



# *e-planet*

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Volume - 13

January - 2015

Issue No. - 1

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**A Multi-disciplinary International Journal  
of  
Ecology, Environment, Agriculture and Allied Sciences**



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Owned by the Organisation for Protection of Ecosystem, Environment and Endangered Species (OPES), Plot No. - 20/ A, Ashok Nagar,  
Bhubaneswar- 751 009, Odisha, India: Published and printed at Sai Enterprises, Naraina Industrial Area, New Delhi - 110028

**Logo Description :** It symbolizes an elephant within an ecological frame of peace and harmony moving towards prosperity and posterity.

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# Sustainable intensification of animal systems in emerging economies

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## ABSTRACT

Increasing global population and the concomitant rise in demand for livestock products is a challenge that the agriculture industry should address. There are three ways to increase production to meet demand: increase land area for agriculture or produce more within the area that is currently used for agriculture. There is little room for expansion and will not be enough for the expected 70% increase in demand. Therefore, the solution lies in increasing production (and reduce waste at the same time). However, higher production may negatively affect the environment; therefore, increases in production must be made in a more sustainable way. Livestock emit greenhouse gases that impacts environmental sustainability directly by belching (methane) and during manure storage and land application (mostly nitrous oxide). While agriculture in developed countries has served well in providing abundant, safe food, even more effort should be done to reduce the carbon footprint. The highest opportunity in increasing productivity lies in developing countries, particularly reducing emissions per unit of product produced. The ultimate goal is sustainable intensification: keeping the production gains made in intensification while continuing to improve the sustainability of our agricultural production systems. But it is also worth recognizing that the success producers have had in intensifying agricultural production has helped to reduce climate change.

**Key words:** Greenhouse gas, methane, livestock, manure.

## INTRODUCTION

It has been generally accepted that the world population is expected to exceed 9 billion people by 2050. Supplying this population with safe, secure, and healthy food will challenge the world's agricultural resources and production systems. This is expected to increase the demand for livestock products, which will be challenging to meet because very little room for land expansion exists. It is expected that the largest growth will be in developing countries and will overtake developed countries in their consumption of livestock products (FAO, 2011). Due to this increasing global demand for livestock products, there are concerns over sustainable animal agriculture practices and

particularly environmental impacts of livestock production (Kebreab *et al.*, 2012). The environment can be impacted in several ways such as degradation of water quantity and quality, air emissions and other emerging issues such as hormones, antibiotics and other chemical pollutants. Godfray *et al.* (2014) summarized the challenges for the food system in the coming decades as (1) population growth including changes in demography, (2) increases in disposable income and expectations and consequent diet change, (3) resource scarcity, particularly water, (4) global change, particularly climate change and its impact on food production, and (5) mitigation of greenhouse gas emission and at the same time adaptation to consequences to climate change.

Livestock industries are a significant source of greenhouse gas (GHG) emissions globally, however, in developing countries with less intensive agricultural practices, livestock contribute proportionally higher compared to developed countries which comprise less than 10% (Fig. 1). Different countries and regions contribute different amounts both in relative to other industries and in absolute terms, which is primarily a function of the number of animals kept and their efficiency of feed utilization (Kebreab *et al.*, 2012). Using a life cycle analysis, which is a tool used by many industries to quantify the environmental impact of products, FAO (2010) compared the total average life cycle emissions across different world regions. The report showed that the highest emissions per unit of product were found in developing regions with sub-Saharan Africa, South Asia, North Africa and the Near East emitting an average of 7.5, 4.6 and 3.7 kg CO<sub>2</sub>-eq. per kg of fat and protein corrected (FPC) milk, respectively. Industrialized regions such as North America and Europe, on the other hand, were found to exhibit the lowest emissions per kg of product.

Tilman *et al.* (2011) estimate that if current trends of greater agricultural intensification in richer nations and greater land clearing (extensification) in poorer nations were to continue, 1 billion ha of land would be cleared globally by 2050, with CO<sub>2</sub>-C equivalent GHG emissions reaching 3 Gt/y and

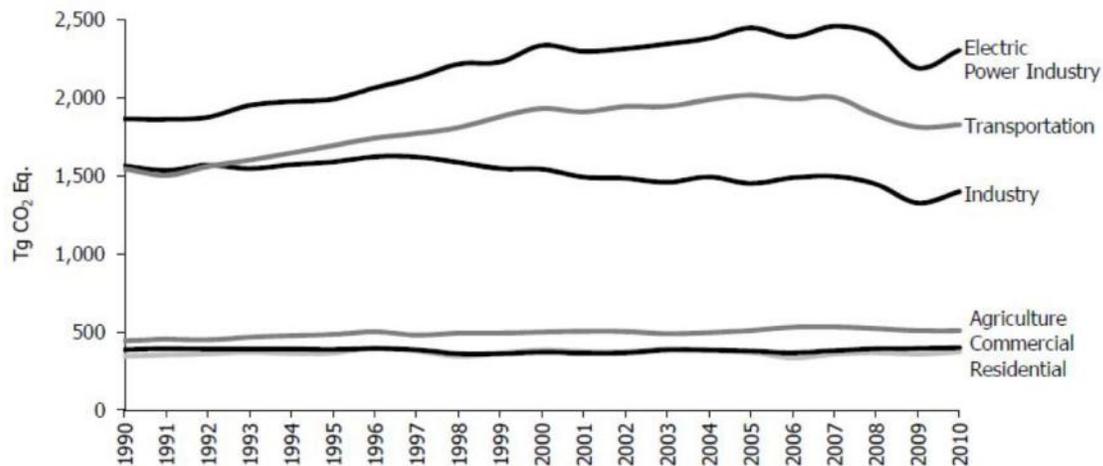
N use 250 Mt/y by then. In contrast, if 2050 crop demand was met by moderate intensification focused on existing croplands of under-yielding nations, adaptation and transfer of high-yielding technologies to these croplands, and global technological improvements, their analyses forecast the need for land clearing to be reduced by ~0.8 billion ha, GHG emissions cut by a third, and global N use reduced by 25 Mt/y.

The objective of the paper is to investigate the environmental sustainability of intensive agriculture and lessons learned to achieve similar level of efficiency in animal production in less intensive systems.

### ENVIRONMENTAL SUSTAINABILITY

Although sustainability is usually seen as the ‘sweet spot’ which lies at the confluence between the environment, economics and social issues, this paper is mostly devoted to environmental sustainability without completely disregarding its implications on the other aspects. Due to limitation of space, the paper also focuses on cattle, particularly, dairy.

The main processes contributing directly to GHG emissions from livestock and thus affecting environmental sustainability are (1) enteric fermentation and (2) manure decomposition – both



**Fig. 1.** Emissions of greenhouse gases in the USA allocated to economic sectors. (From: Inventory of US Greenhouse Gas Emissions and Sinks 1990-2010, US Environmental Protection Agency).

during storage or after application to soil (Kebreab *et al.*, 2006). These processes are the largest sources of methane and nitrous oxide emissions in any animal production system (Fig. 2). Methane is the main GHG gas eructated from cattle, which is 25 times more effective in trapping heat in the atmosphere than carbon dioxide over 100 year period (IPCC, 2006). Beauchemin *et al.* (2010) indicated that enteric methane was the largest contributing GHG in beef production accounting for 63% of total emissions, with about 84% of the enteric methane from the cow-calf herd, mostly from mature cows. In the dairy sector, methane contributes most to the global warming impact of milk - about 52% of the GHG emissions – from both developing and developed countries (FAO, 2010). Most of the lifecycle analyses conducted on emissions from livestock suggest that research into mitigation practices to reduce GHG emissions from cattle should focus on reducing enteric methane production from cows.

Methane production is directly related to level of intake and diet composition. Increased production levels associated with increased feed intake levels reduce methane per unit product in two principal

ways (1) dilution of maintenance effect and (2) decreased proportion of feed converted to methane. Increased intake levels are associated with proportionally less methane production, because higher intake levels are associated with reduced rumen retention times, reduced rumen pH and a consequent shift towards propionic acid formation which reduces methane emissions (Ellis *et al.*, 2008). Considering the energy transformations of a feed, Dijkstra *et al.* (2013) pointed out that the highest gains in efficiency can come from converting gross energy into digestible energy.

### FEED EFFICIENCY

Feed efficiency can simply be defined as a measure of how well cows utilize the ration. An analysis of methane emissions based on milk production in California showed that methane emissions have declined 52% compared to 1950 (Fig. 3). This reflects a 200% increase in milk production due to genetic and nutritional improvements while cow numbers have increased just over 100% since 1950. This means that fewer and fewer numbers of animals are required to meet the growing demand, which is a more sustainable

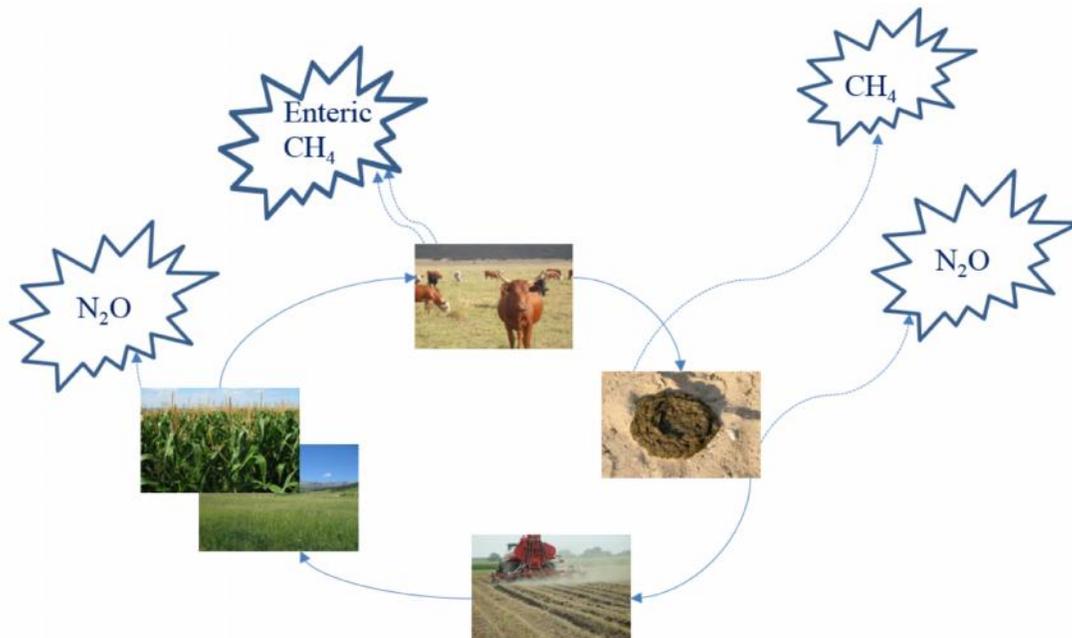
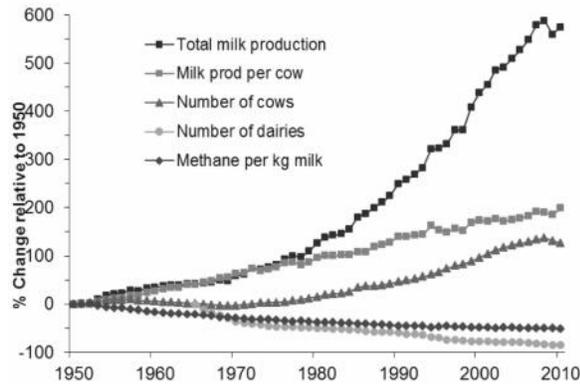


Fig. 2. Main environmental stressors from an agricultural system.



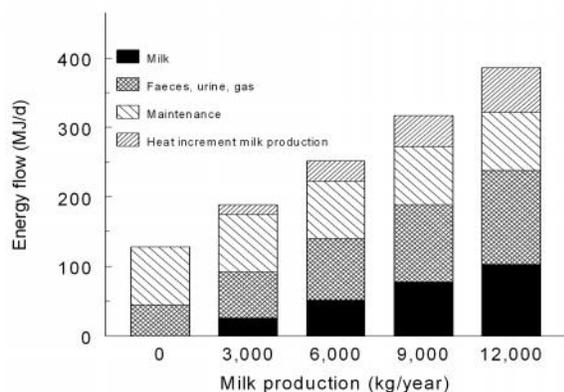
**Fig. 3.** Comparative changes in the California dairy industry from 1950 to 2010 (Adapted from Von Kyserlingk *et al.*, 2013).

system. The maintenance requirements of animals are usually considered to be fixed. High production levels will increase feed conversion efficiency simply because the amount of metabolized feed needed for maintenance processes is diluted out (Fig. 4). An increase in productivity (amount of product per animal) not only offers a pathway to satisfy increasing demands for milk and beef but also a possible mitigation approach to reduce the emission of various pollutants. Moraes *et al.* (2014a) showed the efficiency of energy utilization in beef cattle may be higher in modern beef cattle compared to those from decades ago. Because maintenance energy requirement is assumed not to change as a function of production, whereas the daily energy requirement increases as milk yield increases, the proportion of total energy used for maintenance is reduced (Dijkstra *et al.*, 2013). For example, upon an increase in annual FPC milk production from 6,000 to 10,000 kg/cow, the energy requirements per kg milk (MJ/kg FPC milk) are reduced by 16% and 19% in the Dutch and UK systems, respectively. Similarly, Capper *et al.* (2009) and Capper (2011) showed that modern, high-production level dairy and beef cattle practices require considerably fewer feed resources than low-production level systems several decades ago. Average annual milk production in the USA increased from 2,074 kg/cow in 1944 to 9,193 kg/cow in 2007 and feed input per kg milk was reduced by 77% at the 2007 level

compared with the 1944 level. Average daily growth rate of beef cattle in the USA increased from 0.75 (1977) to 1.08 (2007) kg/head, and feed input per kg gain was reduced by 19% at the 2007 level compared with the 1977 level.

### EFFECT OF DIET ON ENTERIC METHANE EMISSION

Due to cost and time required to measure enteric methane production, prediction equations are widely used to calculate methane emissions. Mathematical equations range from simple fixed conversion values (IPCC 2006; Tier 1) to highly complex process-based models (e.g., Bannink *et al.*, 2011). Various studies have shown that diet has a significant impact on methane emissions mainly due to fiber and fat contents. For example, Moraes *et al.* (2014b) developed a mathematical model that showed a strong positive relationship between dry matter intake and methane emissions. Fiber fractions were also positively related to methane emissions while increased levels of lipids in the diet were associated with reduced enteric methane emissions. Although the IPCC methodology recommends the use of Tier 2 method for estimating emissions, it does not differentiate between diets high in structural carbohydrates (e.g. cows on native pastures) and those based on digestible starch (grain) sources. Therefore, applying the recommended methane conversion factor ( $Y_m$ ) of 6.5% GE across the world would not give a true assessment of efficiency. For example, Kebreab *et al.* (2008) found that the average  $Y_m$  in dairy cows in the US was 5.63% (range 3.78 to 7.43%) and in feedlot cattle the average  $Y_m$  was 3.88% (range 3.36 to 4.56%) for Holstein breeds. In tropical breeds, Kennedy and Charmley (2012) measured methane emissions from Brahman cattle using open-circuit gas exchange. They reported that methane emissions ranged from 5.0 to 7.2% of GE and were linearly related to dry matter intake (DMI). Arias *et al.* (2013) reported that in Chilean conditions the  $Y_m$  ranged from 6.2 to 7.9% GE with the lower value being associated with beef cows fed in feedlot and the higher value with pasture finished cattle. The variation was attributed to differences



**Fig. 4.** Milk production level (kg fat and protein corrected milk (FPCM) per cow per year) and partitioning of feed towards losses in feces, urine and gases, maintenance needs, heat increment for milk production, and milk production in dairy cattle. Adapted from Dijkstra *et al.*, 2013.

in digestibility and contents of fiber. Therefore, intensive systems can reduce methane emissions when considered per product basis.

### Diet digestibility

Although the amount of feed required per unit of milk or meat is reduced when milk production levels increase, the associated higher feed intake levels generally coincide with a reduction in digestibility. The level of reduction in digestibility is usually more pronounced for structural carbohydrates compared with non-structural carbohydrates. For example, Robinson *et al.* (1987) evaluated the effect of feed intake level of a 1/3 hay, 2/3 concentrate diet on apparent digestibility of dairy cattle. Based on the intake and digestibility results reported, the decline in digestion of organic matter (OM), neutral detergent fiber (NDF) and starch was 2.9%, 6.9% and 0.1% per multiple of energy maintenance requirement, respectively. The digestibility of cellulose and hemicellulose are strongly related to methane production more so than soluble carbohydrate. Therefore, improved digestibility of feed in intensive systems increases the feed energy available to the animal, thereby reducing emissions of methane per unit of animal product. Forage preservation, forage composition

and an increase in the concentrate:forage ratio may all reduce the proportion of dietary intake energy lost as methane by up to 30% (Kebreab *et al.*, 2006).

### CONCLUSION

Sustainable intensification of agricultural systems, particularly animal systems in emerging economies have the potential in meeting the increasing global demand for meat and milk. When calculated per unit of product produced, these systems reduce greenhouse gas emissions and environmental impact of increasing productivity.

### ACKNOWLEDGEMENT

The authors acknowledge financial support from the Sesnon Endowed Chair Fund (UC Davis).

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# Climate change and animal health

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## ABSTRACT

Climate change and global warming now being accepted facts have affected all the ecosystems and will do so if left uncontrolled. Climate change, in particular global warming, is likely to greatly affect the health of animals, both directly and indirectly. Direct effects include temperature-related illness and death, and the morbidity of animals during extreme weather events. Indirect impacts follow more intricate pathways and include those deriving from the attempt of animals to adapt to thermal environment or from the influence of climate on microbial populations, distribution of vector-borne diseases, host resistance to infectious agents, feed and water shortages, or food-borne diseases. Climate change may bring about substantial shifts in disease distribution, and outbreaks of severe disease could occur in previously unexposed animal populations. It is also imperative the welfare of animals be considered when developing climate change policies and programmes.

**Key words:** Climate change, animal health, disease.

## INTRODUCTION

Climate change refers to a statistically significant variation in either the mean state of the climate or in its variability, persisting for an extended period (IPCC, 2007). From the years 1906 to 2005, global average temperature has warmed by 0.74°C, and since 1961, sea level has risen on average by approximately 2 mm per year. Arctic sea ice extent has declined by 7.4% per decade and snow cover and glaciers have diminished in both hemispheres. The rate of change in climate is faster now than in any period in the last 1000 years. According to the United Nations Intergovernmental Panel on Climate Change, in 90 years, average global temperatures will increase between 1.8°C and 4.0°C and sea level will rise between 18 and 59 cm. Extremes of the hydrologic cycle (e.g. floods and droughts) are also expected to accompany global warming trends. Climate change is expected to cause an increase in weather-related disasters and extreme weather events, such as droughts, heat waves, storms, desertification, and increases in insect infestations. Long-term changes in climate will jeopardise the future of all animals-including those in oceans, on farms, in forests, in wilderness areas, and in our

homes. All climate change has a significant effect on livestock and other animals through its impact on diseases (Grasty *et al.*, 1999). Animal health is affected by climate change in four ways: heat-related diseases and stress, extreme weather events, adaptation to new environments, and emergence or re-emergence of infectious diseases, especially vector-borne diseases critically dependent on environmental and climatic conditions, vectors, such as biting flies and ticks, are more likely to survive year-round.

## EFFECTS ON HOST

The increase in frequency and intensity of heat waves, the risk of death and serious illness is expected to increase and will be exacerbated by increased temperature and humidity (McCarthy *et al.*, 2001). Under the changing climate animals tend to undergo acclimation. Acclimation to high environmental temperatures involves responses that lead to reduce heat load. The acclimation of the animals to meet the thermal challenges results in the reduction of feed intake and alteration of many physiological functions and the alteration of productive and reproductive efficiency. The decrease in energy intake is due to reduced feed

intake, results in a negative energy balance followed by a decline in the secretion of calorogenic hormones (growth hormone, catecholamines and glucocorticoids in particular). All these events together lead to reduce metabolic heat production and might be responsible for alterations in energy, lipids, protein and mineral metabolism, and liver function (Moore *et al.*, 2005; Sano *et al.*, 1983). Blood glucose is usually reduced in heat-stressed subjects due to reduced hepatic glucose synthesis, the alteration of glucose turnover and the increased glucose demand for energy (Baumgard *et al.*, 2007 and Wheelock *et al.*, 2006). The consequence of the reduction of glucose is the lower availability of glucose for mammary gland lactose synthesis. Lactose is the primary osmo-regulator and thus determinant of milk yield, hence reduction of the glucose availability leads to the reduction of milk yield. Alteration of glucose and lipid metabolism, liver function and oxidative status may also be responsible for the increased sensitivity of heat-stressed animals to metabolic diseases with negative consequences on production, reproduction and infectious disease sensitivities in intensive and extensive livestock production systems.

Under heat stress, the increased respiration rate results in enhanced  $\text{CO}_2$  being exhaled. Hyperventilation induces a decrease in blood  $\text{CO}_2$  and the kidney secretes  $\text{HCO}_3^-$  to maintain this ratio. This reduces the availability of  $\text{HCO}_3^-$  that can be used (via saliva) to buffer and maintain a healthy rumen pH. In addition, panting ruminants drool and drooling reduces the quantity of saliva that would have normally been deposited in the rumen. Furthermore, due to reduced feed intake and reduced forage/ concentrate ratio, heat-stressed ruminants ruminate less and therefore produce less saliva. The reduction in the amount of saliva produced and salivary  $\text{HCO}_3^-$  content, the decreased amount of saliva entering the rumen and the reduced forage intake make the heat-stressed cow much more susceptible to sub-clinical and acute rumen acidosis, which indirectly enhances the risk of other concurrent health and productive problems (laminitis, milk fat depression, etc.).

An important effect of climate change may be on genetic resistance to disease. While animals often have evolved genetic resistance to diseases

to which they are commonly exposed, they may be highly susceptible to “new” diseases. Climate change is expected to bring about substantial shifts in disease distribution, and outbreaks of severe disease could occur in previously unexposed animal populations. Baylis and Githeko (2006) have mentioned that mammalian cellular immunity can be suppressed following exposure to ultraviolet B radiation, which is an expected outcome of stratospheric ozone depletion. So, greenhouse-gas emissions that affect ozone could have an impact on certain animal diseases.

### EFFECTS ON PATHOGENS

Warmer and wetter weather than normal (particularly warmer winters) will increase the risk and occurrence of animal diseases. For a climate change perspective, it is important to assess the extent to which a pathogenic agent is exposed to the conditions outside the body. Higher temperatures may increase the rate of development of pathogens or parasites that spend some of their life cycle outside their animal host, which may lead to larger populations (Harvell *et al.*, 2002). Other pathogens are sensitive to high temperatures and their survival may decrease with climate warming. Temperature signals the release of eggs or larvae by parasites and vectors, embryonic development and hatching rates, the longevity of the free living stage, infectivity to intermediate hosts, the development of either microorganisms or macro parasites in these hosts, the infectivity to definitive hosts, time until maturation and life span and mortality.

Reisen and colleagues found that the strain of West Nile virus (WNV) that entered New York (during the record hot July of 1999) differed from the South African strain in that it required warmer temperatures for efficient transmission. The investigators concluded that during the epidemic summers of 2002 and 2004 in the United States, epicenters of WNV were linked to above-average temperatures. Anomalously hot summer temperatures are also linked to international WNV outbreaks in South Africa and Russia. Bluetongue disease, a viral illness that is fatal to sheep and other ruminants, is spread by *Culicoides* sp. (midges) and

historically, only rarely reached north into Europe. But since 1998, several strains of bluetongue virus have advanced 800 km further into Europe than previously reported. Warming temperatures in the region have allowed enhanced survival of viruses through winter and a northern expansion of the insect vector of the disease. Warmer winter temperatures projected for the future may further increase the geographic range of this serious livestock disease; the warm temperatures of 2007 already have allowed establishment of bluetongue in Northern Europe.

### EFFECTS ON VECTORS

There have been several impacts of climate change on the vectors of disease (midges, flies, ticks, mosquitoes and tsetse are all important vectors of livestock disease in the tropics). Changes in rainfall and temperature regimes may affect both the distribution and the abundance of disease vectors, as can changes in the frequency of extreme events (outbreaks of some mosquito-borne diseases have been linked to El Nino-Southern Oscillation (ENSO), for example). It has also been shown that the ability of some insect vectors to become or remain infected with viruses (such as bluetongue) varies with temperature. The feeding frequency of arthropod vectors may also increase with rises in temperature. As many vectors must feed twice on suitable hosts before transmission is possible (to acquire and then to transmit the infection), warmer temperatures may increase the likelihood of successful disease transmission.

Changes to winds could affect the spread of certain pathogens and vectors. The increasing energy levels in the atmosphere are likely to alter wind patterns and so affect dispersal patterns of flying insects, such as biting midges (*Culicoides* sp.), mosquitoes and the exotic, but nearby, screw-worm (*Chrysomia bezziana*) which poses one of the largest threats to livestock in Australia. Certain existing parasitic diseases may also become more prevalent, or their geographical range may spread, if rainfall increases (Epstein and Mills, 2005). This may contribute to an increase in disease spread of diseases, including zoonotic diseases. Rogers (1996) looked at possible climate change impacts on the distribution of the brown-ear tick, *Rhipicephalus*

*appendiculatus*, and the primary vector of East Coast Fever. Over the past 100 years a distinct trend of increases in the intensity and frequency of events such as storms, typhoons, droughts, sandstorms and floods has been observed in different areas and seasons in Asia (Chen and Li, 2005). The related changes in rainfall, humidity and the El Nino/Southern Oscillation (ENSO) may alter the quality and availability of some vector breeding sites. For example, an outbreak of bovine ephemeral fever (BEF) was observed after a typhoon episode in August 1996 in Chinese Taipei, and resulted from a proliferation of biting midges (Liao *et al.*, 1998). It has been observed that combinations of drought followed by high rainfall have led to wide-spread outbreaks of diseases such as Rift Valley Fever and bluetongue in East Africa and of African horse sickness in the Republic of South Africa.

Lindgren (1998) and Lindgren *et al.* (2000) reported the northward spread of *Ixodes ricinus* in Sweden. The range expansion and increased incidence of tick-borne encephalitis transmitted by the tick *I. ricinus* between the early 1980s and mid-1990s was related to markedly warmer summers and winters in the 1990s compared with the previous three decades (Lindgren *et al.*, 2000). Tropical species such as *Haematobia* sp. and mosquitoes, which transmit many vector-borne diseases, will expand their ranges polewards. Anecdotal reports of expansions of vector distributions are common and include such reports as the spread of *Phlebotomus* sandflies (that transmit *Leishmania*) from mainland France to the British Channel Islands.

Tick vector is affected by the climatic change such as tick borne encephalitis and lyme disease. The ticks may live for several years and their survival, reproduction rate and activity are affected by seasonal climate which indirectly influences the risk of the disease. At the same time, climate change may narrow available habitats, forcing birds of several species to crowd into ever smaller areas of remaining resources and increasing the chance of within-species and cross-species disease transmission. This scenario is a likely explanation for the recent dispersion of highly pathogenic H5N1 avian influenza (Gale *et al.*, 2009; Harvell *et al.*, 2009).

## CONCLUSION

Climate change is considered by many to be the most serious long-term threat to animal health. The faster climate change occurs, the greater will be the risk of damage to the Earth. The extent of such damage may exceed our ability to cope with the consequences. Impacts may include unanticipated emergence of new pathogens/diseases or re-emergence. By assessing the risk of potential disease through the identification of potential pathogens and routes of disease spread, can generate forecasts of disease risk areas and implement surveillance efforts to monitor spread and plan intervention efforts in order to reduce the risk of animal disease. It is also imperative that animal agriculture practices and the welfare of animals be considered when developing climate change policies and programmes, both as potential victims and causes

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# Occurrence of *Hedychium flavescens* Carey ex Roscoe (Zingiberaceae): A new species record for eastern and central India

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## ABSTRACT

*Hedychium* Koenig., the largest genus of Zingiberaceae in India, is mainly distributed in the northeast and southern parts. Southeast Asia happens to be the centre of the present distribution and species diversity and about half of the species occur in the Indo-Chinese region. During the exploration mission for germplasm collection of medicinal plants, the occurrence of secluded populations of *Hedychium flavescens* Carey ex Roscoe was recorded in the semi-evergreen forests of Niyamgiri hills in Odisha. After critical review of published literature on distribution, its wild occurrence from Odisha was found to represent a new distributional record for Eastern and Central India. A detailed morphological description with diagnosis, habitat and ecology, comparison with closely related species and ethno-botanical uses was provided for easy identification and documentation of traditional knowledge. In view of its economic potential, implications relating to its sustainable utilization of the germplasm and crop improvement were discussed.

