restlessly within two days (48 hours). The shape, size and the materials used in the nest were exactly similar to the previous one. They laid one egg each on the morning of 11th, 12th and 13th June. Eggs were hatched on 23rd June. Rest of the events were similar to the previous one.

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**Table 1 :** Important events from nest building to fledgling stage of *P. cafer.*

| Sl No. | Event | Date | Day | Reference figures |
|---|---|---|---|---|
| 1 | Nest Building | 28th-29[th]April | 1st - 2nd | 1,2,3,4 |
| 2 | Egg laying | 3rd-5th May | 6th-8th | 5 |
| 3 | Incubation | 5th-14th May | 8th-17th | — |
| 4 | Hatching | 15th May | 18th | 6 |
| 5 | No feather/Brooding | 15th-20th May | 18th-23th | 7 |
| 6 | Feather/ No Brooding | 21st-25[th]May | 24 th-28th | 8,9,10,14,15,17 |
| 7 | Nestling    Begging for food/mother feed the nestling | 15th-25th | 18th-28th | 11,12 |
| 8 | Opening of eye | 22rd May | 25th | 13 |
| 9 | Fully covered with feather | 23rd May | 27th, 28th | 14,15 |
| 10 | Small Pinnacle on the Head | 25th May | 29th | 17 |
| 11 | Fledgling | 26th and 27[th]May | 29th-30th | 16,17,18 |

**Fig.1 :** Nest building (starting point)

**Fig. 2 :** Nest building ((view after 10h)

**Fig. 3 :** Nest building (view after 30h)

**Fig. 4 :** Nest building (view after 48h)

**Fig.5 :** Three eggs are laid inside the Nest (6th-8th day)

**Fig.6 :** Hatching of an egg (18th day)

**Fig. 7 :** Nestlings with naked body and Closed eye (19th day)

**Fig. 8 :** Appearance of feather (24th day)

**Fig. 9 :** Feather development on 25th day

**Fig. 10 :** Feather development (26th day)

**Fig. 11 :** Nestlings beg for food (18th- 28th day)

**Fig. 12 :** Mother feed the nestling (18th- 28th day)

**Fig. 13 :** Opening of the eye of nestlings (25th day)

**Fig. 14 :** Brown coloured feather cover the entire body (27th day)

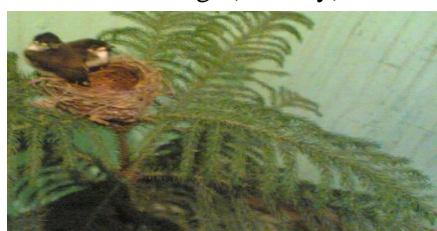**Fig. 16 :** White colour feather on ventral side of the nestling (28th day)

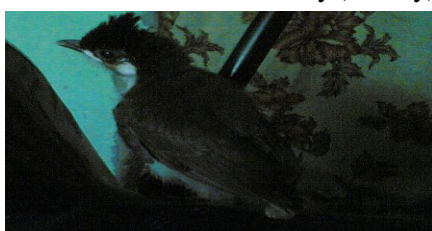**Fig. 16 :** Nestlings are converted into fledgling (29th day)

**Fig. 17 :** Fledgling showing plumage on head (29th day)

**Fig.18 :** Fledgling and the mother sitting on an electric wire (30th day)

# IMPACT ASSESSMENT OF EXTREME CLIMATE VARIABILITY ON LIVELIHOOD OF RICE GROWERS IN MEGHALAYA

**Ram Singh\*, R Saravanan, S M Feroze, L Devarani and Thelma R Paris**

\*School of Social Sciences, College of Post Graduate Studies, Central Agricultural University,

Barapani, Meghalaya - 793 103

*ramsingh.call@gmail.com*

## ABSTRACT

Agriculture sector is affected largely by the negative consequences of extreme climate variability. Rice production has begun to show diminishing returns in recent years. In rural areas of Garo Hills, men and women are highly dependent on biomass such as woods, agricultural crops, wastes and forest resources for their energy and livelihood. However, in the face of climate change, the ability of women and men to obtain these indispensable resources is reduced. Furthermore, changes in the climate usually impact on sectors that are traditionally associated with women. The present study was conducted under International Fund for Agriculture Development (IFAD) funded collaborative research project with International Rice Research Institute (IRRI) to assess the climate change and its impact on men and women farmers' livelihood in the state. It reveals that gender analysis enhances better understanding of what men and women farmer perceive as risks and how they respond to climate changes. Hence, for women, strategies are needed to support their efforts to adapt to climate change. Rice varieties with good yield and stress tolerant varieties should be introduced in farmers' field. Validation and refinement of indigenous traditional knowledge of pest and disease management and their transfer to the farmers' field will improve the production of different crops. Site specific research should be encouraged to identify the micro climate and suitable crop.

**Keywords :** Impact, ECV, livelihood, rice, Meghalaya

## INTRODUCTION

Climate change is one of the most important environmental challenges faced by humanity with its implications on food production, natural ecosystem, freshwater supply, health (Sathaye *et al.*, 2006). Increasing concentration of green house gases in the atmosphere is the cause for global climate change (Thomas, 2011). Agriculture sector is affected largely by the negative consequences of extreme climate variability. It is estimated that every $1^0$C increase in temperature is likely to lead to a 5-10% reduction in yields of some crops (Pachauri, 2010). Complete crop failure usually occurs when severe drought stress takes place during the reproductive stages (Nguyen, 2011). Extreme climate variability (ECV) makes farmers vulnerable to many risks including droughts, floods, diseases on crops and animals, unpredictable market irregularities which adversely affects agricultural labor, food production, farmers' income and often results in abnormal increase in prices of food (Venkateswarlu, 2009, Chand and Raju, 2009 and Sharma *et al.,* 2009). Rice production has begun to show diminishing returns in recent years. Since 2000, world rice production has been less than rice consumption and the deficit has been addressed by drawing on rice from buffer stocks (FAO, 2004). Climate change has significant impacts on fresh water sources affecting the availability of water used for domestic and other work. Women are more vulnerable to the effects of climate change than men (UNDP, 2008 and UN Women Watch, 2009, and Kelkar, 2009).

Meghalaya fall under high rainfall zone with subtropical climate. Still, under influence of global climate change even high rainfall areas are facing drought like situations in the current years. The annual mean maximum temperature is also increasing at a rate of $0.04^0$C per decade in the region (Das, 2009). Hailstorm, thunder and cyclone impacts 16% of the villages where most of the damages are associated with the orchards (Anonymous, 2011). In rural areas of Garo Hills, men and women are highly dependent on biomass such as woods, agricultural crops, wastes and forest resources for their energy and livelihood. However, in the face of

climate change, the ability of women and men to obtain these indispensable resources is reduced. Furthermore, changes in the climate usually impact on sectors that are traditionally associated with women, such as paddy cultivation, plantation, fishing *etc.* (Parikh, 2011). Despite of the increased interest on the impact of climate change on the environment and on agriculture, systematic studies on the impact of extreme climate variability on men and women who are dependent on the natural environment for their livelihoods are scant. Keeping in view the above facts, present paper is an effort in that direction to assess the climate change and its impact on men and women farmers' livelihood in the state.