**Key words:** *Hedychium flavescens*, new record, Niyamgiri hills, Odisha, Eastern and Central India.

## INTRODUCTION

The genus *Hedychium* Koenig. popularly known as “ginger lily” or “butterfly lily” belonging to the tribe Zingibereae of family Zingiberaceae is primarily distributed in tropical to warm-temperate Asia, Madagascar with some species extending to Australia (Wu and Larsen, 2000). The taxonomy and nomenclature of the genus has been controversial since mid-nineteenth century and various workers reported the estimates of species varying from 50 (Wu and Larsen, 2000) to 80 (Sirirugsa and Larsen, 1995). It is estimated that there are 65 valid species (Wood *et al.*, 2000), however, recent reports reveal that out of 175 scientific names of species rank for the genus, only 93 are accepted species names at high confidence level (The plant list 2013). About half of the species occur in the Indo-Chinese region (Sirirugsa and

Larsen, 1995). It is a horticultural and medicinally important genus, highly valued for its ornamental plants having flowers of wide range of colour and fragrances and sugar content of nectar indicative of the way for attracting a number of butterfly and insect pollinators (Sarangthem *et al.*, 2013). Though the flowers are short-lived, many flowers are produced in succession on the inflorescence for a longer period and the genus forms the most beautiful gingers of the family Zingiberaceae. The medicinal efficacies of the essential oil extracted from rhizomes, leaves and flowers of the plants including cercaricidal properties, molluscicidal and antimicrobial activities and anti-inflammatory and analgesic effects are well established (Baby *et al.*, 2007; Shrotriya *et al.*, 2007).

*Hedychium* is the largest genus of Zingiberaceae in India with about 44 taxa

comprising of 31 species and 13 varieties including 19 endemics mainly distributed in northeast and southern parts (Sanoj, 2011). Southeast Asia became the centre of present distribution and species diversity and India and China have the maximum number of taxa harbouring 31 and 29 species respectively (Wu and Larsen, 2000; Sanoj, 2011; Hu and Liu, 2010).

*Hedychium flavescens* Carey ex Roscoe commonly known as wild ginger, cream ginger lily or cream garland lily is native to the eastern Himalayas including northeast India and Nepal (Sarangthem *et al.*, 2013; Dassanayake and Fosberg, 1983; Press *et al.*, 2000; eFloras, 2008; CABI, 2011). It is unknown when it was introduced from its native range to neighboring areas such as Emei Shan (Mount Emei) in Sichuan province of China, southern India and Sri Lanka (Baby *et al.*, 2007; Dassanayake and Fosberg, 1983; eFloras, 2008). It was then widely introduced and naturalized in various tropical countries such as parts of Asia, Africa, Europe, North and Central America, Oceania, Australia, Madagascar, Cook Islands, French Polynesia, New Zealand etc (CABI, 2011; PIER, 2014). However, this species has a confined distributional area in India and was recorded earlier from parts of northeast and south India and not reported elsewhere. The discovery of *H. flavescens* from naturally occurring populations of the dense semi-evergreen forests of Niyamgiri hill range in Odisha forms a new plant record for the flora of Odisha state as well as Eastern and Central India.

Niyamgiri hill range, a natural boundary between Rayagada and Kalahandi districts of Odisha, is one of the floristically rich and biodiversity hot spots of Eastern Ghats harbouring 663 species of vascular plants (Misra and Sahu, 2009). The mountain system comprises of irregular and undulating lofty hills ranging from 400 to 1516m above mean sea level with two flat plateaus at its crest having rich deposits of bauxite. It is wonderfully well-watered by numerous streams and waterfalls that make the region worthy and resourceful forming a congenial niche for the growth of members of Zingiberaceae of high economic/ ethno-botanic importance such

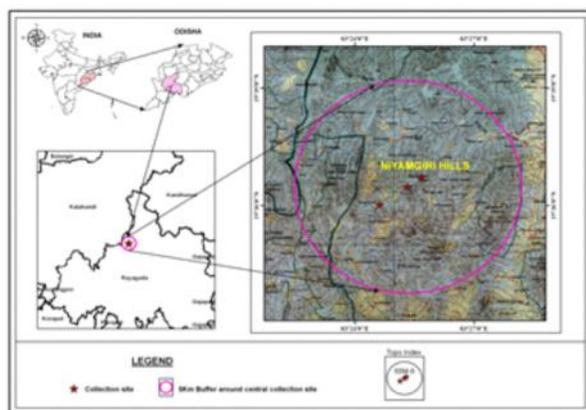
as species of *Alpinia*, *Amomum*, *Costus*, *Curcuma*, *Curcumorpha*, *Hedychium*, *Globba*, *Kaemferia*, and *Zingiber*. The hill range enjoys tropical monsoonal climate with average annual rainfall about 1800 mm with mean maximum temperature 23°C and minimum temperature may sometimes decline to about 5°C during cool periods.

## MATERIALS AND METHODS

While conducting an exploration mission for germplasm collection of medicinal plants in parts of Odisha during 2010, the first author recorded the wild occurrence of few secluded populations of *Hedychium* in Niyamgiri hill ranges in Eastern Ghat zone of Odisha (Fig. 1). A location map highlighting three collection sites was generated using Arc-GIS tools selecting a buffer zone of 5 km radius centered at Torhali village, the median collection site, inside the Niyamgiri hills (Fig. 2). The germplasm samples of matured rhizome propagules of plant species free from insect damage and disease symptoms and without any mechanical injury were collected from the collection sites by selective sampling leaving a part *in-situ*. Further, the rhizomes were vegetatively propagated, multiplied, maintained and the live plants were conserved near a shady drainage site at the field gene bank of the centre (Fig. 3). A set of rhizome propagules of type specimen bearing accession number IC-0612508 was also deposited at *in-vitro* gene bank repository of ICAR-NBPGR, New Delhi for long term conservation. Besides, the plant specimens bearing both vegetative and floral parts were collected, preserved and deposited in the herbarium of ICAR-NBPGR Base Centre, Cuttack, Odisha (Fig. 4) along with one set at the National Herbarium of Cultivated Plants (NHCP), ICAR-NBPGR, New Delhi. The morphological features of the live plants were examined using trinocular lens and dissection microscope and the detailed taxonomic features were described. The herbarium specimens have been critically studied, cross checked and the gross diagnostic characters were compared with the references and images of authentic specimens cited in the relevant literature to confirm the identity of the plant. The photographs of the vegetative and



**Fig. 1.** Wild occurrence of *Hedychium flavescens* population in Niyamgiri hills, Odisha, India.



**Fig. 2.** Location of the collection sites of *Hedychium flavescens*.



**Fig. 3.** *Hedychium flavescens* conserved in the field gene bank, NBPGR, Cuttack.



**Fig. 4.** Herbarium preserved at NBPGR Base Centre, Cuttack.

flowering parts of the plant along with the associated species in natural habitat were taken for reference.

## RESULTS AND DISCUSSION

On critical examination of the vegetative and floral characters of all parts of live plants in the natural habitat and grown at the field gene bank of the centre coupled with the study on herbarium specimens and perusal of literature (Dassanayake and Fosberg, 1983; eFloras, 2008; Drury, 1869; Matthew, 1982; Fischer, 1928), the species was identified as *Hedychium flavescens* Carey ex Roscoe, a species reported in wild state so far only from northeastern and south India (Sarangthem *et al.*, 2013; Drury, 1869; Fischer, 1928; Baker 1892; Nair and Henry, 1983; Pullaiah, 1997; Sasidharan and Sivarajan, 1996; Basak *et al.*, 2014; Devi *et al.*, 2014; Jadhao *et al.*, 2014). On observation of herbarium specimens, Naik and Panigrahi (1961) in their treatise “Genus *Hedychium* in Eastern India” recorded the occurrence of *H. coronarium* var. *subtidum* from Khasi and Jaintia hills, Jorain, Meghalaya in northeast India only. During the present exploration, it was observed that the plant populations were found growing profusely in dense semi-evergreen forests and its occurrence has been recorded along the stream course in three nearby locations within the hill range. Apart from this region,



Fig. 5. Thick and branched rhizome.



Fig. 6. Imbricating, wide, foliaceous bracts.



Fig. 7. Flowers with creamy yellow labellum with a deep yellow patch at base.

there is no authentic record of the natural distribution of this species in India except for references cited in northeastern and south India. Besides, on verification of major published Indian literature regarding distribution, it has not been reported till date in wild state from Central to Eastern India including Odisha (Haines, 1921-25; Saxena and Brahmam, 1995; Singh *et al.*, 2001; Khanna *et al.*, 2001; Verma *et al.*, 1993; Mitra, 1958; Sanyal, 1994; Guhabakshi, 1984). Therefore, the present collection counts as an addition of species to the flora of Odisha and forms a new distributional record for Eastern and Central India. A detail taxonomic description on morphology of different parts of plant species (Figs. 5-7), along with brief notes on diagnosis, habitat and ecology, field photographs and ethno-botanical uses are provided to facilitate its easy identification and further economic exploitation.

#### Taxonomic description

*Hedychium flavescens* Carey ex Roscoe, Monandr. Pl. Scitamin.: t. 50.1824; Drury Handb. Ind. Flora 3: 439. 1869; Trimen Handb. Flora Ceylon 4:245.1898; Lourteig Adansonia 12(1): 123.1972. *Hedychium coronarium* var. *flavescens* (Carey ex Roscoe) Baker in Hooker, Fl. Brit. Ind. 6: 226: 1894. *Hedychium subditum* Turrill, Kew Bull. 370. 1914. *Hedychium coronarium* var. *subditum* (Turill) Naik & Panigrahi., Bull. Bot. Surv. Ind. 3: 71. 1961. *Hedychium coronarium* var.  $\beta$  Thw., Enum. Pl. Zeyl. 319.1864. *Hedychium emeiense* Z.Y.Zhu, Acta Bot. Yunnan. 6: 63. 1984. *Gandasulium peregrinum* (N. E. Br.) Kuntze, Revis. Gen. Pl. 2:690. 1891.

Stout perennial herb; rhizomes thick, fleshy, creeping, branched, 2.5 - 4.0 cm diameter, dull yellowish white within, fragrant; roots many. Leafy shoots up to 3 m high. Leaf sheaths densely pubescent at the insertion; ligule 3-5.5 cm, membranous, pubescent. Leaves sessile, oblong to elliptic-lanceolate, 35-62 cm  $\times$  5-12 cm; base attenuate, apex caudate-acuminate, glabrous above, pubescent below particularly dense pubescent along the midrib beneath. Inflorescence terminal, conical,

dense flowered; spikes oblong-elliptic, tapering at both ends, 12-20 × 5-8 cm; bracts foliaceous, densely imbricate, orbicular-ovate, obtuse, dark-green, concave, sparsely pubescent, rachis concealed; basal ones larger, broadly ovate, 4.5-6.5 × 3.5-5.0 cm; upper ones narrow, 3.5-5.5 × 2.5-3.8 cm; margin membranous, ciliate; each bract subtends a cincinnus of 2-4 flowers; bracteoles tubular, 2.5 × 1.5 cm, membranous, slightly 2-keeled. Flowers fragrant, creamy yellow to pale yellow, open in succession. Calyx tubular, 5-6 cm long, puberulous, tip 3-toothed, unilaterally splitted. Corolla tube slender, up to 10 cm long, lobes linear-lanceolate, 5.0 × 2.0 cm, spreading; labellum large, creamy yellow with deep yellow patch at the base, broadly obcordate, longer than wide, 4-5 × 3.5-4 cm, apex 2-cleft, base tapered into a distinct claw; lateral staminodes petaloid, well developed, spatulate, 4.5-5.0 × 1.5-2.0 cm. Stamen longer than labellum, 5.0-6.0 cm long, deep yellow to light orange, filament slender, anther-thecae more or less parallel. Ovary pubescent; style long, delicate, often lying in the groove in the stamen; stigma centrally raised, margin bearded; capsule not found in live plants after subsequent propagation.

**Phenology:** Flowering occurs in September to November, fruiting not seen.

### Diagnosis

*Hedychium flavescens* is phenotypically resembled to *Hedychium flavum* and *H. coronarium* in respect of specific diagnostic characters such as more or less sessile leaves and dense imbricated bracts, each subtending 2-5 flowers. However, it differs in having leaves pubescent adaxially with dense pubescent on midrib beneath, leaf apex caudate-acuminate and pubescent leaf sheath. Stamen is longer than or sub-equaling the labellum. The color of labellum is creamy yellow to pale yellow with a deep yellow patch at base, labellum longer than wide and apex 2-cleft. Besides, *Hedychium flavescens* has wider bract, dark green in color and autumn flowering whereas *Hedychium flavum* has distinctly narrow bracts, medium green in color and flowers earlier than *Hedychium flavescens*. *Hedychium coronarium* has milky

white labellum with pale yellow to greenish yellow patch at base and the stamen is shorter than labellum.

### Etymology

The genus name *Hedychium* is derived from two ancient Greek words 'hedys' means sweet of pleasant taste or smell and 'chio/ chion' meaning 'snow', which refers to the fragrant white flowers. The name of the species '*flavescens*' refers to the pale yellow, cream yellow or turning yellow color of flowers.

### Habitat and ecology

The population of this plant species was found growing wild along the stream course/ bank and cascades in moist and shady habitats in semi-evergreen forests at an altitude of 500-900m above mean sea level. The plants were confined in the narrow valleys or gorges in secluded habitats almost in undisturbed area of dense forest at different places near to the villages Torhali, Monda and Sutanguni of Niamagiri hill range in the Eastern Ghat zone of Odisha. The spread of this plant community is facilitated by vegetative regeneration of its rhizome along with new red color buds sprouting seasonally which further give rise to leafy shoots. The plant prefers humid tropical climate and extremely moist shady habitat with annual rainfall more than 1500 mm. It was observed that it grows best at 18-24°C maximum and 8-14°C minimum daily temperature. It requires medium to high soil fertility and can thrive in a wide range of soils. It prefers to colonise under full shade beneath the canopy of trees. It has excellent compatibility with semi-evergreen herbaceous elements like *Alocasia macrorrhizos*, *Christella dentata*, *Phrynium placentarium*, *Globba racemosa*, *Cucurma angustifolia*, *Costus speciosus*, *Polygonum hydropiper*, *Centella asiatica*, *Kalanchoe pinnata*, *Curculigo recurvata*, etc. The associated tree species like *Ficus hispida*, *F. benjamina*, *Diospyros malabarica*, *Michelia champaca*, *Artocarpus lacucha*, *Mallotus philippensis*, etc, climbers like *Argyreia nervosa*, *Schefflera venulosa*, *Millettia extensa*, *Cryptolepis buchananii* and shrubs like *Ardisia solanacea*,

*Murraya paniculata*, *Lantana camara*, *Alstonia venenata*, *Homonoia riparia* and *Solanum torvum* occur in this habitat. The growth of grasses is poor due to dense shade, moist floor and profuse ground canopy. In our specimens, the hermaphrodite flowers are sterile and do not produce seeds and the total spread appears to be entirely vegetative.

### Type specimens examined and germplasm collected and conserved

Site: India, Odisha state, Rayagada district (Niyamagiri hill range), Muniguda block, nearby villages: Torhali, Monda and Sutanguni; elevation: 570-900m above mean sea level; R.C.Misra HS. No. 205-206, 1143-1144 and 1161-1162 (herbarium of ICAR-NBPGR Base Centre, Cuttack), dated: 29.03.2010, 20.09.2014 and 30.10.2014 respectively; rhizome germplasm accession IC-0612508 (collection number RCM/01/20); source: natural, wild, indigenous; frequency: rare; soil type: silty loam, littered, soft, slippery, black color; sampling type: rhizome from individual plant by selective sampling; topography: hilly dissected and mountainous; land aspect: narrow valley/ gorges along the stream course/ bank; slope: close depression; type of material: rhizome and live plants.

### Ethno-botanical uses

'Dongria Kandha', a primitive tribal group of people inhabiting the Niyamgiri hills of Odisha, named the plant *Hedychium flavescens* as '*Penu ada*' (*penu* means God, *ada* means ginger). They collect the rhizome or whole plant (Fig. 8) and use in different forms for the treatment of diseases. The rhizome is rubbed on the stone and the paste is applied on sprains, swollen joints and rheumatic affected areas to relieve pain. They also use the paste locally on eczema, scabies and other skin infections. However, no use of rhizome and leaves as food items was reported from local sources. The flowers are used as a component of sacred rituals after the first bloom and offered to their God '*Dharani Penu*' or '*Niyam Raja*' during the autumn festivals and thereafter popularly used in women's hair as ornaments particularly among the



**Fig. 8.** Dongria Kandha tribal man of Niyamgiri, Odisha carrying rhizomes for medicinal use.

newly married ones. The flowers are used as ornaments by the tribes of Manipur too (Devi *et al.*, 2014).

### CONCLUSION

*Hedychium flavescens* renders many scopes to develop as an economically useful crop by finding its use in horticulture, pharmaceutical and socio-cultural sectors. Despite its multifarious economic potential, it is meagerly used as an ornamental and medicinal crop on a very limited scale by the local tribes and still remains as one of the neglected gingers in India due to its restricted distribution and lack of awareness. Its economic potentiality should be publicized and be promoted for cultivation as a perennial ornamental garden crop in India like *Hedychium coronarium* and *H. gardenarium*, because of its showy, fragrant and nectariferous flowers. The species is also facing loss of genetic diversity in the region due to uncontrolled uprooting of rhizomes and whole plants in huge quantities by local inhabitants for socio-economic uses including preparation of traditional medicine. Consequently, there is an urgent need for sustainable management to conserve the germplasm. Besides, conservation of the potential wild species and its efficient utilization will play a vital role in widening the genetic

base of *Hedychium*. Further, its economic and breeding potential along with specific traits/ desirable attributes pertinent to ornamental and adaptability index and pollination biology of this wild ginger lily be investigated in detail for crop improvement studies.

### ACKNOWLEDGEMENT

The first author is grateful to the Director, ICAR-National Bureau of Plant Genetic Resources and Head, Division of Plant Exploration and Germplasm Collection, NBPGR, New Delhi for approving the exploration and germplasm collection programme of medicinal and aromatic plants in parts of Odisha and providing necessary research facilities during the course of investigation.

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# Biomedical waste (BMW) management scenario in SCB Medical College and Hospital, Cuttack, Odisha

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## ABSTRACT

With the advancement in healthcare facilities for effective diagnosis of diseases and subsequent treatment of patients, the enormity as well as the quality of the biomedical wastes generated from such sources have undergone remarkable changes. Improper handling, treatment and disposal of these wastes have been the cause of concern considering the potential health and environmental hazards they inflict on. The SCB Medical College Hospital, Cuttack, the premier medical college of Odisha state, has been catering to the rising healthcare needs of a large populace in diverse specialities. The present study makes an endeavour to generate relevant information on varied aspects of the biomedical waste management scenario of the hospital and propose strategies for resolving effectively the problems associated with the disposal of wastes. The objective is to keep in place an efficient, scientific waste disposal mechanism for effective control of infections and environmental degradation.

**Key words:** Biomedical waste, hazardous waste, waste management, incineration, autoclaving.

## INTRODUCTION

Waste production has been an inevitable outcome of diverse developmental activities undertaken by the mankind. Solid wastes constitute a major chunk of the generated wastes that grow out of proportion steadily imposing a severe challenge to the humanity for their safe handling and disposal, as illustrated in the healthcare sector. Innovations in medical science and advancement in healthcare facilities have contributed significantly to the effective diagnosis of diseases and subsequent treatment of patients. While such facilities have brought about new hopes for combating the ever-increasing health disorders and diseases, the processes of diagnosis, analysis and treatment have led to production of wastes from various sources such as hospitals, nursing homes, clinics, pathological labs, blood banks, etc. These wastes, termed as hospital wastes or biomedical wastes (BMW), refer to on-wastes, biological or non-biological, that are

discarded and will never be used again (Altin *et al.*, 2003).

As MoEF (1998) defines, “bio-medical waste means any waste, which is generated during the diagnosis, treatment or immunization of human beings or animals or in research activities pertaining thereto or in the production or testing of biologicals, and including categories mentioned in Schedule I of the Bio-Medical Waste (Management and Handling) Rules, 1998”. These wastes, generated from various healthcare establishments, can further be categorized into hazardous and non-hazardous wastes basing on their nature (Table 1). However, various terms such as biohazardous, pathological and infectious are often used interchangeably to identify and characterize biomedical wastes without clearly defining their subtle differences and similarities (Anonymous, 2007). Of the total amount of waste generated by health-care activities, about 80% is general waste and the remaining 20% is considered

**Table 1.** Types of biomedical wastes - hazardous and non-hazardous

1. Hazardous wastes	Clinical/ infectious/ medical waste, cytotoxic and cytostatic medicines, batteries, health care chemicals and hazardous properties, radioactive substances, X ray photochemicals.
2. Non-hazardous wastes	Offensive/ hygiene waste, non cytotoxic and cytostatic medicines, domestic waste, packaging waste, recyclable waste, food waste.

Source: RCN, 2007.

hazardous material that may be infectious, toxic or radioactive (WHO, 2011).

Though biomedical wastes constitute a small portion of the total wastes generated in a region, such residues can potentially transmit diseases and present an additional risk to the staff of the healthcare facilities, their patients, and the community as a whole in the absence of proper waste management practices (Baveja *et al.*, 2000; Silva, 2005). Inappropriate disposal of such wastes, including open dumping and improper burning, enhances the risk of spreading infections and exposure to toxic emissions from incomplete combustions (Abor, 2007; Sreejith, 2008). Besides the huge risk to human health, BMW also contribute to grave environmental degradations with far-reaching consequences (Joseph and Krishnan, 2004). The scenario is more severe in developing countries, where sufficient attention is yet to be focused on scientific management of BMW and such wastes are often disposed off collectively along with the municipal solid wastes.

The Cuttack City in Odisha state has acquired the status of a key healthcare hub since pre-independence period for providing contemporary medical treatment facilities. Establishment of the government-run Sriram Chandra Bhanja (SCB) Medical College and Hospital, the first medical college of the State, in the city in 1944 has added further significance to the city as the provider of best medical treatment in the State that attracts patients from far and wide corners of Odisha as well as from the neighbouring states. The objective of the current study was to undertake a thorough investigation into the issue of biomedical waste management in the SCB Medical College and Hospital, Cuttack. The study was aimed at generating relevant information on the BMW

management scenario of the hospital and proposing strategies for resolving effectively specific problems associated with waste management.

### MATERIALS AND METHODS

The investigation was started with a systematic search and review of the existing literature in various books, journals and internet sources with regard to the studies carried out in both national and international spheres on biomedical waste management scenarios in healthcare facilities. A comprehensive survey of the SCB Medical College and Hospital was carried out as a case study of the Cuttack City for drawing an apposite conclusion as to what extent the appropriate standard operating procedures (SOPs) are followed and the provisions of the BMW Rules (1998 and 2011) are abided by. The results obtained from the study were analyzed to find out the current state of BMW management and discussion was made on the scientific disposal of biomedical wastes for enhancing efficiency and compliance with regulatory requirements.

The methodology adopted in the study followed Oweis *et al.* (2005) and Abor (2007).

- Regular visits were made to various departments such as surgical and critical care wards, maternity ward, orthopaedic ward, general medicine ward, operation theatres, casualty, dental wing and laboratories of the hospital to get information about waste generation.
- Personal observations were made from various sources of BMW in the hospital.
- Procedures adopted by the personnel engaged in the collection, segregation, transport and final disposal of the wastes from various sources were recorded with critical analysis.

- A questionnaire, developed basing on the recommendations made by the World Health Organization for evaluation of the management of hospital wastes in developing countries (Prüss *et al.*, 1999; WHO, 2005, 2011), was used for collecting information from the key functionaries of the hospital about their approach towards the BMW.
- Personal interviews were conducted with all the healthcare personnel concerned with waste-related activities of the hospital and operators of waste incinerators.
- The BMW Management Plant located within the hospital was visited on a regular basis to observe the waste management practices adopted by the assigned agency, Mediaid Marketing Services, Bhubaneswar.
- Recorded data on the amount of wastes collected from the hospital on monthly basis were obtained from the agency employed for the purpose.
- Statistical analysis of the collected data was carried out through computer programmes using single factor ANOVA between colour code groups and paired t test for comparison of the means of the two years for each colour code.

## RESULTS

The 1261 bedded SCB Medical College Hospital has under its fold 39 outpatient departments, 23 operation theatres and 24 wards that cater to all types of specialities appropriate for a large academic hospital with a daily outpatient flow of 1250 and average emergency caseload of 200. The results obtained from the investigation with regard to different categories of wastes generated from the hospital are presented in Tables 2 and 3 on month-wise basis and in Fig. 1 on yearly basis for the two years of study. The Figures 2 to 7 illustrate various aspects of waste management scenario.

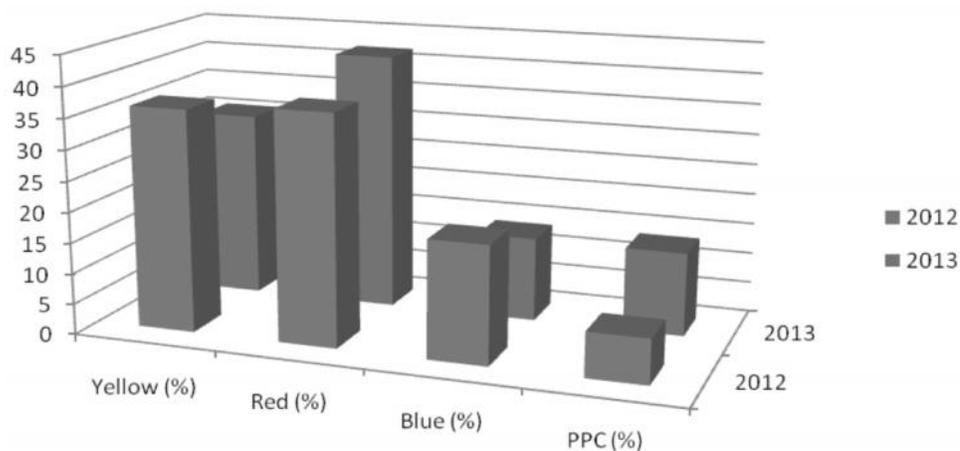
The total amount of BMW generated in the year 2012 was 53936.56 kg, while that in 2013 was 78278.1 kg. This suggests that the total quantity of wastes in 2013 was significantly higher in comparison to that of the year 2012. On the other hand, the waste collected under red code was highest (20,180.12 kg) followed by yellow code (19,553.82 kg), blue code (10,296.73 kg) and PPC (3,905.89 kg) during 2012. The same trend was also maintained in 2013 with red code having the highest wastes (32,888.3 kg) followed by yellow code (23,952 kg), blue code (10,748.9 kg) and PPC (10,688.9 kg). The monthly production of total wastes was higher during 2013 compared to the

**Table 2.** Biomedical wastes (in kg) collected from SCB Medical College & Hospital during the year 2012 in different colour coded bins. (PPC - Puncture proof container)

Month	Yellow	Red	Blue	PPC	Total
January	1915.25	1875.69	891.1	343.4	5025.44
February	1880.25	1885.6	893.35	355.6	5014.8
March	1832.15	1785.03	860.45	338.6	4816.23
April	1753.08	1584.6	874.6	320.5	4532.78
May	1540.15	1569.87	861.5	321.35	4292.87
June	1545.21	1678	858.9	318.69	4400.8
July	1380.24	1752.64	842.57	318.7	4294.15
August	1380.9	1795.06	825.1	320.94	4322
September	1495.78	1605.09	814.4	298.25	4213.52
October	1495.26	1580.57	864.4	303.6	4243.83
November	1520.89	1519.08	860.2	332.7	4232.87
December	1814.66	1548.89	850.16	333.56	4547.27
<b>Total</b>	19553.82 (36.26%)	20180.12 (37.41%)	10296.73 (19.09%)	3905.89 (7.24%)	53936.56 <b>(Grand Total)</b>

**Table 3.** Biomedical wastes (in kg) collected from SCB Medical College & Hospital during the year 2013 in different colour coded bins. (PPC - Puncture proof container)

Month	Yellow	Red	Blue	PPC	Total
January	2090.8	2788	957.8	925.75	6762.35
February	2085.3	2878	892.3	920.83	6776.43
March	1980.3	2896.5	885.6	886.94	6649.34
April	1890.6	2688	886.5	877.86	6342.96
May	1889.2	2518	850.6	879.65	6137.45
June	1989.5	2541	877.1	875.88	6283.48
July	1978.6	2223.4	885.5	880.5	5968
August	1990.3	2684.7	892.1	890.6	6457.7
September	1980.4	2895	896.2	889.65	6661.25
October	1995.6	2804.9	893.5	887.54	6581.54
November	1990.9	2990.8	896.4	887.6	6765.7
December	2090.5	2980	935.3	886.1	6891.9
<b>Total</b>	23952 (30.6%)	32888.3 (42.01%)	10748.9 (13.73%)	10688.9 (13.66%)	78278.1 <b>(Grand Total)</b>

**Fig. 1.** Comparison between percent-wise composition of various categories of wastes produced during the years 2012 and 2013.

corresponding months in 2012. The ANOVA test indicates the variations in the quantity of wastes under different categories to be highly significant in the two years of study [ $F = 361.529$  (2012),  $700.554$  (2013),  $118.819$  (combined);  $p < 0.01$ ]. The t-values (yellow =  $7.30213$ , red =  $13.2317$ , blue =  $4.1155$ , PPC =  $142.1554$ ;  $p < 0.01$ ) suggest significant differences between the quantities of wastes generated during the two years.