## MATERIALS AND METHODS

Present study was conducted under IFAD funded collaborative research project with IRRI during 2011-12 in ten villages of West and South Garo Hills districts of Meghalaya. Northeast India has a predominantly humid sub-tropical climate with hot, humid summer, good monsoon and mild winters. Meghalaya is a hilly strip in the north eastern part of India about 300 km long (east west) and 100 km wide with a total area of about 22,720 km². Meghalaya is dominated by 3 major tribes namely *Khasi, Garo* and *Jaintias*. A sample of 150 male and 150 female was selected to make the 300 sample size of households for the study. This study consists of qualitative and quantitative mixture of data were used in the study. Data were collected from farming households (male and female), through personal interview by using pre-tested well-structured schedule. Apart from this, Participatory Rural Appraisal (PRA) and Focus Group Discussion (FGD) methods (Singh *et al,* 2011) were used in the study.

## RESULTS AND DISCUSSION

### Impact of climate change on rice production

Farmers reported that rice yields have declined over past 20 years (farmers' perception on the basis of their memory) due to drought (Fig. 1). The average productivity of rice in normal year was 3157 kg/ha but in drought years about 53 per cent of loss in yield was reported in the study area. Peng *et al.*, 2004 reported that around 15 per cent rice yield decrease by each 1°C increase in temperature. Although this decline cannot be attributed solely to change in climate, drought definitely has contributed largely to this decline. Farmers had been using traditional rice varieties like Ranjit, Mahsuri and Aijong. More than 50 per cent of the male and female farmers reported

a decrease in area under rice due to drought. Of all the male and female farmers interviewed, 82 and 78 per cent, respectively reported decline in rice yields due to severe drought. A higher proportion of the female respondents reported crop loss during drought. Of all the animals commonly reared, female buffaloes, swan and ducks; followed by pigs, goats, cows and chickens were affected by drought. Within households both male and female members take care of livestock, thus, their observations are same (Table 1).

### Impact of climate change on animal husbandry and household income

Similarly, the livestock sector did not escape from the effect of climate change. Farmer respondents reported that they experience changes in the number of livestock owned in normal and drought years. Incomes from non-farm activities provide the largest share of the household income, followed by income from sales of other crops and wages from off-farm activities during both normal and drought years. The income from different sources was adversely affected during drought year due to failure of crops. The income from fisheries was worst affected followed by incomes from sales of rice, vegetables, animals and other crops during drought years (Table 2). The income from non-farm activity is the life line during Extreme Climate Variability (ECV).

### Mitigation strategies to cope up climate change use of varieties

As mentioned in the earlier sector, rice farming households continued to use their local varieties and traditional farming practices to provide them food supply. However, they are willing to change their varieties based on several criteria (Table 5). The most important criteria is good yields per unit of land. Thus they will only change their variety if the new variety has better or higher yields than their local variety. However, decisions on what rice variety to use mainly depends on the land and soil type particularly for rainfed conditions wherein water drainage and retention are important for crop growth. These findings indicate that there is a need to introduce stress tolerant high yielding rice varieties in these villages and state. Both men and women should be involved in agricultural extension services and training activities to improve rice production practices.

### Sources of information of weather

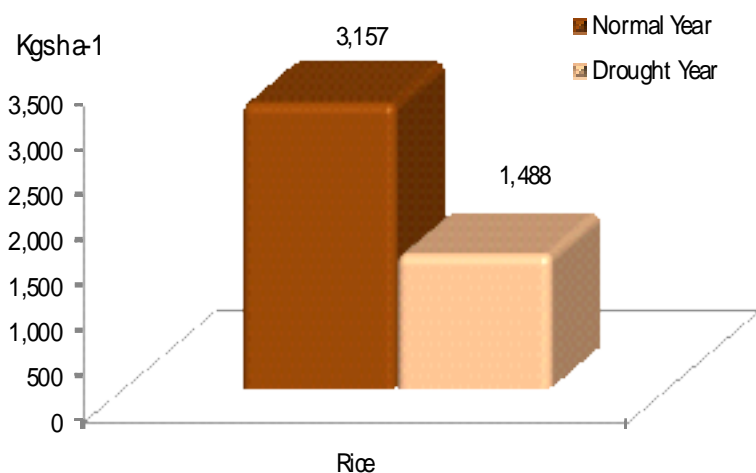Timely crop operations particularly growing rice under rainfed conditions is quite important. However, since

**Fig. 1 :** Average rice yield in normal and drought year

**Table 1 :** Impact of drought on rice production and animal husbandry

| Changes | Per cent of responses | | |
| --- | --- | --- | --- |
| | Men | Women | Overall |
| Decrease in area under rice | 54.0 | 55.0 | 54.5 |
| Decrease in rice yields | 82.0 | 78.0 | 80.0 |
| Crop loss | 62.0 | 83.0 | 72.5 |
| **Occurrence of livestock disease** | | | |
| No | 18.0 | 16.0 | 17.0 |
| Yes | 82.0 | 84.0 | 83.0 |
| All | 100.0 | 100.0 | 100.0 |
| **Higher livestock mortality** | | | |
| No | 18.0 | 22.0 | 20.0 |
| Yes | 82.0 | 78.0 | 80.0 |
| All | 100.0 | 100.0 | 100.0 |

**Table 2 :** Change in the average number of livestock and income by source during normal and drought year

| Livestock | Normal year | Drought year | Change (%) |
| --- | --- | --- | --- |
| Cows | 3.0 | 2.0 | -33.0 |
| Female buffaloes | 2.0 | 0.0 | -100.0 |
| Goats | 3.0 | 2.0 | -33.0 |
| Pigs | 2.0 | 1.0 | -50.0 |
| Chicken | 8.0 | 6.0 | -25.0 |
| Swan | 3.0 | 0.0 | -100.0 |
| Duck | 2.0 | 0.0 | -100.0 |

**Table 3 :** Income by source during normal and draught year - comparison.

| Sale of different item | Normal years | Draught years | Change (%) |
|---|---|---|---|
| **Sources of income ₹** | | | |
| Sales from rice | 755.0 | 293.0 | 61.19.0 |
| Sales from vegetables | 851.0 | 348.0 | 59.11.0 |
| Sales from other crops | 17,279.0 | 8,851.0 | 48.78.0 |
| Sales from large animals | 567.0 | 233.0 | 58.91.0 |
| Sales from small animals/poultry | 51.0 | 24.0 | 52.94.0 |
| Fisheries | 409.0 | 147.0 | 64.06.0 |
| Wages from off-farm income | 11,435.0 | 7,384.0 | 35.43.0 |
| Non-farm | 46,134.0 | 39,394.0 | 14.61.0 |
| Total | 77,481.0 | 56,674.0 | 26.85.0 |