It was noted that waste management practices have been adopted by the SCB Medical College and Hospital with effect from January 2000 keeping in view the objective of complying with the provisions of the BMW (M&H) Rules, 1998. The mission has been to adhere to the prescribed norms and the vision is to emerge as a 'role model hospital' in the field of BMW management and handling at both state and national levels. The process involves

adopting proper segregation, collection, treatment and disposal methods. The hospital has constituted a Waste Management Committee (WMC) comprising of the Superintendent of the Hospital as its Chairman, the Principal as the Deputy Chairman and some members from different departments, including an engineering staff, for effective management of the process.

### **Waste generation**

Both general and biomedical wastes are generated from different sources of the hospital during various types of therapeutic procedures such as surgery, delivery, resection of gangrenous organs, dialysis, injections, autopsy, biopsy, para-clinical examinations, cobalt therapy, chemotherapy, etc. The prime sources of the general wastes include food preparations by the attendants of patients, plastic packets of packaged food products, discarded materials from administrative departments, and wastes from landscaping, construction and repairing works. An agency named Mediaid Marketing Services, Bhubaneswar has been outsourced and engaged by the hospital for collecting wastes from all the wards. It was observed that the BMW is sometimes mixed with regular municipal wastes collected from the hospital campus. The Cuttack Municipal Corporation (CMC) authorities are worried as this has become a regular affair due to the indifference of some hospital staff in segregating biomedical and regular wastes. Data on department-wise waste generation were also not available; however, close observation indicated that Obstetrics and Gynaecology Department produces the largest amount of wastes.

### **Waste segregation**

Wastes from various sources of the hospital are segregated into different categories at the point of generation and also stored in secure environments till their collection. The personnel employed for handling the wastes mostly use personal protective equipments, including protective boots and gloves, but overall gowns are not used. In totality 73 waste collection points exist in the hospital and these are provided with three colour-coded (red, yellow and blue) plastic containers and a puncture proof

container (PPC) for separate collection of wastes, as specified in Table 5. The yellow bin is meant for human body parts and infectious non-plastic wastes (cotton, gauge, plaster, etc.), while the red bin collects non-burnable wastes (infectious plastics, empty containers of antibiotics), the blue bin stores non-infectious plastic wastes (saline and other bottles), the PPC collects sharp wastes such as needles and the black bins collect general municipal wastes. The fluid wastes are segregated from the solids for separate disposal. However, deviations to complete segregation of wastes and violation of standard rules were observed in some cases. Labeling of infectious wastes with biohazard symbol was also observed to be missing at times.

### **Transportation**

The Mediaid Marketing Services manages the on-site and off-site transportation of the BMW from the collection points to the plant site, and their treatment and final disposal. From different segregation points, the BMW in colour-coded polythene bags are collected to common collection points at the basements and then transported in a tricycle van designated for the purpose to the waste management plant. No temporary storage site was observed to be functional in the hospital.

### **Treatment**

The outsourced agency carries out the waste treatment and disposal work. The non-burnable infectious plastic and sharp wastes collected in red and PPC containers are treated with 1% sodium hypochlorite (NaOCl) solution for disinfection; but such treatments are sometimes overlooked. For destruction of the hypodermic needle wastes at the point of their generation, needle syringe terminators (NST) have been provided at all waste production points of the hospital.

### **Disposal**

The hospital has a BMW treatment and disposal (T and D) plant set up in a building of 1500 sq ft area. Here incineration is the key method employed for disposal of the BMW, especially of the infectious (microorganisms), toxic and sharp wastes, and human anatomical remains. A pyrolytic, double



**Fig. 2.** Colour coded waste collection bins.



**Fig. 3.** Wastes loaded into a tricycle for transportation.



**Fig. 4.** Wastes transported into disposal plant.



**Fig. 5.** Microwave used for disinfection of wastes.



**Fig. 6.** Plastic waste shredder.



**Fig. 7.** Incinerator used for waste disposal.

chamber, double burner and fuel fired incinerator having a capacity of 70 kg/cycle/hr, and with automatic loading and other necessary safety devices is operating in the waste management plant of the hospital to dispose of all burnable wastes collected in yellow bags, except the plastics. The non-burnable infectious plastics in red bags and sharp wastes contained in PPC are treated in microwaves for disinfection. There exist two microwaves of 100 liters capacity each with specifications of 20 minutes/cycle, internal temperature of 120°C and frequency of 2450 MHz to disinfect the BMW categories 4 and 7 (sharp wastes except hypodermic needles and infectious plastics). The saline bottles as non-infectious plastics and disinfected plastics and sharps are directly put into a shredder of 100 kg capacity and 45 minutes/cycle for mutilation. The liquid wastes generated in the hospital from various laboratories and housekeeping is sterilized chemically for destruction of infection causing organisms and then discharged into the drains that finally pump out to the existing waste channel for their treatment at Matagajpur, Cuttack.

### Deficiencies in BMW management

The study identified a number of lapses existing in the management of the BMW generated from the hospital. During interaction, the hospital personnel dealing with waste management practices, including the superintendent of the hospital, also admitted the lacunae existing in the system. Some of the vital issues include:

- Strict implementation of the BMW (Management and Handling) Rules 2011.
- The data on the quantity of wastes recorded on daily basis by the outsourced agency appear to be defective and unreliable.
- Complete separation of hazardous and non-hazardous/ municipal wastes still remain to be achieved. Even pathological wastes in some cases were observed to be disposed off along with general wastes.
- Appropriate colour coded bags are occasionally not used for waste separation and a single bag is used for collecting all kinds of wastes. Replacement of bags in all collection sites is not carried out properly.
- Containers carrying specific types of wastes sometimes don't carry labels as prescribed under Schedule III and also information as prescribed in Schedule IV.
- Lapses were observed in regularity of the chemical treatment of wastes with 1% NaOCl and use of needle-syringe terminator.
- Required initiatives for isolating mercury-containing products and other hazardous substances are sometimes wanting and these often intermingle with non-hazardous wastes. Bio-hazard symbol is sometimes not used.
- Since wastes generated from each ward or collection point are not quantified, ascertaining which ward produces maximum or minimum wastes remains unrevealed.
- Knowledge-base of personnel employed in waste collection and disposal processes on infectious diseases such as tetanus, Hepatitis B, Hepatitis C and HIV, and hazardous chemicals remains inadequate.
- The microwave, incinerator and other equipments remain out of order at times and consequently the BMW remains dumped and untreated, that poses health and environmental hazards for the public.
- Provisions for waste minimization and recycling are mostly not in practice.
- Proper training programmes for personnel responsible for waste collection and management are not organized at regular intervals as per the provisions.
- The waste collection personnel sometimes don't use the safety kits, prescribed uniform dress and photo identity cards as required under the rules. Some of the employees were found to have not been covered under insurance against any personal accident that might occur.

## DISCUSSION

Biomedical waste is currently a real health and environmental concern (Spence, 2000; Ndiaye *et al.*, 2012) and improving management of this waste is receiving increased attention throughout the world (Sreejith, 2008). The WHO (1999) estimated that 10-25% of healthcare waste is hazardous with the potential for creating a variety of health problems. Misra and Pandey (2005) opine that in India solid wastes including hazardous wastes, though regulated, are often disposed off indiscriminately posing health and environmental risk, as is evidenced to certain degree at the SCB Medical College & Hospital. The amount of waste generated in hospitals depends upon various factors such as number of beds, types of health services provided, economic, social and cultural status of the patients and the general condition of the area where the hospital is situated (Abor, 2007). Healthcare organizations are obliged to lessen the amount of wastes sent for disposal, because we need to make best use of our resources, reduce the environmental impact of waste, and the landfill space is also running out (RCN, 2014). Because of its hazardous and infections characteristics, proper management of the BMW is of paramount importance. The probable health hazards in the circumstances of ineffective management of BMW are presented in Table 4.

To deal with this ever escalating problem, the Ministry of Environment and Forests (MoEF), Govt. of India, framed the 'Bio-medical Waste (Management and Handling) Rules in 1998 having the 1<sup>st</sup> Amendment to it in 2000 and the 2<sup>nd</sup> Amendment in 2003. However, the MoEF notified a new draft, 'Bio-Medical Waste (Management &

Handling) Rules 2011', on 24<sup>th</sup> August, 2011 under the Environment Protection Act, 1986 to substitute the earlier rules. The generator of BMW has the responsibility to take steps for its appropriate segregation, packaging, labeling, storage, handling, transport and treatment. Wastes collected from various wards result in the production of infectious wastes, contaminated sharps with patients' blood and secretions, radioactive wastes and chemical materials which are considered to be hazardous wastes (Abor, 2007; Prüss *et al.*, 1999). These wastes need efficient, scientific management so as to nullify their adverse impacts on human health and environment. In SCB Medical College & Hospital, the need is to ameliorate the observed deficiencies and reinforce the disposal and management processes.

The present study suggests that the SCB Medical College and Hospital produces huge quantities of wastes, both general and biomedical in nature, generated from different wards of the hospital. The t-values from statistical analysis indicate that the differences between the wastes of the two years for the colour codes yellow, red, blue and PPC were found to be statistically significant ( $p < 0.01$ ). Thus, the enormity of wastes produced during the year 2013 was significantly higher than the quantity of wastes generated in 2012. A study conducted by the WHO (2005) estimated that out of the total amount of waste generated in healthcare facilities, about 85% are actually non-hazardous, 10% are infective, hence hazardous and the remaining 5% are non-infectious, but hazardous. Negligent management of BMW would spread infectious diseases (Table 4) and contaminate the whole living environment prevailing in the hospital creating health hazards. Further, incinerators used

**Table 4.** Health hazards induced due to biomedical wastes

Type of waste	Health hazards
Human / Animal waste/ Soiled waste	HIV, HBV, HCV, Hgic fevers, cholera, salmonellosis, shigellosis, rabies, leptospirosis, anthrax, TB, pneumonia, septicemia
Sharps	HIV, HBV, HCV, Injuries
Cytotoxic/ radioactive waste	Cancer, genetic mutation, birth defect
Chemical waste	Poisonings, dermatitis, conjunctivitis, bronchitis

**Table 5.** Colour coding of waste containers by waste categories and treatment/ disposal mechanisms as per Schedule II of Biomedical Waste (M & H) Rules, 1998

Colour Coding	Type of Container	Waste Category	Treatment/ Disposal
Yellow	Non-chlorinated plastic bags	Nos. 1,2,5,6 Infectious wastes, bandages, gauzes, cotton or any other things in contact with body fluids, human body parts or placenta	Incineration/Deep burial
Red	Non-chlorinated plastic bag / puncture proof Container for sharps	Nos. 3,4,7 Plastic waste such as catheters, injections, syringes, tubings, IV bottles	Chemical Treatment / Autoclaving / Microwaving and followed by mutilation and shredding and disposal in landfills or disposal of recyclable waste
Blue	Non-chlorinated plastic bags / Containers	No. 8 All types of glass bottles and broken glass articles, outdated and discarded medicines	Chemical treatment and discharge into drains for liquids and secured landfill for solids
Black	Non-chlorinated plastic bags	Municipal wastes, syringes without needles, blades, sharps and all metal articles	Disposed as per the Municipal Solid Waste Management Rules

Source: The Bio-Medical Waste (Management and Handling) Rules, 1998

in hospitals are a cause of concern as major contributors of dioxin and other air pollutants. Sreejith (2008) opines that most hospital incinerators were never designed for the disposal of large quantities of waste and consequently have become overloaded, causing air pollution in surrounding areas. Thus, SCB Medical College & Hospital is required to relocate its waste disposal facility to escape from further health predicaments. Hosny and El-Zarka (2008) voice similar concern and state that most of the developed countries have abandoned incineration for associated risks and adopted suitable substitution systems. Though Yadavannavar *et al.* (2010) found in their study that majority of the staff (teaching and non-teaching) was conscious of the measures for safe collection and final disposal of BMW, the present study concludes that they often remain apathetic towards the issue. The BMW management authority has the responsibility to treat and dispose of the wastes with all sincerity, as specified in Table 6.

Biomedical wastes such as infectious or potentially infectious; sharps; cytotoxic; or publicly

sensitive due to the nature of the waste (i.e., human body parts) require special precautions and handling procedures (DENR, 2005); but in SCB Medical College & Hospital procedural lapses in dealing with such wastes were observed sometimes culminating in contamination of general wastes. These act as potential disease transmitting sources. Further, use of gloves, gowns, masks, and other types of personal protective equipments must be made mandatory so as to minimize an individual's risk of exposure to hazardous agents, whether they are physical, chemical, or infectious. Recently, a former superintendent of the hospital was sentenced to four months of simple imprisonment and a fine of Rs 10,000 by the SDJM (Cuttack Sadar) for lapses in biomedical waste management in the hospital. A fine of Rs 5 lakh was also imposed on the Medical College for not adhering to the norms of Bio-medical Wastes (Management and Handling) Rules, 1998. The conviction came in response to a case filed by the Odisha State Pollution Control Board (OSPCB) in 2010 against 21 hospitals including 12 government ones.

**Table 6.** Waste categories, waste types and treatment/ disposal methods as per Schedule II of Biomedical Waste (M and H) Rules, 2011

<b>Waste Category</b>	<b>Waste Type</b>	<b>Treatment &amp; Disposal</b>
Category 1	Human Anatomical waste (human tissues, organs, body parts)	Incineration/deep burial
Category 2	Animal Waste: Animal tissues, organs, body parts carcasses, bleeding parts, fluid, blood and experimental animals used in research, waste generated by veterinary hospitals, colleges, discharge from hospitals, animal houses	Incineration/deep burial
Category 3	Microbiology & Biotechnology Wastes: Wastes from clinical samples, pathology, biochemistry, hematology, blood bank, laboratory cultures, stocks specimens of micro-organisms, live or attenuated vaccines human and animal cell culture used in research and infectious agent from research and industrial laboratories, waste from production of biologicals, toxins, dishes and devices used for transfer of cultures	Disinfection at source by chemical treatment or by Autoclaving / Microwaving / followed by Mutilation / shredding and after treatment final disposal in secured landfills or disposal of recyclable waste (plastic or glass) through registered or authorized recycler
Category 4	Waste Sharps (needles, glass syringes or syringes with fixed needles, scalpels ,blades, glass etc.) that may cause puncture and cuts(Includes both used and unused sharps).	Disinfection (chemical treatment / destruction by needle & tip cutter, autoclaving/microwave and mutilation/shredding and final disposal through CBWTF / landfills
Category 5	Discarded Medicines & Cytotoxic drugs (Wastes comprising of outdated, contaminated and discarded medicines)	Disposal in secured landfills or Incineration
Category 6	Soiled Waste (Items contaminated with blood, & body fluids including cotton, dressings, soiled plaster casts, linens, beddings, other material contaminated with blood)	Incineration
Category 7	Infectious Solid Waste (waste generated from disposable items other than the waste sharps such as tubing's, hand gloves, saline bottles with IV tubes, catheters, glass, intravenous sets etc.	Disinfection by chemical Treatment / autoclaving / Microwaving followed by mutilation / shredding & final disposal through registered recycler
Category 8	Chemical Waste ( Chemicals used in production of biologicals, chemicals used in disinfection as insecticides etc.)	Chemical treatment and discharge into drains for liquids and secured landfill for solids

## RECOMMENDATIONS

In consideration of the current investigation, it would be pertinent to make the following recommendations for reinforcing the scientific management of the biomedical wastes. This is not only applicable to the SCB Medical College and

Hospital, but also to all other healthcare facilities operating in the Cuttack City.

1. The data available on the quantity of wastes generated from the hospitals appear flawed. Hence, the external agency (Mediaid Marketing Services) engaged for collecting the wastes

- should be instructed to employ correct methodology for the measurement or quantification of the wastes collected on daily basis. Further, data on ward or department wise collection of wastes should also be maintained to get a clear and fair picture on waste generation, and ascertain the highest and lowest amount of wastes. This could have implications for resource allocation for proper management of the BMW.
2. Separate containers should be used for collecting various types of wastes as per the BMW Management Rules 1998 and 2011 adhering to the colour codes, which in some cases is not abided by.
  3. Segregation of infectious and non-infectious wastes should be carried out in right earnest. Infectious wastes always need to be collected in red colour coded bag carrying a 'biohazard' symbol.
  4. A compactor needs to be employed to reduce the volume of wastes both at the site of generation and during the transportation thereof (e.g. garbage compacting trucks).
  5. Decontamination of liquid waste should be taken up to remove disease-causing microorganisms before disposal.
  6. A separate initiative should be made for collecting all types of sharps from different departments of the hospital for proper treatment and final disposal, as this can prevent contamination and further health problems.
  7. Incinerator should not have been set up within the hospital in a thickly populated area. A common treatment facility should be established by the Cuttack Municipal Corporation at a suitable location for waste disposal of SCB Medical College and Hospital as well as all other hospitals and healthcare facilities.
  8. Regular training and education programmes should be undertaken for the all personnel of the hospital, from doctors to attendants, and also workers of the private waste management firm to enhance their awareness and knowledge on personal hygiene and potential risks associated with BMW that may create health, safety and environmental hazards, and prevent the transmission of infections.
  9. There must be complete separation of municipal wastes and biomedical wastes, thereby reducing the hospital waste burden for its efficient treatment and disposal.
  10. Health and safety aspects of the hospital staff is the cardinal feature of the biomedical waste management process. Hence, adequate safety measures need to be adopted.
  11. Waste water treatment should be given high priority as it may act as a vital source for spreading of infectious diseases.
  12. Specific officer should remain in charge of the waste management of the hospital. Under his guidance waste collection, segregation, transport final treatment and disposal should be carried out by the private agency. A sub-committee may be constituted for periodic review.

### ACKNOWLEDGEMENT

The authors express their gratitude to the Superintendent of the SCB Medical College Hospital, Cuttack for granting permission to carry out the present study. They also extend their thanks to M/s Mediaid Marketing Services, Bhubaneswar for providing the waste collection data for the two years of investigation.

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# An efficient protocol for micropropagation of indigenous *Musa* cultivars of Odisha

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## ABSTRACT

Odisha has great potential of banana cultivars. They are hardly explored for research work. Therefore, the present study involved the development of highly efficient protocols for micropropagation of four banana cultivars of Odisha, namely *Gaja bantala*, *Paunsia bantala*, *Mendi bantala* and *Gendu Mundia bantala*. Meristem tissue block from the rhizomatous portion was taken as explants. The explants were cultured on Murashige and Skoog (MS) medium supplemented with 0.5 mg l<sup>-1</sup> 6-benzylamino-purine (BAP) + 100mg l<sup>-1</sup> Adenine sulphate for shoot induction. MS + 1 mg l<sup>-1</sup> BAP + 1 mg l<sup>-1</sup> Kinetin + 0.1 mg l<sup>-1</sup> NAA for shoot growth and proliferation. MS + 0.5 mg l<sup>-1</sup> BAP + 0.5 mg l<sup>-1</sup> Kinetin and 0.1 mg l<sup>-1</sup> NAA for shoot elongation. Shoots were rooted when they were transferred to half strength Murashige and Skoog (MS) medium supplemented with 1 mg l<sup>-1</sup> IBA (indole butyric acid). Oxidation of phenolic compounds was controlled by addition of 2 mg l<sup>-1</sup> glycine HCL. Shoot formation and multiplication response was also affected by temperature variations. The best results were obtained at 28°C ± 1°C. Shoot tips coming from different rhizomes behaved differently under *in vitro* conditions. Some of the shoot tips were highly productive while others produced less number of shoots. Acclimatised plants were successfully transplanted and established in the field.

**Key words:** Banana, micropropagation, *in vitro*, MS medium.

## INTRODUCTION

Banana with all its species, varieties or hybrids belong to the genus *Musa*, order Zingiberales and family Musaceae. Banana is a large perennial monocotyledonous herbs characteristically divided into dessert bananas, plantains and cooking bananas (Robinson, 1996; Strosse *et al.*, 2006; Changadeya *et al.*, 2012) with an underground stem called a corm that produces aerial shoots. The roots are initiated from the corm and they range from 50 - 100 cm in length; occasionally sub-horizontal roots reach 3m (Blomme and Ortiz, 2000). The inflorescence is a compound spike of female and male flowers arranged in groups. Each group consists of 2 rows of flowers, one above the other, closely pressed to each other, and the whole collection is covered by a large subtending bract. The all year round fruiting habit of bananas puts the

crop in a superior position in bridging the “hunger gap” between crop harvests. *Musa acuminata* is said to have originated from Malaysia (Daniells *et al.*, 2001), while the hardy *Musa balbisiana* originated from Indochina. It therefore contributes significantly to food and income security of people engaged in its production and trade, particularly in developing countries. Most of the edible cultivars of banana are sterile triploids or tetraploids and propagated mainly by vegetative means. Cultivation of banana needs specific environmental and soil conditions. They are sterile and parthenocarpic; so use of conventional methods for breeding and improvement of banana is difficult to practice (Venkatachalam *et al.*, 2007). To have sufficient planting material for cultivation in acreage with the newly introduced hybrids, it will take years when suckers are used for propagation. Moreover, the plant once infected by pathogen when vegetatively

propagated transfers the pathogen from generation to generation. Meristem culture offers an efficient method for rapid clonal propagation, production of virus free material and germplasm preservation in plants (Cronauer and Krikorian, 1984; Hwang *et al.*, 2000; Helloit *et al.*, 2002). Odisha has great potential for pulpy and juicy banana cultivars but till date they have been hardly explored for research and improvement work. Presently in Odisha, the land-races are cultivated by indigenous method which is more time consuming, laborious and not cost effective. The present study involves the development of *in vitro* propagation protocols of land-races of Odisha, like *Paunsia bantala*, *Gaja bantala*, *Mendi bantala* and *Gendu meudia bantala*.

## MATERIALS AND METHODS

The four banana land-races *Paunsia bantala*, *Gaja bantala*, *Mendi bantala*, *Gendu mundia bantala* were collected from western and coastal belt of Odisha and grown in the nursery of M/S Juan Biotechnology Pvt. Ltd. The suckers collected from the plant were sterilised and advanced for multiplication. For sterilization explants were first washed with running tap water then treated with house hold detergent for five minutes. This was followed by second washing with tap water to remove all the traces of detergent. The explants were then treated with 5% sodium hypochlorite solution for 15 minutes. After discarding sodium hypochlorite the explants were washed 3 times with distilled water to remove all traces of sodium hypochlorite. The sterile explants were then inoculated by proper dissecting and sizing the meristem (1.0- 1.5cm) on MS (Murashige and Skoog, 1962) medium supplemented with 0.5mg<sup>-1</sup> BAP +100mg<sup>-1</sup> Adenine sulphate for shoot induction. The MS medium with (1mg<sup>-1</sup> BAP + 1mg<sup>-1</sup> Kinetin + 0.1mg<sup>-1</sup> NAA) was used for shoot multiplication. The medium used for further shoot elongation were supplemented with 0.5mg<sup>-1</sup> BAP + 0.5mg<sup>-1</sup> Kinetin + 0.1mg<sup>-1</sup> NAA. Half MS medium containing 1 mg<sup>-1</sup> IBA and 3% sucrose was used for root formation. For solidification 0.6% agar was used. Glycine was used as the amino acid. The pH of the medium was adjusted to 5.74-5.8 with

0.1 N solution of NaOH or HCL . The medium was autoclaved at 121°C and 15 lbs / inch<sup>2</sup> pressure for 15 minutes. Cultures were maintained at 2000-3000 lux light intensity. After 3-4 weeks of shoot formation, actively growing cultures were transferred to fresh MS medium in glass bottles for further growth and proliferation. During each sub-culturing all dead or discoloured shoots were removed. Hardening was carried out in glass house under natural light conditions. The effect of different temperature was also studied to determine the optimum temperature for growth of *in vitro* plants.

## RESULTS AND DISCUSSION

### Initiation phase

A block of meristem tissue called sucker was used as starting plant material for the banana clones cultured. Firstly, the size of the explants of the four banana cultivars increased. Then the colour of survived explants changed from creamy white to green within seven to ten days. Some explants became dark coloured and were discarded due to excessive production of phenolic compounds. A supplement of 0.5mg<sup>-1</sup> BAP +100 mg<sup>-1</sup> Adenine sulphate in MS was used for shoot induction. Dissection of these shoot tips in subsequent culture stimulated shoot multiplication.

### Multiplication phase

All the four banana cultivars responded well in their respective culture medium (1mg<sup>-1</sup> BAP + 1mg<sup>-1</sup> Kinetin + 0.1 mg<sup>-1</sup> NAA) for shoot multiplication and (0.5mg<sup>-1</sup> BAP + 0.5 mg<sup>-1</sup> + Kinetin + 0.1 mg<sup>-1</sup> NAA) for further shoot elongation. The maximum number of shoots was noticed in *Paunsia bantala* (6-8) and the minimum in *Mendi bantala* (3-4). Observations including number of shoots, length of shoot and number of leaves were recorded (Table 1). Callus formation was also observed in *Gaja bantala* during this phase. Callus formation was nil in *Paunsia bantala*. Ball like structure was formed at the base of the shoot during shoot multiplication in *Gaja bantala* with 1mg<sup>-1</sup> BAP + 1mg<sup>-1</sup> Kinetin + 0.1mg<sup>-1</sup> NAA. Habib (1994) and Ali (1996) obtained the similar result at 5.0mg<sup>-1</sup> BAP. The

shoot elongation was observed in this phase. The highest shoot length was recorded to be 3.5cm in *Paunsia bantala*.

### Rooting phase

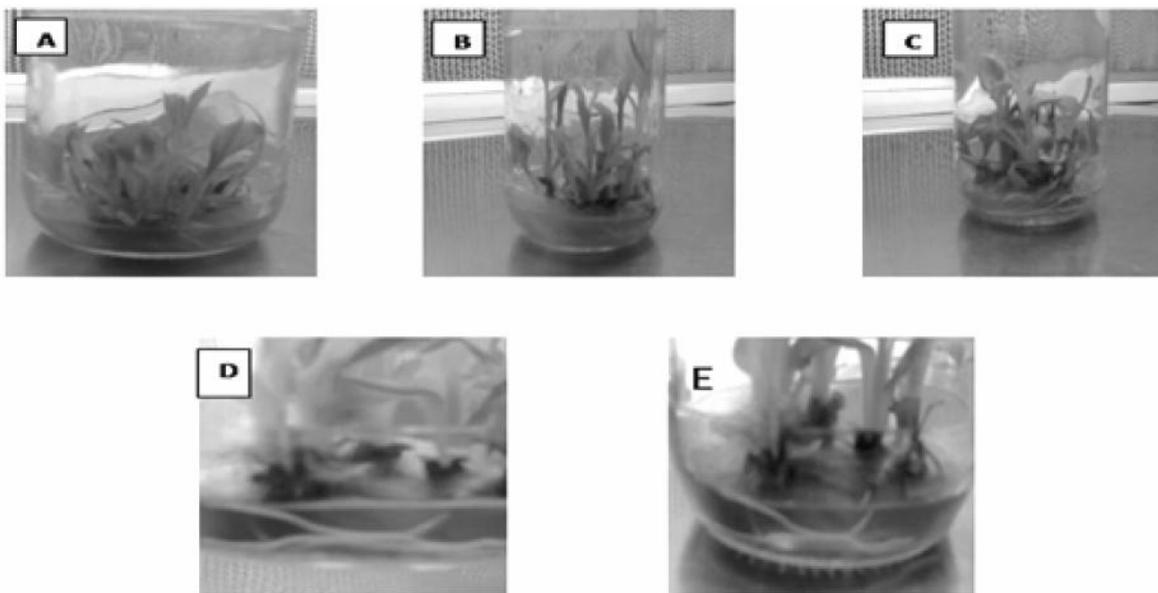
When the shoots of all the cultivars reached minimum of 3cm, they were advanced for rooting. Rooting of the shoots was done in half MS supplemented with 1 mg<sup>l</sup><sup>-1</sup> IBA whereas Al-Amin *et al.* (2009) used mixture of IBA+IAA for rooting. Rootlets were not formed in miniature plantlets and when solid half MS medium was not supplemented by IBA. When the root length of each cultivars was 2.5-3cm, then it was considered for hardening.

### Hardening

Plantlets bearing 4-5 leaves with 3 rootlets were considered for hardening. During primary hardening plantlets were slightly exposed to sunlight and are kept in 95% humidity. The mortality rate during this phase was negligible. During primary hardening, the plantlets reached the height of 5-6 inches. But the rooted plantlets were transplanted to sterile soil (Promix mixture) during the initial stage and propagated in green houses under conditions of about 35μ Epersquarem per s light intensity and 75-80% relative humidity for establishment (Gupta, 1986). Then the plantlets are kept in polybags filled with sand, soil and fertilizer in the ratio of 1:1:1. This

**Table 1.** Standard deviation of length of shoot and number of leaves of *Musa* cultivars

Cultivars of Odisha	No. of explants inoculated	No. of shoots per explant	Length of shoot (cm) (Mean±SD)	No. of leaves (Mean±SD)
<i>Paunsia bantala</i>	3	1	4.268±0.251	4.334±0.577
<i>Gendumundia bantala</i>	3	1	3.867±0.208	3.668±0.568
<i>Gaja bantala</i>	3	2	3.516±0.116	3.324±0.516
<i>Mendi bantala</i>	3	4	3.225±0.103	3.251±0.453



**Fig. 1.** Shoot and root generation in suitable media (A) *Gaja bantala* (B&E) *Paunsia bantala* with shooting and rooting (C) *Gendumundia bantala* (D) *Mendi bantala*.

phase is called secondary hardening. The mortality rate was nil in this phase. The plants were well developed during this phase.

Although, a lot of potential for cultivating landraces of Odisha has been proved but the research for their diversity study and micro propagation is yet at initial stage. The present investigation proved promising for mass propagation of banana through tissue culture for providing healthy and disease free saplings for cultivation of the indigenous cultivars.

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## Going green: Green marketing opportunities and the possible role of internet

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### ABSTRACT

Green marketing requires applying good marketing principles to make green products desirable for consumers. All marketing in the coming times will have to incorporate elements of green marketing and internet will be a boost for this process because of its wide, cost effective reach and less time consumption in the whole process. From an organization's perspective, it is essential that the environmental issues are balanced with primary customer needs and are integrated into all aspects of product life cycle and its marketing. The present paper tries to review and assess the various perspectives of green marketing with special reference to the use of internet for green product promotion.

**Key words:** Green marketing, internet, environment

### INTRODUCTION

Environmental degradation in the form of environmental pollution and global environmental changes which can have potential catastrophic consequences are pressing issues today. 13 out of 20 polluted cities in the world are from India. India ranks third in global greenhouse gas emissions. Two major rivers of India – Ganga and Yamuna are one the most polluted ones. Due to degrading environmental quality, people are now realizing the value of environmental cleanliness and conservation. People are now more aware of environmental friendly products and actions. Due to international obligations and government policies, people now get to choose between environment friendly products, actions and services. Due to the policies and better awareness, business firms too have risen to the occasion and have started responding to environmental challenges by practicing green marketing strategies. Green marketing began in Europe in the early 1980s when certain products

were found to be harmful to the earth's atmosphere. It may be defined as a holistic and responsible strategic management process that identifies, anticipates, satisfies and fulfils stakeholder needs for a reasonable reward that does not adversely affect human or natural environmental well-being (Charter, 1992). Green consumerism has played an important role in ushering corporate environmentalism and making business firms green marketing oriented. Green marketing incorporates a broad range of activities, including product modification, changes to the production process, packaging changes, as well as modifying advertising. For a product to be a green product it should not endanger the health of people or animals, damage the environment at any stage of its life, consume a disproportionate amount of energy and other resources, tested unnecessarily upon an animal and use materials derived from threatened species or environments (Hanas, 2007). For example, eco labeling programs which are typically voluntary, third-party expert assessments of the environmental

impacts of products. The popularity of such marketing approach and its effectiveness is hotly debated. The environmental appeals are actually growing in number—the Energy Star label, for example, now appears on 11,000 different companies' models in 38 product categories. Bureau of Energy Efficiency (BEE), an agency of Ministry of Power, Government of India introduced energy five-star rating for every home appliance in India. The more stars an appliance have, the more it will save electricity consumption and saving on monthly expense. Apart from that, Government of India has also started various environment friendly schemes like Bachat Lamp Yojana, Green Building Code etc.

It appears that all types of consumers, both individual and industrial are becoming more concerned and aware about the natural environment. Under a study of 16 countries during 1992, more than 50 % of consumers in each country, other than Singapore, indicated they were concerned about the environment (Charter, 1992). A study in Australia during 1994 found that 84.6 % of the sample believed all individuals had a responsibility to care for the environment. A recent survey discovered that 94 % of all consumers prefer to do business with companies that demonstrated that they cared about the environment and almost 80 % said they would pay more for environment friendly products (Ottman, 1997). As demands change, many firms see these changes as an opportunity. McDonald's replaced its clam shell packaging with waxed paper because of increased consumer concern relating to polystyrene production and ozone depletion. Xerox introduced a high quality recycled photocopier paper in an attempt to satisfy the demands of firms for less environmentally harmful products. Fab India has introduced certified organic agro products that minimize damage to the environment while providing sustainable livelihood to the local people. Tata hotels and resorts have claimed green practices like waste management by converting waste into compost and energy conservation using of CFL bulbs.

E-commerce and online retailing has seen sudden surge in India since 2011 with total business

around 500 billion INR. Online retailers, Flipkart, Snapdeal, and Amazon India have made huge impact on buying behavior of Indian consumers. By 2020, it is expected to cross 8.8 billion INR in business (Anonymous, 2014). Even traditional retailers are gradually increasing their presence in online retailing. Due to this booming sector, the scope of sale of green products would be a topic of interest. In this review paper, we have emphasized to scrutinize the green marketing opportunity through online retailing in India.

### **GREEN MARKETING OPPORTUNITIES**

Many organizations believe 'Green Marketing' as a possible strategy for promoting their product and achieving a niche in the fast paced world of branding and advertisement. Environmental or green marketing differs from other forms of advertising in some fairly fundamental ways. The environmental impacts of a product are not always apparent and may not affect the purchaser directly and so environmental claims are often more abstract. Also the environmental claims may apply to the full product life cycle and most importantly environmental marketing provides an incentive for manufacturers to achieve significant environmental improvements (Coddington, 1993). Some of the opportunities and benefits are furnished below:

#### **More profits and business opportunities**

Firms may also use green marketing in an attempt to address cost or profit related issues. Disposing of environmentally harmful by-products, such as polychlorinated biphenyl (PCB) contaminated oil are becoming increasingly costly and in some cases difficult. Therefore, firms that can reduce harmful wastes may incur substantial cost savings. When attempting to minimize waste, firms are often forced to re-examine their production processes. In these cases, they often develop more effective production processes that not only reduce waste, but reduce the need for some raw materials. This serves as a double cost savings, since both waste and raw materials are reduced. In other cases, firms attempt to find end-of-pipe solutions, instead of minimizing waste. In these

situations, firms try to find markets or use for their waste materials, where one firm's waste becomes another firm's input of production. For example, an Australian firm which produces acidic waste water as a by-product sells it to a firm involved in neutralizing base materials. Producing environment friendly things require relatively less inputs by creating fewer wastes, using fewer raw materials, and saving energy, too. The last way in which cost or profit issues may affect firms' environmental marketing activities is that new industries may be developed. Either a firm develops a technology for reducing waste and sells it to other firms or a waste recycling or removal industry develops. For example, firms that clean the oil in large industrial condensers increase the life of those condensers, removing the need for replacing the oil, as well as the need to dispose of the waste oil. This reduces operating costs for those owning the condensers and generates revenue for those firms cleaning the oil (Polonsky, 1994).

### **Increased market share and a competitive advantage**

Many marketers now know that being first to the shelf with an environmental innovation brings competitive advantage. With 50 % of the production capacity for phosphate detergents, German-based Henkel pioneered the market for zeolites and claimed market leadership when their consumers shifted to phosphate free detergents. Some companies like Fujitsu have gone on to make products designated as Super Green Products. They meet the preconditions for Green Products and are top class in terms of low energy consumption, 3R (Reduce, Reuse and Recycle) design and technology, non-use of hazardous substances, materials and technology that contribute to the environment and other environmental considerations. In 2004, 12 different products in the category of IT products and communications hardware, semiconductor devices and other electronic components were developed by Fujitsu were designated as Super Green Products (Fujitsu, 2005). Customers' loyalty to the brands keep on changing with the time. Therefore, companies are now

concentrating on their product image to themselves distinct in the market in front of new competitors and project themselves as eco-friendly.

### **Better products and environmental security**

Green products attract the customers because they perceive it of high quality, low on the demands of energy, and non-toxic. In this way they and their families are safe. Creating products that are more in sync with nature also allows us to personally contribute to environmental cleanup and help to secure a better future.

### **Corporate Social Responsibility(CSR)**

Many firms are beginning to realize that they are members of the wider community and therefore must behave in a socially and environmentally responsible fashion. This translates into firms that believe they must achieve socio-environmental objectives as well as profit related objectives. Therefore, corporate social responsibility (CSR) has become deeply integrated into companies' business practices. Firms can take two perspectives from CSR i.e. they can use the fact that they are environmentally responsible as a marketing tool or they can become responsible without promoting this fact. There are examples of firms adopting both strategies. Cosmetic company like Rustic Art heavily promoted their products to be organic, chemical free and environment friendly. While this behavior is a competitive advantage, the firm was established specifically to offer consumers environmentally responsible alternatives to conventional cosmetic products. This aspect is one of the part of CSR activities making companies' socio environmentally responsive rather than simply being a competitive tool. Apart from that companies can undertake social development programs, such as building toilets in remote areas and reframe it as a green practice.

### **CONSUMERS OF GREEN PRODUCTS**

Studies have been done in the last two decades to assess the purchasing behavior towards these green products (Delafrooz *et al.*, 2014). In a study to assess the purchase behavior (Coddington, 1993), it was concluded that 14 % of consumers regularly

**Table 1.** Hierarchies of needs and behaviour: Green consumers

Maslow	Roper
Self- actualization	True – Blue Greens, Opinion leaders, Trend setters, Executives
Esteem needs	Greenback Greens, Intellectually concerned, Not activists, Busy lifestyle, Up-and-coming
Social needs	Sprout Key swing group, Not certain ( environment or economy)
Safety needs	Grouzers, Object to higher prices, Blame others, Excuses for noninvolvement
Physiological needs	Basic Browns, Least environ-active group, Socially and economically disadvantaged

buy products in refillable packaging, 19 % buy products made from or packaged in recycled materials, 28 % avoid buying products in aerosol containers and 29 % use biodegradable, low-phosphate detergents. The study inferred that consumers are turning to green products at the rate of 4-5 % annually. The people who are buying green products or so called green consumers are segmented according to the Roper-S.C. Johnson Segmentation developed in 1990 which is by far the best known segmentation of consumers' environmental attitudes. They identified five categories of green consumers (Coddington, 1993):

- True- Blue Greens are the most actively green consumers. Their actual behavior is consistent with very strong concerns about the environment. They are much environmentally oriented and could be considered leaders of the green movements among the general population. They compromise 11 % of the population.
- Greenback Greens (11 %) are characterized by the fact that their commitment to the environment is mainly manifested by their willingness to pay substantially higher prices for green products.
- Sprouts (26 %) show middling levels of concern both about the environment and behavioral response. The sprouts certainly seem to have green tendencies, but they have yet to exhibit a clearly established pattern of pro-environment behavior.
- Grouzers (24 %) consistently rationalize their lack of pro-environmental behavior by offering all kinds of excuses and criticizing poor performance of others.

- Basic Browns (28 %) simply do not believe individuals can make a difference in solving environmental problems; indeed they do not want to make an effort.

In a study, carried out in Delhi NCR region, a majority of consumers were familiar with concept of green marketing and worried about the environment (Gupta and Abbas, 2013). In another study, more than 60% Indian consumers are familiar with green products with more than 85% consumers said they are good for environment. According to another study (Hanas, 2007), about 12 % of the U.S. Population can be identified as True Greens, consumers who seek out and regularly buy so-called green products. Another 68 % can be classified as Light Greens, consumers who buy green sometimes. However such classification for Indian customers is yet to be done. Reference to the Maslow's hierarchy of needs (Maslow, 1997), Table 1 depicts the division of consumers according to their degree of environmental concern.

### **GREEN MARKETING: THE ROLE OF INTERNET IN PROMOTING THE GREEN PRODUCTS**

The number of internet users has increased tremendously over the past few years. Successful firms have built businesses around people and internet and have been so successful because it offers the users a cheaper, quicker and easier route to getting the desired product. For the firms too it saves the costs and time. Connectivity to web world gives the consumer more power because most of the sites are interactive and moreover the potential customer is looking through the site mostly of interest and desire to purchase or know more about a product whereas in conventional mass media they

are sometimes exposed to advertisements they do not want to see or products they are not interested in. Influencing the consumer decision making process related to green products through internet is very essential for companies looking to promote green consumerism. The first step to influence the consumer decision making process related to green products through internet is a good website design. A good looking, user friendly and affordable website is must to develop the initial interest of the consumer. It should be constantly updated to keep the interest of the viewer alive and ensure maximum hits. Graphic and pictorial representations often capture the interest of people. Web based email marketing software may be attached with the website which send the customized mail through personal mailing if requested. Web links, web promotions or internet advertising have been in vogue in recent years and most recently the concert of search engine registration is a very successful tool for promoting green marketing. By buying advertising space on portals (Yahoo!, Msn etc.) and popular website the visitors are obliged to see the advertisement and they can decide whether to click. The firm which is selling green products pays any search engine to show its link on search results related to green products. For example, if a consumer searches about the green products on the website Google.com, the search results show the sponsored links related to green products on the right side of webpage. Promotion is one of the four elements of an organization's marketing mix. In traditional media the main methods are advertising, sales promotion, merchandising, publicity, and direct marketing. There are some key differences between the internet and traditional advertising and promotional media. Traditional advertising and promotion follows 'one to many' model (Bickerton *et al.*, 2000). There is little or no opportunity to talk back to the advertiser. But internet is interactive and a tiny banner will tell the user very little unless they choose to click through it to find out more about a product. The consumers have more choice and control and so it is very necessary to have a careful approach to market in a new medium. The challenge is to be innovative and not replicate conventional advertising material online.

## **BEHAVIOURAL ASPECTS OF INTERNET MARKETING**

Internet marketing is the process of building and maintaining customer relationships through online activities to facilitate the exchange of ideas, products, and services that satisfy the goals of both parties. Three primary forces are generated by the Internet effect e-marketing such as individualization, information, and interactivity. A study (Koufaris, 2002) examined how emotional and cognitive responses to visiting a web-based store for the first time can influence revisit intention. Another study (Kiang *et al.*, 2000) proposed a scheme for determining e-product characteristics for Internet marketing while a hypothetical customer theme (Ottman, 1997) of common activities that customers want to complete when they visit a particular web site was also prepared. This will examine the relationship between consumers' perceived importance of and satisfaction with Internet shopping (Sang and Young, 2001). Since the web is a fragmented medium containing billions of constantly changing web pages, capturing the attention of potential users is difficult. Although some destinations on the web are branded better from outside the digital world and enjoy consumers' direct clicks, the majority of web pages are still waiting to be discovered. Indeed, Internet marketing has recognized that e-customer advocacy, reactions to stimulated transactions and sensory, cognitive, and emotional experiences are all crucial in building an understanding of customer experience in the design of appropriate marketing programs for securing customer relationships (Hoffman and Novak, 2000).

## **INFORMATION SEARCH: HOW INTERNET BROUGHT CHANGES IN THE 4PS**

Today's online consumer can easily reach an enormous amount of information about products and services from a variety of sources (e.g. search engines, corporate sites, brand sites, third-party sites). At this stage, companies frequently lose consumer attention and consumers easily gain as much information as possible about the company's marketing mix. Information is a main contributor to

consumer economic power and a detractor of firm power. If consumers are brand loyal or information insensitive, they may just focus on distribution—instead of price, product features and promotion. This indicates the brand power of companies created in the early stages of the consumer decision-making process. Presence of the online customer modifies the approach to the marketing mix information. Information search now implies searching for the right product and price, and thus, the right value. The other Ps—Place and Promotion—assist the first two (product and price) to create value. Perhaps no area of green marketing has received as much attention as promotion. In fact, green advertising claims grew so rapidly during the late 1980s that the Federal Trade Commission (FTC) issued guidelines to help reduce consumer confusion and

prevent the false or misleading use of terms such as “recyclable,” “degradable,” and “environmentally friendly” in environmental advertising<sup>3</sup>. The changes in the 4P through internet are summarized in Table 2 (O’Rourke, 2000). Along with that managing the customers’ behavior towards green products are explained in Table 3.

In terms of products, we are reaching high levels of transparency in the business operations of many firms. Similarly, many “choice board” software programs make consumers the main product makers. By removing barriers to entry and transaction costs, the Internet provides for the costless exchange of information. Consumers can simultaneously access comparable products and services with low costs of searching and switching,

**Table 2.** Changes in 4P through internet

4Ps	Consumer benefits	Changes in operations	
		Manufacturer	Retailer
Product	Customerization	Customized products, economies of scope, more investment in R&D	Increased assortment, more investment in consumer services
Price	Transparency	Lower price premium and profit margins	Volume discounts and lower profit margins
Place	Convenience & Expertise	Transparency in ordering and inventory process	Higher inventory costs, warehousing (Disintermediation)
Promotion	Personalization	Increase in acquisition/retention costs, increase in gifting	More investment in store loyalty, in-store merchandising

**Table 3.** Transition of consumers from convention products to green ones (William Bridges Managing Transition Theory, Bridges, 2009)

Stage	Description	Management
Ending, Losing, Letting go	The consumers are suspicious or have ignorance or no knowledge about green products in their lifestyles.	It’s important to acknowledge the customers’ feeling or ignorance towards green products. Therefore, awareness is the key to at least inform about value of green products
Neutral zone	Anxiety or resentment towards green products	Continues provision of knowledge about green products
The new beginning	Openness to accept green products	As customer adopted green product, the challenge is to sustain the customers’ loyalty

giving him/her more options and the ability to easily compare prices, thus increasing consumer bargaining power. Place brought “convenience” and “expertise” to the markets and now the internet revolution has brought the shopping environment (or Place) into one’s own home. The introduction of personalized messages between the firm and consumer has led to a stronger consumer voice and now, the firm needs consumers’ permission to sell its products for first time in business history (Krishnamurthy, 2001). Consumers are gaining greater control over their media selection for purchase decisions. The rise of user-generated content, blogs and community sites has meant greater competition for branded corporate websites.

#### **CASES RELATED TO THE SALE OF GREEN PRODUCTS THROUGH INTERNET**

Kumar, Chattopadhyay and Sharma, 2012 carried out a case study on green supply chain in electronic and electric sector. The study found that the supply chain need to do more in order to make their business practices greener. However, cases related to green marketing through internet is still lacking in India. Therefore, a case of Japanese car Prius is presented here. The Japanese car maker Toyota advertises its hybrid car PRIUS through internet on the Automobile sections of the popular portal like Yahoo and MSN by projecting itself as a green or environmental friendly car. Internet plays an important role, direct mail were sent as email where possible and enable buyers to purchase the Prius online. A Prius specific microsite offers all information e-brochures for download. All adverts bear the Prius web address to make it easy for users to find. Direct mail is one of the first activities with pre-launch awareness letters being sent to existing Toyota customers. Direct mails were also sent to corporate buyers, which ought to be included in Toyota’s database of information of potential customers. Emails were designed to enable to directly pre-order the car and purchase the car online, something that appealed to the pioneer mindset. Meanwhile, high prices at the gas pump, concerns about global warming and an increase in production sent hybrid car sales soaring from

around 9,000 in 2002 to above 2,00,000 in 2005 according to Hybridcars.com. The above website guides the user that how their service can make websites to inform potential customers about green products. In 2005, Proctor and Gamble partnered with the non-profit organization, the Alliance to Save Energy (ASE), in a “viral marketing” campaign to spread news about the money-saving benefits of laundering clothes in cold water with specially formulated Tide Coldwater ASE provided credibility where by auditing and backing P and G’s claims that consumers could save an average of \$ 63 a year if they switched from warm to cold water washes. ASE sent e-mail promotions encouraging consumers to visit Tide.com’s interactive Website and take the “Coldwater Challenge” by registering to receive a free sample. Visitors could calculate how much money they would save by using the detergent, learn other energy-saving laundry tips, and refer email addresses of their friends to take the challenge as well. Tide.com offered an engaging map of the United States where, over time, visitors could track and watch their personal networks grow across the country when their friends logged onto the site to request a free sample.

#### **CONCLUSION**

Most of the firms and corporates have realized that consumers will buy products or avoid their purchase, based upon environmental considerations. At present, firms are in the beginning of the greening process to develop an environmental competitive advantage but as firms further develop their greening activities, they will require additional expertise to enable more substantial environmental improvements. The ability of consumers to access a variety of information sources enhances their voice at the individual and community level, and is changing markets. Green marketing requires applying good marketing principles to make green products desirable for consumers. Rising energy prices, growing pollution and resource consumption in the world, and political pressures to address climate change are driving innovation toward healthier, more-efficient, high-performance products. From an organizational standpoint, environmental

considerations should be integrated into all aspects of marketing - new product development and communications and all points in between. The holistic nature of green also suggests that besides suppliers and retailers new stakeholders be enlisted, including educators, members of the community, regulators, and non governmental organizations.s.

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# Effect of integrated nutrient management on growth and yield of Onion (*Allium cepa* L.)

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## ABSTRACT

A field experiment entitled “effect of integrated nutrient management on growth and yield of onion (*Allium cepa* L.)” was undertaken during Rabi 2011-12 and 2012-13 in farmer’s field of Deogarh district of Odisha. Among the ten treatments taken, the highest plant height (57.17 cm), leaf area (378.13 sq. cm) and total yield (310.92 q ha<sup>-1</sup>) were observed with the application of 100% recommended dose of fertilizer + farmyard manure @ 5t ha<sup>-1</sup> + vermicompost @ 2t ha<sup>-1</sup> + biofertilizer (*Azospirillum* + phosphate solubilising bacteria @ 5:5 kg ha<sup>-1</sup>). The results revealed highest number of leaves (7.52), average bulb weight (81.08g) and BC ratio (2.93:1) with application of 75% recommended dose of fertilizer + farmyard manure @ 5t ha<sup>-1</sup> + vermicompost @ 2t ha<sup>-1</sup> + biofertilizer (*Azospirillum* + PSB @ 5:5 kg ha<sup>-1</sup>).

**Key words:** *Azospirillum*, economics, onion, PSB, vermicompost.

## INTRODUCTION

Onion (*Allium cepa* L.), is an important species belonging to family Alliaceae. It is an indispensable item in every kitchen as vegetable and condiment used to flavour many of the food stuffs. Therefore, onion is popularly known as ‘Queen of the kitchen’. Onion is considered to be the second most important vegetable crop grown in the world next to tomato. In the world, India stands first in area and ranks second to China in production; the total area in India under onion cultivation during 2012-13 was 10.51 lakh hectare with a production of 168.13 lakh tons and productivity of 16 t ha<sup>-1</sup> (Tiwarly, 2014). The steady depletion of native soil fertility and the occurrence of multiple nutrient deficiencies in onion fields have led to the identification of nutrient management as a key factor limiting sustainable onion production (Sharma *et al.*, 2003). Although the use of manures as nutrient sources for vegetables is common, their effectiveness is potentially limited by nutrient release patterns that

are often out of synchrony with crop demand, large variability in source quality and field distribution, and food safety. All of these issues have contributed to experimentation with alternative options. A gradual shift from using purely organic sources to introducing some proportion of inorganic sources is gaining acceptance. This shift has formed the basis for integrated nutrient management (INM), which could involve three nutrient sources: microbial inoculants or biofertilizers including azotobacter, *azospirillum*, and phosphate solubilising bacteria (PSB), inorganic fertilizers and manures. However, INM further prescribes that selected nutrient inputs be used judiciously to ensure optimum supply of all essential nutrients for sustained crop production. Most of the INM studies conducted with onion have lacked the experimental components required to link soil nutrient budgeting with bulb yield response. Onion is a heavy feeder of mineral elements. A crop of 40 t ha<sup>-1</sup> removes approximately 120 kg of N, 50 kg of P<sub>2</sub>O<sub>5</sub> and 160 kg of K<sub>2</sub>O ha<sup>-1</sup> (Tandon and Tiwarly, 2008). Hence, the greater its ability to utilize

nutrients for crop production, the greater is the yield potential. This study was therefore, conducted to find out the best combination of organic manure and inorganic fertilizers on growth and yield of onion.