**Table 4 :** Factors influencing the decision to change rice variety (ies)

| Criteria for changing rice variety | Decision to change rice variety (%) | | |
|---|---|---|---|
| | Male | Female | Overall |
| Good yields | 91.0 | 80.0 | 85.5 |
| Tolerance to stress | 12.0 | 23.0 | 17.5 |
| NGO | 0.0 | 1.0 | 0.5 |
| Suitability to soil type/land type | 57.0 | 77.0 | 67.0 |
| No change | 0.0 | 0.0 | 0.0 |

**Table 5 :** Source of information about weather condition

| Source | Percent (%) | | |
|---|---|---|---|
| | Male | Female | Overall |
| Radio | 6.0 | 21.0 | 13.5 |
| Newspaper | 7.0 | 1.0 | 4.0 |
| Television | 27.0 | 36.0 | 31.5 |
| Neighbor | 69.0 | 46.0 | 57.5 |
| Family member | 65.0 | 75.0 | 70.0 |
| Traditional knowledge | 91.0 | 81.0 | 86.0 |
| Others | 1.0 | 1.0 | 1.0 |

time immemorial men and women farmers have relied on their own knowledge and farming experience in predicting the onset and termination of rainfall. However, they said that nowadays it is more difficult to predict the onset of rains due to climate change. Male farmers through their networks obtain weather information from neighbours/farmers, and relatives followed by television. Women on the other hand, obtain weather information mainly from family members/relatives followed by neighbours, television and radio. Women use the radio more than men in obtaining news and weather information (Table 6). These findings reveal the importance of social networks (neighbours, friends, other farmers) in information exchange. There is opportunity to train women as key agents of change through their social networks in employing adaptation strategies that can help mitigate the negative effects of drought on their livelihoods.

It has been inferred that women suffer from a gamut of issues under adverse climate conditions and a focused debate on gender responsive adaptations has tremendous potential to help them offset adverse impacts of climate change. The findings confirms that there is a gender dimension to the way in which climate variability is experienced and expressed by the farmers in their coping strategies to ensure their livelihoods and food security. Further, extreme climate variability (ECV) affects both the gender but with different consequences according to their roles and responsibilities. More context specific research is needed for effective mitigation and adaptation strategies at the local and national level for the tribal farming communities.

## ACKNOWLEDGEMENT

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# ZERO TILLAGE - A RESOURCE CONSERVING TECHNOLOGY FOR RICE CULTIVATION IN HILLY ECOSYSTEMS OF INDIA

**R Saha\*, V K Mishra and P K Ghosh**

Division of Water Management, ICAR Complex for NEH Region, Umiam, Meghalaya - 793 103

*saharitesh74@rediffmail.com*

## ABSTRACT

Over the past few years or so, globally, rapid strides have been made to evolve and spread resource conservation technologies like zero and reduced tillage systems, better management of crop residues and planting systems, which enhance conservation of water and nutrients. North-east India, being rich in natural resources, still lagging behind in terms of crop productivity and livelihood security because of monocropping based on conventional and traditional agricultural practices. In order to have higher productivity under this situation, various tillage practices are adopted for rice cultivation. Study reveals that farmers can produce higher yields and reduce cost of production ten percent by adopting zero tillage. Coupled with incorporation of organic residues under zero tillage, the technique resulted in significant improvement in soil physical properties and crop yield. In view of widespread resource degradation problems and the need to reduce production costs, increase profitability and make agriculture more competitive, the zero tillage technique will provide a better option for sustainable agriculture and livelihood security.

**Keywords :** Zero tillage, conservation agriculture, hill agriculture, rice

## INTRODUCTION

The Indian agriculture is very complex and carrying out multi-functionalities of providing food, nutrition and ecological security besides employment and livelihood for over 700 million people. Indeed, India has made a marvelous achievement in attaining self-sufficiency in food grain production after Green Revolution which eventually resulted in maintaining all-time high buffer stock in warehouses of our country. Such rosy picture in production trends turned to be bleak in the past few years due to various reasons. Shrinking resources of prime lands, deforestation and accelerated erosion, deterioration of soil physical environment, increasing waterlogging and salinity in canal irrigated areas, declining water table in well-irrigated areas, poor management of rainwater, lower efficiency of inputs such as water, fertilizers and agrochemicals, rapid industrialization coupled with pollution, environmental degradation and material calamities have aggravated the problem. Under such circumstance, there is an imperative need to produce more from less arable land through proper management of basic agricultural resources such as soil, water and biological inputs.

The northeastern part of India is considered as a temperate, humid, high valley zone located between $22^0 05'$ and $29^0 30'$ N latitudes and $87^0 55'$ and $97^0 24'$ E longitudes. Rainfed cropping predominates in this region with average annual rainfall ranging from 1000 to 11,409 mm.with 4 to 6 month wet season is followed by a dry and often frosty winter. Thus, the cropping is largely confined to a single crop per year planted at or before the onset of mansoon. Most of the precipitation occurs between May to October, but there are significant dry spells and water stress arising within the wet season. Agricultural sustainability problems related to soil degradation, fertility decline, and subsequent crop yield decline have arisen throughout this region. The Northeastern hill region of India, being a hot spot region of biodiversity and "a low" fertilizer consumption region, recycling of organic materials is gaining importance particularly in rice-based cropping systems and overall impact of climate change.

Rice (*Oryza sativa* L.) is a staple food crop of people of the northeastern hill region of India and occupies 72 % of total cultivated area. Among different rice cultivation practices, puddled rice in the valley land situation forms a major part of crop cultivation.

Puddling is known to increase the yield of rice due to creation of suitable environment that favours growth of the crop, but it destroys soil structure and in turn influences water holding and transmission capacity of soils (Sharma et al, 1995). Among various factors responsible for low productivity, soil moisture availability regarded as the most limiting factor because crops are very much sensitive to soil moisture stress, particularly at their critical growth stages. Strategies to minimize crop water stress include conservation of soil moisture by increased infiltration, reduced evaporation and maximum exploitation of available soil water. Depletion of soil organic carbon (SOC) and reduction in water table aggregates have been identified as other reasons for the unsustainability of continuous rice cultivation even with adequate NPK application (Nambiar, 1995). For long-term sustainability of the rice-based system, soil organic matter dynamics and soil physical environment need to be better understood and subsequently managed. Conservation agriculture is a practice that reduces soil erosion, sustains soil fertility, improves water management and reduces production costs, making inputs and services affordable to small-scale farmers. Conservation agriculture is defined as a set of practices aimed at achieving the following 3 principles simultaneously: i) maintaining adequate soil cover, ii) disturbing the soil minimally, and iii) ensuring crop rotation and intercropping. It is based on enhancing natural biological processes above and below the ground. Interventions such as mechanical soil tillage are reduced to an absolute minimum, and the use of external inputs such as agrochemicals and nutrients of mineral or organic origin are applied at an optimum level and in a way and quantity that does not interfere with, or disrupt the biological processes (Philip *et al.*, 2007).