## MATERIALS AND METHODS

Field experiments were conducted to find out the best combination of organic manure and inorganic fertilizers on growth and yield of onion at Malehipada village of Reamal block of Deogarh district of Odisha during *rabi* 2011-12 and 2012-13, which is located at 21° 34' 81" North latitude, 84° 64' 06" East longitude and at an altitude of 220 meters above the mean sea level. The experiment was conducted on medium deep black soil and the texture of the soil was clay loam type. pH of the soil of the plots were found to be in the range of 7.32. The average fertility status of experimental site was available N 284 kg ha<sup>-1</sup>, P 28.3 kg ha<sup>-1</sup> and K 445 kg ha<sup>-1</sup>. The experiment was laid out in Randomised Block Design. The experiment consist of ten treatments *viz.*, T<sub>1</sub> – Control, T<sub>2</sub> - 100% recommended dose of fertilizer (NPK @ 150:50:80 kg ha<sup>-1</sup>), T<sub>3</sub> - 100% recommended dose of fertilizer (RDF) + farmyard manure (FYM) @ 10t ha<sup>-1</sup>, T<sub>4</sub> - 100% RDF + FYM @ 5t ha<sup>-1</sup> + vermicompost @ 2t ha<sup>-1</sup>, T<sub>5</sub> - 100% RDF + FYM @ 10t ha<sup>-1</sup> + Biofertilizer (*Azospirillum* + phosphate solubilising bacteria @ 5:5 kg ha<sup>-1</sup>), T<sub>6</sub> - 100% RDF + FYM @ 5t ha<sup>-1</sup> + vermicompost @ 2t ha<sup>-1</sup> + biofertilizer (*Azospirillum* + phosphate solubilising bacteria @ 5:5 kg ha<sup>-1</sup>), T<sub>7</sub> - 75% RDF + FYM @ 10t ha<sup>-1</sup> + biofertilizer (*Azospirillum* + phosphate solubilising bacteria @ 5:5 kg ha<sup>-1</sup>), T<sub>8</sub> - 75% RDF + FYM @ 5t ha<sup>-1</sup> + vermicompost @ 2t ha<sup>-1</sup> + biofertilizer (*Azospirillum* + phosphate solubilising bacteria @ 5:5 kg ha<sup>-1</sup>), T<sub>9</sub> - 50% RDF + FYM @ 10t ha<sup>-1</sup> + biofertilizer (*Azospirillum* + phosphate solubilising bacteria @ 5:5 kg ha<sup>-1</sup>), T<sub>10</sub> - 50% RDF + FYM @ 5t ha<sup>-1</sup> + vermicompost @ 2t ha<sup>-1</sup> + biofertilizer (*Azospirillum* + phosphate solubilising bacteria @ 5:5 kg ha<sup>-1</sup>). 45 days old onion seedlings of variety, Agrifound Light Red were transplanted in the plot with a spacing of 15×10cm. Three hand weeding were carried out at 20, 40 and 60 DAT to control weed. All recommended packages of practices were adopted uniformly to all the treatments except

nutrient management practices to raise a good crop. The data was recorded for vegetative parameters (plant height and number of leaves), yield parameters (average bulb weight and total bulb yield). The observed data were then subjected to statistical analysis (Gomez and Gomez, 1984). The treatment comparisons were made using t-test at 5% level of significance. The economics was calculated on the basis of prevailing local market price of onion bulbs and cost of inputs.

## RESULTS AND DISCUSSION

### Effect on growth parameters

The data presented on vegetative parameters in onion (Table 1) revealed significant variations among the treatments. Significantly highest pooled plant height was recorded in T<sub>6</sub> (57.17cm), closely followed by T<sub>8</sub> (56.94cm), T<sub>5</sub> (55.39cm) and T<sub>7</sub> (54.87cm) than rest of the treatments. Significantly shortest pooled plant height of 37.81 cm was observed in control plots (T<sub>1</sub>). Similar trend was also recorded in pooled number of leaves/plant, significantly maximum in T<sub>8</sub> (7.52) and minimum in T<sub>1</sub> (4.93). Significant variation was observed in pooled leaf area of year 2011-12 and 2012-13, maximum leaf area found with T<sub>6</sub> (378.13 sq. cm.) followed by T<sub>8</sub> (376.92 sq. cm.), T<sub>5</sub> (374.02 sq. cm.), T<sub>7</sub> (371.62 sq. cm.), and T<sub>4</sub> (369.68 sq. cm.). Lowest pooled leaf area was observed with T<sub>1</sub> (265.97 sq. cm.). The results clearly indicated the effect of integrated nutrient management in onion. Similar results were reported by Chandre Gowda *et al.* (2007) and Naik and Gupta (2010).

### Effect on yield and economic parameters

Significant variations were also observed for average bulb weight and total bulb yield in onion (Table 2). The pooled average bulb weight in onion varies from 42.60g (T<sub>1</sub>) to 81.08g (T<sub>8</sub>). Significantly heaviest bulb was recorded in T<sub>8</sub> (81.08g) than rest of the treatments except T<sub>6</sub> (80.08g), T<sub>7</sub> (80.07g), T<sub>5</sub> (77.43g), T<sub>4</sub> (77.30g) and T<sub>3</sub> (74.25g) which were statistically *at par*. The variability was due to effectiveness of integrated nutrient management methods which ultimately increased the nutrient availability for the crop (Arora and Dan, 2003). The

**Table 1.** Effect of integrated nutrient management on growth parameters of onion

Treatment	Plant Height (cm)			No. of Leaves			Leaf Area (sq. cm.)		
	2011-12	2012-13	Pooled	2011-12	2012-13	Pooled	2011-12	2012-13	Pooled
T <sub>1</sub>	39.39	36.23	37.81	5.07	4.80	4.93	271.07	260.87	265.97
T <sub>2</sub>	47.60	46.22	46.91	6.33	5.83	6.08	344.37	337.77	341.07
T <sub>3</sub>	52.26	50.36	51.31	6.73	5.93	6.33	351.80	340.83	346.32
T <sub>4</sub>	53.47	51.28	52.37	6.93	6.60	6.77	375.87	363.50	369.68
T <sub>5</sub>	56.11	54.66	55.39	7.07	6.87	6.97	376.83	371.20	374.02
T <sub>6</sub>	58.05	56.29	57.17	7.33	7.00	7.17	381.73	374.53	378.13
T <sub>7</sub>	54.31	55.43	54.87	7.07	6.97	7.02	375.07	368.17	371.62
T <sub>8</sub>	57.10	56.77	56.94	7.87	7.17	7.52	380.63	373.20	376.92
T <sub>9</sub>	43.79	42.20	43.00	6.20	5.67	5.93	328.97	322.73	325.85
T <sub>10</sub>	43.96	44.59	44.28	6.27	5.73	6.00	333.90	324.87	329.38
CV(%)	6.74	7.15	6.95	6.72	8.37	7.54	8.54	6.48	7.61
SEm (±)	1.97	2.04	1.42	0.26	0.30	0.20	17.36	12.87	9.99
LSD(0.05)	5.86	6.06	4.54	0.77	0.90	0.64	51.58	38.24	31.95

**Table 2.** Effect of integrated nutrient management on yield and economics parameters of onion

Treatment	Average bulb weight (g)			Total bulb yield (q ha <sup>-1</sup> )			BC ratio		
	2011-12	2012-13	Pooled	2011-12	2012-13	Pooled	2011-12	2012-13	Pooled
T <sub>1</sub>	45.33	39.87	42.60	180.00	161.67	170.83	1.67	1.40	1.53
T <sub>2</sub>	68.70	57.80	63.25	248.33	225.00	236.67	2.31	2.02	2.16
T <sub>3</sub>	83.33	65.17	74.25	301.67	268.33	285.00	2.69	2.29	2.49
T <sub>4</sub>	86.20	68.40	77.30	303.33	271.67	287.50	2.66	2.28	2.47
T <sub>5</sub>	85.83	69.03	77.43	323.33	295.00	309.17	2.86	2.53	2.70
T <sub>6</sub>	86.47	73.70	80.08	325.00	296.87	310.93	2.89	2.55	2.72
T <sub>7</sub>	85.47	74.67	80.07	316.73	291.57	304.15	2.83	2.53	2.68
T <sub>8</sub>	86.43	75.73	81.08	320.93	293.43	307.18	3.11	2.76	2.93
T <sub>9</sub>	53.00	44.07	48.53	218.33	205.00	211.67	1.69	1.52	1.61
T <sub>10</sub>	54.40	45.73	50.07	226.67	209.33	218.00	1.82	1.60	1.71
CV(%)	9.72	10.06	9.90	14.58	13.31	14.04	–	–	–
SEm (±)	4.13	3.57	2.73	23.27	19.35	15.13	–	–	–
LSD(0.05)	12.26	10.60	8.72	69.15	57.50	48.41	–	–	–

results also showed that treatment effect were significant in pooled total bulb yield in onion. Significantly highest total bulb yield was recorded in T<sub>6</sub> (310.93q ha<sup>-1</sup>) followed by T<sub>5</sub> (309.17q ha<sup>-1</sup>), T<sub>8</sub> (307.18q ha<sup>-1</sup>), T<sub>7</sub> (304.15 q ha<sup>-1</sup>), T<sub>4</sub> (287.50 q

ha<sup>-1</sup>) and T<sub>3</sub> (285 q ha<sup>-1</sup>) than the rest of the treatments. On the other hand, significantly lowest pooled total yield of 170.83q ha<sup>-1</sup> was recorded in T<sub>1</sub>, the control plot. The results are in agreement with Jamir *et al.* (2013), Singh and Pandey (2006) as well as Tilak and Saxsena (2008).

The result on BC ratio (Table 2) showed variability among different nutrient management applied in onion. The pooled BC ratio estimated in integrated nutrient management practice over control indicated maximum BC ratio of 2.93 in T<sub>8</sub> and minimum 1.53 in T<sub>1</sub>. Similar results were also reported by Jayathilake *et al.* (2003).

The present study exhibited that integrated management practices produced significantly highest number of leaves, average bulb weight, highest BC ratio and more onion bulb yield with either application of 75% recommended dose of fertilizer + farmyard manure @ 5t ha<sup>-1</sup> + vermicompost @ 2t ha<sup>-1</sup> + biofertilizer (*Azospirillum* + phosphate solubilising bacteria @ 5:5 kg ha<sup>-1</sup>) (T<sub>8</sub>) or highest plant height, leaf area and total bulb yield with the application of 100% recommended dose of fertilizer + farmyard manure @ 5t ha<sup>-1</sup> + vermicompost @ 2t ha<sup>-1</sup> + biofertilizer (*Azospirillum* + phosphate solubilising bacteria @ 5:5 kg ha<sup>-1</sup>).

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# Management of pulse beetle *Callosobruchus chinensis* in storage condition using biopesticides

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## ABSTRACT

The pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae) is a stored grain pest of pulses in India. The management of pulse beetle was studied in the P.G. Department of Zoology, Utkal University during 2010-11. The percent of grain damage, weight loss etc. were recorded under the treatment by neem, *Lantana camara*, lemon grass, tulsi, begunia, custard apple, bael and karanja leaf powder. Among the treatments, neem leaf powder was found to be most effective against *Callosobruchus chinensis* at 45 and 90 DAT (days after treatment).

**Key words:** *Callosobruchus chinensis*, pulse beetle, green gram.

## INTRODUCTION

Pulses are playing a vital role in the agriculture economy of our country. The farmers include them in the cropping system realizing their value as a soil fertility improver. These crops have inherent capability to fix atmospheric nitrogen and thereby enrich the soil. In India, pulses are grown on an area of 22 million hectares producing 13 million tons annually. The productivity of the crops has remained static for the last three and half decades with the increasing population. The stagnating pulse production is such that the estimated availability of pulses has gone down from 70.1 gm/day/person in 1951 to 31 gm/day/person in 2008 whereas Indian Council of Medical Research recommends 65 gm/day/person (Ready *et al.*, 2012). Legumes are included in many of the food items all over the world, but their use is particularly wide spread in the tropics and the subtropics. The pulse crops occupy 20 per cent of the total area under food grain in India, but they contribute only 14 per cent of the total food grain production since efforts have not yet been made for the development of high yielding, fertilizer responsive, disease and pest resistant varieties.

The major pest of pulses in storage are the bruchids particularly *Callosobruchus chinensis*, which often appears in the crop in less number just prior to harvest and reaches extremely high in successive generations in the store. The damaged seed exhibits circular holes made by adults during their emergence. The flat whitish oval eggs are laid by female sticking to the seeds. The damaged seeds are unfit for consumption as well as for the seed purposes. The pulse beetle is the most destructive pest of pulses in India. In the management of the pest, utilization of the number of preferred/resistant pulse species and varieties would play an important role. Perfection of this method is however time consuming and difficult to execute. Hence, alternative methods would be controlling the pest by mixing toxic chemicals with food grains which is not recommended. Therefore, the only safe and feasible approach is the treatment of stored grains with non-poisonous organic and/or inorganic substances. These various substances have been tested with different methods of application by different workers (Bhaskar *et al.*, 1976; Jotwani and Sircar, 1967; Luca, 1982, Mummigatti and Ragunathan, 1977 and Singh *et al.*, 2001).

## MATERIALS AND METHODS

The studies on management of *Callosobruchus chinensis* in green gram were carried out during the year 2010-11 in the P.G. Department of Zoology, Utkal University during 2010-11. The main culture medium viz., the seeds of Green gram (*Vigna radiata*) var. PDM-139 was dry cleaned manually and sieved to remove the undesired other seeds and the insects if any. The seeds were taken in air tight galvanized seed bin and fumigated by ethylene di bromide (EDB) in order to eliminate the traces of insects and mite infestation. Further, the seeds were fumigated for very short time period i.e. only for 10-15 minutes then the seeds were removed and dried under sunlight to free the seeds from residual effect of ethylene-bromide. Half kilogram of disinfested seeds was taken in glass jar of (15 X 10) cm size. The moisture content of grain was measured before experimentation by using OSAW digital moisture meter (OSAW Industrial Product Pvt. Ltd., Hariyana, an ISO 9001 N ISO 14001 Co., manufacturer and exporter of Sc. Edn and Agro Industries equipments). For preparation of stock culture, adult beetles of *Callosobruchus chinensis* were collected from infested grains of go-downs using an aspirator. A pair of adults was then released in jar containing conditioned green gram grains. The jars were kept in incubator at 27±5°C temperature and 70±5 per cent relative humidity. After emergence, pairs of freshly emerged adult beetles from this uni-parental culture were taken for experiment. The management of *Callosobruchus chinensis* were undertaken taking undamaged seeds, freshly emerged adult pairs in plastic jar covered with muslin cloth. The per cent of grain damage, per cent of weight loss and per cent of germination were recorded at 45 and 90 days after treatment.

The leaf powder of different plant products viz. neem, *L. camara*, lemon grass, tulsi, begunia, custard apple, bael and karanja were collected from Baliapal of Balasore district of Odisha. Then the plant products were dried in shade and were grinded by electronic grinder to get fine powder. The powder was sieved through 60 mm mesh sieve. The plant

products were mixed with conditioned green gram seeds @ 2 g/100 g of grains separately. Untreated check was also kept simultaneously. Sample of 100 g seeds of green gram from each treatment was transferred to the jar of 4.5 cm length and 3.5 cm diameter size. The treatments were replicated three times. Five pairs of freshly newly emerged *C. chinensis* were released in each jar. Then the jar was covered with muslin cloth and tied with rubber band and kept in room for 90 days. The data obtained on grain damage, per cent weight loss and germination per cent of seeds were recorded. Then the data were finally statistically analyzed by adopting suitable transformation.

## RESULTS AND DISCUSSION

In the laboratory eight plant products treated green gram seeds (@ 2g plant product /100g of green gram seed) and 100 gram of untreated green gram seeds were taken against the stored grain pest, *C. chinensis*. Among the eight plant products tested on grain damage of green gram is presented in Table 1. The percentages of grain damage were calculated by separating and counting the damaged seeds and undamaged seeds means there was not any symptoms of attack of *C. chinensis*. It is revealed that on the basis of percentage of grain damage of green gram seed, neem leaf powder @ 2g/100g of seed was superior to rest of the treatments. The lowest seed damage (2.33%) was observed in neem leaf powder @ 2g/100g of seed. It was at par with begunia leaf powder (3.66%) and bael leaf powder (4.00%). Others treatments viz., lemon grass, tulsi, custard apple, karanja and *Lantana camara* leaf powder @ 2g/100g grain were significantly superior to untreated check. The *L. camara* leaf powder proved to be least effective (5.33% damage) but it was also significantly superior to untreated check. The effectiveness of plant products were as follows neem, begunia, bael, lemon grass, tulsi, custard apple, karanja and *L. camara* leaf powder respectively.

The persistence impact of plant products viz., neem, *L. camara*, lemon grass, tulsi, begunia, custard apple, bael and karanja leaf powder against *C. chinensis* on green gram seeds after one month

**Table 1.** Effect of plant products on grain damage, weight loss and germination of green gram stored for 45 days

Treatments	Per cent grain damage	Per cent wt. loss	Per cent germination
T <sub>1</sub> Neem leaf powder	2.33 <sup>a</sup> (8.74)	1.41 <sup>a</sup> (6.81)	97.66 <sup>bcdef</sup> (81.22)
T <sub>2</sub> Lantana leaf powder	5.33 <sup>bcdefg</sup> (13.26)	2.25 <sup>abcdef</sup> (8.54)	96.00 <sup>ab</sup> (78.49)
T <sub>3</sub> Lemon grass powder	4.33 <sup>abcd</sup> (11.89)	2.11 <sup>abcd</sup> (8.34)	97.33 <sup>bcde</sup> (80.60)
T <sub>4</sub> Tulsi leaf powder	4.33 <sup>abcd</sup> (11.99)	2.08 <sup>abc</sup> (8.29)	96.33 <sup>abc</sup> (78.95)
T <sub>5</sub> Begunia leaf powder	3.66 <sup>ab</sup> (11.01)	1.58 <sup>ab</sup> (6.39)	95.00 <sup>a</sup> (77.09)
T <sub>6</sub> Custard apple powder	4.66 <sup>abcde</sup> (12.45)	2.60 <sup>abcdefgh</sup> (9.24)	96.58 <sup>abcde</sup> (79.34)
T <sub>7</sub> Bael leaf powder	4.00 <sup>abc</sup> (11.47)	2.17 <sup>abcde</sup> (8.45)	98.00 <sup>cdefg</sup> (81.90)
T <sub>8</sub> Karanj leaf powder	5.00 <sup>abcdef</sup> (12.87)	2.33 <sup>abcdefg</sup> (8.67)	98.50 <sup>defgh</sup> (82.24)
T <sub>9</sub> Untreated check	39.33 <sup>h</sup> (38.80)	19.50 <sup>i</sup> (26.08)	99.25 <sup>efghi</sup> (85.30)
SEM±	0.968	0.858	0.646
CD (P=0.05)	(2.88)	(2.55)	(1.92)

of application was recorded and compared against untreated condition. At 45 DAT the minimum per cent seed damage (2.33%) was recorded in the neem leaf powder which was statistically at par with the begunia leaf powder (3.66%) and bael leaf powder (4.00%). Results obtained on the per cent weight loss of green gram seed admixed with different plant products @ 2g/100g grain are presented in Table 1. The results revealed that in untreated green gram seed 19.50 per cent weight loss occurred due to infestation of *C. chinensis* whereas, the seeds admixed with different plant products, the per cent weight loss ranged from 1.41 to 2.60 per cent. Admixing of all the plant products @ 2g/100g grain after the storage of one month showed significantly superior results to untreated check. The effect of all plant products on per cent weight loss was statistically at par. These were 1.58, 2.08, 2.11, 2.17, 2.25, 2.62, 2.60 per cent weight loss

in begunia, tulsi, lemon grass, bael, *L. camara*, karanja and custard apple leaf powder respectively. At 30 DAT the lower and higher value of seed weight loss were obtained from the neem leaf powder treatment (1.41%) and in untreated check (19.50%), respectively. However, begunia leaf powder was less infested (1.58%) but it was statistically at par with remaining treatments. The effect of plant products after one month of storage on germination was also studied by adopting standard germination test in the laboratory and results are presented in Table 1. The data revealed that germination per cent of seeds after 30 days of admixing with plant products @ 2g/100g grain were from 95.00 to 98.50 per cent in different treatments as against 99.25 per cent in untreated check. The minimum germination (95.00%) was recorded in begunia leaf powder treated seeds followed by *L. camara* leaf powder (96.00%) and tulsi leaf powder (96.33%). Other plant products viz. neem, lemon grass, custard apple, bael and karanja leaf powder produced all most similar results. The result of different treatments is presented in Table 2. It is revealed that neem leaf powder @ 2g/100g of seed was superior in controlling the pest. The lowest seed damage (11.70%) was observed in neem leaf powder treatment followed by begunia leaf powder (17.66%), bael leaf powder (20.00%). The other treatments viz. custard apple, tulsi, lemon grass, *L. camara* and karanja leaf powder @ 2g/100g of seed were statistically at par and were found to be significantly superior to untreated check. The *L. camara* leaf powder was least effective (24%). The order of effectiveness of plant products on controlling *C. chinensis* is as follows, viz. neem, begunia, bael, custard apple, tulsi, lemon grass, *L. camara*, karanja leaf powder respectively. The lowest (11.70%) and highest (96.66%) seed damage at 90 DAT were obtained from the treatments viz. neem leaf powder and application free treatment, respectively. The present finding was in accordance with those of Jotwani and Sircar (1967) in relation to potential of neem seed powder. They tested the neem seed powder on four pulses and on each pulse they had observed zero per cent seed damage. Results obtained on the per cent weight loss of green gram seed stored for three months and

admixed with different plant products @ 2 g/100 g of seed are presented in Table 2. It was evident from the studies that in untreated green gram seed, 49.10 per cent weight loss occurred due to infestation of *Callosobruchus chinensis*, whereas, the seed admixed with different plant products, the per cent weight loss varied from 8.30 to 16.12 per cent. The treatment with neem leaf powder showed best result among all the treatments having 8.30 per cent weight loss after the storage of three months. All the treatments showed significantly better results than untreated check. The least effective treatment was tulsi leaf powder (16.12%) but it was also superior to untreated check. The lowest seed weight loss (8.30%) at 90 DAT was recorded in neem leaf powder treatment which was statistically at par with begunia leaf powder (9.72%). However, all the treatments showed statistical superiority over

untreated check (44.46%). Amongst different treatments tulsi leaf powder registered highest (16.12%) seed weight loss. The present observation in relation to neem seed powder was also in confirmation with those of AL-Hemgari (1994). Similarly, this study was in accordance with those of Pandey and Singh (1995) in regard to performance of *Ipomea* powder. The effect of plant products after three months of storage on germination per cent of seed was also studied by adopting standard germination test in the laboratory and results are presented in Table 2. The data revealed that germination percentage of seeds after 90 days of admixing plant products varied from 86.66 to 95.58 per cent in different treatments as against 83.75 per cent in untreated check. The minimum germination (86.66%) was recorded in begunia leaf powder treated seed followed by *Lantana camara* (90.33%), tulsi (93.00%), neem (93.33%), lemon grass (94.00%), custard apple (94.00%), bael (94.16%) and karanja leaf powder (95.58%). At 90 DAT, the minimum (83.75%) and the maximum (95.58 %) germination percentage were noticed on untreated check and karanja leaf powder, respectively with 2g/100g of seed.

**Table 2.** Effect of plant products (@ 2 g/100g seed) on grain damage, weight loss and germination after 90 days storage of Green gram

Treatments	Per cent grain damage	Per cent wt. loss	Per cent germination
T <sub>1</sub> Neem leaf powder	11.70 <sup>a</sup> (19.98)	8.30 <sup>a</sup> (16.72)	93.33 <sup>ef</sup> (75.07)
T <sub>2</sub> Lantana leaf powder	24.33 <sup>defg</sup> (29.53)	14.20 <sup>e</sup> (22.11)	90.33 <sup>c</sup> (71.86)
T <sub>3</sub> Lemon grass powder	23.10 <sup>cdef</sup> (28.71)	15.10 <sup>ef</sup> (22.85)	94.00 <sup>efg</sup> (75.82)
T <sub>4</sub> Tulsi leaf powder	22.33 <sup>cde</sup> (28.18)	16.12 <sup>efg</sup> (23.64)	93.00 <sup>e</sup> (74.65)
T <sub>5</sub> Begunia leaf powder	17.66 <sup>b</sup> (24.84)	9.72 <sup>ab</sup> (18.15)	86.66 <sup>b</sup> (68.59)
T <sub>6</sub> Custard apple powder	21.20 <sup>bcd</sup> (27.39)	11.59 <sup>bcd</sup> (19.89)	94.00 <sup>efg</sup> (75.82)
T <sub>7</sub> Bael leaf powder	20.00 <sup>bc</sup> (26.51)	10.90 <sup>bc</sup> (19.22)	94.16 <sup>efgh</sup> (75.99)
T <sub>8</sub> Karanj leaf powder	27.20 <sup>gh</sup> (31.30)	14.20 <sup>e</sup> (22.06)	95.58 <sup>hi</sup> (77.86)
T <sub>9</sub> Untreated check	96.66 <sup>i</sup> (80.00)	49.10 <sup>h</sup> (44.46)	83.75 <sup>a</sup> (66.37)
SEM ±	1.310	0.834	0.726
CD (P=0.05)	(3.89)	(2.48)	(2.16)

## CONCLUSION

Eight plant products were taken as bio-pesticides on the basis of the performance studied by different researchers time to time earlier. On the basis of their results the active ingredients in the plant product which control *C. chinensis* were not studied, only performance of plant product was studied in the experiment. It was revealed from the experiment that the seed damage caused by the insect at 45 days after treatment with 2g/100g of seed was minimum in neem leaf powder but was maximum in the *Lantana camara* leaf powder treatment. At 90 DAT, the minimum per cent seed damage was recorded in neem leaf powder while maximum seed damage was recorded in karanja leaf powder treatment. The seed weight loss at 45 DAT as minimum with neem leaf powder and maximum with custard apple leaf powder while the minimum seed weight loss noticed at 90 DAT was in neem leaf powder and maximum in tulsi leaf powder treatment. The minimum germination

percentage was observed at 45 DAT in case of begunia leaf powder while maximum was in case of karanja leaf powder. At 90 DAT the minimum germination percentage was observed in begunia leaf powder and maximum in karanja leaf powder. The management parameters indicated that neem, lemon grass and begunia leaf powder could check the pest infestation. But *Lantana camara* leaf powder treatment performed moderately.

#### ACKNOWLEDGEMENT

The authors are thankful to Dr. Devi Prasad Dash, Subject Matter Specialist (Soil Science), Krishi Vigyan Kendra, Balasore for extending his support to carry out the research work in the Soil Science Laboratory during 2010-11 and providing his valuable suggestions.

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# High frequency *in vitro* shoot proliferation in local cultivar of Gerbera

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## ABSTRACT

Study was carried out to standardization of media supplements for shoot proliferation in the local cultivar of gerbera (Common Red) in Department of Floriculture and Landscaping. In capitulum explants, MS + BAP (3.0 mg lt<sup>-1</sup>) +Kinetin (1.0 mg lt<sup>-1</sup>) was the potent combination for production of longer shoots , (2.59 cm) and (7.10 cm during culturing and subculturing ) respectively. MS medium with (3.0 mg lt<sup>-1</sup>) BAP and (80.0 mg lt<sup>-1</sup>) Ads was the most suitable combination for production of maximum multiple shoots/calli mass (6.00) with maximum number of leaves (15.00) during culturing and the treatment produced maximum number of multiple shoots (6.00) and leaves (16.67) during subculturing .The MS medium with (2.0 mg lt<sup>-1</sup>) BAP and (80.0 mg lt<sup>-1</sup>) Ads was most effective for production of highest number of multiple shoots (36.00) during culturing and subculturing ,with probable number of approximate shoots (576.00) in capitulum explants (1/4<sup>th</sup> part capitulum). Among the explants, capitulum explant was most prolific over the leaf bit explant for profuse shoot proliferation.

**Key words:** Gerbera, calli, media, explant.

## INTRODUCTION

Gerbera, a majestic flower used in floral decoration, exhibition and in high class bouquet. It is widely used as a decorative garden plant i.e. they are planted in beds, pots, borders and in rockery for garden display. It is one of the most popular flower gaining importance day by day. Now a days the cv. Common Red is used hugely in landscape garden. Gerbera contributes largely to the floriculture industry by virtue of its yield potential. To produce new and large number of qualitative cultivars in less time, tissue culture technique can be a boon. Tissue culture technique enables manifold increase in number of plants produced per year. The plants produced under tissue culture are most ideal for the large scale production of flowers as they are free from diseases. In recent years, most commercial varieties have been multiplied through tissue culture like, e.g: axillary shoot formation from excised shoot tips (Murashige *et al.*,1974) and capitulum explants (Pierik *et al.*, 1973, 1974) have been reported.Plants

may be regenerated through leaf (Jerzy and Lubomski, 1991 and Hedtrich, 1981) to produce regenerated ones showing no variations. In tissue culture and organogenesis somatic embryogenesis are done thorough callus or non callus phase. The plants regenerated from callus can be useful in mutation breeding. Tissue culture techniques are more preferred in gerbera as because conventionally propagated gerbera are heterozygous and their seedlings are not uniform. So, to produce high quality uniform blooms, *in vitro* propagation is the need of the hour. The earlier works indicated that, depending on cultivars as well as local conditions, the requirements for response, multiplication vary considerably. It is therefore necessary to standardize media supplements for *in vitro* proliferation of gerbera.

## MATERIALS AND METHODS

The present experiment was carried out at Department of Floriculture and Landscaping, College

**Table 1.** Details of concentrations of BAP, Adenine sulphate and Kinetin for shoot proliferation (in capitulum and leaf bits derived calli)

Treatments	Growth promoters and regulators (mg/l)		
	BAP	Ads	Kinetin
	T <sub>1</sub>	–	–
T <sub>2</sub>	1.0	–	1.0
T <sub>3</sub>	2.0	–	1.0
T <sub>4</sub>	3.0	–	1.0
T <sub>5</sub>	1.0	75.0	–
T <sub>6</sub>	2.0	75.0	–
T <sub>7</sub>	3.0	75.0	–
T <sub>8</sub>	1.0	80.0	–
T <sub>9</sub>	2.0	80.0	–
T <sub>10</sub>	3.0	80.0	–

of Agriculture, OUAT, Bhubaneswar to standardize media supplements for *in vitro* proliferation of shoots.

After subculturing of callus, it was transferred to MS medium without any plant bioregulators as control (T<sub>1</sub>) and MS medium containing different concentrations of BAP (1.0,2.0,3.0 mg l<sup>-1</sup>), adenine sulphate (75.0,80.0 mg l<sup>-1</sup>) and Kinetin (1.0mg l<sup>-1</sup>) to increase the rate of proliferation (Table 1). Three replications per treatment and 10 cultures per replication were used. Observations on shoot initiation, shoot proliferation, shoot multiplication, leaf formation, number of multiple shoots/calli mass, shoot length, number of leaves/ multiple shoot and leaf colour were recorded after 35 days of inoculation during culturing of the proliferated calli. After 20 days of first subculturing of proliferated shoots observations on number of multiple shoots/shoot, shoot length (cm), number of leaves/multiple shoot and leaf colour were recorded.

1/4<sup>th</sup> part of the proliferated callus was used for shoot proliferation. So, 4<sup>n</sup> = 4<sup>2</sup> = 16 was multiplied to total number of multiple shoots during culturing and subculturing to get the probable number of approximate shoots/capitulum.

Number of multiple shoots during culturing and subculturing = Number of shoots/callus mass during culturing x Number of multiple shoots/single shoot during subculturing.

Probable number of shoots = 4 x 4 x No. of shoot during culturing and subculturing.

## RESULTS AND DISCUSSION

Organogenesis can be induced from the callus as reported by Chawla (2002). In the present investigation, calli derived from capitulum explant were used for shoot multiplication study with one control treatment and media combinations of BAP (1.0,2.0,3.0 mg l<sup>-1</sup>) with Kinetin (1.0 mg l<sup>-1</sup>) or Ads (75.0 and 80.0 mg l<sup>-1</sup>). All the BAP and Kinetin combinations and higher concentration combinations of BAP and Ads were found to be more effective in earlier shoot initiation, multiplication and formation of more number of leaves. Higher concentrations of BAP and Ads produced maximum number of multiple shoots per calli mass and more number of deep green leaves per multiple shoot. The results obtained in present study were similar to the results by Aswath and Choudhary (2001) where shoot organogenic capacity was higher with cytokinins. All the BAP with Kinetin combinations increased the shoot length. In tobacco, the presence of adenine or KIN in the medium leads to promotions of bud differentiation and development (Chawla, 2002). However, increased concentration of BAP increased the shoot length. But there was no shoot initiation found in MS medium alone without any plant bioregulators. So during first subculturing same combinations of plant bioregulators were used except the control (MS medium alone).

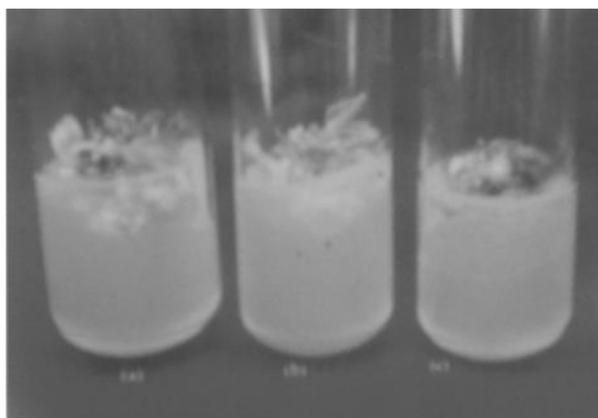
During first subculturing, the same trend was observed in BAP with Ads and BAP with Kinetin combinations. Maximum multiple shoots per single shoot and more number of deep green leaves were observed in MS medium fortified with medium concentration of BAP and higher concentration of Ads. In potato addition of Ads led to shoot proliferation from potato sprouts as reported by Beura (2002) and in tobacco, application of adenine promoted bud differentiation and development



**Plate 1.** Field view of gerbera cv. Common Red planting.



**Plate 2.** cv. Common Red in floriferous condition.



**Plate 3.** Multiple shoot regeneration from callus after 32 days of culturing

- MS +BAP 2.0 mg l<sup>-1</sup> +Ads 80.0 mg l<sup>-1</sup>
- MS +BAP 3.0 mg/l +Ads 75.0 mg l<sup>-1</sup>
- MS + BAP 1.0 mg l<sup>-1</sup> +Ads 80.0 mg l<sup>-1</sup>



**Plate 4.** Shorter shoot regeneration in MS + BAP 2.0 mg l<sup>-1</sup> + Ads 80.0 mg l<sup>-1</sup>



**Plate 5.** Longer shoot regeneration in MS + BAP 3.0 mg l<sup>-1</sup> + Kinetin 1.0 mg l<sup>-1</sup>

(Chawla, 2002). However, longer shoots were produced with BAP and Kinetin combinations, being maximum with higher concentration of BAP during first subculturing of capitulum derived single shoot. The ability of cytokinins to promote the growths of dicotyledons has been reported by Murashige (1974). BAP with Ads induced shoot organogenesis (Plate 3) from callus of the capitulum explants. Addition of BAP proliferated maximum shoots was reported by Barbosa *et al.* (1993) and Modh *et al.* (2002). The multiple shoot formation was more in BAP with Ads supplemented media than in Kinetin. The highest number of probable shoots during culturing and subculturing was 36.00. Number of approximate probable shoots per capitulum explant (1/4<sup>th</sup> part of capitulum explant) could be 576.00 produced in MS media having 2.0 mg l<sup>-1</sup> BAP and 80.0 mg l<sup>-1</sup> Ads. BAP along with Ads, 2ip and GA<sub>3</sub> were used for production of higher number of multiple shoots per explants in shoot tip explants of gerbera but, the shoot length was reduced (Aswath and Choudhary, 2001). Shailaja *et al.* 2004 reported that the suppression of apical dominance leads to the production of more number of multiple shoots with reduced shoot length.

**Table 2.** Efficacy of BAP, Adenine sulphate and Kinetin on shoot initiation, proliferation and multiplication, and leaf formation on callus derived from capitulum explants during culturing

Basal media-MS				Duration-35days			
Treatment	Treatments (mg l <sup>-1</sup> )			Shoot initiation (days)	Shoot proliferation (days)	Shoot multiplication (days)	Total number of days for leaf formation
	BAP	Ads	Kinetin				
T <sub>1</sub>	–	–	–	–	–	–	–
T <sub>2</sub>	1.0	–	1.0	15.33	31.33	32.33	24.00
T <sub>3</sub>	2.0	–	1.0	15.00	26.67	31.67	24.67
T <sub>4</sub>	3.0	–	1.0	15.33	25.00	30.00	22.33
T <sub>5</sub>	1.0	75.0	–	24.33	26.67	32.00	33.33
T <sub>6</sub>	2.0	75.0	–	22.67	25.67	32.00	30.00
T <sub>7</sub>	3.0	75.0	–	20.00	21.67	30.67	27.00
T <sub>8</sub>	1.0	80.0	–	22.33	25.00	30.67	27.67
T <sub>9</sub>	2.0	80.0	–	15.33	23.33	30.00	23.33
T <sub>10</sub>	3.0	80.0	–	18.00	20.33	31.33	25.67
SE(m)±1.5				0.38	0.67	0.62	0.72
CD (P=0.05)				1.11	1.96	1.82	2.09

**Table 3.** Efficacy of BAP, Adenine sulphate and Kinetin on shoot proliferation of callus derived from capitulum explants during culturing

Basal media-MS				Duration-35days			
Treatment	Treatments (mg l <sup>-1</sup> )			Number of multiple shoots/ calli mass	Shoot length (cm)	Total number of leaves/ multiple shoot(s)	Leaf color
	BAP	Ads	Kinetin				
T <sub>1</sub>	1.0	–	1.0	1.00	2.10	3.33	Pale green
T <sub>2</sub>	2.0	–	1.0	1.33	2.46	5.00	Pale green
T <sub>3</sub>	3.0	–	1.0	1.00	2.59	5.33	Pale green
T <sub>4</sub>	1.0	75.0	–	3.00	1.14	7.67	Deep green
T <sub>5</sub>	2.0	75.0	–	4.00	1.25	9.00	Deep green
T <sub>6</sub>	3.0	75.0	–	5.67	1.38	10.67	Deep green
T <sub>7</sub>	1.0	80.0	–	4.67	1.27	12.33	Deep green
T <sub>8</sub>	2.0	80.0	–	6.00	1.60	15.00	Deep green
T <sub>9</sub>	3.0	80.0	–	4.67	1.52	14.67	Deep green
SE (m)±1.5				0.22	0.02	0.27	
CD (P=0.05)				0.64	0.08	0.79	

**Table 4.** Efficacy of BAP, Adenine sulphate and Kinetin on multiple shoot production during 1<sup>st</sup> subculturing of capitulum explants

Basal media-MS				Duration-20days			
Treatment	Treatments (mg l <sup>-1</sup> )			Number of multiple shoots/ single shoot	Shoot length (cm)	Total number of leaves/ multiple shoot(s)	Leaf color
	BAP	Ads	Kinetin				
T <sub>1</sub>	1.0	–	1.0	2.00	6.10	5.00	Pale green
T <sub>2</sub>	2.0	–	1.0	1.67	6.73	6.33	Pale green
T <sub>3</sub>	3.0	–	1.0	1.00	7.10	6.33	Pale green
T <sub>4</sub>	1.0	75.0	–	4.67	4.03	10.67	Deep green
T <sub>5</sub>	2.0	75.0	–	5.00	4.17	12.00	Deep green
T <sub>6</sub>	3.0	75.0	–	6.00	4.17	12.67	Deep green
T <sub>7</sub>	1.0	80.0	–	5.00	3.17	15.33	Deep green
T <sub>8</sub>	2.0	80.0	–	6.00	5.11	16.67	Deep green
T <sub>9</sub>	3.0	80.0	–	5.33	4.50	15.67	Deep green
SE(m)±1.5				0.19	0.12	0.29	
CD (P=0.05)				0.55	0.34	0.85	

**Table 5.** Efficacy of BAP ,Adenine sulphate and Kinetin on multiple shoot regeneration of capitulum explants

Basal media-MS				Duration-170 days	
Treatment	Treatments (mg l <sup>-1</sup> )			Total number of multiple shoots during culturing and subculturing	Probable number of approximate shoots/ capitulum (1/4 <sup>th</sup> part of capitulum)
	BAP	Ads	Kinetin		
T <sub>1</sub>	1.0	-	1.0	2.00	32.00
T <sub>2</sub>	2.0	-	1.0	2.33	37.33
T <sub>3</sub>	3.0	-	1.0	1.00	16.00
T <sub>4</sub>	1.0	75.0	-	13.00	208.00
T <sub>5</sub>	2.0	75.0	-	20.00	320.00
T <sub>6</sub>	3.0	75.0	-	32.00	544.00
T <sub>7</sub>	1.0	80.0	-	23.00	373.33
T <sub>8</sub>	2.0	80.0	-	36.00	576.00
T <sub>9</sub>	3.0	80.0	-	25.00	400.00
SE(m)±1.5				1.37	21.92
CD (P=0.05)				3.98	63.72

In capitulum explants, MS medium with BAP (1.0, 2.0, 3.0 mg/l<sup>-1</sup>) and Kinetin (1.0 mg l<sup>-1</sup>) were the best combinations for, early shoot initiation and production of maximum number of longer shoots (2.59 cm) in BAP (3.0 mg/lit) with Kinetin (1.0 mg l<sup>-1</sup>) during culturing. MS medium fortified with BAP

(2.0 mg l<sup>-1</sup>) and Ads (80.0 mg l<sup>-1</sup>) was the best combination for production of maximum multiple shoots per calli mass (6.00) with maximum number of leaves (15.00). During subculturing, MS medium supplied with (3.0 mg l<sup>-1</sup>) BAP and (1.0 mg l<sup>-1</sup>) Kinetin were the best combinations for production

of longer shoots (7.10 cm). Moreover BAP (2.0 mg  $l^{-1}$ ) with Ads (80.0 mg  $l^{-1}$ ) was the most suitable combination for production of maximum number of multiple shoots (6.00) per single shoot with maximum number of leaves (16.67) per multiple shoot. MS medium supplemented with 2.0 mg  $l^{-1}$  BAP and 80.0 mg  $l^{-1}$  Ads was the best treatment for production of maximum number of multiple shoots (36.00) during culturing and subculturing in capitulum explants. The probable number of approximate shoots per capitulum explant (1/4<sup>th</sup> part of the capitulum) was 576.00.

### CONCLUSION

On the basis of the findings of the present study it is concluded that a local cultivar of gerbera plant can produce approximately 576 number of shoots/capitulum explants within a period of less than 6 months, in contrast to the conventional methods which produces only 5-10 suckers per year. Production of a huge lot of plants is an impossible task in *in vivo* conditions in such a short period of time.

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# Effect of drought management practices on Rice-Horse gram cropping sequence in rainfed upland

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## ABSTRACT

A field experiment was conducted to study the effect of date of sowing and in upland water use efficiency of rice-horse gram cropping sequence under rain fed upland conditions. The experiment was laid out in split-plot design for rice and split-split plot design for horse gram with three replications at the Central Research Station, OUAT, Bhubaneswar during June 2005 to January 2006. Four combinations of two dates of sowing (18 June and 4 July) and two early maturing (80-95 days) rice varieties (ZHU-XI-26 and Vandana) were allotted to the main-plots and five drought management practices (Broadcasting of non treated seeds, Line sowing 4% KCl treated seeds or non treated seeds, Line sowing 4% KCl treated seeds or non treated seeds with 1/4 of N and K out of recommended dose was top dressed after dryspell) in rice to the sub plots. Two liming treatments in horse gram i.e., no lime and 0.2 LR were allotted to the sub-sub plots. Grain yield of rice was reduced by 20.8% delayed sowing by 16 days from 18 June to 4 July. Total uptake of nutrients (57 kg N, 11 kg P and 106 kg K ha<sup>-1</sup>). Horse gram crop sown after harvest of 18 June sown ZHU-XI-26 produced maximum seed yield (1569 kg ha<sup>-1</sup>) and removed the maximum quantity of nutrients (96 kg N, 11 kg P and 61 kg K ha<sup>-1</sup>). Application of paper mill sludge in line (0.2 LR) marginally improved seed yield of horse gram.

## INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most ancient crops being cultivated in 117 countries, hence called as “Global Grain”. It is the main item of the diet of 3.5 billion people (Vivek *et al.*, 2004, Bashir *et al.*, 2010). In India it is grown over an area of 44.1 m ha with a total production of 105m t and a productivity of 2393 kg ha<sup>-1</sup> (Anonymous, 2012). A number of factors like soil moisture stress, delayed sowing, heavy weed infestation, poor native soil fertility status and poor spread of improved upland cultivars have been identified as important constraints in realization of enhanced productivity levels under rainfed upland situations (Mishra,1999).

The defining feature of rainfed upland rice ecosystem is the lack of ponded water at any time during the life cycle, so soils remain aerobic throughout where establishment of rice is risky

(Wade, 2003). The major problems limiting productivity of upland rice are moisture stress, weed infestation and nutrient deficiency (IRCN, 1985). Among these, drought induced moisture stress has been identified as the major yield depressant of upland rice crop in Eastern India which constitutes about 85% of total upland rice area in India (Widawsky and O’Toole, 1996). About 70% of upland rice area in India are drought prone (Singh, 2002). Productivity of the crop fluctuates drastically from year to year due to vagaries of south-west monsoon, occurrence of dry spells and moisture deficit during growing season. Occurrence of dry spell of varying intensity at different growth stages of upland rice resulting in even complete failure is not uncommon. But the ill effects of drought is not equal throughout the life cycle of the plant. Reproductive development of rice is known to be highly vulnerable to water deficit (O’Toole and Moya, 1981; Saini and Westgate, 2000). Keeping

in view the nature of crop, soil and weather conditions, various crop management practices can be integrated together to minimize the adverse effect of moisture stress on upland rice.

Manipulation of sowing time may help the crop to avoid the coincidence of stress at critical period. The crop should be sown in optimum time as that sown too early usually encounters with initial stress, whereas, the delayed sown one suffers much from terminal drought (Behera *et al.*, 1997; Mohapatra *et al.*, 1997). Most of the upland rice soil are deficient in nitrogen. Moisture deficit in upland conditions also prevents rice plant from making full use of applied nitrogen (Gupta and O'Toole, 1986). On the other hand, crop fertilized with over dose of N suffers more from moisture stress than that with normal dose (Arogen and De Datta, 1982). Split application of potassium is beneficial in leaching prone upland soils which may help plants to tolerate stress because of its osmoregulatory property (Das and Zaidi, 2002). So selection of suitable variety, time and method of sowing, seed treatment and timing of application of nitrogen and potassium may help upland rice crop to alleviate the adverse effect of moisture stress whose occurrence during the cropping season is most unpredictable. Liming an acid soil encourages root growth with the added advantages of water and nutrient availability (Bear, 1964). Horse gram crop grown on residual soil moisture has been benefited by residual effect of liming to preceding *Kharif* maize in acid soils (Pradhan and Mishra, 1982). Keeping in this view, a field experiment was conducted at the Central Research Station, OUAT, Bhubaneswar during 2005-06 with the following objectives:

- To study the occurrence of drought in upland rice sown on different dates
- To identify suitable rice variety and crop management practices to mitigate drought
- To study the performance of horse gram with or without liming in rice-horse gram cropping sequence.

#### MATERIALS AND METHODS

Field experiment was carried out during 2005-06 at the Central Research Station, OUAT,

Bhubaneswar. The soil of this plot was loamy sand in texture, poor in nutrient content and low in water holding capacity, slightly acidic in reaction (pH-5.20), low in organic carbon (0.36), available in nitrogen ( $544 \text{ kg ha}^{-1}$ ) in available phosphorus ( $15.60 \text{ kg ha}^{-1}$ ), in available potassium ( $126.70 \text{ kg ha}^{-1}$ ). The experiment was conducted in a split-plot design with three replications, four combinations of two early varieties of rice (ZHU-XI-26 and Vandana) sown in two different dates (18 June and 4 July) were allotted to the main plots and five drought management practices (Broadcasting non treated seeds, line sowing non treated seeds, line sowing 4% KCl treated seeds, line sowing non treated seeds but 1/4 of N and K out of recommended dose was top dressed after dry spell, line sowing 4% KCl treated seeds but 1/4 of N and K out of recommended dose was top dressed after dryspell) in rice sub plots. Two levels of lime treatments (no lime and 0.2 LR) to horse gram following rice were allotted to the sub sub-plots.

Required quantity of seeds as per M3 and M5 treatments were soaked with 4% (analytical grade) solution @1 litre/kg of seeds for 12 hours. The treated seeds were then air dried under shade for five days before sowing. As per the treatments the treated or untreated seeds were sown broadcast or in lines made at 15cm apart. The recommended seed rate of  $100 \text{ kg ha}^{-1}$  was used for the experiment. Horse gram crop was line sown after harvest of rice in different dates at a spacing of 30 cm using a seed rate of  $30 \text{ kg ha}^{-1}$ .

Recommended fertilizer for upland rice was 40 kg N, 20 kg  $\text{P}_2\text{O}_5$  and 20 kg  $\text{K}_2\text{O}$   $\text{ha}^{-1}$  which were applied in the form of urea (46% N), single super phosphate (16%  $\text{P}_2\text{O}_5$ ) and murate of potash (60%  $\text{K}_2\text{O}$ ), respectively. Nitrogen was applied in three splits i.e., 1/4 as basal, 1/2 at 20 days after sowing (DAS) and 1/4 at panicle initiation (PI) stage. Phosphorous was applied as basal and potassium was applied in two splits i.e., 1/2 basal and 1/2 at PI stage. In M4 and M5 treatment 1/4 out of recommended N and K dose was top dressed after dry spell at 42 and 49 DAS of  $D_1$  and  $D_2$  crops respectively. The recommended fertilizer dose of horse gram i.e. 20:40:0 kg of N,  $\text{P}_2\text{O}_5$ ,  $\text{K}_2\text{O}$   $\text{ha}^{-1}$  was

applied as basal. Paper mill sludge (75% CaCO<sub>3</sub> equivalent) at the rate of 0.2 LR was applied in lines at the time of sowing of horse gram taking LR as 4.0 t ha<sup>-1</sup>.

Ten plants selected randomly from the net plot area in both rice and horse gram were used for post harvest studies.

## RESULTS AND DISCUSSION

### Yield of rice

#### Grain yield

The data reveal that delay in sowing by 16 days from 18 June to 4 July significantly decreased the grain yield of upland rice from 3425 to 2712 kg ha<sup>-1</sup>, the percentage reduction was 20.8. Grain yield of rice variety ZHU-XI-26 (3485 kg ha<sup>-1</sup>) was significantly higher than that of Vandana (2651 kg ha<sup>-1</sup>). These data are in a good harmony with those reported by Abou Khalifa *et al.* (2005), Song *et al.* (1990) and El-Khoby (2004). Different drought management practices like line sowing with or without seed treatment and split application of nitrogen and potassium after dry spell did not influence the grain yield of upland rice.

#### Straw yield

Straw yield of upland rice was influenced significantly by date of sowing and varieties. Earlier sowing on 18 June resulted in significantly higher straw yield (6671 kg ha<sup>-1</sup>) than late sowing on 4 July (5859 kg ha<sup>-1</sup>). Similarly, straw yield of rice variety ZHU-XI-26 (6736 kg ha<sup>-1</sup>) was significantly higher than that of Vandana (5795 kg ha<sup>-1</sup>). Straw yield was not influenced by various drought management practices.

#### Harvest index

The harvest index value of upland rice was significantly influenced by date of sowing and varieties. Earlier sowing on 18 June exhibited significantly higher harvest index (33.9%) than late sowing on 4 July (31.6%). Similarly, rice variety ZHU-XI-26 showed higher harvest index (34.1%) than Vandana (31.4%). Harvest index was not

significantly influenced by various drought management practices.

The yield declined, on an average, at a rate of 45 kg grains per a day delay in sowing beyond 18 June (following Rajo Sankranti). This might be due to significant decrease in ear bearing tillers by 36 per m<sup>2</sup> and number of filled spikelets per panicle by 10 in number due to delayed sowing. straw yield decreased only by 12 per cent due to decrease in almost all growth parameters towards the later stage. Several workers (Dinesh Chandra *et al.*, 1991; Behera *et al.*, 1997; Kebede, 2000; Khatua, 2002) have also reported decrease in grain yield of upland rice due to late sowing.

#### Yield of horse gram

Horse gram crop sown on 24 September following harvest on 18 June sown ZHU-XI-26 produced 27.6, 73.2 and 51.1 per cent higher seed yield than the delayed sowings on 1, 8 and 17 October, respectively. This was because of significantly longer plants (81 cm) bearing more number of branches (7.5) and pods (56) per plant with the earliest sown crop (Table 1), although the differences in pod length, seeds per pod and 1,000 seed weight almost similar. This is an agreement with findings of Nagaraju *et al.* (1995). The earliest sown crop has enjoyed higher amount of rainfall (135 mm) and minimum the air temperature was also above 20 degrees centigrade for up to one month of sowing. Plant length, branches and pods per plant of the crops sown after harvest of 4 July sown rice were the minimum because the crops experienced moisture stress from the very beginning as well as the withdrawal of monsoon from October itself caused their forced maturity reducing the duration by about two weeks in spite of low minimum air temperature beyond mid November. Several workers have also reported yield reduction in horse gram due to delayed sowing beyond September (Rafey *et al.*, 1988; Nagraju *et al.*, 1995; Tripathy, 2002).

Averaged over dates of sowing, seed yield of horse gram sown after harvest of ZHU-XI-26 was marginally (9%) higher than when sown after

**Table 1.** Effect of date sowing, variety and drought management practices on grain and straw yield and harvest index (HI) of upland rice

Particular	Yield (kg ha <sup>-1</sup> )		Harvest index (%)	Seed yield (kg ha <sup>-1</sup> )	Bhusa yield (kg ha <sup>-1</sup> )	Harvest index (%)
	Grain	Straw				
Date of sowing						
18 June	3425	6671	33.87	1400	2526	36.38
4 July	2712	5859	31.64	972	2012	33.02
SEm ±	56.2	214.9	0.577	13.3	47.9	0.531
CD (P=0.05)	194	743	2.00	46	166	1.84
Variety						
ZHU-XI-26	3485	6736	34.12	1237	2618	32.00
Vandana	2651	5795	31.40	1134	1920	37.39
SEm ±	56.2	214.9	0.577	13.3	47.9	0.531
CD (P=0.05)	194	743	2.00	46	166	1.84
Drought management						
M1	3094	6160	33.17	1213	2367	34.08
M2	3043	6404	32.20	1158	2317	33.73
M3	3061	6313	32.54	1171	2204	35.20
M4	3019	6330	32.39	1186	2270	34.50
M5	3125	6120	33.48	1201	2188	35.97
SEm ±	81.7	186.2	0.780	32.5	46.2	0.694
CD (P=0.05)	NS	NS	NS	NS	NS	NS

harvest of cv. Vandana because of non significant differences in almost yield attributes except pods per plant. This might be due to a difference of only a week between sowing dates of horse gram following harvest of two rice varieties sown on same date. That too, horse gram crop sown on 8 and 17 October following harvest of two rice varieties sown on 4 July practically germinated on the same date after receipt of a soaking rain on 19 October. The seed yield of 8 October sown horse gram crop was even less than that sown on 17 October after harvesting 4 July sown Vandana. Yield improvement in horse gram due to lime application was also marginal i.e. about only 53 kg ha<sup>-1</sup> due to cumulative effect of almost all growth and yield attributes though the differences were not significant. Dwivedi (1996) has also reported practically no effect of liming on acid tolerant crops like horse gram and ricebean. However, Pradhan and Mishra (1982) have reported that horse gram

crop was benefited even by the residual effect of paper mill sludge applied to the preceding maize crop in an acid soil. This has also induced better root development.

### Nutrient content and nutrient uptake of rice

#### *Nutrient content of rice*

The data in table-reveal that the average nutrients in grain and straw were of upland rice respectively, 1.018 and 0.486 per cent N, 0.235 and 0.072 per cent P and 0.334 and 1.719 percent K. delayed sowing invariable improved the nutrient contents of grain and straw except P content of grain. But the difference was significant only in case of N content straw, P content of grain and K content of straw. Similarly N, P and K contents of grains and straw of cv. Vandana were higher than those of ZHU-XI-26 except N content of grain and straw of upland rice were not influenced significantly by different drought management practices.