## MATERIALS AND METHODS

By definition, zero tillage is also known as conservation tillage system that consists of a one pass operation which places seed and fertilizer into an undisturbed seedbed, packs the furrow and retains adequate surface residues to prevent soil erosion. It involves planting seeds into soil that hasn't been tilled after the harvest of the previous crop. The crop germinates in residual water left by the previous crop, saving up to a million liters of water per hectare.

Zero tillage seeding offers the benefits of retained surface residues and reduced soil water losses. Studies conducted throughout the prairies have shown that zero tillage seeding systems can provide higher spring soil moisture and lower evaporation losses compared with conventional tillage systems. Zero tillage and residue management has been widely acclaimed as a highly effective practice for conservation of soil and water and maintenance of soil quality as compared with conventional, it enhances water consumption though improved infiltration and reduced evaporation (Unger and Ordief, 1985; Mohanty *et al.*, 2006). Moreover, direct seeding protects young seedlings from heat and wind stress during early growth stages. Standing stubble reduces wind speed at ground level and reflects rather than absorbs heat. This is particularly important to crops which at early growth stages are susceptible to hot winds and abrasion from wind blown soil particles. Zero tillage has reduced the demand for water in rice and wheat farming on almost a million hectares of land in India, Pakistan and Bangladesh.

## Methodology for rice cultivation under zero tillage :

### Land Preparation

No Tillage operation, only existing weeds and crop stand were cleared and kept in the field. For weed management, either manual weeding or spraying of herbicide (Glyphosate) @ 2.5 ml / liter/ 10 $m^2$ was done before three weeks of transplanting. All the weed biomass was spreaded over the entire field as mulch and allowed sun dry for a few days to facilitate planting operations. Still some weed existed in the field. Hence, one more herbicide spray was undertaken two weeks before transplanting.

### Transplanting

Transplanting of 25 days old 5-6 number seedlings/ hill with the help of cone type of manual dibbler at spacing of 25-10 cm row-to-row and plant-to-plant in moist field. If soil is marshy/ submerged /muddy direct transplanting without use of dibbler can be done to minimize the labour cost. At the time of transplanting ponding of water in the plot was avoided. After completion of transplanting, 5-6 cm water was maintained through proper bunding.

## Weed management

Rice field should be weed free throughout the cropping season. Here, manual deweeding was taken upmore for better control of the weeds. Where there was severe weed population, application of herbicide- Anilofost ethoxyenlfuron (Rice guard) @ 324 g/ha was undertaken on 15th day of transplantation to save excessive labour cost.

## Water management

Adequate water supply was maintained continually with shallow submergence of 5 ±2 cm throughout the crop growth. In case of limited water supply, submergence must be followed only during tillering and panicle initiation to flowering.

## Manuring

At initial stage (1-3 years), FYM @ 10 t/ha was applied before 15 days of transplanting. After $3^{rd}$ year, no external organic manure was required as crop residue/ weed biomass, incorporated in the field.

## Fertilization

Application of NPK fertilizers was undertaken *viz.* 80, 60, and 40 kg/ha NPK with standard method of application i.e. equally three split application of nitrogenous fertilizer and phosphorus and potassium at the time of transplanting as basal dose.

## Harvesting

After harvest panicles with sickle crop residues were left in the field. If 100% crop residue incorporation is not possible at least 25 – 50 % residue incorporation in the field helps to maintain the physico-chemical and biotic environment of the soil. A field experiment was conducted (2000-2005) with various combinations of tillage practices i.e. Zero tillage; $T_0$, manual spading; $T_1$, power tiller; $T_2$ and desi plough; $T_3$ at ICAR Research Complex for NEH Region, Umiam, Meghalaya (25Ú 41´N, 91Ú 63´E, 980 m above mean sea level). Zero tillage consisting of one-pass operation which placed the seeds and fertilizers into an undisturbed seedbed, packed the furrow and retained adequate surface residues to prevent soil erosion. Manual tillage means simple churning of the top soil with the help of spade. Power tilled plots had 4-pass operation of power tiller and desi plough with traditional single or double passes.

## RESULTS AND DISCUSSION

Periodical observations on soil physico-chemical properties were recorded during the study period and were anlysed.

## Soil fertility Status

Zero tillage practice built up the organic carbon content, available phosphorus and potassium while the conventional tillage practices play the role in reduction of available nutrients (Table1). Improvement in soil physico, chemical properties and crop root development by no tillage enhanced the fertilizer response. The favourable effect of zero tillage on fertility status might be partially due to high amount of earthworm crust. The toxic effect of Fe and Al on rice crop in acid soils also minimized by adoption of zero tillage technique.

## Soil physical properties

Soil Structure : Zero tillage system improves the different physical properties of soil like soil structure; increase the relative proportion of bio-channels, macro-pores and decrease the susceptibility of crusting. It has been observed (Table 1) that the bulk density of soil decreased about 29 per cent, total porosity and soil aggregates increased by 29 and 32 per cent, respectively, over the conventional tillage. The more activities of earthworms and other flora and fauna are also recorded in no tillage plots. High number of earthworm activity is the most important soil requirement to improve the soil structure and porosity of soil for successful application of no tillage system.

**Table 1 :** Effect of tillage practices on soil physico-chemical properties

| Tillage treatments | Bulk density (g/cm³) | Org. Carbon (%) | Available P (kg/ha) | Available K (kg/ha) |
|---|---|---|---|---|
| Zero Tillage; $T_0$ | 0.89 | 2.17 | 11.77 | 141.1 |
| Manual spading; $T_1$ | 1.25 | 1.83 | 9.82 | 124.5 |
| Power tiller; $T_2$ | 1.29 | 1.74 | 9.45 | 124.5 |
| Deshi Plough; $T_3$ | 1.25 | 1.80 | 9.72 | 126.6 |

## Puddling index

The puddling index for different treatments varied between 29.2 and 38.6. The application of organic amendments in the form of crop residue and weed biomass improved the degree of churnability in the soil system. Lower the puddling index value, better is the soil aggregation (Mohanty and Painuli, 2004). Significantly lower puddling index values in the zero tillage plots indicated that organic matter acted as a cementing agent during disintegration of aggregated particles by puddling.

## Soil penetration resistance

Soil penetration resistance (SPR), determined by the strength of the bridging material, which, in turn, is influenced by matric potential (moisture content), varied among the various treatments. Organic residues under zero tillage resulted in accumulation of organic matter, low bulk density and thus soil became more porous in comparison to control plots. Increase in bulk density increases the resistance of soil due to both volumetric compression and linear deformation (Sharma and Bhushan, 2001).

## Soil water retention and transmission characteristics

Soil water retention and transmission characteristics showed that zero tillage increased the soil moisture retention capacity (Fig. 1). The increase in total porosity, particularly micro porosity, due to addition of organic matter probably led to enhancement of the moisture retention capacity (Roychoudhury *et al.*, 1983; Fischer *et al.*, 2002). The basic principle of water transmission pattern in the soil profile was that when the infiltration and hydraulic conductivity rate was high, the surface water percolates down to the soil profile and increased the soil moisture recharge. Therefore, the evapo-transpiration water loss is less from the surface soil under this condition. Whereas, water loss through evapo-transpiration activity was much higher when the infiltration and conductivity rate was less in the soil as the excess water stagnate on surface soil. Saturated hydraulic conductivity ($K_{sat}$), which helps to know about the variation in water transmission pattern in the whole soil profile varied from 3.28 to 3.68 cm/hr among the various

treatments. Faster rate of steady infiltration under conventional tillage system could be ascribed to the minimum disturbance and lack of puddling effect that maintained the continuity of pores under organic treatments. The moisture characteristics curve under various treatments revealed that the magnitude of water content reversed with increasing suction probably due to the differences in release pattern capacity of soil.
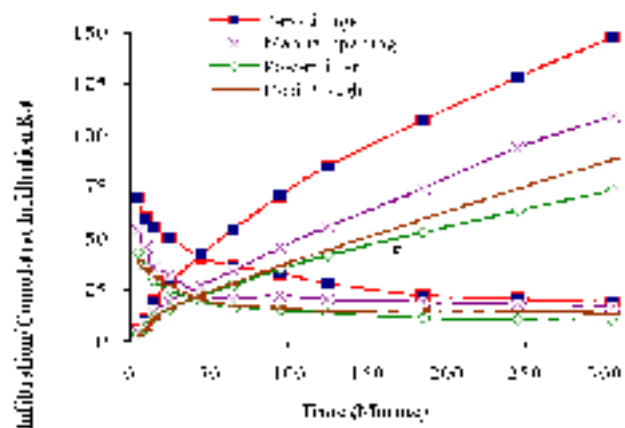


**Fig. 1 :** Effect of tillage practices on infiltration rate (cm/hr) and cumulative infiltration (cm)

Soil erosion was also effectively controlled by zero tillage system.

## Water profile status

Zero tillage practices enhanced 20 per cent water storage at harvest of rice as compared to conventional tillage (Fig. 2). The profile storage moisture can be properly utilized for short duration winter crop like mustard. The high water storage capacity of surface soil in organic-treated plots might have been due to high organic carbon content while higher water rate of transmission may be ascribed to the continuity of micro and macro pores of soil resulting in uniform movement of water within the soil profile. Percolation and evapotranspiration losses indicated that in all the treatments, the water losses were lowest under transplanting stage. Thereafter, it increased marginally in subsequent crop growth stages and became almost constant with marginal fluctuation at flowering and maturity stages. This might be due to the reduction in puddling effect through more root proliferation resulting in higher rate of percolation at advanced stages.
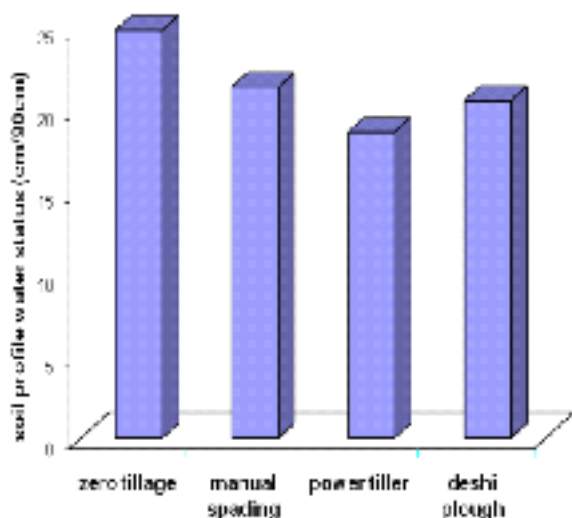
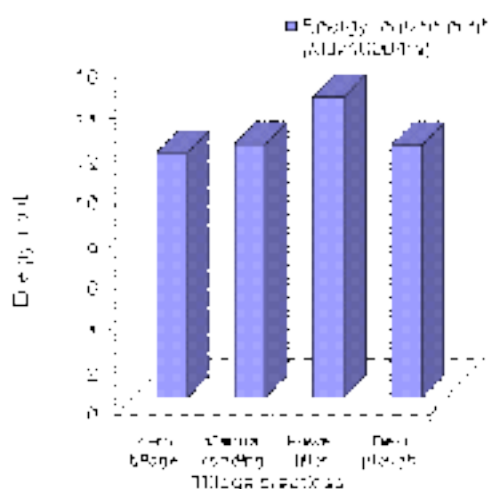**Fig. 2 :** Effect of tillage practices on profile water status



**Fig. 3 :** Energy requirement under various tillage practices

**Douff layer**

The douff layer is the thatch of plant materials on the surface. Zero tillage allow this layer to build up on surface soil. Normaly douff layers suppress weeds and reduce evaporation. It will also create a warmer soil zone giving quicker crop emergence and seedling establishment during early plant growth stage.

**Energy conservation**

The requirement and production of energy in rice cultivation varied from 11.56 x $10^3$ to 14.24 ´$10^3$ and 111.70 ´$10^3$ to 153.10 ´$10^3$ MJ/ha, respectively, for various tillage practices (Fig.3). Through its emphasis on reducing inputs, causes significant saving about 20 per cent in energy requirements without jeopardizing rice production by adoption of no tillage practices in comparison to the conventional tillage. Zero tillage saves energy through reducing

the intensity and frequency of tillage and decreasing fertilizer needs by conserving soil and water (Mishra et al, 2003).

**Crop yield**

Favourable soil environment under zero tillage system produced good rice yield and it was equivalent the yield produced under conventional tillage (Table 2). It was recorded that the yield at initial stages (1-3 year) was lower in zero tillage and thereafter the economic yield of rice increased significantly with improvement in physical, chemical and biological fertility of soil whereas in conventional tilled plots, the yield was almost stabilized. This is due to deteriorating effect of puddling on soil physical, chemical and biological properties (Acharya and Sood, 1992). Incorporation of crop residue also results in improved BD, $K_{sat}$ and MWD (Tripathi, 1992) and increased microbial activity as judged by increased microbial count and biomass and $CO_2$ evolution in soil and increased SOC (Sharma and Prasad, 2001). These improvements in physical, chemical, and biological properties of soils can lead to increased grain yield of rice. Thus, zero tillage is an important technique of low input sustainable agriculture system in high rainfall and susceptible to soil erosion areas.