**Table 3.** Effect of date of sowing, variety and drought management practices on preceding rice and liming to horsegram on nutrient content, uptake and their harvest index in horse gram

Particular	Content (%)									Uptake (kg ha <sup>-1</sup> )									Nutrient harvest index (%)		
	N			P			K			N			P			K			N	P	K
	Seed	Bhusa	Seed	Bhusa	Seed	Bhusa	Seed	Bhusa	Seed	Bhusa	Seed	Bhusa	Seed	Bhusa	Seed	Bhusa	Seed	Bhusa			
Date of sowing (Rice)																					
18 June	3.703	1.533	0.304	0.241	0.573	1.814	46.62	34.88	81.50	3.83	5.48	9.31	7.23	41.40	48.63	57.77	41.88	15.43			
4 July	3.594	1.489	0.295	0.234	0.560	1.775	31.48	26.94	58.42	2.57	4.26	6.82	4.89	32.09	36.98	53.96	38.31	13.65			
SEM±	0.019	0.005	0.001	0.001	0.006	0.019	0.496	0.678	0.877	0.048	0.101	0.118	0.127	0.875	0.942	0.591	0.547	0.275			
CD (P=0.05)	0.066	0.017	0.004	0.003	NS	NS	1.72	2.35	3.03	0.17	0.35	0.41	0.44	3.30	3.26	2.04	1.89	0.95			
Variety (Rice)																					
ZHU-XI-26	3.647	1.506	0.301	0.239	0.571	0.810	40.87	35.64	76.51	3.36	5.66	9.02	6.40	42.47	49.16	53.04	37.26	13.05			
Vandana	3.650	1.516	0.297	0.236	0.562	0.779	37.23	26.18	63.41	3.03	4.08	7.11	5.72	30.73	36.45	58.69	42.93	16.03			
SEM±	0.019	0.005	0.001	0.001	0.006	0.019	0.496	0.480	0.877	0.008	0.101	0.118	0.127	0.875	0.942	0.591	0.547	0.275			
CD (P=0.05)	NS	NS	NS	NS	NS	NS	1.72	1.66	3.03	0.17	0.35	0.41	0.44	3.30	3.26	2.04	1.89	0.95			
Date x Variety (Rice)																					
D <sub>1</sub> V <sub>1</sub>	3.738	1.538	0.304	0.242	0.586	1.859	52.70	43.38	96.08	4.31	6.81	11.12	8.28	52.28	60.56	54.82	38.78	13.78			
D <sub>1</sub> V <sub>2</sub>	3.668	1.528	0.303	0.241	0.559	1.769	40.54	26.37	66.91	3.36	4.15	7.50	6.19	30.52	36.70	60.73	44.97	17.09			
D <sub>2</sub> V <sub>1</sub>	3.556	1.474	0.298	0.237	0.556	1.761	29.04	27.90	56.94	2.42	4.51	6.94	4.51	33.24	37.75	51.26	35.73	12.32			
D <sub>2</sub> V <sub>2</sub>	3.631	1.504	0.292	0.232	0.565	1.790	23.91	25.99	59.90	2.71	4.01	6.72	5.26	30.93	36.20	56.66	40.90	14.97			
SEM±	0.027	0.007	0.002	0.001	0.008	0.027	0.701	0.959	1.24	0.069	0.143	0.167	0.179	1.238	1.333	0.836	0.773	0.389			
CD (P=0.05)	0.093	0.023	NS	NS	NS	NS	2.43	NS	4.29	0.24	0.50	0.58	0.62	4.28	4.61	NS	NS	NS			
Drought management (Rice)																					
M <sub>1</sub>	3.570	1.483	0.299	0.237	0.568	1.797	39.23	31.66	70.89	3.21	5.01	8.22	6.19	38.30	44.49	55.23	39.48	14.16			
M <sub>2</sub>	3.661	1.525	0.302	0.240	0.578	1.830	38.40	31.96	70.36	3.16	5.01	8.17	6.07	38.31	44.38	54.75	39.09	13.97			
M <sub>3</sub>	3.726	1.537	0.291	0.231	0.571	1.811	39.15	30.49	69.64	3.08	4.62	7.70	6.06	36.00	42.06	56.42	40.59	14.86			
M <sub>4</sub>	3.665	1.515	0.303	0.241	0.557	1.767	38.89	30.69	69.58	3.21	4.94	8.15	5.96	36.25	42.20	55.72	39.88	14.44			
M <sub>5</sub>	3.619	1.496	0.301	0.239	0.558	1.768	39.56	29.76	69.32	3.32	4.78	8.09	6.03	34.86	40.89	57.23	41.44	15.28			
SEM±	0.037	0.015	0.003	0.002	0.006	0.019	1.042	0.670	1.32	0.092	0.110	0.157	0.173	0.826	0.874	0.729	0.737	0.386			
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	2.38	2.52	NS	NS	NS			
Liming (Horsegram)																					
No lime	3.644	1.510	0.296	0.235	0.570	1.806	38.20	31.76	69.96	3.09	4.97	8.06	5.96	38.08	44.04	54.51	38.79	13.87			
0.2 LR	3.652	1.513	0.302	0.240	0.563	1.783	39.89	30.06	69.96	3.30	4.37	8.07	6.16	35.41	41.56	57.23	41.40	15.21			
SEM±	0.016	0.007	0.002	0.001	0.004	0.013	0.549	0.663	0.899	0.050	0.114	0.136	0.085	0.849	0.874	0.582	0.577	0.312			
CD (P=0.05)	NS	NS	0.0	0.004	NS	NS	1.57	1.90	NS	0.14	NS	NS	NS	2.43	NS	1.66	1.65	0.89			

### ***Nutrient uptake of rice***

Data on uptake of N, P and K in grains and straw of upland rice shows that the crop sown on 18 June removed 56.6 kg N, 11.1 kg P and 105.7 kg K ha<sup>-1</sup> at harvest as against the corresponding values of 49.4, 8.9 and 96.1 kg ha<sup>-1</sup> by the crop sown on 4 July. Total uptake as well as uptake in grains of all the nutrients by the earlier sown crop were more than 4 July sowing. Rice cv. ZHU-XI-26 removed 58.3 kg N, 11.0 kg P and 106.3 kg K ha<sup>-1</sup> which were 22.1, 20.3 and 11.4 per cent higher than those by Vandana, respectively. But so far as nutrient uptake in grains and straw was concerned, the difference between two varieties was significant in case on N, P and K uptake of grains and K uptake of straw. Nutrient uptake was not significantly influenced by various drought management practices.

### ***Nutrient harvest index***

The proportion of total uptake of nutrients that was removed by grains, was influenced significantly by the dates of sowing and varieties. On an average, nutrient removal through grains of upland rice were 50.4, 61.4 and 8.7 per cent of the total uptake of N, P and K, respectively. Earlier sowing on 18 June improved the proportion of nutrient retention in grains as compared to 4 July. But the difference was not significant in case of nitrogen. Harvest indices for all the primary nutrients were higher in ZHU-XI-26 than Vanadna. Nutrient harvest index was not significantly influenced by various drought management practices.

Nutrient uptake by a crop is a product of its yield and nutrient content in grains and straw. Significantly higher grain and straw yield due to early sowing on 18 June resulted in higher uptake values of all primary nutrients in grain and straw. This is in agreement with the earlier findings of Khatua (2002). The non significant differences in uptake values through straw might be due to significant improvement in nutrient contents of straw in 4 July sown crop. Similarly, upland rice variety ZX-XI-26 with significantly higher grain and straw yield resulted in higher uptake values of N, P and K.

Higher root weight and volume associated with variety might have increased its foraging area resulting in more uptake of nutrients.

### **Nutrient content and nutrient uptake of horse gram**

#### ***Nutrient content***

Data in Table-3 revealed the average nutrient content in seed and bhusa of horse gram were respectively 3.648 and 1.511 per cent N, 0.299 and 0.238 per cent P and 0.566 and 1.795 per cent K. sowing horse gram after the harvest of 18 June sown upland rice invariably improved the nutrient contents of both seeds and bhusa. But the difference was not significant in case of K content. The nutrient content of seed and bhusa of horse gram were not influenced by varieties and drought management practices on preceding rice. Nutrient contents were also not influenced significantly by lime application except of date of sowing and variety of rice.

#### ***Nutrient uptake***

Data on uptake of N, P and K in seeds and bhusa of horse gram show that the crop sown after the harvest of 18 June sown upland rice removed 81.5 kg N, 9.3 kg P and 48.6 kg K ha<sup>-1</sup> at harvest as against the corresponding values of 58.4, 6.8 and 37.0 kg ha<sup>-1</sup> by the crop sown after the harvest of 4 July sown rice. The differences in uptake values due to dates of sowing were significant for all the nutrients both in case of seeds and bhusa. Horse gram sown after the harvest of rice variety ZHU-XI-26, on an average, removed 76.5 kg N, 9.0 kg P and 49.2 kg K ha<sup>-1</sup> which were 20.6, 26.9 and 34.9 per cent higher than those after harvest of Vandana. Horse gram sown after harvest of 18 June sown ZHU-XI-26 removed significantly the maximum amount of 96.1 kg N, 11.1 kg P and 60.6 kg K ha<sup>-1</sup>. Nutrient removal by the crops sown after harvest of 4 July sown ZHU-xi-26 or Vandana were at par with each other. Total uptake of N, P and K were not influenced by drought management practices in rice as well as lime application to horse gram. However, uptake values by seeds were favourably influenced by lime application.

### Nutrient harvest index

The total uptake of N, P and K by horse gram crop about 55.9, 40.1 and 14.5 percent respectively, were retained in the seeds. Nutrient harvest index values of horse gram sown after harvest of 18 June sown rice were more than those sown after 4 July sown rice. Sowing the crop after Vandana rice enhanced the nutrient harvest indices of all the nutrients as compared to ZHU-XI-26. Interaction effect of dates of sowing and varieties as well as drought management practices on preceding rice did not influence the nutrient harvest indices. But, harvest indices for all primary nutrients were higher with lime application than without lime.

Horse gram crop sown after the harvest of 18 June sown upland rice removed higher amount of primary nutrients than when sown following 4 July sown rice. All the nutrient contents were significantly higher in plant parts of earliest sown crop. Horse gram sown after harvest of rice cv. ZHU-XI-26 removed differences in their nutrients than cv Vandana mainly because its higher seed and bhusa yields although differences in their nutrient content were not significant. Liming to horse gram failed to favourably improve the total uptake of all primary nutrients. Nutrient contents except P were also not influenced significantly by lime application in a comparatively dry soil profile due to withdrawal of monsoon almost from October. Increased soil moisture is required for liming to be effective as it accelerates the reaction of lime and facilitates the equilibrium reaction to continue over time allowing greater volume of diluted solution in the surroundings (Bear, 1964).

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# Effect of temperature and relative humidity on incidence of rotting of potato tubers in storage condition

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## ABSTRACT

Studies were conducted on the influence of temperature and relative humidity in storage of potato tubers from the month of February to July in store house of All India Co-ordinated Potato Research Project at Central Farm, Orissa University of Agriculture and Technology for consecutively three years from 2006 to 2008. It was revealed that maximum and minimum temperature as well as minimum relative humidity had positive co-relation while maximum relative humidity had negative co-relation on rotting of potato tubers. However, the study on regression co-efficient indicated 57.5% contribution of these variables in relation to the rotting incidence in storage.

**Key words:** Rotting, potato tuber, storage, weather parameter, co-relation, regression.

## INTRODUCTION

In Odisha, potato is mainly cultivated in winter season. It is the most preferred vegetable of the people of the state round the year. Harvesting of the crop starts from last week of February and continues till the month of March. Potato is perishable in nature. Cold storage facilities are very much essential for storage of tubers. Seasonal production patterns and lack of adequate numbers of cold stores facilities result in market gluts. Most of the farmers compel to sell their produce soon after harvest. Less price at harvest results economic loss to them. Potato prices start increasing from the month of April. Some of the poor and marginal farmers mostly use indigenous storage practices to store their produce for a few months at ambient condition for their consumption and also to sell their produce. Losses due to sprouting and rotting are usually very high under these storage methods (Singh and Srivastav, 1983). The study herein was conducted consecutively for three years to study influence of temperature and relative humidity in rotting of stored potato tubers.

## MATERIALS AND METHODS

Potato crops of three varieties namely Kufri Chandramukhi, Kufri Ashoka and Kufri Jyoti were

raised during the year 2005-06, 2006-07 and 2007-08 at Central Farm of Orissa University of Agriculture and Technology, Bhubaneswar. Healthy seed tubers were planted on 30<sup>th</sup> November in each year and recommended practices were followed. Irrigation was stopped 10 days before harvesting and de-hauling was done. The crop was harvested 80 days after planting. After harvesting cut, deformed, inert materials, insect damaged and diseased tubers were removed from the lot. The potato tubers of three different potato varieties, i.e.  $V_1$  = Kufri Chandramukhi,  $V_2$  = Kufri Ashoka, and  $V_3$  = Kufri Jyoti were categorized into four grades according to weight i.e.  $S_1$  = 40 to 50 gm,  $S_2$  = 51 to 70 gm,  $S_3$  = 71 to 100 gm and  $S_4$  = 101 to 150 gm were used. Two hundred numbers of potato tubers with specified weight of each grade were kept in plastic crates under ambient condition of the experimental store of the All India Coordinated Research Project (AICRP). Each treatment was replicated five times. Periodical observations on per cent of rotting of potato tubers on the basis of different sizes were recorded at 7 days interval. The rotted tubers were sorted out after the observation to reduce the inoculum density by eliminating the source of infection and the rotting per centage was

recorded periodically and analyzed statistically to study influence of temperature and relative humidity in rotting of stored potato tubers from the month of last week of February to July. The tubers were de-sprouted twice during the study.

## RESULTS AND DISCUSSION

The experimental findings revealed that the temperature and relative humidity varied from minimum 18.87 to 26.82 °C to maximum 32.44 to 36.34 °C and the relative humidity varied from minimum 37.40 to 73.86% to maximum 85.45 to 93.20% prevailed during the study period, presented in Table 1. There was 2.55% of rotting of tubers after harvest was recorded in the month of February which increased up to 5.13% in the month of March coinciding the maximum storage temperature of 34.38 °C and minimum temperature of 22.14 °C with maximum relative humidity of 90.40% and minimum relative humidity of 47.67%. There was gradual increase in rotting of stored tubers from the month of February up to the month of July. On the other hand, maximum average temperature increased from 33.2 °C in February to 36.02 °C in May after which it remained around 32 °C during subsequent months. Similarly, the minimum average temperature increased from 18.87 °C in February to 26.82 °C in June. It is interesting to note that the minimum monthly average temperature increased by more than 5 °C during May in comparison to April.

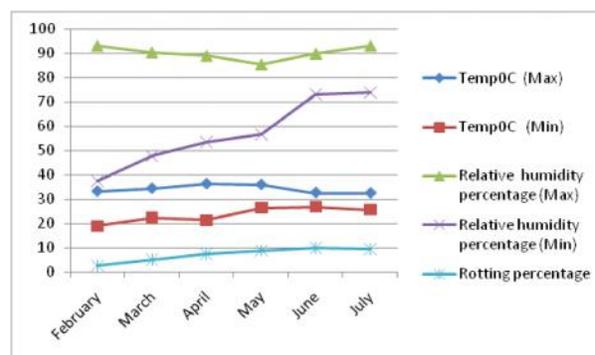
The maximum relative humidity remained above 85% during the entire period of the study. However, minimum relative humidity remained below 73%

during the entire period of the study. However, minimum relative humidity increased from 37.4% during the month of February to 73.86% in the month of July.

The percentage of rotting was shown in increasing order from the month of February to July (Fig.1).

The correlation between the rotting with that of minimum and maximum temperature as well as the minimum and maximum relative humidity was analyzed statistically and depicted in Table 2.

Maximum temperature and minimum temperature influenced rotting of potato tuber significantly having positive relationship. On the other hand maximum relative humidity had negative relationship with rotting while minimum relative humidity had positive relationship with rotting behavior of potato (Meheta and Ezekiel, 2010).



**Fig. 1.** Rotting percentage of potato tubers at different months in storage over three years.

**Table 1.** Incidence of rotting of potato tuber in storage during different months at ambient condition (Avg. of 2006, 2007 and 2008)

Month	Temp°C (Max)	Temp°C (Min)	Relative humidity percentage (Max)	Relative humidity percentage (Min)	Rotting percentage
February	33.20	18.87	93.19	37.40	2.55
March	34.38	22.14	90.40	47.67	5.13
April	36.34	21.28	89.03	53.34	7.46
May	36.02	26.57	85.45	56.65	8.88
June	32.62	26.82	89.95	72.93	9.92
July	32.44	25.66	93.20	73.86	9.35

**Table 2.** Correlation half matrix on storage rot incidence of potato at store house

	ROT	Maximum Temperature	Minimum Temperature	Maximum Relative Humidity	Minimum Relative Humidity
ROT	1.000				
Maximum Temperature	0.151	1.000			
Minimum Temperature	0.662	-0.276	1.000		
Maximum Relative Humidity	-0.413	-0.754	-0.248	1.000	
Minimum Relative Humidity	0.415	-0.588	0.824	0.225	1.000



**Fig. 2.** Ideal size for storage of potatos.



**Fig. 3.** Kufri Chandramukhi variety of potato tubers.



**Fig. 4.** Kufri Ashoka variety of potato tubers.



**Fig. 5.** Kufri Jyoti variety of potato tubers.

From the regression analysis, the linear relationship between the rotting percentage with maximum, minimum temperature and relative humidity was found out with following equation.

$$\text{Rotting} = -85.66 + 1.00 \text{ Max Temp} + 1.12 \text{ Min Temp} + 0.38 \text{ Max RH} - 0.04 \text{ M in RH}$$

$$R^2 = 0.575$$

Increase in maximum temperature contributed 41.67 % towards rotting. On the other hand, increase in minimum temperature contributed 52.25 % towards rotting of potato tubers in storage. Maximum relative humidity contributed 06.00 % and minimum relative humidity contributed less than 1% towards rotting of potato tubers. Further, the four variables together contribute to 57.5 % rotting of potato tubers during storage.

Weather parameters also influenced bacterial wilt, early blight and late blight disease of potato in field condition had already been reported (Biswal

*et al.*, 2003; Bambawale and Bedi, 1982; Behera *et al.*, 2009; Cook *et al.* and Biswas *et al.*, 2013). The rotting of potato in storage under ambient condition and also in cold stores had also studied previously (Bhutani and Khurana, 2005 and Patel *et al.*, 2005; Mathur *et al.*, 1952). The effect of temperature and humidity in potato storage as well as in agriculture produce have been studied by different workers in Europe (Doeswijk *et al.*, 2006; Keesman *et al.*, 2003 and Lukasse *et al.*, 2007).

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# Fish diversity and physico-chemical characteristics of rivers of Bhadrak district, Odisha

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## ABSTRACT

A systematic checklist of freshwater fishes of Bhadrak district of Odisha is provided with a note on physico-chemical characteristics of rivers of the district. A total number of 72 species under 51 genera, 27 families and 8 orders have been recorded. Highest species diversity was observed in Cyprinidae (31.9%) followed by Bagridae (12.5%). The fish fauna includes 1 vulnerable (VU), 4 near threatened (NT), 53 least concern (LC) and 2 data deficient (DD) as per IUCN. The present finding indicates that Bhadrak district is blessed with diverse fish fauna including numerous economically important food fishes and indigenous ornamental fishes. The water quality of the river is considered suitable for fish culture and wildlife propagation.

**Key Words:** Fish diversity, indigenous ornamental fishes, Physico-chemical characteristics, Bhadrak, Odisha

## INTRODUCTION

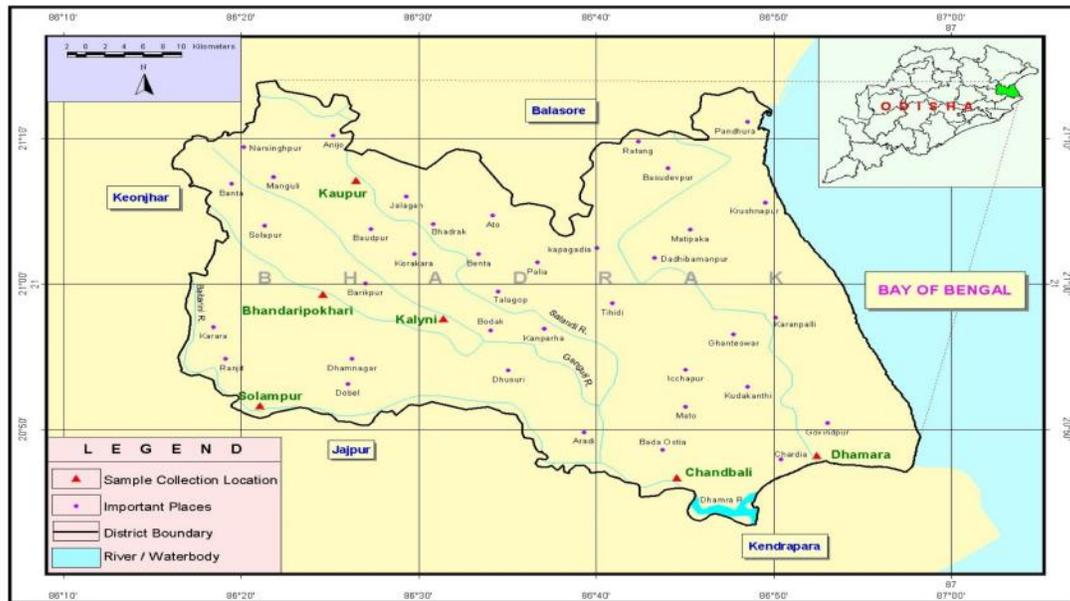
Bhadrak is one of the coastal districts of Odisha which has a total area of 2,505 sq. km. The district lies between 21.0500° N latitude and 86.5167° E longitude. Bhadrak is surrounded by Balasore district in the north, Keonjhar district in the west, Jajpur and Kendrapara in the south and Bay of Bengal in the east (Anonymous, 2010). It is blessed with numerous rivers namely, Salandi, Baitarani, Brahmani, Kansabansa, Gamol, Mantei, Genguti, Kochila, Reba and Kapali. Out of which the Baitarini, Brahmani and Salandi are the major rivers which passes through the district. The average rainfall in the district is 1451.6 mm. The Bhitarkanika National Park is situated in this district on the deltaic region of Baitarini, Brahmani and Salandi rivers. The people in the sea coast areas like Dhamara, Chudamani etc. mainly depend upon fishing for their livelihood (Anonymous, 2013)

The first ever study on marine and freshwater fishes of Odisha were made by Day (1869). Later,

the rich and varied fish fauna of Chilika lake comprising fresh, brackish and marine element has been studied mainly by Chaudury (1916a, 1916b, 1917, 1923), Hora (1923), Menon (1961), Rao (1995). Venkateswarlu *et al.* (1998) studied the fishes of the Mahanadi estuary. However, no detail investigation has been taken up so far on the fish diversity of Bhadrak district of Odisha. In the present study an attempt has been made to study the fish diversity and physico-chemical characteristics of rivers of Bhadrak district of Odisha.

## MATERIALS AND METHODS

Fishes and water samples were collected on quarterly basis from Baitarini, Brahmani and Salandi rivers at 6 stations namely Kaupur, Kalyani, Solampur, Bhandari pokhari, Chandbali and Dhamara of Bhadrak district, Odisha during 2008-2009 (Fig. 1). Fishes were preserved in 10% formalin and identified following Jayaram (1999), Talwar and Jingran (1991) and Talwar and Kacker



**Fig. 1.** Map of Bhadrak district, Odisha showing sampling stations.

(1984). The families have been arranged phylogenetically and species under a genus followed alphabetic sequence. The correct scientific name with local name, abundance and conservation status based on IUCN (2012) are shown against each species. The physico-chemical parameters were analyzed following standard methods APHA (1989) and Trivedi and Goel (1986). The average of four samples for each parameters studied was considered as one reading. The water temperature, dissolved oxygen, pH, were determined in the field and inorganic phosphorus was analyzed in the research laboratory of Central Institute of Freshwater Aquaculture, Bhubaneswar within 48 hours of collection.

## RESULTS AND DISCUSSION

A list of the fish fauna of the Bhadrak district of Odisha recorded during the present survey is represented in Table 1. A total of 72 species of fish belonging to 51 genera and 27 families were identified. Highest diversity was observed in Cypriniformes (26 species, 17 genera and 3 families) followed by Siluriformes (20 species, 13 genera and 7 families), Perciformes (16 species, 12 genus and 11 families), Synbranchiformes (4

species, 3 genera and 2 families), Beloniformes (2 species, 2 genera and 1 family), Osteoglossiformes (2 species, 2 genera and 1 family), Syprinidontiformes (1 species, 1 genera and 1 family), and Pluronectiformes (1 species, 1 genera and 1 family). Among the families highest species diversity was observed in the Cyprinidae (31.9%) followed by Bagridae (12.5%). Out of the 72 species, 20 species namely *Notopterus notopterus*, *Chitala chitala*, *Cirrhinus reba*, *Labeo bata*, *Labeo calbasu*, *Labeo dero*, *Labeo rohita*, *Osteobrama vigorsii*, *Sperata aor*, *Sperata seenghala*, *Rita rita*, *Wallago attu*, *Pangassius pangassius*, *Clarias batrachus*., *Heteropneustes fossilis*, *Anabas testudineus*, *Etroplus suratensis* *Liza tade*, *Channa striata*, were identified as commercially important food fishes which may be cultured *in-situ* as well as *ex-situ* in the water bodies of the district. In the same time, 21 species namely *Barilius berna*, *Barilius bendelisis*, *Barilius vagra*, *Danio rerio*, *Esomus danricus*, *Garra mullya*, *Puntius ticto*, *Puntius sophore*, *Rasbora daniconius*, *Acanthocobitis botia*, *Schistura denisoni dayi*, *Lepidocephalichthys guntea*, *Chaca chaca*, *Apolocheilus panchax*, *Chanda nama*, *Terapon*

**Table 1.** A checklist of fishes known from the Bhadrak district of Odisha with their local name and IUCN status

Scientific Name	Local name	IUCN Status	Remarks
Order: Osteoglossiformes			
<b>Family: Notopteridae</b>			
1. <i>Notopterus notopterus</i> (Pallas)	Fali	LC	Rare
2. <i>Chitala chitala</i> (Hamilton)	Chitala	NT	Rare
Order : Cypriniformes			
<b>Family: Cyprinidae</b>			
3. <i>Amblypharyngodon mola</i> (Hamilton)	Pathari,	LC	Abundant
4. <i>Aspidoparia morar</i> (Hamilton)	Bayi	LC	Rare
5. <i>Barilius barna</i> (Hamilton)	Bahari	LC	Abundant
6. <i>Barilius bendelisis</i> (Hamilton)	Bahari	LC	Abundant
7. <i>Barilius vagra</i> (Hamilton)	Jhalli	LC	Abundant
8. <i>Danio rerio</i> (Hamilton)	Dumala	LC	Abundant
9. <i>Cirrhinus reba</i> (Hamilton)	Mirk	LC	Abundant
10. <i>Devario aequipinnatus</i> (McClelland)	Hubaland	LC	Rare
11. <i>Esomus danricus</i> (Hamilton)	Kulia	LC	Rare
12. <i>Garra mullya</i> (Sykes)	Patharachata	LC	Abundant
13. <i>Labeo bata</i> (Hamilton)	Pohala	LC	Rare
14. <i>Labeo boga</i> (Hamilton)	Pohala	LC	Abundant
15. <i>Labeo calbasu</i> (Hamilton)	Kalabainsi	LC	Most abundant
16. <i>Labeo dero</i> (Hamilton)	Laya	LC	Abundant
17. <i>Labeo fimbriatus</i> (Bloch)	Pedusi	NA	Abundant
18. <i>Labeo rohita</i> (Hamilton)	Rohi	LC	Abundant
19. <i>Laubuca fasciata</i> (Silas)	Jadda	VU	Rare
20. <i>Osteobrama vigorsii</i> (Sykes)	Chilanti	LC	Abundant
21. <i>Puntius sophore</i> (Hamilton)	Sema	LC	Abundant
22. <i>P. ticto</i> (Hamilton)	Patiakerandi	LC	Most abundant
23. <i>Puntius</i> sp.	Kujikerandi		Rare
24. <i>Rasbora daniconius</i> (Hamilton)	Kerandi	LC	Most abundant
25. <i>Salmophasia bacaila</i> (Hamilton)	Kerandi	LC	Abundant
Family: <b>Balitoridae</b>			
26. <i>Acanthocobitis botia</i> (Hamilton)	Gentu	LC	Abundant
27. <i>Schistura denisoni dayi</i> (Hora)	Bali gentu	LC	Rare
Family: <b>Cobitidae</b>			
28. <i>Lepidocephalichthys guntea</i> (Hamilton)	Jimani	LC	Most abundant
Order: Siluriformes			
Family: <b>Bagridae</b>			
29. <i>Sperata aor</i> (Hamilton)	Singla	LC	Rare
30. <i>S. seenghala</i> Sykes	Singhi	LC	Rare
31. <i>Mystus bleekeri</i> (Day)	Kujikantia	LC	Rare
32. <i>M. cavasius</i> (Hamilton)	Baikantia	LC	Abundant
33. <i>M. gulio</i> (Hamilton)	Kantia	LC	Rare