**Fig. 4 :** Rice crop performance under conventional tillage



**Fig. 5 :** Rice crop performance under zero tillage

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**Table 2 :** Effect of tillage practices on rice grain yield

| Tillage practice | Rice grain yields during subsequent years (q/ha) | | | | |
|---|---|---|---|---|---|
| | 2000 | 2001 | 2002 | 2003 | 2004 |
| Zero tillage; $T_0$ | 26.3 | 29.1 | 30.6 | 37.3 | 36.6 |
| Manual spading; $T_1$ | 32.4 | 39.0 | 34.5 | 35.8 | 33.0 |
| Powertiller; $T_2$ | 33.1 | 40.5 | 36.8 | 32.5 | 33.1 |
| Desi plough; $T_3$ | 28.5 | 36.8 | 32.4 | 30.6 | 31.5 |

With zero-tillage technology, farmers can produce higher yields and reduce production costs by up to 10%. They also save on diesel for tractors, and the fertility and structure of the soil improves. The Zero tillage not only favourably moderated the soil rhizosphere and produced higher grain yield in long term aspect but also improved the water economy during dry periods by permitting downward movement of water across the root boundary. Coupled with this improvement in soil physical properties, the incorporation of organic residues resulted in significant increase in rice yield. Moreover, the locally available jungle grasses are equally good as organic amendment, which would also ease the problem of disposal of these grasses during peak monsoon. In this way, they can save a significant portion of production cost. Thus this technology may provide greater opportunity for low cost rice cultivation through better management practices for sustaining crop production in the hilly eco-system.

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# ECONOMIC ANALYSIS OF WASTE HEAT RECOVERY SYSTEM IN SPONGE IRON PLANTS

**C R Mohanty*[1], B B Pani[1] and S K Khuntia[2]**

*[1]Veer Surendra Sai University of Technology, Burla, Sambalpur,

[2]NM Institute of Engineering and Technology, Bhubaneswar

*sujit_khuntia@yahoo.com*

## ABSTRACT

The conservation of electrical energy in making of sponge iron by direct reduction process is of prime importance. The objective of this study is to investigate the feasibility of using a waste heat boiler in Suryaa Sponge Iron Plant, Rajgangpur, Odisha to generate steam using energy recovered from the exhaust gases of a kiln. The energy rejected in the waste gases of a DRI kiln has been shown to be in the region of 480 kWh tonne of sponge iron produced. The waste gases from a DRI kiln provides sufficient potential for electricity generation to satisfy the in-house consumption of pollution control equipments. A cost-benefit analysis indicates that the capital cost of around 4.0 crore rupees for the installation of a waste heat boiler on one 100 TPD kiln would have a simple pay-back period of 11 months.

**Keywords :** Sponge iron; Waste heat recovery systems; Cost-benefit analysis.

## INTRODUCTION

Electrical energy is a major contributor to the overall manufacturing cost in sponge iron making, and its conservation is of prime importance(Pradhan, 2003). Sponge iron making involves the separation of iron from iron ore by reduction in a kiln at appropriate temperature and pressure. Direct reduction is defined as a process used to make solid iron products from ore or pellets using natural gas or a coal-based reductant. The process reaction / steps are carried out in a rotary kiln with iron ore and non coking coal as prime raw materials in requisite proportions along with a little quantity of dolomite acting as a desulphuriser.

The largest single source of energy loss from a kiln is via the waste gas stream, and urgent consideration is being given to methods of reducing the quantity of energy lost in this way, and to recovering heat from the exhaust gases. Ore preheating is potentially the most attractive method of energy recovery, but its effectiveness is often restricted because of practical considerations. However, these plants can produce electrical energy by using a waste heat boiler which is different from a conventional Boiler Plant. The proportion of sponge iron manufactured by the DRI process in India has steadily increased over the past

20 years. The rapid industrialization in the country has increased the energy requirements. On the other hand, the country has limited quantity of fuels reserve such as oil, coal, etc. For this reason, utilization of waste heat recovery systems (WHRSs) seems to be a key concept for clean and sustainable future for the state. The investigations have been directed to technology development in the usage of waste-heat sources as a result of ever increasing in the country energy demand associated with environmental factors. Therefore, the use of the existing technology or development of any other new technology for usage of waste heat sources is needed due to increasing in the country's energy demand associated with environmental factors.

Effective utilization of lost energy in industry is important economically and environmentally not only for Orissa but also for all over the world. Designing efficient and cost effective systems that also meet lower capital and running costs and environmental conditions are the foremost challenges that engineers face. In the world, with finite natural resources and large energy demands, it becomes ever increasingly important to understand the mechanisms that degrade energy and resources and to develop systematic approaches for improving systems and, thus, also reducing the impact on the environment. So, waste-heat recovery systems have been

investigated by numerous researchers. Finishing accounts for a sizeable share of the total amount of energy is consumed by the DRI industry. This study focuses on heat recovery from hot air coming from the kilns for recycling heat is also discussed.

A large number of different techniques for recovering waste heat which can be reduce process operation cost and conserve significant amount of fuel. Waste-heat recovery is likely to be the major conservation method to be adopted in a wide range of industries, and can involve a substantial outlay of capital.

The major energy saving systems are waste gas heat recovery. Process efficiency should be improved before considering waste-heat recovery from any stream or systems; Johnson (1980) describes five factors for the selection of waste-heat recovery exchangers: usability (of available waste heat), temperature, fouling, corrosion, characteristics (of the waste heat stream), and quantity (flow rate of waste-heat stream and desired heat exchanger effectiveness). A process to recover at least 80% of energy from hot flue gas has been developed by Fedele (1984).

Pantz (1979) and Fernadenz (1979) presented technological recovery potential of waste-heat in the industry and developed a methodology for heat recovery techniques. The principal reason for attempting to recover waste heat is economic. All waste heat that is successfully recovered directly substitutes for purchased energy and therefore reduces the consumption of and the cost of that energy. A second potential benefit is realized when waste-heat substitution results in smaller capacity requirements for energy conversion equipment. Thus, the use of waste-heat recovery can reduce capital costs in new installations. The waste-heat recovery reduces the requirement for space-heating energy. This permits a reduction in the capacity of the furnaces or boilers used for heating the plant (Fernadenz Lopez, 1979). In every case of waste-heat recovery, a gratuitous benefit is derived: that of reducing thermal pollution of the environment by an amount exactly equal to the energy recovered, at no direct cost to the recovery (Pradhan, 2003).

In recent years, a great deal of attention is focused on the efficient utilization of energy resources with minimum heat loss. There is a growing interest on second law analysis to minimize the entropy generation in various thermal units and thereby to improve and optimize the design and performance. In this case study, a waste-heat recovery steam generator is considered which consists of an economizer, an evaporator, a super heater and a turbo-generator. The unit produces superheated steam by absorbing heat from the hot flue gases. Energy and environmental studies show that in increase of process efficiency simultaneously with a decrease of thermal pollution. If the feed water is heated with flue gases, the fuel consumption is reduced by about 13%, while the boiler outlet flue gases temperature decreases from 232 to 55.8° C. The combination of feed water and air preheating shows fuel savings of 13.6%, and the outlet flue gases temperature is reduced to 29.78° C (Blank and Garbutt, 1981; Hill and Marshall, 1984).

## MATERIALS AND METHODS

An analysis based on energy concept is carried out in order to find effective working conditions for WHRSs. The study describes an easy-to-follow procedure for energy analysis for WHRSs and how to apply the described procedure to assess the performance, energy destruction and economical contribution, and thus showing the direction for improvement of WHRSs. The scope of this study is to present systematically the possibilities and the efficiencies of heat recovery from hot flue gas.

A case study, based on a furnace producing sponge iron company named Suryaa Sponge Iron Plant, Joda, Keonjhar, Odisha, has been used to demonstrate the advantages and disadvantages of this method of energy recovery in practical and economic terms.

## RESULTS AND DISCUSSION

The off gas getting out of the rotary reactor contained combustible gases such as hydrogen, carbon monoxide, hydrocarbons, volatile organic compounds, and such other gases contained in the volatile matter of coal. Due to their polluting nature, the waste gas can neither be vented into the atmosphere nor can be cleaned with an electrostatic precipitator (ESP) due to combustible characteristics prior to combustion in an after burning chamber (ABC). Apart from these, the off-gas also contained

large quantity of dust and coal fines (coal fines being significant part).

It became essential therefore, to burn the combustibles before they passed through the Electro Static Precipitator (ESP), to a safe limit to avoid explosion in ESP. Further, that was required to be reduced to a safe limit from environmental point of view. The off-gas emerged at about 850-900⁰C from the rotary reactor and entered to the ABC where combustion air is added to the gas stream at a controlled rate taking care that the temperature does not rise more than 1000⁰C, pre-determined based on the dust fusion temperature. There were provisions to cool the gas with either water spray or inert gas in case of exceed of desired temperature.

The gas after ABC was taken to a water tube waste heat recovery boiler. The gas first passed through the radiation chamber of the boiler where the gas was cooled to about 600⁰C by transferring heat to the water wall. The water from the water wall were in natural circulation with the water chest of the boiler drum.

The next stage of gas cooling was in the super heater banks of the waste heat boiler where the gas was cooled by transferring heat to the steam exiting from the boiler drum. The gas cooled to below 400⁰C while the steam was heated to above 500⁰C. This steam was then de-superheated to the desired temperature.

The gas from super heater tube bank passed to the economizer where it heated the boiler feed water from boiler feed pump to the boiler drum. Here the gas was cooled down to below 180⁰C, suitable for cleaning in pressure rating of the boiler maintained at 42 atm or 64 atm or even 100 atm depending on the turbine selected for power generation.

### Typical data of waste gas vis-a vis probable power generation capacity in a 100TPD sponge iron plant

The volme of flue gas generated from a 100TPD kiln was measured from the kiln located inside the plant area. Power drawn by the ID fan and static pressures across it were measured to estimate air flow rates. Average power drawn by the fan motor was about 5.5. kw and discharge fan pressure was 2.4 cm WC.

Assuming fan efficiency of about 40 % and based on above online measurements the estimated fan discharge is about 30637 Nm³/hr.

Fan power output = fan shaft power x Fan efficiency

$$= 5.5 kw \times 0.4 = 2.0 \ kw$$

Fan power output = 2.72 x 10⁻⁵ x Q x P

$$Q = \frac{fan}{2.72x} \frac{power}{10^5} \frac{otput}{P} = 30637 \ m^3/hr$$

where, Q = Air flow in m³/hr, P = cm water column

### Estimation of available gas quantity from kiln exhausts and steam generation potential in Waste Heat Recovery Steam Generator (WHRSG)

Based on above ID fan measurement and estimation of gas flow rates, Table 1 below table presents the average gas flow quantity and its heat recovery potential in a WHRSG. The below estimations are on the basis of present fuel qualities. It has been discussed that post installation of a WHRSG the exhaust gas temperature from the kiln could be further raised (to about 950 ⁰C) by admitting more air in the post combustion chamber. The same has been incorporated for estimating waste heat energy potential. Also, the present coal quality is being reviewed by the management and any improvement in coal quality will also increase the flue gas quantity and from it the heat recovery potential.

**Table 1 :** Typical characteristics of stack gas of a 100TPD kiln

| Sl.No. | Particulars | Value |
|---|---|---|
| 1 | Volumetric flow rate of flue gases after temp correction, Nm²/hr | 31000 |
| 2 | Estimated temperature of flue gases at inlet of waste heat recovery steam generator | 950 ⁰C |
| 3 | Specific heat of flue gases at 950 ⁰C (kcal/kg ⁰C) | 0.28 |
| 4 | Density of Flue Gas kg/m³ | 0.7802 |
| 5 | Mass flow rate of flue gases, kg/hr | 40134 |
| 6 | Average total coal feeding rate in kiln, kg/hr | 4670 |
| 7 | Ratio of air to coal fed, kg/kg fuel | 8.59 |

**Table 2 :** Typical steam generation potential of stack gas of a 100 TPD kiln

| Sl.No. | Particulars | Value |
|---|---|---|
| 1 | Mass flow rate of flue gases, kg/hr | 40134 |
| 2 | Specific heat of flue gases at 950 °C (kcal/kg °C) | 0.28 |
| 3 | Inlet temperature of flue gases at WHRSG (°C) | 950 |
| 4 | Design temperature of flue gases at outlet of WHRSG (°C) | 180 |
| 5 | Waste heat recovery potential (Mkcal/hr) $40134 \times 0.28 \times (950-180) \times 10^6 = 8.65$ |  |
| 6 | Enthalpy of superheated steam at outlet of WHRSG at 67 kg/cm² and 490 °C | 810 |
| 7 | Design feed water temperature / enthalpy (kcal/kg) | 126.5 |
| 8 | Steam generation potential (kg/hr) $8.65/(810-126.5) = 12400$ |  |

The specific steam consumption of the turbine is 3.9 – 4.10 kg/kWh depending on the condition. Thus the power generation potential from each WHRB being 12400/4.10 = 3024 kw h which was 2.0-2.5 MW from each WHR Boiler.

**Table 3 :** Economics of waste heat boiler for 2 MW power generations in a 100 TPD sponge iron plant

|  | Particulars | Costs (in lakhs) |
|---|---|---|
| 1 | Civil work | 20.83 |
| 2 | Structural & plate work | 20.83 |
| 3 | Waste heat recovery boiler | 180.00 |
| 4 | Turbo-generator set | 93.83 |
| 5 | Steam pipes | 15.00 |
| 6 | EOT cranes | 6.00 |
| 7 | Cooling Tower & pump house | 20.00 |
| 8 | DM plant | 3.50 |
| 9 | Electrical & substation | 30.00 |
| 10 | Equipment erection | 10.00 |
|  | Total | 368.75 |

The industry shall generate 14.4 million unit in a year (considering 300 operating days) which shall save around 432 lakhs in a year. The simple payback of capital would be 11 months.

The energy rejected in the waste gases of a DRI kiln has been shown to be in the region of 480 kWh tonne of sponge iron produced. A study of the possibility of using a waste heat boiler to recover energy indicates that the waste gases from a DRI kiln provides sufficient potential for electricity generation to satisfy the in-house consumption of pollution control equipments. A cost-benefit analysis indicates that the capital cost of around 4.0 crore rupees for the installation of a waste heat boiler on one 100 TPD kiln would have a simple pay-back period of 11 months. Various practical factors including optimization of efficiency, the accretion of solids on boiler tubes and the effect of the boiler on the operation of gas cleaning facilities may be investigated.