34.	<i>M. vittatus</i> (Bloch)	Kantia	LC	Rare
35.	<i>M. Menoda</i> (Hamilton)	Guggah	NA	Rare
36.	<i>Rita chrysea</i> Day	Rita	NA	Abundant
37.	<i>R. rita</i> (Hamilton)	Rita macha	LC	Abundant
Family: <b>Siluridae</b>				
38.	<i>Ompok bimaculatus</i> (Bloch)	Pabda	NT	Rare
39.	<i>Wallago attu</i> (Bloch & Schneider)	Balhia	NT	Rare
Family: <b>Schilbeidae</b>				
40.	<i>Ailia coila</i> (Hamilton)	Putuli	NT	Abundant
41.	<i>Clupisoma garua</i> (Hamilton)	Gujri	LC	Rare
42.	<i>Eutropiichthys murius</i> (Hamilton)	Mur	LC	Abundant
43.	<i>Eutropiichthys vacha</i> (Hamilton)	Muribacha	LC	Rare
44.	<i>Pseudeutropius atherinoides</i> (Bloch)	Bopatasi	NA	Abundant
Family: <b>Pangasiidae</b>				
45.	<i>Pangassius pangassius</i> (Hamilton)	Pangas	LC	Abundant
Family: <b>Clariidae</b>				
46.	<i>Clarias</i> sp.	Magur	LC	Rare
Family: <b>Heteropneustidae</b>				
47.	<i>Heteropneustes fossilis</i> (Bloch)	Singi	LC	Rare
Family: <b>Chacidae</b>				
48.	<i>Chaca chaca</i> (Hamilton)	Chaka	LC	Abundant
Order: Beloniformes				
Family: <b>Belonidae</b>				
49.	<i>Strongylura strongylura</i> (van Hasselt)	Gania	NA	Rare
50.	<i>Xenentodon cancila</i> (Hamilton)	Bagania	LC	Rare
Order: Cyprinodontiformes				
Family: <b>Aplocheilidae</b>				
51.	<i>Aplocheilus panchax</i> Hamilton	Dandikiri	LC	Abundant
Order: Synbranchiformes				
Family: <b>Synbranchidae</b>				
52.	<i>Monopterusuchia</i> (Hamilton)	Cuchia	LC	Rare
Order: Perciformes				
Family: <b>Ambassidae</b>				
53.	<i>Ambassis gymnocephalus</i> (Lacepède)	Chandu	LC	Abundant
54.	<i>Chanda nama</i> Hamilton	Guachopi	LC	Abundant
Family: <b>Teraponidae</b>				
55.	<i>Terapon jarbua</i> (Forsskål)	Kunkuni	NA	Abundant
Family: <b>Scatophagidae</b>				
56.	<i>Scatophagus argus</i> (Linnaeus)	Kalileep	NA	Rare
Family: <b>Nandidae</b>				
57.	<i>Nandus nandus</i> (Hamilton)	Khasia	LC	Rare
Family: <b>Badidae</b>				
58.	<i>Badis badis</i> (Hamilton)	Kalamacha	LC	Abundant
Family: <b>Cichlidae</b>				

59. <i>Etroplus suratensis</i> (Bloch)	Kundala	NA	Rare
Family: <b>Mugilidae</b>		-	
60. <i>Liza tade</i> (Forsskål)	Megi	NA	Abundant
Family: <b>Gobiidae</b>			
61. <i>Glossogobius giuris</i> (Hamilton)	Baligarada	LC	Abundant
Family: <b>Anabantidae</b>			
62. <i>Anabas cobojius</i> (Hamilton)	Kau	DD	Rare
63. <i>Anabas testudineus</i> (Bloch)	Rajakau	DD	Rare
Family: <b>Belontiidae</b>			
64. <i>Trichogaster fasciata</i> Bloch & Schneider	Raja kau	LC	Abundant
Family: <b>Channidae</b>			
65. <i>Channa marulius</i> (Hamilton)	Chenga	LC	Abundant
66. <i>Channa punctata</i> (Bloch)	Sahala	LC	Rare
67. <i>Channa striata</i> (Bloch)	Gadisa	LC	Rare
68. <i>Channa</i> sp.	Seola	NA	Abundant
Family: <b>Mastacembelidae</b>			
69. <i>Macrognathus aculeatus</i> (Bloch)	Balitodi	NA	Rare
70. <i>Macrognathus pancalus</i> (Hamilton)	Todi	LC	Rare
71. <i>Mastacembelus armatus</i> (Lacepède)	Gomitodi	LC	Rare
Ord: Pleuronectiformes			
Family: <b>Cynoglossidae</b>			
72. <i>Cynoglossus puncticeps</i> (Richardson)	Patamacha	LC	Rare

LC= Least Concern; NT = Near Threatened; VU= Vulnerable; EN=Endangered; DD = Data Deficient

*jarbua*, *Badis badis*, *Etrophus suratensis*, *Scatophagus argus*, *Nandus nandus*, *Trichogaster fasciata* were identified as ornamental fishes which may be exported for aquarium keeping. As per IUCN (2010) the fish fauna of the study area includes 1 vulnerable, 4 near threatened, 53 least concern, 2 data deficient and 14 not assessed.

The coastal portion of the Bhadrak especially Dhamara and Chandbali areas harbours abundantly brackish water species like, *Scatophagus argus*, *Terapon jarbua*, *Strongylura storgylura*, *Cyanoglossus puncticeps*, *Rita chrysea* etc. On the other hand, hill stream forms like *Acanthocobitis botia*, *Barilius vagra*, *Crossocheilus latius*, *Garra mullya*, *Schistura* spp. etc. were also recorded from Kaupur, Kalyani and Bhandaripokhori of the study area. Therefore, fauna of the district is a mixture of primary freshwater fishes, estuarine fishes and widely distributed forms.

The water bodies of Bhadrak district of Odisha are within the tolerance limits of class 'D' water prescribed by the ISI (1982) for fish culture and wild life propagation. The surface water temperature ranged from 20.8° to 34.2°C with an average value of 27.6°C. The pH value ranged from 6.9 to 8.5 with an average of 7.4. High concentration of dissolved oxygen was observed throughout the study period which ranged from 4.0 to 8.6 mg l<sup>-1</sup> with an average value of 7.8 mg l<sup>-1</sup>, which is within the permissible limit of ISI (1982). However, level of CO<sub>2</sub> was slightly high which ranged from 2.0 to 13.0 mg l<sup>-1</sup> with an average value of 7.4 mg l<sup>-1</sup>. Dissolved inorganic phosphate phosphorus varied from 0.001 to 0.09 mg l<sup>-1</sup>. with an average value of 0.017 mg l<sup>-1</sup>. The eutrophic nature of the water bodies may be attributed to inflow of fertilizers from the surrounding agricultural fields and human inferences.

The present finding indicates that Bhadrak district is blessed with diverse fish fauna including

numerous economically important food and ornamental fishes. The water quality of the rivers and streams of the district are not contaminated as the value of pH and dissolved oxygen(DO) are within the tolerance limit of class 'D' water prescribed for fish culture and wild life propagation. Therefore, attempts may be made to introduce the *in-situ* and *ex-situ* fish cultivation techniques for conservation and sustainable management of fish genetic resources.

### ACKNOWLEDGEMENT

Authors are grateful to the Director, Zoological Survey of India for allowing us to identify some of the species collected from Bhadrak district. Authors are also indebted to the Director, Central Institute of Freshwater Aquaculture, Bhubaneswar for extending support to undertake the research work.

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# Occurrences and distribution of Geographic Sea Hare, *Syphonota geographica*, Adams and Reeve 1850 in Pulicat Lake of Tamilnadu

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## ABSTRACTS

Geographic Sea hare, *Syphonota geographica* (Adams and Reeve, 1850) is a species of sea hare belongs to Opisthobranch molluscs in the family Aplysiidae. It is distributed in Indo-West Pacific oceans, off Western Australia, the Mediterranean, and Red seas. This sea hare resides in estuary, lagoons and bays of sandy substrates. Specimens of geographic sea hare, *Syphonota geographica*, were collected from Pulicat Lake along East Coast of India. The density distribution of sea hare was minimum at a depth of 1 m and maximum of 10 cm (13°33'57"N 80°10'29"E) from sandy soil substrate. Sea hare are considered as a low value catches in India and hence discarded or used for manure and fish feed preparation. However, they are known to possess novel compounds such as anti-cancer, anti-tumor and anti-viral drugs and therefore extensively used in the pharmacological industry in other parts of the world. A literature review on the distribution of this species revealed that this is the first report from Pulicat Lake along Bay of Bengal, East coast of India. A note on the morphological features of this specimen is detailed in the paper.

**Key Words:** Geographic Sea Hare, *Syphonota geographica*, distribution, Pulicat Lake, East coast of India.

## INTRODUCTION

Geographic Sea Hare, *Syphonota geographica* (Adams and Reeve, 1850) is a species of sea hare belongs to Opisthobranch gastropod molluscs in the family of Aplysiidae. This species is otherwise known as *Paraplysia geographica*. *Syphonota geographica* gets its name, the map-like nature of the color pattern on its body surface. This species is widely distributed in the Indo-West Pacific oceans, off Western Australia, and the Mediterranean, Red seas and South Andaman, India (Ramakrishna *et al.*, 2010).

Specimens of geographic sea hare, *S. geographica*, were collected from Pulicat Lake

along East Coast of India. The density distribution of sea hare was minimum at a depth of 1 m and maximum of 10 cm (13°33'57"N 80°10'29"E) from sandy soil. The species lives in lagoons, bays and estuaries on sandy substrates. It was available in lake mouth with a minimum recorded depth of occurrence was recorded to be 1m and a maximum recorded depth was 20 cm in Pulicat Lake. It is a herbivorous sea hare found also on sea weed, *Gracilaria verrucosa* beds.

This is a circumtropical sea hare which is found worldwide in warm temperate to tropical marine environments (Rudman, 1998). Kruczynski and Porter (1969) have listed North Carolina as the

northern limit of this species on the US east coast. In recent times, many workers studied opisthobranch fauna of India. Rajaganapathi *et al.* (2002) studied purification of anti-HIV protein from purple fluid of the sea hare, *Bursatella leachii* de Blainville from India. Sethi *et al.* (2012) reported the occurrence of sea slug, *Kalinga ornata* along the inshore waters of Bay of Bengal off Chennai coast. Sethi *et al.* (2013) reported on new record of the black-margined nudibranch, *Doriprismatica atromarginata* from the inshore waters of Bay of Bengal along Karaikal coast, and occurrence of Wedge Sea hare, *Dolabella auricularia* from Kayalpatinam, Gulf of Mannar, Tamil Nadu, India. (Sethi *et al.*, 2014). New Occurrences of side-gilled slugs, *Pleurobranchus mamillatus* off Tuticorin Coast, Tamil Nadu, India (Sethi *et al.*, 2014) new records of Nudibranch, *Dendrodoris atromaculata* from the Pulicat Lake along Bay of Bengal, Coromandal coast of India. First record of Ragged Sea Hare, *Bursatella leachii* Blainville, 1817 Opisthobranchia :Euopisthobranchia: Aplysiidae) in Pulicat lake, East Coast of India was reported (Sethi *et al.*, 2015). The present finding is the first record of the reported species from the Pulicat Lake along East coast of India.

## MATERIALS AND METHODS

While carrying out a survey on marine molluscs from the Pulicat lake, we have come across of *S. geographica* which was uncommon for this coast until now and the sea slug was caught approximately 3 km southeast off Pulicat lake (13°33'57"N 80°10'29"E) in a sea weed, *Gracilaria verrucosa* beds (Fig.1).

The specimen was identified as *Syphonata geographica*. This geographic sea hare was found in estuaries and tidal pools, and more on seaweed bed in Pulicat Lake (Fig. 1-2). It was available in lake mouth. Minimum recorded depth of occurrence was recorded to be 1m and a maximum recorded depth was 20 cm. It is a herbivorous sea hare found on sea weed *G. verrucosa* beds.

The collected specimens were preserved in 70% (v/v) ethanol and deposited in National Biodiversity

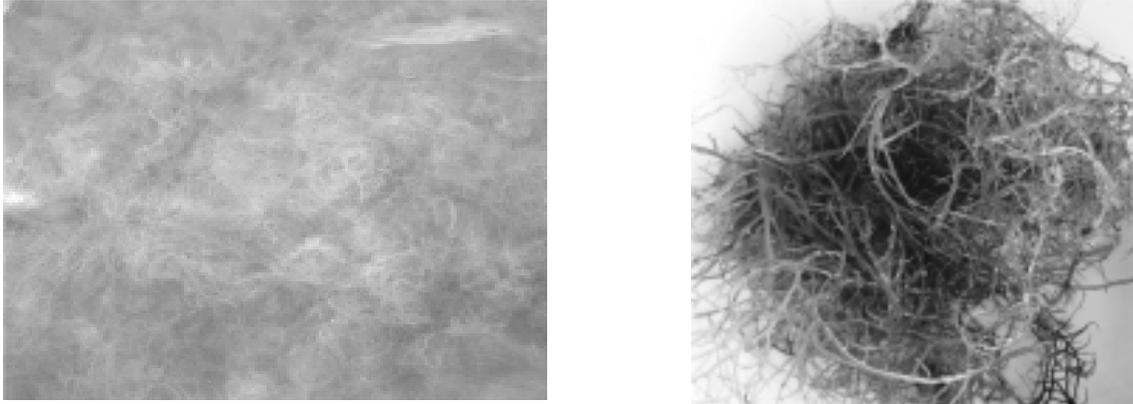
Referral Museum at CMFRI, Kochi. For photographic documentation and detailed study, Cyber-Shot Sony 16.2 Megapixel camera was used.

## RESULTS AND DISCUSSION

### SPECIES DESCRIPTION AND MORPHOMETRIC MEASUREMENT

*Syphonata geographica* is an opisthobranch with Family of Aplysiidae and Order Anaspide, the sea hares. This species differ from other Aplysiidae genera, through the position of the rhinophores (head tentacles position), which are further back, almost between the parapodial lobes. The parapodia fleshy wing like outgrowths, come to a noticeable high point at the top of the animal. The body coloration is whitish to green, with brown specks and a complicated network of white lines with tiny white spots forming patterns of stripes (hence the name termed as 'geographica'). Body is large, heavy and smooth. With two pairs of tentacles: one pair of oral tentacles forming flap at the front of the body. When compared with sea hares of the genus *Aplysia*, sea hares of the genus *Syphonota* group have relatively small rhinophores which are close together and situated further back from the head almost between the long 'wings' or parapodia. The maximum recorded length and weight was 70 mm and 27g respectively (Fig. 2-3).

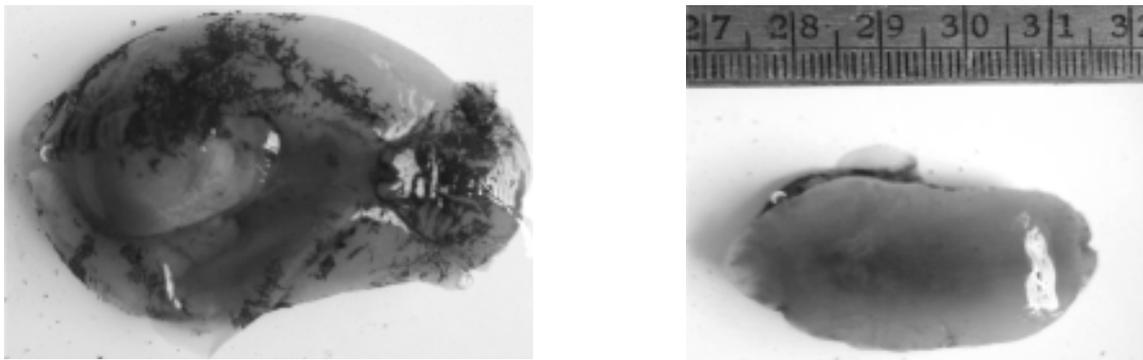
In India Sea hares are not used for domestic food consumption and therefore less popular. Rather the sea hare are considered as a low value fish by-catch and hence discarded or used for manure and fish feed preparation. However, they are well known to possess novel compounds such as anti-viral, anti-cancer, anti-tumor and antibiotics and therefore extensively used in the pharmacological industry world wide. Soblidotin (Dolastatin-10 derivative), Synthadotin/ILX651, Cemadotin, and Kahalalide F are marine natural anti-cancer compounds derived from sea slugs or sea hare which are under various stages of clinical trials (Haefner, 2003). Rajaganapathi *et al.* 2002 isolated an anti-HIV protein, Bursatellanin-P from the purple ink secretion of *B.leachii*, although it remains to be seen whether there will be any tangible biomedical



**Fig. 1.** Natural habitat of Geographic Sea Hare, *Syphonota geographica*, collected from the Pulicat Lake, Bay of Bengal along East coast of India. from *Gracilaria verrucosa* seaweeds beds.



**Fig. 2.** Live Geographic Sea Hare, *Syphonota geographica*, collected from the Pulicat Lake, Bay of Bengal along East coast of India.



**Fig. 3.** Dorsal and Ventral view of Geographic Sea Hare, *Syphonota geographica* from the Pulicat Lake, Bay of Bengal along East coast of India.

and economic benefits derived from this research works.

### ACKNOWLEDGEMENT

The authors are thankful to Dr.Gary Cobb, Sea Slug Forum, Australia and Dr.Deepak Apte, BNHS, Mumbai for providing necessary information and help in the validation of the specimen.

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# Rapid detection and therapeutic management of Parvo virus infected dogs

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## ABSTRACT

The present study was conducted for easy and rapid detection of canine parvo virus infection in dogs exhibiting typical signs of vomiting and hemorrhagic enteritis followed by prompt clinical management of positive cases. Rapid diagnosis using Canine Parvovirus Antigen (CPV) detection kit reported 11 positive cases out of 20 cases exhibiting similar symptoms of hemorrhagic gastroenteritis. The present cases were differentially diagnosed from that of canine corona virus and *Ancylostoma caninum* infections. The symptomatic treatment, includes intensive fluid therapy, intravenous antibiotic, antiemetic, systemic coagulant/haemocoagulase enzyme, vitamin B-complex, were found to be highly effective in treatment of canine parvovirus infection.

**Key words:** Canine parvovirus, clinical management, diagnosis.

## INTRODUCTION

Canine parvovirus, first appeared in 1978 (Kelly, 1978; Appel *et al.*, 1979) is an important emerging disease of dogs that mainly affects the intestinal tract and causes vomiting, diarrhoea, dehydration, anorexia and decreases the ability to fight against the infections (Legendre, 2000). Because of the severity of the disease and its rapid spread within canine population, CPV has aroused a great deal of public interest. The virus does not directly cause death; rather, it causes loss of the lining of the intestinal tract which in turn results in severe dehydration, electrolyte (sodium and potassium) imbalances, and bacteraemia. The clinical form of parvo virus infection may not manifest all the clinical signs and sometimes mimic other diseases such as canine corona virus infection, gastrointestinal ulcers, bacterial infections like *Campylobacter*, *Salmonella*, *Clostridium*, parasites such as whipworms, and hookworms. As a result, the diagnosis of parvovirus infection and clinical management of affected dogs

become difficult. Hence, the CPV infection should be differentiated from the above mentioned disease conditions and a prompt therapeutic management should be carried out. The positive confirmation of CPV infection requires the demonstration of the virus in the faeces or the detection of anti-CPV antibodies in the blood serum. A tentative diagnosis is often based on the presence of a reduced white blood cell count (leukopaenia). However, confirmative diagnosis is made on detection of the viral antigen in feces. Commercially available diagnostic test kits based on sandwich lateral flow immunochromatographic assay have aided a lot in diagnostic aspect, which allows rapid identification and treatment of animals infected with CPV. These tests are quite specific for CPV and relatively sensitive. In the present study attempts were made for rapid diagnosis of parvo virus infection by screening through antigen detection kit (Scanvet parvo, Intas, India) and prompt therapeutic management of the positive cases. Also attempts were made to differentially diagnose CPV from that

of canine corona virus and *Ancylostoma caninum* infections.

### MATERIALS AND METHODS

A total of 20 fecal samples were collected from dogs presented to Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, showing the clinical signs of vomiting and bloody diarrhoea (Fig. 1). The samples were screened for canine parvovirus infection along with corona virus and hook worm infection. Confirmatory diagnosis was based on canine parvovirus antigen detection kit (Scanvet parvo, Intas, India). Also screening of corona virus was conducted by canine corona virus antigen detection kit (Scanvet corona, Intas, India) and microscopic examination of faecal sample was done for screening of hook worm egg.

#### Test Procedure (CPV and CCV)

The faecal swab from the affected dogs was collected by a sterile swab provided with the kit. The swab was put in to the provided diluents buffer and then it was mixed properly. The cassette was placed horizontally and three drops of sample was put into the sample hole (S). The results were interpreted after 5-10 minutes.

*Interpretation:* If one red band appears in the control line with no apparent band in the test line (T), the sample in the above cases was considered negative for CPV (Fig. 2). In case two red bands appeared one in the control line (C) and other in test line (T) that was considered positive for CPV infection (Fig. 3). Similar procedure was followed for rapid detection of corona virus and results were interpreted as stated above (Fig. 4).

#### Clinical Management

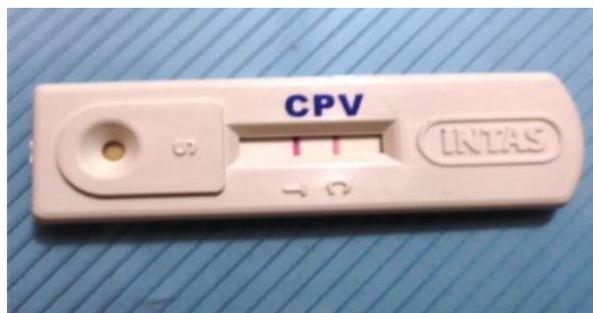
All the positive cases screened by antigen detection kit were treated with Ringer's lactate @ 50-60 ml kg<sup>-1</sup> bwt IV, Ceftriaxone @ 25 mg kg<sup>-1</sup> bwt, I/V, Metoclopramide @ 0.2 mg kg<sup>-1</sup> bwt, I/V, Ranitidine @ 2 mg kg<sup>-1</sup> bwt, I/M, and Haemocoagulase enzyme @ 0.5 ml IM along with B-complex twice daily for five to seven days. Dogs showing poor perfusion were supplemented with



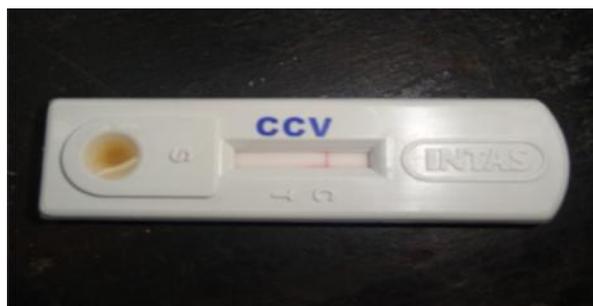
**Fig. 1.** Dog showing bloody diarrhoea.



**Fig. 2.** Canine parvo virus antigen detection kit showing -ve result.



**Fig. 3.** Canine parvo virus antigen detection kit showing +ve result.



**Fig. 4.** Canine corona virus antigen detection kit showing -ve result.

plasma expander with above treatment. Dextrose @ 0.5 mg kg<sup>-1</sup> bwt was given where severe hypoglycemia occurred.

### RESULTS

Out of 20 tested faecal samples 11 (55%) were found to be positive for CPV infection. All the samples were screened negative for corona virus infection and 10 samples were positive for *Ancylostoma caninum*. Interestingly two samples positive for parvo virus also found positive for *Ancylostoma caninum* infection. Single sample screened negative for all the three infections. All the information regarding the screening of various infections is presented in Table 1.

The treatment included intensive fluid therapy, antibiotic, antiemetic, antacid, plasma expander, dextrose, haemocoagulase enzyme and

supplementation of B-vitamins were found to be highly effective in CPV infection. After continuation of above treatment the dogs were recovered within five to seven days.

### DISCUSSION

In the present study the overall incidence of parvo virus was found to be 55% where as 50% cases screened positive for *Ancylostoma caninum* infection. All the suspected dogs screened negative for corona virus infection. The systematic approach in the elimination of the differential diagnosis is based on the fact that vomiting seen in case of CPV is not frequently observed in corona virus infection in dogs (Nwoha, 2011). This higher incidence of CPV can be correlated with the higher sensitivity and specificity of detection method.

The first step in treatment is to correct dehydration and electrolyte imbalances. This requires the administration of intravenous fluids containing electrolytes. Hence, Ringer's lactate and dextrose was included in the therapeutic regimen. This is in accordance with Foster and Smith (2011) who said that recovery of infected puppy is dependent on the effective and adequate fluid replacement. The lactated ringer's solution is better in replacing the lost electrolytes (Nwoha, 2011; Bargujar *et al.*, 2011). Lactate metabolizes in liver to bicarbonates that helps in correction of metabolic acidosis (Bargujar *et al.*, 2011). Administration of balanced electrolyte solution and dextrose were also suggested earlier as the fluid of choice in case of gastroenteritis (Singh *et al.*, 2000). In case of severe blood loss through diarrhoea plasma expanders should be included in the supportive therapy to make up the total blood volume and reduce the chances of hypovolemic shock. In the above study the infected dogs were treated with intravenous antibiotic therapy (Ceftriaxone). Antibiotics are given to prevent or control septicemia due to secondary bacterial complications. Metoclopramide was given as antiemetic to counter check vomiting (Bargujar *et al.*, 2011). Ranitidine is used as an anti histaminic drug that reduces the gastritis. Antispasmodic drugs are used to inhibit the diarrhoea and vomiting that perpetuate the

**Table 1.** Breed wise screening of various infections

Sample no.	Breed	CPV	CCV	<i>Ancylostoma caninum</i>
1	German shepherd	+ve	-ve	-ve
2	Deshi	+ve	-ve	+ve
3	Pug	+ve	-ve	+ve
4	Deshi	-ve	-ve	+ve
5	Deshi	-ve	-ve	+ve
6	Deshi	-ve	-ve	+ve
7	Dalmatians	-ve	-ve	+ve
8	German spitz	-ve	-ve	+ve
9	Labrador retriever	+ve	-ve	-ve
10	German shepherd	+ve	-ve	-ve
11	Deshi	+ve	-ve	-ve
12	Deshi	+ve	-ve	-ve
13	Golden retriever	-ve	-ve	+ve
14	German spitz	-ve	-ve	+ve
15	Deshi	+ve	-ve	-ve
16	Deshi	+ve	-ve	-ve
17	Deshi	+ve	-ve	-ve
18	German shepherd	+ve	-ve	-ve
19	Deshi	-ve	-ve	+ve
20	Doberman	-ve	-ve	-ve

problems. In case of CPV infection the blood clotting is adversely affected that contributes to severe blood loss in stool. Hence, this problem was successfully managed by administration of systemic coagulant or haemocoagulase enzyme. Iron dextrans can be given as a haematinic to boost replacement of blood loss. B complex was used as a good immune booster for quick recovery of the dog (Muley *et al.*, 2009).

### CONCLUSION

Parvoviral enteritis is an endemic disease in our environment because of its hardy nature and also newer strains of the virus. Therefore, dog owners and veterinarian should ensure the proper vaccination of dogs following the vaccine protocol. Along with this clinically suspected dogs can be screened by rapid antigen detection method followed by symptomatic therapy that will aid in early recovery of infected dogs.

### ACKNOWLEDGMENT

The authors are thankful to the Dean and Director Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Bhubaneswar for providing facilities to conduct the experiment.

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