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# PREVALENCE OF BENIGN PROSTATIC HYPERPLASIA AMONG GERIATRIC PETS

**M R Das*, J C Jena, R C Patra, N Sahoo, G S Parida and S Biswal**

Department of Veterinary Medicine College of Veterinary Science and Animal Husbandary,

Orissa University of Agriculture and Technology, Bhubaneswar - 751 003

*dr_mrdas@rediffmail.com*

## ABSTRACT

Examination of 445 geriatric dogs with the history and/or clinical signs of passing small thin tape-shaped faeces, straining during urination and defecation, urinary incontinence, tenesmus, blood dribbling from penis, holding tail away from backward, hind limb paralysis followed by digital rectal examination (DRE) for size, symmetry, surface contour, consistency, movability, pain on palpation and isothermic confirmed Benign Prostatic Hyperplasia(BPH) in 57 geriatric dogs indicating an overall prevalence of 12.8 percent. Break-up of BPH in affected dogs with respect to breed revealed that prevalence was more in large-sized breeds (13.8%) and lowest in toy/small-sized breed (11.9%).

**Keywords :** Prostatic hyperplasia, geriatric, prevalence, testosterone

## INTRODUCTION

Geriatric dogs, particularly in un-neutered intact males, have a greater chance of developing prostate diseases (Read and Bryden, 1995). Almost all breeds are affected with prostate disorders (Rink *et al.*, 1998). According to Krawiec and Heflin (1992), middle and big sized breeds are more prone to development of prostatic disease. Types of prostate abnormalities seen in dogs include benign prostatic hyperplasia (BPH), cysts, abscesses, acute and chronic infections and neoplasia (Paclikova *et al.*, 2006). Though institution of proper therapy is required for an accurate diagnosis, neutering is often recommended as a part of therapy, regardless of the type of prostatic disease present (Krawiec, 1989). Under the study, an attempt was made to record the prevalence of benign prostatic hyperplasia (BPH) among geriatric dogs in Bhubaneswar city.

## MATERIALS AND METHODS

A total of 445 geriatric dogs brought/referred to the Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, OUAT were screened to ascertain the prevalence of benign prostatic hyperplasia during 2011-12. Based on the guideline of Goldston (1989), pet dogs were grouped as giant, large, medium and small-sized dogs if their minimum age was 6, 7, 9 and 11 years, respectively. Selected dogs with the chief complaints/ history/ clinical signs of passing small thin tape-shaped faeces, straining during urination and defecation, urinary incontinence, tenesmus, blood dribbling from penis, holding tail away from backward and hind limb paralysis were segregated for subsequent study with respect to physical characters of prostate through digital rectal examination(DRE) which included size, symmetry, surface contour, consistency, movability, pain on palpation and isothermic.

## RESULTS AND DISCUSSION

It is interesting to note that all the 57 dogs had the prostate bigger than the size of a walnut, when compared to that of apparently healthy dogs. The other characters of prostate recorded in this study were asymmetric, rough, soft, unmoveable, pain on palpation and hyperthermic in 71.9, 75.4, 94.7, 68.4, 96.5 and 70.1 %, respectively. It was found that more than 70% of the affected dogs exhibited bigger size, asymmetric, rough, soft, pain on palpation and hyperthermic prostate gland. Unmoveable prostate were detected in 68.4% of geriatric dogs.

**Table 1 :** Prevalence of benign prostatic hyperplasia in different breeds of geriatric dogs.

| Category with geriatric age | Name of the breeds | No. of geriatric dogs examined | No. of dogs found positive for benign prostatic hyperplasia (%) |
|---|---|---|---|
| Toy/small-sized breed(>11 yrs) | German spitz | 41 | 4 |
| | Dachshund | 28 | 3 |
| | Pomeranian | 19 | 3 |
| | Pug | 16 | 2 |
| | Beagle | 4 | 1 |
| | Cocker spaniel | 18 | 2 |
| | Total | 126 | 15 (11.9%) |
| Medium-sizedbreed(>9 yrs) | Samoyed | 22 | 3 |
| | Dalmatian | 26 | 3 |
| | French Bull Dog | 4 | 1 |
| | Basset hound | 2 | 1 |
| | Non- descript | 45 | 4 |
| | Total | 99 | 12(12.1%) |
| Large-sizedbreed(>7 yrs) | Doberman Pinscher | 34 | 8 |
| | German Shepherd Dog | 42 | 7 |
| | Labrador Retriever | 48 | 5 |
| | Golden Retriever | 19 | 2 |
| | Rottweiler | 14 | 1 |
| | Boxer | 17 | 1 |
| | Total | 174 | 24(13.8%) |
| Giant-sizedbreed(>6 yrs) | Great Dane | 23 | 3 |
| | St. Bernard | 16 | 2 |
| | Bull mastiff | 5 | 1 |
| | Tibetan mastiff | 2 | - |
| | Total | 46 | 6(13.0%) |
| | Grand Total | 445 | 57(12.8%) |

Out of 445 geriatric male dogs of various breeds and differentage groups examined, 57 were found positive for benign prostatic hyperplasia (BPH) reflecting an overall prevalence of 12.8 per cent. Breed-wise break-up showed the prevalence rates of 11.9, 12.1, 13.8 and 13.0 % in toy/ small-sized, medium-sized, large-sized and giant-sized geriatric dogs (Table 1). Ling *et al.* (1983) observed BPH in 16.3 per cent geriatric dogs in California city. Prevalence rate varying from 36.6 to 80.0 % have been observed by Barsanti and Finco, 1984; Dakshinkar *et al.*, 2000; Teske *et al.*, 2002; Powe *et al.* 2004; Mukaratirwa and Chitura , 2007 and Dakshinkar *et al.* , 2008. The patho-physiology behind BPH in intact males refers to avoidance of mating, neutered males, more secretion of testosterone, rapidly changing of oestrogen / androgen ratio when oestrogen predominates and region-based managemental practices (Paclikova et al., 2006).

Breed related susceptibility to BPH has been investigated where highest prevalence was recorded in large-sized breed (13.8%) comprising Doberman, German Shepherd and Labrador dogs. This corroborated the statement made by Smith (2008). Ruel *et al.* (1998) reported that BPH was more commonly encountered in Poodle (12%), York Shire Terrier (8%) and Labrador Retriever (7%). Similar type of breed wise prevalence was also reported by Jayaraja *et al.*(2003) in Tamilnadu and Varshney *et al.*(2010) in Gujarat. Sirinarumitr *et al.* (2001) reported higher prevalence of BPH in the breeds like Doberman, GSD and Labrador. According to Smith (2008), prevalence of BPH seemed to be higher in large-sized breed dogs such as GSD and Doberman.

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